Iron metabolism and peripheral eosinophil count do not correlate in the general population

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Abstract

Iron is a primary component of the human body and exerts many functions, mainly concerning red cells and the immune system. In addition, there is evidence that iron-deficiency anemia is associated with allergic diseases. Type 2 inflammation characterizes allergic diseases. Peripheral eosinophils are a reliable biomarker for type 2 inflammation. Therefore, the present study investigated the possible relationship between iron metabolism and peripheral eosinophils in a large population. Eosinophils also play important roles in immune and tissue homeostasis. A growing body of data suggests tissue eosinophils represent a plastic and heterogeneous population of functional sub-phenotypes, shaped by environmental (systemic and local) factors. The retrospective study included 2,795 subjects who were afferent to the Clinical Chemistry Analysis Laboratory during the year 2022. Men had higher peripheral eosinophil count than women ($p<0.001$). Furthermore, stratifications for sex, eosinophil, and hemoglobin cut-offs showed that men had more eosinophil counts than women. However, there was no correlation between iron biomarkers and peripheral eosinophils. The present study did not demonstrate a clear relationship between iron deficiency and peripheral eosinophil count. Even if this large population may include allergic subjects and patients with different diseases, it seems that iron does not directly affect eosinophil count.

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Introduction

Allergic diseases are impressively common, as their prevalence is about 50% of the general population. Allergy recognizes a type 2 immune mechanism.[1] T helper 2 polarization and consequent increased production of type 2 cytokines, including interleukin-4 (IL-4), IL-5, and IL-13, characterize type 2 inflammation.[2] Type 2 inflammation represents the leading hallmark of allergic disorders. In particular, eosinophilic infiltrate is a valuable biomarker of allergic inflammation.[3,4] As detecting tissue eosinophils is an invasive practice, peripheral eosinophil count can represent a suitable way to identify patients with active inflammation.[5] Namely, there is consistency between tissue and peripheral eosinophils.[6] As a result, blood cell count (for measuring eosinophils) is a routine exam in asthmatic patients as a high level of peripheral eosinophils suggests the presence of sustained bronchial type 2 inflammation.[7] Consistently, peripheral eosinophilia allows the phenotyping of patients who have type 2 asthma.[8] Accordingly, in the context of Precision Medicine, also known as Personalized Medicine (PM), the eosinophil count is a parameter to be considered for the prescriptive appropriateness of biologics (*e.g.*, benralizumab, dupilumab, mepolizumab, and omalizumab) that target key mediators of the type 2 inflammation cascade, including IL-4, IL-5, IL-13, and IgE.[9] Usually, a value of

$0.30\times10^3/\mu L$ peripheral eosinophils is a reliable cut-off for defining candidates for the use of anti-IL-4 and anti-IL-5 biological agents; in addition, this cut-off is independent of age.[10] So, the biologics prescription can meet the criteria of PM.

Moreover, peripheral eosinophil count is also a useful parameter in managing patients with other allergic diseases, including allergic rhinitis, food allergy, and atopic dermatitis.[1]

Iron is a metal belonging to group 8 of the periodic table of elements, the transition metals. Iron is widespread in nature and has important biological activities. In particular, it exerts numerous functions in humans, especially concerning oxygen metabolism through red blood cells, muscle function, and the immune system.[11] Its importance is underlined by the fact that a deficiency or excess of iron leads to significant imbalances in the body's physiology. Furthermore, iron is fundamental during the developmental age, mainly concerning immunity and neuronal functioning.[12] In this regard, a substantial mass of studies documented an association between iron deficiency, primarily anemia, and allergic diseases.[13-16] The more allergic diseases a person suffers, the greater the likelihood of having sideropenic anemia. However, the exact mechanisms contributing to this association have yet to be entirely understood. It has been envisaged that β-lactoglobulin bound to iron and quercetin complexes could prevent allergy by inducing tolerogenic pathways.[17] Moreover, it has been reported that iron deficiency worsens allergic symptoms and promotes allergy development.[13-15] Consistently, iron supplementation improved allergic symptoms and ameliorated response to allergen-specific immunotherapy.[18]

Based on this background, we tested the hypothesis about a possible relationship between iron metabolism biomarkers and peripheral eosinophils in an unselected population undergoing a blood test. In other words, the purpose of this study was to demonstrate whether an iron defect could affect type 2 immunity, mainly concerning the most reliable biomarker, such as circulating eosinophils, also in non-allergic subjects, as the data collected includes outpatients without distinction of pathology. Therefore, this study aims to investigate the possible association between iron biomarkers and eosinophil counts.

