were enumerated in donor tissue in the setting of PGD or non-PGD. The authors are to be congratulated for their protocol of harvesting the tissue and processing the cells in a uniform fashion, freezing the cells, and then performing the flow cytometry concurrently to avoid the confounding factor of variation in flow compensation and other possible cytometer-related differences in the results. Another strength of the analysis is that the flow strategy for cell identification is appropriate based on the current state of the art. Thus, this work adds to a growing body of literature that describes the presence of ILC2s in human diseases. The presence of ILC2s has been described in allergic rhinitis, chronic rhinosinusitis, asthma, atopic dermatitis, pleural effusion, pulmonary fibrosis, psoriasis, and graft versus host disease (10). Although ILC2s are potent producers of cytokines that are critical for the pathophysiology of many diseases, the specific importance of these cells in pathogenesis is unknown given that no methods are currently available to eliminate or suppress the function of these cells without also directly targeting other effector cells. Until such methods are available, investigators are left with the association of ILC2s with a condition, and not a definitive cause-and-effect relationship. ■

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References

1. Monticelli LA, Diamond JM, Saenz SA, Tait Wojno ED, Porteous MK, Cantu E, *et al*. Lung innate lymphoid cell composition is altered in primary graft dysfunction. *Am J Respir Crit Care Med* 2020;201: 63–72.
2. Lanier LL. Plastic fantastic innate lymphoid cells. *J Exp Med* 2019;216: 1726–1727.
3. Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors: II. Characterization of effector cells. *Int J Cancer* 1975;16: 230–239.
4. Kiessling R, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells: characteristics of the killer cell. *Eur J Immunol* 1975; 5:117–121.
5. Mebius RE, Rennert P, Weissman IL. Developing lymph nodes collect CD4+CD3- LTbeta+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity* 1997;7: 493–504.
6. Stier MT, Peebles RS Jr. Innate lymphoid cells and allergic disease. *Ann Allergy Asthma Immunol* 2017;119:480–488.
7. Zaiss DMW, Gause WC, Osborne LC, Artis D. Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. *Immunity* 2015;42:216–226.
8. Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* 1992; 176:287–292.
9. Turner J-E, Morrison PJ, Wilhelm C, Wilson M, Ahlfors H, Renauld J-C, *et al*. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. *J Exp Med* 2013;210:2951–2965.
10. Peebles RS Jr. At the bedside: the emergence of group 2 innate lymphoid cells in human disease. *J Leukoc Biol* 2015;97:469–475.

Early Detection of Pulmonary Vascular Dysfunction in Neonatal Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD), a paramount morbid lung disease typically complicating premature birth, is arguably one of the most vexing clinical problems in the neonatal ICU (NICU). Although the disease is characterized by truncated parenchymal growth, both vascular and alveolar, the pathogenic mechanisms involved remain poorly understood, and the early and late pulmonary complications, particularly development of pulmonary hypertension (PH), are ominous, as they limit survival. Although the definition of BPD is based simply on oxygen dependence at 36 weeks postmenstrual age (PMA), as agreed at a 2001 NIH workshop (1), several pre- and postnatal factors have been linked to its development, including identification of early disrupted pulmonary vascular growth in the form of pulmonary vascular disease (PVD) or PH (2, 3). In fact, these factors may have more relevance to later respiratory complications in early childhood than BPD itself (4) and have led investigators to question the usefulness of the NIH definition of BPD (5). In addition, what has become clearer over the past few years is that early identification of high-risk preterm infants is important for prognostication and possibly early therapy.

In this issue of the *Journal*, Critser and colleagues (pp. 73–82) tested the hypothesis that neonatal cardiac magnetic resonance imaging (MRI) correlates with BPD severity and can predict short-term clinical outcomes, including the need for PH therapies (6). Building on their previous work showing that an MRI scoring system, based on high- and low-signal intensity lung parenchyma, can detect quantifiable BPD structural abnormalities (7), the investigators retrospectively analyzed a mixed cohort of 52 infants with various degrees of BPD severity who underwent MRI between 39 and 47 weeks' PMA on a neonatal-sized, NICU-sited 1.5T MR scanner. MR left ventricular eccentricity index (MR-EI), main pulmonary artery to aorta diameter (PA/AO) ratio, and pulmonary

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arterial blood flow were determined, among several other parameters. Similarly, echocardiograms were obtained, and both MRI and echo indices were compared with BPD severity. Clinical outcomes to be tested against these measurements included NICU and hospital length of stay (LOS), duration of respiratory support, and need for respiratory support at discharge and for PH therapy. The results indicated that, after controlling for confounding factors, MR-EI was associated with LOS and duration of respiratory support, and both increased PA/AO and MR-EI were associated with PH therapy during hospitalization and at discharge. The authors, however, found no correlation between pulmonary arterial blood flow and BPD outcomes. They concluded from their study that specific MRI parameters can provide important clues regarding cardiac morphology that correlate with disease severity and clinical outcomes in neonates with BPD. They specifically highlight that PA/AO ratio and MR-EIs > 1.3 could be used for prognostication for short-term outcomes and potentially for initiation of therapy when the clinical context is appropriate.

