5. Bellani G, Laffey JG, Pham T, Madotto F, Fan E, Brochard L, *et al*.; LUNG SAFE Investigators; ESICM Trials Group. Noninvasive ventilation of patients with acute respiratory distress syndrome: insights from the LUNG SAFE study. *Am J Respir Crit Care Med* 2017;195:67–77.
6. Mehta AB, Douglas IS, Walkey AJ. Evidence-based utilization of noninvasive ventilation and patient outcomes. *Ann Am Thorac Soc* 2017;14:1667–1673.
7. Wunsch H, Angus DC, Harrison DA, Collange O, Fowler R, Hoste EA, *et al*. Variation in critical care services across North America and Western Europe. *Crit Care Med* 2008;36:2787–2793, e1–e9.
8. Wunsch H, Linde-Zwirble WT, Harrison DA, Barnato AE, Rowan KM, Angus DC. Use of intensive care services during terminal hospitalizations in England and the United States. *Am J Respir Crit Care Med* 2009;180:875–880.
9. Wunsch H, Angus DC, Harrison DA, Linde-Zwirble WT, Rowan KM. Comparison of medical admissions to intensive care units in the United States and United Kingdom. *Am J Respir Crit Care Med* 2011; 183:1666–1673.
10. Yarnell CJ, Fu L, Manuel D, Tanuseputro P, Stukel T, Pinto R, *et al*. Association between immigrant status and end-of-life care in Ontario, Canada. *JAMA* 2017;318:1479–1488.
11. Gray A, Goodacre S, Newby DE, Masson M, Sampson F, Nicholl J; 3CPO Trialists. Noninvasive ventilation in acute cardiogenic pulmonary edema. *N Engl J Med* 2008;359:142–151.
12. Xie J, Tong Z, Guan X, Du B, Qiu H. Clinical characteristics of patients who died of coronavirus disease 2019 in China. *JAMA Netw Open* 2020;3:e205619.
13. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, *et al*. Clinical characteristics of covid-19 in New York City. *N Engl J Med* [online ahead of print] 17 Apr 2020; DOI: 10.1056/NEJMc2010419.
14. ICNARC. ICNARC report on COVID-19 in critical care, 10 April 2020 [accessed 2020 Apr 15]. https://www.icnarc.org/.
15. Grasselli G, Pesenti A, Cecconi M. Critical care utilization for the COVID-19 outbreak in Lombardy, Italy: early experience and forecast during an emergency response. *JAMA* 2020;323: 1545–1546.
16. Wunsch H, Linde-Zwirble WT, Angus DC, Hartman ME, Milbrandt EB, Kahn JM. The epidemiology of mechanical ventilation use in the United States. *Crit Care Med* 2010;38:1947–1953.
17. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O''Donnell LF, Chernyak Y, *et al*. Factors associated with hospitalization and critical illness among 4,103 patients with COVID-19 disease in New York city [preprint]. medRxiv; 2020 [accessed 2020 Apr 15]. Available from: https://www.medrxiv.org/content/10.1101/2020.04.08.20057794v1.
18. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395: 1054–1062.
19. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, *et al*.; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;382: 1708–1720.

MicroRNAs as Biomarkers in Corticosteroid-Resistant/Neutrophilic Asthma: Still a Long Way to Go!

Asthma is a complex and heterogeneous disorder characterized by chronic airway inflammation with variable airflow obstruction that affects people of any age. It is associated with an earlier decline of lung function over time and, in some cases, reduced lung function growth during childhood/adolescence (1, 2). Antiinflammatory corticosteroids are the mainstay of asthma treatment from infancy to senescence, often combined with long-acting β-agonists in patients older than 6 years of age (3). In most subjects, corticosteroids allow clinical control of asthma (symptomatic treatment) and are also effective as disease-modifying therapy, inhibiting lung function decline in both children and adults (4, 5).

