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Bronchial hyper-responsiveness and exhaled nitric oxide in chronic obstructive pulmonary disease

Implications for diagnosis, treatment and prognosis

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Summary

Several lung diseases including asthma and chronic obstructive pulmonary disease (COPD) involve chronic inflammation of the airways. Therefore, there is great interest in non-invasive methods assessing airway inflammation. Measurement of bronchial hyper-responsiveness (BHR) and exhaled nitric oxide (NO) are such indirect markers of airway inflammation. Additional information about severity of disease, prognosis and possible response to anti-inflammatory treatment with inhaled corticosteroids can be gained by these methods. However, they are not yet established in assessing patients with COPD in clinical routine.

BHR has long been recognised as a hallmark of asthma. Less is known about prevalence and clinical relevance of BHR in the general population and in COPD patients. Longitudinal studies have shown that BHR in healthy persons is a risk factor for development of respiratory symptoms, asthma and COPD. BHR has also been shown to increase the detrimental effect of cigarette smoke and is associated with a decline in lung function. Furthermore, studies indicate that the presence of BHR is a prognostic factor in COPD. Increased BHR to histamine has been shown to be a predictor for mortality in COPD patients. Based on current guidelines, treatment of patients with severe COPD (GOLD stage III and IV) and regular exacerbations includes therapy with inhaled corticosteroids. Inhaled corticosteroids have been shown to reduce frequency of exacerbations but they have not been shown to modify long-term decline in FEV_1. However, one small study found that BHR to inhaled mannitol could possibly predict responsiveness to inhaled corticosteroids in patients with moderately severe COPD and identify a subgroup of patients that is likely to benefit from this treatment.

Exhaled NO has been shown to correlate with other inflammatory markers and to be elevated in asthma. In COPD patients, data is inconsistent. However, measuring exhaled NO may have a role in the identification of patients with severe, unstable COPD who were shown to have higher NO levels compared to patients with stable COPD. This suggests that exhaled NO might be a method to assess and monitor disease activity in COPD. Possible explanations for the contradictory results are different measurement techniques of exhaled NO and different smoking histories of patients in various studies. Smoking has been found to be a confounding factor by reducing NO levels significantly, an effect which might counteract the potentially increased exhaled NO due to airway inflammation.

In conclusion, measuring BHR and exhaled NO in patients with COPD might provide additional information about disease severity, prognosis and possible response to anti-inflammatory medical treatment. However, to establish these methods in clinical routine in COPD patients, more data is clearly needed.

Key words: chronic obstructive pulmonary disease; exhaled nitric oxide; bronchial hyper-responsiveness; airway inflammation

Introduction

Chronic obstructive pulmonary disease (COPD) is characterised by chronic airflow limitation that is not fully reversible; airflow limitation is usually progressive [1–3]. Like a variety of other lung diseases, COPD is associated with an abnormal inflammatory response to noxious particles or gases [1, 4, 5]. In various parts of the lung an increase in neutrophils, macrophages and T-lym-

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phocytes can be found. An increase in eosinophils can be found in some patients, especially during exacerbations [2, 3]. Therefore, there is great interest in non-invasive methods assessing airway inflammation. Pathological changes characteristic of COPD are found in the central airways (trachea, bronchi), peripheral airways (small bronchi, bronchioli), lung parenchyma and pulmonary vasculature [1–3]. However, especially severe COPD also has systemic features such as cachexia, loss of skeletal muscle mass and weakness [3].

Today, management of COPD patients includes regular clinical assessment (symptoms, history, physical examination) and pulmonary function testing. Arterial blood gas analyses are needed to assess ventilatory status in all patients with a forced expiratory volume in 1 second (FEV_1) <40 to 50% predicted or when clinical signs of respiratory failure or right heart failure are present. A plain chest X-ray can provide information about the presence of emphysema and is indicated as part of the initial workup to exclude other pathologies such as lung cancer [4, 6].

