



Non-invasive tools beyond lung function before and after specific inhalation challenges for diagnosing occupational asthma

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

Received: 7 November 2018 / Accepted: 9 May 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Purpose Increases of fractional exhaled nitric oxide (FeNO), sputum eosinophils, and methacholine responsiveness have been described after specific inhalation challenges (SIC) with occupational allergens, but limited information is available about their comparative performance. It was the aim of the study to assess the diagnostic accuracy of these non-invasive tests before and after SIC for the diagnosis of occupational asthma (OA).

Methods A total of 122 subjects with work-related shortness of breath were included. The ‘gold standard’ was defined as airway obstruction (pulmonary responders) and/or an increase of FeNO of at least 13 ppb after SIC. The results were compared with those obtained using the pulmonary responder status alone as ‘gold standard’.

Results If the pulmonary responder status and/or an increase of FeNO was used as ‘gold standard’ for SIC, 28 out of 39 positives (72%), but also 20 out of 83 negatives (24%) showed an increase of sputum eosinophils and/or bronchial hyperresponsiveness after SIC. If the pulmonary responder status alone was used as ‘gold standard’, an increase of FeNO with a sensitivity of 0.57 and a specificity of 0.82 showed a higher accuracy than increases of sputum eosinophils (0.52/0.75) or bronchial hyperresponsiveness (0.43/0.87). Individual case analyses suggest that a few cases of OA may be detected by increases of sputum eosinophils or bronchial hyperresponsiveness alone, but probably false-positive tests dominate.

Conclusion It is recommended to use both lung function and increase of FeNO as primary effect parameters of SIC. Changes of sputum eosinophils and bronchial hyperresponsiveness after SIC have a low additional diagnostic value, but may be useful in individual cases.

Keywords Occupational asthma · Fractional exhaled nitric oxide · Specific inhalation challenge · Sputum eosinophils · Bronchial hyperresponsiveness

Introduction

Specific inhalation challenges (SIC) with occupational agents in the laboratory are still considered as the ‘gold standard’ for diagnosing occupational asthma (OA) (Vandenplas et al. 2017). Historically, effect parameters of SIC are symptoms and lung function, i.e., spirometry in most countries. It is well known that both false-negative and false-positive tests may occur, but, due to the lack of

an alternative ‘gold standard’, the number of such tests is difficult to assess (Vandenplas et al. 2014). It has been reported that non-invasive methods like serial measurements of fractional exhaled nitric oxide (FeNO), sputum eosinophils, and bronchial hyperresponsiveness before and after SIC may increase the diagnostic accuracy (Quirce et al. 2010). Possibly due to its easy measurement, the most frequently used non-invasive tool is the assessment of FeNO before and after SIC. Two studies reported high specificity of FeNO with low sensitivity, but, in a combined overall diagnostic approach, sensitivity of SIC could be increased (Sastre et al. 2013; Engel et al. 2018). Similar results were obtained with measurements of sputum eosinophils (Vandenplas et al. 2009) and bronchial hyperresponsiveness before and after SIC (Vandenplas et al. 1996; Sastre et al. 2003). Comparative studies are sparse: with the pulmonary

✉ Rolf Merget
merget@ipa-dguv.de

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

responder status as ‘gold standard’ sputum eosinophils demonstrated higher sensitivity and positive predictive value than FeNO with similar specificity and negative predictive value (Lemière et al. 2010). Recently, it was shown that the change in sputum eosinophils yielded a higher diagnostic accuracy compared to changes in methacholine responsiveness, but a combination of both parameters achieved a sensitivity of 0.84 and a specificity of 0.74 for the diagnosis of OA (Racine et al. 2017). In this study, we used two different ‘gold standards’. As shown recently, FeNO measurements before and after SIC increase sensitivity of SIC considerably without relevant loss of specificity (Engel et al. 2018). Due to its simple and objective measurement, we used the pulmonary responder status (assessed by lung function) and/or an increase of FeNO as ‘gold standard’ in a combined approach (primary ‘gold standard’) and evaluated the additional value of measurements of sputum eosinophils and methacholine testing, which are more cumbersome to perform. In addition, we used pulmonary responder status alone as secondary ‘gold standard’.

Methods

Subjects and study design

All subjects with suspected OA referred to our outpatient clinic by the accident insurances or courts who underwent SIC from 2006 to 2016 and agreed to participate were included. Twenty subjects without sputum results were excluded; the remaining 122 subjects were included in the analyses. Of these 122 subjects, 13 did not undergo two methacholine challenges due to technical or personal reasons. There were no further missing values. The investigations were performed on 3 consecutive days as described previously (Engel et al. 2018). Briefly, during the first day (control day), the subjects underwent baseline examinations including skin prick testing (SPT) and methacholine testing as well as repeated pulmonary function tests to document a stable respiratory status. On the second day, SIC with the suspected allergen were performed. FeNO measurements and sputum induction with saline were performed directly before and 24 h after SIC, and methacholine testing was repeated 24 h after SIC. Figure 1 illustrates the sequence of examinations. Out of the 122 subjects investigated in the present study, 97 subjects had already been included in a recent study which examined the use of FeNO measurements only (Engel et al. 2018). The Ethics Committee of the Ruhr University Bochum gave approval to perform the study and all subjects gave informed consent.

