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Iron metabolism in patients with rheumatoid arthritis

W. TAŃSKI[1], M. CHABOWSKI[2,3], B. JANKOWSKA-POLAŃSKA[4], E.A. JANKOWSKA[5]

[1]Department of Internal Medicine, 4th Military Teaching Hospital, Wrocław, Poland
[2]Division of Oncology and Palliative Care, Department of Clinical Nursing, Faculty of Health Science, Wrocław Medical University, Poland
[3]Department of Surgery, 4th Military Teaching Hospital, Wrocław, Poland
[4]Division of Nervous System Diseases, Department of Clinical Nursing, Faculty of Health Science, Wrocław Medical University, Wrocław, Poland
[5]Department of Cardiology, Wrocław Medical University, Faculty of Medicine, Wrocław, Poland

Abstract. – OBJECTIVE: Anemia is the hematological issue that occurs most often as a manifestation in RA. The aim of the study was to assess iron deficiency in RA patients.

PATIENTS AND METHODS: The study was carried out on 62 RA patients treated between 2016 and 2017.

RESULTS: A higher percentage of RA patients compared to the control group had TSAT below 20% (43% *vs*. 5%), ferritin below the reference range (15% *vs*. 7%), sTfR above 1.59 mg/l (26% *vs*. 0%) and hepcidin below 14.5 ng/ml (56% *vs*. 2%). 60% of RA patients had iron deficiency, and 18% – anemia. Correlations were found between reduced levels of ferritin and patients being younger, female, with lower GGT and higher platelet counts. Correlations were also found between iron deficiency and patients being younger, female, having reduced hemoglobin, increased platelet counts, increased GFR, reduced GGT, lower disease activity, and less frequent use of sulfasalazine.

CONCLUSIONS: Iron deficiency is common (64%) in RA patients where there is high disease activity. RA patients had lower transferrin, lower ferritin, lower hepcidin, and higher sTfR. Decreased DAS-28 and reduced hemoglobin were the strongest determinants of iron deficiency.

Key Words:
Rheumatoid arthritis, Iron metabolism, Anemia, Hepcidin, Disease activity score of 28 joints (DAS-28).

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease involving numerous joints and showing systemic symptoms, leading to disability and premature death[1]. The most common hematological problem in RA is anemia, which affects 30-60% of RA patients and significantly complicates the clinical course of the disease. Anemia of chronic disease, which is associated with systemic inflammation, is considered the most common type of anemia in RA patients (>60%)[2]. However, anemia may also be associated with iron, vitamin B_{12} or folic acid deficiency. A patient may suffer from more than one type of anemia simultaneously[3].

Iron deficiency may be absolute or functional (relative). It may be present in anaemic patients, but it may also occur in patients with normal hemoglobin levels. Absolute iron deficiency occurs when the body's iron stores are depleted. It usually results from an insufficient iron intake in the diet, impaired absorption of iron from the gastrointestinal tract or iron loss as a result of bleeding (usually from the gastrointestinal tract, or less often from the urinary or genital tract)[4-6]. Functional iron deficiency is caused by insufficient availability of iron (which is trapped in macrophages) for cellular processes[4-6]. This can be seen when there is inflammation. It has been found that both anemia and iron deficiency (without anemia) in inflammatory diseases other than RA (e.g., heart failure) may be associated with reduced exercise tolerance and a worse prognosis[6]. Moreover, a causal link between iron deficiency and reduced work capacity has been found[7].

Similar to functional iron deficiency, anemia of chronic disease (anemia of inflammation) has multifactorial pathogenesis and is associated with the stimulation of the immune system. When there is inflammation, the activation of

Corresponding Author: Mariusz Chabowski, MD, Ph.D; e-mail: mariusz.chabowski@gmail.com

immune-competent cells and the increased secretion of proinflammatory cytokines impair erythropoiesis (partly because proinflammatory cytokines cause resistance to erythropoietin). This in turn disrupts iron metabolism (partly because proinflammatory cytokines cause iron to be captured by active macrophages, thus making it unavailable to cells that metabolise iron)[4,8-10]. Interleukin 6 (IL-6), which is overexpressed when inflammation is present and which stimulates the production of hepcidin by hepatocytes, plays a key role in these processes[4,8,9,11]. Hepcidin, which binds and internalises ferroportin (the protein which transports iron through cell membranes to the outside of the cell), inhibits the absorption of iron from the gastrointestinal tract and inhibits the release of iron stored by macrophages in the reticuloendothelial system[4,6,12-14].

