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

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Summary

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.

Key words: asthma; chronic obstructive pulmonary disease; primary ciliary dyskinesia; chronic cough; rhinitis; exhaled nitric oxide; hydrocarbons; exhaled breath condensate; airway inflammation; oxidative stress; non-invasive monitoring; clinical practice

Introduction

There has recently been an explosion of interest in the analysis of breath constituents as a way of monitoring inflammation and oxidative stress in the lungs. Here we review whether this novel approach is ready for routine clinical use.

Many lung diseases, including asthma and chronic obstructive pulmonary disease (COPD), involve chronic inflammation and oxidative stress. Yet these are not measured directly in routine clinical practice because of the difficulties in monitoring inflammation [1]. This makes management of asthma, COPD and other lung diseases difficult, because it is based on indirect measurements of airway inflammation such as symptoms and lung function. Symptoms may not accurately reflect the extent of underlying inflammation due to differences in perception, and lung function tests may have little room for improvement in mild asthma. None of these parameters is able to distinguish the effect of different doses of inhaled corticosteroids and both may be affected by bronchodilators. The latter is particularly important because of a recent trend towards use of lower doses of inhaled corticosteroids in combination with long-acting β_2-agonists.

Current invasive (bronchoscopy) or semi-invasive (sputum induction) direct methods to measure airway inflammation are difficult to use repeatedly in clinical practice. The use of sputum induction is limited by its pro-inflammatory effect [2], and a considerable bronchospasm has been reported in moderate (14%) and severe (25%) asthma as the result of the procedure [3].

Non-invasive monitoring may assist in differential diagnosis of lung diseases, assessment of their severity and response to treatment. Because these techniques are completely non-invasive they can be used repeatedly to give information about kinetics, they can be used in patients with severe disease which has been previously difficult to monitor.

Breath analysis is currently a research procedure, but there is increasing evidence that it may have an important place in the diagnosis and management of asthma, COPD, primary ciliary dyskinesia (PCD) and other major lung diseases [4]. This will drive the development of cheaper and more convenient analysers which can be used in a hospital and later in a family practice setting, then eventually to the development of personal monitoring devices for use by patients.

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Exhaled NO

NO is the most extensively studied exhaled marker and abnormalities in exhaled NO have been documented in several lung diseases [4], particularly asthma [5] and in COPD [6, 7].

Standardised [8, 9] measurements of fractional exhaled NO (FE_{NO}) provide a completely non-invasive means of monitoring of airway inflammation and anti-inflammatory treatment in asthma [1]. The changes in serial FE_{NO}, as a loss-of-asthma-control-marker [5], have higher predictive values for diagnosing deterioration of asthma [10] than do single measurements [11, 12].

It can be argued, however, that these changes in FE_{NO} may be due to measurement error and/or the natural variability of airway inflammation over time. Therefore, the use of FE_{NO} in routine clinical practice depends greatly on reproducibility and safety of FE_{NO} measurements. Reproducibility of FE_{NO} measurements was studied by several groups, but either statistical analysis was inappropriate (correlation coefficient) [13], or exhalation flow rate was either not registered [14] or was different from the American Thoracic Society (ATS) Recommendations [15].

Measurement

Equipment for direct exhaled and nasal NO measurements

There are several major manufacturers making commercially available analysers for NO measurements in exhaled breath: NIOX® NO analyser (Aerocrine, Sweden, http://www.aerocrine.com/index.html), LR2000 analyser (Logan Research Ltd, Rochester, UK, http://www.logan-research.co.uk), ECO Physics NO analyser (ECOPHYSICS, Duernten, Switzerland, http://ic.net/~ecophys/index.htm) and Sievers® NO analyser (Ionics Instrument, Boulder, USA, http://www.ionicsinstruments.com/ionics/index.cfm?category_code=NOA).

The NO analyser systems currently used in clinical investigations vary in complexity, but are based on a sensitive chemoluminescence technique with the required accuracy. Most of the analysers consist of a sampling system, a computerised NO analyser with data processing, and user interface. The equipment measures the concentration of NO in sampled air online with high sensitivity in the parts per billion (ppb) range, as well as pressure and flow of the sampled air. The software calculates the concentration of NO during a selected time period, displays measured and calculated data on the monitor and saves the information on disk.

The NIOX® has been approved according to the Medical Device Directive (Ref. no. 41 313 149; Full Quality Assurance System Approval, Annex II of the Directive 93/42/EEC on Medical Devices). The equipment bears the CE marking of conformity. The NIOX® nasal application is intended for research use only.

Currently, NIOX® is the only NO analyser that has been cleared for marketing, a first-of-a-kind, non-invasive test system that measures the concentration of NO in exhaled human breath, in order to make it easier for doctors to monitor a patient's asthma [16].

Remote collection of FENO and nasal NO – a "balloon technique"

Levels of FE_{NO} in a sample of 450 children aged 7–12 years out of a total sample of 2504 school children living in different urban areas were recently determined by a simple "balloon technique" [17]. Exhaled air of the children was sampled in balloons using a sampling device equipped with a "pre-balloon" of 500 ml to exclude dead space volume from the sample, similar to the device used by other authors [18, 19]. In short, after exhalation at 4–6 L/min into a "pre-balloon", exhaled air was collected at a low flow rate of 500 ml/min at 20 cm H_2O back pressure in a foil bag of 1000 ml (Mylar balloon, ABC ballonnen, Zeist, The Netherlands). A low flow rate was chosen to obtain relatively high eNO concentrations, although some other authors have suggested that the ideal flow rate for children is between 30 and 50 ml/s [20], but the number of the studied healthy adolescent subjects was substantially lower (n = 32).

This off-line sampling in balloons may serve as a simple and, hence, attractive method for FE_{NO} oxide measurements in children which differentiates between groups with and without self-reported asthma, allergy and colds. It may also reinforce the power of epidemiological surveys on respiratory health [21].

Recently, a study to test the accuracy of an immediate and delayed off-line technique for measuring nasal NO by comparing it with on-line measurements has been conducted [22]. Comparison of the data using the Bland-Altman analysis has shown that off-line nasal NO measurements can be reliably used in clinical practice, thus obviating the need for patients/subjects to be in close proximity to the analyser.

It is still uncertain, however, if both off-line exhaled and nasal NO measurements can effectively substitute for on-line NO measurements when the latter technique is not practical. One of the serious limitations of this approach is the stability of NO in a balloon. Any bacteria colonizing the inner walls of a balloon can substantially increase the NO levels within a short period of time.

US Food and Drug Administration (FDA) clearance for NIOX® to monitor asthma

On 1st may 2003 the Food and Drug Administration (FDA) cleared for marketing a first-of-a-kind, non-invasive test system NIOX® (Aerocrine, Sweden) that measures the concentration of NO

in exhaled human breath, in order to make it easier for doctors to monitor a patient's asthma [16].

It has been stated that doctors can use the device in their office to evaluate their patient's response to anti-inflammatory treatment. A decrease in FE_{NO} concentration suggests that the anti-inflammatory treatment may be decreasing the lung inflammation associated with asthma. Alternatively, increased levels of FE_{NO} in the breath of people with asthma may indicate whether or not treatment for asthma is working.

FDA cleared the NIOX system based on clinical studies conducted by the manufacturer on 65 patients, both adults and children aged four years and older, with confirmed diagnoses of asthma. The patients were tested with the NIOX system before they began drug treatment and again two weeks later. The studies were conducted at nine medical centres in the United States. The results showed that most patients had a 30–70% decrease of nitric oxide levels after two weeks of treatment with inhaled steroids. In this study, elevated nitric oxide levels above 30 parts per billion correlated with moderate to severe asthma.

Quality control of exhaled and nasal NO measurements

Standardisation of exhaled NO measurements in adults and children

Expiratory flow [15, 23], soft palate closure [24], and dead space air may all influence exhaled NO levels. Therefore, exhaled NO is usually determined during single-breath exhalations against a resistance [8, 25] to prevent contamination with nasal NO.

