



An increase of fractional exhaled nitric oxide after specific inhalation challenge is highly predictive of occupational asthma

Julia Engel¹ · Vera van Kampen¹ · Anne Lotz¹ · Jessica Abramowski¹ · Vitali Gering¹ · Olaf Hagemeyer¹ · Thomas Brüning¹ · Monika Raulf¹ · Rolf Merget¹ 

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Abstract

Purpose An increase of fractional exhaled nitric oxide (FeNO) has been described after specific inhalation challenges (SICs) with occupational allergens, but the clinical role of FeNO measurements before and after SIC is unknown. It was the aim of this study to assess the diagnostic accuracy of FeNO measurements before and after SIC in subjects with suspected occupational asthma (OA).

Methods One hundred forty-eight patients with suspected OA were examined by SIC with various occupational allergens. Subjects were assigned to pulmonary responders, nonresponders or doubtful by standard lung function criteria. FeNO was measured before SIC (baseline) and 24 h afterwards. Subjects with negative or doubtful SIC but increase of FeNO were evaluated individually by an overall expert rating. Effect modifiers of FeNO increases were assessed by regression analyses.

Results Thirty-one patients (21%) were classified as pulmonary responders, 105 (71%) as nonresponders and 12 (8%) as doubtful. With the pulmonary responder status as gold standard an increase of FeNO ≥ 13 ppb showed a specificity of 0.9 and a sensitivity of 0.5. Seventeen subjects with negative or doubtful responder status showed such an increase of FeNO, among them 13 subjects with definitive or probable OA after expert rating. Regression analyses revealed no significant modifiers for the FeNO increase.

Conclusion An increase of FeNO after SIC is highly predictive of OA and should be regarded as an additional criterion for the interpretation of SIC with occupational agents.

Keywords Asthma · Occupational · Fractional exhaled nitric oxide · Specific inhalation challenge · Predictive value

Introduction

Increased levels of fractional exhaled nitric oxide (FeNO) represent a marker of eosinophilic airway inflammation which is a feature of (immunologic) occupational asthma (OA) (Lemiere 2007). As the diagnosis of OA is associated with substantial health and socioeconomic impacts (Vandenplas et al. 2003), an accurate diagnosis is of high importance. Specific inhalation challenges (SICs) in the laboratory are still regarded as the gold standard for establishing the diagnosis of OA. Historically, lung function, especially

forced expiratory volume in 1 s (FEV₁) is the primary outcome parameter.

A moderate number of studies analyzed FeNO measurements before and after SIC with occupational allergens. Concentrations of FeNO are lower in smokers and higher in atopics (Jonaïd et al. 2014), but it is not known to what extent these factors modify also the acute response during SIC. An increase of FeNO of 17.5 ppb after SIC has been considered to provide the optimal sensitivity and specificity associated with positive SIC in an earlier study (Lemiere et al. 2014). However, it is a matter of discussion which level of increase of FeNO after SIC is highly predictive of OA, especially in smokers or atopics. Also there are few data about other modifiers, e.g. it is not known whether FeNO increases depend on the type of the agent, severity of the asthmatic reaction, smoking status or medication use. It is known that FeNO concentrations may increase after SIC, but this is clearly not the

✉ Rolf Merget
merget@ipa-dguv.de

¹ Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

case in all subjects with an asthmatic response (pulmonary responders) (Barbinova and Baur 2006; Piipari et al. 2002), and obviously FeNO may increase also in pulmonary nonresponders (Barbinova and Baur 2006). So far no study made an attempt to explain these “false positive” FeNO increases in pulmonary nonresponders, thus it remains unknown whether an increase of FeNO after SIC is indicative of OA. It has been shown that increases of sputum eosinophils are highly predictive of a positive asthmatic reaction after repetitive SICs (Vandenplas et al. 2009), but studies examining FeNO before and after SIC do not allow to give an estimate of the test accuracy for clinical decision making in individual cases (Ferrazzoni et al. 2009; Mason et al. 2016; Piipari et al. 2002; Pedrosa et al. 2012; Sastre et al. 2013; Walters et al. 2014). It was the aim of this study to assess the diagnostic accuracy of FeNO measurements before and after SIC for clinical decision making and to identify effect modifiers of FeNO increases.

Methods

Subjects and study design

All subjects referred to our outpatient clinic with suspected OA who underwent SIC with putative allergens from 2006 to 2015 and agreed to participate were included in this study. The investigations were performed on three consecutive days, with few exceptions. During the first day (“control day”) the subjects answered a questionnaire and underwent a physical examination, routine laboratory testing, total and specific immunoglobulin E antibodies to occupational allergens (sIgE), electrocardiogram, X-ray of the thorax (if not done earlier), skin prick testing (SPT) with occupational and common environmental allergens, methacholine testing, and repeated pulmonary function tests to document a stable respiratory status. On the second day SIC with the putative allergen was performed. Lung function parameters and FeNO were measured before the challenge (baseline). Spirometry and bodyplethysmography were performed before and after each challenge step as well as until 4 h after the end of SIC (follow-up). Those subjects challenged with low-molecular weight substances like diisocyanates were equipped with a portable spirometer and kept in hospital during night. On the third day, 24 h after exposure, pulmonary function tests and FeNO testing were repeated. Induced sputum was collected immediately before and 24 h after SIC in some subjects. In this study, we report the results of sputum analyses of 11 subjects with doubtful or negative SIC responder status

with increases of FeNO. Sputum collection and analysis were performed as described earlier (Raulf et al. 2015).

