

Research Article

Exhaled Breath Condensate pH as a Non-invasive Measure of Inflammation in Non-CF Bronchiectasis

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Bronchiectasis is characterised by neutrophilic bronchial inflammation. Direct measurement of lung inflammation would be useful to assess disease activity, guide need for treatment, and monitor response. The aim of this study was to test whether exhaled breath condensate (EBC) pH, a simple noninvasive test, provides a clinically useful measure of inflammation in the lungs of patients with bronchiectasis. 96 consecutively referred patients were studied when clinically stable, 20 followed up over two years, and a further 22 patients seen during an exacerbation. Subjects breathed tidally for 10 minutes into a condensing chamber (Ecoscreen, Erich Jaeger, Hoechberg, Germany). pH in EBC was measured immediately using a pH probe. In a representative group of 25 patients samples were deaerated with argon gas. This was to control for variations in pH *ex vivo* by removing CO₂. EBC was acidic in bronchiectasis patients (6.79 ± 0.72) compared to controls (7.08 ± 0.69) and primary ciliary dyskinesia patients (7.24 ± 0.53). pH was related to lung volume but not disease severity. Repeated measures show EBC pH changes with symptoms. EBC is further acidified during an exacerbation of bronchiectasis (6.44 ± 0.72), this acidification persists following treatment (6.09 ± 0.80). EBC pH is not sufficiently sensitive or specific to monitor patients' health status or provide information to inform acute treatment decisions.

1. Introduction

Patients with bronchiectasis have airway dilatation due to a structural abnormality of the bronchial wall that leads to delayed mucociliary clearance and predisposes the lung to bacterial infection [1]. In severe disease, infection is often persistent leading to chronic inflammation causing further damage to the lung tissue [2]. The levels of inflammation in stable state bronchiectasis correlate with a reduction in patient's quality of life [3]. Monitoring inflammation is an important part of management of bronchiectasis patients in order to prevent disease progression and enhance quality of life. Current markers of inflammation such as blood, sputum markers, and bronchoscopy can be indirect, variable and invasive, respectively.

Exhaled breath condensate (EBC) is the collection of breath as a liquid by exhaling through a cooled tube. EBC contains microdroplets of extracellular lining fluid, an important first line of the lung's defence system. EBC is a simple non-invasive test to perform making it an attractive

technique for potentially monitoring oxidative stress and lung inflammation.

A range of markers can be measured in EBC. pH and ammonia are simple markers which are easy to measure. Both are important within the respiratory system as mediators of host defence and have potential to reflect neutrophilic inflammation in the lungs of patients with bronchiectasis. pH shows good reproducibility [4] and has previously been shown to be reduced in 20 patients with bronchiectasis compared with normal controls and mild asthmatics [5]. The reduced pH values correlated with airway neutrophilia, EBC hydrogen peroxide, and FEV₁. pH was reduced further in patients with *Pseudomonas aeruginosa* colonisation suggesting pH may be a potential inflammatory marker in bronchiectasis. Ammonia is a potential regulator of pH [6] and has not been studied previously in EBC of non-CF bronchiectasis patients. We aim to establish the value of EBC pH and its relationship to ammonia as an inflammatory marker in bronchiectasis whilst patients are stable and during an exacerbation of the condition.

1.1. Aim. This paper aims to test the hypothesis that EBC pH will provide clinically useful direct measures of inflammation in the lungs of patients with bronchiectasis.

To address this hypothesis, we assess EBC in a large group of patients with bronchiectasis compared to healthy controls. EBC pH is compared with existing measures of inflammation, EBC ammonia as a potential pH regulator, disease activity and severity, and longitudinal assessments are made following patients up every 6 months for two years. EBC is also measured during and after an acute exacerbation of bronchiectasis requiring IV antibiotics.

2. Methods

2.1. Ethics. This study was approved by the Royal Brompton Hospital ethics committee and subjects gave written informed consent to take part.

2.2. Study Design

2.2.1. Consecutive Referral Cross-Sectional Study. Consecutive patients were studied whilst they attended the hospital for a program of tests designed to investigate the cause and severity of their bronchiectasis. This protocol has been previously described [7]. Patients were asked to perform EBC collection. pH and ammonia were measured and the results compared to those of twenty healthy controls.