In 1997, the European Respiratory Society Task Force on measurement of nitric oxide in fractional exhaled air published recommendations for fractional exhaled and nasal nitric oxide measurement procedures [8]. Two years later the American Thoracic Society and the Medical Section of the American Lung Association presented a document entitled "Recommendations for standardised procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children" [9].

Standardisation issues of exhaled NO measurements in children have been further addressed by both European and American Respiratory Societies [26], providing further evidence that exhaled NO measurements have definitely found their way into clinical research in paediatric respiratory medicine.

Standardisation of nasal NO measurements

Nasal application can be used to determine the concentration of NO in gas sampled from one nostril at a flow rate of 5 ml/s [9]. Our recent study on standardised nasal NO measurements using the NIOX® nasal application has demonstrated reproducible clinical results in both adults and children [27]. Nasal NO measured using an aspiration

Table 1

Reproducibility of different methods of airway inflammation assessment.

ICC	FE_{NO}	EBC	Bronchoscopy		Nasal lavage	Induced sputum (cells, supernatant)			PC_{20}	FEV_1
Interval			T	$CD_{45}Ro^+$		Eos	Neutrophils	Supernatant		
between the										
measurements										
10 minutes	0.96–0.99 (133)									
1 day	0.99 (30)		0.37–0.67 (134)		Eos 0.9 (35) ECP 0.2–0.6 (35)					0.87 (135)
2 days						0.85 (134)	0.57 (134)	ECP 0.82 (134)		
5 days	0.99 (30)									
2–7 days		LTB_4 0.72 (108) LTE_4 0.68 (108) PGE_2 0.82 (108) PGD_2 0.79 (108) $PGF_{2\alpha}$ 0.73 (108)				0.74 (3)		ECP 0.81 (3)	0.74(3)-0.94(136)	0.87 (137)-0.93 (3)
14 days			0.81 (138)	0.7 (138)						0.95 (138)
35 (18–52) days									0.94 (139)	
4–8 weeks			0.41	0.51		0.49 (140)	0.66 (140)	IL-8 0.5 (140)		0.75 (138)

FE_{NO} = exhaled nitric oxide; EBC = exhaled breath condensate; LTB_4 = leukotriene B_4; LTE_4 = leukotriene E_4; PGE_2 = prostaglandin E_2; PGD_2 = prostaglandin D_2; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; ICC = interclass correlation coefficient; * = ICC >0.7 is statistically and clinically significant/repeatable and signifies that the measurements are reproducible; PC_{20} = bronchial hyperreactivity test (20% reduction of FEV_1); ECP = eosinophilic cationic protein; Eos = eosinophil differential count; IL-8 = interleukin-8; $CD_{45}Ro^+$ = memory T cells; T = T cells.

technique [28], aspirating room air through the nasal cavities by means of a Teflon nozzle placed in one nasal vestibule while maintaining velopharyngeal closure using a party "blow-out" toy has shown a considerable variability in the values for nasal NO output in normal children [29].

Exhaled NO measurements: efficacy conclusions

Mean NO levels

We have recently shown that patients with asthma (adults and children, n = 30) had higher $FE_{NO,}$ 32.3 ± 25.9 ppb, than healthy subjects (n = 30), 16.3 ± 8.4 ppb (p <0.005) [30]. These levels are similar to FE_{NO} levels previously reported in both adults [31] and children [32].

Repeatability

The mean pooled standard deviation of standardised FE_{NO} measurements by NIOX® was 1.83 ± 0.75 ppb [30]. It makes FE_{NO} measurements the most reproducible physiological measurement amongst the other standard means of monitoring in respiratory medicine (table 1).

Coefficient of variation

The mean coefficient of variation (CV) within sessions for the studied populations was 9.5 ± 4.7% [30].

Standard deviation

The over-all mean standard deviation based on session means was 2.50 ± 3.0 ppb (fig. 1) [30].

Repeatability of measurement using two measurements per session instead of three measurements

We have shown that the high reproducibility of FE_{NO} measurements in both children and adults may allow medical practitioners to perform two instead of three exhalations and obtain reliable results [30] (table 2). This may be of great advantage, as it will shorten the time needed for the measurement procedure.

Diurnal variation of measurements

No diurnal variation of the FE_{NO} values could be demonstrated. The only group with a statistically significant difference in FE_{NO} morning-afternoon was healthy adults (mean difference: 1.43 ± 1.60 ppb, p <0.02). Out of the 10 subjects, 9 had a higher in FE_{NO} in the morning [30] (fig. 2).

Day-to-day variation of measurements

No significant differences were observed when comparing levels of FE_{NO} between days (table 3). No significant "learning effects" could be demonstrated (table 4).

Feasibility of FE_{NO} measurements

We have demonstrated that standardised exhaled NO measurements were possible in 100% of the subjects (n = 60), both adults and children (7–13 years) [30]: asthmatics had statistically significantly higher FE_{NO} 30.1 ± 23.6 ppb, than healthy subjects 14.6 ± 5.2 (p <0.005). The proportion of successful measurements is strongly associated with the age of the children. Acceptable FE_{NO} measurements were made in 71% of children (n = 137) age 3.8–7.5 years [33].

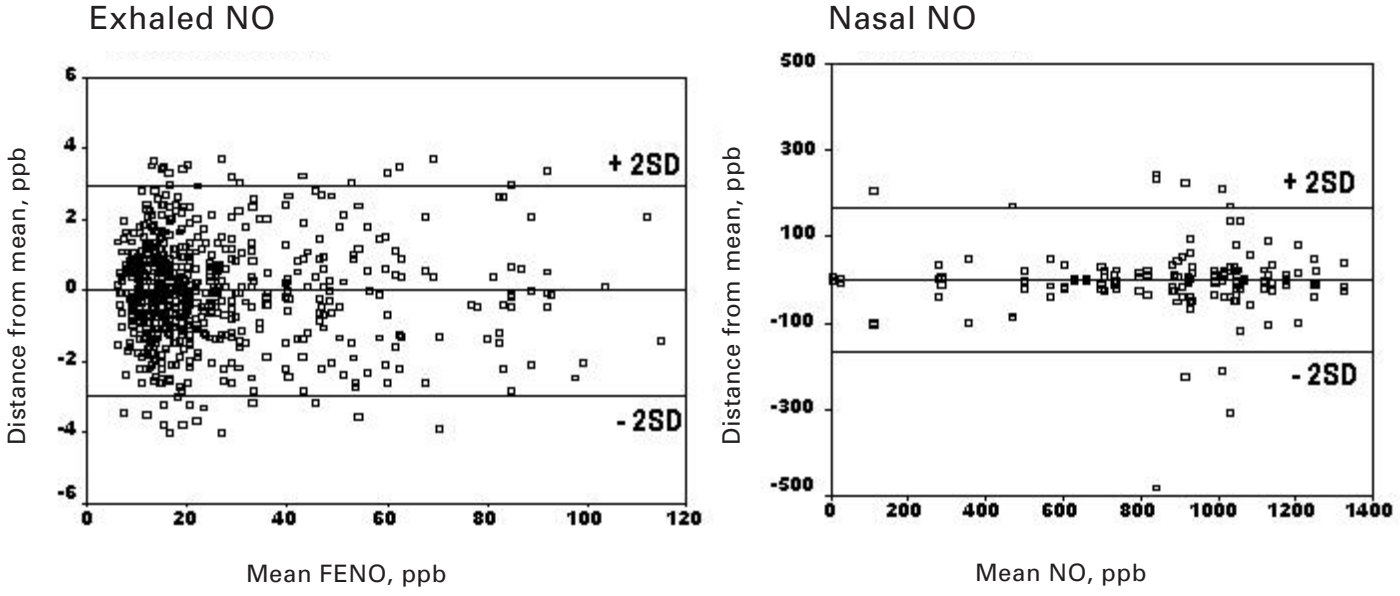
Interestingly, sputum induction is only possible in a proportion of children. Interesingly, only 60% of asthmatic (5–13 years) and 61% of healthy children (5–13 years) [34], and 81% of healthy adults [35] are able to produce sputum after sputum induction.

