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Fractional Exhaled Nitric Oxide in the Interpretation of Specific Inhalational Challenge Tests for Occupational Asthma

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Abstract

Purpose Fractional exhaled nitric oxide (FE_{NO}) measurements are recommended for the assessment of eosinophilic airway inflammation in asthma. Clinically relevant increases in FE_{NO} have been reported 24 h after positive specific inhalational challenge (SIC) tests in occupational asthma. We aimed to determine whether positive SICs could be discriminated from control tests, on the basis of change in FE_{NO}.

Methods We reviewed all positive SICs to a variety of agents performed at our institution 2008–2012 and gathered data on age, sex, asthmatic response (immediate/dual/late), smoking status, inhaled corticosteroid usage, and FE_{NO} pre- and 24-h postcontrol and positive SIC from each worker. Changes in FE_{NO} after positive SICs were compared with control SICs from each worker, by using paired Student's *t* tests.

Results In 16 workers, negative control challenges were associated with mean changes in FE_{NO} of 9 % (95 % CI −1.14 to 19.01) or 1.1 ppb (95 % CI −3.59 to 5.84); 2 of 16 (13 %) workers tested showed increases in FE_{NO} that were clinically relevant based on recent guidelines. Subsequent positive SICs were associated with mean changes in FE_{NO} of 7 % (95 % CI −15.73 to 29.6) or 2.1 ppb (95 % CI −6.07 to 10.19), which were not significantly different to controls; only 2 of 16 (13 %) workers had FE_{NO} changes that were clinically relevant.

Conclusions FE_{NO} changes above the upper confidence limits of ≥20 % or ≥6 ppb may be considered to be

outside the range of normality. However, the majority of workers who had clearly positive SICs to common low molecular weight agents also had no statistically or clinically relevant increase in FE_{NO}. Therefore, change in FE_{NO} does not predict a positive SIC in this group.

Keywords Occupational asthma · Bronchial challenge · Exhaled nitric oxide · Specific inhalational challenge · Asthma · Eosinophilic inflammation

Introduction

Specific inhalational challenge (SIC) is the “gold standard” diagnostic test for occupational asthma (OA), with a high specificity for identifying affected workers [1, 2]. However, results are sometimes equivocal and sensitivity decreases with time away from exposure. Induction of sputum eosinophilia has been demonstrated in both early and late asthmatic responses to SIC with occupational agents [3, 4]. Fractional exhaled nitric oxide (FE_{NO}) is a simple noninvasive method of assessing eosinophilic airway inflammation [5, 6], which correlates well with sputum eosinophilia when inhaled corticosteroid (ICS) use and smoking have been taken into account [7–9]. Recent guidelines recommend the application of FE_{NO} to support a diagnosis of asthma where further objective evidence is required [5]. Clinically relevant increases in FE_{NO} have been seen 24 h after positive SIC [10, 11] and also in symptomatic patients with negative SICs [12]. We aimed to investigate whether FE_{NO} could discriminate positive from control tests for SICs undertaken to a variety of occupational agents, at our specialist occupational lung disease unit.

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Methods

SIC Tests

All SIC tests with occupational agents undertaken between January 2008 and April 2012 at Birmingham Heartlands Hospital Occupational Lung Disease Unit were reviewed by two occupational lung disease physicians. All SICs were performed in a dedicated challenge chamber in the hospital setting according to international guidelines [13]. Workers were electively admitted to hospital from Monday to Friday for a series of SICs to possible causative agents. Each worker undertook one challenge lasting 10–120 min, per morning, until a positive challenge was elicited, beginning on the Monday with challenge to a control agent for which no response was predicted. Workers were blinded to the agent under investigation where possible. The regimes were designed individually, based on the workers' clinical and exposure history. SIC was considered positive if the FEV₁ fell >15 % from baseline on >1 measurement (within 10–30 min for immediate asthmatic reactions and >1 h for late reactions) or fell below the lower 95 % confidence limit for the mean FEV₁ from ≥3 days without occupational exposure immediately before SIC (dual or late asthmatic reactions only; [14]). All spirometry was measured on a Viasys Microlab portable spirometer (Micromedical Ltd., Rochester, Kent, UK) according to European Respiratory Society/American Thoracic Society (ERS/ATS) standards using European Community for Coal and Steel predicted values [15]. For each worker undergoing SIC, data were collected on age, sex, smoking status, and ICS prescription, as well as duration of allergen exposure and baseline and serial FEV₁ measurements, following each control and positive SIC.

