



Exhaled Breath Condensate Articles

published as of May 10, 2005

1. Zacharasiewicz, A., N. Wilson, C. Lex, E. M. Erin, A. M. Li, T. Hansel, M. Khan, and A. Bush.

Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children.

Am J Respir Crit Care Med 171(10):1077-82. 2005.

The use of noninvasive methods of monitoring airway inflammation, such as exhaled nitric oxide (eNO) and induced sputum, has been shown to improve asthma monitoring and optimize treatment in adult patients with asthma. There is a lack of comparable data in children. Forty children with stable asthma eligible for inhaled steroid reduction were reviewed every 8 weeks, and their inhaled steroid dose halved if clinically indicated. eNO, sputum induction combined with bronchial hyperreactivity testing, and exhaled breath condensate collection were performed at each visit to predict success or failure of reduction of inhaled steroids. Thirty of 40 (75%) children tolerated at least one dose reduction, 12 of 40 (30%) were successfully weaned off, and in total, 15 of 40 (38%) children experienced loss of asthma control. Treatment reduction was successful in all children who had no eosinophils in induced sputum before the attempted reduction. Using multiple logistic regression, increased eNO (odds ratio, 6.3; confidence interval, 3.75-10.58) and percentage of sputum eosinophils (odds ratio, 1.38; confidence interval, 1.06-1.81) were significant predictors of failed reduction. These findings suggest that monitoring airway inflammation may be useful in optimizing treatment in children with asthma.

2. Wells, K., J. Vaughan, T. N. Pajewski, S. Hom, L. Ngamtrakulpanit, A. Smith, A. Nguyen, R. Turner, and J. Hunt.

Exhaled breath condensate pH assays are not influenced by oral ammonia.

Thorax 60(1):27-31. 2005.

BACKGROUND: Measurement of pH in exhaled breath condensate (EBC) is robust and simple. Acidic source fluid (airway lining fluid) traps bases while volatilising acids, leading to EBC acidification in many lung diseases. Lower airway ammonia is one determinant of airway lining fluid pH, raising the concern that addition of the base ammonia by contamination from the mouth might confound EBC pH assays. **METHODS:** Three discrete methods were used to limit oral ammonia contamination of EBC collections: endotracheal intubation, oral rinsing, and -40 degrees C condenser temperatures. Separately, ammonia was removed from collected EBC samples by lyophilisation and resuspension. Intraweek and intraday variability of ammonia concentration was determined in 76 subjects, and ammonia and pH from a further 235 samples were graphically compared. Ammonia was assayed spectrophotometrically and pH was assessed after deaeration. **RESULTS:** Data from 1091 samples are presented. Ammonia was reduced in EBC by all methods. Endotracheal intubation decreased EBC ammonia from a mean (SD) of 619 (124) microM to 80 (24) microM ($p < 0.001$, $n = 32$). Oral rinsing before collection also led to a decline in EBC ammonia from 573 (307) microM to 224 (80) microM ($p = 0.016$, $n = 7$). The colder the condensation temperature used, the less ammonia was trapped in the EBC. Lyophilisation removed 99.4 (1.9)% of ammonia. Most importantly, the pH of EBC never decreased after removal of ammonia by any of these methods. Intraweek and intraday coefficients of variation for ammonia were 64 (27)% and 60 (32)%, which is substantially more variable than EBC pH assays. **CONCLUSIONS:** Although ammonia and pH appear to correlate in EBC, the oral ammonia concentration is not an important determinant of EBC pH. No precautions need to be taken to exclude oral ammonia when EBC pH is of interest. The low pH and low ammonia found in EBC from patients with lung diseases appear to be independent effects of volatile compounds arising from the airway.

3. Svensson, S., A. C. Isacson, G. Ljungkvist, K. Toren, and A. C. Olin.

Optimization and validation of an ion chromatographic method for the simultaneous determination of sodium, ammonium and potassium in exhaled breath condensate.

J Chromatogr B Analyt Technol Biomed Life Sci 814(1):173-7. 2005.

An ion chromatographic method with conductivity detection for the simultaneous quantification of sodium, ammonium and potassium in exhaled breath condensate (EBC) was developed and validated. A factorial design was used to optimize the chromatographic conditions, which resulted in baseline separations of the cations within 6 min. The method requires no pre-treatment of EBC samples. The optimized method was used for the intra-individual screening of cations in EBC of 10 healthy volunteers. The LOQs were low (0.3, 0.1 and 0.2 microM for sodium, ammonium and potassium, respectively), compared with levels detected in

healthy volunteers. The responses were linear with good precision, and samples could be stored for at least 10 weeks at refrigerating conditions.

4. Schmidt, S. M., C. E. Muller, and S. K. Wiersbitzky.

Inverse association between Chlamydia pneumoniae respiratory tract infection and initiation of asthma or allergic rhinitis in children.

Pediatr Allergy Immunol 16(2):137-44. 2005.

To evaluate the role of Chlamydia pneumoniae respiratory tract infection on pediatric asthma, allergic rhinitis or atopic eczema initiation, children of three age groups (n=1211) were prospectively studied for a C. pneumoniae infection using throat swabs and polymerase chain reaction (PCR) with enzyme immunoassay (EIA) detection. Infected children (study group, SG) were examined monthly until the agent could not be detected, quantifying persistent infection. They were compared with randomly selected, non-infected children without asthma matched for age, gender and origin (control group, CG) regarding lung function and inflammatory parameters as well as initiation of allergic diseases judged by family doctor diagnosis after, in median, 22 months. At the first follow-up examination, SG children revealed a higher leukotriene B4 (median 36 pg/ml vs. 19, p=0.04) and 8-isoprostane (median 15 pg/ml vs. 12, p=0.04) in breath condensate characterizing neutrophil, agent-related inflammation and oxidative stress in the lower airways. Cysteinyl leukotrienes, important in acute allergic inflammation, were without difference. Local, anti C. pneumoniae secretory immunoglobulin A antibodies were higher in children after C. pneumoniae infection (optical density median 0.7 vs. 0.4, p=0.001) confirming PCR-EIA results. At the final examination, there was no difference in pathological lung function tests, parameters of exhaled breath condensate or eosinophilia of the nasal mucosa. Incidence of asthma (0/55 vs. 5/54, p=0.03) and allergic rhinitis [3/53 vs. 10/52, p=0.04, odds ratio and 95% confidence interval-OR 0.25 (0.06;0.98)] as well as prevalence of asthma [1/56 vs. 9/58, p=0.02, OR 0.1 (0.01;0.81)] and allergic rhinitis [6/56 vs. 16/58, p=0.03, OR 0.32 (0.11;0.88)] were lower in the SG children. There was no association in atopic eczema. Three children with persistent infection revealed a slightly higher incidence in allergic rhinitis without significance than those with single C. pneumoniae detection (1/3 vs. 2/50), however, not to the CG. In conclusion a C. pneumoniae upper respiratory tract infection may be regarded as a protective factor for childhood asthma or allergic rhinitis in a population of kindergarten and school-age children.

5. Ojoo, J. C., S. A. Mulrennan, J. A. Kastelik, A. H. Morice, and A. E. Redington.

Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis.

Thorax 60(1):22-6. 2005.

BACKGROUND: It has been proposed that the pH of airway lining fluid may regulate the fractional exhaled concentration of nitric oxide (Fe(NO)) in respiratory disease. METHODS: Fe(NO), exhaled breath condensate (EBC) pH, and EBC concentrations of nitrite plus nitrate (NO₂/NO₃) were compared in 12 subjects with stable asthma, 18 with stable cystic fibrosis (CF), and 15 healthy control subjects. Eight of the CF patients were studied on a separate occasion at the start of a pulmonary exacerbation. RESULTS: Fe(NO) was significantly greater in asthmatic subjects than in control subjects (mean 35 v 9 ppb, p<0.001). EBC pH, however, was similar in the asthmatic and control groups (median 5.82 v 6.08, p=0.23). Levels of NO₂/NO₃ were on average higher in EBC samples from asthmatic subjects, but the difference was not significant. In patients with stable CF both the Fe(NO) (mean 4 ppb, p<0.001) and EBC pH (median 5.77, p=0.003) were lower than in the control group. Levels of EBC NO₂/NO₃ (median 29.9 microM; p=0.002) in patients with stable CF, in contrast, were significantly higher than in control subjects. During CF exacerbations, EBC pH was further reduced (median 5.30, p=0.017) but Fe(NO) and NO₂/NO₃ were unchanged. CONCLUSIONS: These findings demonstrate a dissociation between EBC pH and Fe(NO) in inflammatory airways disease.

6. Morice, A., C. F. Everett, S. A. Mulrennan, and K. F. Chung.

Exhaled breath condensate in chronic cough.

Thorax 60(3):259; author reply 259. 2005.

7. Marteus, H., D. C. Tornberg, E. Weitzberg, U. Schedin, and K. Alving.

Origin of nitrite and nitrate in nasal and exhaled breath condensate and relation to nitric oxide formation.

Thorax 60(3):219-25. 2005.

BACKGROUND: Raised concentrations of nitrate and nitrite have been found in exhaled breath condensate (EBC) in airway disease, and it has been postulated that this reflects increased nitric oxide (NO) metabolism. However, the chemical and anatomical origin of nitrate and nitrite in the airways has not yet been sufficiently studied. METHODS: The fraction of exhaled NO at an exhalation

flow rate of 50 ml/s (FE(NO)) and nitrite and nitrate in EBC, nasal condensate, and saliva were measured in 17 tracheostomised and 15 non-tracheostomised subjects, all of whom were non-smokers without respiratory disease. Tracheal and oral samples were taken from the tracheostomised subjects and nasal (during velum closure) and oral samples from the non-tracheostomised subjects. Measurements were performed before and after sodium nitrate ingestion (10 mg/kg) and use of antibacterial mouthwash (chlorhexidine 0.2%). RESULTS: In tracheostomised subjects oral FE(NO) increased by 90% ($p < 0.01$) while tracheal FE(NO) was not affected 60 minutes after nitrate ingestion. Oral EBC nitrite levels were increased 23-fold at 60 minutes ($p < 0.001$) whereas the nitrite levels in tracheal EBC showed only a minor increase (fourfold, $p < 0.05$). Nitrate was increased the same amount in oral and tracheal EBC at 60 minutes (2.5-fold, $p < 0.05$). In non-tracheostomised subjects oral FE(NO) and EBC nitrite increased after nitrate ingestion and after chlorhexidine mouthwash they approached baseline levels again ($p < 0.001$). Nasal NO, nitrate, and nitrite were not affected by nitrate intake or mouthwash. At baseline, mouthwash with deionised water did not affect nitrite in oral EBC or saliva, whereas significant reductions were seen after antibacterial mouthwash ($p < 0.05$ and $p < 0.001$, respectively). CONCLUSIONS: Besides the salivary glands, plasma nitrate is taken up by the lower airways but not the nasal airways. Nitrate levels in EBC are thus influenced by dietary intake. Nitrate is reduced to nitrite by bacterial activity which takes place primarily in the oropharyngeal tract of healthy subjects. Only oropharyngeal nitrite seems to contribute to exhaled NO in non-inflamed airways, and there is also a substantial contribution of nitrite from the oropharyngeal tract during standard collection of EBC.

8. Leung, T. F., G. W. Wong, F. W. Ko, C. Y. Li, E. Yung, C. W. Lam, and T. F. Fok.

Analysis of Growth Factors and Inflammatory Cytokines in Exhaled Breath Condensate from Asthmatic Children.

Int Arch Allergy Immunol 137(1):66-72. 2005.

Background: Vascular endothelial growth factor (VEGF), AA isoform of platelet-derived growth factor (PDGF-AA), and epidermal growth factor (EGF) are involved in the pathogenesis of airway inflammation in asthma. These molecules are closely associated with cytokines such as tumor necrosis factor-alpha (TNF-alpha) and interleukin (IL)-4. This study investigates the relation between childhood asthma and levels of these mediators in exhaled breath condensate (EBC). Methods: EBC was collected from asthmatic children and controls using a disposable collection kit, and the concentrations of VEGF, PDGF-AA, EGF, TNF-alpha and IL-4 in EBC were measured using sandwich enzyme immunoassays. Exhaled nitric oxide concentration was measured by a chemiluminescence analyzer. Results: Thirty-five asthmatic patients aged between 7 and 18 years and 11 controls were recruited. Sixteen patients had intermittent asthma (IA) whereas 19 of them suffered from persistent asthma (PA). A significant correlation was found between IL-4 and TNF-alpha in EBC ($\rho = 0.374$, $p = 0.010$). PDGF-AA levels in EBC were higher in subjects with diminished FEV(1) ($p = 0.023$) whereas IL-4 concentrations were increased in asthmatics ($p = 0.007$) as well as subjects with increased plasma total IgE ($p = 0.033$). Patients with PA receiving high-dose inhaled corticosteroid (ICS) had higher EBC IL-4 concentration than those on low-dose ICS ($p = 0.007$). Linear regression revealed that PDGF-AA levels in EBC were negatively associated with FEV(1) percentage ($\beta = -0.459$, $p = 0.006$) among the asthmatic patients. Conclusions: IL-4 in EBC is increased in childhood asthma, and growth factors are detectable in a significant proportion of these children. Increased PDGF-AA is found in asthmatics with more severe airflow limitation. Copyright (c) 2005 S. Karger AG, Basel.

9. Kirschvink, N., D. Marlin, F. Delvaux, J. Leemans, C. Clercx, A. Sparkes, and P. Gustin.

Collection of exhaled breath condensate and analysis of hydrogen peroxide as a potential marker of lower airway inflammation in cats.

Vet J 169(3):385-96. 2005.

The objective of this study was to describe a standardised and non-invasive method for exhaled breath condensate (EBC) collection in cats and to test whether determination of hydrogen peroxide (H(2)O(2)) in EBC might be used as marker of lower airway inflammation. The technique of barometric whole body plethysmography for cats was combined with a system to condense the effluent air from the plethysmograph, allowing simultaneous EBC collection and respiratory pattern measurement. H(2)O(2) was determined spectrophotometrically. Eighteen experimental cats were used to investigate the impact on EBC volume and EBC H(2)O(2) of plethysmograph ventilation rate, collection duration, sample stability, within-day and day-to-day variability. After determination of a standardised EBC collection procedure, correlation analyses between EBC H(2)O(2) and bronchoalveolar lavage (BAL) cytology of healthy and allergen-challenged *Ascaris suum* (AS)-sensitised cats were performed. A significant and positive correlation between EBC H(2)O(2) and bronchoalveolar lavage (BAL) neutrophil% was found in healthy cats ([Formula: see text], [Formula: see text]), whereas in AS-sensitised cats, correlation with BAL eosinophil% was significant ([Formula: see text], [Formula: see text]). H(2)O(2) was increased after an allergen challenge in AS-sensitised cats ([Formula: see text], 0.56 ± 0.12 versus 1.08 ± 0.35 $\mu\text{mol/L}$, [Formula: see text]). This study proposes a non-invasive, well tolerated and repeatable method of EBC collection for cats and suggests that EBC H(2)O(2) might be used as non-invasive biomarker for monitoring lower airway inflammation.

10. Horvath, I.

The exhaled biomarker puzzle: bacteria play their card in the exhaled nitric oxide-exhaled breath condensate nitrite game. *Thorax* 60(3):179-80. 2005.

11. Harrison, C. M., and C. C. Andersen.

Exhaled breath measures of inflammation: are they useful in neonatal chronic lung disease?

Arch Dis Child Fetal Neonatal Ed 90(1):F6-10. 2005.

Neonatal chronic lung disease is a common problem for surviving infants of extreme prematurity. Although the precise pathophysiology is still not known, it is clear that inflammation provides a common link that amplifies the injury to the premature lung. Current invasive measures of pulmonary inflammation include markers in blood and airway effluent, with the cellular composition of tracheal fluid being the "gold standard". In this article available exhaled breath measures, particularly nitric oxide, carbon monoxide, volatile hydrocarbons, and exhaled breath condensate, are reviewed with particular reference to sample collection, analysis, and common pitfalls as they apply to the ventilated premature newborn at risk of chronic lung disease. Although they have great potential, all measures require thorough validation before being used clinically.

12. Gerritsen, W. B., J. Asin, P. Zanen, J. M. van den Bosch, and F. J. Haas.

Markers of inflammation and oxidative stress in exacerbated chronic obstructive pulmonary disease patients.

Respir Med 99(1):84-90. 2005.

COPD is characterised by damage to small airways due to an inflammatory process as well as an imbalance between oxidants and antioxidants. Several cytokines and cell adhesion molecules enhancing a mainly neutrophilic inflammation have been associated with COPD. The aim of the study was to investigate whether inflammation or oxidative markers gave an indication of the course of COPD during an exacerbation. Fourteen patients with moderate to severe COPD admitted to the St. Antonius Hospital because of an exacerbation have been monitored during treatment with prednisolone 50 mg intravenously during 24 h at admission, reduced to 25 mg at day 3 and tapered off with oral prednisolone at day 7. On three separate occasions, day 1, 3 and 7, H₂O₂ in exhaled air, IL-8 and the soluble cell adhesion molecule sICAM and sE-selectin in serum were measured. We compared the patients at day 1 with healthy controls (in both non-smokers and smokers). Furthermore, we examined the changes from the study group in time during therapy. At admission all the markers were raised in comparison with the control groups. During treatment H₂O₂ concentrations in breath condensate declined significantly ($P < 0.001$) as well as IL-8 and sICAM in serum ($P = 0.002$, respectively, $P < 0.001$). There was no significant change in sE-selectin ($P = 0.132$). No significant improvement has been found in spirometry. These data suggest that the markers H₂O₂ in exhaled air, IL-8 and sICAM in serum are suitable markers in monitoring exacerbated COPD.

13. Effros, R. M., J. Su, R. Casaburi, R. Shaker, J. Biller, and M. Dunning.

Utility of exhaled breath condensates in chronic obstructive pulmonary disease: a critical review.

Curr Opin Pulm Med 11(2):135-9. 2005.

PURPOSE OF REVIEW: Evaluation of the utility of exhaled breath condensates in chronic obstructive pulmonary disease. **RECENT FINDINGS:** Exhaled breath condensates have recently been introduced as a simple, noninvasive method of sampling respiratory fluid in inflammatory lung disorders, including chronic obstructive pulmonary disease. Increases in condensate concentrations of at least 12 markers of inflammation have been reported in these disorders. Furthermore, condensate pH appears to be decreased in both chronic obstructive lung disease and bronchial asthma. This has been referred to as acidopnea and could reflect airway acidification by inflammatory cells. Although safer and more convenient than bronchoalveolar lavage, interpretation of condensate data is complicated by uncertainty regarding the source of condensate solutes and by variable dilution of respiratory droplets from condensed water vapor, which represents more than 99.9% of condensate volumes. This dilution can be estimated from the dilution of plasma constituents such as urea or electrolytes. Because the principal buffer in condensate is NH₄, much of which is derived from bacterial degradation of urea in the mouth, condensate pH measurements may not provide accurate estimates of airway pH. Nevertheless, acidification of condensate may be indicative of gastroesophageal reflux, which frequently occurs in obstructive lung diseases and may contribute to cough and bronchospasm. **SUMMARY:** It is too early to tell how useful condensate studies will be to pulmonary investigators and clinicians. Realization of the enormous potential of this approach will require a thorough understanding of the manner in which these solutions are generated and how they should be analyzed.

14. Doniec, Z., D. Nowak, W. Tomalak, K. Pisiewicz, and R. Kurzawa.

Passive smoking does not increase hydrogen peroxide (H₂O₂) levels in exhaled breath condensate in 9-year-old healthy children. *Pediatr Pulmonol* 39(1):41-5. 2005.

Environmental tobacco smoke, also called passive smoking, was shown to have adverse effects on the health of children. Hydrogen peroxide (H₂O₂) is proposed as a sensitive marker of oxidative injury and inflammatory processes in the airways, being increased in adult active cigarette smokers. We tested whether passive smoking had an influence on H₂O₂ exhalation in healthy children. Thirty healthy passive smoking and 24 nonexposed healthy children aged 9 years were included in the study. Exhaled breath condensate (EBC) was obtained by spontaneous tidal volume breathing with EcoScreen (Jaeger, Germany). All subjects underwent flow-volume measurements immediately after EBC collection. Levels of H₂O₂ were measured fluorimetrically with the homovanillic acid method. Lung function did not differ between the passive smoking and nonexposed children groups. In the passive smoking group, EBC H₂O₂ concentration (median and range) was 0.32 (0.00-1.20) microM, and did not differ significantly ($P > 0.05$) from that found in the nonexposed group, i.e., 0.22 (0.00-0.68) microM. Exhaled H₂O₂ did not correlate with spirometric parameters (FEV₁, FEV₁%FVC, and MEF₅₀%FVC) in either group. We conclude that passive smoking does not increase H₂O₂ exhalation in healthy children.

15. De Benedetto, F., A. Aceto, B. Dragani, A. Spacone, S. Formisano, R. Pela, C. F. Donner, and C. M. Sanguinetti.

Long-term oral n-acetylcysteine reduces exhaled hydrogen peroxide in stable COPD.

Pulm Pharmacol Ther 18(1):41-7. 2005.

Oxidative stress caused by airway inflammation is increased in chronic obstructive pulmonary disease (COPD) and may account for the progressive deterioration of structure and function of the respiratory tract observed in this disease. Antioxidant defences of the respiratory tract may be overwhelmed by the oxidant burden in COPD and possibly restored with antioxidant therapy. The level of hydrogen peroxide (H₂O₂) concentration in exhaled air condensate (EAC) is a valuable tool for assessing and monitoring oxidative stress. This study aimed to verify the effect of 2-month oral N-acetylcysteine (NAC) treatment compared to placebo on the H₂O₂ content in EAC of 55 clinically stable COPD patients (48 males), mean age 65.93±9.3 years. After clinical examination, pulmonary function tests, and collection of EAC for the basal (T₀) assay of H₂O₂, patients were randomly allocated to group A (usual therapy plus oral NAC 600 mg b.i.d. for 2 months) or group B (usual therapy plus placebo b.i.d. for 2 months). H₂O₂ assay in EAC was repeated at 15 (T₁₅), 30 (T₃₀), and 60 (T₆₀) days after the start of therapy in each group. All patients were non-smokers or ex smokers for at least 5 years and the two groups were comparable in terms of demographic, respiratory function, and EAC data at baseline. The H₂O₂ level in EAC of group A was significantly decreased at T₁₅ (1.00±0.38 SD microM; $p=0.003$), T₃₀ (0.91±0.44 microM; $p=0.007$), and T₆₀ (0.83±0.41 microM; $p=0.000$) compared to T₀ (1.28±0.61 microM). No significant decrease in H₂O₂ of group B was found at any time point. We conclude that oral NAC 600 mg b.i.d. for 2 months rapidly reduces the oxidant burden in airways of stable COPD patients.

16. Csoma, Z., E. Huszar, E. Vizi, G. Vass, Z. Szabo, I. Herjavec, M. Kollai, and I. Horvath.

Adenosine level in exhaled breath increases during exercise-induced bronchoconstriction.

Eur Respir J 25(5):873-8. 2005.

In asthmatic patients, airway obstruction provoked by exercise challenge is accompanied by an increase in plasma adenosine level. In this study, the current authors investigated if exercise-induced bronchoconstriction was associated with local changes of adenosine concentration in the airways. Oral exhaled breath condensate (EBC) collection (5-min duration) and forced expiratory volume in one second (FEV₁) measurements were performed at rest (baseline) and 4-8 times after treadmill exercise challenge in healthy and asthmatic subjects. Adenosine concentration in EBC was determined by HPLC. Observations indicated that physical exercise results in bronchoconstriction together with a significant increase of adenosine level in EBC in asthmatic patients (mean±sd maximal fall in FEV₁ 27±13%; associated increase in adenosine 110±76% as compared to baseline), but not in healthy control subjects. Exercise-induced changes in adenosine concentration correlated significantly with the fall in FEV₁ values in asthmatic patients. In conclusion, the observed increase in adenosine concentration of oral exhaled breath condensate most probably reflects changes in the airways during exercise-induced bronchoconstriction. Due to its known bronchoconstrictor property in asthma, adenosine may contribute to the development of bronchospasm.

17. Carraro, S., G. Folesani, M. Corradi, S. Zanconato, B. Gaston, and E. Baraldi.

Acid-base equilibrium in exhaled breath condensate of allergic asthmatic children.

Allergy 60(4):476-81. 2005.

BACKGROUND: The dysregulation of airway pH control may have a role in asthma pathophysiology. The measurement of exhaled

breath condensate (EBC) pH and ammonia levels may be used as a noninvasive method to study acid-base status in the airway of asthmatics. **METHODS:** Exhaled breath condensate from 29 allergic stable asthmatic children and 13 healthy controls was collected by cooling exhaled air during tidal breathing. Ammonia was measured by high-performance liquid chromatography with fluorescence detection. pH was measured after deaeration of EBC samples by bubbling with argon. The children also underwent FENO measurement. **RESULTS:** Both pH and ammonia values in EBC were significantly lower in the asthmatics than in the control group [pH: ICS-treated (median and interquartile range) 7.70 (7.62-7.74), steroid-naïve 7.53 (7.41-7.68), controls 7.85 (7.80-7.90), $P < 0.01$ and $P < 0.001$, respectively; ammonia: ICS-treated 476.17 microM (282.50-594.80), steroid-naïve 253.24 microM (173.43-416.08), controls 788.30 microM (587.29-1310.39), $P < 0.05$ and $P < 0.001$, respectively]. Both pH and ammonia values were higher in ICS-treated than in steroid-naïve asthmatic children. There was a significant correlation between EBC pH and ammonia concentrations. **CONCLUSIONS:** These data show that EBC pH values of stable asthmatic children are lower compared with those of healthy controls and positively correlated with ammonia concentrations, supporting the hypothesis that airway acidification may have a role in the pathobiology of allergic asthma.

18. Carraro, S., M. Corradi, S. Zanconato, R. Alinovi, M. F. Pasquale, F. Zacchello, and E. Baraldi.

Exhaled breath condensate cysteinyl leukotrienes are increased in children with exercise-induced bronchoconstriction.

J Allergy Clin Immunol 115(4):764-70. 2005.

BACKGROUND: It is recognized that airway inflammation has a central role in the pathogenesis of asthma, but how it relates to exercise-induced bronchoconstriction (EIB) is not completely understood. **OBJECTIVE:** The aim of our study was to investigate the relationship between EIB and baseline concentrations of cysteinyl leukotrienes (Cys-LTs) and other inflammatory markers in exhaled breath condensate (EBC). **METHODS:** EBC was collected, and the fraction of exhaled nitric oxide (FE NO) was measured in a group of 19 asthmatic children, after which they performed a treadmill exercise test. Fourteen healthy children were enrolled as control subjects. **RESULTS:** The asthmatic children were divided into the EIB group (decrease in FEV₁ $> \text{or} = 12\%$) and the non-EIB group. The EBC was analyzed for the presence of Cys-LTs, leukotriene B₄, and ammonia. Asthmatic patients with EIB (mean FEV₁ decrease, 23% \pm 3%) had higher Cys-LT concentrations than either asthmatic patients without EIB or control subjects (42.2 pg/mL [median] vs 11.7 pg/mL and 5.8 pg/mL; $P < .05$ and $P < .001$, respectively). Ammonia concentrations were lower in both the EIB and non-EIB groups than in control subjects (253.2 microM and 334.6 microM vs 798.4 microM; $P < .01$ and $P < .05$, respectively). No difference in EBC leukotriene B₄ levels was found among the 3 groups. Both asthmatic groups had higher FE NO levels than control subjects ($P < .001$). EBC Cys-LT ($P < .01$; $r = 0.7$) and FE NO ($P < .05$; $r = 0.5$) values both correlated significantly with the postexercise FEV₁ decrease. **CONCLUSION:** this study shows that EBC Cys-LT values are higher in asthmatic children with EIB and correlate with the decrease in FEV₁ after exercise. These findings suggest that the pathways of both Cys-LT and nitric oxide are involved in the pathogenesis of EIB.

19. Cap, P., F. Pehal, J. Chladek, and M. Maly.

Analysis of exhaled leukotrienes in nonasthmatic adult patients with seasonal allergic rhinitis.

Allergy 60(2):171-6. 2005.

BACKGROUND: Leukotrienes (LTs) are increased in exhaled breath condensate (EBC) in patients with asthma. So far no data have been reported about LT levels in nonasthmatic patients with seasonal allergic rhinitis (SAR). The aim of the study was to find out whether the LT levels in EBC were increased in the nonasthmatic adult patients with SAR both during and after the pollen season in comparison with healthy controls and to assess the changes of the LT levels after the pollen season. **METHODS:** Twenty-nine nonasthmatic adult patients with SAR underwent measurement of exhaled LTs in the EBC during and after the pollen season. Leukotrienes B₄, C₄, D₄ and E₄ were analysed by a specific and sensitive gas chromatography/mass spectrometry (GC/MS) assay and compared with 50 healthy nonsmoking controls. Spirometry, skin prick tests and nonspecific IgE were evaluated. **RESULTS:** Leukotrienes concentrations (B₄, E₄) but not D₄) were significantly increased in and after the pollen season in patients with SAR in comparison with healthy controls. In most of the samples, LT C₄ was undetectable. The values of all exhaled LTs were significantly decreased after the pollen season compared with the seasonal baseline: LTB₄ ($P = 0.023$), LTD₄ ($P = 0.020$), LTE₄ ($P = 0.047$). **CONCLUSIONS:** Levels of exhaled LTB₄ and LTE₄ were higher in SAR patients than in healthy controls and decreased after the pollen season as compared with levels in season. The SAR patients with the highest in season LT levels had also the post-season levels elevated and this may be an early marker of inflammatory process in the lower airways despite the absence of clinical symptoms of asthma.

20. Borrill, Z., C. Starkey, J. Vestbo, and D. Singh.

Reproducibility of exhaled breath condensate pH in chronic obstructive pulmonary disease.

Eur Respir J 25(2):269-74. 2005.

Increasingly, exhaled breath condensate (EBC) is being used to sample airway fluid from the lower respiratory tract. EBC pH may be a biomarker of airway inflammation in chronic obstructive pulmonary disease (COPD). In this study, the reproducibility of EBC pH in COPD was investigated. A total of 36 COPD patients and 12 healthy nonsmoking subjects participated in several investigations: duration of argon deaeration, within-sample variability, effect of freezing, leaving samples at room temperature, nose-peg use, within- (WD) and between-day (BD) variability. Analysis of repeated measurements was performed using the Bland-Altman method with limits of agreement (LOA; mean difference \pm 2 SD). Wider LOA indicate greater variability. EBC pH became significantly higher with argon deaeration for \leq 5 min. Variability during sample analysis was minimal; LOA of within-sample variability, freezing for 3 months and leaving at room temperature for 3 h were -0.29-0.45, -0.37-0.42 and -0.13-0.09, respectively. In contrast, variability due to nose-peg use (LOA -1.46-1.99), WD (LOA -1.50-2.48) and BD variability (LOA -2.52-3.02) were higher in COPD. In healthy nonsmoking subjects, nose-peg use (LOA -0.27-0.23), WD (LOA -0.33-0.40) and BD variability (LOA -0.46-0.44) were more reproducible. In conclusion, the variability of exhaled breath condensate pH in chronic obstructive pulmonary disease patients is mainly due to changes in airway pH over time, which are not seen in healthy nonsmoking subjects. Reasons for these fluctuations in exhaled breath condensate pH are unclear and require further investigation.

21. Zanconato, S., S. Carraro, M. Corradi, R. Alinovi, M. F. Pasquale, G. Piacentini, F. Zacchello, and E. Baraldi.

Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma.

J Allergy Clin Immunol 113(2):257-63. 2004.

BACKGROUND: Cysteinyl-leukotrienes (cys-LTs) and 8-isoprostane are biomarkers of airway inflammation and oxidative stress. **OBJECTIVE:** The aim of this study was to evaluate cys-LT and 8-isoprostane levels in exhaled breath condensate (EBC) of children with different degrees of asthma severity. **METHODS:** EBC was collected from 14 steroid-naive children with mild persistent asthma, 13 children with stable mild- to-moderate persistent asthma treated with inhaled corticosteroids (ICS), 9 ICS-treated children with unstable asthma, and 19 healthy children. **RESULTS:** In the three groups of asthmatic children, EBC concentrations of cys-LTs and 8-isoprostane were significantly higher than in control children (steroid-naive asthmatic children: cys-LTs median, 10.8 pg/mL, $P < .001$, 8-isoprostane, 16.2 pg/mL, $P < .001$; ICS-treated stable asthmatic children: cys-LTs, 12.7 pg/mL, $P < .001$, 8-isoprostane, 18.1 pg/mL, $P < .001$; children with unstable asthma: cys-LTs, 106.0 pg/mL, $P < .01$, 8-isoprostane, 29.7 pg/mL, $P < .01$; control children: cys-LTs, 4.3 pg/mL, 8-isoprostane, 3.5 pg/mL). Cys-LT levels were higher in children with unstable asthma than in the other two asthmatic groups ($P < .05$). FE(NO) levels were significantly higher in steroid-naive and in children with unstable asthma compared with ICS-treated children with stable asthma ($P < .01$). **CONCLUSIONS:** Our study shows that EBC cys-LTs and 8-isoprostane concentrations are higher in asthmatic children than in healthy control children, with scattered values in patients with unstable asthma. These findings suggest that EBC eicosanoid measurement may have useful clinical implications for investigating phenotype differences among asthmatic patients.

22. Zacharasiewicz, A., N. Wilson, C. Lex, A. Li, M. Kemp, J. Donovan, J. Hooper, S. A. Kharitonov, and A. Bush.

Repeatability of sodium and chloride in exhaled breath condensates.

Pediatr Pulmonol 37(3):273-5. 2004.

Exhaled breath condensate (EBC) has been proposed as a noninvasive tool to study airway inflammation. The reproducibility of breath condensates was recently questioned. We therefore measured sodium and chloride concentrations in EBC and assessed the repeatability of these measurements in healthy adults and children with airway disease. We investigated technical repeatability and within-day repeatability in five healthy adults, and compared these results with those of 10 asthmatic children and 9 children with cystic fibrosis (CF). We also assessed within-period repeatability in the healthy controls. We report that the variability of measurements was similar for within sample, within day, and between visits, for both normals and children with asthma and CF, and that the major source of variability of sodium and chloride measurements is restricted by the reproducibility of the measurement assay method used. The wide use of EBC is more likely to depend on the development of highly sensitive and reproducible assays, rather than further refinements of the collection technique.

23. Wyse, C. A., T. Preston, P. S. Yam, D. G. Sutton, R. M. Christley, J. W. Hotchkiss, C. A. Mills, A. Glidle, D. R. Cumming, J. M. Cooper, and S. Love.

Current and future uses of breath analysis as a diagnostic tool.

Vet Rec 154(12):353-60. 2004.

The analysis of exhaled breath is a potentially useful method for application in veterinary diagnostics. Breath samples can be easily collected from animals by means of a face mask or collection chamber with minimal disturbance to the animal. After the adminis-

tration of a ^{13}C -labelled compound the recovery of ^{13}C in breath can be used to investigate gastrointestinal and digestive functions. Exhaled hydrogen can be used to assess oro-caecal transit time and malabsorption, and exhaled nitric oxide, carbon monoxide and pentane can be used to assess oxidative stress and inflammation. The analysis of compounds dissolved in the aqueous phase of breath (the exhaled breath condensate) can be used to assess airway inflammation. This review summarises the current status of breath analysis in veterinary medicine, and analyses its potential for assessing animal health and disease.

24. Wyse, C. A., J. Hammond, A. Arteaga, D. Cumming, J. M. Cooper, J. D. McEwan, and P. S. Yam. Collection and analysis of exhaled breath condensate hydrogen peroxide in conscious healthy dogs. *Vet Rec* 155(23):744-6. 2004.

25. Weissmann, N., H. Vogels, R. T. Schermuly, H. A. Ghofrani, J. Hanze, L. Fink, F. Rose, W. Seeger, and F. Grimminger. Measurement of exhaled hydrogen peroxide from rabbit lungs. *Biol Chem* 385(3-4):259-64. 2004.

Exhaled H_2O_2 is considered an indicator of lung inflammatory and oxidative stress. Moreover, H_2O_2 may be involved in signal transduction processes. It is not fully elucidated to what extent (i) H_2O_2 escapes from the intravascular compartment, and (ii) pulmonary H_2O_2 generation and nasopharyngeal H_2O_2 generation contribute to exhaled H_2O_2 . We investigated H_2O_2 concentrations in breath condensate from isolated buffer-perfused and ventilated rabbit lungs, and from both intubated and spontaneously breathing rabbits with a horseradish peroxidase/2',7'-dichlorofluorescein assay. For the perfused lungs, a H_2O_2 concentration of 58 ± 19 nM was found. Addition of H_2O_2 to the buffer fluid resulted in only minute appearance in the exhaled air (<0.001%). Levels of exhaled H_2O_2 in intubated rabbits and perfused lungs were virtually identical. Nearly ten-fold higher levels were detected in spontaneously breathing rabbits. Decreasing the inspired oxygen concentration from 21% to 1% resulted in a tendency toward decreased H_2O_2 exhalation in perfused lungs. In contrast, phorbol-12-myristate-13-acetate (PMA) prompted an approximately 4-fold increase in H_2O_2 exhalation. We conclude that the horseradish peroxidase/2',7'-dichlorofluorescein assay is a feasible technique to measure H_2O_2 in exhaled breath condensate in rabbits. When collecting exhaled air via the tracheal tube, the signal represents pulmonary H_2O_2 generation with the contribution of the remaining body being negligible.

26. Van Hoydonck, P. G., W. A. Wuyts, B. M. Vanaudenaerde, E. G. Schouten, L. J. Dupont, and E. H. Temme. Quantitative analysis of 8-isoprostane and hydrogen peroxide in exhaled breath condensate. *Eur Respir J* 23(2):189-92. 2004.

Exhaled breath condensate (EBC) provides a noninvasive means of sampling the lower respiratory tract. Collection of EBC might be useful in the assessment of airway oxidative stress in smokers. The aim of this study was to determine 8-isoprostane and hydrogen peroxide levels in EBC, and, in addition, to investigate the reproducibility of these measurements. EBC samples were collected from 12 healthy male smokers at three time points within 1 week. 8-isoprostane and H_2O_2 were measured in nonconcentrated EBC using immunochemical and colorimetric assays, respectively. 8-isoprostane and H_2O_2 were detected in only 36 and 47% of all EBC samples, respectively. It was not possible to calculate the within-subject variation in a reliable manner since only three of the 12 smokers exhibited detectable 8-isoprostane concentrations on all three occasions (mean $4.6 \text{ pg} \times \text{mL}^{-1}$; range $3.9\text{-}7.7 \text{ pg} \times \text{mL}^{-1}$), whereas H_2O_2 could not be detected on all three occasions in any of the smokers. Spiking experiments revealed a recovery of 83.5-109.5% for 8-isoprostane and 69.9-129.0%, for H_2O_2 in fresh EBC samples. It was concluded that levels of 8-isoprostane and hydrogen peroxide cannot be reproducibly assessed in exhaled breath condensate from healthy smokers because of their low concentration and/or the lack of sensitivity of the available assays.

