

## Noninvasive methods for assessment of airway inflammation in occupational settings

S. Quirce<sup>1,2</sup>, C. Lemièrè<sup>3</sup>, F. de Blay<sup>4</sup>, V. del Pozo<sup>2,5</sup>, R. Gerth Van Wijk<sup>6</sup>, P. Maestrelli<sup>7</sup>, G. Pauli<sup>4</sup>, P. Pignatti<sup>8</sup>, M. Raulf-Heimsoth<sup>9</sup>, J. Sastre<sup>2,10</sup>, T. Storaas<sup>11</sup> & G. Moscato<sup>8</sup>

<sup>1</sup>Department of Allergy, Hospital La Paz and <sup>2</sup>CIBER of Respiratory Diseases CIBERES, Madrid, Spain; <sup>3</sup>Department of Chest Medicine, Hôpital du Sacré-Coeur, Montreal, Canada; <sup>4</sup>Division of Asthma and Allergy, Department of Chest Diseases, University Hospital Strasbourg, France; <sup>5</sup>Department of Immunology, Fundación Jiménez Díaz-Capio, Madrid, Spain; <sup>6</sup>Section of Allergology, Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands; <sup>7</sup>Department of Environmental Medicine and Public Health, University of Padova, Padova, Italy; <sup>8</sup>Allergy and Immunology Unit, Fondazione Salvatore Maugeri, Institute of Care and Research, Scientific Institute of Pavia & Occupational Immunology and Allergy Laboratory ISPEL, Pavia, Italy; <sup>9</sup>BGFA – Research Institute of Occupational Medicine, German Social Accident Insurance, Ruhr University Bochum, Bochum, Germany; <sup>10</sup>Department of Allergy, Fundación Jiménez Díaz-Capio, Madrid, Spain; <sup>11</sup>Department of Otolaryngology, Head & Neck Surgery and Department of Occupational Medicine, Haukeland University Hospital, Bergen, Norway

**To cite this article:** Quirce S, Lemièrè 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. Noninvasive methods for assessment of airway inflammation in occupational settings. *Allergy* 2010; **65**: 445–458.

### Keywords

asthma; eosinophils; exhaled breath condensate; fractional exhaled nitric oxide; induced sputum; occupational respiratory diseases; rhinitis.

### Correspondence

Santiago Quirce, Hospital La Paz, Department of Allergy, P. Castellana 261, 28046 Madrid Spain. Tel: +34 91 727 70 80 Fax: +34 91 727 70 50 E-mail: squirce@gmail.com

Accepted for publication 15 October 2009

DOI:10.1111/j.1398-9995.2009.02274.x

Edited by: Jean Bousquet

### Abstract

The present document is a consensus statement reached by a panel of experts on noninvasive methods for assessment of airway inflammation in the investigation of occupational respiratory diseases, such as occupational rhinitis, occupational asthma, and nonasthmatic eosinophilic bronchitis. Both the upper and the lower airway inflammation have been reviewed and appraised reinforcing the concept of 'united airway disease' in the occupational settings. The most widely used noninvasive methods to assess bronchial inflammation are covered: induced sputum, fractional exhaled nitric oxide (FeNO) concentration, and exhaled breath condensate. Nasal inflammation may be assessed by noninvasive approaches such as nasal cytology and nasal lavage, which provide information on different aspects of inflammatory processes (cellular *vs* mediators). Key messages and suggestions on the use of noninvasive methods for assessment of airway inflammation in the investigation and diagnosis of occupational airway diseases are issued.

Noninvasive methods for assessing airway inflammation are increasingly used in the investigation and management of asthma and rhinitis. In asthma, the analysis of cells and mediators in induced sputum has been widely applied for studying bronchial inflammation (1). The use of the fractional exhaled nitric oxide (FeNO) concentration as a surrogate marker of eosinophilic airway inflammation has been suggested (2), whereas exhaled breath condensate (EBC) is still under evaluation (3). Nasal inflammation may be assessed by noninvasive methods such as nasal cytology and nasal lavage (NAL), which provide information on different aspects of inflammatory processes (cellular *vs* mediators) (4).

Noninvasive methods for the evaluation of airway inflammation have also been used in the investigation of occupa-

tional respiratory diseases (5–8). However, there is currently no consensus on the role of new means that assess airway inflammation in the evaluation of occupational respiratory disease, such as FeNO and induced sputum (9). There is a need to increase the use and availability of these tests in the investigation of occupational asthma (OA) (10), nonasthmatic eosinophilic bronchitis (NAEB) (11), and occupational rhinitis (12). It should be considered, however, that subjective symptom scores and objective physiological measurements are not necessarily correlated with the severity of airway inflammation. This is probably because of the fact that neural regulation, hyperreactivity, and perception of severity of symptoms play important role in overall severity of the disease.

The main objective of this document, elaborated as a consensus statement, is to issue key messages and consensus suggestions on noninvasive methods for assessment of airway inflammation in the investigation and diagnosis of occupational airway diseases based on existing scientific evidence and the expertise of a panel of physicians coming from different countries. Both upper and lower airway inflammation have been reviewed and appraised reinforcing the concept of 'united airway disease' (4) in the occupational settings.

### Induced sputum analysis

The aim of sputum induction is to collect an adequate sample of secretions from lower airways in subjects who do not produce sputum spontaneously. Inhalation of isotonic or hypertonic solutions administered by nebulization has been demonstrated to induce a small amount of airway secretion that can be expectorated and analyzed (13–15). Some investigators change concentration during the procedure, starting with 3% and subsequently increasing to 4% and 5%, with a cumulative duration of nebulization of 15–20 min. However, it seems that hypertonic saline 3% is as successful as 3–5% given sequentially (16). There is no difference in the cellular composition of sputum induced with either isotonic or hypertonic saline (17), and different saline concentrations do not affect total and differential cell counts (DCC) in induced sputum (16), eosinophil cationic protein (ECP), or histamine level.

Repeating sputum induction 8–24 h after an initial induction can cause an increase in neutrophil levels in the second sputum sample (18). An interval of 48 h between two inductions gave reproducible cell counts in normal subjects (19). However, there are many studies on OA in which induced sputum is performed 24 h after a baseline induction, showing reproducible results on neutrophil counts.

Pretreatment with a short-acting  $\beta_2$ -agonist (e.g. salbutamol) is to be recommended as the standard procedure to prevent excessive bronchoconstriction (13, 15, 20). Salbutamol has no effect on sputum inflammatory cell percentages (16, 20). Methacholine challenge has no influence on sputum inflammatory cell counts (21).

Two methods for processing the expectorate have evolved. The first involves selecting all viscid or denser portions from the expectorated sample with the aid of an inverted microscope or by visual aspect (13, 22). The second approach involves processing the entire expectorate, comprising sputum plus variable amounts of saliva (23). The reproducibility of cell counts has been reported to be lower if squamous cell contamination represents >20% of all recovered cells (24). There is conflicting data as to whether or not DCC differ between the two methods.

It is recommended that sputum be processed as soon as possible or within 2 h to ensure optimum cell counting and staining. Complete homogenization is important and can be achieved by the use of dithiothreitol (DTT) or dithioerythritol (DTE) to break the disulphide bonds in mucin molecules, allowing cells to be released. Once the sputum cells have been obtained, the investigations are directed to obtain DCC, and

**Table 1** Differential cell counts in induced sputum from healthy individuals (mean  $\pm$  SD)

	% cells		
	Belda et al. (25)	Spanevello et al. (26)	Thomas et al. (27)
Neutrophils	37.5 $\pm$ 20.1	27.3 $\pm$ 13.0	47.0 $\pm$ 7.0
Eosinophils	0.4 $\pm$ 0.9	0.6 $\pm$ 0.8	0.3 $\pm$ 0.6
Macrophages	58.8 $\pm$ 21.0	69.2 $\pm$ 13.0	49.0 $\pm$ 25.2
Lymphocytes	1.0 $\pm$ 1.1	1.0 $\pm$ 1.2	1.0 $\pm$ 1.4
Epithelial cells	1.6 $\pm$ 3.9	1.5 $\pm$ 1.8	2.5 $\pm$ 3.2

the most common method used is the stain after cytocentrifugation method. The nonsquamous DCC are expressed as a percentage of the total nonsquamous cells. Several reports have studied induced sputum cell count in healthy adults (25–27). Table 1 summarizes data of DCC in induced sputum from healthy individuals.

Induced sputum cells can be phenotypically analyzed by flow cytometry using monoclonal antibodies to select the single population of analysis (28, 29). DTT has no effect when sputum sample is processed for flow cytometry (28, 29). Even if present in small amount in sputum, lymphocytes are quite stable cells, and their phenotypic identification with the use of monoclonal antibodies anti-CD3, -CD4, -CD8, and -CD19 is accurate. Furthermore, the lymphocyte subset can be evaluated even on frozen sputum samples (30). Sputum T-cell activation has also been characterized in asthmatic patients through the expression of some membrane markers such as CD25, CD69, and CD103 (31). To evaluate Th1 or Th2 skewing of sputum lymphocytes, the expression of intracellular cytokines has been measured by flow cytometry (32). Mameless et al. (33) demonstrated an increase in activated sputum T cells producing interferon (IFN)- $\gamma$  and interleukin (IL)-13 after a specific inhalation challenge (SIC) in subjects with OA confirming the presence of a Th1/Th2 mixed population also in occupational setting. Flow cytometry is useful in determining cells usually present in small amounts in sputum as lymphocytes and basophils (34). However, reference values for sputum cell distribution specifically evaluated by flow cytometry are still lacking. The possibility of applying flow cytometry for the evaluation of airway inflammation in the occupational setting, besides the research field, depends on the identification of specific activation markers, on T cells, B cells, macrophages, eosinophils or neutrophils, useful and significant to highlight an inflammatory response induced after a SIC.

