

Asthma phenotyping: T_H2-high, T_H2-low, and beyond

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The step change of biomedical research during the past years has been the recognition that clinical characteristics and biological mechanisms of disease can no longer be addressed separately. There is little doubt that an integrative multiscale approach linking patient-reported, clinical, physiologic, structural, cellular, and molecular features will get us closer to identification of various disease phenotypes that will allow tailored patient management.¹

There are essentially 2 ways to address this. We are all used to the hypothesis-dependent approach, in which biological pathways that have been shown to be involved in the disease are included in diagnosis and management. A good example in respiratory disease is the presence or absence of eosinophilic inflammation in asthmatic patients,² which is derived from longstanding data showing the involvement of eosinophils in flare-ups of this disease.³ This paradigm has subsequently been fine-tuned by subphenotyping asthma based on T_H cell cytokine profiles that were discovered 25 years ago.⁴ By using these pathways, it has been proposed that asthma can be divided into T_H2 -high and T_H2 -low phenotypes, which can have relevance for relative differences in treatment responsiveness to existing⁵ or newly developed⁶ drugs.

The more recent strategy in linking biological information to clinical phenotyping is the unbiased approach. This is purposely not based on *a priori* hypotheses, thereby avoiding the implicit restriction to including previously discovered biological pathways only. This can be accomplished by high-throughput “-omics” technologies that allow the unsupervised identification of composite molecular fingerprints at the transcriptional, protein, or metabolic level in relation to clinical disease features.⁷ This uses all biological information as sampled from complex matrices, such as blood, sputum, or exhaled air,⁸ allowing discovery of new and previously unrecognized biological networks in relation to inflammatory pathways⁹ and treatment responses.¹⁰ Therefore, at present, both the hypothesis-dependent and unbiased approaches are meant to go hand in hand in the subphenotyping of asthma.¹¹

In the current issue of the *Journal*, Peters et al.¹² have followed the hypothesis-dependent approach by searching for T_H2-high and T_H2-low gene expression in the sputum of asthmatic patients and control subjects. The rationale of this study is obvious because sputum (rather than epithelial brushings⁵) is noninvasively accessible in patients in daily practice. Their study was a *post hoc* analysis of sputum samples of well-characterized subjects from 4 existing clinical trials. The authors examined sputum cell RNA using quantitative PCR for the T_H2 cytokines *IL4*, *IL5*, and *IL13* and for the IL-13-induced epithelial gene transcripts of periostin, chloride channel accessory 1 (*CLCA1*), and *SERPINB2*. Furthermore, they included genes derived from other hypothesis-derived pathways, such as *IL17*, *IFNG*, and genes related to mast cell activation. RNA quality was carefully monitored by using a bioanalyzer, and only those sputum samples with an acceptable RNA integrity number (>5) were used.

As expected, the results showed continuously distributed, partly overlapping gene expression levels between asthmatic patients and control subjects.¹² Nevertheless, gene expression for *IL4*, *IL5*, *IL13*, periostin, and *CLCA1* was significantly upregulated in asthmatic patients compared with control subjects. Most importantly, the data showed elegantly that some asthmatic patients exhibit increased expression of these cytokines, whereas other asthmatic patients do not. Notably, these subgroups were not distinguishable because of the unimodal distribution of gene expression profiles among asthmatic patients, which makes distinction by cut points inevitably arbitrary. To strengthen the concept, the authors compiled T_H2 gene expressions into a composite “T_H2 gene mean score,” which appeared to be a repeatable index within samples but was not invariable over time. Nevertheless, the T_H2 gene mean score was associated with asthma control and spirometry, although again with considerable overlap. Interestingly, receiver operating characteristic analysis demonstrated that sputum eosinophilia is fully specific but not very sensitive for T_H2-high gene expression, indicating that both measures provide complementary information. Taken together, this study shows that when searching for T_H2-high and T_H2-low biological phenotypes at the cytokine level, sputum is a suitable source of material. Combined with sputum eosinophilia, this brings more detailed phenotyping of asthmatic patients closer to clinical research and practice.

Interestingly, the strengths and limitations of the study are somewhat related. First, the data were derived from 4 independent studies with strict and adequate inclusion criteria. However, these studies have not been published. Even though their subject characteristics seem to be comparable (see Table E1 in the study by Peters et al¹²), a study bias cannot be excluded.

Second, sputum represents a limited and variable source of resident and inflammatory cell types from the airways. Hence the cellular source might vary between patients, introducing a confounder when distinguishing gene expression patterns. This is not critical if one considers the effector functions of the expressed genes, but it is critical if these genes are being related to

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a specific cell type, such as here to T_H2 cells. The recently discovered type 2 innate lymphoid cells have a very similar cytokine profile as T_H2 cells,^{13,14} and therefore the findings for T_H2 mediators might also be due to type 2 innate lymphoid cells. On the other hand, concentrating on one particular cell type^{5,11,13} might not always capture the gene expression of interest.