Fractional Exhaled Nitric Oxide (FE_{NO}) Measurements

FE_{NO} was measured immediately pre- and 24-h postcontrol and positive SICs using a Niox Mino handheld machine (Aerocrine AB, Solna, Sweden), at 50 ml/s compliant with ERS/ATS recommendations before spirometry [16]. All measurements were taken in the hospital respiratory physiology laboratory away from the challenge chamber. Abnormal baseline FE_{NO} measurements were considered to be ≥22 ppb in never- or ex-smokers and ≥14 ppb in active smokers [17, 18], a level equivalent with a raised sputum eosinophil count of ≥2.2 %. A clinically relevant change was considered to be an increase in FE_{NO} of >20 % for baseline values >50 ppb, and >10 ppb for values <50 ppb, as recommended in ATS guidelines [5].

Statistical Analysis

Descriptive statistics were used to illustrate all demographic data, pretest FE_{NO} values, and change in FE_{NO} following SIC in both absolute parts per billion (ppb) and percentage change. Paired Student's *t* tests were used to compare mean change in FE_{NO} (in ppb and percentage change) after control and positive SICs. FE_{NO} data also were grouped by the type of asthmatic reaction seen during a positive SIC (immediate, dual, or late), and by whether workers were taking ICS or not, but not by smoking status (*n* = 1). All statistical analysis was performed using Prism version 6 (GraphPad Software Inc., La Jolla, CA, USA) and calculated at the 95 % confidence level. Ethical approval was obtained from the Birmingham East, North and Solihull Research Ethics Committee (Birmingham, UK).

Results

Fifty-one patients underwent SIC during the study period. SIC tests were excluded from analysis if they had missing FE_{NO} measurements or if baseline FE_{NO} measurements were preceded by positive or equivocal SICs on the previous day. Thus, FE_{NO} data pre- and post-SIC were available for 16 positive (6 immediate, 5 dual, 5 late) SICs (Fig. 1). Mean age was 44 [standard deviation (SD) = 12], there were 14 of 16 males (88 %), 1 active smoker, 10 of 16 workers were taking ICS (63 %) and mean baseline FEV₁ measurement was 92 % of predicted (SD = 19) before control challenge.