Questionnaire

Personal data and information about current cigarette smoking status, medical history, asthma prior to exposure to the suspected occupational allergen, exposures at work and medication were obtained by a questionnaire and a physician interview. All subjects complained of work-related shortness of breath.

Allergy testing

SPT were performed with a battery of 20 environmental allergens from various manufacturers and if available with occupational allergens. A positive reaction was assumed if the wheal reaction was ≥ 3 mm. Total IgE was measured in sera by ImmunoCAP (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). sIgE testing was performed if available (ImmunoCAP) and a sIgE concentration of ≥ 0.35 kU/L was considered positive. Atopy was defined by a positive SPT to at least one environmental allergen. Occupational allergens were categorized in three groups according to the size of the allergens: high-molecular weight (HMW) allergens, low-molecular weight allergens (without isocyanates; LMW), and isocyanates.

Lung function and methacholine testing

Pulmonary function measurements (spirometry and bodyplethysmography; MasterLab, CareFusion, Würzburg, Germany) were performed as described earlier (Wanger et al. 2005; Miller et al. 2005). The European Community for Steel and Coal (ECSC) reference values for spirometry were chosen (Quanjer et al. 1993). Methacholine testing was performed with a reservoir method as described earlier (Merget et al. 2005). Repetitive methacholine testing before and after SIC was performed in some subjects and is shown for 11 cases with doubtful or negative SIC responder status with increases of FeNO.

FeNO measurements

FeNO was measured according to international recommendations (American Thoracic Society and European Respiratory Society 2005) at 50 mL/s with NIOX FLEX[®] or NIOX MINO[®] (Aerocrine, Solna, Sweden).

Specific inhalation challenges

SICs were evaluated by spirometry and bodyplethysmography. Asthma medication (steroids and/or

bronchodilators) was withheld for at least 12 h before methacholine testing and 24 h before SIC. Due to the great number of different allergens the methods of SIC were variable. Each SIC was performed according to a multiple step protocol with at least four steps of doubling or quadrupling doses. For water soluble allergens we used commercial extracts if available.

Most soluble allergens were inhaled with 5 or 10 breaths (depending on the allergen) by an APSpro dosimeter (Jaeger, Würzburg, Germany) and a DeVilbiss 646 nebulizer (DeVilbiss Healthcare, Mannheim, Germany). Inhalations were performed as described by the ATS for methacholine challenges, with minor modifications (American Thoracic Society 2000; Merget et al. 2009). Soluble allergens were platinum salts ($n = 14$ tests), molds ($n = 8$), mites ($n = 4$), persulfate salts ($n = 4$), animal hair ($n = 2$), milk ($n = 1$), amylase ($n = 2$), pectin ($n = 1$), washnut ($n = 1$) and rhodium salt ($n = 1$).

Isocyanate challenges were performed according to a 4-step-1-day whole body challenge protocol (Scheidler et al. 2013). Allergens were diphenylmethane-4,4'-diisocyanate [MDI] ($n = 43$), 1,6-hexamethylene diisocyanate [HDI] ($n = 24$), isophoronediiisocyanate [IPDI] ($n = 4$), toluene diisocyanate [TDI] ($n = 3$) and naphthylene-1,5-diisocyanate [NDI] ($n = 2$).

SICs with particulate allergens were performed by a RBG-1000-L dust disperser (Palas GmbH, Karlsruhe, Germany). Allergens were flour ($n = 4$), milk powder ($n = 2$) and cobalt powder ($n = 1$).

Workplace-simulating exposures were performed with hairdressing products ($n = 11$), disinfectants ($n = 5$), latex ($n = 2$), straw and grain ($n = 2$), chicken feathers ($n = 1$), wall color ($n = 1$), primer coat ($n = 1$) and Teflon ($n = 1$).

A positive challenge was assumed if FEV_1 decreased $\geq 20\%$ and sR_t increased $\geq 100\%$ to ≥ 2 kPa s. If only one positivity criterion was fulfilled (either FEV_1 or sR_t) and the test had to be stopped because of symptoms we classified the challenge positive. A challenge was considered negative if there was no decrease of $FEV_1 \geq 20\%$ and no increase of $sR_t \geq 100\%$ to ≥ 2 kPa s. If only FEV_1 decreased $\geq 20\%$ and the test was completed insufficient cooperation was assumed and the challenge considered negative. SICs which could not be assigned to positive or negative, e.g. tests which were completed and fulfilled only one positivity criterion together with sufficient cooperation of the patient were considered doubtful. Each positive reaction that started later than 60 min after the end of exposure was classified as late reaction.

The Ethics Committee of the Ruhr-Universität Bochum gave approval to perform the study and all subjects gave informed consent.

