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Original Article

An erythrocyte macrocytosis by methotrexate is associated with early initiation of biologic or targeted synthetic agents in patients with rheumatoid arthritis

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Objective: An association between increased erythrocyte mean corpuscular volume (MCV) and treatment response in patients with inflammatory arthritis receiving methotrexate (MTX) has been reported. We investigated the frequency of red blood cell (RBC) macrocytosis and its clinical implications regarding the initiation of biological or targeted synthetic disease-modifying anti-rheumatic drugs (b/tsDMARDs) in patients starting MTX for rheumatoid arthritis (RA).

Methods: RBC macrocytosis (MCV >100 fL) and clinical characteristics were retrospectively examined in 1,156 patients starting MTX for RA. Multivariable logistic regression analyses were performed to identify the independent predictors of RBC macrocytosis. The initiation of b/tsDMARDs was assessed using a multivariable Cox proportional hazards regression model.

Results: RBC macrocytosis was observed in 21.6% of RA patients over 35 [8, 89] months following MTX initiation and was persistent in 63.6% of the patients during MTX treatment. Anemia coexisted in only 20.0% of the patients with RBC macrocytosis. The occurrence of RBC macrocytosis was independently associated with age, MTX dose, and concomitant use of sulfasalazine or leflunomide (all p<0.001). A higher dose of MTX and double- or triple-DMARDs therapy were more frequently used in the group with RBC macrocytosis than in the group with normal MCV. Patients experiencing RBC macrocytosis were more likely to use b/tsDMARDs (hazard ratio: 1.45 [95% confidence interval: 1.13, 1.87], p=0.003).

Conclusion: RBC macrocytosis was possibly associated with the use of b/tsDMARD and could be a supplementary marker for assessing MTX resistance.

Keywords: Rheumatoid arthritis, Methotrexate, Erythrocyte, Erythrocyte indices

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by synovial inflammation and hyperplasia, autoantibody production, and joint damage, which can lead to structural deformation and irreversible functional impairments [1]. The introduction of various biological disease-modifying anti-rheumatic drugs (bDMARDs), including tumor necrosis factor inhibitors and interleukin-6 inhibitors, and targeted synthetic disease-modifying anti-rheumatic drugs (tsDMARDs), such as Janus kinase inhibitors, has changed the paradigm of RA treatment. However, methotrexate (MTX) remains the mainstay as the initiating and anchor drug for the treatment of RA, considering its effectiveness, low cost, and affordable safety profile [2,3]. MTX monotherapy has been shown to produce significant therapeutic effects in MTX-naïve patients

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with RA, although they are weaker than those of the combination of MTX with bDMARD or tsDMARD [1,4]. In a review of randomized controlled trials, remission rates less than 50% were obtained with MTX monotherapy, while high remission rates up to 86% were achieved with combination therapy of MTX and bDMARDs or tsDMARDs [4]. The treat-to-target strategy emphasizes early recognition of the disease and proactive titration of treatment to prevent joint damage [5]. Therefore, it is meaningful to discern between subsets of good and poor responders to MTX therapy in clinical practice [3].

The discontinuation of MTX therapy is mainly attributed to toxicity rather than inefficacy, and toxicity can develop despite folic acid supplementation [6,7]. The major adverse effects of MTX include gastrointestinal troubles, hepatotoxicity, pneumonitis, cytopenia, and alopecia [6,7]. Cytopenia is a rare but serious hematologic manifestation and was reported to account for about 5% of adverse effect leading to MTX discontinuation [6,7]. A minor but interesting hematologic finding of MTX treatment is the change of erythrocyte mean corpuscular volume (MCV). An increase in MCV was suggested as a harbinger of hematologic toxicity or a predictor of treatment response in patients with inflammatory arthritis [8-10]. However, the former study included only six patients who developed hematologic toxicity among 23 RA patients receiving MTX [8]. In addition, the cut-off point for MCV increase in later studies was only 3.5 or 3.9 fL at 6 months of MTX therapy, and the differentials of MCV were within the normal range, i.e., 80 to 100 fL [9,10].

A complete blood count is a primary item of routine laboratory monitoring in RA management, and macrocytosis of red blood cells (RBC) (MCV >100 fL) is a readily discernible change. A subset of RA patients with RBC macrocytosis under MTX treatment has not been fully characterized. A particular interesting topic is the clinical implication of RBC macrocytosis in terms of treatment outcome during long-term use of MTX. To address this, we investigated the frequency, duration, and associated factors of RBC macrocytosis in patients with RA commencing MTX-based conventional DMARD (cDMARD) therapy. Moreover, we examined its relationship with regard to the initiation of bDMARD or tsDMARD.

