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Original article

Iron metabolism and peripheral eosinophil count in children with asthma

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Abstract. *Background and aim:* 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. The present study investigated the possible relationship between iron metabolism and peripheral eosinophils in a general population. *Methods:* This retrospective study included 49 children with asthma afferent to a pediatric clinic during the last solar year and evaluated the association between eosinophil count, iron status, and asthma control in 49 children. *Results:* The mean eosinophil count was 346.73 cells/μL. There was a significant difference in age between children with eosinophil counts below and above 300 cells/μL (12.9 vs 10.1 years, p=0.02). This difference was particularly evident in females alone when stratified by eosinophil count and sex (13.4 vs. 9, p=.03). There was a slight but positive correlation between the eosinophil count and the ACT score. Iron status and the ACT score also showed a mild but negative correlation. These results do not support a possible pathogenetic role of iron metabolism on asthma. *Conclusions:* The present study did not demonstrate a relationship between iron deficiency and peripheral eosinophil count or asthma control in children. (www.actabiomedica.it)

Key words: iron, red cells, hemoglobin, ferritin, transferrin, peripheral eosinophils, asthma control

Introduction

Allergic asthma is a common medical condition in childhood. Allergic asthma recognizes a type 2 immune-mediated mechanism (1). T helper 2 polarization, increased production of type 2 cytokines, including interleukin-4 (IL-4), IL-5, and IL-13, and consequent eosinophilic infiltration of airways characterize type 2 inflammation (2). Mainly, eosinophils are valuable biomarkers of allergic inflammation (3,4). As detecting tissue eosinophils is invasive, peripheral eosinophil count can represent a suitable measure of inflammation grade (5). Accordingly, the number of tissue eosinophils correlates well with peripheral count (6). As a result, blood cell count should be a routine exam in asthmatic patients; a high level of peripheral eosinophils may suggest intense bronchial type 2 inflammation (7). Peripheral eosinophilia allows phenotyping of patients with type 2 asthma (8). Also, biologics' prescriptive appropriateness requires considering the eosinophil count (9). Usually, a value of 300 peripheral eosinophils per μL is a reliable cut-off for identifying candidates for anti-IL-4 and anti-IL-5 biological agents (10).

Iron is a widespread metal and exerts essential biological activities concerning oxygen metabolism through red blood cells, muscle function, and the immune system (11). In particular, 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). Some studies reported an association between iron deficiency, primarily anemia, and allergic diseases (13-17). However, the exact mechanisms contributing to this association have yet to be entirely clarified.

Interestingly, it has been reported that iron deficiency worsens allergic symptoms and might promote allergy development (13-15). Consistently, a study demonstrated that iron supplementation improved allergic symptoms and ameliorated response to allergen-specific immunotherapy (18).

Based on this background, we tested the hypothesis that there could be a relationship between iron metabolism biomarkers and peripheral eosinophils in children with asthma. Therefore, this study aimed to compare iron biomarkers with eosinophil counts and asthma control.

Materials and methods

The current study retrospectively analyzed the data of children with asthma consecutively evaluated at the Pediatric Clinic in Pavia. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the local Ethical Committee (protocol number 0003233/22). The inclusion criteria were age between 6 and 16 years and asthma diagnosis performed according to validated criteria. The time frame considered was the calendar year 2022. The parents signed an informed consent. The iron biomarkers included red blood cell count, serum hemoglobin, iron, ferritin, and transferrin. The peripheral eosinophil count was also evaluated.

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

Asthma diagnosis was performed according to the GINA (Global Initiative for Asthma) guidelines (19).

The Asthma Control Test questionnaire consisted of 5 questions with five possible responses, exploring the patient's perception of asthma control (20). The result could range between 0 and 25 or 27, where 25 or 27 is, respectively, the optimal asthma control.

