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ORIGINAL ARTICLE

Assessment of Iron Levels in Settings of Infections in Patients with Normal Hemoglobin

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SUMMARY

Background: **During our daily routine in laboratory, we noticed that in patients with changes in white blood cell (WBC) count, which are accompanied by elevated levels of C-reactive protein (CRP), a decrease in the levels of iron is found, even though the hemoglobin concentration is within the reference range. We aimed to figure out if low levels of iron are related to infections and whether they are signs of anemia and aimed to evaluate the possible correlations between iron levels and WBC and CRP.**

Methods: **We performed a descriptive and retrospective study including 159 outpatients with signs of infections and with normal concentration of hemoglobin, from January 2023 through December 2024. We used Jamovi Statistical Software version 2.3.28. We used the Shapiro-Wilk test for normal distribution and F-test for variances. We tested differences with Mann-Whitney and ANOVA for non-parametric variables between two or more groups, respectively. We performed Pearson's correlations and did the linear regression analysis. A two-sided p-value equal to or less than 0.05 was considered statistically significant.**

Results: **The study included 70 women (44%) and 89 men (56%), with a median age of 27 years (1 - 91 years) and 32 years (1 - 91 years), respectively. Based on one-way ANOVA or Mann-Whitney test for independent samples, we found differences in iron levels between children, adults, and elderly ($p < 0.001$) and differences between normal and elevated levels of WBC ($p = 0.019$). We also found correlations between iron and CRP ($r = -0.255$, $p = 0.001$), iron and WBC ($r = -0.337$, $p < 0.001$), and CRP and WBC ($r = 0.189$, $p = 0.017$). Based on regression analysis, CRP and WBC can both serve as a predictor of serum iron levels, with a probability of 15.2%.**

Conclusions: **We conclude that there is a correlation between iron levels and CRP and WBC and that low levels of iron are related to settings of infections.**

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INTRODUCTION

Iron is a mineral that is naturally present in many foods, added to some food products, and available as a dietary supplement. Iron is an essential part of hemoglobin, an erythrocyte (red blood cell) protein that transfers oxygen from the lungs to the tissues [1]. Hemoglobin concentration (Hb) is usually reported as grams of hemo-

globin per deciliter of blood (g/dL) [2].
The reference ranges for hemoglobin (Hb) concentrations in adults are as follows [3]:
1) Male: 14 - 18 g/dL
2) Female: 12 - 16 g/dL
3) Pregnant female: >11 g/dL
4) Elderly: Slight decrease in values
The reference ranges for Hb concentrations in children are as follows [3]:
1) Newborn: 14 - 24 g/dL
2) 0 - 2 weeks: 12 - 20 g/dL
3) 2 - 6 months: 10 - 17 g/dL
4) 6 months - 1 year: 9.5 - 14 g/dL
5) 1 - 6 years: 9.5 - 14 g/dL
6) 6 - 18 years: 10 - 15.5 g/dL
Hemoglobin concentration is used clinically to figure out the presence of anemia, which is functionally defined as insufficient red blood cell (RBC) mass to adequately deliver oxygen to peripheral tissues [4,5].
Infection is the invasion and growth of germs in the body. The germs may be bacteria, viruses, yeast, fungi, or other microorganisms. Infections can begin anywhere in the body and may spread all the way through it. An infection can cause fever and other health problems, depending on where it occurs in the body [6].
During our daily routine in the laboratory, we noticed that in patients with changes in white blood cells (WBC) count, which are accompanied by elevated levels of C-reactive protein (CRP), a decrease in the levels of iron was found, even though the hemoglobin concentration was within the reference range.
We aimed to figure out if low levels of iron are related to infections and whether they are warning signs of anemia and aimed to evaluate the possible correlations between iron levels with WBC and CRP.

MATERIALS AND METHODS

Our study is a descriptive and retrospective analysis of outpatients' medical records. We searched the laboratory database of outpatients that presented to the Polyclinic Father Luigi Monti and Catholic Hospital "Our Lady of Good Counsel'' Tirana, Albania, with signs of infection and had undergone a complete blood count (CBC), C reactive protein (CRP), and iron levels between January 2023 and December 2024.
To keep the confidentiality of participants' data, we coded the identity of each participant in the study, according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.
The criterium for being part of the study was serum CRP level higher than 5 mg/L.
We collected blood samples through venipuncture using BD Vacutainer k3EDTA 5.4 mg, 3 mL tube for complete blood count and measured on ADVIA 2120i (SIEMENS).
For the measurement of C-reactive protein and iron levels, we collected the blood with BD Vacutainer SST II Advance 3.5 mL tube, and we measured them in Dimension EXL 200 (SIEMENS) with turbidimetry and with bichromatic endpoint method, respectively.

Statistics

We initially organized the data after their collection in an EXCEL database, which for the purpose of statistical analysis was imported, and then the statistical analysis was performed with the program Jamovi Statistical Software version 2.3.28. We used descriptive statistics for all variables, and we studied frequencies. We used the Shapiro-Wilk test for normal distribution. We tested differences with Mann-Whitney and ANOVA for non-parametric variables between two or more groups, respectively. We performed Pearson's correlations; a two-sided p-value of 0.05 or less was considered statistically significant. We employed linear regression analysis, and we used coefficient "t" to measure the statistical significance of an independent variable in explaining the dependent variable and r^2 (coefficient of determination) to measure the percentage of the variation in the dependent variable that is explained by variation in the independent variable.

