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Airway impedance entropy and exacerbations in severe asthma

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

66 patients with severe asthma and 30 healthy control subjects underwent impulse oscillometry at baseline and following bronchodilator administration. On each occasion, airway impedance parameters were measured at 0.2-s intervals for 150 s, yielding a time series that was then subjected to sample entropy (SampEn) analysis.

Airway impedance and SampEn of impedance were increased in asthmatic patients compared with healthy controls. In a logistic regression model, SampEn of the resistance at 5 Hz minus the resistance at 20 Hz, a marker of the fluctuation of the heterogeneity of airway constriction over time, was the variable most strongly associated with the frequent exacerbation phenotype (OR 3.23 for every 0.1 increase in SampEn).

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

KEYWORDS: Airflow obstruction, asthma, entropy, oscillometry

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Acute exacerbations of asthma account for much of the morbidity and mortality associated with this 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 scales 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.* [11] plotted frequency distributions of the

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natural logarithm of respiratory system impedance ($\ln Z_{rs}$), measured six times per second over a 15-min period, and found that both the mean and standard deviation of the $\ln Z_{rs}$ were 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 methacholine (MCh) challenge led to a significant increase in the standard deviation of $\ln Z_{rs}$, 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.* [14], who found that the entropy of airflow time series was reduced in patients with asthma compared with healthy controls, and that reduced entropy was associated with increased severity of airflow obstruction. 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: 1) severe asthma is characterised by altered entropy of airway resistance and reactance; and 2) entropy of impedance time series is related to exacerbation frequency in severe asthma.

METHODS

Subjects

66 patients with severe asthma fulfilling the American Thoracic Society (ATS) criteria for asthma, and with no other respiratory disease, were recruited from Glenfield Hospital (Leicester, UK) outpatients. 33 patients were at Global Initiative for Asthma (GINA) treatment step 4 and 33 at GINA treatment step 5, as previously defined [15]. 30 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 MCh responsiveness. All subjects were over the age of 18 yrs. The Leicestershire and Rutland ethics committee (Leicester) 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 $\geqslant$3 days [16]. Spirometry was performed according to ATS/European Respiratory Society (ERS) guidelines [17]. In particular, long-acting bronchodilators were withheld for 12 h prior to testing and short-acting bronchodilators for 4 h. Sputum induction and cell counting were performed as previously described [18]. Exhaled nitric oxide fraction at an expiratory flow rate of 50 mL·s^{-1} ($F_{eNO_{50}}$) was measured 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 in the 5–35-Hz frequency range, with impulses triggered every 0.2 s for 150 s. At each time point, the resistance (R) and reactance (X) components of impedance were recorded at multiple frequencies from 5 to 35 Hz, yielding time series of each variable containing ~750 data points. IOS was performed both before and after the administration of inhaled salbutamol (400 µg), 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 (table 1): 1) R at 20 Hz (R_{20}), which we interpret as a measure of the mean level of airway constriction within the bronchial tree; 2) R at 5 Hz (R_5) minus R_{20} (R_5–R_{20}), which we interpret as a measure of the heterogeneity of airway narrowing throughout the bronchial tree [21]; and 3) the area under the curve of the reactance spectrum between 5 Hz and resonant frequency (reactance area; A_X), which we interpret as a measure of the heterogeneity of airway closure throughout the bronchial tree [22].

Examples of time series of R_{20} and R_5–R_{20} at baseline are shown in figure 1, 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 nonstationary dynamic time series, as described in detail in the online supplementary material. Within a highly regular system, sequence matches are of greater frequency, implying lower entropy and less complexity. SampEn has emerged as a less biased metric of variability than the alternative measure approximate entropy, and is relatively independent of record length, since it does not incorporate

TABLE 1 Airway impedance biomarkers

Airway impedance biomarker	Clinical interpretation
R_{20}	Mean level of airway constriction
R_5–R_{20}	Heterogeneity of airway constriction
A_X	Heterogeneity of airway closure
SampEn R_{20}	Fluctuation of the mean level of airway constriction over time
SampEn R_5–R_{20}	Fluctuation of the heterogeneity of airway constriction over time
SampEn A_X	Fluctuation of the heterogeneity of airway closure over time

R_{20}: resistance at 20 Hz; R_5–R_{20}: resistance at 5 Hz minus R_{20}; A_X: reactance area; SampEn: sample entropy.

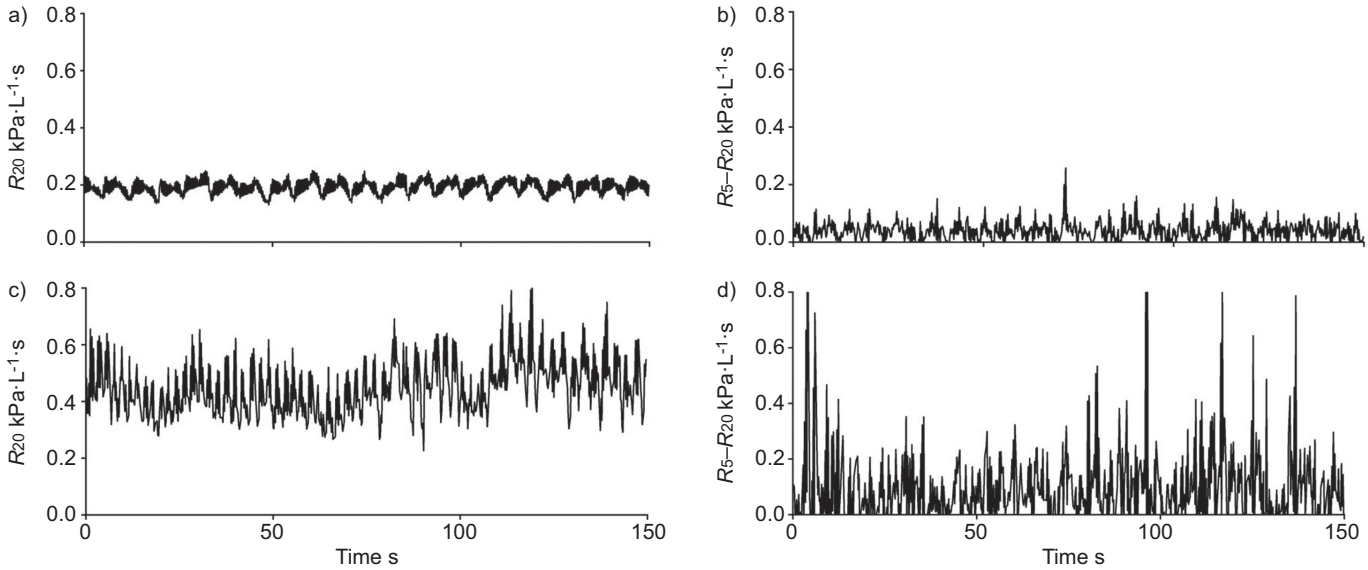

FIGURE 1. Examples of airway impedance time series. a) Resistance at 20 Hz (R_{20}) in a healthy control subject; b) resistance at 5 Hz minus R_{20} (R_5–R_{20}) in a healthy control subject; c) R_{20} in a patient with severe asthma; d) R_5–R_{20} in a patient with severe asthma.

