Respiratory Medicine (2010) **104**, 873–879

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Two variants of occupational asthma separable by exhaled breath nitric oxide level

Vicky C. Moore [a,*], Wasif Anees [a], Maritta S. Jaakkola [b,c], Cedd B.S.G. Burge [a], Alastair S. Robertson [a,d], P. Sherwood Burge [a]

[a] *Occupational Lung Disease Unit, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham B9 5SS, UK*
[b] *Respiratory Medicine Unit, Division of Internal Medicine, University of Oulu, Oulu, Finland*
[c] *Occupational and Environmental Medicine, University of Birmingham, Birmingham, UK*
[d] *Occupational Health Department, University Hospitals Birmingham, Birmingham, UK*

Received 27 August 2009; accepted 8 January 2010
Available online 2 February 2010

KEYWORDS
Occupational asthma;
Peak expiratory flow;
Non-specific bronchial reactivity;
Exhaled nitric oxide

Summary
Exhaled nitric oxide (FE_{NO}) has been used as a marker of asthmatic inflammation in non-occupational asthma, but some asthmatics have a normal FE_{NO}. In this study we investigated whether, normal FE_{NO} variants have less reactivity in methacholine challenge and smaller peak expiratory flow (PEF) responses than high FE_{NO} variants in a group of occupational asthmatics.
Methods: We measured FE_{NO} and PD_{20} in methacholine challenge in 60 workers currently exposed to occupational agents, who were referred consecutively to a specialist occupational lung disease clinic and whose serial PEF records confirmed occupational asthma. Bronchial responsiveness (PD_{20} in methacholine challenge) and the degree of PEF change to occupational exposures, (measured by calculating diurnal variation and the area between curves score of the serial PEF record in Oasys), were compared between those with normal and raised FE_{NO}. Potential confounding factors such as smoking, atopy and inhaled corticosteroid use were adjusted for.
Results: There was a significant correlation between FE_{NO} and bronchial hyper-responsiveness in methacholine challenge ($p = 0.011$), after controlling for confounders. Reactivity to methacholine was significantly lower in the normal FE_{NO} group compared to the raised FE_{NO} group ($p = 0.035$). The two FE_{NO} variants did not differ significantly according to the causal agent, the magnitude of the response in PEF to the asthmagen at work, or diurnal variation.
Conclusions: Occupational asthma patients present as two different variants based on FE_{NO}. The group with normal FE_{NO} have less reactivity in methacholine challenge, while the PEF changes in relation to work are similar.

* Corresponding author at. Occupational Lung Disease Unit, Birmingham Heartlands Hospital, Department of Respiratory Medicine, Bordesley Green East, Birmingham B9 5SS, UK. Tel.: +44 121 424 2745; fax: +44 121 772 0292.
E-mail address: vicky.c.moore@heartofengland.nhs.uk (V.C. Moore).

0954-6111/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2010.01.007

Introduction

Measurement of exhaled breath nitric oxide (FE_{NO}) has been promoted as a measure of airway inflammation in asthma.[1–4] It has been shown to be correlated with sputum eosinophilia and non-specific reactivity in asthmatics[2,4–12] but has the advantages of being less invasive for the patient and less labour intensive for the clinician. However, some symptomatic asthmatics have been reported to have normal levels of FE_{NO}[7,12–14] even when factors such as inhaled corticosteroid therapy and smoking have been accounted for. In the diagnosis of occupational asthma, one of the best first line investigations for occupational asthma is serial peak expiratory flow (PEF) monitoring and is recommended by several guidelines.[15,16] It has been suggested previously that using changes in sputum eosinophil counts between periods of exposure and non-exposure increases the sensitivity and specificity of serial PEF measurement in the diagnosis of occupational asthma.[17] Specific inhalation challenge tests to occupational agents have resulted in a mean increase of exhaled nitric oxide levels.[18–21] However, some workers with positive challenges have not showed changes. We have previously found a strong positive correlation between exhaled nitric oxide level and sputum eosinophil count in workers with occupational asthma exposed to low molecular weight agents and a relationship between sputum eosinophilia and non-specific reactivity.[7] The study suggested that workers can be separated into two variants, those with eosinophilic airways inflammation and those with non-eosinophilic inflammation and that they would also be separable by FE_{NO} due to the strong relationship between the two indices. The aim of this study was to see whether our retrospective analysis could be confirmed with a prospective group, and whether the magnitude of PEF response to occupational exposure is related to FE_{NO}.