Results were compared with age, gender, height, weight, patient's sputum (including, sputum volume, purulence, eosinophil counts, and bacteriology), peripheral blood inflammatory markers (white cell count, neutrophils, ESR and CRP), quality of life (as judged by SGRQ score), exercise capacity (measure by shuttle walking test), exhaled nitric oxide (FE_{NO}), lung function (FEV_1 and % predicted), and disease severity on CT scan.

2.2.2. Exacerbation Study. EBC was collected within 24 hours of admission to hospital, for an infective exacerbation of bronchiectasis requiring intravenous antibiotics, and within 24 hours of discharge. Antibiotics were chosen by a physician who was guided by current or previous sputum sensitivities. The time of discharge was dependent on clinical response to treatment.

2.2.3. Two Year Longitudinal Study. Patients were recruited from the outpatient department when stable. They were seen every 6 months and each time they visited clinic for the following two years.

At each visit the patient completed EBC collection, FE_{NO} measurement, FEV_1 , and a SGRQ. Other tests such as blood markers of inflammation and sputum bacteriology were performed when clinically indicated. Treatment was also given when clinically indicated.

2.3. Study Subjects. Patients with bronchiectasis were recruited from the infection firm at the Royal Brompton hospital. Bronchiectasis was confirmed by high-resolution CT scan according to standard criteria [8].

Hospital staff of similar age and gender to the bronchiectasis group were recruited as normal controls. Study group demographics are shown in Table 1.

2.4. Experimental Tests

2.4.1. EBC Collection. Subjects were asked to sit breathing tidally for 10 minutes through a non-rebreathing valve with saliva trap into a condensing chamber. (Ecoscreen, Erich Jaeger, Hoechberg, Germany). The exhaled breath was cooled in a metal chamber so that it condensed. It was then collected as a liquid in an inert disposable collection vessel at the bottom of the metal chamber. Patients were instructed to remove their mouth away from the mouthpiece if they needed to cough, yawn, or sneeze, if subjects needed to swallow saliva or if their breathing became uncomfortable. Nose clips were not used.

2.4.2. pH Measurement. The condensate was stirred manually and pH was measured immediately using a small combination pH probe (BDH, Poole, UK) and bench pH meter (Hanna Instruments, UK). The bench meter was calibrated before each measurement with 4.01, 7.01, and 10.01 standards and adjusted according to the temperature of the sample, measured with a small hand-held thermometer. Reproducibility of this measurement was checked once a day over three days in a group of 10 patients. All patients were able to perform the EBC measurement every day for three days. EBC pH reliability coefficient was 0.66 and nonsignificant variation was calculated to be ± 0.56 . In a representative group of 25 patients, samples were deaerated with argon gas. This was to control for variations in pH *ex vivo* by removing CO_2 . This results in a pH which is stable for a long time frame [9]. Argon gas (BOC speciality gases, UK) was bubbled through the sample at a rate of 350 mL/min until the pH of the sample stabilised for more than 30 seconds. This was achieved using a series of regulators and a flow controller linked to a small inert plastic tube which was inserted directly into the condensate.

2.4.3. Ammonia Measurement. Ammonia content was measured using an NH_4^+ electrode in conjunction with a double junction reference electrode (Thermo Orion, USA) connected to a bench mV meter (Hanna Instruments, UK). An anilate addition method was used as described by Gaston et al. (2002) [10].

2.5. Data Analysis. The data was evaluated using commercially available statistical analysis software (SPSS). Differences were considered to be significant when probability values were less than or equal to 0.05. Where data was normally distributed, values are reported as mean and standard deviation. Where data distributions differed from normal values are reported as median and variation is reported by interquartile range. The natural log of the value was used to normalise the data distribution for statistical analysis. A student's *t*-test or ANOVA was used to compare groups and linear regression analysis used to assess relationship between variables.

TABLE 1: Study group demographics.

	Consecutive referrals with bronchiectasis	Two-year longitudinal assessment	Exacerbation of bronchiectasis	Control subjects
<i>n</i>	63	20	20	30
Age in years median (IQR)	50 (15)	52 (8)	54 (7.5)	42 (24)
Male (% group)	19 (30)	10 (50)	10 (32)	8 (27)
Smoking status (non/ex/current)	54/9/0	20/0/0	19/1/0	30/0/0
FEV ₁ % pred	70.9 (19.7)	60.7 (26.0)	N/A	N/A
SGRQ total	50.9 (25.9)	41.9 (13.0)	N/A	N/A

SGRQ: St George's Respiratory Questionnaire.

