ERJ Express. Published on March 9, 2012 as doi: 10.1183/09031936.00228611

Airway impedance entropy and exacerbations in severe asthma

[1]Sherif Gonem*, [1]Imraan Umar*, [2]Daniel Burke, [1]Dhananjay Desai, [1]Steven Corkill, [2]John Owers-Bradley, [1]Christopher E Brightling, [1]Salman Siddiqui

[1]Institute for Lung Health, Department of Infection, Immunity & Inflammation, University of Leicester, United Kingdom

[2]School of Physics and Astronomy, University of Nottingham, Nottingham, United Kingdom

*Co-first authors

Address for correspondence:

Dr. Sherif Gonem

Respiratory Medicine

Glenfield Hospital

Leicester

LE3 9QP, UK

Tel: (+44) (0)116 250 2842

E-mail: sg330@le.ac.uk

Abstract

Background

Variability of peak flow measurements has been related to clinical outcomes in asthma. We hypothesized that the entropy, or information content, of airway impedance over short time scales may predict asthma exacerbation frequency.

Methods

Sixty-six patients with severe asthma and thirty healthy control subjects underwent impulse oscillometry at baseline, following a deep exhalation manoeuvre, and following bronchodilator administration. On each occasion, airway impedance parameters were measured at 0.2 second intervals for 150 seconds, yielding a time series, which was then subjected to Sample Entropy analysis.

Results

Airway impedance, and Sample Entropy of impedance, was increased in asthmatic patients compared to healthy controls. In a logistic regression model, Sample Entropy of R5-R20, a marker of the fluctuation of the heterogeneity of airway constriction over time, was the variable most strongly associated with the frequent exacerbation phenotype (odds ratio of 3.23 for every 0.1 increase in Sample Entropy).

Conclusion

Increased airway impedance and Sample Entropy of impedance is associated with the frequent exacerbation phenotype. Prospective studies are required to assess their predictive value.

Key words asthma, oscillometry, airflow obstruction, entropy

Introduction

Acute exacerbations of asthma account for much of the morbidity and mortality associated with the condition [1]. However, there is no currently available biomarker that can accurately predict the risk of future exacerbations. Previous studies have suggested that a geometrically self-similar airway tree may confer increased risk of asthma exacerbations and that fatal asthma is associated with a reduction in the structural complexity of the airway tree [2]. Similarly, the ventilation heterogeneity observed in asthma follows power law behaviour which predicts catastrophic closure of small airways [3]. Therefore, characterising structural complexity may have utility in predicting asthma exacerbations.

It has been speculated that the temporal variability in lung function may also exhibit self-similarity at multiple time scales [4]. This would suggest that monitoring lung function over short time-scales may provide insights into lung function variability over longer time-scale of weeks to months, thus providing a more practical predictive tool for exacerbations. A number of tools have been utilised to characterise time series properties of physiological signals, including those that predict scaling and power law behaviour of information over multiple time scales, and those that predict the probability of information repeating itself within a time series [5,6]. Fluctuations and power law behaviour observed in a time series of lung function measurements such as peak expiratory flow (PEF) may predict poor asthma control or exacerbations [7,8]. Thamrin *et al* [9] found that the degree of long-range correlation (self-similarity at different temporal length scales) in PEF measurements appeared to provide additional predictive information with respect to exacerbations in mild to moderate asthma, but less so in severe asthma.

The forced oscillation technique (FOT) [10] provides an ideal tool for measuring airway function over time, as it allows the respiratory system to be interrogated at a high temporal resolution by delivering forced oscillations to the airways and measuring the impedance (incorporating resistance and reactance components) of the respiratory system. Dynamics of FOT time series over very short time scales (e.g. minutes) may provide additional information that predicts the behaviour of the airways over longer time scales. For instance, Que *et al* plotted frequency distributions of the natural logarithm of respiratory system impedance (lnZrs), measured six times per second over a fifteen-minute period, and found that both the mean and standard deviation (SD) of lnZrs was higher in patients with asthma than in healthy controls. Furthermore, in healthy controls unloading of the airway smooth muscle (ASM) induced by adoption of a supine posture in conjunction with increased ASM activation induced by MCh challenge led to a significant increase in the SD of lnZrs, recapitulating the fluctuating behaviour observed in the asthmatic airway [11]. The authors concluded that asthma may be associated with not only generalised airway narrowing but also an increased appearance of statistically unlikely airway configurations.

Entropy, a measure of increased irregularity and statistically unlikely configurations, has been utilised to characterise a variety of physiological signals [12]. Entropy measurements give a statistical probability that a series of points within a physiological signal will repeat themselves at a subsequent time point within a given tolerance [13]. The entropy of FOT time series was recently investigated by Veiga *et al*, who found that the entropy of airflow time series was reduced in patients with asthma compared to healthy controls, and that reduced entropy was associated with

increased severity of airflow obstruction [14]. However, the possible associations between the entropy of airway impedance measurements and other clinically important patient-centred outcomes in asthma, including asthma control and exacerbations, have not been evaluated.

