Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis

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Submitted 2 October 2008; accepted in final form 19 March 2009

Exhaled breath condensate (EBC) biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. Am J Physiol Lung Cell Mol Physiol 296: L987–L993, 2009. First published March 20, 2009; doi:10.1152/ajplung.90512.2008.—Exhaled breath condensate (EBC) biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. Am J Physiol Lung Cell Mol Physiol 296: L987–L993, 2009. First published March 20, 2009; doi:10.1152/ajplung.90512.2008.—Exhaled breath condensate (EBC) analyses promise simple and noninvasive methods to measure airway biomarkers but pose considerable methodological challenges. We utilized mass spectrometry to measure EBC purine biomarkers adenosine and AMP plus urea to control for dilutional variability in two studies: 1) a cross-sectional analysis of 28 healthy, 40 cystic fibrosis (CF), and 11 asthmatic children; and 2) a longitudinal analysis of 26 CF children before and after treatment of a pulmonary exacerbation. EBC adenosine, AMP, and urea were readily detected and quantified by mass spectrometry, and analysis suggested significant dilutional variability. Using biomarker-to-urea ratios to control for dilution, the EBC AMP-to-urea ratio was elevated in CF (median 1.3, interquartile range [IQR] 0.7–2.3) vs. control (median 0.75, IQR 0.3–1.4; P < 0.05), and the adenosine-to-urea ratio was elevated in asthma (median 1.5, IQR 0.9–2.9) vs. control (median 0.4, IQR 0.2–1.6; P < 0.05). Changes in EBC purine-to-urea ratios correlated with changes in percent predicted forced expiratory volume in 1 s (FEV1) (r = –0.53 AMP/urea, r = –0.55 adenosine/urea; P < 0.01 for both) after CF exacerbation treatment. Similar results were observed using dilution factors calculated from serum-to-EBC urea ratios or EBC electrolytes, and the comparable ratios of EBC electrolytes to urea in CF and control (median 3.2, IQR 1.6–6.0 CF; median 5.5, IQR 1.4–7.7 control) validated use of airway urea as an EBC dilution marker. These results show that mass spectrometric analyses can be applied to measurement of purines in EBC and demonstrate that EBC adenosine-to-urea and AMP-to-urea ratios are potential noninvasive biomarkers of airways disease.

Adenosine; adenosine monophosphate; urea

Cystic fibrosis (CF) and asthma are characterized by persistent airway inflammation associated with significant morbidity and mortality (18, 26). Simple and noninvasive biomarkers of this inflammation are urgently needed to monitor disease progression, identify exacerbations, and evaluate the efficacy of novel therapies (27). Such biomarkers are particularly needed for young children with CF, in whom lower airway secretions are difficult to obtain.

Multiple studies suggest that biomarkers of airway disease can be measured in exhaled breath condensate (EBC), a simple and noninvasive method to obtain airway secretions (3, 15, 22, 27). However, the fraction of airway secretions within EBC has been shown to be very low and highly variable, posing considerable challenges to analysis (7, 8). Although several methods to control for this dilution have been validated, including measures of EBC electrolytes or urea (7, 8), the practical difficulties of measuring both a biomarker and a dilution marker within the relatively small volume EBC have limited widespread use of dilution correction.

We have recently demonstrated that mass spectrometry can be utilized to perform quantitative analyses of EBC biomarkers simultaneously with urea to control for dilutional variability. These studies focused on adenosine and AMP as biomarkers, adenylnitrites that are released onto the airway surfaces by resident airway cells and can act as signaling molecules to regulate host defenses (4). Several studies suggest that airway release of adenylnitrites is altered by disease, and elevated levels of airway adenosine have been reported in subjects with asthma (6, 16). In addition, airway secretions from subjects with CF exhibit elevated levels of AMP that are correlated with the severity of neutrophilic inflammation (10). This correlation could reflect contribution of neutrophils to extracellular purine levels, since this inflammatory cell releases ATP, which is metabolized primarily to AMP (19, 20) as part of a purinergic signaling pathway critical for neutrophil chemotaxis (5). However, other mechanisms underlying the relationship between purines and neutrophils have not been excluded.