This study deals with a challenging neonatal clinical problem (i.e., BPD), which carries a heavy burden in terms of morbidity and mortality. Despite the relatively small size of patients, the authors were able to reach several important conclusions and opened a novel field of imaging with prognostic relevance for a disease that has long been difficult to manage, from both a diagnostic and a therapeutic standpoint. Importantly, the investigators demonstrate that MRI is feasible in a NICU setting and appears to be more reliable than echocardiography in detecting the severity of BPD and its short-term clinical outcomes. The performance of simple imaging biomarkers such as the PA/AO ratio and MRI-EI appears compelling, and their potential clinical use would be quite novel and clinically relevant in this patient population. However, the questions not clearly answered, owing probably to the retrospective nature of this study, are whether MRI is *1*) useful for the diagnosis of PH or PVD (a common and serious complication of BPD) or just a predictor of outcomes, and *2*) indeed superior to the more cost-effective echocardiography, the preferred modality for PVD diagnosis in high-risk infants. The authors, however, state that comparing the two imaging modalities was not the purpose of their study. In addition, the analysis is limited to relatively short in-hospital outcomes, such as LOS, duration of respiratory support, and initiation of PH therapy. Mourani and colleagues (8) recently demonstrated that early evidence of PVD in preterm infants, based on simple echocardiographic criteria (i.e., one of the following: systolic pulmonary arterial pressure > 40 mm Hg by tricuspid jet velocity, PA/systemic systolic pressure > 0.5, cardiac shunt with bidirectional or right-to-left flow, or any degree of pressure flattening) obtained at 7 days and 36 weeks PMA in a large (i.e., 221 subjects) population of preterm infants, can be detected. They further showed that PVD, in combination with other perinatal factors such as gestational diabetes or mechanical ventilatory support, was a strong predictor for late respiratory disease during childhood (up to 24 mo of age), defined as a diagnosis of asthma or reactive airways disease, a physician diagnosis of bronchiolitis or pneumonia, or hospitalization for a respiratory illness.

Although the study by Crister and colleagues opens a new area of diagnostic investigation in this debilitating disease, it has several notable limitations that were acknowledged by the authors, including the retrospective nature of the analysis, the lack of similar comparator measurements in a group of healthy neonate control subjects (comparisons were made to healthy neonates and neonates with mild BPD), and the relatively small and heterogeneous population of patients from a single center (6). In addition, the diagnosis of PH and, therefore, the decision to start therapy was based on a combination of clinical information and echocardiographic evaluation. This suggests that some patients may have been overdiagnosed, considering the relative lack of accuracy of echocardiography, whereas in others PVD may have been missed if the imaging study was unrevealing (i.e., false negative, although less likely). Right heart catheterization for confirmation of PVD is rarely performed in this vulnerable population, as opposed to adults for whom this test is necessary for the diagnosis. For this study, the authors used evidence of PH based on echocardiographic criteria previously defined by Mourani and colleagues for PVD (8). Other notable limitations include the fact that, although MRI and echocardiographic images were analyzed by readers blinded to the patient identity, there were no attempts at checking accuracy and reproducibility of the imaging parameters (PA/AO and MR-EI) with inter- and intraobserver measurements.

In closing, Crister and colleagues should be commended for pioneering a new avenue of investigation for the early detection of preterm infants at risk for a challenging disease with often somber outcomes (6). However, although this study proposes interesting novel imaging parameters as potential tools for early diagnosis and prognostication, these findings should clearly be validated in prospectively designed, large multicenter studies in which short- and long-term outcomes should both be evaluated. ■

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References

1. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;163:1723–1729.
2. Abman SH. Bronchopulmonary dysplasia: "a vascular hypothesis". *Am J Respir Crit Care Med* 2001;164:1755–1756.
3. Mourani PM, Sontag MK, Younoszai A, Miller JI, Kinsella JP, Baker CD, *et al*. Early pulmonary vascular disease in preterm infants at risk for bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2015;191: 87–95.
4. Morrow LA, Wagner BD, Ingram DA, Poindexter BB, Schibler K, Cotten CM, *et al*. Antenatal determinants of bronchopulmonary dysplasia and late respiratory disease in preterm infants. *Am J Respir Crit Care Med* 2017;196:364–374.
5. Poindexter BB, Feng R, Schmidt B, Aschner JL, Ballard RA, Hamvas A, *et al*.; Prematurity and Respiratory Outcomes Program. Comparisons and limitations of current definitions of bronchopulmonary dysplasia for the prematurity and respiratory outcomes program. *Ann Am Thorac Soc* 2015;12:1822–1830.
6. Critser PJ, Higano NS, Tkach JA, Olson ES, Spielberg DR, Kingma PS, *et al*. Cardiac magnetic resonance imaging evaluation of neonatal

bronchopulmonary dysplasia–associated pulmonary hypertension. *Am J Respir Crit Care Med* 2020;201:73–82.
7. Higano NS, Spielberg DR, Fleck RJ, Schapiro AH, Walkup LL, Hahn AD, *et al*. Neonatal pulmonary magnetic resonance imaging of bronchopulmonary dysplasia predicts short-term clinical outcomes. *Am J Respir Crit Care Med* 2018;198:1302–1311.
8. Mourani PM, Mandell EW, Meier M, Younoszai A, Brinton JT, Wagner BD, *et al*. Early pulmonary vascular disease in preterm infants is associated with late respiratory outcomes in childhood. *Am J Respir Crit Care Med* 2019;199:1020–1027.