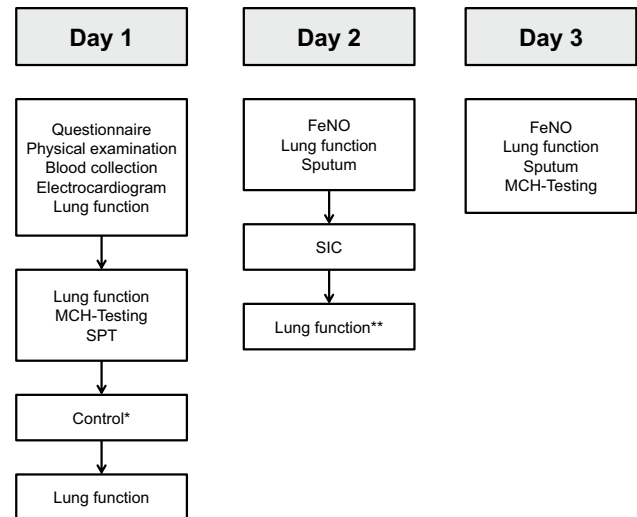


Fig. 1 Schematic flowchart for sequence of examinations of performing specific inhalation challenges. *Inhalation with control substance for SIC with particulate substances; **Follow-up to 4 h post-SIC

Procedures

Subjects answered a questionnaire about personal data, smoking habits (current cigarette smoking or not), medical history, and exposures at work. The temporal context of asthma symptoms was described by latency, defined as time between the beginning of exposure to the onset of asthma symptoms and by time without exposure to diagnosis.

Spirometry and body plethysmography were performed as described earlier (Miller et al. 2005; Wanger et al. 2005) with MasterLab (CareFusion, Würzburg, Germany). Reference values for spirometry of the European Community for Steel and Coal (ECSC) were chosen (Quanjer et al. 1993).

Methacholine testing was performed as previously described with a reservoir method (Merget et al. 2005), whereat bronchial hyperresponsiveness was assumed if FEV_1 decreased $\geq 20\%$ from baseline ($PD_{20}FEV_1$) and/or sR_t doubled and increased to ≥ 2 kPa s ($PD_{100}sR_t+$) with a cumulative dose of ≤ 300 μ g methacholine.

All subjects underwent SPT with a battery of 20 environmental allergens from various manufacturers, and if available, allergen-specific sensitizations were tested by SPT with the suspected occupational allergen. Atopy was defined by a positive SPT (wheal ≥ 3 mm) with any environmental allergen. Specific allergen sensitization was defined by a positive SPT and/or a positive ImmunoCAP (≥ 0.35 kU/L; ThermoFisher Scientific Inc, Uppsala, Sweden) with the suspected occupational allergen.

Induced sputum was collected after inhalation of isotonic (0.9%) saline aerosol, generated by an ultrasonic

nebulizer (Pari Boy, PARI GmbH, Starnberg, Germany) for six times 5 min (Bacci et al. 1996), processed as described earlier (Raulf-Heimsoth et al. 2011; Raulf et al. 2015), and total cell count and differential cytology were assessed. Absolute numbers and percentages of eosinophils and neutrophils were used for analyses.

Due to the great number of different allergens, the methods of allergen application were manifold (Engel et al. 2018). SIC were evaluated by spirometry, body plethysmography, and FeNO increase (measured with NIOX MINO®, Aerocrine, Solna, Sweden, according to the international recommendations (American Thoracic Society et al. 2005)). Asthma medication (steroids and/or bronchodilators) was interrupted for at least 12 h prior to methacholine testing and 36 h to SIC. Multiple step protocols with at least four steps of doubling or quadrupling doses were used for SIC whereby the minimum and maximum doses differed depending on the allergen. Allergens are presented in the appendix. Spirometry and body plethysmography were performed before and after each step as well as until 4 h after the end of SIC (follow-up). Pulmonary responders were defined as subjects with a decrease of $FEV_1 \geq 20\%$ from baseline and doubling of sR_t with an increase to ≥ 2 kPa s, the remainders as pulmonary nonresponders. A positive reaction beginning during the exposure or during the first 60 min after the end of SIC was defined as immediate reaction whereby a late reaction was classified as positive reaction that started later than 60 min after the end of exposure.

For FeNO increase during SIC, a cut-off of ≥ 13 ppb has been described to be highly predictive of OA in a recent study (Engel et al. 2018). Thus, in the present study, a FeNO increase ≥ 13 ppb was used as additional positivity criterion. Pulmonary responders and nonresponders with a FeNO increase ≥ 13 ppb were defined as SIC positive (group A). If no criterion (neither pulmonary response nor a FeNO increase ≥ 13 ppb) was fulfilled SIC was assigned negative (groups B and C). Subjects with negative SIC but increase of bronchial hyperresponsiveness and/or sputum eosinophils were assigned to group B, those subjects without any changes after SIC to group C (Fig. 2). Subjects of group B and subjects of group C with sensitizations to occupational allergens were evaluated individually by an overall expert rating taking into account all available information and examinations like specific allergen sensitization, serial FeNO measurements at work and at home, near positive SIC reactions, or improvement of symptoms after exposure cessation.