self-matches within the time series into the calculation of conditional probability [13]. We interpret SampEn of the core airway impedance markers (R_{20}, R_5–R_{20} and A_X) to be a measure of fluctuation as a function of time (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 supplementary material.

Statistical analysis

Statistical analysis was performed using Prism version 5 (GraphPad, San Diego, CA, USA) and SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Colour maps were produced using Matlab R2007b (Mathworks Inc., Boston, MA, USA). Parametric data are presented as mean±SEM, data that were log-normally distributed were log transformed and are presented as geometric mean (95% CI), and nonparametric data are presented as median (interquartile range). Unmatched groups were compared using one-way ANOVA with Bonferroni's correction or unpaired t-tests for normally distributed data, Chi-squared test or Fisher's exact test for ratios, unpaired t-tests of log-transformed data for log-normally distributed data, and the Kruskal-Wallis test with Dunn's correction or the Mann–Whitney U-test for nonparametric data. Matched groups were compared using Friedman's test with Dunn's correction. A p-value of <0.05 was taken as the threshold for statistical significance. Spearman's correlation coefficient was used to determine the degree of correlation between impedance parameters, and principal component analysis with varimax rotation and Kaiser normalisation was used 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 (BMI). 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 (R_5–R_{20}) and closure (A_X) at baseline were significantly raised in both asthma groups compared with healthy controls, as shown in table 3 (R_5–R_{20}: healthy controls 0.035 kPa·L^{-1}·s, GINA 4 asthma 0.08 kPa·L^{-1}·s ($p<0.01$), GINA 5 asthma 0.14 kPa·L^{-1}·s ($p<0.001$); A_X: healthy controls 0.33 kPa·L^{-1}, GINA 4 asthma 0.935 kPa·L^{-1} ($p<0.05$), GINA 5 asthma 1.8 kPa·L^{-1} ($p<0.001$)). The mean level of airway constriction (R_{20}) was also raised in the asthma groups compared with controls, but this effect was much less pronounced and only reached statistical significance for healthy controls *versus* GINA 5 asthma patients. The GINA 4 and 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 A_X 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 with 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 fewer than two exacerbations

TABLE 2 Clinical and demographic characteristics

	Healthy	Asthma	
		GINA step 4	GINA step 5
Subjects	30	33	33
Age yrs	47.0±2.2	51.0±2.3	56.5±1.9*
Males/females	12/18	16/17	15/18
BMI kg·m^{-2}	25.9±0.6	30.0±1.0*	31.2±1.6*
Smoking pack-yrs	7.3±2.3	5.3±2.1	8.5±2.3
Post-BD FEV1 % pred	105.9±3.1	86.4±3.8*	76.0±3.5*
Post-BD FEV1/FVC %	80.9±1.1	70.0±2.1*	65.2±1.9*
Duration of disease yrs	NA	23.5±3.1	33.2±3.0^{f}
BDP-equivalent ICS dose# µg	NA	1920 (1000–2000)	1920 (960–2000)
Oral prednisolone dose# mg	NA	0	10 (5–15)^{f}
Sputum eosinophil count¶ % geometric mean (95% CI)	NA	3.0 (1.6–5.9)	1.4 (0.7–2.6)
Sputum neutrophil count %	NA	61.9±4.9	65.6±4.2
$F_{eNO_{50}}$# ppb	NA	21 (14–49)	19.5 (14.2–30.7)
Exacerbations in previous year#	NA	2 (1–3)	2 (1–4)
ACQ score+	NA	1.7±0.1	2.5±0.2^{f}
AQLQ score§	NA	5.2±0.2	4.5±0.2^{f}

Data are presented as n, mean±SE or median (interquartile range), unless otherwise stated. Groups were compared using one-way ANOVA with Bonferroni correction or t-tests for normally distributed data and Chi-squared test for ratios, unless otherwise stated. GINA: Global Initiative for Asthma; BMI: body mass index; BD: bronchodilator; FEV1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; BDP: beclomethasone dipropionate; ICS: inhaled corticosteroid; $F_{eNO_{50}}$: exhaled nitric oxide fraction measured at a flow rate of 50 mL·s^{-1}; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; NA: not applicable. #: Kruskal–Wallis test with Dunn correction or Mann–Whitney U-test for non-parametric data; ¶: t-test of log-transformed data for log-normally distributed data; +: on a scale of 0 (best) to 6 (worst); §: on a scale of 1 (worst) to 7 (best); f: p<0.05 between GINA step 4 and GINA step 5 groups. *: p<0.05 compared with control group.

during the previous year (n=25), and those who had frequent exacerbations, defined as two or more 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 1 s (FEV1) % predicted or FEV1/forced vital capacity ratio, sputum eosinophil or neutrophil counts, $F_{eNO_{50}}$ or AQLQ score. However, frequent exacerbators were significantly more likely to be female than infrequent exacerbators, had a significantly increased mean BMI and had a higher mean ACQ score.

