

Editorial

Nitrosative stress in patients with asthma–chronic obstructive pulmonary disease overlap

Peter J. Barnes, FRS, FMedSci London, United Kingdom

Key words: Asthma–chronic obstructive pulmonary disease overlap, oxidative stress, nitrosative stress, nitrotyrosine

Asthma and chronic obstructive pulmonary disease (COPD) are markedly different airway diseases, but sometimes they overlap, with some asthmatic patients showing some features of COPD (fixed airway obstruction, mixed neutrophilic/eosinophilic airway inflammation, and relatively poor response to corticosteroids) and some patients with COPD having features of asthma (atopy, history of asthma, greater airway reversibility, increased blood and sputum eosinophil counts, and a clinical response to corticosteroids).

The prevalence of asthma-COPD overlap (ACO) depends on how it is defined, but it is relatively common in clinical practice, affecting 15% to 20% of patients with asthma and COPD. Some have questioned the value of labeling patients with ACO, and it is better to describe the phenotypes within the ACO spectrum, which are mainly smoking asthmatic patients and patients with eosinophilic COPD.¹ Because patients with ACO have usually been excluded from studies of asthma and COPD mechanisms and therapies, there is still a relative lack of understanding of the underlying disease mechanisms. The mechanisms of eosinophilia among some patients with COPD is not well understood and might be a reflection of coexisting asthma. However, the relatively poor response to anti-IL-5 therapies in patients with COPD might indicate that different mechanisms of eosinophilia are at play.² More is understood about the mechanisms of smoking asthma, where smoking-induced neutrophilic inflammation is imposed on T2 inflammation with eosinophils, resulting in fixed airway obstruction and reduced responsiveness to corticosteroids.³ Various airway and blood biomarkers of neutrophilic and eosinophilic inflammation might distinguish the different phenotypes of ACO.⁴

The study by Kyogoku et al⁵ from Japan has provided further insight into the smoking asthmatic phenotype of ACO and extends their previous studies in asthma and COPD to show that there is an increase in nitrosative stress in the airways, as measured by a greater number of sputum cells (macrophages and neutrophils) expressing 3-nitrotyrosine (3-NT). The patients

classified as having ACO in the study were elderly asthmatic patients with a history of cigarette smoking. There were no other significant differences with the age-matched asthmatic patients, and both groups were treated with inhaled corticosteroids. Increased 3-NT levels can be derived from the rapid interaction between superoxide anions and free nitric oxide (NO) resulting in formation of the highly reactive peroxynitrite ions, which then nitrate tyrosine residues of proteins. This tyrosine nitration might result in their altered function (Fig 1). For example, peroxynitrite inhibits the enzymatic function of histone deacetylase 2 through nitration of tyrosine residues at the enzyme's catalytic site, resulting in decreased activity, which might lead to enhanced inflammatory gene expression and reduced responsiveness to corticosteroids.⁶

It is likely that oxidative stress is increased to a greater extent in smoking compared with nonsmoking asthmatic patients. Superoxide dismutase activity in sputum was reduced more in patients with ACO than in asthmatic control subjects in this study, which would potentially increase superoxide anions to a great extent, thus generating more peroxynitrite. Myeloperoxidase released from activated neutrophils in the airways can also generate 3-NT through the oxidation of nitrite, and therefore 3-NT is correlated with the increased sputum neutrophil counts seen in smoking asthmatic patients.

Nitrosative stress describes increased production of reactive nitrogen species, such as nitrite and peroxynitrite, which can result in increased or amplified inflammation, as well as structural changes in the airways. For example, peroxynitrite activates matrix metalloproteinase 9, which is increased in patients with severe and smoking asthma. Nitrosative stress induces inflammation, leading to recruitment of neutrophils into the airways, and these neutrophils generate more superoxide anions, which generate more peroxynitrite and 3-NT, whereas neutrophil myeloperoxidase release generates additional 3-NT, resulting in persistent inflammation.