There is evidence that low hemoglobin levels in RA patients are significantly associated with disability, the activity and duration of the disease and damage to joints and joint pain[15]. Treatment for anemia in RA patients includes iron supplementation, blood transfusions and the use of erythropoiesis-stimulating agents. Importantly, the treatment of the underlying inflammatory condition itself may lead to an increase in hemoglobin levels[16]. Biological treatments used in RA patients, e.g., infliximab (an anti-TNF-a antibody), tocilizumab (an anti-IL-6 receptor antibody) and anakinra (an anti-IL-1 antibody), not only effectively inhibit the progression of joint involvement, but may also prevent anemia[17].

The main aim of this study was to assess the prevalence of iron deficiency in RA patients using standard parameters (ferritin, transferrin saturation) and new biomarkers (soluble transferrin receptor, hepcidin) measured in the peripheral blood of patients with and without anemia.

Specific Objectives

1. A comparison of iron metabolism in RA patients and healthy controls using the aforementioned parameters.
2. The analysis of correlations between iron metabolism parameters and hemoglobin levels as well as red blood cell parameters in RA patients.
3. The analysis of correlations between iron deficiency and the clinical condition of RA patients, especially regarding disease activity, inflammation parameters, and comorbidities.

Patients and Methods

This retrospective and observational study was conducted on 62 RA patients aged 52±15 who had been treated in the Department of Internal Medicine of the 4th Military Teaching Hospital in Wroclaw between 2016 and 2017. The control group comprised 58 healthy individuals aged 56±9. 28 patients with RA were treated with monoclonal antibodies against IL-6 receptor (tocilizumab).

Inclusion criteria:

a) age ≥18;
b) RA diagnosed according to the ACR criteria;
c) time from RA diagnosis ≥6 months;
d) active RA (DAS-28>5.1);
e) informed written consent to participate in the study.

Exclusion criteria:

a) treatment with iron, erythropoietin analogues or blood transfusions during the previous 12 months.

A clinical and laboratory assessment was done for each RA patient. The following tests and examinations were carried out:

a) a physical examination, anthropometric measurements (e.g., BMI - body mass index) and the patient's medical history (history of RA, comorbidities and medication taken);

b) a Disease Activity Score of 28 joints (DAS-28)[18] – the system is commonly used in the daily rheumatology assessment of RA patients and helps make decisions about any modifications to the treatment. It involves determining the number of tenders (TEN28) and swollen (SW28) joints out of 28 peripheral joints (including 10 proximal interphalangeal joints, 10 metacarpophalangeal joints, 2 wrist joints, 2 elbow joints, 2 shoulder joints and 2 knee joints), together with the patient's assessment of their general health status using a 100-mm visual analogue scale (VAS), as well as checking their C-reactive protein (CRP) levels. DAS-28 is used both to determine RA activity and to assess clinical improvement parameters.

The DAS-28 score is calculated according to the following formula[19]: $DAS\text{-}28 = 0.56 \times \sqrt{(TEN28)} + 0.28 \times \sqrt{(SW28)} + 0.70 \times \ln(ESR) + 0.014 \times (VAS)$

RA activity is defined as follows: DAS-28 >5.1 - high disease activity, DAS-28 ≤5.1 - >3.2 - moderate disease activity, DAS-28 ≤3.2 - ≥2.6 - low disease activity, DAS-28 < 2.6 - remission;

c) laboratory tests to assess the severity of the inflammation (ESR – erythrocyte sedimentation rate, CRP – C reactive protein) by means of standard laboratory methods;
d) laboratory tests to determine the presence or severity of comorbidities, i.e., a complete blood count, creatinine, uric acid, AST (aspartate aminotransferase), ALT (alanine aminotransferase), GGT (gamma-glutamyl transpeptidase), bilirubin, TSH (thyroid-stimulating hormone), lipid profile;
e) laboratory tests to assess iron metabolism: iron, TIBC (Total Iron Binding Capacity), TSAT (transferrin saturation), ferritin, sTfR - soluble transferrin receptor (the levels determined by immunonephelometry), and hepcidin (the levels determined by the kit of BACHEM AG; Cat. No. S-1337. Hepcidin – 25);
f) the serum level of IL-6, a proinflammatory cytokine.

Iron deficiency was defined as serum ferritin<100 µg/L or serum ferritin between 100 µg/L and 299 µg/L with TSAT < 20%. Anemia was defined as hemoglobin levels below 12 g/dL for women and below 13 g/dL for men[20].

The study was approved by the Bioethics Committee at the Military Institute of Medicine in Warsaw (No. 34/WIM/2015).