Statistical analyses

Continuous variables were described by medians and ranges. Differences between subjects with positive, doubtful and negative SICs were tested pairwise with the Mann–Whitney-*U*-test for continuous variables and with the Chi-square test for categorical variables. Differences of baseline FeNO and FeNO increases between subjects with and without specific sensitizations were tested with the Mann–Whitney-*U*-test. Correlations between parameters were calculated with Spearman's rank correlation test. To find a classification rule based on the FeNO measurements, data of 105 subjects with a negative SIC and 31 subjects with a positive SIC were taken for receiver-operating characteristic (ROC) curves, and the maximum Youden Indexes (sensitivity + specificity – 1) were calculated for the whole group and after stratification for smoking, atopy or ICS treatment to determine the optimal cut-off for a positive FeNO increase. In a second approach the cut-off was defined at a fixed specificity of 0.9. A logistic regression model was used to define modifying factors. The dependent variables were the optimal cut-offs for a significant FeNO increase as defined by ROC-curves. Independent variables were medication with inhaled corticosteroids, type of agent, smoking status, atopy and baseline FeNO.

The statistical analysis was done with SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Out of 148 subjects with suspected OA, 31 subjects showed positive and 12 subjects doubtful asthmatic reactions after SICs (Table 1). The most important differences in characteristics and clinical data between subjects with positive and negative pulmonary responder status were lower age, earlier occurrence of symptoms, higher total IgE, higher post SIC FeNO and higher rates of atopy, hyperresponsiveness and specific sensitization in the pulmonary responders. Interestingly subjects with a doubtful SIC showed significantly higher total IgE and post SIC FeNO than subjects with a negative SIC. However, the median FeNO values at baseline showed no significant differences between the groups of subjects with positive, negative or doubtful SICs.

The agents which induced a positive SIC could be assigned to the three types of agents in about equal numbers, whereas there was a wide range of different agents in each group (Table 2). Most SICs were performed with isocyanates (51%), but these tests accounted for only 36% of positive SIC results. Positive and doubtful SICs were almost exclusively associated with immediate reactions. Late reactions were observed in five cases (four pulmonary responders and one subject with doubtful responder status).

Table 1 Personal and clinical data according to pulmonary responder status

	SIC result			P values*	Total (n = 148)
	Positive (P) (n = 31)	Doubtful (D) (n = 12)	Negative (N) (n = 105)		
Age: years [median (range)]	34 (20–58)	42 (27–74)	46 (20–64)	P–N 0.01	43 (20–74)
Male gender: n (%)	25 (81)	10 (83)	59 (56)	P–N 0.01	94 (64)
Atopy: n (%)	21 (68)	6 (55)	43 (41)	P–N 0.01	70 (48)
Smoking habits					
Current smokers: n (%)	13 (42)	1 (8)	26 (25)	P–D 0.04	40 (27)
Non- or exsmokers: n (%)	18 (58)	11 (92)	79 (75)		108 (73)
Beginning of exposure to onset of symptoms: years [median (range)]	3 (0–27)	7 (0–50)	6 (0–49)	P–D 0.01 P–N 0.04	5 (0–50)
End of exposure to diagnosis: years [median (range)]	0.5 (0–2.5)	0.9 (0–5.8)	0.5 (0–20)		0.5 (0–20)
ICS treatment: n (%)	13 (43)	8 (73)	51 (49)		72 (49)
Hyperresponsiveness: n (%)	23 (77)	7 (64)	42 (40)	P–N < 0.001	72 (49)
Type of agent					
HMW: n (%)	10 (32)	3 (25)	16 (15)		29 (20)
LMW: n (%)	10 (32)	4 (33)	29 (28)		43 (29)
Isocyanates: n (%)	11 (36)	5 (42)	60 (57)		76 (51)
Total IgE: kU/L [median (range)]	166 (2–1274)	110 (12–4506)	35 (2–5000)	P–N < 0.001 D–N 0.04	53 (2–5000)
Specific allergen sensitization: n (%)	18 (58)	3 (25)	11 (11)	P–D 0.05 P–N < 0.001	32 (22)
Type of reaction			–		
Immediate: n (%)	3.25 (86)	11 (92)			36 (88)
Late: n (%)	4 (14)	1 (8)			5 (12)
Baseline FEV ₁ %pred [median (range)]	96 (67–118)	92 (71–128)	100 (66–133)		98 (66–133)
Max. fall in FEV ₁ %Baseline [median (range)]	32 (19–74)	25 (9–32)	–	P–D 0.02	–
Time to max. fall of FEV ₁ : min [median (range)]	20 (0–360)	0 (0–1440)	–		–
Baseline sR _i : kPa s [median (range)]	0.9 (0.6–2.6)	0.8 (0.5–2.3)	0.7 (0.4–2.8)	P–N 0.002	0.8 (0.4–2.8)
Max. increase of sR _i : kPa s [median (range)]	2.3 (0–8.1)	1.2 (0–2.7)	–	P–D 0.009	–
Time to max. of sR _i : min [median (range)]	10 (0–360)	0 (0–1440)	–		–
Baseline FeNO: ppb [median (range)]	23 (3–151)	19 (8–119)	14 (3–158)		15 (3–158)
FeNO post SIC: ppb [median (range)]	41 (5–156)	36 (9–156)	17 (6–193)	D–N 0.02 P–N < 0.001	21 (5–193)