In the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (U-BIOPRED) cohort of patients with severe asthma, as expected, current smokers and also ex-smokers showed evidence for increased airway oxidative stress, with increased expression of NADPH oxidase (NOX) 2 in airway epithelial cells and increased urinary 8-isoprostane levels.⁷ These patients had lower fraction of exhaled nitric oxide (FENO) values than nonsmoking asthmatic patients, despite a greater degree of airway inflammation. This is likely to be due to the combination of free NO in the airways with superoxide anions generated by NOX2. In the study by Kyogoku et al,⁵ FENO values were only slightly increased in asthmatic patients and patients with ACO, which might reflect a reduction in response to inhaled corticosteroids but also to increased superoxide anions in the patients with ACO, which scavenge the free NO. For this reason, FENO is not a useful biomarker of inflammation in these patients because it is

From the National Heart & Lung Institute, Imperial College.

Disclosure of potential conflict of interest: The author declares that he has no relevant conflicts of interest.

Received for publication June 25, 2019; revised July 23, 2019; accepted for publication July 25, 2019.

Corresponding author: Peter J. Barnes, FRS, FMedSci, Airway Disease Section, NHLI, Dovehouse Street, London SW3 6LY, United Kingdom. E-mail: p.j.barnes@imperial.ac.uk.

J Allergy Clin Immunol 2019;■■■:■■■-■■■.

0091-6749/\$36.00

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<https://doi.org/10.1016/j.jaci.2019.07.040>

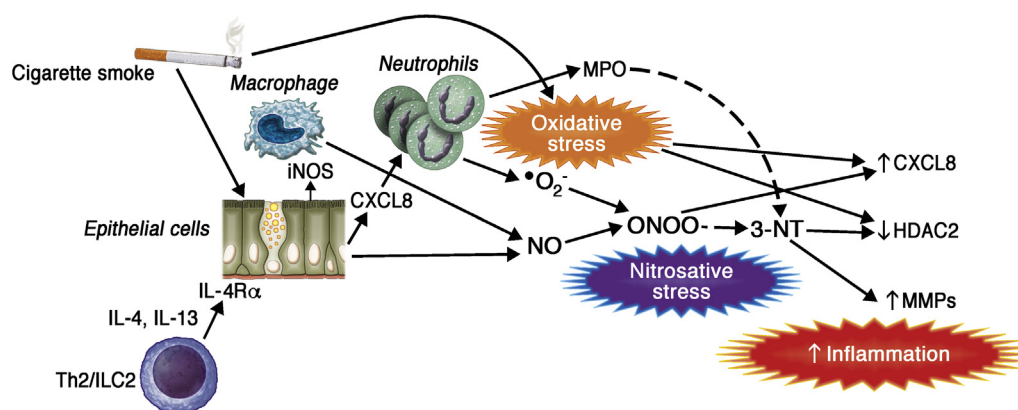


FIG 1. Increased oxidative and nitrosative stress in smoking patients with asthma. Cigarette smoke and neutrophils release reactive oxygen species, including superoxide anions (O_2^-). Inducible nitric oxide synthase (*iNOS*) is induced in airway epithelial cells and macrophages by IL-4 and IL-13 secreted by T_H2 and type 2 innate lymphoid cells (*ILC2*) through the IL-4 receptor (IL-4R α), resulting in formation of NO in the airways. NO and O_2^- avidly combine to form peroxynitrite anions (*ONOO* $^-$), which account for nitrosative stress. Both O_2^- and *ONOO* $^-$ stimulate the release of inflammatory mediators, such as CXCL8, which recruits neutrophils into the airways. *ONOO* $^-$ forms nitrotyrosine (*NT*) adducts on various proteins. Myeloperoxidase (*MPO*) released from activated neutrophils in the airways also generates NT. Tyrosine nitration results in reduced levels of histone deacetylase 2 (*HDAC2*) and increased levels of matrix metalloproteinases (*MMPs*). O_2^- also reduces *HDAC2* levels, resulting in corticosteroid resistance and amplified inflammation.

reduced by oxidative stress in the same way that FENO values are reduced in normal smokers and patients with COPD.