Statistical Analysis

Continuous variables with normal distribution were taken as the mean and standard deviation, whereas variables with skewed distribution were presented as a median with upper and lower quartiles. The distribution of continuous variables was tested using the Kolmogorov-Smirnov test. The significance of differences for continuous variables with normal distribution was tested using a Student's *t*-test for unpaired (independent) samples. The significance of differences for continuous variables with skewed distribution was tested using the Mann-Witney test. The significance of differences for categorical variables was tested using the Chi-square test. Correlations between continuous variables with normal distribution were tested using Pearson correlation coefficients, whereas correlations between continuous variables with skewed distribution were tested using Spearman's rank correlation coefficients. Correlations between continuous variables describing iron metabolism (serum ferritin level, TSAT and serum levels of hepcidin and soluble transferrin receptor), as well as hemoglobin levels and clinical and laboratory parameters were analysed using single-factor and multi-factor linear models. Iron deficiency was a dependent variable, whereas other clinical and laboratory parameters were independent variables. Explanatory variables (clinical and laboratory parameters) which were statistically significantly correlated with the prevalence of iron deficiency in single-factor models were included in logistic multi-factor models. In all analyses, $p<0.05$ denoted statistical significance.

Results

The majority of RA patients were women (84%). The mean BMI was higher in healthy controls than in RA patients. RA patients had the following comorbidities: hypertension (32%), diabetes (6%), asthma (6%), ischemic heart disease (5%), atrial fibrillation (2%). Both groups were compared in terms of selected laboratory parameters and the following was found in RA patients: lower triglyceride levels, lower uric acid levels, higher CRP levels, higher platelet counts and higher GFR. It was found that, compared with the control group, RA patients had a higher prevalence of anemia (5% vs. 18%), lower hemoglobin levels, lower hematocrit levels, lower MCV, MCH and MCHC values and higher RDW values. Two RA patients had vitamin B12 deficiency, and one RA patient had folic acid deficiency. A higher percentage of RA patients compared with the control group had TSAT values below 20% (43% *vs.* 5%), ferritin levels below the reference range (15% *vs.* 7%), soluble transferrin receptor levels above 1.59 mg/l (26% *vs.* 0%) and hepcidin levels below 14.5 ng/ml (56% *vs.*2%). Sixty-four percent of the RA patients had iron deficiency (Table I).

A positive RF was present in 55% of the RA patients and anti-CCP (anti-cyclic citrullinated peptide) antibodies were found in 75% of the patients. Ninety-one percent of the RA patients showed high disease activity, as assessed by DAS-28 (>5.1). The mean duration of the disease in the RA patients was 5.5 years. At the time of their inclusion in the study, 10% of the patients were taking sulfasalazine at therapeutic doses, 77% methotrexate at therapeutic doses and 42% glucocorticoids at low doses (Table II).

The assessment of correlations between ferritin levels and the selected parameters showed that reduced ferritin levels were observed in younger participants and occurred more frequently in

Table I. Clinical characteristics, basic laboratory parameters, hematological parameters and iron metabolism parameters in RA patients and healthy controls.

Variable	RA patients, n = 62	Control group, n = 58	p
Age [years]	52 ± 15	56 ± 9	0.07
Sex, male, n (%)	10 (16)	35 (60)	< 0.001
BMI - body mass index [kg/m^2]	25.5 ± 4.6	27.3 ± 4.5	< 0.05
Hypertension, n (%)	20 (32)	0	–
Diabetes mellitus, n (%)	4 (6)	0	–
Atrial fibrillation, n (%)	1 (2)	0	–
Ischemic heart disease, n (%)	3 (5)	0	–
Asthma, n (%)	4 (6)	0	–
Triglycerides [mg/dL]	97 ± 40	184 ± 72	< 0.0001
GFR – glomerular filtration rate [mL/min/1.73 m^2]	95 ± 19	87 ± 23	< 0.05
Uric acid [mg/dL]	4.8 ± 1.3	5.7 ± 1.1	< 0.001
CRP – C-reactive protein [mg/L]	3.5 (1.3-17.1)	0.8 (0.5-1.3)	< 0.0001
Platelet count [G/L]	294 ±90	246 ± 67	< 0.01
Hemoglobin [g/dL]	13.3 ± 1.3	14.3 ± 1.2	< 0.001
Anemia[$], n (%)	11 (18)	3 (5)	< 0.05
RBC [T/L]	4.5 ± 0.4	4.8 ± 0.4	< 0.001
Hematocrit [%]	40.4 ± 5.7	42.2 ± 3.2	< 0.001
MCV [fL]	90.7 ± 5.9	88.4 ± 3.9	< 0.05
MCH < 26 pg (f), < 27 pg (m), n (%)	4 (6)	0	< 0.05
MCHC [g/dL]	32.3 ± 1.2	33.9 ± 1.1	< 0.001
RDW [%]	14.5 ± 1.3	12.9 ± 0.6	< 0.001
Vitamin B12 < 200 pg/mL, n (%)	2 (3)	–	–
Folic acid < 3 ng/mL, n (%)	1 (2)	–	–
TSAT < 20 %, n (%)	26 (43)	3 (5)	< 0.001
Ferritin < 30 µg/L, n (%)	9 (15)	4 (7)	0.17
Hepcidin < 1 4.5 ng/mL, n (%)	32 (56)	1 (2)	< 0.0001
sTfR > 1.59 mg/L, n (%)	16 (26.2)	0	< 0.0001
Iron deficiency[¥], n (%)	39 (64)	–	–

