

# Airway surface liquid pH in paediatric patients with cystic fibrosis

## PH del líquido de la vía aérea en pacientes pediátricos con fibrosis quística

Amalui Vasquez-Perez<sup>1,2,3,a\*</sup>, Montserrat Bosque-García<sup>2,3,b</sup>, Nuria Rudi-Sola<sup>3,4,c</sup>,  
Ester Parada-Ricart<sup>1,2,d</sup>, Ricardo Closa-Monasterolo<sup>2,e</sup>

<sup>1</sup>Servicio de Pediatría, Unidad de Pneumología Pediátrica, Hospital Universitari Joan XXIII, Tarragona; <sup>2</sup>Institut d'Investigació Sanitaria Pere Virgili, Universitat Rovira i Virgili, Tarragona; <sup>3</sup>Servicio de Pediatría, Unidad de Fibrosis Quística, Consorcio Corporación Sanitaria Parc Taulí, Sabadell; <sup>4</sup>Servicio de Farmacia, Hospital General de Granollers, Granollers. España

<sup>a</sup>0000-0002-3967-6361; <sup>b</sup>0000-0001-5426-4034; <sup>c</sup>0000-0003-1288-8046; <sup>d</sup>0000-0002-1696-036X; <sup>e</sup>0000-0002-9963-4163

### Abstract

**Background:** Cystic fibrosis (CF) patients have more acidic airway surface liquid (ASL), which can denature antimicrobial defensins. Induced sputum is non-invasive and is as representative as bronchoalveolar lavage. **Objectives:** The objectives of this study were to analyse the ASL pH obtained by induced sputum and assess the relationship between clinical features in paediatric CF patients. **Methods:** This is a prospective observational study in CF paediatric patients. Sputum was induced in a patient by inhaling 4.5% hypertonic saline, the sputum was collected into a sterile container for pathological analysis, and the pH was measured from the liquid part (ASL) in a gas machine. **Results:** A total of 27 patients were included in the study: mean age (11.96 ± 3.9) years, mean sweat test (99.38 ± 17.76) ng/L, common mutation Del508F (N24, 88.8%), mean FEV1% 91.94% ± 12.6%, Staphylococcus colonization 14 (51.9%), normal chest CT 8 (29.6%), air trapping 12 (44.4%), bronchiectasis 6 (22.2%), and mean ASL pH 6.72 ± 0.06 (n = 15). A significant correlation was found between a higher sweat test and lower ASL pH (R = 0.683, p = 0.005). There were no differences between altered chest CT (p = 0.199) and positive Staphylococcus aureus (p = 0.17). **Conclusion:** This is the first publication that use induced sputum to obtain the ASL pH in CF patients. The ASL pH in CF patients is usually acidic and correlated with altered transmembrane function conductance.

**Keywords:** Airway surface liquid pH. Cystic fibrosis. Infections. Sweat test. CFTR. Children.

### Resumen

**Antecedentes:** Los pacientes con fibrosis quística (FQ) tienen el líquido de la vía aérea (ASL) ácido, provocando desnaturalización de defensinas antimicrobianas. El esputo inducido es reproducible, no invasivo y la muestra es equiparable al lavado broncoalveolar. **Objetivo:** Evaluar el pH ASL obtenido por esputo inducido y analizar sus implicaciones clínicas, en niños FQ. **Métodos:** Estudio observacional prospectivo desarrollado en niños FQ. El esputo se indujo mediante inhalación de solución salina hipertónica 4,5%. Se recolectó el esputo y posterior al análisis patológico el ASL se colocó en una jeringa y se midió pH con una máquina de gases. **Resultados:** Se incluyeron 27 pacientes: edad (11,96 ± 3,9) años, test del sudor (99,38 ± 17,76) ng/L, variante común Del508F (N24, 88,8%), FEV1% 91,94% ± 12,6%, Staphylococcus 14 (51,9%), TAC

### \*Correspondence:

Amalui Vasquez Perez  
E-mail: amaluivanesa@hotmail.com

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pulmonar normal 8 (29,6%), atrapamiento 12 (44,4%), bronquiectasias 6 (22,2%), pH ASL  $6,72 \pm 0,06$  ( $n = 15$ ). Se observó correlación significativa entre niveles elevados del test del sudor y pH ASL ácido ( $R = 0,683$ ,  $p = 0,005$ ). No hubo correlación entre pH ASL y TAC pulmonar alterado ( $p = 0,199$ ) o *S. aureus* positivo ( $p = 0,17$ ). **Conclusiones:** Esta es la primera publicación que utiliza esputo inducido para medir pH del ASL en FQ. El pH ASL se correlacionó con la alteración del gen de conductancia transmembranal.