All six impedance and impedance entropy parameters were significantly raised at baseline in frequent exacerbators compared with infrequent exacerbators, as shown in table 6. For example, median $R5$–$R20$ was 0.07 kPa·L^{-1}·s in infrequent

TABLE 3 Impedance measurements in healthy subjects and patients with asthma

	$R5$–$R20$ kPa·L^{-1}·s	Ax kPa·L^{-1}	$R20$ kPa·L^{-1}·s
Healthy controls			
Baseline	0.035 (0.020–0.053)	0.330 (0.230–0.595)	0.310 (0.260–0.363)
Post-BD	0.040 (0.020–0.050)	0.290 (0.175–0.390)###	0.285 (0.250–0.340)
Asthma			
GINA step 4			
Baseline	0.080 (0.060–0.120)**	0.935 (0.438–1.270)*	0.355 (0.293–0.448)
Post-BD	0.070 (0.045–0.125)	0.585 (0.325–1.263)	0.340 (0.290–0.390)
GINA step 5			
Baseline	0.140 (0.095–0.305)###	1.800 (0.613–3.640)***	0.390 (0.340–0.475)***
Post-BD	0.130 (0.070–0.210)##	1.305 (0.643–2.620)##	0.40 (0.320–0.485)

Data are presented as median (interquartile range). $R5$–$R20$: resistance at 5 Hz minus resistance at 20 Hz; Ax: reactance area; $R20$: resistance at 20 Hz; BD: bronchodilator; GINA: Global Initiative for Asthma. *: p<0.05 compared with control group (using Kruskal–Wallis test with Dunn's correction); **: p<0.01 compared with control group (using Kruskal–Wallis test with Dunn's correction); ***: p<0.001 compared with control group (using Kruskal–Wallis test with Dunn's correction); ##: p<0.01 compared with baseline (using Friedman's test with Dunn's correction); ###: p<0.001 compared with baseline (using Friedman's test with Dunn's correction).

TABLE 4 Sample entropy (SampEn) of impedance measurements in healthy subjects and patients with asthma

	SampEn R_5–R_{20}	SampEn A_X	SampEn R_{20}
Healthy controls			
Baseline	0.008 (0.001–0.025)	0.42 (0.22–0.81)	0.025 (0.007–0.044)
Post-BD	0.004 (0.001–0.021)	0.39 (0.19–0.59)	0.021 (0.007–0.060)
Asthma			
GINA step 4			
Baseline	0.042 (0.003–0.100)**	1.05 (0.47–1.27)##	0.079 (0.017–0.156)¶
Post-BD	0.027 (0.007–0.069)	0.80 (0.52–1.08)¶	0.038 (0.020–0.133)
GINA step 5			
Baseline	0.150 (0.055–0.300)###	1.19 (0.80–1.45)###	0.129 (0.053–0.291)###
Post-BD	0.094 (0.017–0.190)¶¶	1.09 (0.69–1.37)¶	0.096 (0.036–0.283)

Data are presented as median (interquartile range). R_5–R_{20}: resistance at 5 Hz minus resistance at 20 Hz; A_X: reactance area; R_{20}: resistance at 20 Hz; BD: bronchodilator; GINA: Global Initiative for Asthma. **: $p<0.01$ between GINA step 4 and GINA step 5 groups (using Kruskal–Wallis test with Dunn's correction); ##: $p<0.01$ compared with control group (using Kruskal-Wallis test with Dunn's correction); ###: $p<0.001$ compared with control group (using Kruskal-Wallis test with Dunn's correction); ¶: $p<0.05$ compared with baseline (using Friedman's test with Dunn's correction); ¶¶: $p<0.01$ compared with baseline (using Friedman's test with Dunn's correction).

and 0.13 kPa·L^{-1}·s in frequent exacerbators ($p=0.0065$), while median SampEn R_5–R_{20} was 0.014 in infrequent and 0.114 in frequent exacerbators ($p=0.0004$). However, these differences were much less pronounced following bronchodilator administration (table S1).

SampEn R5–R20 *is independently associated with frequent exacerbations in severe asthma*

Close correlations were found between impedance and impedance entropy measurements, with $r>0.5$ and $p<0.01$ for every combination of parameters, as shown in table S2 and figure S4. Principal component 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 S3.

A logistic regression model was constructed with the presence or absence of frequent exacerbations, defined as two or more 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

TABLE 5 Clinical features of asthma patients who had infrequent and frequent exacerbations

	Patients with infrequent# exacerbations	Patients with frequent¶ exacerbations
Subjects	25	41
Age yrs	51.7±2.8	55.0±1.7
Males/females*	16/9	15/26
BMI* kg·m^{-2}	28.1±1.3	32.1±1.2
GINA step 4/5	16/9	17/24
Smoking pack-yrs	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 yrs	26.8±4.2	29.3±2.6
Sputum eosinophil count+ % geometric mean (95% CI)	2.5 (1.1–5.4)	1.8 (1.0–3.2)
Sputum neutrophil count %	67.9±4.8	61.2±4.2
$F_{eNO_{50}}$§ ppb median (interquartile range)	20.5 (13.7–44.5)	20.9 (14.4–33.2)
ACQ score*,ƒ	1.7 (0.2)	2.3 (0.2)
AQLQ score##	5.2 (0.2)	4.6 (0.2)

Data are presented as n or mean±SE, unless otherwise stated. Groups compared using unpaired t-tests for normally distributed data and Fisher's exact test for ratios. BMI: body mass index; GINA: Global Initiative for Asthma; BD: bronchodilator; FEV_1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; $F_{eNO_{50}}$: exhaled nitric oxide fraction measured at a flow rate of 50 mL·s^{-1}; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire. #: fewer than two exacerbations during the previous year; ¶: two or more exacerbations during the previous year; +: unpaired t-test of log-transformed data for log-normally distributed data; §: Mann–Whitney U-test for nonparametric data; ƒ: on a scale of 0 (best) to 6 (worst); ##: on a scale of 1 (worst) to 7 (best). *: $p<0.05$ between groups.

TABLE 6 Baseline impedance measurements of asthma patients who had infrequent and frequent exacerbations

Impedance parameter	Patients with infrequent# exacerbations	Patients with frequent¶ exacerbations	p-value+
Subjects n	25	41	
***R*5–*R*20 kPa·L^{-1}·s**	0.070 (0.040–0.130)	0.130 (0.075–0.260)	0.0065
SampEn *R*5–*R*20	0.014 (0.003–0.101)	0.114 (0.057–0.281)	0.0004
***Ax* kPa·L^{-1}**	0.700 (0.345–1.345)	1.270 (0.860–3.100)	0.0041
SampEn *Ax*	0.922 (0.417–1.212)	1.216 (0.992–1.454)	0.0041
***R*20 kPa·L^{-1}·s**	0.320 (0.285–0.405)	0.410 (0.340–0.485)	0.0034
SampEn *R*20 kPa·L^{-1}·s	0.057 (0.014–0.139)	0.136 (0.059–0.282)	0.0044

Data are presented as median (interquartile range), unless otherwise stated. *R*5–*R*20: resistance at 5 Hz minus resistance at 20 Hz; SampEn: sample entropy; *Ax*: reactance area; *R*20: resistance at 20 Hz. #: fewer than two exacerbations during the previous year; ¶: two or more exacerbations during the previous year; +: Mann–Whitney U-test.

dataset (table S4), was that incorporating SampEn *R*5–*R*20. 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 *R*5–*R*20 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 *R*5–*R*20.