[$]Anemia: hemoglobin < 12 g/dL for women and < 13 g/dL for men. ¥Iron deficiency: ferritin < 100 µg/L or ferritin 100-299 µg/L and TSAT < 20%. RA – rheumatoid arthritis; RBC – red blood cells – erythrocytes; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin/mean cell hemoglobin; MCHC – mean corpuscular hemoglobin concentration; RDW – red cell distribution width; PHRC – percentage of circulating hypochromic red blood cells; CHR – content of hemoglobin in reticulocytes; TSAT – transferrin saturation; sTfR – soluble transferrin receptor.

women, participants without hypertension, participants without diabetes, participants with lower triglyceride levels, a higher GFR (glomerular filtration rate), lower GGT levels, lower uric acid levels and higher platelet counts. No correlations were found between ferritin levels and parameters

Table II. Laboratory parameters for RA and treatment used in RA patients.

Variable	RA patients, n = 62
RF, [IU/mL]	25.2 (9.75-75.5)
RF > 16 IU/mL, n (%)	32 (55)
Anti-CCP antibodies [IU/mL]	260 (15-5000)
Anti-CCP antibodies > 17 IU/mL, n (%)	44 (75)
DAS-28 [score]	6.5 (6.3-6.8)
DAS-28 > 5.1, n (%)	60 (91)
Period from diagnosis of RA [years]	5.5 (3.0 - 10.0)
Sulfasalazine, n (%)	6 (10)
Sulfasalazine, daily dose [mg]	2500 (2000-3000)
Methotrexate, n (%)	38 (77)
Methotrexate, weekly dose [mg]	25 (20-25)
Glucocorticoids, n (%)	26 (42)
Glucocorticoids, daily dose& [mg]	5 (5.0 - 7.5)

[&]Glucocorticoids: dose of steroids expressed as a prednisone-equivalent dose. RA – rheumatoid arthritis; RF – rheumatoid factor; Anti-CCP antibodies – anti-cyclic citrullinated peptide antibodies; DAS-28 – disease activity score.

describing disease activity and the treatment used in RA patients (Table III). The multi-factor model describing relationships between ferritin levels and the clinical and laboratory parameters in RA patients revealed correlations between reduced ferritin levels and younger patients, female, and/or having lower GGT levels and increased platelet counts (Table IV).

The assessment of correlations between transferrin saturation and the parameters studied showed that TSAT values were lower in participants with a higher BMI and elevated CRP levels while they were higher in participants with elevated bilirubin and AST levels. No correlations were found between TSAT values and parameters describing disease activity and the treatment being used for RA patients. The multi-factor model describing the relationships between TSAT values and the clinical and laboratory parameters in RA patients revealed correlations between reduced TSAT values and reduced hemoglobin levels, as well as lower AST levels, higher BMI values and higher CRP levels.

The assessment of correlations between hepcidin levels and the selected parameters showed that lower hepcidin levels correlated with younger patients, lower triglyceride levels, lower uric acid levels, and lower CRP and IL-6 levels. A correlation was found between elevated hepcidin levels and parameters describing disease activity (DAS-28 > 5.1 and higher anti-CCP antibody levels) and the treatment being used for RA patients. A correlation was also found between elevated hepcidin levels and a higher daily dose of glucocorticoids. The multi-factor model describing the relationships between hepcidin levels and clinical and laboratory parameters in RA patients revealed correlations between lower hepcidin levels and lower triglyceride levels, lower uric acid levels and lower anti-CCP antibody levels.

The analysis of correlations between soluble transferrin receptor levels and the parameters studied showed that higher sTfR levels correlated with elevated CRP levels, higher platelet counts and the use of glucocorticoids in RA patients. The multi-factor model describing the relationships between sTfR levels and clinical and laboratory parameters in RA patients revealed correlations between increased sTfR levels and reduced hemoglobin levels.