Since cigarette smoking is the major risk factor for COPD, smoking cessation is the most effective way to reduce developing and progression of COPD. Pharmacological therapy is used to reduce symptoms as well as frequency and severity of exacerbations, improve exercise capacity and health status. At present, none of the existing medications for COPD have been shown to modify the long-term decline in lung function that is the hallmark of this disease [1, 4].

Based on current guidelines, there should be a stepwise increase in treatment, depending on the severity of the disease. Short- and long-acting bronchodilators (such as beta2-agonists and anticholinergics) are central to the symptomatic management of COPD. Inhaled glucocorticoids can be given in more severe COPD (GOLD stage III and IV). They do not modify long-term decline in FEV_1 but have been shown to reduce frequency of exacerbations and improve health status in a subgroup of patients [1, 4].

Measurement of BHR and exhaled NO are not yet established in assessing patients with COPD in clinical routine. However, there is evidence that additional information about severity of disease, prognosis and possible response to anti-inflammatory medical treatment can be gained by these methods.

Bronchial hyper-responsiveness (BHR)

Background

Bronchial hyper-responsiveness (BHR) is defined as excessive narrowing of the airways to various inhaled stimuli. It can be reproduced in a lung function laboratory by administration of standardised bronchial provocation tests [7]. BHR is characteristic feature of asthma; it is related to the severity of the disease and has been shown to reflect airway inflammation [8]. It is increasingly being recognised as a clinical endpoint for therapeutic intervention [9]. So far, less is known about the mechanisms and/or prevalence of BHR in patients with COPD [10].

Bronchial provocation tests

There are two types of bronchial provocation tests used to identify BHR: “direct” and “indirect” tests.

Direct tests involve challenge with pharmacological agents such as histamine and methacholine (an analogue of acetylcholine) which act directly on receptors of bronchial smooth muscle to cause contraction. These tests were first used over 50 years ago [11] and are well established for identifying BHR [7, 9, 12].

Indirect tests include physical stimuli (such as exercise, eucapnic hyperventilation, cold air, hypertonic saline or distilled water) and certain chemical stimuli (such as mannitol and adenosine monophosphate) to cause the airway to narrow. These stimuli are thought to cause bronchoconstriciton indirectly by releasing a variety of mediators from inflammatory cells (eg mast cells) within the airway and/or by stimulating neural pathways. These mediators then act on their specific receptors on bronchial smooth muscle to cause contraction and airway narrowing [7, 8, 12, 13].

Since in direct challenge tests the pharmacological agent acts directly on a specific receptor to cause bronchial smooth muscle cell contraction, it does not depend on the presence of inflammatory cells and their mediators. In contrast, indirect tests depend on the presence of inflammatory mediators that are released probably in response to changes in cell volume from osmotic stress. Therefore, a response to an indirect test is indicative that inflammatory cells are present in sufficient numbers to have a significant concentration of mediators to which the bronchial smooth muscle is responsive. Hence, indirect tests can give complementary information to the direct tests [7, 12].

However, both types of bronchial provocation tests depend on the ability of the patient to perform acceptable spirometric manoeuvres since change in FEV_1 is the primary outcome measure. Unacceptable manoeuvres may result in false positive or false negative results. Contraindications of bronchial provocation tests are conditions that put the patient to increased risk or discomfort such as severe airflow limitation (FEV_1 <50% predicted or <1,0 L), acute myocardial infarction or cerebrovas-

cular stroke in the last 3 month, uncontrolled hypertension (systolic BP >200 or diastolic BP >100 mm Hg) or known aortic aneurysm [9].

Prevalence of BHR in general population and in COPD patients

BHR is common in general population samples with prevalence in different studies from 6 to 35% [14]. Even though BHR is generally accompanied by respiratory symptoms (such as wheezing, cough and shortness of breath), population studies have shown that a significant proportion of individuals with BHR do not have any respiratory symptoms. The prevalence of individuals with asymptomatic or "silent" BHR varies in different studies from 2.2 to 14.3% [14]. In the SAPALDIA study, a random population sample (18–60 years) recruited from eight areas of Switzerland was investigated. At baseline, 17% of subjects were found to have BHR, of which 51% were asymptomatic [15].

