

Monitoring eosinophils to guide therapy with biologics in asthma: does the compartment matter?

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1 | INTRODUCTION

Eosinophils, since their initial description by Thomas Wharton Jones in 1846, and staining characteristics by Paul Ehrlich in 1879, have been associated with asthma.¹ They are produced in the bone marrow, from pluripotential stem cells, which first differentiate, largely regulated by a transcription factor GATA-1, into a hybrid precursor for both basophils and eosinophils, and then into a separate eosinophil lineage. The eosinophilopoietins IL-3, GM-CSF and notably IL-5 regulate their further expansion and migration out of the bone marrow, and into the circulation. Circulating eosinophils subsequently interact with the endothelium by processes involving rolling, adhesion and diapedesis. Depending on the target organ, eosinophils cross the endothelium into tissues by a regulated process involving the coordinated interaction between networks involving cytokines such as IL-13, the chemokine eotaxin-1, eosinophil adhesion molecules ($\alpha 4\beta 1$, $\alpha 4\beta 7$, $\alpha m\beta 2$, $\alpha L\beta 2$), and adhesion receptors on the endothelium (MAdCAM-1, VCAM-1 and ICAM-1). Under homeostatic conditions, eosinophils traffic into the thymus, mammary gland, uterus and most prominently into the gastrointestinal tract.²

Thus, in a disease such as asthma, long associated with mobilization of eosinophils from the bone marrow, it is not surprising that eosinophil numbers may be increased in blood and sputum in patients with asthma. The development of biologics targeting

IL-5 and IL-13 has provided opportunities to treat patients with asthma whose severity or symptoms are driven by eosinophil biology. Most studies evaluating these biologics were conducted by selecting patients with raised blood eosinophil numbers, and not by enumerating numbers in the airway, as the latter process is cumbersome. There is ongoing controversy as to which would be a better strategy.

This commentary examines whether blood eosinophil number is an adequate method to choose those patients who may be good responders to anti-eosinophil biologics and to monitor the response to therapy.

2 | PRO: BLOOD EOSINOPHIL NUMBERS: THE MIRROR OF THE EOSINOPHIL COMPARTMENT IN HEALTH AND DISEASE

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- The role of eosinophils in allergic inflammation is well recognized, but these cells have evolved for millions of years before the evolution of T2 inflammation.
- In homeostasis, these cells are found in multiple healthy tissues including the lung parenchyma, but the function of these resident eosinophils is unknown.

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- As eosinophils are produced by the bone marrow^{2,3} and are distributed through the blood, the number of blood eosinophils is associated with the numbers of cells found in the tissues in health and disease. Indeed, the extent of T2 inflammation is mirrored by higher numbers of eosinophils in the peripheral blood.² Stratification on blood eosinophil numbers identifies patients likely to respond to IL-5(R) targeted therapy (see for a review³) with a cut-off value of 150 eosinophils/ μ l.⁴(Figure 1)
- Sputum eosinophilia is not specific for airway disease. It is also increased in other eosinophil-associated pathologies such as Crohn's disease.⁵ Besides, the number of sputum eosinophils is mainly not reproducible.⁶
- Blood eosinophil numbers can easily and quantitatively be determined in any hospital laboratory. This is in marked contrast to sputum eosinophils where adequate determination of absolute eosinophil numbers is impossible, because of the requirement of complex processing of sputum samples (in a specialized laboratory) and the lack of an adequate determination of sputum volume.
- No experimental data support the often made suggestion that sputum cells represent tissue eosinophils. In fact, the scarce data available show that tissue eosinophils exhibit a different phenotype than sputum cells.⁷ Surely a cut-off value of the eosinophil percentage in sputum obtained from the *central* airways, cannot easily be translated to values found in the *lower* airways, that are more relevant for asthma. Therefore, blood eosinophils as a reflection of the total eosinophil compartment en route to tissues provide much better, reproducible and quantifiable information.⁴
- Eosinophils ending up in the sputum exhibit an activated phenotype because of the process of adhesion and trans-endothelial/stromal/epithelial migration rather than induced by the disease process per se. Subtle (pre)-activation of eosinophils by disease-associated signals can therefore only be measured in the peripheral blood.
- It is clear that total eosinophil numbers in blood and tissue are determined by two independent differentiation pathways (see Figure 1): IL-5 dependent inflammatory eosinophils and IL-5-independent-resident cells.⁸ The total number of eosinophils in blood and tissues will be the summation of the numbers of both phenotypes. It is essential to develop markers for both phenotypes as the number of the individual phenotypes will hold important information about the total eosinophil compartment. More research around this critical issue is warranted.
- Therefore, the specific determination of the absolute number of *inflammatory* eosinophils in blood will be a great step forward. Until then, several large field studies have already shown that total blood eosinophil count, which can be quantified accurately and simply, is a critical marker for the success of treatment of asthmatics with eosinophil-targeted drugs.^{3,4}
- In conclusion, sputum induction and analysis are **inadequate** for the accurate determination of asthma phenotype and/or severity. The determination of the numbers of resident and inflammatory eosinophils in the peripheral blood is an ideal immunological instrument to identify asthma patients eligible for treatment with biologicals particularly when the resident and inflammatory eosinophils can be quantified individually.