27. Szkudlarek, U., A. Zdziechowski, K. Witkowski, M. Kasielski, M. Luczynska, R. Luczynski, A. Sarniak, and D. Nowak. Effect of inhaled N-acetylcysteine on hydrogen peroxide exhalation in healthy subjects. *Pulm Pharmacol Ther* 17(3):155-62. 2004.

N-acetylcysteine (NAC) has antioxidant properties and its oral administration decreased H_2O_2 exhalation in patients with chronic obstructive pulmonary disease. In this study we tested whether inhaled NAC could suppress H_2O_2 levels in exhaled breath condensate (EBC) of eight healthy subjects that have never smoked (never-smokers). Original NAC solution (ACC vial, 300 mg NAC in 3 ml solvent), NAC-placebo (vehicle), sterile 0.9% NaCl or distilled water were nebulized via the pneumatic De Vilbiss nebulizer once daily every 7 days and H_2O_2 and thiols exhalation was measured just before, 30 min and 3 h after the end of drug administration. Additional in vitro experiments were performed to evaluate NAC stability during nebulization, reactivity with H_2O_2 and possible H_2O_2 generation in aqueous NAC solutions. NAC almost completely abolished H_2O_2 exhalation

30 min after inhalation (0.02 ± 0.04 vs. 0.21 ± 0.09 μM , $p < 0.001$). However, 3 h later the H_2O_2 levels raised 1.8-fold from baseline ($p < 0.01$). Other inhaled solutions did not affect H_2O_2 levels. Mean thiol concentration in EBC rose ($p < 0.05$) after treatment with NAC and reached 1.03 ± 0.48 μM at 3 h. Although, 25 and 50 mM NAC completely inhibited H_2O_2 -peroxidase-luminol-dependent chemiluminescence, detectable amounts of H_2O_2 were generated in NAC solutions. It was accompanied by moderate loss of -SH groups. Catalase and ascorbic acid prevented H_2O_2 formation in NAC solutions. In conclusion inhaled NAC revealed biphasic effect on H_2O_2 exhalation in healthy subjects, which depends on direct H_2O_2 scavenging and H_2O_2 generation related to drug oxidation. The net result of these processes may determine anti- or pro-oxidant action of inhaled NAC.

28. Svensson, S., A. C. Olin, M. Larstad, G. Ljungkvist, and K. Toren.

Determination of hydrogen peroxide in exhaled breath condensate by flow injection analysis with fluorescence detection.

J Chromatogr B Analyt Technol Biomed Life Sci 809(2):199-203. 2004.

A method for the determination of hydrogen peroxide in exhaled breath condensate (EBC) by automated flow injection analysis (FIA) with fluorescence detection was developed and validated. In the enzymatic assay a fluorescent dimer of para-hydroxyphenyl acetic acid (HPAA) was formed by the redox coupling reaction between hydrogen peroxide and horseradish peroxidase (HRP). The calibration curve of hydrogen peroxide was linear over a range of 40-5000 nM. The coefficient of variation (CV) for within-day precision was 1-3%; for between-day precision, it was 2-5% over the validated range. The assay requires a small sample aliquot (150 μl) and no incubation time, and has an analytical runtime of < 2 min. It is therefore suitable for larger studies. The method was used to detect hydrogen peroxide in EBC of asthmatic patients and healthy volunteers. A statistically significant difference was found between patients with asthma ($n = 19$) and control subjects without asthma ($n = 19$), 780 nM versus 480 nM ($P = 0.03$).

29. Sparkes, A. H., E. J. Mardell, C. Deaton, N. Kirschvink, and D. Marlin.

Exhaled breath condensate (EBC) collection in cats--description of a non-invasive technique to investigate airway disease.

J Feline Med Surg 6(5):335-8. 2004.

Exhaled breath condensate has been collected in other species and used as a non-invasive method of evaluating airway disease by measurement of various markers in the fluid, including hydrogen peroxide, nitric oxide, leukotrienes and prostaglandins. We describe a novel technique for the collection of exhaled breath condensate from cats, which enabled collection of fluid and measurement of its hydrogen peroxide concentration. Further studies will be needed to establish the value of this technique in the investigation of feline respiratory disease.

30. Sanak, M., B. Kielbasa, G. Bochenek, and A. Szczeklik.

Exhaled eicosanoids following oral aspirin challenge in asthmatic patients.

Clin Exp Allergy 34(12):1899-904. 2004.

BACKGROUND: Biochemical analysis of expiratory breath condensate is an emerging non-invasive technique for assessment of airway inflammation. OBJECTIVE: We wondered whether application of expiratory breath condensate could facilitate diagnosis of aspirin-intolerant asthma and reproduce eicosanoids mediators' abnormalities described in this disease. METHODS: We measured prostaglandins (PGs) E(2), F(2 α), 9 α 11 β F(2) and iso-F(2) by gas-chromatography/mass-spectrometry and cysteinyl leukotrienes (cys-LTs) by radioimmunoassay in breath condensates of asthmatic patients undergoing oral aspirin challenge. Fourteen patients with aspirin-induced asthma and 20 aspirin-tolerating asthmatics, most of them on chronic inhaled corticotherapy, were studied and compared with 10 healthy subjects. Additionally, plasma 9 α 11 β PGF(2), the metabolite of PGD(2) and urinary leukotriene (LT) E(4) were measured before and following the challenge. RESULTS: At baseline, PG did not differ between the groups, except for lower 9 α 11 β PGF(2) in aspirin-intolerant asthma. Their concentrations were not changed by the challenge. Breath condensate cys-LTs were similar in the groups studied at base, and after aspirin challenge increased only in aspirin-intolerant patients. Elevated baseline urinary LTE(4) and its further increase following aspirin challenge was highly diagnostic for aspirin-intolerant asthma. The discriminatory value of cys-LTs increase in breath condensates was lower (72.8%) than either basal (99%) or post-challenge increase (94%) of urinary LTE(4). CONCLUSIONS: In asthmatic patients on chronic corticotherapy measurement of urinary LTE(4) excretion rather than cys-LTs in breath condensate is of greater value for diagnosis of aspirin hypersensitivity.

31. Rysz, J., M. Kasielski, J. Apanasiewicz, M. Krol, A. Woznicki, M. Luciak, and D. Nowak.

Increased hydrogen peroxide in the exhaled breath of uraemic patients unaffected by haemodialysis.

Nephrol Dial Transplant 19(1):158-63. 2004.

BACKGROUND: Uraemia is accompanied by conditions favouring the rise of H₂O₂ activity in body fluids. This results from the increased release of H₂O₂ by polymorphonuclear leukocytes and decreased plasma glutathione peroxidase activity. The purpose of this study was to determine if patients on chronic haemodialysis (HD) exhale more H₂O₂ than healthy individuals, and if dialysis affects breath H₂O₂ content. **METHODS:** We studied 29 chronic HD patients (mean age 49 +/- 11 years) and 40 healthy persons (mean age 44 +/- 9 years). H₂O₂, which is volatile, was measured fluorimetrically with the homovanillic acid method in the exhaled breath condensate (EBC) of the study cohort. EBC was collected immediately before and after the HD session and also at 20 and 60 min of HD treatment (n = 14) and once in controls. Peak expiratory flow (PEF), white blood cell (WBC) count, PaO₂ and circulatory cyclic guanosine monophosphate (cGMP), IL-6 and IL-8 concentrations were measured concomitantly. Finally, H₂O₂ diffusion through the dialyser cuprophane membrane was determined in an in vitro experiment. **RESULTS:** At baseline, EBC H₂O₂ concentration was 22 times higher in HD patients than in controls (2.92 +/- 4.64 vs 0.16 +/- 0.13 microM, P < 0.001). Although the maximum decrease in PEF (431 +/- 52 vs 398 +/- 56 l/min, P < 0.01) and WBC count (6.72 +/- 1.02 vs 3.82 +/- 1.51 x 10³/ microl, P < 0.01) occurred at 20 min after the start of HD, no significant changes in breath H₂O₂ levels were noted throughout the session. Plasma IL-6 and IL-8 levels remained unchanged whereas cGMP rose 1.3 times at 60 min (P < 0.01). In vitro, H₂O₂ rapidly diffused through the cuprophane membrane. **CONCLUSION:** Chronic HD patients exhale more H₂O₂ than healthy subjects. Although no change of breath H₂O₂ concentration was observed during HD, as H₂O₂ easily diffuses through the dialyser membrane, it is not possible to rule out that HD stimulates H₂O₂ generation.

32. Rosias, P. P., E. Dompeling, H. J. Hendriks, J. W. Heijmans, R. A. Donckerwolcke, and Q. Jobsis.

Exhaled breath condensate in children: pearls and pitfalls.

Pediatr Allergy Immunol 15(1):4-19. 2004.

Exhaled breath condensate (EBC) is a rapidly growing field of research in respiratory medicine. Airway inflammation is a central feature of chronic lung diseases, like asthma, cystic fibrosis, bronchopulmonary dysplasia and primary ciliary dyskinesia. EBC may be a useful technique for non-invasive assessment of markers of airway inflammation. The non-invasive character of EBC "inflammometry" and the general lack of appropriate techniques makes it particularly interesting for paediatrics. We provide a detailed update on the methods currently used for EBC collection and measurement of mediators. We emphasize on paediatric data. The apparent simplicity of the EBC method must not be overstated, as numerous methodological pitfalls have yet to overcome. Comparison and interpretation of data on this rapidly growing field of research is mainly hampered by the lack of standardization and the lack of specific high-sensitivity immunochemical or colorimetric assays. The initiative of the European Respiratory Society to institute a task force on this topic is a first step towards a uniform technique of EBC. Meanwhile, when using this technique or when interpreting research data, one should be fully aware of the possible methodological pitfalls.

33. Rosias, P. P., E. Dompeling, M. A. Dentener, H. J. Pennings, H. J. Hendriks, M. P. Van Iersel, and Q. Jobsis.

Childhood asthma: exhaled markers of airway inflammation, asthma control score, and lung function tests.

Pediatr Pulmonol 38(2):107-14. 2004.

Exhaled markers of airway inflammation become increasingly important in the management of childhood asthma. The aims of the present study are: 1) to compare exhaled markers of inflammation (nitric oxide, carbon monoxide, and acidity of breath condensate) with conventional asthma measures (lung function tests and asthma control score) in childhood asthma; and 2) to investigate the detectability of albumin, CRP, IL-6, IL-8, TNF-alpha, sICAM-1, and sTNF-R75 in the exhaled breath condensate (EBC) of asthmatic children. Thirty-two children with mild to moderate persistent asthma and healthy controls aged 6-12 years were studied. We measured exhaled NO and CO, and subsequently EBC was collected. Inflammatory mediators in EBC were measured using an enzyme-linked immunosorbent assay. Respiratory symptoms and asthma control were assessed using the asthma control questionnaire (ACQ) of Juniper et al. (*Eur Respir J* 1999;14:902-907). Exhaled NO showed a significant correlation with exhaled CO (r = 0.59, P < 0.05) and FEV1 (r = -0.59, P < 0.05), but not with ACQ score (r = 0.48, P = 0.06). Exhaled CO was correlated with prebronchodilator FEV1 (r = -0.45, P < 0.05), but not with asthma control (r = 0.18, P = 0.35). Acidity of EBC was significantly lower in asthmatic children than in healthy controls (P < 0.05), but did not correlate with any of the conventional asthma measures. We were not able to demonstrate the presence of CRP, IL-6, IL-8, TNF-alpha, sICAM-1, and sTNF-R75 in EBC. Albumin was found in two EBC samples of asthmatic children. We conclude that exhaled NO had a better correlation with lung function parameters and asthma control than exhaled CO and acidity of EBC, in mild to moderate persistent childhood asthma. However, exhaled NO, CO, and deaerated pH of EBC did not differ between asthmatic children and controls, possibly because of a too homogeneous and well-controlled study population. To further evaluate the clinical utility of exhaled markers in monitoring childhood asthma, more studies are required on a wider range of asthma severity, and preferably with repeated measurements of markers and of asthma control.

34. Rosias, P., C. Robroeks, J. Hendriks, E. Dompeling, and Q. Jobsis.
Exhaled breath condensate: a space odyssey, where no one has gone before.
Eur Respir J 24(1):189-90; author reply 190. 2004.

35. Rahman, I., and S. K. Biswas.
Non-invasive biomarkers of oxidative stress: reproducibility and methodological issues.
Redox Rep 9(3):125-43. 2004.

Oxidative stress is the hallmark of various chronic inflammatory lung diseases. Increased concentrations of reactive oxygen species (ROS) in the lungs of such patients are reflected by elevated concentrations of oxidative stress markers in the breath, airways, lung tissue and blood. Traditionally, the measurement of these biomarkers has involved invasive procedures to procure the samples or to examine the affected compartments, to the patient's discomfort. As a consequence, there is a need for less or non-invasive approaches to measure oxidative stress. The collection of exhaled breath condensate (EBC) has recently emerged as a non-invasive sampling method for real-time analysis and evaluation of oxidative stress biomarkers in the lower respiratory tract airways. The biomarkers of oxidative stress such as H₂O₂, F₂-isoprostanes, malondialdehyde, 4-hydroxy-2-nonenal, antioxidants, glutathione and nitrosative stress such as nitrate/nitrite and nitrosated species have been successfully measured in EBC. The reproducibility, sensitivity and specificity of the methodologies used in the measurements of EBC oxidative stress biomarkers are discussed. Oxidative stress biomarkers also have been measured for various antioxidants in disease prognosis. EBC is currently used as a research and diagnostic tool in free radical research, yielding information on redox disturbance and the degree and type of inflammation in the lung. It is expected that EBC can be exploited to detect specific levels of biomarkers and monitor disease severity in response to appropriate prescribed therapy/treatment.

36. Niimi, A., L. T. Nguyen, O. Usmani, B. Mann, and K. F. Chung.
Reduced pH and chloride levels in exhaled breath condensate of patients with chronic cough.
Thorax 59(7):608-12. 2004.

BACKGROUND: Increased hydrogen and reduced chloride ionic environments of the airways are conducive to the stimulation of cough. However, the constituents of the local milieu of the airways of patients with chronic cough are unknown. METHODS: The pH and chloride levels in exhaled breath condensate and capsaicin cough threshold (C5) were measured in 50 patients with chronic cough and in 16 healthy controls. pH and chloride measurements were repeated after capsaicin challenge in those with cough. The cause of cough was asthma (n = 13), postnasal drip/rhinitis (n = 7), gastro-oesophageal reflux (n = 5), bronchiectasis (n = 5), but remained unidentified in 20. RESULTS: Compared with controls, patients with chronic cough had lower pH (mean 7.9 v 8.3, 95% CI of difference -0.5 to -0.2, p < 0.0001), chloride levels (median 4 v 6 mmol/l, 95% CI -3.1 to -0.2, p = 0.007), and C5 (median 3.9 v 125 micro M, 95% CI -270.0 to -17.6, p = 0.002). The pH levels were different in the six subgroups including controls, and were reduced in all diagnostic subgroups of patients with cough compared with controls but did not differ between them. Chloride levels were significantly different in the six subgroups but were lower than controls in only the gastro-oesophageal reflux subgroup. There was a weak but significant correlation between chloride levels and C5 when all participants were analysed together, but not between pH and C5 or chloride levels. pH and chloride levels did not change after capsaicin challenge. CONCLUSIONS: The epithelial lining fluid of patients with chronic cough has a reduced pH and reduced chloride levels which could contribute to the enhanced cough reflex.

37. Neubauer, B., U. Schotte, N. Struck, N. Langfeldt, and T. S. Mutzbauer.
Leukotriene-B4 concentrations in breathing condensate before and after simulated deep dives.
Undersea Hyperb Med 31(2):217-24. 2004.

During diving the respiratory tract is exposed to occupational hazards (increased oxygen partial pressure, pulmonary vessel engorgement during submersion, inert gas micro embolism during decompression). Leukotriene-B4 [LTB₄] concentrations in the exhaled breath mirrors the inflammatory activity of the airways if the respiratory tract has been exposed to occupational hazards. In this study LTB₄-concentrations in the exhaled breath and spirometry data obtained before and after simulated dives helped to elucidate any contributions by hyperbaric exposure to impaired lung function and to separate effects of ambient pressure from those of submersion and increased oxygen partial pressure. Thirty two healthy subjects carried out dives in a hyperbaric chamber using a cross over design to 600 kPa ambient pressure with and without submersion and a dry exposure to pure oxygen at 120 kPa ambient pressure (durations: 43 min). Pre-dive and four hours after surfacing the exhaled breath was collected non-invasively. Condensate was measured by a standard enzyme immuno-assay for LTB₄ in parallel with lung function values (FVC, FEV₁, MEF 25-75). Pre-exposure baseline values of LTB₄-concentrations and lung function values were in the normal range. Post-exposure values

did not differ significantly from the baseline values. The data gave no evidence of any inflammatory activity in the subjects' airways after hyperbaric exposure.

38. Montuschi, P., S. Martello, M. Felli, C. Mondino, and M. Chiarotti.

Ion trap liquid chromatography/tandem mass spectrometry analysis of leukotriene B4 in exhaled breath condensate.

Rapid Commun Mass Spectrom 18(22):2723-9. 2004.

The objective of this study is the measurement of leukotriene B7 (LTB4), a potent inflammatory mediator, in exhaled breath condensate by using liquid chromatography/mass spectrometry (LC/MS and LC/MS/MS). Condensation of exhaled breath is a non-invasive method to collect airway secretions. Deuterated (d4)-LTB4 was used as internal standard. The MS and MS/MS behavior of LTB4 and LTB4-d4 was studied by electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in both positive and negative ion polarity mode. Preliminary results show that monitoring negative ions in ESI mode has the best sensitivity for both LTB4 and LTB4-d4. Therefore, negative ESI was chosen, and the [M-H]⁻ ions at m/z 335 and 339 were selected for quantification. The lower limit of quantification for LTB4, expressed as the lowest point of the calibration curve, was 100 pg/mL. Using this technique, we measured LTB4 in exhaled breath condensate in two healthy subjects, four asthmatic patients on anti-inflammatory treatment, and four asthmatic patients who were not on anti-inflammatory drugs. Exhaled LTB4 concentrations were detected only in asthmatic patients who were not on anti-inflammatory therapy. This method is potentially useful for non-invasive assessment of airway inflammation, but the sensitivity of the technique needs to be improved.

39. Mondino, C., G. Ciabattoni, P. Koch, R. Pistelli, A. Trove, P. J. Barnes, and P. Montuschi.

Effects of inhaled corticosteroids on exhaled leukotrienes and prostanoids in asthmatic children.

J Allergy Clin Immunol 114(4):761-7. 2004.

BACKGROUND: Lipid mediators play an important pathophysiologic role in atopic asthmatic children, but their role in the airways of atopic nonasthmatic children is unknown. OBJECTIVE: We sought (1) to measure leukotriene (LT) E 4, LTB 4, 8-isoprostane, prostaglandin E 2, and thromboxane B 2 concentrations in exhaled breath condensate in atopic asthmatic and atopic nonasthmatic children; (2) to measure exhaled nitric oxide (NO) as an independent marker of airway inflammation; and (3) to study the effect of inhaled corticosteroids on exhaled eicosanoids. METHODS: Twenty healthy children, 20 atopic nonasthmatic children, 30 steroid-naive atopic asthmatic children, and 25 atopic asthmatic children receiving inhaled corticosteroids were included in a cross-sectional study. An open-label study with inhaled fluticasone (100 microg twice a day for 4 weeks) was undertaken in 14 steroid-naive atopic asthmatic children. RESULTS: Compared with control subjects, exhaled LTE 4 (P <.001), LTB 4 (P <.001), and 8-isoprostane (P <.001) levels were increased in both steroid-naive and steroid-treated atopic asthmatic children but not in atopic nonasthmatic children (LTE 4, P=.14; LTB 4, P=.23; and 8-isoprostane, P=.52). Exhaled NO levels were increased in steroid-naive atopic asthmatic children (P <.001) and, to a lesser extent, in atopic nonasthmatic children (P <.01). Inhaled fluticasone reduced exhaled NO (53%, P <.0001) and, to a lesser extent, LTE 4 (18%, P <.01) levels but not LTB 4, prostaglandin E 2, or 8-isoprostane levels in steroid-naive asthmatic children. Conclusions Exhaled LTE 4, LTB 4, and 8-isoprostane levels are increased in atopic asthmatic children but not in atopic nonasthmatic children. In contrast to exhaled NO, these markers seem to be relatively resistant to inhaled corticosteroids.

40. Moloney, E. D., S. E. Mumby, R. Gajdocsi, J. H. Cranshaw, S. A. Kharitonov, G. J. Quinlan, and M. J. Griffiths.

Exhaled breath condensate detects markers of pulmonary inflammation after cardiothoracic surgery.

Am J Respir Crit Care Med 169(1):64-9. 2004.

Cardiac surgery using cardiopulmonary by-pass and, to a greater extent, lung resection, causes acute lung injury that is usually subclinical. Analysis of mediators in exhaled breath condensate is a promising means of monitoring inflammation in a variety of airway diseases but the contribution of the airway lining fluid from the lower respiratory tract is uncertain. We compared the analysis of markers of lung injury in exhaled breath condensate and bronchoalveolar lavage in endotracheally intubated patients before and after coronary artery bypass graft surgery with cardiopulmonary bypass and lobectomy. The neutrophil count and leukotriene B4 concentration in bronchoalveolar lavage fluid rose after coronary artery bypass graft surgery (p < 0.05), but there was no significant change in leukotriene B4, hydrogen peroxide, or hydrogen ion concentrations in exhaled breath condensate. By contrast, after lobectomy, the concentration in exhaled breath condensate of leukotriene B4, hydrogen peroxide and hydrogen ions rose significantly (p < 0.05). Exhaled breath condensate is a safe, noninvasive method of sampling the milieu of the distal lung and is sufficiently sensitive to detect markers of inflammation and oxidative stress in patients after lobectomy, but not after the milder insult associated with cardiac surgery.

41. McCafferty, J. B., T. A. Bradshaw, S. Tate, A. P. Greening, and J. A. Innes.

Effects of breathing pattern and inspired air conditions on breath condensate volume, pH, nitrite, and protein concentrations. *Thorax* 59(8):694-8. 2004.

BACKGROUND: The effects of breathing pattern and inspired air conditions on the volume and content of exhaled breath condensate (EBC) were investigated. **METHODS:** Total exhaled water (TEW), EBC volume, pH, nitrite and protein concentrations were measured in three groups of 10 healthy subjects breathing into a condenser at different target minute ventilations (V_m), tidal volumes (V_t), and inspired air conditions. **RESULTS:** The volumes of both TEW and EBC increased significantly with V_m . For V_m 7.5, 15 and 22.5 l/min, mean (SD) EBC was 627 (258) microl, 1019 (313) microl, and 1358 (364) microl, respectively ($p < 0.001$) and TEW was 1879 (378) microl, 2986 (496) microl, and 4679 (700) microl, respectively ($p < 0.001$). TEW was significantly higher than EBC, reflecting a condenser efficiency of 40% at a target V_m of 7.5 l/min which reduced to 29% at V_m 22.5 l/min. Lower V_t gave less TEW than higher V_t (26.6 v 30.7 microl/l, mean difference 4.1 (95% CI 2.6 to 5.6), $p < 0.001$) and a smaller EBC volume (4.3 v 7.6 microl/l, mean difference 3.4 (95% CI 2.3 to 4.5), $p < 0.001$). Cooler and drier inspired air yielded less water vapour and less breath condensate than standard conditions ($p < 0.05$). Changes in the breathing pattern had no effect on EBC protein and nitrite concentrations and pH. **CONCLUSION:** These results show that condensate volume can be increased by using high V_t and increased V_m without compromising the dilution of the sample.

42. Majewska, E., M. Kasielski, R. Luczynski, G. Bartosz, P. Bialasiewicz, and D. Nowak.

Elevated exhalation of hydrogen peroxide and thiobarbituric acid reactive substances in patients with community acquired pneumonia.

Respir Med 98(7):669-76. 2004.

BACKGROUND: Bacterial pneumonia involves influx of activated phagocytes into distal airways. These cells release oxidants including H_2O_2 , that may be exhaled or induce peroxidative damage to lung tissues with formation of thiobarbituric reactive substances (TBARs). **STUDY OBJECTIVES:** To determine whether concentrations of H_2O_2 and TBARs in exhaled breath condensate (EBC) is elevated and correlate with systemic response to pneumonia during 10 days of hospital treatment. **DESIGN:** The concentration of H_2O_2 and TBARs was measured in EBC of 43 inpatients with community acquired pneumonia (CAP) and 20 healthy never smoked subjects over 10 days and were accompanied by monitoring of WBC count, serum concentration of C-reactive protein (CRP) and peroxy radical-trapping capacity. **RESULTS:** Patients with CAP exhaled 4.6-, 3.7-, 3.9-, 3.3-times more H_2O_2 than healthy controls at 1st, 3rd, 5th and 10th day of treatment ($P < 0.05$), respectively. EBC concentrations of TBARs were elevated at 1st and 3rd day. H_2O_2 and TBARs levels decreased along with treatment course. Correlation ($P < 0.05$) was found between H_2O_2 levels and CRP and WBC count ($r = 0.31$) at 1st day and between TBARs and CRP at 5th ($r = 0.34$) and 10th day ($r = 0.46$). The mean H_2O_2 exhalation estimated over ten days of treatment correlated with pneumonic chest X-ray score ($r = 0.42$), CRP levels ($r = 0.46$) and WBC count ($r = 0.33$) at admission ($P < 0.05$). **CONCLUSIONS:** Pneumonia is accompanied by oxidative stress in airways that moderately correlates with intensity of systemic inflammatory response. Determination of H_2O_2 in EBC may be helpful for non-invasive monitoring of oxidants production during lower respiratory tract infection.

43. Leung, T. F., G. W. Wong, F. W. Ko, C. W. Lam, and T. F. Fok.

Increased macrophage-derived chemokine in exhaled breath condensate and plasma from children with asthma.

Clin Exp Allergy 34(5):786-91. 2004.

BACKGROUND: Type 2 helper T lymphocyte-specific chemokines including macrophage-derived chemokine (MDC), thymus and activation-regulated chemokine (TARC) and eotaxin are important mediators for allergic airway inflammation. **OBJECTIVE:** We investigated whether these chemokines can be detected in exhaled breath condensate (EBC) and their relation to childhood asthma. **METHODS:** Asthmatics recruited from paediatric clinics of a university teaching hospital were classified into intermittent asthma (IA) and persistent asthma (PA) according to Global Initiative for Asthma guidelines. EBC was collected by a disposable collection kit, whereas fractional exhaled nitric oxide (FENO) was measured by a chemiluminescence analyser. Concentrations of MDC, TARC and eotaxin in both EBC and plasma were measured using sandwich enzyme immunoassay. The intra-subject reproducibility of exhaled chemokine measurements was determined by co-efficients of variation (CV). **RESULTS:** Forty-eight patients with PA, 36 children with IA and 18 controls were recruited. MDC and eotaxin were present in EBC from nearly all subjects, whereas TARC could be measured in EBC from 33 (32%) subjects only. The median MDC concentration in EBC was higher in PA (117 pg/mL) as compared with IA (106 pg/mL) and controls (105 pg/mL; $P = 0.003$ for both). The median plasma MDC concentration in PA (648 pg/mL) was also higher than that in IA (520 pg/mL; $P = 0.002$) and controls (490 pg/mL; $P = 0.008$). The median plasma TARC concentration was also increased in PA as compared with IA (72 pg/mL vs. 35 pg/mL; $P = 0.004$). MDC concentrations in EBC were lower in patients with PA who received high-dose inhaled corticosteroid ($P = 0.005$). FENO was significantly higher in asthmatics than controls ($P < 0.0001$), but it was not associated with chemokines in EBC or plasma. The mean (range) CV for measuring MDC, TARC and

eotaxin in EBC (n=6) were 5.5 (2.0-7.2%), 8.8 (3.6-14.4%) and 5.2 (2.8-7.9%), respectively. CONCLUSIONS: Our results suggest that MDC in EBC and MDC and TARC in plasma are increased in children with PA as compared with IA or control. MDC concentrations in EBC are suppressed in patients on high-dose inhaled corticosteroid treatment.

44. Leung, T. F., C. Y. Li, C. W. Lam, C. S. Au, E. Yung, I. H. Chan, G. W. Wong, and T. F. Fok.
The relation between obesity and asthmatic airway inflammation.

Pediatr Allergy Immunol 15(4):344-50. 2004.

Epidemiologic studies suggest increased asthma prevalence in obese subjects. However, the relation between obesity and airway inflammation remains unclear. This cross-sectional study aims to investigate the relation between obesity indices and exhaled nitric oxide (ENO) and leukotriene B(4) (LTB(4)) in children with asthma. Asthmatic patients aged 7-18 yr old were recruited. Weight-for-height Z score was calculated from anthropometry. ENO was measured by online single-breath method using a chemiluminescence analyzer, whereas LTB(4) concentrations in exhaled breath condensate (EBC) were quantified using competitive enzyme immunoassay. Ninety-two asthmatics and 23 controls were recruited. The mean ENO and LTB(4) concentrations in EBC were higher in asthmatic patients (87 p.p.b. and 40.5 pg/ml) than controls (25 p.p.b. and 18.7 pg/ml) ($p < 0.0001$ for both). Obesity, as defined by weight $>120\%$ median weight-for-height, was not associated with any alteration in ENO or LTB(4) concentrations in patients with asthma. Besides, these inflammatory markers did not differ between asthmatics in the highest and lowest quartiles of weight-for-height Z score. On multivariate analysis, ENO showed significant correlation with age ($\beta = 0.511$, $p < 0.0001$), peripheral blood eosinophil count ($\beta = 0.222$, $p = 0.019$), plasma total IgE concentration ($\beta = 0.187$, $p = 0.050$) and forced expiratory volume in 1-s (FEV(1); $\beta = -0.221$, $p = 0.014$). None of the factors was associated with LTB(4) concentration in EBC. In conclusion, ENO and LTB(4) concentration in EBC are increased in childhood asthma. However, these inflammatory markers did not differ between obese and non-obese children with asthma.

45. Khasina, M. A., S. A. Dvinskaia, S. I. Beloglazova, M. Khasina, and T. F. Puchinskaia.

[Vapor condensate of exhaled air in evaluating the impaired metabolism of the bronchopulmonary system in nonspecific lung diseases].

Klin Lab Diagn(5):15-7. 2004.

The degree of metabolic rehabilitation of the bronchopulmonary system was evaluated in non-specific pulmonary diseases, like pneumonia or chronic obstructive bronchitis, by using the data of biochemical testing of the exhaled-air vapor condensate. Nine parameters were investigated, i.e. enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase, alkaline phosphatase (AP), gamma-glutamyl amino-transpeptidase (GGT) as well as parameters of protein metabolism - common protein, seromuroid (SC), C-reactive protein and urea. AST, ALT, AP, GGT, SC and urea were acknowledged as the most informative parameters. The results are indicative of that the recovery of metabolic processes in the bronchopulmonary system was not completed.

46. Kharitonov, S. A.

Exhaled markers of inflammatory lung diseases: ready for routine monitoring?

Swiss Med Wkly 134(13-14):175-92. 2004.

Assessing airway inflammation is important for investigating the underlying mechanisms of many lung diseases, including asthma and chronic obstructive pulmonary disease (COPD). Yet these are not measured directly in routine clinical practice because of the difficulties in monitoring inflammation. The presence and type of airway inflammation can be difficult to detect clinically, and may result in delays in initiating appropriate therapy. Non-invasive monitoring may assist in differential diagnosis of lung diseases, assessment of their severity and response to treatment. There is increasing evidence that breath analysis may have an important place in the diagnosis and clinical management of asthma, COPD, primary ciliary dyskinesia (PCD) and other major lung disease. The article reviews whether current noninvasive measurements of exhaled gases, such as nitric oxide (NO), hydrocarbons, inflammatory markers exhaled breath condensate (EBC) are ready for routine use in clinical practice.

47. Holz, O., and R. A. Jorres.

[Non-invasive methods for monitoring airway inflammation: a comparison of expenditures, gain and clinical value].

Pneumologie 58(7):510-5. 2004.

Among the noninvasive procedures for the assessment of airway inflammation, the analysis of spontaneous sputum is currently the only method, the expenses of which are covered by health insurance in Germany. It can easily be used for semiquantitative

cytological analyses by practising pneumologists. Recent data also indicate the usefulness of sputum induction, particularly in asthma diagnosis and therapy control, and demonstrate its capability of reducing total costs per patient. In contrast to sputum analysis, the measurement of exhaled nitric oxide (NO) yields a read-out without time delay. NO as associated with eosinophils also seems suitable for monitoring airway inflammation. The number of studies regarding NO, both its pathophysiological role and clinical use, is far greater than that regarding any other marker of exhaled air. Measurements are easy and fast, but the costs of analysers are still prohibitive in clinical practice. The analysis of other compounds of exhaled air, particularly those of exhaled breath condensate (EBC), offers fascinating perspectives, owing to the scope of markers that might be measured, and could enable the assessment of multivariate profiles that are useful for diagnosis and therapy control. Currently, however, the method still faces methodological questions, and data indicating its usefulness and cost-efficiency in clinical practice are scarce. Compared to NO, the expenses per measurement in clinical use are mainly due to the costs per marker detection after sampling, as well as storage and transport of samples. The on-site analysis of pH in the EBC could be a first step to circumvent this obstacle.

48. Hitka, P., M. Cerny, M. Vizek, J. Wilhelm, and P. Zoban.
Assessment of exhaled gases in ventilated preterm infants.
Physiol Res 53(5):561-4. 2004.

Hydrogen peroxide (H₂O₂) production in exhaled air was measured in ventilated preterm newborns at 5, 24 and 48 hours after delivery, using originally designed method of exhaled breath condensate (EBC) collection. H₂O₂ production in expired gas was 812±/−34 pmol/20 min during the first measurement and then declined to 389±/−21 at 24 hours and 259±/−26 pmol/20 min at 48 hours.

49. Griese, M.
Exhaled breath condensate.
Pediatr Pulmonol Suppl 26:14-5. 2004.

50. Goldoni, M., S. Catalani, G. De Palma, P. Manini, O. Acampa, M. Corradi, R. Bergonzi, P. Apostoli, and A. Mutti.
Exhaled breath condensate as a suitable matrix to assess lung dose and effects in workers exposed to cobalt and tungsten.
Environ Health Perspect 112(13):1293-8. 2004.

The aim of the present study was to investigate whether exhaled breath condensate (EBC), a fluid formed by cooling exhaled air, can be used as a suitable matrix to assess target tissue dose and effects of inhaled cobalt and tungsten, using EBC malondialdehyde (MDA) as a biomarker of pulmonary oxidative stress. Thirty-three workers exposed to Co and W in workshops producing either diamond tools or hard-metal mechanical parts participated in this study. Two EBC and urinary samples were collected: one before and one at the end of the work shift. Controls were selected among nonexposed workers. Co, W, and MDA in EBC were analyzed with analytical methods based on mass spectrometric reference techniques. In the EBC from controls, Co was detectable at ultratrace levels, whereas W was undetectable. In exposed workers, EBC Co ranged from a few to several hundred nanomoles per liter. Corresponding W levels ranged from undetectable to several tens of nanomoles per liter. A parallel trend was observed for much higher urinary levels. Both Co and W in biological media were higher at the end of the work shift in comparison with preexposure values. In EBC, MDA levels were increased depending on Co concentration and were enhanced by coexposure to W. Such a correlation between EBC MDA and both Co and W levels was not observed with urinary concentration of either element. These results suggest the potential usefulness of EBC to complete and integrate biomonitoring and health surveillance procedures among workers exposed to mixtures of transition elements and hard metals. Key words: cobalt, exhaled breath condensate, hard metals, lung, malondialdehyde, oxidative stress, tungsten.

51. Gianazza, E., L. Allegra, E. Bucchioni, I. Eberini, L. Puglisi, F. Blasi, C. Terzano, R. Wait, and C. R. Sirtori.
Increased keratin content detected by proteomic analysis of exhaled breath condensate from healthy persons who smoke.
Am J Med 117(1):51-4. 2004.

52. Gessner, C., H. Kuhn, K. Toepfer, S. Hammerschmidt, J. Schauer, and H. Wirtz.
Detection of p53 gene mutations in exhaled breath condensate of non-small cell lung cancer patients.
Lung Cancer 43(2):215-22. 2004.

Early diagnosis of lung carcinoma is greatly desired. A potential source of early information regarding the process of cancerisation

in the airways is exhaled breath condensate (EBC). The direct approach to detecting cancerisation is examining DNA from the area of chronic damage, i.e. airways and lung parenchyma. We therefore investigated DNA in EBC of patients with NSCLC and healthy volunteers. Human DNA was amplified by PCR in exhaled breath condensate and used to detect p53 mutations. A PCR of the beta-actin gene fragment was used to detect human DNA in each of the EBC samples. In 65.7% of the samples, the beta-actin gene was found. Extracted DNA as well as native EBC were equally suited as starting material for amplification. Mutations of the p53 gene were investigated in all EBC samples of NSCLC patients. p53 exons 5-8 were amplified using nested PCR and subsequently sequenced. Mutations were found in four of the patients (n=11; 36.4%) while no mutation was found in volunteers (n=10). Mutations detected in EBC were also compared with those of corresponding tumor tissue. Different point mutations in EBC and tumor tissue were revealed in all cases. Our findings demonstrate that exhaled breath condensate may be used for analysis of somatic gene mutations in an area of direct tobacco-related DNA damage.

53. Gessner, C., S. Hammerschmidt, H. Kuhn, and H. Wirtz.
[Expired diagnosis?—the potential of exhaled breath analysis].
Pneumologie 58(4):230-7. 2004.

Analysis of breath condensate is an innovative approach to biochemical information from the lung. It provides a new tool to estimate and characterize the burden of oxidative and inflammatory processes in the airways/lung. Clinical applications in asthma, COPD and CF can be envisioned as well as determining organ-specific inflammation in mechanically ventilated patients or monitoring patients with transplanted lungs. However, besides inflammation other important areas have begun to be evaluated, such as the demonstration of p53 mutations in NSCLC patients or an increased ratio of EBC nitrite to tidal volume in mechanically stressed lungs. Of course a careful validation of each and every parameter is paramount to the use in clinical applications. The comparison to BAL is oftentimes called for but at the same time is not the comparison to a gold standard because of the well-known problems of BAL. The scope of this review is a summary of facts and theories concerning exhaled breath condensate generation, collection and analysis but at the same time the representation of the many aspects that remain to be resolved.

54. Garey, K. W., M. M. Neuhauser, R. A. Robbins, L. H. Danziger, and I. Rubinstein.
Markers of inflammation in exhaled breath condensate of young healthy smokers.
Chest 125(1):22-6. 2004.