MATERIALS AND METHODS

Patients

A total of 2,211 RA patients who fulfilled the 2010 RA classification criteria [11] and had received care at The Catholic University of Korea, St. Vincent's Hospital between 2000 and 2023 were included in the study. Clinical and laboratory data as well as radiographic images, were retrieved from the medical records of the patients. Patients with known folate or B12 deficiency, renal insufficiency, and active diseases including thyroid dysfunction, infection, cancer, hematologic disease, lung disease, ischemic heart disease, and heart failure were excluded due to the potential effects on MCV. Older patients with onset age >65 years were excluded because of the high prevalence of anemia of other causes, diverse comorbid conditions, and vulnerability to diverse anemia-precipitating factors [12]. Patients with severe anemia (hemoglobin <10.0 g/dL) and MCV >100 fL at baseline were also excluded. Hematologic changes probably due to gastrointestinal bleeding, fracture, operation, dental procedures, vaccination, and malnutrition were excluded from evaluation. Significant MCV elevation was defined if the MCV elevation was detected on at least two consecutive tests and was not explainable by the discernible medical conditions. Some patients showed single-time, temporary MCV elevation, but we did not count them as having significant MCV elevation. Finally, 1,156 patients with RA initiating MTX-based DMARDs therapy were included, and all patients used folic acid as a supplemental medicine. We defined the last follow-up date as the last laboratory date just before MTX discontinuation if MTX discontinued and as the last follow-up laboratory date if MTX continued. The total follow-up duration was 82 [42, 136] months, and the laboratory test interval was 2.79 [1.47, 5.26] months. The study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of The Catholic University of Korea, St. Vincent's Hospital (IRB no. VC24RISI0069).

Laboratory tests

MCV is calculated by dividing the hematocrit by the RBC counts and ranged from 80 to 100 fL. RBC macrocytosis was defined as MCV greater than 100 fL. Anti-citrullinated peptide antibody (ACPA) was detected using a chemiluminescent microparticle immunoassay (Abbott Laboratories, Abbott park, IL, USA), and a positive reading was defined as an ACPA level greater than 5 U/mL. The maximum limit of the APCA assay was 200 U/mL. The rheumatoid factor (RF) titers were measured using the latex agglutination test (Beckman Coulter, Brea, CA, USA) with a cutoff value of 14 U/mL.

Table 1. Baseline characteristics of the study subjects by RBC macrocytosis

	All (n=1,156)	RBC		p-value
		Macrocytosis (n=250)	Normal (n=906)	
Female	917 (79.3)	181 (72.4)	736 (81.2)	0.003
Age at diagnosis (yr)	49 [42, 56]	52 [45, 58]	48 [41, 55]	<0.001
BMI				0.613
Underweight	70 (6.1)	17 (6.8)	53 (5.8)	
Normal	779 (67.4)	173 (69.2)	606 (66.9)	
Overweight	243 (21.0)	50 (20.0)	193 (21.3)	
Obese	64 (5.5)	10 (4.0)	54 (6.0)	
Smoking*	228 (19.7)	65 (26.0)	163 (18.0)	0.006
Diabetes	126 (10.9)	38 (15.2)	88 (9.7)	0.019
Hypertension	331 (28.6)	84 (33.6)	247 (27.3)	0.060
Dyslipidemia	542 (46.9)	138 (55.2)	404 (44.6)	0.004
ESR (mm/h)	38 [24, 60]	41 [26, 64]	38 [23, 59]	0.126
CRP (mg/dL)	0.54 [0.15, 1.71]	0.6 [0.2, 2.0]	0.5 [0.1, 1.6]	0.129
IgM RF				
Positive	945 (81.7)	203 (81.2)	742 (81.9)	0.872
Titer (IU/mL)	54.3 [21.6, 144.0]	57.2 [21.6, 169.0]	53.8 [21.6, 139.5]	0.373
ACPA				
Positive	972 (84.1)	205 (82.0)	767 (84.7)	0.358
Titer (IU/mL)	82.7 [18.1, 200.0]	64.2 [10.8, 172.7]	91.1 [21.4, 200.0]	0.040
SHS (units)				0.459
Erosion	189 (16.5)	43 (17.3)	146 (16.3)	0.793
DAS28-CRP (units)	2.92 [2.47, 3.35]	2.9 [2.4, 3.4]	2.9 [2.5, 3.3]	0.907
cDMARDs at baseline				
MTX monotherapy	173 (15.0)	45 (18.0)	128 (14.1)	0.156
MTX dose (mg)	10.0 [7.5, 10.0]	10.0 [7.5, 10.0]	10.0 [7.5, 10.0]	0.368
HCQ	794 (68.7)	165 (66.0)	629 (69.4)	>0.99
SSZ	371 (32.1)	69 (27.6)	302 (33.3)	0.339
LFNM	196 (17.0)	40 (16.0)	156 (17.2)	0.719
cDMARDs at RBC macrocytosis				
MTX monotherapy	418 (36.2)	64 (25.6)	354 (39.1)	<0.001
MTX dose (mg)	10.0 [7.5, 10.0]	10.0 [10.0, 12.5]	10.0 [7.5, 10.0]	<0.001
HCQ	455 (39.4)	98 (39.2)	357 (39.4)	>0.99
SSZ	124 (10.7)	42 (16.8)	82 (9.1)	0.001
LFNM	382 (33.0)	111 (44.4)	271 (29.9)	<0.001
TCLM	4 (0.3)	0 (0.0)	4 (0.4)	0.657
bDMARDs or tsDMARDs†	328 (28.4)	92 (36.8)	236 (26.0)	0.001