IFN Therapy in Airway Disease: Is Prophylaxis a New Approach in Exacerbation Prevention?

It is widely accepted that respiratory virus infections precipitate the great majority of acute asthma attacks (exacerbations) in all age groups (1). Evidence that viruses precipitate the great majority of acute exacerbations of chronic obstructive pulmonary disease (COPD) is also strong, in that the great majority of patients report colds before onset of exacerbations (2), colds are powerful predictors of exacerbation risk and severity (3), viruses are detected in exacerbations at rates up to 67% (4), it is increasingly recognized that many bacterial exacerbations result from secondary bacterial infection after initial virus infection (5, 6), and experimental virus infection induces exacerbation in ∼95% of infected volunteers with COPD (7).

Although not found in all studies (8), there is now abundant evidence that asthma is frequently accompanied by broadly impaired antiviral immunity in both adults (9–15) and children (16–18). Most of these studies report deficiencies in IFN-β and IFN-λ induction by virus infection of bronchial epithelial cells (bronchial epithelial cells do not produce IFN-α), as well as deficiencies in IFN-α, IFN-β, and IFN-λ induction by virus infection of macrophages/dendritic cells. These data clearly implicate IFN deficiency in the pathogenesis of asthma attacks. This interpretation is also strongly supported by the recent elegant demonstration that low IFN response gene expression in children with asthma strongly predicts future exacerbation risk (19).

Deficient virus-induced IFN responses have also been reported in BAL cells (7) and primary bronchial epithelial cells (PBECs) in COPD (20). Rhinovirus challenge studies in COPD have confirmed that increased virus replication is observed in both upper and lower respiratory tracts *in vivo* (7, 21).

These data have generated great interest in the potential of exogenous IFN therapy as an acute interventional treatment to prevent early symptomatic colds progressing to virus-induced asthma and COPD exacerbations. Djukanović and colleagues investigated the effect of inhaled IFN-β/placebo treatment for 14 days, initiated within 24 hours of diary-verified cold/influenza symptoms, in 134 patients with British Thoracic Society step 2–5 asthma with a history of at least one cold-related asthma exacerbation requiring oral corticosteroids and/or antibiotics in the last 24 months (22). There was no significant effect on the primary endpoint of asthma symptoms in the 7 days after treatment initiation; however, it should be noted that the colds did not result in a clinically meaningful worsening of asthma symptoms in the placebo-treated subjects; thus there was likely no clinically meaningful worsening of asthma symptoms for the IFN-β treatment to impact upon (22). There was a significant effect of IFN-β on the secondary endpoint of morning peak flow (mean difference, 19.5 L/min; $P = 0.03$), on CCL4 levels in sputum supernatants ($P = 0.035$), and on induction of antiviral activity in the lung (increased sputum ISG [IFN-stimulated gene] *OAS1* and *MX1* expression; $P = 0.0003$ and 0.0001).

In a preplanned subgroup analysis of 54 subjects with British Thoracic Society step 4–5 asthma, in whom colds did result in clinically meaningful worsening of asthma symptoms in the placebo group, there was a clinically meaningful and statistically significant improvement in symptoms ($P = 0.004$), and the percentage of patients with clinically meaningful worsening of asthma symptoms was lower for IFN-β (17%) compared with placebo (50%; $P = 0.012$). The improvement with treatment in morning peak flow was also greater (mean difference, 31.4 L/min; $P = 0.03$). Five patients receiving placebo, but only one receiving IFN-β, required oral corticosteroids/antibiotics.

In the INEXAS (A Study in Asthma Patients to Evaluate Efficacy, Safety and Tolerability of 14 Days Once Daily Inhaled Interferon Beta-1a After the Onset of Symptoms of an Upper Respiratory Tract Infection) phase 2 trial, 121 subjects with Global Initiative for Asthma step 4–5 asthma, with ⩾2 cold-related severe exacerbations in the last 2 years, were randomly assigned to inhaled IFN-β/placebo treatment for 14 days within 48 hours of cold/influenza symptoms (23). The primary outcome, severe exacerbations, was unexpectedly rare, with only 7 and 5 patients in the IFN and placebo groups, respectively, resulting in the trial being stopped early. As in the previous study, there was a significant improvement in morning peak flow with IFN-β ($P = 0.01$).

These studies together indicate that IFN-β-treated asthma exacerbations did show some evidence of efficacy, especially in subjects in whom clinically meaningful worsening of asthma symptoms occurred after colds. However, both studies suffered

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