Statistical analyses

Whereas pulmonary responder status and FeNO defined the (primary) ‘gold standard’, changes in sputum eosinophils and bronchial hyperresponsiveness were chosen as effect

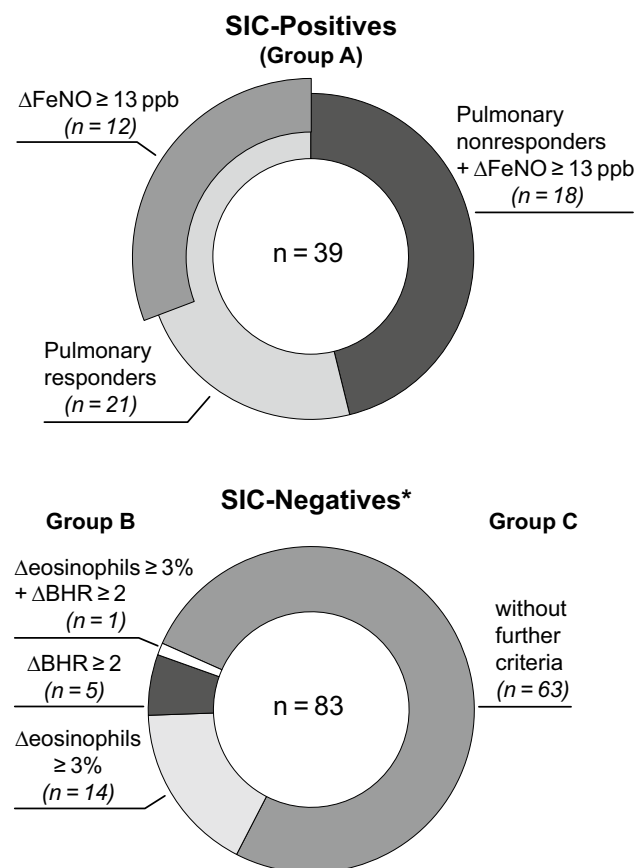


Fig. 2 Group assignment considering significant changes in FeNO, bronchial hyperresponsiveness, and sputum eosinophils. *SIC negatives were defined as pulmonary nonresponders without FeNO increase ≥ 13 ppb

parameters. Between-group differences were analyzed by Mann–Whitney *U* test for continuous variables and by Chi-square test for categorical variables whereby they were considered significant at a *p* value of ≤ 0.05 . Changes in FeNO (Δ FeNO) and sputum eosinophils (Δ sputum eosinophils) from before to after SIC were calculated by subtracting the value before SIC from the value after SIC. A receiver-operating characteristic (ROC) curve was built and the maximum Youden Index (sensitivity + specificity – 1) was calculated to determine the optimal cut-off for a significant increase in sputum eosinophils. To define the discriminatory ability of Δ sputum eosinophils the area under the curve (AUC) was determined. A significant increase of bronchial hyperresponsiveness was assumed if the ratio of $PD_{20}FEV_1$ before SIC/ $PD_{20}FEV_1$ after SIC was ≥ 2 . In subjects who did not show a significant reaction in methacholine testing before SIC but after SIC, $PD_{20}FEV_1$ before SIC was presumed 500 μ g and the ratio of $PD_{20}FEV_1$ before SIC/ $PD_{20}FEV_1$ after SIC was calculated. If bronchial hyperresponsiveness after SIC was not measured due to airway obstruction or medication ($n = 4$), we assumed an increase of bronchial

hyperresponsiveness. The statistical analyses were done with the software SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Eighty-three of the 122 subjects (68%) did not show a significant change of pulmonary function and/or FeNO during SIC (SIC negatives, Table 1; groups B and C). Thirty-nine subjects (32%) were considered SIC positives (group A); among them, 21 pulmonary responders (12 of them with an increase of FeNO ≥ 13 ppb) and 18 pulmonary nonresponders with a FeNO increase ≥ 13 ppb (Table 1, Fig. 2). SIC positives were younger ($p=0.04$), showed higher total IgE ($p=0.001$), a higher rate of atopy ($p=0.02$), and specific allergen sensitization ($p=0.002$) than SIC negatives (groups B and C). Post-SIC sputum eosinophils as well as the increases of sputum eosinophils and of bronchial hyperresponsiveness after SIC were significantly higher in SIC positives ($p<0.001$).

To determine the diagnostic accuracy of sputum eosinophils, an ROC curve was built and the area under the curve (AUC) was calculated with 0.72 [95% confidence interval (CI) 0.6–0.8]. Youden Index was generated and proposed a change of eosinophils in sputum after SIC of at least 3% as optimal cut-off with a sensitivity of 0.51 and a specificity of 0.87 (Fig. 3).

Among the 39 SIC positives, 15 subjects (38%) showed an increase of sputum eosinophils without an increase of bronchial hyperresponsiveness. Seven SIC positives (18%) demonstrated an increase of bronchial hyperresponsiveness alone and further 6 (15%) showed significant changes of both criteria after SIC. Out of the 83 SIC negatives, 20 subjects (24%) demonstrated significant increases of sputum eosinophils and/or bronchial hyperresponsiveness (Table 1, group B). In detail: 14 subjects (17%) showed an increase of sputum eosinophils alone, 5 subjects (6%) of bronchial hyperresponsiveness alone, and 1 subject (1%) showed significant changes of both criteria after SIC (Fig. 2; Table 2). Allergens which induced significant changes of eosinophil counts or bronchial hyperresponsiveness were manifold with a preponderance of low-molecular-weight (LMW) allergens. This result represents roughly the allergen distribution in our whole-study population (Table 1; a list of allergens is presented in the appendix). Out of the 20 subjects of group B, overall expert rating resulted in a diagnosis of OA in only one subject (Table 2). Seven subjects (35%) were rated doubtful; in these cases, a clear decision was impossible. Three subjects showed a positive result in specific allergen sensitization. One of them received a positive overall rating and two were rated doubtful. Overall, OA was considered as possible diagnosis in 8 of the 83 pulmonary nonresponders

without increases of FeNO ≥ 13 ppb after SIC, this represents a possible false-negative classification of about 10%.