Values are presented as number (%) or median [interquartile range]. ACPA: anti-citrullinated peptide antibody, bDMARD: biological disease-modifying anti-rheumatic drugs, BMI: body mass index, CRP: C-reactive protein, cDMARD: conventional disease-modifying anti-rheumatic drugs, ESR: erythrocyte sedimentation rate, HCQ: hydroxychloroquine, MTX: methotrexate, LFNM: leflunomide, RBC: red blood cell, RF: rheumatoid factor, SHS: van der Heijde modified Sharp score, SSZ: sulfasalazine, TCLM: tacrolimus, tsDMARD: targeted synthetic disease-modifying anti-rheumatic drugs, DAS28: Disease Activity Score 28-joints, IgM: immunoglobulin M. *Include ex- and current smokers. †Biologic DMARDs (bDMARDs) include tumor necrosis factor inhibitors (etanercept, adalimumab, infliximab, golimumab), tocilizumab, abatacept, and rituximab. Targeted synthetic DMARDs (tsDMARDs) include tofacitinib, baricitinib, and upadacitinib.

Statistical analyses

For data with continuous distribution, the results were presented as medians with interquartile ranges. Between-group comparisons were performed using Student's t-test or Mann–Whitney U-test. Categorical or dichotomous variables were expressed as frequencies and percentages and were compared using the chi-square test or Fisher's exact test. Multivariate logistic regression model was used to identify the independent predictors of RBC macrocytosis. The odds ratio (OR) and corresponding 95% confidence interval (CI) were presented. The initiation of bDMARDs or tsDMARDs was assessed via a Kaplan–Meier analysis and was compared using log-rank tests. To identify significant predictors of bDMARD or tsDMARD initiation, a multivariable Cox proportional hazards regression model with hazard ratio (HR) and corresponding 95% CIs was conducted using clinically relevant variables. A two-sided $p<0.05$ was considered statistically significant. All statistical analyses were performed using the R platform (version 4.3.1; The R Project for Statistical Computing, www.r-project.org).

RESULTS

Characteristics of the study population

In total, 250 (21.6%) patients were identified to have RBC macrocytosis during MTX treatment. The baseline demographics and disease characteristics comparing groups of patients with RBC macrocytosis and with normal MCV are summarized in Table 1. The proportion of females in the group with RBC macrocytosis was smaller than that with normal MCV (72.4% vs. 81.2%, p=0.003) and the median age at disease diagnosis was higher in the group with RBC macrocytosis (52 [45, 58] vs. 48 [41, 55] years, p<0.001). Smoking, diabetes, and dyslipidemia were more frequent in the group with RBC macrocytosis (all p<0.05).

The positive rate of RF and ACPA did not differ between the two groups. However, the median ACPA titer in the group with RBC macrocytosis was lower (64.2 [10.8, 172.7] vs. 91.1 [21.4, 200.0] U/mL, p=0.018) than that in patients with normal MCV. The median RF titer was comparable between the two groups (57.2 [21.6, 169.0] vs. 53.8 [21.6, 139.5] IU/mL, p=0.373). There was no significant difference in erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels, radiographic joint damage, or Disease Activity Score 28-joints (DAS28)-CRP at baseline between the two groups.