INTRODUCTION: Although a strong correlation exists between long-term cigarette smoking, pulmonary inflammation, and COPD, efforts to identify populations at risk of acquiring COPD have so far been unsuccessful. To this end, noninvasive detection and monitoring of biomarkers of pulmonary inflammation in young healthy smokers may assist in this task. STUDY OBJECTIVES: The purpose of this study was to determine the concentrations of total protein, nitrites, interleukin (IL)-1beta, and tumor necrosis factor (TNF)-alpha, and neutrophil chemotactic activity in exhaled breath condensate (EBC) collected from healthy college student smokers and nonsmokers. DESIGN: EBC was collected from 20 volunteers (9 nonsmokers and 11 smokers) during tidal breathing for 20 min. EBC was also collected from smokers 30 min after smoking one filtered cigarette. The concentrations of total protein, nitrite, IL-1beta, and TNF-alpha in EBC was determined by enzyme-linked immunosorbent assay. Neutrophil chemotactic activity in EBC was determined in vitro using the blind-well technique. RESULTS: The concentrations of total protein and nitrite, and neutrophil chemotactic activity were significantly higher in EBC of smokers in comparison to nonsmokers ($p < 0.05$). The concentrations of total protein and nitrite in the condensate of smokers did not change significantly after smoking one cigarette. The concentrations of IL-1beta and TNF-alpha in EBC were similar in nonsmokers and smokers. CONCLUSIONS: Concentrations of certain inflammatory mediators and neutrophil chemotactic activity are increased in EBC of young healthy smokers. Collection and analysis of EBC may assist in early detection of cigarette smoke-induced pulmonary inflammation and identifying populations at risk for acquiring COPD.

55. Effros, R. M.
Exhaled breath condensate pH.
Eur Respir J 23(6):961-2; author reply 962. 2004.

56. Effros, R. M.
Exhaled breath condensate acidification in acute lung injury.
Respir Med 98(7):682; author reply 683. 2004.

57. Dwyer, T. M.

Sampling airway surface liquid: non-volatiles in the exhaled breath condensate.

Lung 182(4):241-50. 2004.

Exhaled breath condensate (EBC) samples contain molecules that have no appreciable vapor pressure; such molecules likely derive from droplets of airway fluid. We analyzed EBC gathered from a total of 62 healthy volunteers in order to quantify the volume of airway liquid that was the source of the non-volatiles; saliva was analyzed as a reference secretion. EBC urea averaged 0.52 +/- 0.12 micromol/L (n = 18), an 8,600-fold dilution from predicted blood urea nitrogen levels. Protein averaged 2.3 +/- 0.3 microg/ml (n = 31), three orders of magnitude less than in saliva (1.4 +/- 0.1 mg/ml, n = 15). EBC ammonia was 6.6 +/- 0.6 mmol/L (1/15 that of saliva) and EBC ammonium ion was 0.90 +/- 0.19 micromol/L, concentrations that are incompatible with an 8,600-fold dilution from a biological source. Thus, urea-derived dilution factors may be used to interpret EBC non-volatile molecules, but not EBC volatiles.

58. Corradi, M., P. Pignatti, P. Manini, R. Andreoli, M. Goldoni, M. Poppa, G. Moscato, B. Balbi, and A. Mutti.

Comparison between exhaled and sputum oxidative stress biomarkers in chronic airway inflammation.

Eur Respir J 24(6):1011-7. 2004.

The aim of the present study was to compare aldehyde levels resulting from lipid peroxidation in exhaled breath condensate (EBC) and induced sputum (IS) supernatant of subjects with asthma and chronic obstructive pulmonary disease (COPD). Aldehydes (malondialdehyde (MDA), acrolein, n-hexanal (C6), n-heptanal (C7), n-nonanal (C9), 4-hydroxynonenal (HNE) and 4-hydroxyhexenal (HHE)) in both biological fluids were measured by liquid chromatography-tandem mass spectrometry. MDA concentrations in sputum were 132.5 nM (82.5-268.8) and 23.7 nM (9-53.7) in EBC. Similarly, C6, C7 and C9 concentrations in IS were 1.5-4.7-fold higher than in EBC. Acrolein levels were 131.1 nM (55.6-264.6) in IS and 45.3 nM (14.4-127.1) in EBC. The concentrations of HNE and HHE in IS were not significantly different from the levels in EBC. Aldehyde levels in EBC did not show any correlation with aldehyde levels in IS or with differential sputum cellular count. In COPD, MDA in EBC, but not its IS counterpart, was negatively correlated with the severity of disease. In conclusion, the data presented here show that aldehydes can be detected in both exhaled breath condensate and supernatant of induced sputum, but that their relative concentrations are different and not correlated with each other. Therefore, with regard to lipid peroxidation products, exhaled breath condensate and induced sputum must be considered as independent techniques.

59. Carpagnano, G. E., O. Resta, M. P. Foschino-Barbaro, A. Spanevello, A. Stefano, G. Di Gioia, G. Serviddio, and E. Gramiccioni.

Exhaled Interleukine-6 and 8-isoprostane in chronic obstructive pulmonary disease: effect of carbocysteine lysine salt monohydrate (SCMC-Lys).

Eur J Pharmacol 505(1-3):169-75. 2004.

Chronic obstructive pulmonary disease (COPD) is characterized by an airways inflammation and by an enhanced generation of reactive oxygen species. The aim of our study was to assess the inflammation and the oxidative stress in airways of COPD patients with acute exacerbation of disease and in stability. Furthermore, we investigated the anti-inflammatory and antioxidant effects of 6 months treatment with carbocysteine lysine salt monohydrate (SCMC-Lys) in COPD. We studied 30 mild acute COPD, 10 mild stable COPD and 15 healthy subjects. 8-isoprostane and Interleukine-6 were measured in their breath condensate through immunoassay. Significantly higher concentrations of exhaled 8-isoprostane and Interleukine-6 were found in acute COPD patients compared to stable COPD and healthy controls (21.8 +/- 5.1 vs. 13.2 +/- 2.0 vs. 4.7 +/- 1.8 pg/ml and 7.4 +/- 0.9 vs. 5.8 +/- 0.2 vs. 2.7 +/- 0.6 pg/ml, p < 0.0001). COPD patients treated with SCMC-Lys showed a marked reduction of exhaled 8-isoprostane and Interleukine-6 (8.9 +/- 1.5 and 4.6 +/- 0.8 pg/ml, p < 0.0001). These findings suggest that there is an increase of 8-isoprostane and Interleukine-6 concentrations in the breath condensate of COPD patients compared to healthy controls especially during acute exacerbations of the disease. Moreover, we showed an anti-inflammatory and antioxidant effect of short-term administration of SCMC-Lys in COPD, suggesting the importance of a further placebo-controlled study that should evaluate the effects of this drug.

60. Carpagnano, G. E., S. A. Kharitonov, M. P. Foschino-Barbaro, O. Resta, E. Gramiccioni, and P. J. Barnes.

Supplementary oxygen in healthy subjects and those with COPD increases oxidative stress and airway inflammation.

Thorax 59(12):1016-9. 2004.

BACKGROUND: Hyperoxia increases oxidative stress through the generation of reactive oxygen species and may therefore enhance inflammation in the lungs. The aim of this study was to investigate whether short term supplementary oxygen (28%) increases oxidative stress and inflammation in the airways by measuring 8-isoprostane and interleukin 6 (IL-6) concentrations in exhaled breath condensate. METHODS: Twenty three healthy subjects (12 men, mean (SD) age 48 (7) years) and 23 patients with

chronic obstructive pulmonary disease (COPD; 15 men, mean (SD) age 56 (5) years) were studied. 8-isoprostane and IL-6 concentrations were measured by immunoassay. RESULTS: Increased concentrations of 8-isoprostane and IL-6 were found in all subjects after breathing 28% oxygen for 1 hour. In healthy subjects the concentrations of 8-isoprostane and IL-6 were 10.9 (2.9) pg/ml and 4.9 (0.8) pg/ml, respectively, compared with baseline concentrations of 6.1 (1.3) pg/ml and 2.9 (0.6) pg/ml, and in patients with COPD the concentrations were 27.9 (3.1) pg/ml and 8.3 (1.2) pg/ml, respectively, compared with baseline concentrations of 18.9 (3.6) pg/ml and 6.3 (0.6) pg/ml. By contrast, breathing air through the same face mask for 1 hour had no significant effects on 8-isoprostane or IL-6 concentrations in normal subjects or those with COPD. CONCLUSIONS: These findings suggest that short term supplementary oxygen may enhance oxidative stress and inflammation in the airways. Whether this happens with long term oxygen therapy needs to be determined.

61. Carpagnano, G. E., M. P. Foschino-Barbaro, O. Resta, E. Gramiccioni, and F. Carpagnano.

Endothelin-1 is increased in the breath condensate of patients with non-small-cell lung cancer.

Oncology 66(3):180-4. 2004.

One recent line of cancer research is currently directed to the study of growth factors. Of increasing interest is endothelin-1 (ET-1), a mitogenic factor already investigated in several human cancer cell lines, which has been found to participate in the development and progression of tumours. This peptide has an important role also in non-small-cell lung cancer (NSCLC) where ET-1 expression has been found in 100% of cell lines. OBJECTIVES: The aim of this study was to measure ET-1 concentrations in the airways of patients with NSCLC using a completely non-invasive procedure--the breath condensate--and to verify the involvement of this peptide in the growth of lung tumours. METHODS: We enrolled 30 patients (17 men, median age 63 years; range 53-74) with histological evidence of NSCLC and 15 healthy controls (9 men, median age 59 years; range 52-70). ET-1 was measured in the exhaled breath condensate by means of a specific enzyme immunoassay kit. RESULTS: Higher concentrations of exhaled ET-1 were found in NSCLC patients (8.3 +/- 0.7 pg/ml) compared to controls (5.2 +/- 0.5 pg/ml, $p < 0.0001$). A statistically significant difference was observed between patients with distant metastases (stage IV) of NSCLC (8.9 +/- 0.6 pg/ml) and those with locoregional disease (stage I-III) (7.9 +/- 0.5 pg/ml). A significant reduction in ET-1 levels was found in 14 patients after surgical removal of the tumour either associated with or without adjuvant chemotherapy (6.3 +/- 0.5 vs. 7.9 +/- 0.4 pg/ml, $p < 0.0001$). CONCLUSIONS: These findings suggest that the measurement of ET-1 in the breath condensate of patients with NSCLC could be proposed as a marker for early detection of NSCLC as well as for monitoring reduction or progression of the neoplasm in the follow-up of treated patients.

62. Carpagnano, G. E., P. J. Barnes, J. Francis, N. Wilson, A. Bush, and S. A. Kharitonov.

Breath condensate pH in children with cystic fibrosis and asthma: a new noninvasive marker of airway inflammation?

Chest 125(6):2005-10. 2004.

STUDY OBJECTIVES: The noninvasive assessment and monitoring of airway inflammation could be important in respiratory disease. The pH of exhaled breath condensate (EBC) is a promising marker. Although pH has been measured in the EBC of adults with inflammatory airway diseases, no study has measured this in children. DESIGN: This study aimed to assess whether there is a change in pH in the EBC of children with cystic fibrosis (CF) and asthma, and to try to determine whether pH could be used as a marker of airway inflammation. Furthermore, the relationships among EBC pH, severity of disease, and oxidative stress were studied. PATIENTS AND METHODS: We studied 20 children with CF (mean [± SEM] age, 7 ± 3 years), 20 children with asthma (mean age, 7 ± 2 years), and 15 age-matched healthy children (mean age, 7 ± 2 years). The pH of EBC was measured using a pH meter. MEASUREMENTS AND RESULTS: Lower pH values were observed in the EBC of children with CF and asthma compared to control subjects (mean pH, 7.23 ± 0.03 and 7.42 ± 0.01 vs 7.85 ± 0.02, respectively). Furthermore, relationships among EBC pH, severity of asthma, and the presence of an infective exacerbation of CF was found. There was a negative correlation between exhaled pH and exhaled leukotriene B(4) concentrations ($r = -0.5$; $p < 0.005$). CONCLUSION: We conclude that the measurement of EBC pH may be useful in the evaluation of airway inflammation in children with asthma and CF.

63. Cap, P., and F. Pehal.

[Examination of exhaled breath condensate in patients with asthma and chronic obstructive pulmonary diseases].

Cas Lek Cesk 143(11):742-6; discussion 746-7. 2004.

Considerable interest of specialists all over the world has focused on the measurement of the markers of inflammation and oxidative stress in the exhaled breath condensate in patients with asthma or chronic obstructive pulmonary diseases recently. Use of exhaled condensate is based on the hypothesis that aerosol particles exhaled in human breath reflect the composition of the bronchoalveolar extracellular lining fluid. The standard collection of the material requires condensation of exhaled air and the samples have to be kept in biologically inert containers. Measurement of the very low concentrations of selected substances re-

quires very sensitive analytical methods. The examination of exhaled breath condensate is absolutely non-invasive method, which can be repeated as often as needed and it is extremely well tolerated both by children and seniors. Markers in the condensate enable detection and quantification of the inflammation process, the disease monitoring, and assessment of the response to the treatment. The breath condensate diagnostics is a new progressive method and in the patients with asthma and chronic obstructive pulmonary disease it can bring complementary information to the very sensitive method of determination of exhaled nitric oxide.

64. Cap, P., J. Chladek, F. Pehal, M. Maly, V. Petru, P. J. Barnes, and P. Montuschi.

Gas chromatography/mass spectrometry analysis of exhaled leukotrienes in asthmatic patients.

Thorax 59(6):465-70. 2004.

BACKGROUND: Leukotriene-like immunoreactivity has been detected in exhaled breath condensate (EBC), but definitive evidence for the presence of leukotrienes (LTs) in this biological fluid is not available. A study was undertaken to determine whether LTC(4), LTD(4), LTE(4), and LTB(4) are measurable in EBC by gas chromatography/mass spectrometry and to quantify exhaled LTs in adults and children with asthma and in control subjects. METHODS: Twenty eight adults and 33 children with mild to moderate persistent asthma treated with inhaled corticosteroids and age matched healthy controls (50 adults and 50 children) were studied. LTC(4), LTD(4), LTE(4), and LTB(4) in EBC were measured by gas chromatography/mass spectrometry. RESULTS: LTD(4), LTE(4), and LTB(4) were detectable in all samples. Concentrations of LTC(4) in EBC were either close to or below the detection limit of 1 pg/ml. Median exhaled LTD(4), LTE(4), and LTB(4) concentrations in asthmatic adults were increased 4.1-fold ($p < 0.001$), 1.8-fold ($p < 0.01$), and 2.6-fold ($p < 0.001$), respectively, compared with values in healthy adults. Median exhaled LTD(4), LTE(4), and LTB(4) concentrations in asthmatic children were increased 2.8-fold ($p < 0.001$), 1.3-fold ($p < 0.001$), and 1.6-fold ($p < 0.001$), respectively, compared with those in healthy children. In patients with asthma there was a correlation between exhaled LTD(4) and LTE(4) in both adults ($r = 0.87$, $p < 0.0001$) and children ($r = 0.78$, $p < 0.0001$). CONCLUSIONS: Gas chromatography/mass spectrometry can be used to accurately quantify exhaled LTs which are increased in asthmatic adults and children compared with controls.

65. Bucchioni, E., Z. Csoma, L. Allegra, K. F. Chung, P. J. Barnes, and S. A. Kharitonov.

Adenosine 5'-monophosphate increases levels of leukotrienes in breath condensate in asthma.

Respir Med 98(7):651-5. 2004.

Hyperresponsiveness (AHR) is a key physiological abnormality in asthma. In clinical and research studies AHR is measured bronchial challenge, with methacholine (MCh), but more recently with adenosine-5'-monophosphate (AMP). In the search for markers of airway inflammation in asthmatic patients, we measured the concentrations of histamine and cysteinyl-leukotrienes (cys-LTs) before and after MCh and AMP challenges in the exhaled breath condensate of 13 patients with mild asthma (FEV1 78.5%pred) and nine healthy non-smokers, using specific enzyme immunoassays. With methacholine challenge we did not find any differences between asthmatics and normal subjects in the pre- and post-challenge concentrations of cys-LTs: 27.2+/-1.4 vs. 29.2+/-1.2 pg/ml and 26.3+/-2.2 vs. 27.5+/-4.2 pg/ml, respectively or histamine: 5.1+/-0.4 vs. 5.1+/-0.6 nM and 4.5+/-0.4 vs. 4.4+/-0.3 nM; $P > 0.05$). In asthmatic patients cys-LT levels were significantly higher after AMP challenge (56.2+/-9.7 vs. 31.7+/-6.9 pg/ml; $P < 0.05$); but there was no difference in healthy subjects (27.2+/-4.6 vs. 30.3+/-4.7 pg/ml). There was no difference in histamine concentrations in asthmatic (5.9+/-1.8 vs. 4.5+/-0.5 nM), or healthy subjects (5.5+/-0.4 vs. 5.7+/-0.9 nM) after AMP challenge. In conclusion, our results show that the cys-LTs are increased in exhaled breath condensate after AMP challenge, which may indicate that the AMP acts indirectly by releasing cys-LTs from primed mast cells. The detection of LTs and histamine in exhaled breath condensate may be useful in monitoring asthma.

66. Bodini, A., D. Peroni, L. Vicentini, A. Loiacono, E. Baraldi, L. Ghio, M. Corradi, R. Alinovi, A. L. Boner, and G. L. Piacentini.

Exhaled breath condensate eicosanoids and sputum eosinophils in asthmatic children: a pilot study.

Pediatr Allergy Immunol 15(1):26-31. 2004.

Cysteinyl leukotrienes (cys-LTs), LTB4 and 8-isoprostane are increased in the exhaled breath condensate (EBC) from asthmatic patients. The aim of this study was to investigate whether the measurement of cys-LTs, LTB4 and 8-isoprostane in EBC can reflect the level of airway inflammation assessed by induced sputum in asthmatic children sensitized to house dust mite (HDM) during natural avoidance of HDM allergens. Twelve children were evaluated at the time of admission (T0) and after 3 months of stay (T1) at the Istituto Pio XII (Misurina, Italian Dolomites 1756 m). Sputum eosinophil percentage and measurement of cys-LTs, LTB4 and 8-isoprostanes in the breath condensate at T0 and T1 were evaluated. Eosinophil percentage in induced sputum was 8.5 +/- 1.1% at T0 and 3.5 +/- 0.4% at T1 ($p = 0.011$). Neutrophil percentage in sputum was 1.1 +/- 0.5% at T0 and 1.5 +/- 1.0% at T1 (ns). Cys-LTs mean level was 14.24 +/- 4.53 pg/ml at T0 and 4.65 +/- 0.68 pg/ml at T1 ($p = 0.0125$). LTB4 level was 2.36 +/- 0.19 pg/ml at T0 and

2.41 +/- 0.23 pg/ml at T1 (ns). 8-Isoprostane level reduced from 17.47 +/- 3.18 pg/ml at T0 to 7.36 +/- 3.26 pg/ml at T1 (p = 0.003). This study shows that exhaled cys-LTs and 8-isoprostane, as well as eosinophil percentage in induced sputum, are reduced after allergen avoidance in asthmatic children suggesting a potential application of EBC for the non-invasive evaluation of airway inflammation in asthma in allergic asthmatic children.

67. Vaughan, J., L. Ngamtrakulpanit, T. N. Pajewski, R. Turner, T. A. Nguyen, A. Smith, P. Urban, S. Hom, B. Gaston, and J. Hunt. Exhaled breath condensate pH is a robust and reproducible assay of airway acidity. *Eur Respir J* 22(6):889-94. 2003.

Exhaled breath condensate (EBC) pH is low in several lung diseases and it normalises with therapy. The current study examined factors relevant to EBC pH monitoring. Intraday and intraweek variability were studied in 76 subjects. The pH of EBC collected orally and from isolated lower airways was compared in an additional 32 subjects. Effects of ventilatory pattern (hyperventilation/hypoventilation), airway obstruction after methacholine, temperature (-44 to +13 degrees C) and duration of collection (2-7 min), and duration of sample storage (up to 2 yrs) were examined. All samples were collected with a disposable condensing device, and de-aerated with argon until pH measurement stabilised. Mean EBC pH (n=76 subjects, total samples=741) was 7.7 +/- 0.49 (mean +/- SD). Mean intraweek and intraday coefficients of variation were 4.5% and 3.5%. Control of EBC pH appears to be at the level of the lower airway. Temperature of collection, duration of collection and storage, acute airway obstruction, subject age, saliva pH, and profound hyperventilation and hypoventilation had no effect on EBC pH. The current authors conclude that in health, exhaled breath condensate pH is slightly alkaline, held in a narrow range, and is controlled by lower airway source fluid. Measurement of exhaled breath condensate pH is a simple, robust, reproducible and relevant marker of disease.

68. Vass, G., E. Huszar, E. Barat, M. Valyon, D. Kiss, I. Penzes, M. Augusztinovicz, and I. Horvath. Comparison of nasal and oral inhalation during exhaled breath condensate collection. *Am J Respir Crit Care Med* 167(6):850-5. 2003.

Analysis of exhaled breath condensate is a method for noninvasive assessment of the lung. Condensate can be collected with a nose clip (subjects inhale and exhale via the mouth) or without it (subjects inhale via the nose and exhale via the mouth), but the mode of inhalation may influence condensate volume and mediator levels. We compared condensate volume and adenosine, ammonia, and thromboxane B2 levels in young healthy volunteers (n = 25) in samples collected for 10 minutes from subjects with or without a nose clip. Patients with allergic rhinitis (n = 8) were also studied to assess the effect of upper airway inflammation on mediator levels. Adenosine, ammonia, and thromboxane B2 levels were determined by HPLC, spectrophotometry, and radioimmunoassay, respectively. Volume of condensate was significantly higher without nose clip than that with nose clip (mean +/- SD, 2321 +/- 736 microl and 1746 +/- 400 microl, respectively; p = 0.0001). We found no significant difference in any mediator levels between these two collection modes in healthy volunteers, but adenosine showed a tendency to differ between oral and nasal inhalation in patients with allergic rhinitis. Our data indicate that whereas a greater volume of condensate can be obtained when subjects inhale through their noses, the mode of inhalation does not influence mediator levels in young healthy volunteers, but may affect these levels in patients with allergic rhinitis.

69. Vass, G., E. Huszar, E. Barat, and I. Horvath. [Exhaled breath condensate and its analysis--a new method in pulmonology]. *Orv Hetil* 144(51):2517-24. 2003.

In the middle of the nineties a new, non-invasive method for investigation of the lung aroused the interest of many researchers: the exhaled breath condensate. It shows the extent of the interest that in the last five years more than 80 original articles have been published in this theme. Many substances are found in the expired breath which are detectable in the liquid that we obtain by cooling (= condensing) the exhaled breath. The advantages of this method are that it is non-invasive, convenient, it could be performed with mechanically ventilated patients as well as with children. The most studied substance is the hydrogen-peroxide, which is the marker of oxidative stress, and its level in condensate is elevated in numerous inflammatory diseases. 8-isoprostane was also studied a lot, which is another marker of oxidative stress. Numerous substances could be even measured in condensate, so the decay-product of nitric-oxide (nitrite, nitrate, nitrotyrosine), further nitrosothiol, adenosine, ammonia, different ions, leukotrienes, cytokines; recently even other feature of condensate is examined, such as its pH. The different mediators could help us to know better the diseases, support the diagnosis, follow the treatment or the disease. In this study the authors attempt to present the most important knowledge till now.

70. van Beurden, W. J., M. J. van den Bosch, W. C. Janssen, F. W. Smeenk, P. N. Dekhuijzen, and G. A. Harff.

Fluorimetric analysis of hydrogen peroxide with automated measurement.

Clin Lab 49(11-12):637-43. 2003.

In the pathophysiology of chronic obstructive pulmonary disease (COPD) oxidative stress plays an important role, which can be determined by measuring hydrogen peroxide. Hydrogen peroxide can be measured fluorimetrically in exhaled breath condensate (EBC), however, not standardized. The objective of this study was to investigate the sensitivity and reproducibility of measuring the hydrogen peroxide concentration in EBC of COPD patients using an automated flow injection device with varying flow rates and measurements. **METHODS:** 10 microl p-hydroxyphenylacetic acid (1.0 mmol/l) and 10 microl horseradish peroxidase (15 mU/l) were manually added to several hydrogen peroxide containing solutions and EBC of patients suffering from COPD. The fluorescence of the reaction product was measured with an automated sampler, flow injection and scanning fluorescence detector, excitation wave 295 nm, emission wave 405 nm, at different flow rates. The degree of fluorescence was expressed as either the area under the curve or the peak value. **RESULTS:** A flow rate of 1 ml/min gave the best results. There were no significant differences in calibration curves or detection limits using area under the curve or peak value (respectively 0.007 and 0.005 micromol/l) (flow rate 1.0 ml/min). The mean volume of EBC was 2.8 ml, the mean hydrogen peroxide concentration in the patient group was 0.2 micromol/l and the standard deviation of duplication 0.009 micromol/. **CONCLUSIONS:** The low detection limit may be explained by using flow injection, because it measures the fluorescence over a period of time. It is important to choose an appropriate flow rate. There is no difference in the detection limit between measuring the fluorescence as area under the curve or as peak value.

71. van Beurden, W. J., F. W. Smeenk, G. A. Harff, and P. N. Dekhuijzen.

Markers of inflammation and oxidative stress during lower respiratory tract infections in COPD patients.

Monaldi Arch Chest Dis 59(4):273-80. 2003.

BACKGROUND: Lower respiratory tract infections (LRTI) occur frequently in patients with Chronic Obstructive Pulmonary Disease (COPD), and are a major cause of morbidity, mortality and health care utilization. The aim of this study was to investigate if non- or less invasive markers of inflammation and oxidative stress can predict the course of the infections. **METHODS:** Twenty-five COPD patients who were admitted to hospital with a LRTI were included. Within 24 hours after admittance, spirometry (FEV₁, FVC, MEF₅₀), measurement of hydrogen peroxide (H₂O₂) in exhaled breath condensate (EBC), symptom scores and analyses of ESR, CRP, ECP, and MPO in serum were performed. All patients were treated with intravenous dexamethasone, nebulised salbutamol/ipratropium and, if needed, antibiotics. The tests were repeated at day 2, 3, 7 and 30. **RESULTS:** Complete data of the first four visits were collected in 19 patients. The H₂O₂ concentration and spirometry parameters did not change significantly during the study period. CRP, ESR and MPO levels decreased significantly during treatment, while the other serum inflammatory parameters did not change. There were no significant correlations between H₂O₂ concentration, spirometry and serum inflammatory parameters. **CONCLUSIONS:** In conclusion, this study showed no significant changes in H₂O₂ concentration in EBC, or spirometry during treatment of a LRTI in COPD patients. In contrast, several serum inflammatory markers did decrease during hospitalization, thus providing a simple tool to monitor exacerbations.

72. van Beurden, W. J., G. A. Harff, P. N. Dekhuijzen, S. M. van der Poel-Smet, and F. W. Smeenk.

Effects of inhaled corticosteroids with different lung deposition on exhaled hydrogen peroxide in stable COPD patients.

Respiration 70(3):242-8. 2003.

BACKGROUND: The effects of inhaled corticosteroids (ICS) on markers of oxidative stress in patients with stable COPD are unclear. **OBJECTIVES:** The aim was to investigate the effect of ICS on exhaled H₂O₂ in stable COPD patients and to compare ICS with different lung deposition. **METHODS:** Forty-one stable patients with moderate COPD (FEV₁ approximately 60% predicted) were randomized to sequence 1; first HFA-134a beclomethasone dipropionate (HFA-BDP, an ICS with more peripheral deposition) 400 microg b.i.d., then fluticasone propionate (FP, an ICS with more central deposition) 375 microg b.i.d. (n = 20) or sequence 2; first FP, then HFA-BDP (n = 21). Both 4-week treatment periods were preceded by a 4-week washout period. After each period, the concentration of H₂O₂ in exhaled breath condensate was measured. **RESULTS:** The H₂O₂ concentration decreased significantly after the first treatment period in both sequence 1 and 2 (p < 0.05, p = 0.01, respectively). In neither sequence was there a return to baseline values after the second washout, indicating a carry-over effect. The concentrations remained low in both sequences during the second treatment period. **CONCLUSIONS:** Both ICS appeared to reduce exhaled H₂O₂ in stable COPD patients. However, this study showed no difference between ICS with different deposition patterns, which in part may be due to the carry-over effect.

73. Szkudlarek, U., L. Maria, M. Kasielski, S. Kaucka, and D. Nowak.

Exhaled hydrogen peroxide correlates with the release of reactive oxygen species by blood phagocytes in healthy subjects.

Respir Med 97(6):718-25. 2003.

Various cells including polymorphonuclear leukocytes, alveolar macrophages and type-II pneumocytes may be a source of exhaled hydrogen peroxide (H₂O₂) in airways of humans. H₂O₂ can convert into hydroxyl radicals leading to peroxidative damage of airways structures and formation of volatile thiobarbituric acid-reactive substances (TBARs). We tested whether exhalation of H₂O₂ and TBARs by healthy subjects depends on reactive oxygen species generation from blood phagocytes. The expired breath condensate (EBC) and blood specimens were collected from 41 healthy, never smoked subjects (mean age 20.7 +/- 0.8 years, 18 men, 23 women) and then the EBC concentration of H₂O₂ and TBARs and 2 x 10⁻⁵ M fMLP-provoked whole blood chemiluminescence response was measured. The mean concentration of H₂O₂ and TBARs in EBC was 0.28 +/- 0.17 and 0.04 +/- 0.13 microM with ratio of positive readings reaching 36/41 and 4/41, respectively. The chemiluminescence response to n-formyl-methionyl-leucyl-phenylalanine stimulation was obtained in all cases and the following parameters were estimated: basal chemiluminescence (bCl); peak chemiluminescence (pCl); absolute light emission (aCl); and peaktime. H₂O₂ levels in EBC positively correlated (Spearman test) with bCl (r=0.41, P<0.01), pCl (r=0.47, P<0.01), aCl (r=0.49, P<0.001), peaktime (r=0.52, P<0.001) in the whole group and with bCl (r=0.56, P<0.01), pCl (r=0.67, P<0.01), aCl (r=0.66, P<0.01) in men and with aCl (r=0.41, P<0.05) and peaktime (r=0.48, P<0.05) in women. No association between exhaled TBARs and blood phagocytes activity was found. These results indicate that H₂O₂ exhalation in healthy never smoked subjects depends on ability of blood phagocytes to generate reactive oxygen species.

74. Spicuzza, L., G. U. Di Maria, and R. Polosa.

Adenosine levels in the exhaled breath condensate: a potential surrogate marker of airway inflammation.

Eur Respir J 22(2):392; author reply 392-3. 2003.

75. Sandrini, A., I. M. Ferreira, J. R. Jardim, N. Zamel, and K. R. Chapman.

Effect of nasal triamcinolone acetonide on lower airway inflammatory markers in patients with allergic rhinitis.

J Allergy Clin Immunol 111(2):313-20. 2003.

BACKGROUND: Allergic rhinitis (AR) and asthma are commonly associated, and similar underlying inflammatory processes link both diseases. AR, even in the absence of asthma, is associated with increased levels of exhaled nitric oxide (ENO) and hydrogen peroxide (H₂O₂) in exhaled breath condensate, 2 noninvasive markers of lower airway inflammation. OBJECTIVE: We sought to evaluate the effect of treatment with the nasal steroid triamcinolone acetonide on ENO and exhaled H₂O₂ in subjects with AR. METHODS: We allocated 23 subjects in a randomized, double-blind, parallel-controlled fashion to 4-week treatment with triamcinolone acetonide (220 microg/d) or matching placebo. RESULTS: ENO levels were greater in the subgroup with concomitant asthma (16/23 subjects) and decreased significantly with triamcinolone acetonide treatment in this subgroup of patients in comparison with patients receiving placebo. Breath condensate levels of H₂O₂ were higher in patients with AR without asthma than in those with asthma but decreased significantly with triamcinolone acetonide treatment in both subgroups. No changes were observed in bronchial hyperresponsiveness, nasal and asthma symptoms, or peak expiratory flow with active treatment or placebo. CONCLUSION: We conclude that treatment of AR with triamcinolone acetonide results in decrease of 2 noninvasive markers of lower airway inflammation, ENO and H₂O₂, supporting that upper and lower airway inflammation should be seen as a continuum in subjects with AR with and without asthma. ENO might be a more specific marker of the lower airway inflammation present in asthma.

76. Sandrini, A., I. M. Ferreira, C. Gutierrez, J. R. Jardim, N. Zamel, and K. R. Chapman.

Effect of montelukast on exhaled nitric oxide and nonvolatile markers of inflammation in mild asthma.

Chest 124(4):1334-40. 2003.

STUDY OBJECTIVES: Leukotriene receptor antagonists appear to exert anti-inflammatory activity in asthma. We undertook the present study to evaluate the effect of montelukast on levels of exhaled nitric oxide (ENO) and two inflammatory markers, hydrogen peroxide (H₂O₂), and cysteinyl leukotrienes (cys-LTs), in the exhaled breath condensate of subjects with mild asthma. PATIENTS: Twenty stable subjects with mild asthma (15 women and 5 men; mean [± SD] age, 34.8 ± 12.6 years) were included in the study. INTERVENTION: A 1-week run-in period was followed by 2 weeks of treatment (with montelukast or placebo) that was administered in randomized, double-blind, crossover fashion. One week of washout followed each treatment arm. RESULTS: Montelukast significantly reduced the levels of ENO from baseline (median, 52.5 parts per billion [ppb]; 25th to 75th percentile, 37.8 to 101.8 ppb) during the entire treatment period (ie, day 1 to day 14), with the effect measurable as early as day 1 (median, 45.9 ppb; 25th to 75th percentile, 29.3 to 92.5 ppb) and with the maximal effect being observed on day 7 (median, 35.7 ppb; 25th to 75th percentile, 27.6 to 66.6 ppb). The levels of ENO did not change significantly with placebo therapy. Montelukast improved symptom score and reduced peak expiratory flow (PEF) variability. Changes in PEF variability correlated positively with changes in ENO (r =

0.46; $p = 0.04$). No significant changes in FEV(1) or concentration of H₂O(2) in the exhaled breath condensate were observed. Levels of cys-LTs were undetectable in the exhaled breath condensate. CONCLUSIONS: We concluded that montelukast reduces the levels of ENO in patients with mild asthma, a finding that is compatible with an anti-inflammatory effect of montelukast, and that ENO appears to be more sensitive in detecting this effect than FEV(1) and H₂O(2) levels in the exhaled breath condensate.

77. Sampson, A. P., E. Pizzichini, and H. Bisgaard.

Effects of cysteinyl leukotrienes and leukotriene receptor antagonists on markers of inflammation.

J Allergy Clin Immunol 111(1 Suppl):S49-59; discussion S59-61. 2003.

The understanding that asthma pathophysiology includes an inflammatory component has spurred the more aggressive use of anti-inflammatory therapies and created a need for effective tools to measure inflammation. Biomarkers of airway inflammation proposed are obtained by methods that are direct but highly invasive (bronchial biopsy, bronchoalveolar lavage), moderately direct, and less invasive (indirect sputum, exhaled air, breath condensate) or indirect and least invasive (blood, urine). Several studies described in this review have implicated the cysteinyl leukotrienes (CysLTs) as inflammatory mediators in a wide range of diseases, implying that their biological activities reach far beyond acute bronchoconstriction, the activity traditionally ascribed to them. The validity of examining sputum for "biomarkers" has improved the understanding of asthma pathophysiology, optimization of asthma treatment and management, and investigation of the relation between CysLTs and airway inflammation in asthma. Nitric oxide is also a surrogate marker of asthma and reflects airway inflammation. The anti-inflammatory effects of the leukotriene receptor antagonists and the markers of their activity continue to grow.

78. Rosenbaum, A., and P. H. Breen.

Novel, adjustable, clinical bymixer measures mixed expired gas concentrations in anesthesia circle circuit.

Anesth Analg 97(5):1414-20. 2003.

We have introduced a novel, parallel design into a new clinical bymixer (patent pending), named for the bypass of a constant fraction of total flow through a mixing chamber. Over a wide range of tidal volumes (300-1200 mL), frequency (6-20 breaths/min), and PCO₂ (6-50 mm Hg), the bymixer provided accurate measurement of mixed expired gas fractions in the ventilation circuit compared with an expired gas collection in a metabolic lung bench setup (average slope, 1.00; average y intercept, -0.01; average coefficient of determination, $R(2) = 0.9988$). Simple changes in mixing chamber volume provided adjustable bymixer response times. The fast bymixer response (time constant, 6.4 s) should allow measurements to be updated every 20 s (where 95% response occurs by three time constants). The new clinical bymixer is constructed from standard anesthesia circuit components, attaches easily to the anesthesia machine inspired outlet and expired inlet ports, is simple to clean and sterilize, and has no reservoir to trap condensed water vapor from expired gas. The new clinical bymixer may facilitate indirect calorimetry (CO₂ elimination, VCO₂), and oxygen uptake, VCO₂) during anesthesia and the noninvasive detection of metabolic upset (e.g., onset of anaerobic metabolism) and critical events (e.g., pulmonary embolism). IMPLICATIONS: A new clinical bymixer (inline mixing chamber) provides a fast response and accurate measurements of mixed expired gas fractions in the anesthesia circle circuit. A novel parallel design facilitates adjustable response, easy cleaning, and construction from standard airway circuit components. The new clinical bymixer may facilitate widespread introduction of indirect calorimetry during anesthesia.

79. Rahman, I., and F. Kelly.

Biomarkers in breath condensate: a promising new non-invasive technique in free radical research.

Free Radic Res 37(12):1253-66. 2003.

Oxidative stress is associated with a range of inflammatory lung diseases including asthma, adult respiratory distress syndrome, idiopathic pulmonary fibrosis, pneumonia, lung transplantation, chronic obstructive pulmonary disease, cystic fibrosis, bronchiectasis and lung cancer. Increased concentrations of reactive oxygen species (ROS) in the airways of such patients are reflected by elevated concentrations of oxidative stress markers in the breath, airways, lung tissue and blood. Traditionally, the measurement of these biomarkers has involved invasive procedures to procure the samples, or examine the compartments. As a consequence, there is a need for less invasive approaches to measure oxidative stress. Analysis of breath hydrocarbons has partly fulfilled this need, however only gas phase volatile constituents can be assessed by this approach. The collection of exhaled breath condensate (EBC) is a simple, non-invasive approach, which comprehensively samples the lower respiratory tract. It is currently used as a research and diagnostic tool in the free radical field, yielding information on redox disturbance and the degree and type of inflammation in the lung. With further technical developments, such an approach may ultimately have a role in the clinic, in helping to diagnose specific lung diseases. EBC can be exploited to assess a spectrum of potential biomarkers, thus generating a "finger print" characteristic of the disease. By assessing the nature of oxidative stress in this manner, the most appropriate therapy can be

selected and the response to treatment monitored.

80. Mutti, A., M. Corradi, and I. Rubinstein.

Reporting data on exhaled breath condensate.

Am J Respir Crit Care Med 168(6):719; author reply 719. 2003.

81. Montuschi, P., E. Ragazzoni, S. Valente, G. Corbo, C. Mondino, G. Ciappi, and G. Ciabattoni.

Validation of 8-isoprostane and prostaglandin E(2) measurements in exhaled breath condensate.

Inflamm Res 52(12):502-7. 2003.

