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OBJECTIVE COUGH FREQUENCY, AIRWAY INFLAMMATION AND DISEASE CONTROL IN ASTHMA

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Abbreviations List

ACQ; Asthma Control Questionnaire, BHR; Bronchial hyper-responsiveness, BTS; British Thoracic Society, DRR; Dose-response ratio to inhaled methacholine, ECP; eosinophilic cationic protein, eNO; Exhaled nitric oxide, FEV_1; Forced expiratory volume in one second, $GABA_B$; gamma-aminobutyric acid, GINA; Global Initiative for Asthma, IL-8; interleukin-8, LTB_4; leukotriene B_4, LTC_4; leukotriene C_4, MPO; myeloperoxidase, PGE_2; prostaglandin E_2, 8-iso; 8-isoprostane, PD_{20}; the dose causing a 20% drop from baseline FEV_1 in response to inhaled methacholine, P2X3; purinergic receptor type 2 subunit X3, TRPV1; transient receptor potential type-1, TRPA1; transient receptor potential ankyrin type-1,

ABSTRACT

Background: Cough is recognised as an important troublesome symptom in the diagnosis and monitoring of asthma. Asthma control is thought to be determined by the degree of airway inflammation and hyper-responsiveness but how these relate to cough frequency is unclear.

Objective: To investigate the relationships between objective cough frequency, disease control, airflow obstruction and airway inflammation in asthma.

Methods: Participants with asthma underwent 24 hour ambulatory cough monitoring, exhaled nitric oxide, spirometry, methacholine challenge and sputum induction (cell counts and inflammatory mediator levels). Asthma control was assessed by GINA classification and the Asthma Control Questionnaire (ACQ).

Results: Eighty-nine subjects with asthma (mean age 57 years (±SD 12); 57% female) were recruited. According to GINA criteria, 18 (20.2%) patients were classified as controlled, 39 (43.8%) partly controlled and 32 (36%) uncontrolled; median (range) ACQ score was 1 (0.0-4.4). ACQ-6 correlated with 24hr cough frequency ($r=0.40$; $p<0.001$) and patients with uncontrolled asthma (GINA) had higher median 24hr cough frequency (4.2c/h, range 0.3-27.6) compared with partially controlled and controlled asthma (1.8c/h, range 0.2-25.3 and 1.7c/h range 0.3-6.7, $p=0.01$ and $p=0.002$ respectively). Measures of airway inflammation were not significantly different between GINA categories and were not correlated with ACQ. In multivariate analyses, increasing cough frequency and worsening FEV1 independently predicted measures of asthma control.

Conclusion: Ambulatory cough frequency monitoring provides an objective assessment of asthma symptoms that correlates with standard measures of asthma control, but not airflow obstruction or airway inflammation. Moreover, cough frequency and airflow obstruction represent independent dimensions of asthma control.

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways, estimated to affect 300 million people worldwide[1]. The aim of asthma treatment is to achieve and maintain control of the clinical manifestations of asthma; this includes current symptoms but also reducing the risk of future exacerbations. However despite an increase in our understanding of the mechanisms underlying asthma and the availability of effective treatments, optimal asthma control is often not achieved[2]. Several tools are currently in use for describing asthma control; a categorical classification based on expert opinion has been suggested by the Global Initiative for Asthma (GINA) and clinical trials have commonly used the Asthma Control Questionnaire (ACQ)[3] which gives a numerical score and is responsive to treatment[4-6]. These tools rely on patient recall of symptom frequency, severity, and medication use over several weeks. Such reporting is inevitably influenced by a range of external factors unrelated to asthma, for example, vigilance, mood, and social interactions, as well as memory. A more objective measure of asthma symptoms may therefore be a useful tool for assessing control and treatment responses.