Materials and Methods

The current study retrospectively analyzed the database of the Clinical Chemistry Laboratory, Fondazione IRCCS Policlinico San Matteo (Pavia, Italy), concerning the blood samples of outpatients. The inclusion criteria were the contextual presence of iron biomarkers and peripheral eosinophil count investigated in the same subject. There were no exclusion criteria.

The time frame considered was the calendar year 2022. We have data on the anamnesis of some outpatients, but they are very different (*e.g.*, from renal transplantation to the child with suspected atopy) as our aim was to verify if the anemia could be related to the value of eosinophils.

The patients or the parents of children signed an informed consent concerning the willingness to accept the possibility of their data being used anonymously for research purposes. The study procedure was approved by the Internal Review Board (08042/10).

The iron biomarkers included red blood cell count, serum hemoglobin, iron, ferritin, and transferrin. The peripheral eosinophil count was also evaluated. Blood was drawn in the morning.

All parameters were measured at the Clinical Chemistry Analysis Laboratory. Venous blood samples were collected in ethylenediaminetetraacetic acid tubes (VacutainerTM, Becton Dickinson Vacutainer Systems, Plymouth, UK) for the analysis of blood count parameters. Cell blood count was performed using Beckman Coulter, DXH 800 analyzer. Serum transferrin and iron concentrations were measured using automated immunoturbidometric and colorimetric methods, respectively (Siemens, Advia Chemistry XPT). Serum ferritin was measured using a chemiluminescence method (Siemens, Advia Centaur XPT).

The statistical analysis was performed using Stata v17.0. program. Categorical variables are described as counts and percentages and compared between groups with a chi-square test. Quantitative variables are described as mean and standard deviation if not normally distributed (Shapiro-Wilks test). They are compared between groups with a t-test for independent samples or a Mann-Whitney test, as appropriate. Association between quantitative variables was assessed. Correlations were calculated by the Pearson test.

Results

The present study analyzed the laboratory data of 2,795 subjects who underwent a blood draw also to measure iron metabolism.

Table 1 reports the demographic data. The mean age was 31 years; there were 1,598 (57.8%) women and 1,197 (42.8%) men. The analysis proceeded by stratifying the population based on gender. Comparing sexes, women were younger and had lower peripheral eosinophils, red cells, hemoglobin, ferritin, and transferrin levels than men ($p<0.001$ for all).

The second step was to stratify the subjects considering the eosinophil cut-off of $0.30\times10^3/\mu L$, separately evaluating women and men. Table 2 reports the results. In women, the subjects with high eosinophils (>0.30) were younger and had lower red cells than subjects with ≤0.30 eosinophils ($p<0.001$ for both). In men, the subjects with high eosinophils (>0.30) were younger and had lower red cells and ferritin levels but more elevated serum transferrin ($p<0.001$, 0.004, <0.001, and <0.001, respectively).

The third step considered the stratification by sex of subjects regarding the hemoglobin cut-off of 12 g/dL in women and 13 g/dL

Table 1. Demographic data observed in 2795 subjects. Data are expressed as mean±standard deviation.

