

BIOMARKERS

Is a Single Blood Eosinophil Count a Reliable Marker for “Eosinophilic Asthma?”

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Introduction. “Eosinophilic asthma” refers to an asthma phenotype characterized by predominance of eosinophils in the bronchial airways and corticosteroid responsiveness. Recent clinical trials of eosinophil-blocking agents have utilized a blood eosinophil count of 300 or 400 eosinophils/mm³ or higher to identify subjects with moderate to severe asthma. We observed multiple instances of counts which varied widely in the same patient within the same day. **Objectives.** To determine whether there is significant variability in blood eosinophil counts taken throughout the day in the same patients with moderate asthma. **Methods.** Twelve subjects had serial blood eosinophil counts obtained within a 24-hour period. **Results.** Twelve subjects were enrolled: seven subjects had moderate asthma, three subjects had mild asthma, and two control subjects had no asthma. The variability of blood eosinophil counts ranged from 17% to 396%. No specific diurnal pattern was found among the subjects. The highest variability were seen in three moderate asthmatics (396%, 170%, and 154%) and one mild asthmatic (164%) while the other subjects had variability of 84% or less. **Conclusions.** This study showed significant variability in blood eosinophil counts within a 24-hour period in the same subjects. The highest variability was seen in moderate asthmatics. These findings would appear to place the utility of a single eosinophil count in question.

Keywords asthma, blood eosinophil counts, eosinophils, variability

INTRODUCTION

“Eosinophilic asthma” refers to an asthma phenotype characterized by predominance of eosinophils in the bronchial airways and corticosteroid responsiveness (1). Persistence of eosinophils in the airways despite inhaled steroids has been associated with severe asthma (2, 3). Increased asthma exacerbations appear to be associated with higher airway eosinophilia (4).

Clinical research trials for new asthma medications are increasingly using blood eosinophil counts as a marker to identify patients with eosinophilic asthma for inclusion in trials for agents that target eosinophil effects such as mepolizumab and reslizumab (5, 6). In our research center, at least three clinical trials have required, as an inclusion criterion, a level of 300 or 400 eosinophils/mm³ and higher to identify subjects with moderate to severe asthma who would qualify for the studies. These trials usually allow for only a one-time collection with no parameters for the timing with potential subjects being screened out if their blood eosinophil count is lower than the designated cutoff.

While sputum eosinophils (7–9) are considered a more accurate reflection of tissue eosinophilia, the collection of viable samples can be time-consuming, expensive, and difficult. By contrast, blood eosinophil counts are routinely measured as part of a full blood count and are inexpensive and easily collected which make them an attractive alternative for pharmaceutical companies conducting clinical trials. Peripheral blood eosinophils have been shown to correlate with asthma activity (10–12). Recent literature

now recognizes blood eosinophils as a biomarker that can be used to monitor systemic biological effects of pharmacologic and immunologic intervention in asthma (13).

In our clinical practice and research center, we have observed multiple instances of blood eosinophil counts which varied widely in the same patient within a short period of time. The same patient could be accepted or rejected from enrolment into a clinical trial depending on which result was used. While this variability has been discussed in the literature, it appears that it is still not widely recognized in the clinical research field where it has enormous implications in subject selection and subsequent drug approval. We report our observations on the significant liability or variability in blood eosinophil counts taken throughout the day in the same patients with moderate asthma.

METHODS

Twelve subjects from an allergy and asthma private practice office participated in this study. These subjects were already known to the practice and had received a complete history and physical examination at least once in the previous 3 months. The subjects’ medical records were reviewed with particular attention to their atopic history. A complete physical examination was performed on the day of the study. All subjects remained on their ongoing medication regimen.

The study approval was received from the University of California Los Angeles (UCLA) Institutional Review Board (IRB). All subjects signed an IRB-approved consent form.

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TABLE 2.—Variability of blood eosinophil counts.

		Variability (%)
MB	Moderate asthma	104
TH	Moderate asthma	396
JH	Moderate asthma	154
JS	Moderate asthma	70
JDZ	Moderate asthma	84
MRB	Moderate asthma	40
SSS	Moderate asthma	170
SS	Mild asthma	36
RL	Mild asthma	17
AW	Mild asthma	164
SLS	AR, no asthma	63
SSH	Non-atopic	51

154% (JH), 70% (JS), 84% (JDZ), 40% (MRB), and 170% (SSS). In the three mild asthmatics, variation was 36% (SS), 17% (RL), and 164% (AW). SLS with allergic rhinitis and no asthma had variation of 63% while the non-atopic subject SSH had 51%.

Nine subjects had their highest count at or before noon while three subjects had their highest count in the afternoon or evening. No specific diurnal pattern was found among the subjects.