The other common phenotype of ACO is the patient with a diagnosis of COPD who has increased eosinophil counts in sputum or blood. These patients might have greater reversibility, more frequent exacerbations, and a better response to corticosteroids in reducing COPD exacerbations.² It is likely that these patients would also have an increase in 3-NT levels in sputum cells, as reported in patients with COPD.⁸

Increased oxidative stress in the lungs might play a key role in driving the chronic inflammation, corticosteroid resistance, and cellular senescence seen in patients with COPD. It is derived not only from exposure to cigarette smoke, which contains multiple oxidants and reactive nitrogen species, but is also found in ex-smokers to a similar degree. The oxidative stress in ex-smokers is likely to be derived from the increased production of reactive oxygen species by activated inflammatory cells in the lung, particularly neutrophils and macrophages. Mitochondria are now believed to be an important source of endogenous oxidants in patients with COPD, and there is marked dysfunction of mitochondrial function in cells from patients with COPD, particularly airway epithelial cells. Increased oxidative stress is also likely in smoking asthmatic patients who have increased numbers of activated neutrophils in the airways.

The increase in oxidative stress in patients with COPD is amplified by a reduction in levels of endogenous intracellular and extracellular antioxidants, which might be determined by genetic polymorphisms of various antioxidant genes in some patients or due to defective function in key transcription factors, such as Nrf2 and FOXO3a, which regulate multiple antioxidant genes.⁹ FOXO3a regulates superoxide dismutase, levels of which are reduced in patients with COPD but also found to be reduced in smoking asthmatic patients in the current study. Kyogoku et al⁵ demonstrated that levels of reactive persulfides, such as cysteine persulfide and polysulfides, which are potent scavengers of reactive

oxygen species, were also reduced in smoking asthmatic patients compared with nonsmoking asthmatic patients and control subjects, as this group had previously demonstrated in patients with COPD. The reduction in persulfide levels was correlated with the increase in numbers of 3-NT cells. Surprisingly, levels of the enzymes cystathionine- β -synthase and cystathionine- γ -lyase, which generate persulfides, were increased rather than decreased as expected, and this could be due to their upregulation as a compensatory response in response to peroxynitrite ions.

The clinical implications of these observations is that new approaches to treatment might be needed.¹⁰ Obviously smoking cessation is important in reducing the oxidative and nitrosative burden to the lungs, but oxidative and nitrosative stress in smoking asthmatic patients persists after smoking cessation. Both oxidative and nitrosative stress reduce corticosteroid responsiveness through impairment of histone deacetylase 2 activity and expression, and even high doses of inhaled corticosteroids might be insufficient to adequately control airway inflammation. A logical approach is to reduce oxidative and nitrosative stress by effective antioxidants or by peroxynitrite scavenger drugs, but these have thus far proved to be ineffective in patients because the burden of oxidative and nitrosative stress in the lungs might be too high. Because mitochondria are an important source of oxidative stress, at least in patients with COPD, mitochondria-targeted antioxidants might be more effective in the future and might be more effective than targeting NOX enzymes. The reduction in endogenous persulfide levels, despite increased expression of their synthetic enzymes, could be addressed by hydrogen sulfide donor compounds that are currently in development. Finally, the neutrophilic inflammation characteristic of the smoking asthma variant of ACO might be addressed by drugs targeting neutrophilic inflammation, such as phosphodiesterase 4 inhibitors, p38 mitogen-activated protein kinase inhibitors, or CXCR2 antagonists, although these drugs have thus far not been shown to be very effective or well tolerated in patients with neutrophilic lung diseases.

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