Eur Respir J 2003; 21: 37–42
DOI: 10.1183/09031936.03.00027603
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European Respiratory Journal
ISSN 0903-1936

Airway surface pH in subjects with cystic fibrosis

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Airway surface pH in subjects with cystic fibrosis. D. McShane, J.C. Davies, M.G. Davies, A. Bush, D.M. Geddes, E.W.F.W. Alton. ©ERS Journals Ltd 2003.

ABSTRACT: The cystic fibrosis (CF) transmembrane conductance regulator protein can transport bicarbonate and may therefore regulate airway surface (AS) pH. Disturbances of AS pH could contribute to the pathophysiology of CF lung disease.

Five studies were carried out including the following: study 1) nasal pH measurements were made in 25 CF and 10 non-CF adults using an antimony pH probe. Mean nasal pH was significantly lower in the CF group. Nasal potential difference may have been a confounding factor; study 2) in a fresh cohort of CF and non-CF subjects, no significant difference was found between the two groups using a gold pH probe; study 3) simultaneous nasal pH measurements were made in 15 CF and 15 non-CF adults using both probes. In the CF group, there was a trend for the antimony probe to read lower than the gold probe. In the non-CF group, the antimony probe read higher. The pH difference noted in study 1 related to technical factors; study 4) the effect of acute changes in serum acid/base balance on nasal pH was assessed in five non-CF adults. Nasal pH was not altered by either acute respiratory acidosis or alkalosis; study 5) nasal and lower airway pH was measured in five CF and six non-CF children. No difference was found between the groups. There was a correlation between nasal and lower airway pH.

The authors conclude that airway surface pH does not differ between cystic fibrosis and noncystic fibrosis subjects and therefore, cystic fibrosis transmembrane conductance regulator may not play a major role in airway surface pH *in vivo*.

Eur Respir J 2003; 21: 37–42.

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Keywords: Airway pH, bicarbonate, bronchoscopy, cystic fibrosis

Received: April 3 2002
Accepted after revision: September 9 2002

This work was supported by the Cystic Fibrosis Research Trust and by a Wellcome Trust Senior Clinical Fellowship (E.W.F.W.A.).

Cystic fibrosis (CF) is characterised by thick, viscous secretions, chronic bacterial infection and inflammation of the airways. The clinical phenotype is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. One function of CFTR is as a cyclic adenosine monophosphate-dependent chloride channel expressed by many epithelial cells [1]. However, how this or the many other described functions of CFTR relate to pathophysiology is unclear. The low volume hypothesis considers that the lack of chloride secretion plus excessive sodium absorption leads to impaired mucociliary clearance and hence chronic bacterial infection [2]. In contrast the high salt hypothesis suggests that abnormally elevated sodium and chloride concentrations in airway surface liquid (ASL) impair innate defence mechanisms [3].

In addition to its role in ion transport, CFTR has many other functions. CF cells express increased levels of asialoglycolipids, such as asialoGM1, a receptor for *Pseudomonas aeruginosa* and *Staphylococcus aureus* [4, 5]. This increased bacterial adherence has been shown to lead to a proportionately increased release of pro-inflammatory cytokines [6], a characteristic hallmark of CF. Furthermore, secreted glycolipids and mucins are over-sulphated, the degree of which correlates with both CFTR mutations [7] and severity of lung disease [8].

CFTR-mediated bicarbonate secretion has recently been identified [9–11]. CFTR mutations could thus alter ASL pH which in turn could alter a number of innate defence processes. For example, both ciliary function [12, 13] and mucus viscosity have been shown to be pH dependent [14]. Optimal ciliary function is favoured by an alkaline rather than an acidic environment [12]. Additionally, mucin solubility is enhanced by a high pH [15] and inhibition of bicarbonate secretion has been shown in an animal model to result in total occlusion of the submucosal glands by mucin [14]. Such occlusion, with associated glandular hypertrophy, is one of the first signs of lung pathology in newborn children with CF [16], although this has not been a consistent finding in all studies [17]. In addition to these effects on mucociliary clearance, an acidic environment has the potential to adversely affect other aspects of host defence and inflammation. Activation and adhesion of neutrophils [18] and interleukin-8 production by epithelial cell lines [19] have been shown to increase in conditions of low pH. Inflammatory cells are more likely to undergo necrosis than apoptosis in an acid environment [20, 21], which could contribute to tissue damage from the release of toxic cell contents. In addition to effects on the host, environmental pH has been shown to influence bacterial virulence factors. Lipopolysaccharide (LPS) possesses increased pro-inflammatory properties and Gram-negative bacteria have increased resistance

