

Disconnect between sputum neutrophils and other measures of airway inflammation in asthma

Joseph R. Arron, MD, PhD,^a David F. Choy, PhD,^a Michel Laviolette, MD,^b Steven G. Kelsen, MD,^c Ammar Hatab, MD,^d Richard Leigh, MD, PhD,^e Neil C. Thomson, MBChB, MD,^f Eugene R. Bleeker, MD,^g Ron Olivenstein, MD,^h Pedro C. Avila, MD,ⁱ Nizar N. Jarjour, MD,^j Mario Castro, MD, MPH,^k Gail M. Gauvreau, MD,^l James T. Good, MD,^m Joel N. Kline, MD,ⁿ Adel Mansur, MD,^o Irvin Mayers, MD,^p Liam G. Heaney, MD,^q Qutayba Hamid, MD, PhD,^r Jeffrey M. Harris, MD, PhD,^a

^aGenentech Research and Early Development, South San Francisco, CA, USA

^bIUCPQ, Université Laval, Québec, QC, Canada

^cDepartment of Medicine, Temple University School of Medicine, Philadelphia PA, USA

^dWest Coast Clinical Trials LLC, Costa Mesa, CA, USA

^eFaculty of Medicine, University of Calgary, Calgary, AB, Canada

^fInstitute of Infection, Immunity, and Inflammation, University of Glasgow, Glasgow, UK

^gCenter for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, NC, USA

^hMontréal Chest Institute, Montréal, QC, Canada

ⁱDepartment of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

^jDepartment of Medicine, University of Wisconsin-Madison, Madison, WI, USA

^kDepartment of Medicine, Washington University School of Medicine, St. Louis, MO, USA

^lDepartment of Respiriology, Faculty of Health Sciences, McMaster University, Hamilton ON, Canada

^mDepartment of Medicine, National Jewish Health, Denver, CO

ⁿDepartment of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA, USA

^oRespiratory Department, Birmingham Heartlands Hospital, Birmingham, UK

^pDepartment of Medicine, University of Alberta, Edmonton, AB, Canada

^qCentre for Infection and Immunity, Queens University Belfast, Belfast, UK

^rMeakins-Christie Laboratories, McGill University, Montréal, QC, Canada

Corresponding author:

Jeffrey M. Harris, MD, PhD

Genentech, Inc.

1 DNA Way, MS 453b

South San Francisco, CA 94080

Tel: (650) 467-7154

FAX: 866-858-7006

Email: harris.jeffrey@gene.com

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Take-home message: Defining asthma phenotypes solely according to sputum granulocyte proportions may be misleading.

Key words: asthma, eosinophil, neutrophil, sputum, bronchoscopy

Abbreviations used

ACQ: asthma control questionnaire

BAL: bronchoalveolar lavage

BOBCAT: Bronchoscopic exploratory research study of Biomarkers in Corticosteroid-refractory Asthma

FeNO: fraction of exhaled nitric oxide

FEV₁: forced expiratory volume in one second

FP: fluticasone propionate

ICS: inhaled corticosteroid

LABA: long-acting β_2 -adrenergic agonist

PC₂₀: provocative concentration resulting in a decline of 20% FEV₁

r_s: Spearman's rank correlation

To the editor:

Asthma heterogeneity has been described by the nature and intensity of granulocytic infiltration into the airways[1, 2]. Sputum, endobronchial biopsies, and bronchoalveolar lavage (BAL) sample different anatomical regions of the airways. Few studies have directly compared the inflammatory cell infiltrates in these regions within a large cohort of moderate-severe asthma patients using standard techniques.

In the BOBCAT study, we sampled multiple airway compartments concurrently, enabling us to evaluate relationships between granulocytic infiltrates within and between compartments in a large cohort of moderate-severe adult asthma patients. This prospective multicenter observational study was conducted in 4 visits over a 4-6 week period as described previously[3].

Included patients had moderate-severe persistent asthma (FEV_1 40%-80% predicted and ACQ score[4] >1.5), and evidence within the last 5 years of >12% post-bronchodilator reversibility or PC_{20} methacholine ≤ 8 mg/mL despite high-dose ICS (≥ 1000 μ g/d fluticasone propionate (FP) equivalent) with or without LABA therapy. Key exclusion criteria included initiation or increase in systemic steroid use 30 days prior to screening, use of chronic or recent (within 30 days) immunosuppressive therapies, or other active lung disease. Patients had a prior established diagnosis of moderate-to-severe asthma for ≥ 6 months prior to screening while receiving a stable dose regimen

(>6 weeks) of a high-dose ICS. Allowed concomitant medications included leukotriene receptor antagonists and oral corticosteroids.

Seventy-eight patients with confirmed moderate-severe asthma were enrolled at 18 sites; 67 (86%) completed the study. All patients had persistently impaired lung function and uncontrolled symptoms despite high dose ICS treatment of ≥ 1000 $\mu\text{g}/\text{day}$ FP equivalent. Eleven patients (14%) did not complete the study, due to an adverse event (n=1), physician's decision (n=5), subject's decision (n=3), or sponsor's decision (n=2). One patient experienced increased bronchospasm following bronchoscopy requiring additional observation and oral corticosteroid treatment prior to being discharged home from the final study visit.

We enumerated sputum, tissue, and BAL eosinophils and neutrophils using standard techniques as described previously [5, 6]. Both granulocyte types spanned broad ranges and were unimodally distributed in each compartment (Figure 1). The majority of non-squamous cells in sputum from most subjects were neutrophils, while a much smaller percentage of sputum cells were eosinophils. The majority of BAL cells were macrophages (data not shown), with eosinophils and neutrophils comprising less than 10% of total BAL cells in most cases.

