Nonetheless, the findings support the observed clinical effectiveness of both inhaled glucocorticosteroids (14) and leukotriene receptor antagonists (15) against exercise-induced bronchoconstriction and are thought provoking with respect to the concerted role of epithelial cells, mast cells, and eosinophils in driving T2 and non-T2 airway inflammation as mechanisms responsible for indirect AHR. The clinical implications of these data with respect to treating exercise-induced bronchoconstriction are unclear. ■

Beth E. Davis, Ph.D.
Department of Medicine
University of Saskatchewan
Saskatoon, Saskatchewan, Canada

Gail M. Gauvreau, Ph.D.
Department of Medicine
McMaster University
Hamilton, Ontario, Canada

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CEACAM6: A Novel Marker of Chronic Obstructive Pulmonary Disease Susceptibility?

Chronic obstructive pulmonary disease (COPD) is a progressive disease typified by airflow obstruction, lung inflammation, and airway remodeling. COPD is a major global health burden and is predicted to be the leading cause of death worldwide by the year 2030. Current therapeutic strategies are sparse and largely ineffective (1, 2). Although cigarette smoke (CS) exposure is the primary risk factor for COPD development, only 25% of people who smoke develop COPD, highlighting the importance of predisposing genetic and epigenetic factors and of environmental exposures as key contributors to COPD pathogenesis (3–5). Long-term imbalances in oxidative stress and inflammation are believed to drive the airway remodeling and alveolar destruction observed in chronic bronchitis and emphysema (1). Likewise, extrapulmonary manifestations such as cardiovascular disease, osteoporosis, and skeletal muscle wasting are also linked to oxidative stress and inflammation. The uncovering of novel therapeutic strategies to treat patients with COPD depends on understanding how these dysfunctions mechanistically support the pathogenesis of COPD.

Increases in cellular nitric oxide, induced by inflammation, result in a type of oxidative stress known as nitrosative stress, which is marked by increases in 3-nitrotyrosine (3-NT) and nitrogen dioxide.

3-NT immunostaining and amounts of inducible nitric oxide synthase (iNOS) are abnormally increased in inflammatory cells present in sputum from patients with COPD and directly correlate with disease severity (1, 6, 7). These findings, which persist well after cessation of smoking, result in vascular damage and remodeling as a long-term consequence (8). Notably, studies in the murine chronic smoke exposure model show that blocking of nitrosative stress via inhibition of iNOS protects against development of emphysema (9, 10).

Heme oxygenase-1 (HO-1) is a key enzyme in heme biosynthesis and is known to be regulated by increases in various types of oxidative stress, including nitric oxide accumulation. Lung protein levels of HO-1 increase in the lung in response to oxidative stress and have been linked to disease states of asthma and COPD (11–16). In the smoke-induced rat emphysema model, augmenting HO-1 levels by delivery of hemin blunted the oxidative stress response and protected the development of emphysema (12). As such, the identification of HO-1 regulators represents an area of investigation with great potential to uncover new therapies in COPD.

In this issue of the *Journal* (pp. 1576–1590) Wu and colleagues report a series of studies that shed light on the contribution of nitrosative stress to COPD pathogenesis and the potential to leverage inherent stress defenses as novel therapies (17). They used human tissue and sputum samples to assess the level of 3-NT, as a marker of nitrosative stress across nonsmokers, smokers without COPD, and smokers with COPD. Disease severity by Global Initiative for Chronic Obstructive Lung Disease stage criteria positively correlated with 3-NT levels in sputum, lung tissue homogenates, and isolated human alveolar epithelial cells. Notably, they found that elevated 3-NT levels specifically in the alveolar septa predicted the degree of emphysema observed histologically. Because oxidative stress is a known consequence of CS exposure, the investigators took their data to suggest that smokers with no to mild COPD have protective mechanisms to blunt the long-term accumulation of reactive nitrogen species and the development of emphysema.

To determine the molecular mechanisms mitigating the development of COPD in a subset of smokers, they developed an *in vitro* system to generate CS extract–resistant cells. They used the A549 cell line, which derives from alveolar basal epithelial cells to generate the resistant phenotype. After treatment with CS extract, cellular morphology and proliferation were distinct between resistant and control cells. Notably, 3-NT, nitric oxide, and superoxide levels remained low in the resistant cells, supporting the hypothesis that they are primed for antioxidant functions. The resistant phenotype was not explained by alterations in either NOXO1 or iNOS, key regulators of the oxidative stress response.

Exploration of the antioxidant activity of the resistant cells revealed that HO-1 was upregulated, as both mRNA and protein within the A549 cells were resistant to CS extract. Further studies showed that the resistant state depended on the presence of HO-1, as loss of HO-1 increased cellular susceptibility to CS extract–induced nitrosative stress. Transcriptomics analysis of control and resistant cells identified several genes that were downregulated in the resistant state. Importantly, in resistant cells, increased HO-1 protein levels correlated inversely with *CEACAM6*, a molecule previously shown to support the development of multiple lung pathologies (18). Overexpression of *CEACAM6* in resistant cells obliterated the protective gain of HO-1 protein, and 3-NT levels climbed. These data position CEACAM6 as a critical determinant of the antioxidant response in the alveolar epithelial cells *in vitro*.