to antimicrobial peptides when grown at low pH. Interestingly, such modifications of LPS have been found in *P. aeruginosa* cultured from the lungs of CF patients [22]. Thus potentially, alterations in airway surface pH may explain many of the phenotypic hallmarks of CF and may lead to new treatment options.

Airway surface pH has been previously measured in the nose, and both the equipment and methodology are well established [23, 24]. Previous studies have reported that nasal pH varies with race [25] and the presence of rhinitis [26]. Acidification of airway vapour condensate has been shown to occur in acute asthma and is reversible with steroid therapy [21], suggesting a link with inflammation. Further pH measurements in the lower airways have been undertaken in a study assessing the degree of tracheal aspiration secondary to gastro-oesophageal reflux [27]. This group has also used this technique to detect microaspiration in CF adults, although mean baseline pH was not reported precluding comparison with their reported non-CF values [28]. Finally, *in vivo* murine studies have reported no difference in ASL pH between CF (CFTR-null) and non-CF (wild type) mice [29]. However a comparison has not been made in humans.

The possibility that the lack of CFTR-mediated bicarbonate secretion may play a key role in the pathophysiology of CF, led the current authors to compare the airway surface (AS) pH in CF and non-CF subjects. Here, five linked studies comparing two pH probes, measuring the effects of acute hyper- and hypocapnea on AS pH and comparing upper and lower AS pH, are reported.

Methods

Subjects

Adult subjects were recruited from the Royal Brompton Hospital (CF), London, UK, and from within the Dept of Paediatric Respiratory Medicine and Dept of Gene Therapy (controls). Eighteen CF adult subjects were homozygous for ΔF508, eight were heterozygous for this deletion and the genotype was undetermined in the remaining 12. Children (CF and non-CF) were recruited from fibreoptic bronchoscopy lists (table 1). The study was approved by the Ethics Committee of the Royal Brompton and Harefield NHS Trust and subjects or parents gave informed consent.

Studies 1, 2 and 3: nasal pH measurements

pH measurements were made using either a monocrystalline antimony catheter (studies 1 and 3) or an in-gold combined pH-glass electrode (studies 2 and 3) (Synectics Medical Ltd, Middlesex, UK), calibrated according to manufacturers instructions. Both probes are used clinically for the diagnosis of gastro-oesophageal reflux and are designed for repeated use. Initial studies were performed with the antimony probe (study 1), which has an external reference electrode. Due to concerns that this could allow pH readings to be affected by potential difference (PD), which is abnormal in CF, data were collected from a new cohort of subjects with the gold probe (study 2) and in some cases with both (study 3).

Initially, pH was measured at 1 cm intervals along the floor of the nose; all further measurements were made at 4 cm, a position known to discriminate clearly between CF and non-CF subjects with respect to PD [28]. pH was recorded on a Synectics Medical Digitrapper Mark II (gold probe) or Mark III (antimony probe) after 15 s of stability. In study 3, readings were taken from the same nostril with the order of probes varied randomly. In all cases, recordings were excluded from analysis if the probe was covered in mucus on removal. All patients were asked to blow their nose prior to insertion of the pH probe.