3. Results

3.1. EBC in Consecutive Referrals Compared to Healthy Controls. Ninety six consecutive referrals had EBC collected and pH and ammonia measurements taken. Their results were compared to those of 20 healthy controls of similar age and gender. Patients with non-CF, non-PCD bronchiectasis have acidified breath condensate. These results are shown in Figure 1. Patients with bronchiectasis caused by primary ciliary dyskinesia (PCD) had pH similar to normal controls. This could be related to low exhaled nitric oxide in this group 6.1 (4.5) ppb versus 13.9 (7.0) in controls ($P < 0.05$). 22 patients did not have evidence of bronchiectasis on CT scan. Their EBC pH was not significantly different to those patients with bronchiectasis.

There was no significant difference in ammonia levels between bronchiectasis patients 0.13 mM (0.20) and normal controls 0.21 mM (0.31), $P = 0.31$. However, there was a weak correlation between EBC ammonia and pH $r = 0.42$, $P = 0.02$.

Twelve subjects did not produce a condensate sample. In three of these subjects the measurement was repeated on a separate day, and still no condensate was collected. This may be due to nose breathing despite instruction to breath through the mouth.

EBC measurements of pH and ammonia were compared with a number of demographic factors and measures of disease activity and severity, as described in Section 2.2.1. There was no relationship between any of the factors measured and EBC pH or ammonia levels.

These studies were repeated in a group of 25 patients in which EBC was deaerated with argon gas. This was to control for variations in pH ex vivo by removing CO₂. This results in a more stable pH result [9]. When EBC was deaerated, a correlation between EBC pH and height and consequently a weak relationship with FEV₁ ($r = 0.43$, $P = 0.05$) and a difference in pH between genders were revealed. The group consisted of eleven males and fourteen females. Females had significantly more acidic pH mean (SD) 7.20 (0.23) than the males 7.49 (0.21), $P = 0.02$ (shown in Figure 2). In the control group in which EBC was deaerated ($n = 20$), there was no difference in pH between males and females and no relationship with height ($r = 0.08$, $P = 0.78$). Male reported $n = 7$ mean (SD) 7.43 (0.18), and female $n = 13$ mean (SD) 7.63 (0.16), $P = 0.11$.

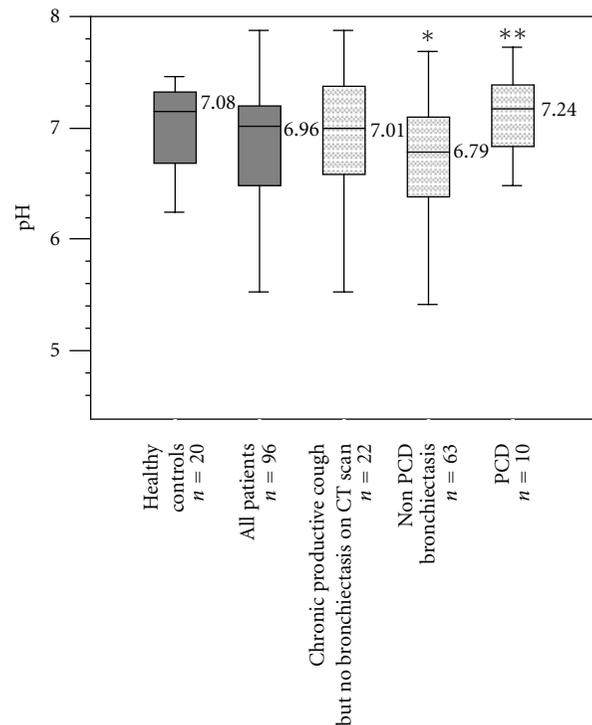


FIGURE 1: pH of EBC in patients with bronchiectasis compared to primary ciliary dyskinesia, chronic cough, and normal controls. * $P = 0.04$ when comparing non-PCD bronchiectasis patients with healthy controls. ** $P = 0.01$ when comparing PCD patients with non-PCD bronchiectasis patients. 1 patient with CF was not included in the graphical representation of the breakdown of all patients.