Acceptability and experience of the method

The overall impression from all 60 subjects whose FE_{NO} was measured using NIOX® [30] was that they had received adequate information re-

Figure 1

Bland-Altman analysis for the repeatability of FE_{NO} and nasal NO values.



garding the device and the measurement procedure, the measurements were easy to perform and could be accepted as a routine practice in a clinical setting (table 5).

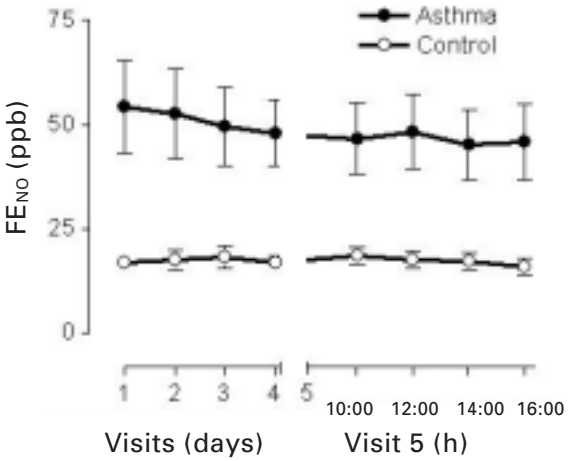


Figure 2

Means and standard errors of means for fractional exhaled NO (FE_{NO}) in healthy and asthmatic adult subjects during successive sessions [30].

Nasal NO measurements: efficacy conclusions

Mean NO levels

The mean NO level for the total (n = 49) population in one of our recent studies was 837 ppb [27]. The mean NO levels in the children were lower than in the adults (751 and 897 ppb). These levels of nasal NO are similar to those previously found in adults [15] and children [36].

Repeatability

The repeatability of nasal NO levels measured with NIOX was studied and the coefficient of variation was 12.5% (95% C.I. 11.0–14.7%) for the total population.

Coefficient of variation

Healthy adults had a significantly better repeatability (9.8%) than healthy children (16.6%, p <0.008) (fig. 3) [27].

Table 2

Mean distance from session mean of FE_{NO} measured during one or two exhalations in studied population groups.

Subjects	Difference between FENO of single exhalation and session mean (3 exhalations), ppb			Difference between mean FE_{NO} of two exhalations and session mean (3 exhalations), ppb		
		N	Difference ± SD		N	Difference ± SD
Normal						
Adults	FE_{NO} 1 – FE_{NO} M	75	0.15 ± 1.52	FE_{NO} 1 – FE_{NO} M	75	0.10 ± 0.64
	FE_{NO} 2 – FE_{NO} M	75	0.34 ± 1.18	FE_{NO} 2 – FE_{NO} M	74	0.18 ± 0.59
	FE_{NO} 3 – FE_{NO} M	74	0.20 ± 1.30	FE_{NO} 3 – FE_{NO} M	74	0.08 ± 0.77
Children	FE_{NO} 1 – FE_{NO} M	40	0.32 ± 2.17	FE_{NO} 1 – FE_{NO} M	40	0.03 ± 0.87
	FE_{NO} 2 – FE_{NO} M	40	0.26 ± 1.46	FE_{NO} 2 – FE_{NO} M	38	0.12 ± 0.75
	FE_{NO} 3 – FE_{NO} M	38	0.07 ± 1.78	FE_{NO} 3 – FE_{NO} M	38	0.15 ± 1.11
Asthma						
Adults	FE_{NO} 1 – FE_{NO} M	70	0.28 ± 2.90	FE_{NO} 1 – FE_{NO} M	70	0.07 ± 1.19
	FE_{NO} 2 – FE_{NO} M	70	0.42 ± 2.27	FE_{NO} 2 – FE_{NO} M	69	0.21 ± 1.14
	FE_{NO} 3 – FE_{NO} M	69	0.13 ± 2.39	FE_{NO} 3 – FE_{NO} M	69	0.14 ± 1.46
Children	FE_{NO} 1 – FE_{NO} M	40	0.31 ± 1.80	FE_{NO} 1 – FE_{NO} M	40	0.21 ± 1.22
	FE_{NO} 2 – FE_{NO} M	40	0.72 ± 2.11	FE_{NO} 2 – FE_{NO} M	40	0.36 ± 1.06
	FE_{NO} 3 – FE_{NO} M	40	0.42 ± 2.44	FE_{NO} 3 – FE_{NO} M	40	0.15 ± 0.90

FE_{NO} 1, 2, 3 = FE_{NO} measured at the first, second and third exhalation; FE_{NO} M = mean FE_{NO} from all three exhalations in a session; FE_{NO} M 1&2 or 1&3 or 2&3 = mean FE_{NO} of measurements 1&2 or 1&3 or 2&3, respectively; N = number of measurements.

Table 3

Reproducibility of FE_{NO} measurements.

Subjects	Measurements							
	Visits				Visit 5*			
	1	2	3	4	9–10 a.m.	11–12 a.m.	2–3 p.m.	4–5 p.m.
Normal								
Adults	19.0 ± 6.72	17.4 ± 7.20	18.1 ± 8.88	18.5 ± 7.18	18.5 ± 6.81	17.5 ± 6.35	17.2 ± 6.58	15.8 ± 5.96
Children					15.9 ± 9.74		15.2 ± 8.75	
Asthma								
Adults	54.2 ± 33.04	52.5 ± 32.30	49.5 ± 28.15	47.9 ± 23.80	46.5 ± 25.95	48.3 ± 26.78	45.1 ± 24.99	45.8 ± 27.67
Children					25.4 ± 24.06		24.3 ± 20.49	

Data presented are means and standard deviations; * = visit 5 in adults and visit 1 in children.

Table 4

Statistical comparison of the reproducibility of FE_{NO} measurements between 1st & 2nd *vs.* 1st & 4th and morning *vs.* Afternoon visits.

Subjects	Intraclass Correlation Coefficient and 95% CI		
	Visits		Visit 5*
	1 vs. 2	1 vs. 4	9–10 a.m. vs. 2–3 p.m.
Normal			
Adults	0.94 (0.77–0.99)	0.94 (0.74–0.98)	0.98 (0.91–0.99)
Children			0.99 (0.98–0.996)
Asthma			
Adults	0.94 (0.75–0.99)	0.90 (0.57–0.98)	0.99 (0.98–0.999)
Children			0.99 (0.98–0.996)

Data presented are means and 95% confidence intervals (CI);
* = visit 5 in adults and visit 1 in children.

Table 5

Acceptability of exhaled and nasal NO measurements in routine clinical practice.

Questions	Exhaled NO (n = 60)*		Nasal NO (n = 55)**	
	Answers		Answers	
	Yes	No	Yes	No
Information adequate and easy to understand?	100	–	100	–
Performance easy?	100	–	87.3	12.7
Discomfort or stress?	1.6	98.3	1.8	98.2
Accept as routine?	95	5	100	–

Data = %; * = ref [30]; ** = ref [27]

Repeatability of NO measurement using two measurements per session instead of three measurements per session

When using two measurements per session instead of three the overall CV was 10.5% (95% C.I. 8.8–13.1%) [27].

Feasibility of measurements

The mean breathhold length needed to obtain a steady NO plateau for all subject categories combined was 20.4 ± 6.01 s [27]. The average number of attempts needed to obtain three approved NO measurements was 5.4 (range 3–13) for the total population. There were no significant differences between the different subject categories.

Acceptability of method

Most subjects found the measurements easy to perform and all of them would accept to do the examination as a routine (table 5) [27].

Clinical areas for routine use of exhaled and nasal NO

Asthma

Increased levels of exhaled NO have been widely documented in patients with asthma [25]. The increased levels of exhaled NO in asthma have a predominant lower airway origin [15] and are most likely due to activation of NOS2 in airway epithelial and inflammatory cells [37, 38], with a small contribution from NOS1 [39].