Table 1. – Data on all paediatric patients studied

Subject	Sex	Age yrs	Indication	Route	BAL culture
Cystic fibrosis					
No. 1	F	5	Insertion of portacath	ETT	*S. maltophilia*
No. 2	F	14	Refractory wheeze	LM	Negative
No. 3	F	3	RUL collapse	Nose	*S. epidermidis* *A. fumigatus*
No. 4	M	16	Refractory wheeze	ETT	*S. maltophilia*
No. 5	F	13	RLL collapse	ETT	*P. aeruginosa* *A. fumigatus*
Noncystic fibrosis					
No. 1	F	15	Haemoptysis	Nose	Negative
No. 2	F	3	Recurrent croup	Nose	*B. catarrhalis*
No. 3	F	5	Cough	LM	*B. catarrhalis*
No. 4	F	14	Haemoptysis	LM	Negative
No. 5	M	10	Cough	Nose	*B-haemolytic streptococcus*
No. 6	F	12	Recurrent respiratory tract infections	Nose	Negative

BAL: bronchoalveolar lavage; M: male; F: female; RUL: right upper lobe; RLL: right lower lobe; ETT: endotracheal tube; LM: laryngeal mask; *S. maltophilia*: *Stenotrophomonas maltophilia*; *S. epidermidis*: *Staphylococcus epidermidis*; *A. fumigatus*: *Aspergillus fumigatus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *B. catarrhalis*: *Branhamella catarrhalis*.

Study 4: the effect of acute changes in serum acid/base balance on nasal pH

Before embarking upon assessment of lower airway pH in patients under general anaesthetic in whom the carbon dioxide pressure (PCO_2) would not be monitored, the effect of acute respiratory acidosis/alkalosis on airway pH was assessed. An antimony pH probe was secured inside the nostril of non-CF adults. Oxygen was delivered *via* a nasal cannula into the other nostril to maintain saturations $\geqslant 95\%$. Nasal pH, transcutaneous PCO_2 (TINA Radiometer, TCM3; Radiometer Ltd, Copenhagen, Denmark) and oxygen saturations were recorded every 15 s throughout. After 1 min of tidal breathing, subjects rebreathed from a modified Douglas bag until their PCO_2 had risen to at least 1 kPa above baseline. Following a return of PCO_2 to baseline levels, they hyperventilated until their PCO_2 fell to at least 1 kPa below baseline. The mean of three pH measurements at the time of maximal and minimal PCO_2 was calculated.

Study 5: lower airway pH measurements

All lower airway measurements were undertaken with an inhaled general anaesthetic. Topical anaesthesia was not applied to the larynx before introduction of the bronchoscope. All measurements were made with either a 2.8 or 3.6 bronchoscope. The gold probe was attached to the outside of the bronchoscope with sterile tape, protruding 0.5 cm beyond the end to allow visualisation and introduced through the nostril, laryngeal mask or endotracheal tube (table 1) depending on the size of the child and the availability of an artificial airway. Where possible, pH was recorded in the trachea, main bronchus, and second and third generation bronchi. Mucus and sites of previous lavage were avoided. Nasal pH was then measured for comparison.

Statistics

The Mann-Whitney U-test was used to compare means between groups and the Wilcoxon rank-sum test to compare the means of paired data. The null hypothesis was rejected at $p<0.05$. For convenience, data are expressed as mean±SEM.

Results

Nasal pH measurements

Study 1. In 12 subjects (six CF; six non-CF), values at the edge of the nostril were similar in both groups (CF pH 5.6±0.1; non-CF pH 5.5±0.1). As the probe was advanced along the floor of the nose, the pH rose (fig. 1). Following this, nasal pH measurements were obtained with the antimony probe in 25 CF and 10 non-CF adults. At 4–5 cm, the site at which nasal PD is measured, the mean pH in the CF group was significantly lower than that of the non-CF group (CF 6.2±0.1; non-CF 6.7±0.13; $p<0.05$).

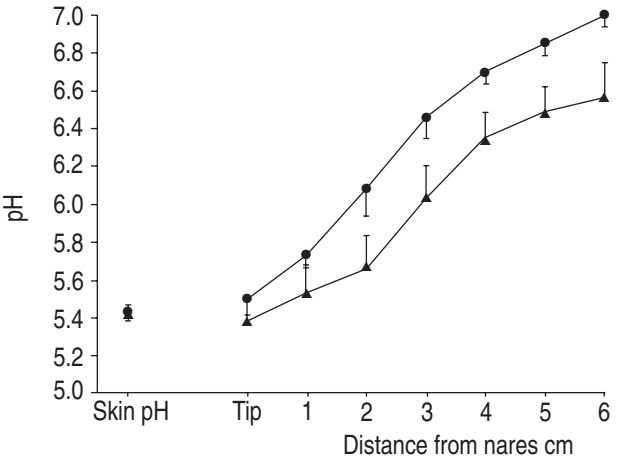

Fig. 1. – Nasal pH measured with an antimony probe at different distances from the nares. ●: non-cystic fibrosis (n=6); ▲: cystic fibrosis (n=6).