The assessment of correlations between hemoglobin levels and the selected parameters showed that reduced hemoglobin levels were more frequently found in female participants, patients suffering from heart failure, and those with lower triglyceride levels and lower uric acid levels. Elevated hemoglobin levels were correlated with higher CRP and IL-6 levels and higher platelet counts. No correlations were found between hemoglobin levels and parameters describing disease activity and the treatment being used for RA patients. The multi-factor model describing the relationships between hemoglobin levels and clinical and laboratory parameters in RA patients revealed correlations between reduced hemoglobin levels and reduced uric acid levels, increased IL-6 levels and female participants.

In single- and multi-factor models in RA patients with and without iron deficiency, the strongest correlations were found between iron deficiency and participant being younger and/or female, or having reduced hemoglobin levels, increased platelet counts, increased GFR, reduced GGT levels, lower disease activity (assessed by DAS-28) and using sulfasalazine less frequently (Table V).

Discussion

Surprisingly, despite the pathophysiological relationship between iron deficiency and anemia, as well as inflammation, data on iron metabolism in RA patients is quite enigmatic. The available studies focus only on anemia in the context of iron deficiency in RA patients[21,22] and do not take into account new, more specific, biomarkers (e.g., soluble transferrin receptor or hepcidin) in the assessment of iron metabolism[4-6]. It is also unclear whether anti-inflammatory treatment (e.g., blocking the IL-6 pathway) may, at least partly, normalise iron levels in RA patients.

It has been demonstrated that iron deficiency, defined on the basis of serum ferritin levels and transferrin saturation, is very common in RA patients both with and without anemia. It was also found that RA patients with iron deficiency have very low serum hepcidin levels, which indicates that those patients predominantly have absolute rather than functional (relative) iron deficiency, as one would expect.

To date, iron metabolism in RA patients has generally been analysed in the literature only in the context of anemia[2,10,18,19,23,24]. Anemia is commonly reported in RA patients and its level of prevalence is 33-60% according to various publications[2,10]. The current study demonstrated that only 18% of the RA patients who were anal-

Table III. Correlations between iron metabolism and hemoglobin parameters and clinical and laboratory parameters in RA patients – single-factor models.

