the total airway count is related to disease progression in COPD (8), and further work to explain this is needed. Possibly, the inclusion of oscillometric variables, which seem to identify impaired exercise performance in smokers, would be useful here (6).

Whatever its limitations, the CanCOLD exercise data set is a remarkable achievement that takes our understanding of exercise limitation in COPD to a new level. Clearly, mechanical constraints associated with dynamic increase in end-expiratory lung volume during exercise restrict performance in patients with well-established COPD. However, there is now evidence that ventilatory inefficiency plays a role in those with relatively preserved lung function and even in those for whom airflow obstruction has yet to develop. This study broadens our understanding of impaired exercise performance, suggests new approaches to its understanding, and will no doubt be succeeded by more exciting data. It is a fine addition to the long and honorable history of Canadian research into the pathophysiology of COPD. ■

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References

1. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. *N Engl J Med* 1968;278: 1355–1360.
2. Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med* 1987;106:196–204.
3. Hargreave FE, Leigh R. Induced sputum, eosinophilic bronchitis, and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:S53–S57.
4. O'Donnell DE, Laveneziana P, Webb K, Neder JA. Chronic obstructive pulmonary disease: clinical integrative physiology. *Clin Chest Med* 2014; 35:51–69.
5. Elbehairy AF, Ciavaglia CE, Webb KA, Guenette JA, Jensen D, Mourad SM, *et al.*; Canadian Respiratory Research Network. Pulmonary gas exchange abnormalities in mild chronic obstructive pulmonary disease. Implications for dyspnea and exercise intolerance. *Am J Respir Crit Care Med* 2015;191: 1384–1394.
6. Elbehairy AF, Guenette JA, Faisal A, Ciavaglia CE, Webb KA, Jensen D, *et al.*; Canadian Respiratory Research Network. Mechanisms of exertional dyspnoea in symptomatic smokers without COPD. *Eur Respir J* 2016;48:694–705.
7. Bourbeau J, Tan WC, Benedetti A, Aaron SD, Chapman KR, Coxson HO, *et al.*; Cancold Study Group. Canadian Cohort Obstructive Lung Disease (CanCOLD): Fulfilling the need for longitudinal observational studies in COPD. *COPD* 2014;11:125–132.
8. Kirby M, Tanabe N, Tan WC, Zhou G, Obeidat M, Hague CJ, *et al.*; CanCOLD Collaborative Research Group; Canadian Respiratory Research Network; CanCOLD Collaborative Research Group, the Canadian Respiratory Research Network. Total airway count on computed tomography and the risk of chronic obstructive pulmonary disease progression. Findings from a population-based study. *Am J Respir Crit Care Med* 2018;197:56–65.
9. Tan WC, Bourbeau J, Nadeau G, Wang W, Barnes N, Landis SH, *et al.*; CanCOLD Collaborative Research Group. High eosinophil counts predict decline in FEV_1: results from the CanCOLD study. *Eur Respir J* 2021;57:2000838.
10. Phillips DB, Elbehairy AF, James MD, Vincent SG, Milne KM, de-Torres JP, *et al.*; CanCOLD Collaborative Research Group and the Canadian Respiratory Research Network. Impaired ventilatory efficiency, dyspnea and exercise intolerance in chronic obstructive pulmonary disease: results from the CanCOLD Study. *Am J Respir Crit Care Med* 2022;205:1391–1402.
11. Lewthwaite H, Elsewify O, Niro F, Bourbeau J, Guenette JA, Maltais F, *et al.*; CanCOLD Collaborative Research Group; Canadian Respiratory Research Network. Normative cardiopulmonary exercise test responses at the ventilatory threshold in Canadian adults 40 to 80 years of age. *Chest* 2021; 159:1922–1933.
12. Aaron SD, Tan WC, Bourbeau J, Sin DD, Loves RH, MacNeil J, *et al.*; Canadian Respiratory Research Network. Diagnostic instability and reversals of chronic obstructive pulmonary disease diagnosis in individuals with mild to moderate airflow obstruction. *Am J Respir Crit Care Med* 2017;196:306–314.
13. Smith BM, Kirby M, Hoffman EA, Kronmal RA, Aaron SD, Allen NB, *et al.*; MESA Lung, CanCOLD, and SPIROMICS investigators. Association of dysanapsis with chronic obstructive pulmonary disease among older adults. *JAMA* 2020;323:2268–2280.