An array of mediators can be measured in induced sputum supernatant by using immunoassays (13, 14, 35). The mediators can reflect different aspects of airway inflammation (35) and remodeling including eosinophil activation (e.g. ECP), mast cell activation (e.g. tryptase), cytokine production (e.g. IL-5), and microvascular leakage (e.g. albumin and fibrinogen). An important issue is the possibility that induction itself or subsequent sputum processing activates airway inflammatory cells (36). In particular, the effect of interference by

sulfhydryl-reducing reagents, such as DTE or DTT, is important (37). However, DTT does not appear to interfere with the assays of IL-5, histamine, immunoglobulin A, fibrinogen, albumin, or tryptase, or appreciably to interfere with immunocytochemical staining of granulocyte-macrophage colony stimulating factor, tumor necrosis factor- $\alpha$ , IL-8 or most lymphocyte surface markers; it may slightly decrease staining of EG2 and HLA-DR (35, 38), and ECP data are controversial (35, 39).

Eicosanoids are lipidic mediators whose levels are important in asthmatic status, PGE<sub>2</sub> could be measured repeatable, and interference in the cysteinyl-leukotrienes assays by DTT is unlikely because concentrations were not significantly different in sputum treated with and without DTT (40). The use of a protein-rich milieu during the incubation of sputum with DTT has been demonstrated to reduce the detrimental effect of DTT both on cells and on soluble mediators (41).

Key messages:

- Reference values for sputum cells detected with flow cytometry are still lacking.
- There is a need to identify the relationships between cell activation markers (new or classic ones) and the inflammatory pattern arising from exposure to specific causative agents to benefit from the use of flow cytometry in sputum cell evaluation.

### Use of induced sputum and exhaled nitric oxide in the investigation of OA and related conditions

#### Changes in sputum cell counts during specific inhalation challenges

SIC remain the reference tests for diagnosing OA. However, those tests are sometimes difficult to interpret, especially when the patients are unable to perform reliable spirometric maneuvers. The addition of an objective measure to SIC is therefore likely to improve the diagnosis of OA. Furthermore, analysis of sputum cells may be useful in the investigation of the effects of occupational agents upon experimental exposures because it provides direct information on type, intensity, and time course of airway inflammation. The occurrence of an asthmatic reaction during allergen inhalation challenges or during asthma exacerbations is most of the time accompanied by an increase in sputum eosinophil counts (42). However, occupational agents differ from common inhalants because many of them are chemicals with irritant properties. Inhaling these agents may induce a different type of airway inflammation compared with common inhalants.

#### Changes in sputum cell counts after exposure to high and low molecular weight agents in subjects with occupational asthma

High molecular weight agents are proteins that usually induce an immediate or a dual asthmatic reaction. Similar to the airway inflammation found after common allergen inhalation challenges, an increase in sputum eosinophils has been observed after SIC to a number of high molecular weight

agents: cereals (6), oil seed rape flour (43), *Lathyrus sativus* flour (44), *Lepidoglyphus destructor* (45), spores of *Pleurotus ostreatus* (46), or tampico fibers found in agave leaves (47). The exposure to low molecular weight agents, which are chemicals that often induce delayed asthmatic reactions, can also induce sputum eosinophilia. For example, isocyanates (5), acrylates (48, 49), red cedar (50), exotic woods (51), persulfate (52), manganese (53), or styrene (34) have been shown to induce an increase in sputum eosinophil counts. Exposure to manganese (53) and styrene (34) can also induce an increase in sputum basophils counts. Overexpression of LTC<sub>4</sub>, relative underproduction of PGE<sub>2</sub>, and greater eosinophilia in induced sputum was observed in patients with positive SIC to high or low molecular weight occupational agents 24 h after the challenge (54).

A neutrophilic airway inflammation has been reported after exposure to isocyanates (55, 56). The factors that influence the type of airway inflammation induced by the exposure to occupational agents are unclear. The concentration and the length of exposure to these agents may play a role (55). Few studies have looked at the impact of exposure to occupational agents in healthy subjects. Exposure to isocyanates (5), acrylates or flour (6) does not seem to induce eosinophilic or neutrophilic inflammation in healthy subjects, but a neutrophilic inflammatory response has been found in the induced sputum of healthy subjects after short-term exposure to irritant agents such as ozone (57), diesel exhaust (58), and endotoxin (59).

#### Interpretation of changes in sputum cell counts during specific inhalation challenges

Using sputum DCC during the investigation of OA can improve its diagnosis by bringing an additional objective measure to this investigation. Although the reliability of FEV<sub>1</sub> and PC<sub>20</sub> can be affected by inadequate spirometric maneuvers, the presence or the absence of airway inflammation cannot be affected by improper maneuvers during sputum collection.

The best timing for the collection of induced sputum with respect to the exposure to occupational agents is likely to be 7–24 h after exposure. Indeed, an increase in sputum eosinophils has been shown to occur 7 h after exposure to occupational agents and persist 24 h after exposure (50). It should be considered that the time course of sputum influx may be different when other cell types are assessed.

The magnitude of increase in sputum eosinophil counts occurring after exposure to occupational agents that should be regarded as clinically significant is not clearly established. An increase in absolute eosinophil counts of  $0.26 \times 10^6/\text{ml}$  compared with baseline values yields a sensitivity of 82% and a specificity of 91.7% for predicting a 20% fall in FEV<sub>1</sub> (6). A 2% cutoff increase in sputum eosinophil counts after SIC is considered to be the most discriminant value associated with an asthmatic response (6–8).

The increase in sputum eosinophil counts tends to occur with levels of experimental exposures lower than those necessary to elicit the asthmatic reactions induced by high and low

molecular weight agents (60). Vandenas et al. (61) showed that an increase in sputum eosinophil counts greater than 3% after the first day of exposure during SIC was the most accurate parameter for predicting the development of an asthmatic response on subsequent exposures with a sensitivity of 67% and a specificity of 97%. Therefore, an important increase in the sputum eosinophil counts in the absence of an FEV<sub>1</sub> fall should incite pursuing the investigation (9, 11).

The lack of increase in sputum eosinophil counts after exposure to occupational agents should not rule out the diagnosis of OA. Indeed, some subjects can experience a 20% fall in FEV<sub>1</sub> without showing sputum eosinophilia (50), whereas others can experience a 20% fall in FEV<sub>1</sub> accompanied by an important increase in airway inflammation without airway hyperresponsiveness to methacholine (62, 63). Interfering factors that can modify the sputum cell response should be considered in the interpretation. Treatment with corticosteroids may blunt eosinophil influx, endotoxin contamination may favor sputum neutrophilia, and relatively high levels of exposure to chemicals may produce irritant effects.

In conclusion, sputum cell counts bring an additional objective measure in the investigation by SIC. Further studies are needed to improve the interpretation of the changes in sputum cell counts occurring after exposure to occupational agents.

#### Changes in induced sputum in response to various work exposures

The investigation of OA can be made by performing SIC in the laboratory or by monitoring the functional and inflammatory changes during periods at and away from the workplace. The majority of studies have described the changes in sputum cell counts observed in response to occupational exposures during the performance of SIC in the laboratory. However, during these tests, the workers are exposed to a single specific substance, which probably differs from the workplace where the workers are exposed to multiple agents often combining irritants and sensitizing properties.

The studies that have assessed the changes in induced sputum at the workplace are scarce. One of the first studies that investigated the changes in sputum cell counts between periods at and away from the workplace studied subjects with OA and asthmatics without OA working in the same environment (7). The workers were mostly exposed to low molecular weight agents. The diagnosis of OA was made if their asthma symptoms were worse at work and if there was either a FEV<sub>1</sub> fall  $\geq 20\%$  or a fourfold change or more in PC<sub>20</sub> between periods at and away from work. Sputum induction was performed at the end of periods at work and away from work. The subjects with OA had a large increase in sputum ECP when at work, which resolved when they were removed from their workplace. According to the inclusion criteria, the study population comprised subjects with OA who had very large changes in FEV<sub>1</sub> and PC<sub>20</sub> between periods at and away from work. However, this population differs from the majority of subjects with OA who do not show such large functional changes between periods at and off work, espe-

cially if they are treated with inhaled corticosteroids and long-acting beta<sub>2</sub> agonists. Therefore, such large changes in sputum eosinophil counts may not be always observed in clinical practice. Another study looked at the changes in sputum cell counts in subjects suspected to have OA because of high and low molecular weight agents, whose diagnosis was subsequently confirmed by SIC (8). When at work, the subjects with OA had a significant increase in sputum eosinophils, whereas the group with negative SIC had higher neutrophil counts compared with the periods away from work. The changes in sputum eosinophil counts were smaller than those observed in the previous study (7). Anees et al. (64) examined the changes in induced sputum in subjects with OA because of low molecular weight agents while working. No comparison with periods away from work was made. Thirty-eight workers were investigated. Only 14 had sputum eosinophils greater than 2.2% when at work. However, the diagnosis of OA was not based upon the same criteria for all subjects. The authors reported that the workers had a sputum neutrophilia (59% of neutrophils). However, the important variability in sputum neutrophil counts makes difficult to consider this sputum neutrophil count as a significant increase from a normal count without having a comparison between periods at and away from work. Indeed, the normal values of neutrophils reported in healthy subjects vary from  $27.3 \pm 13.0$  to  $47.7 \pm 7.0\%$  (65).

Another case of sputum neutrophilia was reported in a worker exposed to metal working fluid who had a marked increase in neutrophils when at work (82%), which resolved after periods away from work (56%) (66). The sputum findings were mirrored by corresponding changes in spirometry and PC<sub>20</sub> methacholine.

In conclusion, although subjects with OA seem to show predominantly an eosinophilic airway inflammation when at work, neutrophilic inflammation has been also described. The determinants leading to an eosinophilic or a neutrophilic response are poorly understood. Further research is needed to investigate whether or not the type of inflammation observed is related to the OA severity or prognosis.

#### Key messages:

- The majority of subjects with OA show an eosinophilic airway inflammation after exposure to occupational agents during SIC.
- An increase in sputum eosinophil counts greater than 3% after SIC often precedes the occurrence of functional changes on subsequent exposures.
- An isolated increase in sputum eosinophil count of at least 2% without functional changes should incite to pursue the investigation by increasing the duration of exposure.