Variables	Basic value	Analysed subgroups	Ferritin [µg/L]	TSAT [%]	Hepcidin [ng/mL]	sTfR [mg/L]	Haemoglobin [g/dL]
Age [years]	*r* *p*		0.38 < 0.01	0.04 0.79	0.38 < 0.01	0.001 1.00	-0.03 0.79
Sex, male, n (%)	Median (Q1-Q3) or mean ± SD median (Q1-Q3) or mean ± SD *p*	Male Women	189 (112-364) 74 (38-111) <0.001	30.7 ± 14.5 23.0 ± 13.4 0.11	22.7 (8.1-27.8) 12.9 (6.9-21.0) 0.17	1.43 (1.11-1.61) 1.36 (1.13-1.58) 0.82	14.2 ± 1.9 13.1±1.0 <0.05
BMI [kg/m²]	*r* *p*		-0.03 0.82	-0.2829 0.03	-0.01 0.97	0.19 0.14	0.08 0.54
Hypertension, n (%)	Median (Q1-Q3) or mean ± SD median (Q1-Q3) or mean±SD *p*	Yes No	149.0 (86.0-220.5) 72.0 (33.0-105.0) <0.001	24.8 ± 12.7 24.0 ± 14.3 1.0	18.5 (10.6-22.5) 12.7 (4.3-19.9) 0.09	1.41 (1.26- 1.81) 1.31 (1.10-1.58) 0.63	13.3 ± 1.1 13.3 ± 1.3 0.91
Diabetes, n (%)	Median (Q1-Q3) or mean±SD median (Q1-Q3) or mean±SD *p*	Yes No	188.5 (161.5-288.0) 82.0 (41.0-122.0) < 0.05	24.0 ± 7.8 24.3±14.1 0.97	17.5 (10.5-24.3) 12.9 (7.1-21.5) 0.54	1.26 (1.04-1.80) 1.36 (1.14-1.61) 0.89	13.2±1.0 13.3±1.3 0.95
Heart failure, n (%)	Median (Q1-Q3) or mean ± SD median (Q1-Q3) or mean±SD *p*	Yes No	100.0 (82.0-364.0) 88.5 (41.0-146.0) 0.30	20.7 ± 10.1 24.5 ± 13.9 0.65	12.8 (6.9-46.7) 13.9 (7.1-21.5) 0.53	1.91 (1.04-2.04) 1.36 (1.13-1.58) 0.38	11.8±0.5 3.3 ± 1.2 1 < 0.05
Triglycerides [mg/dL]	*r* *p*		0.41 < 0.01	0.05 0.70	0.27 < 0.05	-0.07 0.61	0.30 < 0.05
GFR [mL/min/ 1.73 m²]	r *p*		-0.29 < 0.05	-0.02 0.86	-0.19 0.17	-0.02 0.90	-0.06 0.66
Total bilirubin [mg/dL]	r *p*		0.07 0.59	0.36 < 0.01	0.01 0.94	0.01 0.97	0.18 0.16
AST [IU/L]	*r* *p*		0.14 0.29	0.29 < 0.05	0.12 0.37	-0.09 0.50	0.18 0.15
GGT [IU/L] [mg/dL]	*r* *p*		0.32 < 0.05	0.02 0.86	0.18 0.18	0.12 0.37	0.15 0.25
Uric acid [mg/dL]	*r* *p*		0.39 < 0.01	0.09 0.49	0.34 < 0.05	-0.22 0.10	0.32 < 0.05
CRP [mg/L]	*r* *p*		0.15 0.25	-0.33 < 0.01	0.30 < 0.05	0.27 < 0.05	-0.28 < 0.05
IL-6 [pg/mL]	*r* *p*		0.03 0.80	-0.06 0.65	0.29 < 0.05	0.19 0.15	-0.42 < 0.01
Platelet count [G/L]	r *p*		-0.36 < 0.01	-0.22 0.09	-0.05 0.74	0.27 <0.05	-0.27 <0.05
Anti-CCP antibodies [IU/mL]	*r* *p*		-0.01 0.94	0.10 0.45	0.28 <0.05	-0.07 0.60	-0.04 0.75
DAS-28 >5.1, n (%)*	Median (Q1-Q3) or mean±SD median (Q1-Q3) or mean±SD *p*	Yes No	128 (43-152) 100 (100-100) 0.85	24.5±13.9 16.7 ± 0 0.58	13.8 (7.1-21.5) 47.7 (47.7-47.7) <0.0001	1.36 (1.11-1.61) 1.22 (1.22-1.22) 0.85	13.3 ± 1.3 12.5 ± 0 0.52

RA – rheumatoid arthritis; BMI – body mass index; GFR – glomerular filtration rate; AST – aspartate aminotransferase; GGT – gamma-glutamyl transpeptidase; CRP – C-reactive protein; IL-6 – interleukin 6; Anti-CCP antibodies – anti-cyclic citrullinated peptide antibodies; DAS-28 – disease activity score. *In the case of the variable analysed, there were no patients with a given diagnosis or category in the group concerned.

Table IV. Correlations between iron metabolism and hemoglobin parameters and clinical and laboratory parameters in RA patients – multi-factor models.

Dependent variables	Independent variables	Standardised β	*p*	Adjusted R^2
Ferritin [1 log μg/L]	Age [years]	0.27	< 0.05	0.41
	Sex [male *vs* female]	0.37	< 0.01	
	GGT [log IU/L]	0.28	< 0.05	
	Platelet count [G/L]	-0.26	< 0.05	
TSAT [%]	BMI [kg/m²]	-0.27	< 0.05	0.29
	AST [log IU/L]	0.23	< 0.05	
	CRP [mg/L]	-0.24	< 0.05	
	Hemoglobin [g/dL]	0.28	< 0.05	
Hepcidin [1 log ng/dL]				
	Triglycerides [mg/dL]	0.29	< 0.05	0.32
	Uric acid [mg/dL]	0.33	< 0.05	
	Anti-CCP antibodies [log UI/L]	0.46	< 0.001	
sTfR [1 log mg/L]	Hemoglobin [kg/m^2]	-0.35	< 0.01	0.11
Hemoglobin [g/dL]	Uric acid [mg/dL]	0.26	< 0.05	0.29
	IL-6 [pg/mL]	-0.40	<0.01	
	Sex [male *vs* female]	-0.25	< 0.05	

TSAT – transferrin saturation; sTfR – soluble transferrin receptor; GGT – gamma-glutamyl transpeptidase; BMI – body mass index; AST – aspartate aminotransferase; CRP – C-reactive protein; Anti-CCP antibodies – anti-cyclic citrullinated peptide antibodies; IL-6 – interleukin 6.

ysed had anemia, whereas the prevalence of iron deficiency in those patients was over three times higher (64%). The pathomechanism of anemia in RA patients is complex and multifactorial and is linked to chronic inflammation and the deficiency/loss of particular components of erythropoiesis, including iron deficiency[10,25]. It was shown that anemia in RA patients leads to the underlying condition taking a more severe course and to there being more advanced damage to the joint structure[2,24,26].

