# Monitoring eosinophils to guide therapy with Biologics in Asthma: does the compartment matter?

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**Abstract**

The role of eosinophils in allergic inflammation is well recognized. In homeostasis these cells are found in multiple healthy tissues including the lung parenchyma, but the function of these resident eosinophils is unknown. Circulating eosinophils are easily quantifiable and have been used to define "eosinophilic phenotype", and to select patients who are likely to respond to anti-eosinophil and anti-Th2—directed therapies. However, presence of eosinophils in circulation may not necessarily indicate that the eosinophils are key effector cells for an airway disease such as asthma and this may be reason for not all patients responding well to anti-IL5 therapies despite normalization of blood eosinophils. This pro-con commentary examines the role of enumerating circulating vs luminal (sputum) eosinophils (and their activation status) not only to initiate therapies with monoclonal antibodies, but to monitor their clinical response while on therapy.

## Blood eosinophil numbers: the mirror of the eosinophil compartment in health and disease.

Leo Koenderman and Marwan Hassani

- 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.
- 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 extend of T2-inflammation is mirrored by higher numbers of eosinophils in het peripheral blood (2). Stratification on blood eosinophil numbers identifies patients likely to respond to IL-5(R) targeted therapy (see for a review (3)).**(figure 1)**
- Sputum eosinophilia is not specific for airway disease. It is also increased in other eosinophil associated pathologies such as Crohn's disease[4]. Besides, the number of sputum eosinophils is mainly not reproducible (5).
- 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 (6). 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 provides much better, reproducible and quantifiable information.

- 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): IL-5 dependent (2) inflammatory eosinophils and IL-5-independent resident cells (7). 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).
- 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.

**Blood eosinophil numbers are of limited use to guide biologics: asthma is an airway disease!**

Manali Mukherjee and Parameswaran Nair

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 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.
- 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 (for example, as in hypereosinophilic syndromes), they cause minimal asthma symptoms (9). 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 are poor in the very severe patients who are on oral glucocorticosteroids (OCS) (10), with higher blood eosinophil counts (>600>>500>400>300/μL) 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 (11). The annualized relative reduction in exacerbations (AAER) is greater (up to 70%) in those with higher blood eosinophil counts, particularly over 500 or 600/μL, as these are the patients who are likely to have sputum eosinophilia as well. The response in the most severe OCS-dependent patients are less, and this partly dependent on the dose, route of administration, and mechanisms of action of the biologics (12).
- While accepting that raised baseline blood eosinophil counts (of >400/μL) may be helpful to predict response to an anti-Th2 biologic (albeit up to 70% AAER), the have limited role to monitor 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 (13), there was an overall suboptimal

response in 43%. An absolute blood eosinophil count of 530/μL at baseline (that had 70% sensitivity and 90% specificity to predict [?]3% sputum eosinophils) best predicted a clinical response. Of the 129 patients in whom paired blood and sputum eosinophils were available after at least 4 months of treatment, there were 65 sub-optimal responders. 78% of them had sputum eosinophils [?]3%. Blood eosinophils were [?]400/μL 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 relate to poor processing techniques, specifically related to poor dispersal, and not properly selecting out the squamous cells. The 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 associated 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 [?]400/μL 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.

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**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/µl[8].

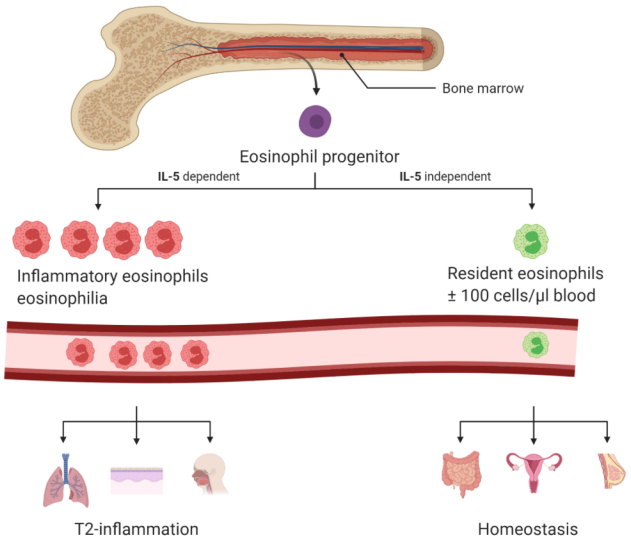


**Figure 2:** Correlation between blood and sputum eosinophils in severe asthmatics after at least 4 months of treatment with mepolizumab (100 mg SC Q4W) or reslizumab (3 mg/kg IV Q4W). Dotted lines represent cut-off upper normal limit for sputum (3%) and blood eosinophils (0.4/µL). Red dots represent sub-optimal responders, while black dots represent good responders.

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