#### Use of FeNO in the investigation of occupational asthma

Nitric oxide (NO) is produced in the respiratory tract by activation of NO synthase in various cell types and is detectable in exhaled air. Fractional concentrations of exhaled NO (FeNO) can be measured online with fixed or portable instruments, or exhaled air can be collected for offline FeNO measurements. Recommendations for standardized proce-

dures of measurement of FeNO have been published by the European Respiratory Society (ERS) and the American Thoracic Society (ATS) (2).

FeNO is elevated in untreated asthma and falls after corticosteroid treatment. Although correlations between FeNO levels and percentages of eosinophils in induced sputum have been consistently demonstrated, in subjects treated with inhaled corticosteroids and in severe asthma, the correlation between FeNO and sputum eosinophils appears to be poor (67). Compared with induced sputum, assessment of FeNO is totally noninvasive, quick, and relatively simple to perform. However, elevated FeNO is not specific for asthma and eosinophilic inflammation because it has been found in other diseases and several conditions may influence exhaled NO (2).

Some studies examined the usefulness of FeNO in the investigation of OA, but with inconsistent results (50, 68–77). No significant changes in FeNO were observed in asthmatic reactions induced by western red cedar (50). Only patients with low basal FeNO showed an elevation of FeNO after a significant bronchoconstriction induced by various occupational agents in the work of Piipari et al. (72). In subjects exposed to isocyanates with respiratory symptoms, those with a positive SIC exhibited a greater increase in FeNO than subjects with negative challenge (73). However, a remarkable proportion (28%) of SIC-negative subjects showed an increase in FeNO > 50% of baseline values. Increase in FeNO was observed in the majority of subjects who had an asthmatic reaction after SIC with latex, but also in 35% of subjects with latex-induced rhinitis (68). In contrast, no significant relationship between FeNO and workplace exposure was detected in subjects with self-reported symptoms to latex (77). FeNO in subjects sensitized to lupin in the workplace was not different from that measured in nonsensitized subjects (76). Exposure to laboratory animals tended to increase FeNO in sensitized workers (74, 75). Higher concentrations of FeNO were detected in nonsmoking aluminium potroom workers with asthma-like symptoms, but not in symptomatic smokers (70). A recent study showed that isocyanate-induced asthmatic reactions were associated with a consistent increase in FeNO which was maximal at 48 h postexposure, whereas FeNO did not vary with isocyanate exposure in occupational rhinitis and in nonsensitized subjects (78).

A study carried out in farmers, bakers, and health care workers showed increased FeNO levels only 24 h after SIC, along with a rise in the proportion of eosinophils in induced sputum and in NAL fluid in the cases with diagnosed OA. A significant correlation was found between FeNO level at 24 h after SIC and the percentage of eosinophils in nasal fluids before and 4 and 24 h after SIC, as well as in sputum before and 24 h after SIC in subjects with diagnosed OA (71).

Concentrations of FeNO were shown to decrease in farmers with OA after an educational intervention aiming to decrease their level of exposure to the offending agent (79).

There are some issues that should be considered in the interpretation of the conflicting results obtained by the studies which analyzed FeNO after SIC with occupational agents. One is the insufficient duration of monitoring of patients.

Indeed, maximum increase in FeNO occurred 48 h after allergen challenge with *D. pteronyssinus* in atopic subjects with dual asthmatic responses (80), while the last measurement of FeNO in occupational studies was obtained 20–24 h after challenge (50, 68, 69, 72, 73). Secondly, corticosteroids inhibit the induction of NO synthase, and FeNO falls after treatment with oral or inhaled corticosteroids in subjects with asthma (2). In the studies which included patients on steroid treatment at the time of the test, FeNO response might have been blunted (50, 68, 73). Finally, an increase in NO production in the presence of bronchoconstriction might have been underestimated. Because FeNO is measured by a constant mouth flow, the reduction in the volume of conducting airways as a consequence of bronchoconstriction will lead to an increase in airflow within the conducting airways. When velocities are increased, the exhaled gas has less residence time in the airways, and thus less time for the airway epithelium and inflammatory cells to load the bolus of expirate with NO, resulting in lower exhaled NO concentrations (81). This explanation is consistent with the observation that airway constriction induced by histamine challenge is associated briefly with a reduction in exhaled NO levels (82).

Key messages:

- Although the measurement of FeNO has some advantages over the analysis of induced sputum in OA, the interpretation of increased FeNO is more difficult than sputum DCC because it is less specific and several confounding factors may influence the results.
- Several investigations of FeNO using SIC gave conflicting results. Studies in the workplace/natural setting are limited, and prospective studies are not available.

#### Special considerations on nonasthmatic eosinophilic bronchitis

NAEB was first described by Gibson et al. in 1989 (83), and it is now considered a relatively common cause of chronic cough (84). This disorder is characterized by the presence of eosinophilic airway inflammation, similar to that seen in asthma. However, in contrast to asthma, NAEB is not associated with variable airflow limitation or airway hyperresponsiveness (84). The differences in airway physiology are related to the differences in the localization of mast cells within the airway wall, with airway smooth muscle infiltration occurring in patients with asthma (85). It has also been found that the concentrations of PGE<sub>2</sub> in induced sputum are significantly higher in patients with NAEB than in patients with asthma (29).

The usefulness of FeNO measurements in the diagnosis of NAEB has been examined in a few papers. Oh et al. (86) have reported the role of FeNO for the investigation of chronic cough, especially of NAEB. The FeNO and induced sputum eosinophils were significantly higher in the asthma and NAEB groups than those in the other groups. FeNO levels were significantly correlated with induced sputum eosinophils in the asthma and NAEB groups. In the nonasthmatic groups, the sensitivity and specificity of FeNO for detecting NAEB, using 31.7 ppb as the FeNO cutoff point, were 86%

and 76%, respectively. The positive and negative predictive values were 47% and 95%, respectively. Berlyne et al. (87) measured FeNO in NAEB patients, irrespective of the use or nonuse of corticosteroid therapy, and reported elevated FeNO levels that were significantly higher than those observed in patients with asthma. Brightling et al. (88) found significantly higher FeNO levels in subjects with NAEB or asthma than in normal controls. Sato et al. (89), however, have reported that patients with NAEB (sensitized to cedar pollen) do not show increased FeNO levels.

NAEB may arise from exposure to occupational agents, and this condition has been labeled occupational eosinophilic bronchitis (90). In fact, NAEB can be regarded as a variant syndrome of OA when it develops as a consequence of work exposures, and work-related changes in sputum eosinophil counts are significant and reproducible (11, 91). Exposure to several occupational allergens or sensitizers has been shown to induce occupational NAEB: acrylates (90), egg lysozyme (11), epoxy resin hardener (92), latex (93), mushroom spores (94), welding fumes, formaldehyde (95), chloramine (96), isocyanates, wheat flour (97), and fungal  $\alpha$ -amylase (98). Diagnostic criteria for occupational NAEB have been published (Table 2) (11).

The cough of patients with NAEB usually responds well to treatment with inhaled corticosteroids, but the dose and duration of treatment differ between patients (83, 84). For patients with occupational NAEB, avoidance of the causal allergen or occupational sensitizer is the best treatment (84). The condition can be transient, episodic, or persistent unless treated. Although it has been reported that patients with NAEB may develop asthma or progressive chronic airflow limitation, the available data suggest that the most likely outcome is that NAEB usually turns into a persistent condition (99).

#### Key messages:

- NAEB may arise from exposure to occupational agents, and it is characterized by persistent cough and sputum eosinophilia that are work-related.
- FeNO measurement may be useful as part of the initial evaluation for chronic cough, especially for the exclusion of NAEB.
- Although the cough and airway eosinophilia usually respond well to inhaled corticosteroids, avoidance of the

**Table 2** Diagnostic criteria for occupational eosinophilic bronchitis (adapted from ref. 11)

Isolated persistent cough (lasting more than 3 weeks) that worsens at work and improves away from work
Sputum eosinophilia $\geq 3\%$ in sputum
Increases in sputum eosinophils are related to exposure to the offending agent (either at work or after specific inhalation challenge in the laboratory)
Spirometric parameters are normal and are not significantly affected by exposure to the offending agent
Absence of bronchial hyperresponsiveness to methacholine both at work and away from work
Other causes of chronic cough are ruled out

causative occupational allergen or sensitizer is the best treatment

#### Exhaled breath condensate

For evaluation of airway inflammation, the collection of EBC is a useful and noninvasive method (3). A wide range of volatile substances in gas phase and nonvolatile compounds from the respiratory tract can be collected in condensed water during the sampling without affecting airway function or inflammation. EBC can be sampled from individuals on multiple occasions, with robust and easy to handle condensing devices, allowing monitoring the time course of an inflammatory response as well as the response to pharmacological therapy and follow-up in longitudinal studies. One indication is epidemiological surveys or screening for work-related lung alterations. Another approach is to use compounds of EBC as a tool to verify symptoms claimed to result of environmental exposure. Therefore, low priced and easy to handle equipment suitable for field studies are preferred.

Recommendations for EBC collection and the potential pitfalls of the technique are summarized in the ATS/ERS Task Force report (100). The fact that a variety of condenser designs are used limits the comparison of the results of different studies, and it has to be considered that the EBC collection device and collecting circumstances are potential confounding factors. Sputum induction seems to modify the levels of biomarker in EBC, therefore it is recommended to collect EBC before the assessment of the inflammatory response caused by the induction of sputum (101).

Measurements of biomarkers of effect in EBC are suggested as a way of exploring adverse effects in the context of air pollution exposure. In asymptomatic welders, e.g., increased concentrations of hydrogen peroxide in EBC were detected independent of the different metal fumes and gases generated according to material and method used for the welding process (102). The inflammatory status of the airways was modulated by the exposure profile, and in welders exposed to cadmium, chromium, iron, lead, and nickel, EBC pH was lower than in welders processing aluminium and iron at the workplace. Ferrazzoni et al. (78) demonstrated that isocyanate-induced asthmatic reactions are not associated with acidification of EBC. Aside investigating local inflammatory effects, EBC in this context might simply be useful in monitoring the exposure to occupational toxic elements. A good correlation between chromium levels and biomarkers of oxidative stress ( $H_2O_2$ , malondialdehyde) could be observed in EBC of otherwise asymptomatic chrome-plating workers (103).

