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RESEARCH ARTICLE

Biomarkers of alveolar epithelial injury and endothelial dysfunction are associated with scores of pulmonary edema in invasively ventilated patients

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Abstract

Pulmonary edema is a central hallmark of acute respiratory distress syndrome (ARDS). Endothelial dysfunction and epithelial injury contribute to alveolar-capillary permeability but their differential contribution to pulmonary edema development remains understudied. Plasma levels of surfactant protein-D (SP-D), soluble receptor for advanced glycation end products (sRAGE), and angiopoietin-2 (Ang-2) were measured in a prospective, multicenter cohort of invasively ventilated patients. Pulmonary edema was quantified using the radiographic assessment of lung edema (RALE) and global lung ultrasound (LUS) score. Variables were collected within 48 h after intubation. Linear regression was used to examine the association of the biomarkers with pulmonary edema. In 362 patients, higher SP-D, sRAGE, and Ang-2 concentrations were significantly associated with higher RALE and global LUS scores. After stratification by ARDS subgroups (pulmonary, nonpulmonary, COVID, non-COVID), the positive association of SP-D levels with pulmonary edema remained, whereas sRAGE and Ang-2 showed less consistent associations throughout the subgroups. In a multivariable analysis, SP-D levels were most strongly associated with pulmonary edema when combined with sRAGE (RALE score: $\beta_{SP\text{-}D}$ = 6.79 units/log10 pg/mL, β_{sRAGE} = 3.84 units/log10 pg/mL, R^2 = 0.23; global LUS score: $\beta_{SP\text{-}D}$ = 3.28 units/log10 pg/mL, β_{sRAGE} = 2.06 units/log10 pg/mL, R^2 = 0.086), whereas Ang-2 did not further improve the model. Biomarkers of epithelial injury and endothelial dysfunction were associated with pulmonary edema in invasively ventilated patients. SP-D and sRAGE showed the strongest association, suggesting that epithelial injury may form a final common pathway in the alveolar-capillary barrier dysfunction underlying pulmonary edema.

ARDS; endothelial dysfunction; epithelial injury; pulmonary edema; vascular permeability

INTRODUCTION

The acute respiratory distress syndrome (ARDS) is a leading cause of acute respiratory failure in critically ill patients. Permeability of the pulmonary vasculature is secondary to disruption of the alveolar-capillary barrier due to inflammatory epithelial injury and endothelial dysfunction (1, 2). Plasma biomarkers can be used as surrogates for these two processes and may aid our understanding of ARDS subphenotypes (3). For example, patients with ARDS of pulmonary origin, including patients with COVID-19 ARDS, have higher levels of epithelial injury markers, whereas ARDS caused by nonpulmonary conditions is characterized by elevated biomarkers of endothelial dysfunction (4, 5). The differential contribution of alveolar epithelial injury and endothelial dysfunction to the development of pulmonary edema within subgroups of invasively ventilated critically ill patients remains understudied.

Several plasma biomarkers have been identified as useful indicators of pathophysiological processes in ARDS. The soluble isoform of the alveolar epithelial membrane-bound receptor for advanced glycation end products (sRAGE) is a validated biomarker for pneumocyte type I injury. Plasma levels of sRAGE have shown to be increased in patients at risk for ARDS and are associated with increased mortality (6). Surfactant protein-D (SP-D) is a marker of pneumocyte type 2 injury particularly associated with ARDS of pulmonary origin (7) and raised levels of SP-D may be a potential diagnostic biomarker for ARDS (8). Angiopoietin-2 (Ang-2) is a growth factor that binds to the Tie2 receptor on the endothelial surface and is a biomarker of endothelial dysfunction.

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Elevated plasma levels of Ang-2 have been associated with increased vascular permeability (9) and the development of nonhydrostatic pulmonary edema (1, 10). Higher plasma concentrations of sRAGE and Ang-2 have been suggested to be causally related to ARDS via Mendelian randomization studies (6, 11). Although epithelial injury and endothelial dysfunction have been independently associated with the development of capillary barrier dysfunction (7, 9), their relative association with alveolar edema remains understudied.

Pulmonary edema can be visualized and quantified using chest X-ray and lung ultrasound (LUS). To systematically assess edema on chest X-ray, the radiographic assessment of lung edema (RALE) score was recently introduced (12). The RALE score has a high interrater agreement (12, 13) and good correlation with postmortem lung gravimetry and ARDS prognosis (13). On LUS, B-lines (ultrasonographic artifacts generated by the loss of aeration) indicate the presence and extent of alveolar-interstitial edema (14). The global LUS score can be calculated as a semiquantitative measure of pulmonary edema (15).

To gain a better understanding of the relative contribution of molecular drivers of pulmonary edema in invasively ventilated patients and specifically in patients with ARDS, the aim of this study was to assess the association between alveolar epithelial injury and endothelial dysfunction and the extent of pulmonary edema, quantified by the RALE and global LUS score. We hypothesized that sRAGE, SP-D, and Ang-2 plasma levels are associated with pulmonary edema in patients receiving invasive ventilation. Furthermore, based on previous findings that biomarker levels differ among ARDS subgroups, we postulated that endothelial dysfunction would have a stronger association with edema in patients with nonpulmonary causes of ARDS, whereas alveolar epithelial injury would be more strongly associated with pulmonary edema in patients with pulmonary ARDS, including COVID-19 related ARDS. The latter presents with a distinct clinical image of severe alveolar edema combined with pulmonary microthrombosis (16), indicating both epithelial and endothelial injury. The rationale for investigating COVID ARDS separately was thus to examine potential differences in the studied associations compared with other forms of pulmonary ARDS.