In this study, we assessed the utility of our mass spectrometric analysis of EBC purines and urea in two studies: 1) a cross-sectional study of healthy children compared with children with asthma or CF; and 2) a longitudinal CF exacerbation study of CF subjects at the beginning and end of treatment of a pulmonary exacerbation. EBC was collected and analyzed simultaneously with urea to control for dilutional variability. Using biomarker-to-urea ratios to control for dilution, the EBC AMP-to-urea ratio was elevated in CF (median 1.3, interquartile range [IQR] 0.7–2.3) vs. control (median 0.75, IQR 0.3–1.4; P < 0.05), and the adenosine-to-urea ratio was elevated in asthma (median 1.5, IQR 0.9–2.9) vs. control (median 0.4, IQR 0.2–1.6; P < 0.05). Changes in EBC purine-to-urea ratios correlated with changes in percent predicted forced expiratory volume in 1 s (FEV1) (r = –0.53 AMP/urea, r = –0.55 adenosine/urea; P < 0.01 for both) after CF exacerbation treatment. Similar results were observed using dilution factors calculated from serum-to-EBC urea ratios or EBC electrolytes, and the comparable ratios of EBC electrolytes to urea in CF and control (median 3.2, IQR 1.6–6.0 CF; median 5.5, IQR 1.4–7.7 control) validated use of airway urea as an EBC dilution marker. These results show that mass spectrometric analyses can be applied to measurement of purines in EBC and demonstrate that EBC adenosine-to-urea and AMP-to-urea ratios are potential noninvasive biomarkers of airways disease.
asthma. The 2 asthma subjects who did not have mild disease were classified in accordance with the National Heart, Lung and Blood Institute guidelines for mild persistent disease for asthma.

### Subject demographics

<table>
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<th>Study</th>
<th>Control</th>
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<tbody>
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<td>40</td>
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<tr>
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<tr>
<td>Sex, % male</td>
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<tr>
<td>Number prescribed ICS†</td>
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<td>Number enrolled</td>
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<tr>
<td>Not detected</td>
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<tr>
<td>Number analyzed</td>
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<tr>
<td>BUN, mg/dl</td>
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</table>

Values are means ± SD. *Mild disease defined as percent predicted forced expiratory volume in 1 s (FEV₁) >80% for cystic fibrosis (CF) by National Heart, Lung and Blood Institute guidelines for mild persistent disease for asthma. The 2 asthma subjects who did not have mild disease were classified as having moderate persistent asthma. †ICS, inhaled corticosteroids, either fluticasone (dose range 176–500 μg daily) or budesonide (dose range 0.25–0.5 mg daily). Two subjects with CF and 1 subject with asthma had received oral corticosteroids (10-20 mg prednisone daily) before the study visit. BUN, blood urea nitrogen.

subjects were recruited from the Children’s Pulmonology Clinic at UNC during regularly scheduled visits. CF was diagnosed using Cystic Fibrosis Foundation criteria (25); asthma was a clinical diagnosis made by the subject’s pediatric pulmonologist. For the CF exacerbation study, CF subjects were recruited within 48 h of admission to the inpatient unit for intravenous antibiotics to treat an pulmonary exacerbation with treatment plan determined by the attending CF physician. EBC was collected at the time of recruitment and again within 48 h of discontinuation of antibiotics. Seventeen CF subjects were included in both the cross-sectional and CF exacerbation studies. All studies were approved by the institutional review board, and informed consent was obtained.

### Laboratory values

Spirometry was obtained using a flow-based SensorMedics Vmax Spirometer (SensorMedics, Yorba Linda, CA), and percent predicted values were calculated using National Health and Nutrition Examination Survey (NHANES) III normative data (13). Serum blood urea nitrogen (BUN) was analyzed by the UNC Hospitals core laboratory using standard procedures.

**EBC collection.** EBC was collected using the RTube device (Respiratory Research, Charlottesville, VA). Subjects exhaled through the RTube for 7 min with the chiller sleeve at −20°C at the start of collection. This relatively short collection time facilitated participation of younger subjects and was sufficient to generate the necessary volume of condensate. No nose clips were used. EBC volumes collected in the cross-sectional study averaged 770 ± 220 μl for control, 640 ± 160 μl for CF, and 470 ± 180 μl for asthma subjects; EBC volumes in the CF exacerbation study averaged 610 ± 220 and 730 ± 240 μl at the start and end of antibiotic treatment, respectively. EBC (which remained in liquid form during collection) was extracted from the RTube per the manufacturer’s recommendation, divided into 300-μl aliquots, and frozen at −80°C until analysis. Although salivary contamination was not specifically excluded, EBC collected using the RTube has been found to be free of salivary contamination when collected under supervised conditions such as those of this study (15).