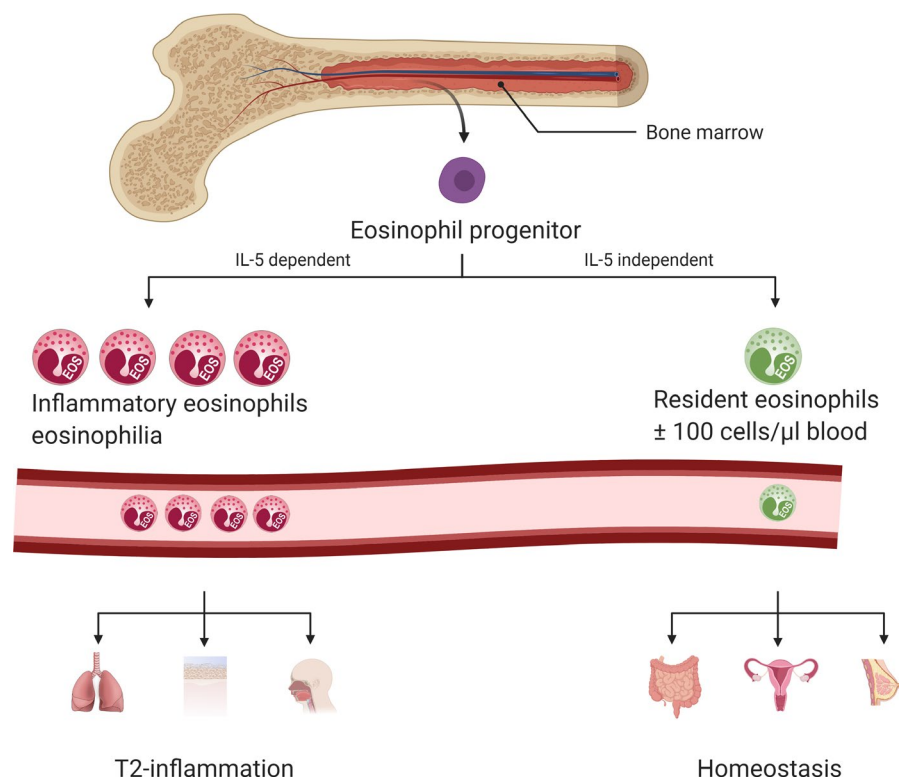


FIGURE 1 model for the eosinophil compartment in humans. Eosinophils are produced in the bone marrow and released into the blood as mature cells. Two separate differentiation paths seem to be present: one IL-5 dependent,² leading to inflammatory cells and one IL-5 independent,⁷ leading to resident cells. In contrast to inflammatory cells, resident eosinophils are present in healthy individuals at rather stable numbers of ± 100 cells/ μ l⁸ Made with the use of Biorender software. [Color figure can be viewed at wileyonlinelibrary.com]

3 | CON: BLOOD EOSINOPHIL NUMBERS ARE OF LIMITED USE TO GUIDE BIOLOGICS: ASTHMA IS AN AIRWAY DISEASE!