We chose to include patients without ARDS in this analysis, as the Berlin definition remains an imperfect classification for a complex clinical syndrome such as ARDS (17). Patients with ARDS and patients at risk for developing ARDS show considerable overlap in extravascular lung water (18), which is reflected in the currently debated update to the Berlin criteria to include patients on high-flow nasal oxygen (17, 19). A part of the patients classified as not having ARDS will have pulmonary edema and be at risk of developing ARDS. Hence we included this group with the aim to capture a more complete patient population and to reduce the spread of the data.

METHODS

Study Design and Ethical Considerations

This study is a predefined study within the prospective observational DARTS project (Diagnosis of acute respiratory distress syndrome by bedside exhaled breath octane measurements in invasively ventilated patients; trialregister.nl identifier NL8226), that included invasively ventilated patients on mixed intensive care units (ICUs) at two university hospitals [Amsterdam University Medical Centers (AUMC), location AMC and Maastricht University Medical Center+ (MUMC+) in the Netherlands] between March 2019 and March 2021. The Institutional Review Board (IRB) of the AUMC, MUMC+, and the biobank approved the study (IRB identifiers W18_311#18.358, 2019-1137 and 2018_287#A201921), and written informed consent for the use of clinical data, imaging, and blood samples was obtained from patients or their legal representatives. The protocol of the DARTS study has been published (20).

Population

The DARTS study consecutively included patients who were admitted to the ICU and expected to be invasively ventilated for at least 24 h. Exclusion criteria included invasive ventilation for more than 48 h in the week before inclusion and refusal of patients or their legal representatives to participate in the study. The current secondary analysis included patients of whom plasma SP-D, sRAGE, Ang-2, and global LUS and/or RALE scores were available within 48 h after intubation.

Measurements

Plasma biomarkers.

The remainder of the blood used in an arterial blood gas analysis (~2 mL) was collected for plasma biomarker analysis. The samples were centrifuged (1,500 *g* for 15 min), after which the plasma was frozen at −80°C for future analysis. Biomarkers were measured using Luminex multiplex assay (R&D systems, Abington, UK) and Bioplex 200 (Bio-Rad, Hercules, California) according to the manufacturer's instructions.

Lung ultrasound and lung ultrasound score.

Lung ultrasound examination was performed using standard ultrasound machines: *1*) LOGIQ e, GE Healthcare, Milwaukee, *2*) Lumify, Philips Ultrasound, Inc. Bothell, or *3*) MyLabGamma, Esaote, Genoa, Italy. A linear array transducer (5.0–12.0 MHz) was used to examine six regions per hemithorax, according to previously described protocols (16, 18). To determine the regions, the chest is divided into anterior, anterolateral, and posterior of the axillary line. The first two examined points are located anteriorly, points three and four anterolaterally, and points five and six posteriorly. The most pathologic finding was used to determine the aeration profile defined as follows: A = A lines or <3 B lines, B1 ≥ 2 well-spaced B lines occupying <50% of the intercostal space, B2 = coalescent B lines occupying >50% of the intercostal space, and C = lung consolidation >2 cm in diameter (21). Each regional image was scored according to aeration pattern (A = 0 points, B1 = 1 point, B2 = 2 points, and C = 3 points). The sum of the individual scores was used to calculate the LUS aeration score, ranging from 0 to 36.

Chest X-ray and radiographic assessment of lung edema score.

The RALE score was determined as previously described (12). In short, image quality was first assessed as adequate, borderline (doubtful image quality and/or interfering co-

morbidities such as pleural effusion or subcutaneous emphysema), or unusable (poor image quality and/or interfering comorbidities). Subsequently, images were assessed for the extent and density of consolidations. The four lung quadrants were scored by assigning points for percentage of consolidation (0% = 0, <25% = 1, 25%–50% = 2, 50%–75% = 3, and >75% = 4) and density (hazy = 1, moderate = 2, and dense = 3). Finally, the points were multiplied per quadrant and added to result in a RALE score from 0 to 48. Examiners were trained in determining the RALE score by absolving a curriculum with an expert (CZ) until reaching an intraclass correlation coefficient (ICC) with the expert of $\geq$ 0.90. All available baseline chest X-rays acquired within 48 h after intubation were assessed by one examiner (DFLF). A second examiner (LNA) verified the interobserver agreement by random selection of 50 images, yielding an ICC of 0.78 (95% CI 0.65–0.87). Images previously rated unusable or borderline (51 images) were reviewed by three raters (D. F. L. Filippini, L. N. Atmowihardjo, and M. R. Smit) in a consensus meeting, and eventually, 13 images were not scored due to poor quality. All reviewers were blinded for other patient data.

If a patient had >1 chest X-ray or LUS measurement available within the 48 h window, the first score was chosen. In case of the biomarkers, first, the highest value was selected, and if biomarker measurements of separate time points were selected in one patient, the value measured on the first time point was chosen.

Definitions

ARDS.

ARDS was defined according to the Berlin criteria (22). To limit the influence of interobserver disagreement, an expert panel consisting of three independent physicians scored chest X-rays and computed tomography (CT) images for confidence of ARDS diagnosis, aside from also considering the patients' clinical characteristics (see Supplemental Material for an explanation of the scoring and classification method).

Pulmonary ARDS was defined as originating from a pulmonary insult such as pneumonia, aspiration of gastric contents, submersion, lung contusion, or smoke inhalation. Nonpulmonary ARDS was defined as ARDS from an extrapulmonary inflammatory origin such as a severe infection, sepsis, as a consequence of burns, trauma, or as a reaction to blood transfusion or medication. COVID-19-related ARDS (from here on referred to as COVID-ARDS) refers to a distinct etiology of pulmonary ARDS caused by infection with SARS-CoV-2.

