Title

Cigarette Smoke Exposure and Acute Respiratory Distress Syndrome in Sepsis: Epidemiology, Clinical Features, and Biologic Markers.

Journal

American Journal of Respiratory and Critical Care Medicine, 205(8)

ISSN

1073-449X

Authors

Moazed, Farzad
Hendrickson, Carolyn
Jauregui, Alejandra
et al.

Publication Date

2022-04-15

DOI

10.1164/rccm.202105-1098oc

Peer reviewed

ORIGINAL ARTICLE

Cigarette Smoke Exposure and Acute Respiratory Distress Syndrome in Sepsis

Epidemiology, Clinical Features, and Biologic Markers

Farzad Moazed[1,2,3], Carolyn Hendrickson[1], Alejandra Jauregui[1], Jeffrey Gotts[1,2], Amanda Conroy[4], Kevin Delucchi[2,5], Hanjing Zhuo[6], Mikhaela Arambulo[7], Kathryn Vessel[1], Serena Ke[1], Thomas Deiss[1], Amy Ni[1], Aleksandra Leligdowicz[1], Jason Abbott[1], Mitchell J. Cohen[8], Pratik Sinha[1], Antonio Gomez[1], Kirsten Kangelaris[1], Lucy Kornblith[4], Michael Matthay[1,6,7], Neal Benowitz[1,2], Kathleen Liu[1], and Carolyn S. Calfee[1,2,6,7]

[1]Department of Medicine, [2]Center for Tobacco Control Research and Education, [5]Department of Psychiatry, [6]Cardiovascular Research Institute, and [7]Department of Anesthesia, University of California San Francisco, San Francisco, California; [3]Department of Medicine, Highland Hospital, Oakland, California; [4]Department of Surgery, Zuckerberg San Francisco General Hospital, San Francisco, California; and [8]Department of Surgery, University of Colorado Denver, Denver, Colorado

ORCID ID: 0000-0001-6055-4644 (A.L.).

Abstract

Rationale: Cigarette smoke exposure is associated with an increased risk of developing acute respiratory distress syndrome (ARDS) in trauma, transfusion, and nonpulmonary sepsis. It is unknown whether this relationship exists in the general sepsis population. Furthermore, it is unknown if patients with ARDS have differences in underlying biology based on smoking status.

Objectives: To assess the relationship between cigarette smoke exposure and ARDS in sepsis and identify tobacco-related biomarkers of lung injury.

Methods: We studied a prospective cohort of 592 patients with sepsis from 2009 to 2017. Plasma cotinine and urine NNAL [urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol] were measured to categorize smoking status. Plasma biomarkers of inflammation and lung injury were measured, including in a smaller cohort of trauma patients with ARDS to increase generalizability.

Measurements and Main Results: Passive and active smoking were associated with increased odds of developing ARDS in patients with sepsis. Among patients with sepsis and ARDS, active cigarette smokers were younger and had lower severity of illness than nonsmokers. Patients with ARDS with cigarette smoke exposure had lower plasma levels of IL-8 ($P = 0.01$) and sTNFR-1 (soluble tumor necrosis factor 1; $P = 0.01$) compared with those without exposure. Similar biomarker patterns were observed in blunt trauma patients with ARDS.

Conclusions: Passive and active smoking are associated with an increased risk of developing ARDS in patients with pulmonary and nonpulmonary sepsis. Among patients with ARDS, those with cigarette smoke exposure have less systemic inflammation, while active smokers also have lower severity of illness compared with nonsmokers, suggesting that smoking contributes to biological heterogeneity in ARDS.

Keywords: ARDS; cigarette smoking; biomarkers

Cigarette smoke exposure has been identified as a risk factor for acute respiratory distress syndrome (ARDS) in a variety of populations. In blunt trauma patients, we reported that active and passive cigarette smoke exposure is associated with an increased risk of ARDS (1); likewise, smoking is associated with an increased risk of ARDS in nonpulmonary sepsis (2) and after blood transfusion (3), as well as with primary graft dysfunction (4), a form of ARDS that occurs within 72 hours of lung

(Received in original form May 4, 2021; accepted in final form January 19, 2022)

Supported by NHLBI grants R01 HL110969, R35 HL140026 (C.S.C.) and HL136844 (F.M.); U.S. Department of Defense grant W911NF-10-1-0384 (M.J.C.); U.S. Food and Drug Administration/NIH P50 grant CA180890 (C.S.C.); and National Institute on Drug Abuse grant P30 DA012393 and National Center for Research Resources grant S10 RR026437 (N.B.).

Author Contributions: Study concept and design: F.M., M.J.C., N.B., and C.S.C. Acquisition of data: F.M., C.H., A.J., J.G., A.C., M.A., K.V., S.K., T.D., A.N., A.L., J.A., P.S., A.G., K.K., L.K., N.B., M.M., K.L., and C.S.C. Interpretation of data: all authors. Drafting of the manuscript: F.M. and C.S.C. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: F.M., K.D., H.Z., and C.S.C. Guarantor: F.M. Approval of final manuscript: all authors.

Correspondence and requests for reprints should be addressed to Farzad Moazed, M.D., Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of California San Francisco, 505 Parnassus Avenue, San Francisco, CA 94143-0111. E-mail: farzad.moazed@ucsf.edu.

This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Crit Care Med Vol 205, Iss 8, pp 927–935, Apr 15, 2022

Originally Published in Press as DOI: 10.1164/rccm.202105-1098OC on January 20, 2022
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Although cigarette smoke exposure is associated with an increased risk of acute respiratory distress syndrome (ARDS), it is unknown whether this relationship exists in the general sepsis population. Furthermore, it is unknown if patients with ARDS have differences in underlying biology based on smoking status.