Supporting the translational relevance of these findings, Wu and colleagues noted elevated *CEACAM6* expression and a reduction in HO-1 levels in the alveolar septa of patients with COPD compared with smokers without COPD (17). Using an *ex vivo* model of CS extract exposure that leveraged human precision-cut lung slice technology, overexpression of *CEACAM6* in healthy donor tissue increased both 3-NT levels and cell death in response to CS extract exposure. In addition, in peripheral blood mononuclear cells from patients with severe COPD, 3-NT levels correlated positively with CECAM6 and inversely with HO-1.

These highly compelling data from Wu and colleagues highlight the importance of nitrosative stress in the pathogenesis of COPD (17). They also identify a novel link between CEACAM6 and HO-1, whereby CEACAM6 post-transcriptionally regulates HO-1. Their findings emphasize the importance of HO-1 to stabilize oxidative stress in response to CS and potentially to prevent the development of the severe COPD phenotype. The confirmation of their *in vitro* findings in both lung tissue and peripheral blood mononuclear cells from patients with COPD supports the notion that CEACAM6 may serve as a biomarker of COPD severity. How the presence of alveolar CEACAM6 impacts the nitrosative stress in adjacent cell types (e.g., endothelial cells) is critical to understanding whether CEACAM6 could also be a potential therapeutic target. Notably, humanized CEACAM6 blocking antibodies were previously generated as a potential therapy in cancers originating in the epithelia (19). Further studies examining the utility of these antibodies in the *in vitro* and *ex vivo* COPD models and as a potential therapy for the treatment of COPD are of great interest. ∎

Alexandra C. Racanelli, M.D., Ph.D.
Augustine M. K. Choi, M.D.
Division of Pulmonary and Critical Care Medicine, Joan and Sanford I Weill Department of Medicine, Weill Cornell Medicine
New York, New York, USA
and
New York Presbyterian Hospital-Weill Cornell Medical Center
New York, New York, USA

ORCID ID: 0000-0002-8414-097X (A.C.R.).

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Improving Acute Respiratory Distress Syndrome Diagnosis
Is Lung Ultrasound the Answer?

The acute respiratory distress syndrome (ARDS) is a clinical syndrome that lacks a gold standard diagnostic test. In the absence of a gold standard, the Berlin definition of ARDS attempts to capture the clinical, laboratory, and radiographic features that best represent the underlying conceptual model of ARDS as an acute diffuse lung injury that manifests with alveolar flooding, impaired gas exchange, and acute respiratory failure (1). ARDS is underdiagnosed clinically (2), a problem that has been attributed to poor interobserver reliability in applying the Berlin definition (3). Variability in identification of the fundamental diagnostic criterion of bilateral opacities consistent with pulmonary edema on the chest radiograph is particularly problematic (3). Although chest computed tomography (CT) may be a more reliable imaging modality for diagnosis of ARDS, CT imaging can be difficult to obtain in critically ill patients, has not been rigorously validated for diagnosis of ARDS, and is not available in resource-constrained clinical settings.

Lung ultrasound has been proposed as an alternative imaging modality for ARDS diagnosis. Lung ultrasound can be readily applied at the bedside without the need for patient transport and is frequently available in resource-constrained settings, even when other imaging modalities are not. The use of lung ultrasound to test for bilateral opacities was formally proposed in the Kigali modification of the Berlin definition (4). In that study, several modifications of the Berlin definition were deployed, including substitution of $Sp_{O_2}/F_{I_{O_2}}$ for $Pa_{O_2}/F_{I_{O_2}}$, elimination of the requirement for positive pressure ventilation, and use of lung ultrasound to test for bilateral opacities, defined as the presence of B-lines or consolidation without associated effusion in at least one area on each side of the chest. Using the Kigali definition, the hospital prevalence of ARDS in a large urban hospital in Rwanda was 4% with a mortality of 50%; none of these patients were captured by the Berlin definition. The Kigali definition was externally validated in a large European teaching hospital (5) and found to be overly sensitive compared with the Berlin definition, largely because of the lack of specificity of the ultrasound criteria. However, beyond these studies, there has been minimal large-scale validation of lung ultrasound as a diagnostic tool for ARDS.

In this issue of the *Journal*, Smit and colleagues (pp. 1591–1601) report a multicenter study designed to systematically derive and validate a more quantitative lung ultrasound score for the diagnosis of ARDS (6). The authors are to be congratulated for a thoughtfully designed and implemented study. Consecutive mechanically ventilated patients underwent a 12-region lung ultrasound examination to quantify the presence and severity of B-lines, consolidation, pleural effusions, abnormal pleural lines, and several other features of lung morphology. These findings were then compared with expert ARDS diagnosis using all features of the Berlin definition (clinical history, blood gas analysis, chest CT if available, and chest radiograph). Determination of ARDS status was made by a panel of expert clinicians who applied an ARDS certainty score (7), an approach that has been shown to modestly improve interobserver agreement for the Berlin