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CORRESPONDENCE

The effect of gas standardisation on exhaled breath condensate pH

To the Editors:

We have read with interest the American Thoracic Society/European Respiratory Society Task Force document on exhaled breath condensate (EBC) [1]. EBC pH is emerging as a potential biomarker in respiratory disease. Gas standardisation (or de-aeration) of EBC with argon is commonly performed to remove carbon dioxide prior to pH measurement [2–4]. It has been argued that CO_2 is unwelcome ''noise'' in the sample and, although the completeness of CO_2 removal has not been confirmed, gas-standardised EBC pH is stable and provides reproducible measurements [3, 4]. However, some authors regard CO_2 as a relevant component of EBC and have measured pH without gas standardisation [5, 6]. These variations in methodology make comparison between studies difficult.

We recently reported a mean change in pH after gas standardisation of 0.94 [4], and this pH was stable at room temperature. Despite this rise in EBC pH, samples from patients with respiratory disease may remain acidic. Furthermore, gas standardisation may have little effect on the pH of very acidic samples [3, 7]. These observations indicate that the effect of gas standardisation is variable, and that there may still be stable acids present in the sample after presumed removal of CO_2. Therefore, we measured EBC pH pre- and post-gas standardisation to investigate the relative contributions of CO_2 and other acids to EBC pH. We also investigated the stability of EBC pH samples left at room temperature without gas standardisation.

EBC was collected from a total of 30 chronic obstructive pulmonary disease (COPD; 19 males, mean age 63 yrs, 15 current smokers, mean forced expiratory volume in one second (FEV_1) 62% predicted) and 20 asthma patients (nine males, mean age 50 yrs, zero current smokers, mean FEV_1 96% pred). pH was measured prior to and following gas standardisation with argon as previously described [5]. In samples collected from six COPD patients, pH was measured immediately and after 30 min and 3 h standing at room temperature without gas standardisation. Informed consent was obtained and the local ethics committee approved the study.

The mean (95% confidence interval (CI)) increase in pH post-argon was 0.91 (0.81–1.01; $p<0.00001$) and 0.92 (0.77–1.06; $p<0.00001$; fig. 1) in asthma and COPD, respectively. In asthma, there was a significant correlation between pre-argon pH and subsequent change in pH ($r=-0.77$; $p<0.0001$), which was described by the equation $y=-0.58x+4.8$. Six samples from COPD patients had a pre-argon pH of <6, with the change in pH post-argon ranging -0.05–1.75. Exclusion of these six samples resulted in a similar correlation ($r=-0.67$; $p=0.0004$) and equation ($y=-0.6x+4.9$). The pH of samples measured after standing at room temperature was significantly higher than those measured immediately; the mean (95% CI) increase was 0.20 (0.11–0.28) after 30 min and 0.67 (0.47–0.86) after 3 h.

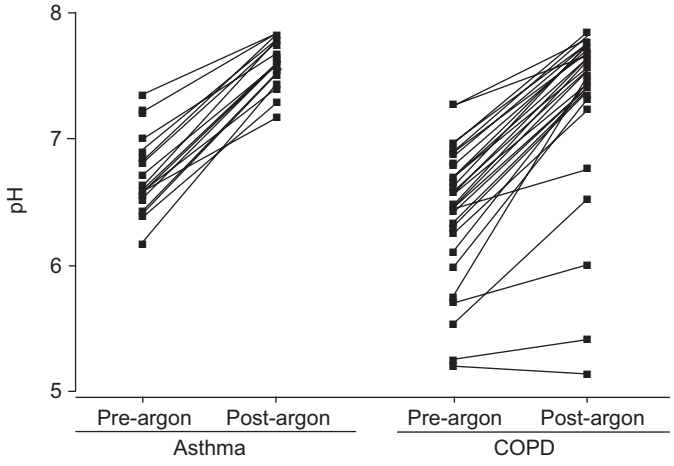

FIGURE 1. Exhaled breath condensate pH pre- and post-argon gas standardisation in asthma and chronic obstructive pulmonary disease (COPD) patients.

EBC pH is unstable at room temperature, presumably because CO_2 diffuses out of solution. This raises practical difficulties, as pH measurements may vary if not performed immediately. In contrast, the pH of argon de-aerated EBC samples is stable at room temperature [4].

Gas standardisation caused a significant increase in EBC pH, with a mean change of ~1, which is similar to a previous study [8]. However, we have shown that the effects of gas standardisation are more complex and can be related to the pre-argon value. At pre-argon pH >6, the effect is predictable and described by similar equations in asthma and COPD patients. Gas standardisation removes more CO_2 in samples with lower pre-argon pH values.