DISCUSSION

We have shown for the first time that increased heterogeneity of airway constriction and closure (*R*5–*R*20 and *Ax*), and increased fluctuation of these biomarkers over time (SampEn) are associated with an exacerbation-prone phenotype in patients with severe asthma. This association appears to be strongest with SampEn *R*5–*R*20, 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 (*R*5–*R*20) 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 *R* and *X* 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.

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

Predictor variable	OR for frequent exacerbations (95% CI)	p-value
ACQ score#	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 *R*5–*R*20	3.23 (1.242–8.4)	0.016

Logistic regression: having frequent exacerbations (two or more in the previous year) was compared with the baseline category of having infrequent exacerbations (fewer than two in the previous year). Odds ratios are for a one-point increase in Asthma Control Questionnaire (ACQ) score, for female sex compared with the baseline category of male sex, for a 1-kg·m^{-2} increase in body mass index (BMI) and for a 0.1 increase in sample entropy (SampEn) of resistance at 5 Hz (*R*5) minus resistance at 20 Hz (*R*20). #: on a scale of 0 (best) to 6 (worst).

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 *R* at low oscillation frequencies [25]. This frequency dependence of *R* is thought to be due to heterogeneous airway constriction, since mathematical modelling indicates that even severe homogeneous constriction would produce an elevated baseline *R* but not frequency dependence [25]. Furthermore, an image-functional modelling approach has suggested that the ventilation defects and frequency-dependence of *R* 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 *R*20 to represent general airway resistance and *R*5–*R*20 to represent frequency-dependence of resistance, a marker of heterogeneous airway constriction. *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 *R*5–*R*20 and *Ax*, and to a lesser extent *R*20, in patients with severe asthma compared with 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 *versus* control comparison. Close correlations were seen between impedance measurements and their respective SampEn parameters, as shown in table S2 and figure S4, 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 previously been associated with asthma exacerbations, although a recent large observational study of asthma outcomes [29] did not find female sex to be a significant predictor of having had a recent severe exacerbation. Interestingly, we found that markers of eosinophilic airway inflammation were no higher in patients with frequent compared with 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 was numerically lower in our patients with frequent exacerbations than those without frequent exacerbations (78.2 *versus* 85.8% pred), this result did not reach statistical significance. In contrast, all three of the impedance parameters at baseline, namely R_5–R_{20}, A_X and R_{20}, were significantly higher in those with frequent exacerbations compared with those without. Very similar results were obtained for the SampEn of impedance parameters. However, as shown in table S1, 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.* [33], who found that pre-bronchodilator impedance parameters could more effectively discriminate between children with well-controlled and poorly-controlled asthma than post-bronchodilator values.

A logistic regression model (table 6) showed that SampEn R_5–R_{20} 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 (table S4). This suggests that calculation of the SampEn 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 used 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 (R_{20}, R_5–R_{20} and A_X). Inverse modelling approaches [21, 22] and *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 de-trended 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.

STATEMENT OF INTEREST

Statements of interest for S. Siddiqui and C. E. Brightling can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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Airway impedance complexity and exacerbations in severe asthma

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Online supplement

- **i) Supplementary details of sample entropy calculations**
- **ii) Supplementary data tables**
- **iii) Supplementary figure (Figure S4)**

i) Supplementary details of sample entropy calculations

Sample entropy, SampEn, is a useful statistical measure for analysing the dynamics of non-stationary time series. Sample entropy is defined as the negative natural logarithm of an estimate of the conditional probability (*CP*) that, within the time series, a sequence of length *m-1* that matches elsewhere in the series will also match pointwise at the next point, within a tolerance, *r*.

$$SampEn(m, r) = -\ln(CP)$$

Where *m* is the template size and *r* is the tolerance.

Conditional probability is the probability of one event occurring given that a separate event has already occurred. For example consider rolling two dice. The probability of rolling a total of 12, (Outcome $\acute{A}$), is 1/36. However if the dice are rolled separately and one of the dice lands on a 6 (Outcome $\acute{B}$), the probability of outcome $\acute{A}$ changes: it is now 1/6. The general rule for this is:

$$P\left(\acute{A}\middle|\acute{B}\right) = \frac{P\ (\acute{A} \cap \acute{B})}{P\ (\acute{B})}$$

Where $P\ \left(\acute{A} \cap \acute{B}\right)$ is the joint probability of $\acute{A}$ and $\acute{B}$ occurring, and $P\left(\acute{A}\middle|\acute{B}\right)$ is the conditional probability of $\acute{A}$ given $\acute{B}$ has already occurred.

For sample entropy, event A is that a sequence of length *m*, will match pointwise within tolerance *r* and event B is that a sequence of length *m-1* will match pointwise within *r*. So:

$$CP = P\ (A|B) = \frac{P\ (A \cap B)}{P\ (B)}$$

Now any sequence that matches for *m* points, will also match for *m-1* points, so we can say $A \in B$, (meaning event A is a subset of event B). From set theory $P(A \cap B)$ can be reduced to just $P(A)$, So:

$$CP = \frac{P(A)}{P(B)}$$

Now the exact probability of A or B cannot be known with full certainty, so an estimate of these numbers must be used. These will respectively be referred to as A_m and B_{m-1}. These approximations can be made by counting the number of times the sequences of length m or $m-1$ match, divided by the number possibilities where they could match.

Figure S1 shows a non-stationary time series u[i] for i=1 to 61. From this figure it can be seen how a three-component template (u[1-3] or the solid black, green and red points) can be matched to subsequent templates (u[20-22]; and u[49-51]).