Asthma diagnosis

An elevation of exhaled NO is not specific for asthma, but an increased level may be useful in differentiating asthma from other causes of chronic cough [40]. The diagnostic value of exhaled NO measurements to differentiate between healthy subjects with or without respiratory symptoms and patients with confirmed asthma has been recently analysed by Dupont *et al.* [41] with 90% specificity and 95% positive predictive value when exhaled NO >15 ppb is used as a cutoff for asthma. The intraindividual coefficient of variation (CV) of exhaled NO in normal subjects was 15.8% within

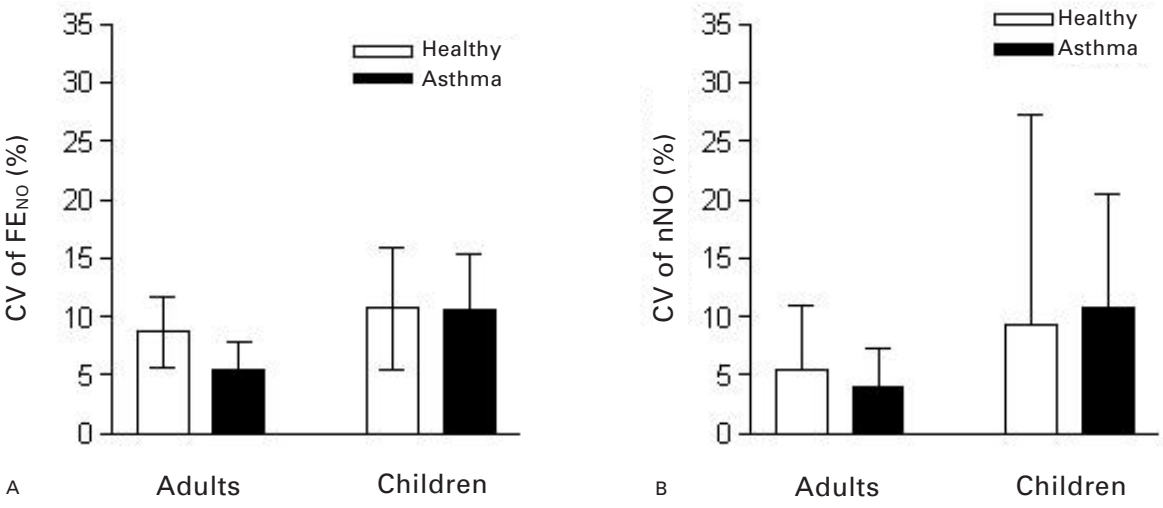


Figure 3

Repeatability of FE_{NO} (Panel A) and nasal NO (Panel B) measurements. Data are coefficient of variation (CV, %) [30].

an interval of 7 days, and 16.8% within 23 days, suggesting that the change of exhaled NO by 30–35% or more within the interval of 1–3 weeks would be abnormal [42].

More recent data suggest that FE_{NO} at a cut-off level of 16 ppb has a specificity for the diagnosis of asthma of 90% and a positive predictive value of >90% [31], suggesting that the simple and absolutely noninvasive measurement of exhaled NO can be used as an additional diagnostic tool for the screening of patients with a suspected diagnosis of asthma.

Recently, a systematic comparison to confirm the diagnostic utility of exhaled NO and induced sputum has revealed that sensitivity and specificity of the conventional tests (peak flow measurements, spirometry, and changes in these parameters after a trial of steroid) were lower (0–47%) than for exhaled NO (88%) and sputum eosinophils (86%) [43]. Overall, the diagnostic accuracy when using exhaled NO and sputum eosinophils was significantly greater. Therefore, exhaled NO measurements and induced sputum analysis are superior to conventional approaches, with exhaled nitric oxide being most advantageous because the test is quick and easy to perform.

Detection and monitoring of latent airway inflammation

Symptoms of atopic asthma often begin in early childhood and mostly improve or even seem to disappear at puberty, but will relapse later in life. This persistent but latent airway inflammation, known as airway remodeling, leads to the thickening of the airway wall and may account for bronchial hyperresponsiveness, which could have a substantial impact on the progression of asthma.

Elevated exhaled NO, blood eosinophil cell counts, and bronchial response to adenosine-5'-monophosphate correlated significantly with the quantity of tissue eosinophils in the bronchoscopy samples from adolescents in clinical remission of atopic asthma [44, 45]. This signifies that airway inflammation and remodeling are both ongoing processes even in subjects in clinical remission, and may be detected and monitored by routine exhaled NO measurements in clinic. Therefore, subjects with subclinical airway inflammation and elevated exhaled NO levels could benefit from anti-inflammatory treatments.

Detection of probable asthma in preschool children

Respiratory function and airway inflammation can be evaluated in preschool children with special techniques, but their relative power in identifying young children with asthma has not been studied. Exhaled NO is shown to be superior to baseline respiratory function and bronchodilator responsiveness in identifying preschool children with probable asthma. The analysis of receiver operating characteristics (ROC) showed that FE_{NO} provided the best power for discriminating between children (age 3.8–7.5 years) with probable asthma and healthy controls, with a sensitivity of 86% and specificity of 92% at the cut off level of 1.5 SD above predicted [33].

Prediction of future asthma

Another potential use of exhaled NO levels in patient management is the prediction of future asthma. An elevated exhaled NO may be found in patients with "subclinical" forms of asthma (normal lung function, negative bronchodilator tests, and elevated sputum eosinophilic cationic protein concentrations) [46, 47]. Elevated levels of NO in patients with "subclinical asthma" are not in conflict with the specificity of exhaled NO as a marker to diagnose asthma, as lack of current asthma symptoms does not exclude the diagnosis of asthma. Perhaps, this subclinical airway inflammation, which is reflected by elevated levels of exhaled NO in adolescent asymptomatic patients with asthma remission, should be treated with corticosteroids to prevent the risk of becoming clinically manifest again. This category of patients with "subclinical" forms of asthma, especially children, may be predisposed to develop asthma in the future [48]. This may be studied in epidemiological studies, in which the reservoir collection of exhaled NO has proved to be useful [49, 50]. Airway responsiveness measurements (PC_{20}) in this "high risk" group make the combination of exhaled NO and PC_{20} a more specific test for allergic asthma. This has recently been demonstrated in a study of over 8000 adolescents in Norway [51]. Because of the non-invasive character and practicality of exhaled and nasal NO measurements they may be used cost effectively for screening large populations.

Asthma control

We are edging closer to the answer to this very important practical question: what is the clinical value of eNO measurements in asthma [52], especially thanks to the publication by Jones et al. [10] that demonstrated that exhaled NO measurements have a positive predictive value between 80 and 90% for predicting and diagnosing loss of control in asthma, and are as useful as induced sputum eosinophils and airway hyperresponsiveness to hypertonic saline but with the enormous advantage that they are easy to perform.

This is the largest longitudinal study (11 weeks) to date in which the utility of *repeated* (once a week for 7 weeks) eNO, symptoms, and spirometry measurements has been explored in 78 predominantly atopic asthmatics. Patients maintained a good lung function (FEV_1 92% predicted) on inhaled corticosteroids of 630 µg/day (range 100–1600 µg beclomethasone equivalent) during a 4-week run-in period before their steroid treatment was stopped. Although a placebo-controlled study would be a better choice, the current design was simple and sufficient to pick-up 78% of the patients with the deterioration of their asthma within 6 weeks after the cessation of steroid treatment.

The median time to loss of control was 17 days, and the most frequent criteria of the loss were fall in peak expiratory flow and symptoms.

An advantage of eNO as a "loss-of-control-marker" [5] is that increase in eNO and asthma symptoms may be seen before any significant deterioration in airway hyperresponsiveness, sputum eosinophils or lung function during asthma exacerbation induced by steroid reduction [11, 12]. Exhaled NO levels were (median, quartiles) 11 (9–21) ppb in children who had good asthma control, 15 ppb (11–26) in those who had acceptable asthma control, and 28 ppb (19–33) with insufficiently controlled asthma [53], suggesting NO measurements may be useful for monitoring paediatric asthma in clinic.

Serial vs. single FE_{NO} measurements in assessment of asthma control

Monitoring of asthma may be less conclusive when single baseline eNO measurements instead of serial assessments were used, as a single baseline assessment of either exhaled NO [12, 54] or sputum eosinophils [54] had a low power to predict asthma deterioration during the reduction of steroid treatment.

