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Exercise induced eosinophil responses: normal cell counts with a marked decrease in responsiveness

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To the Editor,

Type II inflammation is characterized by elevated blood eosinophils which makes these cells an important diagnostic and treatment target in, for instance, severe asthma. Therefore, blood eosinophil numbers are a main inclusion criterion for many clinical studies that investigated the treatment of eosinophilic asthma with anti-IL5(Rα). However, there is no consensus on cut-off values for blood eosinophils during inclusion, as evidenced by a high variability between studies, ranging from 150 to 400 cells/μL. Moreover, the range of blood eosinophils in a healthy population, without confounding factors for increased blood eosinophils, is 30 – 330 cells/μL in males and 30 – 310 cells/μL in females. This implies that the cut-off values used for clinical studies greatly overlap with blood eosinophil counts that are found in the healthy population. This inherently poses a problem as eosinophil blood counts seem to be inadequate to use for diagnosing eosinophilic disease.

This overlap in eosinophil counts between patients and the healthy population limits the application of eosinophil numbers for discriminating between health and several inflammatory diseases. A more promising approach in diagnosing eosinophilic disease is to combine eosinophil numbers with their activation status. Unfortunately, there is surprisingly little evidence that blood eosinophil counts correlate with their activation status and/or responsiveness in vivo in disease. This lack of correlation can be caused by ex vivo activation and/or their homing to the lung leaving behind non-activated cells in the blood.

To circumvent ex vivo activation, we analyzed blood eosinophils activation status directly after venipuncture with a fast, automated, point-of-care, mobile flow cytometer (AQUIOS CL, Beckman Coulter). As exercise can be used as a model to modulate eosinophil numbers in a healthy setting, we studied whether eosinophil blood counts correlate with their activation status and their responsiveness to formyl peptides in a cohort of long-distance runners participating in a mass-participation trail run (22, 29 or 43 km). The study was approved by medical research ethics committee Oost-Nederland (NL79864.091.22). After written informed was obtained, venous blood samples were collected from 35 athletes before, directly after and 24 hours after exercise. The eosinophil activation status was assessed by combining automated flow cytometry with a 5-dimensional algorithm-based gating.

An acute leukocytosis with eosinopenia was present directly post-exercise, which is in agreement with previous research. These numbers normalized 24 hours after exercise (**figure 1A, 1C**). Compared to before exercise, eosinophils showed a more activated phenotype (increased CD11b and decreased CD62L) directly after

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exercise which also normalized within 24 hours. In marked contrast to acute inflammation, such as caused by SARS-CoV2 infection, this eosinopenia directly after exercise did not lead to refractoriness to fNLF-stimulation. However, after the normalization of eosinophil counts 24 hours after exercise, the eosinophils were refractory for activation by fNLF (**figure 1B, 1C**). This clearly showed a complete dissociation between blood eosinophil numbers and their relative activation status.

Our results illustrate that the eosinophil blood compartment is not adequately characterized by solely counting cell numbers ('quantity') as normalized numbers do not necessarily reflect normalization of their activation status ('quality'). This finding is not limited to measuring the state of type II immunity in eosinophilic disease, but probably also applies to other infectious/inflammatory conditions and to non-pathological settings. Our data call for a re-evaluation of using blood eosinophil counts as an adequate representation of the eosinophil compartment's state. Until recently, determining the activation status of the eosinophil compartment was complicated by ex vivo artifacts already starting at the moment of venipuncture. Now with the availability of fast, automated, point-of-care flow cytometry, it is feasible to measure both the *quantity* and *quality* of eosinophils in a wide scope of health and disease settings.

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Φιγυρε 1. A) *Τοταλ ωηιτε βλοοδ ςελλ ανδ βλοοδ εοσινοπηιλ ςουντς ιν τραιλ-ρυν παρτιςιπαντς ατ τηρεε διφφερεντ τιμεποιντς αρουνδ α τραιλ-ρυν. Τηε σηαδεδ αρεα'ς ινδιςατε γενεραλ ρεφερενςε αλυες φορ τοταλ ωηιτε βλοοδ ςελλ (ΩΒ˜· 4 – 10 ξ 10⁹/Λ) ανδ εοσινοπηιλ ςουντς (0.03 – 0.33 ξ 10⁹/Λ).* ***B)*** *Τηε περςενταγε οφ ˜Δ11β^βριγητ ανδ ˜Δ62Λ^βριγητ βλοοδ εοσινοπηιλς ατ τηρεε διφφερεντ τιμεποιντς αρουνδ τηε τραιλ-ρυν. φΝΛΦ-/φΝΛΦ+ σαμπλες ωερε μεασυρεδ ιν τηε αβσενςε/πρεσενςε οφ τηε φορμψλπεπτιδε (10 μΜ). Στατιστιςαλ σιγνιφιςανςε ωας δετερμινεδ ωιτη α μιξεδ-εφφεςτς μοδελ ωιτη Γεισσερ-Γρεενηουσε ςορρεςτιον ανδ Τυκεψ'ς μυλτιπλε ςομπαριςονς τεστ.˜)* *Μεδιαν (ιντερχυαρτιλε ρανγε) αλυες οφ τηε δατα ιν πανελς****A*** *ανδ* ***B***

References

Authors

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Author contribution statement

Conceptualization: BJ, SJ, AM, WV, TE, LK. Methodology: BJ, SJ, TE, LK. Data analysis: BJ. Visualization: BJ. First draft writing: BJ, LK. Revision and approval of final paper: all authors.

Conflict of interests

The AQUIOS CL® "Load & Go" flow cytometer is provided by the company Beckman Coulter Life Sciences, Miami, FL, USA. This company had no role in the study's design, the data analysis, the article's preparation, or the decision to submit the article for publication. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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