At baseline, 173 patients (15.0%) began with MTX monotherapy, and 983 patients (85.0%) began with MTX-based double or triple DMARDs therapy. There was no significant difference in numbers or frequencies in the use of initial DMARDs between the two groups. However, at the first time of RBC macrocytosis, the rate of MTX monotherapy was increased compared with that at baseline but lower than that in the group with normal MCV at the last follow-up (25.6% vs. 39.1%, p<0.001). The dose of MTX in a group with RBC macrocytosis was significantly higher than that in the group with normal MCV (10.0 [10.0, 12.5] vs. 10.0 [7.5, 10.0] mg, p<0.001), although the differential was small (about 2.5 mg). The rates of combination with sulfasalazine and leflunomide were significantly higher in the group with RBC macrocytosis (16.8% vs. 9.1%, p=0.001 and 44.4% vs. 29.9%, p<0.001, respectively). Initiation of bDMARDs or tsDMARDs is an option if the disease activity is not appropri-

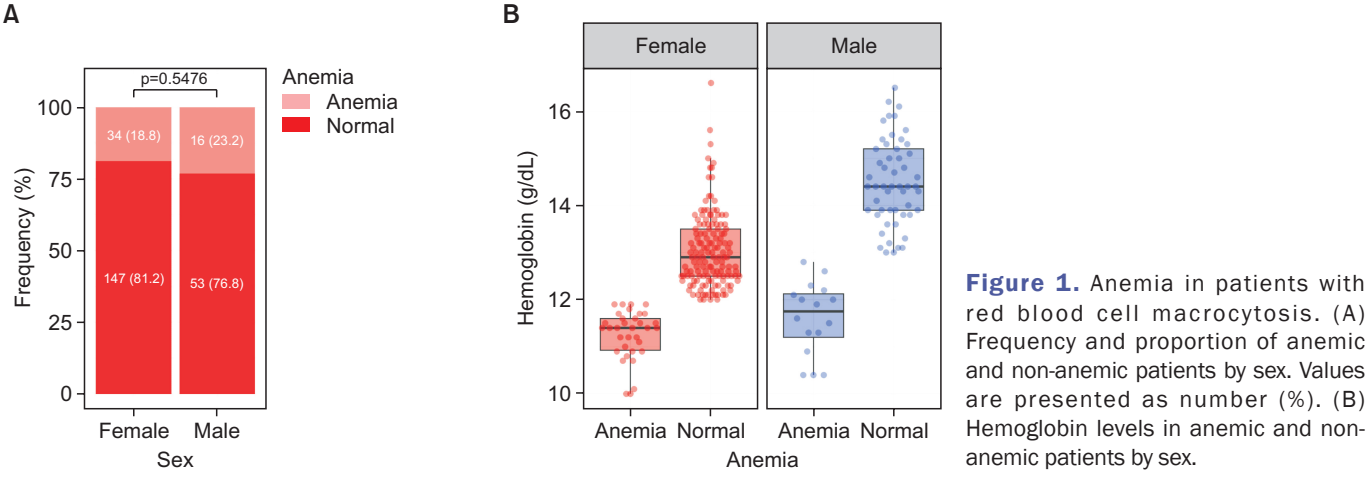

Figure 1. Anemia in patients with red blood cell macrocytosis. (A) Frequency and proportion of anemic and non-anemic patients by sex. Values are presented as number (%). (B) Hemoglobin levels in anemic and non-anemic patients by sex.

ately controlled despite MTX-based multiple combination cDMARDs therapy for longer than 6 months [2]. The rate of use of bDMARDs or tsDMARDs at follow-up was higher in the group with RBC macrocytosis (36.8% vs. 26.0%, p=0.001).

Development of erythrocyte macrocytosis and association with anemia

Baseline MCV of the patients with RBC macrocytosis and normal MCV was 91.6 [88.9, 93.8] and 91.2 [88.2, 93.6] fL (p=0.341), respectively. In patients with RBC macrocytosis, the median time of development was 35 [8, 89] months after initiation of MTX. Female patients occupied 72.4% (n=181) of the group with RBC macrocytosis. Anemia was comorbid in 20.0% of all patients (female: 18.8%, male: 23.2%) (Figure 1A). The mean hemoglobin levels in the anemic patients were 11.3 g/dL in females and 11.6 g/dL in males, and there was no case of severe anemia (hemoglobin <10 g/dL) (Figure 1B). RBC macrocytosis was persistent in 63.6% (n=159) of patients during MTX-based therapy, and the median duration at the last laboratory follow-up was 37 [2, 72] months. RBC macrocytosis was temporary in 36.4% (n=91) of patients and resolved within 13 [3, 19] months.

Independent risk factors for development of red blood cell macrocytosis

To identify the risk factors of RBC macrocytosis in the study population, we performed a multivariate logistic regression analysis (Figure 2A). Variables including male sex, age at diagnosis, diabetes, hypertension, smoking, ESR, CRP, DAS28-CRP, RF positive, ACPA positive, MTX dose, concomitant use of sulfasalazine, and concomitant use of leflunomide were included into the model. Age at diagnosis, MTX dose, and concomitant use of sulfasalazine or leflunomide were found to be independently associated with RBC macrocytosis development (all p<0.05). Patients taking MTX 10.0 and 12.5 mg occupied the largest (56.0%, n=140) and second (25.2%, n=63) largest proportions of the group with RBC macrocytosis, while MTX 7.5 and 10.0 mg were the most frequent prescriptions in the group with normal MCV ($p<2.996\times10^{-7}$) (Figure 2B).

