



Letter

Variability of blood eosinophil count as an asthma biomarker

In several recent clinical trials, the efficacy of novel targeted therapeutics, such as the monoclonal antibodies for interleukin (IL) 5 (eg, mepolizumab), IL-13 (eg, lebrikizumab), and IL-4 receptor (eg, dupilumab), was demonstrated mainly in a subgroup of patients with eosinophilic or T_H2 endotype asthma.^{1–4} In these studies, sputum eosinophil counts, serum periostin level, and blood eosinophil counts identified the patients who were likely to respond. Furthermore, a post hoc analysis of patients with chronic obstructive pulmonary disease revealed that increased blood eosinophilia predicted response to the addition of inhaled corticosteroid.⁵ The blood eosinophil count is an attractive biomarker because of the ease of availability and its ability to identify which patients with asthma should receive novel therapeutics. However, is the blood eosinophil count a stable and reliable biomarker?

Since 2008, we have recruited 82 individuals who have donated blood on at least 4 separate occasions (at least 8 weeks apart) for the purification of eosinophils and other blood cells for a total of 749 blood donations. Individuals were characterized initially with an extensive medical history, spirometry, exhaled nitric oxide, aeroallergen skin prick testing, and manual blood eosinophil count. At each donation visit, typically between 7 and 8 AM, an update to the medical history (including medication changes), symptom questionnaire, exhaled nitric oxide, and eosinophil count was obtained. If the individuals had an acute infection, were taking oral corticosteroids, or were receiving increased doses of inhaled steroids, they were not allowed to donate. Individuals were classified as having asthma only (13%), allergic rhinitis only (23%), or both allergic rhinitis and asthma (63%) based on self-report with confirmation based on medical history, prescribed medications, skin prick testing, and/or spirometry. Of these individuals, 55% were male, and the mean age at first donation was 34 years (range, 18–53 years). On the basis of the availability of repeated blood eosinophil counts, we sought to determine its variability and consider the suitability of eosinophil count as a biomarker using a linear mixed-effect model.

Eosinophil counts were greater in individuals with asthma alone (geometric mean, 254 cells/mm³; 95% confidence interval [CI], 189–342 cells/mm³) and asthma with allergic rhinitis (244 cells/mm³; 95% CI, 213–280 cells/mm³) compared with those with allergic rhinitis alone (174 cells/mm³; 95% CI, 139–219 cells/mm³) ($P = .05$ and $P = .01$, respectively). The within-subject geometric coefficient of variation was 39.7%; in other words, a typical eosinophil count in any given individual is approximately 40% higher or lower than that individual's mean. This variability

was similar in each of the clinical diagnoses categories: 42.1% for allergic rhinitis only, 35.4% for asthma only, and 39.7% for allergic rhinitis and asthma. As shown in Figure 1, there was a wide range of blood eosinophil counts for each study participant. When considering blood eosinophil cutoffs of 150 cells/mm³, most participants (57%) exhibited eosinophil counts both above and below the respective cutoff. To determine whether some of the variability in eosinophil counts could be attributed to seasonal allergies, the blood eosinophil counts for individuals with at least 1 documented allergen sensitivity were examined in relation to the month of sample collection (data not shown). Eosinophil counts varied slightly with the collection month, with several pairwise comparisons of samples drawn in each month demonstrating differences, particularly when compared with October (data not shown). However, considerable variability remained within each month, indicating the contribution of season on the eosinophil count variability is minimal; only 2% of the within-subject variability in eosinophil counts could be attributed to the month of collection. We performed a similar analysis to consider the effects of inhaled corticosteroid use and found less than 1% of the within-subject variability in eosinophil counts could be attributed to inhaled corticosteroid use.

In recent clinical trials of mepolizumab, different eosinophil counts have been used as a cutoff as part of the inclusion criteria, specifically, 150 cells/mm³ or 300 cells/mm³.^{1,4} The sensitivity and specificity were calculated for the initial blood draw, correctly categorizing the geometric mean eosinophil count of the individual's subsequent blood draws above or below the respective cutoffs of 150 cells/mm³ (sensitivity, 83%; specificity, 75%) and 300 cells/mm³ (sensitivity, 78%; specificity, 92%). Therefore, because of the variability in eosinophil counts, 18 of 23 patients (78%) with a mean eosinophil count greater than or equal to 300 cells/mm³ on a single blood draw had an initial count greater than or equal to 300 cells/mm³, and 58 of 70 patients (83%) with a mean count greater than or equal to 150 cells/mm³ had an initial count greater than or equal to 150 cells/mm³. In other words, roughly 1 in 6 patients whose mean count was at or above the 150-cells/mm³ cutoff value had initial counts below that cutoff value. These data suggest that the use of a single eosinophil count as a criterion for eligibility for specific therapeutics is insufficient to identify all patients with potential to benefit.

The patients examined in this study predominantly had mild asthma. Therefore, it remains a possibility that patients with more severe asthma, who are the expected population to receive targeted biologic therapies, will exhibit more consistent blood eosinophil counts. Furthermore, our cohort enriches for patients with higher eosinophil counts to provide greater numbers of cells for eosinophil purifications. As a result, we speculate that

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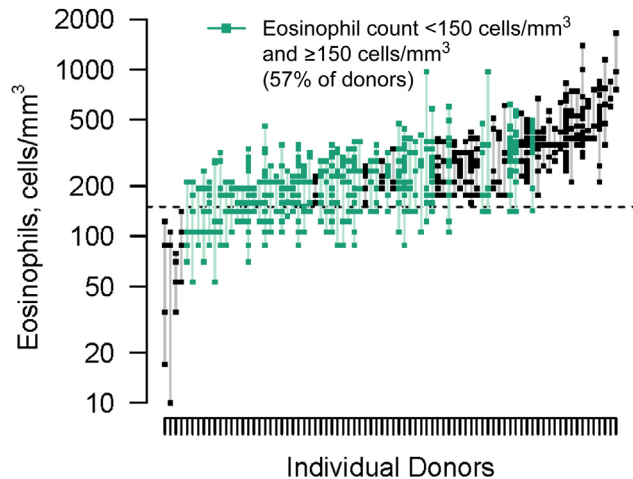


Figure 1. Blood eosinophil counts. Each individual is shown with blood eosinophil counts from each of their donations represented as individual dots. Dashed line denotes a cutoff of 150 cells/mm³. Individuals with donations of eosinophil counts both above and below the cutoff are depicted in green.

our analyses overestimate the likelihood of identifying an eosinophil count above a certain cutoff. The prospect of using the blood eosinophil count as a biomarker for eosinophilic asthma was also examined by Katz et al.⁶ They observed that 98 of 111 patients (88%) with a postscreening mean eosinophil count above 150 cells/mm³ had an initial screening count above 150 cells/mm³. Similarly, in our cohort, the sensitivity of 83% for the 150-cells/mm³ cutoff suggests that 1 of 6 blood draws in individuals with a mean eosinophil count at or above 150 cells/mm³ may report an initial count below the cutoff. Spector and Tan⁷ also examined the blood eosinophil count as a biomarker and observed a great deal of variability that could not be attributed to diurnal variation or seasonality. Therefore, repeated measurements of the peripheral blood eosinophil count may be necessary to definitively identify patients who could benefit

from the novel T_H2 endotype–targeted therapeutics currently available and in development.

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