However, a clinically relevant proportion of individuals with asthma do not respond to corticosteroid treatment, even when administered at high doses. Severe steroid-resistant asthma affects 5–10% of adult patients, who disproportionately account for 50–80% of all asthma-associated healthcare costs (6). The epidemiology and prevalence of severe steroid-resistant asthma in children are unclear (7). In adults, severe asthma is classified based on the inflammatory profile as T2 high and T2 low. The latter is often characterized by neutrophilic inflammation, an indication of steroid resistance.

The molecular mechanisms leading to corticosteroid resistance are various and only partially understood (6). Their identification could pave the way for new treatment targets in asthma. Even better, unraveling the risk factors associated with the development of corticosteroid resistance over time could allow early targeted interventions and the implementation of preventive precision medicine ("precision prevention"). Complex interactions between genetic and environmental factors regulate corticosteroid resistance. The genetic factors include microRNAs (miRNAs), which are are small noncoding RNAs that intervene in gene expression regulation during inflammatory and immune responses, and are recognized as possible genetic modulators of steroid sensitivity in asthma (6).

In this issue of the *Journal*, Gomez and colleagues (pp. 51–64) and Li and colleagues (pp. 65–72) provide additional data about the role of miRNAs in corticosteroid-resistant asthma in children and neutrophilic adults, respectively (8, 9).

Li and coworkers identified seven circulating miRNAs associated with treatment response, quantified as the change in FEV_1% predicted after 4 years, in a cohort of nearly 500

Originally Published in Press as DOI: 10.1164/rccm.202004-1216ED on April 30, 2020

children with asthma who were selected from the CAMP (Childhood Asthma Management Program) study and were receiving either budesonide or placebo. Based on an adjusted analysis, the miRNAs with the greatest effects on treatment response were miR-155-5p and miR-532-5p. These two miRNAs had different effects: increasing levels of circulating miR-155-5p were associated with an increased response to budesonide, whereas the opposite was true for miR-532-5p. In an attempt to validate these biological effects, the authors intriguingly observed that miR-155-5p inhibited, rather than enhanced, the transrepressive effects of corticosteroids on IL-1β expression in lung epithelial cells *in vitro*. Also, circulating miR-155-5p levels predicted treatment response with an area under the receiver operating characteristic curve of 0.85. However, the prediction data were obtained using only the (unspecified) highest versus lowest quartiles of response, leaving a large gray zone. In the absence of a complete analysis, we wonder whether including the highest versus lowest quartiles of miRNA levels in the same model could have been more informative in terms of prediction? With this in mind, it is still encouraging to find that long-term treatment response can be predicted as early as in the first decade of life with the use of circulating biomarkers, at least in a subpopulation of patients selected *a posteriori*. The opposite effects observed in intracellular versus extracellular compartments for the same miRNA are intriguing and raise new questions. It would be informative to reproduce the experimental approach using primary cells from individuals with asthma and control subjects, and using stimuli more "typical" of the asthmatic response in children, such as T2 cytokines. Similar approaches should also be applied to other structural and immune cell types that are more likely involved in the asthmatic response to identify the targets of miRNA-155-5p.

Gomez and colleagues used RNA sequencing and complex bioinformatics to match miRNA networks with clinical phenotypes of the disease and with specific patterns of mRNA expression in sputum cells from 62 subjects with mild-to-severe asthma and 9 control subjects. They found a miRNA network (the "nely" network) associated with sputum neutrophilia and lymphocytosis, reduced FEV1% predicted and quality of life, and increased hospitalizations in the previous year. Among the 12 nely miRNAs identified, the one that most closely correlated with these clinical aspects was miR-223-3p, which the authors showed to be expressed in neutrophils and lymphocytes. However, they did not exclude the possibility that other cell types express this miRNA. Clustering the patients in two groups based on their expression of nely miRNAs revealed that, with similar ICS doses, subjects with asthma and high nely miRNA expression had reduced FEV_1% predicted both before and after bronchodilation. Lastly, the authors identified patterns of mRNA expression linked to the T-helper cell type 17 (Th17) response, TLR, and unfolded protein response/endoplasmic reticulum stress associated with the nely miRNA network. Overall, these data confirm previous findings and suggest that miRNA could play an important role in neutrophil biology and neutrophil–lymphocyte signaling. Severe steroid-resistant asthma has been associated clinically with increased sputum and bronchial neutrophilia, high exacerbation rates, and decreased quality of life (10–12). An increased expression of miR-223-3p has already been described in neutrophilic asthma (13). Also, the Th17 and unfolded protein responses are increasingly recognized as mechanisms of corticosteroid resistance in asthma (14, 15). Several questions remain to be answered. Is there a role for such miRNA in disease pathobiology, or is it simply a marker of neutrophilia? Do circulating neutrophils express the same miRNA pattern? Can we prove biologically what has been inferred bioinformatically? These are all questions that deserve to be answered in the future.