What This Study Adds to the Field: This study demonstrates that cigarette smoke exposure is associated with an increased risk of developing ARDS among patients with sepsis, including both those with nonpulmonary and pulmonary sepsis. In addition, patients with cigarette smoke exposure who develop ARDS have lower severity of illness and less systemic inflammation. Taken together, these findings suggest that patients with cigarette smoke exposure are particularly susceptible to developing ARDS and thus may require a weaker "second hit" to do so and that cigarette smoke exposure may contribute to biologic heterogeneity in ARDS.

transplant. These epidemiologic studies are supported by findings that cigarette smoke exposure enhances inflammation, pulmonary edema, endothelial dysfunction, and vascular permeability in mouse models of acute lung injury (5–8). However, it is unknown whether smoking is associated with ARDS in a broader cohort of patients with sepsis, including those with pulmonary sepsis, and whether the biological phenotype of cigarette smokers who develop ARDS differs from nonsmokers.

Cigarette smoking may contribute to biological and clinical heterogeneity in ARDS. Organ donor smoking is associated with increased oxidative stress in patients with primary graft dysfunction (9). In addition, smoking is associated with increased pulmonary inflammation and lung injury in human lungs rejected for transplant (10) and with increased pulmonary inflammation and alveolar-capillary membrane permeability in a human model of acute lung injury (11). Furthermore, our group reported that smokers might develop ARDS with lower clinical severity of illness compared with nonsmokers (12). Further studies are needed to assess the impact of smoking in patients with ARDS to more accurately measure the public health burden of acute cigarette smoke exposure as well as identify potential tobacco-related biomarkers of acute lung injury, which may inform regulation of tobacco products.

In this study, we tested the epidemiologic relationship between cigarette smoke exposure and the development of ARDS in a prospective cohort of hospitalized patients with sepsis. Furthermore, we used plasma biomarkers of lung injury and inflammation in patients with ARDS to understand pathways of tobacco-related lung injury and identify biomarkers that might be useful for tobacco product regulation. We hypothesized that cigarette smoke exposure would be associated with an increased risk of ARDS in patients with sepsis and with an increase in biomarkers of inflammation and lung injury. Some results have been previously reported in the form of an abstract (13).

Methods

Study Population

The primary cohort was comprised of 592 patients with sepsis at the University of California San Francisco (UCSF) Moffitt-Long Hospital and Zuckerberg San Francisco General Hospital that were prospectively enrolled between 2009 and 2017 as part of the Early Assessment of Renal and Lung Injury (EARLI) cohort. Data collection methods for this cohort have been previously described (14). Patients admitted via the emergency department within 24 hours with sepsis were eligible for enrollment (Figure 1). The primary parent cohort was comprised of patients admitted to the ICU from the emergency department. For this project, we also enrolled 82 patients admitted from the emergency department to other hospital locations to capture a wider spectrum of illness severity. Because enrollment for this project began in 2009, sepsis was defined by Sepsis-2 criteria, including meeting two or more systemic inflammatory response syndrome criteria in the setting of physician-adjudicated diagnosis of infection. Blood and urine samples were obtained within 24 hours of hospital admission and stored at −80°C. Comprehensive demographic and clinical data were obtained until discharge or death. Severity of illness was measured using Acute Physiology and Chronic Health Evaluation (APACHE) III scores. Alcohol misuse history was obtained using the validated Alcohol Use Disorders Identification Test (15) or chart history, with a positive from either classified as alcohol misuse. ARDS adjudication was performed by a two-physician review of patient data during the first 5 days of admission according to both the American-European Consensus Conference and Berlin definitions (16); adjudicating physicians were blinded to patient smoking status. Patients with equivocal ARDS status, who could not be clearly categorized as either with or without ARDS, were excluded *a priori* to enhance detection of epidemiological risk factors, as in prior studies (17). The American-European Consensus Conference definition was used for most analyses; sensitivity analyses using the Berlin definition did not significantly change results (data not shown). UCSF's Institutional Review Board approved the research protocol and granted a waiver of initial consent for blood sampling as a minimal risk intervention. Informed consent was subsequently obtained from patients or surrogates for continued study participation. A waiver of consent was approved for patients unable to consent for whom no surrogate was identified and for those who died prior to being approached for consent.

To assess the generalizability of our biomarker analyses, we also analyzed data from a second cohort of 69 critically ill blunt trauma patients with ARDS, selected based on sample availability from an observational prospective cohort at Zuckerberg San Francisco General Hospital, a level one trauma center affiliated with UCSF. Data collection methods for this cohort have been described previously (18). Patients meeting criteria for full trauma activation underwent blood draw within 10 minutes of arrival to the emergency department and 24 hours later. In this cohort, we previously reported that both active and passive cigarette smoke exposure were associated with an increased risk of ARDS after blunt trauma (19). UCSF's Institutional Review Board approved the protocol and granted a waiver of initial consent for blood sampling as a minimal risk intervention. Informed consent was subsequently obtained from patients or

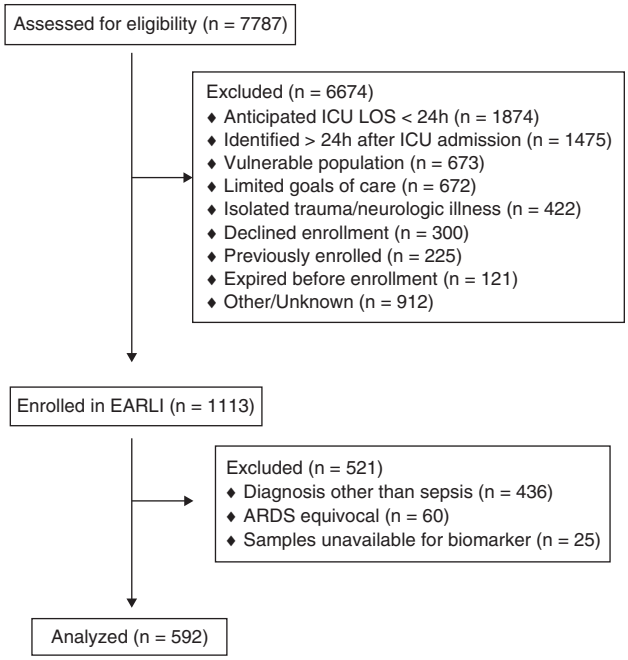

Figure 1. Study flowchart for EARLI cohort. ARDS = acute respiratory distress syndrome; EARLI = Early Assessment of Renal and Lung Injury; LOS = length of stay.

surrogates for continued study participation as previously described (1).