Categorical variables are described as counts and percentages and compared between groups with the chi-square test. Quantitative variables are expressed as mean and standard deviation (sd) 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. The Pearson test calculated correlations. The statistical analysis was performed using Stata v17.0. Program.

The STROBE checklist was used for this study (21).

Results

The present study analyzed the data of 49 children with asthma.

Table 1 reports the demographic, biological, and clinical data.

The mean age was 11.89 years; there were 18 females and 31 males. The mean eosinophil count was 346.73 cells/μL; the mean hemoglobin level was 13.6 g/dL; the mean serum iron concentration was 72 μg/dL; the mean ferritin level was 32 ng/mL; the mean transferrin level was 287 mg/dL; and the mean ACT score was 21.73. There was no difference between the sexes for all considered parameters.

The analysis proceeded by stratifying the population, considering the eosinophil cut-off of 300 cells/μL. Table 2 reports the results: there was a significant

Table 1. Demographic, biological, and clinical data of pediatric patients with asthma.

	Global population	Females	Reference values	Males	Reference values	p value
Sex	49	18		31		
Age (years)	11.89 (3.67)	11.22 (4.53)		12.29 (3.09)		0.33
Eosinophils (-0)	346.73 (328.9)	436,11 (478.8)	0.1-0.5 x 10^3/µl	294,84 (188.7)	0.1-0.5 x 10^3/µl	0.14
Saturation* (%) *Iron-binding capacity	17.67 (8.51)	16.25 (8.21)	>16 %	18.49 (8.7)	>16 %	0.38
Hemoglobin (g/dl)	13.6 (1.39)	13.2 (1.11)	up to 6 years: 11-13 g/dl up to 12 years: 11-15 g/dl	13.8 (1.50)	up to 6 years: 11-13 g/dl up to 12 years: 11-15 g/dl	0.12
Iron (µg/dl)	72 (30.30)	66 (31.51)	25-156 µg/l	76 (29.58)	31-144 µg/l	0.31
Ferritin (ng/mL)	32 (19.13)	34 (21.96)	7-140 µg/ml	30 (17.45)	7-140 µg/ml	0.44
Transferrin (mg/dl)	287 (33.2)	286 (35.6)	200-400 mg/dl	287 (32.3)	200-400 mg/dl	0.85
Asthma Control Test (ACT)	21.73 (3.51)	22.11 (2.76)		21.35 (3.89)		0.47

Data are expressed as mean (standard deviation).

Table 2. Stratification of the hematological parameters considering the sex and number of eosinophils (eosinophil cut-offs: ≤300 and >300 cells/µL). Data are expressed as mean and standard l deviation.

	≤300 cells/µL	>300 cells/µL	p value	≤300 cells/µL	>300 cells/µL	p value	≤300 cells/µL	>300 cells/µL	p value
Sex	Global population	Global population		F (9)	F (9)		M (18)	M (13)	
Age (years)	12.9 (4.24)	10.1 (2.36)	0.02	13.4 (5.10)	9 (2.54)	0.03	12.6 (3.88)	11.7 (1.42)	0.43
Saturation*(%) *Iron-binding capacity	17.44 (8.98)	17.95 (8.08)	0.83	13.11 (7.52)	19.4 (8.02)	0.10	19.61 (9.05)	16.94 (8.28)	0.41
Hemoglobin (g/dL)	13.7 (1.69)	13.4 (0.91)	0.47	12.7 (1.03)	13.6 (1.07)	0.11	14.2 (1.78)	13.3 (0.80)	0.10
Iron (µg/dL)	71 (31.36)	72 (29.63)	0.84	53 (24.32)	79 (33.82)	0.08	80 (31.19)	68 (26.93)	0.84
Ferritin (ng/mL)	27 (14.27)	36 (23.12)	0.09	26 (15.76)	42 (25.27)	0.13	28 (13.93)	33 (21.7)	0.44
Transferrin (mg/dL)	286.40 (37.1)	286.77 (28.5)	0.96	288 (40.16)	282 (32.42)	0.75	285 (36.64)	289 (26.51)	0.73
Asthma Control Test (ACT)	22 (3.45)	21.18 (3.61)	0.42	22 (3.52)	22 (1.88)	0.62	22 (3.52)	20 (4.28)	0.21

F: female; M: male.

difference concerning age (12.9 vs 10.1 years, p=0.02).