Likelihood of initiation of bDMARDs or tsDMARDs

The lack of early response to MTX-based treatment and the initiation of b/tsDMARD are surrogate indexes of poor long-term outcomes [2,13]. The cumulative incidence of b/tsDMARD initiation was compared between patients with and without RBC macrocytosis over 20 years (Figure 3A). The risk of b/tsDMARD initiation was significantly different between the two groups (log-rank test, p=0.021). The HR for initiation of b/tsDMARDs was calculated using Cox regression and adjusted for sex, age at diagnosis, body weight, diabetes, hypertension, smoking status, ESR, CRP, DAS28-CRP, RF positivity, ACPA positivity, and erosive change at baseline. RBC macrocytosis (HR: 1.45 [95% CI: 1.13, 1.87], p=0.003) and DAS28-CRP (HR: 1.28 [95% CI: 1.05, 1.56], p=0.004) were independently associated with initiation of b/tsDMARDs (Figure 3B). Excluding the patients concomitantly taking sulfasalazine or leflunomide,

A

Variable	Crude OR	Adjusted OR	p-value
Male sex	1.65 (1.19, 2.28)	1.10 (0.71, 1.69)	0.669
Age at diagnosis	1.04 (1.02, 1.06)	1.04 (1.02, 1.06)	<0.001
Diabetes	1.66 (1.10, 2.51)	1.31 (0.84, 2.05)	0.242
Hypertension	1.35 (1.00, 1.82)	1.06 (0.76, 1.47)	0.739
Smoking	1.60 (1.15, 2.23)	1.18 (0.77, 1.82)	0.454
ESR	1.0038 (0.9989, 1.0087)	1.0019 (0.9958, 1.0079)	0.548
CRP	0.9991 (0.9830, 1.0155)	0.9953 (0.9646, 1.0266)	0.718
DAS28-CRP	1.04 (0.84, 1.30)	0.89 (0.69, 1.17)	0.411
RF positive	0.95 (0.66, 1.36)	1.13 (0.71, 1.79)	0.609
ACPA positive	0.82 (0.56, 1.18)	0.69 (0.43, 1.10)	0.120
MTX dose	1.18 (1.10, 1.26)	1.20 (1.11, 1.29)	<0.001
SSZ	2.03 (1.36, 3.04)	2.69 (1.73, 4.16)	<0.001
LFNM	1.86 (1.39, 2.48)	1.87 (1.36, 2.57)	<0.001

B

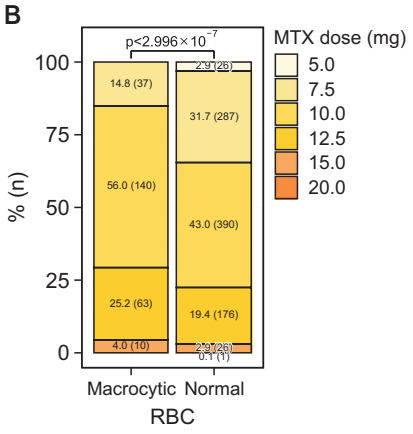

Figure 2. Factors associated with red blood cell macrocytosis. (A) Multivariate linear regression analysis for development of RBC macrocytosis. (B) Distribution of patients with RBC macrocytosis and normal MCV by MTX dose. ACPA: anti-citrullinated peptide antibody, CRP: C-reactive protein, DAS28: Disease Activity Score 28-joints, ESR: erythrocyte sedimentation rate, LFNM: leflunomide, MTX: methotrexate, OR: odds ratio, RBC: red blood cell, RF: rheumatoid factor, SSZ: sulfasalazine, MCV: mean corpuscular volume.