OBJECTIVE: To qualitatively validate radioimmunoassays for 8-isoprostane and prostaglandin (PG) E(2) in exhaled breath condensate. **SUBJECTS:** Twenty-two subjects with different lung diseases attended the outpatient clinic on one occasion for exhaled breath condensate collection. **METHODS:** Samples were pooled together and purified by reverse phase high performance liquid chromatography (RP-HPLC). The eluted fractions were assayed for 8-isoprostane-like immunoreactivity and PGE(2)-like immunoreactivity by radioimmunoassays. In addition, simultaneous measurements of exhaled breath condensate unextracted samples with two anti-8-isoprostane and anti-PGE(2) sera with different cross-reactivity were performed. **RESULTS:** A single peak of 8-isoprostane-like immunoreactivity and PGE(2)-like immunoreactivity co-eluting with 8-isoprostane (retention time: 13 min) and PGE(2) (retention time: 21 min) standards, respectively, was identified by radioimmunoassays. Testing with two different antisera showed similar results for both 8-isoprostane-like immunoreactivity (limits of agreement = 4.5 pg/ml and - 4.1 pg/ml, n = 12) and PGE(2)-like immunoreactivity (limits of agreement = 6.1 pg/ml and - 6.1 pg/ml, n = 12). **CONCLUSION:** This study provides evidence for the specificity of the radioimmunoassays for 8-isoprostane and PGE(2) in exhaled breath condensate. This is critical for proposing these markers as a non-invasive way for monitoring airway inflammation.

82. Montuschi, P., E. Ragazzoni, S. Valente, G. Corbo, C. Mondino, G. Ciappi, P. J. Barnes, and G. Ciabattoni.

Validation of leukotriene B4 measurements in exhaled breath condensate.

Inflamm Res 52(2):69-73. 2003.

OBJECTIVE: To qualitatively validate an enzyme immunoassay to measure leukotriene B4 in exhaled breath condensate. Exhaled breath condensate is a new non-invasive method to monitor airway inflammation. **SUBJECTS:** Twenty-two subjects with different lung diseases attended the outpatient clinic on one occasion for exhaled breath condensate collection. **METHODS:** Samples were pooled together and purified by reverse-phase high-performance liquid chromatography. The fractions eluted were assayed for leukotriene B4 by enzyme immunoassay. **RESULTS:** A single peak of leukotriene B4-like immunoreactivity co-eluting with leukotriene B4 standard (retention time: 24 min) was identified by enzyme immunoassay. Reverse phase-high performance liquid chromatography peak of leukotriene B4 was clearly separated from those of 6-trans-leukotriene B4 (retention time: 14 min) and leukotriene B5 (retention time: 18 min) for which the antiserum used in the enzyme immunoassay had the highest cross-reactivity. Leukotriene B4 recovery was 64%. **CONCLUSIONS:** This study provides evidence for the presence of leukotriene B4 in the exhaled breath condensate and the specificity of the enzyme immunoassay used.

83. Montuschi, P., S. A. Kharitonov, G. Ciabattoni, and P. J. Barnes.

Exhaled leukotrienes and prostaglandins in COPD.

Thorax 58(7):585-8. 2003.

BACKGROUND: The role of eicosanoids, including leukotrienes (LTs) and prostaglandins (PGs), in chronic obstructive pulmonary disease (COPD) is uncertain. The aim of this study was to investigate whether eicosanoids are measurable in exhaled breath condensate (EBC), a non-invasive method of collecting airway secretions, in patients with stable mild to moderate COPD, and to show possible differences in their concentrations compared with control subjects. **METHODS:** LTB(4), LTE(4), PGE(2), PGD(2)-methoxime, PGF(2 α), and thromboxane B(2) (TxB(2)) were measured in EBC in 15 healthy ex-smokers, 20 steroid naive patients with COPD who were ex-smokers, and in 25 patients with COPD who were ex-smokers and who were treated with inhaled corticosteroids. The study was of cross sectional design and all subjects were matched for age and smoking habit. **RESULTS:** LTB(4) and PGE(2) concentrations were increased in steroid naive (LTB(4): median 100.6 (range 73.5-145.0) pg/ml, p<0.001; PGE(2): 98.0 (range 57.0-128.4) pg/ml, p<0.001) and steroid treated patients with COPD (LTB(4): 99.0 (range 57.9-170.5) pg/ml, p<0.001; PGE(2): 93.6 (range 52.8-157.0) pg/ml, p<0.001) compared with control subjects (LTB(4): 38.1 (range 31.2-53.6) pg/ml; PGE(2): 44.3 (range 30.2-52.1) pg/ml). Both groups of patients had similar concentrations of exhaled LTB(4) (p=0.43) and PGE(2) (p=0.59). When measurable, LTE(4) and PGD(2)-methoxime concentrations were similar in COPD patients and controls, whereas PGF(2 α) concentrations were

increased in the former. TxB(2)-LI was undetectable in any of the subjects. CONCLUSIONS: There is a selective increase in exhaled LTB(4) and PGE(2) in patients with COPD which may be relatively resistant to inhaled corticosteroid therapy.

84. Makarevich, A. E., and D. L. Ivashkevich.

The importance of exhaled air condensate in assessing the oxidant-antioxidant system in patients with chronic obstructive pulmonary disease.

Wiad Lek 56(1-2):19-23. 2003.

The aim of this study was to investigate the relationship between selected lipid peroxidation products as markers of oxidative stress and antioxidant defense capacity in exhaled air condensate in comparison to blood and COPD severity. We detected that the increase of lipids peroxidation products in exhaled air condensate (less in blood) was accompanied by the reduction of total antioxidant capacity (antiradical activity and ceruloplasmin) according to the severity of COPD. Thus, the level of malondialdehyde significantly increased both in blood and exhaled air condensate in COPD_{1,2,3} (by 24%, 86%, 100% and 58%, 92%, 2,3 times respectively). Antiradical activity level was significantly decreased both in blood and exhaled air condensate in patients with COPD_{2,3} by 24%, 51% and 23.31% respectively. The results of this study clearly demonstrated the imbalance in oxidant-antioxidant system in advanced COPD.

85. Luczynska, M., U. Szkudlarek, B. Dziankowska-Bartkowiak, E. Waszczykowska, M. Kasielski, A. Sypa-Jedrzejowska, and D. Nowak. Elevated exhalation of hydrogen peroxide in patients with systemic sclerosis.

Eur J Clin Invest 33(3):274-9. 2003.

BACKGROUND: Systemic sclerosis is accompanied by an influx of activated phagocytes into distal airways. These cells release H₂O₂, which may evaporate from the airways surface and be detected in expired breath condensate. We tested whether patients with systemic sclerosis exhale more H₂O₂ than healthy subjects and whether breath condensate H₂O₂ levels correlate with some clinical parameters. MATERIAL AND METHODS: H₂O₂ was measured fluorimetrically in the expired breath condensate of 27 patients (22 women, five men, mean age 49 +/- 13.1 years) with systemic sclerosis and 27 age- and sex- matched healthy controls. RESULTS: Exhaled H₂O₂ levels were 3.5-fold higher (0.88 +/- 0.62 microM vs. 0.25 +/- 0.17 microM, P < 0.001) in the patients with systemic sclerosis than in the controls. Treatment with cyclophosphamide and/or prednisone (29 +/- 50 months, range 3-168 months) did not significantly decrease H₂O₂ exhalation (0.78 +/- 0.50 microM, n= 10 vs. 0.94 +/- 0.67 microM, n= 17, P > 0.05). No significant difference was found between patients with limited and diffuse scleroderma (1.03 +/- 0.69 microM, n= 17 vs. 0.63 +/- 0.41 microM, n= 10, P > 0.05). H₂O₂ levels correlated with disease duration (r = 0.38, P < 0.05) and time from the first Raynaud's episode (r = 0.44, P < 0.05). CONCLUSIONS: Patients with systemic sclerosis exhale more H₂O₂ than healthy controls, suggesting involvement of reactive oxygen species in disease processes. Lack of significant intergroups differences in H₂O₂ levels may have resulted from the small number of patients analyzed.

86. Latzin, P., J. Beck, A. Bartenstein, and M. Griese.

Comparison of exhaled breath condensate from nasal and oral collection.

Eur J Med Res 8(11):505-10. 2003.

BACKGROUND: Analysis of exhaled breath condensate may provide new insights into pulmonary inflammatory processes. A new collection method via suction of nasally expired air especially suitable for younger children was presented recently. Here we compare this nasal suction method with the more widely used oral collection method regarding the amount of condensate collected as well as the concentrations of hydrogen peroxide (H₂O₂), nitrite and nitrate, respectively. MATERIALS AND METHODS: Exhaled breath condensate was collected from 11 healthy adults for the measurements of the amount of condensate and H₂O₂ concentration and from 17 children for the measurements of nitrite and nitrate. Condensate was collected via nasal suction and oral exhalation from each subject. RESULTS: Overall, no differences between both collection methods were found for all variables assessed except the concentration of H₂O₂, whereas the latter closely correlated (Spearman r = 0.88, p = 0.0007) between both collection methods. No correlation was found for the amount of condensate collected and the concentration of nitrite and nitrate. The Bland-Altman limits of agreement scattered over a wide range with clinical impact, proving significant differences between both collection methods for all variables measured. CONCLUSIONS: Although nasal and oral collection method proved again suitable for the collection of exhaled breath condensate, the variability of the results obtained precludes the interchangeable usage of the inflammatory markers assessed here.

87. Kawamoto, H., and M. Kambe.

[Pulmonary function tests].

Rinsho Byori 51(6):568-73. 2003.

The spirometry has been most valuable pulmonary function test and it defines pulmonary physiology. But the spirometry has not been widely used by general physicians in Japan. The spirometry is effort-dependent test, so, they seem to keep it at a distance. It's desirable that pulmonary function tests are effort-independent. We introduce some effort-independent pulmonary function tests and refer to analysis of exhaled breath condensate.

88. Kawamoto, H., and M. Kambe.

[Effort-independent pulmonary function tests].

Rinsho Byori 51(8):782-9. 2003.

Many pulmonary function tests require forced expiration, and the precision of the tests depends on the effort of the subjects. In elderly people, the reproducibility of test results may be inadequate because of insufficiency of the subjects' effort, and the diagnosis of COPD, which is frequently observed in elderly people, is often difficult. To improve the accuracy of the diagnosis, pulmonary function tests that do not require effort are needed. In this study, effortless pulmonary function tests (examinations of the cardio-genic oscillation, negative expiratory pressure, exhaled temperature, and exhaled breath condensate), the application of which to various respiratory disorders (i.e., sleep apnea syndrome, COPD, bronchial asthma) is attempted, are presented.

89. Horvath, I.

Exhaled breath condensate contains more than only volatiles.

Eur Respir J 22(1):187-8; author reply 188. 2003.

90. Hitka, P., M. Vizek, and J. Wilhelm.

Hypoxia and reoxygenation increase H₂O₂ production in rats.

Exp Lung Res 29(8):585-92. 2003.

To test the effect of transition from sustained hypoxia to normoxia on production of reactive oxygen species (ROS) in lungs, the authors measured hydrogen peroxide (H₂O₂) output in the expired air of rats breathing hypoxic, normoxic, and hyperoxic gas mixtures at the end of exposure to 72 hours of hypoxia. Twenty-one male Wistar rats (200 to 280 g) were randomly assigned to 1 of 3 groups. First two groups (experimental) were kept for 3 days in normobaric hypoxic chamber (F(1)O(2) 0.1), rats of the third group (controls) breathed air. The rats were then anesthetized, intubated, placed in the plethysmograph, and their ventilation measured. Two periods of exhaled breath condensate (EBC) collection, each lasting 1 hour, were then performed to assay H₂O₂ output. The controls breathed during both samplings air, the first experimental group breathed during first sampling period hypoxic mixture (F(1)O(2) 0.1; SH-H measurement) and then, during second period, air (SH-H-A measurement), the second experimental group breathed first air (SH-A measurement) and then hyperoxic mixture (F(1)O(2) 1.0; SH-A-O(2) measurement). Concentration of H₂O₂ in the EBC was assayed by chemiluminescence. H₂O₂ production in the control group was low and similar in both measurements (20±10 and 13±5 pmol/h, mean±SEM). Exposure to 72 hours of hypoxia increased the H₂O₂ production to 105±18 pmol/h (SH-H). Transition from hypoxia to normoxia resulted in an increase in the H₂O₂ production (SH-A 421±24 pmol/h, and SH-H-A 366±19 pmol/h). Following transition from air breathing to hyperoxia did not affect the H₂O₂ production (SH-A-O(2) 373±25 pmol/h). The results showed that sustained hypoxia and transition from sustained hypoxia to normoxia increased H₂O₂ formation in the lungs.

91. Griese, M., J. Noss, and P. Schramel.

Elemental and ion composition of exhaled air condensate in cystic fibrosis.

J Cyst Fibros 2(3):136-42. 2003.

BACKGROUND: In cystic fibrosis (CF) the exact ion composition of the airway surface fluid is still debated and it is not clear if it differs from healthy subjects. The air that we exhale contains small droplets, which are generated by shear forces from the airway surface fluid and very likely mirror its ion composition. We hypothesized that differences between CF-patients and healthy controls would be reflected by differences in their exhaled air. METHODS: In nasally collected exhaled breath condensate from 20 children and young adults with cystic fibrosis and 20 healthy subjects, the elements and anions were determined by optical emission spectroscopy and ion-exchange chromatography. RESULTS: The concentrations of the major components Na and Cl⁻ did not differ, Zn was higher and NO₃⁻ was lower in CF-patients. During a given time period, CF-patients produced a slightly larger volume of breath condensate and they exhaled more Na, K and Zn. Fluoride was detected in half of all samples, whereas copper, iron, magnesium,

phosphorus and sulfur were present only sporadically, with no differences. CONCLUSIONS: These data detail the composition of exhaled breath condensate and suggest a similar Na and Cl- concentration in CF-airway surface fluid as in healthy subjects.

92. Gessner, C., S. Hammerschmidt, H. Kuhn, H. J. Seyfarth, U. Sack, L. Engelmann, J. Schauer, and H. Wirtz.

Exhaled breath condensate acidification in acute lung injury.

Respir Med 97(11):1188-94. 2003.

Lung injury in ventilated lungs may occur due to local or systemic disease and is usually caused by or accompanied by inflammatory processes. Recently, acidification of exhaled breath condensate pH (EBC-pH) has been suggested as marker of inflammation in airway disease. We investigated pH, ammonia, Lactate, pCO₂, HCO₃⁻, IL-6 and IL-8 in EBC of 35 ventilated patients (AECC-classification: ARDS: 15, ALI: 12, no lung injury: 8). EBC-pH was decreased in ventilated patients compared to volunteers (5.85 +/- 0.32 vs. 7.46 +/- 0.48; P < 0.0001). NH₄⁺, lactate, HCO₃⁻, pCO₂, IL-6 and IL-8 were analyzed in EBC and correlated with EBC-pH. We observed correlations of EBC-pH with markers of local (EBC IL-6: r = -0.71, P < 0.0001, EBC IL-8: r = -0.68, P < 0.0001) but not of systemic inflammation (serum IL-6, serum IL-8) and with indices of severity of lung injury (Murray's Lung Injury Severity Score; r = -0.73, P < 0.0001, paO₂/FiO₂; r = 0.54, P < 0.001). Among factors potentially contributing to pH of EBC, EBC-lactate and EBC-NH₄⁺ were found to correlate with EBC-pH. Inflammation-induced disturbances of regulatory mechanisms, such as glutaminase systems may result in EBC acidification. EBC-pH is suggested to represent a marker of acute lung injury caused by or accompanied by pulmonary inflammation.

93. Gessner, C., S. Hammerschmidt, H. Kuhn, T. Lange, L. Engelmann, J. Schauer, and H. Wirtz.

Exhaled breath condensate nitrite and its relation to tidal volume in acute lung injury.

Chest 124(3):1046-52. 2003.

STUDY OBJECTIVE: Mechanical ventilation may damage the lung. Low tidal volume (VT) is protective, but VT is scaled to body weight (BW) and may be high in functionally small ARDS lungs. We hypothesized that exhaled breath condensate (EBC) nitrite (NO₂(-)) concentration may increase with lung distension. DESIGN: Prospective, noncontrolled study. SETTING: University hospital and medical ICU. PATIENTS: Thirty-five ICU patients requiring mechanical ventilation (severe pneumonia, n = 31; exacerbated COPD, n = 4). Patients were scored according to American and European Consensus Conference on ARDS criteria (AECC) [no lung injury, n = 7; acute lung injury, n = 13; ARDS, n = 15], as well as the Murray lung injury severity score (LISS) [score 0, n = 3; score 0.1 to 2.5, n = 19; score > 2.5, n = 13]. INTERVENTIONS: EBC was collected and analyzed for NO₂(-), interleukin (IL)-6, and IL-8. Serum was analyzed for IL-6, IL-8, and procalcitonin. RESULTS: and measurements: EBC NO₂(-) correlated well with VT (milliliters per kilogram of BW; r = 0.79, p < 0.0001) and expiratory minute volume (r = 0.60, p < 0.0001) but not with other ventilatory parameters or parameters of pulmonary (EBC IL-6, EBC IL-8) or systemic (serum IL-6, IL-8, and procalcitonin) inflammation. The ratio of EBC NO₂(-) and the size of the VT correlated directly with lung injury (AECC, r = 0.66, p < 0.0001; LISS, r = 0.84, p < 0.0001). CONCLUSION: EBC NO₂(-) increased linearly with VT. The ratio of EBC NO₂(-) to VT is assumed to reflect NO₂(-) release at a given VT. An increase in this ratio indicates an inappropriate increase of NO₂(-) production most likely due to mechanical stress of the remaining open lung units in injured lungs. We conclude that the EBC NO₂(-)/VT ratio may help to identify situations of critical mechanical stress.

94. Gel'tser, B. I., E. E. Peteshova, E. A. Kochetkova, and E. V. Eliseeva.

[The test for nitric oxide metabolites in exhaled air condensate as a method of assessing NO-reactivity of the airways in patients with bronchial asthma].

Ter Arkh 75(10):91-4. 2003.

AIM: To estimate airways NO-reactivity in response to their stimulation with fenoterol in different clinical forms of bronchial asthma (BA). MATERIAL AND METHODS: The study included 73 patients with BA: mild disease was in 21, moderate--in 24 and severe--in 28 patients. Severe BA patients were divided into two subgroups: with a stable course (n = 15) and unstable course (n = 13). NO-producing function of the airways was estimated by concentration of stable NO-metabolites (mNO)(NO₂, NO₃) in exhaled air condensate. RESULTS: Spontaneous NO-producing activity of the airways increases and reaches maximum in severe unstable asthma. Fenoterol-stimulated NO-production was minimal in mild BA while the most significant augmentation of mNO was observed in unstable BA. Basal level of mNO and velocity parameters of external respiration function correlated. CONCLUSION: Estimation of NO-reactivity of the airways in the test with fenoterol with calculation of the index of airways NO-reactivity provides additional information about respiratory system condition in BA patients which may be used in clinical pulmonology.

95. Fozard, J. R.

The case for a role for adenosine in asthma: almost convincing?

Curr Opin Pharmacol 3(3):264-9. 2003.

Mice rendered adenosine deaminase-deficient manifest an 'asthma' phenotype in the lungs that includes mast cell degranulation, eosinophilia, mucus hypersecretion and bronchial hyperresponsiveness. These changes can be reversed by enzyme therapy with adenosine deaminase, and attenuated by theophylline. Theophylline also blocks the pro-inflammatory effects of adenosine in allergen-challenged mice. Adenosine A_{2A} receptors are an essential part of the physiological negative feedback mechanism for limitation and termination of both tissue-specific and systemic inflammatory responses. In recent clinical studies, increases in plasma adenosine have been shown to accompany exercise-induced asthma, and adenosine concentrations in exhaled breath condensate are increased in asthmatics. These new data provide support for a key role for adenosine in asthma, which has become increasingly persuasive in recent years. The evidence is now convincing, and the time has come for the asthma community to give its full support to the design and evaluation of molecules that mimic or block the biological effects of adenosine as potential novel therapeutics for this condition.

96. Effros, R. M., J. Biller, B. Foss, K. Hoagland, M. B. Dunning, D. Castillo, M. Bosbous, F. Sun, and R. Shaker.

A simple method for estimating respiratory solute dilution in exhaled breath condensates.

Am J Respir Crit Care Med 168(12):1500-5. 2003.

Exhaled breath condensates have been widely used to detect inflammatory mediators in the fluid that covers airway surfaces of patients with inflammatory lung disorders. This approach is much less invasive than bronchoalveolar lavage, but respiratory droplets are markedly diluted by large and variable amounts of water vapor. We estimated the dilution of respiratory droplets by comparing concentrations of nonvolatile, reference indicators (total nonvolatile cations, urea or conductivity) in 18 normal subjects with normal plasma concentrations by assuming similar concentrations in the respiratory fluid and plasma. The volatile cation, NH₄⁺ (most of which is delivered as NH₃ gas from the mouth), represented 93 ± 3% (SEM) of the condensate cations. More than 99% of the NH₄⁺ was removed by lyophilization, making it possible to use conductivity to estimate total nonvolatile ionic concentrations and facilitating analysis of urea. Conductivity was significantly correlated with electrolyte and urea concentrations. Estimates of dilution based on total cations, conductivity, and urea were not significantly different (cations: 20,472 ± 2,516; conductivity: 21,019 ± 2,427; and urea: 18,818 ± 2,402). These observations suggest that the conductivity of lyophilized samples can be used as an inexpensive, simple, and reliable method for estimating dilution of nonvolatile, hydrophilic mediators in condensates.

97. Csoma, Z., A. Bush, N. M. Wilson, L. Donnelly, B. Balint, P. J. Barnes, and S. A. Kharitonov.

Nitric oxide metabolites are not reduced in exhaled breath condensate of patients with primary ciliary dyskinesia.

Chest 124(2):633-8. 2003.

STUDY OBJECTIVES: To investigate whether nitric oxide (NO) metabolites would be reduced in children affected by primary ciliary dyskinesia (PCD). **DESIGN:** Single-center observational study. **PATIENTS:** Fifteen children with PCD (seven boys; mean [± SEM] age, 10.3 ± 0.7 years; mean FEV₁, 73 ± 2.1% predicted) were recruited along with 14 healthy age-matched subjects (seven boys; mean age, 11.5 ± 0.4 years; mean FEV₁, 103 ± 5% predicted). **INTERVENTIONS:** We assessed the levels of nitrite (NO₂(-)), NO₂(-)/NO₃(-) (NO₂(-)/NO₃(-)), and S-nitrosothiol in exhaled breath condensate, exhaled NO, and nasal NO from children with PCD compared to those in healthy children. **MEASUREMENTS AND RESULTS:** The mean exhaled and nasal NO levels were markedly decreased in children with PCD compared to those without PCD (3.2 ± 0.2 vs 8.5 ± 0.9 parts per billion [ppb], respectively [p < 0.0001]; 59.6 ± 12.2 vs 505.5 ± 66.8 ppb, respectively [p < 0.001]). Despite the lower levels of exhaled NO in children with PCD, no differences were found in the mean levels of NO₂(-) (2.9 ± 0.4 vs 3.5 ± 0.3 microM, respectively), NO₂(-)/NO₃(-) (35.2 ± 5.0 vs 34.3 ± 4.5 microM, respectively), or S-nitrosothiol (1.0 ± 0.2 vs 0.6 ± 0.1 microM, respectively) between children with PCD and healthy subjects. **CONCLUSION:** These findings suggest that NO synthase activity may not be decreased as much as might be expected on the basis of low exhaled and nasal NO levels.

98. Corradi, M., I. Rubinstein, R. Andreoli, P. Manini, A. Caglieri, D. Poli, R. Alinovi, and A. Mutti.

Aldehydes in exhaled breath condensate of patients with chronic obstructive pulmonary disease.

Am J Respir Crit Care Med 167(10):1380-6. 2003.

The aims of the present study were (1) to evaluate whether individual aldehydes resulting from lipid peroxidation can be measured in exhaled breath condensate, (2) to assess the influence of sampling procedures on aldehyde concentrations, and (3) to compare aldehyde levels of patients with stable, moderate to severe, chronic obstructive pulmonary disease with those of smok-

ing and nonsmoking control subjects. Aldehydes (malondialdehyde, hexanal, heptanal, and nonanal) were measured by liquid chromatography-tandem mass spectrometry in all samples and overlapping results were obtained by different sampling procedures. Malondialdehyde (57.2 +/- 2.4 nmol/L), hexanal (63.5 +/- 4.4 nmol/L), and heptanal (26.6 +/- 3.9 nmol/L) were increased in patients as compared with nonsmoking control subjects (17.7 +/- 5.5 nmol/L, $p < 0.0001$; 14.2 +/- 3.5 nmol/L, $p = 0.004$; and 18.7 +/- 0.9 nmol/L, $p = 0.002$, respectively). Only malondialdehyde was increased in patients compared with smoking control subjects (35.6 +/- 4.0 nmol/L, $p = 0.0007$). In conclusion, different classes of aldehydes were identified in exhaled breath condensate of humans. Whereas all aldehydes but nonanal were lower in control subjects as compared with other groups, only malondialdehyde distinguished smoking control subjects from patients with chronic obstructive pulmonary disease and could be envisaged as a biomarker potentially useful to monitor the disease and its response to therapy.

99. Corradi, M., A. Pesci, R. Casana, R. Alinovi, M. Goldoni, M. V. Vettori, and A. Cuomo.

Nitrate in exhaled breath condensate of patients with different airway diseases.

Nitric Oxide 8(1):26-30. 2003.

There is an increasing interest in the measurement of nitric oxide (NO) in the airways. NO is a free radical that reacts rapidly with reactive oxygen species in aqueous solution to form peroxynitrite which can then break down to nitrite (NO(2)(-)) and nitrate (NO(3)(-)). NO(3)(-) is considered a stable oxidative end product of NO metabolism. The aim of this study was to assay NO(3)(-) in exhaled breath condensate (EBC) of normal nonsmoking and smoking subjects, asthmatics, patients with obstructive pulmonary disease (COPD), and patients with community-acquired pneumonia (CAP). EBC was collected using a glass condenser and samples were assayed for NO(3)(-) by ion chromatography followed by conductivity measurement. NO(3)(-) was detectable in EBC of all subjects. NO(3)(-) was elevated in smokers [median (range)] [62.5 (9.6-158.0) microM] and in asthmatics [68.0 (25.8-194.6) microM] compared to controls [9.6 (2.6-119.4) microM; $p=0.003$ and $p=0.006$, respectively], whereas NO(3)(-) was not elevated in COPD patients [24.1 (1.9-337.0) microM]. The concentration of NO(3)(-) in patients with CAP [243.4 (26.1-584.5) microM] was higher than that in controls ($p=0.002$) and NO(3)(-) values decreased after treatment and recovery from illness [40.0 (4.1-167.0) microM, $p=0.009$]. This study shows that NO(3)(-) is detectable in EBC of healthy subjects and it varies in patients with inflammatory airway diseases.

100. Corradi, M., G. Folesani, R. Andreoli, P. Manini, A. Bodini, G. Piacentini, S. Carraro, S. Zanconato, and E. Baraldi.

Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation.

Am J Respir Crit Care Med 167(3):395-9. 2003.

Oxidative stress is implicated in the pathogenesis of asthma, and clinical studies show an imbalance in the level of oxidants to the level of antioxidants in subjects with asthma. Aldehydes and glutathione are examples of biomarkers of oxidant-induced damage and antioxidant status in asthma, respectively. In the study, we applied analytical techniques based on liquid chromatography for the assessment of aldehydes and glutathione in the exhaled breath condensate of children with asthma and in control subjects without asthma. Twelve subjects with asthma were evaluated at exacerbation and after 5 days of therapy with prednisone. At exacerbation, malondialdehyde levels were higher in patients with asthma (30.2 +/- 2.4 nM) than in control subjects (19.4 +/- 1.9 nM, $p = 0.002$) and were reduced after steroid therapy (18.5 +/- 1.6 nM, $p = 0.001$). At exacerbation, glutathione levels were lower in subjects with asthma (5.96 +/- 0.6 nM) than in control subjects (14.1 +/- 0.8 nM, $p < 0.0001$) and were increased after the therapy (8.44 +/- 1.2 nM, $p = 0.04$). Malondialdehyde and glutathione both in subjects with asthma and control subjects were negatively correlated ($r = -0.5$, $p = 0.001$). The study shows that aldehydes and glutathione are detectable in the exhaled breath condensate of children with asthma and healthy children and that their levels are modified during asthma exacerbation and after a 5-day course of therapy with oral prednisone.

101. Cheah, F. C., B. A. Darlow, and C. C. Winterbourn.

Problems associated with collecting breath condensate for the measurement of exhaled hydrogen peroxide from neonates on respiratory support.

Biol Neonate 84(4):338-41. 2003.

We developed a bedside method for collecting exhaled breath condensate (EBC) from neonates who were ventilated or receiving nasal continuous positive airway pressure (CPAP) and analyzed their EBC for hydrogen peroxide levels. A sufficient volume for analysis could be collected over 25-40 min from neonates on the ventilator and nasal CPAP (medians 5.3 and 2.7 ml, respectively). There was no significant difference between hydrogen peroxide levels from neonates on a ventilator or CPAP (median 0.28 vs. 0.38 microM, $p = 0.06$) and these were no different from a background with the ventilator or CPAP system alone (median for each 0.31 microM). The dilution of breath condensate by humidified gases plus the existence of background hydrogen peroxide resulted in this collecting setup being insufficiently sensitive to use for the detection of exhaled hydrogen peroxide in infants who were

ventilated or on nasal CPAP.

102. Carpagnano, G. E., S. A. Kharitonov, A. U. Wells, P. Pantelidis, R. M. Du Bois, and P. J. Barnes.

Increased vitronectin and endothelin-1 in the breath condensate of patients with fibrosing lung disease.

Respiration 70(2):154-60. 2003.

BACKGROUND: Non-specific interstitial pneumonia (NSIP) and fibrosing alveolitis associated with systemic sclerosis (FASSc) are diseases of unknown aetiology that are characterised by the accumulation of mononuclear cells, followed by the progressive deposition of collagen within the interstitium and subsequent destruction of lung airspace. Better understanding of mediators involved in fibrosis may be useful for early diagnosis and in clinical monitoring of disease progression. **OBJECTIVE:** The aim of this study was to investigate the presence of two profibrotic markers, the vitronectin and the endothelin-1 (ET-1) in the airways of NSIP and FASSc patients. **METHODS:** Ten NSIP (6 males, age 57 +/- 2 years) and 15 FASSc (8 males, age 55 +/- 4 years) patients were recruited along with 10 normal subjects (4 male, age 52 +/- 2 years). Vitronectin and ET-1 concentrations were measured in their breath condensate, using a specific enzyme immunoassay. **RESULTS:** Higher levels of vitronectin and ET-1 were observed in NSIP and FASSc patients [median 92.8 (91.7-93.9) microg/ml; median 8.3 (7.9-9.3) pg/ml] than in control subjects [median 80.3 (89.3-91.4) microg/ml; $p < 0.01$; median 5.3 (4.9-5.9) pg/ml, $p < 0.0001$]. We also found increased concentrations of vitronectin in patients with clinical deterioration compared to those remaining stable and in ex-smokers compared to non-smokers and, increased vitronectin and ET-1 in patients treated with steroids compared to untreated patients. **CONCLUSION:** These findings justify further studies of vitronectin and ET-1 levels in exhaled breath condensate, as a means of monitoring activity and predicting progression of pulmonary fibrosis.

103. Carpagnano, G. E., S. A. Kharitonov, O. Resta, M. P. Foschino-Barbaro, E. Gramiccioni, and P. J. Barnes.

8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy.

Chest 124(4):1386-92. 2003.

STUDY OBJECTIVES: Obstructive sleep apnea (OSA) is characterized by recurrent apnea during sleep that may compromise oxidative balance. Oxidative stress is increased in the blood and in the airways of OSA patients. **DESIGN:** The aim of this study was to investigate whether oxidative stress is determined by nocturnal apneas and could be reduced by CPAP therapy, and whether there is a relation between local and systemic oxidative stress in these patients. **PATIENTS AND METHODS:** Eighteen patients with OSA (13 men; mean [+/- SD] age, 48 +/- 3 years) and 12 healthy age-matched and weight-matched subjects (8 men; mean age, 46 +/- 7 years) were recruited. 8-Isoprostane was measured in exhaled breath condensate and blood by a specific enzyme immunoassay. **Measurements and results:** Higher concentrations of 8-isoprostane were found in the morning exhaled condensate (9.5 +/- 1.9 pg/mL) and plasma (9.7 +/- 1.5 pg/mL) of OSA patients compared to healthy obese subjects (6.7 +/- 0.2 and 7.1 +/- 0.3 pg/mL, respectively; $p < 0.0001$). Elevated mean concentrations of exhaled 8-isoprostane were observed in the OSA patients at 8:00 AM (9.5 +/- 1.9 pg/mL) but not at 8:00 PM (7.6 +/- 0.8 pg/mL; $p < 0.0005$), and a significant reduction was seen after continuous positive airway pressure (CPAP) therapy (7.7 +/- 0.9 pg/mL; before treatment, 9.6 +/- 1.7 pg/mL; $p < 0.005$). A positive correlation was found between morning exhaled 8-isoprostane levels and the apnea-hypopnea index ($r = 0.8$; $p < 0.0001$), and 8-isoprostane levels and neck circumference ($r = 0.6$; $p < 0.0001$). **CONCLUSIONS:** These findings suggest that systemic and local oxidative stress are increased in OSA patients, and that they are higher after nocturnal apnea and reduced by CPAP therapy.

104. Carpagnano, G. E., S. A. Kharitonov, M. P. Foschino-Barbaro, O. Resta, E. Gramiccioni, and P. J. Barnes.

Increased inflammatory markers in the exhaled breath condensate of cigarette smokers.

Eur Respir J 21(4):589-93. 2003.

Cigarette smoking induces an inflammatory response in the airways that may play a key role in the pathogenesis of chronic obstructive pulmonary disease. Noninvasive markers of inflammation may, therefore, be useful in monitoring the airways of smokers as well as in the screening of subjects at high risk of developing airway obstruction. The aim of the present study was to determine whether the concentrations of the pro-inflammatory cytokine, interleukin (IL)-6, is increased in the exhaled breath condensate of smokers and whether the number of cigarettes smoked has any influence on the exhaled concentrations. The possibility that exhaled IL-6 levels are related to exhaled carbon monoxide (CO) and lung function has also been explored. Another inflammatory marker, leukotriene (LT), was also measured. Twenty-one smokers (39 +/- 7 yrs, 13 male) and 14 nonsmokers (45 +/- 6 yrs, eight male) were recruited. IL-6 and LTB4 levels in the breath condensate were measured with an immunoassay kit and exhaled CO examined by means of a modified electrochemical sensor. Higher IL-6 and exhaled CO concentrations were found in current smokers (5.6 +/- 1.4 pg x mL(-1) and 16.7 +/- 5.5 parts per million (ppm)) than in nonsmokers (2.6 +/- 0.2 pg x mL(-1) and 2.1 +/- 0.6 ppm). Elevated

concentrations of LTB₄ were also observed in smokers compared to nonsmokers (9.4±0.4 pg x mL⁻¹) versus 6.1±0.3 pg x mL⁻¹). In addition, there was a correlation between IL-6 concentrations, the number of cigarettes smoked per day, exhaled CO, LTB₄ and lung function. Exhaled interleukin-6 and leukotriene B₄ levels may be useful noninvasive markers of airway inflammation in cigarette smokers.

105. Carpagnano, G. E., P. J. Barnes, D. M. Geddes, M. E. Hodson, and S. A. Kharitonov.
Increased leukotriene B₄ and interleukin-6 in exhaled breath condensate in cystic fibrosis.
Am J Respir Crit Care Med 167(8):1109-12. 2003.

Chronic neutrophilic airway inflammation is an important feature of cystic fibrosis (CF). Noninvasive inflammatory markers may be useful in monitoring CF. Leukotriene B₄ (LTB₄) and interleukin (IL)-6 are inflammatory mediators that are increased in chronic neutrophilic inflammation. The aim of this study was to assess whether LTB₄ and IL-6 were increased in exhaled breath condensate of CF patients and whether they could be used to monitor inflammation. Twenty patients with CF (13 males, age of 28 ± 9 years) were recruited together with 15 age-matched healthy subjects (8 males, age 35 ± 7 years). LTB₄ and IL-6 levels were markedly elevated in patients with acute exacerbations (28.8 ± 4.3 and 8.7 ± 0.4 pg/ml) compared with control subjects (6.8 ± 0.7 and 2.6 ± 0.1 pg/ml, $p < 0.0001$). We also observed a decrease of exhaled LTB₄ and IL-6 concentrations after antibiotic treatment in six patients who were followed until clinically stable (31.1 ± 4.4 and 9.5 ± 0.4 pg/ml vs. 18.8 ± 0.8 and 6.4 ± 0.2 pg/ml, respectively) and an increase in 15 CF patients infected with *Pseudomonas aeruginosa* (34.3 ± 5.0 and 9.3 ± 0.3 pg/ml) compared with those infected with other bacteria (18.3 ± 0.7 and 6.9 ± 0.5 pg/ml). These findings suggest that LTB₄ and IL-6 levels are increased in exhaled breath condensate of patients with CF during exacerbation and could be used to monitor airway inflammation in these patients.

106. Bucchioni, E., S. A. Kharitonov, L. Allegra, and P. J. Barnes.
High levels of interleukin-6 in the exhaled breath condensate of patients with COPD.
Respir Med 97(12):1299-302. 2003.

BACKGROUND: Chronic obstructive pulmonary disease (COPD) is characterised by chronic inflammation of the respiratory tract. METHODS: We investigated the presence of interleukin-6 (IL-6: a cytokine secreted by monocytes/macrophages, T cells, B cells, fibroblasts, bone marrow stromal cells, keratinocytes and endothelial cells) in the exhaled breath condensate of 16 exsmokers with moderate COPD, 12 healthy non-smokers. IL-6 was measured by means of a specific enzyme immunoassay. RESULTS: IL-6 levels were detectable in all of the subjects, but were higher in the COPD patients (8.0 ± 0.1 pg/ml; $P < 0.0001$) than in the healthy non-smokers (4.9 ± 0.2 pg/ml) with a correlation in this group between age and IL-6 levels ($r = 0.597$; $P < 0.05$). CONCLUSIONS: The increased IL-6 levels in exhaled breath condensate may reflect airway inflammation in patients with COPD.

107. Biernacki, W. A., S. A. Kharitonov, and P. J. Barnes.
Increased leukotriene B₄ and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD.
Thorax 58(4):294-8. 2003.

BACKGROUND: Exacerbations are an important feature of chronic obstructive pulmonary disease (COPD), accounting for a large proportion of health care costs. They are associated with increased airway inflammation and oxidative stress. METHODS: Concentrations of leukotriene B₄ (LTB₄), a marker of inflammation, and 8-isoprostane, a marker of oxidative stress, were measured in the exhaled breath condensate of 21 patients (11 M) with COPD during an exacerbation and 2 weeks after treatment with antibiotics. In 12 patients who had no further exacerbations these markers were also measured after 2 months. RESULTS: LTB₄ concentrations were raised during the COPD exacerbation (mean (SE) 15.8 (1.1) pg/ml) and fell after treatment with antibiotics to 9.9 (0.9) pg/ml ($p < 0.0001$). In 12 patients the level of LTB₄ fell further from 10.6 (1.1) pg/ml to 8.5 (0.8) pg/ml ($p < 0.005$) after 2 months. In 12 normal age matched subjects the LTB₄ levels were 7.7 (0.5) pg/ml. Concentrations of 8-isoprostane were also increased during the exacerbation (13.0 (0.9) pg/ml) and fell after antibiotic treatment to 9.0 (0.6) pg/ml ($p < 0.0001$). In 12 patients there was a further fall from 9.3 (0.7) pg/ml to 6.0 (0.7) pg/ml ($p < 0.001$) after 2 months compared with normal subjects (6.2 (0.4) pg/ml). CONCLUSIONS: Non-invasive markers of inflammation and oxidative stress are increased during an infective exacerbation of COPD and only slowly recover after treatment with antibiotics.