Endpoints

The primary endpoint of this study was the individual association of SP-D, sRAGE, and Ang-2 with the global LUS score and with the RALE score obtained within the first 48 h after intubation. As secondary endpoints, we studied the association of the biomarkers with pulmonary edema in the following subgroups: *1*) in pulmonary versus nonpulmonary ARDS, and *2*) in COVID-ARDS versus non-COVID ARDS.

Statistical Analysis

Categorical data are expressed as numbers and percentages. Continuous data are expressed as mean ± standard deviation (SD) or median ± interquartile range (IQR). Differences between categorical variables were tested using the chi-square test. Differences between continuous variables were analyzed depending on parametric or nonparametric distribution using a *t* test or one-way ANOVA, or a Mann–Whitney *U* test or Kruskal–Wallis test, respectively. Tests were two-sided with a significance level of 0.05. We tested the degree of collinearity between the predictor variables by calculating the variance inflation factor (VIF). All statistical analyses were performed using R studio, version 4.0.3.

For analysis of the primary end point, linear regression was performed with the ^{10}log transformed plasma concentrations of SP-D, sRAGE, and Ang-2 as the independent variables and the global LUS or RALE score as the dependent variables. Moderation was examined by testing statistical significance of the interaction term in the regression model. The regression coefficient *beta*, which represents the change of the dependent variable per unit change of the predictor, was provided as measure of the effect size of the linear regression analysis. In the analysis of the primary outcome, we corrected *P* values for multiple comparisons using the Benjamini–Hochberg correction. For analysis of the secondary endpoints, the groups were stratified according to ARDS etiology (pulmonary versus nonpulmonary) and COVID-ARDS versus non-COVID ARDS. Last, plasma SP-D, sRAGE, and Ang-2 levels were included as predictor variables to evaluate their independent association with pulmonary edema. A log likelihood ratio test was performed to determine the strength of independent biomarker associations with pulmonary edema through comparing the models' goodness of fit.

A sensitivity analysis of the primary outcome, excluding patients admitted without respiratory pathology (i.e., planned surgery and neurosurgery patients without respiratory infection or aspiration upon admission), was also performed.

RESULTS

Patient Population

Three hundred sixty-two patients (70%) of the total 519 patients who were included in the DARTS project fulfilled inclusion criteria for this analysis based on the availability of biomarker measurements within 48 h after intubation (Supplemental Fig. S1). Of these patients, 137 (38%) fulfilled the Berlin criteria for ARDS and 225 (62%) did not. The distribution between ARDS of pulmonary versus nonpulmonary origin was 101 (74%) versus 36 (26%), respectively (Supplemental Fig. S1). Within the group of patients with ARDS, COVID-19 accounted for 45 (33%) cases (Supplemental Fig. S1). Patient characteristics are shown in Table 1.

Alveolar Epithelial Injury and the Association with Pulmonary Edema

Patients with ARDS, and specifically ARDS of pulmonary origin, had significantly higher levels of alveolar epithelial injury plasma biomarkers than patients without ARDS or patients with nonpulmonary ARDS (Supplemental Fig. S2, *A* and *B*). Notably, patients with COVID ARDS showed significantly higher SP-D levels compared with patients with ARDS due to other causes, whereas no such difference was found for sRAGE concentrations (Supplemental Fig. S2, *A* and *B*).

Table 1. *Patient characteristics stratified for ARDS*

	No ARDS *n* = 225	ARDS *n* = 137	*P* Value
Patient characteristics			
Age, yr, mean (SD)	61 (16)	63 (14)	0.42
Male, *n* (%)	155 (68.9)	95 (69.3)	1.0
BMI, kg/m^2, median [IQR]	26.1 [23.1, 29.7]	26.6 [23.7, 29.4]	0.42
Admission characteristics			
Admission type, *n* (%)			0.004
Medical	158 (70.2)	117 (85.4)	
Emergency surgical	37 (16.4)	10 (7.3)	
Planned surgical	30 (13.3)	10 (7.3)	
LIPS, median [IQR]	4.5 [3, 6]	6 [4.5, 7]	<0.001
Apache II score, median [IQR]	21 [15, 26]	20 [14, 24]	0.071
SOFA score, median [IQR]	9 [7, 11]	8 [6, 11]	0.094
Comorbidities, *n* (%)			
History of COPD	23 (10.2)	7 (5.1)	0.13
History of renal failure	17 (7.6)	6 (4.4)	0.33
History of heart failure	37 (16.4)	6 (4.4)	0.001
Ventilation and gas exchange			
Pa_{O_2}/FI_{O_2}, mmHg, median [IQR]	229 [156, 315]	120 [94, 158]	<0.001
Driving pressure, cmH_2O, median [IQR]	13.7 (5.2)	15.6 (6.1)	0.002
PEEP, cmH_2O, median [IQR]	7 [5, 8]	10 [8, 12]	<0.001
Imaging			
RALE score, median [IQR]	13 [8, 18]	20 [15, 27]	<0.001
Global LUS score, median [IQR]	5 [2, 9]	13 [8, 18]	<0.001
Outcomes			
ICU length of stay, days, median [IQR]	6 [3, 12]	9 [5, 16]	0.006
ICU mortality, *n* (%)	68 (31.6)	54 (40.9)	0.10

Apache II, acute physiology and chronic health evaluation score; ARDS, acute respiratory distress syndrome; BMI, body mass index; COPD, chronic obstructive pulmonary disease; FI_{O_2}, fraction of inspired oxygen; ICU, intensive care unit; IQR, interquartile range; LIPS, lung injury severity score; LUS, lung ultrasound; Pa_{O_2}, partial pressure of oxygen; PEEP, positive end-expiratory pressure; RALE, radiographic assessment of lung edema; SOFA, sequential organ failure assessment score at admission.