SIC specific inhalation challenge, ICS inhaled corticosteroids, HMW high-molecular weight allergens, LMW low-molecular weight allergens excluding isocyanates, FEV₁ forced expiratory volume in one second, sR_i specific airway resistance, FeNO fractional exhaled nitric oxide

*Only significant results are shown ($p < 0.05$); P–D positives versus doubtfulls, P–N positives versus negatives, D–N doubtfulls versus negatives

Six subjects showed a maximum fall in FEV₁ > 50% from baseline. All these strong asthmatic reactions showed a good immediate reversibility after medication (systemic corticosteroids and inhaled short-acting beta-agonists), in no case intensive care was necessary.

Youden Index defined the cut-offs for post challenge increase of FeNO from baseline as 10.5 ppb for the whole group with a sensitivity of 0.55 and a specificity of 0.88 [area under the curve (AUC) 0.71; 95% CI 0.59–0.83] (Table 3). After stratification for smoking, atopy or ICS

treatment, sensitivities varied between 0.38 and 0.80 and specificities between 0.82 and 0.98. As high specificity (only few false positive results) was desirable and due to the low number of subjects in the subgroups, we decided to set the specificity at 0.9, resulting in a FeNO increase ≥ 13 ppb as cut-off for all subjects (Fig. 1), despite some variability between the cut-offs within subgroups. Absolute changes in FeNO performed slightly better than relative changes (data not shown).

Table 2 SIC results according to pulmonary responder status and type of agent

Type of agent	SIC result			
	Positive (<i>n</i> = 31)	Doubtful (<i>n</i> = 12)	Negative (<i>n</i> = 105)	Total (<i>n</i> = 148)
HMW	Amylase ^b (<i>n</i> = 2)	Latex ^a (<i>n</i> = 1)	Flour ^c (<i>n</i> = 3)	
	Animal hair ^b (<i>n</i> = 2)	Mites ^b (<i>n</i> = 1)	Mites ^b (<i>n</i> = 1)	
	Mites ^b (<i>n</i> = 2)	Milk powder ^c (<i>n</i> = 1)	Latex ^a (<i>n</i> = 1)	
	Molds ^b (<i>n</i> = 2)		Pectin ^b (<i>n</i> = 1)	
	Wash nut ^b (<i>n</i> = 1)		Feathers ^a (<i>n</i> = 1)	
	Flour ^c (<i>n</i> = 1)		Milk ^b (<i>n</i> = 1)	
			Straw, grain ^a (<i>n</i> = 2)	
			Molds ^b (<i>n</i> = 6)	
	Total (<i>n</i> = 10)	Total (<i>n</i> = 3)	Total (<i>n</i> = 16)	(<i>n</i> = 29)
LMW	Platinum salt ^b (<i>n</i> = 6)	Platinum salt ^b (<i>n</i> = 2)	Hairdressing products ^a (<i>n</i> = 9)	
	Persulfate salt ^b (<i>n</i> = 1)	Hairdressing products ^a (<i>n</i> = 1)	Platinum salt ^b (<i>n</i> = 6)	
	Cobalt powder ^a (<i>n</i> = 1)	MMA ^c (<i>n</i> = 1)	Disinfectants ^a (<i>n</i> = 5)	
	Rhodium salt ^b (<i>n</i> = 1)		Persulfate salt ^b (<i>n</i> = 3)	
	Hairdressing products ^a (<i>n</i> = 1)		MMA ^c (<i>n</i> = 1)	
			Cyanacrylates ^c (<i>n</i> = 1)	
			Paint ^a (<i>n</i> = 1)	
			Formaldehyde ^c (<i>n</i> = 1)	
			Primer coating ^a (<i>n</i> = 1)	
			Teflon ^a (<i>n</i> = 1)	
	Total (<i>n</i> = 10)	Total (<i>n</i> = 4)	Total (<i>n</i> = 29)	(<i>n</i> = 43)
Isocyanates ^c	MDI (<i>n</i> = 10)	MDI (<i>n</i> = 3)	MDI (<i>n</i> = 30)	
	NDI (<i>n</i> = 1)	HDI (<i>n</i> = 2)	HDI (<i>n</i> = 22)	
			IPDI (<i>n</i> = 4)	
			TDI (<i>n</i> = 3)	
			NDI (<i>n</i> = 1)	
	Total (<i>n</i> = 11)	Total (<i>n</i> = 5)	Total (<i>n</i> = 60)	(<i>n</i> = 76)

SIC procedure: ^aworkplace simulation (without air monitoring), ^binhaled nebulized solutions with defined allergen concentrations, ^cstandardized whole body exposures