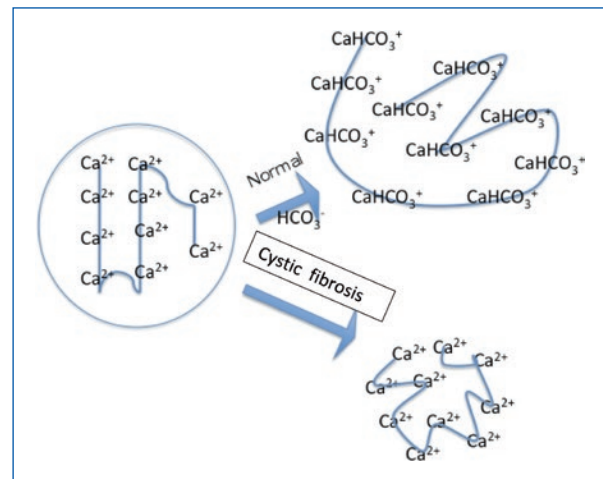
**Palabras claves:** pH del líquido de la vía aérea. Fibrosis quística. Infección. Test del sudor. CFTR. Niños.

## Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder caused by a mutation in a gene located on chromosome 7q31.2. This gene encodes a *transmembrane conductance regulatory protein* called CFTR, whose chlorine channel is adenosine triphosphate (ATP) and adenosine monophosphate cyclase-dependent (AMPC-dependent)<sup>1</sup>.

This gene mutation produces a CFTR defect that leads to an electrolyte imbalance in the apical membrane of epithelial cells, causing an ion alteration with the hyosecretion of chlorine and bicarbonate and the hyperabsorption of sodium and water<sup>2-4</sup>. For this reason, patients with CF have a more acidic and viscous airway surface liquid (ASL) than healthy individuals. Recent studies have shown that, under normal conditions, high concentrations of bicarbonate are required for mucin molecules to expand properly by creating a gel matrix that lubricates the epithelial surface, which acts as a barrier against pathogens (Fig. 1)<sup>4,5</sup>. Bicarbonate hyosecretion causes a drop in the ASL pH, which leads to a decrease in the bactericidal capacity of ASL and contributes to the potential progression of lung disease<sup>2</sup>. This is because the acidic pH of ASL can denature some defensins, which are antimicrobial proteins with neutral pH or alkaline activity. This decrease in pH in turn decreases the bactericidal capacity of defensins, increases mucus viscosity, and facilitates bacterial growth<sup>6-9</sup>.

The complexity of sampling human ASL *in vivo* limits the results. To date, the samples used to quantify the pH of ASL are exhaled breath condensate (EBC)<sup>10,11</sup>, direct pH measurements by bronchoscopy and bronchoalveolar lavage (BAL)<sup>9,12</sup>, nasal epithelium<sup>13</sup>, primary airway cell culture models of pigs<sup>2,14</sup> or humans<sup>15-17</sup>, and spontaneous sputum<sup>4,18</sup>, among others. At present, there is no publication about the utility of measuring ASL pH using induced sputum samples. However, it is well known that induced sputum is a non-invasive, easy, and reproducible technique and provides similar information as BAL<sup>9,19</sup>.



**Figure 1.** MUC2 mucin granules. The presence of  $\text{HCO}_3^-$  reacts with  $\text{Ca}_2^+$  condensed in mucin granules producing  $\text{CaHCO}_3^+$  which results in expanded mucin.

The objectives of our study were to analyse the ASL pH obtained by induced sputum technique, compare our results with another publications that measure ASL pH, and assess the relationship between the ASL pH value and its clinical features in a sample of paediatric CF patients without any type of *Pseudomonas* colonization.

## Materials and methods

### Study population

CF patients aged<sup>6-18</sup> years who were treated at the CF Unit of a third-level Hospital between September 2017 and March 2018 were screened for enrolment. The inclusion/exclusion criteria are summarized in Table 1, and written consent for inclusion in the study was obtained from the caregivers.

### Type of study

This is a prospective descriptive observational study, and it was approved by the ethical committee of the

**Table 1.** Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> <li>– Patients of both sexes, over 6 years of age, diagnosed with CF.</li> <li>– Pulmonary function &gt; 40% of the forced expiratory volume in the first second (FEV1%).</li> <li>– Patients who gave their informed consent or whose legal guardians gave informed consent to participate in the study.</li> </ul>	<ul style="list-style-type: none"> <li>– Basal oxygen saturation &lt; 92% or supplemental home oxygen needs.</li> <li>– Chronic colonization by <i>Pseudomonas</i>.</li> <li>– Massive haemoptysis.</li> <li>– Patients who were unable to follow the study's assessments according to protocol.</li> <li>– Any circumstance that, at the physician's discretion, may pose a clinical risk or harm to the patient during participation in the study or that interferes with the assessments of the study.</li> </ul>

CF: cystic fibrosis; FEV1%: non-bronchodilated forced spirometry in the first second.