The advantage of repetitive FE_{NO} measurements has been studied and an increase in FE_{NO} and asthma symptoms was seen before any significant deterioration in airway hyperresponsiveness, sputum eosinophils or LF during asthma exacerbation induced by steroid reduction [11, 12]. These data suggest that FE_{NO} may be used as a loss-of-control-marker in asthma [5].

The finding that *changes* in FE_{NO} measured over time have higher predictive values, sensitivities and specificities both for predicting and diagnosing loss of control than did single measurements [10], clearly indicates the need for repeated tests. When measured longitudinally the *changes* in FE_{NO} correlated significantly not only with changes in sputum eosinophils and hyperresponsiveness, but also with lung function and asthma symptoms.

Methodologically, standardised FE_{NO} measurements [9] have obvious advantages over sputum induction and airway hyperresponsiveness or any other bronchial provocation tests due to their simplicity, reproducibility [30] and entirely non-invasive nature. It is vital that this technique can be used repeatedly in patients with severe disease and to assess disease in children, making it a suitable test for use in the clinical as well as the research practice [10, 52]. Because the technique is non-invasive it is possible to make repeated measurements without disturbing the system, in contrast to the invasive or semi-invasive procedures currently used. Individual FE_{NO} values, like individual peak expiratory flows, should be established and monitored, and when the levels are above or below a certain reference level, steroid treatment should be either reduced or increased.

We clearly need further clinical research on exhaled NO to be able to tailor strategies for effective treatment and early intervention in asthma. As exhaled NO analysers become more widely available and miniaturized, it is likely that this measurement will become routine in monitoring asthma control, particularly in patients with unstable and difficult to control asthma.

FE_{NO} measurements to study the effects of inhaled corticosteroids

Onset of action

There have been no direct measurements of acute inhaled corticosteroids (ICS) effects on airway inflammation and microvascular permeability in asthma and COPD.

Exhaled NO behaves as a "rapid response" marker that is extremely sensitive to steroid treatment, because it may be significantly reduced even 6 hours after a single dose of nebulised budesonide [55], or within 2 to 3 days [56, 57] after regular treatment with ICS. We observed that the onset of action of inhaled budesonide on exhaled NO was dose-dependent, both within the initial phase (first 3 to 5 days of treatment) and during treatment weeks 1, 2 and 3 [56].

Cessation of action

An important question is how quickly exhaled NO levels recover when steroid treatment is stopped. We have shown that exhaled NO levels recovered rapidly during the first 3 to 5 days in all patients who stopped inhaled budesonide, and recovery was complete by the end of the first week of treatment [56].

Dose-dependent effect

We have shown that the acute reduction in exhaled NO (within the first 3 to 5 days of treatment) and the chronic reduction (days 7 to 21) are dose dependent in patients with mild asthma who are treated with low doses of budesonide [56]. Serial exhaled NO measurements, as we recently suggested [1], may therefore be useful in studying the onset and duration of action of ICS, as well as in monitoring patient compliance.

Effects of combination treatment

Combination inhalers (ICS plus LABA) are going to be used as the first-line treatment in asthma. Recently, it has been shown that combination treatment produces a clinically significant improvement in health status and reduces daily symptoms in COPD. It is important, however, to monitor the underlying airway and alveolar inflammation in both diseases, independently of patients' lung function and symptoms, which are affected by LABAs. Surrogate markers may help us to see whether there is an additional anti-inflammatory effect of combination treatment in these patients in the clinic.

There is evidence that symptom-driven dosing with combination inhalers may be useful in the future, as long as the dose of the steroid can be de-

termined by the degree of symptoms at a particular time. We suggest that the high sensitivity of exhaled NO may be used to adjust doses of combination therapy based on control of inflammation in asthma. This is important because a LABA may control symptoms and, therefore, mask underlying inflammation that is not adequately suppressed by corticosteroids. Portable, simple and inexpensive exhaled NO analysers (based on measurements other than the chemiluminescence principle of NO detection) could be available in the next 1–2 years.

Effects of other treatments

Inhaled β_2-agonists

Neither short-acting β_2-agonists nor LABAs reduce exhaled NO [58]. This is consistent with the fact that they do not have any anti-inflammatory effects in asthma.

Leukotriene antagonists

It is difficult to assess the anti-inflammatory action of compounds that have no bronchodilator action and none of the profound immunomodulatory effects of corticosteroids. Nevertheless, some noninvasive inflammatory markers may be used in clinical studies to test the efficacy of leukotriene antagonists. Pranlukast blocks the increase in exhaled NO when ICSs are withdrawn, and montelukast rapidly reduces exhaled NO by 15% to 30% in children with asthma [59]. Zafirlukast, which is as effective as formoterol in maintaining asthma control, causes a significant reduction in exhaled NO [60].

COPD

Exhaled NO in stable COPD [61] is lower than in either smoking or non-smoking asthmatics, despite an abundant iNOS and nitrotyrosine positive sputum cells in COPD patients as compared with healthy smokers [62]. This may be due to the fact that tobacco smoking down-regulates eNOS and decreases endothelial arginine content [63], in addition to the depletion of NO by formation of peroxynitrite, as a result of oxidative stress in COPD. On the other hand, severe airway inflammation, prevalence of neutrophilic inflammation, oxidant/antioxidant imbalance and high iNOS presence in sputum cells [62], alveolar macrophages, alveolar walls, bronchial epithelium and vascular smooth muscles of COPD patients [62, 64] may outweigh the effect of smoking on exhaled NO [6].

Monitoring of COPD exacerbations

Patients with unstable COPD, however, have high NO levels compared with stable smokers or ex-smokers with COPD [6], which may be explained by increased neutrophilic inflammation and oxidant/antioxidant imbalance. Eosinophils that are capable of expressing NOS2 and producing NO are present in exacerbations of COPD [65]. Exacerbations are an important feature of COPD and are often triggered by viral or bacterial infection. Elevated exhaled NO levels are found in patients with lower respiratory tract inflammation and chronic bronchitis [66].

COPD patients with coexistent asthma or pulmonary hypertension

A small proportion of patients with COPD appears to respond to corticosteroids and these patients, who are likely to have coexistent asthma, have an increased proportion of eosinophils in induced sputum [67]. These patients also have an increased exhaled NO [68]. This suggests that exhaled NO may be useful in predicting which COPD patients will respond to long-term inhaled corticosteroid treatment.

Pulmonary hypertension has the opposite effect, as COPD patients with *Cor Pulmonale* have low exhaled NO levels [69], which may reflect their impaired endothelial NO release.

Monitoring of small airway inflammation in COPD by multiple exhalation flow technique (MEFT)

We have demonstrated that peripheral airways/alveolar region is the predominant source of elevated exhaled NO in COPD [70]. In contrast, increased exhaled NO levels in asthma are mainly of larger airways/bronchial origin [70]. Prevalence of alveolar-derived NO in COPD is possibly related to the iNOS in macrophages, alveolar walls and bronchial epithelium of COPD patients [64].

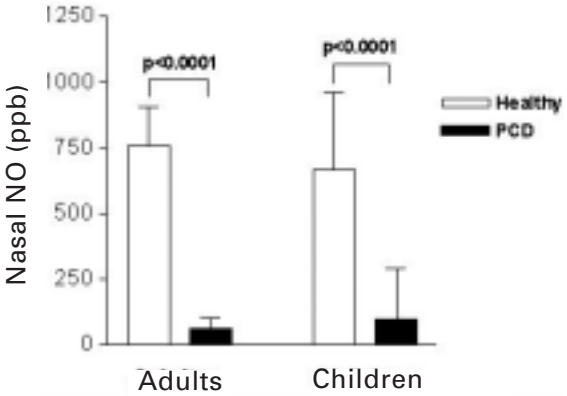
Primary ciliary diskinesia

Primary ciliary dyskinesia (PCD) presents to general practitioners with symptoms pertinent to a variety of specialists because of the involvement of ciliated epithelium in the upper/lower respiratory tract, ears, eyes and genital tract. There is no easy, reliable screening test for PCD, and thus, the majority of patients remain undiagnosed.