108. Baraldi, E., L. Ghio, V. Piovan, S. Carraro, F. Zacchello, and S. Zanconato.
Safety and success of exhaled breath condensate collection in asthma.
Arch Dis Child 88(4):358-60. 2003.

BACKGROUND: Exhaled breath condensate (EBC) is a rapidly expanding area of research to study airway inflammation through the detection of volatile and non-volatile substances in the airways. **AIMS:** To determine the safety and feasibility of EBC procedure in a group of children with asthma of varying severity. **METHODS:** In a cross sectional study of children aged 4-17 years, 18 healthy and 91 asthmatic children (69 in stable condition and 22 with asthma exacerbation) underwent the EBC procedure. Outcomes assessed included completion of the procedure, decrease in FEV₁, change in fractional exhaled nitric oxide (FE(NO)), and adverse effects. No pretreatment with beta₂ agonists was given. All children were able to successfully complete the EBC procedure. **RESULTS:** Median fall in FEV₁ after the procedure was -1% (IQR -3.5, 1.8) in asthmatics and was comparable to that observed in healthy children. In only one asthmatic child did the drop in FEV₁ exceed 12%. No significant changes in FE(NO) were observed after EBC. **CONCLUSION:** This study suggests that EBC is a simple and well tolerated method for evaluating biological samples from the lower airway. The procedure was safe in children with asthma exacerbation, and the success rate was 100% in children aged 4 years and above.

109. Baraldi, E., L. Ghio, V. Piovon, S. Carraro, G. Ciabattoni, P. J. Barnes, and P. Montuschi.

Increased exhaled 8-isoprostane in childhood asthma.

Chest 124(1):25-31. 2003.

STUDY OBJECTIVE: To quantify lung oxidative stress in asthmatic children by measuring concentrations of 8-isoprostane, a marker of oxidative stress, in exhaled breath condensate (EBC), which is a noninvasive method of sampling airway secretions. Secondary objectives were as follows: (1) to measure levels of exhaled prostaglandin (PG) E₂, since impaired PGE₂ production has been implicated in the pathogenesis of asthma in adults; and (2) to measure levels of fractional exhaled nitric oxide (FeNO), which is a marker of airway inflammation. **DESIGN:** Single-center, cross-sectional study. **PATIENTS:** Twelve healthy children, 12 steroid-naïve asthmatic children, and 30 children in stable condition with mild-to-moderate persistent asthma who were being treated with inhaled corticosteroids (ICSs) [average dose, 300 micro g per day] were studied. **INTERVENTIONS:** Subjects attended the outpatient clinic on one occasion for the collection of EBC and FeNO measurements. **Measurements and results:** 8-Isoprostane and PGE₂ concentrations in EBC were measured with specific radioimmunoassays. FeNO was measured online by a chemiluminescence analyzer. 8-Isoprostane was detectable in the EBC of healthy children (mean [± SEM], 34.2 ± 4.5 pg/mL), and its concentrations were increased in both steroid-naïve asthmatic children (mean, 56.4 ± 7.7 pg/mL; $p < 0.01$) and steroid-treated asthmatic children (mean, 47.2 ± 2.3 pg/mL; $p < 0.05$). There was no difference in exhaled 8-isoprostane concentrations between the two groups of asthmatic children ($p = 0.14$). By contrast, exhaled PGE₂ concentrations were similar among the three study groups ($p = 0.56$). FeNO levels were higher in steroid-naïve children with asthma (49.2 ± 9.6 parts per billion [ppb]; $p < 0.05$) and, to a lesser extent, in steroid-treated asthmatic children (37.8 ± 6.6 ppb; $p < 0.05$) compared with healthy children (15.2 ± 1.7 ppb). **CONCLUSIONS:** Lung oxidative stress is increased in children who are in stable condition with asthma, as reflected by increased exhaled 8-isoprostane concentrations. This increase seems to be relatively resistant to treatment with ICSs. Decreased PGE₂ lung production is unlikely to play a pathophysiologic role in childhood asthma.

110. Baraldi, E., S. Carraro, R. Alinovi, A. Pesci, L. Ghio, A. Bodini, G. Piacentini, F. Zacchello, and S. Zanconato.

Cysteinyl leukotrienes and 8-isoprostane in exhaled breath condensate of children with asthma exacerbations.

Thorax 58(6):505-9. 2003.

BACKGROUND: Cysteinyl leukotrienes (Cys-LTs) and isoprostanes are inflammatory metabolites derived from arachidonic acid whose levels are increased in the airways of asthmatic patients. Isoprostanes are relatively stable and specific for lipid peroxidation, which makes them potentially reliable biomarkers for oxidative stress. A study was undertaken to evaluate the effect of a course of oral steroids on Cys-LT and 8-isoprostane levels in exhaled breath condensate of children with an asthma exacerbation. **METHODS:** Exhaled breath condensate was collected and fractional exhaled nitric oxide (FE(NO)) and spirometric parameters were measured before and after a 5 day course of oral prednisone (1 mg/kg/day) in 15 asthmatic children with an asthma exacerbation. Cys-LT and 8-isoprostane concentrations were measured using an enzyme immunoassay. FE(NO) was measured using a chemiluminescence analyser. Exhaled breath condensate was also collected from 10 healthy children. **RESULTS:** Before prednisone treatment both Cys-LT and 8-isoprostane concentrations were higher in asthmatic subjects (Cys-LTs, 12.7 pg/ml (IQR 5.4-15.6); 8-isoprostane, 12.0 pg/ml (9.4-29.5)) than in healthy children (Cys-LTs, 4.3 pg/ml (2.0-5.7), $p = 0.002$; 8-isoprostane, 2.6 pg/ml (2.1-3.0), $p < 0.001$). After prednisone treatment there was a significant decrease in both Cys-LT (5.2 pg/ml (3.9-8.8), $p = 0.005$) and 8-isoprostane (8.4 pg/ml (5.4-11.6), $p = 0.04$) concentrations, but 8-isoprostane levels remained higher than in controls ($p < 0.001$). FE(NO) levels, which fell significantly after prednisone treatment ($p < 0.001$), did not correlate significantly with either Cys-LT or 8-isoprostane concentrations. **CONCLUSION:** After a 5 day course of oral prednisone there is a reduction in Cys-LT and 8-isoprostane levels in EBC of children with an asthma exacerbation, although 8-isoprostane levels remain higher than in controls. This finding suggests that corticosteroids may not be fully effective in reducing oxidative stress in children with an exacerbation of asthma.

111. Andreoli, R., P. Manini, M. Corradi, A. Mutti, and W. M. Niessen.

Determination of patterns of biologically relevant aldehydes in exhaled breath condensate of healthy subjects by liquid chromatography/atmospheric chemical ionization tandem mass spectrometry.

Rapid Commun Mass Spectrom 17(7):637-45. 2003.

A method for the simultaneous determination of several classes of aldehydes in exhaled breath condensate (EBC) was developed using liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (LC/APCI-MS/MS). EBC is a biological matrix obtained by a relatively new, simple and noninvasive technique and provides an indirect assessment of pulmonary status. The measurement of aldehydes in EBC represents a biomarker of the effect of oxidative stress caused by smoke, disease, or strong oxidants like ozone. Malondialdehyde (MDA), acrolein, alpha,beta-unsaturated hydroxylated aldehydes [namely 4-hydroxyhexenal (4-HHE) and 4-hydroxynonenal (4-HNE)], and saturated aldehydes (n-hexanal, n-heptanal and n-nonanal) were measured in EBC after derivatization with 2,4-dinitrophenylhydrazine (DNPH). Atmospheric pressure chemical ionization of the analytes was obtained in positive-ion mode for MDA, and in negative-ion mode for acrolein, 4-HHE, 4-HNE, and saturated aldehydes. DNPH derivatives were separated on a C18 column using variable proportions of 20 mM aqueous acetic acid and methanol. Linearity was established over 4-5 orders of magnitude and limits of detection were in the 0.3-1.0 nM range. Intra-day and inter-day precision were in the 1.3-9.9% range for all the compounds. MDA, acrolein and n-alkanals were detectable in all EBC samples, whereas the highly reactive 4-HHE and 4-HNE were found in only a few samples. Statistically significant higher concentrations of MDA, acrolein and n-hexanal were found in EBC from smokers.

112. Agarwal, A. R., J. Mih, and S. C. George.

Expression of matrix proteins in an in vitro model of airway remodeling in asthma.

Allergy Asthma Proc 24(1):35-42. 2003.

Acute asthma is characterized by a decrease in the pH of the exhaled breath condensate and bronchoconstriction. These perturbations may injure the epithelium in a chronic, intermittent pattern, leading to subepithelial fibrosis. We used an in vitro three-dimensional model of the bronchial mucosa to elucidate the response to a repeated chemical or physical insult to the epithelium in the postcontraction phase. We used enzyme-linked immunosorbent assay and reverse transcriptase--polymerase chain reaction to assess the production of the following proteins: matrix metalloproteinase (MMP) 3, MMP-9, tissue inhibitor of MMP-1, transforming growth factor beta 1, thrombospondin 1, tenascin, and fibronectin. The presence of the epithelium enhanced the degree of tissue contraction (50.1 +/- 4.4% of original area versus 75.4 +/- 2.3%). In the absence of injury, tenascin, fibronectin, MMP-3, and tissue inhibitor of MMP-1 are actively expressed. However, the chronic chemical wound markedly inhibited the expression of all proteins. We conclude that the epithelium, wound type, and age of the tissue (contracting versus postcontraction) impact the expression of key proteins in an in vitro model of subepithelial fibrosis in asthma.

113. Wilson, N.

Measurement of airway inflammation in asthma.

Curr Opin Pulm Med 8(1):25-32. 2002.

Chronic airway inflammation is considered responsible for symptoms and disorders of airway function associated with asthma. This process is the target of anti-inflammatory therapy, so a number of standardized, noninvasive techniques have been developed to assess it. More recent approaches include the measurement of exhaled gases and nonvolatile substances in breath condensate. Results from studies using a wide variety of inflammatory markers have shown group differences between patients with asthma and healthy control subjects, but evidence for the diagnostic use of these markers in individual patients is scarce. Similarly, despite many studies demonstrating some correlation between markers of airway inflammation and a measure of disease control, none has yet convincingly shown a place for the use of these markers in an individual with corticosteroid-treated asthma. However, application of these markers continues to further our understanding of the disease process and provides the potential for more appropriate, customized therapy.

114. van Beurden, W. J., G. A. Harff, P. N. Dekhuijzen, M. J. van den Bosch, J. P. Creemers, and F. W. Smeenk.

An efficient and reproducible method for measuring hydrogen peroxide in exhaled breath condensate.

Respir Med 96(3):197-203. 2002.

We investigated the sensitivity and reproducibility of a test procedure for measuring hydrogen peroxide (H₂O₂) in exhaled breath condensate and the effect of storage of the condensate on the H₂O₂ concentration, and compared the results to previous studies. Twenty stable COPD patients breathed into our collecting device twice for a period of 10 min. The total exhaled air vol-

ume (EAV) and condensate volume were measured both times and the H₂O₂ concentration of the condensate was determined fluorimetrically. The concentration was measured again after freezing the reaction product at -70 degrees C for a period of 10, 20 and 40 days. We collected 2-5 ml condensate in 10 min. The EAV and condensate volumes were strongly correlated. There was no significant difference between the mean H₂O₂ concentration of the first and second test. We obtained a detect on limit for the H₂O₂ concentration of 0.02 micromoll(-1). The H₂O₂ concentration appeared to remain stable for a period up to 40 days of freezing. Compared to previous studies we developed a more efficient breath condensate collecting device and obtained a lower H₂O₂ detection limit. The measurement of exhaled H₂O₂ was reproducible. In addition, storage of the samples up to 40 days showed no changes in H₂O₂ concentration.

115. van Beurden, W. J., P. N. Dekhuijzen, G. A. Harff, and F. W. Smeenk.

Variability of exhaled hydrogen peroxide in stable COPD patients and matched healthy controls.

Respiration 69(3):211-6. 2002.

BACKGROUND: Because inflammation induces oxidative stress, exhaled hydrogen peroxide (H₂O₂), which is a marker of oxidative stress, may be used as a non-invasive marker of airway inflammation in chronic obstructive pulmonary disease (COPD). There are no data on the circadian variability of exhaled H₂O₂ in COPD patients. **OBJECTIVE:** The aim of this study was to investigate the variability of the H₂O₂ concentration in breath condensate of stable COPD patients and of matched healthy control subjects. **METHODS:** We included 20 patients with stable mild COPD (forced expiratory volume in 1 s approximately 70% of predicted) and 20 healthy subjects, matched for age, sex and pack-years, all smokers or ex-smokers. Breath condensate was collected and its H₂O₂ concentration determined fluorometrically three times on day 0 (9 and 12 a.m., and 3 p.m.) and once on days 1, 2, 3, 8 and 21. **RESULTS:** The mean H₂O₂ concentration increased significantly during the day in both the patient and control groups (p = 0.02 and p < 0.01, respectively). Over a longer period up to 21 days, the mean concentration did not change in both groups. There was no significant difference between patients and controls. The mean coefficient of variation over 21 days was 45% in the patient group and 43% in the control group (p = 0.8). **CONCLUSIONS:** The exhaled H₂O₂ concentration increased significantly during the day in both stable COPD patients and controls. Over a period of 3 weeks, the mean H₂O₂ concentration did not change and the variability within the subjects was similar in both groups.

116. Tate, S., G. MacGregor, M. Davis, J. A. Innes, and A. P. Greening.

Airways in cystic fibrosis are acidified: detection by exhaled breath condensate.

Thorax 57(11):926-9. 2002.

BACKGROUND: The loss of cystic fibrosis transmembrane conductance regulator (CFTR) mediated chloride conductance does not fully explain the diverse pathologies evident in patients with cystic fibrosis (CF). Bicarbonate (HCO₃⁻) secretion is also impaired in CFTR expressing tissues and CFTR is thought to regulate HCO₃⁻ secretion at the apical membrane of epithelial cells. We hypothesised that the epithelial lining fluid (ELF) of patients with CF would be acidified and that this may be worsened during an infective exacerbation due to the increased inflammatory burden. **METHODS:** pH and nitrite levels in exhaled breath condensate (EBC) from 12 healthy non-smoking controls and 30 patients with CF (11 of whom were in an infective exacerbation) were measured. A further nine patients were studied before and after intravenous antibiotic treatment for an exacerbation of CF. **RESULTS:** The pH of EBC was significantly lower in patients with stable CF than in controls (5.88 (0.32) v 6.15 (0.16), p=0.017), and was further reduced in CF patients with an exacerbation (5.32 (0.38), p=0.001) compared with stable CF patients. EBC pH increased significantly following antibiotic treatment from 5.27 (0.42) to 5.71 (0.42), p=0.049. Nitrite levels in EBC were increased in CF patients with an exacerbation compared with control subjects (4.4 (4.0) micro m v 1.6 (1.6) micro m p=0.047). No correlation was found between EBC pH and nitrite levels. **CONCLUSIONS:** These findings support the hypothesis that airway acidification occurs in CF. This acidity is in part a function of inflammation as the pH of the EBC of patients increased significantly with treatment of an exacerbation, although not to control levels. Acidic pH of the ELF may play a role in the pathophysiology of CF lung disease and requires further investigation.

117. Shahid, S. K., S. A. Kharitonov, N. M. Wilson, A. Bush, and P. J. Barnes.

Increased interleukin-4 and decreased interferon-gamma in exhaled breath condensate of children with asthma.

Am J Respir Crit Care Med 165(9):1290-3. 2002.

Exhaled breath condensate analysis for noninvasive quantification of airway inflammation in asthma is a potentially useful research tool in children. There is an imbalance between T-helper (Th)-2 cells, which secrete interleukin (IL)-4, and Th1 cells, which secrete interferon (IFN)-gamma, in asthma. We measured concentrations of IL-4 and IFN-gamma in breath condensates of 37 children (11 normal, 12 steroid-naive, and 14 steroid-treated children with asthma). Exhaled IFN-gamma was significantly lower in

steroid-naïve and steroid-treated children with asthma compared with normal control subjects (3.7 +/- 0.2 versus 5.1 +/- 0.4 pg/ml, $p < 0.01$ and 4.1 versus 5.1 pg/ml, $p < 0.05$). By contrast, mean exhaled IL-4 was elevated in asthma (53.7 +/- 4.2 pg/ml) compared with normal children (35.7 +/- 6.2 pg/ml, $p < 0.05$) and concentrations were lower with steroid treatment (37.5 +/- 5.6 pg/ml, $p < 0.05$). Exhaled IL-4 was significantly lower in children with asthma on more than 600 microg inhaled steroid/day. The IL-4/IFN-gamma ratio was significantly greater in children with asthma compared with control children and the children with asthma on inhaled steroid therapy. We have shown for the first time that IFN-gamma and IL-4 can be assayed in exhaled breath condensate and shows an increased ratio of IL-4/IFN-gamma, consistent with predominance of Th2 cells in airways of children with asthma. Exhaled breath condensate analysis may have a useful role in studying allergic inflammation in childhood asthma.

118. Paredi, P., S. A. Kharitonov, and P. J. Barnes.

Analysis of expired air for oxidation products.

Am J Respir Crit Care Med 166(12 Pt 2):S31-7. 2002.

Chronic inflammation is a critical feature of chronic obstructive pulmonary disease, cystic fibrosis, and asthma. This inflammation is associated with the increased production of reactive oxygen species or oxidative stress in the lungs. Oxidative stress may have several adverse effects and may amplify the inflammatory process; however, monitoring oxidative stress is difficult and may not be reflected by changes in blood markers. We have therefore developed several noninvasive markers in the exhaled breath that may indicate oxidative stress in the lungs, and we studied these in relationship to the severity of chronic inflammatory lung diseases. We analyzed the exhaled breath for the content of nitric oxide as a marker of inflammation, carbon monoxide as a marker of oxidative stress, and ethane, which is one of the end products of lipid peroxidation. In addition, we measured the concentration of markers of oxidative stress such as isoprostanes in exhaled breath condensate. Our results confirm that there are increased inflammation, oxidative stress, and lipid peroxidation in lung disease, as shown by elevated levels of nitric oxide, carbon monoxide, and ethane, respectively. The finding of lower levels of these gases in patients on steroid treatment and of higher levels in those with more severe lung disease, as assessed by lung function tests and clinical symptoms, reinforces the hypothesis that the noninvasive measurement of exhaled gases maybe useful in monitoring the underlying pathologic pathways of lung disease. Longitudinal studies are required to assess the clinical usefulness of these measurements in the monitoring of chronic inflammatory lung disease.

119. Neubauer, B., N. Struck, T. S. Mutzbauer, U. Schotte, N. Langfeldt, and K. Tetzlaff.

Leukotriene-B4 concentrations in exhaled breath condensate and lung function after thirty minutes of breathing technically dried compressed air.

Int Marit Health 53(1-4):93-101. 2002.

In previous studies it had been shown that leukotriene-B4 [LTB4] concentrations in the exhaled breath mirror the inflammatory activity of the airways if the respiratory tract has been exposed to occupational hazards. In diving the respiratory tract is exposed to cold and dry air and the nasopharynx, as the site of breathing-gas warming and humidification, is bypassed. The aim of the present study was to obtain LTB4-concentrations in the exhaled breath and spirometric data of 17 healthy subjects before and after thirty minutes of technically dried air breathing at normobar ambient pressure. The exhaled breath was collected non-invasively, via a permanently cooled expiration tube. The condensate was measured by a standard enzyme immunoassay for LTB4. Lung function values (FVC, FEV1, MEF 25, MEF 50) were simultaneously obtained by spirometry. The measured pre- and post-exposure LTB4-concentrations as well as the lung function values were in the normal range. The present data gave no evidence for any inflammatory activity in the subjects' airways after thirty minutes breathing technically dried air.

120. Nayeri, F., E. Millinger, I. Nilsson, Zetterstrom, L. Brudin, and P. Forsberg.

Exhaled breath condensate and serum levels of hepatocyte growth factor in pneumonia.

Respir Med 96(2):115-9. 2002.

Hepatocyte growth factor (HGF) is a protein produced by mesenchymal cells in many organs, which can stimulate epithelial growth. An enhanced production and concentration of HGF is observed after injuries. The lung is one of the major sources of HGF. By cooling exhaled air, a condensate is formed containing molecules from bronchi and alveoli. In order to investigate HGF-concentration and time course in pneumonia, paired serum and exhaled breath condensate was collected from 10 patients with pneumonia, 10 patients with non-respiratory infections and 11 healthy controls. The concentration of HGF was measured by an immunoassay kit. In the acute phase HGF-levels in breath condensate and serum were significantly higher in the patients with pneumonia compared to the control groups. Similar concentrations in breath condensate were seen in healthy controls and in patients with non-respiratory infections. In the patients with pneumonia a decrease in serum HGF was seen already after 4-7 days while HGF val-

ues in breath condensate remained elevated even after 4-6 weeks. These results might imply local production of HGF in the lungs and a long repair and healing process after pneumonia.

121. Montuschi, P., J. A. Nightingale, S. A. Kharitonov, and P. J. Barnes.

Ozone-induced increase in exhaled 8-isoprostane in healthy subjects is resistant to inhaled budesonide.

Free Radic Biol Med 33(10):1403-8. 2002.

The aim of this study was to quantify lung oxidant stress after short-term ozone exposure as reflected by 8-isoprostane concentrations in exhaled breath condensate (EBC) and to investigate the effects of inhaled budesonide on this response. 8-Isoprostane is a prostaglandin-F(2 alpha) isomer that is formed *in vivo* by free radical-catalyzed peroxidation of arachidonic acid. EBC is a noninvasive method to collect airway secretions. We undertook a double-blind, randomized, placebo-controlled, crossover study with inhaled budesonide (800 microg) or placebo twice daily for 2 weeks prior to ozone exposure (400 parts per billion) for 2 h in nine healthy nonsmokers. Exhaled 8-isoprostane was measured by an enzyme immunoassay. 8-Isoprostane was increased 4 h after ozone exposure compared to pre-exposure values in both placebo (36.9 +/- 3.9 pg/ml, mean +/- SEM, vs. 16.9 +/- 0.7 pg/ml; $p < .001$) and budesonide groups (33.4 +/- 2.6 pg/ml vs. 15.8 +/- 0.3 pg/ml; $p < .001$). Pretreatment with budesonide did not affect the increases in 8-isoprostane (mean differences 3.4 pg/ml, 95% CI -8.9 to 15.7, $p = .54$). Short-term ozone exposure causes acute increase in lung oxidative stress as reflected by exhaled 8-isoprostane. This increase is resistant to pretreatment with a high dose of inhaled budesonide.

122. Montuschi, P., and P. J. Barnes.

Exhaled leukotrienes and prostaglandins in asthma.

J Allergy Clin Immunol 109(4):615-20. 2002.

BACKGROUND: Most of the studies investigating the role of leukotrienes (LTs) and prostaglandins (PGs) in asthma have used invasive (eg, bronchoalveolar lavage fluid) or semi-invasive (eg, sputum induction) techniques. Others have measured eicosanoids in plasma or urine, probably reflecting systemic rather than lung inflammation. Collection of exhaled breath condensate (EBC) is a noninvasive method to collect airway secretions. **OBJECTIVE:** We sought to investigate whether eicosanoids are measurable in EBC, to show possible differences in their concentrations in asthmatic patients and healthy subjects, and to investigate whether exhaled eicosanoids correlate with exhaled nitric oxide (NO), a marker of airway inflammation. **METHODS:** Twelve healthy nonsmokers and 15 steroid-naive patients with mild asthma were studied. Subjects attended on one occasion for pulmonary function tests, collection of EBC, and exhaled NO measurements. Exhaled LTB(4)-like immunoreactivity, LTE(4)-like immunoreactivity, PGE(2)-like immunoreactivity, PGD(2)-methoxime, PGF(2)(alpha)-like immunoreactivity, and thromboxane B(2)-like immunoreactivity were measured by means of enzyme immunoassays. **RESULTS:** LTE(4)-like immunoreactivity and LTB(4)-like immunoreactivity were detectable in EBC in healthy subjects, and their levels in asthmatic patients were increased about 3-fold ($P < .0001$) and 2-fold ($P < .0005$), respectively. Exhaled NO was increased in asthmatic patients compared with healthy subjects ($P < .0001$). There was a correlation between exhaled LTB(4) and exhaled NO ($r = 0.56$, $P < .04$) in patients with asthma. When measurable, prostanoid levels were similar in asthmatic patients and control subjects. **CONCLUSIONS:** Exhaled LTE(4) and LTB(4) are increased in steroid-naive patients with mild asthma. EBC may be proved to be a novel method to monitor airway inflammation in asthma.

123. Montuschi, P., and P. J. Barnes.

Analysis of exhaled breath condensate for monitoring airway inflammation.

Trends Pharmacol Sci 23(5):232-7. 2002.

Several inflammatory mediators have been identified in the exhaled breath condensate (EBC) that is formed by breathing through a cooling system. Analysis of EBC is a noninvasive method that allows repeat measurements of lung inflammation and is potentially useful for monitoring drug therapy. Characterization of the profiles of exhaled markers could help to discriminate between different inflammatory lung diseases; thus, EBC might be a novel, noninvasive approach to monitoring lung diseases. However, several methodological issues, such as standardization of sample collection and validation of analytical techniques, need to be addressed before this method can be applied clinically. Controlled studies are needed to establish the utility of EBC markers for guiding pharmacological treatment in inflammatory lung diseases.

124. Montuschi, P.

Indirect monitoring of lung inflammation.

Nat Rev Drug Discov 1(3):238-42. 2002.

The assessment of airway inflammation by non-invasive methods could provide a signal to start anti-inflammatory treatment before the onset of symptoms and the impairment of lung function. It could also be useful in the follow-up of patients with lung disease, and for guiding drug treatment. Measuring inflammatory markers in exhaled breath condensate is potentially the easiest way to quantify lung inflammation. The clinical applicability of this method could facilitate the practice of respiratory medicine.

125. Latzin, P., and M. Griese.

Exhaled hydrogen peroxide, nitrite and nitric oxide in healthy children: decrease of hydrogen peroxide by atmospheric nitric oxide. *Eur J Med Res* 7(8):353-8. 2002.

Hydrogen peroxide (H₂O₂) and nitrite (NO₂⁻) in exhaled breath condensate have recently been suggested as non-invasive markers of airway inflammation. The goal of this study was to clarify the role of factors that may potentially influence the measurement of H₂O₂ and nitrite and to look for possible correlations among these inflammatory markers. H₂O₂ and nitrite values were assessed fluorometrically in breath condensate of 102 healthy children (age 4-18 years) and a detailed status of atopy (including history, lung function and skin prick test) was taken in all children. To find out the role of atmospheric nitric oxide, eNO and envNO were measured via chemiluminescence in association with the sampling of the breath condensate. Median (interquartile range) H₂O₂ was 0.51 (0.26 - 0.74) microM and nitrite was 3.3 (2.7 4.1) microM. A significant negative correlation between H₂O₂ and envNO was observed ($r = -0.50$; $p < 0.0001$). eNO was independent of envNO at our envNO range up to 56 ppb. No further correlation was found. The inflammatory markers in exhaled breath condensate H₂O₂, nitrite and eNO are not interrelated to each other in healthy children. Whereas eNO was not dependent on envNO values, high envNO values must be taken into account when measuring H₂O₂ in exhaled breath condensate.

126. Kharitonov, S. A., L. E. Donnelly, P. Montuschi, M. Corradi, J. V. Collins, and P. J. Barnes.

Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. *Thorax* 57(10):889-96. 2002.

BACKGROUND: Dose dependent anti-inflammatory effects of inhaled corticosteroids in asthma are difficult to demonstrate in clinical practice. The anti-inflammatory effect of low dose inhaled budesonide on non-invasive exhaled markers of inflammation and oxidative stress were assessed in patients with mild asthma. **METHODS:** 28 patients entered a double blind, placebo controlled, parallel group study and were randomly given either 100 or 400 micro g budesonide or placebo once daily, inhaled from a dry powder inhaler (Turbohaler), for 3 weeks followed by 1 week without treatment. Exhaled nitric oxide (NO), exhaled carbon monoxide (CO), nitrite/nitrate, S-nitrosothiols, and 8-isoprostanes in exhaled breath condensate were measured four times during weeks 1 and 4, and once a week during weeks 2 and 3. **RESULTS:** A dose-dependent speed of onset and cessation of action of budesonide was seen on exhaled NO and asthma symptoms. Treatment with 400 micro g/day reduced exhaled NO faster (-2.06 (0.37) ppb/day) than 100 micro g/day (-0.51 (0.35) ppb/day; $p < 0.01$). The mean difference between the effect of 100 and 400 micro g budesonide was -1.55 ppb/day (95% CI -2.50 to -0.60). Pretreatment NO levels were positively related to the subsequent speed of reduction during the first 3-5 days of treatment. Faster recovery of exhaled NO was seen after stopping treatment with budesonide 400 micro g/day (1.89 (1.43) ppb/day) than 100 micro g/day (0.49 (0.34) ppb/day, $p < 0.01$). The mean difference between the effect of 100 and 400 micro g budesonide was 1.40 ppb/day (95% CI -0.49 to 2.31). Symptom improvement was dose-dependent, although symptoms returned faster in patients treated with 400 micro g/day. A significant reduction in exhaled nitrite/nitrate and S-nitrosothiols after budesonide treatment was not dose-dependent. There were no significant changes in exhaled CO or 8-isoprostanes in breath condensate. **CONCLUSION:** Measurement of exhaled NO levels can indicate a dose-dependent onset and cessation of anti-inflammatory action of inhaled corticosteroids in patients with mild asthma.

127. Kharitonov, S. A., and P. J. Barnes.

Biomarkers of some pulmonary diseases in exhaled breath.

Biomarkers 7(1):1-32. 2002.

Analysis of various biomarkers in exhaled breath allows completely non-invasive monitoring of inflammation and oxidative stress in the respiratory tract in inflammatory lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), bronchiectasis and interstitial lung diseases. The technique is simple to perform, may be repeated frequently, and can be applied to children, including neonates, and patients with severe disease in whom more invasive procedures are not possible. Several volatile chemicals can be measured in the breath (nitric oxide, carbon monoxide, ammonia), and many non-volatile molecules (mediators, oxidation and nitration products, proteins) may be measured in exhaled breath condensate. Exhaled breath analysis may be used to quantify inflammation and oxidative stress in the respiratory tract, in differential diagnosis of airway disease and in the monitoring of therapy. Most progress has been made with exhaled nitric oxide (NO), which is increased in atopic

asthma, is correlated with other inflammatory indices and is reduced by treatment with corticosteroids and antileukotrienes, but not (beta 2-agonists). In contrast, exhaled NO is normal in COPD, reduced in CF and diagnostically low in primary ciliary dyskinesia. Exhaled carbon monoxide (CO) is increased in asthma, COPD and CF. Increased concentrations of 8-isoprostane, hydrogen peroxide, nitrite and 3-nitrotyrosine are found in exhaled breath condensate in inflammatory lung diseases. Furthermore, increased levels of lipid mediators are found in these diseases, with a differential pattern depending on the nature of the disease process. In the future it is likely that smaller and more sensitive analyzers will extend the discriminatory value of exhaled breath analysis and that these techniques may be available to diagnose and monitor respiratory diseases in the general practice and home setting.

128. Huszar, E., G. Vass, E. Vizi, Z. Csoma, E. Barat, G. Molnar Vilagos, I. Herjavec, and I. Horvath. Adenosine in exhaled breath condensate in healthy volunteers and in patients with asthma. *Eur Respir J* 20(6):1393-8. 2002.

Persistent airway inflammation may require the use of different markers for monitoring airway inflammation. In this study, the authors investigated whether adenosine, which may be produced in allergic inflammatory conditions, could be measured with good reproducibility in exhaled breath condensate (EBC), and whether its concentration was elevated in patients with asthma. EBC adenosine and exhaled nitric oxide (eNO), a noninvasive marker of asthmatic airway inflammation, were measured in 40 healthy volunteers and 43 patients with allergic bronchial asthma. Repeatability of adenosine measurement was checked in 20 pairs of samples collected from healthy control subjects. Adenosine was detectable in all EBC samples by the applied high-performance liquid chromatographic method. The mean difference between repeated measurements of adenosine was -0.1 nM and all differences were within the coefficient of repeatability. Adenosine concentration was higher in steroid-naive patients (n=23) compared with healthy control subjects and steroid-treated patients (n=20). In patients with worsening symptoms of asthma (n=23), adenosine concentration was elevated compared with those in a stable condition (n=20). Furthermore, adenosine concentrations were related to eNO levels in asthmatic patients. These results, showing good reproducibility of adenosine measurements and increased adenosine concentrations in steroid-naive patients and in patients with worsening of asthmatic symptoms, indicate that adenosine measurement in exhaled breath condensate might be an acceptable novel method to investigate the role of local production of adenosine in the airways.

129. Hunt, J. F., E. Erwin, L. Palmer, J. Vaughan, N. Malhotra, T. A. Platts-Mills, and B. Gaston. Expression and activity of pH-regulatory glutaminase in the human airway epithelium. *Am J Respir Crit Care Med* 165(1):101-7. 2002.

Fluid condensed from the breath of patients with acute asthma is acidic. Several features of asthma pathophysiology can be initiated by exposure of the airway to acid. In renal tubular epithelium, glutaminase produces ammonia to buffer urinary acid excretion. We hypothesized that human airway epithelium could also express glutaminase. Here, we demonstrate that human airway epithelial cells in vitro have biochemical evidence for glutaminase activity and express mRNA for two glutaminase isoforms (KGA and GAC). Glutaminase activity increased in response to acidic stress (media pH 5.8) and was associated with both increased culture medium pH and improved cell survival. In contrast, activity was inhibited by interferon-gamma and tumor necrosis factor-alpha. Glutaminase protein was expressed in the human airway in vivo. Further, ammonia levels in the breath condensate of subjects with acute asthma were low (30 microM [range: 0-233], n = 18, age 23 +/- 2.5 yr) compared with control subjects (327 microM [14-1,220], n = 24, age 24 +/- 2.4 yr, p < 0.001), and correlated with condensate pH (r = 0.58, p < 0.001). These data demonstrate that glutaminase is expressed and active in the human airway epithelium and may be relevant both to the regulation of airway pH and to the pathophysiology of acute asthmatic airway inflammation.

130. Hunt, J.

Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease.

J Allergy Clin Immunol 110(1):28-34. 2002.

Exhaled breath condensate (EBC) contains aerosolized airway lining fluid and volatile compounds that provide noninvasive indications of ongoing biochemical and inflammatory activities in the lung. Rapid increase in interest in EBC has resulted from the recognition that in lung disease this easily sampled fluid has measurable characteristics that differ prominently from health. These assays have provided evidence of airway and lung redox deviation, acid-base status, and degree and type of inflammation in acute and chronic asthma, chronic obstructive pulmonary disease, adult respiratory distress syndrome, occupational diseases, and cystic fibrosis. Characterized by uncertain and variable degrees of dilution, EBC does not provide precise assessment of individual solute concentrations within native airway lining fluid. However, it can provide useful information when concentrations differ substantially between health and disease or are based on ratios of solutes found in the sample. Because they can be used to measure the

targets of modern therapy, EBC assays are likely to become integral components of future clinical studies, and after further technical work is accomplished, they might be used to diagnose and monitor therapy in individual patients.

131. Griese, M., J. Noss, and C. von Bredow.

Protein pattern of exhaled breath condensate and saliva.

Proteomics 2(6):690-6. 2002.

The proteins recovered in exhaled breath condensate (EBC) might be used to non-invasively monitor respiratory diseases. However, the range of proteins and their source are still unresolved and contamination by saliva or a similar protein pattern in the nasal and bronchial compartments may make interpretation of the data difficult. We studied nasal EBC (collected through a "free of touch" technique by negative pressure), oral tidal, and oral forced EBC (collected through a rebreathing valve as a saliva trap connected to tubing submerged into ice) and matched saliva samples from five healthy adult subjects. The protein samples were separated by two-dimensional electrophoresis and the silver stained gels were analyzed by Melanie 2 software. In both nasal and oral EBC, three spots (72 kDa/isoelectric point (pI) 6.6-7.0, 66 kDa/pI 5.9-6.7 and 45-48 kDa/pI 8.0-8.6) were consistently present in all subjects. Several other proteins were only sporadically detected. Despite improbable saliva contamination (no phosphorus contamination in the same oral and nasal EBC, no amylase activity in 10 pairs of nasal and oral EBC collected by the same technique), on average 63% and 71% of the spots identified in oral and nasal EBC were also found in the matched individual saliva samples. Compared to saliva, the range and amount of protein in all types of EBC was very small. Even when collected free of saliva contamination the majority of proteins present in EBC was also found in saliva, suggesting that these proteins are present in both compartments, e.g. saliva and secretions of the lower airspaces. The quantification and identification of specific proteins in the various compartments is warranted in future studies to determine the practical value of EBC.

132. Gordon, S. M., L. A. Wallace, M. C. Brinkman, P. J. Callahan, and D. V. Kenny.

Volatile organic compounds as breath biomarkers for active and passive smoking.

Environ Health Perspect 110(7):689-98. 2002.

We used real-time breath measurement technology to investigate the suitability of some volatile organic compounds (VOCs) as breath biomarkers for active and passive smoking and to measure actual exposures and resulting breath concentrations for persons exposed to tobacco smoke. Experiments were conducted with five smoker/nonsmoker pairs. The target VOCs included benzene, 1,3-butadiene, and the cigarette smoke biomarker 2,5-dimethylfuran. This study includes what we believe to be the first measurements of 1,3-butadiene in smokers' and nonsmokers' breath. The 1,3-butadiene and 2,5-dimethylfuran peak levels in the smokers' breath were similar (360 and 376 microg/m³, respectively); the average benzene peak level was 522 microg/m³. We found higher peak values of the target chemicals and shorter residence times in the body than previously reported, probably because of the improved time resolution made possible by the continuous breath measurement method. The real-time breath analyzer also showed the presence of the chemicals after exposure in the breath of the nonsmokers, but at greatly reduced levels. Single breath samples collected in evacuated canisters and analyzed independently with gas chromatography/mass spectrometry confirmed the presence of the target compounds in the postexposure breath of the nonsmokers but indicated that there was some contamination of the breath analyzer measurements. This was likely caused by desorption of organics from condensed tar in the analyzer tubing and on the quartz fiber filter used to remove particles. We used the decay data from the smokers to estimate residence times for the target chemicals. A two-compartment exponential model generally gave a better fit to the experimental decay data from the smokers than a single-compartment model. Residence times for benzene, 1,3-butadiene, and 2,5-dimethylfuran ranged from 0.5 (1,3-butadiene) to 0.9 min (benzene) for tau₁ and were essentially constant (14 min) for tau₂. These findings will be useful in models of environmental tobacco smoke exposure and risk.