We hypothesised that:

i) Severe asthma is characterised by altered entropy of airway resistance and reactance.

ii) Entropy of impedance time series is related to exacerbation frequency in severe asthma.

Methods

Subjects

Sixty-six patients with severe asthma fulfilling the American Thoracic Society (ATS) criteria for asthma, and with no other respiratory disease, were recruited from Glenfield Hospital outpatients. Thirty-three patients were at Global Initiative for Asthma (GINA) treatment step 4, and thirty-three at GINA treatment step 5, as previously defined [15]. Thirty healthy control subjects with similar demographics were recruited from hospital staff and by local advertising. Healthy subjects had no history of respiratory disease, and had normal spirometry and methacholine responsiveness. All subjects were over the age of 18 years. The Leicestershire and

Rutland ethics committee approved the study and all subjects gave written informed consent.

Subject characterisation

Patients with asthma completed the Asthma Control Questionnaire (ACQ) and Asthma Quality of Life Questionnaire (AQLQ). The number of severe asthma exacerbations suffered in the previous year was recorded. Severe exacerbations were defined as an acute worsening of asthma symptoms requiring treatment with high-dose systemic corticosteroids for at least three days [16]. Spirometry was performed according to ATS / European Respiratory Society (ERS) guidelines [17]. In particular, long-acting bronchodilators were withheld for 12 hours prior to testing, and short-acting bronchodilators for 4 hours. Sputum induction and cell count was performed as previously described [18]. Exhaled nitric oxide concentration was measured with an expiratory flow rate of 50 ml/s ($FeNO_{50}$) using a chemiluminescence analyser (NIOX; Aerocrine, Stockholm, Sweden), according to ATS/ERS guidelines [19].

Measurement of respiratory impedance

Impedance testing was undertaken using a Jaeger MasterScreen Impulse Oscillometry (IOS) system (Viasys Healthcare GmbH, Hoechberg, Germany), according to standard guidelines [20]. IOS was performed at the 5-35 Hz frequency range, with impulses triggered every 0.2s for 150s. At each time point, the resistance (R) and reactance (X) components of impedance were recorded at multiple frequencies from 5 Hz to 35 Hz, thus yielding time series of each variable containing approximately 750 data points. IOS was performed both before and after the administration of inhaled salbutamol (400 micrograms), delivered via a metered-dose inhaler and spacer.

A variety of measures of airway calibre were derived from the mean values of the 750 data points captured (summarised in Table 1):

(i) The resistance at 20 Hz (R20), which we interpret as a measure of the mean level of airway constriction within the bronchial tree.

(ii) The resistance at 5 Hz minus resistance at 20 Hz (R5-R20), which we interpret as a measure of the heterogeneity of airway narrowing throughout the bronchial tree [21].

(iii) The area under the curve of the reactance spectrum between 5 Hz and resonant frequency (reactance area, AX), which we interpret as a measure of theheterogeneity of airway closure throughout the bronchial tree [22].

Examples of time series of R20 and R5-R20 at baseline are shown in Figures 1a-d, in a patient with severe asthma and a healthy control subject.

Sample entropy analysis

We used the Sample Entropy (SampEn) algorithm to evaluate complexity in the respiratory impedance time series, using a custom program downloaded from the PhysioNet online resource [23]. This measure relies on the identification of recurrent patterns within a non-stationary dynamic time series, as described in detail in the online supplement. Within a highly regular system, sequence matches are of greater frequency, implying lower entropy and less complexity, and vice versa. Sample entropy has emerged as a less biased metric of variability than the alternative measure approximate entropy (ApEn), and is relatively independent of record length since it does not incorporate self-matches within the time series into the calculation of

conditional probability [13]. We interpret SampEn of the core airway impedance markers (R20, R5-R20 and AX) to be a measure of fluctuation as a function of time (*see* Table 1). A higher value for SampEn equates to greater levels of temporal fluctuation, and vice versa. Further details may be found within the online supplement.

Statistical analysis

Statistical analysis was performed using GraphPad version 5 (Prism, San Diego, California, USA) and SPSS version 16.0 (SPSS Inc, Chicago, Illinois, USA). Colour maps were produced using Matlab R2007b (Mathworks Inc., Boston, Massachusetts, USA). Parametric data was expressed as mean (SEM), data that was log-normally distributed was log transformed and expressed as geometric mean (95% confidence interval), and non-parametric data was expressed as median (interquartile range). Unmatched groups were compared using one-way analysis of variance with Bonferroni's correction or Student's T test for normally distributed data, Chi-squared test or Fisher's exact test for ratios, Student's T test of log-transformed data for log-normally distributed data, and the Kruskal-Wallis test with Dunn's correction or Mann-Whitney U test for non-parametric data. Matched groups were compared using Friedman's test with Dunn's correction. A value of $p < 0.05$ was taken as the threshold for statistical significance. Spearman's correlation coefficient was utilised to determine the degree of correlation between impedance parameters, and principal components analysis with varimax rotation and Kaiser normalisation was utilised as a

data reduction tool. Logistic regression analysis was performed using block entry with all selected independent variables entered at the first step.