It has been decisively shown that measurement of nasal NO can be used in clinical practice in various specialities to screen suspected patients for PCD, both adults [36, 71] and children [72] (Fig. 4). Such low values of exhaled and nasal NO are not seen in any other condition and are therefore of diagnostic value. Measurement of exhaled NO might be used as a screening procedure to detect PCD amongst patients with recurrent chest infections or male infertility due to immotile spermatozoa, and the diagnosis of PCD is then confirmed by the saccharine test, nasal nitric oxide, ciliary beat frequency and electron microscopy [73].

Despite the lower levels of exhaled NO in children with PCD, no differences were found in the mean levels of NO metabolites in exhaled breath condensate [74], suggesting that detection of NO in exhaled and nasal breath, but not in the EBC, may be the method of choice in the diagnosis of PCD.

Figure 4



Chronic cough

Increased levels of exhaled NO accompany not all forms of airway inflammation. Patients with chronic cough that is not attributable to asthma have lower NO values as compared with healthy volunteers and patients with asthma [40, 75], including those with cough due to gastro-oesophageal reflux [76]. Measurement of exhaled NO may therefore be a useful screening procedure for patients with chronic cough and would readily identify those patients with cough due to asthma [40].

Rhinitis

The levels of NO derived from the upper respiratory tract are over 100-fold higher than from lower airways. This fact is mostly due to its high production in human paranasal sinuses [77] which is due to high basal activity of constitutively expressed forms of NOS2 [78].

Elevated nasal NO has been reported in allergic and perennial rhinitis [79, 80], which is reduced by treatment with nasal corticosteroids [80]. Similar results are seen in children with allergic rhinitis [81]. In addition, exhaled NO is also significantly elevated in allergic rhinitis in the non-pollen season and is increased further in the pollen season [82]. However, the differences between the levels of nasal NO in rhinitis compared with normal subjects is much less marked than the differences between exhaled NO between patients with asthma and normal subjects because of the very high baseline values. This makes nasal NO less useful for diagnosis and monitoring treatment in rhinitis than exhaled NO in asthma.

Nasal and lower airway levels of NO in prematurely born infants

Nasal and lower airway NO measurements were possible in prematurely born infants on days 1, 3, 5, 7, 14, 21 and 28 after birth [83]. Nasal NO was sampled directly from the nasal space and lower airway. NO was sampled from a catheter positioned so that its tip lay at the lower end of the endotracheal tube. Interestingly, even in very immature infants examined in the first day after birth, nasal NO levels were greater than those from the lower airway. Nasal and lower airway NO levels did not correlate significantly with gestational age, but lower airway NO levels correlated with postnatal age ($r = 0.86$, $p = 0.014$).

Therefore, nasal and exhaled NO can be measured very early in life and may be used to screen infants for PCD and other hereditary conditions with abnormal NO production.

Exhaled and nasal NO in the current asthma guidelines

Non-invasive investigations into the degree of bronchial inflammation performed by measuring the NO in exhaled air has been included into the Dutch Guideline "Treating asthma in children for pediatric pulmonologists (2nd revised edition). I. Diagnosis and prevention" [84].

FE_{NO} in childhood asthma research and education (CARE) network

The NHLBI's Childhood Asthma Research and Education (CARE) Network measured FE_{NO} in children aged 6 to 17 years with mild to moderate persistent asthma [85]. These findings suggest that FE_{NO} provides information about the asthmatic state that is consistent with information from other biomarkers associated with inflammation and this noninvasive technique that could be used in onsite management decisions for the care of asthmatic children.

FE_{NO} may replace some current methods of asthma diagnosis

Exhaled NO may be used to identify subjects with atopy, because non-atopic asthmatics have normal exhaled NO [86], and elevated nasal NO is also related to the size of skin test reactivity in asymptomatic asthmatic subjects [87]. This may denote "subclinical" airway inflammation.

Bronchial reactivity and exhaled NO are not often used to monitor control and severity of asthma in clinical practice. There is accumulating evidence that exhaled NO alone can replace PC_{20} provocation [88] and skin prick tests for creening patients, both children and adults, for an abnormal bronchial reactivity and atopy.

FE_{NO} in epidemiologic studies

It has been shown that a 10 microg/m^3 increase in particular matter, both outdoor and indoor, was associated with increases in exhaled NO collected offline into a Mylar balloon [21]. This suggests that exhaled NO can be used as an assessment tool in epidemiologic studies of health effects of air pollution.

Multiple exhalation flow technique (MEFT): novel method to measure small airway FE_{NO}

A novel method of measuring exhaled NO at several exhalation flow rates has recently been described which can used to approximate alveolar and airway NO production [89]. NO is continuously formed in the airways. Mixing during exhalation between the NO produced by the alveoli and

the conducting airways, explains its flow dependency [23] and accumulation during a breath-hold [15]. A relatively simple and robust two-compartment model of NO has been developed that is capable of simulating many important features of NO exchange in the lungs [90].

This new approach may be a simple, effective, and reproducible technique for determining the relative contribution of the airways and alveoli to exhaled NO.

Advantages of NO measurements

Reproducibility

Reproducibility of FE_{NO} measurements within a single day in both adults (ICC 0.94) and children (ICC 0.94) is superior to any conventional methods of airway inflammation monitoring in asthma (table 1). This adds significantly to other major advantages of FE_{NO} measurements, such as their strong association with airway inflammation [1], even in non-symptomatic asthma patients [44], their high sensitivity to steroid treatment [91], insensitivity to β_2-agonists [1], and non-invasiveness.

Repeated FE_{NO} measurements, therefore, can be used much more frequently and will not disturb the system, in contrast to the invasive or semi-invasive procedures currently used in clinical research to monitor inflammation status [1].

Simplicity and practicality

There are several important practical implications for the FE_{NO} measurements regarding the data comparison of spirometry *vs.* FE_{NO} examination. Firstly, we have shown that high reproducibility of FE_{NO} measurements in both children and adults may allow medical practitioners to perform two instead of three exhalations in order to obtain the reliable results (table 2). This may be of great advantage, as it will shorten the time needed for the measurement procedure.

Secondly, because FE_{NO} measurements with NIOX are fully automated and incorrect exhalation manoeuvres by a patient (shorter than 10 s or above the certain limits of the exhaled flow) will not be accepted by the analyser, the staff training procedure can be minimal. Finally, the advantage of FE_{NO} measurements is that it does not require an extra encouragement, as it may be in case of PEF measurements. Indeed, a significant difference may be seen between unobserved and encouraged PEF readings [92].

Minimal training for medical staff

The Bland and Altman analysis of FE_{NO} measurements between the sessions separated by either hours or days allowed us to investigate whether there was a "learning effect" (implying that the subsequent, but not the first FE_{NO} measurements are the most reliable). We did not find any such "learning effect" or systematic error of serial FE_{NO} measurements [30]. The simplicity and high reproducibility of FE_{NO} measurements in our study are probably the major reasons for this. In our study, a single nurse made all the FE_{NO} measurements after she was given a short tutorial.

The simplicity of the standardised FE_{NO} measurements was further enhanced by the design of the NIOX® system, which controls the exhalation parameters and ensures that the measurements will not be accepted unless they are performed according to the guidelines [9]. The youngest child in our study was a 7-year-old boy who, in fact, was unable to answer question 4 (table 5): would he accept to do FE_{NO} examination as part of a routine visit to his doctor, but was able to perform the test.

The mean pooled SD of standardised FE_{NO} measurements was 2.1 ± 1.25 ppb [30]. These results suggest that if a patient exhaled FE_{NO} levels would change more than 4 ppb between sessions; this is more likely due to the inflammatory process rather than to an inaccuracy of the NIOX device. This finding is valuable for potential use of FE_{NO} in routine clinical practice. Short-term monitoring, when the measurements of airway inflammation are made more often, for example every day or twice a day as in the case of PEF, is particularly important. This is because of a recent trend towards the use of lower doses of inhaled corticosteroids in combination with LABA when anti-inflammatory and clinical effect of combination treatment may be seen within hours and days. Another example is the use of specific inducible NO synthase (iNOS) inhibitors, which may potentially be an additional treatment of severe asthma, chronic obstructive pulmonary disease or arthritis, when the effect of iNOS inhibitors may be seen within minutes or hours [1].