Recently, a hypothesis has been proposed that the main factor leading to the development of anemia in the course of chronic conditions (e.g., chronic kidney disease, RA) is excessive inflammatory activation. This has been linked, among other things, to the production of proinflammatory cytokines, especially IL-6, which leads to an increase in the production of hepcidin in the liver[15,27,28]. Models of chronic conditions in which the activation of inflammation is an important pathophysiological feature have provided data suggesting that IL-6-mediated overproduction of hepcidin in the liver leads to the development of so-called functional (relative) iron deficiency. This means that there are iron stores in the body, but the element is unavailable for cell metabolic processes[4,29-31]. This phenomenon was reported in patients with chronic kidney disease; it was observed that patients with chronic kidney disease and iron deficiency have very high serum hepcidin levels[32]. It should be stressed that in the case of RA, these are only theoretical assumptions. It is still unclear to what extent excessive inflammatory activation (linked to the overexpression of IL-6 and hepcidin) observed in RA patients leads to functional (relative) iron deficiency and to what extent iron deficiency in RA patients constitutes absolute iron deficiency (as may be indicated by reduced levels of serum hepcidin). It should be noted that while it has theoretically been suggested that iron deficiency in patients with heart failure is primarily functional (relative), this has not yet been confirmed. However, there is data indicating that patients with iron deficiency have reduced (rather than increased) serum hepcidin levels. This was observed in patients with stable heart failure, as well as those with acute heart failure. Also, there is no correlation between increased IL-6 levels and increased serum hepcidin levels in this group of patients[33,34].

In the RA patients who took part in this study, iron metabolism was analysed using classic parameters that have been in use for many years now (serum ferritin levels and transferrin saturation) and new biomarkers (serum soluble transferrin receptor and hepcidin levels)[23,35,36]. There are numerous different definitions of iron deficiency in the literature. Given the practical implications (relating to e.g., the qualification of patients for iron supplementation treatment), the definition of

Table V. Comparison of the clinical characteristics and basic laboratory parameters in patients with and without iron deficiency and determinants of iron deficiency (single- and multi-factor models) in RA patients.

Variable	RA patients without ID, n = 22	RA patients with ID, n = 39	*p*	Category/unit	Single-factor model				Multi-factor model			
					OR	± 95% CI	χ^2	*p*	OR	± 95% CI	χ^2	*p*
Age [years]	57 ± 14	49 ± 16	0.05	5 years	0.83	0.69-1.01	3.54	0.06	0.95	0.91-1.00	4.31	0.04
Sex, male, n (%)	10 (45)	0	< 0.001	£	-	-	-	-	-	-	-	-
GFR [mL/min/1.73 m^2]	89 ± 19	99 ± 18	< 0.05	10 mL/min/1.73 m^2	1.4	1.01-1.93	4.27	0.04	-	-	-	-
GGT [IU/L]	26 (21-43)	20 (18-29)	< 0.05	1 log IU/L	0.31	0.09-1.04	3.74	0.05	-	-	-	-
Platelet count [G/L]	259 ± 67	316 ± 96	< 0.05	10 g/L	1.12	1.02-1.23	5.47	0.02	-	-	-	-
Hemoglobin [g/dL]	13.7 ± 1.5	13.0 ± 1.0	< 0.05	g/dL	0.63	0.39-1.01	3.83	0.05	0.54	0.31-0.95	4.82	0.03
DAS-28 [score]	6.7 (6.5-6.8)	6.5 (5.8-6.8)	< 0.05	1 point	0.22	0.06-0.84	5.08	0.03	0.25	0.06-0.96	4.23	0.04
Sulfasalazine, n (%)	5 (23)	1 (3)	< 0.05	yes *vs.* no	0.09	0.01-0.86	4.53	0.03	-	-	-	-

£ - it is impossible to build a logistic model due to 0 or 1 in one of the categories analysed. RA – rheumatoid arthritis; ID – iron deficiency; GFR – glomerular filtration rate; GGT – gamma-glutamyl transpeptidase; DAS-28 – disease activity score.

iron deficiency used in the case of RA patients participating in this study was the one that is used in patients with chronic conditions, such as heart failure and chronic kidney disease[6]. Iron deficiency was found in 64% of RA patients, while for women it was 76%. Thus, iron deficiency was common among RA patients and its prevalence was several times higher than the prevalence of anemia. It needs to be stressed that the prevalence of iron deficiency in RA patients with anemia was similar to the prevalence of iron deficiency in RA patients without anemia (66% and 55%, respectively). The study clearly indicates that the scale of the iron deficiency problem is significantly greater compared to that of anemia. Therefore, it is surprising that iron deficiency has, until now, not been taken into account in the clinical assessment of RA patients.