The highest variability were seen in three moderate asthmatics (396%, 170%, and 154%) and one mild asthmatic (164%) while the other subjects had variability of 84% or less.

DISCUSSION

This small study has shown significant variability in blood eosinophil counts within a 24-hour period in the same subjects. The variations in counts ranged from 17% to 396%. The highest counts were seen in moderate asthmatics. These findings place the utility of a single eosinophil count obtained during the usual daytime hours in question.

In animal studies, diurnal variations in eosinophil counts have been observed. Halberg and Visscher (15) studied four inbred strains and one hybrid group of mice and observed a regular diurnal variation in eosinophil count with the highest levels in the morning and lowest levels at midnight showing less than one third of the morning level. Another study in mice showed maximum eosinophil levels at midday and lowest levels at midnight (16).

A review of the literature shows that diurnal variation of eosinophil counts in humans has been recognized and has most often described with the lowest counts being seen in the morning and the highest ones observed in the evening. However, there have been very few reports regarding this issue in the last three decades.

Older literature from the 1940s to 1950s revealed a great interest in the variability of eosinophil counts, which were used at the time to test adrenal corticosteroid production and function and even to measure the severity of stress on individuals (17). Donato and Strumia (18)

and Tatai and Ogawa (19) reported diurnal variations with the lowest eosinophil levels at noon. Some studies show decreasing levels until noon and increasing levels for the rest of the day while other studies showed variations but did not identify specific patterns. In 1956, Acland studied the variations in eosinophil counts prospectively in 20 healthy male volunteers during "normal working days" with samples obtained at 10 am, 11 am, 12 noon, 2 pm, 3 pm, and 4 pm for 3 consecutive days. The author concluded that there were considerable hourly changes in eosinophil counts but did not find a consistent diurnal pattern similar to earlier reports with low levels in the morning and increasing levels in the afternoon (20). Several other studies have shown variability of eosinophil counts in children and adults (21–23). A more recent study reported diurnal variation of up to 40% in healthy subjects (24).

Peripheral blood tissue eosinophils are derived primarily from the bone marrow. The half-life of eosinophils in the circulation is approximately 18 hours. The mean blood transit time is 26 hours, but this is extended by cytokines that promote eosinophil survival in asthma and other eosinophilic conditions (25).

Variability in eosinophil counts has been frequently attributed to the lowering effect of cortisol hormones. Cortisol levels are highest in the morning and lower at night with eosinophil counts consequently being lower in the morning and highest at night (26). However, this has not been consistently observed in studies with the opposite pattern being seen in many studies including this one.

In our study, the highest levels were seen before noon in 9 out of 12 subjects. This does not coincide with the expected diurnal variation of cortisol. Nine subjects had variations of more than 50%, which is much higher than the 40% previously reported by Winkel et al. (24). Counts varied widely throughout the day with no obvious allergic triggers. The subjects were not observed to have increased symptoms (e.g., sneezing or chest tightness) or engage in activities (e.g., going outside to a garden, exercise) that could be correlated with the changes in the counts. All subjects with moderate asthma continued on their ongoing medication regimens which included at least an inhaled corticosteroid with or without a LABA.

There are interesting questions raised by the observation of the greatest variability observed in the subjects with moderate asthma (except for one mild asthmatic). Does the greater variability imply a correlation with severity of asthma? Could the variability of serial counts be used as a marker to monitor a patient's progress and response to medications such as ICS?

This *preliminary* observational study is limited by its small sample size which leads to issues of selection bias and lack of controls. Many subjects did not stay in the office for the entire study period and were exposed to outside environmental conditions between blood draws. Compliance with ICS dosing was not monitored directly and serial pulmonary function was not performed to coincide with blood draws. Further studies in a larger population are needed to expand our findings. Variables

that would need to be controlled include: time of day, allergen sensitivity, asthma triggers, concomitant medications and time of dosing, level of asthma control, and pulmonary function. The influence of cortisol levels, cytokine levels, and asthma phenotype would also need to be elucidated.

CONCLUSIONS

The variability or fluctuations in blood eosinophil counts have very significant implications for clinical research and need to be recognized urgently. Pharmaceutical companies may already be making inappropriate decisions regarding "eosinophilic asthma" using a single blood eosinophil count of 300 or 400/mm³ or higher as an inclusion criterion. On the basis of our results, potential subjects could be enrolled or rejected based on which count is captured during the same day. This would affect subject selection and lead to questions regarding study validity and subsequent drug approval. We are hoping that these preliminary findings can focus attention from investigators, pharmaceutical companies as well as regulatory bodies (NIH, FDA) so that larger studies can be designed and conducted to expand these findings.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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