Down to a T: The Functional Importance of Lymphopenia in Severe COVID-19

The emergence of the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019 has led to a pandemic that has had widespread detrimental effects on populations globally. Ample evidence now supports that immunopathology associated with severe coronavirus disease (COVID-19) relates to a key role for inflammatory mediators such as TNF-α (tumor necrosis factor α) and IL-6 that are augmented systemically as part of a "cytokine storm" (1, 2) and can be targeted by immunomodulatory therapies, including dexamethasone and JAK (Janus kinase)-inhibitors.

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From the very earliest case series emerging from Wuhan, it became clear that reduced circulating lymphocyte numbers (lymphopenia) was a hallmark feature of severe hospitalized COVID-19 cases (3). More recently, this abnormality has been shown to correlate with hyperinflammation (4) and adverse disease outcomes, including requirement for invasive ventilation and mortality (5, 6). Lymphopenia has been recognized for many years to be a feature in a range of severe viral infections, but the mechanisms driving this abnormality, particularly in the context of SARS-COV-2, are poorly understood. Recent studies have elucidated that SARS-CoV-2–associated lymphopenia may be related to a dysregulation of T-cell homeostasis (7), but the precise mechanism governing this alteration and relationships to disease severity are unknown. A better understanding of these mechanisms is key to facilitating new therapies to target these abnormalities and improve outcomes.

In this issue of the *Journal*, Popescu and colleagues (pp. 1403–1418) report an in-depth functional evaluation of lymphopenia in COVID-19 (8). The authors initially conducted an analysis of a multihospital cohort comprising 148 patients with severe COVID-19. Similar to other studies in the literature, absolute lymphocyte counts were reduced in severe COVID-19 and were associated with 30-day mortality. Flow cytometric analysis showed a significant reduction in CD4+ T-cell frequencies in subjects with severe COVID-19 compared with those with mild disease or healthy control subjects.

Having established a predominant T-cell lymphopenia in severe COVID19, the authors then proceeded to conduct an *in vitro* evaluation of T-cell phenotype and function. Using S1 (SARS-COV-2 spike 1) protein as a stimulus, they showed that specific TNF-α responses predominate in CD4+ T cells with a disproportionate increase evident in severe compared with mild COVID-19. Moreover, S1-specific CD4+TNF-α+ responses and circulating TNF-α concentrations were inversely correlated with CD4+ lymphopenia. The authors then sought to move from correlation to addressing causation by examining the plausible hypothesis that CD4+ T-cell proliferation may be impaired in severe COVID-19 as a contributory factor to lymphopenia. Accordingly, S1-specific CD4+ T-cell *in vitro* proliferation was profoundly impaired in severe versus mild disease, an effect that was rescued by exogenous IL-2 administration.

TNF-α is well recognized to have both proliferative and inhibitory effects upon T cells (9), and the authors therefore reasoned that S1-specific TNF-α production by CD4+ T cells may have negative effects upon the proliferative capacity of these cells. In keeping with this, TNF-α blockade with infliximab rescued the impaired CD4+ proliferative responses in severe COVID-19. Furthermore, the receptor TNFR1 (which is expressed by CD4+ T cells) was markedly upregulated in this cell type in severe compared with mild COVID-19 subjects, and TNFR1 blockade similarly restored CD4+ proliferative responses. The authors further elucidated that TNF-α secretion from CD4+ T cells contributes to T-cell lymphopenia by inducing activation-induced cell death (AICD) and apoptosis because Annexin V expression (a marker of apoptotic cells) was increased in severe COVID-19 and could be inhibited in S1-activated CD4+ T cells by neutralization of the apoptosis-inducing factors Fas and TRAIL.