Cough is an important symptom in asthma because it predicts disease severity[7, 8] , poor prognosis[9], and is a common[10] troublesome symptom[11]. Unlike wheezing, breathlessness and chest tightness, coughing is readily objectively quantified using ambulatory monitoring systems[12]. We have previously shown that objective cough counts are elevated in asthma patients compared with healthy controls, but are poorly represented by patient reports of cough[13]. However, it is not known whether the objective measurement of cough frequency is a useful marker of asthma control. Furthermore, it is unknown whether cough frequency is related to elements of asthma pathophysiology such as airflow obstruction and inflammation. Therefore, the aim of this study was to examine in a group of patients with asthma not selected for cough, the relationships between objective cough frequency, asthma control (GINA classification and ACQ) and measures of airway obstruction and inflammation.

MATERIALS AND METHODS

Subjects

We studied subjects with asthma recruited from a longitudinal cohort of adult asthma patients established in 1997[14, 15]. Inclusion criteria were: physician diagnosis of asthma, age ≥16 years, symptoms of asthma in the preceding 12 months and minimum treatment with a short-acting bronchodilator. Ethical approval was obtained from the regional Research Ethics Committee (06/Q1403/110) and subjects provided written informed consent.

Study Procedures

All subjects attended the North West Lung Research Centre, University Hospital of South Manchester on two occasions, at least one week apart and within a two week period. Prior to both visits subjects withheld medication as follows: short acting bronchodilators for six hours; long acting bronchodilators, theophyllines and leukotriene receptor antagonists for 12 hours; antihistamines and inhaled steroid for 48 hours.

At visit 1, subjects performed exhaled nitric oxide (eNO) (NIOX, Aerocrine, Sweden) followed by spirometry (Jaeger Viasys Healthcare, Germany) and reversibility of forced expiratory volume in one second (FEV_1) to 400µg salbutamol determined according to American Thoracic Society criteria[16]. Reversible airflow obstruction was defined by an increase in FEV_1 of ≥12%. Sputum was induced using 3%, 4% and 5% saline sequentially via an ultrasonic nebulizer (Sonix 2000, Clement Clarke, Harlow, UK). Samples were processed as previously described[17]. A total of 400 non-squamous cells were counted by a fully trained observer and were expressed both as a percentage and as a number of cells per gram of selected sputum. Competitive or sandwich ELISA's were performed to measure inflammatory mediators; leukotriene B_4 (LTB_4), prostaglandin E_2 (PGE_2) 8-isoprostane (8-iso) and leukotriene C_4 (LTC_4), interleukin-8 (IL-8), myeloperoxidase (MPO) and eosinophilic cationic protein (ECP). Subjects completed the Asthma Control

Questionnaire (ACQ-7)[3]. Subjects were categorised into levels of control according to GINA guidelines using information given in questionnaires, clinical history and pulmonary function. Finally, subjects underwent ambulatory 24 hour cough monitoring using the VitaloJAK cough recorder as previously described[18, 19].Recordings were analysed using an audio editing package (Audition 3.0, Adobe systems Inc., San Jose, CA). The number of cough sounds was manually counted and expressed as coughs per hour (c/h).

At visit 2, bronchial hyper-responsiveness (BHR) was assessed using the 5-breath dosimeter method (KoKo, Ferraris, Hertford, UK) according to published guidelines, and defined by the dose causing a 20% drop from baseline FEV_1 in response to methacholine (PD_{20})[20] . Skin Prick Testing was performed and atopy was defined by the presence of at least one positive skin prick test to common inhaled allergens (House dust mite, cat, dog, grass, mixed moulds)

Statistical Analysis

Data were analysed using SPSS Version 20.0 (IBM Corp., NY). Overall 24 hour and daytime cough rates were log transformed prior to analysis and geometric mean (95%CI) summary data given. Spearman rank correlations were used throughout. Non-parametric data were compared using the Mann- Whitney U test. Multinomial logistic regression and general linear models were used to investigate possible predictors of GINA control category and ACQ score respectively. Only parameters correlated with control in the univariate analysis ($p<0.1$) were included in the models and non-statistically significant variables were sequentially removed from each model to determine the model which explained the greatest proportion of variance in asthma control. As the ACQ-7 includes a score for % predicted FEV_1, (that would inevitably be correlated with the % predicted FEV_1), we opted to also use the ACQ-6 score for the general linear models, which only includes scores for symptoms and reliever medication use.