Results

Subject demographics

The clinical and demographic characteristics of the study population are shown in Table 2. The groups were well-matched for sex but differed significantly with respect to age and body mass index. However, there were no significant differences between the two asthmatic GINA 4 and GINA 5 cohorts.

Severe asthma is characterised by increased heterogeneous airway constriction and closure

Median values of heterogeneous airway constriction (R5-R20 [$kPaL^{-1}s$]) and closure (AX [$kPaL^{-1}$]) at baseline were significantly raised in both asthma groups compared to healthy controls, as shown in Table 3 (healthy = 0.035, GINA 4 asthma = 0.08 [$p < 0.01$ vs healthy], GINA 5 asthma = 0.14 [$p < 0.001$ vs healthy] for R5-R20; healthy = 0.33, GINA 4 asthma = 0.935 [$p < 0.05$ vs healthy], GINA 5 asthma = 1.8 [$p < 0.001$ vs healthy] for AX). The mean level of airway constriction (R20) was also raised in the asthma groups compared to controls, but this effect was much less pronounced and only reached statistical significance for healthy vs GINA 5 asthma. The GINA 4 and GINA 5 asthma groups did not differ significantly with respect to any parameter.

Severe asthma is characterised by increased fluctuation (entropy) of airway impedance

Results relating to the entropy of impedance measurements closely mirrored those of the impedance measurements themselves, as shown in Table 4. There was a progressive increase in the median baseline values of each parameter moving from the control to the GINA 4 asthma to the GINA 5 asthma groups. For instance, median SampEn of AX at baseline was 0.42 in controls, 1.05 in GINA 4 asthma and 1.19 in GINA 5 asthma ($p < 0.0001$). These observations suggest that the heterogeneity of both airway constriction and closure persists over short time scales in patients with asthma. In contrast the mean level of airway narrowing did not fluctuate significantly in patients with asthma when compared to healthy controls.

Airway impedance and entropy measures are associated with frequent exacerbations in severe asthma

The patients with asthma were divided into those who had infrequent exacerbations, defined as < 2 exacerbations during the previous year (n = 25), and those who had frequent exacerbations, defined as ≥ 2 exacerbations during the previous year (n = 41), as shown in Table 5. ,The two groups did not differ significantly with respect to age, GINA category, smoking history, duration of disease, post-bronchodilator forced expiratory volume in one second (FEV_1) (% pred.) or FEV_1/forced vital capacity (FVC) ratio, sputum eosinophil or neutrophil counts, $FeNO_{50}$ or AQLQ. However, frequent exacerbators were significantly more likely to be female than infrequent exacerbators, had a significantly increased mean body mass index (BMI), and had a higher mean ACQ score.

All six impedance and impedance entropy parameters were significantly raised at baseline in frequent exacerbators compared to infrequent exacerbators, as shown in Table 6. For example, median R5-R20 was 0.07 $kPaL^{-1}s$ in infrequent and 0.13 $kPaL^{-1}s$ in frequent exacerbators ($p = 0.0065$), while median SampEn of R5-R20 was 0.014 in infrequent and 0.114 in frequent exacerbators ($p = 0.0004$). However, these differences were much less pronounced following bronchodilator administration (data shown in Table E1 in the online supplement).

Sample entropy of R5-R20 is independently associated with frequent exacerbations in severe asthma

Close correlations were found between impedance and impedance entropy measurements, with Spearman's rho > 0.5 and $p < 0.01$ for every combination of parameters, as shown in Table E2 and Figure E4 in the online supplement. Principal components analysis of all the continuous variables that were associated with frequent exacerbations showed that most impedance and impedance entropy measurements loaded onto the same factor, as shown in Table E3 in the online supplement.

A logistic regression model was constructed with the presence or absence of frequent exacerbations, defined as ≥ 2 exacerbations within the previous year, as the dependent variable. Sex, BMI and ACQ score were entered into the model as independent variables, as well as one of the impedance or impedance entropy parameters. The most favourable model, chosen on the basis of the greatest predictive value in this dataset (data shown in Table E4 in the online supplement), was that incorporating SampEn of R5-R20. The parameters of this model, which correctly classified 74.2% of asthma patients as having frequent or infrequent exacerbations, are shown in Table

7. SampEn of R5-R20 was the only variable significantly associated with frequent exacerbations in this model ($p = 0.016$), with an odds ratio of 3.23 for every 0.1 increase in SampEn of R5-R20.