133. Formanek, W., D. Inci, R. P. Lauener, J. H. Wildhaber, U. Frey, and G. L. Hall.

Elevated nitrite in breath condensates of children with respiratory disease.

Eur Respir J 19(3):487-91. 2002.

The aim of the study was to determine the differences in nitrite, in the exhaled breath condensates of healthy children and those children with asthma, cystic fibrosis (CF) and nonasthmatic, episodic cough. Breath condensates were obtained from 66 children (43 males:23 females, 3.1-16 yrs) and included 29 asthmatics, 12 clinically stable CF patients, 12 children with cough but not asthma and 13 healthy volunteers. The collected condensate was assayed colourimetrically using the Griess reaction to determine nitrite concentrations. Patients with CF (median: 5-95% percentiles; 2.02: 0.43-6.37 microM) or asthma (2.10: 0.63-5.45 microM) had significantly higher levels of nitrite compared to healthy subjects (0.41: 0.13-1.83 microM; p<0.05) or subjects with cough (0.75: 0.03-1.75 microM; p<0.05). Airway inflammation, as assessed by the nitrite in breath condensates, is present in children with

asthma and cystic fibrosis, but not those children with nonasthmatic, episodic cough. Nitrite can be conveniently, cheaply and rapidly measured in breath condensates of children as young as 3 yrs of age, and may prove useful for the assessment of airway inflammation in children with respiratory disease.

134. Emelyanov, A., G. Fedoseev, O. Krasnoschekova, A. Abulimity, T. Trendeleva, and P.J. Barnes.
Treatment of asthma with lipid extract of New Zealand green-lipped mussel: a randomised clinical trial.
Eur Respir J 20(3):596-600. 2002.

Asthma is a chronic inflammatory disease of the airways mediated, at least in part, by leukotrienes and other lipid mediators. Experimental studies have shown that lipid extract of New Zealand green-lipped mussel, *Perna canaliculus*, is effective in inhibiting 5'-lipoxygenase and cyclo-oxygenase pathways responsible for production of eicosanoids, including leukotrienes and prostaglandins. The aim of this study was to assess its effect on symptoms, peak expiratory flow (PEF) and hydrogen peroxide (H₂O₂) in expired breath condensate as a marker of airway inflammation in patients with steroid-naïve atopic asthma in a double-blind randomised, placebo-controlled clinical trial. Forty six patients with atopic asthma received two capsules of lipid extract (Lyprinol) or placebo b.i.d for 8 weeks. Each capsule of lipid extract contained 50 mg omega-3 polyunsaturated fatty acids and 100 mg olive oil, whereas placebo capsules contained only 150 mg olive oil. There was a significant decrease in daytime wheeze, the concentration of exhaled H₂O₂ and an increase in morning PEF in the lipid extract group compared to the placebo group. There were no significant side-effects. The authors conclude that lipid extract of New Zealand green-lipped mussel may have some beneficial effect in patients with atopic asthma.

135. Effros, R. M., K. W. Hoagland, M. Bosbous, D. Castillo, B. Foss, M. Dunning, M. Gare, W. Lin, and F. Sun.
Dilution of respiratory solutes in exhaled condensates.
Am J Respir Crit Care Med 165(5):663-9. 2002.

Most exhaled water is produced as gaseous water vapor, which can be collected in cooled condensers. The presence of nonvolatile solutes in these condensates suggests that droplets of respiratory fluid (RF) have also been collected. However, calculation of RF solute concentrations from condensates requires estimation of the dilution of RF droplets by water vapor. We used condensate electrolyte concentrations to calculate the dilution of RF droplets in condensates from 20 normal subjects. The total ionic concentration (conductivity) was 497 plus minus 68 (mean plus minus SEM) μM . Of this, 229 plus minus 43 μM was NH₄(+), but little NH₄(+) was collected from subjects with tracheostomies, indicating oral formation. The Na⁺ concentration in condensate ([Na⁺](cond)) averaged 242 plus minus 43 μM . Large variations in [Na⁺](cond) correlated well with variations of K⁺ in condensate ([K⁺](cond)) and Cl⁻ in condensate ([Cl⁻](cond)), and were attributed to differences in respiratory droplet dilution. Dividing condensate values of ([Na⁺] + [K⁺]) by those of plasma indicated that RF represented between 0.01% and 2.00% of condensate volumes. Calculated values for Na⁺, K⁺, Cl⁻, lactate, and protein in RF were [Na⁺](RF) = 91 +/- 8 mM, [K⁺](RF) = 60 +/- 11 mM, [Cl⁻](RF) = 102 +/- 17 mM, [lactate](RF) = 44 +/- 17 mM, and [protein](RF) = 7.63 +/- 1.82 g/dl, respectively.

136. Disse, B.
Clinical evaluation of new therapies for treatment of mucus hypersecretion in respiratory diseases.
Novartis Found Symp 248:254-72; discussion 272-6, 277-82. 2002.

In the past mucoactive drugs in airway diseases have been identified and profiled in symptom-based animal experiments and in clinical trials along related lines (cough and expectoration). Presently available drugs of this class are not generally accepted by licensing authorities worldwide and no new molecule clinically profiled as a mucoactive drug has been brought to regulatory approval in the past 20 years. Among regulatory guidelines only the CPMP 1999 'points to consider' on drug development in chronic obstructive pulmonary disease (COPD) advises for mucoactive drug development by suggesting that an indication for symptomatic treatment may be established on the basis of a symptom-related primary endpoint that should be justified as for its importance and supported by a co-primary lung function endpoint. Quality and safety of the new drug must be documented in long-term studies and the indication and use clearly described based on established or adequately profiled new primary endpoints in two pivotal studies. Published trials on mucoactive drugs have used a variety of endpoints. These include mucus hypersecretion-related symptoms by questionnaire, expectorated volume and dry weight, and mucus viscosity, elasticity and transportability. Most methods and endpoints are not validated and a positive standard of treatment is not established. New surrogate markers of efficacy for shorter term trials, e.g. induced or spontaneous sputum based assays (cellularity, mucus antigens), exhaled breath (NO), breath condensate (eicosanoids) or airway biopsy are only partially validated and the risk of false positive or negative phase II results is appreciably high. On the other hand, lung function measurements including airway hyper-reactivity assessment and typical phase III (long-term) endpoints like dyspnoea ratings, health status assessments, incidence of exacerbations and lung

function decline over time are validated endpoints and offer a high likelihood of regulatory acceptance. Proof for no depression of lung mucociliary clearance is an important safety endpoint.

137. Csoma, Z., S. A. Kharitonov, B. Balint, A. Bush, N. M. Wilson, and P. J. Barnes.

Increased leukotrienes in exhaled breath condensate in childhood asthma.

Am J Respir Crit Care Med 166(10):1345-9. 2002.

Cysteinyl leukotrienes (cys-LTs; LTC₄, LTD₄, and LTE₄) are generated predominantly by mast cells and eosinophils and induce airway smooth muscle contraction, microvascular leakage, and mucous hypersecretion whereas leukotriene B₄ (LTB₄) is a potent chemoattractant of neutrophils. We measured cys-LTs and LTB₄ in exhaled breath condensate from children aged 7-14 years including healthy nonatopic children (n = 11) and children with mild intermittent asthma (steroid naive, n = 11), mild persistent asthma (low-dose inhaled steroid treatment, n = 13), or moderate to severe persistent asthma (high-dose inhaled steroid treatment, n = 13). Exhaled LTB₄ levels were increased in patients with mild and moderate to severe persistent asthma compared with patients with mild intermittent asthma (126.0 +/- 8.8 and 131.9 +/- 7.1 versus 52.7 +/- 3.8 pg/ml, p < 0.001 and p < 0.0001) and normal subjects (126.0 +/- 8.8 and 131.9 +/- 7.1 versus 47.9 +/- 4.1 pg/ml, p < 0.0001). Elevated exhaled cys-LT levels were found in patients with mild and moderate to severe persistent asthma compared with normal subjects (27.9 +/- 2.8 and 31.5 +/- 4.5 versus 18.5 +/- 0.5 pg/ml, p < 0.01 and p < 0.05). There was an inverse correlation between exhaled cys-LTs and LTB₄ in patients with mild persistent asthma. We conclude that exhaled cys-LTs and LTB₄ may be noninvasive markers of airway inflammation in pediatric asthma.

138. Corradi, M., R. Alinovi, M. Goldoni, M. Vettori, G. Folesani, P. Mozzoni, S. Cavazzini, E. Bergamaschi, L. Rossi, and A. Mutti.

Biomarkers of oxidative stress after controlled human exposure to ozone.

Toxicol Lett 134(1-3):219-25. 2002.

This study was aimed at evaluating whether controlled short-term exposure to ozone (O₃) induces changes in biomarkers of lung inflammation and oxidative stress in exhaled breath condensate (EBC) and blood of healthy subjects. Twenty-two volunteers were exposed to 0.1 ppm of O₃ for 2 h while performing moderate intermittent exercise. EBC and blood were collected before, immediately after and 18 h after exposure. Changes in biomarkers were measured both in EBC and blood, without significant alterations of lung function tests. Changes in EBC, but not in blood, were mainly accounted for by a subgroup of 'susceptible' individuals bearing the wild genotype for NAD(P)H:quinone oxidoreductase (NQO1) and the null genotype for glutathione-S-transferase M1 (GSTM1). Thus, a single 2-h exposure to 0.1 ppm of O₃ induces changes in biomarkers of inflammation and oxidative stress. Polymorphic NQO1 and GSTM1 act as modifier of the lung response to O₃.

139. Carpagnano, G. E., O. Resta, M. P. Foschino-Barbaro, E. Gramiccioni, and F. Carpagnano.

Interleukin-6 is increased in breath condensate of patients with non-small cell lung cancer.

Int J Biol Markers 17(2):141-5. 2002.

Despite recent advances in the diagnosis and treatment of non-small cell lung cancer (NSCLC), most patients still present with advanced stage disease at the time of diagnosis. Recent studies suggest that IL-6 is involved in the development of lung cancer. The aim of the present study was to investigate whether the measurement of IL-6 levels in the breath condensate of NSCLC patients could be used to bring forward the moment of diagnosis and to monitor the progression of the disease. Twenty patients with histological evidence of NSCLC (14 men and 6 women, age 63 +/- 8 years) and 15 healthy controls (8 men and 7 women, age 45 +/- 6 years) were enrolled in the study. IL6 was measured in the exhaled breath condensate of patients and controls by means of a specific enzyme immunoassay kit. Higher concentrations of exhaled IL-6 were found in NSCLC patients (9.6 +/- 0.3 pg/mL) than in controls (3.5 +/- 0.2 pg/mL). A statistically significant difference was observed between patients with NSCLC at different stages: higher concentrations of IL-6 (10.9 +/- 0.5 pg/mL) were found in patients with metastatic disease than in those with stage III (9.7 +/- 0.4 pg/mL), stage II (8.9 +/- 0.3 pg/mL) and stage I disease (7.9 +/- 0.3 pg/mL). These findings suggest that the measurement of IL-6 in the breath condensate of patients with NSCLC could be proposed as a parameter to take into account in early diagnosis and disease monitoring.

140. Carpagnano, G. E., S. A. Kharitonov, O. Resta, M. P. Foschino-Barbaro, E. Gramiccioni, and P. J. Barnes.

Increased 8-isoprostane and interleukin-6 in breath condensate of obstructive sleep apnea patients.

Chest 122(4):1162-7. 2002.

STUDY OBJECTIVES: Obstructive sleep apnea (OSA) is characterized by repeated episodes of upper airways obstruction during

sleep that result in episodes of hypoxia. An increase of systemic biomarkers of inflammation and oxidative stress has been found in patients with OSA and obesity. DESIGN: The aim of this study was to measure the levels of markers of inflammation (interleukin [IL]-6) and oxidative stress (8-isoprostane) in the exhaled breath condensate of OSA and obese patients. PATIENTS AND METHODS: Eighteen OSA patients (13 men; mean [\pm SEM] age, 44 \pm 7 years), 10 obese subjects (4 men; mean age, 39 \pm 8 years), and 15 healthy age-matched subjects (8 men; mean age, 42 \pm 4 years) were recruited. IL-6 and 8-isoprostane were measured in exhaled breath condensate by a specific enzyme immunoassay kit. Measurements and results: Higher concentrations of IL-6 were found in OSA patients (8.7 \pm 0.3 pg/mL) than in healthy control subjects (1.6 \pm 0.1 pg/mL; $p < 0.0001$). Obese subjects also had higher levels than healthy control subjects, but lower levels than OSA patients (2.1 \pm 0.2 pg/mL, $p < 0.05$ and $p < 0.0001$ respectively). Furthermore, 8-isoprostane levels were found to be higher in OSA patients (7.4 \pm 0.7 pg/mL) than in obese subjects (5 \pm 0.3 pg/mL; $p = 0.4$) and healthy subjects (4.5 \pm 0.5 pg/mL; $p < 0.005$). We found a positive correlation between these two markers and neck circumference and apnea/hypopnea index. CONCLUSIONS: These findings suggest that inflammation and oxidative stress are characteristic in the airways of OSA patients but not in obese subjects, and that their levels depend on the severity of the OSA. The measurement of IL-6 and 8-isoprostane levels may prove to be useful in screening and monitoring obese patients who have a high risk of developing OSA.

141. Antczak, A., P. Montuschi, S. Kharitonov, P. Gorski, and P. J. Barnes.

Increased exhaled cysteinyl-leukotrienes and 8-isoprostane in aspirin-induced asthma.

Am J Respir Crit Care Med 166(3):301-6. 2002.

The pathogenesis of aspirin-induced asthma (AIA) has not yet been clearly elucidated, although eicosanoid metabolites appear to play an important role. We hypothesized that levels of eicosanoids in exhaled air condensate are abnormal in patients with AIA and that they change in patients receiving steroid therapy. We measured cysteinyl-leukotrienes (cys-LTs), prostaglandin E(2) (PGE(2)), and leukotriene B(4) (LTB(4)), and also 8-isoprostane as a marker of oxidative stress, by enzyme immunoassay in exhaled breath condensate from patients with AIA (17 steroid naive; mean age, 41 \pm 23 years; FEV(1), 63%pred), 26 patients with aspirin-tolerant asthma (ATA) (11 steroid naive; mean age, 47 \pm 18 years; FEV(1), 69%pred), and 16 healthy subjects (mean age, 45 \pm 17 years; FEV(1), 93%pred). Cys-LTs were significantly higher in steroid-naive patients with AIA compared with steroid-naive patients with ATA and healthy subjects (152.3 \pm 30.4 and 36.6 \pm 7.1 versus 19.4 \pm 2.8 pg/ml; $p < 0.05$ and $p < 0.05$, respectively). Steroid-naive patients with AIA also had higher levels of 8-isoprostane than normal subjects (131.8 \pm 31.0 versus 21.9 \pm 4.5 pg/ml; $p < 0.05$). There were significantly lower levels of both cys-LTs and 8-isoprostanes in steroid-treated patients with AIA. There was no difference in either the PGE(2) or LTB(4) level between the patient groups. This is the first study to show that cys-LTs and 8-isoprostanes are elevated in expired breath condensate of steroid-naive patients with AIA, and that cys-LTs are decreased in steroid-treated patients. Exhaled PGE(2) levels are not reduced, so that it is unlikely that a deficiency of PGE(2) is an important mechanism, whereas exhaled LTB(4) levels are unchanged, indicating an abnormality beyond 5-lipoxygenase.

142. Antczak, A., and P. Gorski.

Markers of pulmonary diseases in exhaled breath condensate.

Int J Occup Med Environ Health 15(4):317-23. 2002.

Exhaled breath condensate has been more and more extensively used as a novel and non-invasive method to study airway inflammation. It is simple to perform, very well tolerated by patients and no adverse events have been reported so far. Serial measurements can be made with no harmful effects on patients, which is of extreme value in occupational medicine. Exhaled breath condensate has been obtained from both adult and children patients suffering from various pulmonary diseases such as asthma, cystic fibrosis, chronic obstructive pulmonary disease, and interstitial lung diseases. Several markers and mediators are detectable in breath condensate: hydrogen peroxide, thiobarbituric acid-reactive substances, isoprostanes, prostaglandins and leukotrienes. Nitric oxide-related markers have also been studied in the condensate. There is increasing body of evidence that changes in condensate markers reflect local abnormalities of airway lining fluid.

143. Zappacosta, B., S. Persichilli, F. Mormile, A. Minucci, A. Russo, B. Giardina, and P. De Sole.

A fast chemiluminescent method for H(2)O(2) measurement in exhaled breath condensate.

Clin Chim Acta 310(2):187-91. 2001.

BACKGROUND: Breath condensate can give useful information on volatile compounds produced at alveolar level. Actual concentration of H(2)O(2) in breath condensate is dependent on its production at alveolar level and on the efficacy of the detoxifying systems, catalase, glutathione peroxidase, etc. METHODS: In the present paper, a simple chemiluminescent method for the determination of the H(2)O(2) collected in exhaled breath is shown and data of both smokers and nonsmokers volunteers are presented.

RESULTS: The chemiluminescent response is linear up to 100 micromol/l H₂O₂. The analytical sensitivity is about 0.01 micromol/l. Most of the nonsmokers have a H₂O₂ content lower than 0.05 micromol/l, while smokers have a content ranging from 0.1 to 0.6 micromol/l.

144. Ochiai, N., M. Takino, S. Daishima, and D. B. Cardin.

Analysis of volatile sulphur compounds in breath by gas chromatography-mass spectrometry using a three-stage cryogenic trapping preconcentration system.

J Chromatogr B Biomed Sci Appl 762(1):67-75. 2001.

A method for the determination of trace volatile sulphur compounds (VSCs) including methanethiol, dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) at low ppbv (volume/volume) in breath has been developed using a large volume preconcentration technique prior to capillary GC-MS analysis. The breath sample was collected in a 6-1 fused-silica-lined stainless steel canister and introduced into the three-stage cryogenic trapping preconcentration system by GC-MS in the total ion monitoring (scan) mode. The water condensation effect of breath sample inside the canister, which is due to the difference between human body temperature and laboratory temperature, was examined. The condensed water in the fused-silica-lined canister at 24 degrees C did not affect the recoveries of VSCs within 12 h. As this three-stage cryogenic trapping preconcentration technique made it possible to remove excess water [relative humidity (RH) >95%] and carbon dioxide (3.8%) without loss of the VSCs, more than 400 ml of the breath sample could be concentrated. The detection limits of methanethiol, DMS and DMDS in a breath sample using this method were 0.13, 0.09 and 0.15 ppbv, respectively.

145. Nowak, D., S. Kalucka, P. Bialasiewicz, and M. Krol.

Exhalation of H₂O₂ and thiobarbituric acid reactive substances (TBARs) by healthy subjects.

Free Radic Biol Med 30(2):178-86. 2001.

Enhanced exhalation of H₂O₂ and TBARs have been reported in various inflammatory lung diseases. This may reflect activated phagocytes influx and free radical generation in the airways. However, to apply these compounds as markers of oxidative stress it is necessary to understand factors influencing their exhalation in healthy subjects. We investigated the concentration of H₂O₂ and TBARs in expired breath condensate (EBC) of 58 healthy volunteers. EBC was collected seven times every 4 h during 24 h and three times every 7 d during 2 consecutive weeks. The H₂O₂ exhalation revealed diurnal variation with two-peak values 0.45 +/- 0.29 microM and 0.43 +/- 0.22 microM at 12:00 and 24:00 h. The lowest concentrations, 0.26 +/- 0.13 microM and 0.25 +/- 0.26 microM, were found at 20:00 and 8:00 h. Cigarette smokers exhaled about 2.4 times more H₂O₂ than never smoked subjects. Moreover, in contrast to nonsmokers, cigarette smokers' H₂O₂ exhalation was stable over 2 week observation. The mean H₂O₂ concentration estimated over the whole 2 week period was higher in subjects above 40 years regardless of smoking habit, and it positively correlated with age in never smoked subjects (p < .004). Smoking of one cigarette caused 1.8-fold rise in H₂O₂ exhalation (p < .01). The baseline H₂O₂ levels correlated with cumulative cigarette consumption (p < .05) and MEF 25% of predicted (p < .05). Neither moderate exercise nor one puff of salbutamol nor ipratropium influenced significantly the concentration of H₂O₂ and TBARs in EBC. Only 4 of 120 EBC specimens from never smoked subjects revealed detectable levels of TBARs. Cigarette smokers exhaled more TBARs (p < .05) than never smoked volunteers. Our results indicate that healthy never smoked subjects exhale H₂O₂ with diurnal variation and significant changes over 2 week observation. Cigarette smoking enhanced H₂O₂ generation in the airways. These results could be useful for planning studies with exhaled H₂O₂ as a marker of airway inflammation. Occasional detection of TBARs in EBC of never smoked persons may be a result of sufficient antioxidant activity in the airways that protects tissues from peroxidative damage.

146. Mutlu, G. M., K. W. Garey, R. A. Robbins, L. H. Danziger, and I. Rubinstein.

Collection and analysis of exhaled breath condensate in humans.

Am J Respir Crit Care Med 164(5):731-7. 2001.

147. McRae, K., M. De Perrot, S. Fischer, T. K. Waddell, M. Liu, and S. Keshavjee.

Detection of IL-10 in the exhaled breath condensate, plasma and tissue during ischemia-reperfusion injury in experimental lung transplantation.

J Heart Lung Transplant 20(2):184. 2001.

148. Linnane, S. J., A. G. Thin, V. M. Keatings, J. B. Moynihan, P. McLoughlin, and M. X. FitzGerald.

Glucocorticoid treatment reduces exhaled nitric oxide in cystic fibrosis patients.

Eur Respir J 17(6):1267-70. 2001.

In cystic fibrosis (CF), low concentrations of exhaled nitric oxide (NO) and reduced expression of inducible nitric oxide synthase (iNOS) in airway epithelium have been reported. However, abundant iNOS expression has been found in the subepithelial tissues and elevated concentrations of NO metabolites in breath condensate and sputum. These conflicting results may be explained by increased scavenging of NO by superoxide radicals, resulting in rapid conversion to peroxynitrite, so that only a small proportion of the NO produced in the lung tissue reaches the airway lumen. If iNOS were active in the CF lung, exhaled NO would be further reduced by glucocorticoid treatment. CF patients (n = 13) were recruited to a double-blind, placebo-controlled study with crossover. Treatment comprised prednisolone or placebo for 5 days with a 9 day washout. After each treatment, exhaled NO was measured, spirometry performed and blood collected for measurement of serum nitrogen dioxide/nitrous oxide (NO₂/NO₃). Ten patients (8 male) completed the study. Following prednisolone treatment (mean +/- SD) exhaled NO concentration (3.1 +/- 1.6 parts per billion (ppb)) was significantly reduced versus placebo treatment (4.9 +/- 4.2 ppb; p<0.05, Wilcoxon signed-rank test). Spirometric indices and serum NO₂/NO₃ concentration were unchanged. These findings support the hypothesis that glucocorticoids suppress nitric oxide production in cystic fibrosis airways by reducing inducible nitric oxide synthase expression or by inhibiting recruitment of neutrophils, cells which express inducible nitric oxide synthase.

149. Kirsanov, A. I., A. F. Dolgodvorov, V. G. Leont'ev, I. A. Gorbacheva, V. D. Romanova, L. S. Velichko, and V. V. Aleksandrov.

[Chemical element levels in various human biological media].

Klin Lab Diagn(3):16-20. 2001.

Analysis of element composition (sodium, potassium, magnesium, calcium, zinc, copper, iron, total sulfur and its compounds, chloride, and inorganic phosphorus) in 40 normal subjects (22 women and 18 men aged 17-30 years) showed that the composition is specific for each of the analyzed media (blood, plasma, erythrocytes, urine, saliva, exhaled air condensate). Element composition of exhaled air condensate is described for the first time. Analysis of element composition in 102 patients with asthma demonstrated characteristic shifts in the concentrations of some macro- and trace elements, which confirms the clinical significance of analysis and correction of deviations in the element composition of biological media.

150. Kharitonov, S. A., and P. J. Barnes.

Exhaled markers of inflammation.

Curr Opin Allergy Clin Immunol 1(3):217-24. 2001.

Exhaled markers of inflammation allow completely noninvasive monitoring of inflammation and oxidative stress in the respiratory tract in inflammatory lung diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis, bronchiectasis and interstitial lung diseases. Such noninvasive techniques are simple to perform, may be repeated frequently and can be applied in children, including neonates and patients with severe disease in whom more invasive procedures are not possible. Several volatile chemicals can be measured in the breath (nitric oxide, carbon monoxide, hydrocarbons), and many nonvolatile molecules (mediators, oxidation and nitration products, proteins) may be measured in exhaled breath condensate.

151. Kasielski, M., and D. Nowak.

Long-term administration of N-acetylcysteine decreases hydrogen peroxide exhalation in subjects with chronic obstructive pulmonary disease.

Respir Med 95(6):448-56. 2001.

Patients with chronic obstructive pulmonary disease (COPD) exhale more hydrogen peroxide (H₂O₂) and lipid peroxidation products than healthy subjects. This may reflect oxidative stress in the airways that plays important role in the development and progression of COPD. N-acetylcysteine (NAC), a mucolytic drug, possesses antioxidant properties as it is a precursor of reduced glutathione that together with glutathione peroxidase may decompose H₂O₂ and lipid peroxides. We aimed to determine the effect of NAC, 600 mg effervescent tablets (Fluimucil), once a day for 12 months, and placebo on the concentration of H₂O₂ and thiobarbituric acid reactive substances (TBARs) in expired breath condensate and serum levels of two lipid peroxidation products (TBARs, lipid peroxides) in patients with COPD. The study was performed as a double-blind, double-dummy comparison between active drug and placebo in two parallel groups. Forty-four outpatients with stable COPD (22 in the NAC group and 22 in the placebo group) completed the study. Specimens of expired breath condensate and serum were collected at the randomization visit and then every 3 months over 1 year. The concentration of TBARs and H₂O₂ in expired breath condensate was measured spectrofluorimetrically by the thiobarbituric acid and homovanillic acid methods, respectively. Serum levels of lipid peroxides were

determined spectrophotometrically after extraction with butanol and pyridine. Initially, H₂O₂ exhalation did not differ between the placebo and NAC groups up to 6 months of treatment. After this the significant differences were observed. After 9 and 12 months of treatment NAC group exhaled 2.3-fold (0.17±0.33 microM vs. 0.41±0.26 microM, P<0.04) [median 0.01 microM, quartile range (qr)=0.22 vs. median 0.15 microM, qr =0.43] and 2.6-fold (0.15±0.23 microM vs. 0.40±0.25 microM, P<0.05) median = 0.00 microM, qr = 0.23 vs. median = 0.36 microM, qr = 0.51] less H₂O₂ than placebo receivers, respectively. No significant effect of NAC administration on TBARS exhalation and serum levels of TBARS and lipid peroxides were noted over the whole treatment period. Also no significant associations between exhaled H₂O₂ and concentrations of lipid peroxidation products were noted in both treatment groups at any time-point. These results indicate that long-term oral administration of NAC attenuates H₂O₂ formation in the airways of COPD subjects and prove anti-oxidant action of drug. However, further studies are necessary to estimate the clinical significance of this finding.

152. Jobsis, R. Q., S. L. Schellekens, A. Fakkkel-Kroesbergen, R. H. Raatgeep, and J. C. de Jongste.

Hydrogen peroxide in breath condensate during a common cold.

Mediators Inflamm 10(6):351-4. 2001.

BACKGROUND: Hydrogen peroxide (H₂O₂) in exhaled air condensate is elevated in inflammatory disorders of the lower respiratory tract. It is unknown whether viral colds contribute to exhaled H₂O₂. AIM: To assess exhaled H₂O₂ during and after a common cold. METHODS: We examined H₂O₂ in the breath condensate of 20 normal subjects with acute symptoms of a common cold and after recovery 2 weeks later and, similarly, in 10 subjects without infection. H₂O₂ was measured with a fluorimetric assay. RESULTS: At the time of infection exhaled H₂O₂ (median, ranges) was 0.20 microM (0.03-1.2 microM), and this decreased to 0.09 microM (< 0.01-0.40 microM) after recovery (p = 0.006). There was no significant difference in lung function (forced vital capacity and forced expiratory volume in 1 sec) during and after colds. In the controls, exhaled H₂O₂ did not change over a 2-week period. CONCLUSIONS: H₂O₂ in exhaled air condensate is elevated during a common cold, and returns to normal within 2 weeks of recovery in healthy subjects. Hence, symptomatic upper respiratory tract infection may act as a confounder in studies of H₂O₂ as a marker of chronic lower airway inflammation.

153. Horvath, I., W. MacNee, F. J. Kelly, P. N. Dekhuijzen, M. Phillips, G. Doring, A. M. Choi, M. Yamaya, F. H. Bach, D. Willis, L. E. Donnelly, K. F. Chung, and P. J. Barnes.

"Haemoxygenase-1 induction and exhaled markers of oxidative stress in lung diseases"; summary of the ERS Research Seminar in Budapest, Hungary, September, 1999.

Eur Respir J 18(2):420-30. 2001.

In recent years, there has been increasing interest in noninvasive monitoring of airway inflammation and oxidative stress. Several volatile and nonvolatile substances can be measured in exhaled breath and have been suggested as potential biomarkers of these events. Exhaled gases, including carbon monoxide (CO), alkanes (ethane, pentane), and substances measured in breath condensate, such as hydrogen peroxide (H₂O₂) and isoprostanes were all suggested as potential markers of oxidative stress in the lung. A European Respiratory Society (ERS) International Research Seminar entitled "Haemoxygenase-1 induction and exhaled markers of oxidative stress in lung diseases" was organized by the Airway Regulation and Provocation Group of the Clinical Allergy and Immunology Assembly in Budapest, Hungary in September, 1999 to integrate the latest knowledge on these issues and accelerate further improvement in this area. During this 2-day event several issues were raised about: the use and standardization of measurements in exhaled breath; problems of measuring expired H₂O₂ and other mediators in breath condensate; role and regulation of haemoxygenase (HO)-1 in the lung; and conditions and factors influencing exhaled CO. This report is a summary of the main presentations at the seminar, together with the current areas of research in this rapidly expanding field.

154. Griese, M., P. Latzin, and J. Beck.

A noninvasive method to collect nasally exhaled air condensate in humans of all ages.

Eur J Clin Invest 31(10):915-20. 2001.

BACKGROUND: The analysis of exhaled breath condensate may provide valuable insights into inflammatory and other metabolic processes of the lungs. However, its collection by active exhalation with conventional methods is cumbersome, demands a substantial level of co-operation with high motivation and is very difficult or impossible in children younger than about 4-6 years or in the elderly. A comfortable, noninvasive and efficient method is desirable. DESIGN AND PATIENTS: For collection a high-performance pump connected to a cold trap and nasal prongs were used. The volume of the condensate collected was assessed in 141 children of all ages and five adults. As an example for a low molecular component, H₂O₂ a marker of oxidative stress, was determined fluorometrically. RESULTS: On average, in healthy children from 4 weeks to 18 years of age, 84.0 (79.4, 87.3) microL min⁻¹ of

nasally exhaled air condensate were collected. The volume obtained was about 45% less in 1-6-year-old children, increased linearly with collection time, and averaged about 20-30% of the exhaled water vapour. The concentration of H₂O₂ in the healthy children was 0.49 (0.48, 0.61) microM and did not depend on age, the time of the day, family, or personal history of atopy and sex. CONCLUSIONS: The method described is generally applicable, comfortable, noninvasive, safe and efficient and allows the collection of nasally exhaled air condensate for the evaluation of metabolic processes of the lungs.

155. Ganas, K., S. Loukides, G. Papatheodorou, P. Panagou, and N. Kalogeropoulos.

Total nitrite/nitrate in expired breath condensate of patients with asthma.

Respir Med 95(8):649-54. 2001.

Production of nitric oxide (NO) is generally increased during inflammatory diseases including asthma. The eventual fate of NO is oxidation to nitrite (NO₂) and nitrate (NO₃), both of which are end-products of NO metabolism. Hydrogen Peroxide (H₂O₂) is increased in exhaled breath condensate of asthmatic subjects and may be used as a non-invasive marker of oxidative stress. NO has in some cases been shown to attenuate oxidant-induced lung injury. Total NO₂/NO₃ concentration and H₂O₂ levels were measured in expired breath condensate in 50 clinically stable asthmatics [all males, all atopics, mean age 22 (3) SD yrs, forced expiratory volume in 1 sec (FEV₁) 91 (10)% predicted, PD₂₀ to histamine 0.262 (0.16) mg 20 on inhaled steroids, 20 smokers, all steroid-naive] and in 10 normal, non-atopic subjects [all males, age 23 (4) yrs, FEV₁ 101 (14)% predicted, PD₂₀ to histamine 1.3 (0.55) mg]. NO₂/NO₃ levels were significantly higher in patients with asthma than in normal subjects (1.08, 95% CI 0.86-1.3 microM vs. 0.6; 95% CI 0.46-0.8, P < 0.001). Patients who were on inhaled steroids had significantly lower values compared to steroid-naive (0.71, 95% CI 0.55-0.87 microM vs. 1.33, 95% CI 1-1.65 microM, P < 0.001). Similar results were observed between smokers and non-smokers (1.11, 95% CI 0.74-1.47 microM vs. 1.77, 95% CI 1.1-2.4 microM, P < 0.0001). There was a significant positive correlation between NO₂/NO₃ levels and H₂O₂ concentration in expired breath condensate (r = 0.48, P < 0.0001). No correlation was observed between NO₂/NO₃ levels, airway obstruction and bronchial hyper-reactivity as assessed by PD₂₀ to histamine. Total NO₂/NO₃ levels in expired breath condensate are raised in patients with stable asthma and are significantly related to oxidative stress as assessed by H₂O₂ concentration. Measurement of expired breath NO₂/NO₃ and H₂O₂ levels may be clinically useful in the management of oxidation and inflammation mediated lung injury.

156. Ferreira, I. M., M. S. Hazari, C. Gutierrez, N. Zamel, and K. R. Chapman.

Exhaled nitric oxide and hydrogen peroxide in patients with chronic obstructive pulmonary disease: effects of inhaled beclomethasone.

Am J Respir Crit Care Med 164(6):1012-5. 2001.

There is controversy about the role of inhaled corticosteroids in chronic obstructive pulmonary disease (COPD). Although they appear to have little impact on airways obstruction or its progression, their use may reduce the frequency and/or severity of exacerbations in a subset of patients. We undertook the following study to determine the impact of inhaled corticosteroid on two noninvasive markers of airways inflammation. We assigned 20 stable nonsmoking patients with COPD in random, double-blind crossover fashion to two 2-wk treatment periods with inhaled beclomethasone 500 microg twice daily or matching placebo, followed by a 2-wk washout period. We measured exhaled nitric oxide (ENO), breath condensate H₂O₂, and flow volume spirometry at weekly intervals. Median baseline ENO was 26.2 (19.3 to 54.8) ppb and fell significantly following 1 and 2 wk of beclomethasone (-10.6 ppb, p = 0.002, and -6.3 ppb, p = 0.013, respectively) but was unchanged by placebo inhalation. Breath condensate H₂O₂ levels did not change significantly with inhaled beclomethasone or placebo. Although there were no significant changes in FEV₁ with BDP therapy, there was a moderate inverse correlation between changes in ENO and changes in FEV₁ (r -0.50). We conclude that inhaled beclomethasone reduces ENO levels in stable nonsmoking patients with COPD, a finding compatible with an antiinflammatory mechanism of action.

157. Emelyanov, A., G. Fedoseev, A. Abulimity, K. Rudinski, A. Fedoulov, A. Karabanov, and P. J. Barnes.

Elevated concentrations of exhaled hydrogen peroxide in asthmatic patients.

Chest 120(4):1136-9. 2001.

BACKGROUND: Airway inflammation is important in the development and progression of asthma. Activation of inflammatory cells induces a respiratory burst resulting in the production of reactive oxygen species, such as H₂O₂. The aim of this study was to measure the concentration of H₂O₂ in exhaled breath condensate and its correlation with airway obstruction, airway hyper-responsiveness, and concentration of eosinophil cationic protein (ECP) in serum in 70 steroid-naive, atopic patients with unstable asthma (20 men; age range, 18 to 62 years) and 17 normal subjects (7 men; age range, 19 to 34 years). METHODS: Exhaled H₂O₂ was measured using a colorimetric assay, and the concentration of ECP in serum was measured using radioimmunoassay. Airway

hyperresponsiveness was expressed as the provocative concentration of inhaled histamine causing a 20% fall in FEV(1) (PC(20)). RESULTS: In patients with asthma, the mean H(2)O(2) concentration was significantly elevated compared to values in normal subjects: 0.127 +/- 0.083 mol/L vs 0.024 +/- 0.016 mol/L (p < 0.001). There was a significant correlation among H(2)O(2) concentration, FEV(1), PC(20), and ECP in serum. CONCLUSION: We conclude that exhaled H(2)O(2) is significantly elevated in asthmatic patients. This is correlated with disease severity and indirect markers of airway inflammation. Measurement of exhaled H(2)O(2) may be useful to assess airway inflammation and oxidative stress in asthmatic patients.

158. Corradi, M., P. Montuschi, L. E. Donnelly, A. Pesci, S. A. Kharitonov, and P. J. Barnes.
Increased nitrosothiols in exhaled breath condensate in inflammatory airway diseases.
Am J Respir Crit Care Med 163(4):854-8. 2001.

Nitrosothiols (RS-NOs) are formed by interaction of nitric oxide (NO) with glutathione and may limit the detrimental effect of NO. Because NO generation is increased in airway inflammation, we have measured RS-NOs in exhaled breath condensate in patients with asthma, cystic fibrosis, or chronic obstructive pulmonary disease (COPD). We also measured exhaled NO and nitrite (NO(2-)) in the same subjects. RS-NOs were detectable in exhaled breath condensate of all subjects. RS-NOs were higher in subjects with severe asthma (0.81 +/- 0.06 microM) when compared with normal control subjects (0.11 +/- 0.02 microM, p < 0.01) and with subjects with mild asthma (0.08 +/- 0.01 microM, p < 0.01). Elevated RS-NOs values were also found in patients with cystic fibrosis (0.35 +/- 0.07 microM, p < 0.01), in those with COPD (0.24 +/- 0.04 microM, p < 0.01) and in smokers (0.46 +/- 0.09 microM, p < 0.01). In current smokers there was a correlation (r = 0.8, p < 0.05) between RS-NOs values and smoking history (pack/year). We also found elevated concentrations of NO(2-) in patients with severe asthma, cystic fibrosis, or COPD, but not in smokers or patients with mild asthma. This suggests that exhaled NO(2-) is less sensitive than exhaled RS-NOs. This study has shown that RS-NOs are detectable in exhaled breath condensate of healthy subjects and are increased in patients with inflammatory airway diseases. As RS-NOs concentrations in exhaled breath condensate vary in the different airway diseases and increase with the severity of asthma, their measurement may have clinical relevance as a noninvasive biomarker of nitrosative stress.

159. Balint, B., S. A. Kharitonov, T. Hanazawa, L. E. Donnelly, P. L. Shah, M. E. Hodson, and P. J. Barnes.
Increased nitrotyrosine in exhaled breath condensate in cystic fibrosis.
Eur Respir J 17(6):1201-7. 2001.