Methods

Study population

Consecutive workers referred to the Occupational Lung Disease Clinic, Birmingham, UK between November 2001 and December 2004 were recruited who had performed an exhaled nitric oxide measurement (FE_{NO}), methacholine challenge test and serial PEF record while still exposed at work. Sixty subjects whose serial PEF measurements showed occupational asthma while exposed to the causative agent and who had a diagnosis of occupational asthma formed the study population. The study was approved by the East Birmingham Local Ethics Committee (reference 929).

Measurements

Workers were requested to record PEF every 2 h from waking to going to bed on work days and days away from work for a total of 4 weeks. The best of 3 PEF readings were recorded on each occasion, provided that the best 2 readings were within 20 L/min of each other. Records were plotted, linearised[22] (if recorded on a non-linear PEF meter) and analysed by the Oasys computer program.[23] Those with a work effect index score $\geq$2.51, (that was used as a cut-off point for definite occupational effect)[23] were included in this analysis.

Spirometry, FE_{NO} and non-specific bronchial reactivity in methacholine challenge were performed within 24 h of work exposure after withholding treatment with long acting β-agonists for 24 h (including combined steroid and long acting β-agonists inhalers), short-acting β-agonists for 6 h and tiotropium for 36 h as part of their routine clinic visit.

Spirometry was performed on either a wedge bellows Vitalograph spirometer or on the Jaeger pulmonary function system according to ERS/ATS standards.[24] Non-specific bronchial reactivity to methacholine was measured using the Yan technique.[25] FE_{NO} was measured during exhalation at 50 ml/s using the Niox from Aerocrine, which requires values from two readings to be within 10% as recommended by the ATS/ERS[26] and performed before spirometry. The Oasys program[23] was used to calculate diurnal variation on days at and away from work and the area between curves (ABC) score based on mean PEF on work days and days away from work plotted by waking time (Fig. 1).[27]

Workers were split into normal and raised nitric oxide level groups based on an eosinophil cut off of 2.2% which was used in our previous study to separate eosinophilic and non-eosinophilic variants.[7] A cut off of 14.7 ppb for smokers and 22.1 ppb for non-smokers (equivalent to $<$ or $\geq$2.2% sputum eosinophilia) was selected from a regression analysis of all our previous combined measurements of sputum eosinophils and FE_{NO}. These values were then used to

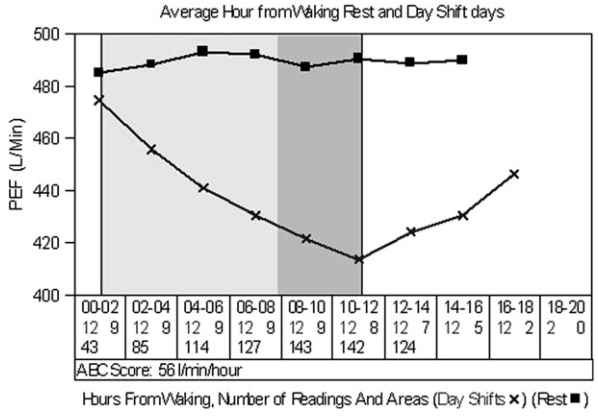

Figure 1 The ABC plot of a worker exposed to chrome from stainless steel welding. He has normal methacholine reactivity ($>$4800 mcg) and an FE_{NO} of 6.1 ppb. The plot has a 56 L/min/h difference between the mean curves of PEF on work and rest days. In the bottom panel, the first row of numbers is the time from waking in 2-hourly sections, e.g. 00—02; 02—04, etc. The second row shows the number of readings used for the mean PEF curves in each 2-hourly section (left side shows work readings and right side shows rest readings). The third row shows the area between the curves for each 2-hourly section which are then used to calculate the ABC score which is in L/min/h. A score of $\geq$15 L/min/h has a sensitivity of 69% and specificity of 100% for occupational asthma diagnosis.[27]

separate workers into those with normal FE_{NO} and those with raised FE_{NO} levels.