RESULTS

Subjects

We studied 89 subjects with asthma, summarised in Table 1. Out of 88 subjects who underwent reversibility testing (one subject with β_2-agonist intolerance not tested), 23 (26.1%) had bronchodilator reversibility, and 37 (50.7%) of 73 subjects tested had a positive methacholine challenge; testing was not carried out in 16 subjects (three subjects did not attend; one subject refused; two subjects had used a β_2-agonist and 10 subjects were below the FEV_1 safety cut-off for the test). A total of 45 subjects had bronchodilator reversibility and/or positive methacholine challenge, and 32 (44% of those tested) had neither. Subjects were evenly distributed between British Thoracic Society (BTS) Steps 1-4 (e-Table 1).

Asthma Control

According to GINA criteria, 18 (20.2%) patients were classified as controlled, 39 (43.8%) were partly controlled and 32 (36%) uncontrolled. GINA category was not influenced by gender but uncontrolled patients were older than partly controlled and controlled (62.4yrs (±9.0) versus 54.8(±12.1) and 54(±13.7), p=0.019). ACQ-7 data were available for 88 (98.9%) subjects [median (range) score 1 (0.0-4.4)]. There was also a correlation between ACQ score and age (r=0.22; p=0.04). Uncontrolled patients were treated with higher doses of ICS (p<0.001) and there was a positive correlation between ACQ score and ICS dose (r = 0.39, p<0.001). Smoking status did not influence measures of asthma control (ACQ score or GINA control).

Objective Cough Rates

Ambulatory cough monitoring was performed in 86 subjects. Night time data was missing in four patients due to battery failure. Cough rate varied widely between subjects [geometric mean (95% CI) 2.5 c/h (0.2-27.6)] and were higher by day than by night [median day (range) 3.7 c/h, (0.2 – 41.3) night 0.5 (0-29.6), p<0.001]. There were no gender differences in cough frequency (log 24hour cough rate), nor was this influenced by

age, BMI, FEV_1 percentage predicted, log eNO or dose-response ratio to methacholine. There was a tendency for the small number of current smokers to have a higher cough frequency than ex-smokers or non-smokers (p=0.09). Thirteen subjects were on ACE inhibitors but cough frequency was not raised in these patients (ACE inhibitor geometric mean 2.8c/h [95% CI 1.5-5.2] versus no ACE inhibitor 2.4c/h [1.8-3.2]); p=0.64).

Airway Inflammation

The geometric mean of eNO was 23.2 ppb (95% CI 19.9-27.1). There was a negative correlation between eNO and ICS dose (r=-0.29; p=0.007).

Induced sputum data were available for 55 (61.8%) subjects. The inter-observer agreement for counts of 400 non squamous cells were: eosinophil mean difference 3.3 cells (equivalent to 0.8% of total count; 95% CI -9.5 to 16.0), and neutrophil mean difference 7.6 cells (equivalent to 1.9% of total count; 95% CI -41.5 to 56.6). Geometric mean of percentage sputum eosinophils was 2.1% (95% CI 1.5-2.9) and a median $0.11x10^{-6}$ cells/g of sputum (IQR 0.01-$0.11x10^{-6}$). A total of 30 (54.5%) subjects had increased sputum eosinophils (i.e. ≥2%). Median percentage of sputum neutrophils was 68% (57.3-77.3) with 2.25 $x10^{-6}$ cells/g of sputum (IQR 0.76-2.96 $x10^{-6}$).

Levels of airway inflammatory mediators were measured in subjects with satisfactory cell counts, see e-Table 2. There were correlations between sputum ECP levels and eosinophil counts (percentage: r=0.43, p=0.002) as well as between sputum IL-8 and neutrophils (percentage: r=0.44, p=0.001). There were no significant correlations between objective cough frequency and sputum eosinophils or neutrophils (e-Table 3). For sputum inflammatory mediators only 8-isoprostane showed a correlation with objective cough frequency (e-Table 4).