Hospital (code PED-FQ-2016, CIR2015/077, 2015–2016), in Eudra CT and in EU Clinical Trials Register (code PED-FQ-2016, 2016-004033-25).

### Work plan

An appointment was set up for every patient, and a medical history review, physical exam, non-bronchodilated forced spirometry in the first second (FEV1%), CFQ-R quality of life<sup>20,21</sup>, and control Leicester Cough Questionnaires were carried out<sup>22</sup>. At baseline, sputum was induced according to the Belda methodology<sup>19</sup>, by inhaling 4.5% hypertonic saline for 7 min and repeating it 3–5 times (depending on the quality and the quantity of the sample obtained) through a mouthpiece without a valve or nose clip. Subjects were asked to blow their nose, rinse their mouth, and swallow water to minimize contamination from postnasal drip and saliva. After the third nebulization, they were instructed to cough sputum into a sterile container. The obtained sputum was divided into two containers: one container was sent to the pathology laboratory and the second container was sent to the microbiology laboratory for bacteriological and fungal culture. Opaque or dense portions that appeared different from saliva and that were free of squamous cell contamination under an inverted microscope were separated from the sample and centrifuged. The liquid part (ASL) was placed in a 1 mL syringe, and within 5 min, the pH was measured in a RAPIDPoint®500 gas machine (SIEMENS®). The solid portion was sent for cell analysis.

Once the results of the ASL pH were obtained by induced sputum technique, we compared the results obtained in our study with another methods in previously published studies, and a literature search was performed in PubMed following the PRISMA method protocol using the keywords: pH, ASL, and cystic fibrosis. We included papers with patients of all ages, clinical trials, observational studies with and without a

control group, human and animal model studies, and *in vivo* and *ex vivo* studies, published in English language, from 2002 to the present day.

The study variables can be seen in [Table 2](#).

### Statistical analysis

Discrete qualitative and quantitative variables were described using absolute frequencies and percentages. Normally distributed variables were reported as the mean and standard deviation, and non-normally distributed variables were reported as the median, minimum, and maximum. The Spearman/Pearson correlation coefficient was used to compare variables. Statistical significance was considered at a p-value of < 0.05.

### Results

A total of 27 patients were included in the study, and their general features are listed in [Table 2](#). The mean age was  $11.96 \pm 3.9$  years, and the male/female ratio was 1.4:1. The mean BMI z-score was  $-0.11 \pm 1.34$  (minimum of  $-2.5$  and maximum of  $5$ ). Notably, 25 patients (88%) were diagnosed with neonatal screening and 2 (12%) per clinical presentation. The mean sweat test was  $99.38 \pm 17.76$  ng/L. The most common mutation was Del508F, which was present in 24 (88.8%) patients, and 7 (25.9%) of those patients were homozygous. The second most common mutation was the N1303K genotype, which was present in 3 (11.1%) patients who were heterozygous. Notably, 19 (70.4%) patients had pancreatic insufficiency. The mean vitamins concentrations were as follows: vitamin A:  $0.41 \pm 0.39$  mg/L, vitamin D:  $28.7 \pm 8.7$  ng/mL, and vitamin E:  $12 \pm 6.8$  µg/mL. The mean TPr was  $1 \pm 0.1$ .

To assess the clinical condition of patients, 27 non-bronchodilated forced spirometry were performed, and the mean FEV1% was  $91.94\% \pm 12.6\%$ . Two patients