To evaluate whether subjects of group B show distinct phenotypes we compared them with groups A and C (Table 1). Subjects of group B were older ($p=0.012$), showed lower total IgE ($p=0.013$), lower baseline FeNO values ($p=0.045$), and higher sputum neutrophil counts 24 h after SIC (13.5% vs. 37.5% (median), $p=0.049$) than group A. Although not significant, group B had fewer atopics, more non-smokers, less specific sensitizations, and higher baseline neutrophil values than group A. Subjects of group B also showed significantly higher sputum neutrophil numbers after SIC (37.5% vs. 12.5% (median), $p=0.009$) than those of group C. However, group B did not show significant differences in neutrophil increase compared to either groups A or C (Table 1).

Six of the seven SIC negative subjects with specific sensitizations to occupational allergens were considered doubtful or probable OA, among them three subjects with increases of sputum eosinophils.

Table 3 demonstrates a summary of sensitivities, specificities, and predictive values of sputum eosinophil counts and bronchial hyperresponsiveness separately and in combination as additional tools to the reference parameters for diagnosing OA. A change of sputum eosinophils $\geq 3\%$ showed a sensitivity of 0.51 and specificity of 0.86, whereas the increase of bronchial hyperresponsiveness showed a clearly lower sensitivity (0.38) with similar specificity (0.92). If lung function criteria alone were used as ‘gold standard’, the diagnostic accuracy of increases of FeNO was slightly superior to increases of sputum eosinophil counts and bronchial hyperresponsiveness for diagnosing OA. A combination of these parameters improves specificity, but is accompanied by a substantial loss of sensitivity.

Discussion

Studies with the aim to compare efficiencies of various diagnostic tests need a valid ‘gold standard’. Most previous studies about the accuracy of non-invasive tests like increases of FeNO, sputum eosinophils, and bronchial hyperresponsiveness for the diagnosis of OA used lung function, i.e., the pulmonary responder status as ‘gold standard’, although it is well known that false-positive and false-negative tests occur (Vandenplas et al. 2014). There are a few studies which used the results of repetitive SICs as ‘gold standard’: Whereas two studies represent case series with a few additional cases of OA that were detected only after repetitive SICs (Vandenplas et al. 1996; Sastre et al. 2003), one larger study of 68 subjects showed that an increase of sputum eosinophils $> 3\%$ had a sensitivity of 0.67 and a specificity of 0.97 (Vandenplas et al. 2009). Although the number of subjects who were

Table 1 Personal and clinical data

	SIC positives (<i>n</i> = 39)		SIC negatives (<i>n</i> = 83)		<i>p</i> value [§]	Total (<i>n</i> = 122)
	Group A ^a (<i>n</i> = 39)	Group B ^b (<i>n</i> = 20)	Group C ^c (<i>n</i> = 63)			
Age: years [median (range)]	42 (20–61)	53 (20–63)	47 (22–69)	AB:0.012		47 (20–69)
Male gender: <i>n</i> (%)	26 (67)	12 (60)	41 (65)	ns		79 (65)
Atopy: <i>n</i> (%)	23 (59)	7 (37)	22 (37)	AC:0.029		52 (44)
Current smokers: <i>n</i> (%)	11 (28)	2 (10)	14 (22)	ns		27 (22)
Never- or ex-smokers: <i>n</i> (%)	28 (72)	18 (90)	49 (78)			95 (78)
Beginning of exposure to onset of symptoms: years [median (range)]	4 (0–41)	1.8 (0–28)	6 (0–42)	ns		4 (0–42)
End of exposure to diagnosis: years [median (range)]	0.5 (0–2)	0.9 (0–6)	0.75 (0–15)	ns		0.75 (0–15)
ICS treatment: <i>n</i> (%)	14 (37)	11 (55)	35 (57)	ns		60 (50)
HMW allergens: <i>n</i> (%)	9 (23)	4 (20)	6 (10)	ns		19 (16)
LMW allergens: <i>n</i> (%)	30 (77)	16 (80)	57 (90)			103 (84)
Total IgE: kU/L [median (range)]	106 (4–1274)	24 (5–536)	24 (2–5000)	AB:0.013 AC:0.02		40 (2–5000)
Specific allergen sensitization: <i>n</i> (%)	13 (33)	3 (16)	4 (7)	AC:0.001		20 (16)
Pulmonary responders: <i>n</i> (%)	21 (54)	0	0	nd ^d		21 (17)
Immediate reaction: <i>n</i> (%)	15 (38)	0	0	nd ^d		15 (12)
Late reaction: <i>n</i> (%)	4 (10)	0	0			4 (3)
Baseline FEV ₁ : %pred [median (range)]	100 (67–128)	103 (81–133)	97 (64–127)	ns		99 (64–133)
Baseline sR _i : kPa s [median (range)]	0.9 (0.4–2.6)	0.8 (0.4–2.4)	0.8 (0.1–2.8)	ns		0.8 (0.1–2.8)
Baseline bronchial hyperresponsiveness: <i>n</i> (%)	20 (54)	6 (30)	29 (47)	ns		55 (46)
Increase of bronchial hyperresponsiveness: <i>n</i> (%)	13 (38)	6 (35)	0	nd ^d		19 (17)
Baseline FeNO: ppb [median (range)]	24 (3–151)	14 (6–97)	14 (5–119)	AB:0.045 AC:0.01		15 (3–151)
Post-SIC FeNO: ppb [median (range)]	54 (6–156)	15 (9–83)	16 (6–126)	AB:<0.001 AC:<0.001		20 (6–156)
Δ FeNO: ppb [median (range)]	19 (–42–127)	2.5 (–14–8)	1 (–12–10)	AB:<0.001 AC:<0.001		3.5 (–42–127)
Δ FeNO ≥ 13 ppb: <i>n</i> (%)	30 (76.9)	0	0	nd ^d		30 (24.6)
Baseline sputum eosinophils: % [median (range)]	1 (0–23)	0.25 (0–6.5)	0.5 (0–29)	ns		0.5 (0–29)
Post-SIC sputum eosinophils: % [median (range)]	5.5 (0–47.5)	4.7 (0–11.5)	0.5 (0–16.5)	AC:<0.001 BC:<0.001		1.5 (0–47.5)
Δ Sputum eosinophils: % [median (range)]	3.3 (–3.5–47.5)	4.4 (0–10)	0.0 (–20.5–2.5)	AC:<0.001 BC:<0.001		0.0 (–20.5–47.5)
Δ Sputum eosinophils ≥ 3%: <i>n</i> (%)	21 (53.8)	15 (75)	0	nd ^d		36 (29.5)
Baseline sputum neutrophils: % [median (range)]	14.5 (0–76.5)	27.5 (0–82.7)	18.5 (0–78)	ns		17.8 (0–82.7)
Post-SIC sputum neutrophils: % [median (range)]	13.5 (0–59.5)	37.5 (0–69.5)	12.5 (0–72.5)	AB:0.049 BC:0.009		14 (0–72.5)
Δ Sputum neutrophils: % [median (range)]	0 (–34–54)	1 (–22–70)	–1.5 (–78–67)	ns		–0.5 (–78–70)