RESULTS

From January 2023 through December 2024, 1,836 patients had at least a CRP test performed in our laboratory, out of which 772 patients had a CRP value > 5 mg/L, and only 159 patients also had an iron level test done and had normal values of hemoglobin according to age and gender classification. Our study comprised a total of 159 patients, out of which 70 (44%) were females and 89 (56%) were males, with a median age of 27 years (1 - 82 years) for females and 32 years (1 - 91 years) for males.
The key characteristics of the patients according to age group classification are summarized in Table 1. Patients were divided into three groups: children (< 18 years), adults (18 - 65 years), and elderly (> 65 years). Since one of the groups had less than 30 outpatients (n < 30) and the data were not normally distributed, we used the median instead of the mean to show the central tendency and the IQR (interquartile range, 25th percentile and 75th percentile) instead of the standard deviation to measure the variability.
We found that the most affected age group was children with 42%, where we found the highest value of white blood cells and the lowest value of iron, followed by adults with 41.5% and elderly with 16.5%, where we found the highest value of CRP.
Notably, the median value of white blood cells was within the reference range, while the median value of iron was below the reference for all groups.
We performed one-way ANOVA to test the differences of iron levels between children, adults, and elderly and found a statistically significant difference of iron be-

Table 1. Descriptive statistics according to age group classification.

	Groups	WBC (x 10^9/L)	CRP (mg/L)	Iron (µmol/L)
n	adults	66	66	66
	children	67	67	67
	elderly	26	26	26
Median	adults	10.3	26.5	6.8
	children	12.6	23.5	2.86
	elderly	9.86	27.8	7.16
Minimum	adults	3.09	6.5	1.43
	children	4.57	6	0.54
	elderly	4.08	7.1	1.97
Maximum	adults	20.5	220	27.9
	children	32.0	195	17.2
	elderly	21.0	273	21.5
25th percentile	adults	7.60	13.1	4.17
	children	9.84	15.8	1.97
	elderly	7.09	15.7	3.63
75th percentile	adults	12.7	50.4	9.4
	children	18.0	55.5	4.92
	elderly	11.1	72.5	11.3
Shapiro-Wilk p	adults	0.101	< 0.001	< 0.001
	children	0.006	< 0.001	< 0.001
	elderly	0.030	< 0.001	0.017

Table 2. Pearson's correlation.

		CRP	Iron	WBC
CRP	Pearson correlation "r"	1	-0.255	0.189
	Sig. (2-tailed) "p"		0.001	0.017
	n	159	159	159
Iron	Pearson correlation "r"	-0.255	1	-0.337
	Sig. (2-tailed) "p"	0.001		< 0.001
	n	159	159	159
WBC	Pearson correlation "r"	0.189	-0.337	1
	Sig. (2-tailed) "p"	0.017	< 0.001	
	n	159	159	159

Table 3. Regression analysis.

Independent variable	b	m	t	r^2	p	Dependent variable
CRP	7.33	-0.0254	-3.30	0.065	0.001	Iron
WBC	9.93	-0.316	-4.49	0.114	< 0.001	Iron
CRP and WBC	10.39	-0.02 and -0.28	-3.99	0.152	< 0.001	Iron

tween those age groups ($p < 0.001$). In the same way, we used the Mann-Whitney test and found differences in iron levels between normal and elevated white blood cells levels ($p = 0.019$).

According to Pearson's correlation (Table 2), we can state the following: iron had a negative correlation with CRP ($r = -0.255$, $p = 0.001$) and WBC ($r = -0.337$, $p < 0.001$). The magnitude, or strength, of the association was weak ($0 < |r| < 0.4$) between both iron and CRP and iron and WBC.

Similarly, CRP and WBC shared a positive correlation ($r = 0.189$, $p = 0.017$), and the magnitude, or strength, of the association was also weak ($0 < |r| < 0.4$).

For the correlation of iron with CRP and WBC that had turned out to be statistically significant, we performed the regression analysis procedure to see if the variables had a predictive effect on each other (Table 3).

According to Table 3, CRP, as an independent variable, can serve as a predictor of iron ($t = -3.30$, $p = 0.001$). By estimating r^2, we quantified the percentage of this predictive effect. This means that if we find an increased value of CRP, the probability of finding a decreased value of iron is calculated to be 6.5%. Meanwhile, WBC ($t = -4.49$, $p < 0.001$), as independent variable, can serve as predictor of iron with a probability of 11.4%. And if both variables (CRP and WBC) are used, the predictor effect increases by 15.2%.

Linear regression also provides an equation that can be used to predict the value of a response variable based on the value of the predictor variable.

The formula for simple linear regression is $Y = mX + b$, where Y is the response (dependent) variable, X is the predictor (independent) variable, m is the estimated slope, and b is the estimated intercept.