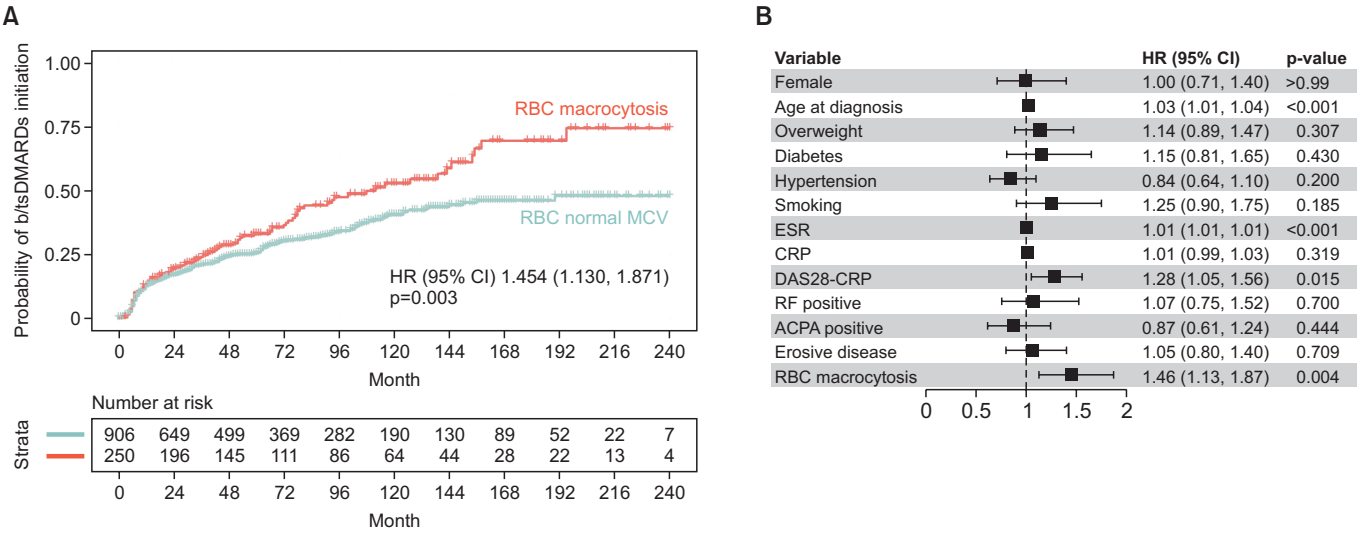

Figure 3. Cumulative incidence of the initiation of biologic or targeted synthetic DMARDs by the presence of RBC macrocytosis. (A) Kaplan–Meyer plot for probability of b/tsDMARDs initiation by RBC macrocytosis. (B) Forest plot of HRs of b/tsDMARD initiation. ACPA: anti-citrullinated peptide antibody, CRP: C-reactive protein, DAS28: Disease Activity Score 28-joints, ESR: erythrocyte sedimentation rate, HR: hazard ratio, CI: confidence interval, LFNM: leflunomide, MTX: methotrexate, RBC: red blood cell, RF: rheumatoid factor, SSZ: sulfasalazine, b/tsDMARDs: biological or targeted synthetic disease-modifying anti-rheumatic drugs, MCV: mean corpuscular volume.

patients with RBC macrocytosis (n=101) and normal MCV (n=556) remained. The rate of use of b/tsDMARDs at follow-up was higher in the group with RBC macrocytosis (44.6% [n=45] vs. 32.9% [n=183], p=0.032). b/tsDMARDs initiated after development of RBC macrocytosis in 57 patients (62.0%), while RBC macrocytosis was observed after b/tsDMARDs therapy in 35 patients (38.0%). All patients were on MTX during the follow-up period. The median interval between development of RBC macrocytosis and initiation of b/tsDMARDs was 21 [–10, 67] months.

DISCUSSION

In the present study, RBC macrocytosis was observed in approximately one-fifth of RA patients using MTX, and it varied in onset time following MTX initiation and duration. The development of RBC macrocytosis was associated with age, MTX dose, and concomitant use of sulfasalazine or leflunomide. Patients experiencing RBC macrocytosis had a higher risk for initiation of b/tsDMARDs.

MTX is a folate antagonist that inhibits purine synthesis and competes with folate for cellular uptake via the folate transporter [3,14]. MTX is converted into polyglutamated MTX, an active form, within cells. MTX polyglutamation occurs in erythrocytes as well as T lymphocytes [3,14]. In one study, a low baseline folate level was associated with a poor response to MTX at a 3-month follow-up [15]. In addition, erythrocyte folate levels were positively correlated with erythrocyte MTX levels [16]. This indicated that physiologic or metabolic changes in RBC can be caused by MTX even with folic acid supplementation and might be linked with clinical status in patients with RA receiving MTX.

In recent studies, an accelerated increase in MCV at 6-month follow-up after commencing MTX therapy was associated with improved clinical outcomes in patients with RA and psoriatic arthritis [9,10]. In the early stage (i.e., immediately after initiation of MTX therapy) active transport of MTX into cells and relative depletion of folate can effectively control the active immune cells in good responders and cause RBC macrocytosis as a collateral effect. However, the increase of MCV in good responders was only 5 fL, and MCV change was mostly within the normal range. It might be difficult to perceive such a minor change in real clinical practice, and overt MCV change to a level above the upper normal limit in long-term MTX therapy would have more clinical implications.

