

Asthma biomarkers: what constitutes a 'gold standard'?

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Many studies have assessed sputum eosinophil percentage as a phenotypic descriptor in asthma patients. Adjusting inhaled corticosteroid (ICS) dose based on sputum eosinophilia can lead to better clinical outcomes than using empirical clinical guidelines alone.^{1,2} While techniques for collecting and analysing sputum eosinophils are available in specialty clinics, sputum analysis remains time consuming, onerous to the patient, labour intensive and is not always successful, which precludes its broader clinical generalisability to primary care settings where most asthma patients are managed. Thus, there is a need for less invasive and more technically straightforward means of phenotyping asthma patients to enable therapeutic management along biological, rather than empirical, guidelines. In particular, accurate phenotyping of severe asthma patients refractory to ICS treatment has proven to be critical for demonstrating the efficacy of emerging experimental therapies that target specific inflammatory pathways.³

Sterk and colleagues compared three biomarkers, peripheral blood eosinophils, fractional exhaled nitric oxide (FeNO) and serum periostin levels, for assessing how reliably each reflects sputum eosinophilia in a cross-sectional study.⁴ They report that blood eosinophils performed favourably, with a receiver operating characteristic area under the curve of 0.89 in a test cohort and 0.85 in a replication cohort. Using a threshold of 270 eosinophils/ μL of blood, they report positive and negative predictive values of 79% and 91% for predicting sputum eosinophilia of $\geq 3\%$, which is in line with a recent similar study in *Clinical and Experimental Allergy* by Zhang and colleagues who identified 260 eosinophils/ μL of blood as an optimal cut-off.⁵ Overall, the finding that blood eosinophil counts are a reasonably accurate biomarker of sputum eosinophil

percentage in two rigorously conducted independent studies is encouraging, as blood eosinophil counts are readily obtained from a complete blood count with differential, a test that is convenient and widely available on automated haematology analysers, which are locally calibrated at most clinical laboratories.

While Sterk and colleagues found that FeNO levels were also significantly predictive of sputum eosinophilia, they did not observe a significant relationship between serum periostin and sputum eosinophilia, a finding at odds with previous work from our groups.^{6,7} Periostin is a matricellular protein secreted from stromal cells in response to various stimuli, most notably TGF β , interleukin (IL)-4 and IL-13. Elevated peripheral blood levels of periostin have been described in asthma as well as other disease states including systemic sclerosis, idiopathic pulmonary fibrosis, atopic dermatitis and cancer.^{8,9} Among asthma patients, periostin gene and protein expression levels are elevated in bronchial tissue and are highly correlated with the expression of the type 2 cytokines IL-5 and IL-13 and with eosinophilic airway inflammation.^{10,11} Independently developed serum periostin assays in multiple patient populations in exploratory studies and randomised placebo-controlled clinical trials (RCTs) have shown that serum periostin levels are positively correlated with FeNO and eosinophils in sputum, bronchial mucosal tissue and peripheral blood in asthma patients.^{3,6,7} Serum periostin, blood eosinophils and FeNO were prognostic for increased rates of severe asthma exacerbations in the placebo arm of a large clinical trial and predicted increased clinical benefit from treatment with omalizumab (anti-IgE).¹² Elevated serum periostin levels identified patients more likely to demonstrate improvement in lung function and reduction in exacerbation rates in 3 different RCTs of two independent anti-IL-13 therapies (lebrikizumab and tralokinumab) in over 1100 moderate-severe asthma patients, using two independently developed periostin assays.¹³⁻¹⁶ Lebrikizumab treatment reduced the distributions of serum periostin and FeNO levels in moderate-severe asthmatics to those

observed in healthy non-asthmatic subjects, underscoring the mechanistic link between IL-13 and these biomarkers.³

As one of the goals of Sterk and colleagues was to 'externally validate' serum periostin as a surrogate biomarker of sputum eosinophilia in asthmatic patients, it is important to try to understand the discrepancies between their findings and previously published results from independent groups. They cite the Standards for Reporting of Diagnostic Accuracy (STARD) guidelines¹⁷ as a pretext to externally validate the diagnostic accuracy of blood eosinophils, FeNO and serum periostin in identifying asthmatic patients with sputum eosinophilia. A critical component of the STARD guidelines is Item 8, which pertains to technical specifications for measurements, index tests and reference standards. There are several levels of regulatory qualification for clinical biomarker tests, the most relevant designation being *in vitro* diagnostic (IVD) devices. An IVD device has been defined by the Global Harmonization Task Force as 'a device, whether used alone or in combination, intended by the manufacturer for the in-vitro examination of specimens derived from the human body solely or principally to provide information for diagnostic, monitoring or compatibility purposes. This includes reagents, calibrators, control materials, specimen receptacles, software, and related instruments or apparatus or other articles' (<http://www.imdrf.org/docs/ghtf/final/sg1/technical-docs/ghtf-sg1-n071-2012-definition-of-terms-120516.pdf>). Of the biomarkers assessed in this article, FeNO and blood eosinophils were measured using devices that meet this standard, whereas sputum eosinophils and serum periostin were measured by assays that do not meet this standard. Presently, there are no commercially available serum periostin or sputum eosinophil assays that are regulated as IVD devices. It should be noted that blood eosinophils, even if measured on an IVD device, are not indicated for the diagnosis of sputum eosinophilia in asthma patients. Additionally, there are many makes and models of automated haematology counters; they are not all calibrated identically nor do they have the same reference ranges.