Discussion

We have shown for the first time that increased heterogeneity of airway constriction and closure (R5-R20 and AX), and increased fluctuation of the above biomarkers over time (SampEn), is associated with an exacerbation-prone phenotype in patients with severe asthma. This association appears to be strongest with SampEn of R5-R20, a putative marker of time-varying fluctuation of heterogeneous airway constriction. We have established that impedance time series entropy measurements are closely correlated with raw impedance values, suggesting that the raw values alone may provide useful prognostic information, but that time series analysis could be of additional value. Our data suggest that airway closure and narrowing (in particular, heterogeneous time fluctuation behaviour) identifies patients at the greatest risk of exacerbations. Indeed, a 0.1 increase in sample entropy of heterogeneous airway constriction (R5-R20) was associated with a 3.2 fold increase in the risk of an exacerbation. It has previously been shown that minimal heterogeneity of airway constriction can result in catastrophic shifts in ventilation to particular lung regions [24]. We speculate that the fluctuations in resistance and reactance we observed in patients with severe asthma represent such step-wise shifts in airway patency, occurring over a time-scale of seconds, and that patients who exhibit such fluctuations may be constantly on the cusp of asthma exacerbations. In such patients, small

perturbations in smooth muscle tone, for instance caused by minor allergen exposure or a viral upper respiratory tract infection, may result in life-threatening airway constriction and closure.

Significance of the impedance parameters R20, R5-R20 and AX

It has previously been shown that in severe asthma, the general level of airway resistance is raised, and that there is an additional increase in resistance at low oscillation frequencies [25]. This frequency dependence of resistance is thought to be due to heterogeneous airway constriction, since mathematical modelling indicates that even severe homogeneous constriction would produce an elevated baseline resistance but not frequency dependence [25]. Furthermore, an image-functional modelling approach has suggested that the ventilation defects and frequency-dependence of resistance and elastance seen in asthma can only be explained by small airway constriction, or a combination of large and small airway constriction [26]. In this study, we used R20 to represent general airway resistance and R5-R20 to represent frequency-dependence of resistance, a marker of heterogeneous airway constriction. Reactance area (AX)is thought to be a marker of airway closure, since such closure results in peripheral airway capacitive properties not being measured, thus increasing the effective elastance of the respiratory system [22].

Impedance and impedance entropy parameters in healthy subjects and patients with asthma

In line with previous studies [27,28], we found significant increases in R5-R20 and AX, and to a lesser extent R20, in patients with severe asthma compared to healthy

controls, suggesting that the baseline level of airway narrowing, and the accompanying heterogeneity of narrowing and closure, are increased in patients with severe asthma. We were unable to demonstrate a difference in these parameters between patients with severe asthma established on maintenance oral corticosteroids (GINA treatment step 5) and patients on high-dose inhaled corticosteroids (GINA treatment step 4), suggesting that these processes may be resistant to corticosteroid therapy.

The SampEn parameters were higher at baseline in the asthma groups than in the control group, although this only reached statistical significance for the GINA 5 vs control comparison. Since impedance and airflow are inversely related, our results are concordant with those of Veiga *et al* [14], who found that the entropy of airflow time series was lower in patients with asthma than in healthy controls. Close correlations were seen between impedance measurements and their respective SampEn parameters, as shown in Table E2 and Figure E4 in the online supplement, suggesting that entropy of airway impedance is strongly associated with baseline airway calibre.

Determinants of the exacerbation-prone asthma phenotype

In our cohort of patients with asthma, we found that those who had suffered two or more exacerbations in the previous year were significantly more likely to be female, and had a significantly greater BMI and ACQ score. Obesity [29,30] and reduced asthma control [29] have been previously associated with asthma exacerbations, although a recent large observational study of asthma outcomes [29] did not find female sex to be a significant predictor for having had a recent severe exacerbation. Interestingly, we found that markers of eosinophilic airway inflammation were no higher in patients with frequent compared to infrequent exacerbations, suggesting that

the excess exacerbations in the former group were due to factors other than uncontrolled eosinophilic airway inflammation. Of note, all patients in this study were being treated with high-dose inhaled or long-term oral corticosteroids, which would be expected to suppress eosinophilic airway inflammation [31].

Previous studies have shown that fixed airflow obstruction is a risk factor for frequent exacerbations [32]. Although post-bronchodilator FEV_1 (% pred.) was numerically lower in our patients with frequent exacerbations than those without frequent exacerbations (78.2 vs 85.8), this result did not reach statistical significance. In contrast, all three of the impedance parameters at baseline, namely R5-R20, AX and R20, were significantly higher in those with frequent exacerbations compared to those without. Very similar results were obtained for the SampEn of impedance parameters. However, as shown in Table E1, post-bronchodilator values of the parameters were much less discriminatory, suggesting that pre-bronchodilator values may be more valuable as predictors of clinical outcome. This is concordant with the results of Shi *et al*, who found that pre-bronchodilator impedance parameters could more effectively discriminate between children with well-controlled and poorly-controlled asthma than post-bronchodilator values [33].

A logistic regression model (*see* Table 6) showed that SampEn of R5-R20 was independently associated with frequent exacerbations. However, substitution of this with any one of the other impedance or impedance entropy parameters resulted in similar model performance, as shown in Table E4 in the online supplement. This suggests that calculation of the Sample Entropy of impedance time series may provide

only a small amount of additional information over and above the mean values of the impedance parameters themselves.