Tailoring individual treatment and cost reduction in clinical practice

Dose adjustment in both clinical practice and clinical research is an important issue, in which high reproducibility of FE_{NO} measurements and sensitivity of FE_{NO} to corticosteroids may substantially reduce the cost of medical care and research. Recently, we have demonstrated a dose-dependent onset and cessation of anti-inflammatory action of inhaled corticosteroids on FE_{NO} and asthma symptoms in a small number (n = 28) of mild asthma patients who were treated with 100 or 400 µg budesonide, or placebo once daily for 3 weeks in a double-blind, placebo-controlled, parallel group study [56].

Improving efficacy and cost reduction of clinical trials

Sample-size determination is often an important step in planning such studies. According to our data [30] only a small number (between 7 and 20) of asthmatic patients, either adults or children, will be required to demonstrate a 25 to 80% effect of a studied drug in a clinical trial. Based on the knowledge of the individual variability of FE_{NO} measurements, like individual peak expiratory flows, individual FE_{NO} values should be established and monitored, and when the levels are above or below a certain reference level, steroid treatment should be either reduced or increased [52].

Limitations of NO measurements

Exhaled NO

The value of particular markers will depend on the availability of reliable, fast and inexpensive detector systems. NO chemiluminescence analysers are currently relatively expensive and are mainly available in academic research laboratories. However, advances in technology have now resulted in smaller devices that are easier to use and cheaper. This will increase the availability of the measurement that will further reduce the price as exhaled NO analysers become routine lung function measurements. Eventually it may be possible to introduce such analysers in family practice and even into patients' homes, so that patients themselves will be able to monitor their own markers and adjust their treatment accordingly.

Nasal NO

The high background levels of nasal NO from constitutive sources in the nose may blunt smaller increases in mucosal NO output; as observed when an intranasal administration of the NOS inhibitor NG-nitro-L-arginine-methyl ester (L-NAME) caused only a small (26–37%) reduction in nasal NO in patients with allergic rhinitis [93]. In contrast, inhaled L-NAME may cause a profound (60–70%) reduction in exhaled NO [94]. It seems that the methods for measurement of nasal NO need to be substantially improved and standardised before they can be used to monitor allergic rhinitis and its treatment.

Exhaled hydrocarbons

Increased levels of volatile hydrocarbons in exhaled breath could be used as biochemical markers of exposure to cigarette smoke and oxidative damage caused by smoking, as pentane [95] and isoprene [96] are increased in normal smokers [97], and ethane in smoking COPD patients [98].

Measurement

Lipid peroxidation is assessed by measuring its secondary reaction products, such as chemiluminescent and fluorescent molecular products, lipid hydroperoxides, conjugated dienes, aldehydes, malonaldehyde or thiobarbituric acid-reactive substances and aliphatic hydrocarbons [99].

Exhaled hydrocarbons are measured by gas chromatography. The repeatability for exhaled air samples was 7, 10 and 12% for ethane, pentane and isoprene, respectively [100]. This method could, with minor modifications, be used to determine other low-molecular hydrocarbons in exhaled air as well. One of the serious limitations of the method is that it requires rather large sample volumes (500 ml) [100].

Standardisation of exhaled hydrocarbons measurements

Although ethane and pentane, for example, are among the numerous end-products of lipid peroxidation, they represent only a small and possibly variable proportion of the total amount of peroxidized polyunsaturated fatty acids. To date, the number of studies utilizing the hydrocarbon breath test as a marker of lipid peroxidation in humans is small [1, 101]. Technical difficulties are among the main reasons for the limited use of this method. An appropriate washout period, the use of the right materials, the scrupulous avoidance of air contamination, adequate preinjection concentrations of the samples, and a sensitive gas chromatographic technique enable the accurate and reproducible measurement of hydrocarbons in human breath.

When the hydrocarbon breath test is standardised for clinical use it will likely provide a non-invasive and extremely sensitive instrument for the assessment of oxidative stress status in adults as well as in children in the future.

Exhaled breath condensate

Exhaled breath condensate is collected by cooling or freezing exhaled air, a totally noninvasive technique. Although the collection procedure has not been standardised, there is strong evidence that abnormalities in EBC composition may reflect biochemical changes of airway lining fluid [4]. Potentially, EBC can be used to measure the targets of modern therapy in clinical trials and to monitor asthma and COPD in the clinic.

Collection and measurement

Several methods of condensate collection have been described. The most common approach is to ask the patient to breathe tidally via a mouthpiece through a non re-breathing valve in which inspiratory and expiratory air is separated. During expiration the exhaled air flows through a condenser which is cooled to 0 °C by melting ice [102], or to –10 [103], or –20 °C [104] by a refrigerated circuit, and breath condensate is then collected into a cooled collection vessel. A low temperature may be important for preserving labile markers as lipid mediators during the collection period, which usually takes between 10 and 15 minutes to obtain 1–3 ml of condensate. Exhaled condensate may be stored at –70 °C and is subsequently analysed by gas chromatography and/or extraction spectrophotometry, or by immunoassays (ELISA).

Equipment

Expired breath condensate may be collected by using a glass condensing device, with an inner glass chamber which contains ice and is suspended in a larger chamber [56, 102]. Condensate is formed on the outside surface of the inside glass that is separated from ambient air. Approximately 1 ml of EBC is collected within 15 minutes and stored at –70 °C. Recently, most of the centres use commercially available condenser EcoScreen (Jaeger, Germany) that allows to collect 2–3 ml of EBC (–20 °C) within 10 minutes, and is equipped with a spirometer to register the volume of exhaled air [32, 74, 103].

There are reports, however, that cysLT were undetectable in EBC (–30 °C) collected with Cryocond (Boehringer Ingelheim, Burlington, ON, Canada) [105]. It is unclear if this was due to the technical characteristics of Cryocond, as cysLT are easily detectable in EBC collected by EcoScreen in patients with asthma, both children [106] and adults [107], and COPD [108].

Validation of measurements

There are only a few studies that attempted to validate enzyme immunoassays used to measure various markers in EBC. Exhaled LTB_4 in EBC (recovery was 64%) in patients with different lung diseases was validated by reverse phase-high performance liquid chromatography (HPLC) [109], and exhaled nitrotyrosine measurements in patients with asthma [107] and cystic fibrosis [110] were also validated by HPLC.

Standardisation of the EBC collection and markers/mediators measurements

Despite the fact that the first studies identifying pulmonary surfactant in the EBC were published in Russia in the 1980s [111, 112], the EBC method has not been standardised. Several rather complicated and impractical methods have been proposed to standardise the EBC collection and estimate dilution, including estimation of dilution based on total cations [113], conductivity of lyophilised samples and urea [114]. Controlled studies are needed to establish the utility of EBC markers for monitoring airway inflammation and guiding pharmacological treatment in COPD and other lung diseases.

There is an ongoing joint workshop of the European Respiratory and American Thoracic Societies on EBC standardisation that may help to resolve some standardisation issues and simplify the procedure to make it ready for clinical use.

Repeatability of measurements

Although several papers describe some aspects of the repeatability of 8-isoprostanes (coefficient of variation, CV = 4.4%) [115], several leukotrienes (LTB_4, intraclass correlation coefficient, ICC = 0.72; LTE_4, ICC = 0.68) [108] and prostaglandins (PGE_2, ICC = 0.82; PGD_2, ICC = 0.79; 0.73 for $PGF_{2\alpha}$, ICC = 0.73) [108] (table 1), there is no formal study to date addressing this question. Often, the repeatability of exhaled markers in EBC was studied in a rather small number of normal subjects [115].

EBC collected in patients on respiratory support

Cardiac surgery using cardio-pulmonary bypass and, to a greater extent lung resection, cause acute lung injury that is usually sub-clinical. 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 have demonstrated that LTB_4, H_2O_2 and hydrogen ions rose significantly in EBC in patients after lobectomy, but not after the milder insult associated with cardiac surgery [116].