Characteristics of the workers such as smoking history, atopy (defined as at least one positive skin prick test of $\geq$3 mm wheal to a common environmental allergen using saline and histamine as negative and positive controls) and inhaled corticosteroid treatment were recorded. Inhaled corticosteroids were classified into groups according to the GINA guidelines[28] for analysis against FE_{NO}.

Statistical analysis

Data was analysed by using FE_{NO} as a continuous variable and also by grouping the workers into two variants based on their FE_{NO} level. Physiological data were not normally distributed, so reactivity to methacholine and nitric oxide levels were log transformed. Subjects who had a $PD_{20} > 4800$ μg (the highest dose used) in methacholine challenge had their percent fall in FEV_1 extrapolated to give a PD_{20} value. Differences in physiological parameters between groups were assessed using a Mann Whitney *U* test or Chi-square test for non-parametric data and either independent *t*-test or one-way ANOVA for parametric data (age, FEV_1 percent predicted, ABC PEF score and log transformed reactivity to methacholine and nitric oxide). Multiple linear regression was used for controlling for variables potentially confounding the relation between FE_{NO} and bronchial hyper-responsiveness. Pearson correlation was used to compare reactivity to methacholine and nitric oxide levels when using both as continuous data. The Yates' continuity correction was used when at least one cell count was <5 when performing the Chi-square statistic. SPSS version 15 was used for all statistics.

Results

Workers had a mean age of 44 years and 83% were males. Mean FE_{NO} levels were similar between atopics and non-atopics ($p = 0.521$), males and females ($p = 0.183$) and those with an FEV_1 percent predicted of <80% or >80% ($p = 0.547$). There were eighteen workers at step 4 of the GINA treatment pathway, eleven at step 3, eight at step 2 and twenty-three on inhaled short-acting beta agonists only. There was no difference in log FE_{NO} between these groups ($p = 0.591$). Current smokers had significantly lower nitric oxide levels ($p = 0.013$) compared to ex or never smokers. Those who showed bronchial hyper-responsiveness in methacholine challenge had a significantly higher FE_{NO} ($p = 0.006$). Table 1 shows statistical comparisons of characteristics and physiological parameters between raised and normal FE_{NO} groups, using the different cut-off points for smokers and ex or never smokers.

There was a significant positive correlation between reactivity to methacholine and nitric oxide level when both were analysed as continuous data (Pearson correlation $= -0.320$; $p = 0.013$). When controlling for smoking, inhaled corticosteroid use and atopy (the main determinants of nitric oxide levels) in multiple linear regression, there was still a significant relationship ($R^2 = 0.221$; $p = 0.009$). Fig. 2 shows the relationships split by current smokers and ex or never smokers.

Correlations between nitric oxide level and ABC score (as a measure of PEF response) were analysed using multiple linear regression controlling for smoking, inhaled corticosteroid use and atopy. There was not a significant relationship ($p = 0.781$). The ABC score was also compared between those with raised and normal FE_{NO} levels in a group of non-smokers who were not taking inhaled corticosteroids. The ABC score was similar ($p = 0.912$). Diurnal variation in PEF was also similar between the two groups ($p = 0.653$).

Workers were analysed for differences in the raised and normal nitric oxide groups according to causative agents (Table 2). There were no differences between those with raised and normal FE_{NO} for high versus low molecular weight agents ($p = 0.898$).

Discussion

In our study of 60 patients with occupational asthma confirmed by their PEF record, we found that occupational asthma patients can be divided into two variants by FE_{NO} level and that the group with raised FE_{NO} has significantly more reactivity in methacholine challenge. The two variants do not differ significantly according to atopy, causative agents of occupational asthma, inhaled steroid use, or FEV_1 percent predicted, indicating that these did not explain the relation between FE_{NO} and bronchial hyper-responsiveness.