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- There is no dispute that eosinophils are associated with asthma and that their numbers in circulation may increase with asthma symptoms and severity. There is also no dispute that there are number of processes, in addition to IL-5, that may contribute to the recruitment of these cells out of the bone marrow, and into target organs.
- However, their numbers in circulation are not specific to asthma. Other allergic conditions such as rhinitis, sinusitis and food allergies, and most importantly, atopic dermatitis could determine the eosinophil numbers even when asthma is mild. With respect to age, there is no significant effect on the number of eosinophils; however, activation states, particularly in response to IL-5, may be less in older patients.⁹ Again, peripheral blood eosinophilia rarely reflects airway eosinophilia in the paediatric population¹⁰
- Circulating eosinophils traffic to the airway and spill over into the lumen, release their cationic proteins and other contents including DNA, and these contribute to airflow obstruction. When eosinophils are confined to the circulation, however raised their numbers may be (eg as in hypereosinophilic syndromes), they cause minimal asthma symptoms.¹¹ Of course, circulating eosinophil numbers may reflect the numbers that may eventually track into the lumen, but in general, they are not activated. This correlation between blood and airway (sputum) eosinophil numbers is poor in the very severe patients who are on oral glucocorticosteroids (OCS),¹² with higher blood eosinophil counts (>600 » 500 > 400>300/ μ L) being associated with higher sputum eosinophil counts. Further, given their diurnal fluctuation, lack of specificity for asthma and generally non-activated state, circulating eosinophil number may simply be an indicator of Th2 biology, rather than the eosinophil being the effector cell.
- Therefore, it is not surprising that asthmatic patients with raised blood eosinophil counts respond well to anti-Th2 biologics.¹³ The annualized relative reduction in exacerbations (annualized asthma exacerbation rate or AAER) is greater (up to 70%) in those with higher blood eosinophil counts, particularly over 500 or 600/ μ L, as these are the patients who are likely to have sputum eosinophilia as well. The response in the most severe OCS-dependent patients is less, and this partly dependent on the dose, route of administration and mechanisms of action of the biologics.¹⁴
- While accepting that in adults with asthma, a raised baseline blood eosinophil counts (of >400/ μ L) may be helpful to predict response to an anti-Th2 biologic (albeit up to 70% AAER), they have a limited role in monitoring response to treatment. As we recently reported in 250 patients with baseline blood eosinophilia who were treated with either mepolizumab or reslizumab for at least 4 months,¹⁵ there was an overall suboptimal response

in 43%. Suboptimal response was defined as described.¹⁵ Of the 129 patients in whom paired blood and sputum eosinophils were available after at least 4 months of treatment, there were 65 suboptimal responders. 78% of them had sputum eosinophils \geq 3%. Blood eosinophils were \geq 400/ μ L in only 7 patients (Figure 2). This discordance is likely due to in situ eosinophilopoiesis resulting from inadequate neutralization of airway IL-5 largely from ILC2 cells. Sputum eosinophil counts are reproducible and reliable. Reports that they have poor measurement properties are related to poor processing techniques, specifically related to poor dispersal, and not properly selecting out the squamous cells. The