**Table 2.** General features of the study population

General features	Results	n
Mean age: years $\pm$	11.96 $\pm$ 3.96	27
Gender: M:F	1.4:1	16/11
Sweat test: mean $\pm$	99.1 nmol/L $\pm$ 16.5	27
Neonatal screening: %	88	25
Variants: %		
Del508F homozygous	25.9	7
Del508F heterozygous	62.9	17
N1303K heterozygous	11.1	3
BMI z-score: mean	-0.11 $\pm$ 1.34	27
FEV1%: mean $\pm$	91.94 $\pm$ 12.6	27
< 70%: mean $\pm$	63.5 $\pm$ 5.5	2
70-80%: mean $\pm$	76.3 $\pm$ 3	3
> 80%: mean $\pm$	96.6 $\pm$ 15.5	22
ASL pH: mean $\pm$	6.72 $\pm$ 0.06	15
Cellularity: %		
Neutrophils: mean $\pm$	79.75 $\pm$ 6.9	7
Eosinophils: mean $\pm$	9.3 $\pm$ 4.8	2
Bacterial culture: %		
Negative	40.7	11
<i>Staphylococcus aureus</i>	51.9	14
Other	7.4	2
Chest tomography: %	96.3	26
Normal	29.6	8
Air trapping	44.4	12
Bronchiectasis	22.2	6
Vitamins:		
A: mg/L	0.41 $\pm$ 0.39	27
D: ng/mL	28.7 $\pm$ 8.7	27
E: $\mu$ g/mL	12 $\pm$ 6.8	27
Coagulation (TPPr)	1 $\pm$ 0.1	24
Faecal elastasa: $\mu$ g/g mean $\pm$ (min-max)	184 $\pm$ 302 (0-1020)	27
Lipid digestion test: (g) mean $\pm$ (min-max)	8.73 $\pm$ 6.6 (1-26)	23
Pancreatic function: %		
Pancreatic sufficient	29.6	8
Pancreatic insufficient	70.4	19
Exacerbations in the previous year: mean $\pm$	1.77 $\pm$ 1.42	27

BMI: body mass index; FEV1%: not bronchodilated forced spirometry in the first second; ASL pH: airway surface liquid pH.

had a FEV1% < 80 (mean 63.5%  $\pm$  5.5%), 3 patients had FEV1% between 70% and 80% (mean 76.3%  $\pm$  3%), and 22 patients had FEV1% > 80 (mean 96.6%  $\pm$  15%). The mean exacerbation in the previous year was 1.77  $\pm$  1.42. Notably, 14 (51.9%) patients were chronically colonized by *Staphylococcus* spp., and 26 chest CT scans were performed with 8 (29.6%) being normal, 12 (44.4%) having air trapping, and 6 (22.2%) having bronchiectasis. For prior use of inhaled daily treatment, 12 (44.4%) patients used SSH7%, and 13 (48.1%) patients used SSH7% + dornase alfa. Notably,

**Table 3.** Mean values of the different domains in the Leicester Cough Questionnaire

Domain	Result: mean $\pm$ SD (min-max)
Physical	6.34 $\pm$ 0.5 (5-7)
Psychological	6.4 $\pm$ 0.66 (5.28-7)
Social	6.43 $\pm$ 0.8 (4-7)

**Table 4.** Mean values of the different domains in the CFQ-R Quality of Life Questionnaire

Items	Results: media $\pm$ SD	n
Physical	91.46 $\pm$ 11.7	17
Emotional	88.37 $\pm$ 10	17
Vitality	73 $\pm$ 13.2	11
School	65 $\pm$ 16	5
Food	83.66 $\pm$ 29	17
Treatment	65.36 $\pm$ 21.83	17
Body	85.4 $\pm$ 13.88	16
Health	79.8 $\pm$ 14.7	11
Social	71.57 $\pm$ 12.18	11
Weight	83.33 $\pm$ 22.47	12
Role	90.28 $\pm$ 12.2	6
Respiratory	79.9 $\pm$ 14.35	17
Digestive	90.2 $\pm$ 20.36	17

15 (55.6%) patients used ICS as a daily treatment, and 3 (11.1%) patients used oral azithromycin 10 mg/kg/day 3 days a week.

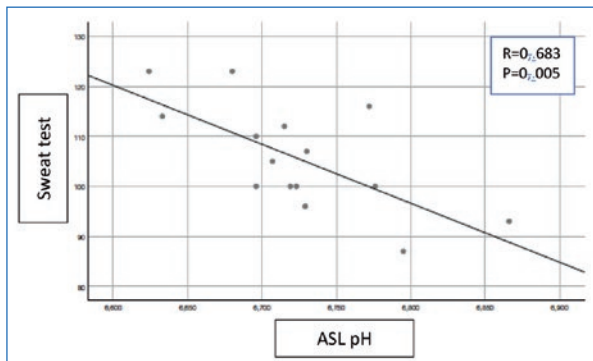
A total of 17 (62.9%) Leicester Cough Questionnaires were distributed, and the following results were obtained: a mean of 6.34  $\pm$  0.5 in the physical domain, a mean of 6.4  $\pm$  0.66 in the psychological domain, and a mean of 6.43  $\pm$  0.8 in the social domain (Table 3). Patients also completed 17 (62.9%) CFQ-R questionnaires, and the results can be seen in Table 4.

The mean pH value of the ASL was 6.72  $\pm$  0.06 (n = 15; two patients entered in the trial withdrew their consent to perform the induced sputum), and the cellularity of the induced sputum samples had a neutrophilic predominance with a mean of 79.9%  $\pm$  6.9% (n = 7).