Study 2

Because of the potential confounding influence of the external reference electrode, nasal pH was measured in a further 38 CF (19 males, mean age 26 yrs, mean forced expiratory volume in one second 45% predicted) and 36 non-CF (9 males, mean age 31 yrs) adults at 4–5 cm using the gold probe. In contrast to the above findings, no significant difference was seen (CF 6.8±0.10; non-CF 6.6±0.1) (fig. 2). Similar to other studies, there was no sex difference (males 6.6±0.1; females 6.7±0.1).

Study 3

To investigate further the discrepancy between these results, nasal pH was measured in a new cohort of subjects (15 CF, 15 non-CF) using both probes. The

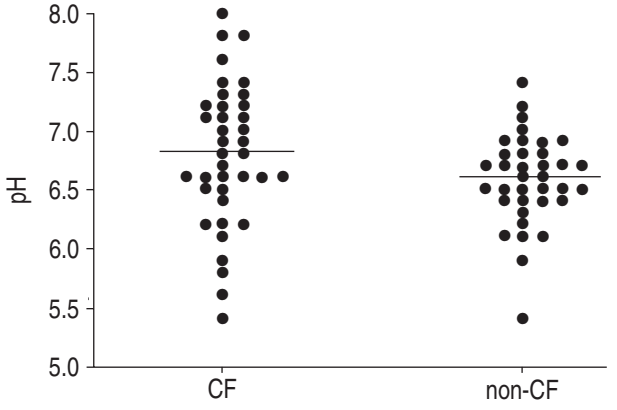

Fig. 2. – Nasal pH measured with a gold probe at a depth of 4 cm in cystic fibrosis (CF) (n=38) and non-CF (n=36) adults. No significant difference was found between the groups (CF 6.8±0.2, non-CF 6.6±0.1).

order of probes was varied between subjects and there was no significant difference in mean nasal pH when comparing first readings to second for either probe (first reading 6.8±0.1; second reading 6.7±0.1). The median time difference between readings was 20 min (range 10–390 min). CF subjects showed a trend towards a lower pH recording with the antimony than the gold probe (6.6±0.2 *versus* 7.0±0.2, respectively; p=0.05). Conversely, in the non-CF subjects, mean pH was significantly higher ($p<0.05$) with the antimony than with the gold probe (6.9±0.2 *versus* 6.5±0.2, respectively). Thus, the use of an antimony probe produces a CF/non-CF difference that is not seen with the gold probe.

Study 4: the effect of acute changes in serum acid/base balance on nasal pH

Duplicate studies were performed on five non-CF volunteers. The maximum P_{CO_2} achieved was 7.5 kPa (range 6.0–7.5) and the minimum 2.3 kPa (range 2.3–3.1). For the purposes of analysis, the means of three pH measurements at the time of both maximum and minimum P_{CO_2} were obtained for each individual. The mean observed increase in P_{CO_2} was 1.5±0.1 kPa, whilst the mean drop was 2.2±0.2 kPa. Over this range the pH did not change significantly (0.2±0.2).

Thus, acute alterations in systemic P_{CO_2} had no significant effect on airway surface pH.

Study 5: lower airway pH

Lower airway pH was successfully recorded in 11 children (table 1). In one child lack of manoeuvrability of the combined bronchoscope and pH probe would not allow passage beyond the larynx.

The mean pH for the lower airways was 7.1±0.2 for CF subjects, and 7.1±0.1 for non-CF subjects. The coefficient of variability for all readings was 2.4% (range 0–8.4%) and did not differ significantly between the groups. However in four children (one CF; three non-CF) a more marked variability was noted in the recordings at different sites (fig. 3). Interestingly, these patients had the most acidic pH values obtained by the group.

