2. Hartl S, Lopez-Campos JL, Pozo-Rodriguez F, Castro-Acosta A, Studnicka M, Kaiser B, *et al*. Risk of death and readmission of hospital-admitted COPD exacerbations: European COPD audit. *Eur Respir J* 2016;47:113–121.
3. Aaron SD, Vandemheen KL, Maltais F, Field SK, Sin DD, Bourbeau J, *et al*. TNFα antagonists for acute exacerbations of COPD: a randomised double-blind controlled trial. *Thorax* 2013;68:142–148.
4. Mackay AJ, Patel ARC, Singh R, Sapsford RJ, Donaldson GC, Prasad N, *et al*. Randomized double-blind controlled trial of roflumilast at acute exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2017;196:656–659.
5. Seemungal TA, Donaldson GC, Bhowmik A, Jeffries DJ, Wedzicha JA. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;161:1608–1613.
6. Donaldson GC, Law M, Kowlessar B, Singh R, Brill SE, Allinson JP, *et al*. Impact of prolonged exacerbation recovery in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2015;192:943–950.
7. Hurst JR, Donaldson GC, Quint JK, Goldring JJP, Baghai-Ravary R, Wedzicha JA. Temporal clustering of exacerbations in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2009;179:369–374.
8. Seemungal TA, Wilkinson TM, Hurst JR, Perera WR, Sapsford RJ, Wedzicha JA. Long-term erythromycin therapy is associated with decreased chronic obstructive pulmonary disease exacerbations. *Am J Respir Crit Care Med* 2008;178:1139–1147.
9. Albert RK, Connett J, Bailey WC, Casaburi R, Cooper JA Jr, Criner GJ, *et al*.; COPD Clinical Research Network. Azithromycin for prevention of exacerbations of COPD. *N Engl J Med* 2011;365:689–698.
10. Krishnan JK, Voelker H, Connett JE, Niewoehner DE, Albert RK, Scanlon PD, *et al*.; COPD Clinical Research Network Investigators. Effect of daily azithromycin therapy and adherence on readmission risk in COPD. *Eur Respir J* 2019;53:1801377.
11. Segal LN, Clemente JC, Wu BG, Wikoff WR, Gao Z, Li Y, *et al*. Randomised, double-blind, placebo-controlled trial with azithromycin selects for anti-inflammatory microbial metabolites in the emphysematous lung. *Thorax* 2017;72:13–22.
12. Vermeersch K, Gabrovska M, Aumann J, Demedts IK, Corhay J-L, Marchand E, *et al*. Azithromycin during acute chronic obstructive pulmonary disease exacerbations requiring hospitalization (BACE): a multicenter, randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med* 2019;200:857–868.
13. Donaldson GC, Goldring JJ, Wedzicha JA. Influence of season on exacerbation characteristics in patients with COPD. *Chest* 2012;141:94–100.

NET Gain for Sepsis Research: A New Approach to Assess Neutrophil Function in Patients

Neutrophil extracellular trap (NET) formation, a feature of neutrophils that involves extracellular release of a DNA web with attached histones and proteolytic enzymes, plays a critical role in the immune response to infection by trapping and preventing the dissemination of pathogens (1, 2). However, it is now well recognized that the release of NETs can also contribute to tissue injury in several pathologic conditions, including acute lung injury (3), thrombosis (4), and sepsis (5). Thanks to decades of research, we now have a deep understanding of the characteristics of NETs and subsequent effects on different organ systems in experimental models. However, there is still a gap in our knowledge regarding how we can use this information to improve clinical outcomes, especially during a critical illness. Although many assays have been developed to detect circulating NET components, including cell-free DNA, MPO (myeloperoxidase), and histones (6–8), these markers of already released NETs are not specific and may not always correlate with disease severity or outcomes. Furthermore, they are subject to degradation and clearance, which limits their potential to provide meaningful clinical information.

In an elegant study in this issue of the *Journal*, Abrams and colleagues (pp. 869–880) developed a novel assay to test the potential of plasma from patients with sepsis to stimulate healthy human neutrophils to release NETs (9). They then used this approach to prospectively test the association between plasma NET-forming capacity and clinical outcomes of ICU patients with sepsis. Using this new method, the authors discovered that the NET-forming capacity of plasma was independently associated with disease severity, the development of disseminated intravascular coagulation, organ injury, and mortality during critical illness. Importantly, the NET-forming capacity of plasma does not seem to be dependent on the neutrophil donor, and no plasma from healthy donors stimulated NETs. The assay procedure is relatively simple and straightforward, assuming that someone with the necessary expertise in neutrophil isolation, immunofluorescence, and microscopy, as well as fresh donor neutrophils, would be available when needed. An inability to meet these requirements could be a potential shortcoming of the assay. In addition, the time requirement of the assay (at least 4 h for stimulation of neutrophils, in addition to sample collection, neutrophil isolation, staining, and imaging) may not necessarily be an improvement from the predictive scoring mechanisms already in place, especially considering that the investigators found no significant improvement in the predictive capacity of this assay compared with Acute Physiology and Chronic Health Evaluation (APACHE) II or Sequential Organ Failure Assessment (SOFA). Nevertheless, this outside-the-box approach could provide additional insight into patient outcomes, as well as underlying pathological processes during a critical illness.