	Global population	Women (N. 1,598)	Men (N. 1,197)	p value
Age (years)	27 (±26)	29 (±27)	24.18 (±23.98)	<0.001
Eosinophils (cells×$10^3/\mu L$)	0.22 (±0.23)	0.19 (±0.18)	0.27 (±0.26)	<0.001
RBC ($10^6/\mu L$)	4.54 (±0.62)	4.45 (±0.57)	4.64 (±0.66)	<0.001
Hemoglobin (g/dL)	12.9 (±1.62)	12.7 (±1.49)	13.1 (±1.74)	<0.001
Iron (μg/dL)	77 (±39)	76 (±38)	77 (±39)	0.84
Ferritin (ng/mL)	93 (±265)	77 (±201)	115 (±330)	<0.001
Transferrin (mg/dL)	267 (±52)	271 (±53)	263 (±52)	<0.001

in men and the eosinophil cut-off of 0.30×10³/μL. Table 3 reports the results. In women, subjects with hemoglobin ≥12 g/dL had a more frequent high eosinophil count of 18% *vs*. 11% (p=0.002); in this subgroup, 18% had low hemoglobin levels. Considering the absolute eosinophil counts, women with low hemoglobin had fewer eosinophils than women with normal hemoglobin levels (p=0.002). In men, subjects with hemoglobin ≥13 g/dL had more frequently, but not significantly, high eosinophil count: 35% *vs*. 25%; in this subgroup, 18% of subjects had low hemoglobin levels. Considering the absolute eosinophil counts, men with low hemoglobin had more eosinophils than men with normal hemoglobin levels (p=0.05). Comparing the sexes, men with low hemoglobin had more frequently high eosinophil count: 44% *vs*. 18% (p<0.0001).

The last step was to correlate the parameters among them. Table 4 reports the Pearson's correlation coefficients (r values). There was no significant relationship between eosinophils and any other investigated parameters.

Discussion

Iron is a primary constituent of red cells, and iron-deficient anemia implicates several detrimental effects. In particular, there is evidence that sideropenic anemia is related to allergic diseases concerning prevalence and severity.[13-16] Consistently, iron supplementation may improve allergic issues.[17-18] However, these studies examined the selected populations composed of allergic patients. For this reason, we investigated the possible relationship between iron metabolism and eosinophil count in a general population of subjects. The findings showed that there was a significant difference between sexes as regards these parameters, mainly concerning eosinophils and red cells, including hemoglobin, ferritin, and transferrin levels. However, the findings were inconsistent as the ferritin and transferrin levels showed conflicting trajectories. Interestingly, the mean age of the population was relatively young, mostly men.

Table 2. Hematological parameters considering the sex and number of eosinophils after stratification (eosinophil cut-off of ≤0.30 and >0.30 cells×10³/μL). Data are expressed as mean+standard deviation.

	≤0.30 cells×10³/μL	>0.30 cells×10³/μL	P value	≤0.30 cells×10³/μL	>0.30 cells×10³/μL	P value
Sex (n)	♀ (1338)	♀ (260)		♂ (844)	♂ (353)	
Age (years)	36 (±28)	22 (±26)	<0.001	33 (±28)	15 (±19)	<0.001
RBC (10⁶/μL)	4.42 (±0.59)	4.61 (±0.44)	<0.001	4.60 (±0.73)	4.73 (±0.50)	0.004
Hemoglobin (g/dL)	12.6 (±1.6)	12.8 (±1.2)	0.03	13.2 (±1.9)	13 (±1.4)	0.12
Iron (μg/dL)	77 (±39)	76 (±35)	0.86	77 (±39)	77 (±39)	0.81
Ferritin (ng/mL)	82 (±211)	56 (±145)	0.14	133 (±25)	76 (±39)	<0.001
Transferrin (mg/dL)	269 (±54)	277 (±45)	0.07	258 (±55)	272 (±44)	<0.001

RBC, red blood cells.

Table 3. Stratification of patients considering Hb values, sex, and eosinophils (≤0.30 and >0.30 cells×10³/μL) as continuous variable.