Table 1 Characteristics of the two variants of occupational asthma separated by FE_{NO} level and smoking.

	Normal FE_{NO} (smokers <14.7 ppb; never/ex <22.1 ppb) $n = 25$	Raised FE_{NO} (smokers ≥14.7 ppb; never/ex ≥22.1 ppb) $n = 35$	*p* value
Mean Age (SD)	43.4 (9.7)	45.3 (10.1)	0.469
% Male	84.0	82.9	0.907
Mean FEV_1% predicted (SD)	90.7 (21.8)	88.5 (18.1)	0.665
% Atopic	56.0	62.9	0.593
% Using ICS	54.2	65.7	0.372
Mean ABC PEF score (SD)	38.5 (23.9)	29.6 (24.9)	0.196
Mean PEF work diurnal variation (SD)	17.8 (9.4)	20.5 (12.4)	0.653
Mean PD20 in methacholine challenge μg (SD)	5730 (4975)	3883 (5048)	0.035

ICS = inhaled corticosteroids

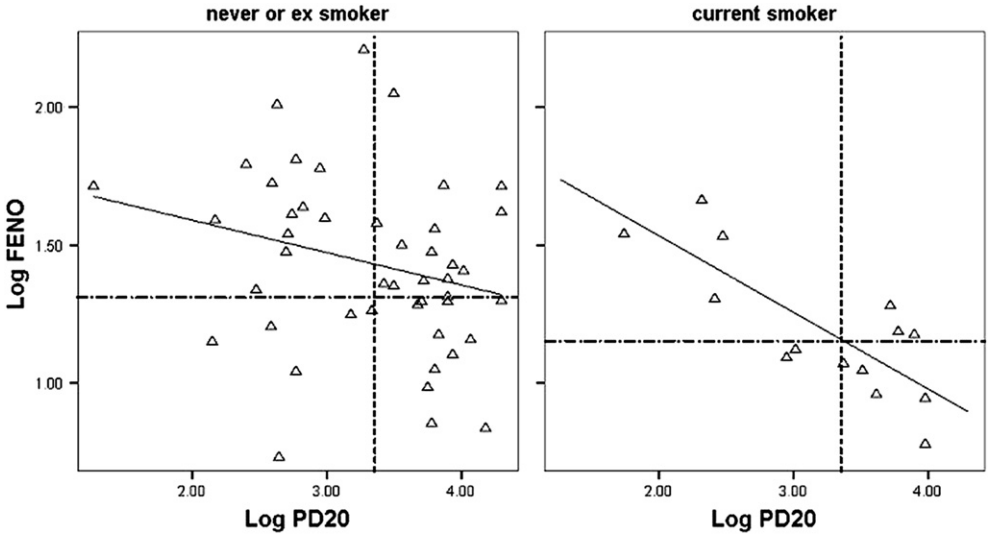

Figure 2 Scatter diagram of correlation between exhaled FE_{NO} and reactivity in methacholine challenge separated by smoking. - - - - - - - - - - - - indicates the cut off for normal methacholine reactivity —·—·—·—·—·— indicates the cut off separating normal and raised FE_{NO} levels.

Both FE_{NO} groups were similar with respect to changes in PEF in response to occupational exposure, as small and large changes in mean PEF and low and high diurnal variation were seen equally in both normal and raised FE_{NO} groups.

Our results are compatible with others but our interpretation differs. Several groups have shown that the mean FE_{NO} increases with exposure in occupational settings, and that there is a relationship between FE_{NO} and non-specific bronchial reactivity in occupational and non-occupational groups.[8–12,18–21] Barbinova and Baur found that 52% of occupational asthmatics who had non-specific bronchial hyper-responsiveness had a >50% increase in FE_{NO} post specific inhalation challenge test compared to 20% with normal hyper-responsiveness.[19] The mean changes have however been driven by a subset who show changes, the subgroup without changes in FE_{NO} have not been analysed separately by others.