Predictors of Asthma Control

GINA Categories

Cough frequency and FEV_1 significantly differed between GINA categories (p=0.003, p<0.001 respectively), see Figure 1A and B. Subjects in the uncontrolled group had higher cough rates than both partially controlled (p=0.01), and controlled (p=0.002). This was also the case for daytime cough counts, (p=0.004 and p=0.002) and nocturnal cough counts (p=0.004 and p=0.04). Subjects with uncontrolled asthma had greater airflow obstruction than both partly controlled (p=0.002) and controlled (p<0.001). Although sputum eosinophils increased with worsening asthma control, the differences between groups did not reach statistical significance (Figure 1D). There were no statistically significant differences in sputum neutrophils (% or cells $x10^6$/g sputum) (Figure 1C), levels of airway inflammatory mediators (e-Table 5), methacholine challenge or log eNO between GINA control categories.

In a multinomial logistic regression model with GINA control category as the outcome variable, higher log 24hr cough frequency (p<0.001) and lower % FEV1 (p<0.001) were independently predictive of GINA category. Numbers in each group were too small for odds ratios to be calculated.

Asthma Control Questionnaire (ACQ)

There were positive correlations between ACQ-6 and 24hr cough frequency (r=0.40; p<0.001) (see Figure 2), daytime cough rate, (r=0.40; p<0.001) and overnight cough rate (r=0.30; p=0.006). There was a correlation between ACQ-6 and logeNO (r=0.24, p=0.03) and borderline correlation with % predicted FEV_1 (r=-0.19, p=0.07). There were no statistically significant correlations between ACQ-6 and percentage sputum eosinophils/neutrophils (% or cells $x10^6$/g sputum) (see table 2), inflammatory mediators, or methacholine challenge (data not shown).

In the general linear model, log ACQ-6 was only significantly predicted by log 24hr cough rate (p=0.008) which accounted for 17% of the variance. When the model was repeated with ACQ-7 (including % predicted FEV_1) as the outcome variable, log total cough rate

(p=0.004) and FEV_1 percent predicted (p=0.001) independently predicted asthma control, accounting for 37% of the variance.

DISCUSSION

Until recently, few studies have sought to objectively quantify cough in patients with asthma. Our previous work found objective measures of cough were poorly represented by subjective patient reporting of cough severity and were also not related to standard measures of asthma such as FEV_1, methacholine responsiveness or eNO.[13] To the best of our knowledge, this study is the first to examine the relationships between objective cough frequency, asthma control, airflow obstruction, and airway inflammation. We have demonstrated that increasing cough rates are associated with worse asthma control as assessed by both the GINA criteria and the ACQ score. In addition, we found that objective cough frequency and airflow obstruction independently predicted disease control, whereas measures of airway inflammation did not.

Bronchial hyper-responsiveness (BHR) and airway inflammation are acknowledged as important components of asthma pathophysiology but how they relate to asthma control is less clear. Previously published data suggest BHR and inflammation do not discriminate well between different levels of asthma control[21, 22]. Furthermore, treatment algorithms which incorporate measures of sputum eosinophils[23, 24] or BHR [25] to titrate treatment, improved exacerbation rates and reduced airway re-modelling respectively, but failed to show improvements in asthma control. Similarly disappointing were the outcomes based on eNO algorithms when used as an adjunct or as an alternative to standard clinical management[26-28]; at best, only a reduction in the inhaled steroid dose was achieved. Together these observations suggest that there may be other important determinants of day to day asthma control.

This study is consistent with a cluster analysis of asthmatics[29] which showed some groups of asthmatics demonstrated discordance between airway inflammation and symptoms, i.e. high levels of symptoms despite low levels of eosinophilic inflammation (discordant symptoms) or high levels of eosinophilic inflammation but low expression of symptoms (discordant inflammation). Our data relating symptoms to inflammatory cells and mediators similarly failed to show any overall relationship, even when the symptom of cough was quantified objectively and for mediators with known tussive effects, e.g. PGE_2. This data would therefore suggest that inflammation does not directly trigger asthma symptoms and that other mechanisms may play an important role. Airway nerves are crucial to the development of symptoms such as cough, shortness of breath, chest tightness and pain. These symptoms are dependent on nerve activation and transmission to the central nervous system predominantly via vagal afferents, hence, these symptoms are blocked or inhibited by vagal anaesthesia or transection[30]. We speculate that neuronal dysfunction in asthma could be the missing link in explaining the discordance between airway inflammation and symptoms.