Based on that, we can predict the possible iron serum level based on the measured CRP level, WBC values, and both variables through the formulas:

Iron = [(-0.0254) x CRP] + 7.33

Iron = [(-0.316) x WBC] + 9.93

Iron = [(-0.02) x CRP] + [(-0.28) x WBC] + 10.39.

DISCUSSION

In our daily routine, we encountered many cases of patients who, due to a viral or bacterial infection, presented to a clinician, who, along with the clinical evaluation, also requests other, more extensive, laboratory examinations to better assess the patients' health status. We have noticed that in patients with signs of viral or bacterial infection, serum iron levels are reduced, and hemoglobin levels are normal according to age and gender classification. This led us to conduct this study; to evaluate the possible correlations between serum iron levels and CRP to see if low serum iron levels are warning signs of anemia or if it is the infection that lowers the serum iron levels in the blood.

Various articles in the literature have concluded that the use of iron as a cofactor in basic metabolic pathways is essential to both pathogenic microorganisms and their host [7]. Another finding is that to combat invading bacteria, animals go into an iron-withholding mode, and some invading bacteria respond by producing specific iron chelators-siderophores that remove the iron from the host sources [8]. Crucial cellular processes such as DNA synthesis and the generation of ATP require iron. Viruses depend on iron to efficiently replicate within living host cells. Some viruses selectively infect iron-acquiring cells or influence the cellular iron metabolism via human hemochromatosis protein (HFE) or hepcidin [9,10].

In our study, the female-male distribution was almost the same, 44% and 56% respectively; a result that reflects the lack of gender differences in the prevalence of infections and their association with reduced serum iron levels, in contrast to the prevalence of iron deficiency anemia, where a female predominance is seen. The Global Burden of Disease Study 2021 reported that the prevalence of anemia was 17.5% in men and 31.2% in women [11], and the National Health and Nutrition Examination Survey published in December 2024 reported that the prevalence of anemia was higher in females (13.0%) than it was in males (5.5%) [12].

The median age of the study population was relatively young, 27 years for females and 32 years for males. Results from the Gutenberg Health Study show that the prevalence of iron deficiency is markedly increased in younger, menopausal women and older men [13].

The most affected age group was children with 42%, with more than 1/3 of them, 28 from 67 patients, being children under 2 years old, which reflects their vulnerability to infections, whether bacterial or viral. Earlier articles confirm our results that infections are the most common cause of acute illness in children. The most common ones are respiratory infections, which peak when the child starts to go to school or out-of-home day care [14].

In the elderly age group, we found the maximum value of CRP and the median of CRP higher compared to the other two age groups, children and adults. We believe that these results are related to the fact that the serum values of C-reactive protein increase with increasing age [15]. Low-grade inflammation also takes part in the aging process (Vasto et al., 2007) [16], and CRP levels rise with age, even when no acute illness is present (Ferrucci et al., 2005) [17].

The results of this study highlight statistically significant differences between the group of patients with normal WBC values and those with increased WBC. This supports our hypothesis that infections are associated with decreased serum iron levels. The hypoferremia of infection was documented in seminal studies by Cartwright et al. in the 1940s, who noted a precipitous drop in plasma iron levels upon intramuscular inoculation of canines with *Staphylococcus aureus* [18]. During the acute phase of an infection, a pro-inflammatory cytokine response causes a decrease in intestinal iron absorption and decreased release from body iron stores.

This is called functional iron deficiency (FID) [19]. According to Patteril et al., FID was independently associated only with abnormal white blood cell count (WBC < 4 or $> 11 \times 10^9$ /L) at admission to ICU, $p = 0.007$, but not with positive cultures [20].

The correlation found by us between iron levels and CRP was also reported in Hashimoto et al.´s Japanese study, who showed that a small elevation in CRP within its normal range was associated with a significant decrease in serum iron [21]. Another article of 2012 revealed a negative correlation of CRP and iron levels in children with severe pneumonia [22]. More recent studies confirm the negative correlation of CRP and iron levels [23], and the regression analysis made by them showed that CRP contributed significantly to the prediction of serum iron levels [24], when the prediction effect was 6.5%.

This study showed that there is a weak statistically significant negative correlation between iron and WBC, where in 11.4% of patients iron values can be predicted by WBC. These findings had also been reported by Moro et al. [25] and Ghio et al. [26].

The correlation between CRP and WBC is known in the literature, because both variables are considered indirect markers of generalized body response to inflammatory cytokine stimulation [27]. This is why these variables correlate with each other in different infections from orofacial infections [28] to pneumonia [29], acute pancreatitis [30], acute appendicitis [31], even in apparently healthy populations [32], and in critically ill patients with functional iron deficiency [33].

CONCLUSION

We found correlations between iron levels and WBC and CRP and concluded that low levels of iron are related to infections and that they are no sign of anemia. The results of our study indicate that when performing laboratory tests to evaluate a viral or bacterial infection, we should not use that same blood sample to perform other laboratory examinations, especially not when related to anemia.

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Personal savings of the authors were used for this study.

Declaration of Interest:
The authors have no conflicts of interest to declare.

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