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2 **Determinants of exhaled breath condensate pH in a large asthma population.**

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10

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71

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89 Dr. Hunt is a shareholder in Respiratory Research, Inc. and in Airbase Pharmaceuticals. Dr.

90 Hunt is cofounder of Respiratory Research, Inc, which manufactures EBC collection

91 equipment and has financial interest in EBC pH testing. He is an inventor of EBC pH

92 assays. His University owns IP on EBC pH testing. He speaks regarding EBC pH

93 testing (no honorarium). He advises for Pulse Health, Inc, a company that studies
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98

99

100

101 **ABSTRACT**

102

103 Background: Exhaled breath condensate (EBC) pH is 2 log orders below normal during acute
104 asthma exacerbations and returns to normal with anti-inflammatory therapy. However, the
105 determinants of exhaled breath condensate pH, particularly in stable asthma, are poorly
106 understood.

107

108 Objectives: We hypothesized that patients with severe asthma would have low EBC pH; and
109 that there would also be an asthma subpopulation of patients with characteristically low values.

110

111 Methods: We studied the association of exhaled breath condensate pH with clinical
112 characteristics in 572 stable subjects enrolled in the Severe Asthma Research Program: these
113 included 250 subjects with severe asthma; 291 with non-severe asthma; and 31 healthy controls.

114

115 Results: Overall, EBC in this population of stable, treated study subjects was not lower in severe
116 asthma (8.02 interquartile range 7.61 – 8.41) or non severe asthma (7.90; 7.52 – 8.20) than in
117 controls (7.9; 7.40 – 8.20). However, in asthma the data clustered below and above pH 6.5.

118 Subjects in the subpopulation with pH below 6.5 had lower fraction of exhaled NO values

119 ($FE_{NO} = 22.6 \pm 18.1$ ppb) than those with $pH \geq 6.5$ (39.9 ± 40.2 ppb; $p < 0.0001$). By multiple

120 linear regression, low EBC pH was associated with high body mass index; high bronchoalveolar

121 lavage neutrophil counts; low pre-bronchodilator FEV_1 ratio; high allergy symptoms; race other

122 than Caucasian and gastroesophageal reflux (GER) symptoms.

123

124 Conclusion: Asthma is a complex syndrome. Subjects who are not experiencing an
125 exacerbation but have low exhaled breath condensate pH appear to be a unique subpopulation.

126

127

128

129

130 **ABBREVIATIONS**

131	AQLQ	Asthma quality of life questionnaire
132	BAL	Broncho alveolar lavage
133	BMI	Body mass index
134	EBC	Exhaled breath condensate
135	FE _{NO}	Fraction of exhaled nitric oxide
136	GER	Gastroesophageal reflux
137	GSNO	S-nitrosoglutathione
138	NO	Nitric oxide
139	SARP	Severe Asthma Research Program

140 **INTRODUCTION**

141
142 The airway can be acidified in a variety of inflammatory lung diseases¹⁻¹⁰. The airway
143 epithelium has a pH buffering function analogous to that of the renal tubular epithelium: acid
144 originating in the airway--or inhaled--is buffered by pH regulatory enzymes^{2,6,11,13}. In turn, these
145 enzymes are regulated. For example, airway buffering by glutaminase may be inhibited by Th1
146 cytokines, possibly for innate host defense by airway acid¹¹⁻¹³. In cystic fibrosis, the airway is
147 chronically somewhat acidic^{8,10}. In the case of asthma, on the other hand whereas the proximal
148 airway is acidified minimally--if at all--at baseline^{1,14,15}; it becomes more acidic^{12,13} in the
149 context of acute exacerbations caused by intercurrent viral infections^{1,16}. Here, we tested the
150 hypothesis that there is a subpopulation in stable, severe asthma with low exhaled breath
151 condensate (EBC) pH. This primary outcome variable was studied in a large cohort of subjects
152 in the Severe Asthma Research Program (SARP).

153
154 Determinants of EBC pH are multifactorial^{1,11,18-20}. Changes in deaerated EBC pH reflect
155 changes in subglottic airway acid production and buffering^{2,9,17-18}. For example, EBC pH values
156 measured through an endotracheal tube after intubation are the same as those measured through a
157 mouthpiece before intubation¹⁷; and asthmatic subjects intubated for respiratory failure have low
158 EBC pH that normalizes with corticosteroid treatment⁹. Of note, EBC pH values do not vary
159 with bronchodilator administration, hyperventilation or hypoventilation^{14,17}.

160
161 Asthma is likely not exclusively one homogeneous disease, but is a collection of different
162 biochemical and immune responses to environmental challenges that have, as a common

163 pathway, airway inflammation and reversible bronchoconstriction^{21,22}. We report that low pH is
164 associated with phenotypic features such as high body mass index (BMI), low baseline pre-drug
165 FEV₁, high airway neutrophil percent identified by bronchoalveolar lavage (BAL), non-
166 Caucasian race and gastroesophageal reflux (GER) symptoms. Further, we confirm^{1,5,23} that
167 there is a cluster of subjects with EBC pH values less than 6.5; and that these have a low fraction
168 of exhaled nitric oxide (F_ENO) and, particularly in severe asthma, increased allergy symptoms.
169 We conclude that low EBC pH may ultimately be useful as a biomarker to identify 1) specific
170 subpopulations of asthma patients; and 2) exacerbations of asthma^{1,16}. EBC pH does not
171 discriminate between severe and non-severe subgroups of stable asthma, but rather is one
172 biomarker that may be useful for identifying an asthma subpopulation.