SIC specific inhalation challenge; animal hair: cow and mouse, HMW high-molecular weight allergens, LMW low-molecular weight allergens excluding isocyanates, MMA methyl methacrylate, MDI diphenylmethane-4,4'-diisocyanate, NDI naphthylene-1,5-diisocyanate, HDI 1,6-hexamethylene diisocyanate, IPDI isophorone diisocyanate, TDI toluene diisocyanate

Thirty-two subjects presented an increase in FeNO ≥ 13 ppb, among them 16 pulmonary responders, 11

Table 3 Cut-offs, sensitivities and specificities of the differences in FeNO concentrations before and after SIC with the pulmonary responder status as the gold standard in the whole group and after stratification for smoking, atopy and ICS treatment

Analysis	Parameter	Whole group	Current smoking		Atopy		ICS treatment	
			Yes	No	Yes	No	Yes	No
ROC curve: maximum Youden Index	AUC	0.71	0.77	0.66	0.60	0.9	0.78	0.68
	(95% CI)	(0.59–0.83)	(0.59–0.96)	(0.49–0.83)	(0.43–0.77)	(0.79–1.0)	(0.60–0.96)	(0.51–0.85)
	Cut-off (optimal)	10.5 ppb	7 ppb	27.5 ppb	16.5 ppb	12 ppb	12.5 ppb	10.5 ppb
	Sensitivity	0.55	0.77	0.44	0.38	0.80	0.54	0.59
Specificity: 0.9	Specificity	0.88	0.89	0.95	0.91	0.92	0.98	0.82
	Cut-off	13 ppb	12.5 ppb	13.5 ppb	16.5 ppb	10.5 ppb	9.5 ppb	27 ppb
	Sensitivity	0.52	0.62	0.44	0.38	0.80	0.54	0.41

Evaluation was performed using the cut-offs which were obtained by the maximum Youden Index and in addition with a fixed specificity of 0.9. Only subjects with positive and negative pulmonary responder status were considered (*n* = 136)

ROC receiver-operating characteristic, AUC area under the curve

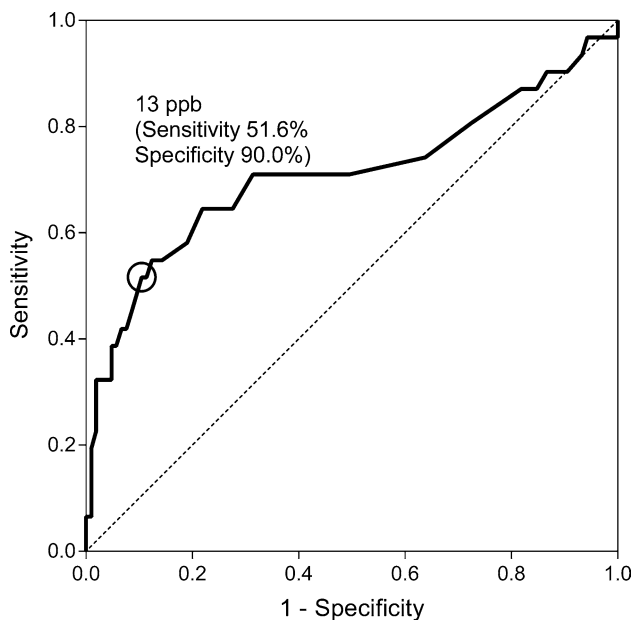


Fig. 1 ROC-plot for the total group showing differences in FeNO concentrations before and after SIC with the pulmonary responder status as the gold standard. Only SIC positive and negative subjects were considered ($n=136$). A cut-off of a FeNO increase ≥ 13 ppb resulted in a specificity of 0.9 (circle)

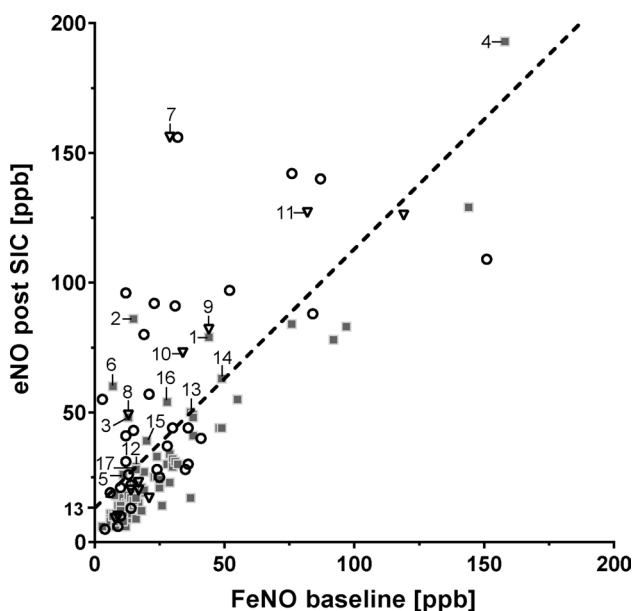


Fig. 2 Baseline and post SIC FeNO concentrations for all subjects ($n=148$). Circles represent SIC positive ($n=31$), triangles doubtful ($n=12$), and (filled) squares SIC negative subjects ($n=105$). The dashed line shows the cut-off for a FeNO increase of 13 ppb. The numbers indicate subjects with an increase of FeNO in SIC negative and doubtful subjects (numbers correspond to Table 4)