Measurements

Plasma cotinine, a nicotine metabolite with an average half-life of 16 hours that quantifies cigarette smoke exposure (20), was measured on baseline samples in both cohorts via liquid chromatography–tandem mass spectrometry (21, 22). The limit of quantification was 0.02 ng/ml. Patients with undetectable cotinine levels were assigned a level of 0 ng/ml. Based on previous analyses, we used a cutoff of 3.08 ng/ml to identify active smokers (23). Passive smokers were defined as those with plasma cotinine levels between 0.02 and 3.08 ng/ml.

Urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), another well-validated tobacco-specific marker of cigarette smoke exposure with a half-life of 10 to 18 days, was measured using liquid chromatography–tandem mass spectrometry, as in our previous work (2), in the main cohort (EARLI) only. The limit of quantitation was 0.25 pg/ml (24). Patients with undetectable NNAL levels were assigned a level of 0 pg/ml. Urine NNAL levels were normalized to urine creatinine to adjust for urine concentration (24). Based on prior analyses, we used a cutoff of 47 pg/mg to identify active smokers (20). Passive smokers were defined as those with urine NNAL levels between 0.25 and 47 pg/mg creatinine.

Due to the relatively short half-life of plasma cotinine and the potential for samples to be drawn up to 24 hours after ICU admission in our main cohort (EARLI), we used a combined biomarker approach to categorize patients into nonsmokers, passive smokers, and active smokers. If there was discordance among biomarker-based smoking categories, patient smoking status was categorized using the biomarker reflecting the highest exposure, as in prior studies (20) (Table E1). For example, if a patient's NNAL level reflected active smoking, while a cotinine level suggested passive smoking, that patient was categorized as an active smoker. In the trauma cohort, we used only plasma cotinine given the very short travel times to the emergency department (median transit time, 25 min), where samples were subsequently collected within 10 minutes.

Plasma biomarkers. Based on prespecified hypotheses and results of testing in animal models (6), in the EARLI cohort, we measured IL-4, IL-6, IL-8, sTNF-r1 (soluble TNF [tumor necrosis factor]-receptor 1), ang-2 (angiopoietin 2), MMP-8 (matrix metalloproteinase-8) and MMP-9, vWF (von Willebrand factor), ICAM-1 (intercellular adhesion molecule-1), MCP-2 (monocyte chemoattractant protein-2), MIP-1α (macrophage inflammatory protein 1α), VEGF (vascular endothelial growth factor), RAGE (receptor for advanced glycation end products), and SPD (surfactant protein D) via custom MAGPIX multiplex assay (Bio-Techne/R&D Systems) in plasma samples from enrollment. In the trauma cohort, we measured IL-6, IL-8, and s-TNFr1 via Multiplex Luminex Assay (Thermo Fisher Scientific) and RAGE (Bio-Techne/R&D Systems) via enzyme-linked immunosorbent assay in plasma drawn both upon arrival to the emergency department as well as 24 hours later. Levels below or above the limit of quantification were assigned the limit of detection.

Statistical Analysis

Continuously distributed variables were compared using Student's *t* test, or ANOVA if more than two groups, and displayed as mean ± SD. Nonnormally distributed variables were compared using the Mann-Whitney *U* test, or Kruskal-Wallis test if more than two groups, and displayed as median with interquartile range. Pearson's chi-squared test or Fisher's exact test were used for comparisons of proportions between groups. In the sepsis cohort, we adjusted for age, sex, alcohol misuse, diabetes, race, APACHE III, and source of sepsis in multivariate logistic regression to assess the relationship between cigarette smoking status and ARDS. In comparing biomarker levels by smoking status in the sepsis cohort, we used multivariate regression adjusting for age, sex, race, and source of sepsis; in the trauma cohort, we used a parallel approach with multivariate regression adjusting for age, sex, and race. Potential confounders were selected *a priori* based on known relevance to smoking and/or ARDS. Log transformation using a small positive constant was used as needed to fulfill all assumptions required for linear regression testing. A P value ≤ 0.05 was considered statistically significant. A Bonferroni adjustment was used to adjust for multiple comparisons. Statistical analyses were performed with STATA 15.0 (StataCorp LP).

Results

Sepsis Cohort—Demographics and Epidemiology

In the EARLI cohort ($N = 592$), active smokers were younger, more likely to be male, African American or White, misuse

alcohol, and have pulmonary sepsis (Table 1), compared with nonsmokers and passive smokers. These findings were similar to our previous studies in trauma (19). Severity of illness measured by APACHE III, vasopressor use, and clinical outcomes such as mortality and ventilator-free days did not differ by smoking status. Pa_{O_2}:$F_{I_{O_2}}$ was similar across smoking categories.

In the overall cohort, the proportion of patients who developed ARDS differed by smoking status (active, 51%; passive, 48%; nonsmokers, 36%; chi-square $P = 0.007$). In pairwise comparisons, a significantly higher proportion of active smokers ($n = 48/94$ [51%]; $P = 0.01$) and passive smokers ($n = 100/207$ [48%]; $P = 0.008$) developed ARDS compared with nonsmokers ($n = 106/291$ [36%]). In patients with detectable cotinine, we did not observe a dose–response relationship between cotinine and ARDS development. Similarly, there was no difference in odds of developing ARDS between active and passive smokers, suggesting a threshold effect rather than a dose–response relationship.

Both passive and active smokers had increased odds of developing ARDS in multivariate analyses adjusting for age, sex, alcohol misuse, diabetes, race, APACHE III, and source of sepsis compared with nonsmokers (Table 2). Multivariate regression analyses including marijuana, methamphetamine, heroin, and cocaine use did not change these findings (data not shown). Notably, in patients with a pulmonary source of sepsis ($n = 283$), the odds of developing ARDS were increased in both passive (odds ratio, 1.9; $P = 0.04$) and active smokers (odds ratio, 2.4; $P = 0.03$) in multivariate models, which we had not observed in prior studies of sepsis. Findings were substantively unchanged when using the Berlin definition of ARDS (Table E2). Since patients with undetectable NNAL and detectable cotinine might fit a pattern more consistent with vaping, we performed sensitivity analyses excluding these patients ($n = 35$), which did not significantly affect the findings (data not shown).