173 **METHODS**

174

175 The Severe Asthma Research Program is a multi-center collaborative study sponsored by the
176 National Heart, Lung and Blood Institute²⁵. The goal of the study is to characterize as fully as
177 possible the distinguishing features of severe asthma, as defined by the American Thoracic
178 Society Consensus Meeting and adopted by the SARP Steering Committee^{24,25}. All severe
179 asthmatic subjects are chronically maintained on high dose-inhaled and/or systemic
180 corticosteroid treatment—and remain chronically symptomatic—when compared with a large
181 cohort of non-severe asthmatics, and with a smaller cohort of control subjects enrolled for
182 comparison^{24,25}. The methods of evaluation have been published²⁵. All subjects undergo lung
183 function testing—including maximum bronchodilator challenge; they also undergo an extensive,
184 questionnaire-based history, including allergy history, and those subjects who do not have a
185 specific contraindication undergo methacholine challenge and flexible bronchoscopy. Subjects
186 also undergo allergen skin testing (Greer, Lenoir, NC) for tree mix (white ash, American beech,
187 red birch, American elm, shagbark hickory, red oak, cottonwood), Timothy grass, ragweed,
188 *Alternaria*, *Cladosporium*, *Aspergillus*, *Dermatophagoides farinae* and *pteronysis*, weed mix
189 (cocklebur, lambs quarter, pigweed, English plantain, Russian thistle), dog, cat, and cockroach.
190 Most subjects in the first phase of SARP, including all patients at the Virginia, Wake Forest,
191 Emory, Cleveland and Wisconsin sites—where there was a specific interest in EBC analysis—
192 underwent EBC analysis. The protocol was approved by the Institution Review Board of each
193 participating institution.

194

195 **EBC Collection**

196
197 Exhaled breath condensate was collected over 5-10 minutes at baseline on the initial visit of the
198 SARP protocol as previously described. Samples were collected using the R-tube (Respiratory
199 Research, Inc., Charlottesville, VA) at a collection temperature of -20°C. For this study, EBC
200 was shipped on dry ice at variable time intervals to the University of Virginia for deaeration (at
201 ambient temperature²⁶ pH measurement by investigators blinded to the identity of characteristics
202 of the subject. Note that deaeration and storage at -20°C yields reproducible pH results, even in
203 intercontinental shipping^{17,27,28} across pH range 5-8 (n = 15): repeat assays give an $r^2 = 0.975^{27}$.
204 Samples were deaerated using Argon as previously described for 7 minutes and stable pH
205 recorded as previously described¹.

206

207 **Data Analysis**

208

209 All SARP data are collected, stored and analyzed in the SARP Data Coordinating Center at
210 National Jewish Hospital, in Denver. Data were further analyzed in the University of Virginia
211 Department of Public Health Sciences.

212

213 By univariate analysis, we studied the relationship between a continuous pH value and risk
214 factors. We computed the correlation between pH value and continuous risk factors (e.g., FEV₁
215 and BMI, numbers of positive skin tests, presence of bronchoalveolar lavage [BAL] neutrophils).
216 We carried out *t* test and non-parametric rank-sum test for two sample comparison (e.g.,
217 gastroesophageal reflux) and ANOVA for categorical variables with more than two levels (e.g.,
218 allergy symptoms in spring and winter). We then investigated using a multiple linear regression

219 to study the independent relationship of the continuous pH value with these risk factors,
220 adjusting for other confounding variables. We studied variables associated with dichotomized
221 pH determined by 1) the strongest positive and negative predictive value for identifying
222 gastroesophageal reflux (GER) symptoms (identified by receiver-operator curve [ROC]
223 analysis); and 2) distributions observed to be derived at pH 6.5. The statistical analyses were
224 carried out in SAS 9.1 (SAS Institute Inc., Cary, NC). The plots were drawn in S-Plus software
225 (Insightful Corp., Seattle, WA). Results are presented as mean, \pm SD; or median and range.

226 **RESULTS**

227

228 EBC was collected from 573 subjects 6 through 70 years old between July, 2003 and April,
229 2007. This cohort included 249 subjects with severe asthma (age 37.8 ± 15.6 ; 39% male), 293
230 subjects with non-severe asthma (age 32.2 ± 13.4 ; 32% male) and 31 healthy controls (age 28.7
231 ± 9.6 ; 32% male).

232

233 In this cohort, EBC pH has a median of 7.94, with inter-quartile range (7.55, 8.30). Since the pH
234 value is not normally distributed, we used the Wilcoxon rank-sum test to perform the two-sample
235 comparison. The deaerated pH of subjects with asthma (Median: 7.94; interquartile range: 7.56-
236 8.30) did not differ from that of controls (7.90; 7.40-8.20, $p=0.80$); and, if anything, pH in non-
237 severe asthma (7.90; 7.52-8.20) was lower than in severe asthma (8.02; 7.61-8.41). Patients with
238 asthma had a bimodal distribution, with a subpopulation having low pH (Figure 1).

239

240 We initially studied the relationship between a continuous pH value and other risk factors by
241 univariate analysis . We found that low pH value was significantly correlated with GER
242 symptoms ($p = 0.007$), race (non-white; $p = 0.002$), history of pneumonia ($p = 0.04$), allergic
243 symptoms without cold or flu ($p = 0.04$), acute or recurrent sinusitis ($p = 0.03$), nasal polyps ($p =$
244 0.01), not taking steroid ($p = 0.02$), receiving anti-IgE therapy ($p = 0.04$), and more severe
245 allergy symptoms, particularly in the spring ($p = 0.0001$). Low pH value was also associated
246 with high BMI ($p = 0.0009$), low baseline pre-bronchodilator FEV₁ ($p = 0.04$), low asthma
247 quality of life questionnaire (AQLQ) Emotion Score and low AQLQ Environmental Score ($p =$
248 0.002).

249 We then adopted multiple linear regression to study the relationship between the continuous pH
250 level and risk factors (Table I). We included all the variables which were significant in the
251 univariate analysis. In this multiple linear regression model including all subjects who
252 underwent bronchoscopy (n = 161; overall $R^2 = 0.32$), we found that pH value was negatively
253 associated with BAL neutrophil count, BMI, baseline pre-bronchodilator FEV₁, and GER
254 symptoms; and positively associated with pre-bronchodilator FEV₁. Caucasian patients had a
255 0.42 unit higher pH value than other races (Table I) (p=0.0006). These differences were not
256 affected by a priori NHLBI guidelines—or defined asthma severity assignment. Of note, the
257 association of low pH with GER symptoms was confounded by a strong association between
258 GER and BMI (p < 0.0001). In terms of pathophysiology, it is reasonable to speculate that high
259 BMI could lead to GER which, in turn, could contribute to low EBC pH.