There was a significant correlation between nasal and lower airway pH measurements (n=11, $r^2=0.7$, $p<0.01$) for all data (fig. 4).

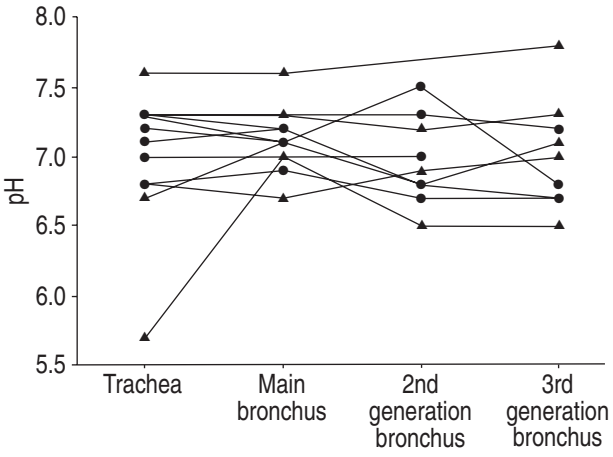

Fig. 3. – Lower airway pH was measured bronchoscopically with the gold probe. ●: non-cystic fibrosis; ▲: cystic fibrosis. Measurements for each individual have been plotted.

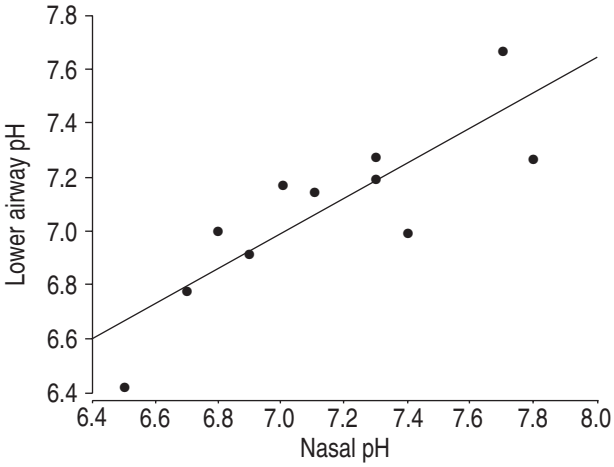

Fig. 4. – Correlation of upper and lower airway pH. A positive correlation between nasal pH and mean lower airway pH was identified. $r^2=0.7$, $p<0.01$.

Discussion

This study suggests that *in vivo* there is no significant difference between CF and non-CF nasal or lower airway pH. The genotype of the CF subjects did not appear to be an important variable. Airway surface pH remained stable despite acute changes in serum acid/base balance. Finally, a difference in pH measurements made by antimony and gold pH probes was demonstrated, suggesting that in the context of an altered potential difference in CF, only the gold probe provides relevant measurements.

Nasal pH measurements were made with an oesophageal pH probe. Placement of this probe could result in stimulation of the nasal mucosa with subsequent release of alkaline nasal secretions onto the airway surface. To reduce the impact of this, measurements were only recorded after a period of stability, which could take up to a minute to achieve. A further potential limitation of this technique is that the probe could be inadvertently placed into a nasal mucus plug. All patients were asked to clear their nose of secretions by blowing it prior to any measurements. Data were excluded from three subjects as the tip of the probe was found to be covered in mucus upon removal from the nasal cavity. There is a possibility that mucus may be removed from the probe upon its withdrawal from the nasal cavity and that these numbers, therefore, underestimate this problem. However the authors believe that this is unlikely since in both groups of subjects, nasal pH tended to be

acidic rather than alkaline. Additionally, the range of pH values observed in the nose and indeed the lower airways compares well with previously reported values from patients with tracheostomies [27].