The effect of gas standardisation in samples with pre-argon pH <6 was unpredictable. GESSNER *et al.* [7] also noted that argon de-aeration may have very little effect on the pH of samples with an initial pH of <6. This suggests that either dissolved CO_2 is not being removed, or that the pH is mainly determined by other acids. If we assume that the change in pH after gas standardisation is a surrogate for CO_2 concentration, it has been shown that this varies between individuals. Acidic exhaled breath condensate pH could be caused by airway acidification at any level from the mouth to the alveoli. The

source and nature of exhaled breath condensate acidification remain controversial [9, 10]. The use of gas standardisation allows us to differentiate the probable contribution of CO_2 and that of other, as yet unknown, acids to exhaled breath condensate pH.

Z.L. Borrill, J.A. Smith, J. Naylor, A.A. Woodcock and D. Singh

Medicines Evaluation Unit, North West Lung Centre, South Manchester University NHS Trust, Wythenshawe Hospital, Manchester, UK.

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From the authors:

We appreciate the opportunity to reply to the letter by Z.L. Borrill and coworkers regarding the European Respiratory Society (ERS)/American Thoracic Society (ATS) exhaled breath condensate (EBC) Task Force report. EBC pH measurement is indeed a rapidly growing field of research with the promise of providing previously unknown information about the airways. The measurement is simple, can be performed sample by sample and there is no problem with the detection limit. EBC, however, is a diluted fluid with a low buffering capacity, which makes its pH value sensitive to changes in CO_2 content. Although the CO_2 concentration of fresh EBC samples is probably similar to that found in the airway lining fluid, this CO_2 concentration will change spontaneously due to the rapid interaction with environmental air after sampling. That is the main reason why deaeration of EBC samples is recommended to decrease the level of uncertainty when measuring EBC pH and to obtain more standardised readings. All of this appears to be so simple that, until recently, nobody dared to measure EBC partial pressure of carbon dioxide (P_{CO_2}) or at least to publish data on it.

It is interesting to observe the indirect approach that Z.L. Borill and coworkers use to estimate the CO_2 content of the EBC and its relative contribution to EBC pH by analysing the relationship between baseline pH values and pH changes caused by deaeration.

To prove the above concept (deaeration results in EBC pH change by causing a decrease in P_{CO_2} of the samples), there is a simply direct way, *i.e.* by measuring EBC pH together with CO_2 concentration in the samples. Therefore, we collected EBC samples from 17 healthy subjects (10 male; mean age 25 yrs; lung function values and exhaled nitric oxide concentration in normal range; all never-smokers) and performed measurements of pH and P_{CO_2} by a blood gas analyser directly after sampling (within 30 min) and after 10 min of deaeration using argon. Data are hereby given as mean$\pm$SD. The pre-deaeration pH was 6.70 ± 0.24 with a P_{CO_2} of 2.11 ± 0.92 kPa. Argon deaeration caused a decrease in EBC P_{CO_2} to 0.33 ± 0.09 kPa but never to 0 kPa ($p<0.0001$ compared with pre-argon value), and a significant increase of the pH to 7.67 ± 0.20 ($p<0.0001$; figs 1 and 2). There was a significant relationship between the changes in EBC P_{CO_2} and that of pH. Furthermore, in line with the observation by Z.L. Borrill and coworkers, there was a relationship between baseline EBC pH and the observed pH increase ($r^2=0.53$; $p=0.001$). The observed increase in pH (mean increase of 0.97) in our healthy subjects was similar to that observed by Z.L. Borrill and coworkers in asthmatic and

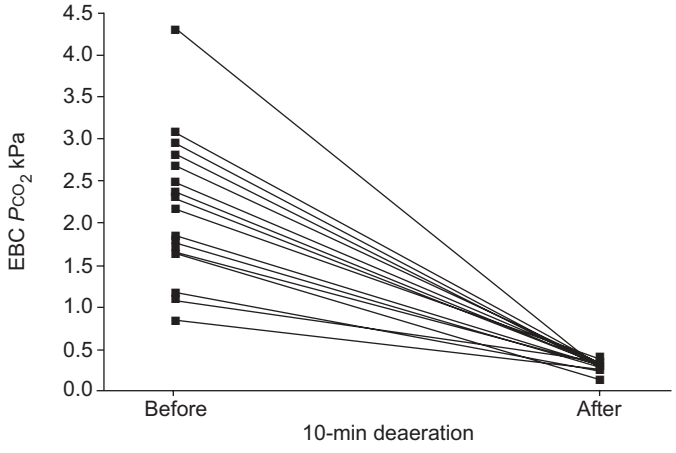

FIGURE 1. The effect of gas standardisation on exhaled breath condensate (EBC) partial pressure of carbon dioxide (P_{CO_2}).