Among patients who developed ARDS ($n = 254$), active smokers were younger, more likely to be male, African American, misuse alcohol, and have pulmonary sepsis (Table 3). Active smokers with ARDS also had significantly lower APACHE III scores compared with nonsmokers (93 vs. 107; $P = 0.04$) but not passive smokers (93 vs. 100; $P = 0.3$). There was no difference in APACHE III scores between passive smokers and nonsmokers with ARDS (100 vs. 107; $P = 0.2$). As in prior studies (12), smokers with ARDS had significantly lower mortality and more ventilator-free days than nonsmokers or passive smokers with ARDS.

Sepsis Cohort—Plasma Biomarkers

We next compared plasma biomarkers of lung injury and inflammation in patients

Table 1. Overall Demographics of Sepsis Cohort (EARLI)

	Nonsmoker ($n = 291$)	Passive Smokers ($n = 207$)	Active Smokers ($n = 94$)	*P* Value
Age, mean ± SD, yr	69 ± 16	66 ± 16	58 ± 13	<0.0001
Sex, M, *n* (%)	150 (52)	108 (52)	62 (66)	<0.001
Race, *n* (%)				0.001
White	140 (48)	101 (49)	59 (63)	
Asian	93 (32)	51 (25)	10 (11)	
African American	23 (8)	33 (16)	20 (21)	
Other	35 (12)	22 (11)	5 (5)	
Alcohol misuse, *n* (%)				<0.001
Yes	14 (5)	19 (9)	30 (32)	
No	277 (95)	188 (91)	64 (68)	
Diabetes, *n* (%)	89 (31)	53 (26)	19 (20)	0.12
Malignancy, *n* (%)	55 (19)	54 (26)	9 (10)	0.003
Sepsis source, *n* (%)				0.06
Pulmonary	130 (45)	96 (46)	57 (61)	
Nonpulmonary	121 (42)	93 (45)	31 (33)	
Both	31 (11)	11 (5)	5 (5)	
Unknown	9 (3)	7 (3)	1 (1)	
Location, *n* (%)				0.02
ICU	239 (82)	187 (90)	84 (89)	
Floor	52 (18)	20 (10)	10 (11)	
APACHE III, mean ± SD	86 ± 39	90 ± 40	86 ± 0	0.59
Vasopressor use, *n* (%)	123 (42)	83 (40)	39 (41)	0.91
Intubated, *n* (%)*	117 (40)	107 (52)	56 (60)	0.001
ARDS, *n* (%)	106 (36)	100 (48)	48 (51)	0.007
P:F ratio†, median (IQR)	175 (112–259)	165 (95–260)	192 (116–252)	0.62
Ventilator-free days‡, median (IQR)	14 (0–25)	16 (0–25)	22 (0–26)	0.09
60-d hospital mortality, *n* (%)	77 (26)	60 (29)	20 (21)	0.34

Definition of abbreviations: APACHE III = Acute Physiology and Chronic Health Evaluation III; ARDS = acute respiratory distress syndrome; EARLI = Early Assessment of Renal and Lung Injury; IQR = interquartile range; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; P:F = Pa_{O_2}:$F_{I_{O_2}}$.

Smoking status was determined with a combined biomarker approach using both plasma cotinine and urine NNAL.

*During admission.

†Day 1 on enrollment.

‡Calculated in a 28-day timeframe, only on patients who were ever intubated. Patients who died were assigned 0.

Table 2. Odds of Developing ARDS by Smoking Status in Main Sepsis Cohort (EARLI)

	Univariate		Multivariate*	
Smoking Status†	**OR (95% CI)**	***P* Value**	**OR (95% CI)**	***P* Value**
Passive smokers ($n = 207$)	1.6 (1.1–2.3)	0.008	1.8 (1.2–2.7)	0.007
Active smokers ($n = 94$)	1.8 (1.1–2.9)	0.01	2.1 (1.2–3.7)	0.01

Definition of abbreviations: APACHE III = Acute Physiology and Chronic Health Evaluation III; ARDS = acute respiratory distress syndrome; CI = confidence interval; EARLI = Early Assessment of Renal and Lung Injury; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; OR = odds ratio.

*Age, sex, race, diabetes, alcohol misuse, APACHE III, and source of sepsis.

†Smoking status was determined with a combined biomarker approach using both plasma cotinine and urine NNAL. All analyses were performed with nonsmokers ($n = 291$) as the reference group.

who developed ARDS in our sepsis cohort ($n = 254$). Given that passive and active smoking were associated with similar increases in the risk of ARDS, we combined passive and active smokers and categorized them as patients with cigarette smoke exposure. For this analysis, we focused on patients who developed ARDS on Day 1 or 2 of the study (210/254 patients). Compared with nonsmokers with ARDS ($n = 91$), patients with ARDS with cigarette smoke exposure ($n = 119$) had significantly decreased levels of plasma IL-8 (28 vs. 44 pg/ml; $P = 0.01$) and sTNFr1 (4,147 vs. 5,584 pg/ml; $P = 0.01$), with a similar trend for RAGE (3,263 vs. 4,026 pg/ml; $P = 0.07$) but no significant differences in the other biomarkers tested (Table 4). Multivariate regression analyses adjusting for age, sex, race, and source of sepsis did not attenuate differences in plasma IL-8 (0.02) but increased the variance for sTNFr1 ($P = 0.07$). Bonferroni adjustment increased the *P* value for all biomarkers, including IL-8 ($P = 0.15$) and sTNFr-1 ($P = 0.15$).

Trauma cohort—validation of biological findings. To assess the generalizability of our findings, we measured a limited set of plasma biomarkers in a separate cohort of critically ill blunt trauma patients with ARDS ($n = 69$). Patients with ARDS and cigarette smoke exposure were more likely to be male and less likely to have diabetes than nonexposed patients with ARDS, with no significant difference in injury severity score, shock, or alcohol misuse (Table E3).