However, it is obvious that iron metabolism is also associated with the effectiveness of erythropoiesis in patients with chronic conditions[37]. In the current study, correlations were found between reduced hemoglobin levels as well as reduced red blood cell parameters (MCV – mean cell volume, MCH – mean cell hemoglobin, CHR – reticulocyte hemoglobin content) and parameters describing iron metabolism (reduced serum ferritin and hepcidin levels, increased serum sTfR levels and reduced transferrin saturation) in the RA patients being studied. In the multi-factor model, reduced hemoglobin levels and increased DAS-28 scores were the two strongest determinants of iron deficiency in RA patients. Our study shows that, as expected, there is a correlation between iron metabolism and erythropoiesis in RA patients. However, iron deficiency is also common in RA patients displaying parameters indicative of normal erythropoiesis. This clinically important observation proves that normal hemoglobin levels do not preclude iron deficiency. Thus, in order to diagnose iron deficiency in an RA patient, iron metabolism parameters should be assessed in addition to hemoglobin levels and other blood cell parameters.

There was a slight discrepancy in our study. Iron deficiency was more prevalent in patients with RA than in the control subjects, but lower disease activity was a significant risk factor for iron deficiency in patients with RA. Iron supplementation is probably more often administered in the advanced stages of RA. Therefore, iron deficiency is less severe. And conversely, iron supplementation is probably less often administered in the early stages of RA.

In the current study, RA patients had lower serum ferritin levels compared with the healthy controls. This was the case even though inflammatory activation in RA patients was higher compared with the healthy controls, which may indirectly indicate the presence of a large absolute iron deficiency independent of a concurrent inflammatory process. Similar differences were also observed as regards serum hepcidin levels. RA patients, regardless of the presence of iron deficiency, had lower serum hepcidin levels compared with the healthy controls. Moreover, RA patients with iron deficiency had lower serum hepcidin levels than RA patients without iron deficiency. No clear correlations were found between iron metabolism parameters and inflammation parameters (CRP, IL-6) in RA patients. The above data does not confirm that there is a link between inflammatory activation and the pathogenesis of iron deficiency in RA patients, or at least it appears that inflammatory activation does not play a dominant role in the pathogenesis of iron deficiency in RA patients.

Limitations of the Study

We acknowledge some limitations to this study. The study group was small and was based on a single-center analysis. The power analysis for sample size calculation was not done. Future randomised and prospective studies should be performed in order to evaluate the iron deficiency in RA patients.

The novelty of this study is the attempt to explain iron deficiency in RA patients by means of new and specific biomarkers (e.g., serum soluble transferrin receptor or hepcidin). The analysis of the relationship between anemia in the course of chronic conditions and excessive inflammatory activation was carried out. It was stressed that the range of iron deficiency was not only a question of anemia. A correlation was proved between iron metabolism and erythropoiesis in RA patients.

Conclusions

1. Iron deficiency is common in RA patients with high disease activity (DAS-28 > 5.1). It is present in 64% of these patients. Iron deficiency is comparably common in RA patients both with and without anemia (66% *vs.* 55%).
2. RA patients had lower transferrin saturation, lower ferritin and hepcidin levels and higher

serum sTfR levels. RA patients with iron deficiency had lower serum hepcidin levels compared with RA patients without iron deficiency.

3. In RA patients, correlations were found between reduced hemoglobin levels as well as reduced red blood cell parameters (MCV, MCH, CHR) and parameters describing iron metabolism (reduced serum ferritin and hepcidin levels, increased serum sTfR levels and reduced transferrin saturation).
4. Decreased DAS-28 scores and reduced hemoglobin levels were the two strongest determinants of iron deficiency in RA patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval and Consent to Participate

The study was approved by the Commission of Bioethics at the Military Institute of Medicine in Warsaw (No 34/WIM/2015).

Consent for Publication

Written informed consent in Polish for the publication of this paper was obtained from all the patients.

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Authors' Contribution

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Authors' Contribution

WT wrote the manuscript. MC helped to draft the manuscript. BJP participated in the design of the study and arranged the manuscript. EAJ participated in the design and coordination of the paper. All the authors read and approved the final manuscript.

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