**LC-MS/MS analysis of EBC.** Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed using a previously described method (11). Briefly, 15 μl of an internal standard solution (50 μM [15N]urea, 50 nM [13C,15N]adenosine, and 250 nM [15N]AMP) was added to 300 μl of EBC, and samples were lyophilized to dryness overnight and resuspended in 15 μl HPLC grade water. Ten microliters of resuspended samples was injected into an LC-MS/MS system consisting of an Acquity solvent manager and a TSQ Quantum Ultra Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA). An Atlantis T3 C18, 1.8-μm column (Waters, Milford, MA) was operated with a gradient of 5% methanol-0.1% formic acid to 80% methanol-0.1% formic acid over 4 min. Purines and urea were detected in positive mode electrospray ionization (ESI)-LC-MS/MS by selected reaction monitoring of the mass-to-charge ratio (m/z) transitions 61→44 for urea, 268→136 for adenosine, and 348→136 for AMP as previously described (11).

**Statistical analysis.** Purine concentrations from EBC studies were analyzed as ratios to urea or through calculation of dilution factors as described in the text. Because these data did not follow a normal distribution (P < 0.05 by D’Agostino and Pearson omnibus normality test), they were analyzed using nonparametric procedures: Mann-Whitney for comparison between groups, Wilcoxon matched pairs for paired data comparisons, Spearman coefficients for correlations, and Kruskal-Wallis for ANOVA with Dunn’s multiple comparison for posttest comparisons. All demographic measures in Table 1 are presented as means ± SD, and all EBC measures are graphed as medians ± interquartile range (IQR).

### RESULTS

**EBC purines are elevated in CF: the cross-sectional study.** To determine whether airway purines were altered in respiratory disease, we measured EBC purines and urea using a highly sensitive and reliable LC-MS/MS-based protocol (Fig. 1; Ref. 11). EBC was collected from 34 healthy controls, 45 children with CF, and 14 children with asthma as disease controls during regular clinic visits (cross-sectional study, Table 1). Purines and urea successfully measured from a total of 28 healthy control, 40 CF, and 11 asthma EBCs (Table 1).

As expected from previous studies of EBC dilution, we observed a wide variability in purines and urea concentrations in these samples (Fig. 2A). The EBC concentrations of the dilution marker urea were significantly different among the groups and highly correlated with both adenosine and AMP (r = 0.65 and 0.66, respectively; P < 0.05), suggesting that much of the measured variability in purine concentrations resulted from variable dilution of airways secretions within the condensate. Therefore, we examined ratios of EBC constituents as an established method to control for dilution (15, 23). Using ratios (nanomolar purine to micromolar urea), we found that the AMP-to-urea ratio was significantly elevated in CF (median 1.3, IQR 0.7–2.0) compared with healthy control (median 0.75, IQR 0.3–1.4; Fig. 2B). In addition, we observed an elevation in the EBC adenosine-to-urea ratio in subjects with asthma (median 1.5, IQR 0.9–2.9) compared with control (median 0.4, IQR 0.2–1.6), in agreement with previous studies.
in this disease (6, 16). The adenosine-to-urea ratio in CF subjects (median 1.0, IQR 0.5–1.8) displayed a trend toward a modest increase ($P < 0.10$) relative to control. There were no significant differences between asthma and CF in either ratio ($P > 0.1$). In the CF subjects, we did not observe any relationship between the adenosine-to-urea or the AMP-to-urea ratio and either the presence of pathogens on culture or with lung function [percent predicted forced expiratory volume in 1 s (FEV1)] obtained at the time of the EBC.

A significant fraction of the CF population was prescribed inhaled corticosteroids (ICS), a treatment that has been shown to influence adenosine in EBC (16). Therefore, we examined EBC purine ratios in the CF subjects relative to treatment with ICS (Fig. 2C). Within the CF subjects prescribed ICS, the EBC adenosine-to-urea ratio (median 1.1, IQR 0.88–2.2) was elevated relative to healthy controls ($P < 0.03$). Within the CF subjects not prescribed ICS, the EBC adenosine-to-urea ratio (median 0.70, IQR 0.41–1.4) was not significantly different from either healthy controls ($P = 0.13$) or CF subjects prescribed ICS ($P = 0.08$). In contrast, EBC AMP-to-urea values were similar between both CF groups (median 1.3, IQR 0.7–2.9 prescribed ICS, median 1.3, IQR 1.3–2.1 not prescribed ICS; $P = 0.95$), and AMP-to-urea remained elevated relative to control even after excluding all CF subjects prescribed ICS ($P < 0.02$).