In COPD patients, the Lung Health Study (a multicentre trial designed to evaluate early intervention in COPD) found BHR to methacholine in 63% of men and 87% of women. The authors stated the hypothesis that an important determinant of BHR is airway calibre and that gender differences in airway calibre result in the female participants being more likely to demonstrate BHR than men [16].

Impact of smoking and smoking cessation on BHR

Epidemiologic studies have shown that BHR is more common in smokers [17, 18]. A population based study in Australia with 876 subjects found a prevalence of BHR of 11.4% with more smokers in the groups with hyper-responsiveness (26%) and intermediate hyper-responsiveness (35%) than in the normal group (19%) [18]. An English study in 511 randomly selected subjects found similar results: the prevalence of BHR to histamine in this study was 14%, current cigarette smoking showed a strong association with BHR in the population above the age of 40 years [17].

A large study with 3993 participants (based on the ECRHS population study) investigated the change in BHR over time. Over a median follow up of 8.9 years the authors found an overall increase of BHR. The increase in BHR was found in continuing and re-starting smokers, average non-smokers showed no change and recent and long-term quitters showed little change in BHR. Therefore smoking seems to be a risk factor for increasing BHR over time [19]. In COPD patients, similar results were found. 4201 participants of the Lung Health Study underwent methacholine challenge testing both at study entry and after 5 years. The study sample showed an overall increase of BHR over the 5-year period. Smoking status was shown to have a large effect on change of BHR over time in COPD patients. Continuous smokers almost had twice the increase in BHR of intermittent smokers and showed more than three-fold increase in BHR compared to sustained quitters [20].

Smoking cessation is the only effective treatment for avoiding or reducing the progression of COPD [21]. However, the effect of smoking cessation on BHR is unclear with contradictory results in different studies and with different bronchial provocation tests [22, 62]. Three longitudinal studies investigated the change of BHR to methacholine or carbachol before and after smoking cessation in 17 patients [23], 13 patients [24] and 10 patients [25]. They all found no significant change in BHR after smoking cessation. Another study included 33 COPD patients in a 1-year smoking cessation programme in which 15 out of 33 patients successfully quit smoking. In this study BHR to both methacholine and adenosine monophosphate was shown to improve after 1 year of smoking cessation [26]. Limitation of these longitudinal studies are the small numbers of patients and the short follow-up time after smoking cessation of several months to maximum one year. There are no long-term studies available.

BHR – a risk factor for the development of respiratory symptoms, COPD and loss of lung function

A large longitudinal Dutch study found a positive association between BHR and the development of respiratory symptoms (chronic cough, chronic phlegm, dyspnoea, persistent wheeze, asthmatic attacks, bronchitis) and a negative association with the resolution of these symptoms. These associations were independent of smoking status [27]. These results were confirmed in a recent study from Brutsche et al. [15] which studied 5825 participants of the SAPALDIA cohort study 11 years after baseline. The authors found that initially asymptomatic BHR was associated with the development of respiratory symptoms, asthma and COPD. BHR was not only found to be risk factor for the development of COPD, current smokers with BHR also showed the highest annual losses of FEV_1 [15]. These findings indicate that BHR is an important predictor of progression of airway obstruction especially in continuing smokers. Similar results were found by Tashkin et al. [28] who studied change in lung function after 1 and 5 years in 5733 smokers with mild to moderate airflow obstruction who underwent methacholine challenge tests at baseline. BHR was found to be a strong predictor of progression of airway obstruction in continuing smokers with early COPD.