ICS inhaled corticosteroids, HMW high molecular weight, LMW low molecular weight, FEV₁ forced expiratory volume in one second, sR_i specific airway resistance, FeNO fractional exhaled nitric oxide, Δ FeNO difference in FeNO values after minus before specific inhalation challenge, Δ sputum eosinophils difference in sputum eosinophil counts after minus before specific inhalation challenge, increase of bronchial hyperresponsiveness ratio PD₂₀FEV₁ before/after SIC ≥ 2, ns not significant

^aSIC positives: pulmonary response (FEV₁ decrease ≥ 20% and sR_i increase ≥ 100% to ≥ 2 kPa s) and/or Δ FeNO ≥ 13 ppb

^bNegative SIC but significant increases of sputum eosinophils and/or bronchial hyperresponsiveness (subjects shown in detail in Table 2)

^cNegative SIC without significant increases of sputum eosinophils and/or bronchial hyperresponsiveness

^dnd: calculation of *p* value was not done, because parameters were used for group assignment

[§]Only significant results are shown (*p* < 0.05)

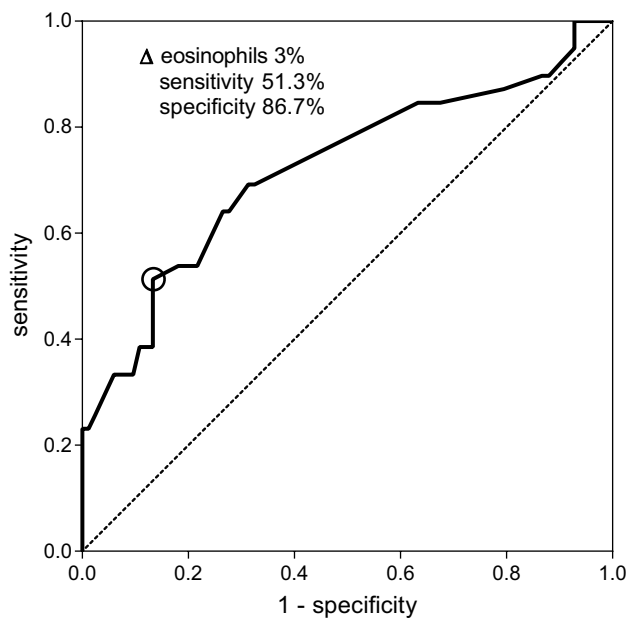


Fig. 3 Receiver-operating characteristic (ROC) plot showing sputum eosinophil changes before/after SIC. As ‘gold standard’ for SIC, pulmonary responder status (see Methods) and/or an increase of FeNO ≥ 13 ppb was used. The circle indicates an increase of sputum eosinophils of 3%

detected with a positive SIC only after subsequent testing was low ($n=6$), it is clear from that study that specificity of eosinophil increases should be rather high. This finding was more or less corroborated in the present study with specificities of 0.75 and 0.87 with both ‘gold standards’.

In this study, we used a combination of pulmonary responder status and/or an increase of FeNO as ‘gold standard’. This approach was stipulated by a previous study that showed that an increase of FeNO is highly predictive of OA and minimizes the number of false-negative SIC results (Engel et al. 2018). The number of false-positive increases of FeNO was very low (3 out of 117 (2.6%) negative or doubtful SICs). Here, an overall expert rating was used as the ‘gold standard’. Thus, the true number of false-positive FeNO increases is actually not known, but should be very low. Although the performances of the three non-invasive tools were similar in this study, we included FeNO measurements in the ‘gold standard’ for SIC evaluation, because FeNO is easy to perform and well standardized.