nonresponders and 5 doubtfuls (Fig. 2). Not all pulmonary responders showed an increase of FeNO after SIC which explained the moderate sensitivities. However, the moderate sensitivity was associated with a high specificity, thus a detailed analysis was performed in subjects with negative or doubtful SIC but with an increase of FeNO ≥ 13 ppb. Characteristics of the 17 subjects with doubtful or negative pulmonary responder status, but with significant increases of FeNO according to different cut-off criteria are presented in Table 4. Ten of them were classified subsequently positive, and 3 further subjects were rated as probable OA because further evidence for OA was available. Only four subjects had no further evidence for OA. Interestingly, the “false negative” pulmonary responders had been challenged with LMW agents or isocyanates, with one exception (flour). Among the four true negatives there was one subject with very high baseline FeNO (158 ppb), which increased by 35 ppb, but there was no further evidence for OA. The overall rating of subjects with doubtful responder status without positive FeNO increases ($n=7$) yielded 2 cases with probable OA (data not shown).

Of the 15 pulmonary responders without a FeNO increase ≥ 13 ppb five subjects were challenged with LMW allergens (including isocyanates) and 10 subjects with HMW allergens (data not shown).

Baseline FeNO was moderately associated with total IgE ($r=0.254$) and was higher in subjects with specific sensitizations ($p=0.006$). However, there were neither correlations between baseline FeNO nor between FeNO increase and total IgE. Subjects with and without specific sensitizations to occupational allergens did not show significant differences of FeNO increase.

Regression analyses did not show relevant modifying factors for a FeNO increase of ≥ 13 ppb (Table 5), and similar results were obtained with the further cut-offs as dependent variables (data not shown).

Discussion

Recent reviews of diagnostic tests in occupational asthma stated that there are conflicting results concerning the usefulness of FeNO measurements before and after SIC (Vandenplas et al. 2014) and that further studies are needed (Pralong and Cartier 2017). The major new finding of this study is that a combination of lung function and FeNO as outcomes of SIC minimizes false negative SIC results, and that increases of FeNO after SIC are highly predictive of OA, with a low number of false positive FeNO increases (in this study below 10%).

It is a strength of this study that additional information like sputum analyses, measurements of bronchial

Table 4 Characteristics of 17 subjects with doubtful or negative pulmonary responder status, but with significant increases of FeNO

Subject	Allergen	Smoking habit	FeNO base-line/24 h (ppb)	Fall of FEV ₁ (%baseline)	Increase of sR _t baseline/maximal (kPa s)	Pulmonary responder	Evidence for OA	Overall rating
Cut-off criteria: FeNO increase of at least 13 ppb and Youden Index								
1	Pt salt	Nonsmoker	44/79	5	0.7/0.9	Negative	SIC not completed due to cough and skin rash, increase of hyperresponsiveness (PD ₂₀ FEV ₁ from 136 to 44 µg), SPT + ve earlier, sputum not obtainable	OA
2 ^a	Pt salt	Nonsmoker	15/86	8	0.9/1.0	Negative	SIC not completed due to cough and dyspnea, increase of sputum eosinophils (from 1 to 10%) and hyperresponsiveness (PD ₂₀ FEV ₁ from nonresponsive to 270 µg), SPT negative, patch test + ve, symptom-free after exposure cessation	OA
3	Isocyanates	Nonsmoker	13/48	16	0.5/1.3	Negative	SIC not completed due to hyperventilation, serial FeNO and FEV ₁ measurements at home and at work indicate OA, sputum not obtainable	OA
4	Isocyanates	Nonsmoker	158/193	5	0.9/1.0	Negative	None (sputum not obtainable; no significant change in hyperresponsiveness; uneventful continuing exposure for 2 years)	No OA
5	Isocyanates	Smoker	11/26	5	0.39/0.55	Negative	Increase of sputum eosinophils (from 1.5 to 6.5%), no hyperresponsiveness, uneventful continuing exposure for 6 months	No OA
6	Persulfates	Smoker	7/60	17	1.3/1.5	Negative	Near positive SIC (spirometry), increase of sputum eosinophils (from 3 to 5%) and hyperresponsiveness (PD ₁₀₀ sR _t + from nonresponsive to 107 µg), patch test + ve, symptom-free after exposure cessation	OA
7	Pt salt	Nonsmoker	29/156	22	0.5/0.8	Doubtful	No asthma symptoms, doubtful SIC, increase of eosinophils in sputum (from 0 to 3%), SPT + ve	Beginning OA
8	Pt salt	Nonsmoker	13/49	25	0.9/1.6	Doubtful	Doubtful SIC, SPT + ve earlier, symptom-free after exposure cessation, sputum not obtainable	OA
9	Isocyanates	Nonsmoker	44/82	32	0.6/1.7	Doubtful	Doubtful SIC, FeNO increase reproduced 2 years afterwards, symptom-free after exposure cessation, sputum nd	OA
10	Isocyanates	Nonsmoker	34/73	26	0.6/1.8	Doubtful	Doubtful SIC with isolated late reaction, dyspnea and increase of sR _t on the day after SIC, increase of sputum eosinophils (from 1 to 40%)	OA
11	Isocyanates	Nonsmoker	82/127	18	0.6/3.1	Doubtful	Doubtful SIC with dyspnea and cough, increase of sputum eosinophils (from 11 to 38%) and hyperresponsiveness (PD ₁₀₀ sR _t + from 236 to 30 µg)	OA
Cut-off criterion: FeNO increase of at least 13 ppb (without Youden Index)								
12	Isocyanates	Nonsmoker	16/29	4	0.75/1.04	Negative	SIC without symptoms and significant changes in pulmonary function, extended period of FeNO increase for 5 days after SIC, increase of sputum neutrophils (from 2 to 56%), and hyperresponsiveness (PD ₁₀₀ sR _t + from nonresponsive to 142 µg), symptom-free after exposure cessation	Probably OA