In the total population, increased levels of SP-D and sRAGE were significantly associated with higher scores of pulmonary edema (Fig. 1, Table 2). Before stratification into subgroups, the interaction by ARDS and ARDS etiology on the biomarker associations with the radiological scores was tested. It showed significant interaction terms in some of the subgroups (Supplemental Table S6), indicating differences in associations among them and justifying further subgroup exploration.

After stratification, SP-D concentrations remained significantly associated with the RALE score in all subgroups (Fig. 2*A* and Supplemental Table S2*A*), whereas this was not the case for the association with the global LUS score (Fig. 3*A* and Supplemental Table S2*B*). sRAGE concentrations were positively associated with the RALE score in all subgroups except for patients with nonpulmonary ARDS (Fig. 2*B* and Supplemental Table S2*A*). The association of sRAGE with the global LUS score did not reach statistical significance in any of the ARDS subgroups (Fig. 3*B* and Supplemental Table S2*B*).

Endothelial Dysfunction and the Association with Pulmonary Edema

There was no significant difference in Ang-2 plasma concentration between patients with ARDS and patients without ARDS (Supplemental Fig. S2*C*). Within the ARDS population, patients with a nonpulmonary etiology and ARDS unrelated to COVID-19 had the highest median Ang-2 levels (Supplemental Fig. S2*C*).

In the total population, there was a significant association of Ang-2 plasma concentration with an increased RALE score and global LUS score (Fig. 1, Table 2). After stratification, the association between Ang-2 levels and both pulmonary edema scores only reached statistical significance in patients without ARDS (Supplemental Fig. S3*C* and Supplemental Table S2).

The sensitivity analysis (n = 322) that excluded patients without respiratory pathology upon admission did not show significant differences in the results of the primary outcome (Supplemental Table S5).

No distinct differences in the studied associations were observed between COVID-ARDS and other forms of pulmonary ARDS, which is likely due to the overlap between the two groups (Fig. 1).

Independent Associations of SP-D, sRAGE, and Ang-2 with Pulmonary Edema

When considered independently, a higher plasma SP-D concentration was most strongly associated with the RALE and the global LUS score in the total population (R^2 of 0.20 and 0.076, respectively, Table 2), followed by an increase in sRAGE levels (R^2 of 0.13 and 0.056, Table 2). The addition of sRAGE as a covariate to SP-D levels significantly improved the explained variance of the model with the RALE and the global LUS score, as well as improving the fit characteristics on top of SP-D alone (Table 3, log likelihood ratio test $P < 0.001$). Addition of Ang-2 did not lead to further improvement.

Within the predefined subgroups of patients with nonpulmonary and non-COVID ARDS, an increase of SP-D concentration alone accounted for the highest explained variance in the models of association with the RALE score.