The data set contained several elevated values that could be considered outliers based on visual examination of the data or statistical considerations (values $>4$ SD from the mean). Exclusion of theses outliers (3 control, 3 CF, 2 asthma) did not significantly alter our findings, since we continued to observe
significant differences in the adenosine-to-urea ratios between control and asthma (median 0.30, IQR 0.15–0.90 control; median 1.4, IQR 0.85–1.6 asthma; \( P < 0.05 \)) and in the AMP-to-urea ratios between control and CF (median 0.60, IQR 0.25–1.2 control; median 1.2, IQR 0.68–1.9 CF; \( P < 0.05 \)).

**EBC purines in the CF exacerbation group.** Since treatment of CF exacerbations reduces neutrophilic airway inflammation (24), we hypothesized that treatment of exacerbations would reduce EBC AMP-to-urea ratios. We tested this hypothesis by enrolling CF subjects in the CF exacerbation study, from which EBC purines and urea were successfully measured in 26 EBC collection pairs. For this group, treatment averaged 20.1 ± 8.2 days, and lung function increased significantly after treatment (percent predicted FEV\(_1\) 69.8 ± 24.0 start antibiotics, 80.2 ± 22.3 end antibiotics; \( P < 0.001 \)).

Contrary to our predictions, neither the AMP-to-urea nor any other ratio changed significantly after treatment (Fig. 3A). However, we noted that the treatment response, as assessed by changes in lung function, varied considerably among subjects. Therefore, we examined the relationship between variability in treatment response (as indexed by changes in lung function) and changes in EBC purine ratios. These studies revealed a significant negative correlation between changes in percent predicted FEV\(_1\) and changes in the AMP-to-urea ratio (Fig. 3B) and a similar correlation between change in percent predicted FEV\(_1\) and the adenosine-to-urea ratio (Fig. 3C). Overall, these data suggest that EBC purine ratios track the therapeutic response to treatment in CF.

**Alternate methods to control for EBC dilution.** Although use of purine-to-urea ratios to control for dilution has the advantage of simplicity, this method does not account for variations in airway urea levels between subjects. These variations can theoretically be controlled using measures of serum urea, which are believed to provide a reasonable estimate of airway urea levels (7). We obtained serum urea values measured on admission in all subjects in the CF exacerbation study (Table 1), which were then used to calculate dilution factors. As expected, we observed variation in serum urea values, with a coefficient of variation of 29%. The calculated dilution factors were similar at the start and end of antibiotic treatment (25,300 ± 5,000 start, 26,500 ± 5,000 end; \( P = 0.8 \)), and the magnitude of dilution was consistent with previous reports (9).

Using dilution factor-corrected AMP concentrations, we continued to observe a correlation between changes in EBC AMP and changes in percent predicted FEV\(_1\) in the CF exacerbation study (Fig. 4A). Interestingly, the measured coefficients of variation were slightly higher for dilution factor-corrected purine levels for purine-to-urea ratios (coefficients of variation 1.25 corrected adenosine, 1.08 adenosine/urea; 1.24 corrected AMP, 0.94 AMP/urea). Thus, despite our predictions, the use of corrected dilution factors did not reduce the data variability.

The validity of urea as a dilution marker assumes that airway urea values are not significantly affected by disease pathology, which has been demonstrated in COPD (9) but not CF. To determine whether urea levels were altered in CF, we compared EBC urea concentrations to EBC concentrations of the electrolytes sodium plus potassium as an independent dilution marker. We hypothesized that CF-induced variations in airway urea values would be reflected in altered EBC electrolyte-to-urea ratios since serum electrolytes show little variation (coefficient of variation of sodium plus potassium 1.3% in the