Nevertheless, there is an urgent need for the standardization of the collection technique as well as the assessment and the evaluation of the already existing biomarkers (in parallel with the assessment of new mediators in different disease states) to establish normal values of biomarkers. Such research followed by cross-day variation studies, longitudinal follow-ups, and clinical trials using EBC biomarker analysis may lead to the introduction of EBC in clinical practice and

into the routine use for the diagnosis of work-related respiratory diseases.

Key messages:

- EBC collection is a noninvasive and repeatable tool, and the EBC analysis can reflect oxidative stress, acidification, and inflammation in the airways.
- EBC analysis may be useful in occupational studies on a group level (using the same method) and in individuals when serving as their own controls.
- Based on methodological limitations, lack of standardization and difficulties in the interpretation of the data (e.g. calculation of several confounding factors), EBC collection and analysis are foremost research tools and not yet suitable for the clinical diagnosis setting.

### Assessment of nasal inflammation

The verification of nasal inflammation is a key aspect of diagnosing rhinitis, but how to do it objectively in the individual is still a difficult task, in occupational rhinitis, as in other types of rhinitis (12). Symptom scoring is used in nasal provocation testing (104). Indirectly, the effect of nasal inflammation may be assessed by measurement of nasal patency (105). However, subjective symptom scores and nasal patency measurements are not necessarily correlated with severity of nasal inflammation; so, they may be related to disease severity independently of inflammation.

Nasal mucosal blood flow may be investigated by laser Doppler technique or Xenon wash-out methods (106). In this section, the main focus will be on nasal NO (nNO), NAL methods, and inflammatory markers, but also nasal cytology will be appraised.

### Nasal nitric oxide and occupational rhinitis

In patients with allergic rhinitis, increased nNO levels have been demonstrated (107). Enhanced inducible NOS (iNOS) expression within the nasal epithelium may generate these levels. Enhanced iNOS expression is considered as a consequence of persistent nasal inflammation. Paranasal sinuses—more than nasal epithelium—are the source of high levels of nNO. In sinuses, NO is continuously produced at levels of 25–30 ppm (108).

If nNO can be considered as a valid marker for nasal inflammation, assessment of NO might be helpful in characterizing patients with work-related rhinitis. Patients with work-related rhinitis might be distinguished from colleagues without disease. Ideally, fluctuations in NO should be associated with variations in exposure to occupational allergens of other stimuli. Finally, NO might be a tool to assess the outcome of a nasal challenge test or a provocation at the workplace. However, before accepting measurement of nNO as a tool to evaluate work-related disorders of the upper airways, nNO measurement should be validated in well-characterized conditions such as allergic rhinitis.

Standard operation procedures have been established to measure NO in both upper and lower airways (2). Normal levels of nNO may range from 400 to 900 ppb. High levels

may reflect nasal inflammation, whereas low levels may be seen in conditions such as nasal blockage, polyps, and cystic fibrosis. Levels below 105 ppb may be predictive for primary ciliary dyskinesia (108).

The level of nNO measured in allergic rhinitis may be increased by nasal inflammation. However, nasal blockage and secretions will occlude the ostia of the paranasal sinuses thereby lowering nNO levels. These opposite phenomena may explain why in several studies nNO is increased in allergic rhinitis (109), whereas in other studies, no differences in nNO levels have been seen between patients with allergic rhinitis and healthy subjects (110). Also, the high background level of nNO may mask small fluctuations or increase. The effect of nasal blockage also explains why nNO decreases during the acute phase of the nasal challenge (111).

Only a few studies focus on determination of nNO in occupational allergy. In one study among laboratory workers, it was shown that exhaled NO was raised in those with laboratory animal allergy symptoms compared with asymptomatic subjects (75). A trend of increased NO by allergic status was observed; asymptomatic, to early laboratory animal allergy, to asthma. Symptomatic subjects also had raised nNO vs asymptomatic subjects (mean difference 378 ppb,  $P < 0.05$ ) (75). In two studies, nNO was measured in NAL instead of exhaled air. In a study among paper-mill workers, there was no statistically significant relationship between nNO concentration and nitrate in NAL fluid or nasal symptoms (112). In another study, the association between the development of rhinitis reactions during workplace-related challenge tests with latex and nasal allergic inflammation was studied. The NO derivative concentrations in NAL fluid were significantly increased 6 h postchallenge compared with the prechallenge values (113).

In conclusion, a few studies suggest that measurement of nNO might be helpful in the diagnostic workup of occupational allergy. However, at this stage, determination of nNO in patients with rhinitis is an experimental technique hampered by the opposite effects arising from nasal blockage and the level of nasal inflammation. Notwithstanding, nNO measurements could be used as objective (intraindividual) measure of nasal inflammation in selected patients with occupational rhinitis after excluding those with rhinosinusitis, nasal polyps, ciliary dyskinesia, and other factors that may increase or decrease nNO levels.

### Nasal lavage methods

NAL has been extensively used in experimental/laboratory research to elucidate the luminal cell recruitment, cell activation, and plasma protein extravasation in the nasal mucosa, not only under natural challenge conditions, but also when using a wide range of different stimuli. The method has been less used under clinical and epidemiologic circumstances, and the sources of variability and the repeatability of the findings are poorly substantiated under field conditions (114).

Two main methods are being used; a 'head-back' with the palate closing off the nasopharynx, or a 'head-forward' method, or modifications of these (115, 116). Both methods

are very well tolerated. The first (head-back) is quickly performed, relying on the co-operation between the investigator and the subject, and where the crucial point is whether the subject manages to close off the nasopharynx (117). The latter method with the use of the nasal pool device has the advantage that a group may be investigated at the same time under guidance of the investigator, but where some will have problems keeping the pressure on the nasal pool container throughout the 5 min. Modifications of a 'head-forward'/nasal pool technique have replaced the compressible container with a syringe connected to a rubber tube or Foley catheter and equipped with an inflatable balloon serving as a nasal adapter (118). There are great differences in dwelling times of the fluid in the nasal cavities in all the different methods used.

In an experimental model, Belda et al. (119) compared the two methods 'head-back'/Naclerio and 'head-forward'/Greiff-Grünberg. The modified Greiff-Grünberg method gave higher and more repeatable total cell counts and, in subjects with rhinitis, more reproducible ECP levels compared with the Naclerio method. Both methods were able to discriminate between healthy and rhinitics.

The NAL fluid once collected has to be kept cold. After measuring volume/weight, the lavage fluid is centrifuged between 400 and 1000 g for 5–20 min. The sediment or cell pellet may be used for cytology, see later. The supernatant is stored as aliquots of 0.5–1.0 ml at –20 to –70 °C until assay is performed (114).

#### Nasal lavage and inflammatory markers

Many different mediators, cytokines, and chemokines have been measured in NAL studies in occupational settings (Table 3). Which one of the many inflammatory markers to use will partly depend on the purpose of the investigation; research, nasal provocation testing, detecting health effects of occupational exposures, or monitoring the effect of interventions.

ECP is one of the most studied markers of inflammation, also in terms of repeatability (see later), and may serve as a general marker of mucosal inflammation, both in processes of eosinophil and neutrophil activation, and regardless of whether the subjects are atopic or nonatopic (114, 120, 121). A key feature of mucosal inflammation is the exudation of plasma proteins such as albumin,  $\alpha_2$ -macroglobulin, and others, which can be monitored by analysis of plasma proteins in nasal lavages (122). The measurement of a marker of this process should provide a single integrated measurement of inflammation that reflects the underlying tissue processes (114).

Histamine is rapidly metabolized by histaminases and N-methyl transferase, thus the mast cell degranulation products tryptase or the prostaglandin PGD<sub>2</sub> are recommended as markers of mast cell activation (123). The most used marker of neutrophil activation is the myeloperoxidase (124).

#### Nasal and mucosal responsiveness/reactivity

Nasal hyperresponsiveness may be seen as one of the characteristics of nasal inflammation. The method of monitoring by

nasal lavages the ability of histamine challenge to produce plasma exudation gives the opportunity to verify nasal exudative hyperresponsiveness, as demonstrated in bakers (120, 125).

#### Repeatability and validity of nasal lavage

The response after histamine or allergen challenge has been found reproducible when measured as N-alpha-tosyl-L-arginine-methyl esterase (TAME) esterase, albumin, or ECP in NAL (114). The intrasubject variance compared with the intersubject variance in ECP levels in NAL has been shown to differ whether the study subjects are children, healthy volunteers or with rhinitis, short-term or follow-up over a longer period, and with gender (119, 125). Some authors have proposed cutoff values defining a significant change in eosinophil counts in NAL (117, 125). The short-term reproducibility of cell counts in NAL has revealed satisfactory intrasubject variance (125, 126).

There have been attempts to define norm values for nasal mucosal ECP, and other methods of collecting mucosal outputs than NAL have been advocated. The problem especially with the dilution effect in NAL has been addressed (127), which may be solved by adding an exogenous dilution marker to the washing fluid, such as inulin, radioactive albumin, and lithium chloride. Other factors may also affect levels of cellular markers and mediators in NAL, like steroid treatment for eosinophil activation markers, or other treatments and environmental conditions (127) that are beyond the scope of this paper.

#### Nasal lavage used in field studies

NAL has most often been used in an experimental design in research purposes, or as part of the response parameters in provocation testing. The NAL method is far less applied as a means of objectively monitoring nasal disease in 'natural' exposure settings. Douwes et al. (128) did a field study with pre and postshift NAL ('head-back') in workers of a compost plant visited on two occasions 1 year apart. In a field study in Bergen, Norway, the exposure and NAL ('head-forward') data suggested a dose-response relation between exposure to flour dust and plasma exudation/ECP levels in the nasal mucosa (120). Other of the few studies performed at the work place include the study by Granstrand et al. (129) on wood-surface coating industry workers using a 'head-back' method. It is difficult to find studies comparing the levels of indices of inflammation at work, and when the subjects have had a longer period away from work.