Table 4 (continued)

Subject	Allergen	Smoking habit	FeNO baseline/24 h (ppb)	Fall of FEV_1 (%baseline)	Increase of sR_i baseline/maximal (kPa s)	Pulmonary responder	Evidence for OA	Overall rating
13	Isocyanates	Nonsmoker	37/50	4	0.91/1.48	Negative	SIC with weakly positive late reaction by only 2 months of symptomatic occupational exposure, dyspnea and cough in the morning after SIC, increase of sputum eosinophils (from 0 to 8%)	OA
14	Flour	Nonsmoker	49/63	15	0.73/1.06	Negative	SIC with nasal symptoms, increase of sputum eosinophils (from 9 to 13%) and hyperresponsiveness ($PD_{100}sR_i$ + from 143 to 44 μ g and $PD_{20}FEV_1$ from 243 to 25 μ g), serial FeNO and FEV_1 measurements at home and at work indicate OA	Probably OA
15	Isocyanates	Nonsmoker	20/39	8	0.53/1.03	Negative	SIC with dyspnea and cough, nonsignificant decrease of paO_2 , no change in sputum eosinophils, no hyperresponsiveness	No OA
16	Isocyanates	Nonsmoker	28/54	10	0.85/1.28	Negative	SIC with increase of sputum eosinophils (from 4 to 35%) and hyperresponsiveness ($PD_{100}sR_i$ + from nonresponsive to 609 μ g), decrease of paO_2 from 10 to 8.5 kPa after SIC	Probably OA
Cut-off criterion: FeNO increase according to Youden Index								
17	Hairdressing products	Smoker	16/28	None	0.8/0.82	Negative	None (sputum nd, no change in hyperresponsiveness, serial FeNO and FEV_1 measurements at home and at work do not indicate OA)	No OA

Only four subjects (nos. 4, 5, 15, 17) had no evidence for OA

Youden Index criteria: FeNO increase of ≥ 7 ppb (smokers) and ≥ 27.5 ppb (nonsmokers)

nd not done, OA occupational asthma, FEV_1 forced expiratory volume in 1 s, sR_i specific airway resistance, SIC specific inhalation challenge, $PD_{20}FEV_1$ cumulative dose of methacholine provoking a 20% decline in FEV_1 , SPT skin prick test, $PD_{100}sR_i$ + cumulative dose of methacholine provoking a doubling of specific airway resistance to ≥ 2 kPa s

^aPublished case report; Merget et al. (2015)

Table 5 Factors associated with a change in FeNO ≥ 13 ppb after SIC

	Δ FeNO < 13 ppb	Δ FeNO ≥ 13 ppb	OR (95% CI)	P value
Female	42	12	Reference	
Male	66	20	0.69 (0.26–1.87)	0.47
Type of allergen: LMW	30	11	Reference	
Type of allergen: HMW	23	1	0.11 (0.01–1.08)	0.058
Type of allergen: isocyanates	55	20	1.63 (0.58–4.63)	0.36
Nonsmoking	48	18	Reference	
Smoking	27	10	1.25 (0.45–3.45)	0.67
Ex smoking	33	4	0.42 (0.12–1.51)	0.19
No atopy	60	15	Reference	
Atopy	48	17	1.86 (0.75–4.64)	0.18
No ICS treatment	51	21	Reference	
ICS treatment	57	11	0.53 (0.22–1.30)	0.17
Baseline FeNO			1.02 (1.0–1.03)	0.08

SIC specific inhalation challenge, FEV_1 forced expiratory volume in 1 s, ICS inhaled corticosteroids, LMW allergens low-molecular weight allergens excluding isocyanates, HMW allergens high-molecular weight allergens

hyperresponsiveness or of FeNO during holidays or clinical longitudinal observations were available in some subjects, but it is a weakness that such information were not available for all subjects and that these subjects were selected. Thus, the resulting expert rating that was performed in this study may be criticized, but it is difficult to obtain a valid gold standard for OA. Repetitive SICs which have been performed by others (Vandenplas et al. 2009) were not considered in our diagnostic repertoire. Although this study clearly shows an additional value of FeNO measurements for a correct diagnosis of OA, an estimation of the precise number of false negative SICs that can be avoided with the additional use of FeNO measurements should be made with caution. Also the increased number of such cases among subjects with OA due to isocyanates (in this study 7 out of the 10 false negative SICs) has to be considered.