We first analyzed plasma obtained within 10 minutes (0 h) of arrival to the emergency department. At 0h, there were no differences in plasma IL-6, IL-8, TNFr-1, or RAGE levels between patients with and without smoke exposure. Next, we compared biomarkers in plasma obtained approximately 24 hours after presentation to the emergency department. Patients with ARDS and cigarette smoke exposure had significantly decreased levels of plasma sTNFR-1 (852 vs. 2,448 pg/ml; $P = 0.04$) and

Table 3. Demographics of Patients with ARDS from Sepsis Cohort (EARLI) ($N = 254$)

	Nonsmokers ($n = 106$)	**Passive Smokers ($n = 100$)**	**Active Smokers ($n = 48$)**	***P* Value**
Age, mean ± SD, yr	74 ± 15	66 ± 16	59 ± 12	<0.0001
Sex, M, *n* (%)	57 (54)	43 (43)	31 (65)	0.004
Race, *n* (%)				0.005
White	53 (50)	47 (47)	29 (60)	
Asian	36 (34)	26 (26)	5 (10)	
African American	10 (9)	13 (13)	12 (25)	
Other	7 (7)	14 (14)	2 (4)	
Alcohol misuse, *n* (%)				<0.001
Yes	5 (5)	9 (9)	15 (31)	
No	101 (95)	91 (91)	33 (69)	
Diabetes, *n* (%)	34 (32)	28 (28)	9 (19)	0.23
Malignancy, *n* (%)	16 (15)	25 (25)	6 (13)	0.09
Sepsis source, *n* (%)				0.05
Pulmonary	59 (56)	59 (59)	35 (73)	
Nonpulmonary	22 (21)	28 (28)	11 (23)	
Both	19 (18)	8 (8)	2 (4)	
Unknown	6 (6)	5 (5)	0 (0)	
APACHE III, mean ± SD	107 ± 41	100 ± 40	93 ± 40	0.11
Vasopressor use, *n* (%)	60 (57)	51 (51)	23 (48)	0.57
Ventilator-free days*, median (IQR)	0 (0–22)	4 (0–23)	24 (3–26)	0.0009
60-d hospital mortality, *n* (%)	48 (45)	38 (38)	9 (19)	0.02

Definition of abbreviations: APACHE III = Acute Physiology and Chronic Health Evaluation III; ARDS = acute respiratory distress syndrome; EARLI = Early Assessment of Renal and Lung Injury; IQR = interquartile range.

*Calculated in a 28-day timeframe, only on patients who were ever intubated. Patients who died were assigned 0.

Table 4. Plasma Biomarkers at 24 Hours after Emergency Department Presentation by Smoke Exposure Status in Patients with ARDS (AECC Definition)

	Sepsis Cohort (EARLI) (*n* = *210*)				Trauma Cohort (*n* = *69*)		
	Nonexposed [*Median (IQR)*] (*n* = *91*)	Smoke Exposed [*Median (IQR)*] (*n* = *119*)	*P* Value	Adjusted *P* Value*	Nonexposed [*Median (IQR)*] (*n* = *10*)	Smoke Exposed [*Median (IQR)*] (*n* = *59*)	*P* Value
IL-8	44 (16–873)	28 (7–175)	0.01	0.15	25 (17–1,353)	10 (0–58)	0.03
sTNFr-1	5,584 (3,398–10,168)	4,147 (1,899–9,038)	0.01	0.15	2,448 (593–2,605)	852 (664–1,175)	0.04
RAGE	4,026 (2,299–7,527)	3,263 (1,840–6,019)	0.07	1.0	1,353 (1,072–1,834)	1,203 (837–2,146)	0.80
IL-6	281 (59–2,691)	133 (33–1,638)	0.09	1.0	159 (74–1,316)	137 (74–214)	0.28
MIP-1α	140 (101–266)	154 (101–276)	0.95	1.0	—†	—†	—
VEGF	28 (19–55)	33 (20–61)	0.13	1.0	—†	—†	—
CXCL-9	1,679 (988–3,439)	2,044 (1,019–3,610)	0.58	1.0	—†	—†	—
SP-D	7,891 (3,302–20,313)	7,967 (4,596–18,904)	0.50	1.0	—†	—†	—
IL-4	44 (25–44)	44 (29–44)	0.32	1.0	—†	—†	—
MCP-2	54 (31–182)	47 (23–172)	0.29	1.0	—†	—†	—
vWF	121 (64–278)	108 (42–259)	0.33	1.0	—†	—†	—
Ang-2	6,101 (3,870–11,908)	6,361 (3,464–13,693)	0.76	1.0	—†	—†	—
MMP-8	8,136 (2,387–21,531)	8,852 (2,853–21,292)	0.63	1.0	—†	—†	—
MMP-9	32,615 (10,757–89,011)	39,490 (14,397–154,384)	0.14	1.0	—†	—†	—
ICAM-1	699,763 (455,601–1,222,377)	692,860 (372,068–1,374,366)	0.97	1.0	—†	—†	—

Definition of abbreviations: AECC = American-European Consensus Conference; Ang = angiopoietin; ARDS = acute respiratory distress syndrome; CXCL-9 = C-X-C motif chemokine ligand 9; EARLI = Early Assessment of Renal and Lung Injury; ICAM-1 = intercellular adhesion molecule-1; IQR = interquartile range; MCP-2 = monocyte chemoattractant protein-2; MIP-1α = macrophage inflammatory protein 1α; MMP = matrix metalloproteinase; RAGE = receptor for advanced glycation end products; SP-D = surfactant protein D; sTNFr-1 = soluble tumor necrosis factor receptor 1; VEGF = vascular endothelial growth factor; vWF = von Willebrand factor.
All values are shown in pg/ml.
*Bonferroni correction.
†Not measured.

IL-8 (10 vs. 25 pg/ml; $P = 0.03$) compared with unexposed patients with ARDS, but not IL-6 (137 vs. 159 pg/ml; $P = 0.28$) or RAGE (1,203 vs. 1,353 pg/ml; $P = 0.80$). A multivariate regression model adjusting for age, sex, and race increased the variance of these findings for IL-8 ($P = 0.13$) and sTNFr-1 ($P = 0.056$). There were no differences in plasma IL-6, IL-8, sTNFr-1, or RAGE between passive and active smokers. These results suggest that cigarette smoke exposure was associated with decreased systemic inflammation in patients who developed ARDS but that these differences were not simply reflective of baseline differences immediately after trauma.