Two possible explanations for the lack of concordance between serum periostin and sputum eosinophilia are: 1) periostin assay used and 2) the clinical features of the cohorts examined. The R&D Systems periostin assay technical performance as reported by the authors had intra-assay and inter-assay coefficients

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of variability (CVs) of 12.3% and 17.4%, respectively. In our proprietary assays we have reported assay CVs of 2.8%–5.3% or 1.5%–3.0%, respectively, across a broad range of periostin concentrations in human serum.^{6, 18} Although the authors have attempted to optimise the R&D assay for both serum and EDTA-anticoagulated plasma, these matrices are complex and a given biomarker assay may behave differently in each matrix. Blood biomarkers may have undergone post-translational modifications that affect the interpretability of the assay relative to a recombinant standard. As periostin can exist in multiple differentially spliced isoforms,¹⁹ which are present in different proportions in different tissues and disease states,^{20, 21} the potential for confounding results due to different mixtures of isoforms in a biological sample must be accounted for. Accordingly, the authors report that there was a significant correlation between sputum eosinophil percentage and serum periostin levels using the Elecsys Periostin assay (under development by Roche Diagnostics), but not with the R&D assay, although neither assay showed significant effects in the ROC analyses. Furthermore, the range and distribution of periostin levels were substantially different between the two assays; a direct comparison of individual samples run on both assays is not shown. Regarding asthma severity, the populations described in the present study were generally less severe and on lower steroid doses than those described in prior periostin studies, which may result in different relationships between airway inflammation and biomarker distributions. This study does not fully resolve these issues and as the authors suggest, it will be of interest to assess these biomarkers in larger multi-center cohorts representing a range of severity such as U-BIOPRED.

Larger questions remain: why are sputum eosinophils the ‘gold standard’? What is the significance of applying a cut-off of 2% or 3% to sputum eosinophils when their levels are continuously distributed across the population? Given the temporal variability in airway inflammation, what is the significance of a single cross-sectional measurement of sputum eosinophil percentage? And what pathophysiological mechanisms of asthma do sputum eosinophils actually reflect? There is a significant but weak correlation between sputum eosinophil percentage and blood eosinophil counts, but the correlations between eosinophils in different airway compartments including bronchial mucosal tissue and

bronchoalveolar lavage (BAL) fluid are also weak.²² We have shown that serum periostin levels are related to both sputum and bronchial tissue eosinophil levels, and these effects are additive.⁵ Therefore, a systemic biomarker that effectively integrates the aggregate inflammation across the total airway tissue may be a better indicator of a given subject’s inflammatory state than an isolated sputum, biopsy or BAL sample. Unfortunately, such a highly sensitive and specific biomarker may not exist. Asthma patients often have comorbidities such as atopic dermatitis or rhinosinusitis that can contribute to the peripheral blood pool of eosinophils or periostin, or there may be genetic and/or environmental influences on these biomarkers.

All this is to say that defining an airway phenotype in asthma is important, but only if there is an intended use for that phenotypic information. If the decision is to initiate, escalate, or decrease the dose of inhaled or systemic corticosteroids so as to reduce the risk of asthma exacerbations, evidence suggests that titrating steroid doses to sputum eosinophil or FeNO levels^{1, 2, 23} may reduce the rate of exacerbations in a population of patients although we are not yet at the point of being able to confidently predict benefit or risk for any given individual based on biomarkers. The results presented by the Sterk⁴ and Zhang⁵ groups suggest that future studies should assess the utility of blood eosinophil counts for similar purposes. For investigational therapies targeting inflammatory cytokines such as IL-5 and IL-13, consideration should be paid to the biological activities of therapeutic targets as they relate to biomarkers. IL-5 leads to eosinophil haematopoiesis and mobilisation from the bone marrow but does not directly induce the expression of periostin or inducible nitric oxide synthase (iNOS). IL-13 acts directly on airway structural cells to induce periostin and iNOS expression and therefore may be more directly and mechanistically linked to serum periostin and FeNO than IL-5. IL-13 does not directly induce eosinophil production, but does induce the expression of chemoattractants in structural cells of the airway that can recruit and retain eosinophils in bronchial tissue. IL-5 and IL-13 are highly coregulated *in vivo* in human asthmatic airway tissue. Thus, IL-5 and IL-13 likely conspire to promote systemic and tissue eosinophilia, with IL-5 leading to eosinophil production and IL-13 leading to eosinophil recruitment.³

