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Toward Personalized Medicine in Bronchiolitis

Nothing essential has changed in the definition of bronchiolitis since its description nearly 80 years ago (1, 2). The syndrome affects infants and young children at different ages, presents with a few overlapping symptoms of varying intensity and other manifestations often associated with specific pathogens (e.g., fever and pharyngitis in influenza or wheezing with respiratory syncytial virus), and is caused by infection with one or a combination of half a dozen viruses with different inflammatory, antiviral, and/or atopic phenotypes (3). Conceived as a single entity, a number of candidate drugs failed as therapeutics against it in randomized trials (4). To date, management recommendations for hospitalized patients are based on supportive therapy (2). Etiologic testing is discouraged (2).

Times are changing. Paradigm shifts usually announce themselves through periods characterized by coexisting contradictory practices, concepts, and models. We are living in the dawn of the omics era and precision medicine, and steadily moving away from the illusion of the panacea (one medicine that cures all diseases) to re(de)fine our diagnoses and provide tailored, personalized treatments. We are only now coming to terms with the idea that bronchiolitis is, in fact, a constellation of clinical manifestations arbitrarily considered a single disease, instead conformed by several distinct pathophysiological entities. In this issue of the *Journal*, Jones and colleagues (pp. 1537–1549) perform transcriptomics profiling of peripheral blood mononuclear cells and nasal mucosal scrapings from 26 infants and 27 young children who suffered acute bronchiolitis, and provide evidence supporting the need to discriminate apples from oranges. Samples were obtained during acute infection and at convalescence to characterize the immune-inflammatory response networks associated with the entities within the syndrome (5). Adding nasal mucosal scrapings expanded the analysis to two biological sources of data, whereas previous reports limited their sampling to either blood or nasal scrapings (6–8).

The first message emerging from these observations is that, during acute bronchiolitis, IFN seems to matter, particularly for infants (most of them infected with respiratory syncytial virus [RSV]). Infants with bronchiolitis exhibit hyperactivation of type I IFN transcription pathways, validated at the protein level (5). During RSV infection, IFN is seldom found in respiratory secretions, and consequently has been speculated to play a minor role in disease pathogenesis (9). However, type I IFN levels peak early after infection, and RSV bronchiolitis has a long incubation period followed by a prodromal phase. Therefore, samples may have been collected too late in earlier studies to detect its antiviral effects (9). For the same reason, and given the nature of study design, the observed up-regulation of IFN during acute illness does not imply a positive association between levels and severity. Earlier works in asthma nicely show that timing is everything when

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assaying IFN, which may be suppressed or abundant at different stages of disease (10, 11).

Responses in older children skewed toward inflammation-associated regulators, remodeling/repair-associated drivers, and Th2 signaling. These subjects also experienced more frequent infections with rhinoviruses and enteroviruses (5). Through careful statistical analysis of the data, the authors show that age is the main driving force behind the diversity of the inflammatory response profiles. When in clinic or at the hospital, older children with severe bronchiolitis often reflect a group we intuitively (and from our reading of the literature) expect to see again with long-term respiratory complications (12). But even in this relatively small group of young children, the authors employ an N-of-1 pathways analysis to recover singular patterns that would otherwise get averaged out, and identify a second subset of subjects exhibiting infant-equivalent hyper-upregulation of IFN pathways. Evidently, there is more diversity in the pathophysiology of bronchiolitis than we often acknowledge.

This interesting study offers a glimpse of a complex picture. As recognized by the authors, a limited sample size, unknown duration of symptoms when sampling during acute illness, heterogeneous cell populations in peripheral blood mononuclear cell and nasal mucosal scraping samples, and absence of a replicate population limit the discriminating depth in subgroups. Previous work in a multicenter U.S. cohort of 921 infants used latent class analysis, based on clinical factors and viral etiologies, to discriminate three severe bronchiolitis profiles: a larger subgroup (49%) resembling classic RSV bronchiolitis presentations, a third of the subjects with very severe disease, and 15% most often infected with rhinoviruses and presenting with higher eosinophil counts and high cathelicidin levels, and at increased risk for recurrent wheezing (13). An additional, fourth profile of nonwheezing patients with milder illness was identified in two other cohorts in the United States and Finland, using a clustering approach (14). Another study clustered intubated RSV-infected children by phenotyping dendritic cells in blood and BAL fluid (15). Weak antiviral plasmacytoid dendritic cell responses were characteristic of an endotype grouping preterm and infants older than 4 months (15). Recently, using urine for untargeted metabolomic analyses based on mass spectrometry in 52 Italian children with bronchiolitis, major involvement of the citric acid cycle discriminated those prone to subsequently experience recurrent wheezing (16).

The N-of-1 pathways analysis in the present work and other approaches discussed here set the stage for evaluating larger cohorts, sampled more often, and subjected to next-generation sequencing technologies that enable single-cell RNA sequencing. Unsupervised machine learning analysis of disease trajectories in these populations would link to transcriptome patterns and uncover hidden endotypes, akin to the provocative recent advances in asthma. Then, the syndrome we have been calling bronchiolitis for 80 years may evolve into a discrete group of disease endotypes with specific diagnostic tests, finer prognostic tools, and tailored therapeutics. And the search for the panacea will be finally abandoned. ■

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Is Myocardial Fibrosis Impairing Right Heart Function?