Discussion

This study indicates that both active and passive cigarette smoke exposure are associated with an increased risk of developing ARDS in patients with both pulmonary and nonpulmonary sepsis. To our knowledge, this is the first report that identifies an increased risk of developing ARDS among patients with cigarette smoke exposure who have a pulmonary source of sepsis. Given that cigarette smoking is associated with an increased risk of pulmonary infection (24) and that pulmonary sepsis is in turn associated with the highest risk of ARDS among patients with sepsis (25), this finding has significant epidemiological and public health importance. Furthermore, patients with cigarette smoke exposure who go on to develop ARDS have less systemic inflammation, while active smokers also have lower severity of illness compared with nonsmokers. These findings are consistent with the hypothesis drawn from preclinical models (8, 26) that patients with cigarette smoke exposure more readily develop ARDS in the setting of a "second hit" compared with nonsmokers. Given the significant mortality, morbidity, reduction in quality of life, and healthcare costs that are associated with ARDS (27–29), the finding that patients with cigarette smoke exposure, including those with low levels of exposure, develop ARDS at a higher rate despite younger age and less systemic inflammation has significant implications for public health and U.S. Food and Drug Administration regulation, highlighting both the acute pulmonary toxicities and public health costs of cigarette smoke exposure.

In our prior work in human experimental models of acute lung injury, we found that cigarette smokers develop exaggerated pulmonary inflammation, alveolar epithelial injury, and alveolar-capillary membrane permeability compared with nonsmokers in response to an identical inflammatory stimulus (11). While at first glance this may seem to conflict with our current findings, it is important to note that in our sepsis cohort, patients with ARDS with cigarette smoke exposure trended toward lower severity of illness compared with nonexposed patients as measured by APACHE III, whereas, in our experimental human model of acute lung injury, all subjects received an identical inflammatory stimulus. In a sensitivity analysis, when we limited our sample to patients with APACHE III >100, there was no difference in plasma biomarkers between those with and without smoke exposure (data not shown). Additional studies of patients with ARDS are needed to assess whether the relationship between cigarette smoke exposure and decreased inflammation extends to the pulmonary compartment rather than the systemic compartment alone.

Although we detected differences in some biomarkers between patients with and

without cigarette smoke exposure, we did not identify any biomarkers that were specifically elevated in tobacco-related lung injury. This finding may be due to a variety of factors, including differences in age and severity of illness between smokers and nonsmokers in our cohort. It may also reflect limitations of human and animal models of ARDS, in which we have previously identified biomarkers that were specifically elevated in tobacco-related lung injury (6). Given the significant inflammatory changes that occur in the plasma of patients with sepsis, it may be difficult to identify tobacco-specific biomarkers in the plasma. Future work should include studies of the pulmonary compartment to assess the presence of tobacco-specific biomarkers that could be used for regulatory science and the testing of novel tobacco products.

While it is well known that cigarette smoke has proinflammatory properties, it also possesses antiinflammatory properties via endogenous cholinergic antiinflammatory pathways (30). Several studies have found that activation of α-7 nicotinic receptors by nicotine is associated with decreased inflammation and improved survival in animal models of sepsis (31–33). Despite the potential role of these receptors in modulating inflammation in sepsis, less is known about their effects in ARDS, although there is some evidence that they may decrease inflammation in acute lung injury (34–36). Although less likely, it is plausible that the decreased systemic inflammation we found in patients with ARDS with cigarette smoke exposure may be due to the antiinflammatory effects of nicotine rather than differences in severity of illness or susceptibility to developing ARDS in these patients. Notably, among patients with ARDS in our main sepsis cohort, active smokers had significantly lower 60-day hospital mortality compared with nonsmokers and passive smokers, which could reflect antiinflammatory properties of cigarette smoke. However, after adjustment for severity of illness and baseline demographics, these differences were no longer significant (data not shown). We note that we observed a strikingly similar pattern in prior analyses of patients with ARDS enrolled in a multicenter United States–based randomized controlled trial (12). Thus, we hypothesize that differences by smoking status in the mortality of patients with ARDS are more likely to reflect the predisposition for active smokers to develop ARDS at younger ages, with fewer comorbidities and lower severity of illness. Further studies are needed to evaluate whether α-7 nicotinic receptors may represent a therapeutic target in ARDS.

Our findings have potential implications for clinical trials for patients at risk for or with ARDS. Numerous randomized controlled trials in patients with ARDS that have studied the effect of antiinflammatory therapies such as glucocorticoids (37, 38) or statins (39, 40) have failed to identify a benefit. Notably, cigarette smokers have been shown to have a less robust response to certain antiinflammatory therapies (41, 42). Furthermore, our group's prior work has found that 36% of patients tested from two large multicenter ARDS trials had biomarker evidence of active smoking, with an additional 41% of patients showing evidence of passive cigarette smoke exposure (12). Given that cigarette smokers with ARDS may have less systemic inflammation than nonsmokers and have been shown to have less robust responses to certain antiinflammatory therapies, it is possible that cigarette smoking may contribute to the inefficacy of antiinflammatory therapies in patients with ARDS. Future clinical trials in patients with ARDS should consider this potential confounder in their design and analysis.

This study has several strengths. First, we used well-validated biomarkers to classify cigarette smoke exposure. Prior studies suggest that biomarkers of cigarette smoke exposure provide a more accurate assessment of true exposure in critically ill populations (20). Second, we used two distinct cohorts to test our biologic hypotheses and observed similar findings in each, strengthening generalizability. Third, our cohort included patients outside of the ICU ($n = 82$), 18% of whom went on to develop ARDS. The inclusion of both ICU- and floor-level patients increases generalizability. Fourth, in our trauma cohort, we had baseline samples available immediately after injury, which indicated that the differences we observed in inflammation related to smoking status were not present at baseline but developed over time. Finally, this prospective cohort study was specifically designed to test the hypothesis that cigarette smoking would be associated with ARDS in patients with sepsis and included physician phenotyping of each patient for both sepsis and ARDS, including a two-physician review of all chest radiographs, collection of thousands of biospecimens, and careful measurement of hypothesis-directed biomarkers both of tobacco use and lung injury.