Sputum eosinophil counts have been reported to perform better to predict OA than FeNO (Lemière et al. 2010). This result could not be corroborated in this study: the test efficiency of FeNO was slightly higher than of increases of sputum eosinophils or bronchial hyperresponsiveness if the pulmonary responder status alone was used as ‘gold standard’.

It is a major result of this study that 20 out of 83 (24%) pulmonary nonresponders without an increase of FeNO

showed either an increase in sputum eosinophils or bronchial hyperresponsiveness. In one case (No 1 in Table 2), it is probable that the inhaled allergen dose was too low, but additional seven subjects (8%) who were considered doubtful indicate that OA may be suspected in spite of a pulmonary nonresponder status and a lack of a FeNO increase. It should be noted that the differences of performance between increases of FeNO and sputum eosinophils were minor in the present study, but, due to its much easier measurement, FeNO is the preferable parameter. We consider this finding compatible with the previous studies.

It is of importance how a significant increase of the parameters is defined. In the present study, we used an increase of FeNO of ≥ 13 ppb as cut-off, which has been established in a recent study (Engel et al. 2018), and is almost identical with a cut-off proposed by others (Lemière et al. 2014). The cut-off for sputum eosinophil increase of 3% was assessed by ROC plot and fitted well with the previous recommendations (Vandenplas et al. 2009). For bronchial hyperresponsiveness, we used a somewhat arbitrary cut-off (i.e., doubling of the provocation dose for a significant change in lung function), but this was due to the lacking data in the literature concerning the combined use of spirometry and body plethysmography (which we used for high specificity). The finding that sensitivities were rather low for all parameters irrespective of the reference is a further indicator that the choice of the cut-off for each parameter is not a relevant confounder of the overall results and the conclusions of this study.

In this study, SIC positives as defined by the primary ‘gold standard’ were younger, showed higher total IgE, a higher rate of atopy, and specific allergen sensitization. This finding might be due to selection, but we consider atopy, specific sensitization, and elevated baseline FeNO as plausible predictors of a positive SIC. Whereas atopy and baseline FeNO cannot be used as diagnostic tools due to low specificity, specific sensitization should have a high predictive value for a positive SIC. Actually, six of the seven SIC negative subjects with specific sensitizations were considered doubtful or probable OA; among them, three subjects with increases of sputum eosinophils. This indicates that sputum eosinophils may be more sensitive than FeNO in a few cases.

Also the fact that SIC negative subjects who showed increases of sputum eosinophils or bronchial hyperresponsiveness were younger and had higher total IgE-factors which were associated with positive SIC-indicates that the primary ‘gold standard’ was not ideal, and that increases of sputum eosinophils or bronchial hyperresponsiveness may be predictive of OA in individual cases if lung function or FeNO are negative.

Subjects with OA showed neither increased numbers of baseline neutrophils nor increased neutrophil numbers after SIC. Whereas SIC did not affect neutrophil numbers,

Table 2 Characteristics of 20 subjects (group B) with negative SIC but significant increases of sputum eosinophils and/or bronchial hyperresponsiveness (BHR)

No.	Age (years)	Agent	Specific sensitization	Sputum eosinophils before SIC (%)	Sputum eosinophils after SIC (%)	Sputum neutrophils before SIC (%)	Sputum neutrophils after SIC (%)	BHR	Increase of BHR	Overall rating
Significant increase of sputum eosinophils $\geq 3\%$										
1	55	Gammarus	Yes ^a	0	10	2.5	30	No	nd	Positive
2	63	Molds	Yes ^a	0.5	3.5	31	44.5	No	No	Doubtful
3	20	Persulfate	Yes ^a	2.5	8.5	27.5	31	No	No	Doubtful
4	42	Isocyanates	No	0	5.5	53	38	No	No	Doubtful
5	59	Isocyanates	No	0	7.5	73	64.5	Yes	nd	Doubtful
6	47	Isocyanates	No	1.5	4.5	28.5	46.5	No	No	Doubtful
7	57	Isocyanates	No	0	4.5	7.5	44.5	Yes	No	Negative
8	61	Isocyanates	No	0.6	4.9	82.7	60.8	No	No	Negative
9	55	Isocyanates	No	0	6	0	69.5	No	No	Negative
10	59	Isocyanates	No	3.5	11	21.5	61.5	No	No	Negative
11	49	Disinfectant	No	1	4	34.5	50	No	No	Negative
12	53	Disinfectant	nd	6.5	11.5	37	38	Yes	No	Negative
13	57	Flour	No	2	10	33	28	No	No	Negative
14	47	Teflon	No	0	3	2	0	No	nd	Negative
Significant increase of bronchial hyperresponsiveness $PD_{20}FEV_1$ before/after SIC ≥ 2										
15	42	Isocyanates	No	0.5	1.5	4	0	Yes	Yes	Doubtful
16	42	Isocyanates	No	0	0.3	28	37	No	Yes	Negative
17	40	Primer coating	No	0	0	0	0	No	Yes	Negative
18	43	Cyanacrylate	No	0	0	4.5	2	Yes	Yes	Negative
19	60	Coriander and feathers	No	0.5	0.5	27.5	26	Yes	Yes	Negative
Significant increase of sputum eosinophils and of bronchial hyperresponsiveness										
20	52	Isocyanates	No	0	7	0.5	1.5	No	Yes	Doubtful

nd not done

^aPositive specific sensitization in skin prick test

Table 3 Sensitivities, specificities, and predictive values of different criteria according to different ‘gold standards’ for positive SIC

