smokers compared with nonsmokers in several transcriptomic data sets of lung samples from healthy never- and ever-smokers and patients with chronic obstructive pulmonary disease. Also, they report an increase in ACE2-producing goblet cells in ever-smoker versus never-smoker lungs. These findings have putatively important implications for patients with COVID-19 because ACE2 has been shown to be the receptor used by SARS-CoV-2 to enter the host cells (3) and yet seem in contrast with the consolidated epidemiological data worldwide indicating a low prevalence of active smokers among patients with COVID-19.

Cigarette smoke induces epigenetic modifications of the bronchial epithelium, leading to mucous (goblet) cell metaplasia. As goblet cells are a major source of ACE2 in the lung, this could, in part, justify the increased levels of ACE2 found by Cai and colleagues in lungs of smokers. However, goblet cells are also the main source of mucous, which provides an essential first host barrier to inhaled pathogens that can prevent pathogen invasion and subsequent infection.

Additional factors could play a role in the interaction between active smoking and SARS-CoV-2.

First, naturally occurring structural changes in the ACE2 allelic variants can interfere with the intermolecular interactions of such variants with SARS-CoV-2 spike protein (4). It is conceivable that, upon cigarette smoke (or nicotine?) stimulation, some ACE2 allelic variants that inhibit the SARS-CoV-2 binding may undergo positive selection.

Second, nicotine interacts with many components of the RAS (renin–angiotensin system) in multiple organ systems. In the ACE/AT-II (angiotensin II)/AT_1R (angiotensin$_1$ receptor) arm, nicotine increases the expression and/or activity of renin, ACE, and AT_1R, whereas, in the compensatory ACE2/angiotensin (1–7) arm, nicotine downregulates the expression and/or activity of ACE2 and AT_2R (5). How these findings fit with the ones from Cai and colleagues is worth investigation. Interestingly, activation of nicotinic receptors can lead to enhanced protease activation that may cleave and activate the spike protein of SARS-CoV for membrane fusion (5). This effect may counterbalance the increase in ACE2 levels observed in the lungs of smokers by Cai and colleagues.

Third, ACE2 knockout mice exposed to cigarette smoke exhibit increased pulmonary inflammation with activation of metalloproteinases (6) that could, in part, contribute to the inactivation or modification of ACE2 in the lungs of the smokers.

Last, though it is possible that cigarette smoke increases the ACE2 expression by the bronchial epithelium, thus facilitating the entry of SARS-CoV-2, this does not necessarily translate into a higher risk for developing COVID-19 pneumonia.

To conclude, what is unchallengeable is that cigarette smoke is detrimental for the lungs in several ways, and further studies are needed to clarify the reasons behind the reported low prevalence of current smokers among hospitalized patients with COVID-19. The effect of current smoking on SARS-CoV-2 infection is a delicate and complex topic that should be addressed meticulously before delivering messages that could be misinterpreted. ∎

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Reply to Polverino

From the Authors:

We thank our reader, Dr. Polverino, for the interest in our work and intriguing opinions. The major opinion of Dr. Polverino is that patients with coronavirus disease (COVID-19) include fewer than expected numbers of smokers. However, Dr. Polverino cites a recent study showing a 1.8-fold higher risk for death among current smokers (1). The overall lower-than-expected prevalence of smoking reported in retrospective and/or observational databases is most likely because of incomplete or incorrect information about smoking patterns. Indeed, some early reports did not include smoking demographics in patients with severe COVID-19 (2, 3), suggesting that smoking history may be overlooked in these patients. Therefore, "the

consolidated epidemiological data worldwide" claimed by Dr. Polverino is not "consolidated" but probably instead the result of not recording or reporting smoking history correctly, as we indicated above. In our opinion, "a low prevalence of active smokers among patients with COVID-19" has been translated too quickly in public news with potentially harmful consequences. An alarming number of non–peer-reviewed and shoddy ecological studies of smoking and coronavirus have appeared online, which can be harmful. In contrast, an increased risk for severe COVID-19 is very well documented to be associated with a history of smoking (1, 4–6), although making a special distinction between former, active, or never-smokers is important because chronic obstructive pulmonary disease (COPD) remains highly underdiagnosed (7).

Our reader also raised a concern regarding the role of goblet cells in COPD, stating that mucus "…provides an essential first host barrier to inhaled pathogens…," implying that the increased mucus seen in COPD protects from airway infections, especially viral infections. However, no citations are provided, and we know of no evidence to support this concept. In contrast to this opinion, increased mucus production is a common mechanism leading to alterations of the lung microbiota of smokers with chronic bronchitis, pulmonary function of whom regularly and highly predictably deteriorates after acute disease exacerbations that are precipitated by intercurrent infections caused by viruses, bacteria, and fungi (5, 8–10).