Exhaled nitric oxide (ENO), a marker of inflammation in airway diseases is decreased in cystic fibrosis (CF) patients, perhaps because nitric oxide (NO) is metabolized to oxidative end-products. A stable product, 3-nitrotyrosine, may indicate local formation of reactive nitrogen species. Whether NO metabolites in exhaled breath condensate may be increased in CF patients was investigated. The fractional concentration of ENO (Feno), nitrotyrosine and oxides of nitrogen in exhaled breath condensate from 36 stable CF patients were compared to 14 normal subjects using an enzyme immunoassay and fluorescence assay. Nitrotyrosine levels in breath condensate were increased significantly in stable CF patients, compared with normal subjects (25.3 +/- 1.5 versus 6.3 +/- 0.8 ng x mL(-1), p < 0.0001). There was an inverse correlation between the levels of nitrotyrosine and the severity of lung disease. Feno levels were significantly lower in CF patients than in normal subjects (4.4 +/- 0.3 versus 5.6 +/- 0.4 (parts per billion), p < 0.05). No correlation was found between nitrotyrosine and Feno levels in CF. There was no significant difference in the levels of nitrite and nitrate between CF patients and normals. The elevation in nitrotyrosine may reflect increased formation of reactive nitrogen species such as peroxy nitrite or direct nitration by granulocyte peroxidases, indicating increased oxidative stress in airways of cystic fibrosis patients.

160. Balint, B., L. E. Donnelly, T. Hanazawa, S. A. Kharitonov, and P. J. Barnes.
Increased nitric oxide metabolites in exhaled breath condensate after exposure to tobacco smoke.
Thorax 56(6):456-61. 2001.

BACKGROUND: Cigarette smoking reduces the level of exhaled nitric oxide (NO) in healthy subjects, although the mechanism is unclear. NO is a highly reactive molecule which can be oxidised or complexed with other biomolecules, depending on the microenvironment. The stable oxidation end products of NO metabolism are nitrite and nitrate. This study investigated the effect of smoking on NO metabolites in exhaled breath condensate. METHODS: Fifteen healthy current smokers were recruited together with 14 healthy non-smokers. Measurement of exhaled NO, lung function, and collection of exhaled breath condensate were performed. Nitrite, nitrite + nitrate, S-nitrosothiols, and nitrotyrosine levels were measured. The effect of inhaling two cigarettes in smokers was also evaluated. The mean level of exhaled NO in smokers was significantly lower than in non-smokers (4.3 (0.3) ppb v 5.5 (0.5) ppb, p < 0.05). RESULTS: There was no difference in the levels of nitrite, nitrite + nitrate, S-nitrosothiols, and nitrotyrosine in the exhaled breath condensate at the baseline visit between smokers and non-smokers. After smoking, nitrite + nitrate levels were

significantly but transiently increased (from 20.2 (2.8) microM to 29.8 (3.4) microM, $p < 0.05$). There was no significant change in the levels of exhaled NO, nitrite, S-nitrosothiols, or nitrotyrosine 30 and 90 minutes after smoking. CONCLUSIONS: These findings suggest that acute smoking can increase the level of nitrate, but not nitrite, S-nitrosothiols, or nitrotyrosine in breath condensate. The deleterious effect of oxidant radicals induced by smoking may contribute to the epithelial damage of airways seen in smokers.

161. Schleiss, M. B., O. Holz, M. Behnke, K. Richter, H. Magnussen, and R. A. Jorres.

The concentration of hydrogen peroxide in exhaled air depends on expiratory flow rate.

Eur Respir J 16(6):1115-8. 2000.

Hydrogen peroxide (H₂O₂) is known to be detectable in exhaled air. The present study aimed to determine whether the concentration of exhaled H₂O₂ depends on expiratory flow rate in order to make inferences on the site of its production within the lung. Breath condensate was collected in cooled Teflon tubes, at three different expiratory flow rates, in 15 healthy or mild asthmatic subjects. Tests were repeated 2-5 times to assess reproducibility. Mean \pm SEM concentrations of H₂O₂ at flow rates of 140, 69 and 48 mL.s⁻¹ were 0.12 \pm 0.02, 0.19 \pm 0.02 and 0.32 \pm 0.03 microM, respectively. These values differed significantly from each other ($p < 0.001$). For comparison, average coefficients of variability within repeated measurements at each of the three flow rates were 68, 62 and 82%, respectively. These data demonstrate that the concentration of exhaled hydrogen peroxide depends on expiratory flow rate. Since flow dependence is an indicator of production within the airways, this result suggests that, to a large extent, the exhaled hydrogen peroxide originates within the airways. However, even under strictly controlled conditions, a high degree of variability persists, which may limit the usefulness of exhaled hydrogen peroxide as a marker of airway inflammation.

162. Reinhold, P., G. Becher, and M. Rothe.

Evaluation of the measurement of leukotriene B₄ concentrations in exhaled condensate as a noninvasive method for assessing mediators of inflammation in the lungs of calves.

Am J Vet Res 61(7):742-9. 2000.

OBJECTIVE: To determine whether measurement of an inflammatory mediator in exhaled condensate could provide a noninvasive method for evaluating lungs of calves. ANIMALS: 84 calves \leq 2 months old. PROCEDURE: Concentration of leukotriene B₄ (LTB₄) was evaluated in the exhaled condensate of healthy calves and calves with experimentally induced respiratory tract infections. For collection of samples of exhaled condensate, the total amount of exhaled air was directed into a cooled double-jacketed tube. Each tube was sealed and stored at -80 C. The LTB₄ concentration was measured, using an ELISA. RESULTS: In exhaled condensates of clinically healthy calves, normally distributed and highly reproducible LTB₄ concentrations (mean \pm SD, 116.1 \pm 55.4 pg/ml) were measured. After experimentally induced infection with *Pasteurella multocida* serovar D, LTB₄ in exhaled condensate increased significantly (mean, 179% increase), compared with basal concentrations before infection; this increase in LTB₄ was significantly correlated with deterioration in lung function. In 2 of 4 calves experimentally infected with bovine respiratory syncytial virus, the LTB₄ concentration in exhaled condensate increased (300 to 400% increase), compared with baseline values, which was associated with development of bronchial hyperresponsiveness after infection. CONCLUSIONS AND CLINICAL RELEVANCE: Collection of exhaled condensate is tolerated well by calves and is an acceptable method for obtaining fluid from exhaled air originating from the lungs. This method provides alternatives for diagnosing and evaluating treatment of naturally acquired and experimentally induced diseases of the lungs and airways in calves.

163. Nightingale, J. A., D. F. Rogers, K. F. Chung, and P. J. Barnes.

No effect of inhaled budesonide on the response to inhaled ozone in normal subjects.

Am J Respir Crit Care Med 161(2 Pt 1):479-86. 2000.

Inhalation of ozone in normal subjects causes a neutrophilic inflammatory response in the airways. Pretreatment with inhaled corticosteroids reduces the inflammatory response to inhaled ozone in dogs. We undertook a double-blind, randomized, placebo-controlled, crossover study to investigate the effects of 2 wk of treatment with inhaled budesonide 800 microg twice daily or placebo prior to ozone exposure in humans. Fifteen (six male; mean age, 31.1 \pm 2.1 yr) healthy nonsmokers were exposed to 400 parts per billion (ppb) ozone for 2 h with intermittent exercise. Spirometry, exhaled carbon monoxide (CO) and nitric oxide (NO) levels, measurement of methacholine reactivity, and collection of exhaled air condensate and induced sputum samples were performed at baseline, preexposure, and at intervals up to 24 h postexposure. Ozone exposure led to significant decreases in spirometry and increased methacholine reactivity and sputum neutrophils and myeloperoxidase (MPO). There were no changes in exhaled NO and CO levels, or exhaled breath nitrite after ozone exposure. There were no differences in any of the parameters after treatment with budesonide compared with placebo, and no differences in the response to ozone between treatment groups. We conclude that a high dose of inhaled corticosteroid does not protect against the effects of ozone exposure in normal subjects.

164. Montuschi, P., S. A. Kharitonov, G. Ciabattoni, M. Corradi, L. van Rensen, D. M. Geddes, M. E. Hodson, and P. J. Barnes. Exhaled 8-isoprostane as a new non-invasive biomarker of oxidative stress in cystic fibrosis. *Thorax* 55(3):205-9. 2000.

BACKGROUND: Cystic fibrosis is characterised by oxidative stress in the airways. Isoprostanes are prostaglandin isomers formed by free radical catalysed peroxidation of arachidonic acid. 8-Isoprostane is increased in interstitial lung diseases, asthma, chronic obstructive pulmonary disease, and adult respiratory distress syndrome. Exhaled nitric oxide (NO) and carbon monoxide (CO) are biomarkers of inflammation and oxidative stress in the airways, respectively. **METHODS:** Concentrations of 8-isoprostane in the breath condensate of 10 normal subjects and 19 patients with stable cystic fibrosis were measured using an enzyme immunoassay (EIA). Breath condensate is a non-invasive method of collecting airway secretions. Exhaled nitric oxide (NO) and carbon monoxide (CO) levels were measured by a chemiluminescence analyser. **RESULTS:** Concentrations of 8-isoprostane in the breath condensate of patients with stable cystic fibrosis were increased about threefold compared with normal subjects (42.7 (4.5) pg/ml vs 15.2 (1.7) pg/ml; $p < 0.005$, 95% CI 14.6 to 40.9). 8-Isoprostane concentrations were negatively correlated with forced expiratory volume in one second in patients with cystic fibrosis ($r = -0.61$; $p < 0.005$). Exhaled CO was also increased in patients with cystic fibrosis compared with normal subjects (6.7 (1.2) ppm vs 2.9 (0.3) ppm; $p < 0.05$, 95% CI 0.2 to 7.4). 8-Isoprostane concentrations were significantly correlated with CO levels ($r = 0.66$; $p < 0.002$). **CONCLUSIONS:** The results of this study show that oxidative stress is increased in cystic fibrosis and may be quantified by measuring 8-isoprostane concentrations in breath condensate.

165. Montuschi, P., J. V. Collins, G. Ciabattoni, N. Lazzeri, M. Corradi, S. A. Kharitonov, and P. J. Barnes. Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med* 162(3 Pt 1):1175-7. 2000.

Most of the studies linking chronic obstructive pulmonary disease (COPD) with oxidative stress are in vitro, using invasive techniques, or measuring systemic oxidative stress. The aim of this study was to quantify oxidative stress in the lungs in patients with COPD and in healthy smokers, as reflected by 8-isoprostane concentrations in breath condensate. This is a noninvasive method to collect airway secretions. 8-Isoprostane is a prostaglandin-F(2 α) isomer that is formed in vivo by free radical-catalyzed peroxidation of arachidonic acid. We also studied the acute effect of smoking on exhaled 8-isoprostane in healthy smokers. Exhaled 8-isoprostane was measured by a specific enzyme immunoassay in 10 healthy nonsmokers and 12 smokers, 25 COPD ex-smokers, and 15 COPD current smokers. 8-Isoprostane concentrations were similar in COPD ex-smokers (40 +/- 3.1 pg/ml) and current smokers (45 +/- 3.6 pg/ml) and were increased about 1.8-fold compared with healthy smokers (24 +/- 2.6 pg/ml, $p < 0.001$), who had 2.2-fold higher 8-isoprostane than healthy nonsmokers (10.8 +/- 0.8 pg/ml, $p < 0.05$). Smoking caused an acute increase in exhaled 8-isoprostane by about 50%. Our study shows that free radical production is increased in patients with COPD and that smoking causes an acute increase in oxidative stress.

166. Lases, E. C., V. A. Duurkens, W. B. Gerritsen, and F. J. Haas. Oxidative stress after lung resection therapy: A pilot study. *Chest* 117(4):999-1003. 2000.

STUDY OBJECTIVES: To investigate whether oxidative stress occurs following lobectomy and pneumonectomy and to evaluate whether markers of oxidative stress might be of value in the assessment of the diagnosis, course, and prognosis of postoperative complications. **DESIGN:** A prospective study. **SETTING:** A specialized thoracic surgical unit in a large referral hospital. **PATIENTS:** Twenty-eight patients with lung carcinoma undergoing thoracotomy. **MEASUREMENTS:** Exhaled H(2)O(2) concentrations in breath condensate were measured by spectrophotometry, while malondialdehyde (MDA) levels in urine samples collected every 24 h were measured by reversed-phase, ion-pair high-performance liquid chromatography using ultraviolet detection. **RESULTS:** Our results show increased H(2)O(2) and MDA levels in lobectomy patients compared with pneumonectomy patients. A strong correlation was found between the levels of H(2)O(2) and MDA. **CONCLUSION:** The present data support the hypothesis that oxidative stress may occur following pulmonary resection.

167. Jobsis, Q., H. C. Raatgeep, S. L. Schellekens, A. Kroesbergen, W. C. Hop, and J. C. de Jongste. Hydrogen peroxide and nitric oxide in exhaled air of children with cystic fibrosis during antibiotic treatment. *Eur Respir J* 16(1):95-100. 2000.

Cystic fibrosis (CF) patients characteristically have severe chronic airway inflammation associated with bacterial infection. A noninvasive marker of airway inflammation could be a useful guide to treatment of CF lung disease. The aim of this study was to assess

whether measurement of hydrogen peroxide (H₂O₂) and nitric oxide (NO) in exhaled air can serve to monitor the effect of treatment with antibiotics in CF-children with acute infective pulmonary exacerbations. Sixteen CF-patients (mean age 12.3 yrs) with exacerbation of their lung infection were treated with intravenous antibiotics in an uncontrolled study. During treatment, H₂O₂ in exhaled air condensate was measured twice a week. In addition, serial NO measurements were performed in nine patients. During antibiotic treatment the median H₂O₂ concentration in exhaled air condensate decreased significantly from 0.28 microM (range 0.07-1.20 microM) to 0.16 microM (range 0.05-0.24 microM, p=0.002) and the mean forced expiratory volume in one second significantly increased from 55% predicted to 75% pred (p=0.001). In individual subjects, changes of H₂O₂ and FEV1 between pairs of serial measurements correlated weakly (p=0.08). Data on exhaled NO were inconclusive; exhaled NO did not change systematically during treatment. It is concluded that cystic fibrosis patients with an acute pulmonary exacerbation have abnormally high concentrations of hydrogen peroxide, but not of nitric oxide, in exhaled air, which decrease during intravenous antibiotic treatment. Further controlled studies should establish if exhaled hydrogen peroxide, may serve as a noninvasive parameter of airway inflammation to guide antibiotic treatment in cystic fibrosis lung disease.

168. Hunt, J. F., K. Fang, R. Malik, A. Snyder, N. Malhotra, T. A. Platts-Mills, and B. Gaston.

Endogenous airway acidification. Implications for asthma pathophysiology.

Am J Respir Crit Care Med 161(3 Pt 1):694-9. 2000.

Airway concentrations of many reactive nitrogen and oxygen species are high in asthma. The stability and bioactivities of these species are pH-dependent; however, the pH of the airway during acute asthma has not previously been studied. As with gastric and urinary acidification, asthmatic airway acidification could be expected dramatically to alter the concentrations and bioactivities/cytotoxicities of endogenous nitrogen oxides. Here, we demonstrate that the pH of deaerated exhaled airway vapor condensate is over two log orders lower in patients with acute asthma (5.23 +/- 0.21, n = 22) than in control subjects (7.65 +/- 0.20, n = 19, p < 0.001) and normalizes with corticosteroid therapy. Values are highly reproducible, unaffected by salivary or therapeutic artifact, and identical to samples taken directly from the lower airway. Further, at these low pH values, the endogenous airway compound, nitrite, is converted to nitric oxide (NO) in quantities sufficient largely to account for the concentrations of NO in asthmatic expired air, and eosinophils undergo accelerated necrosis. We speculate that airway pH may be an important determinant of expired NO concentration and airway inflammation, and suggest that regulation of airway pH has a previously unsuspected role in asthma pathophysiology.

169. Hanazawa, T., S. A. Kharitonov, and P. J. Barnes.

Increased nitrotyrosine in exhaled breath condensate of patients with asthma.

Am J Respir Crit Care Med 162(4 Pt 1):1273-6. 2000.

The reaction of nitric oxide (NO) and superoxide anions (O₂(-)) in the airway results in the formation of peroxynitrite, a highly reactive oxidant species. Peroxynitrite reacts with tyrosine residues in proteins to form the stable product nitrotyrosine. We investigated whether nitrotyrosine in exhaled breath condensates may be increased in patients with asthma. Four groups of nonsmoking subjects were studied. We measured exhaled NO, nitrotyrosine, and leukotrienes concentrations in breath condensate in healthy nonatopic subjects (n = 15) and in patients with mild asthma (steroid naive, n = 15), moderate asthma (inhaled steroid treatment, n = 12), and severe asthma (oral steroid treatment, n = 12). Exhaled NO was increased significantly in patients with mild (19.2 +/- 2.7 ppb, p < 0.01) and moderate asthma (14.0 +/- 1.53 ppb, p < 0.05), as compared with normal control (6.58 +/- 0.61 ppb). The levels of LTC(4)/D(4)/E(4) and LTB(4) were increased significantly in patients with moderate and severe asthma treated with steroids. Nitrotyrosine concentrations were detectable (6.3 +/- 0.8 ng/ml) in breath condensate of normal subjects, and were increased significantly in patients with mild asthma (15.3 +/- 2.0 ng/ml, p < 0.01). However, the levels of nitrotyrosine in exhaled condensate were lower in patients with moderate (5.0 +/- 0.6 ng/ml) and severe asthma (3.3 +/- 0.6 ng/ml, p < 0.05). There was a significant correlation between nitrotyrosine in breath condensate and exhaled NO in patients with mild asthma (r = 0.65, p < 0.05). We conclude that nitrotyrosine formation in exhaled breath condensates may be a marker of oxidative stress in airways of asthma.

170. Gibson, P. G., R. L. Henry, and P. Thomas.

Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate.

Eur Respir J 16(5):1008-15. 2000.

Noninvasive markers of airway inflammation are needed for use in research and clinical practice in childhood asthma. Induced sputum and exhaled nitric oxide are well established as direct markers of inflammation for use in asthma research. Sputum can be induced from children of >6 yrs using inhalation of hypertonic saline, and, if appropriate, can be combined with an assessment of airway responsiveness to hypertonic saline. The success rate of sputum induction in children is 68-100%. Most studies

have processed sputum using the plug selection method, and show that the dominant cell in sputum from normal children is the macrophage, and that the upper normal limit for sputum eosinophils in children is 2.5%. The inflammatory response in childhood asthma is characterized by elevated numbers of sputum eosinophils, and eosinophil cationic protein concentration, as well as increased nitric oxide and hydrogen peroxide levels in exhaled breath. Sputum eosinophils correlate with objective markers of disease severity in steroid-naïve children with asthma, and in severe asthma. Inflammatory marker levels are lower in children using glucocorticosteroids. Induced sputum and exhaled gases are important markers of inflammation in childhood asthma. The clinical utility of these markers warrants further study.

171. De Benedetto, F., A. Aceto, B. Dragani, A. Spacone, S. Formisano, R. Cocco, and C. M. Sanguinetti.

Validation of a new technique to assess exhaled hydrogen peroxide: results from normals and COPD patients.

Monaldi Arch Chest Dis 55(3):185-8. 2000.

Chronic airways inflammation in chronic obstructive pulmonary disease (COPD) induces the activation of several cell types with delivery of proteases and reactive oxygen species (ROS). Assessing oxidant content in the exhaled air of COPD patients has proven useful in monitoring airway inflammation. The present study was designed to confirm the usefulness of exhaled hydrogen peroxide concentration determination in COPD patients using a new technique which allows longer storage of the expired air condensate before the H₂O₂ assay. The technique was applied in 13 healthy nonsmoking subjects (six male, age range 22-40 yrs) and in seven patients (five male, age range 58-81 yrs) with mild or moderate COPD. Subjects breathed into a one-valve mouthpiece, and the exhaled air was directed into a vial kept at 0 degree C. After approximately 15 min of quiet breathing, 1 mL of expired air condensate was collected. An aliquot, 450 microL, of this sample was immediately added to an equal volume of a reaction mixture containing 2 mM 3,5,3',5'-tetramethylbenzidine and 40 microL of enzyme stock solution (0.5 mg.mL⁻¹). After 15 min, 45 microL sulphuric acid was added (1 N final concentration), resulting in a reaction mixture pH of 1.0. After a further 10-min incubation, H₂O₂ concentration determination was performed spectrophotometrically at 450 nm. This solution, as well as the H₂O₂ assay, was stable for > or = 24 h if the sample was kept in the dark and at 4 degrees C. There was high stability on repeated measures, with a coefficient of variation equal to zero. The mean +/- SD H₂O₂ level in exhaled air from normal subjects was 0.12 +/- 0.09 microM, whereas it was significantly increased in COPD patients (0.50 +/- 0.11 microM; p = 0.0001 compared to healthy subjects). In three healthy control subjects, a normal H₂O₂ level in expired air increased to 0.70-0.80 microM during an acute upper respiratory tract infection. This new technique of hydrogen peroxide assay in expired air condensate greatly minimizes the inaccuracy deriving from the instability of hydrogen peroxide. The preliminary results obtained using this technique provide direct evidence for increased reactive oxygen species production in the airways of stable chronic obstructive pulmonary disease patients. However, the specificity of the procedure could be reduced by the interference of upper respiratory tract infections.

172. Antczak, A., Z. Kurmanowska, M. Kasielski, and D. Nowak.

Inhaled glucocorticosteroids decrease hydrogen peroxide level in expired air condensate in asthmatic patients.

Respir Med 94(5):416-21. 2000.

H₂O₂ is elevated in the exhaled air condensate in several inflammatory disorders of the lung, including bronchial asthma, and thus may reflect inflammatory processes in the airways. Exhaled H₂O₂ may be used to guide the anti-inflammatory treatment of patients with asthma. Therefore in this study we analysed the effect of inhaled glucocorticosteroid beclomethasone for 4 weeks on H₂O₂ level in the exhaled air condensate. Seventeen asthmatics and 10 healthy subjects were included to the study. Eleven patients were given inhaled beclomethasone and six were given placebo (3M Health Care). In all patients pulmonary function tests were performed. H₂O₂ in the expired air condensate was measured spectrofluorimetrically (homovanillic acid method). Inhaled beclomethasone significantly decreased H₂O₂ in the expired air condensate in the active-treatment group, with a fall from baseline on day 1 which remained on day 43 (follow-up) (P<0.05). Exhaled H₂O₂ in the active-treatment group was significantly lower than that in placebo group (P<0.05). A negative correlation between H₂O₂ and forced expiratory volume in 1 sec (FEV₁) on day 29 was observed. The decrease in exhaled H₂O₂ in the active-treatment group was accompanied by an improvement in pulmonary function tests results. Inhaled glucocorticosteroids reduce the level of H₂O₂ in the expired air condensate of asthmatic patients over a 4-week period and this may reflect their anti-inflammatory activity in lung diseases.

173. Thibeault, D. W., M. H. Rezaiekhaliq, I. Ekekezie, and W. E. Truog.

Compressed air as a source of inhaled oxidants in intensive care units.

Am J Perinatol 16(9):497-501. 1999.

Exhaled gas from mechanically ventilated preterm infants was found to have similar oxidant concentrations, regardless of lung disease, leading to the hypothesis that wall outlet gases were an oxidant source. Oxidants in compressed room air and oxygen

from wall outlets were assessed in three hospitals. Samples were collected by flowing wall outlet gas through a heated humidifier and an ice-packed condenser. Nitric oxide (NO) was measured in intensive care room air and in compressed air with and without a charcoal filter using a Sievers NOA280 nitric oxide analyzer (Boulder, CO). Oxidants were measured by spectrophotometry and expressed as nMol equivalents of H₂O₂/mL. The quantity of oxidant was also expressed as amount of Vitamin C (nMol/mL) added until the oxidant was nondetectable. This quantity of Vitamin C was also expressed in Trolox Equivalent Antioxidant Capacity (TEAC) units (mMol/L). Free and total chlorine were measured with a Chlorine Photometer. Oxidants were not found in compressed oxygen and were only found in compressed air when the compression method used tap water. At a compressed room air gas flow of 1.5 L/min, the total volume of condensate was 20.2 +/- 1 mL/hr. The oxidant concentration was 1.52 +/- 0.09 nMol/mL equivalents of H₂O₂/mL of sample and 30.8 +/- 1.2 nMol/hr; 17.9% of that found in tap water. Oxidant reduction required 2.05 +/- 0.12 nMol/mL vitamin C, (1.78 +/- 0.1 x 10⁻³) TEAC units). Free and total chlorine in tap water were 0.3 +/- 0.02 mg/mL and 2.9 +/- 0.002 mg/mL, respectively. Outlet gas contained 0.4 +/- 0.06 mg/mL and 0.07 + 0.01 mg/mL total and free chlorine, respectively; both 14% of tap water. When a charcoal filter was installed in the hospital with oxidants in compressed air, oxidants were completely removed. Nursery room air contained 12.4 +/- 0.5 ppb NO; compressed wall air without a charcoal filter, 8.1 +/- 0.1 ppb and compressed air with a charcoal filter 12.5 +/- 0.5 ppb. A charcoal filter does not remove NO. (Table 3) We recommend that all compressed air methods using tap water have charcoal filters at the compression site and the gases be assessed periodically for oxidants.

174. Reinhold, P., A. Langenberg, G. Becher, and M. Rothe.

[Breath condensate—a medium obtained by a noninvasive method for the detection of inflammation mediators of the lung]. *Berl Munch Tierarztl Wochenschr* 112(6-7):254-9. 1999.

Collection of exhaled condensate (freezing of expired air under conditions of spontaneous breathing) is a non-invasive method permitting the collection of material originating from the lung and the lower respiratory tract so that it can be used for diagnostic examinations. In order to be able to evaluate the diagnostic evidence of exhaled condensate samples in cases of respiratory disease of the calf, leukotriene B₄ (LTB₄) in bovine exhaled condensate was determined. The influence of the breathing pattern and body temperature on the quantity of condensate to be collected was tested in a total of 49 exhaled condensate samples. It became obvious that the exhaled condensate quantity obtained per time unit is dependent on the ventilation volume per time unit (minute volume). In exhaled condensate samples from 35 clinically healthy calves, LTB₄ concentrations of less than 250 pg/mL exhaled condensate were detected. A total of 14 exhaled condensate samples from 7 calves was analyzed before and after experimental respiratory infection with *Pasteurella multocida* D. In parallel to the analysis of LTB₄ in exhaled condensate, the lung function of the calves was examined by means of impulse oscillometry. The increase of LTB₄ in the exhaled condensate post infection correlated significantly ($p < \text{or} = 0.05$) with decreases of respiratory reactance. The determination of LTB₄ concentrations in exhaled condensate seems to be suitable, in principle, for the detection of inflammations in the respiratory system of the calf. Further studies are needed for the evaluation of the diagnostic validity of the method.

175. Nightingale, J. A., D. F. Rogers, and P. J. Barnes.

Effect of inhaled ozone on exhaled nitric oxide, pulmonary function, and induced sputum in normal and asthmatic subjects. *Thorax* 54(12):1061-9. 1999.

BACKGROUND: Nitric oxide (NO) may have a role in the pathophysiology of tissue injury in response to inhaled ozone in animals. METHODS: A double blind, randomised, placebo controlled, crossover study was undertaken to investigate the effects of inhaled ozone in 10 normal and 10 atopic asthmatic volunteers. Subjects were exposed to 200 ppb ozone or clean air for four hours with intermittent exercise, followed by hourly measurement of spirometric parameters and exhaled NO for four hours. Nasal NO and methacholine reactivity were measured and exhaled breath condensate and induced sputum samples were collected four and 24 hours after exposure. RESULTS: Exposure to ozone caused a fall in forced expiratory volume in one second (FEV₁) of 7% in normal subjects ($p < 0.05$) and 9% in asthmatic subjects ($p < 0.005$). There was a 39% increase in sputum neutrophils at four hours in normal subjects ($p < 0.05$) and a 35% increase at four hours in asthmatic subjects, remaining high at 24 hours ($p < 0.005$ and $p < 0.05$, respectively). There were no differences between normal and asthmatic subjects. There were no changes in methacholine reactivity, exhaled or nasal NO, nitrite levels in exhaled breath condensate, or sputum supernatant concentrations of interleukin 8, tumour necrosis factor alpha, or granulocyte-macrophage colony stimulating factor in either group. CONCLUSIONS: Exposure to 200 ppb ozone leads to a neutrophil inflammatory response in normal and asthmatic subjects but no changes in exhaled NO or nitrite levels.

176. Namazbaeva, Z. I., G. A. Kulkybaev, D. M. Dzhangozina, M. A. Mukasheva, and N. M. Zhanbastinova.

[The informational significance of metal bioaccumulation in the hair of preschool children].

Gig Sanit(1):34-6. 1999.

Hair and urine metal accumulation was studied in healthy children living in different pollution areas. The levels of biogenic amines, the activity of the enzymes LDH, AP, and cholinesterase were also determined in the exhaled air condensate (EAC) and urine.

High accumulation of metals, low EAC levels of biogenic amines, high activity of the urine enzymes were observed in the children residing in high polluted areas. At the same time these changes were less marked in the moderately polluted areas. The revealed changes allow one to select children at risk and to make health-promoting measures.

177. Mumby, S., R. Block, A. J. Petros, and J. M. Gutteridge.

Hydrogen peroxide and catalase are inversely related in adult patients undergoing cardiopulmonary bypass: implications for antioxidant protection.

Redox Rep 4(1-2):49-52. 1999.

Adult patients undergoing cardiopulmonary bypass (CPB) surgery are subjected to increased oxidative stress and show a spectrum of lung injury. Increased levels of hydrogen peroxide (H₂O₂) are often seen during episodes of oxidative stress, such as the use of high FiO₂s, and this molecule plays a key role in the formation of highly damaging oxidants such as the hydroxyl radical. Oxidative damage to plasma proteins was assessed by measuring free thiol groups, and antioxidant protection against H₂O₂ by measuring catalase activity. CPB patients (n = 39) receiving either 100% or 50% oxygen at the end of bypass were studied by measuring levels of H₂O₂ in breath condensate and levels of catalase in their plasma, and comparing these to pre-bypass levels. Post-bypass, all CPB patients exhaled significantly lower levels of H₂O₂ (P < 0.0001) at a time when they had significantly increased activity (0.809 +/- 0.11 versus 1.688 +/- 0.18 U/mg protein) of catalase in their plasma. There were no significant differences in these parameters between the 100% and 50% oxygen groups. At a time when oxidative stress is greatest, there appears to be a corresponding plasma increase in the antioxidant catalase. Whether this change is fortuitous or a response to oxidative stress is at present under consideration.

178. Montuschi, P., M. Corradi, G. Ciabattini, J. Nightingale, S. A. Kharitonov, and P. J. Barnes.

Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients.

Am J Respir Crit Care Med 160(1):216-20. 1999.

Oxidative stress has an important role in the pathogenesis of asthma. 8-Isoprostane is a prostaglandin (PG)-F₂-like compound belonging to the F₂ isoprostane class that is produced in vivo by the free radical-catalyzed peroxidation of arachidonic acid. 8-Isoprostane is a biomarker of oxidative stress, and its concentration is increased in the bronchoalveolar lavage fluid of patients with interstitial lung diseases. We measured 8-isoprostane concentrations in exhaled breath condensate in healthy subjects and in patients with mild (steroid naive, n = 12), moderate (inhaled steroid treatment, n = 17), and severe asthma (oral steroid treatment, n = 15). We also measured exhaled carbon monoxide (CO) and nitric oxide (NO), which may also reflect oxidative stress in the airways. 8-Isoprostane was detectable in breath condensate of normal subjects (15.8 +/- 1.6 pg/ml), and was increased in the breath condensate of patients with mild (33.7 +/- 2.8, p < 0.001), moderate (38.3 +/- 3.7 pg/ml, p < 0.001), and severe asthma (48.9 +/- 5.0 pg/ml, p < 0.001). There was a positive correlation (r = 0.68, p < 0.05) of 8-isoprostane with NO, but not with CO, in the exhaled air of patients with mild asthma, but not in that of patients with moderate or severe asthma. There was no correlation between 8-isoprostane and lung function tests in any group of patients. Our study shows that oxidative stress is increased in asthmatic subjects as reflected by 8-isoprostane concentrations in breath condensate.

179. Heard, S. O., K. Longtine, I. Toth, J. C. Puyana, B. Potenza, and N. Smyrniotis.

The influence of liposome-encapsulated prostaglandin E1 on hydrogen peroxide concentrations in the exhaled breath of patients with the acute respiratory distress syndrome.

Anesth Analg 89(2):353-7. 1999.

Hydrogen peroxide (H₂O₂) levels are increased in the exhaled breath of patients with the acute respiratory distress syndrome (ARDS). Because liposome-encapsulated prostaglandin E1 (PGE1) downregulates the CD11/CD18 receptor of the neutrophil, thereby limiting endothelial adhesion, the use of this drug should decrease the excretion of H₂O₂ in the expiratory condensate of patients with ARDS. Patients > 11 yr of age with ARDS (diffuse, patchy infiltrates by chest radiograph; Pao₂/fraction of inspired oxygen [P/F] ratio < or = 200 mm Hg; pulmonary capillary wedge pressure < or = 18 mm Hg; and the requirement for mechanical ventilation) were randomized to receive placebo (n = 14) or escalating doses (0.15-3.6 micrograms/kg) of liposomal PGE1 (n = 14) every 6 h for up to 7 days. Condensate was collected every morning from the expiratory tubing that was submerged in an ice

saltwater bath (-5 degrees C). H₂O₂ levels were measured by using a horseradish peroxidase assay. Other data collected included white blood cell count and P/F ratios. There was no significant difference in the concentration of H₂O₂ in the expiratory condensate between the liposomal PGE1 group and the control group either before (0.99 +/- 0.52 vs 0.93 +/- 0.48 mumol/L) or during treatment (1.04 +/- 0.45 vs 0.76 +/- 0.25 mumol/L). Liposomal PGE1 treatment improved the P/F ratio and decreased the white blood cell count over time. Despite its ability to downregulate the CD11/CD18 neutrophil receptor, liposomal PGE1 did not reduce exhaled H₂O₂ excretion. Implications: White blood cells (WBC) are thought to be part of the cause of the acute respiratory distress syndrome, a lung disease. WBC in the lung produce hydrogen peroxide, which is exhaled. Liposomal PGE1 inhibits WBC function but was found to have no effect in decreasing exhaled hydrogen peroxide in patients with the acute respiratory distress syndrome.

180. Dzhangozina, D. M., G. A. Kulkybaev, and B. M. Salimbaeva.

[Parameters of oxidative metabolism, neuro-humoral and hormonal regulation in the condensed exhaled air in early stages of anthracosilicosis].

Med Tr Prom Ekol(8):13-6. 1999.

For early diagnosis of anthracosilicosis, the authors studied oxidation parameters, neuromediators and hormones in expired air condensate in coal miners. Premorbid stage of anthracosilicosis is characterized by activated lipids peroxidation, high levels of neuromediators (acetylcholine, catecholamines), tissue hormones (histamine, serotonin) and lower contents of adaptive hormones (cortisol, triiodothyronine, thyroxine).

181. Prokhorova, M. N., T. S. Briuzgina, T. R. Umanets, V. Sokolova Ia, and S. N. Reva.

[The use of noninvasive biological means in assessing lipids in children].

Klin Lab Diagn(7):13-5. 1998.

Gas chromatographic analysis of the lipid fatty acid spectrum of blood (serum and plasma), sweat, and exhaled air condensate in children with acute pneumonia and dermatitis caused by alimentary allergies showed good correlation of the results. Therefore, biological objects obtained by noninvasive methods can be used for studies of lipid metabolism in children.

182. Loukides, S., I. Horvath, T. Wodehouse, P. J. Cole, and P. J. Barnes.

Elevated levels of expired breath hydrogen peroxide in bronchiectasis.

Am J Respir Crit Care Med 158(3):991-4. 1998.

Airway inflammation is important in the development and progression of many lung diseases, including bronchiectasis. Activation of inflammatory cells such as neutrophils, eosinophils, and macrophages induces a respiratory burst resulting in the production of reactive oxygen species such as hydrogen peroxide (H₂O₂). We have measured exhaled H₂O₂ in patients with documented bronchiectasis and investigated whether the concentration of H₂O₂ is related to the disease severity, as defined by lung function. We also investigated whether the concentrations of expired H₂O₂ were different in bronchiectatic patients who received inhaled corticosteroids compared with steroid-naive patients. In 37 patients with bronchiectasis (mean age, 45 +/- 2.5 yr; FEV₁, 59 +/- 3% pred), mean H₂O₂ concentration in exhaled breath condensate was significantly elevated as compared with the values in 25 age-matched (mean age, 42 +/- 2 yr) normal subjects (0.87 +/- 0.01 versus 0.26 +/- 0.04 microM, p < 0.001). There was a significant negative correlation between H₂O₂ and FEV₁ (r = -0.76, p < 0.0001). Patients treated with inhaled corticosteroids had values of H₂O₂ similar to those of steroid-naive patients (0.8 +/- 0.1 versus 0.9 +/- 0.1, p > 0.05). We conclude that H₂O₂ is elevated in exhaled air condensate of patients with bronchiectasis and is correlated with disease severity. Measurement of H₂O₂ may be used as a simple noninvasive method to monitor airway inflammation and oxidative stress.

183. Khyshiktueva, N. A., and B. S. Khyshiktuev.

[Prenatal diagnosis of fetal hypoxia based on lipid peroxidation values in exhaled air condensate].

Klin Lab Diagn(1):21-2. 1998.

Lipid peroxidation values were measured in the serum and expired air of pregnant women. The levels of diene conjugates, secondary intermediates of free-radical processes, and malonic dialdehyde were increased in exhaled air condensate of pregnant women who gave birth to babies with grave hypoxia, as against women with healthy babies or babies with slight hypoxia. All the studied values were more demonstrative in the expirate than in the serum. Hence, grave fetal and neonatal hypoxia can be diagnosed before delivery by examining the exhaled air condensate of pregnant women.

184. Jobsis, Q., H. C. Raatgeep, S. L. Schellekens, W. C. Hop, P. W. Hermans, and J. C. de Jongste.

Hydrogen peroxide in exhaled air of healthy children: reference values.

Eur Respir J 12(2):483-5. 1998.

An increased content of hydrogen peroxide (H₂O₂), a marker of inflammation, has been described in the condensate of exhaled air from adults and children with inflammatory lung disorders, including asthma. However, the normal range of [H₂O₂] in the exhaled air condensate from healthy children has not been established. Therefore, the aim of this study was to determine the reference range of exhaled [H₂O₂] in healthy school-aged children. Ninety-three healthy nonsmoking children (48 female and 45 male, mean age 10 yrs, range 8-13 yrs), with a negative history for allergy, eczema or respiratory disease and with a normal lung function, participated. Exhaled air condensate was examined fluorimetrically for the presence of H₂O₂. In addition, the reproducibility of [H₂O₂] within subjects and between days and the stability of [H₂O₂] during storage at -20 degrees C were assessed. The median [H₂O₂] in the exhaled air condensate of all children was 0.13 microM, with a 2.5-97.5% reference range of <0.01-0.48 microM. No significant difference existed between males and females. There was no correlation between exhaled [H₂O₂] and age or lung function. Repeated [H₂O₂] measurements on 2 consecutive days showed satisfactory within-subject reproducibility and [H₂O₂] in stored samples remained stable for at least 1 month at -20 degrees C. In conclusion, this study provides reference data for exhaled hydrogen peroxide in a large group of healthy children. The observed levels were lower than those reported previously for healthy adults and were independent of age, sex and lung function.