A discrepancy was identified between the antimony and gold pH probe readings. In order to investigate this further, pH measurements were taken sequentially from the same nostril after a minimum of 10 min. Previous researchers have demonstrated crosstalk between nostrils, such that stimulation of the nasal mucosa from one nostril will result in the release of alkaline nasal secretions in the other [23]. In this study, the current authors found that the order in which either probe was used, that is either first or second, did not affect the pH recorded. Importantly, it was demonstrated that the antimony pH probe reads a lower pH in the CF group compared to the non-CF group, which was not seen with the gold probe. The abnormal nasal PD of CF patients [30] may render the measurements of the antimony probe inaccurate, and the authors would therefore recommend the gold probe be used for any further studies of airway pH.

Measurement of lower airway pH proved challenging. In preparation for lower airway readings, it was demonstrated that nasal pH is stable despite acute changes in serum acid/base balance possibly related to the presence of buffers on the airway surface. Recent work has suggested that ammonia produced by the glutaminase enzyme system may play an important role in airway pH homeostasis [31]. A disturbance of acid/base status could quite conceivably occur during any general anaesthetic and in particular during bronchoscopy when the airway is obstructed. These results confirmed the *ex-vivo* findings of KYLE *et al.* [32]. Briefly, using excised ferret trachea in which both cut ends were sealed, KYLE *et al.* [32] demonstrated the stability of ASL pH despite increasing concentrations of CO_2, both within the external bathing fluid and the tracheal lumen. The findings of KYLE *et al* [32] are in contrast to JAYARAMAN *et al.* [33] who used a murine model to assess the effects of acute respiratory acidosis on ASL pH. Hypoventilation of the mice increased arterial PCO_2 significantly, failed to cause a significant drop in arterial pH but reduced ASL pH. Species differences may account for the contrasting conclusions. For example, the diffusion capacities and buffer systems may differ between mouse, ferret and indeed humans. In the study by JAYARAMAN *et al.* [33], the resting murine arterial pH was 7.2, considerably more acidic than resting human blood pH. Despite this, both JAYARAMAN *et al.* [33] and the current group found the mean lower airway surface pH to be 7.1. The present study is thus in keeping with data from the ferret, whose airways more closely resemble those of man.

Lower airway measurements were made under general anaesthetic. Gas induction with sevoflurane was used in all patients and anaesthesia was maintained with this agent throughout the procedure. There is no evidence to support acidification of the airway secondary to this agent. Furthermore, it is likely that had there been an effect this would be seen in both CF and non-CF subjects. The authors were aware that local anaesthetic applied to the vocal cords prior to instrumentation could cause a change in airway surface pH and for this reason it was avoided in all patients. The increased cross-sectional diameter of the combined bronchoscope and pH probe limited the size of airway in which measurements could be made and also technically made manipulation of the bronchoscope within the airway difficult. pH readings were taken at individual sites after only a 15-s period of stability, as previously defined, and all readings were completed within 5 min. Nasal mucus was observed to have an alkaline pH during the measurements and during episodes of rhinitis nasal secretions become alkaline [26]. Although the current authors tried to avoid areas covered in mucus in the lower airways, accurate placement of the pH probe in the peripheral airways was difficult and this may account for the apparent trend towards an increase in pH observed in the third generation airways of the CF patients. Further measurements are necessary in order to clarify this.

The CF children attending the current authors' unit undergo bronchoscopy infrequently and recruitment for this study was therefore limited. It was also difficult to find matched controls for lower airway pH measurements in this group, as the non-CF children that undergo bronchoscopy may be less likely to have inflamed or infected airways similar to those of the CF children. The four most acidic lower airway measurements were from a mix of children (three non-CF, one CF). Only one out of these three non-CF children was infected as demonstrated by positive bacteriological culture of bronchoalveolar lavage fluid. It is unlikely the lack of difference in pH between CF and non-CF subjects is therefore a consequence of differing levels of inflammation.

In conclusion, no difference in the pH of the upper and lower airways between cystic fibrosis and non-cystic fibrosis subjects was found. The authors would suggest that the cystic fibrosis transmembrane regulator plays a relatively minor role in airway surface pH homeostasis. Finally, it is recommended that the gold probe is used for future airway pH studies.

Acknowledgements. The authors would like to thank the patients and colleagues who took part in this study.

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