This suggests that EBC is a safe, non-invasive method of sampling the milieu of the distal lung and is sufficiently sensitive to detect markers of inflammation and oxidative stress in adults. A different observation has been reported in neonates who were ventilated or receiving nasal continuous positive airway pressure (CPAP). Despite the fact that a sufficient volume of EBC was collected for analysis over 25–40 min from neonates on the ventila-

tor and nasal CPAP (medians 5.3 and 2.7 ml, respectively), no significant difference between H_2O_2 in EBC from neonates from a background with the ventilator or CPAP system alone was found [117]. The dilution of breath condensate by humidified gases plus the existence of background H_2O_2 resulted in this collecting setup being insufficiently sensitive to detect exhaled H_2O_2 in infants who were ventilated or on nasal CPAP.

Safety, success rate and feasibility of EBC collection

EBC collection is an intrinsically safe procedure which can be successfully applied in asthmatic and healthy children aged 4 and above with 100% success rate and negligible fall in FEV_1 after the procedure [118].

Potential clinical areas of use of some markers in exhaled breath condensate

Hydrogen peroxide

Activation of inflammatory cells, including neutrophils, macrophages, and eosinophils, results in increased production of O_2^- and formation of hydrogen peroxide (H_2O_2). Because H_2O_2 is soluble, increased H_2O_2 in the airway equilibrates with air and can be detected in EBC. Thus, exhaled H_2O_2 has potential as a marker of oxidative stress in the lungs. H_2O_2 has been detected in EBC in healthy adults, and increased concentrations have been detected in asthmatics [102].

Cigarette smoking causes an influx of neutrophils and other inflammatory cells into the lower airways, and 5-fold higher levels of H_2O_2 have been found in EBC of smokers compared with nonsmokers. In patients with stable COPD, levels of exhaled H_2O_2 are higher than in normal subjects and are further increased during exacerbations.

Tyrosine, nitrotyrosine, nitrite, nitrate, and reactive nitrogen species

A significant proportion of NO is consumed by chemical reactions in the lung, leading to the formation of nitrite, nitrate, and *S*-nitrosothiol in the lung epithelial lining fluid. Elevated levels of *S*-nitrosothiols in EBC have been demonstrated in patients with asthma or COPD [119], increased nitrotyrosine in asthmatic airway epithelium has been inferred from immunostaining of lung biopsies [38], and elevated levels of free nitrotyrosine have been observed in EBC from asthmatics [107].

Hydrogen ions

Elevated levels of lactic acid have been found in exhaled condensate in patients with acute bronchitis [120], and a low pH of exhaled condensate is reported in patients with acute asthma [121]. Exhaled pH is free of salivary, nasal, and gastric contamination and is not influenced by either airflow obstruction or by inhaled albuterol, but is increased by corticosteroid therapy.

Mean intraweek and intraday coefficients of variation of pH in healthy subjects has been shown to be between 4.5% and 3.5% [122]. The pH levels were not affected by duration of collection and storage, acute airway obstruction, subject age, saliva pH, profound hyperventilation and hypoventilation [122].

It can be argued, however, that without knowing about dilution of respiratory droplets in water vapor, the interpretation of condensate data, especially of pH, as it may be contaminated by ammonia generated in the mouth [123], may still be problematic [124]. Simple and reliable methods of detecting alterations in droplet dilution need to be developed, for example sodium plus potassium [113].

Eicosanoids

Exhaled prostaglandins, for example PGE_2 and $PGF_{2\alpha}$, are detectable in EBC and are markedly increased in patients with COPD, whereas they are not significantly elevated in asthma [125, 126]. In contrast, thromboxane B_2 is increased in asthma but is not detectable in normal subjects or in patients with COPD [127].

Detectable levels of the leukotrienes LTB_4, LTC_4, LTD_4, LTE_4, and LTF_4 have been reported in EBC of asthmatic and normal subjects [106, 128]. The levels of LTE_4, LTC_4, and LTD_4 in EBC are elevated significantly in patients with moderate and severe asthma [128], and steroid withdrawal in moderate asthma leads to worsening of asthma and further increases in exhaled NO and the concentration of LTB_4, LTE_4, LTC_4, and LTD_4 in EBC [129]. Concentrations of LTB_4 are increased in EBC of patients with stable COPD [127], COPD exacerbations, [130] or moderate or severe asthma [128]. This suggests that LTB_4 may also be involved in exacerbations of asthma and may contribute to neutrophil recruitment.

Levels of 8-isoprostane are approximately doubled in patients with mild asthma compared with normal subjects, and they are increased by about 3-fold in patients with severe asthma, irrespective of treatment with corticosteroids [131]. The relationship to asthma severity is a useful aspect of this marker, in contrast to exhaled NO. A relative lack of effect of corticosteroids on exhaled 8-isoprostane in patients with mild asthma has been confirmed in a placebo-controlled study with 2 different doses of inhaled corticosteroids (ICSs) [56]. This provides evidence that ICSs may not be very effective in reducing oxidative stress. The concentration of 8-isoprostane in EBC is also increased in normal cigarette smokers and, to a much greater extent, in COPD patients [132]. Interestingly, exhaled 8-isoprostane is increased to a similar extent in COPD patients who are ex-smokers as in smoking COPD patients, indicating that the exhaled isoprostanes in COPD patients are largely derived from oxidative stress from airway inflammation, rather than from cigarette smoking.

Conclusion

Exhaled markers of inflammatory lung disease that are ready for routine monitoring

Exhaled NO

Exhaled NO analysis has an enormous potential as a non-invasive means of monitoring of airway particular in childhood and adult asthma. This standardised technique is simple, reproducible, acceptable for patients to perform and is ready for routine monitroing.

Advances in technology have now resulted in smaller devices that are easier to use and cheaper. It may be possible to introduce such analysers in the very near future in family practice and even into patients' homes, so that patients themselves will be able to monitor their own markers and adjust their treatment accordingly.

Exhaled markers of inflammatory lung disease that are not ready for routine monitoring

Exhaled breath condensate

The value of this non-invasive and promising approach will depend on the standardisation of the collection, availability of reliable, fast and inexpensive detector systems that will overcome the current high variability of the measurements and its high cost.

Other exhaled gases

Measurement of some of the other exhaled markers, such as hydrocarbons, is much more difficult using present technology, but it may also be possible to develop much smaller and cheaper detectors that would make this measurement more readily available.

Future directions

At the moment single exhaled markers are usually evaluated in isolation, but as indicated above markers are affected differently in different diseases, and different markers vary in their sensitivity to certain manoeuvres, such as the effect of therapy.

These differences may be exploited in the future as more markers are characterised, so that each disease may have a characteristic profile or fingerprint of different markers that may be diagnostic. Treatments too may impose a characteristic effect on these markers and this may improve the specificity of treatment in the future, particularly as more potent and specific treatments become available.

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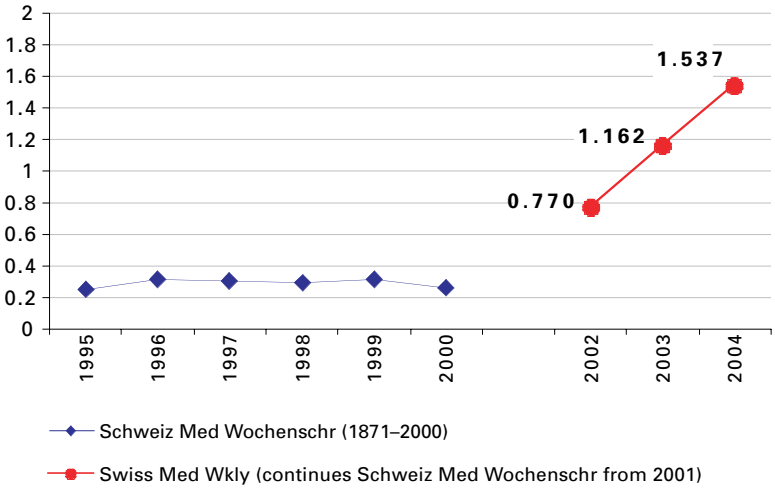

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