Upon comparing the ASL pH with the CFTR function variables, we observed a statistically significant results between a higher sweat test value and a lower ASL pH (R = 0.683, p = 0.005) (Table 5, Fig. 2). There were

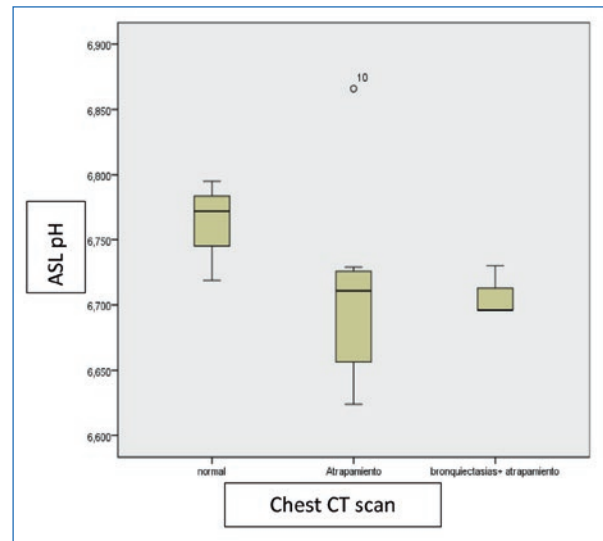
**Table 5.** Relationship among patients with sweat tests, variants, and ASL pH

ID Patient	Variants	Sweat test (nmol/L)	ASL pH
1	Del508F/R334W	93	6.866
3	Del508F/E92K	105	6.707
4	Del508F/E92K	112	6.715
5	Del508F/Del508F	107	6.73
6	Del508F/Del508F	110	6.696
7	Del508F/G85V	123	6.624
8	Del508F/R347P	100	6.723
9	Del508F/Del508F	100	6.696
10	Del508F/G542X	114	6.633
11	Del508F/2347Gdel	100	6.776
12	Del508F/N1303K	123	6.68
13	G542X/L206W	87	6.795
14	Del508F/R1066C	100	6.719
16	Del508F/Del508F	116	6.772
17	Del508F/6542X	96	6.729

**Figure 2.** Correlation between the sweat test and ASL pH in CF patients.

no statistically significant differences in the ASL pH among the pancreatic function variables. To compare the pH value of the ASL with the different clinical variables, we obtained no statistically significant results: chest CT ( $p = 0.199$ ) (Fig. 3), sputum culture ( $p = 0.17$ ) (Fig. 4), lung function ( $p = 0.45$ ), Leicester Cough Questionnaire domains (physical domain  $p = 0.67$ , psychological domain  $p = 0.63$ , and social domain  $p = 0.42$ ), or the CFQ-R Quality of Life Questionnaire (all  $p$ -values  $> 0.05$ ).

For the results of the literature research, 12 ASL pH studies were included and are summarized in Table 6. Two (16.7%) studies measured the ASL pH by EBC and the mean value was  $5.825 \pm 0.07$ ; 1 (8.3%) by

**Figure 3.** Relationship between the ASL pH and altered chest CT in CF patients. There was a tendency to observe a more acidic ASL pH in patients with altered chest CT ( $pH 6.70 \pm 0.06$ ) versus normal CT ( $pH 6.76 \pm 0.03$ ).

bronchoscopy and the mean value was  $7.1 \pm 0.1$ ; 1 (8.3%) by BAL and the mean value was  $6.98 \pm 0.15$ ; 1 (8.3%) by nasal catheter and the mean value was  $5.2 \pm 0.3$ ; 2 (16.7%) by bronchial epithelial pig cells cultures and the mean value was  $6.97 \pm 0.1$ ; 3 (25%) by bronchial epithelial human cell cultures and the mean value was  $7.02 \pm 0.54$ ; 1 (8.3%) by spontaneous sputum from the upper ASL and the mean value was 6.3, and 1 (8.3%) from the lower ASL and the mean value was  $7.33 \pm 0.36$ . In our study, the mean ASL pH value measured by induced sputum was  $6.72 \pm 0.06$ .