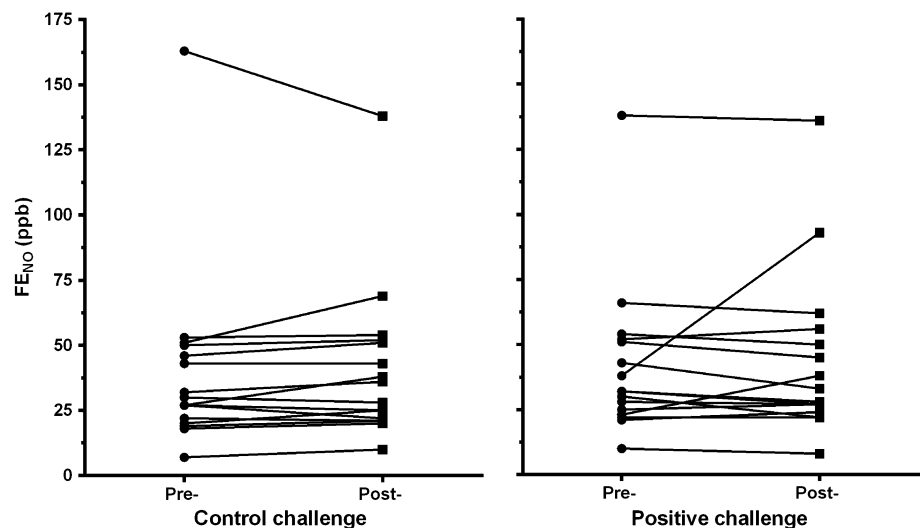
Control SICs

Materials tested were unused metalworking fluid (MWF) (6), lactose and inert dusts (3), alcohol hand gels (2), and resins without catalysts (2). In addition, three workers were exposed to agents known to cause OA (an isocyanate, methyl methacrylate, and activated styrene). All control tests were negative and are detailed in Table 1. Mean prechallenge FE_{NO} was 39.7 ppb (SD = 36) and 12 of 16 patients (75 %) had a raised baseline FE_{NO}. After control challenge, two patients (13 %) had a clinically relevant increase in FE_{NO} heated starch powder (worker 7) and styrene filler with peroxide catalyst (worker 15). Mean change in FE_{NO} after control challenge was 9 % (SD = 19; 95 % CI −1.14 to 19.01) or 1.1 ppb (SD = 9; 95 % CI −3.59 to 5.84).

Positive SICs

Positive SICs were observed to a wide range of agents: isocyanates (3), used MWFs (3), metals (3), hand gels (2),

Fig. 1 Pre- and postchallenge FE_{NO} (ppb) for all control and positive SICs ($n = 16$)



and others (5), including two (lime and cardboard dust) that were probably acting as irritants causing OA with latency (Table 2). Mean prechallenge FE_{NO} was 41.6 ppb (SD = 30) and 14 of 16 patients (88 %) had a raised baseline FE_{NO} . Two workers, one with a dual reaction to potassium dichromate (worker 4) and the other a late reaction to MDI (worker 15), showed a clinically relevant increase in FE_{NO} , and both lay above the upper 95 % confidence limits for control exposures, for percentage and actual ppb change (Fig. 2). Of all agents tested, 1 of 2 potassium dichromate, 0 of 3 used MWF, 1 of 3 MDI, and 0

of 2 alcohol gels showed clinically relevant increases in FE_{NO} (rest too few to group). Mean change in FE_{NO} after positive SIC was 7 % (SD = 43; 95 % CI -15.73 to 29.6) or 2.1 ppb (SD = 15; 95 % CI -6.07 to 10.19).

Comparisons Between Groups

There was no significant difference between mean change in FE_{NO} after positive SIC compared with control SIC, either in absolute ppb ($p = 0.82$) or percentage change ($p = 0.85$). When positive SICs were grouped by asthmatic

Table 1 Control SICs with respective changes in FEV_1 and FE_{NO} after challenge

Patient number	Control exposure	Length of exposure (min)	Start FEV_1 (L)	Maximum immediate change FEV_1 (%)	Maximum late change FEV_1 (%)	Initial FE_{NO} prechallenge (ppb)	Change in FE_{NO} 24-h postchallenge; ppb (% pretest FE_{NO})
1	Methyl methacrylate	70	2.59	-10.04	-3.09	46	5 (11)
2	Lactose	70	3.49	-4.01	-6.02	53	1 (2)
3	New Hysol G (MWF)	70	2.97	2.02	3.7	32	4 (13)
4	New Hysol G (MWF)	60	2.44	2.46	-5.74	22	-1 (-5)
5	New HD59 (MWF)	70	4.74	-2.95	-0.42	27	-2 (-7)
6	Spirigel alcohol gel	70	2.86	-12.24	-2.45	19	2 (11)
7	Heated starch powder	60	2.06	2.91	15.53	51	18 (35)
8	New Hysol G (MWF)	70	3.67	3	-1.63	50	2 (4)
9	New Hysol G (MWF)	70	4.41	2.27	3.63	20	5 (25)
10	New Hocut 808 (MWF)	70	2.3	-3.48	-1.3	43	0 (0)
11	Brick dust	70	2.84	-11.27	3.17	18	2 (11)
12	Purell alcohol gel	70	2.82	-5.32	1.06	7	3 (43)
13	Epoxy paint without thinner	40	2.6	-3.85	1.15	30	-2 (-7)
14	Polyol	70	2.77	-5.05	-13.4	163	-25 (-15)
15	U-pol (styrene and peroxide catalyst)	70	4.43	-5.19	-6.09	27	11 (41)
16	MDI	70	3.66	-7.1	-7.1	27	-5 (-19)

MWF metalworking fluid, MDI methylene diphenyl diisocyanate

Table 2 Positive SICs with respective changes in FEV₁ and FE_{NO} after challenge