We set out to study a 'real life' asthma population and inclusion criteria for the study reflected this; patients had a physician diagnosis of asthma but were not selected for cough, reversibility or BHR as a pre-requisite. However, subjects were recruited from a previously established much larger ASMAL (*AS*sessment of *M*anchester *A*sthmatics *L*ongitudinally) cohort established in 1996 where over 88% had evidence of BHR at baseline[31]. Over 80% of our patients were established on inhaled corticosteroid treatment and the sensitivity of methacholine challenge in the diagnosis of asthma is reduced by taking this treatment[32]. Furthermore, compared with the tidal breathing method, the 5 breath dosimeter method may underestimate BHR due to the protective bronchodilator effect of deep breathing[33]. Finally, although this study found an association between objective cough frequency and asthma control, further interventional studies would be

required to confirm objective cough frequency is a useful and sensitive marker of improved control.

CONCLUSIONS

Data from this study suggest objective measurements of cough provide unique information about asthma control, not fully captured by ACQ or GINA questionnaires. Importantly cough frequency reflected asthma control independent of airflow obstruction and inflammation. The inclusion of measures of cough frequency in future asthma studies offers an objective measure of day to day control that may be better for assessing the impact of novel asthma therapies.

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Author's contributions: Concept and design; P.M, I.S, B.I, A.A.W, N.C.T, S.J.F, J.A.S. Data generation; P.M, B.I, L.Y, L.J, I.D. Statistical analysis and modelling; P.M, I.S and J.A.S. All authors reviewed the manuscript and approved the final draft.

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Other contributions: We thank Dr Elizabeth Juniper for permission to use the Asthma Control Questionnaire (ACQ) in this study.

Additional information: The e-Tables can be found in the Supplemental Materials section of the online article.

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Tables

Table 1 Subject Characteristics

Characteristic		Asthma Subjects (n=89)
Age, years*		57.3 (±11.9)
Gender, % Female		57.3
BMI, kg/m^2*		28.6 (±5.3)
Smoker, %	Never	52.8
	Ex	39.3
	Current	7.9
Pack Year History**		0.0 (0.0-67.5)
Asthma Duration. Years**		29.0 (10.0-68.0)
Atopy		83.5%
FEV_1, % predicted*		86.4 (±22.1)
ICS Dose, µg BDP**		800 (0-4000)

Data quoted as * mean (±SD); ** median (range)

411 **Table 2: Correlation between ACQ-6, cough rates and sputum cell counts**
412

Parameter			**ACQ-6**
Cough rate (coughs/hour, c/h)	Day		**r=0.40** **p<0.001**
	Night		**r=0.30** **p=0.006**
	Total		**r=0.40** **p<0.001**
Sputum cell counts	Eosinophil	%	r=0.12 p=0.37
		cells x10^6.g sputum	r=0.12 p=0.37
	Neutrophil	%	r=-0.10 p=0.45
		cells x 10^6/g sputum	r=-0.10 p=0.45

413 All correlations Spearman Rank

414

FIGURE LEGENDS

Figure 1: Comparison of controlled, partially controlled and uncontrolled asthma patients (GINA classification) A) Objective cough frequency over 24hrs; B) FEV_1 percent predicted; C) Percent sputum neutrophils and D) Percent sputum eosinophils.