longitudinal study), and airway electrolyte concentrations are similar in CF and healthy airways (17). Sufficient volume for EBC electrolyte analysis was available in a subset of samples (\( n = 9 \) control and 8 CF from the cross-sectional study and 9 paired samples from the CF exacerbation study). In these samples, EBC urea and EBC electrolyte levels were significantly correlated (Fig. 4B), consistent with the hypothesis that both assess dilution of airway secretions. Furthermore, the electrolyte-to-urea ratios in control EBCs were similar to those in CF EBCs from both the cross-sectional and CF exacerbation studies (Fig. 4C), suggesting that airway urea levels were not significantly altered in CF. Assuming an airway fluid isosmotic to normal plasma, we calculated that electrolyte-based dilution factors were 18,000 ± 1,600, similar to the dilution factors previously assessed using urea. Using these factors,
increase in EBC AMP-to-urea in samples from CF relative to airway secretions within EBC. Our primary finding was an increase in EBC AMP-to-urea in samples from CF relative to control subjects, correlated with changes in lung function, and EBC urea was observed, suggesting that both urea and electrolytes were functioning as dilution markers. The simplest interpretation of these results is that EBC AMP-to-urea is a biomarker of neutrophilic airway inflammation, which has been shown to be increased in CF compared to control and effective treatment of an exacerbation. This explanation is consistent with the reported accumulation of extracellular AMP by activated neutrophils and the reported correlation between AMP and neutrophilic inflammation in other airway samples. However, since we did not directly assess an established marker of neutrophilic airway inflammation, we cannot rule out the possibility that the elevated EBC AMP-to-urea ratios in CF reflect some other aspect of CF airways disease. Furthermore, we cannot definitively exclude the possibility that salivary contamination influenced our results, although such contamination has not been previously observed with the RTube under supervised collections such as those of our study. Although the presence of such contamination could contribute to the outliers in our data set, we note that our significant findings for CF and asthma are not altered by exclusion of these outliers.

We also observed an increase in the EBC adenosine-to-urea ratio in subjects with asthma. This finding is not unexpected since elevated levels of airway adenosine in asthma have been previously reported. Interestingly, we observed a trend toward increased EBC adenosine-to-urea within our CF cohort, although previous studies had suggested that airway adenosine is not elevated in CF. It is possible that the EBC adenosine-to-urea ratio is affected by the subset of CF subjects with asthma, a relatively common comorbidity in CF populations. Indeed, we found that the EBC adenosine-to-urea ratio was elevated in the subset of CF subjects prescribed ICS, suggestive of a physician diagnosis of comorbid asthma. Although further investigation is needed, these findings support previous studies suggesting that adenosine and AMP track different aspects of airway inflammation found in asthma and CF, respectively.

Although the elevated EBC AMP-to-urea ratio in CF was consistent with our predictions, the ~2-fold differences between CF and control were much less than those previously observed in sputa or bronchoalveolar lavage fluid (BALF; Ref. 10). The reasons for this discrepancy are not clear. It is possible that continued purine release and metabolism during isolation and processing of sputum or lavage fluid may have exaggerated differences between groups. Alternatively, the airway secretions contained within EBC may differ from those represented in sputum or BALF. The source of airway secretions in EBC is not well-understood but has been postulated to represent entraining of airway surface liquid microdroplets generated from turbulent airway flow. This mechanism would suggest a large number of patent airways throughout the lung contribute to droplet formation, whereas sputum and lavage fluid are generally derived from the most diseased airways. Furthermore, it is possible that microdroplet formation is reduced in the most diseased airways such that these are underrepresented within the condensate, a hypothesis consistent with our observation that the EBC urea concentrations are lower in subjects with airways disease (see Fig. 2A). Increased metabolism of

DISCUSSION

Our results demonstrate successful application of the EBC biomarker approach to analysis of airways disease in children, using mass spectrometry to simultaneously measure purine biomarkers and urea to control for known variable dilution of airway secretions within EBC. Our primary finding was an increase in EBC AMP-to-urea in samples from CF relative to control and a relationship between EBC AMP-to-urea levels and treatment response to an acute exacerbation. These findings suggest that the EBC AMP-to-urea has potential to serve as a biomarker of CF airways disease.