Limitations of the study

We have shown that increased airway impedance parameters are associated with frequent asthma exacerbations. However, our conclusions are based on retrospective data, and thus prospective studies are required in order to validate the predictive capacity of the impedance measurements. Nevertheless, our results suggest that airway impedance measurements represent a marker of exacerbation risk that may be utilised in clinical practice or as an outcome measure in clinical trials.

In addition, further work is required to interpret the structural basis of the impedance parameters derived from impulse oscillometry (R20, R5-R20 and AX). Inverse modelling approaches [21,22] as well as *ex vivo* airway models may provide a deeper understanding.

Finally, in this study we chose to examine the sample entropy of impedance time series as a marker of the temporal fluctuation of airway calibre. However, a number of other techniques exist to analyse time series data, including detrended fluctuation analysis, a measure of long-range scaling [5,6], and dynamic systems analysis [34], and it is possible that one or more of these alternative techniques may provide greater predictive power. Future studies should investigate this possibility and further refine these novel fluctuation biomarkers against clinically important patient-centred outcomes.

.

References

1) Dougherty RH, Fahy JV. Acute exacerbations of asthma: epidemiology, biology and the exacerbation-prone phenotype. *Clin Exp Allergy*. 2009; **39**(2): 193-202.

2) Boser SR, Park H, Perry SF, Ménache MG, Green FH. Fractal geometry of airway remodeling in human asthma. *Am J Respir Crit Care Med*. 2005; **172**(7): 817-23.

3) Mauroy B, Filoche M, Weibel ER, Sapoval B. An optimal bronchial tree may be dangerous. *Nature*. 2004; **427**(6975): 633-6.

4) Frey U, Maksym G, Suki B. Temporal complexity in clinical manifestations of lung disease. *J Appl Physiol*. 2011; **110**(6): 1723-31.

5) Perkiömäki JS, Mäkikallio TH, Huikuri HV. Fractal and complexity measures of heart rate variability. *Clin Exp Hypertens*. 2005; **27**(2-3): 149-58.

6) Seely AJ, Macklem PT. Complex systems and the technology of variability analysis. *Crit Care*. 2004; **8**(6): R367-84.

7) Frey U. Predicting asthma control and exacerbations: chronic asthma as a complex dynamic model. *Curr Opin Allergy Clin Immunol*. 2007; **7**: 223-30.

8) Frey U, Brodbeck T, Majumdar A, Taylor DR, Town GI, Silverman M, Suki B. Risk of severe asthma episodes predicted from fluctuation analysis of airway function. *Nature*. 2005; **438**(7068): 667-670.

9) Thamrin C, Nydegger R, Stern G, Chanez P, Wenzel SE, Watt RA, FitzPatrick S, Taylor DR, Frey U. Associations between fluctuations in lung function and asthma control in two populations with differing asthma severity. *Thorax*. 2011 May 20. [Epub ahead of print].

10) Goldman MD, Saadeh C, Ross D. Clinical applications of forced oscillation to assess peripheral airway function. *Respir Physiol Neurobiol*. 2005; **148**: 179-94.

11) Que CL, Kenyon CM, Olivenstein R, Macklem PT, Maksym GN. Homeokinesis and short-term variability of human airway caliber. *J Appl Physiol*. 2001; **91**(3): 1131-41.

12) Thamrin C, Frey U. Complexity and respiratory growth: a developing story. *J Appl Physiol*. 2009; **106**(3): 753-4.

13) Richman JS, Moorman JR. Physiological time-series analysis using approximate entropy and sample entropy. *Am J Physiol Heart Circ Physiol*. 2000; **278**(6): H2039-49.

14) Veiga J, Lopes AJ, Jansen JM, Melo PL. Airflow pattern complexity and airway obstruction in asthma. *J Appl Physiol*. 2011; **111**(2): 412-9.

15) *Global Strategy for Asthma Management and Prevention*. Global Initiative for Asthma (GINA) 2011. Available from: http://www.ginasthma.org/.

16) Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, de Jongste JC, Kerstjens HA, Lazarus SC, Levy ML, O'Byrne PM, Partridge MR, Pavord ID, Sears MR, Sterk PJ, Stoloff SW, Sullivan SD, Szefler SJ, Thomas MD, Wenzel SE; American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med*. 2009; **180**(1): 59-99.

17) Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J; ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J*. 2005; **26**(2): 319-38.

18) Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax*. 1997; **52**: 498-501.

19) American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline

measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med*. 2005; **171**(8): 912-930.

20) Oostveen E, MacLeod D, Lorino H, Farré R, Hantos Z, Desager K, Marchal F; ERS Task Force on Respiratory Impedance Measurements. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J*. 2003; **22**(6): 1026-41.

21) Kaminsky DA, Irvin CG, Lundblad L, Moriya HT, Lang S, Allen J, Viola T, Lynn M, Bates JH. Oscillation mechanics of the human lung periphery in asthma. *J Appl Physiol*. 2004; **97**(5): 1849-58.

22) Thorpe CW, Bates JH. Effect of stochastic heterogeneity on lung impedance during acute bronchoconstriction: a model analysis. *J Appl Physiol*. 1997; **82**(5): 1616-25.