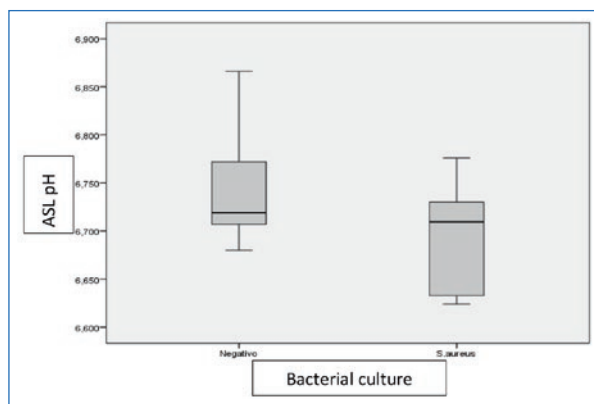
## Discussion

In our study, we developed a novel strategy to measure the ASL pH in CF patients obtained by sputum induction according to the Belda protocol<sup>19</sup> measured directly from the liquid part of the centrifugated sample of the induced sputum, and this methodology is easier, less invasive, reproducible, and shows similar results to other complex techniques, such as BAL or bronchial cell cultures. The mean ASL pH obtained by sputum induction was  $6.72 \pm 0.06$ , and our results confirm that the ASL pH in CF paediatric patients tends to be acidic.

This low pH is related to the CFTR function and not to the clinical condition as we have not found statistically significant results when comparing the induced sputum ASL pH with different variables (bacterial culture, CT

**Table 6.** Summary of published articles describing ASL pH in CF patients

Author	Journal	Year	Technique	Sample	Model	N	pH
Tate et al. <sup>10</sup>	Thorax	2002	EBC	ASL	Adults	30	5.88 ± 0.32
Ojoo et al. <sup>11</sup>	Thorax	2005	EBC	ASL	Adults	18	5.77 (4.81-6.99)
McShane et al. <sup>12</sup>	Eur Respir J	2003	Bronchoscopy	Lower ASL	Children	5	7.1 ± 0.1
Schultz et al. <sup>9</sup>	Nat Commun	2017	Bronchoscopy with BAL	Lower ASL	Children	30	6.98 ± 0.15
Alaiwa et al. <sup>13</sup>	J. Cyst Fibros	2014	Nasal mobidium catheter	Nasal ASL	Neonates	7	5.2 ± 0.3
Pezzulo et al. <sup>2</sup>	Nature	2013	Fluorescent pH indicator	ASL of bronchial epithelial cells cultures	Pigs	7	6.94 ± 0.05
Tang et al. <sup>14</sup>	J. Clin Invest	2016	Fluorescent pH indicator	ASL of bronchial epithelial cells cultures	Pigs	4	7 ± 0.2
Coakley et al. <sup>15</sup>	Proc Natl Acad Sci USA	2003	Micro-combination pH electrode	ASL of bronchial epithelial cells cultures	Adults	20	7.4
Garland et al. <sup>16</sup>	Proc Natl Acad Sci USA	2013	Micro-combination pH electrode	ASL of bronchial epithelial cells cultures	Adults	7	6.4
Simonin et al. <sup>17</sup>	Sci. Rep	2019	Micro-combination pH electrode	ASL of bronchial epithelial cells cultures	Adults	4	7.26
Grandjean et al. <sup>18</sup>	Medicine	2014	pH strips test	Upper ASL (spontaneous sputum)	Adults	54	6.3
Souza et al.	Clin Drug Investig	2020	pH meter ST2100®	Lower ASL (spontaneous sputum)	Adults	12	7.33 ± 0.361
Vasquez et al.	Rev Pat Resp	2023	pH meter RAPIDPoint® 500	Lower ASL (induced sputum)	Children	16	6.72 ± 0.06



**Figure 4.** Relationship between the ASL pH and positive *Staphylococcus aureus* culture in CF patients. There was a tendency to have a more acidic ASL pH in patients with positive culture (pH 6.69 ± 0.05) versus negative culture (pH 6.74 ± 0.05).

scan, and lung function). However, we found a tendency to have a more acidic ASL pH in patients with altered chest CT (pH 6.70 ± 0.06) versus normal CT (pH 6.76 ± 0.03) (Fig. 3). This was also observed in patients with positive *Staphylococcus aureus* sputum culture (pH

6.697 ± 0.05) versus negative culture (6.74 ± 0.05). These findings are similar to those of Simonin et al.<sup>17</sup>, who showed that the bacterial growth of *S. aureus* cultures from bronchial epithelial cells at different pH values increased significantly in CF cells versus healthy bronchial epithelial cells, and the bacterial growth was 1.5 times higher in CF bronchial cells than in healthy bronchial cells. Likewise, we did not find significant differences among the ASL pH in patients with altered chest CT or low pulmonary function. Monitoring lung physiology is critical in the clinical management of CF patients. Spirometry provides a functional assessment of total lung function. However, it does not provide local anatomical information as a chest CT would, which is the current “gold standard” method for monitoring anatomical changes in CF patients. Nevertheless, it is well established that patients could have a good FEV1% with altered chest CT<sup>27</sup>.

We found a significant statistically correlation between the CFTR function, higher sweat test value, and the lower ASL pH. We believe that this is because ASL pH value depends more on the gradient of the CFTR than on the clinical status of the CF patients. However, we consider the acidity of the ASL pH as an

important factor in the patient's prognosis and survival because it is related to the sweat test, which is one of the parameters that best measure the CFTR gradient.