In conclusion, NAL is well tolerated, rather simple, and rapid to perform. NAL is a useful method for the detection of nasal inflammation in occupational settings where comparison can be made using test subjects as their own controls and may be used to confirm the diagnosis of occupational rhinitis in challenge testing. The lack of norm values and standardization makes the method less useful to single out the diseased individual from a group. On a group level, NAL may be useful monitoring effects of exposure and interventions and is an excellent research tool.

**Table 3** Inflammatory markers used in nasal lavage in occupational settings

Authors (ref.)	Inflammatory markers	Study design	Occupation/ subjects	Exposure	Number of subjects
Nielsen et al., 1994 (134)	Tryptase, ECP, TAME	Case-control	Weapon industry?	Acid anhydrides	43 + 27
Hauser et al., 1995 (126)	PMN cell counts	Field study	Boilermakers, utility workers	Fuel oil ash (Vanadium)	37 (49)
Åhman et al., 1995 (135)	Albumin, tryptase, ECP, cells	Case-control	Industrial arts teachers	Wood dust	24 + 24
Gorski et al., 1998 (125)	ECP, tryptase, albumin, total protein	Case-control challenge	Bakers	Flour	100 + 40
Raulf-Heimsoth et al., 2000 (113)	ECP, NO derivative, IL-5, IL-8, tryptase, sICAM, cells	Case-control challenge	Health care workers and physicians	Latex	32 + 6
Hellgren et al., 2001 (136)	IL-8	Case-control	Soft paper millers	Soft paper dust	37 + 36
Palczynski et al., 2001 (137)	Tryptase, ECP, albumin	Case-control challenge	Health care workers	Glutaraldehyde	21 + 10
Lund et al., 2002 (138)	ECP, MPO, IL-6, IL-8, TNF- $\alpha$ , PGE <sub>2</sub> , PGF <sub>2<math>\alpha</math></sub> , PGD <sub>2</sub> , LTB <sub>4</sub> , peptide LT, GSH, GSSG, uric acid, total protein	Experimental challenge	Healthy volunteers	Hydrogen Fluoride	10
Littorin et al., 2002 (139)	Albumin, ECP, MPO, cells	Case-control	Automobile factory	Isocyanates (heating polyurethane)	38 + 9
Larsson et al., 2002 (140)	IL-6, IL-8, cells	Case-control	Swine farming	Dust aerosols, endotoxin	7 + 9
Palczynski et al., 2003 (141)	Tryptase, ECP, albumin	Case-control	Health care workers	Chloramine	13 + 6
Priha et al., 2004 (142)	IL-6, IL-8, TNF- $\alpha$ , IFN- $\gamma$	Case-control	Furniture makers	Medium-density fiber/wood-dust	45 + 15
Tuomainen et al., 2006 (143)	IL-4, IL-6, IL-12, TNF- $\alpha$ , NO	Experimental challenge	Asthmatics/Nonasthmatics	Degraded PVC products	10 (5 + 5)
Raulf-Heimsoth et al., 2007 (144)	IL-1 $\beta$ , IL-5, IL-6, IL-8, TNF- $\alpha$ , NO, albumin, total protein	Cross-sectional cross-shift case-control	Asphalt workers	Bitumen	74 + 49
Storaas et al., 2007 (120)	$\alpha_2$ M, ECP	Cross-sectional field	Bakers	Flour	183
Diab et al., 2008 (145)	Albumin	Case-control challenge	Hairdressers	Persulfate	29 + 12
Bakke et al., 2008 (146)	ECP, MPO, lysozyme, albumin	Cross-sectional field	University staff	Indoor air	173
Gaughan et al., 2008 (147)	ECP, MPO, albumin	Cohort follow-up	Wildland firefighters	Aldehydes, respirable particles, CO	58
Holmström et al., 2008 (148)	ECP, MPO, tryptase, albumin	Case-control	Farmers (milk, grain producers, swine)		53 + 15
Krakowiak et al., 2008 (149)	IL-18	Case-control challenge	Bakers	Flour	19 + 9

$\alpha_2$ M,  $\alpha_2$ -macroglobuline; ECP, eosinophil cationic protein; PMN, polymorphonuclear cells; TAME, N-alpha-tosyl-L-arginine-methyl esterase; MPO, myeloperoxidase; NO, nitric oxide derivative; sICAM, soluble intercellular adhesion molecule-1; TNF- $\alpha$ , tumor necrosis factor; PGE<sub>2</sub>/PGF<sub>2 $\alpha$</sub> /PGD<sub>2</sub>, prostaglandins; LTB<sub>4</sub>/peptide LT, Leukotriens; GSH, reduced glutathione; GSSG, oxidized glutathione; IFN- $\gamma$ , interferon- $\gamma$ .

### Nasal cytology

Several techniques have been elaborated to harvest nasal cells. When doing NAL, the cell pellet portion may be used, and at least eosinophils are readily identified (114, 115). Secretions can be blown onto paper or plastic wrap, and then

placed on a glass slide. The processing of nasal blown secretions to solubilize mucus, as performed for sputum, has recently been demonstrated to allow reproducible data, at least for nasal eosinophils (130). The disadvantages with these techniques are that the cells originate only from the secretions, and do not necessarily reflect the present pato-

physiologic processes in the epithelium, and some subjects will have problems with blowing the nose effectively enough. With a plastic curette scraping the surface in the middle-third of the inferior turbinate, a nasal specimen of both the secretions and the surface epithelium may be easily obtained (131). Nasal brushing utilizes a small plastic-coated steel wire brush with nylon bristles, and is placed between the septum and the inferior turbinate, and rotated while being removed (132). Both scraping and brushing methods have the advantage of adequacy of specimen but may cause a slight irritation.

Meltzer in the review by Howarth et al. (114) has detailed the most used processing methods and has provided guides to the grading and interpreting of the nasal cytograms. A study by Raulf-Heimsoth et al. (113) demonstrates the possibility of combining the NAL (for mediator analysis) and brushing (for cytograms) methods in an occupational setting, each method revealing different aspects of the inflammatory process. The sole use of nasal brushing has been used in a

5 years follow-up of Swiss customs officers exposed to diesel engine emission (133).

Pignatti et al. (130) have recently shown that the evaluation of nasal blown secretions in occupational setting might be useful in monitoring eosinophilic inflammation after a specific nasal provocation test. These authors suggest a 4% and/or  $1 \times 10^4$  eosinophils/ml cutoff for a significant postchallenge eosinophil increase.

Key messages:

- NAL is a useful method in occupational settings on a group level and in the individual when the subject acts as his/her own control.
- Nasal cytology and NAL may both be used as a clinical tool to objectively measure nasal inflammation, and the reliability of both methods depends on the sampling technique and sample analysis (cell counts vs cellular marker measurements).
- nNO is foremost a research tool, and as yet not suitable in the clinical setting.

## References

1. Pavord ID, Sterk PJ, Hargreave FE, Kips JC, Inman MD, Louis R et al. Clinical applications of assessment of airway inflammation using induced sputum. *Eur Respir J Suppl* 2002;**37**:40s–43s.
2. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;**171**:912–930.
3. Hoffmeyer F, Raulf-Heimsoth M, Brüning T. Exhaled breath condensate and airway inflammation. *Curr Opin Allergy Clin Immunol* 2009;**9**:16–22.
4. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;**63**(Suppl. 86): 8–160.
5. Maestrelli P, Calcagni PG, Saetta M, Di Stefano A, Hosselet JJ, Santonastaso A et al. Sputum eosinophilia after asthmatic responses induced by isocyanates in sensitized subjects. *Clin Exp Allergy* 1994;**24**:29–34.
6. Lemièrè C, Chaboillez S, Malo JL, Cartier A. Changes in sputum cell counts after exposure to occupational agents: what do they mean? *J Allergy Clin Immunol* 2001;**107**:1063–1068.
7. Lemièrè C, Pizzichini M, Balkissoon R, Clelland L, Efthimiadis A, O'Shaughnessy D et al. Diagnosing occupational asthma: use of induced sputum. *Eur Respir J* 1999;**13**:482–488.
8. Girard F, Chaboillez S, Cartier A, Côté J, Hargreave F, Labrecque M et al. An effective strategy for diagnosing occupational asthma: use of induced sputum. *Am J Respir Crit Care Med* 2004;**170**:845–850.
9. Lemièrè C. Induced sputum and exhaled nitric oxide as noninvasive markers of airway inflammation from work exposures. *Curr Opin Allergy Clin Immunol* 2007;**7**: 133–137.
10. Malo JL, Newman Taylor A. Defining occupational asthma and confirming the diagnosis: what do experts suggest? *Occup Environ Med* 2007;**64**:359–360.
11. Quirce S. Eosinophilic bronchitis in the workplace. *Curr Opin Allergy Clin Immunol* 2004;**4**:87–91.
12. EAACI Task Force on Occupational Rhinitis. Moscato G, Vandenplas O, Gerth Van Wijk R, Malo JL, Quirce S et al. Occupational rhinitis. *Allergy* 2008;**63**:969–980.
13. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992;**47**:25–29.
14. Paggiaro PL, Chanez P, Holz O, Indz PW, Djukanovic R, Maestrelli P et al. Sputum induction. *Eur Respir J* 2002;**20**(Suppl. 37):3s–8s.
15. Iredale MJ, Wanklyn SA, Phillips IP, Krausz T, Ind PW. Non-invasive assessment of bronchial inflammation in asthma: no correlation between eosinophilia of induced sputum and bronchial responsiveness to inhaled hypertonic saline. *Clin Exp Allergy* 1994;**24**:940–945.
16. Popov TA, Pizzichini MM, Pizzichini E, Kolendowicz R, Punthakee Z, Dolovich J et al. Some technical factors influencing the induction of sputum for cell analysis. *Eur Respir J* 1995;**8**:559–565.
17. Bacci E, Cianchetti S, Paggiaro PL, Carnevali S, Bancalari L, Dente FL et al. Comparison between hypertonic and isotonic saline-induced sputum in the evaluation of airway inflammation in subjects with moderate asthma. *Clin Exp Allergy* 1996;**26**:1395–1400.
18. Holz O, Richter K, Jorres RA, Speckin P, Mucke M, Magnussen H. Changes in sputum composition between two inductions performed on consecutive days. *Thorax* 1998;**53**:83–86.
19. Cianchetti S, Bacci E, Ruocco L, Bartoli ML, Carnevali S, Dente FL et al. Salbutamol pretreatment does not change eosinophil percentage and eosinophilic cationic protein concentration in hypertonic saline-induced sputum in asthmatic subjects. *Clin Exp Allergy* 1999;**29**:712–718.
20. Purokivi M, Randell J, Hirvonen MR, Tukiainen H. Reproducibility of measurements of exhaled NO, and cell count and cytokine concentrations in induced sputum. *Eur Respir J* 2000;**16**:242–246.
21. Fahy JV, Boushey HA, Lazarus SC, Mauger EA, Cherniack RM, Chinchilli VM et al. NHLBI Asthma Clinical Research Network. Safety and reproducibility of sputum induction in asthmatic subjects in a multicenter study. *Am J Respir Crit Care Med* 2001;**163**:1470–1475.
22. Efthimiadis A, Spanevello A, Hamid Q, Kelly MM, Linden M, Louis R et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ

- hybridisation. *Eur Respir J* 2002; **20**(Suppl. 37):19s–23s.
23. Hunter CJ, Ward R, Woltmann G, Wardlaw AJ, Pavord ID. The safety and success rate of sputum induction using a low output ultrasonic nebuliser. *Respir Med* 1999; **93**:345–348.
  24. de la Fuente PT, Romagnoli M, Godard P, Bousquet J, Chanez P. Safety of inducing sputum in patients with asthma of varying severity. *Am J Respir Crit Care Med* 1998; **157**:1127–1130.
  25. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 2000; **161**:475–478.
  26. Spanevello A, Confalonieri M, Sulotto F, Romano F, Balzano G, Migliori GB et al. Induced sputum cellularity. Reference values and distribution in normal volunteers. *Am J Respir Crit Care Med* 2000; **162**:1172–1174.
  27. Thomas RA, Green RH, Brightling CE, Birring SS, Parker D, Wardlaw AJ et al. The influence of age on induced sputum differential cell counts in normal subjects. *Chest* 2004; **126**:1811–1814.
  28. Loppow D, Böttcher M, Gercken G, Magnussen H, Jörres RA. Flow cytometric analysis of the effect of dithiothreitol on leukocyte surface markers. *Eur Respir J* 2000; **16**:324–329.
  29. Sastre B, Fernández-Nieto M, Mollá R, López E, Lahoz C, Sastre J et al. Increased prostaglandin E2 levels in the airway of patients with eosinophilic bronchitis. *Allergy* 2008; **63**:58–66.
  30. Jaksztat E, Holz O, Paasch K, Kelly MM, Hargreave FE, Cox G et al. Effect of freezing of sputum samples on flow cytometric analysis of lymphocyte subsets. *Eur Respir J* 2004; **24**:309–312.
  31. Leckie MJ, Jenkins GR, Khan J, Smith SJ, Walker C, Barnes PJ et al. Sputum T lymphocytes in asthma, COPD and healthy subjects have the phenotype of activated intraepithelial T cells (CD69+ CD103+). *Thorax* 2003; **58**:23–29.
  32. Boniface S, Koscher V, Mamessier E, El Biaze M, Dupuy P, Lorec AM et al. Assessment of T lymphocyte cytokine production in induced sputum from asthmatics: a flow cytometry study. *Clin Exp Allergy* 2003; **33**:1238–1243.
  33. Mamessier E, Milhe F, Guillot C, Birnbaum J, Dupuy P, Lorec AM et al. T-cell activation in occupational asthma and rhinitis. *Allergy* 2007; **62**:162–169.
  34. Fernández-Nieto M, Quirce S, Fraj J, del Pozo V, Seoane C, Sastre B et al. Airway inflammation in occupational asthma caused by styrene. *J Allergy Clin Immunol* 2006; **117**:948–950.
  35. Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax* 1997; **52**:498–501.
  36. Pavord I. Sputum induction to assess airway inflammation: is it an inflammatory stimulus? *Thorax* 1998; **53**:79–80.
  37. Efthimiadis A, Pizzichini MM, Pizzichini E, Dolovich J, Hargreave FE. Induced sputum cell and fluid-phase indices of inflammation: comparison of treatment with dithiothreitol vs phosphate-buffered saline. *Eur Respir J* 1997; **10**:1336–1340.
  38. Girgis-Gabardo A, Kanai N, Denburg JA, Hergreave FE, Jordana M, Dolovich J. Immunocytochemical detection of granulocyte-macrophage colony-stimulating factor and eosinophil cationic protein in sputum cells. *J Allergy Clin Immunol* 1994; **93**:945–947.
  39. Louis R, Shute J, Goldring K, Perks B, Lau LCK, Radermecher M et al. The effect of processing on inflammatory markers in induced sputum. *Eur Respir J* 1999; **13**:660–667.
  40. Pavord ID, Ward R, Woltmann G, Wardlaw AJ, Sheller JR, Dworski R. Induced sputum eicosanoid concentrations in asthma. *Am J Respir Crit Care Med* 1999; **160**:1905–1909.
  41. Pignatti P, Delmastro M, Perfetti L, Bossi A, Balestrino A, Di Stefano A et al. Is dithiothreitol affecting cells and soluble mediators during sputum processing? A modified methodology to process sputum. *J Allergy Clin Immunol* 2002; **110**:667–668.
  42. Pin I, Freitag A, O'Byrne P, Girgis-Gavardo A, Watson R, Dolovich J et al. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. *Am Rev Respir Dis* 1992; **145**:1265–1269.
  43. Alvarez M, Estrada J, Gozalo F, Fernandez-Rojo F, Barber D. Oilseed rape flour: another allergen causing occupational asthma among farmers. *Allergy* 2001; **56**:185.
  44. Antón Gironés M, de la Hoz Caballer B, Muñoz Martín T, Cuevas Agustín M, Sánchez-Cano M. Occupational rhinoconjunctivitis and asthma by exposure to *Lathyrus sativus* flour. *Allergol Immunopathol* 2005; **33**:326–328.
  45. Alvarez M, Castillo R, Rey A, Ortega N, Blanco C, Carrillo T. Occupational asthma in a grain worker due to *Lepidoglyphus destructor*, assessed by bronchial provocation test and induced sputum. *Allergy* 1999; **54**:884–889.
  46. Vereda A, Quirce S, Fernández-Nieto M, Bartolomé B, Sastre J. Occupational asthma due to spores of *Pleurotus ostreatus*. *Allergy* 2007; **62**:211–212.
  47. Quirce S, Fernández-Nieto M, Pastor C, Sastre B, Sastre J. Occupational asthma due to tampico fiber from agave leaves. *Allergy* 2008; **63**:943–945.
  48. Quirce S, Baeza M, Tornero P, Blasco A, Barranco R, Sastre J. Occupational asthma caused by exposure to cyanoacrylate. *Allergy* 2001; **56**:446–449.
  49. Reig Rincón de Arellano I, Cimarra Alvarez-Lovell M, Robledo Echarren T, Fernández-Nieto M, Quirce Gancedo S, Seoane Plata C et al. Occupational asthma due to acrylates in a graphic arts worker. *Allergol Immunopathol (Madr)* 2006; **34**:32–36.
  50. Obata H, Cittrick M, Chan H, Chan-Yeung M. Sputum eosinophils and exhaled nitric oxide during late asthmatic reaction in patients with Western red cedar asthma. *Eur Respir J* 1999; **13**:489–495.
  51. Quirce S, Parra A, Anton E, Fernandez-Nieto M, Jerez J, Sastre J. Occupational asthma caused by tali and jatoba wood dusts. *J Allergy Clin Immunol* 2004; **113**:361–363.
  52. Moscato G, Pignatti P, Yacoub M, Romano C, Spezia S, Perfetti L. Occupational asthma and occupational rhinitis in hairdressers. *Chest* 2005; **128**:3590–3598.
  53. Wittczak T, Dudek W, Krakowiak A, Walusiak J, Palczynski C. Occupational asthma due to manganese exposure: a case report. *Int J Occup Med Environ Health* 2008; **21**:81–83.
  54. Fernández-Nieto M, Sastre B, Sastre J, Lahoz C, Quirce S, Madero M et al. Changes in sputum eicosanoids and inflammatory markers after inhalation challenges with occupational agents. *Chest* 2009; **136**:1308–1315.
  55. Lemiére C, Romeo P, Chabouillez S, Tremblay C, Malo J. Airway inflammation and functional changes after exposure to different concentrations of isocyanates. *J Allergy Clin Immunol* 2002; **110**:641–646.
  56. Park H, Jung K, Kim H, Nahm D, Kang K. Neutrophil activation following TDI bronchial challenges to the airway secretion from subjects with TDI-induced asthma. *Clin Exp Allergy* 1999; **29**:1395–13401.
  57. Fahy JV, Wong HH, Liu JT, Boushey HA. Analysis of induced sputum after air and ozone exposures in healthy subjects. *Environ Res* 1995; **70**:77–83.
  58. Stenfors N, Nordenhall C, Salvi SS, Mudway I, Soderberg M, Blomberg A et al. Different airway inflammatory responses in asthmatic and healthy humans exposed to diesel. *Eur Respir J* 2004; **23**:82–86.