The concept that ACE2 (angiotensin-converting enzyme 2) variants that inhibit severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binding may be upregulated (positively selected) because of cigarette smoking is an interesting one, but it is one that is not supported by any direct data and, furthermore, not supported by any genetic regulatory mechanism that we are aware of.

Nicotine has been shown to downregulate expression of ACE2, but the concept that "…activation of nicotinic receptors can lead to enhanced protease activation that may cleave and activate S (the spike protein) of SARS-CoV for membrane fusion" is not supported by any scientific studies to our knowledge, and in our animal model of chronic exposure to nicotine via electronic cigarettes, we did not detect any changes in ACE2 expression (data not shown). Furthermore, the statement that lung metalloproteinases upregulated in the ACE2-deficient mouse can feed back to inactivate or modify ACE2 in smokers is a non sequitur.

Dr. Polverino also acknowledges that our careful interrogation of data sets revealed increased mRNA expression of ACE2 in former and active smokers but provides an opinion that was not claimed in our letter (11). Though we showed significant associations that we believe will provide one useful piece of the puzzle rather than speculations about COVID-19–related pneumonia, we did not state that increased ACE2 expression translates into worse or higher risk for COVID-19 pneumonia. It is now becoming clear that the presence of ACE2 marks tissues vulnerable to infection (12). We have thus discussed that smoking may increase risk for viral binding and entry of SARS-CoV-2 in lungs of smokers. Obviously, the pathway from discovery to guiding medical decisions or making public recommendations is usually not based on a single study. As indicated in our manuscript, further mechanistic studies are needed to understand the upregulation of ACE2 in lung tissues by smoking.

Finally, we agree with Dr. Polverino that cigarette smoking is detrimental. Investigators need to gauge the right level of evidence before delivering messages and aim for messages that will do more good than harm, which is the baseline for research studies as well as scientific responses. In terms of communicating research results to the public, if someone overstates our study finding and translates this into a message that smoking increases your chance of SARS-CoV-2 infection, then this will be just one more good reason to stop smoking. ∎

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Positron Emission Tomography: A Novel Approach to Detect Pulmonary Artery Hypertension at the Early Stage?

To the Editor:

Pulmonary arterial hypertension (PAH) is a severe disease with a poor prognosis, and both early diagnosis and early treatment are crucial to improve outcomes (1). However, methods for early diagnosis are limited, especially at the stage of PAH development before the onset of elevated pulmonary arterial pressure. Macrophages play an important role in the development and progression of PAH (2). In a recent study published in the *Journal*, Park and colleagues tracked macrophages in the lung using positron emission tomography (PET) with ^{68}Ga-2-(p-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid mannosylated human serum albumin in patients with PAH and animal models (3). Some of the results of these studies were previously reported in the form of an abstract (4).

Via PET scans, Park and colleagues showed that the density of mannose receptor, a marker for macrophages, was significantly higher in patients with PAH and monocrotaline-induced rat PAH models (3). In addition, pulmonary hypertension–targeted therapy with sildenafil or macitentan reduced the density of mannose receptor in the lungs of rats with PAH. These results suggest that the density of lung macrophage infiltration could reflect the severity of PAH. Interestingly, this study also indicates that at the early stage, only 1 week after injection of monocrotaline, a higher density of mannose receptor was found in the rat lungs. This suggests that using PET to monitor lung macrophages may predict the development of PAH even before the onset of high pulmonary arterial pressure, because in the first week after injection of monocrotaline, the rats showed only a mild elevation of pulmonary arterial pressure, which did not meet the standard for PAH diagnosis. Based on these results, Park and colleagues concluded that lung macrophage detection via PET scans

could be used as a diagnostic and monitoring tool for PAH. However, this study raised several concerns. First, PAH is a rare disease, and PET is an expensive diagnostic approach with very limited availability; thus, it may not be realistic to use this technique to diagnose PAH in low-risk populations. However, in individuals at high risk for PAH, such as those with connective tissue disease or HIV infection, PET may be a useful approach for diagnosing PAH, especially at the early stage before the onset of elevated pulmonary arterial pressure. Second, the detection of mannose receptor to monitor macrophage infiltration is questionable because mannose receptor is expressed not only in macrophages but also in other cell types in the lung, especially tracheal smooth muscle cells (5). It would be important to know whether this receptor is upregulated in macrophages in PAH before concluding that macrophage infiltration is increased in the lung during PAH, as the increased PET signal could be a result of increased mannose receptor expression, and not the number of macrophages. Third, the presence of an inflammatory lung disease, such as interstitial lung disease, may affect macrophage infiltration, as shown by Park and colleagues, which may affect diagnostic specificity.

Overall, this study is very interesting in that it demonstrates dynamic changes in mannose receptor in the lung at different time points of PAH development and therapy, and provides solid evidence that macrophage infiltration (mannose receptor elevation) is associated with PAH development and progression. Further studies, especially in humans, are needed to provide more data to evaluate the specificity and sensitivity of lung mannose receptor density for the diagnosis and evaluation of PAH. ∎

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