260
261 In secondary analyses, we observed natural clusters of EBC pH in asthma above and below 6.5
262 (Figures 1 and 2). This distribution had been previously reported^{1,5,23}. We therefore also
263 compared patient characteristics dichotomized above and below pH 6.5 (Table II). We found
264 that F_ENO was lower in those subjects with EBC pH < 6.5 (p < 0.0001), consistent with a previous
265 report²³. Very low EBC pH was also associated with low methacholine PC₂₀ (p=0.03), and, with
266 severe asthma, allergic symptoms in the spring (p=0.006) and winter (p=0.03). There was a
267 trend toward association with number of positive allergy skin tests, but this did not achieve
268 significance (Table IIA).

269 **DISCUSSION**

270
271 Asthma is increasingly appreciated to be a multifactorial symptom complex^{21,22,29}. Genetic and
272 metabolomic markers may ultimately be important tools for patient classification leading to more
273 targeted treatment. For example, it is envisioned that response to therapies such as β -2 agonists,
274 corticosteroids and leukotriene-modifiers may be at least in part genetically determined: optimal
275 treatment could be targeted based on genetics and/or biochemical markers for responsiveness to
276 these treatments²⁹.

277
278 EBC pH is a metabolomic marker that is low (acidic) during acute asthma exacerbations.
279 Further, decreased airway pH recapitulates many features of asthma^{1,2,7,30-33}. Airway pH appears
280 to be normally distributed in the general population, measured at a retail outlet store³⁴, though
281 outliers with low pH might be explained if some of the subjects had unreported asthma, GER or
282 intercurrent infections^{11,13,16,20}. Here, we report that EBC pH in a large cohort of well-
283 characterized, severe and non-severe asthma is, on the whole, normal. In fact, values for severe
284 subjects were higher than those previously reported for normal African American subjects²³,
285 possibly suggesting a corticosteroid treatment effect. Consistent with a previous report³³, EBC
286 pH was higher in Caucasians than non-Caucasians.

287
288 In secondary analysis, we found that—among subjects with asthma—there was a cluster of
289 subjects with pH < 6.5. There was also a previously observed threshold at 7.8, but this was not
290 nearly as distinct in our population of closely monitored, heavily treated patients as that at 6.5.
291 This threshold at pH 6.5 has previously noted^{1,5,23}. It could reflect the point between pK_a's of

292 weak acids and bases. Strikingly, the variable most strongly associated with pH < 6.5 was low
293 $F_{E_{NO}}$, consistent with a previous report that $F_{E_{NO}}$ decreases with pH below pH 6.5²³. $F_{E_{NO}}$ has
294 been used as a nonspecific marker of inflammation, so the robust association of low $F_{E_{NO}}$ with
295 low pH may be counterintuitive. However, this association is not completely surprising for two
296 reasons. First, pH < 6.5 approaches the pK_a of nitrite⁷. *In vitro* and *in vivo*, low airway lining
297 fluid pH can lead to nitrite protonation, forming nitrous acid, which rapidly leads to NO
298 formation^{1,7,10}. Though this will acutely increase $F_{E_{NO}}$ ⁷, the result of chronically low pH will be
299 depletion of nitrite and other airway nitrogen oxides^{1,7,35}. Additionally, S-Nitrosoglutathione
300 (GSNO) metabolism increases $F_{E_{NO}}$ acutely *in vivo*, but depletes airway GSNO levels
301 chronically, decreasing $F_{E_{NO}}$; indeed, GSNO metabolism is increased in many patients with
302 asthma^{32,33,36-38,41}, SNP's in GSNO metabolic enzyme, GSNO reductase, are predictive of
303 asthma^{38,43}, and mice deficient in GSNO reductase are protected from experimental asthma³⁹.
304 Note in this regard that GSNO reductase activity can be induced by allergen exposure, and can
305 result in increased methacholine responsiveness³⁶; and both increased allergic symptoms in
306 general—note that samples were obtained in and out of season—and low methacholine PC₂₀
307 were characteristics of this low pH, low- $F_{E_{NO}}$ phenotype. Increased GSNO reductase activity in
308 asthma can be associated not only with decreased airway nitrogen oxide levels, but also with
309 increased production of formic acid, an important determinant of asthmatic airway
310 acidification^{19,40,42}. Therefore, the subset of patients with increased GSNO reductase activity
311 might be predicted to have low $F_{E_{NO}}$ and low EBC pH. One way or another, this subset appears
312 to be biochemically unique; and may be a subset in which the metabolomically targeted therapy
313 (for example, with inhaled base^{7,35}) may be particularly beneficial.
314

315 It should be noted that airway EBC pH was unrelated to inhaled corticosteroid dose. However,
316 the use of corticosteroids will raise EBC pH¹ acutely during exacerbation. The severe asthmatic
317 group, defined by high dose inhaled or systemic corticosteroid use, had, overall, a tendency to
318 have a slightly higher pH.

319
320 EBC pH was lower in patients with GER symptoms. A subpopulation of patients with chronic
321 cough who have consistently low EBC pH on serial studies at home appear to be those patients
322 whose cough can be successfully treated with high dose proton pump inhibitor therapy¹⁹.

323 However, the finding is based on a subjective questionnaire. Prospective studies will be needed
324 to determine whether a specific EBC pH will be useful in predicting responses to antacid-class
325 therapies in asthma.

326
327 In multiple linear regression low pH was also independently associated with high BAL
328 neutrophil counts, high body mass index (BMI), low log blood eosinophil count, low baseline
329 pre-drug FEV₁. With regard to the association between EBC pH and BAL neutrophilia, note that
330 this relationship 1) suggests—but does not prove—that distal airway/alveolar lung lining fluid
331 may influence EBC pH values; and 2) does not demonstrate conclusively that EBC pH is not
332 influenced by other cells in mixed cellular populations. Evidence suggests that the distal airway,
333 which is sampled by BAL, may be more acidic than the proximal airway³⁵. In the aggregate,
334 subjects with allergies and allergy symptoms could theoretically receive more corticosteroid
335 treatments—and/or have better adherence—leading to higher BMI, more GER, lower lung
336 function and/or relatively more airway neutrophilia. A chronic, undetected infection could also
337 play a role in the neutrophilic inflammation and low pH^{9,13,16}. Low EBC pH was associated with

338 modest evidence for flow limitation, consistent with a previous report⁵, though a decrease in
339 FEV₁, per se, does not cause a change in EBC pH³⁹ and the negative relationship between pH
340 and FEV₁/FVC ratio suggests that low pH does not predict obstruction. The determinants of
341 airway pH in stable asthma appear to be multifactorial.