Finally, Popescu and colleagues took a further important step by attempting to interrogate whether their identified mechanisms in peripheral T cells also occur locally within the lungs. They conducted a preliminary analysis in samples from a single individual undergoing bilateral lung transplantation for end-stage post–COVID-19 lung fibrosis and observed similar findings within pulmonary cells with reduced CD4+/CD8+ ratios and increased S1-specific TNF-α production in CD4 versus CD8+ cells. These findings support that similar mechanistic processes occur in the lungs, although further studies of larger numbers of patients with comparisons between severe and nonsevere disease are now required for future validation.

Overall, the authors should be commended on a well-conducted study that significantly advances our understanding of immune perturbations in COVID-19 by identifying a subset of T cells that may play a key role in driving disease severity. Future studies may seek to evaluate whether the same mechanisms are common to different SARS-CoV-2 variant strains including the currently widespread Omicron variant. Although pharmacological manipulation of the pathways identified could rescue *in vitro* T-cell proliferation in the study by Popescu and colleagues, whether targeting this mechanism would extrapolate to tangible improvements in clinical outcomes remains unclear. TNF-α can have opposing effects, acting as a regulator of both apoptosis but also cell survival (10), and therefore, it is feasible that TNF-α inhibition, although potentially beneficial for T-cell proliferation, may have distinct effects on other cell types that could be counterproductive or detrimental. Future studies may seek to examine this functionally within an *in vivo* model either using a human ACE2-transgenic mouse strain (11) or through infecting wild-type mice with a mouse-adapted SARS-CoV-2 strain, which has been recently shown to recapitulate key aspects of human disease including early lymphopenia and elevated TNF-α (12).

Anti–TNF-α therapy has been championed as a potentially useful therapy for COVID-19 hyperinflammation (13), and observational studies suggest that patients with chronic inflammatory diseases on long-term therapy with these agents have a reduced probability of severe COVID-19 (14). Ultimately a well-controlled clinical trial examining clinically relevant endpoints will provide the most robust justification to guide practice, and such studies are currently ongoing (e.g., NCT04705844), with the results eagerly awaited. The study by Popescu and colleagues raises optimism that such interventions will show clinical benefit, but further studies to confirm this are now needed. ■

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References

1. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, *et al.* An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med* 2020;26:1636–1643.
2. Thwaites RS, Sanchez Sevilla Uruchurtu A, Siggins MK, Liew F, Russell CD, Moore SC, *et al.*; ISARIC4C investigators. Inflammatory profiles across the spectrum of disease reveal a distinct role for GM-CSF in severe COVID-19. *Sci Immunol* 2021;6:eabg9873.

3. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al.* Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020;323: 1061–1069.
4. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, *et al.* Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol* 2020;11:827.
5. Lee J, Park SS, Kim TY, Lee DG, Kim DW. Lymphopenia as a biological predictor of outcomes in COVID-19 patients: a nationwide cohort study. *Cancers (Basel)* 2021;13:471.
6. Niu J, Sareli C, Mayer D, Visbal A, Sareli A. Lymphopenia as a predictor for adverse clinical outcomes in hospitalized patients with COVID-19: a single center retrospective study of 4485 cases. *J Clin Med* 2022;11:700.
7. Adamo S, Chevrier S, Cervia C, Zurbuchen Y, Raeber ME, Yang L, *et al.* Profound dysregulation of T cell homeostasis and function in patients with severe COVID-19. *Allergy* 2021;76:2866–2881.
8. Popescu I, Snyder ME, Iasella CJ, Hannan SJ, Koshy R, Burke R, *et al.* CD4$^+$ T-cell dysfunction in severe COVID-19 disease is tumor necrosis factor-α/tumor necrosis factor receptor 1–dependent. *Am J Respir Crit Care Med* 2022;205:1403–1418.
9. Mehta AK, Gracias DT, Croft M. TNF activity and T cells. *Cytokine* 2018; 101:14–18.
10. Mak TW, Yeh WC. Signaling for survival and apoptosis in the immune system. *Arthritis Res* 2002;4:S243–S252.
11. Winkler ES, Bailey AL, Kafai NM, Nair S, McCune BT, Yu J, *et al.* SARS-CoV-2 infection in the lungs of human ACE2 transgenic mice causes severe inflammation, immune cell infiltration, and compromised respiratory function [preprint]. bioRxiv; 2020 [accessed 2022 Mar 24]. Available from: https://www.biorxiv.org/content/10.1101/2020.07.09.196188v1.
12. Gawish R, Starkl P, Pimenov L, Hladik A, Lakovits K, Oberndorfer F, *et al.* ACE2 is the critical in vivo receptor for SARS-CoV-2 in a novel COVID-19 mouse model with TNF- and IFNγ-driven immunopathology. *eLife* 2022;11:e74623.
13. Robinson PC, Richards D, Tanner HL, Feldmann M. Accumulating evidence suggests anti-TNF therapy needs to be given trial priority in COVID-19 treatment. *Lancet Rheumatol* 2020;2: e653–e655.
14. Kokkotis G, Kitsou K, Xynogalas I, Spoulou V, Magiorkinis G, Trontzas I, *et al.* Systematic review with meta-analysis: COVID-19 outcomes in patients receiving anti-TNF treatments. *Aliment Pharmacol Ther* 2022; 55:154–167.