This study was designed as a follow on to the original Anees et al. study.[7] The original observation was from a retrospective analysis, whereas the current paper is wholly prospective data. We started with the hypothesis generated by our previous study that there were two variants of occupational asthma separated by FE_{NO} values that were raised or within normal ranges while exposed, and hypothesised that the response to occupational exposures might differ. By analysing this prospective group, we have confirmed that the two variants differ in non-specific bronchial reactivity, but have not found differences in either the agents responsible for the occupational asthma nor the responses seen in the workplace measured through serial PEFs. The results indicate that FE_{NO} can be used without the need to measure sputum eosinophilia, the former being a simple and cost effective clinical measurement and the latter a much more time-intensive process. There are centres around the world who

Table 2 Causative occupational exposures by normal and raised FE_{NO} levels.

Type of occupational exposure	Normal FE_{NO} (smokers <14.7 ppb; never/ex <22.1 ppb) $n = 25$	Raised FE_{NO} (smokers ≥14.7 ppb; never/ex ≥22.1 ppb) $n = 35$	p value
Metals	9	11	0.711
Biocides	3	6	0.855
Metal-working fluid	1	5	0.383
Isocyanates	3	6	0.855
Adhesives	2	2	1.000
Plastics	0	1	1.000
Other low molecular weight agents	3	0	0.133
High molecular weight agents	4	4	0.898
Low molecular weight agents	21	31	

believe that increased non-specific bronchial reactivity is essential for the diagnosis of occupational asthma. In our experience, normal non-specific reactivity is found in ~30% of workers currently exposed who have occupational asthma. The results therefore support the inclusion of workers with normal non-specific bronchial reactivity within the family of occupational asthma due to sensitisation. Our PEF response results agree with other studies that have also not shown any correlation between FE_{NO} and the magnitude of lung function (mainly FEV_1) in non-occupational asthma.[9–11]

We think that these two variants of occupational asthma separable by the FE_{NO} level may be related to different types of inflammation in the airways, the raised FE_{NO} being related to eosinophilic inflammation and the normal FE_{NO} perhaps to neutrophilic or other types of inflammation, which has also been proposed by Taylor et al.[29] This hypothesis is supported by our previous finding that raised FE_{NO} was significantly correlated with sputum eosinophilia.[7] Others have also found a linkage between eosinophilia and raised nitric oxide levels.[2,4,6] We originally hypothesised that the occupational asthmatics with large changes in PEF related to work exposure were more likely to have a raised FE_{NO} than the group with small changes; this however was not supported by our data. Whether these two variants of occupational asthma according to FE_{NO} level have implications for prognosis or treatment of the disease needs to be addressed in future studies. One of the factors relating to prognosis (FEV1% predicted) showed similar means for those with raised and normal FE_{NO} levels indicating that prognostic factors may only explain a small amount of the differences in the two variants. This outcome was significantly different in the original retrospective cohort, but other prognostic factors (length of symptomatic exposure and time from first exposure to disease onset) were similar between eosinophilic and non-eosinophilic groups.

A number of studies have shown that inhaled corticosteroid use results in a fall in FE_{NO} levels in patients with asthma.[20,30–36] As the group with a raised FE_{NO} were on more inhaled ICS than the normal group, we were unable to find a correlation between ICS use and FE_{NO}. A small number of patients may have been misclassified in the normal FE_{NO} group because of this. Workers taking combination inhalers (steroid and long acting beta agonists) would have withheld therapy for 36 h prior to the clinic appointment for uncompromised non-specific reactivity measurements which may have led to higher FE_{NO} levels in this group. We also found that atopics and non-atopics had similar FE_{NO} levels whereas other groups studying asthmatics have found a difference.[9,10,37–39] This may be due to the fact that our cohort is a group of occupational asthmatics which may be acting differently to general asthmatics.

Validity issues

All workers in our study had PEF records showing a work-rest pattern compatible with occupational asthma and Oasys score >2.51 (sensitivity of 76% and specificity of 94% for occupational asthma[23]). Workers were recruited consecutively and were currently exposed to the suspected occupational agent at the time of all investigations. There were 12 workers with normal FE_{NO} levels who had a normal reactivity to methacholine and an FEV_1 percent predicted >80%. Although some may regard these subjects as not having occupational asthma, all of them did fulfil the usual definitions of asthma requiring airflow obstruction which varies over short periods of time (here within 24 h of occupational exposures) and their mean diurnal variation at work was 15%. All workers also had a clear, relevant symptom history compatible with occupational asthma and many were exposed to well known causative agents. In addition, 3 had positive specific inhalation challenge tests to the relevant occupational allergen.