Third, the concept of using the T_H2 gene mean score represents a nice attempt to obtain robustness in transcriptomic data. Nevertheless, the within-patient, between-sample reproducibility over time was rather modest, showing that only 8 of 13 patients had persistently high or low T_H2 expression over months. This might be discouraging, but it cannot be excluded that the presence or absence of stability of the T_H2 gene mean score provides a phenotypic signal by itself. Hence rather than using classification based on arbitrary cut points, the level and dynamic behavior of T_H2 gene expression might be of greater interest.

Finally, patients on and off steroids were equally distributed among the T_H2 -high and T_H2 -low subgroups. The authors interpreted this as robustness of the measure regardless of steroid treatment. However, it is highly likely that the genes of interest are modified by steroids within subjects, particularly in the T_H2 -high subgroup. Therefore confounding by steroids has yet to be investigated in longitudinal studies.

Although specifics were not provided, the determination of the T_H2 gene mean score is as labor intensive and demanding as the determination of sputum eosinophil counts, and thus general implementation of this assay or these assays combined in clinical practice will be limited. Also, for a cutoff value of 0.8% for eosinophils to be reliable, an even more demanding sputum cell count of approximately 1000 cells will be required. In view of this, it would have been of interest to see in Fig 6 in the article by Peters et al¹² how serum periostin levels performed as a biomarker of the T_H2 gene inflammation.

The marked interrelation of the various transcripts selected on the basis of the T_H2 -high signature is indicative of their interdependence. This calls for further analyses in sputum of related processes, such as involvement of type 2 innate lymphoid cells,¹³ by measuring IL-33, IL-25, and thymic stromal lymphopoietin transcripts, or rather proteins, because these are the effector molecules. However, analyses of apparently unrelated processes can provide insight into the dynamic interdependence of processes as well and might explain the observed fluctuation of the T_H2 gene mean score over time within patients.

What is the take-home message? Sputum provides a continuous measure of T_H2 gene expression that provides phenotypic

information complementary to sputum eosinophil counts. There is good evidence that these hypothesis-dependent biomarkers can be used for tailoring treatments that are derived from the same hypothesis, in this case derived from the T_H2 paradigm in asthmatic patients.^{2,5,6} Now essentially novel therapeutic strategies need to be derived from the more recent hypothesis-independent approaches that allow discovery beyond existing paradigms, for which the standardized procedures¹⁵ and first data in asthmatic patients are now available.^{10,11}

REFERENCES

1. Bousquet J, Anto JM, Sterk PJ, Adcock IM, Chung KF, Roca J, et al. Systems medicine and integrated care to combat chronic noncommunicable diseases. *Genome Med* 2011;3:43.
2. Petsky HL, Cates CJ, Lasserson TJ, Li AM, Kynaston JA, Chang AB. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). *Thorax* 2012;67:199-208.
3. De Monchy JG, Kauffman HF, Venge P, Koeter GH, Jansen HM, Sluiter HJ, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985;131:373-6.
4. Corrigan CJ, Hartnell A, Kay AB. T lymphocyte activation in acute severe asthma. *Lancet* 1988;8595:1129-32.
5. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;180:388-95.
6. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088-98.
7. Chen R, Mias GI, Li-Pook-Than J, Jiang L, Lam HYK, Chen R, et al. Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* 2012;148:1293-307.
8. Wheelock CE, Goss VM, Balgoma D, Nicholas B, Brandsma J, Skipp PJ, et al. Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. *Eur Respir J* 2013;42:802-25.
9. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol* 2011;127:153-60.
10. Yick CY, Zwirnerman AH, Kunst PW, Grünberg K, Mauad T, Fluiter K, et al. Glucocorticoid-induced changes in gene expression of airway smooth muscle in patients with asthma. *Am J Respir Crit Care Med* 2013;187:1076-84.
11. Auffray C, Adcock IM, Chung KF, Djukanovic R, Sterk PJ. An integrative systems biology approach to understanding pulmonary diseases. *Chest* 2010;137:1410-7.
12. Peters MC, Mekonnen ZK, Yan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify Th2-high and Th2-low subtypes of asthma. *J Allergy Clin Immunol* ■■■■■■■■.
13. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol* 2011;12:21-7.
14. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012;36:451-63.
15. Sung J, Wang Y, Chandrasekaran S, Witten DM, Price ND. Molecular signatures from omics data: from chaos to consensus. *Biotechnol J* 2012;7:946-57.