When they further investigated the NET-forming capacity of individual plasma samples, the authors identified IL-8 as a key component. In fact, blocking IL-8 using an antibody or receptor antagonist, or downstream mitogen-activated protein kinase signaling, removed the ability of patient plasma to induce NETs,

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although IL-8 levels alone could not predict disseminated intravascular coagulation or mortality. This finding is of particular interest because IL-8 receptor antagonists are currently being tested in clinical trials. Although these antagonists seem to be well tolerated in healthy humans (10) and have shown promise in murine models of sepsis (11), it remains to be determined how they will fare in critically ill patients. Of potential concern, IL-8 receptor antagonists can block the signaling of several ligands in multiple cell types, which could result in off-target effects during systemic inflammation. Nonetheless, this study has brought the importance of IL-8–induced NET formation during critical illness to light, and elicits further investigation into targeting this pathway therapeutically.

Looking beyond critical illness, this assay has the potential to be used for broader applications, as NETs are known to play a role in various diseases, including autoimmune disease (12), diabetes (13), atherosclerosis (14), and cancer (15). It would be interesting to determine whether this assay could assist in the early detection of some of these more chronic conditions or help improve outcomes. Expanding the possibilities of this approach even further, it would be worthwhile to consider whether additional functional measures (i.e., other functions of neutrophils, such as respiratory burst, or other cell types) could be tested using patient plasma samples to predict clinical outcomes. This unique way of thinking has the potential to be far-reaching.

The novel approach of using NETs as predictive biomarkers raises a few important questions. First, does NET formation cause worse outcomes during critical illness, or is it solely an indicator of enhanced inflammation? If NET formation contributes to disease progression, is it possible to intervene to inhibit or reverse the outcome? What other factors in plasma contribute to NET formation, and do these factors differ among patients or pathological stimuli? Given that therapies targeting cytokines and NETs have shown varied results, this assay could potentially help to inform the use of specific therapies based on a patient's own plasma sample, resulting in a more personalized, targeted approach. The capacity of this approach to predict complications of disease might also improve prevention strategies for higher-risk patients. Importantly, this strategy has the potential to reveal new therapeutic targets using human clinical data, complementing studies of therapeutic targets discovered using preclinical animal models. In conclusion, although the novel approach proposed by Abrams and colleagues, which uses the NET-forming capacity of plasma to predict patient outcomes in critical illness, does not provide a direct measure of NETs or NET-induced injury, it is a great step toward understanding the role of NETs in sepsis and may help to inform potential therapies for critical illness and patient care in the ICU. ■

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References

1. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, *et al*. Neutrophil extracellular traps kill bacteria. *Science* 2004;303:1532–1535.
2. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* 2012;12:324–333.
3. Lefrançais E, Mallavia B, Zhuo H, Calfee CS, Looney MR. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. *JCI Insight* 2018;3:98178.
4. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. *Blood* 2014;123:2768–2776.
5. McDonald B, Davis RP, Kim SJ, Tse M, Esmon CT, Kolaczkowska E, *et al*. Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. *Blood* 2017;129:1357–1367.
6. Hirose T, Hamaguchi S, Matsumoto N, Irisawa T, Seki M, Tasaki O, *et al*. Presence of neutrophil extracellular traps and citrullinated histone H3 in the bloodstream of critically ill patients. *PLoS One* 2014;9:e111755.
7. Wang H, Sha L-L, Ma T-T, Zhang L-X, Chen M, Zhao M-H. Circulating level of neutrophil extracellular traps is not a useful biomarker for assessing disease activity in antineutrophil cytoplasmic antibody-associated vasculitis. *PLoS One* 2016;11:e0148197.
8. Garnacho-Montero J, Huici-Moreno MJ, Gutiérrez-Pizarraya A, López I, Márquez-Vácaro JA, Macher H, *et al*. Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin, and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. *Crit Care* 2014;18:R116.
9. Abrams ST, Morton B, Alhamdi Y, Alsabani M, Lane S, Welters ID, *et al*. A novel assay for neutrophil extracellular trap formation independently predicts disseminated intravascular coagulation and mortality in critically ill patients. *Am J Respir Crit Care Med* 2019;200:869–880.
10. Cullberg M, Arfvidsson C, Larsson B, Malmgren A, Mitchell P, Wählby Hamrén U, *et al*. Pharmacokinetics of the oral selective CXCR2 antagonist AZD5069: a summary of eight phase I studies in healthy volunteers. *Drugs R D* 2018;18:149–159.
11. Wang M, Zhong D, Dong P, Song Y. Blocking CXCR1/2 contributes to amelioration of lipopolysaccharide-induced sepsis by downregulating substance P. *J Cell Biochem* [online ahead of print] 30 Aug 2018; DOI: 10.1002/jcb.27507.
12. Lee KH, Kronbichler A, Park DD, Park Y, Moon H, Kim H, *et al*. Neutrophil extracellular traps (NETs) in autoimmune diseases: a comprehensive review. *Autoimmun Rev* 2017;16:1160–1173.
13. Wong SL, Demers M, Martinod K, Gallant M, Wang Y, Goldfine AB, *et al*. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat Med* 2015;21:815–819.
14. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation: neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science* 2015;349:316–320.
15. Cedervall J, Zhang Y, Huang H, Zhang L, Femel J, Dimberg A, *et al*. Neutrophil extracellular traps accumulate in peripheral blood vessels and compromise organ function in tumor-bearing animals. *Cancer Res* 2015;75:2653–2662.