Emerging Complexity in the Biomarkers of Exacerbation-Prone Asthma

During the past years, maintenance treatment with controller drugs such as inhaled steroids and, in particular, new biologics have shown clinically significant efficacy in suppressing exacerbation rates in patients with asthma (1). This has substantially changed asthma management from being a largely reactive approach during acute episodes toward being a more prophylactic strategy. The success in suppressing exacerbations in patients with asthma is remarkable, as these emerging events are predominantly caused by unanticipated respiratory (rhino)virus infections. The biological pathways leading to an acute asthma episode are extremely complex, involving an array of pathogen–host interactions at the degree of the virus, the airway microbiome, the innate and adaptive immune responses, a broad spectrum of inflammatory mechanisms, and multiple elements of airway narrowing (2). The real questions are "Which biological pathways are predominating in patients with frequent exacerbations, and can any predictive biomarkers be delineated?"

In this issue of the *Journal*, Peters and colleagues (pp. 973–982) present a prospective follow-up study focused on these questions with two aims: first, to identify clinical and biological variables that are associated with exacerbation-prone asthma and, second, to build a biomarker prediction model for exacerbation rates (3). The strength of the study certainly includes the longitudinal design of the well-characterized SARP-3 (Severe Asthma Research Program-3) population. By using an acute episode requiring ⩾3 days of systemic steroids as their definition of an exacerbation, the authors classified 21% of patients as being exacerbation prone, 41% as being exacerbation resistant, and 38% as having intermittent exacerbations during 3 years of prospective follow-up (3).

As expected, prior exacerbations turned out to increase the probability of subsequent exacerbations, and the observed exacerbation rates were also associated with older age, female sex, higher body mass index, worse asthma symptoms, lower spirometric function, higher doses of inhaled steroids, more frequent gastroesophageal reflux, nasal polyposis, diabetes, hypertension, depression, and oral-steroid treatment (3). This confirms and extends the findings of early cross-sectional studies (4). Interestingly, when examining cellular and molecular factors, it appeared that circulating white blood cells, neutrophils, and IL-6 concentrations were highest in the exacerbation-prone patients, whereas eosinophil counts (in blood or sputum) and exhaled nitric oxide did not differ according to exacerbation rate (3).

The lack of association between eosinophils and exacerbations was unexpected, as the authors had already decided *a priori* to include circulating eosinophils and IL-6 in their (binomial regression) prediction model, on the basis of their previously published cross-sectional observations (5). In doing so, when controlling for clinical and therapeutic covariates, it appeared that a 1-SD increase in baseline blood eosinophils or IL-6 significantly elevated the incident-rate ratio of exacerbations by 1.2 or 1.3, respectively. These are modest effect sizes that certainly need external validation.

What do we learn from this study? The data show that the biomarker story of frequent exacerbations is getting increasingly complex. It may not be surprising that systemic neutrophil counts and IL-6 are positively associated with exacerbations that are largely virus driven (2). However, carry-over effects from previous exacerbations cannot be excluded here. Still, the present data suggest that innate immune pathways and perhaps metabolic dysfunction (indicated by associated diabetes and hypertension) are involved in elevated susceptibility for asthma exacerbations.

As is often the case, the unexpected finding is a major one: namely, the exacerbation rate during longitudinal follow-up was not associated with circulating or sputum eosinophil counts. Blood eosinophils have repeatedly emerged as a risk factor for exacerbations in cross-sectional analyses (4–6), but although such associations have been confirmed in some (6, 7) longitudinal studies on this topic, they have not been confirmed in all such longitudinal studies (8), and this includes the present one (3). This may be linked to differences in asthma severity, phenotype, and treatment, but it certainly suggests that prospective assessment provides its own information in search for asthma biomarkers. Still, despite the lack of association between eosinophils and exacerbation frequency in the present study (3), the authors built their binomial regression model by adding blood eosinophils to IL-6 as potential predictors. Is this correct?