Patient number	Positive challenge exposure	Length of exposure (min)	Start FEV ₁ (L)	Immediate, dual or late asthmatic reaction	Maximum immediate change FEV ₁ (%)	Maximum late change FEV ₁ (%)	Initial FE _{NO} prechallenge (ppb)	Change in FE _{NO} 24-h postchallenge; ppb (% pretest FE _{NO})
1	C-solve (solvent degreaser)	30	2.48	Immediate	-31.45	-3.23	51	-6 (-12)
2	Sand and lime	10	3.51	Immediate	-20.2	-4.56	54	-4 (-7)
3	Potassium dichromate	35	2.99	Immediate	-17.73	-7.02	30	-8 (-27)
4	Potassium dichromate	35	2.52	Dual	-23.81	-25.79	23	15 (65)
5	Used MWF	10	4.82	Immediate	-28.63	-2.28	25	2 (8)
6	Softalind (alcohol gel denatonium)	33	2.63	Dual	-20.2	-17.17	32	-5 (-16)
7	Cardboard dust (high concentration)	70	2.45	Immediate	-15.51	-9.39	66	-4 (-6)
8	Used MWF	50	3.63	Dual	-16.25	-17.91	52	4 (8)
9	Cobalt chloride	35	4.73	Equivocal late; Stenton +ve	-4.86	-11.21 ^a	32	-4 (-13)
10	Used MWF	70	2.4	Late	-7.08	-17.5	43	-10 (-23)
11	Pond water	70	3.2	Late	-6.56	-23.13	21	3 (14)
12	Softalind (alcohol gel denatonium)	30	2.93	Dual	-23.21	-13.99	10	-2 (-20)
13	Epoxy paint thinner (polyamidoamine)	30	2.6	Immediate	-20.38	-3.08	28	-1 (-4)
14	MDI	50	2.92	Dual	-34.3	-47.3	138	-2 (-1)
15	MDI	120	4.31	Late	-6.73	-18.79	38	55 (145)
16	MDI	120	3.64	Equivocal dual; Stenton +ve	-14.01 ^b	-9.89 ^b	22	0 (0)

MWF metalworking fluid, MDI methylene diphenyl diisocyanate

^a Patient 9 had a positive SIC to cobalt chloride 10 mg/ml with a late fall in FEV₁ of 350 ml, below the lower 95 % confidence limit for unexposed FEV₁

^b Patient 16 had a positive SIC to methylene diphenyl diisocyanate (MDI) with a late fall in FEV₁ of 260 ml, below the lower 95 % confidence limit for unexposed FEV₁

reaction, there were no differences compared with control challenges: immediate asthmatic reactions: $n = 6$, $p = 0.15$ (% change), $p = 0.11$ (ppb); dual or late asthmatic reactions: $n = 10$; $p = 0.7$ (% change), $p = 0.3$ (ppb). When grouped by ICS prescription, there were no significant differences between positive and controls SICs: workers taking ICS: $n = 10$, $p = 0.69$ (% change), $p = 0.34$ (ppb); workers not taking ICS: ($n = 6$) $p = 0.94$ (% change), $p = 0.37$ (ppb).

Discussion

In 16 workers undertaking SICs to low molecular weight occupational agents, negative control challenges were associated with mean changes in FE_{NO} of 9 % (95 % CI -1.14 to 19.01) or 1.1 ppb (95 % CI -3.59 to 5.84); 2 of

16 workers tested showed increases in FE_{NO} that were clinically relevant. Subsequent positive SICs were associated with mean changes in FE_{NO} of 7 % (95 % CI -15.73 to 29.6) or 2.1 ppb (95 % CI -6.07 to 10.19), which were not significantly different to controls; only 2 of 16 workers had FE_{NO} changes that were clinically relevant.

This study uses 3 years' experience of SIC testing in a tertiary referral unit for occupational lung disease, where SIC tests are performed routinely in a clinical setting, according to international guidelines [13]. To the best of our knowledge, it is the only study of FE_{NO} measurements in SIC testing that uses paired within-subject comparisons of control and positive challenges.