This simulated time series contains many three-component sequences, but u[1-3] has been chosen for the purpose of demonstration. Let us call r_1 the number of single values that match any of points u[1], u[2] or u[3], r_2 the number of consecutive values or double runs that match pointwise either u[1-2] or u[2-3], and r_3 the number of triple runs (3 points in a row) that match pointwise for u[1-3]. There are 7 matches of run length 1 (u[5], [8], [10], [23], [24], [42] and [43]), 1 of length 2 (u[20, 21]) and 2 template matches of length 3 (u[20-22] and [49-51]) that need to be added to the corresponding counts, r_1, r_2 and r_3. A special case is needed when a run ends at the last point in the data, where the A_m counters are incremented but the B_{m-1} counters are not. This process is repeated for $N-k+1$ sequences of all sizes from $k = 1$ to $k = N-1$, where k is the sequence length or run length, and N is the length of the entire time series. Let us suppose that all other templates of all sizes return no single, double or triple run matches or any other run match of any size, and therefore offer no further contribution to r_1, r_2, and r_3. (Although from inspection, such a supposition is clearly not the case.) $r_1=8$, $r_2=1$ and $r_3=2$. However, each triple run match contains 3 individual matches and 2 double matches that are not accounted for since they are not an isolated run. For

example, within the triple run match u[49-51], u[49,50] matches u[1,2] and u[50,51] matches u[2,3]. To account for this a new counter, *M*, is introduced such that:

$$M_1 = r_1 + 2r_2 + 3r_3 + \cdots + (N-1)r_N$$

$$M_2 = r_2 + 2r_3 + 3r_4 + \cdots + (N-2)r_N$$

$$M_k = r_k + 2r_{k+1} + 3r_{k+2} + \cdots + (N-k)r_N$$

Now, M_k contains the number complete matches (not just the number of runs) of size k, in the entire time series. Where we had $r_1=8$, $r_2=1$ *and* $r_3=2$; we can calculate $M_1=16$, $M_2=3$, $M_3=2$, assuming there are no matches for runs longer than $k=3$. If every single point matched there would be $\frac{(N-k)(N-k+1)}{2}$ matches. This is the maximum of M_k and the number of times the entropy algorithm checks the data series for a match of size *k*. We can therefore use this to estimate the probability of finding a match of size m since the probability of an outcome is defined as $\frac{number\ of\ successful\ outcomes}{number\ of\ trials}$ as the number of trials tends to infinity. Hence for a large time series this will be a good approximation.

The probability of a match of size *k* is:

$$A_k = \frac{2\,M_k}{(N-k)(N-k+1)}$$

and likewise

$$B_{k-1} = \frac{2\,M_{k-1}}{(N-[k-1])(N-k+1)}$$

CP was defined earlier as the ratio $\frac{A_m}{B_{m-1}}$, which will have a few common factors that cancel out, thus:

$$CP = P(_{k=m}|_{k=m-1}) = \frac{A_m}{B_{m-1}} = \frac{\frac{2\,M_k}{(N-k)(N-k+1)}}{\frac{2\,M_{k-1}}{(N-[k-1])(N-m+1)}} = \frac{M_m(N-m+1)}{M_{m-1}(N-m)}$$

In our example, template size, *m=3*; so *k=1, 2, 3; N=61; r=0.01;* $M_1=16$, $M_2=3$, $M_3=2$:

$$A_3 = \frac{2 \times 2}{(61-3)(61-3+1)} = \frac{4}{58 \times 59} = 0.0012$$

$$B_2 = \frac{2 \times 3}{(61-2)(61-3+1)} = \frac{6}{59 \times 59} = 0.0017$$

so

$$CP = P(_{k=3}|_{k=2}) \ = \frac{A_3}{B_2} = 0.6782$$

and

$$SampEn(m = 3, r = 0.01) = \ -\ln(CP) = \ -\ln(0.6782) = \ 0.3884$$

Selection of the optimal template size (m) and tolerance (r) was performed with the aim of maximising the distinction between control and asthma groups, as follows:

Typically, r is between 15-30% of standard deviation (σ). Sample entropy calculations were made using all template sizes from 2-5 and tolerance sizes from 0.1 to 0.5 with increments of 0.1, as shown in Figure S2.

As can be seen from the figure, all template sizes and tolerances produce data such that $\mu_{as} > \mu_{co}$, but the difference between them is greatest for the dark red regions of the graph, noticeably at r=0.3, m=3. There is a greater distinction between the two groups at these values than at, for example, r=0.2, m=2.

In order to determine the behaviour of the SampEn algorithm with respect to other periodic functions, the sample entropy of the sine function at different amplitudes, frequencies and sample frequencies was calculated using two combinations of tolerance and template size, as shown in Figure S3. On these heat maps, sinusoid amplitude is shown on the y axis while the ratio of sinusoid frequency to sampling frequency is shown on the x axis. The vertical streaks occur where the number of points per cycle is almost integer, which shows how dependent sample entropy is on symmetry. Adding noise to the data would remove these streaks.

ii) Supplementary data tables

Table S1: Post-bronchodilator impedance measurements of asthma patients who had infrequent (< 2 / year) and frequent (≥ 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 (0.050 – 0.145)	0.120 (0.055 – 0.185)	0.149
SampEn of R5-R20	0.033 (0.004 – 0.078)	0.064 (0.014 – 0.155)	0.088
AX ($kPaL^{-1}$)	0.520 (0.315 – 1.405)	1.020 (0.530 – 2.325)	0.058
SampEn of AX	0.659 (0.539 – 1.120)	1.076 (0.769 – 1.316)	0.059
R20 ($kPaL^{-1}s$)	0.310 (0.265 – 0.390)	0.380 (0.335 – 0.465)	**0.015**
SampEn of R20	0.036 (0.019 – 0.088)	0.107 (0.029 – 0.223)	**0.017**

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 S2: Correlation between impedance and impedance entropy parameters in patients with asthma

	R5-R20	AX	R20	SampEn of R5-R20	SampEn of AX	SampEn of R20
R5-R20	1.000					
AX	.899	1.000				
R20	.569	.674	1.000			
SampEn of R5-R20	.788	.806	.718	1.000		
SampEn of AX	.691	.790	.600	.795	1.000	
SampEn of R20	.588	.614	.717	.793	.622	1.000

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

Correlation calculated using Spearman's rho.