3.2. Exacerbation of Bronchiectasis. EBC pH was acidic in 20 patients with bronchiectasis on admission to hospital for an exacerbation of their condition. The median (IQR) pH was 6.44 (0.72). pH did not normalise following treatment (as shown in Figure 3). Predictive value of pH for an exacerbation was calculated as 19% sensitive and 92% specific at pH < 5.85 . There was no relationship between EBC pH, ammonia, or FE_{NO} at the time patients were admitted to hospital. At the time of discharge there was a negative correlation between FE_{NO} and EBC pH $r = -0.48$, $P = 0.02$. Blood inflammatory markers were elevated at admission and significantly reduced at discharge. There was no change in EBC ammonia (data not shown).

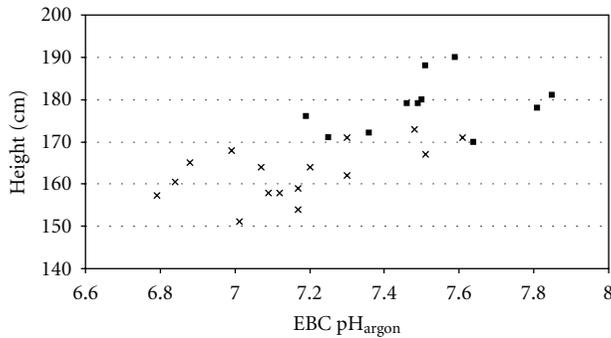


FIGURE 2: Increased pH in EBC deaerated with argon gas from patients with bronchiectasis according to their height and gender. \times = female \blacksquare = male. $r = 0.68$, $P = 0.003$.

3.3. *Two-Year Longitudinal Assessment.* EBC pH was measured in 20 patients over a course of 2 years. EBC pH changed in relation to SGRQ total score over the course of the study: β coefficient = -0.007 , $P = 0.017$. This was due to a relationship between pH and the symptom component $\beta = -0.007$, $P = 0.014$ of the questionnaire. There was no relationship with lung function.

4. Discussion

A large group of patients with well-defined bronchiectasis in terms of aetiology, bacteriology, and disease severity was established and EBC pH and ammonia were successfully measured. EBC can easily be collected in patients with symptoms of bronchiectasis regardless of disease activity or severity. EBC pH is more acidic in bronchiectasis than in healthy controls of similar age and gender. However, acidification was not related to structural damage, as patients with bronchial suppuration but no structural damage had similar EBC pH and there was no difference in EBC pH in patients with more severe bronchiectasis, judged by CT and lung function. This acidification is important since many processes in the respiratory system are pH dependant. These include bronchoconstriction and several host defence mechanisms which may be of importance when considering the bronchiectatic lung. For example, low pH results in impairment of ciliary motility, increase of airway mucus viscosity, and damage of the airway epithelium [11–15].

EBC pH was similar in patients with primary ciliary dyskinesia to normal controls and was less acidic when compared with other causes of bronchiectasis. This may represent less inflammation in the primary ciliary dyskinesia disease group compared to other patients. Alternatively, it may be related to low FE_{NO} in this condition and thus explain one of the sources of low pH in the other patients where FE_{NO} is raised [16]. Available nitrite can be converted to nitrous acid which lowers pH. Where there is no available nitric oxide as in PCD then nitrous acid may not be formed and the expirate may be less acidic. Indeed there was a negative correlation between FE_{NO} and EBC pH at discharge after treatment for an infective exacerbation of bronchiectasis. This relationship was not present in stable patients or at the start of an exacerbation. This suggests both EBC pH and FE_{NO} may be related

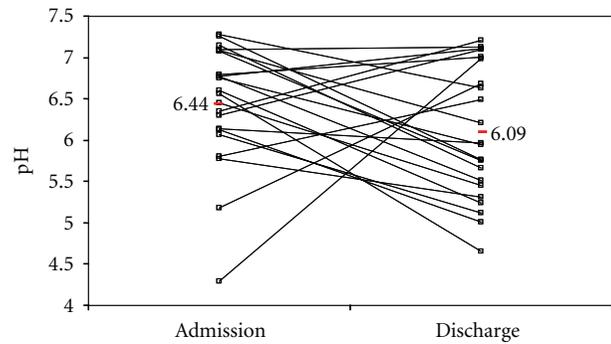


FIGURE 3: No change in pH of EBC in patients within 24 hours of admission to hospital for an exacerbation of bronchiectasis and at discharge from hospital following a course of intravenous antibiotics.

once the exacerbation and other potential contributing factors have been brought under control by treatment.