Within each compartment, eosinophils and neutrophils were significantly inter-correlated. However, these correlations were positive in biopsy tissue and BAL, but negative in sputum (Figure 1). The correlation within any single compartment was

greatest in biopsy tissue ($r_S=0.68$, $p<10^{-4}$). Across compartments, sputum eosinophils significantly, albeit weakly, positively correlated with both biopsy tissue eosinophils ($r_S=0.36$, $p<0.05$) and BAL eosinophils ($r_S=0.33$, $p<0.05$), while neutrophils were not intercorrelated across any compartments. Sputum neutrophils were negatively correlated with tissue eosinophils ($r_S=-0.37$, $p<0.01$) and BAL eosinophils ($r_S=-0.34$, $p<0.05$). These findings show generally positive relationships between eosinophils and neutrophils within and across airway compartments with the exception of sputum neutrophil percentage.

Serum periostin and FeNO are positively correlated with sputum and biopsy eosinophils in BOBCAT[3]. Both serum periostin and FeNO trend toward a positive correlation with biopsy neutrophils ($r_S=0.25$, $p=0.06$ for periostin; $r_S=0.23$, $p=0.08$ for FeNO), and exhibit significantly negative correlations with sputum neutrophils ($r_S=-0.31$ for periostin; $r_S=-0.35$ for FeNO, $p<0.05$ for each). None of the airway measures nor blood biomarkers exhibited significant correlations with lung function as assessed by FEV₁ or asthma control as assessed by ACQ (not shown).

Significantly elevated airway neutrophils are not typically observed in mild-moderate asthma patients not taking ICS or on low-dose ICS, but are often seen in severe asthma on high-dose steroids[7, 8], suggesting that chronic ICS treatment is related to elevated airway neutrophils. Our observations add to these findings by showing that, in patients with moderate-severe asthma, mucosal neutrophils are particularly elevated in subjects with tissue eosinophilia despite ICS treatment, and this relationship scales continuously.

Although eosinophil and neutrophil percentages in sputum are measured on a continuous scale, a desire to classify asthma into discrete subsets has yielded “cutoffs” for eosinophilia around 2-3%, whereas “cutoffs” for neutrophilia range from 40% to over 60%[1, 2, 7, 9, 10]. This is because eosinophils are typically absent in nonasthmatic subjects, while a substantial proportion of sputum cells are neutrophils even in healthy subjects[9]. Since a considerable fraction of sputum is composed of neutrophils, an increase in the proportion of another cell subset may come at the expense of neutrophil percentage, setting up a propensity for inherently negative correlations between proportions of the two cell types. While changes in the relative proportion of a minority component of the cellular content of a sample (e.g., sputum eosinophils) may be informative, caution must be exercised in interpreting the relevance of changes in the relative proportion of a component representing the majority or significant plurality of the cellular content of a sample (e.g., sputum neutrophils). Consistent with these considerations, sputum eosinophils exhibited substantial proportional variability but remained a minority component of sputum cells and correlated with tissue and BAL eosinophils, serum periostin, blood eosinophils, and FeNO[3]. Sputum neutrophils exhibited less proportional variability in this study and largely appeared to vary as a negative function of sputum eosinophil percentage (Figure 1).

The weakness of the positive correlations observed between eosinophils across sputum, biopsies, and BAL may be due to several factors, including: 1) the three compartments sample different regions of the airways; 2) factors mediating

transmigration of granulocytes through bronchial mucosal tissue into the airway lumen are poorly understood; 3) the techniques for enumerating and reporting granulocytes in each compartment vary; and 4) each assessment in this study is cross-sectional, with sputum sampling taking place close to, but not contemporaneous with bronchoscopy. Biomarkers that integrate total inflammatory burden systemically may present a means of circumventing the variability across airway compartments. As previously reported for the BOBCAT cohort, patients with low eosinophils in both sputum and bronchial tissue had the lowest serum periostin levels, while subjects with elevated eosinophils in either one or the other compartment had intermediate periostin levels, and subjects with elevated eosinophils in both sputum and tissue had the highest periostin levels [3]. Therefore, sampling multiple airway compartments and/or systemic biomarkers may be more sensitive means to ascertain whether a given asthma patient has eosinophilic airway inflammation that may be missed by sampling only one compartment.

BOBCAT represents a large, well-characterized cohort of moderate-severe asthma patients resistant to high dose ICS with intensive airway sampling. Our key findings are: 1) Sputum, biopsy, and BAL eosinophils are imperfectly but positively correlated, suggesting that eosinophilia in each of those compartments may be informative in moderate-to-severe asthma. 2) While neutrophils are positively correlated with eosinophils in biopsies and BAL, they are negatively correlated with eosinophils in sputum. Whether this is a technical artifact or a biologically relevant finding is unclear, but given recent attention devoted to “neutrophilic” phenotypes in severe asthma, should be investigated further, particularly in the context of interventional studies of ICS

and new investigational asthma therapies. 3) Eosinophils and neutrophils relate to each other on a continuous scale, which suggests that defining discrete “cutoffs” for airway phenotypes should be undertaken with caution. Future studies should also examine the longitudinal variability of granulocytic infiltration, as well as the ability of these phenotypic variables to predict outcomes in interventional studies.

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FIGURE LEGEND

Figure 1. Relationships between eosinophil and neutrophil levels in each airway compartment. Eosinophil and neutrophil counts (A) or percentages of nonsquamous cells (B-C) are plotted in (A) endobronchial biopsy tissue, (B) induced sputum, and (C) bronchoalveolar lavage (BAL) fluid. r_s , Spearman's rank correlation.