RBC macrocytosis was significantly associated with age, MTX dose, and concomitant use of sulfasalazine or leflunomide. A higher dose of MTX had a higher probability of adverse effects and a more powerful therapeutic effect. In our study, more than half of the patients with RBC macrocytosis were prescribed MTX at 10 mg. There is a tendency for Asian patients with RA

to take a lower dose of MTX compared with European or American patients because of concern for a higher risk of adverse effects [2,17-19]. However, it seems that RBC macrocytosis can occur at any therapeutic dose of MTX. Sulfasalazine enters into cells via the folate transporter [20] and could boost intracellular folate depletion, resulting in RBC macrocytosis. Leflunomide blocks pyrimidine synthesis and can synergize with MTX to inhibit DNA synthesis, aggravating erythrocyte macrocytosis [21,22].

This study showed that patients experiencing RBC macrocytosis in the course of MTX-based treatment were more likely to have a higher risk for b/tsDMARD initiation. In patients experiencing RBC macrocytosis, the MTX dose was generally higher than that in patients with normal MCV, and three-fourths of the patients were treated with double or triple combination therapy. Thus, it is considered that patients experiencing RBC macrocytosis displayed a tendency to have higher resistance to MTX therapy.

An intriguing association between RBC macrocytosis and MTX resistance could be attributed to the genetic variations in MTX metabolic pathway genes influencing MTX response. MTX polyglutamates can inhibit 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC), reducing synthesis of adenosine and guanidine precursors from the pentose-phosphate pathway [3,14]. The net result is reduced DNA synthesis and subsequent cytostasis. It is well-known that the ATIC G allele is strongly associated with non-responsiveness to MTX [23,24]. It is plausible that MTX polyglutamates maintain inhibition of dihydrofolate reductase and thymidylate synthetase for nucleotide production but may be unable to fully activate anti-inflammatory adenosine signaling due to ATIC polymorphism.

Our study has some limitations. First, a selection or attrition bias might exist because the clinical data were retrospectively collected. Second, laboratory anemia evaluation was not performed in all patients with RBC macrocytosis because four-fifths of the patients did not have anemia at the time of RBC macrocytosis. Third, we were unable to evaluate disease activity at the time point or in the period of RBC macrocytosis because DAS28 was not consistently recorded during the period of RBC macrocytosis. Fourth, the ACPA titer levels cannot be accurately estimated because the maximum ACPA measurement was set to 200 U/mL. Fifth, hidden confounding factors affecting MCV change (e.g., alcohol intake or use of proton pump inhibitors) [10,25] could exist and might have affected the results. However, the chances would be equally distributed in patients with RBC macrocytosis and normal MCV, and their effects may be not large.

Anemia is a common problem affecting patients with RA in terms of disease severity and quality of life [26-28]. The estimated prevalence of mild anemia ranged between 33% and 60%, and iron-deficient anemia and anemia of chronic inflammation were the major causes [26,27]. If anemia is absent, erythrocyte profiles, including MCV, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, can be overlooked.

CONCLUSION

In our analysis, RBC macrocytosis was observed in a considerable portion of the non-anemic patients (80% of the patients with RBC macrocytosis). RBC macrocytosis was possibly associated with the use of b/tsDMARD and could be a supplementary marker for assessing MTX resistance. Further research on the mechanistic and physiologic linkages between erythrocyte MCV change and the intracellular action of MTX could contribute to a better understanding of the association between RBC macrocytosis and MTX resistance in RA.

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None.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

KJK conceived and designed the study; KJK carried out data collection; KJK performed computational analysis; KJK and IWB wrote the manuscript; all authors participated in discussion and interpreting the results; KJK supervised all aspects of the project; all authors read and approved the final manuscript.