The third step considered stratification by the eosinophil cut-off of 300 cells/µL and the subjects' sex. Table 2 reports the results: there was only a significant difference in age in females alone (13.4 in female patients with eosinophil count below 300 cells/µL vs 9, p=.03).

Table 3. Correlations between the various parameters and eosinophil counts. Data are expressed as r value.

	Total group	Eosinophils <300 cells/μL	Eosinophils > 300 cells/μL
Ferritin (ng/mL)	0.09	0.59	-0.20
Iron (μg/dL)	0.10	0.25	0.11
Transferrin (mg/dL)	-0.20	-0.41	-0.36
Hemoglobin (g/dL)	-0.02	0.004	0.14
Asthma Control Test (ACT)	0.0067	0.13	0.13
Saturation*(%) *Iron-binding capacity	0.14	0.31	0.17

After stratification for cut-off values, the final step considered the correlations among the variables, eosinophil counts, and ACT scores.

Eosinophil count correlations

There was a moderate (r=0.59) positive correlation between ferritin levels and eosinophil count in patients with low eosinophils (<300 cells/μL). There was a mild (r=0.25) positive correlation between iron concentration and eosinophil count in patients with low eosinophils (<300 cells/μL). There was a moderate (r=-041) negative correlation between transferrin levels and eosinophil count in patients with low eosinophils (<300 cells/μL), and there was a mild (r=-0.35) negative correlation between transferrin levels and eosinophil count in patients with high eosinophils (>300 cells/μL) (Table 3 and Figure 1a and 1b). Finally, there was a mild (r=0.31) positive correlation between saturation and eosinophil count in patients with low eosinophils (<300 cells/μL).

ACT score correlations

There were mild correlations between ACT scores and iron concentrations (r=0.21) in patients with low eosinophils (<300 cells/μL) and transferrin levels (r=-0.28) in patients with high eosinophils (>300 cells/μL) and hemoglobin levels (r=0.22) and also in patients with low eosinophils (r=0.29).

Discussion and conclusions

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 improved allergic issues (17,18).

The present study, conducted in a group of asthmatic children, showed some correlations between iron biomarkers and eosinophil counts. In particular, these correlations were significant only after stratification. In patients with low eosinophil count (<300 cells/μL), eosinophils positively correlated with ferritin and iron but negatively with transferrin. However, these correlations are partially conflicting. There was a negative correlation between eosinophils and transferrin in patients with high eosinophils (>300 cells/μL). These correlations seem to have poor clinical relevance.

Considering ACT scores, there were some correlations with iron biomarkers, but the strength of the correlation was generally slight.

As a result, the present study showed that iron metabolism scarcely affected eosinophil count and asthma control, assessed by the ACT questionnaire. These findings were also conflicting, as the correlations were positive and negative. Therefore, in children with asthma, iron metabolism exerts a modest effect on type 2 inflammation and asthma control.

These results do not support a possible pathogenetic role of iron metabolism on asthma.

However, the present study had some limitations, including the limited number of patients and the need for a longitudinal observation.

Therefore, further studies should be conducted to confirm these preliminary findings.

In conclusion, the current study showed that iron metabolism per se seems not to influence the peripheral eosinophil count and asthma control in children with asthma.