Many articles describing results from pulmonary hypertension studies begin with a statement like this: "Severe pulmonary hypertension is terrible and patients die from right heart failure." For many years, this statement had become a mantra without much attention having been paid to the outcome of the pulmonary vascular diseases. Thankfully, this situation has changed. A recent workshop report sponsored by the American Thoracic society has moved right ventricular (RV) function and right heart failure into the limelight (1). One of the many unresolved questions that have been raised in this report is the following: Is myocardial fibrosis of functional importance in the setting of chronic pulmonary hypertension and right ventricular stress? The question is an important one and is exactly the subject of a collaborative multiinstitutional investigation published in this issue of the *Journal* by Crnkovic and colleagues (pp. 1550–1560) (2). If we can prevent the development of RV fibrosis and reverse established RV fibrosis, we perhaps might prevent RV failure. It is generally believed that fibrosis is bad, whether it is in the liver and kidney or in the context of interstitial lung diseases. Obviously, if we wish to prevent RV fibrosis, we need to understand the cellular and molecular mechanisms underlying tissue fibrosis. Teleological reasoning would have it that the injury that offsets the tissue homeostasis triggers a wound-healing program, and part of this program is fibrosis. Crnkovic and colleagues employ pulmonary artery banding in mice and the Sugen/hypoxia and monocrotaline rat models to stress the RV and generate RV fibrosis. The authors found that pirfenidone treatment of pulmonary artery-banded mice reduced the amount of fibrosis but did not restore RV function. Because the pro-fibrotic galectin-3 and elevation of circulating galectin-3 have been linked to the development of left heart failure and RV dysfunction (3–5), the authors focused their attention on galectin-3 to explain the (lack of) importance of RV fibrosis in RV failure.

The presented data appear at odds with a growing body of evidence linking fibrosis to poor function of the pressure-overloaded right heart. RV diastolic stiffness in pulmonary arterial hypertension is partially mediated by interstitial fibrosis (6) and is related to poor clinical outcomes (7). This mimics the clinical situation in left heart failure, where the pattern and extent of myocardial fibrosis is associated with reduced left ventricular ejection fraction and predicts adverse outcomes (8). In most clinical studies, myocardial fibrosis is quantified using late gadolinium enhancement, and histological proof of the validity of this concept is readily available (9). The list of drugs that concomitantly improve RV function and decrease fibrosis in experimental models is long and includes beta-adrenergic blockers (10), iloprost (11), p38 MAPK inhibition (12), pirfenidone (13), and nintedanib (14). In most of these studies, RV effects were partly mediated by a drug-induced decrease in pulmonary vascular resistance. But iloprost and p38 MAPK inhibition were tested in rats after pulmonary artery banding, isolating RV afterload from the pulmonary vasculature. In those studies, proof of a mechanistic link between fibrosis and RV dysfunction was to a certain degree circumstantial. RV fibrosis seems "guilty by association" to the development of RV failure. However, the lack of association between a drop in fibrosis and a change in RV function in the study by Crnkovic is similarly insufficient to completely dismiss fibrosis as a contributor to RV dysfunction. As such, the boldness of the title of the paper: "Therapy of right ventricular fibrosis does not ameliorate right ventricular dysfunction RV" may seem a bit of an overreach.

One simple explanation for Crnkovic's findings may be that an expected improvement in RV function was offset by adverse effects of pirfenidone unrelated to the drug's antifibrotic action. But on a deeper level, the difficulty of mechanistically linking fibrosis to function or dysfunction of the RV exemplifies a general problem in preclinical cardiac failure research: a reductionist attempt to explain RV dysfunction by one type of cellular response (hypertrophy, fibrosis, inflammation, capillary rarefaction, etc.) is unlikely to solve the puzzle. Under conditions of pressure overload, it is obvious that a certain degree of hypertrophy is necessary to increase contractility. Likewise, a certain response of the extracellular matrix is needed to provide a scaffold for the remodeling myocardium. The capillary network will need to adapt to the changing oxygen demand. The success of RV adaptation will depend on the "quality" of hypertrophy (contractile proteins), fibrosis (perivascular and diffuse type, linking of extracellular matrix proteins), inflammation (type of activity of immune cells), and endothelium (leakiness, facilitation of diffusion). Moreover, the overall quality of RV adaptation will depend on the exact matching of these different processes. As such, there is no "good" or "bad" hypertrophy, fibrosis, inflammation, or capillary rarefaction. Pirfenidone treatment in the studies by Crnkovic did not result in a repair of capillary rarefaction. As such, it is possible that the benefit of reduced fibrosis was negated by the persistence of myocardial ischemia. In contrast, fibrosis reduction during carvedilol treatment occurred alongside an improvement in capillary density and did result in RV functional improvement (10).

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