Several papers describing the ASL pH in CF airways have been published from 2000 to the present, and they measured the ASL pH by different techniques by including animal and human cell culture samples<sup>10,14-17,24,25</sup>, where the ASL pH means were  $6.97 \pm 0.1$  and  $7.02 \pm 0.54$ , respectively; EBC<sup>10,11</sup>, where the ASL pH mean was  $5.825 \pm 0.07$ ; and spontaneous sputum sample collection<sup>4,18</sup>, where the ASL pH means were as follows: upper  $6.3$  and lower  $7.33 \pm 0.36$  SD. When comparing our ASL pH results with the results of the other studies, we note that the values range from a minimum of  $5.2$  in the case of neonatal nasal ASL or EBC to a maximum of  $7.33$  for adult spontaneous sputum samples. Our values are within the ranges reported in previous studies. In our study, the mean ASL pH was  $6.72$ , which was obtained by induced sputum. These results are very similar to the previous results obtained by more invasive techniques such as bronchoscopy with BAL or with bronchial epithelial cell cultures. However, these results do not appear to be comparable with the values obtained by EBC or nasal catheter. Nevertheless, we have not found any previous studies that analysed the ASL pH in CF children using induced sputum, although there are some studies, such as Schultz's study<sup>9</sup>, which analysed the pH of the ASL from BAL samples, and these results were very similar to ours.

Our results support the use of the induced sputum method as a technique that is less invasive than BAL but is as effective for the measurement of ASL pH.

We believe that no correlation was found because the acidic ASL pH is the result of an ion alteration caused by the CFTR defect, leading to a low pH, which facilitates bacterial growth and contributes to impairments in lung function. However, pH is not the only factor that contributes to lung deterioration in CF patients.

Therefore, children with CF will have a more acidic ASL pH, which could contribute to the pathophysiology of their disease because this acidity will cause pulmonary impairment and possibly lower survival.

Despite this, our study has some limitations. As a rare disease, the population included is small, which contributes to a lower statistical power. The absence of a "gold standard" method for measuring ASL pH makes it difficult to validate our results, although, at the same time, it encourages us to develop this interesting line of research that would serve as a great help in the management and prognosis of this disease.

## Conclusion

This is the first publication that used induced sputum as a technique to obtain the ASL pH in CF patients. Induced sputum could be considered a new method for measuring the ASL pH in CF patients. The mean ASL pH value obtained in our paediatric CF patients not colonized by *Pseudomonas aeruginosa* was  $6.72 \pm 0.06$ , and these results are similar to those of other studies that measured the CF airway pH with more invasive techniques. However, more studies are necessary to validate this technique.

## Funding

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## Conflicts of interest

The authors declare no conflicts of interest.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

**Confidentiality of data.** The authors declare that no patient data appear in this article.

**Right to privacy and informed consent.** The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

**Use of artificial intelligence for generating text.** The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

## Bibliography

1. Vankeerberghen A, Cuppens H, Cassiman JJ. The cystic fibrosis transmembranconductance regulator: An intriguing protein with pleiotropic functions. *Journal of Cystic Fibrosis*. 2002;1(1):13-29.
2. Pezzulo AA, Tang XX, Hoegger MJ, Alaiwa MHA, Ramachandran S, Moninger TO, et al. NIH Public Access. *Nature*. 2013;487(7405):109-13.
3. Choi JY, Muallem D, Lee MG, Thomas PJ, Muallem S. Aberrant CFTR-dependent HCO<sub>3</sub>-transport in mutation associated with cystic fibrosis. *Nature*. 2001;410(6824):94-7.