Criterion/criteria	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Primary ‘gold standard’ for positive SIC: lung function criteria (sR_l and FEV_1) and/or increase of $FeNO \geq 13$ ppb				
Δ Sputum eosinophils	0.51	0.86	0.64	0.79
Δ bronchial hyperresponsiveness	0.38	0.92	0.68	0.76
Δ Sputum eosinophils and Δ bronchial hyperresponsiveness	0.17	0.98	0.85	0.72
Secondary ‘gold standard’ for positive SIC: lung function criteria (sR_l and FEV_1) only				
Δ Sputum eosinophils	0.52	0.75	0.30	0.88
Δ Bronchial hyperresponsiveness	0.43	0.87	0.36	0.90
Δ FeNO	0.57	0.82	0.40	0.90
Δ Sputum eosinophils and Δ bronchial hyperresponsiveness	0.18	0.95	0.42	0.87
Δ FeNO and Δ sputum eosinophils	0.33	0.90	0.41	0.86
Δ FeNO and Δ bronchial hyperresponsiveness	0.25	0.93	0.40	0.87
Δ FeNO and Δ sputum eosinophils and Δ bronchial hyperresponsiveness	0.12	0.96	0.40	0.86

Δ sputum eosinophils difference of sputum eosinophils after minus before SIC $\geq 3\%$, Δ bronchial hyperresponsiveness $PD_{20}FEV_1$ before/after SIC ≥ 2 , Δ FeNO difference of FeNO after minus before SIC ≥ 13 ppb

neutrophils at baseline as well as after SIC were higher in group B. This might be indicative of (chronic) neutrophilic bronchitis. The question whether increases of eosinophils or hyperresponsiveness after SIC occur more often in subjects with neutrophilic bronchitis or whether there is a distinct phenotype of OA cannot be answered with the available data. As SICs were performed only with substances of definite or possible immunologic mechanism, we cannot answer the question whether non-immunologic reactions may be accompanied by changes of non-invasive parameters. Interestingly, SICs with disinfectants, hairdressing substances, or sodium sulphite did not show any such changes.

It is a strength of this study that a high number of subjects were included who completed all tests with a wide variety of different allergens. Thus, selection due to missing data and confounding due to testing with selected allergens is considered minor. Differences between this study and previous results with respect to the diagnostic accuracy of FeNO and sputum eosinophils (Lemière et al. 2010) are also considered minor and may be explained by differences in sputum collection or subject selection.

Medication was withheld from the first day onwards to obtain similar exposure conditions of the subjects. This might have made the patients more susceptible and, thus, might have facilitated false-positive SICs. This cannot be excluded in view of the rather high percentage of pulmonary responders without any change of the non-invasive parameters, but we consider this less likely due to a lack of an increase of bronchial hyperresponsiveness in most pulmonary nonresponders. Also repetitive sputum induction before SIC and 24 h afterwards is not considered to affect reactions, because, in contrast to many other researchers, we

used no hypertonic saline for sputum induction. Here, again, no change of the non-invasive parameters was seen in most pulmonary nonresponders.

In summary, this study corroborates the previous results that serial measurements of non-invasive parameters like FeNO, sputum eosinophils, or bronchial hyperresponsiveness may increase the accuracy for diagnosing OA. Although each of these parameters yields similar results, FeNO is less cumbersome to perform and thus should be preferred. Increases of sputum eosinophil counts and bronchial hyperresponsiveness without lung functional response and/or FeNO increase may indicate OA in a few cases.

Acknowledgements We thank the patients for their participation and all involved technical assistants of the Institute for Prevention and Occupational Medicine (IPA) for their excellent assistance. Authors’ contributions were as follows. Study design: R. Merget and J. Engel; data collection: J. Engel, R. Merget, O. Hagemeyer, and M. Raulf; data analysis: J. Engel, and V. Gering; writing: J. Engel, R. Merget, and V. van Kampen; data interpretation: J. Engel, R. Merget, M. Raulf, and V. van Kampen; critical review: V. Gering, O. Hagemeyer, T. Brüning, and M. Raulf.

Funding This research was performed in the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance. For this study, we received no further specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

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

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.

Appendix

Association between SIC results and type of agent

Type of agent	Positives ^a (<i>n</i> = 39)	Negatives (<i>n</i> = 83)	Total (<i>n</i> = 122)
HMW	Enzymes <i>n</i> = 1 Animal dander = 2 Molds <i>n</i> = 1 Wash nut <i>n</i> = 1 Flour <i>n</i> = 3 Plants <i>n</i> = 1	Molds <i>n</i> = 1 Flour <i>n</i> = 1 Pectin <i>n</i> = 1 Feathers <i>n</i> = 1 Milk protein <i>n</i> = 2 Straw, grain <i>n</i> = 1 Heated cotton mat <i>n</i> = 1 Gammarus <i>n</i> = 1 Latex <i>n</i> = 1	Total <i>n</i> = 19
LMW	Total <i>n</i> = 9 Platinum salts <i>n</i> = 7 Persulfate salts <i>n</i> = 2 Cobalt powder <i>n</i> = 1	Hairdressing products <i>n</i> = 5 Platinum salts <i>n</i> = 3 Disinfectants <i>n</i> = 5 Acrylates <i>n</i> = 2 Primer coating <i>n</i> = 1 Teflon <i>n</i> = 1 Adhesive <i>n</i> = 1 Sodium sulphite <i>n</i> = 1 Persulfate salts <i>n</i> = 2	Total <i>n</i> = 31
Isocyanates	Total <i>n</i> = 10 MDI <i>n</i> = 18 NDI <i>n</i> = 1 IPDI <i>n</i> = 1	Total <i>n</i> = 21 MDI <i>n</i> = 26 HDI <i>n</i> = 21 IPDI <i>n</i> = 2 TDI <i>n</i> = 3	Total <i>n</i> = 72