342

343 It will be important prospectively to study the associations identified in this study, particularly
344 including the subpopulations with 1) low EBC and low FE_{NO}; and 2) low EBC pH and increased
345 BAL neutrophils. These subpopulations may be amenable to targeted, steroid-sparing therapy.
346 Indeed, it may be worthwhile to consider interventional trials--for example, with antacid therapy,
347 with inhaled alkaline buffer, with inhaled nitrogen oxides and/or with exercise and weight loss--
348 in which patients are stratified by EBC pH.

349

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355 SERzurum Study design, manuscript preparation

356 AFitzpatrick Study design, data collection, manuscript preparation

357 SMantri Data analysis and interpretation

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367 WNJarjour Study design, data collection

368 WMoore Study design, data collection, data analysis

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373 **REFERENCES**

374

375 1. Hunt J, Fang K, Malik R, Snyder A, et al. Endogenous airway acidification: Implications
376 for asthma pathophysiology. *Am J Respir Crit Care Med.* 2000;161:694-699.

377 2. Hunt JF, Gaston B, Ricciardolo F. Acid stress in the airway. *J Allerg Clin Immunol.*
378 2004;84:731-765.

379 3. Jack CI, Calverley PM, Donnelly RJ, et al. Simultaneous tracheal and oesophageal pH
380 measurements in asthmatic patients with gastro-oesophageal reflux. *Thorax.* 1995;50:201-204.

381 4. Karnad DR, Mhaisekar DG, Moralwar KV. Respiratory mucus pH in tracheostomized
382 intensive care unit patients: Effects of colonization and pneumonia. *Crit Care Med.* 1990;18:699-
383 701.

384 5. Kostikas K, Papatheodorou G, Ganas K, et al. pH in expired breath condensate of
385 patients with inflammatory airway diseases. *Am J Respir Crit Care Med.* 2002;165:1364-1370.

386 6. Paget-Brown A, Hunt J F, Gaston B. Tracheal fluid pH in human preterm infants. *Eur*
387 *Respir J.* 2007;30:840-842.

388 7. Gaston B, Kelly R, Urban P, et al. Buffering airway acid decreases exhaled nitric oxide
389 in asthma. *J Allergy Clin Immunol.* 2006;118:817-822.

390 8. Tate S, MacGregor G, Davis M, et al. Airways in cystic fibrosis are acidified: detection
391 by exhaled breath condensate. *Thorax.* 2002;57:926-929.

392 9. Walsh BK, Mackey DJ, Pajewski T, et al. Exhaled-breath condensate pH can be safely
393 and continuously monitored in mechanically ventilated patients. *Respir Care.* 2006;51:1125-
394 1131.

- 395 10. Yoon SS, Coakley R, Lau GW, et al. Anaerobic killing of mucoid *Pseudomonas*
396 *aeruginosa* by acidified nitrite under conditions characteristic of the cystic fibrosis airway. *J*
397 *Clin Invest*. 2006;116:436-446.
- 398 11. Hunt J, Erwin E, Palmer L, et al. Expression and activity of pH-regulatory glutaminase
399 in the human airway epithelium. *Am J Respir Crit Care Med*. 2002;165:101-107.
- 400 12. Gaston B, Adjei A, Amoah AG, et al. Breath condensate pH and ammonia levels are low
401 in patients with active pulmonary tuberculosis. *Conference of the Am Thorac Soc*. 2005:A574.
- 402 13. Carraro S, Doherty J, Zaman K, et al. S-nitrosothiols regulate cell-surface pH buffering
403 by airway epithelial cells during the human immune response to rhinovirus. *Am J Physiol Lung*
404 *Cell Molec Physiol*. 2006;290:827-832.
- 405 14. Nicolaou NC, Lowe LA, Murray CS, et al. Exhaled breath condensate pH and childhood
406 asthma. Unselected birth cohort study. *Am J Respir Crit Care Med*. 2006;174:254-259.
- 407 15. Coop C, Hagan LL, Dice JP. Exhaled breath condensate pH in the evaluation of asthma.
408 *Allergy Asthma Proc*. 2008;29:51-54.
- 409 16. Hunt J, Ngamtrakulpanit L, Vaughan J, et al. Airway acid-base disturbance in rhinovirus
410 cold. *Eur Respir J*. 2003;45:566S.
- 411 17. Vaughan J, Ngamtrakulpanit L, Pajewski TN, et al. Exhaled breath condensate pH is a
412 robust and reproducible assay of airway acidity. *Eur Respir J*. 2003;22:889-894.
- 413 18. Wells K, Vaughan J, Pajewski TN, et al. Exhaled breath condensate pH assays are not
414 influenced by oral ammonia. *Thorax*. 2005;60:27-31.
- 415 19. Greenwald R, Fitzpatrick AR, Erzurum S, et al. The severe asthma phenotype is
416 characterized by elevated levels of formate in exhaled breath condensate. *Am J Respir Crit Care*
417 *Med*. [abstract] 2009.

- 418
- 419 20. Hunt J, Yu Y, Burns J, et al. Identification of acid reflux cough using serial assays of
420 exhaled breath condensate pH. *Cough*. 2006;2:3-6.
- 421 21. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet*. 2006; 368:804-
422 813.
- 423 22. Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in
424 adults. *Lancet*. 2006;368:780-793.
- 425 23. Hauswirth DW, Sundy JS, Mervin-Blake S, et al. Normative values for exhaled breath
426 condensate pH and its relationship to exhaled nitric oxide in healthy African Americans. *J*
427 *Allergy Clin Immunol*. 2008;122:101-106.
- 428 24. Wenzel SE, Busse WW, The National Heart, Lung, and Blood Institute's Severe Asthma
429 Research Program. Severe asthma: lessons from the Severe Asthma Research Program. *J Allergy*
430 *Clin Immunol*. 2007;119:14-21.
- 431 25. Moore WC, Bleecker ER, Curran-Everett DC, et al. Characterization of the severe
432 asthma phenotype by the NHLBI severe asthma research program. *J Allergy Clin Immunol*.
433 2007;119:405-413.
- 434 26. Koczulla AR, Noeske S, Herr C, et al. Ambient temperature impacts on pH of exhaled
435 breath condensate. *Respirol*. 2010;15:155-159.
- 436 27. Ngamtrakulpanit L, Yuanlin Y, Adjei A, Amoah G, Gaston B, Hunt J. Identification of
437 intrinsic airway acidification in pulmonary tuberculosis. *Glob Public Health*, 2010; in press.
- 438 28. Kullman T, Barta I, Horvath I. Effect of freezing on exhaled breath condensate pH.
439 *Respir Med*. 2007;101:2566.