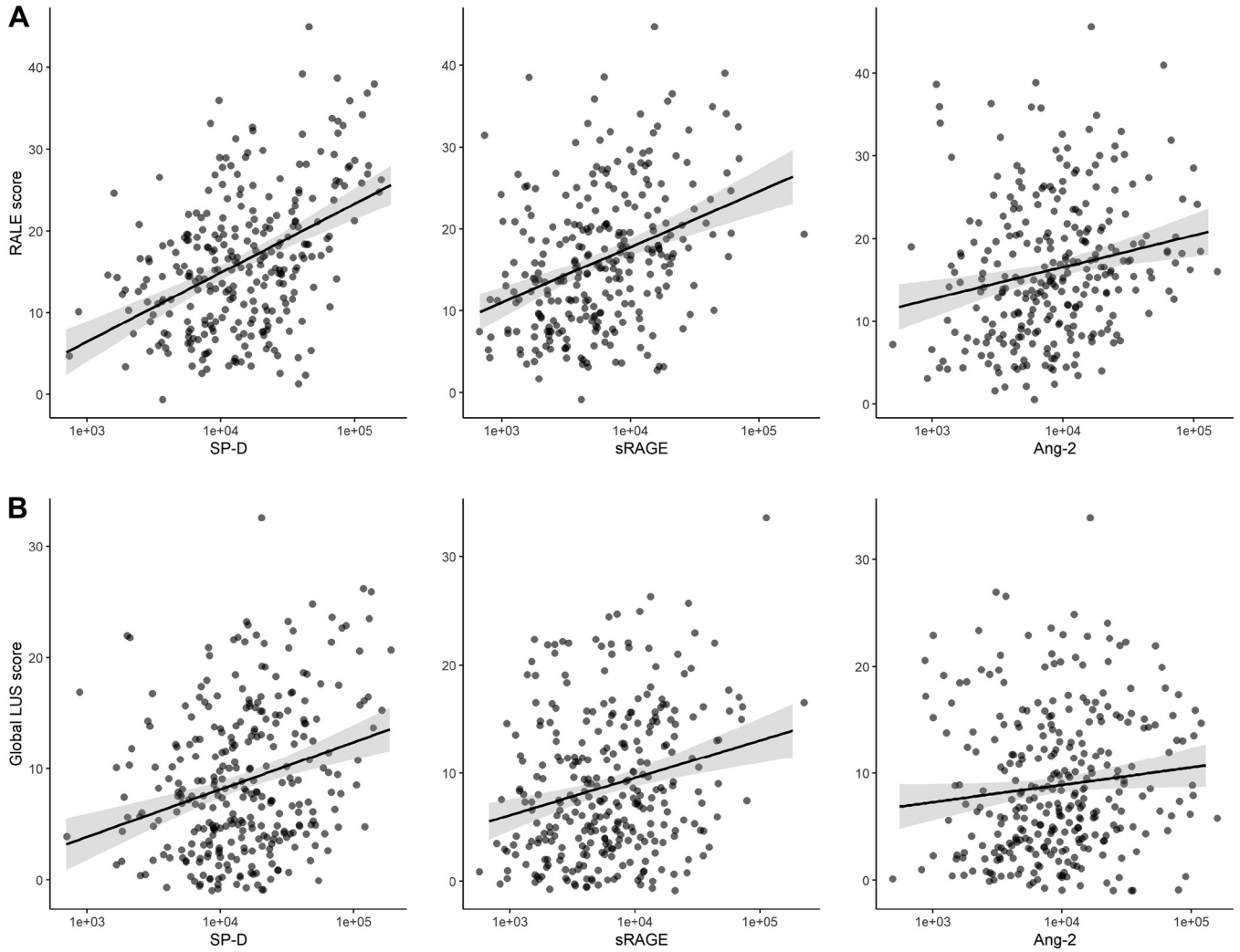

Figure 1. Association of plasma biomarkers [surfactant protein (SP)-D, soluble receptor for advanced glycation end products (sRAGE), and angiopoietin (Ang)-2] with the radiographic assessment of lung edema (RALE) score (*A*) and the global lung ultrasound (LUS) score (*B*) in the total population (n = 362). Increases in surfactant protein (SP)-D, soluble receptor for advanced glycation end products (sRAGE), and angiopoietin (Ang)-2 were all significantly associated with an increase of the RALE score [$\beta_{SP\text{-}D}$ (95% CI) = 8.4 units/log10 pg/mL (6.4–10.4), β_{sRAGE} (95% CI) = 6.8 units/log10 pg/mL (4.7–8.9), $\beta_{Ang\text{-}2}$ (95% CI) = 3.9 units/log10 pg/mL (1.7–6.1), all $P < 0.001$] and the global LUS score [$\beta_{SP\text{-}D}$ (95% CI) = 4.3 units/log10 pg/mL (2.6–5.9), β_{sRAGE} (95% CI) = 3.5 units/log10 pg/mL (1.9–5.1), $\beta_{Ang\text{-}2}$ (95% CI) = 1.6 units/log10 pg/mL (0.02–3.2), all $P < 0.05$]. Plasma biomarkers are represented as units/log10 pg/mL.

In the other subgroups, the addition of sRAGE improved model fit (Supplemental Table S4, *A–D*).

An addition of Ang-2 further improved the models in patients without ARDS and in patients with COVID ARDS (Supplemental Table S3*B* and Supplemental Table S4*C*, respectively).

Testing for collinearity between the biomarkers resulted in a VIF of <2 for all predictor variables, indicating low to moderate correlation and a low risk of the effects of collinearity on the interpretation of results.

DISCUSSION

In this study, we investigated the association between biomarkers of alveolar epithelial injury and endothelial dysfunction with scores of pulmonary edema during the first 48

Table 2. *Association between biomarkers and the RALE and global LUS score in the total population*

	RALE Score				Global LUS Score			
	β	95% CI	Adjusted *P* Value	Adjusted R^2	β	95% CI	Adjusted *P* Value	Adjusted R^2
SP-D	8.43	6.45–10.42	<0.001	0.20	4.26	2.59–5.92	<0.001	0.076
sRAGE	6.81	4.71–8.90	<0.001	0.13	3.45	1.86–5.05	<0.001	0.056
Ang-2	3.86	1.67–6.05	0.001	0.041	1.62	0.02–3.23	0.048	0.013

Linear regression model output of the association of biomarkers with the radiographic assessment of lung edema (RALE) and the global lung ultrasound (LUS) score in the total population.