59. Thorn J, Rylander R. Inflammatory response after inhalation of bacterial endotoxin assessed by the induced sputum technique. *Thorax* 1998;**53**:1047–1052.
60. Lemièrè C, Chaboilliez S, Trudeau C, Taha R, Maghni K, Martin J et al. Characterization of airway inflammation after repeated exposures to occupational agents. *J Allergy Clin Immunol* 2000;**106**:1163–1170.
61. Vandenplas O, D'Alpaos V, Thimpont J, Huaux F, Lison D, Renaud J. Sputum eosinophilia: an early marker of bronchial response to occupational agents. *Allergy* 2009;**64**:754–761.
62. Lemièrè C, Weytjens K, Cartier A, Malo J. Late asthmatic reaction with airway inflammation but without airway hyperresponsiveness. *Clin Exp Allergy* 2000;**30**:415–417.
63. Yacoub MR, Perfetti L, Pignatti P, Frascaroli M, Caminati M, Moscato G. Usefulness of induced sputum in investigating occupational asthma with normal responsiveness to methacholine: a case report. *J Allergy Clin Immunol* 2008;**122**:831–832.
64. Anees W, Huggins V, Pavord I, Robertson A, Burge P. Occupational asthma due to low molecular weight agents: eosinophilic and non-eosinophilic variants. *Thorax* 2002;**57**:231–236.
65. Balbi B, Pignatti P, Corradi M, Baiardi P, Bianchi L, Brunetti G et al. Bronchoalveolar lavage, sputum and exhaled clinically relevant inflammatory markers: values in healthy adults. *Eur Respir J* 2007;**30**:769–781.
66. Leigh R, Hargreave F. Occupational neutrophilic asthma. *Can Respir J* 1999;**6**:194–196.
67. Lemièrè C, Ernst P, Olivenstein R, Yamauchi Y, Govindaraju K, Ludwig MS et al. Airway inflammation assessed by invasive and noninvasive means in severe asthma: eosinophilic and noneosinophilic phenotypes. *J Allergy Clin Immunol* 2006;**118**:1033–1039.
68. Baur X, Barbinova L. Latex allergen exposure increases exhaled nitric oxide in symptomatic healthcare workers. *Eur Respir J* 2005;**25**:309–316.
69. Allmers H, Chen Z, Barbinova L, Marczynski B, Kirschmann V, Baur X. Challenge from methacholine, natural rubber latex, or 4,4-diphenylmethane diisocyanate in workers with suspected sensitization affects exhaled nitric oxide (change in exhaled NO levels after allergen challenges). *Int Arch Occup Environ Health* 2000;**73**:181–186.
70. Lund MB, Oksnel PI, Hamre R, Kongerud J. Increased nitric oxide in exhaled air: an early marker of asthma in non-smoking aluminium potroom workers? *Occup Environ Med* 2000;**57**:274–278.
71. Swierczyńska-Machura D, Krakowiak A, Wiszniewska M, Dudek W, Walusiak J, Paczyński C. Exhaled nitric oxide levels after specific inhalatory challenge test in subjects with diagnosed occupational asthma. *Int J Occup Med Environ Health* 2008;**21**:219–225.
72. Piipari R, Piirila P, Keskinen H, Tuppurainen M, Sovijarvi A, Nordman H. Exhaled nitric oxide in specific challenge tests to assess occupational asthma. *Eur Respir J* 2002;**20**:1532–1537.
73. Barbinova L, Baur X. Increase in exhaled nitric oxide (eNO) after work-related isocyanate exposure. *Int Arch Occup Environ Health* 2006;**79**:387–395.
74. Hewitt RS, Smith AD, Cowan JO, Schofield JC, Herbison GP, Taylor DR. Serial exhaled nitric oxide measurements in the assessment of laboratory animal allergy. *J Asthma* 2008;**45**:101–107.
75. Adishes LA, Kharitonov SA, Yates DH, Snashell DC, Newman-Taylor AJ, Barnes PJ. Exhaled and nasal nitric oxide is increased in laboratory animal allergy. *Clin Exp Allergy* 1998;**28**:876–880.
76. Campbell CP, Jackson AS, Johnson AR, Thomas PS, Yates DH. Occupational sensitization to lupin in the workplace: occupational asthma, rhinitis, and work-aggravated asthma. *J Allergy Clin Immunol* 2007;**119**:1133–1139.
77. Tan K, Bruce C, Birkhead A, Thomas PS. Nasal and exhaled nitric oxide response to occupational latex exposure. *Allergy* 2001;**56**:627–632.
78. Ferrazzoni S, Scarpa MC, Guarnieri G, Corradi M, Mutti A, Maestrelli P. Exhaled nitric oxide and breath condensate pH in asthmatic reactions induced by isocyanates. *Chest* 2009;**136**:155–162.
79. Dressel H, Gross C, de la Motte D, Sultz J, Jörres RA, Nowak D. Educational intervention decreases exhaled nitric oxide in farmers with occupational asthma. *Eur Respir J* 2007;**30**:545–548.
80. Ricciardolo FLM, Timmers MC, Sont JK, Folkerts G, Sterk PJ. Effect of bradikinin on allergen induced increase in exhaled nitric oxide in asthma. *Thorax* 2003;**58**:840–845.
81. Maestrelli P, Ferrazzoni S, Visentin A, Marian E, Dal Borgo D, Accordino R et al. Measurement of exhaled nitric oxide in healthy adults. *Sarcoidosis* 2007;**24**:65–69.
82. Ho LP, Wood FT, Robson A, Innes JA, Greening AP. The current single inhalation method of measuring exhaled nitric oxide is affected by airway calibre. *Eur Respir J* 2000;**15**:1009–1013.
83. Gibson PG, Dolovich J, Denburg EH, Ramsdale EH, Hargreave FE. Chronic cough: eosinophilic bronchitis without asthma. *Lancet* 1989;**1**:1346–1348.
84. Brightling CE. Chronic cough due to non-asthmatic eosinophilic bronchitis: ACCP evidence-based clinical practice guidelines. *Chest* 2006;**129**(1 Suppl.):116S–121S.
85. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002;**346**:1699–1705.
86. Oh MJ, Lee JY, Lee BJ, Choi DC. Exhaled nitric oxide measurement is useful for the exclusion of non-asthmatic eosinophilic bronchitis in chronic cough patients. *Chest* 2008;**134**:990–995.
87. Berlyne GS, Parameswaran K, Kamada D, Eftimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. *J Allergy Clin Immunol* 2000;**106**:638–644.
88. Brightling CE, Symon FA, Birring SS, Bradding P, Wardlaw AJ, Pavord ID. Comparison of airway immunopathology of eosinophilic bronchitis and asthma. *Thorax* 2003;**58**:528–532.
89. Sato S, Saito J, Sato Y, Ishii T, Xintao W, Tanino Y et al. Clinical usefulness of fractional exhaled nitric oxide for diagnosing prolonged cough. *Respir Med* 2008;**102**:1452–1459.
90. Lemièrè C, Eftimiadis A, Hargreave FE. Occupational eosinophilic bronchitis without asthma: an unknown occupational airway disease. *J Allergy Clin Immunol* 1997;**100**:852–853.
91. Vandenplas O, Malo JL. Definitions and type of work-related asthma: a nosological approach. *Eur Respir J* 2003;**21**:706–712.
92. Kobayashi O. A case of eosinophilic bronchitis due to epoxy resin system hardener, methle endo methylene tetrahydro phthalic anhydride. *Aerugi* 1994;**43**:660–662.
93. Quirce S, Fernández-Nieto M, de Miguel J, Sastre J. Chronic cough due to latex-induced eosinophilic bronchitis. *J Allergy Clin Immunol* 2001;**108**:143.
94. Tanaka H, Saikai T, Sugawara H, Takeya I, Tsunematsu K, Matsuura A et al. Work-place-related chronic cough on a mushroom farm. *Chest* 2002;**122**:1080–1085.
95. Yacoub MR, Malo JL, Labrecque M, Cartier A, Lemièrè C. Occupational eosinophilic bronchitis. *Allergy* 2005;**60**:1542–1544.
96. Krakowiak AM, Dudek W, Ruta U, Palczynski C. Occupational eosinophilic bronchitis without asthma due to chlor-