There is a wide spectrum of prediction models for asthma exacerbations (9). The selection of variables in developing those is certainly not straight forward. We strongly recommend following the TRIPOD (Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis) statement (10) and the most recent guidance by STRATOS (Strengthening Analytical Thinking for Observational Studies) (11). What would have been the results of a prediction model using the actual variables that appeared to be associated with exacerbation rate in the first part of the present study? The sensitivity analysis introducing those factors as covariates into the model on the basis of eosinophils and IL-6 may not suffice to examine their own predictive value, regardless of eosinophil counts.

Phenotypic differences among patients regarding the cellular and molecular factors contributing to exacerbations are a likely driver of the observed inconsistencies among various studies. In medicine, we may have been late in realizing that biology is fundamentally complex, with large degrees of freedom and interactions between multiscale networks and information circuits, which are nonlinear, emergent, and partly random, thereby being relatively unpredictable (12) (Figure 1). This indicates that pattern recognition and machine learning need to be added to conventional statistical approaches to get a grip on

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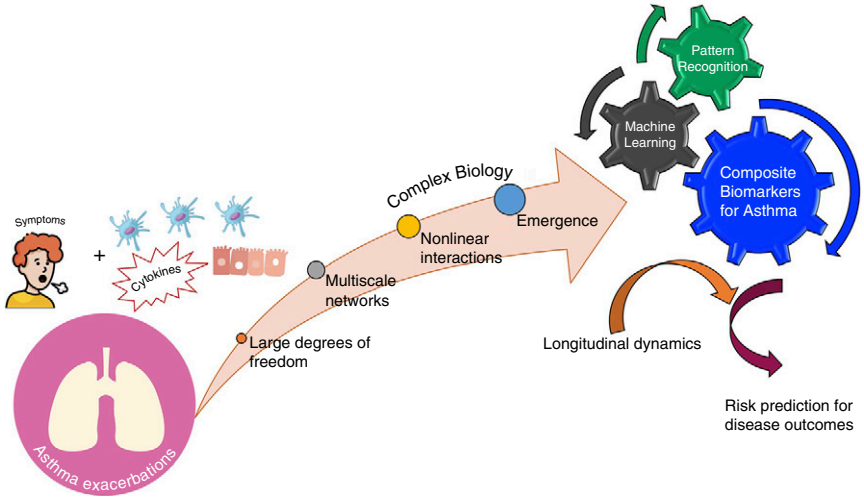

Figure 1. Schematic showing the fundamental complexity of biology in asthma and the need for composite biomarkers to predict exacerbation risk. Conventional statistical approaches need to be supplemented with artificial intelligence and longitudinal time-series analyses to capture its informational organization, complex interactions, and dynamics for optimization of prediction models.

the informational organization and, thereby, the biomarkers in asthma. It will provide competence without full comprehension, which is similar to several other areas of complexity.

Particularly relevant to exacerbations is the recent observation that the dynamics of biomarkers turn out to be different between patients with asthma and control subjects, also in response to respiratory rhinovirus infection (13). This strongly indicates that fluctuation analysis of biomarker time series, rather than linear, single-time-point assessments, is required to identify biomarkers of exacerbation-prone asthma. In fact, the prospective follow-up studies by SARP-3 (3, 14), Novel START (Novel Symbicort Turbuhaler Asthma Reliever Therapy) (6), Hi-CARAT (Hokkaido-based Investigative Cohort Analysis for Refractory Asthma) (8), ADEPT (Airways Disease Endotyping for Personalized Therapeutics) (14) and U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome) (14) will be highly suited to test and collectively validate whether the temporal behavior of composite biomarkers provides a complementary phenotypic signal that is relevant for identification of frequent exacerbators.

At this stage, the only secure prediction is that predicting asthma exacerbations requires capturing information patterns by *1*) repeated assessment of *2*) composite biomarkers, which is becoming a realistic option (15). All of this is needed for more effective prophylaxis in patients with frequent exacerbations and perhaps beyond. Consequently, teasing out the complexity of asthma (even without full comprehension) may eventually allow true disease modification rather than mere control. ■

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Endothelial Oxygen Sensing in Alveolar Maintenance

In this issue of the *Journal*, Pasupneti and colleagues (pp. 983–995) explore the role of HIF (hypoxia-inducible factor) in the pathogenesis and treatment of emphysema (1).