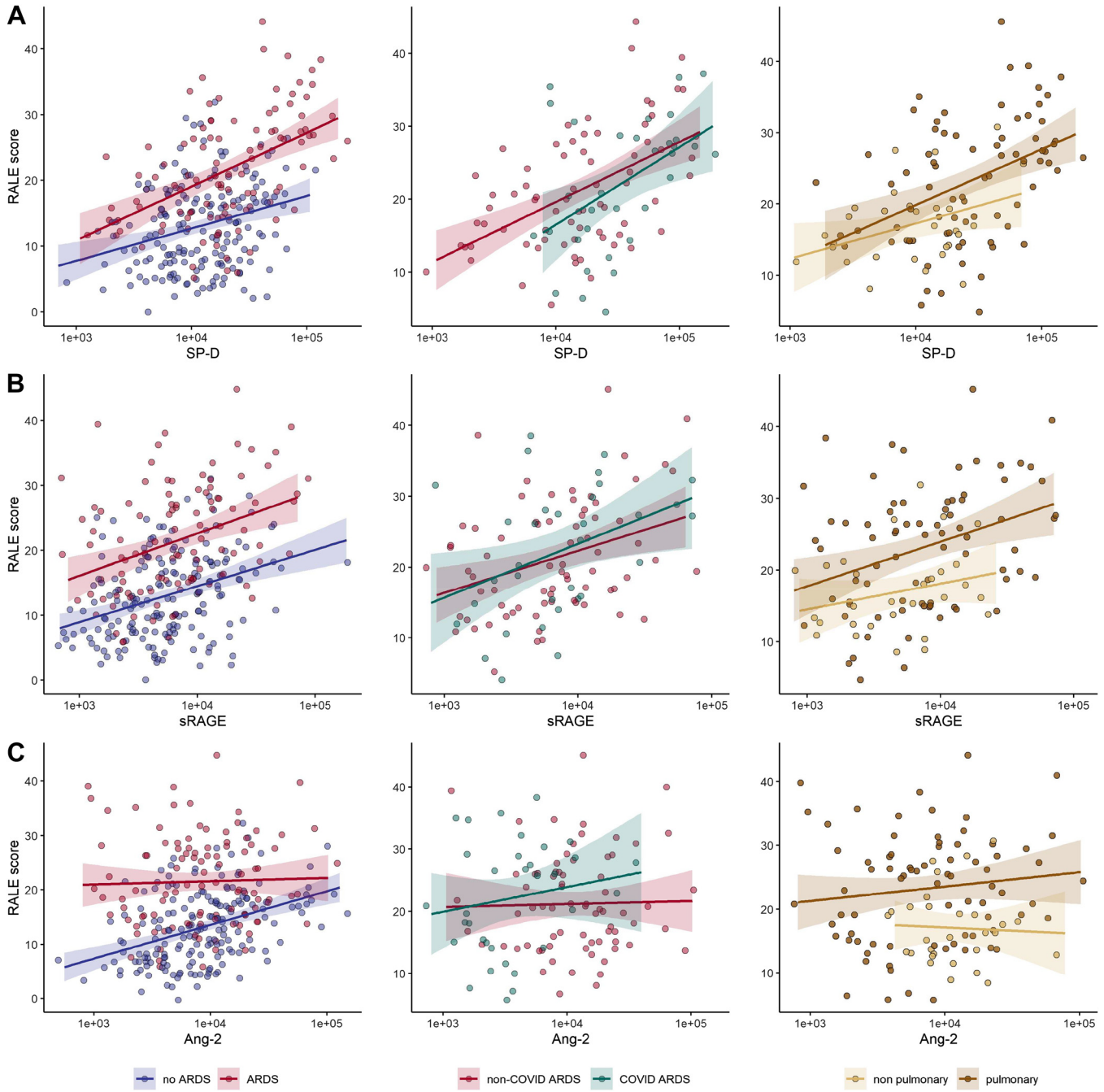

Figure 2. Association of surfactant protein (SP)-D (*A*), soluble receptor for advanced glycation end products (sRAGE; *B*), and angiopoietin (Ang)-2 (*C*) with the radiographic assessment of lung edema (RALE) score. Statically significant associations of the predictors with the RALE score: SP-D: in patients with and without ARDS [β (95% CI) = 8.17 units/log10 pg/mL (5.36–10.99) and 4.97 units/log10 pg/mL (2.49–7.46), both $P < 0.001$], COVID and non-COVID ARDS [β (95% CI) = 10.63 units/log10 pg/mL (3.52–17.73), $P = 0.004$ and 8.23 units/log10 pg/mL (5.07–11.39), $P < 0.001$], and pulmonary and nonpulmonary ARDS [β (95% CI) = 7.83 units/log10 pg/mL (3.98–11.69), $P < 0.001$ and 4.96 units/log10 pg/mL (0.42–9.50), $P = 0.04$]. sRAGE: in patients with and without ARDS [β (95% CI) = 6.50 units/log10 pg/mL (3.21–9.78) and 5.62 units/log10 pg/mL (3.44–7.80), both $P < 0.001$], COVID and non-COVID ARDS [β (95% CI) = 7.59 units/log10 pg/mL (1.33–13.84), $P = 0.017$ and 5.93 units/log10 pg/mL (2.04–9.82), $P = 0.004$], and pulmonary ARDS [β (95% CI) = 6.18 units/log10 pg/mL (2.23–10.13), $P = 0.003$]. Ang-2: in patients without ARDS [β (95% CI) = 6.19 units/log10 pg/mL (4.09–8.28), $P < 0.001$]. Tested associations did not reach statistical significance in the other subgroups. Plasma biomarkers are represented as units/log10 pg/mL. ARDS, acute respiratory distress syndrome.

h after the start of invasive ventilation. The main findings of this study are: *1*) biomarkers of alveolar epithelial injury and endothelial dysfunction are associated with pulmonary edema, quantified by the RALE and global LUS score, and *2*) from the studied biomarkers, SP-D is most strongly associated with pulmonary edema, irrespective of the selected subgroup.

The association of elevated plasma concentrations of SP-D, sRAGE, and Ang-2 with both surrogate scores of pulmonary edema seems to reflect the relationship between the