- 440 29. Weiss ST, Litonjua AA, Lange C, et al. Overview of the pharmacogenetics of asthma
441 treatment. *Pharmacogenomics J*. 2006;6:311-326.
- 442 30. Ahmed T, Ali J, al-Sharif A. Effect of alkali nebulization on bronchoconstriction in
443 acute bronchial asthma. *Respir Med*. 1993;87:235-236.
- 444 31. Holma B. Influence of buffer capacity and pH-dependent rheological properties of
445 respiratory mucus on health effects due to acidic pollution. *Sci Total Environ*. 1985;41:101-123.
- 446 32. Honma S, Tanaka H, Teramoto S, et al. Effects of naturally-occurring acid fog on
447 inflammatory mediators in airway and pulmonary functions in asthmatic patients. *Respir Med*.
448 2000;94:935-942.
- 449 33. Martinez D, Vermeulen M, von Euw E, Sabatté J, et al. Extracellular acidosis triggers
450 the maturation of human dendritic cells and the production of IL-12. *J Immunol*. 2007;179:1950-
451 1959.
- 452 34. Paget-Brown AO, Ngamtrakulpanit L, Smith A, et al. Normative data for pH of exhaled
453 breath condensate. *Chest*. 2006;129:426-430.
- 454 35. Shin H-W, Shelley DA, Fitzpatrick A, Gaston B, George SC. Airway nitric oxide is reduced
455 following phosphate buffered saline inhalation in asthma. *J Appl Physiol*. 2007;102:1028-33.
- 456 36. Snyder A, McPherson ME, Hunt JF, et al. Acute effects of aerosolized S-
457 nitrosoglutathione in cystic fibrosis. *Am J Respir Crit Care Med*. 2002;165:922-926.
- 458 37. Gaston B, Sears S, Woods J, et al. Bronchodilator S-nitrosothiol deficiency in asthmatic
459 respiratory failure. *Lancet*. 1998;351:1317-1319.
- 460 38. Wu H, Romieu I, Sienna-Monge JJ, et al. Genetic variation in S-nitrosoglutathione
461 reductase (GSNOR) and childhood asthma. *J Allergy Clin Immunol*. 2007;120:322-328.

- 462 39. Que L, Liu L, Yan Y, et al. Protection from experimental asthma by an endogenous
463 bronchodilator. *Science*. 2005;308:1618-1620.
- 464 40. Henderson EM, Gaston B. SNOR and wheeze: The asthma enzyme? *Trends Mol Med*.
465 2005;11:481-484.
- 466 41. Staab CA, Hellgren M, Höög J-O. Dual functions of alcohol dehydrogenase 3:
467 implications with focus on formaldehyde dehydrogenase and S-nitrosoglutathione reductase
468 activities. *Cell Mol Life Sci*. 2008;65:3950-3960.
- 469 42. Que LG, Yang Z, Stamler JS, et al. S-Nitrosoglutathione reductase. *Am J Respir Crit*
470 *Care Med*. 2009;180:226-231.
- 471 43. Moore PE, Yang Z, Stamler JS, et al. Genetic variants of GSNOR and ADRB2 influence
472 response to Albuterol in African-American children with severe asthma. *Pediatr Pulmonol*.
473 2009;44:649-654.

474 **FIGURE LEGENDS**

475

476

477 **Figure 1.** Exhaled breath pH condensate population distribution in normal controls, (red), mild-
478 moderate (blue dashed line) and severe asthma (dotted line). Note the distribution of some
479 asthma subjects substantially below pH 6.5. Individual subjects are shown as hash marks on the
480 X-axis.

481

482 **Figure 2.** The relation between pH and phenotypic features. A. Body mass index; B: Fraction of
483 bronchoalveolar lavage neutrophils; C: FEV₁; D: GER symptoms. Asthma severity is denoted by
484 the following: green dot: control; blue X: not severe; red circle: severe. Note that there is a
485 natural distribution of these data above and below pH 6.5.

486

487

488 **Figure 3.** The relationship between pH and allergy symptoms in spring and winter. Asthma
489 severity is denoted by different symbols: red dot: normal; blue X: not severe; green circle:
490 severe.

491

492

493 **Figure 4.** The relationship between pH and response to GERD symptoms by questionnaire
494 (yes/no). Asthma severity is denoted by different symbols: red: normal; blue: not severe; green:
495 severe.

496 **TABLE I. Relation of pH and risk factors (N=161, overall R²=0.32) using multiple linear variable**
 497 **analysis.**

	Estimate*	SE	P value
Baseline pre-bronchodilator FEV ₁	0.16	0.06	0.001
Bronchoalveolar lavage neutrophil (%)	-0.037	0.010	0.0005
BMI	-0.018	0.008	0.03
GER Symptoms	-0.41	0.15	0.009
Race (Caucasian vs. non-Caucasian)	0.42 ⁺	0.12	0.0006

498 *Slope of relationship (continuous variables) or difference (dichotomous variables)

499 ⁺Caucasian pH was 0.42 units higher than non-Caucasian (primarily African-American)

500

501

502 **TABLE IIA. Relation of dichotomous pH (cutpoint 6.5) and continuous risk factors**

503

	pH≤6.5 (Mean, SD) (Total N=39)	pH >6.5 (Mean, SD) (Total N=533)	P value
BAL pure neutrophils percent	11.4 (15.3)*	2.6 (4.1)**	0.18
BAL eosinophils percent	0.99 (1.18)*	0.97 (2.13)**	0.98
Body mass index	32.1 (11.4)	28.6 (8.1)	0.07
Baseline pre-bronchodilator FEV ₁	2.30 (0.74)	2.45 (0.92)	0.24
Baseline pre-bronchodilator FEV ₁ /FVC ratio	0.73 (0.10)	0.72 (0.12)	0.34
Methacholine PC20	4.1 (5.1)	6.8 (13.5)	0.03
AQLQ Emotion Score	4.3 (1.7)	4.7 (1.7)	0.21
AQLQ Environmental Score	4.1 (1.5)	4.6 (1.6)	0.08
IgE	253 (436)	344 (761)	0.29
Log eosinophis: Blood	-0.72 (0.51)	-0.67 (0.50)	0.63
# of positive skin tests	2.4 (2.9)	3.2 (3.0)	0.09
Age at diagnosis of asthma	13.1 (11.6)	13.7 (14.0)	0.78
Age of onset of allergic symptoms without cold or flu	9.8 (8.1)	11.3 (10.9)	0.32
Average fraction of exhaled nitric oxide value	22.6 (18.1)	39.8 (40.2)	<.0001

504

505 * Only 7 subjects had data available.