All correlations were statistically significant ($p < 0.01$).

Table S3: Principal components analysis of variables associated with the frequent exacerbation phenotype

Variable	Component 1	Component 2
R5-R20	**.847**	.331
AX	**.881**	.250
R20	**.705**	.355
SampEn of R5-R20	**.822**	.279
SampEn of AX	**.805**	-.075
SampEn of R20	.483	.630
ACQ score	.122	**.734**
BMI	.108	**.750**

R5-R20 = resistance at 5 Hz minus resistance at 20 Hz; AX = reactance area; R20 = resistance at 20 Hz; SampEn = sample entropy; ACQ = Asthma Control Questionnaire; BMI = body mass index. Loading scores of each variable onto each component are shown. Variables that load strongly (loading score > |0.7|) onto each factor are highlighted.

Table S4: Comparison of logistic regression models to predict the frequent exacerbation phenotype

Impedance parameter	p value for impedance parameter within the model	Percentage of patients correctly classified by model
R5-R20	0.054	69.2
AX	0.099	64.6
R20	**0.042**	69.2
SampEn of R5-R20	**0.016**	74.2
SampEn of AX	**0.009**	66.1
SampEn of R20	0.100	67.7

Logistic regression models were constructed with the dependent variable being the absence (baseline) or occurrence of ≥ 2 exacerbations in the previous year. The models incorporated sex, body mass index, Asthma Control Questionnaire score and one of the impedance parameters listed above as independent variables. Statistically significant p values (< 0.05) are highlighted.

Figure legends

Figure S1: Sample Entropy analysis of a simulated time series

See text for explanation.

Figure S2: Determination of optimal template size and tolerance for the calculation of sample entropy

Sample Entropy calculations were made using all template sizes from 2-5 and tolerance sizes from 0.1 to 0.5 with increments of 0.1. All template sizes and tolerances produce data such that the mean value for asthma (μ_{as}) > mean value for controls (μ_{co}), but the difference between them is greatest for the dark red regions of the graph, noticeably at r=0.3, m=3. There is a greater distinction between the two groups at these values than at, for example, r=0.2, m=2. Data is normalised for the standard deviation (σ) of controls.

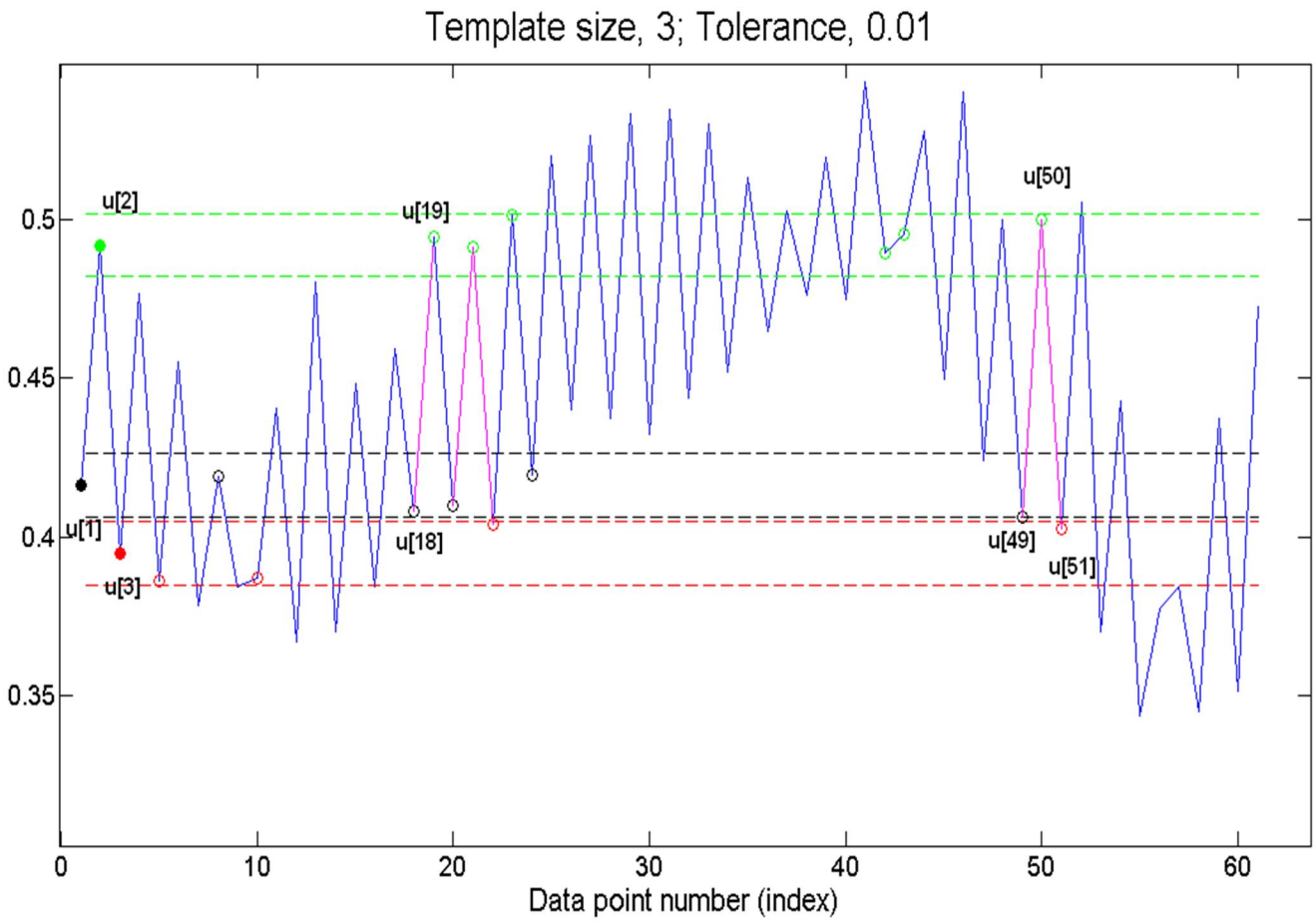
Figure S3: Sample entropy of the sine function