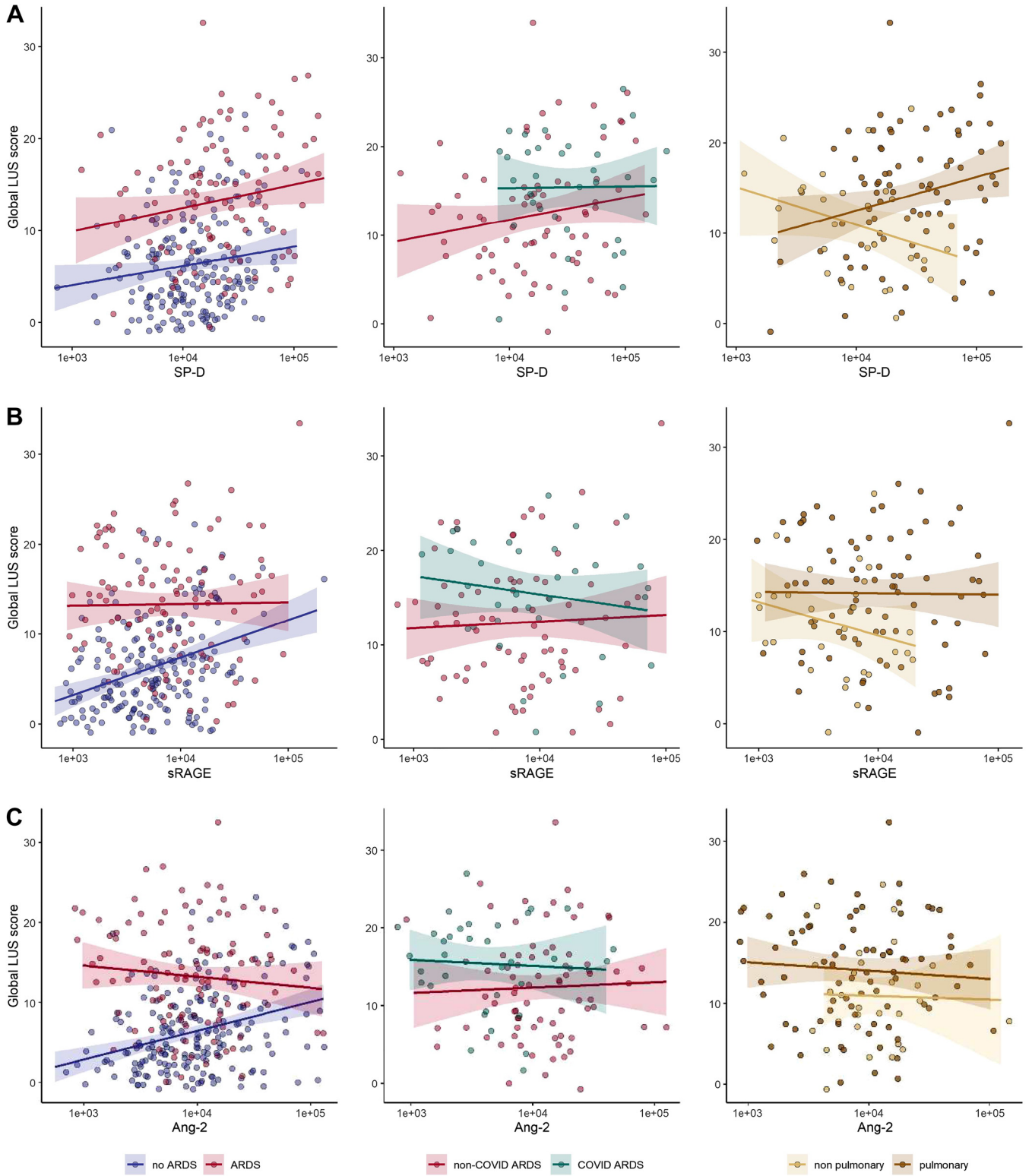

Figure 3. Association of surfactant protein (SP)-D (*A*), soluble receptor for advanced glycation end products (sRAGE; *B*), and angiopoietin (Ang)-2 (*C*) with the global lung ultrasound (LUS) score. Statically significant associations of the predictors with the global LUS score: SP-D: in patients without ARDS [β (95% CI) = 2.04 units/log10 pg/mL (0.13–3.95), $P = 0.04$] and in patients with pulmonary ARDS [β (95% CI) = 3.71 units/log10 pg/mL (0.40–7.02), $P = 0.03$]. sRAGE and Ang-2: in patients without ARDS [β (95% CI) = 4.15 units/log10 pg/mL (2.56–5.73) and 3.59 units/log10 pg/mL (2.14–5.04), respectively, both $P < 0.001$]. Tested associations did not reach statistical significance in the other subgroups. Plasma biomarkers are represented as units/log10 pg/mL. ARDS, acute respiratory distress syndrome.

Table 3. *Association between combinations of biomarkers and the RALE and global LUS score in the total population*

	RALE Score				Global LUS Score			
	β	95% CI	Adjusted *P* Value	Adjusted R^2	β	95% CI	Adjusted *P* Value	Adjusted R^2
SP-D	8.43	6.45–10.42	<0.001	0.20	4.26	2.59–5.92	<0.001	0.08
SP-D + sRAGE								
SP-D	6.79	4.62–8.96	<0.001	0.23	3.28	1.42–5.14	0.001	0.086
sRAGE	3.84	1.65–6.03	<0.001		2.06	0.29–3.82	0.034	
SP-D + sRAGE + Ang-2								
SP-D	6.95	4.75–9.15	<0.001	0.22	3.28	1.40–5.15	0.001	0.083
sRAGE	3.14	0.43–5.86	0.028		2.10	−0.01–4.20	0.06	
Ang-2	1.10	−1.39–3.55	0.39		−0.07	−1.94–1.81	0.95	

Linear regression model output of the independent associations of biomarkers with the radiographic assessment of lung edema (RALE) and the global lung ultrasound (LUS) score in the total population. *P* values were corrected for multiple testing using the Benjamini–Hochberg method.

molecular drivers underlying alveolar-capillary injury and the clinical hallmark of increased pulmonary vascular permeability in ARDS. Although Ang-2 was previously shown to be positively associated with the pulmonary leak index and transpulmonary extravascular lung water in patients with ARDS (10), the current study showed a significant association of both epithelial and endothelial injury biomarkers with two bedside-derived imaging scores of pulmonary edema. These findings add to the growing body of evidence linking biomarkers in patients with ARDS to clinical traits such as pulmonary edema. Moreover, the exploration of the biomarkers' differential contribution to pulmonary edema development seems to imply distinct roles of epithelial and endothelial injury in this process.

Ample preclinical and some clinical evidence indicates that vascular endothelial dysfunction is associated with edema formation (9, 10, 23–25). Ang-2 plays a role in sensitizing the pulmonary endothelium to inflammatory insults (26) and reflects increased endothelial permeability in sepsis and ARDS (9–11, 27). Because of the existing evidence, it was surprising that in this study, Ang-2 was not significantly associated with pulmonary edema in patients with ARDS. We suggest two possible explanations for this. First, Ang-2 levels may not only reflect endothelial dysfunction in the lung specifically but reflect systemic dysfunction of the vasculature (26, 28, 29). Therefore, increases in Ang-2 due to concomitant nonpulmonary organ injury might have masked the contribution of increased Ang-2 levels to pulmonary edema formation. Second, it is possible that in this study population, the relative contribution of endothelial dysfunction to pulmonary edema formation is smaller than that of epithelial injury.