Some non-inflammatory patient factors influenced the EBC measurement. There is a relationship between EBC pH and patient height which may relate to lung size. EBC pH was more acidic in patients with reduced FEV_1 although there was no relationship with $FEV_1\%$ predicted. Also there is a difference between pH in males and females, females having more acidic EBC pH. These should be considered and corrected for if the measurement is to be used as a clinical tool. Height is related closely to lung size and volume and therefore there may be a larger surface area over which acids can be buffered.

Patients were asked to breathe into the condenser for 10 minutes. The volume of condensate differed from 0 mL to up to 5 mL. Therefore it could be considered that the concentration of the compounds of interest may be altered by the amount of water vapour produced to cause dilution. This could vary according to the temperature of the condenser which was not constant, the breathing pattern, and lung volumes of the subject. A method to assess dilution of the condensate has been proposed [17]. However another group have shown pH to be stable regardless of dilution with airway water vapour, temperature collection, age of subject, or breathing pattern during collection [9].

Longitudinal measurements showed that within individuals the change in EBC pH related to symptoms (SGRQ symptom score) and EBC pH is further acidified during an exacerbation of bronchiectasis, but not by more than the normal variation in a stable patient over 3 days (0.56). The specificity and sensitivity for EBC pH to predict an exacerbation and indicate the need for treatment were low, suggesting that EBC pH does not provide a clinically useful tool for measuring inflammation in bronchiectasis.

There is no difference in EBC ammonia between patients with bronchiectasis and healthy volunteers. In addition there is no correlation between EBC ammonia and any other measurements of disease activity or inflammation in bronchiectasis, and no change during an exacerbation. These findings suggest that EBC ammonia would not provide a clinically useful direct measure of inflammation in the lungs of patients with bronchiectasis. However ammonia levels correlate

with EBC pH and this may provide information about the mechanism for reduced pH in patients with bronchiectasis [5].

Significant acidification of EBC pH has been described in other inflammatory airway diseases, such as asthma [6, 18], COPD [6], and CF [4] compared to healthy control subjects. This acidification relates to oxidative stress and can normalise with corticosteroid or antibiotic treatment. There was no correlation between EBC pH and disease severity on lung function, suggesting EBC is not a sensitive marker of disease severity in bronchiectasis. Likewise in CF no relationship between FEV₁ and EBC pH has been found [4]. In contrast, a relationship in COPD and in asthma between pH and lung function has been identified [6]. This may be related to the area that is being assessed whilst measuring EBC. Tidal breathing into the condenser is likely to result in the majority of the aerosolised droplets originating from the proximal airways. It may be in bronchiectasis and CF that other parts of the lung are acidified with worsening lung disease but that this acidification is not measured using the EBC method described. EBC is most likely generated from the proximal airways due to the gentle tidal breathing manoeuvre with which it is collected. Therefore, EBC pH may be related to patient symptom score as a reflection of cough. Many bronchiectasis patients following an exacerbation continue to have an irritable cough and bronchial hyperactivity. This has been thought to be due to damaged epithelium but would be aggravated by the acidic pH which persists after treatment of the exacerbation [19].

In summary EBC is acidified in bronchiectasis patients compared with healthy controls. EBC pH relates to lung volume in patients with bronchiectasis but is not associated with severity of disease. Repeated measures over two years show change in EBC pH correlate with change in health status, which may be related to the relationship between cough and pH. EBC is further acidified during an exacerbation of bronchiectasis which persists following treatment. This could contribute to persistence of cough following an exacerbation. The drawbacks of EBC analysis lie in the anatomical sources of the compounds measured in EBC are not well defined and dilution of the condensate may affect results. EBC pH is not sufficiently specific or sensitive to monitor inflammatory status or provide information to inform acute treatment decisions.

Competing Interests

The authors declare no conflict of interests.

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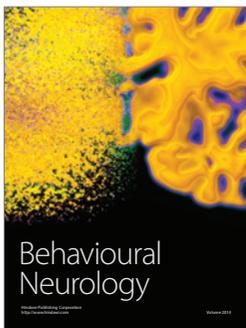
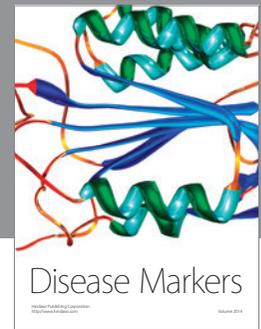
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