506 ** Only 157 subjects had data available.

507 **TABLE IIB. Relation of dichotomous pH (cutpoint 6.5) to discrete risk factors**

508

	pH≤6.5 (N, %) (Total N=39)	pH >6.5 (N, %) (Total N=533)	P value
Pneumonia history	20 (53%)	218 (42%)	0.23
Visited ER for breathing problems in last 12 months	10 (26%)	161 (30%)	0.72
Use theophylline	2 (5%)	40 (8%)	0.76
Asthma symptoms caused by physical activity	23 (61%)	307 (61%)	>0.99
Asthma symptoms caused by animal exposure	24 (63%)	301 (62%)	>0.99
Asthma symptoms caused by physical exercise	26 (70%)	416 (83%)	0.07
Asthma symptoms caused by respiratory infections	37 (95%)	451 (90%)	0.41
Allergic symptoms without cold or flu	35 (90%)	455 (90%)	>0.99
Gastroesophageal reflux symptoms	10 (28%)	109 (21%)	0.40
History of cats in the home	8 (21%)	104 (20%)	0.84
History of rodents in the home	0 (0%)	12 (2%)	>0.99
History of acute or recurrent sinusitis	18 (47%)	205 (39%)	0.31
History of CPAP or BiPAP use	1 (3%)	15 (3%)	>0.99
History of immunotherapy for asthma	2 (5%)	23 (5%)	0.69
History of nasal polyp	5 (14%)	63 (12%)	0.79
Contraceptive use	3 (13%)	74 (24%)	0.31
Herbal or natural suppl. Treatment	0 (0%)	25 (6%)	0.24
Allergy symptoms in spring			0.006
None	3 (8%)	43 (8%)	
Mild	4 (10%)	148 (29%)	
Moderate	15 (38%)	218 (42%)	
Severe	17 (44%)	109 (21%)	
Allergy symptoms in winter			0.03
None	5 (13%)	110 (21%)	
Mild	8 (21%)	160 (31%)	
Moderate	11 (28%)	152 (29%)	
Severe	15 (38%)	94 (18%)	
Taking steroid	31 (79%)	394 (74%)	0.57
Taking oral steroid	9 (24%)	102 (20%)	0.54
Anti-Ige therapy	3 (8%)	23 (4%)	0.41
Use leukotriene modifiers	14 (37%)	190 (37%)	>0.99
Any long-acting beta-agonist use	25 (66%)	333 (66%)	>0.99
Race			0.23
White	21 (54%)	336 (63%)	
Black	16 (41%)	150 (28%)	
Other	2 (5%)	48 (9%)	

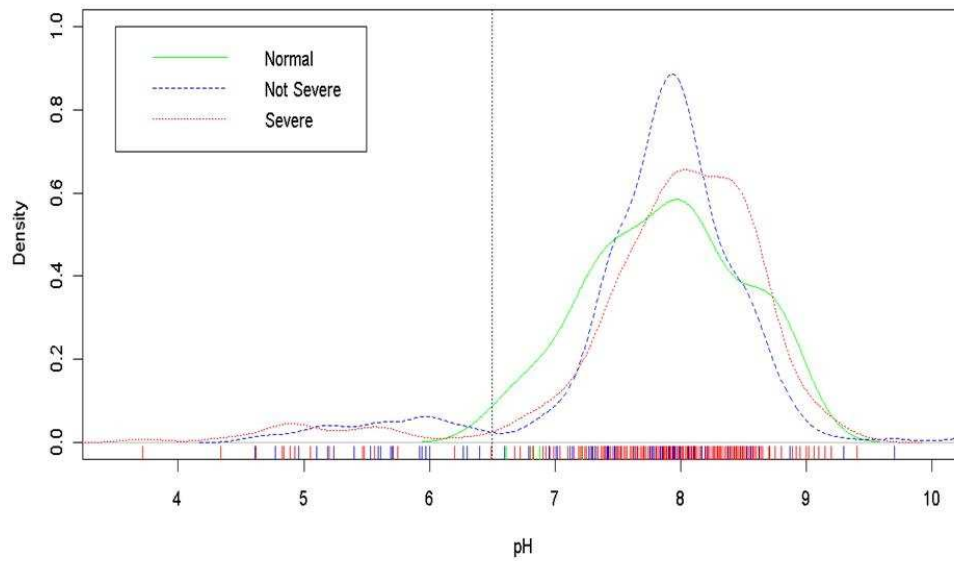
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510 NA: no normal control patients have pH≤6.5