Both plasma SP-D and sRAGE levels were more strongly associated with pulmonary edema than plasma Ang-2 concentration. Based on these findings, one might speculate that epithelial injury may act as the initial driver of pulmonary edema, preceding the contribution of endothelial dysfunction. Indeed, clinical studies examining the temporal characteristics of biomarkers in ARDS support this finding by showing a rise of epithelial injury markers during the first days after intubation (24, 28, 30–32). Interestingly, contrary to our initial hypothesis and despite finding significantly higher levels of epithelial versus endothelial injury biomarkers in pulmonary versus nonpulmonary ARDS, respectively, the epithelial injury markers were significantly associated with pulmonary edema in all ARDS subgroups, also outperforming plasma Ang-2 in nonpulmonary and non-COVID related ARDS. Although we cannot infer causality based on the current data, this surprising finding may indicate a common role of epithelial injury in pulmonary edema development in ARDS of pulmonary and nonpulmonary etiologies.

Of the epithelial injury markers, SP-D was most strongly associated with pulmonary edema, demonstrating an independent association with the RALE and global LUS score in the total population and in all predefined ARDS subgroups. SP-D is produced in the type II pneumocytes and bronchiolar cells, and loss of integrity of the alveolar-capillary membrane results in SP-D leakage into the systemic compartment (27, 33). sRAGE on the other hand, although an alveolar epithelial injury marker, can also be elevated in septic shock without ARDS, trauma, and renal injury (34–36). In addition, it plays a proinflammatory role in the innate immune response and can be found in several nonpulmonary sites (25, 27). Hence, of the two epithelial markers, sRAGE may be less suited than SP-D to predict pulmonary edema.

Last, biomarker association was more marked with pulmonary edema quantified by the RALE score compared with the global LUS score. Two aspects may play a role here. First, technical aspects of chest radiography and lung ultrasound result in inherent differences. The RALE score quantifies loss of aeration in the entire lung, and the global LUS score only focusses on the subpleural region. The RALE score was validated for the detection of pulmonary edema (12) and it requires the rater to differentiate between opacifications attributed to alveolar edema and parenchymal consolidations when assigning points. The global LUS score on the other hand quantifies the loss of aeration, without differentiating between underlying causes. Thereby, quantification of loss of aeration by the global LUS score may be more susceptible to overestimate pulmonary edema. Although the use of the global LUS score in ARDS research should by no means be dismissed, this possibly explains its weaker correlation with plasma biomarkers. A limitation of both scores is that a ceiling effect may occur when scoring severe edema, as the scores will inherently reach a point at which they will less accurately discriminate between increasing degrees of opacification. This may affect the reliability of scoring patients with severe edema.

The main strengths of this study are the inclusion of consecutive patients and the combination of different biomarkers

and radiological tools for pulmonary edema quantification. However, some limitations of this study should be acknowledged. First, only invasively ventilated patients were included, excluding patients on high-flow nasal oxygen who can also have pulmonary edema (19). Second, this study only examined single time point biomarker samples collected in the first 48 h after intubation. Previous research shows that in patients with ARDS, epithelial and endothelial biomarker concentrations change in the course of time (24, 34). Multiple time points would have allowed an investigation of the effect of potential temporal changes in biomarker levels on their association with pulmonary edema. Third, plasma markers are mere surrogates for alveolar-capillary barrier injury, and the biological processes influencing biomarker concentrations are still incompletely understood. Last, the sample size limited the power of the subgroup analyses, and the moderate strength of associations determined by correlation analysis limit the significance of the findings. Thus, it needs to be underscored that this work is of hypothesis-generating nature.

Our findings have implications for future research aimed at understanding ARDS pathophysiology and heterogeneity. SP-D should be considered more widely as a biomarker of pulmonary edema and could be used as a surrogate if the identified associations are independently confirmed in serial analyses. Furthermore, there seems to be an etiology-independent relation between pulmonary edema and SP-D concentration, suggesting that this could be a biomarker of a final common pathway in alveolar-capillary barrier injury. The presented data also implies that plasma Ang-2 concentrations alone are unlikely to be a good representative of the endothelial dysfunction contributing to alveolar-capillary barrier injury, given the poor association with pulmonary edema.

Conclusions

In invasively ventilated ICU patients, the epithelial injury markers SP-D and sRAGE showed a stronger association with pulmonary edema, quantified by the RALE and global LUS score, than the endothelial dysfunction marker Ang-2. The finding that an elevated plasma SP-D concentration is associated with pulmonary edema irrespective of underlying etiology suggests that epithelial injury may form a common pathway associated with pulmonary edema development.

DATA AVAILABILITY

Data will be made available upon reasonable request.

SUPPLEMENTAL DATA

Supplemental Figs. S1 and S2 and Supplemental Tables S1–S6: https://doi.org/10.6084/m9.figshare.20060552.

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The funder had no role in the study design, data collection, analysis, or data interpretation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.N.A. and L.D.J.B. conceived and designed research; N.F.L.H. and L.A.H. performed experiments; L.N.A. analyzed data; L.N.A. interpreted results of experiments; L.N.A. prepared figures; L.N.A. drafted manuscript; L.N.A., N.F.LH., M.R.S., L.A.H., D.F.L.F., C.Z., M.J.S., R.M.S., D.C.J.J.B., J.A., and L.D.J.B. edited and revised manuscript; L.N.A. and L.D.J.B. approved final version of manuscript.

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