Animal dander 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

^aSIC Pulmonary responders and/or increase of FeNO $\geq$ 13ppb

References

American Thoracic Society, European Respiratory Society (2005) ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med* 171:912–930

- Bacci E, Cianchetti S, Paggiaro PL, Carnevali S, Bancalari L, Dente FL, Di Franco A, Giannini D, Vagaggini B, Giuntini C (1996) Comparison between hypertonic and isotonic saline-induced sputum in the evaluation of airway inflammation in subjects with moderate asthma. *Clin Exp Allergy* 26:1395–1400
- Engel J, van Kampen V, Lotz A, Abramowski J, Gering V, Hagemeyer O, Brüning T, Raulf M, Merget R (2018) An increase of fractional exhaled nitric oxide after specific inhalation challenge is highly predictive of occupational asthma. *Int Arch Occup Environ Health* 91:799–809
- Lemière C, D'Alpaos V, Chaboillez S, César M, Wattiez M, Chirys S, Vandenplas O (2010) Investigation of occupational asthma: sputum cell counts or exhaled nitric oxide? *Chest* 137:617–622
- Lemière C, Nguyen S, Sava F, D'Alpaos V, Huaux F, Vandenplas O (2014) Occupational asthma phenotypes identified by increased fractional exhaled nitric oxide after exposure to causal agents. *J Allergy Clin Immunol* 134:1063–1067
- Merget R, Heinze E, Neumann L, Taeger D, Brüning T (2005) Comparison of the PARI Provotest II reservoir method with the ATS dosimeter method to assess bronchial hyperresponsiveness to methacholine. In: Proceedings of the 45th annual conference of the German Society for Occupational and Environmental Medicine (DGAUM), pp 624–625 [in German]
- Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J (2005) Standardisation of spirometry. *Eur Respir J* 26:319–338
- Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC (1993) Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests European community for steel and coal. *Eur Respir J* 6(Suppl 16):5–40
- Quirce S, Lemière C, de Blay F, del Pozo V, Gerth Van Wijk R, Maestrelli P, Pauli G, Pignatti P, Raulf-Heimsoth M, Sastre J, Storaas T, Moscato G (2010) Noninvasive methods for assessment of airway inflammation in occupational settings. *Allergy* 65:445–458
- Racine G, Castano R, Cartier A, Lemiere C (2017) Diagnostic accuracy of inflammatory markers for diagnosing occupational asthma. *J Allergy Clin Immunol Pract* 5:1371–1377
- Raulf M, Hoffmeyer F, van Kampen V, Deckert A, Brüning T, Bünger J (2015) Cellular and soluble inflammatory markers in induced sputum of composting plant workers. *Adv Exp Med Biol* 858:19–29
- Raulf-Heimsoth M, Pesch B, Kendzia B, Spickenheuer A, Bramer R, Marczynski B, Merget R, Brüning T (2011) Irritative effects of vapours and aerosols of bitumen on the airways assessed by non-invasive methods. *Arch Toxicol* 85(Suppl 1):S41–S52
- Sastre J, Fernández-Nieto M, Novalbos A, De Las Heras M, Cuesta J, Quirce S (2003) Need for monitoring nonspecific bronchial hyperresponsiveness before and after isocyanate inhalation challenge. *Chest* 123:1276–1279
- Sastre J, Costa C, Garcia del Potro M, Aguado E, Mahillo I, Fernandez-Nieto M (2013) Changes in exhaled nitric oxide after inhalation challenge with occupational agents. *J Invest Allergol Clin Immunol* 23:421–427
- Vandenplas O, Delwiche JP, Jamart J, Van de Weyer R (1996) Increase in non-specific bronchial hyperresponsiveness as an early marker of bronchial response to occupational agents during specific inhalation challenges. *Thorax* 51:472–478
- Vandenplas O, D'Alpaos V, Heymans J, Jamart J, Thimpont J, Huaux F, Lison D, Renauld JC (2009) Sputum eosinophilia: an early marker of bronchial response to occupational agents. *Allergy* 64:754–761
- Vandenplas O, Suojalehto H, Aasen TB, Baur X, Burge PS, de Blay F, Fishwick D, Hoyle J, Maestrelli P, Muñoz X, Moscato G, Sastre J, Sigsgaard T, Suuronen K, Walusiak-Skorupa J, Cullinan P, ERS Task Force on Specific Inhalation Challenges with Occupational Agents (2014) Specific inhalation challenge in

- the diagnosis of occupational asthma: consensus statement. *Eur Respir J* 43:1573–1587
- Vandenplas O, Suojalehto H, Cullinan P (2017) Diagnosing occupational asthma. *Clin Exp Allergy* 47:6–18
- Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson D, Macintyre N, McKay R, Miller MR, Navajas D, Pellegrino R, Viegi G (2005) Standardisation of the measurement of lung volumes. *Eur Respir J* 26:511–522
- Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.