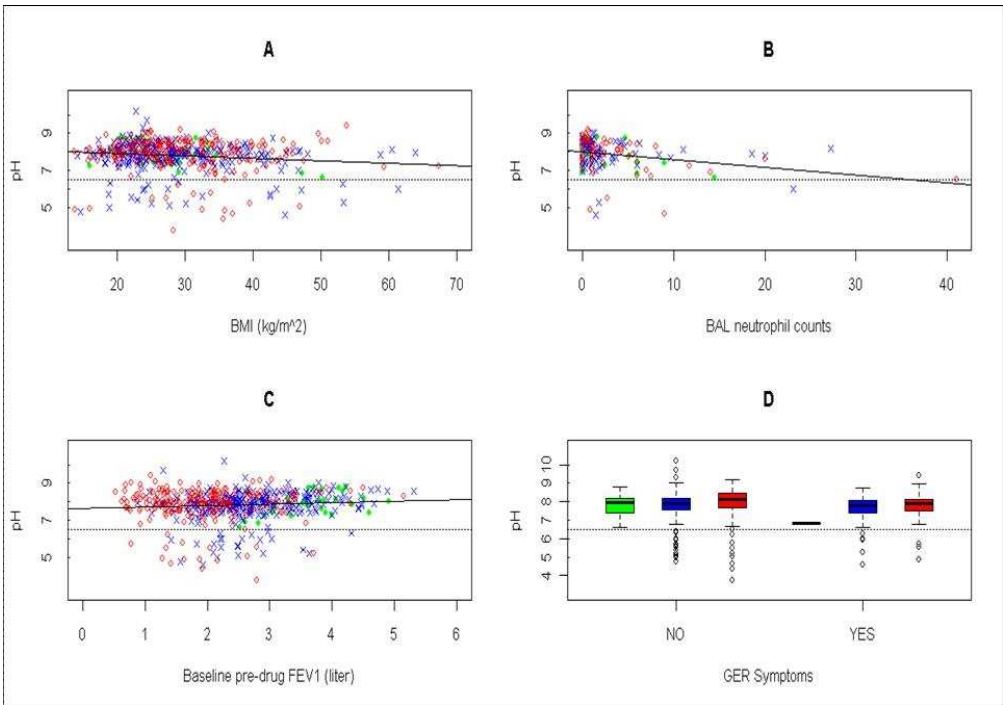
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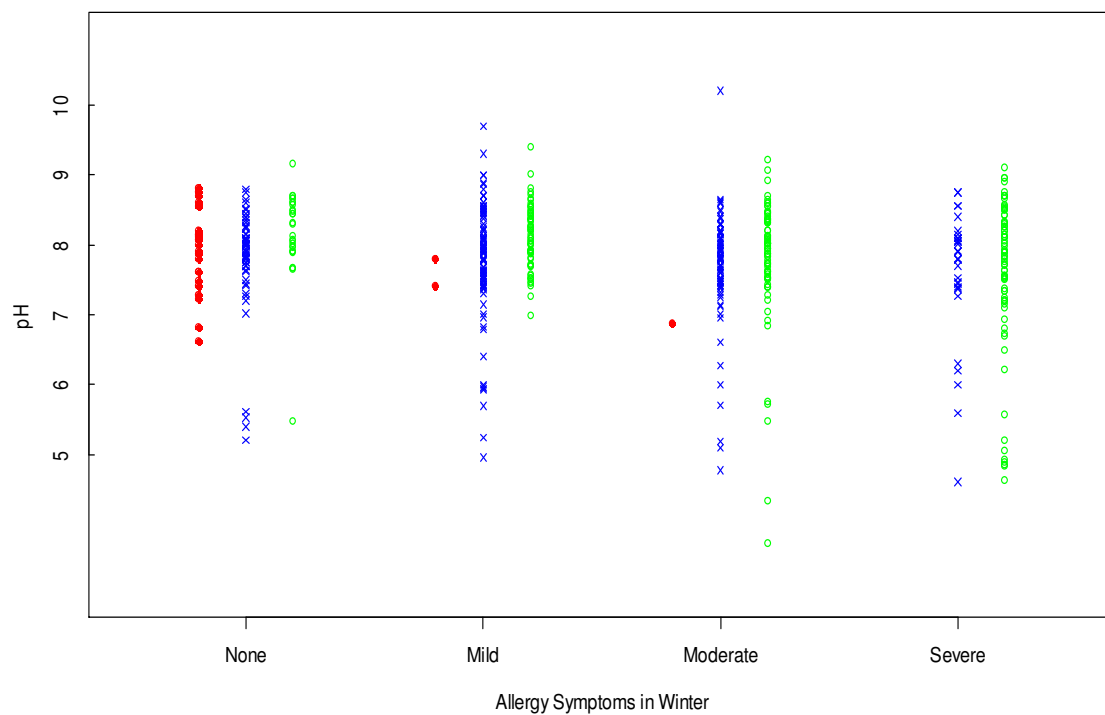
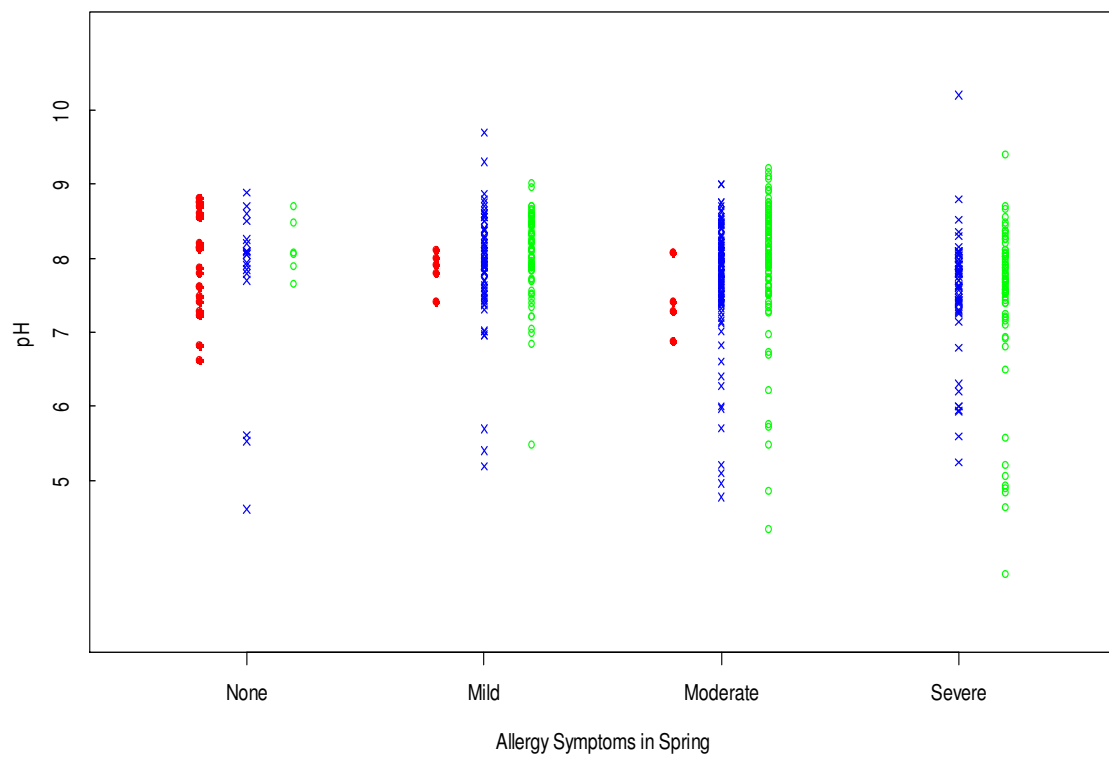


Figure3.