More research is clearly warranted to clarify the role of miRNAs in asthma, and the current data are too limited to speculate about their possible use in clinical practice. Although the novel findings of these studies provide evidence in support of the potential use of miRNAs in disease subendotyping and asthma management, they need to be confirmed in other cohorts, and many mechanistical issues remain to be addressed. The dichotomous function observed intracellularly versus extracellularly for some miRNAs, along with their potential pleiotropic role in physiological processes, raises doubts concerning their use as drug targets at present. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Fabio L. M. Ricciardolo, M.D., Ph.D.
Vitina Carriero, Ph.D.
Department of Clinical and Biological Sciences
University of Turin
Turin, Italy

Michela Bullone, D.V.M., Ph.D.
Department of Veterinary Sciences
University of Turin
Turin, Italy

ORCID IDs: 0000-0003-1826-5018 (F.L.M.R.); 0000-0003-1866-5206 (V.C.); 0000-0002-9094-6734 (M.B.).

References

1. McGeachie MJ, Yates KP, Zhou X, Guo F, Sternberg AL, Van Natta ML, *et al*. Patterns of growth and decline in lung function in persistent childhood asthma. *N Engl J Med* 2016;374: 1842–1852.
2. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998; 339:1194–1200.
3. Global Initiative for Asthma GINA guidelines. The global strategy for asthma management and prevention. 2019 [accessed 2020 Apr]. Available from: https://www.ginasthma.org/.
4. Szefler S, Weiss S, Tonascia J, Adkinson NF, Bender B, Cherniack R, *et al*.; Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med* 2000;343:1054–1063.
5. Lange P, Scharling H, Ulrik CS, Vestbo J. Inhaled corticosteroids and decline of lung function in community residents with asthma. *Thorax* 2006;61:100–104.
6. Wadhwa R, Dua K, Adcock IM, Horvat JC, Kim RY, Hansbro PM. Cellular mechanisms underlying steroid-resistant asthma. *Eur Respir Rev* 2019;28:190096. [Published erratum appears in Eur Respir Rev 28: 195096.]

7. Ahmed H, Turner S. Severe asthma in children-a review of definitions, epidemiology, and treatment options in 2019. *Pediatr Pulmonol* 2019; 54:778–787.
8. Gomez JL, Chen A, Diaz MP, Zirn N, Gupta A, Britto C, *et al*. A network of sputum microRNAs is associated with neutrophilic airway inflammation in asthma. *Am J Respir Crit Care Med* 2020; 202:51–64.
9. Li J, Panganiban R, Kho AT, McGeachie MJ, Farnam L, Chase RP, *et al*. Circulating microRNAs and treatment response in childhood asthma. *Am J Respir Crit Care Med* 2020;202:65–72.
10. Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, *et al*.; National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol* 2014;133: 1557–1563.e5.
11. Ricciardolo FLM, Sorbello V, Folino A, Gallo F, Massaglia GM, Favatà G, *et al*. Identification of IL-17F/frequent exacerbator endotype in asthma. *J Allergy Clin Immunol* 2017;140:395–406.
12. Bullone M, Carriero V, Bertolini F, Folino A, Mannelli A, Di Stefano A, *et al*. Elevated serum IgE, oral corticosteroid dependence and IL-17/22 expression in highly neutrophilic asthma. *Eur Respir J* 2019; 54:1900068.
13. Maes T, Cobos FA, Schleich F, Sorbello V, Henket M, De Preter K, *et al*. Asthma inflammatory phenotypes show differential microRNA expression in sputum. *J Allergy Clin Immunol* 2016;137:1433–1446.
14. Sorbello V, Ciprandi G, Di Stefano A, Massaglia GM, Favatà G, Conticello S, *et al*. Nasal IL-17F is related to bronchial IL-17F/neutrophilia and exacerbations in stable atopic severe asthma. *Allergy* 2015;70:236–240.
15. Jeong JS, Kim SR, Cho SH, Lee YC. A novel insight on endotyping heterogeneous severe asthma based on endoplasmic reticulum stress: beyond the "type 2/non-type 2 dichotomy". *Int J Mol Sci* 2019;20:713.