185. Ignatova, G. L., I. A. Volchegorskii, E. G. Volkova, E. L. Kazachkov, and O. L. Kolesnikov.

[Lipid peroxidation processes in chronic bronchitis].

Ter Arkh 70(3):36-7. 1998.

AIM: Comparison of the levels of lipid peroxidation (LPO) products in condensate of the exhaled air (CEA) and in the biopsy samples from the inflammation focus. MATERIALS AND METHODS: Extraction spectrophotometry was used to measure LPO products in CEA and biopsies from 30 males aged 30-60 years suffering from chronic bronchitis and 30 healthy controls of the same age. RESULTS: There was activation of local accumulation of isopropanol-soluble LPO products in the bronchopulmonary system accompanied by lowered content of lipoperoxides and high antioxidant activity in CEA. CONCLUSION: Chronic bronchitis is characterized by multidirectional shifts in LPO in the inflammation focus and CEA.

186. Howes, D., R. Wilson, and C. T. James.

The fate of ingested glyceran esters of condensed castor oil fatty acids [polyglycerol polyricinoleate (PGPR)] in the rat.

Food Chem Toxicol 36(9-10):719-38. 1998.

Samples of the emulsifier polyglycerol polyricinoleate (PGPR) were synthesized using the radiolabelled precursors [1-¹⁴C]glycerol ([¹⁴C]polyglycerol PGPR), [9,10-³H] or [12-³H]ricinoleic acid ([³H] PGPR) or [1-¹⁴C]stearic acid ([¹⁴C]stearyl PGPR). The absorption, tissue distribution, metabolism and excretion of these ¹⁴C- or tritium-labelled PGPR samples administered to rats was studied. The effects of intestinal and porcine pancreatic lipases on PGPR preparations were examined. Rats were dosed with [1-¹⁴C]glycerol, [¹⁴C]polyglycerol and ([¹⁴C]polyglycerol)PGPR by gavage and their urine, faeces and expired CO₂ monitored for ¹⁴C. The results from the [1-¹⁴C]glycerol treated animals showed extensive metabolism of glycerol. For [¹⁴C]polyglycerols, the lower polyglycerols were preferentially absorbed from the intestine and were excreted unchanged in the urine while the higher polyglycerols were found in the faeces. After 4 days, 93% of the dose of polyglycerols was recovered, of which some 30% was found in the urine and 60% in the faeces. Traces of ¹⁴C activity were found in depot fat and liver. The excretory pattern and urinary metabolites from ([¹⁴C]polyglycerol) PGPR was very similar to that of [¹⁴C]polyglycerol. Analysis of urinary and faecal ¹⁴C material indicated that the PGPR polymer was digested to give free polyglycerol and polyricinoleic acid. PGPR was synthesised incorporating [1-¹⁴C]stearic into polyricinoleic acid which was then esterified with polyglycerol. The resulting [¹⁴C]PGPR or [1-¹⁴C] stearic acid in a dietary slurry was administered to groups of fed or starved rats by gavage. The results indicated complete digestion of PGPR and absorption of the fatty acids. The ¹⁴C-material absorbed was extensively laid down in depot fat and some metabolism to ¹⁴CO₂ was demonstrated. The fate of the stearic acid was similar whether dosed alone or incorporated into the PGPR polymer. Samples of PGPR were synthesized containing ³H-labelled ricinoleic acid. The resulting [³H]PGPR was intubated into rats as a component of a dietary slurry. The results indicated that the polymer is extensively digested and 90% of the administered tritium is absorbed. The absorbed material was extensively metabolized within 24 hr so that large amounts of tritium were present in the aqueous phase of the tissues examined. After 24 hr, less than 5% of the administered material was present as lipid material, of which a large proportion was as non-hydroxy fatty acids. No traces of polymer material were found in the tissues examined. In vitro digestion of PGPR by porcine pancreatic lipase and rat intestinal fractions was demonstrated. The results indicate very extensive digestion of the PGPR polymer to polyglycerols and fatty acids. The fatty acids are metabolized extensively. The mono-, di- and triglycerols are extensively absorbed from the intestinal tract and rapidly excreted in the urine unchanged but the hexa-, penta- and higher

polyglycerols are essentially not absorbed and excreted in the faeces unchanged.

187. Ho, L. P., J. A. Innes, and A. P. Greening.

Nitrite levels in breath condensate of patients with cystic fibrosis is elevated in contrast to exhaled nitric oxide.

Thorax 53(8):680-4. 1998.

BACKGROUND: Nitric oxide (NO) is released by activated macrophages, neutrophils, and stimulated bronchial epithelial cells. Exhaled NO has been shown to be increased in patients with asthma and has been put forward as a marker of airways inflammation. However, we have found that exhaled NO is not raised in patients with cystic fibrosis, even during infective pulmonary exacerbation. One reason for this may be that excess airway secretions may prevent diffusion of gaseous NO into the airway lumen. We hypothesised that exhaled NO may not reflect total NO production in chronically suppurative airways and investigated nitrite as another marker of NO production. **METHODS:** Breath condensate nitrite concentration and exhaled NO levels were measured in 21 clinically stable patients with cystic fibrosis of mean age 26 years and mean FEV1 57% and 12 healthy normal volunteers of mean age 31 years. Breath condensate was collected with a validated method which excluded saliva and nasal air contamination and nitrite levels were measured using the Griess reaction. Exhaled NO was measured using a sensitive chemiluminescence analyser (LR2000) at an exhalation rate of 250 ml/s. Fourteen patients with cystic fibrosis had circulating plasma leucocyte levels and differential analysis performed on the day of breath collection. **RESULTS:** Nitrite levels were significantly higher in patients with cystic fibrosis than in normal subjects (median 1.93 microM compared with 0.33 microM). This correlated positively with circulating plasma leucocytes and neutrophils ($r = 0.6$). In contrast, exhaled NO values were not significantly different from the normal range (median 3.8 ppb vs 4.4 ppb). There was no correlation between breath condensate nitrite and lung function and between breath condensate nitrite and exhaled NO. **CONCLUSIONS:** Nitrite levels in breath condensate were raised in stable patients with cystic fibrosis in contrast to exhaled NO. This suggests that nitrite levels may be a more useful measure of NO production and possibly airways inflammation in suppurative airways and that exhaled NO may not reflect total NO production.

188. Gichka, S. G., T. S. Briuzgina, and S. N. Reva.

[The gas chromatographic analysis of the fatty acids in the expired air in ischemic heart dis].

Klin Lab Diagn(11):5-6. 1998.

Lipids of exhaled air condensate (EAC) and sweat are analyzed by gas chromatography in coronary patients with myocardial infarction complicated by lung edema. Changes of lipid fatty acid spectrum in EAC and sweat were uniform: the levels of linoleic and arachidonic acids were increased, which can aggravate the disease and lead to complications.

189. Gessner, C.

[Detection of mutations of the K-ras gene in condensed breath of patients with non-small-cell lung carcinoma (NSCLC) as a possible noninvasive screening method].

Pneumologie 52(7):426-7. 1998.

190. Carpenter, C. T., P. V. Price, and B. W. Christman.

Exhaled breath condensate isoprostanes are elevated in patients with acute lung injury or ARDS.

Chest 114(6):1653-9. 1998.

BACKGROUND: Oxidant stress is a purported mechanism of tissue damage in patients with ARDS and acute lung injury (ALI). Isoprostanes, prostanoid compounds primarily formed nonenzymatically via lipid peroxidation, are precise markers of in vivo oxidant stress. Plasma levels of metabolites of 8-iso-prostaglandin-F2alpha (8-iso-PGF2alpha) correlate with outcome in patients with ARDS. **OBJECTIVE:** To examine exhaled breath condensate levels of 8-iso-PGF2alpha as a noninvasive quantification of pulmonary oxidant stress in patients with, or at risk for, ARDS/ALI. **METHODS:** Breath condensate was collected from 22 patients with, or at risk for, ARDS/ALI by placing Tygon tubing submerged in an ice bath in line with the expiratory limb of the ventilator circuit. Ten patients without lung disease, who were intubated while undergoing minor surgical procedures, served as control subjects. Between 1 and 3 mL of condensate was collected over a 30- to 60-min period, then immediately frozen and stored at -70 degrees C until analysis. The 8-iso-PGF2alpha was purified and derivatized, then quantified by stable isotope dilution in conjunction with gas chromatography/mass spectrometry. **RESULTS:** The mean level of exhaled 8-iso-PGF2alpha in the patients with ALI/ARDS, 87 +/- 28 pg/mL, was significantly higher than the mean in the normal group, 7 +/- 4 pg/mL ($p = 0.007$). The 8-iso-PGF2alpha levels were greater than two standard deviations above the mean of the normal group in 12 of 22 patients with or at risk for ARDS/ALI. **CONCLUSIONS:** These results provide further evidence that lipid peroxidation does occur in patients with ARDS/ALI. The measure-

ment of exhaled isoprostanes provides a novel, noninvasive method to quantify oxidant stress in such patients.

191. Sterk, P.J.

Non-invasive monitoring of bronchial inflammation in asthma.

Schweiz Med Wochenschr 127(41):1686-92. 1997.

The present consensus on the diagnosis and treatment of asthma relies on symptoms and lung function measurements for the monitoring of disease severity. Even though this probably remains the cornerstone of asthma management, the rapidly increasing insight into the pathogenesis and pathophysiology of the disease is presently leading to the development of more direct measurements of airway inflammation, which may provide potentially relevant information on its clinical course and prognosis. However, at present none of these has sufficiently been validated for current use in monitoring patients with asthma. First, there are new ways of looking at symptoms and lung function. Careful measurements of symptoms by visual analogue scale (VAS) are suggesting that inflammatory activity within the airways can be subjectively perceived, a sensitivity which may be blunted in patients with brittle asthma. In addition, modern physiological parameters, such as the degree of bronchodilatation following a deep breath (M/P-ratio), are strongly associated with airway inflammation. Second, there are multiple cellular and/or soluble markers of inflammation in peripheral blood (using PCR, in situ hybridization, flow cytometry, or circulating mediators and cytokines) and in urine (LTE₄, EPX). Recently this has been extended by similar measurements in hypertonic saline-induced sputum (cell differentials and specific stainings on cytopins, flow cytometry, and levels of e.g. ECP, IL-5, IL-8). Finally, mediators and cytokines in the condensate of exhaled air (H₂O₂, leukotrienes, IL-5?) as well as exhaled NO are currently under evaluation. Adding such markers of airway inflammation as guides in asthma therapy is potentially useful. As a first step towards such a new approach we have recently shown that adding the reduction of airway hyperresponsiveness to the aims of asthma therapy leads to a better clinical as well as histological outcome after two years of treatment. In conclusion, there are new and exciting perspectives in the monitoring of disease severity in asthma in the future. Longitudinal studies presently ongoing will elucidate which parameter is potentially most useful in guiding asthma management.

192. Jobsis, Q., H. C. Raatgeep, P.W. Hermans, and J. C. de Jongste.

Hydrogen peroxide in exhaled air is increased in stable asthmatic children.

Eur Respir J 10(3):519-21. 1997.

Exhaled air condensate provides a noninvasive means of obtaining samples from the lower respiratory tract. Hydrogen peroxide (H₂O₂) in exhaled air has been proposed as a marker of airway inflammation. We hypothesized that in stable asthmatic children the H₂O₂ concentration in exhaled air condensate may be elevated as a result of airway inflammation. In a cross-sectional study, 66 allergic asthmatic children (of whom, 41 were treated with inhaled steroids) and 21 healthy controls exhaled through a cold trap. The resulting condensate was examined fluorimetrically for the presence of H₂O₂. All subjects were clinically stable, non-smokers, without infection. The median H₂O₂ level in the exhaled air condensate of the asthmatic patients was significantly higher than in healthy controls (0.60 and 0.15 micromol, respectively; $p < 0.05$), largely because of high values in the stable asthmatic children who did not use anti-inflammatory treatment (0.8 micromol; $p < 0.01$ compared to controls). We conclude that hydrogen peroxide is elevated in exhaled air condensate of children with stable asthma, and may reflect airway inflammation.

193. Iatsenko, V. P., T. S. Briuzgina, V. E. Khomenko, and S. N. Reva.

[Gas chromatographic analysis of lipids in exhaled air condensate in children with bronchopulmonary diseases].

Klin Lab Diagn(4):16-7. 1997.

The lipid fatty-acid spectrum in exhaled air condensate (EAC) was analyzed in children with bronchopulmonary diseases by gas chromatography. The detected shifts in the fatty-acid composition of EAC lipids may be used for validating a therapeutic approach and monitoring the treatment efficacy.

194. Becher, G., K. Winsel, E. Beck, G. Neubauer, and E. Stresemann.

[Breath condensate as a method of noninvasive assessment of inflammation mediators from the lower airways].

Pneumologie 51 Suppl 2:456-9. 1997.

The detection of mediators from the lower airways still depends on invasive or provoking sampling techniques like bronchoalveolar lavage (BAL) or induced sputum, respectively. Both methods affect the specimen itself. In contrast, the breathing condensate opens the possibility to get native specimens from lower airways during breathing at rest. The breathing condensate was obtained by freezing of exhaled air. The equipment was developed in the FILT Res. Soc. Ltd. The method is applied for a patent. Leukotriene

B4 and Leukotriene C4D4E4F4 were measured in the exhalation of asthmatics, patients with different airway disorders and healthy volunteers. In an additional study the condensate was obtained before and after of a non-specific bronchial challenge test. In asthmatics a close correlation between leukotriene concentration of the condensate and the degree of asthmatic disease according to "International Consensus Report" was found, but no correlation to lung function tests. Within a bronchial challenge test applying histamine the release of leukotrienes was shown to be more sensitive to the challenge test than a lung function test. The results of the study indicate new diagnostic possibilities in lung diseases using the detection of non volatile substances in the exhaled air.

195. Komar, S. I., E. N. Korobeinikova, and E. V. Evdokimova.
[Lipids in the exhaled air condensate of pneumonia patients].
Klin Lab Diagn(6):24-7. 1996.

Lipid composition of exhaled air condensate (EAC) was assessed by thin layer chromatography in patients with pneumonia and normal subjects. In parallel with this, diene conjugates and diene ketones were assessed by spectrophotometry. Berhan's principle was for the first time used for noninvasive obtaining of EAC from each lung separately. Decreased percentage of phospholipids and increased level of free fatty acids in EAC of patients with massive pneumonia may be an evidence of disordered pulmonary surfactant, and an increase of the intermediate products of lipid peroxidation indicate the cause of these changes.

196. Khyshiktuev, B. S., N. A. Khyshiktueva, and V. N. Ivanov.
[Methods of measuring lipid peroxidation products in exhaled air condensate and their clinical significance].
Klin Lab Diagn(3):13-5. 1996.

The authors propose simple, sufficiently sensitive, and reproducible methods for measuring lipid peroxidation products in exhaled air condensate. The content of diene conjugates, ketodienes, and conjugated trienes in the isopropanol phase of lipid extract is recommended to be measured. The methods were tried in patients with chronic nonspecific pulmonary diseases and lung cancer and proved to be valuable for the differential diagnosis of malignant and nontumorous processes in the respiratory organs.

197. Khyshiktuev, B. S., N. A. Khyshiktueva, and S. D. Darenskaia.
[The effect of surgery on free-radical reactions in patients with lung cancer].
Vopr Onkol 42(6):23-7. 1996.

The effect of surgery on free-radical reactions in patients with lung cancer was studied vis-a-vis extent of intervention. To this end, blood components and exhaled air condensate were monitored. Surgery was found to aggravate the imbalance in the "lipid peroxidation-antioxidants" system in those operated on. The data obtained make a case for application of antioxidants in complex treatment of lung cancer. Said parameters were evaluated versus incidence of early postoperative lung-pleura complications. Pre-operative criteria for prognosis of postoperative complications have been worked out.

198. Groves, W. A., and E. T. Zellers.
Investigation of organic vapor losses to condensed water vapor in Tedlar bags used for exhaled-breath sampling.
Am Ind Hyg Assoc J 57(3):257-63. 1996.

This study evaluated the potential loss of organic vapors to condensed water in bags used for breath sampling. "Wet" test atmospheres were prepared by transferring nitrogen into 1-L Tedlar bags and spiking with water and each of several organic solvents (methanol, acetone, 2-butanone, m-xylene, 1,1,1 trichloroethane, and perchloroethylene) to yield atmospheres representative of a 500-mL breath sample collected at 37 degrees C and cooled to 25 degrees C. Vapor concentrations were compared with those in bags prepared without water. Differences between mean concentrations in wet and dry bags were significant only for methanol, which yielded a mean wet-bag concentration approximately 10% lower than for dry bags. In a second series of experiments the same initial concentration of solvent vapor was generated in 1 dry bag and 5 bags containing 1 to 20 times the amount of water expected to condense in a 500-mL breath sample. Significant differences between wet and dry bag concentrations were seen for methanol, acetone, and 2-butanone; however, the quantity of water required to produce this difference in the acetone and 2-butanone bags exceeded three times that expected to condense in a breath sample. Results were in good agreement with predictions based on Henry's law. In both experiments permeation of water vapor out of the bags led to a decrease in the quantity of condensed water, thereby reducing the extent of organic vapor partitioning. These results indicate that solvent vapor loss to condensed water is not likely to be significant under typical conditions.

199. Goncharova, V. A., L. V. Borisenko, E. K. Dotsenko, and M. A. Pokhaznikova.

[Kallikrein-kinin indices and biological composition of exhaled condensate in acute bronchitis patients with varying disease course].

Klin Med (Mosk) 74(7):46-8. 1996.

Parameters of blood kallikrein-kinin system (KKS) and biochemical composition of humor condensated from the exhaled air have been evaluated in 87 patients with acute bronchitis (36 cases with lingering course and 31 with recurrent). The progression of acute bronchitis is associated with elevated blood concentrations of acute-phase proteins, KKS activation in the blood and high serotonin and lactic acid content in the humor condensated from the exhaled air. Lingering and recurrent course of the disease are characterized by more pronounced reduction in inhibitory activity of kallikrein, in activity of angiotensin-converting enzyme and emergence of noradrenalin in high concentration.

200. Dekhuijzen, P. N., K. K. Aben, I. Dekker, L. P. Aarts, P. L. Wielders, C. L. van Herwaarden, and A. Bast.

Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease.

Am J Respir Crit Care Med 154(3 Pt 1):813-6. 1996.

An imbalance between oxidative stress and antioxidative capacity is thought to play an important role in the development and progression of chronic obstructive pulmonary disease (COPD). To assess the lung oxidative status in patients with COPD, we studied whether exhaled hydrogen peroxide (H₂O₂) is increased in breath condensate of patients with stable COPD (n = 12, mean FEV₁ 51% pred) and in patients with exacerbated COPD (n = 19, actual FEV₁ 36% pred) compared with a healthy control group (n = 10, FEV₁ 108% pred). Expired breath condensate during 15 min of tidal breathing was collected by cooling. The concentration of H₂O₂ was measured spectrophotometrically by means of horse radish peroxidase-catalyzed oxidation of tetramethylbenzidine. Concentrations of H₂O₂ (mean +/- SEM) were significantly elevated at 0.205 +/- 0.054 microM in patients with stable COPD compared with 0.029 +/- 0.012 microM in the control group (p < 0.05) and were further increased to 0.600 +/- 0.075 microM in patients with acutely exacerbated COPD (p < 0.001 compared with patients with stable COPD). Patients with pulmonary infiltrates on chest radiograph showed similar values compared with patients without obvious infiltrates. These findings demonstrate that patients with stable COPD exhibit increased oxidant production in the airways and that oxidant production increases further during exacerbations.

201. Hunt, J., R. E. Byrns, L. J. Ignarro, and B. Gaston.

Condensed expirate nitrite as a home marker for acute asthma.

Lancet 346(8984):1235-6. 1995.

202. Emel'ianov, A. V., and V. I. Trofimov.

[Effects of glucocorticoid therapy on mineral metabolism indicators in patients with bronchial asthma].

Klin Med (Mosk) 73(2):23-5. 1995.

Magnesium and calcium levels in the serum, red cells, lymphocytes, circadian urine and exhaled air condensate were measured in 40 patients with hormone-dependent bronchial asthma (BA) and 15 BA patients receiving glucocorticoids in inhalations (beclamete, becotide) and injections (hydrocortisone, prednisolone, dexasone). Nuclear absorption spectrophotometry (AAC-508, Hitachi, Japan) and selective ionometry (Mycrolyte, Kone, Finland) were employed. Short-term (10-14 days) intravenous infusions and inhalations of steroids in medium therapeutic doses do not change much homeostasis of two-valent cations. Complications resultant from disturbance of mineral metabolism developed more frequently in BA patients on long-term (4 years more longer) oral glucocorticoids. Increased renal and bronchial excretion of magnesium and calcium in patients with hormone-dependent BA having complications of glucocorticoid therapy suggests that derangement of mineral metabolism is involved in the onset of steroid osteopathy and myopathy.

203. Emel'ianov, A. V., M. A. Petrova, O. V. Lavrova, L. I. Guleva, A. F. Dolgodvorov, and G. B. Fedoseev.

[Disorders in mineral metabolism at different stages of the development of bronchial asthma].

Ter Arkh 67(8):45-7. 1995.

Magnesium and calcium concentrations were measured by nuclear-absorption spectrometry (AAC-508, Hitachi, Japan) and selective ionometry (Mycrolyte, Kone, Finland) in the serum, red cells, lymphocytes, 24-h urine and exhaled air humor condensate in 23, 31 and 23 sibs (parents and their children) with preasthma, atopic bronchial asthma, free of these diseases, respectively. All the patients had magnesium deficiency and calcium hyperconcentrations in the blood cells, low urine excretion of magnesium. Para-

thormone and calcitonin serum concentrations underwent no changes. It is suggested that in contrast to changes of endobronchial homeostasis of bivalent cations, those of electrolyte cell composition are primary and appear prior to clinical manifestations of bronchial asthma.

204. Khyshiktyev, B. S., N. A. Khyshiktueva, V. N. Ivanov, S. D. Darenskaia, and S. V. Novikov.

[Diagnostic value of investigating exhaled air condensate in lung cancer].

Vopr Onkol 40(4-6):161-4. 1994.

Parameters of lipid peroxidation and the levels of calcium ions in blood plasma and exhaled air condensate (EAC) were studied in 47 patients with lung cancer. The changes in EAC turned out to be more specific than those of blood plasma. The level of lipoperoxidation in condensate in cancer patients was lower than in healthy subjects. However, calcium cations concentrations in both blood and EAC were relatively higher, the latter difference being significant. The investigation pointed to a greater diagnostic value of EAC measurements, as compared with blood plasma, in lung cancer patients.

205. Scheideler, L., H. G. Manke, U. Schwulera, O. Inacker, and H. Hammerle.

Detection of nonvolatile macromolecules in breath. A possible diagnostic tool?

Am Rev Respir Dis 148(3):778-84. 1993.

The analysis of parameters in bronchoalveolar extracellular lining secretions has come into greater use in the diagnosis of diseases of the lung and respiratory passages. The bronchoalveolar lavage (BAL) method is thus used for sampling alveolar fluids or bronchial secretions. However, this method is invasive and therefore cannot be routinely employed for probe sampling. Based on the hypothesis that aerosol particles excreted in human breath reflect the composition of the bronchoalveolar extracellular lining fluid, experiments were performed to concentrate and analyze these aerosols directly using a noninvasive technique. Human exhaled air was directed through a set of cool traps and the condensate of 200 to 400 exhalations examined for nonvolatile components, such as proteins. In experiments conducted with volunteers, the amount of proteins in the breath condensate of 8 healthy individuals (of a total of 10) amounted to between 4 micrograms and 1.4 mg. The proteins were separated by two-dimensional polyacrylamide gel electrophoresis (PAGE) and compared to saliva samples of the respective volunteers. The results suggest that the proteins detected in breath originate partially from the naso-oropharyngeal tract and partially from lower regions of the airways. In clinical tests, the exhaled air of 13 patients suffering from various diseases of the respiratory tract was sampled and analyzed by immunoassays for inflammation parameters, such as interleukin-1 beta (IL-1 beta), soluble interleukin-2 receptor protein, light chain (sIL-2R), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-alpha). In these tests, up to 370 pg IL-1 beta, 120 pg TNF-alpha, and 2,159 U sIL-2R per ml were measured in the breath condensate. (ABSTRACT TRUNCATED AT 250 WORDS)

206. Wilson, W. C., J. F. Swetland, J. L. Benumof, P. Laborde, and R. Taylor.

General anesthesia and exhaled breath hydrogen peroxide.

Anesthesiology 76(5):703-10. 1992.

To study the role of free radical formation on the impairment of pulmonary function seen with general anesthesia, we measured the hydrogen peroxide (H₂O₂) concentration in the exhaled breath condensate of 27 patients. Patients were divided into three study groups: a healthy patient group (group 1, n = 15) consisting of ASA physical status 1 and 2 patients undergoing elective noncardiothoracic surgery; a specific anesthetic event group (group 2, n = 6) composed of patients undergoing cardiopulmonary bypass (CPB); and a positive control group (group 3, n = 6) consisting of patients with the adult respiratory distress syndrome (ARDS). The exhaled breath condensate was collected by diverting exhaled breath through a glass condensation coil submerged in an ice/salt water bath. The exhaled breath condensate samples were then assayed using a spectrophotometric method. In group 1, samples were collected before and after the induction of general anesthesia with intravenous drugs, and before and after the administration of the inhalational anesthetics isoflurane (1.5%) (n = 7) or N₂O (70%) (n = 8). In group 2, samples were collected pre- and post-CPB, and in group 3, when specific diagnostic criteria for ARDS were met. There was no significantly detectable H₂O₂ (not significantly different from zero) in any of the samples from the group 1 patients. Similarly, group 2 patients had exhaled breath H₂O₂ concentrations near zero except for one patient who was positive for the lupus anticoagulant. Group 3 patients had a mean (+/- SE) exhaled breath H₂O₂ concentration of 0.55 (+/- 0.08) microM, which was significantly greater than zero (P less than 0.001). (ABSTRACT TRUNCATED AT 250 WORDS)

207. von Pohle, W. R., J. D. Anholm, and J. McMillan.

Carbon dioxide and oxygen partial pressure in expiratory water condensate are equivalent to mixed expired carbon dioxide and

oxygen.

Chest 101(6):1601-4. 1992.

This study was to determine whether the PCONCO₂ and PCONO₂ which collect in the expiratory trap of a ventilator circuit are equivalent to PECO₂ and PEO₂. Fifty studies were performed in 34 mechanically ventilated male patients. Five milliliters of condensate fluid were collected and PECO₂ and PEO₂ were measured. Exhaled gases were collected simultaneously with condensate fluid for 5 min in a meteorologic balloon and FECO₂ and FEO₂ were measured; PECO₂ and PEO₂ were then calculated. The mean PECO₂ was not significantly different from PCONCO₂ nor was the PCONO₂ significantly different from the condensate PCONO₂. There was a high correlation between mixed expired PECO₂ and PCONCO₂ as well as PEO₂ and PCONO₂. These data indicate expiratory PCONCO₂ and PCONO₂ provide a valid reflection of PECO₂ and PEO₂. The PCONCO₂ and PCONO₂ measured in a clinical blood gas analyzer are accurate and may be used in calculation of VD/VT and in metabolic assessments.

208. Aleksandrov, O. V., O. V. Dobrynina, O. A. Sevrunova, I. S. Ezhova, and S. P. Grigor'ev.

[A method for research on pulmonary surfactant in the vapor condensate of exhaled air].

Ter Arkh 64(10):105-7. 1992.

209. Rybakova, E. V., V. M. Sidel'nikov, T. S. Briuzgina, and E. Kravchenko.

[The fatty acid spectrum and free cholesterol level in exhaled air condensate].

Lab Delo (4):74-5. 1991.

Gas chromatographic measurement of fatty acid spectrum and free cholesterol level in exhaled air condensate of children suffering from asthma has revealed a deficiency of polyunsaturated fatty acids and lipids at the expense of linoleic and arachidonic acids.

210. Iakovleva, O. A., and E. V. Tertyshnaia.

[The effect of endogenous factors and physical loading on the formation of moisture excretion and detoxication by the lungs].

Ter Arkh 63(12):11-4. 1991.

Overall 101 students (a control group), 68 persons from an ambulatory group with exogenous factors at risk for pulmonary diseases, and 122 patients with exacerbation of nonspecific pulmonary diseases were examined for the volume of exhaled air condensate, the content in it of medium-weight molecules and protein by means of two methods (according to Lowry and by adsorption using polysorb). The students were found to have the worst parameters. Exacerbation of the disease led to their increase. Exercise blocked transport of water, protein and medium-weight molecules to the condensate, whose intensity depended on the age, sex and body mass. This stresses the importance of exogenous factors in the maintenance of moisture excretion and detoxication by the lungs. It is recommended that the new methodological approach to assessing the response to exercise according to moisture excretion function of the lungs may be applied.

211. Fedoseev, G. B., A. V. Emel'ianov, K. K. Malakauskas, V. A. Goncharova, T. M. Sinitsina, M. D. Didur, V. L. Emanuel, and L. V. Krunchak.

[The therapeutic potentials of magnesium sulfate in bronchial asthma].

Ter Arkh 63(12):27-9. 1991.

Twelve patients with preasthma (PA) and 27 bronchial asthma (BA) patients were examined for the effect of magnesium sulphate aerosol on bronchial sensitivity and reactivity to acetylcholine (ACC) and graded physical exercise (GPE) as well as on bronchial patency recorded by general plethysmography and pneumotachography. Inhalation of the drug was established to exert no bronchodilatory action but reduced, as compared with placebo, nonspecific hyperreactivity of the bronchial tree and blocked the ACC induced increment of histamine in the exhaled air condensate. The data obtained allow bronchial smooth muscles and mast cells to be regarded as targets for magnesium sulphate. The drug properties discovered may serve a prerequisite for its use in the combined treatment of BA patients and those with PA.

212. Chaialo, P. P., I. E. Kolpakov, G. M. Chobot'ko, E. I. Stepanova, and I. L. Dobrutskaia.

[Use of some biochemical and physico-chemical indices for the evaluation of lung function in healthy school-age children].

Pediatriia(6):42-5. 1991.

The biochemical and physicochemical characteristics of nonrespiratory pulmonary functions were determined in 255 children of school age according to the condensate of exhaled air. As compared to the younger children, those belonging to the senior

age groups manifested an increase of specific respiratory moisture loss. As compared to winter, specific respiratory moisture loss in summer was found to be decreased; the condensate of exhaled air was discovered to have a higher content of total protein, elevated concentration of surface active substances in the lung surfactant, and to show a certain tendency towards reduction of the intensity of the processes of free radical oxidation. No sex-associated differences were established between the characteristics under study.

213. Brimkulov, N. N.

[The alpine climatotherapy of bronchial asthma patients].

Ter Arkh 63(8):25-30. 1991.

As many as 132 patients with bronchial asthma were examined for the clinical, functional and laboratory parameters before and during alpine climatotherapy at a height of 3200 m above the sea level (the Tyuya-Ashu pass, the northern Tien Shan). In addition to the improvement of the general status, the patients manifested amelioration of ventilation and decreased responsiveness of the bronchial tree by the end of alpine climatotherapy. Favourable alterations in the immune parameters together with appreciable stimulation of steroidogenesis in the adrenals were discovered. Investigation into surface activity of the exhaled air condensate revealed activation of the surfactant system. These data and endoscopy with bronchoalveolar lavage confirmed the reduction of the inflammatory lesions in the tracheobronchial tree. Therefore, alpine climatotherapy produces a favourable effect on the main mechanisms of the disease development and can be used on a wider basis for the treatment of patients suffering from bronchial asthma.

214. Khyshiktuev, B. S., V. N. Ivanov, N. V. Solov'eva, and M. Z. Zhits.

[Indices of lipid peroxidation in the condensate of exhaled air--a simple and objective method of estimating injuries to the pulmonary surfactant system].

Lab Delo(5):18-22. 1990.

A method for assessing injury to the pulmonary surfactant system is suggested, based on studies of lipid peroxidation parameters in exhaled air condensate. Free-radical processes in pulmonary surfactants were examined in normal subjects and in patients with various forms of chronic nonspecific diseases of the respiratory organs. The findings evidence that a disease essentially intensifies lipid peroxidation in the alveolar lining, this being paralleled by reduced antiradical defense. Such an approach to studies of pulmonary surfactant system injuries is marked for its simplicity and atraumatism, this recommending it for mass clinical check-ups. Use of this method is promising as regards the disease prognosis and assessment of the treatment efficacy, as well as detection of subjects at high risk of developing bronchopulmonary diseases.

215. Goncharova, V. A., D. T. Mamedov, and E. K. Dotsenko.

[Biologically active substance levels in exhaled air from patients with pre-asthma and bronchial asthma].

Sov Med(5):22-4. 1989.

Acetylcholine, serotonin, and histamine levels have been measured in exhaled air humor condensate in 65 patients aged 18 to 60, 44 ones with bronchial asthma and 21 with preasthma. Nonuniform release of biogenic amines and acetylcholine in pulmonologic patients depended on the severity of the inflammatory process in the bronchi, hypoxemia, bronchial obstruction, and reactivity of the bronchi examined during cold air breathing.

216. Turnbull, T. L., R. G. Hart, G. R. Strange, M. A. Cooper, R. Lindblad, J. M. Watkins, and C. M. Ferraro.

Emergency department screening for unsuspected carbon monoxide exposure.

Ann Emerg Med 17(5):478-83. 1988.

Carbon monoxide (CO) is the leading toxic cause of death in the United States today. Unsuspected exposure to this gas will sometimes result in clinically significant, but undiagnosed, toxicity. A high incidence of such unsuspected exposures would make screening for these worthwhile among high-risk populations. We conducted a two-part study to determine the value of screening for unsuspected CO exposure in a population of patients presenting to an emergency department. The first part of our study involved the prospective screening of ED patients using CO breath analysis, regardless of their chief complaint. In the second part, COHGB levels of all patients who underwent arterial blood gas analysis during the study period were reviewed retrospectively. Of 1,038 patients screened by this combined approach, only 29 (2.8%) had abnormal CO breath readings and/or COHGB levels. Of a condensed subgroup of 152 patients defined retrospectively by chief complaint, eight (5.3%) had abnormal values. We conclude that routine screening of ED patients for unsuspected CO exposure is not practical. Although yield increases when patients are

screened in a more selective manner on the basis of chief complaint, such an increase still does not appear to justify the screening process.

217. Kurik, M. V., L. V. Rolik, N. V. Parkhomenko, L. I. Tarakhan, and N. V. Savitskaia.
[Physical properties of a condensate of exhaled air in chronic bronchitis patients].
Vrach Delo(7):37-9. 1987.

218. Iakovleva, O. A., A. P. Kuchuk, N. Baiak, and E. V. Zubko.
[Significance of exhaled air condensate in the evaluation of the fluid-secreting function of the lungs].
Vrach Delo(10):91-2. 1987.

219. Phillips, M., P. Hensley, R. A. Balter, and S. L. Cohan.
An improved method for collecting breath for the assay of acetaldehyde.
Alcohol Clin Exp Res 8(3):293-6. 1984.
An improved method is described for the collection of breath for the subsequent assay of acetaldehyde and other volatile components. Breath is collected in a Pyrex gas-collecting tube sealed at both ends with Teflon taps. Prior to collection or assay of the samples, this tube is heated to 72 degrees C; breath is sampled for assay by piercing a rubber septum on a sideport with the needle of a similarly heated gas-tight syringe, and injected into a gas chromatograph (GC). The advantages of this system are: (1) Avoidance of the artefacts encountered in the assay of acetaldehyde in the blood; (2) suitability for sample collection at a site remote from the GC laboratory; (3) avoidance of sample loss by leakage, contamination, or partitioning into water condensed from breath; and (4) compatibility with a "nondedicated" GC lacking any special gas-collecting circuitry. A typical study of a normal human volunteer is described, demonstrating the rise and fall of the concentration of acetaldehyde and ethanol in the breath following the ingestion of an oral dose of ethanol.

220. Christopher, K. L., L. D. Saravolatz, T. L. Bush, and W. A. Conway.
The potential role of respiratory therapy equipment in cross infection. A study using a canine model for pneumonia.
Am Rev Respir Dis 128(2):271-5. 1983.
Experimental *Pseudomonas aeruginosa* pneumonia was induced in 8 dogs that had radiation-induced leukopenia. Three dogs were supported by mechanical ventilation (MV), 3 received continuous heated aerosol therapy (CHAT), and 2 did not receive respiratory therapy and served as control animals. The animals were studied in a carefully controlled environment until they succumbed to infection or they were killed at 24 h. An air sampler was used to collect exhaled *P. aeruginosa* aerosols at distances as far as 15 feet from the dogs at multiple time intervals. Water condensate in the tubing of MV and CHAT equipment was collected and cultured at the same intervals. Results showed that all dogs had multilobar *P. aeruginosa* pneumonia at necropsy. Control dogs did not exhale aerosols containing *P. aeruginosa*. Animals that were supported by MV, exhaled contaminated aerosols, but organisms could not be recovered at distances greater than 2 feet. In contrast, aerosols containing *P. aeruginosa* were recovered at distances as far as 15 feet from the animals receiving CHAT. Furthermore, as much as 1L of water condensate was collected in a 24-h period from tubing associated with MV and CHAT. Although the nebulizers and humidifiers remained sterile, tubing condensate was contaminated with as much as 10(7) colony-forming units per ml of *P. aeruginosa* in 5 of the 6 animals receiving either MV or CHAT. Contamination of tubing by *P. aeruginosa* was present as early as 8 to 12 h. This study identifies potential sources for cross infection through an airborne route for CHAT or from direct contact with contaminated tubing.

221. Sidorenko, G. I., E. I. Zborovskii, and D. I. Levina.
[Surface-active properties of the exhaled air condensate (a new method of studying lung function)].
Ter Arkh 52(3):65-8. 1980.

222. Holzer, G., J. Oro, and W. Bertsch.
Gas chromatographic-mass spectrometric evaluation of exhaled tobacco smoke.
J Chromatogr 126:771-85. 1976.

The impact of cigarette smoking on the distribution of organic substances in ambient air has been determined for the intermedi-

ate volatility range. A simple sampling procedure was employed, involving gas-solid adsorption onto an organic polymer followed by direct thermal elution onto a glass capillary column. Aliphatic and substituted aromatic hydrocarbons are predominant in urban atmospheres. Depending on location and weather conditions the total concentration of such volatiles can differ by as much as a factor of 20. This high background variation makes it difficult to analyze for trace substances with low odor threshold values, such as encountered in cigarette smoke. Standard cigarettes were smoked in a relatively small room, having no air filtration system. Air samples of approximately 3.51 were taken. The amount of volatiles added to air by cigarette smoking is insignificant. Substances were analyzed and identified by gas chromatography and gas chromatography-mass spectrometry with glass capillary columns. Many compounds reported in cigarette smoke condensate have been confirmed.