This study also has limitations. First, the sample size in the trauma cohort was modest. Despite this, we identified a significant relationship between cigarette smoke exposure and inflammation in ARDS. When we performed multivariate regression, we observed an increase in variance. However, we think the findings are unlikely to be due to chance alone, given their concordance with the findings from the sepsis patients. Second, our study was limited to the systemic compartment of patients with ARDS. Additional studies that explore the relationship between cigarette smoke exposure and inflammation in the pulmonary compartment are needed to better understand the role of cigarette smoke exposure in impacting biological heterogeneity in ARDS. Third, as in all observational studies, we cannot rule out unobserved potential confounders that may have affected our findings, although we did adjust for a number of potential confounders, including alcohol and drug use. Finally, when we corrected our analyses of plasma biomarkers for multiple comparisons, the *P* values were increased, and the findings were no longer statistically significant. However, given that these biomarker analyses were hypothesis-directed based on our prior animal and human experimental studies of the biological effects of smoke exposure (5, 6, 11), including effects on inflammatory pathways, lung injury, and endothelial function, in addition to prior literature on key biological pathways in ARDS (43, 44), and consistent with findings in our prior work and across both cohorts in this study, a Bonferroni correction may be overly conservative. Nevertheless, it is possible that some of the biomarker findings are due to chance.

In conclusion, this study has two major findings: first, cigarette smoke exposure is associated with an increased risk of ARDS development among patients with both pulmonary and nonpulmonary sepsis; and second, among patients with sepsis-related ARDS, cigarette smoke exposure is associated with younger age, decreased systemic inflammation, and a trend toward decreased overall severity of illness. These findings are consistent with the hypothesis that a weaker second hit is required for both

passive and active smokers to develop ARDS compared with nonsmokers. This increased susceptibility to developing ARDS, with its significant health, financial, and quality of life costs, has significant implications for public health and highlights yet another area in which cigarette smoking creates a significant societal burden. Furthermore, the biological heterogeneity that cigarette smoke exposure contributes to among patients with ARDS may have an impact on preventative and treatment strategies in patients with ARDS. Although further studies are needed to assess whether our findings extend to the pulmonary compartment of patients with ARDS, this study highlights the impact of environmental exposures on biological heterogeneity in ARDS. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Calfee CS, Matthay MA, Eisner MD, Benowitz N, Call M, Pittet JF, *et al.* Active and passive cigarette smoking and acute lung injury after severe blunt trauma. *Am J Respir Crit Care Med* 2011;183:1660–1665.
2. Calfee CS, Matthay MA, Kangelaris KN, Siew ED, Janz DR, Bernard GR, *et al.* Cigarette smoke exposure and the acute respiratory distress syndrome. *Crit Care Med* 2015;43:1790–1797.
3. Toy P, Gajic O, Bacchetti P, Looney MR, Gropper MA, Hubmayr R, *et al.*; TRALI Study Group. Transfusion-related acute lung injury: incidence and risk factors. *Blood* 2012;119:1757–1767.
4. Diamond JM, Lee JC, Kawut SM, Shah RJ, Localio AR, Bellamy SL, *et al.*; Lung Transplant Outcomes Group. Clinical risk factors for primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med* 2013; 187:527–534.
5. Gotts JE, Chun L, Abbott J, Fang X, Takasaka N, Nishimura SL, *et al.* Cigarette smoke exposure worsens acute lung injury in antibiotic-treated bacterial pneumonia in mice. *Am J Physiol Lung Cell Mol Physiol* 2018; 315:L25–L40.
6. Gotts JE, Abbott J, Fang X, Yanagisawa H, Takasaka N, Nishimura SL, *et al.* Cigarette smoke exposure worsens endotoxin-induced lung injury and pulmonary edema in mice. *Nicotine Tob Res* 2017;19:1033–1039.
7. Lu Q, Sakhatskyy P, Grinnell K, Newton J, Ortiz M, Wang Y, *et al.* Cigarette smoke causes lung vascular barrier dysfunction via oxidative stress-mediated inhibition of RhoA and focal adhesion kinase. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L847–L857.
8. Sakhatskyy P, Wang Z, Borgas D, Lomas-Neira J, Chen Y, Ayala A, *et al.* Double-hit mouse model of cigarette smoke priming for acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2017;312:L56–L67.
9. Diamond JM, Porteous MK, Jackson Roberts L II, Wickersham N, Rushefski M, Kawut SM, *et al.* The relationship between plasma lipid peroxidation products and primary graft dysfunction after lung transplantation is modified by donor smoking and reperfusion hyperoxia. *J Heart Lung Transplant* 2016;35:500–507.
10. Ware LB, Lee JW, Wickersham N, Nguyen J, Matthay MA, Calfee CS; California Transplant Donor Network. Donor smoking is associated with pulmonary edema, inflammation and epithelial dysfunction in ex vivo human donor lungs. *Am J Transplant* 2014;14:2295–2302.
11. Moazed F, Burnham EL, Vandivier RW, O'Kane CM, Shyamsundar M, Hamid U, *et al.* Cigarette smokers have exaggerated alveolar barrier disruption in response to lipopolysaccharide inhalation. *Thorax* 2016; 71:1130–1136.
12. Hsieh SJ, Zhuo H, Benowitz NL, Thompson BT, Liu KD, Matthay MA, *et al.*; National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome Network; National Heart Lung and Blood Institute Acute Respiratory Distress Syndrome Network. Prevalence and impact of active and passive cigarette smoking in acute respiratory distress syndrome. *Crit Care Med* 2014;42:2058–2068.
13. Moazed F, Hendrickson C, Jauregui A, Vessel K, Deiss T, Ke S, *et al.* Cigarette smoking is associated with clinical and biological heterogeneity in patients with the acute respiratory distress syndrome. American Thoracic Society International Conference. Dallas; 2019.
14. Kangelaris KN, Prakash A, Liu KD, Aouizerat B, Woodruff PG, Erle DJ, *et al.* Increased expression of neutrophil-related genes in patients with early sepsis-induced ARDS. *Am J Physiol Lung Cell Mol Physiol* 2015; 308:L1102–L1113.
15. Donovan DM, Dunn CW, Rivara FP, Jurkovich GJ, Ries RR, Gentilello LM. Comparison of trauma center patient self-reports and proxy reports on the alcohol use identification test (AUDIT). *J Trauma* 2004;56: 873–882.
16. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, *et al.*; ARDS Definition Task Force. Acute respiratory distress syndrome: the Berlin definition. *JAMA* 2012;307:2526–2533.
17. Shah CV, Lanken PN, Localio AR, Gallop R, Bellamy S, Ma SF, *et al.* An alternative method of acute lung injury classification for use in observational studies. *Chest* 2010;138:1054–1061.
18. Brohi K, Cohen MJ, Ganter MT, Matthay MA, Mackersie RC, Pittet JF. Acute traumatic coagulopathy: initiated by hypoperfusion: modulated through the protein C pathway? *Ann Surg* 2007;245:812–818.
19. Moazed F, Hendrickson C, Conroy A, Kornblith LZ, Benowitz NL, Delucchi K, *et al.* Cigarette smoking and ARDS after blunt trauma: the influence of changing smoking patterns and resuscitation practices. *Chest* 2020;158:1490–1498.
20. Hsieh SJ, Ware LB, Eisner MD, Yu L, Jacob P III, Havel C, *et al.* Biomarkers increase detection of active smoking and secondhand smoke exposure in critically ill patients. *Crit Care Med* 2011;39:40–45.
21. Dempsey D, Tutka P, Jacob P III, Allen F, Schoedel K, Tyndale RF, *et al.* Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin Pharmacol Ther* 2004;76:64–72.
22. Jacob P III, Havel C, Lee DH, Yu L, Eisner MD, Benowitz NL. Subpicogram per milliliter determination of the tobacco-specific carcinogen metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in human urine using liquid chromatography-tandem mass spectrometry. *Anal Chem* 2008;80:8115–8121.
23. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. *Am J Epidemiol* 2009;169:236–248.
24. Arcavi L, Benowitz NL. Cigarette smoking and infection. *Arch Intern Med* 2004;164:2206–2216.
25. Sheu CC, Gong MN, Zhai R, Bajwa EK, Chen F, Thompson BT, *et al.* The influence of infection sites on development and mortality of ARDS. *Intensive Care Med* 2010;36:963–970.
26. Lu Q, Gottlieb E, Rounds S. Effects of cigarette smoke on pulmonary endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2018;314: L743–L756.
27. Ruhl AP, Huang M, Colantuoni E, Karmarkar T, Dinglas VD, Hopkins RO, *et al.*; With the National Institutes of Health, National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome Network. Healthcare utilization and costs in ARDS survivors: a 1-year longitudinal national US multicenter study. *Intensive Care Med* 2017; 43:980–991.
28. Cheung AM, Tansey CM, Tomlinson G, Diaz-Granados N, Matté A, Barr A, *et al.* Two-year outcomes, health care use, and costs of survivors of acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2006; 174:538–544.
29. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, *et al.* Incidence and outcomes of acute lung injury. *N Engl J Med* 2005; 353:1685–1693.
30. Tracey KJ. Reflex control of immunity. *Nat Rev Immunol* 2009;9:418–428.
31. Wang H, Liao H, Ochani M, Justiniani M, Lin X, Yang L, *et al.* Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med* 2004;10:1216–1221.
32. Pavlov VA, Ochani M, Yang LH, Gallowitsch-Puerta M, Ochani K, Lin X, *et al.* Selective alpha7-nicotinic acetylcholine receptor agonist GTS-21 improves survival in murine endotoxemia and severe sepsis. *Crit Care Med* 2007;35:1139–1144.
33. van Westerloo DJ, Giebelen IA, Florquin S, Daalhuisen J, Bruno MJ, de Vos AF, *et al.* The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis* 2005;191:2138–2148.