23) Goldberger AL, Amaral LA, Glass L, Hausdorff JM, Ivanov PC, Mark RG, Mietus JE, Moody GB, Peng CK, Stanley HE. PhysioBank, PhysioToolkit, and PhysioNet: components of a new research resource for complex physiologic signals. *Circulation*. 2000; **101**(23): E215-20.

24) Venegas JG, Winkler T, Musch G, Vidal Melo MF, Layfield D, Tgavalekos N, Fischman AJ, Callahan RJ, Bellani G, Harris RS. Self-organized patchiness in asthma as a prelude to catastrophic shifts. *Nature*. 2005; **434**(7034): 777-82.

25) Lutchen KR, Jensen A, Atileh H, Kaczka DW, Israel E, Suki B, Ingenito EP. Airway constriction pattern is a central component of asthma severity: the role of deep inspirations. *Am J Respir Crit Care Med*. 2001; **164**(2): 207-15.

26) Tgavalekos NT, Tawhai M, Harris RS, Musch G, Vidal-Melo M, Venegas JG, Lutchen KR. Identifying airways responsible for heterogeneous ventilation and mechanical dysfunction in asthma: an image functional modeling approach. *J Appl Physiol*. 2005; 99(6): 2388-97.

27) Kanda S, Fujimoto K, Komatsu Y, Yasuo M, Hanaoka M, Kubo K. Evaluation of respiratory impedance in asthma and COPD by an impulse oscillation system. *Intern Med*. 2010; **49**(1): 23-30.

28) Park JW, Lee YW, Jung YH, Park SE, Hong CS. Impulse oscillometry for estimation of airway obstruction and bronchodilation in adults with mild obstructive asthma. *Ann Allergy Asthma Immunol*. 2007; **98**(6): 546-52.

29) Miller MK, Lee JH, Miller DP, Wenzel SE; TENOR Study Group. Recent asthma exacerbations: a key predictor of future exacerbations. *Respir Med*. 2007; **101**(3): 481-9.

30) Mosen DM, Schatz M, Magid DJ, Camargo CA Jr. The relationship between obesity and asthma severity and control in adults. *J Allergy Clin Immunol*. 2008; **122**(3): 507-11.

31) Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, Bradding P, Wardlaw AJ, Pavord ID. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax*. 2007; **62**: 1043-9.

32) Contoli M, Baraldo S, Marku B, Casolari P, Marwick JA, Turato G, Romagnoli M, Caramori G, Saetta M, Fabbri LM, Papi A. Fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease: 5-year follow-up. *J Allergy Clin Immunol*. 2010; **125**(4): 830-7.

33) Shi Y, Aledia AS, Tatavoosian AV, Vijayalakshmi S, Galant SP, George SC. Relating small airways to asthma control by using impulse oscillometry in children. *J Allergy Clin Immunol*. 2011 Dec 17. [Epub ahead of print]

34) Muskulus M, Slats AM, Sterk PJ, Verduyn-Lunel S. Fluctuations and determinism of respiratory impedance in asthma and chronic obstructive pulmonary disease. *J Appl Physiol*. 2010; **109**(6): 1582-91.

Table 1: Airway Impedance Biomarkers

Airway Impedance biomarker	Clinical interpretation
R20	Mean level of airway constriction
R5-R20	Heterogeneity of airway constriction
AX	Heterogeneity of airway closure
SampEn R20	Fluctuation of the mean level of airway constriction over time
SampEn R5-R20	Fluctuation of the heterogeneity of airway constriction over time
SampEn AX	Fluctuation of the heterogeneity of airway closure over time

R20 = resistance at 20 Hz; R5-R20 = resistance at 5 Hz minus resistance at 20 Hz; AX = reactance area; SampEn = sample entropy.

Table 2: Clinical and demographic characteristics

	Healthy (n=30)	GINA 4 asthma (n=33)	GINA 5 asthma (n=33)
Age (years)	47.0 (2.2)	51.0 (2.3)	56.5 (1.9)‡
Sex (M:F)	12:18	16:17	15:18
BMI (kg/m^2)	25.9 (0.6)	30.0 (1.0)‡	31.2 (1.6)‡
Smoking (pack years)	7.3 (2.3)	5.3 (2.1)	8.5 (2.3)
Post-BD FEV_1 (% pred.)	105.9 (3.1)	86.4 (3.8)‡	76.0 (3.5)‡
Post-BD FEV_1/FVC (%)	80.9 (1.1)	70.0 (2.1)‡	65.2 (1.9)‡
Duration of disease (years)	N/A	23.5 (3.1)	33.2 (3.0)¶
ICS dose (BDP equivalent [µg])*	N/A	1920 (1000 – 2000)	1920 (960 – 2000)
Oral prednisolone dose (mg)*	N/A	0	10 (5 – 15)¶
Sputum eosinophil count (%)†	N/A	3.0 (1.6 – 5.9)	1.4 (0.7 – 2.6)
Sputum neutrophil count (%)	N/A	61.9 (4.9)	65.6 (4.2)
$FeNO_{50}$ (ppb)*	N/A	21 (14 – 49)	19.5 (14.2 – 30.7)
Exacerbations in previous year*	N/A	2 (1 – 3)	2 (1 – 4)
ACQ score (scale 0 [best] – 6 [worst])	N/A	1.7 (0.1)	2.5 (0.2)¶
AQLQ score (scale 1 [worst] – 7 [best])	N/A	5.2 (0.2)	4.5 (0.2)¶