Using a cut off for FE_{NO} may have it's limitations, however we believe that by choosing a previously validated cut off based on sputum eosinophilia, this problem has been addressed. With a sample size increase, we may have seen more difference between groups, although looking at the data we feel this is unlikely.

Conclusions

We have identified two variants of occupational asthma which cannot be separated according to the degree of asthmatic reaction induced by workplace exposures or the agents that they are exposed to, but can be separated by measurement of exhaled nitric oxide whilst symptomatic. The group with raised FE_{NO} levels have greater reactivity to methacholine compared to those with normal FE_{NO}. This could reflect different types of airway inflammation in these two groups. Whether they differ in prognosis remains a question to be addressed in future studies.

Competing interest

Conflicts of interest: Authors Vicky Moore, Maritta Jaakkola, Cedd Burge, Wasif Anees and Alastair Robertson have no conflicts to disclose for this manuscript. Professor Burge promotes and disseminates the use of serial measurements of peak expiratory flow for the diagnosis of occupational asthma. His department receives some monies from grants, donations and legal fees to support the research, but he has no personal financial interest.

Funding

This project was funded by a COLT Foundation PhD fellowship for Vicky Moore.

Acknowledgments

Vicky Moore is the principal data collector, data analyser and principal author for this study. Wasif Anees proposed the original concept, contributed to the collection of data, revised the paper and approved the final version. Professor Burge and Maritta Jaakkola have contributed to the design of the study, interpreted data, revised the paper and approved the final version. Cedd Burge has contributed to

the analysis of data, revised the paper and approved the final version. Alastair Robertson has contributed to the collection of data, revised the paper and approved the final version.