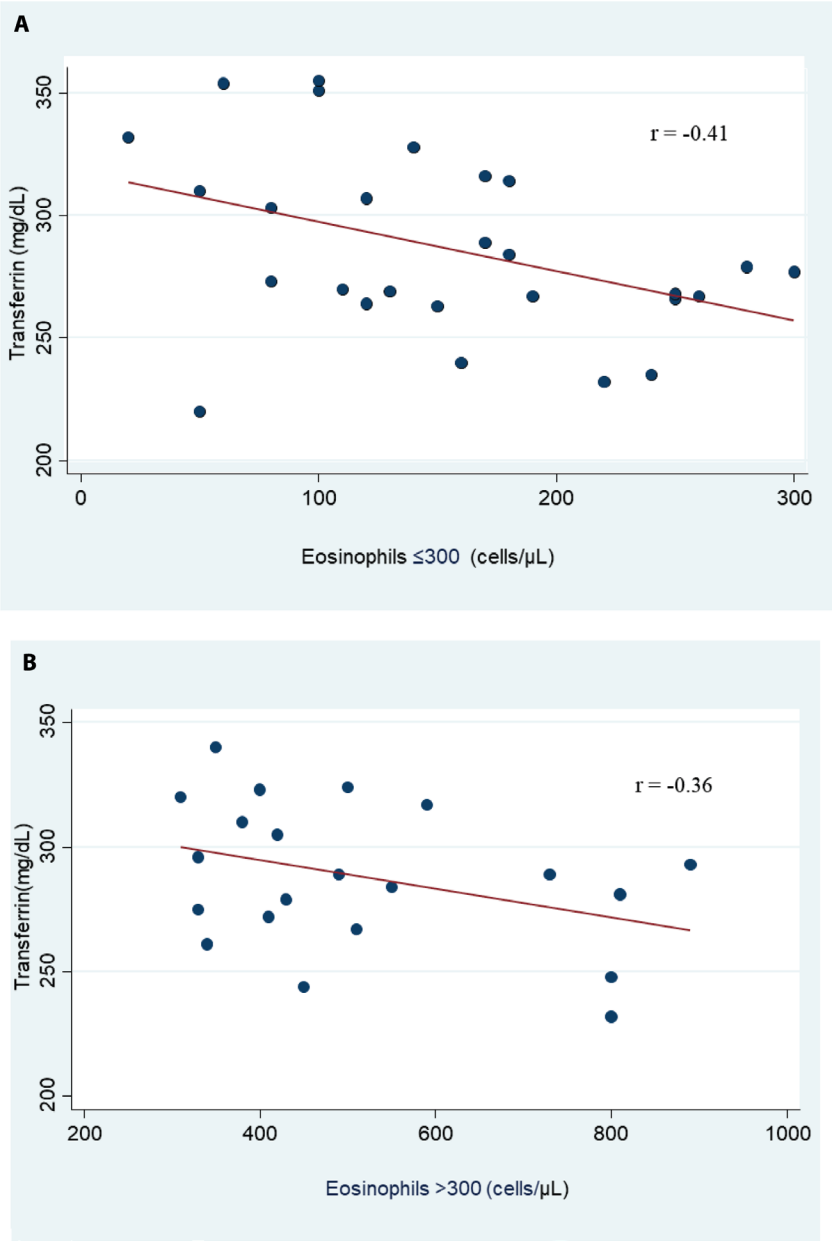

Figure 1. Correlations between transferrin and eosinophils with the stratification (eosinophil cut-off of ≤0.30 and >0.30 cells×10^3/µL). Data are expressed as Pearson's coefficient (r-value) a: r= -0-41 p= 0.03; b: r= -0-36 p= 0.10

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Authors Contribution: AL: Conceptualization, Validation, Writing – Original draft. MDA: Conceptualization, Data curation,

Formal Analysis, Project administration. FB: Data curation, Formal Analysis, Visualization. ADS: Data curation, Formal Analysis. MDF, GT, MV: Investigation, Resources. GC: Conceptualization, Data curation, Project administration, Validation, Writing – Original draft. GLM: Conceptualization, Validation.

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