34. Pinheiro NM, Santana FP, Almeida RR, Guerreiro M, Martins MA, Caperuto LC, *et al.* Acute lung injury is reduced by the α7nAChR agonist PNU-282987 through changes in the macrophage profile. *FASEB J* 2017;31:320–332.
35. Su X, Lee JW, Matthay ZA, Mednick G, Uchida T, Fang X, *et al.* Activation of the alpha7 nAChR reduces acid-induced acute lung injury in mice and rats. *Am J Respir Cell Mol Biol* 2007;37:186–192.
36. Ni YF, Tian F, Lu ZF, Yang GD, Fu HY, Wang J, *et al.* Protective effect of nicotine on lipopolysaccharide-induced acute lung injury in mice. *Respiration* 2011;81:39–46.
37. Bernard GR, Luce JM, Sprung CL, Rinaldo JE, Tate RM, Sibbald WJ, *et al.* High-dose corticosteroids in patients with the adult respiratory distress syndrome. *N Engl J Med* 1987;317:1565–1570.
38. Steinberg KP, Hudson LD, Goodman RB, Hough CL, Lanken PN, Hyzy R, *et al.*; National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. *N Engl J Med* 2006;354:1671–1684.
39. McAuley DF, Laffey JG, O'Kane CM, Perkins GD, Mullan B, Trinder TJ, *et al.*; HARP-2 Investigators; Irish Critical Care Trials Group. Simvastatin in the acute respiratory distress syndrome. *N Engl J Med* 2014;371:1695–1703.
40. Truwit JD, Bernard GR, Steingrub J, Matthay MA, Liu KD, Albertson TE, *et al.*; National Heart, Lung, and Blood Institute ARDS Clinical Trials Network. Rosuvastatin for sepsis-associated acute respiratory distress syndrome. *N Engl J Med* 2014;370:2191–2200.
41. Simon JA, Lin F, Hulley SB, Blanche PJ, Waters D, Shiboski S, *et al.* Phenotypic predictors of response to simvastatin therapy among African-Americans and Caucasians: the Cholesterol and Pharmacogenetics (CAP) Study. *Am J Cardiol* 2006;97: 843–850.
42. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med* 2003;168:1308–1311.
43. Meyer NJ, Gattinoni L, Calfee CS. Acute respiratory distress syndrome. *Lancet* 2021;398:622–637.
44. Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, *et al.* Acute respiratory distress syndrome. *Nat Rev Dis Primers* 2019;5:18.