The precise relationships among molecular pathways, biomarkers, pathologies and treatments in asthma remain to be determined and will require interventional therapeutic studies in conjunction with the assessment of invasive and non-invasive biomarkers. As our armamentarium of biomarkers and therapeutic options expands, we must remember that the ultimate goal of these ongoing and future investigations is not to identify the strongest correlations between biomarkers, but to develop the best tools to identify therapeutic interventions most appropriate for managing the clinical presentation of individual patients.

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REFERENCES

- Green RH, Brightling CE, McKenna S, *et al.* Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;**360**:1715–21.
- Jayaram L, Pizzichini MM, Cook RJ, *et al.* Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006;**27**:483–94.
- Arron JR, Choy DF, Scheerens H, *et al.* Noninvasive biomarkers that predict treatment benefit from biologic therapies in asthma. *Ann Am Thorac Soc* 2013;**10**(Suppl):S206–13.
- Wagener AH, de Nijs SB, Lutter R, *et al.* External validation of blood eosinophils, FeNO, and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 2015;**70**:115–20.
- Zhang XY, Simpson JL, Powell H, *et al.* Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clin Exp Allergy* 2014;**44**:1137–45.
- Jia G, Erickson RW, Choy DF, *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol.* 2012;**130**:647–54.e10.

- 7 Kim MA, Izuhara K, Ohta S, *et al.* Association of serum periostin with aspirin-exacerbated respiratory disease. *Ann Allergy Asthma Immunol* 2014;113:314–20.
- 8 Conway SJ, Izuhara K, Kudo Y, *et al.* The role of periostin in tissue remodeling across health and disease. *Cell Mol Life Sci* 2014;71:1279–88.
- 9 Izuhara K, Arima K, Ohta S, *et al.* Periostin in allergic inflammation. *Allergol Int* 2014;63:143–51.
- 10 Woodruff PG, Modrek B, Choy DF, *et al.* T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;180:388–95.
- 11 Sidhu SS, Yuan S, Innes AL, *et al.* Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci U S A* 2010;107:14170–5.
- 12 Hanania NA, Wenzel S, Rosen K, *et al.* Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013;187:804–11.
- 13 Corren J, Lemanske RF, Hanania NA, *et al.* Lebrilizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088–98.
- 14 McClintock D, Corren J, Hanania NA, *et al.* Lebrilizumab, an anti-IL-13 monoclonal antibody, reduces severe asthma exacerbations over 32 weeks in adults with inadequately controlled asthma. *Am J Respir Crit Care Med* 2012;185:A3959.
- 15 Hanania NA, Noonan MJ, Corren J, *et al.* Efficacy and Safety of lebrilizumab in severe uncontrolled asthma: results from the LUTE and VERSE phase II randomized, double-blind, placebo-controlled trials. *J Allergy Clin Immunol* 2014;133:AB402.
- 16 Brightling CE, She D, Ranade K, *et al.* Efficacy and safety of tralokinumab, an anti-IL13 monoclonal antibody, in a phase 2b study of uncontrolled severe asthma. *Am J Respir Crit Care Med* 2014;189:A6670.
- 17 Bossuyt PM, Reitsma JB, Bruns DE, *et al.* Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. *Ann Intern Med* 2003;138:40–4.
- 18 Kou K, Okawa T, Yamaguchi Y, *et al.* Periostin levels correlate with disease severity and chronicity in patients with atopic dermatitis. *Br J Dermatol* 2014;171:283–91.
- 19 Hoersch S, Andrade-Navarro MA. Periostin shows increased evolutionary plasticity in its alternatively spliced region. *BMC Evol Biol* 2010;10:30.
- 20 Nance T, Smith KS, Anaya V, *et al.* Transcriptome analysis reveals differential splicing events in IPF lung tissue. *PLoS one* 2014;9:e97550.
- 21 Yamada S, Tauchi T, Awata T, *et al.* Characterization of a novel periodontal ligament-specific periostin isoform. *J Dent Res* 2014;93:891–7.
- 22 Arron JR, Choy DF, Laviolette M, *et al.* Disconnect between sputum neutrophils and other measures of airway inflammation in asthma. *Eur Respir J* 2014;43:627–9.
- 23 Powell H, Murphy VE, Taylor DR, *et al.* Management of asthma in pregnancy guided by measurement of fraction of exhaled nitric oxide: a double-blind, randomised controlled trial. *Lancet* 2011;378:983–90.