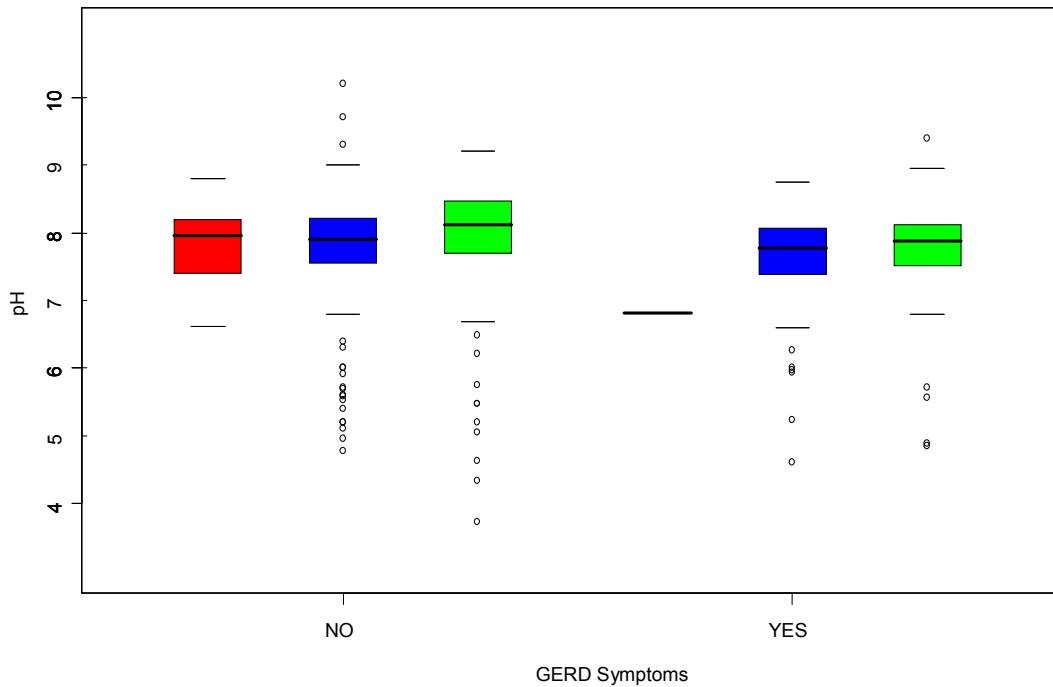


Figure 4.

“Online Data Supplement”

Determinants of exhaled breath condensate pH in a large asthma population.

Lei Liu, W. Gerald Teague, Serpil Erzurum, Anne Fitzpatrick, Sneha Mantri, Raed A. Dweik, Eugene R. Bleecker, William W. Busse, William J. Calhoun Mario Castro, Kian Fan Chung, Douglas Curran-Everett, Elliot Israel, W Nizar Jarjour, Wendy Moore, Stephen P. Peters, Annette Hastie, Sally Wenzel, John Hunt, Benjamin Gaston for the National Heart Lung Blood Institute Severe Asthma Research Program (SARP).

Table E1A. Relation of pH and continuous risk factors: univariate analysis.

Risk Factors	Correlation	P value
BAL neutrophil percent	-0.24	0.002
BAL eosinophils differential counts	0.03	0.72
Body mass index	-0.13	0.001
Baseline pre-bronchodilator FEV ₁	0.08	0.06
Baseline pre-bronchodilator FEV ₁ /FVC ratio	-0.08	0.05
Methacholine PC ₂₀	0.03	0.50
AQLQ Emotion Score	0.09	0.05
AQLQ Environmental Score	0.11	0.009
IgE	0.03	0.55
Log eosinophis: Blood	0.08	0.08
# of positive allergy skin tests	-0.10	0.02
Age at diagnosis of asthma	0.004	0.92
Age of onset of allergic symptoms without cold or flu	0.02	0.69
Average F _E NO	-0.10	0.02

Table E1B. Relation of pH and discrete risk factors” imovariate analysis.

Risk factors	Presence of Risk Factor	Absence of Risk Factor	P value
	No. Subjects (%) pH Mean (SD)	No. Subjects (%) pH Mean (SD)	
Pneumonia history	7.74 (0.88)	7.88 (0.76)	0.05
Visited ER for breathing problems in last 12 months	7.82 (0.81)	7.81 (0.85)	0.81
Use theophylline	8.02 (0.93)	7.81 (0.81)	0.16
Asthma symptoms caused by physical activity	7.82 (0.81)	7.78 (0.81)	0.54
Asthma symptoms caused by animal exposure	7.79 (0.85)	7.79 (0.80)	0.96
Asthma symptoms caused by physical exercise	7.73 (0.78)	7.70 (0.97)	0.21
Asthma symptoms caused by respiratory infections	7.80 (0.84)	7.79 (0.73)	0.92
Allergic symptoms without cold or flu	7.79 (0.82)	7.92 (0.88)	0.29
Gastroesophageal reflux symptoms	7.65 (0.81)	7.88 (0.79)	0.007
History of cats in the home	7.71 (0.88)	7.83 (0.80)	0.21
History of rodents in the home	8.08 (0.40)	7.80 (0.82)	0.04*
History of acute or recurrent sinusitis	7.67 (0.78)	7.87 (0.83)	0.15
History of CPAP or BiPAP use	7.80 (0.69)	7.82 (0.82)	0.91
History of immunotherapy for asthma	7.83 (0.82)	7.78 (0.83)	0.77
History of nasal polyp	7.99 (0.87)	7.80 (0.80)	0.09
Contraceptive use	7.87 (0.62)	7.74 (0.87)	0.14
Herbal or natural supplement treatment	7.92 (0.53)	7.73 (0.81)	0.11

*: This may be caused by the small sample size in the YES group (N=12).

Determinants of exhaled breath condensate pH in a large asthma population

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