- amine exposure. *Occup Med (Lond)* 2005;**55**:396–398.
97. Di Stefano F, Di Giampaolo L, Verna N, Di Gioacchino M. Occupational eosinophilic bronchitis in a foundry worker exposed to isocyanates and a baker exposed to flour. *Thorax* 2007;**62**:368–370.
  98. Barranco P, Fernández-Nieto M, del Pozo V, Sastre B, Larco JJ, Quirce S. Nonasthmatic eosinophilic bronchitis in a baker caused by fungal alpha-amylase and wheat flour. *J Investig Allergol Clin Immunol* 2008;**18**:494–495.
  99. Berry MA, Hargadon B, McKenna S, Shaw D, Green RH, Brightling CE et al. Observational study of the natural history of eosinophilic bronchitis. *Clin Exp Allergy* 2005;**35**:598–601.
  100. Horvath I, Hunt J, Barnes PJ, on behalf of the ERS/ATS Task Force on Exhaled breath condensate. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005;**26**:523–548.
  101. Koutsokera A, Loukides S, Gourgoulanis KI, Kostikas K. Biomarkers in the exhaled breath condensate of healthy adults: mapping the path towards reference values. *Curr Med Chem* 2008;**15**:620–630.
  102. Fireman E, Lerman Y, Stark M, Schwartz Y, Ganor E, Grinberg N et al. Detection of occult lung impairment in welders by induced sputum particles and breath oxidation. *Am J Ind Med* 2008;**51**:503–511.
  103. Caglieri A, Goldoni M, Acampa O, Andreoli R, Vettori MV, Corradi M et al. The effect of inhaled chromium on different exhaled breath condensate biomarkers among chrome-plating workers. *Environ Health Perspect* 2006;**114**:542–546.
  104. Airaksinen LK, Tuomi TO, Tuppurainen MO, Lauerma AI, Toskala EM. Inhalation challenge test in the diagnosis of occupational rhinitis. *Am J Rhinol* 2008;**22**:38–46.
  105. Nathan RA, Eccles R, Howarth PH, Steinsvag SK, Togias A. Objective monitoring of nasal patency and nasal physiology in rhinitis. *J Allergy Clin Immunol* 2005;**115**:S442–S459.
  106. Olsson P. A comparison between the <sup>133</sup>Xe washout and laser Doppler techniques for estimation of nasal mucosal blood flow in humans. *Acta Otolaryngol* 1986;**102**:106–112.
  107. Kharitonov SA, Rajakulasingam K, O'Connor B, Durham SR, Barnes PJ. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 1997;**99**:58–64.
  108. Scadding G. Nitric oxide in the airways. *Curr Opin Otolaryngol Head Neck Surg* 2007;**15**:258–263.
  109. Arnal JF, Didier A, Rami J, M'Rini C, Charlet JP, Serrano E et al. Nasal nitric oxide is increased in allergic rhinitis. *Clin Exp Allergy* 1997;**27**:358–362.
  110. Palm JP, Alving K, Lundberg JO. Characterization of airway nitric oxide in allergic rhinitis: the effect of intranasal administration of L-NAME. *Allergy* 2003;**58**:885–892.
  111. Boot JD, de Kam ML, Mascelli MA, Miller B, van Wijk RG, de Groot H et al. Nasal nitric oxide: longitudinal reproducibility and the effects of a nasal allergen challenge in patients with allergic rhinitis. *Allergy* 2007;**62**:378–384.
  112. Olin AC, Hellgren J, Karlsson G, Ljungkvist G, Nolkranz K, Toren K. Nasal nitric oxide and its relationship to nasal symptoms, smoking and nasal nitrate. *Rhinology* 1998;**36**:117–121.
  113. Raulf-Heimsoth M, Wirtz C, Papenfuss F, Baur X. Nasal lavage mediator profile and cellular composition of nasal brushing material during latex challenge tests. *Clin Exp Allergy* 2000;**30**:110–121.
  114. Howarth PH, Persson CG, Meltzer EO, Jacobson MR, Durham SR, Silkoff PE. Objective monitoring of nasal airway inflammation in rhinitis. *J Allergy Clin Immunol* 2005;**115**:S414–S441.
  115. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF Jr, Meyers DA, Norman PS et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;**128**:597–602.
  116. Greiff L, Pipkorn U, Alkner U, Persson CG. The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy* 1990;**20**:253–259.
  117. Castano R, Theriault G, Maghni K, Ghezzi H, Malo JL, Gauthrin D. Reproducibility of nasal lavage in the context of the inhalation challenge investigation of occupational rhinitis. *Am J Rhinol* 2008;**22**:271–275.
  118. Rondón C, Romero JJ, López S, Antúnez C, Martín-Casañe E, Torres MJ et al. Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis. *J Allergy Clin Immunol* 2007;**119**:899–905.
  119. Belda J, Parameswaran K, Keith PK, Hargreave FE. Repeatability and validity of cell and fluid-phase measurements in nasal fluid: a comparison of two methods of nasal lavage. *Clin Exp Allergy* 2001;**31**:1111–1115.
  120. Storaas T, Irgens A, Florvaag E, Steinsvåg SK, Ardal L, Do TV et al. Nasal indices of eosinophilic and exudative inflammation in bakery-workers. *Clin Physiol Funct Imaging* 2007;**27**:23–29.
  121. Heldal KK, Halstensen AS, Thorn J, Djupesland P, Wouters I, Eduard W et al. Upper airway inflammation in waste handlers exposed to bioaerosols. *Occup Environ Med* 2003;**60**:444–450.
  122. Greiff L, Andersson M, Persson CG. Nasal secretions/exudations: collection and approaches to analysis. In: Rogers D, Donnelly L, editors. *Methods in Molecular Medicine: Human Airway Inflammation*. Totowa, NJ: Humana Press Inc, 2001:61–73.
  123. de Graaf-in t Veld C, Garrelds IM, Koenders S, Gerth van Wijk R. Relationship between nasal hyperreactivity, mediators and eosinophils in patients with perennial allergic rhinitis and controls. *Clin Exp Allergy* 1996;**26**:903–908.
  124. Steerenberg PA, Fischer PH, Gmelig Meyling F, Willighagen J, Geerse E, van de Vliet H et al. Nasal lavage as tool for health effect assessment of photochemical air pollution. *Hum Exp Toxicol* 1996;**15**:111–119.
  125. Gorski P, Krakowiak A, Pazdrak K, Palczynski C, Ruta U, Walusiak J. Nasal challenge test in the diagnosis of allergic respiratory diseases in subjects occupationally exposed to a high molecular allergen (flour). *Occup Med (Lond)* 1998;**48**:91–97.
  126. Hauser R, Garcia-Closas M, Kelsey KT, Christiani DC. Variability of nasal lavage polymorphonuclear leukocyte counts in unexposed subjects: its potential utility for epidemiology. *Arch Environ Health* 1994;**49**:267–272.
  127. Riechelmann H, Deutschle T, Friemel E, Gross HJ, Bachem M. Biological markers in nasal secretions. *Eur Respir J* 2003;**21**:600–605.
  128. Douwes J, Wouters I, Dubbeld H, van Zwieten L, Steerenberg P, Doekes G et al. Upper airway inflammation assessed by nasal lavage in compost workers: a relation with bio-aerosol exposure. *Am J Ind Med* 2000;**37**:459–468.
  129. Granstrand P, Nylander-French L, Holmstrom M. Biomarkers of nasal inflammation in wood-surface coating industry workers. *Am J Ind Med* 1998;**33**:392–399.
  130. Pignatti P, Pala G, Pisati M, Perfetti L, Banchieri G, Moscato G. Nasal blown secretion evaluation in specific occupational nasal challenges. *Int Arch Occup Environ Health* 2009; DOI: 10.1007/s00420-009-0459-9.
  131. Meltzer EO. Evaluating rhinitis: clinical, rhinomanometric, and cytologic assessments. *J Allergy Clin Immunol* 1988;**82**:900–908.
  132. Pipkorn U, Karlsson G, Enerback L. A brush method to harvest cells from the nasal mucosa for microscopic and bio-

- chemical analysis. *J Immunol Methods* 1988;**112**:37–42.
133. Gluck U, Schutz R, Gebbers JO. Cytopathology of the nasal mucosa in chronic exposure to diesel engine emission: a five-year survey of Swiss customs officers. *Environ Health Perspect* 2003;**111**:925–929.
  134. Nielsen J, Welinder H, Ottosson H, Benschryd I, Venge P, Skerfving S. Nasal challenge shows pathogenetic relevance of specific IgE serum antibodies for nasal symptoms caused by hexahydrophthalic anhydride. *Clin Exp Allergy* 1994;**24**:440–449.
  135. Ahman M, Holmstrom M, Ingelman-Sundberg H. Inflammatory markers in nasal lavage fluid from Industrial Arts teachers. *Am J Ind Med* 1995;**28**:541–550.
  136. Hellgren J, Eriksson C, Karlsson G, Hagberg S, Olin AC, Toren K. Nasal symptoms among workers exposed to soft paper dust. *Int Arch Occup Environ Health* 2001;**74**:129–132.
  137. Palczynski C, Walusiak J, Ruta U, Gorski P. Occupational asthma and rhinitis due to glutaraldehyde: changes in nasal lavage fluid after specific inhalatory challenge test. *Allergy* 2001;**56**:1186–1191.
  138. Lund K, Refsnes M, Ramis I, Dunster C, Boe J, Skovlund E et al. Human exposure to hydrogen fluoride induces acute neutrophilic, eicosanoid, and antioxidant changes in nasal lavage fluid. *Inhal Toxicol* 2002;**14**:119–132.
  139. Littorin M, Welinder H, Skarping G, Dalene M, Skerfving S. Exposure and nasal inflammation in workers heating polyurethane. *Int Arch Occup Environ Health* 2002;**75**:468–474.
  140. Larsson BM, Larsson K, Malmberg P, Palmberg L. Airways inflammation after exposure in a swine confinement building during cleaning procedure. *Am J Ind Med* 2002;**41**:250–258.
  141. Paczyński C, Walusiak J, Krakowiak A, Szymczak W, Wittczak T, Ruta U et al. Nasal lavage fluid examination in diagnostics of occupational allergy to chloramine. *Int J Occup Med Environ Health* 2003;**16**:231–240.
  142. Priha E, Pennanen S, Rantio T, Uitti J, Liesivuori J. Exposure to and acute effects of medium-density fiber board dust. *J Occup Environ Hyg* 2004;**1**:738–744.
  143. Tuomainen A, Stark H, Seuri M, Hirvonen MR, Linnainmaa M, Sieppi A et al. Experimental PVC material challenge in subjects with occupational PVC exposure. *Environ Health Perspect* 2006;**114**:1409–1413.
  144. Raulf-Heimsoth M, Pesch B, Schott K, Kappler M, Preuss R, Marczynski B et al. Irritative effects of fumes and aerosols of bitumen on the airways: results of a cross-shift study. *Arch Toxicol* 2007;**81**:35–44.
  145. Diab KK, Truedsson L, Albin M, Nielsen J. Persulphate challenge in female hairdressers with nasal hyperreactivity suggests immune cell, but no IgE reaction. *Int Arch Occup Environ Health* 2009;**82**:771–777.
  146. Bakke JV, Wieslander G, Norback D, Moen BE. Atopy, symptoms and indoor environmental perceptions, tear film stability, nasal patency and lavage biomarkers in university staff. *Int Arch Occup Environ Health* 2008;**81**:861–872.
  147. Gaughan DM, Cox-Ganser JM, Enright PL, Castellan RM, Wagner GR, Hobbs GR et al. Acute upper and lower respiratory effects in wildland firefighters. *J Occup Environ Med* 2008;**50**:1019–1028.
  148. Holmstrom M, Thelin A, Kolmodin-Hedman B, Van Hage M. Nasal complaints and signs of disease in farmers—a methodological study. *Acta Otolaryngol* 2008;**128**:193–200.
  149. Krakowiak A, Walusiak J, Krawczyk P, Wiszniewska M, Dudek W, Wittczak T et al. IL-18 levels in nasal lavage after inhalatory challenge test with flour in bakers diagnosed with occupational asthma. *Int J Occup Med Environ Health* 2008;**21**:165–172.