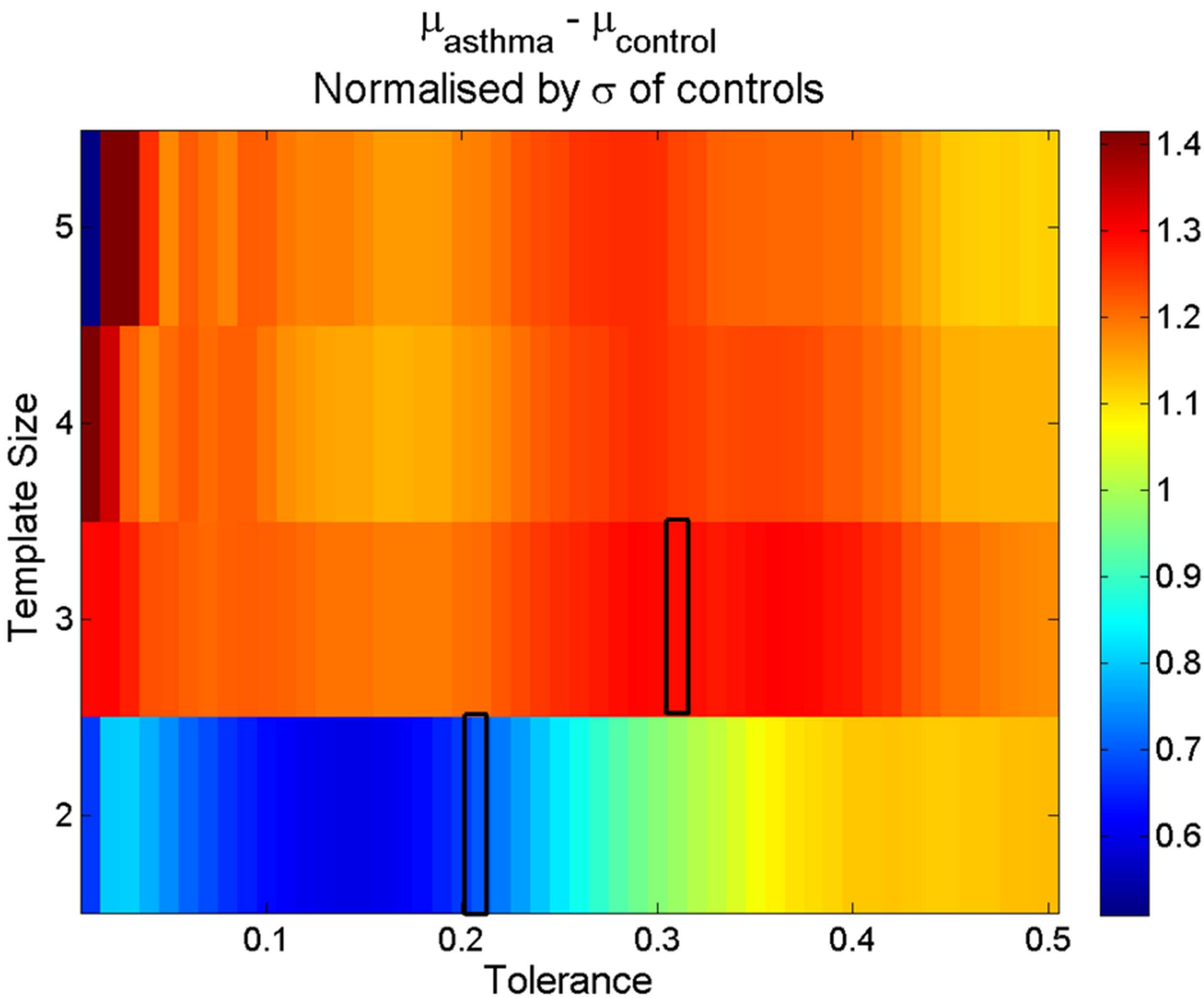
The sample entropy of the sine function at different amplitudes, frequencies and sample frequencies is displayed using two combinations of tolerance and template size. In panel A, a tolerance of 0.2 and template size of 3 is used, and in panel B, a tolerance of 0.3 and a template size of 2. Sinusoid amplitude is shown on the y axis while the ratio of sinusoid frequency to sampling frequency is shown on the x axis.

Figure S4: Correlations between impedance and Sample Entropy of impedance measurements in patients with asthma

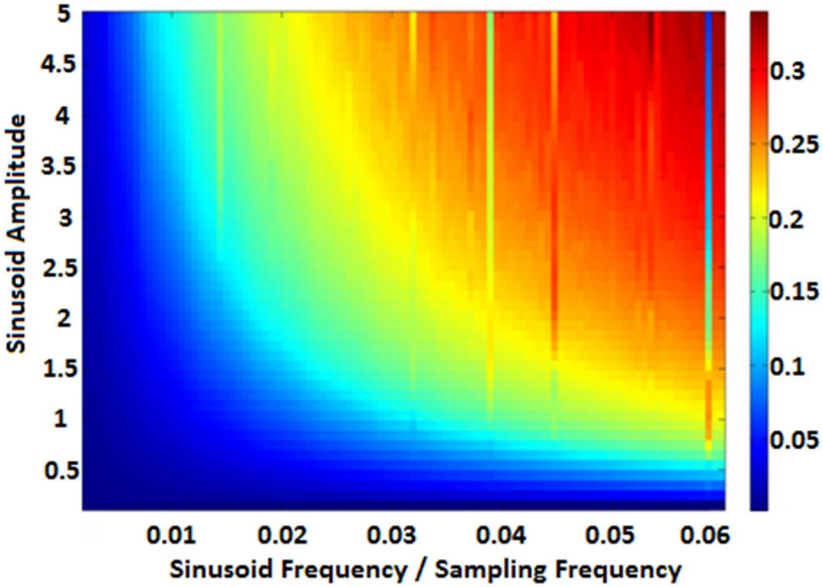
Panels A and B show the linear relationship between R20 and R5-R20, and the respective Sample Entropy values (Spearman's rho = 0.717 and 0.788 respectively).

Panels C and D show the linear relationship between $\log_e(AX)$ and Sample Entropy of AX.