The role of FE_{NO} in occupational challenge testing remains unproven, although several other prospective studies have investigated FE_{NO} changes after SIC in this setting [10, 12, 19–22]. Pedrosa et al. [19] showed a

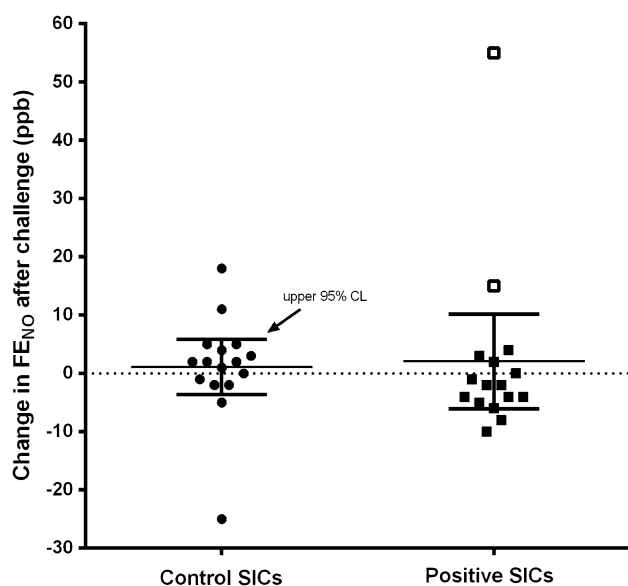


Fig. 2 Absolute change in FE_{NO} after SICs, in ppb. Control and positive SICs are shown with mean and 95 % confidence limits. Individual values of FE_{NO} from positive SICs that are above the upper confidence limit for control exposures are *highlighted*

significant 14 ppb FE_{NO} change 24 h after positive SIC ($n = 21$) compared with negative tests ($n = 13$), and retrospective receiver–operator curve analysis achieved a sensitivity of 81 % and specificity of 92 % in identifying a positive result, from a 12 % increase in FE_{NO} . Three other studies have demonstrated an increase in FE_{NO} 24 h after exposure to high and low molecular weight occupational agents, which correlated well with sputum eosinophil measurements [20–22]. The results of our study differ from these other studies, as increase in FE_{NO} was rarely seen after positive SIC, and as such it is important to consider confounding factors: (i) FE_{NO} is affected by measurement technique, exhalation flow rate, and the analyzer used [5]. In our study, all FE_{NO} measurements were performed according to ERS/ATS guidelines [16] using the Niox Mino handheld machine, whose clinical performance has been validated against static analyzers [23]. (ii) Active smoking inhibits FE_{NO} [5] and this has been illustrated in the occupational setting: atopic bakers who are active smokers have lower baseline FE_{NO} measurements than atopic nonsmokers [24]; however, there was only one smoker in our study. (iii) The timing of our FE_{NO} measurement after SIC was optimal: FE_{NO} can be elevated up to 72 h after SIC in asthmatic subjects [25, 26], and we excluded results that were obtained 24–48 h after a previous positive or equivocal reaction to allow for this. The maximal response is ~ 10 h postexposure [27, 28], and most studies in the occupational setting have used post-exposure measurements at 20–24 h. The main limitation of

this study is the relatively small number of SICs available for analysis.

A previous study from our unit [18] showed that the majority of cases of OA due to low molecular weight agents are of the noneosinophilic (predominantly neutrophilic) variant, and only 37 % of cases demonstrated sputum eosinophilia (>2.2 %). Nonavailability of sputum cell counts prevented us from characterizing workers into eosinophilic and noneosinophilic variants for analysis. However, 88 % of workers in this study had raised baseline FE_{NO} levels suggestive of an eosinophilic variant OA, and we did not see significant FE_{NO} changes after positive SIC in this group.

In workers undertaking SICs to predominantly low molecular weight occupational agents, negative control challenges were associated with mean changes in FE_{NO} of 9 % or 1.1 ppb. FE_{NO} changes above the upper confidence limits of ≥ 20 % or ≥ 6 ppb may be considered to be outside the range of normality. The majority of workers who had clearly positive SICs to common low molecular weight agents also had no statistically or clinically relevant increase in FE_{NO} , and therefore by implication, change in FE_{NO} does not predict a positive SIC in this group.

Conflict of interest There are no conflicts of interest on the part of any of the authors.

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