GINA = Global Initiative for Asthma; BMI = body mass index; BD = bronchodilator; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; $FeNO_{50}$ = fractional exhaled nitric oxide at 50 ml/s flow; ppb = parts per billion; ICS = inhaled corticosteroid; BDP = beclometasone dipropionate; AQLQ = Asthma Quality of Life Questionnaire; ACQ = Asthma Control Questionnaire.

Data is expressed as mean (standard error), ratios, *median (interquartile range) or †geometric mean (95% confidence interval). Groups compared using one-way analysis of variance with Bonferroni correction or Student's T test for normally distributed data, Chi-squared test for ratios, †Student's T test of log-transformed data for log-normally distributed data and *Kruskal-Wallis test with Dunn correction or Mann-Whitney U test for non-parametric data.

‡Statistically significant difference compared to control group ($p < 0.05$).

¶Statistically significant difference between GINA 4 and GINA 5 groups ($p < 0.05$).

Table 3: Impedance measurements in healthy subjects and patients with asthma

	R5-R20 ($kPaL^{-1}s$)	AX ($kPaL^{-1}$)	R20 ($kPaL^{-1}s$)
Healthy (baseline)	0.035 (0.020 – 0.053)	0.330 (0.230 – 0.595)	0.310 (0.260 – 0.363)
Healthy (post-bronchodilator)	0.040 (0.020 – 0.050)	***0.290 (0.175 – 0.390)	0.285 (0.250 – 0.340)
GINA 4 asthma (baseline)	††0.080 (0.060 – 0.120)	†0.935 (0.438 – 1.270)	0.355 (0.293 – 0.448)
GINA 4 asthma (post-bronchodilator)	0.070 (0.045 – 0.125)	0.585 (0.325 – 1.263)	0.340 (0.290 – 0.390)
GINA 5 asthma (baseline)	†††0.140 (0.095 – 0.305)	†††1.800 (0.613 – 3.640)	†††0.390 (0.340 – 0.475)
GINA 5 asthma (post-bronchodilator)	**0.130 (0.070 – 0.210)	**1.305 (0.643 – 2.620)	0.40 (0.320 – 0.485)

R5-R20 = resistance at 5 Hz minus resistance at 20 Hz; AX = reactance area; R20 = resistance at 20 Hz; GINA = Global Initiative for Asthma.

Data expressed as median (interquartile range).

Significant differences from baseline values following bronchodilator (using Friedman's test with Dunn's correction) denoted *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Significant differences in baseline values between control and asthma groups (using Kruskal-Wallis test with Dunn's correction) denoted †$p < 0.05$; ††$p < 0.01$; †††$p < 0.001$.

No significant differences were found between baseline values in GINA 4 and GINA 5 asthma groups.

Table 4: Entropy of impedance measurements in healthy subjects and patients with asthma

	SampEn of R5-R20	SampEn of AX	SampEn of R20
Healthy (baseline)	0.008 (0.001 – 0.025)	0.42 (0.22 – 0.81)	0.025 (0.007 – 0.044)
Healthy (post-bronchodilator)	0.004 (0.001 – 0.021)	0.39 (0.19 – 0.59)	0.021 (0.007 – 0.060)
GINA 4 asthma (baseline)	‡‡0.042 (0.003 – 0.100)	††1.05 (0.47 – 1.27)	†0.079 (0.017 – 0.156)
GINA 4 asthma (post-bronchodilator)	0.027 (0.007 – 0.069)	*0.80 (0.52 – 1.08)	0.038 (0.020 – 0.133)
GINA 5 asthma (baseline)	†††0.150 (0.055 – 0.300)	†††1.19 (0.80 – 1.45)	†††0.129 (0.053 – 0.291)
GINA 5 asthma (post-bronchodilator)	**0.094 (0.017 – 0.190)	*1.09 (0.69 – 1.37)	0.096 (0.036 – 0.283)

SampEn = sample entropy; R5-R20 = resistance at 5 Hz minus resistance at 20 Hz; AX = reactance area; R20 = resistance at 20 Hz; GINA = Global Initiative for Asthma.

Data expressed as median (interquartile range).

Significant differences from baseline values following bronchodilator (using Friedman's test with Dunn's correction) denoted *$p < 0.05$; **$p < 0.01$.

Significant differences in baseline values between control and asthma groups (using Kruskal-Wallis test with Dunn's correction) denoted †$p < 0.05$; ††$p < 0.01$; †††$p < 0.001$.