Fig. 4. Alternate methods to control for EBC dilution. A: airway concentrations of adenosine and AMP values in the CF exacerbation group were estimated using serum and EBC urea values to calculate a dilution factor for airway secretions with EBC. Corrected AMP levels remained significantly correlated with changes in lung function (n = 25). B: EBC electrolytes (sodium plus potassium) were measured as an alternative dilution marker in 35 samples from control subjects (n = 9), CF subjects during the cross-sectional study (n = 8), and CF subjects during CF exacerbation study (n = 18). A significant correlation between EBC electrolytes (Na+K) and EBC urea was observed, suggesting that both urea and electrolytes were functioning as dilution markers. C: the ratio of electrolytes to urea measured in EBC from control subjects was similar to the ratio measured in EBC from CF subjects enrolled from the cross-sectional (Clinic), the CF exacerbation (ABX), and in all CF values combined (All), suggesting that airway urea levels are not significantly altered by CF lung disease.

Airway AMP levels in the cross-sectional CF group averaged 7.2 ± 1.3 μM CF, higher than the 4.4 ± 1.0 μM average for control, although the difference did not reach statistical significance in this small sample (P = 0.11).
purines in EBC could also account for the differences but seems unlikely since we have previously demonstrated that ATP is stable within EBC (10), and our use of isotopically labeled internal standards controlled for metabolism occurring during specimen processing. The discrepancies between purines levels in EBC, sputum, and BALF highlight the need to better understand the physiological mechanisms and sites of generation for each of these airway samples.

Consistent with many previous reports (2, 8, 9, 11), we found that the fraction of airway secretions contained within EBC was highly variable as assessed using either EBC urea or electrolytes as dilution markers. These findings strongly reinforce the need to control for dilution when reporting nonvolatile EBC biomarkers. Our methodology has the advantage of allowing simultaneous measurement of both a dilution marker (urea) and biomarkers (purines) within one relatively small sample volume. Although use of urea as a dilution marker has been a topic of controversy, much of this controversy stems from studies of BALF in which diffusion of urea into the lavage fluid during the procedure can significantly confound results (14). Since EBC collection does not modify airway surface conditions during collection, such limitations should not apply (7, 9). Our results provide further validation for urea as dilution marker in EBC and suggest that a simple ratio of biomarker to urea may suffice to control for dilution. Although use of a simple ratio does not control for potential variations in airway urea, we found that use of serum urea measurements to control for individual variations in airway urea did not alter our conclusions and actually increased variability within the data set. These data suggest that variations in airway concentrations of urea and other dilution markers are not driven primarily by variations in their serum concentrations but may reflect other influences that are not well understood. We conclude that a biomarker-to-urea ratio may be sufficient to control for variable dilution in EBC, allowing measurement of airway biomarkers controlled for dilution without need for even a minimally invasive blood draw.

Using the dilution factors calculated using electrolytes or urea, we estimated that the average adenosine and AMP concentrations on airway surfaces in healthy children are in the low (1–5) micromolar range. Interestingly, these estimates were significantly lower than the 60 μM airway adenosine concentrations derived from a previous study of BALF (6). The reasons for this discrepancy are not clear, although we note that the osmotic and mechanical forces generated during lavage could confound evaluation of purines and/or urea (14, 28). Our airway adenosine concentration estimated from EBC are similar to the reported EC50 values for many adenosine receptors (A1, A2A, and A3) found on resident airway and inflammatory cells (12, 21), suggesting that even the modest increases in adenosine concentration observed in this study could have physiological impact.

In summary, our data suggest that EBC purines are biomarkers of airway disease when controlled for variable dilution and more specifically that the EBC AMP-to-urea ratio may serve as a biomarker of airways disease in children with CF. With further investigation, this finding could form the basis for a simple and noninvasive method to assess the disease severity and the response to treatment. Furthermore, the use of a flexible mass spectrometry-based assay allowed for simultaneous measurement of a dilution marker and offers the potential to add other biomarkers to the EBC analysis in the future. However, our results also underscore the fact that additional investigation is needed to validate the EBC approach to assessing airway biomarkers in CF or other diseases.

GRANTS

C. R. Esther, Jr., was supported by the UNC Program for Translational Science and the Cystic Fibrosis Foundation (CFF Grants ESTHER07A and ESTHER07G). C. R. Esther, Jr., G. Boysen, and J. W. Swenberg were supported by National Institute of Environmental Health Sciences Grant P30 ES-10126. R. C. Boucher was supported by National Heart, Lung, and Blood Institute Grants HL-54322, HL-084934, and P50 HL-084934 [Specialized Centers of Clinically Oriented Research (SCCOR)].

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