	♀ Hb<12 g/dL	♀ Hb≥12 g/dL	♂ Hb<13 g/dL	♂ Hb≥13 g/dL
Total number (%)	427 (27%)	1171 (73%)	522 (44%)	675 (56%)
Eosinophils ≤0.30 cells×10³/μL (total n and %)	378 (89%)	960 (82%)	354 (75%)	490 (65%)
Eosinophils >0.30 cells×10³/μL (total n and %)	49 (11%)	211 (18%)	168 (25%)	185 (35%)
P value	0.002		0.07	
% of subjects with low Hb in the subgroup with eosinophils >0.30 cells×10³/μL	18%		44%	
P value (comparison between sexes)	<0.0001			
Mean (SD eosinophils in the subgroup with eosinophils >0.30 cells×10³/μL)	160.4 (±167)	199.4 (±189)	283 (±270)	253 (±256)
P value	0.002		0.05	

SD, standard deviation.

Table 4. Correlations between the various parameters and after stratification (eosinophil cut-off of ≤0.30 and >0.30 cells×10³/μL). Data are expressed as Pearson's coefficient (r value).

	Eosinophils (whole population)	Eosinophils (subjects with ≤0.30 cells×10³/μL)	Eosinophils (subjects with >0.30 cells×10³/μL)
Ferritin (ng/mL)	-0.07	-0.12	-0.02
Iron (μg/dL)	-0.004	0.00	-0.00
Transferrin (mg/dL)	0.09	0.09	0.03
Hemoglobin (g/dL)	0.03	0.07	-0.01
RBC (10⁶/μL)	0.16	0.18	0.06

RBC, red blood cells.

After stratification for eosinophil cut-off, subjects with high eosinophil count (>0.30 cells×10^3/μL) were younger, mostly the men (mean age 15 years). The high prevalence of allergic diseases in young subjects might explain this outcome. However, there was no information about allergic diseases. Notably, subjects with high eosinophil count had high red cell counts, both women and men. Consistently, transferrin levels were higher in these subgroups. However, ferritin levels were lower in male subjects alone. These discrepancies could depend on the hormonal impact on iron metabolism.[19,20] In addition, considering the stratification for hemoglobin cut-offs (separately per sex), both women and men with normal hemoglobin levels had more frequently high eosinophils counts. Intriguingly, men with low hemoglobin levels had frequently higher eosinophil counts than women. However, this finding is conflicting with the evident behavior of peripheral eosinophils that are higher in men.[21]

Anyway, the most relevant outcome provided by this study was the lack of an association between anemia and low eosinophil count. Considering the hemoglobin value, it was evident that only 27% of women and 44% of men had values below the reference values with low eosinophil counts. However, this finding could depend on the fact that the population was not selected for allergy. Therefore, it could be speculated that the relationship between anemia and allergic disease is peculiar. Furthermore, it is noteworthy that the cited studies did not evaluate peripheral eosinophils.[13-16] Therefore, we are conducting a survey on allergic children to confirm these findings.

The present study had some principal limitations, including the selected population, as it represented a group of patients undergoing a blood test. The most common reasons for blood testing were health check-ups or to confirm clinical diagnosis in most patients. As a result, it is reasonable to think that several recruited subjects had some medical condition that could affect eosinophil count. Namely, eosinophilia may occur in several situations, including parasitosis, immune disorders, malignancy, hormonal imbalance, autoimmunity, connective tissue disease, rheumatologic disease, and as a consequence of medical treatments.[22] Unfortunately, the lack of information about possible comorbidities, mainly concerning allergic diseases and concomitant treatments, does not allow us to draw appropriate conclusions. In addition, there was no mechanistic investigation.

However, the sample represents a large population afferent to an analysis laboratory and the size was considerable. In addition, the present study was original as there was no similar study addressing this issue. In fact, the main field of research has been allergic diseases.[13-18] Therefore, the present study sought to expand knowledge on this topic by analyzing a more generalized sample of people. For this reason, it was planned to evaluate a large sample of people. This new approach could, therefore, expand the field of observation beyond the model of allergic diseases.

Although a definitive conclusion cannot be driven, the current study indicates that iron metabolism *per se* might not influence the peripheral eosinophil count. On the contrary, sex directly impacts peripheral eosinophils, mainly in men. However, there is a need to perform further studies to confirm such findings as well as to provide mechanistic insight.

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