As already described in previous studies we have found a lack of FeNO increases after SIC in about half of the pulmonary responders and a considerable number of FeNO increases among pulmonary nonresponders. Whereas the former is unequivocal (Barbinova and Baur 2006; Piipari et al. 2002) the latter has not been found consistently (Piipari et al. 2002; Pedrosa et al. 2012), is poorly understood (Barbinova and Baur 2006), or is not addressed specifically (Lemiere et al. 2014; Sastre et al. 2013; Walters et al. 2014). Single cases without an asthmatic reaction, but an increase of sputum eosinophils after SIC with various allergens have been described earlier. These subjects were thought to suffer from *eosinophilic bronchitis* (Barranco et al. 2008; Lemiere et al. 1997; Quirce 2004). Also in the present study, such cases with negative functional response, but increases of FeNO or sputum eosinophils were observed. However, it was difficult to separate false negative SICs in subjects with OA

from subjects with possible *eosinophilic bronchitis*. Also false positive increases of eosinophils have to be considered. Overall, we consider the number of cases with *eosinophilic bronchitis* in this study as low.

Several recommendations have been made concerning the definition of a significant increase of FeNO. In a recent study with a high number of study subjects, an increase of 17.5 ppb was considered optimal after analyses of ROC curves (Lemiere et al. 2014). Stratification according to smoking, atopy and ICS treatment resulted in variable cut-offs which were somewhat arbitrary due to the shapes of the ROC curves. As these modifiers had also no significant effects in multivariate analyses, we decided to use one cut-off for the whole group. Since the optimal cut-off in the whole group which was determined by maximum Youden Index resulted in a specificity of 0.88, we chose a cut-off at a fixed specificity of 0.9 (≥ 13 ppb). The results with this cut-off were comparable with those of the cut-off of 17.5 ppb proposed recently by others (Lemiere et al. 2014), however, with the lower cut-off of 13 ppb we detected 4 additional subjects, among them one subject with definite and two subjects with probable OA according to expert rating. The cut-off used in this study is also very close to further recommendations (Piipari et al. 2002; Pedrosa et al. 2012).

Further strengths of this study are the high number of subjects and the widespread spectrum of allergens. Unfortunately, the number of positive SIC with HMW substances was underrepresented in the present study. This is a consequence of selection and also due to the fact that SIC were rarely performed in subjects with unequivocal sensitization and a history suggestive of OA.

Previous studies assigned subjects to a positive or a negative SIC. In the present study, we included a “doubtful”

group. Doubtfuls were subjects with non-matching results of spirometry and body plethysmography which is used frequently in Germany due to its advantages of lower importance of the subjects' cooperation and no need of deep inhalations and forced expirations that may be difficult to obtain after pronounced asthmatic reactions. Although the number of doubtful tests was low, it is of interest that five of 12 doubtful tests obtained a final positive overall rating, all with a FeNO increases ≥ 13 ppb. Thus, FeNO measurements are of particular value if lung function does not allow a valid interpretation of SIC, if SIC are near-positive, or if SIC were not completed due to, e.g. hyperventilation.

In the present study no modifiers of a FeNO increase could be detected. Whereas it is clear that baseline FeNO is lower in smokers and higher in atopics, this is less clear for the increase of FeNO after SIC. Only few studies examined effect modifiers by regression analyses, and descriptive analyses were often performed in a low number of subjects, thus the statistical power to detect significant modifiers remains low. However, it was not the primary aim of this study to identify modifiers. A recent study which addressed this question concluded that the only modifier was the type of agent, HMW substances inducing an increase of FeNO more consistently than LMW substances (Lemiere et al. 2014). In the present study, the type of agent was not a significant modifier, but this finding is limited by the low number of positive SICs with HMW substances in this study.

Interestingly, baseline FeNO was higher in subjects with specific sensitizations to occupational allergens, but FeNO increase was not. The latter can be explained by the high number of subjects with isocyanate asthma who did not show sensitization by SPT or in vitro tests, but demonstrated FeNO increases in many cases. Also FeNO increase was not correlated with 'markers' of allergy like total IgE, but the low number of subjects with FeNO increase limits further interpretation.

Overall, the present study strongly supports the recommendation to include FeNO measurements before and after SIC as a further important tool in the diagnosis of OA. We consider the high specificity of a FeNO increase in line with previous studies, but—to our knowledge—none of these studies took a closer look on subjects with doubtful or negative SIC defined by lung function criteria. As sensitivity of FeNO measurements is low, it should not be used as a single tool, but be combined with lung function. There are few comparative studies of FeNO with further noninvasive parameters, but the assessment of induced sputum eosinophils has been reported to be superior to FeNO measurements (Lemiere 2007). Considering the high specificity of a FeNO increase in this study it is recommended to reproduce this finding and to study whether sputum analyses or measurements of bronchial hyperresponsiveness before and after SIC add relevant information.

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Compliance with ethical standards

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants for being included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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