Figure 2: Correlation between ACQ-6 score and total cough rate (c/hr)

SUPPLEMENTARY MATERIAL

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Abbreviations

ECP; eosinophilic cationic protein, ICS; inhaled corticosteroids, IL-8; interleukin-8, LTB_4; leukotriene B_4, LTC_4; leukotriene C_4, MPO; myeloperoxidase, PGE_2; prostaglandin E_2, 8-iso; 8-isoprostane

Supplementary Results

e-Table 1: Patient therapy according to British Thoracic Society Asthma Treatment Steps

BTS Treatment Step	1	Inhaled short-acting β_2-agonist as required	22.5%
	2	ICS 200-800 mcg/day	27.0%
	3	ICS plus inhaled long-acting β_2-agonist	24.7%
	4	ICS up to 2000 mcg/day and /or addition of a fourth drug e.g. leukotriene receptor antagonist, or slow-release theophylline, β_2-agonist tablet	24.7%
	5	Systemic corticosteroids	1.1%

e-Table 2: Sputum Inflammatory Mediator Levels in Asthma Subjects

Inflammatory Mediator in Sputum	Asthma
LTB_4 (pg/ml)	816.4 (240.4-6063.2) n=55
PGE_2 (pg/ml)	2331.0 (669.0-6032.0) n=55
8-Iso (pg/ml)	43.4 (17.8-373.6) n=55
LTC_4 (pg/ml)	447.2 (8.0-2624.0) n=55
IL-8 (pg/ml)	852.4 (94.8-4400.0) n=54
ECP (ng/ml)	101.2 (1.2-220.0) n=53
MPO (ng/ml)	118.7 (1.0-446.4) n=54

e-Table 3: Correlations between sputum cell types and objective cough rates in subjects with asthma.

Parameter		Daytime Cough Rate	Night time Cough Rate	Overall Cough Rate
Eosinophil	*%*	r=0.13 p=0.37	r=0.17 p=0.21	r=0.16 p=0.26
	cells x 10^{-6}/g sputum	r=0.12 p=0.41	r=0.19 p=0.16	r=0.14 p=0.31
Neutrophil	*%*	r=-0.10 p=0.47	r=-0.01 p=0.92	r=-0.09 p=0.50
	cells x 10^{-6}/g sputum	r=-0.07 p=0.63	r=0.10 p=0.47	r=-0.06 p=0.66

All correlations Spearman Rank

e-Table 4: Correlations between Cough Rates and Sputum Inflammatory Mediators in Asthma Subjects

Mediator	Daytime Cough Rate	Night time Cough Rate	Total Cough Rate
LTB_4	r=0.08 p=0.55	r=0.13 p=0.37	r=0.09 p=0.54
PGE_2	r=-0.19 p=0.17	r=-0.02 p=0.89	r=-0.19 p=0.17
8-iso	**r=0.35** **p=0.009**	r=0.04 p=0.76	**r=0.34** **p=0.01**
LTC_4	r=0.16 p=0.25	r=0.06 p=0.68	r=0.17 p=0.22
IL-8	r=-0.19 p=0.18	r=0.08 p=0.56	r=-0.18 p=0.20
ECP	r=0.12 p=0.40	r=0.23 p=0.10	r=0.12 p=0.39
MPO	r=-0.07 p=0.61	r=0.13 p=0.37	r=-0.07 p=0.63

All correlations Spearman Rank

e-Table 5: Sputum Inflammatory Mediators across different GINA Categories

Inflammatory Mediators in Sputum	GINA Control			P-value
	Controlled	Partly Controlled	Uncontrolled	
	Median (range)	Median (range)	Median (range)	
LTB4 (pg/ml)	**1030.6** (432-3280)	**970.4** (240.4-6732)	**718.8** (278.4-2805)	**0.437**
PGE2 (pg/ml)	**2235** (832-3827)	**2353.5** (103.4-5968)	**2288.5** (33.4-6032)	**0.681**
8-iso (pg/ml)	**45.3** (21.4-111.4)	**55.8** (18.8-700)	**59.9** (16.4-373.6)	**0.815**
LTC-4 (pg/ml)	**424.4** (75.6-1783)	**475.2** (8.0-2664)	**342.8** (38.0-2624)	**0.334**
IL-8 (pg/ml)	**685.2** (43.6-3532)	**522.4** (8.8-4400)	**946.3** (4.40-4400)	**0.116**
ECP (ng/ml)	**36.0** (4.4-170)	**53.60** (1.20-220)	**106.8** (4.8-220)	**0.163**
MPO (ng/ml)	**96.8** (23.2-260)	**87.75** (0.80-506)	**166.4** (4.30-446.4)	**0.863**