neutrophils and occlude the pulmonary vasculature in a caspase 1– and IL-1–dependent manner.

These findings are intriguing because they shed new light on platelet-derived EVs in SCD and link platelet-derived EVs to previously described roles of platelet–neutrophil aggregates in ACS. The authors postulate that platelet EVs may potentiate the formation of heterotypic platelet–neutrophil aggregates, resulting in additional EV shedding in a positive feedback loop. These investigations also bring to light the potential that additional TLRs may contribute to activation of the inflammasome, platelet EV shedding, and the formation of platelet–neutrophil aggregates in SCD, an important area for further investigation. For example, TLR9 is known to bind free heme (generated during hemolysis, a prominent feature of SCD). Gram-positive bacteria and porins (components of encapsulated organism cell walls) are ligands for TLR2. Thus, additional TLRs could be targets for future investigations. In addition, the role of EVs derived from red blood cells and endothelial cells in driving vaso-occlusive crisis remains incompletely understood. Finally, these findings indicate that inhibiting the shedding of platelet EVs may be of therapeutic benefit in SCD, particularly with regard to prevention or treatment of ACS. Indeed, these novel data may function as a catalyst to employ pharmacologically available inhibitors of IL-1B (anakinra), TLR4 (eritoran), and caspase 1 (VX-765) in clinical trials of vaso-occlusive crisis in patients with SCD. ■

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References

1. Vats R, Brzoska T, Bennewitz MF, Jimenez MA, Pradhan-Sundd T, Tutuncuoglu E, *et al*. Platelet extracellular vesicles drive inflammasome–IL-1β-dependent lung injury in sickle cell disease. *Am J Respir Crit Care Med* 2020;201:33–46.
2. Rumbaut RE, Thiagarajan P. Platelet-vessel wall interactions in hemostasis and thrombosis. San Rafael, CA: Morgan & Claypool Life Sciences; 2010.
3. Coenen DM, Mastenbroek TG, Cosemans JMEM. Platelet interaction with activated endothelium: mechanistic insights from microfluidics. *Blood* 2017;130:2819–2828.
4. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. *Blood* 2014;123:2759–2767.
5. Middleton EA, Rondina MT, Schwertz H, Zimmerman GA. Amicus or adversary revisited: platelets in acute lung injury and acute respiratory distress syndrome. *Am J Respir Cell Mol Biol* 2018;59:18–35.
6. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. *N Engl J Med* 2017; 376:1561–1573.
7. Anea CB, Lyon M, Lee IA, Gonzales JN, Adeyemi A, Falls G, *et al*. Pulmonary platelet thrombi and vascular pathology in acute chest syndrome in patients with sickle cell disease. *Am J Hematol* 2016;91:173–178.
8. Bennewitz MF, Jimenez MA, Vats R, Tutuncuoglu E, Jonassaint J, Kato GJ, *et al*. Lung vaso-occlusion in sickle cell disease mediated by arteriolar neutrophil-platelet microemboli. *JCI Insight* 2017;2:e89761.
9. Zhang G, Han J, Welch EJ, Ye RD, Voyno-Yasenetskaya TA, Malik AB, *et al*. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. *J Immunol* 2009;182:7997–8004.
10. Claushuis TAM, Van Der Veen AIP, Horn J, Schultz MJ, Houtkooper RH, Van 't Veer C, *et al*. Platelet Toll-like receptor expression and activation induced by lipopolysaccharide and sepsis. *Platelets* 2019;30:296–304.
11. Brown GT, McIntyre TM. Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1β-rich microparticles. *J Immunol* 2011;186:5489–5496.
12. Ståhl AL, Sartz L, Nelsson A, Békássy ZD, Karpman D. Shiga toxin and lipopolysaccharide induce platelet-leukocyte aggregates and tissue factor release, a thrombotic mechanism in hemolytic uremic syndrome. *PLoS One* 2009;4:e6990.

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Discovering Causal Mechanistic Pathways in Sepsis-associated Acute Respiratory Distress Syndrome

In this issue of the *Journal*, Jones and colleagues (pp. 47–56) find that sRAGE (soluble receptor for advanced glycation end products) blood levels are related to sepsis-associated acute respiratory distress syndrome (ARDS) (1). Moreover, they make the bold claim that sRAGE causally contributes to sepsis-associated ARDS. This is an important step to take, for multiple reasons. First, the sepsis and ARDS fields are littered with hundreds of reports of an association between inflammatory pathway molecule measurements and clinical outcomes (2). Although they are interesting, these associations have not led to the introduction of new therapies to prevent or ameliorate sepsis-induced lung injury/ARDS or any other clinically important outcome (3). Second, identification of a true causal pathway points the field toward a plausible intervention strategy and relevant biomarkers,

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as modulation of a causal pathway would result in altered clinically relevant outcomes, not just a change in a blood cytokine level. Third, claiming causality is bold because it invites us to examine the methodology that supports the inference of causality (4). Understanding when the data support causal inference and when they do not is a key issue that many previous association studies have not adequately addressed. This may have contributed to the critical care medicine field embarking on inadequately supported interventional approaches, as indicated by the many failed sepsis-related clinical trials (5). Examining this methodology also makes us ask, what additional information should we seek, and what are the best next research steps? Fourth, using the current study by Jones and colleagues as a "proof of principle," it is clear that we may be able to extract more knowledge from a thoughtful combination of clinical and basic biological measures from our current and growing patient datasets.