4. Gomez CCS, Parazzi PLF, Clinckspoor KJ, Mauch RM, Pessine FBT, Levy CE, et al. Safety, Tolerability, and Effects of Sodium Bicarbonate Inhalation in Cystic Fibrosis. *Clinical Drug Investigation* [Internet]. 2020;40(2):105-17. Available from: <https://doi.org/10.1007/s40261-019-00861-x>
5. Cutting GR. Cystic fibrosis genetics: From molecular understanding to clinical application. *Nature Reviews Genetics*. 2015;16(1):45-56.
6. Borowitz D. CFTR, bicarbonate, and the pathophysiology of cystic fibrosis. *Pediatric Pulmonology*. 2015;50(April):S24-30.
7. Garnett JP, Gray MA, Tarran R, Brodlie M, Ward C, Baker EH, et al. Elevated Paracellular Glucose Flux Cystic Fibrosis Airway Epithelial Monolayers Is an Important Factor for Pseudomonas aeruginosa Growth. *PLoS ONE*. 2013;8(10).
8. Heltsh SL, Mayer-Hamblett N, Burns JL, Khan U, Baines A, Ramsey BW, et al. Pseudomonas aeruginosa in cystic fibrosis patients with G551D-CFTR treated with ivacaftor. *Clinical Infectious Diseases*. 2015;60(5):703-12.
9. Schultz A, Puvvadi R, Borisov SM, Shaw NC, Klimant I, Berry LJ, et al. Airway surface liquid pH is not acidic in children with cystic fibrosis. *Nature Communications*. 2017;8(1).
10. Tate S, MacGregor G, Davis M, Innes JA, Greening AP. Airways in cystic fibrosis are acidified: Detection by exhaled breath condensate. *Thorax*. 2002;57(11):926-9.
11. Ojoo JC, Mulrennan SA, Kastelik JA, Morice AH, Redington AE. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. *Thorax*. 2005;60(1):22-6.
12. McShane D, Davies JC, Davies MG, Bush A, Geddes DM, Alton EFWF. Airway surface pH in subjects with cystic fibrosis. *European Respiratory Journal*. 2003;21(1):37-42.
13. Abou Alaiwa MH, Beer AM, Pezzulo AA, Launspach JL, Horan RA, Stoltz DA, et al. Neonates with cystic fibrosis have a reduced nasal liquid pH; A small pilot study. *Journal of Cystic Fibrosis*. 2014;13(4):373-7.
14. Tang XX, Ostedgaard LS, Hoegger MJ, Moninger TO, Karp PH, McMenimen JD, et al. Acidic pH increases airway surface liquid viscosity in cystic fibrosis. *Journal of Clinical Investigation*. 2016;126(3):879-91.
15. Coakley RD, Grubb BR, Paradiso AM, Gatzky JT, Johnson LG, Kreda SM, et al. Abnormal surface liquid pH regulation by cultured cystic fibrosis bronchial epithelium. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;100(26):16083-8.
16. Garland AL, Walton WG, Coakley RD, Tan CD, Gilmore RC, Hobbs CA, et al. Molecular basis for pH-dependent mucosal dehydration in cystic fibrosis airways. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110(40):15973-8.
17. Simonin J, Bille E, Crambert G, Noel S, Dreano E, Edwards A, et al. Airway surface liquid acidification initiates host defense abnormalities in Cystic Fibrosis. *Scientific Reports*. 2019;9(1):1-11.
18. Grandjean Lapiere S, Phelippeau M, Hakimi C, Didier Q, Reynaud-Gaubert M, Dubus JC, Drancourt M. Cystic fibrosis respiratory tract salt concentration: An Exploratory Cohort Study. *Medicine (Baltimore)*. 2017 Nov;96(47):e8423.
19. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. *American Journal of Respiratory and Critical Care Medicine*. 2000;161(2 1):475-8.
20. Oliveira G, Oliveira C, Gaspar I, Cruz I, Dorado A, Pérez-Ruiz E, et al. Validation of the Spanish version of the revised quality of life questionnaire for cystic fibrosis in adolescents and adults (CFQR 14+ Spain). *Bronconeumology Archives*. 2010;46(4):165-75.
21. Quittner AL, Buu A, Messer MA, Modi AC, Watrous M. Development and validation of the cystic fibrosis questionnaire in the United States: A health-related quality-of-life measure for cystic fibrosis. *Chest*. 2005; 128(4):2347-54.
22. Corral T, Percegon J, López N, Valiente A, Garriga M, Seborga M, et al. Validation of the Spanish version of the Leicester Cough Questionnaire in children with cystic fibrosis. *Bronconeumology Archives*. 2016;52(2):63-9.
23. Casan P; Burgos F. *SEPAR Procedure Manual (37 edic)*. 37th, chapter ed. *Manual Separ De Procedimientos*. 2015. 7-24.
24. Saint-Criq V, Haq IJ, Gardner AI, Garnett JP, Ward C, Brodlie M, et al. Real-time, semi-automated fluorescent measurement of the airway surface liquid pH of primary human airway epithelial cells. *Journal of Visualized Experiments*. 2019;2019(148):1-9.
25. Coakley RD, Grubb BR, Paradiso AM, Gatzky JT, Johnson LG, Kreda SM, et al. Abnormal surface liquid pH regulation by cultured cystic fibrosis bronchial epithelium. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;100(26):16083-8.
26. Koodziej M, by Veer MJ, Cholewa M, Egan GF, Thompson BR. Lung function imaging methods in Cystic Fibrosis pulmonary disease. *Respiratory Research*. 2017;18(1):1-11.