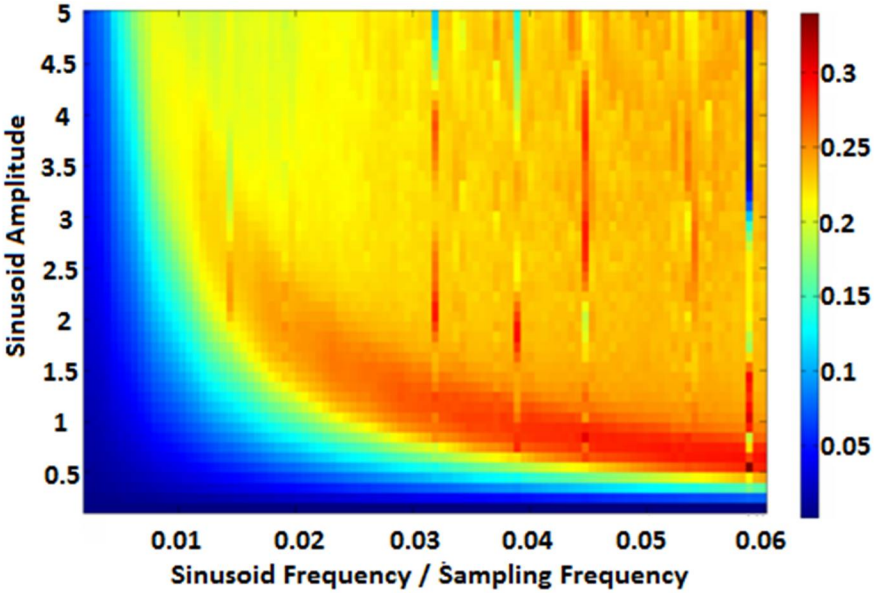

Template size, 3; Tolerance, 0.01
u[2]
u[19]
u[50]
u[1]
u[3]
u[18]
u[49]
u[51]
0.5
0.45
0.4
0.35
0
10
20
30
40
50
60
Data point number (index)


$\mu_{asthma} - \mu_{control}$
Normalised by σ of controls
Template Size
5
4
3
2
0.1
0.2
0.3
0.4
0.5
Tolerance
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6

A


Sinusoid Amplitude
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0.01
0.02
0.03
0.04
0.05
0.06
Sinusoid Frequency / Sampling Frequency
0.3
0.25
0.2
0.15
0.1
0.05

B


Sinusoid Amplitude
5
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Sinusoid Frequency / Sampling Frequency
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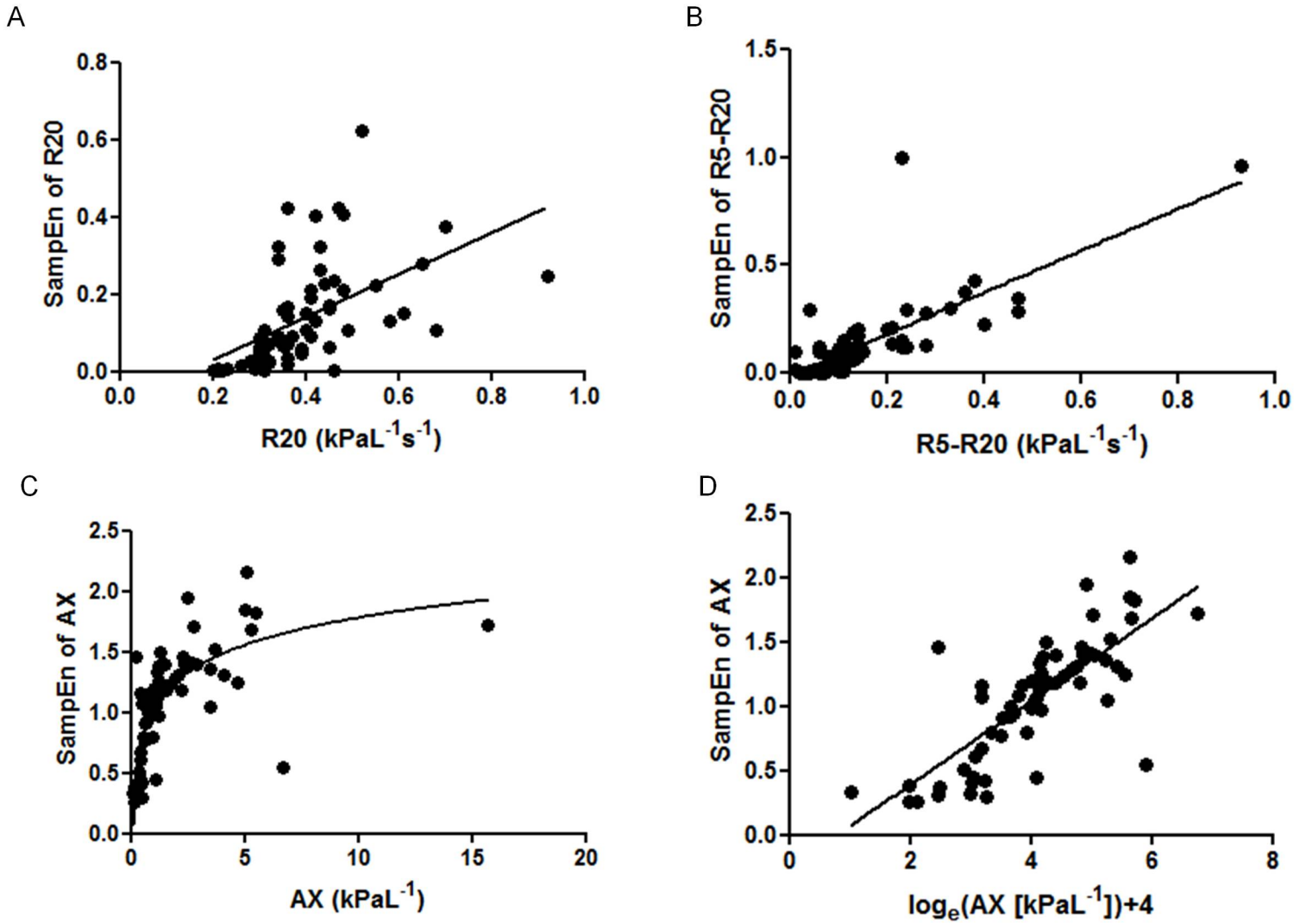
A
SampEn of R20
R20 (kPaL-1s-1)
B
SampEn of R5-R20
R5-R20 (kPaL-1s-1)
C
SampEn of AX
AX (kPaL-1)
D
SampEn of AX
loge(AX [kPaL-1])+4