Alpha-1 Antitrypsin Deficiency: The Learning Goes On

The pathophysiology of chronic obstructive pulmonary disease (COPD) is widely accepted to reflect an abnormal inflammatory response to the inhalation of toxic agents, especially cigarette smoke. This is supported by the development of multiple transgenic and knockout mice that either develop "emphysema-like" changes spontaneously or become more resistant or susceptible to cigarette smoke exposure.

These studies have their origin in the observation some 60 years ago of several subjects with a circulating deficiency of alpha-1 antitrypsin (AAT) associated with severe early onset basal panlobular emphysema. Once recognized as a clinical and familial entity, targeted testing confirmed the association. Index was the term used for those identified by testing symptomatic subjects and nonindex for those identified by chance or family screening. Nonindex cases were generally younger with better preserved lung function, suggesting an earlier stage of evolution of the disease.

Because AAT was recognized as an inhibitor of neutrophil elastase (NE), which can replicate many of the features of COPD, the concept of an NE/AAT imbalance became, and remains, the cornerstone of the pathophysiology of COPD and, specifically, AAT deficiency.

Logically, supplementation of AAT should restore the normal NE/AAT physiological balance in the lung. In the early 1980s, AAT was purified from human blood and shown to increase recipient AAT in both blood and the lungs (1), falling over seven days to levels still above baseline (~12 μM). It was argued that this was still protective because other AAT variants with levels above this were not at increased risk for COPD (2). *In vitro* experiments confirmed excess connective tissue damage with ZZ plasma was greatly abrogated by MM plasma and decreasing ambient level with SZ; MS and MZ plasma had a similar effect, enabling physiological NE activity to take place while preventing excessive damage (3). The authors concluded a target threshold of 11 μM was largely "protective."

The initial patients with AAT deficiency were homozygous for the Z variant gene and these subjects had typical blood levels of AAT of <8 μM (i.e., below the putative protective threshold).

Although such patients represent the vast majority of subjects receiving augmentation therapy, studies have identified multiple variants of AAT, although most have AAT levels in the normal range (17–47 μM).

The S variant has a mild reduction in blood level and SZ heterozygotes have AAT levels that range from the protective threshold of 11 to 28 μM, suggesting at least some may be at risk and benefit from augmentation therapy. Currently, such patients are being treated (as well as MZ heterozygotes who have even higher AAT levels) at great cost (~$80 million/yr in the United States alone) despite lack of demonstrable benefit.

Whether SZ patients are at increased risk of COPD has long been contentious, with some studies saying yes (4) and some no (5). The problem of perceived risk largely relates to acquisition bias. Patients with established COPD are usually tested if young, with severe disease, and perhaps a limited smoking and/or family history of COPD. Family testing identifies further deficient subjects and more COPD. However, COPD can run in families without AAT deficiency, suggesting unknown genetic concordance as well as common social and environmental risk factors. Thus, whether an abnormal AAT genotype is an association with or cause of COPD remains uncertain.

Originally Published in Press as DOI: 10.1164/rccm.202004-0922ED on April 28, 2020