Is sRAGE, among Many Inflammatory Biomarkers, a Good Starting Point?

Many blood biomarkers have been reported to be associated with the development of ARDS (6). Among these, the investigators chose to study sRAGE (1). The investigators support this as a reasonable choice by referring to the substantial body of evidence already in place that demonstrates that RAGE is found in the right place (lung [7]), at the right time (when an inflammatory response is induced [8]), doing the right thing (potentiates the inflammatory response [9]), and with the right effect on perturbation (RAGE knockout mice have better clinically related outcomes [10]) to allow the hypothesis of causality to be considered. But does this model-derived knowledge translate to the clinically relevant outcome of sepsis-associated ARDS in humans? Evidence of causality in humans is therefore critically important.

Approaches to Causal Inference

The most common approach to test for causality is to intentionally modify the putative causal agent and then measure the effect. This works in animal models (e.g., RAGE knockout mice [10]) but typically is very challenging and/or very expensive to do in humans (e.g., clinical trials of experimental interventions in humans). A more recently applied approach to infer causality from observational data is to use instrumental variables (4). For example, if a new tobacco tax (the statistical instrument that reduces smoking) reduces the incidence of lung cancer, we can infer that smoking causally contributes to lung cancer because there is no other plausible way that a tobacco tax could have this effect (11). When genetic variation is used as the statistical instrument, the instrumental variable approach is often called Mendelian randomization, because the genotype is "randomly" assigned at conception and is not influenced by a host of confounding issues (measurement and environmental factors, considered broadly) (4).

Rather than using a single genetic variant, these investigators used multiple genetic variants, associated to varying degrees with sRAGE levels, as statistical instruments. They then tested for the associations that allow for causal inference. First, the investigators confirmed that sRAGE is associated with sepsis-associated ARDS. Then, they found that sRAGE genetic instruments that were associated with greater differences in sRAGE levels were also associated with a greater effect on sepsis-associated ARDS, and genetic instruments that were associated with smaller differences in sRAGE levels were proportionally associated with a smaller effect on sepsis-associated ARDS (Figure 2 in Reference 1). Based on these findings, the investigators reasoned (by the Mendelian randomization flow of logic) that the sRAGE genetic instruments could only be plausibly associated with sRAGE levels and sepsis-associated ARDS in this way if sRAGE causally contributed to sepsis-associated ARDS.

Examining Limitations of the Methodology and Next Steps

From previous work, it is clear that the RAGE pathway modulates the septic inflammatory response (7–10). The current data build on that knowledge and support the hypothesis that the RAGE pathway causally contributes to the development of sepsis-associated ARDS in humans. But this is just a start. Was it really sRAGE? It seems that sRAGE genetic instruments would also be effective in reflecting a molecule directly upstream within the RAGE pathway that may be the true causal contributor, with sRAGE being a proportional but noncausal byproduct. In addition, as these investigators point out, this analysis supports the hypothesis of causality but does not define the underlying mechanism of causality, indicating a need for future investigations. Furthermore, it would be helpful to validate the genetic scores independently to reduce the potential for type 1 error when nearly a million variants were considered. Would the same genetic instruments predict a somewhat similar effect on sRAGE levels in separate populations, and would the same genetic instruments yield similar associations with the development of ARDS in an analysis of a completely separate population? The limited overlap of genetic variants (and even genes) identified in European ancestry and African ancestry populations highlight this concern. Effect size is an even more important issue that this statistical methodology starts to address; that is, would an intervention impacting the RAGE pathway have a sufficiently large effect to meaningfully alter clinically relevant outcomes?

A very positive outcome of this study is that sRAGE, as an intermediate phenotype, becomes a powerful tool in the route toward developing novel therapeutic interventions. A phase 2 trial of a novel intervention likely would not be powered to detect a statistically significant difference in a relevant clinical outcome. However, a relatively small phase 2 trial may be sufficiently powerful to detect a significant difference in sRAGE levels that, based on the current work, may predict clinical benefit. The simple measurement of sRAGE levels opens up the therapeutic development field to investigators who do not have the same financial resources that big pharma may have. Simple but creative ideas can also be tested; for example, do different ventilation or resuscitation strategies alter sRAGE?