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REFERENCES

1. Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, et al. Rheumatoid arthritis. Nat Rev Dis Primers 2018;4:18001.
2. Smolen JS, Landewé RBM, Bergstra SA, Kerschbaumer A, Sepriano A, Aletaha D, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. Ann Rheum Dis 2023;82:3-18.
3. Brown PM, Pratt AG, Isaacs JD. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. Nat Rev Rheumatol 2016;12:731-42.
4. Chatzidionysiou K, Sfikakis PP. Low rates of remission with methotrexate monotherapy in rheumatoid arthritis: review of randomised controlled trials could point towards a paradigm shift. RMD Open 2019;5:e000993.
5. van Vollenhoven R. Treat-to-target in rheumatoid arthritis - are we there yet? Nat Rev Rheumatol 2019;15:180-6.
6. Salliot C, van der Heijde D. Long-term safety of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic literature research. Ann Rheum Dis 2009;68:1100-4.
7. Wang W, Zhou H, Liu L. Side effects of methotrexate therapy for rheumatoid arthritis: a systematic review. Eur J Med Chem 2018;158:502-16.
8. Weinblatt ME, Fraser P. Elevated mean corpuscular volume as a predictor of hematologic toxicity due to methotrexate therapy. Arthritis Rheum 1989;32:1592-6.
9. Shipa MRA, Langley L, Sacks B, Yeoh SA, Mainuddin MD, Mukerjee D, et al. Increased erythrocyte mean corpuscular volume by methotrexate predicts clinical response in psoriatic arthritis. Rheumatology (Oxford) 2022;61:e270-3.
10. Shipa MRA, Yeoh SA, Embleton-Thirsk A, Mukerjee D, Ehrenstein MR. The synergistic efficacy of hydroxychloroquine with methotrexate is accompanied by increased erythrocyte mean corpuscular volume. Rheumatology (Oxford) 2022;61:787-93.
11. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69:1580-8.
12. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and management. Blood 2018;131:505-14.
13. Teitsma XM, Jacobs JWG, Welsing PMJ, de Jong PHP, Hazes JMW, Weel AEAM, et al. Inadequate response to treat-to-target methotrexate therapy in patients with new-onset rheumatoid arthritis: development and validation of clinical predictors. Ann Rheum Dis 2018;77:1261-7.
14. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol 2020;16:145-54.
15. de Rotte MC, de Jong PH, Pluijm SM, Calasan MB, Barendregt PJ, van Zeben D, et al. Association of low baseline levels of erythrocyte folate with treatment nonresponse at three months in rheumatoid arthritis patients receiving methotrexate. Arthritis Rheum 2013;65:2803-13.
16. Stamp LK, O'Donnell JL, Chapman PT, Zhang M, Frampton C, James J, et al. Determinants of red blood cell methotrexate polyglutamate concentrations in rheumatoid arthritis patients receiving long-term methotrexate treatment. Arthritis Rheum 2009;60:2248-56.
17. Kim MJ, Park EH, Shin A, Ha YJ, Lee YJ, Lee EB, et al. Assessment on treatments with conventional synthetic disease-modifying drugs before initiating biologics in patients with rheumatoid arthritis in Korea: a population-based study. J Rheum Dis 2022;29:79-88.
18. Kameda H, Yamaoka K, Yamanishi Y, Tada M, Koike R, Nakajima A, et al. Japan College of Rheumatology guidance for the use of methotrexate in patients with rheumatoid arthritis: secondary publication. Mod Rheumatol 2023;34:1-10.
19. Fraenkel L, Bathon JM, England BR, St Clair EW, Arayssi T, Carandang K, et al. 2021 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Rheumatol 2021;73:1108-23.
20. Jansen G, van der Heijden J, Oerlemans R, Lems WF, Ifergan I, Scheper RJ, et al. Sulfasalazine is a potent inhibitor of the reduced folate carrier: implications for combination therapies with methotrexate in rheumatoid arthritis. Arthritis Rheum 2004;50:2130-9.
21. Torrez M, Chabot-Richards D, Babu D, Lockhart E, Foucar K. How I investigate acquired megaloblastic anemia. Int J Lab Hematol 2022;44:236-47.
22. Toyokawa Y, Kingetsu I, Yasuda C, Yasuda J, Yoshida K, Kurosaka D, et al. Pancytopenia, including macrocytic anemia, associated with leflunomide in a rheumatoid arthritis patient. Mod Rheumatol 2007;17:436-40.
23. Dedmon LE. The genetics of rheumatoid arthritis. Rheumatology (Oxford) 2020;59:2661-70.
24. Lee YH, Song GG. A meta-analysis of the association between the ATIC 347 C/G polymorphism and methotrexate responsiveness and toxicity in rheumatoid arthritis. Semin Arthritis Rheum 2024;64:152337.
25. Kaczmarczyk O, Przybylska-Feluś M, Piątek-Guziewicz A, Wcisło K, Krośniak M, Kryczyk-Kozioł J, et al. Effect of long-term proton pump inhibitor therapy on complete blood count parameters and selected trace elements: a pilot study. Pol Arch Intern Med 2020;130:179-86.
26. Bloxham E, Vagadia V, Scott K, Francis G, Saravanan V, Heycock C, et al. Anaemia in rheumatoid arthritis: can we afford to ignore it? Postgrad Med J 2011;87:596-600.
27. Wilson A, Yu HT, Goodnough LT, Nissenson AR. Prevalence and outcomes of anemia in rheumatoid arthritis: a systematic review of the literature. Am J Med 2004;116(Suppl 7A):50S-7.
28. Möller B, Scherer A, Förger F, Villiger PM, Finckh A. Anaemia may add information to standardised disease activity assessment to predict radiographic damage in rheumatoid arthritis: a prospective cohort study. Ann Rheum Dis 2014;73:691-6.