References

1. Kharatinov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;**343**:133–5.
2. Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005;**35**: 1175–9.
3. Payne DNR. Nitric oxide in allergic airway inflammation. *Curr Opin Allergy Clin Immunol* 2003;**3**:133–7.
4. Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JDM, Ennis M, et al. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. *Thorax* 2002;**57**:383–7.
5. Jatakanon A, Lim S, Kharatinov SA, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;**53**:91–5.
6. Silkoff PE, Lent AMM, Busacker AAB, Katial RKM, Balzar S, Strand M, et al. Exhaled nitric oxide identifies the persistent eosinophilic phenotype in severe refractory asthma. *J Allergy Clin Immunol* 2005;**116**:1249–55.
7. Anees W, Huggins V, Pavord ID, Robertson AS, Burge PS. Occupational asthma due to low molecular weight agents: eosinophilic and non-eosinophilic variants. *Thorax* 2002;**57**: 231–6.
8. Berkman N, Avital A, Breuer R, Bardach E, Springer C, Godfrey S. Exhaled nitric oxide in the diagnosis of asthma: comparison with bronchial provocation tests. *Thorax* 2005;**60**: 383–8.
9. Franklin PJP, Stick SMP, Le Souef PNM, Ayres JGM, Turner SWM. Measuring exhaled nitric oxide levels in adults: the importance of atopy and airway responsiveness. *Chest* 2004;**126**:1540–5.
10. Franklin PJ, Turner SW, Le Souef PN, Stick SM. Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. *Thorax* 2003;**58**:1048–52.
11. Langley SJM, Goldthorpe S, Custovic A, Woodcock A. Relationship among pulmonary function, bronchial reactivity, and exhaled nitric oxide in a large group of asthmatic patients. *Ann Allergy Asthma Immunol* 2003;**91**:398–404.
12. Haahtela T, Malmberg P, Moreira A. Mechanisms of asthma in Olympic athletes – practical implications. *Allergy* 2008;**63**: 685–94.
13. Zietkowski Z, Bodzenta-Lukaszyk A, Tomasiak MM, Skiepko R, Szmitkowski M. Comparison of exhaled nitric oxide measurement with conventional tests in steroid-naive asthma patients. *J Investig Allergol Clin Immunol* 2006;**16**:239–46.
14. Henriksen AH, Lingaas-Holmen T, Sue-Chu M, Bjermer L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in a large population survey. *Eur Respir J* 2000;**15**:849–55.
15. Nicholson PJ, Cullinan P, Newman Taylor AJ, Burge PS, Boyle C. Evidence based guidelines for the prevention, identification, and management of occupational asthma. *Occup Environ Med* 2005;**62**:290–9.
16. British Thoracic Society, Scottish Intercollegiate guidelines Network. British guideline on the management of asthma. *Thorax* 2008;**63**(Suppl. 4):iv1–iv121.
17. Girard F, Chaboillez S, Cartier A, Cote J, Hargreave FE, Labrecque M, et al. An effective strategy for diagnosing occupational asthma: use of induced sputum. *Am J Respir Crit Care Med* 2004;**170**:845–50.
18. Swierczynska-Machura D, Krakowiak A, Wiszniewska M, Dudek W, Walusiak J, Palczynski C. Exhaled nitric oxide levels after specific inhalatory challenge test in subjects with diagnosed occupational asthma. *Int J Occup Med Environ Health* 2008;**21**:219–25.
19. Barbinova L, Baur X. Increase in exhaled nitric oxide (eNO) after work-related isocyanate exposure. *Int Arch Occup Environ Health* 2006;**79**:387–95.
20. Baur X, Barbinova L. Latex allergen exposure increases exhaled nitric oxide in symptomatic healthcare workers. *Eur Respir J* 2005;**25**:309–16.
21. Piipari R, Piirila P, Keskinen H, Tuppurainen M, Sovijarvi A, Nordman H. Exhaled nitric oxide in specific challenge tests to assess occupational asthma. *Eur Respir J* 2002;**20**: 1532–7.
22. Miller MR, Dickinson SA, Hitchings DJ. The accuracy of portable peak flow meters. *Thorax* 1992;**47**:904–9.
23. Gannon PFG, Newton DT, Belcher J, Pantin CF, Burge PS. Development of OASYS-2, a system for the analysis of serial measurements of peak expiratory flow in workers with suspected occupational asthma. *Thorax* 1996;**51**: 484–9.
24. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005;**26**:319–38.
25. Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;**38**:760–5.
26. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;**171**:912–30.
27. Moore VC, Jaakkola MS, Burge CBSG, Robertson AS, Pantin CFA, Vellore AD, et al. A new diagnostic score for occupational asthma; the area between the curves (ABC score) of PEF on days at and away from work. *Chest* 2009;**135**: 307–14.
28. Global strategy for asthma management and prevention, global initiative for asthma (GINA). Available from, http://www.ginasthmaorg.2008; 2008.
29. Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006;**61**:817–27.
30. Kharitonov SA, Yates DH, Chung KF, Barnes PJ. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. *Eur Respir J* 1996;**9**: 196–201.
31. Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. *Thorax* 1999;**54**: 108–14.
32. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. *Chest* 2001; **119**:1322–8.
33. Beck-Ripp J, Griese M, Arenz S, Koring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. *Eur Respir J* 2002;**19**: 1015–9.
34. Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. *Thorax* 2002;**57**: 889–96.
35. Jones SL, Herbison P, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, et al. Exhaled NO and assessment of anti-

inflammatory effects of inhaled steroid: dose-response relationship. *Eur Respir J* 2002;**20**:601—8.
36. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. *Am J Respir Crit Care Med* 2005; **172**:831—6.
37. Olin AC, Rosengren A, Thelle DS, Lissner L, Bake B, Toren K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. *Chest* 2006;**130**:1319—25.
38. Ho LP, Wood FT, Robson A, Innes JA, Greening AP. Atopy influences exhaled nitric oxide levels in adult asthmatics. *Chest* 2000;**118**:1327—31.
39. Gratziou C, Lignos M, Dassiou M, Roussos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. *Eur Respir J* 1999;**14**:897—901.