Significant differences in baseline values between GINA 4 and GINA 5 groups (using Kruskal-Wallis test with Dunn's correction) denoted ‡‡$p < 0.01$.

Table 5: Clinical features of asthma patients who had infrequent (< 2 / year) and frequent (≥ 2 / year) exacerbations

	Patients with infrequent exacerbations (n = 25)	Patients with frequent exacerbations (n = 41)

Age (years)	51.7 (2.8)	55.0 (1.7)
Sex (M:F)‡	16:9	15:26
BMI (kg/m^2)‡	28.1 (1.3)	32.1 (1.2)
GINA category (4:5)	16:9	17:24
Smoking (pack years)	6.2 (2.7)	7.4 (1.9)
Post-BD FEV_1 (% pred.)	85.8 (4.4)	78.2 (3.3)
Post-BD FEV_1/FVC (%)	67.0 (2.5)	67.9 (1.7)
Duration of disease (years)	26.8 (4.2)	29.3 (2.6)
Sputum eosinophil count (%)*	2.5 (1.1 – 5.4)	1.8 (1.0 – 3.2)
Sputum neutrophil count (%)	67.9 (4.8)	61.2 (4.2)
$FeNO_{50}$ (ppb)†	20.5 (13.7 – 44.5)	20.9 (14.4 – 33.2)
ACQ score (scale 0 [best] – 6 [worst])‡	1.7 (0.2)	2.3 (0.2)
AQLQ score (scale 1 [worst] – 7 [best])	5.2 (0.2)	4.6 (0.2)

BMI = body mass index; GINA = Global Initiative for Asthma; BD = bronchodilator; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; $FeNO_{50}$ = fractional exhaled nitric oxide at 50 ml/s flow; ppb = parts per billion; AQLQ = Asthma Quality of Life Questionnaire; ACQ = Asthma Control Questionnaire.

Data is expressed as mean (standard error), ratios, *geometric mean (95% confidence interval) or †median (interquartile range).

Groups compared using Student's T test for normally distributed data, Fisher's exact test for ratios, *Student's T test of log-transformed data for log-normally distributed data and †Mann-Whitney U test for non-parametric data.

‡Statistically significant differences between groups ($p < 0.05$).

Table 6: Baseline impedance measurements of asthma patients who had frequent (≥ 2 / year) and infrequent (< 2 / year) exacerbations

Impedance parameter	Patients with infrequent exacerbations (n = 25)	Patients with frequent exacerbations (n = 41)	p value
R5-R20 ($kPaL^{-1}s$)	0.070 (0.040 – 0.130)	0.130 (0.075 – 0.260)	**0.0065**

SampEn of R5-R20	0.014 (0.003 – 0.101)	0.114 (0.057 – 0.281)	**0.0004**
AX ($kPaL^{-1}$)	0.700 (0.345 – 1.345)	1.270 (0.860 – 3.100)	**0.0041**
SampEn of AX	0.922 (0.417 – 1.212)	1.216 (0.992 – 1.454)	**0.0041**
R20 ($kPaL^{-1}s$)	0.320 (0.285 – 0.405)	0.410 (0.340 – 0.485)	**0.0034**
SampEn of R20 ($kPaL^{-1}s$)	0.057 (0.014 – 0.139)	0.136 (0.059 – 0.282)	**0.0044**

SampEn = sample entropy; R5-R20 = resistance at 5 Hz minus resistance at 20 Hz; AX = reactance area; R20 = resistance at 20 Hz.

Data expressed as median (interquartile range). Groups compared using Mann-Whitney U test. Statistically significant p values (< 0.05) are highlighted.

Table 7: Logistic regression analysis of predictors of the exacerbation-prone phenotype in severe asthma

Predictor variable	OR for frequent exacerbations (95% CI for OR)*	p value

ACQ score (scale 0 [best] – 6 [worst])	1.227 (0.628 – 2.4)	0.549
Sex	2.618 (0.781 – 8.783)	0.119
BMI (kg/m^2)	1.027 (0.939 – 1.123)	0.562
SampEn of R5-R20	3.23 (1.242 – 8.4)	**0.016**

OR = odds ratio; CI = confidence interval; ACQ = Asthma Control Questionnaire; BMI = body mass index; SampEn = sample entropy; R5-R20 = resistance at 5 Hz minus resistance at 20 Hz.

*Logistic regression: Having frequent exacerbations (≥ 2 in the previous year) was compared with the baseline category of having infrequent exacerbations (< 2 in the previous year). Odds ratios expressed are for a one point increase in ACQ score, for female sex compared with the baseline category of male sex, for a 1 kg/m^2 increase in BMI, and for a 0.1 increase in SampEn of R5-R20.

Figure legend

Figure 1: Examples of airway impedance time series

Panel A: R20 in a healthy control subject; Panel B: R5-R20 in a healthy control subject; Panel C: R20 in a patient with severe asthma; Panel D: R5-R20 in a patient with severe asthma.

R20 = resistance at 20 Hz. R5-R20 = resistance at 5 Hz minus resistance at 20 Hz.