Proof of Principle

Stepping outside of the RAGE pathway, this study demonstrates that the large datasets that are being developed can be powerful discovery tools pointing toward potential new therapeutic strategies (12). These valuable datasets optimally would contain clinically relevant physiological and outcome data, intermediate phenotype data (e.g., protein, gene expression, and other omic measurements), and genotype measurements (13). There is no fundamental reason why even complex multipathway approaches and precision medicine approaches (i.e., biomarker-defined subpopulations) could not be examined, so long as the study populations are very large. ■

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References

1. Jones TK, Feng R, Kerchberger VE, Reilly JP, Anderson BJ, Shashaty MGS, *et al*. Plasma sRAGE acts as a genetically regulated causal intermediate in sepsis-associated acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2020;201:47–56.
2. Walley KR. Biomarkers in sepsis. *Curr Infect Dis Rep* 2013;15:413–420.
3. Russell JA, Spronk P, Walley KR. Using multiple 'omics strategies for novel therapies in sepsis. *Intensive Care Med* 2018;44:509–511.
4. Thomas DC, Conti DV. Commentary: the concept of 'Mendelian randomization'. *Int J Epidemiol* 2004;33:21–25.
5. Marshall JC. Why have clinical trials in sepsis failed? *Trends Mol Med* 2014;20:195–203.
6. Spadaro S, Park M, Turrini C, Tunstall T, Thwaites R, Mauri T, *et al*. Biomarkers for acute respiratory distress syndrome and prospects for personalised medicine. *J Inflamm (Lond)* 2019;16:1.
7. Nakamura T, Sato E, Fujiwara N, Kawagoe Y, Maeda S, Yamagishi S. Increased levels of soluble receptor for advanced glycation end products (sRAGE) and high mobility group box 1 (HMGB1) are associated with death in patients with acute respiratory distress syndrome. *Clin Biochem* 2011;44:601–604.
8. Guo WA, Knight PR, Raghavendran K. The receptor for advanced glycation end products and acute lung injury/acute respiratory distress syndrome. *Intensive Care Med* 2012;38:1588–1598.
9. Han SH, Kim YH, Mook-Jung I. RAGE: the beneficial and deleterious effects by diverse mechanisms of actions. *Mol Cells* 2011;31: 91–97.
10. Reynolds PR, Schmitt RE, Kasteler SD, Sturrock A, Sanders K, Bierhaus A, *et al*. Receptors for advanced glycation end-products targeting protect against hyperoxia-induced lung injury in mice. *Am J Respir Cell Mol Biol* 2010;42:545–551.
11. Walley KR, Boyd JH, Kong HJ, Russell JA. Low low-density lipoprotein levels are associated with, but do not causally contribute to, increased mortality in sepsis. *Crit Care Med* 2019; 47:463–466.
12. Au Yeung SL, Luo S, Schooling CM. The impact of glycated hemoglobin on risk of hypertension: a Mendelian randomization study using UK Biobank. *J Hypertens* [online ahead of print] 1 Aug 2019; DOI: 10.1097/HJH.0000000000002210.
13. Sutherland AM, Walley KR. Bench-to-bedside review: association of genetic variation with sepsis. *Crit Care* 2009;13:210.

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Asbestos, Pleural Plaques, and Lung Cancer: Untangling the Relationships

Asbestos exposure remains an important public health and clinical problem; industrial use has been significantly reduced but not eliminated (1). Significant risks of asbestosis, lung cancer, malignant mesothelioma, and other effects continue for many former exposed workers and family members with prior paraoccupational exposure. Pleural plaques, or localized thickening of the parietal pleura, are the most common consequence of asbestos exposure (2). Assessing the relationship between pleural plaques and lung cancer risk is particularly timely now that low-dose computed tomography (LDCT) screening of high-risk tobacco smoking–exposed populations has been demonstrated to reduce mortality. High-resolution computed tomographic screening policy largely focuses on tobacco smokers and inadequately addresses persons with significant risk from asbestos or other occupational carcinogens.

Brims and colleagues (3) in this issue of the *Journal* (pp. 57–62) provide very important data on the relationship of pleural plaques to lung cancer risk. Asbestos exposure causes lung cancer and produces pleural plaques. The paper by Brims and colleagues addresses the important question, "Among persons with known moderate-heavy asbestos exposure, do those with pleural plaques have increased lung cancer risk relative to similarly exposed persons without plaques?" All participants were known to have significant asbestos exposure. The investigators used Cox regression analyses to assess whether pleural plaques were associated with an elevated hazard ratio (HR) for lung cancer. The analyses were adjusted for asbestos exposure, sex, tobacco smoking, and the presence of asbestosis. Pleural plaque status was determined from the most recent radiographic imaging (either CT or chest radiography) or the most recent imaging at least 1 year before cancer diagnosis.

The authors conclude that plaques *per se*, when adjusted for the extent of asbestos exposure and other risk factors, do not enhance the risk of lung cancer. This has important implications for patient counseling and selecting participants to optimize the benefit–risk relationship for individuals and programmatic cost-effectiveness of LDCT screening.

This study has unique strengths. The results were consistent in two distinct, well-defined cohorts. Western Australian crocidolite asbestos miners and community members with extensive residential exposure comprise the first cohort. The second cohort is a nationwide collection of workers in occupations well known to have extensive exposure. The Australian surveillance program is particularly effective at accurately assessing each participant's individual cumulative exposure (4–7). The long latency between initial asbestos exposure and development of malignancy requires long-term studies; the Australian program includes annual

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