Similar results could be shown in non-smoking and primarily asymptomatic adults of the SAPALDIA cohort who were exposed to environmental tobacco smoke. Exposure to environmental tobacco smoke was found to be associated with the development of respiratory symptoms and subjects with additional BHR at baseline were at the highest risk of developing respiratory symptoms over a period of 11 years [29].

BHR as a predictor of treatment-response to inhaled corticosteroids?

Based on current guidelines, patients with severe COPD (stage III and IV) and regular exacerbations should be treated with inhaled corticosteroids (ICS) [4]. Besides reducing frequency of exacerbation, ICS have been shown to reduce BHR in COPD patients [30]. However, ICS have not been shown to modify long term decline in FEV_1 [1, 4, 30].

In an attempt to identify a subgroup of patients showing an improvement of lung function after treatment with ICS, a trial period of oral corticosteroids was performed. The response to the trial was assessed by spirometry; patients with significant reversibility in airways obstruction after the steroid trial were considered to be responders to ICS [31]. However, the steroid trial did not seem to be a very reliable predictor. The study of Burge et al. [32] found in 524 patients that improvement in FEV_1 after therapy with inhaled fluticasone propionate could not be predicted based on the response of pre-treatment with oral steroids.

However, there is evidence that BHR to inhaled mannitol could possibly predict responsiveness to ICS in a subgroup of COPD patients. In a pilot study, 30 patients with mild to moderately severe COPD were challenged with mannitol before a treatment period with ICS. Lung function improved significantly in mannitol positive patients, whereas it remained unchanged in mannitol negative patients [13]. Larger studies to verify these results are in preparation.

BHR as a prognostic factor in COPD

Severity of COPD is classified according to GOLD guidelines based on FEV_1 values and respiratory symptoms of the patients [2, 4]. The prognosis however seems to be influenced by additional factors. Celli et al. [33] could show a multidimensional grading system including four factors (body-mass index, degree of airflow obstruction, dyspnoea, exercise capacity measured by the six-minute walk test) to be a better predictor of the risk of death than FEV_1 alone.

There is evidence that the presence of BHR also is a prognostic factor in COPD patients. Hospers et al. [34] followed up 2008 individuals from several epidemiologic studies for a mean of 23.6 years in whom a histamine challenge test had been performed. 619 (30.8%) of the 2008 participants had a BHR to histamine at the start of the study. It was found that BHR to histamine was significantly associated with mortality from COPD, especially in smokers, but also in non-smokers.

Nitric oxide (NO)

Background

Nitric oxide (NO) is produced endogenously in several cell types (eg epithelial and vascular endothelial cells, macrophages, eosinophils, neutrophils) of the human respiratory tract. In the oral cavity, NO is also formed via bacterial reduction of salivary nitrate to nitrite and subsequently via further chemical reduction of nitrite to NO [35]. NO can act as a dilator of bronchial and vascular smooth muscle, a neurotransmitter and an immune response mediator. It is a free radical with a short half-life (1-5 s) that reacts rapidly with other molecules such as oxygen or superoxide radicals. NO is detectable in exhaled air with non-invasive methods which makes repeated sampling possible [36–39].

Measurement of NO

There are two different approaches for measurement of exhaled NO: "online measurement" refers to exhaled NO testing with a real-time display of NO breath profiles, whereas "offline measurement" refers to collection of exhaled gas into a reservoir for delayed analysis [40]. The standardisation of techniques makes it possible to compare the results of different clinical trials [40].

NO – a marker for airway inflammation

Several studies have demonstrated a significant relationship between changes in exhaled NO levels and other markers of inflammation in the airways. Consequently there is growing interest in the use of exhaled NO in the management of airway diseases involving airway inflammation as a non-invasive biomarker [41]. Several studies show increased exhaled NO levels in patients with asthma [42–46] and a fall of NO after treatment with corticosteroids [40, 47, 48]. Exhaled NO has been shown to correlate significantly with BHR in a population of young adults and in a random sample of allergic children [49, 50].