Back to BaSICS: Early Treatments Matter in Critical Illness

Intravenous fluid is the most common therapy received by critically ill adults. Recent randomized trials have examined the optimal volume and composition of intravenous fluid in critical illness. Because the effects of fluid on physiology and outcomes may be greatest during the earliest phases of critical illness, randomized trials of fluid volume have predominantly focused on initial management in the emergency department or operating room before ICU admission (1, 2). In contrast, trials examining fluid composition have frequently controlled fluid therapy only after ICU admission (3–5), an approach that could predispose to finding no difference between trial groups either by missing the phase of illness in which patients receive the most fluid (decreasing "separation between groups") or by exposing patients to the nonassigned fluid before enrollment ("contamination"). This trial design consideration may apply to the four large trials that recently compared balanced crystalloids versus saline in acutely ill adults (6–9). The two trials in which fluid composition was controlled in the emergency department before admission reported a benefit to the use of balanced crystalloids (6, 7), whereas the two trials in which fluid composition was controlled only after ICU admission reported no statistically significant difference (8, 9). Could fluid therapy early in critical illness be a key determinant of the effect of fluid composition on outcomes?

In this issue of the *Journal*, Zampieri and colleagues (pp. 1419–1428) address this question through a secondary analysis of one of the two large trials of balanced crystalloids versus saline that enrolled patients in the ICU (10). BaSICS (Balanced Solutions in Intensive Care Study) was a multicenter, randomized trial comparing balanced crystalloids versus saline among 10,520 patients in the ICU (8). Most patients were enrolled within 1 day after ICU admission, and 68% had received balanced crystalloids, saline, or both before enrollment. The primary outcome, 90-day mortality, did not significantly differ between the balanced crystalloid and saline groups (hazard ratio, 0.97; 95% confidence interval, 0.9–1.05).

This secondary analysis of the BaSICS trial examined whether the type of fluid patients received in the 24 hours before enrollment modified the effect of trial group assignment on mortality. Patients were categorized as having received only balanced crystalloid, only saline, a mix of both, or no recorded fluid before enrollment. The authors hypothesized that among patients who had received only balanced crystalloids before enrollment, those randomized to balanced crystalloids would experience lower 90-day mortality than those randomized to saline.

The results confirmed the authors' hypothesis. Among the 3,202 patients who had received only balanced crystalloids before enrollment, mortality was 16% in the balanced crystalloid group and 20% in the saline group, and the probability that balanced crystalloids decreased mortality compared with saline was 92%. This high probability of benefit from balanced crystalloids was consistent across planned admissions (97%), unplanned admissions with sepsis (96%), and unplanned admissions without sepsis or traumatic brain injury (94%). As in the original trial (8),

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