HIF is a transcription factor that orchestrates oxygen homeostasis. HIF stability is regulated by the oxygen-sensitive PHD (prolyl hydroxylase domain) enzymes, with the hydroxylated HIF-α subunit ultimately targeted for proteasomal degradation. In hypoxia, HIF-α escapes degradation and activates the transcription of genes that enable adaptation to reduced oxygen availability, including aspects of systems physiology that optimize oxygen delivery (2).

Invertebrates express a single HIF-α homolog, and the appearance of the HIF-2α paralog coincides with the evolution of complex oxygen delivery systems incorporating the lungs and vasculature. HIF-2 is abundantly expressed in these tissues and appears to have a particular role in the regulation of the systemic and pulmonary circulation (3). Chronic hypoxia induces pulmonary vasoconstriction and vascular remodeling, resulting in pulmonary hypertension, but mice with genetic inactivation of HIF-2 are protected from these effects (4). Although less well explored, hypoxia also stimulates proliferation of airway epithelial cells (5), which is similarly HIF-2 dependent (6).

Although originally defined as a hypoxia-inducible system, there is evidence that HIF also contributes to the maintenance of oxygen delivery systems in steady-state conditions. Genetic inactivation of HIF-α isoforms in mice has phenotypic consequences evident without hypoxia exposure or ischemic injury. Mice lacking *Hif-2α* have impaired iron absorption and erythropoietin production (7, 8), resulting in significant anemia following induced postnatal deletion of *Hif-2α*, which excludes confounding developmental effects (9). This might reflect incomplete HIF-α degradation, even in normoxia, or physiological hypoxic niches that result in HIF-α stabilization, such as the intestinal epithelium and renal interstitium, where imbalances in blood flow and $\dot{V}O_2$ result in marked oxygen gradients.

This study by Pasupneti and colleagues provides evidence that HIF-2 contributes to the steady-state maintenance of alveolar architecture. They used an inducible form of genetic recombination to specifically knock out *Hif-2α* in the endothelial cells of adult mice, which then developed features of emphysema over the subsequent 14–28 days. This included evidence of pneumocyte apoptosis, airspace enlargement, and obstructive ventilatory failure, which were not observed in mice with conditional *Hif-1α* deletion, demonstrating a HIF-2–specific function in alveolar maintenance (1).

It is perhaps surprising that this function is intrinsic to endothelial HIF-2, although the pulmonary vascular endothelium may be well placed to sense inadequate pulmonary function and oxygen delivery. Most of the airway epithelium is exposed to high oxygen tensions, close to ambient levels. In contrast, the pulmonary arterioles conduct deoxygenated blood to gas exchange sites, exposing the endothelium to a relatively hypoxic environment. This is illustrated by the effects of carbon monoxide, which impairs the oxygen transport capacity of Hb and strongly induces HIF-2 in the pulmonary endothelium (10). The physiological function of HIF-2–dependent survival signals may be localized, coupling the growth and survival of the alveolar epithelium and endothelium to optimize the gas exchange surface. Alternatively, it may have a general role in matching lung capacity to systemic $\dot{V}O_2$. The compensatory lung growth observed in many species after pneumonectomy is modulated by oxygen availability, with hypoxia stimulating growth (11). In this context, it is significant that the current study does not restrict HIF-2 inactivation to the lungs and the alveolar maintenance signals may be generated in the systemic circulation.

The mechanism of this process remains unclear, although the authors propose impaired paracrine signaling. Reduced expression of the mitogenic hormone HGF (hepatocyte growth factor) was noted in the lungs of *Hif-2α* deficient mice, suggesting this might act as an endothelial-derived growth factor supporting pneumocyte survival (1). Interestingly, HGF is also implicated in

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