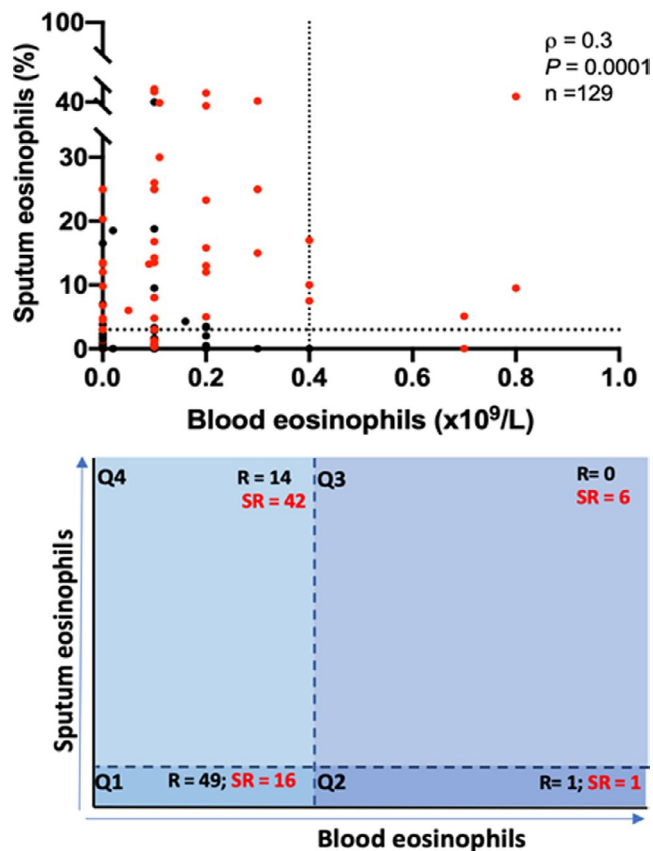


FIGURE 2 Correlation using Spearman rank test between blood and sputum eosinophils in severe asthmatics after at least 4 months of treatment with mepolizumab (100 mg SC Q4 W) or reslizumab (3 mg/kg IV Q4 W). Dotted lines represent cut-off upper normal limit for sputum (3%) and blood eosinophils (0.4 cells $\times 10^9$ /L). Red dots represent suboptimal responders, while black dots represent good responders. Top panel is a plot of the data, where each symbol indicates individual patient data point. Some data points may be superimposed. Bottom plot is a schematic presentation of the numbers in the four quartiles (Q1 = sputum eos < 3%; blood eos < 0.4; Q2 = sputum eos < 3%; blood eos \geq 0.4; Q3 = sputum eos \geq 3%; blood eos \geq 0.4; and Q4 = sputum eos \geq 3%; blood eos < 0.4). Suboptimal response is determined by one of three clinical criteria (i) failure to reduce maintenance ICS/OCS by 50%; (ii) ACQ \geq 1.5; and (iii) failure to reduce exacerbation by 50%, and one biological criterion: failure to reduce sputum and/or blood eosinophils after at least 4 injections [Color figure can be viewed at wileyonlinelibrary.com]

method is simple and should be easy to set up in any tertiary centre that looks after patients with severe asthma.

- Blood eosinophil count may be mis-leading also to monitor anti-IL4/IL-13 therapies. While associated with clinical improvement, dupilumab treatment is accompanied with an increase in circulating blood eosinophil count in almost all patients (putative mechanisms include preventing egress of eosinophils from circulation into tissues by blocking VCAM, endothelial miRNA1, etc).
- In summary, while circulating blood eosinophil count of $\geq 400/\mu\text{L}$ may be helpful to identify a Th2 immune biology to initiate therapy with an anti-Th2 mAb, it has very limited value to monitor response. Normal blood eosinophil counts on anti-IL5 mAbs may be associated with poor asthma control and sputum eosinophilia. Conversely, raised blood eosinophil counts on anti-IL4R mAb may be associated with good asthma control.

ACKNOWLEDGMENTS

Dr Nair was supported by the Frederick E. Hargreave Teva Innovation Chair in Airway Diseases. Dr Mukherjee was supported by a Canadian Institutes of Health Research post-doctoral fellowship. The data in the manuscript was supported by a grant from the Canadian Institute of Health Research.

CONFLICT OF INTEREST

LK reports grants from GlaxoSmithKline and non-financial support from Beckman Coulter. MH declares no conflicts of interests. MM reports grants from Methapharm Specialty Pharmaceuticals, Canadian Institute of Health Research, Canadian Asthma Allergy and Immunology Foundation and personal fees from AstraZeneca. PN reports grants and/or personal fees from AZ, Novartis, Teva, Sanofi, Roche, Novartis, Merck and Equillium.

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