NO and smoking

An important confounding factor of exhaled NO is cigarette smoking, which is associated with reduced exhaled NO levels [36, 40, 42, 51–57]. One study measured nitrate (NO3-), a stable oxidative end product of NO metabolism in 49 patients and found it to be increased in smokers compared to non-smokers. The authors speculate that the high NO3- levels might be related to an increase of scavenged NO gas into NO3-, probably due to the high concentration of reactive oxygen species generated in cigarette smoke. This hypothesis could explain the low NO levels observed in the other studies in exhaled air of smokers [44]. Another study demonstrated a rise in exhaled NO several weeks after smoking cessation which sug-

gests, that the effects of cigarette smoking on exhaled NO are reversible [53].

The decreased NO levels in smokers are considered to be a result of several factors, including down-regulation of NO synthase by the high NO concentrations in cigarette smoke, inactivation of NO by oxidants in cigarette smoke like superoxide anions, and finally tobacco induced toxic damage to NO-producing cells [51, 56, 58].

Since exhaled NO is strongly affected by cigarette smoking, its usefulness in current smokers with COPD is limited. In this patient group it is possible that the potentially increased exhaled NO due to airway inflammation is counteracted by the inhibitory effect of smoking on endogenous NO production [54].

NO in COPD

Since COPD is characterised by airway inflammation, there is interest to evaluate exhaled NO levels in these patients. However, the data is inconsistent showing increased [36, 54] and decreased [59] levels of NO in COPD patients compared to control subjects. Other studies have reported no difference of NO levels [42, 44] in COPD patients compared to healthy controls. Possible explanations for these contradictory results are confounding factors such as different smoking histories and measurement techniques of exhaled NO in the various studies [38].

Several studies analysed a potential correlation between exhaled NO levels and severity of lung function impairment assessed by FEV_1, but no consistent results could be found. Some studies found a positive correlation between NO and FEV_1 [36, 59], others found a negative correlation [54, 60] or no correlation between the two parameters [56, 61].

However, NO may have a role in the identification of patients with severe, unstable COPD showing higher NO levels compared to patients with stable COPD [51, 56, 61]. Maziak et al. [51] found significantly higher NO levels in a group of patients with exacerbated or severe (FEV_1 <35% predicted) COPD than in patients with stable COPD or in smokers without COPD. Agusti et al. [61] conducted a study with 17 patients and found significantly higher NO levels during an exacerbation than in the clinically stable phase of the disease several months later when NO levels were no longer different from control values of healthy subjects. Bhowmik et al. [56] confirmed these findings in a prospective cohort study including 79 outpatients with COPD. Paired stable and exacerbation readings could be obtained in 67 exacerbations from 38 patients. Exhaled NO levels during acute exacerbations were significantly higher than during a stable phase of COPD. These results suggest that exhaled NO may be a method of assessing and monitoring disease activity in COPD.

Conclusion

To date, non-invasive methods assessing airway inflammation in COPD patients are not routinely used in clinical practice. However, they might provide additional information about disease severity and prognosis and might be useful for assessing response to anti-inflammatory treatment with ICS.

BHR has been shown to be an independent risk factor for development of respiratory symptoms, asthma and COPD. In addition, BHR is a predictor for mortality and disease progression in COPD. Based on a pilot study, BHR to inhaled mannitol could possibly predict responsiveness to inhaled corticosteroids in moderately severe COPD patients.

The role and clinical use of exhaled NO measurements have not yet been fully established in COPD patients since studies report conflicting results. Confounding factors such as treatment with ICS, different smoking histories and different measurement methodologies can influence exhaled NO values. Therefore, interpretation of NO levels in COPD patients is difficult. However, NO levels have been shown to be higher in COPD patients during exacerbations than during a stable phase. Therefore, measuring exhaled NO may have a role in assessing disease activity in COPD patients in the future.

In conclusion, measuring BHR and exhaled NO might be useful to monitor patients with COPD. However, to establish these methods in clinical routine in COPD patients, more data is clearly needed.

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