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Research article

Association between Elevated Mean Corpuscular Volume and C-reactive protein in Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that affects many tissues and organs, but principally attacks the joints producing an inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints. Various conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) have been used to reduce disease activity. Methotrexate (MTX) is currently the most frequently used in the treatment of RA, and its hematological toxicity is well documented. Meanwhile, as for the other csDMARDs, hematological toxicity remains to be fully investigated. In the clinical practice we sometimes encounter large mean corpuscular volume (MCV) and low disease activity in RA patients treated with MTX. Additionally, we also encounter large MCV in RA patients without using MTX. The purpose of our study was to investigate the association between MCV and disease activity, and the association between MCV and vitamins associated with hematopoiesis. In this study a total of 70 patients with RA were evaluated. All patients were treated without MTX. These 70 patients were analyzed for the correlation between disease activity and other variables. Significant inverse correlations were present between CRP and age, and MCV and hemoglobin. However, serum levels of folic acid and vitamin B12 were not significantly correlated, respectively. The correlation between CRP quintile and other variants was tested by Spearman rank-order correlation coefficient. Significant inverse correlations were present between CRP and MCV. As for MCV, the fluctuation was within the normal range, but the more the CRP increased, the more MCV decreased. In contrast, the other variants including sex, age, hemoglobin, folic acid and vitamin B12 were found not to be significant. Our data suggested that change of MCV was depend on the inflammation and was not related to the level of folic acid and vitamin B12 .

Keywords: arthritis-rheumatoid, mean corpuscular volume, DMARDs

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that can affect many tissues and organs [1, 2]. Anemia is a common comorbidity in individuals with RA. There are few studies of frequency and type of anemia in RA since the introduction of biologic drugs and a more active anti-rheumatic therapy. Methotrexate (MTX) has been widely used in the treatment of RA, and its hematological toxicity is well documented.

Meanwhile, as for the other conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs), hematological toxicity remains to be fully investigated. In the clinical practice we sometimes encounter large mean corpuscular volume (MCV) and low disease activity in RA patients treated with MTX. Additionally, we also encounter large MCV in RA patients without using MTX. Salazosulfapyridine (SASP) also widely used and one of the key drugs in the treatment of RA. Proton-coupled folate trans-porter/heme carrier protein 1 (PCFT/HCP1) has been identified as a transporter that mediates the translocation of folates across the cellular membrane and suggested to be the possible molecular entity of the carrier-mediated intestinal folate transport system. SASP inhibited the PCFT/HCP1 system [3]. In addition, SASP is a potent inhibitor of the reduced folate carrier and during SASP treatment, the use of SASP may lead to the onset of (sub) clinical folate deficiency [4]. The patients treated with csDMARDs without MTX were also reported to having uncommon side effects of anemia with an elevation in MCV [5]. In these cases, it may be different from MTX that an elevated MCV is not hematological toxicity. If a patient has been taking a csDMARD at a constant dosage for a long period of time, then the risk of toxicity is considered to be low. On the other hand, in patients with active RA, anemia is often present. Many types of anemia were associated with RA. Vitamin B12 and folic acid deficiency are reported to be linked to impaired nutrition and mal-absorption and to be more prevalent among patients with RA [6-10]. These deficiencies may be related to the disease activity of RA. In the clinical practice, we sometimes encounter large MCV and low activity in patients with RA without using MTX. Based on previous reports, the purpose of our study was to investigate the association between MCV and disease activity such as C-reactive protein (CRP) in RA patients.

Materials and Methods

Subjects

A total of 70 outpatients with RA treated in Niigata University Hospital were evaluated in this study. Each patient satisfied the 1987 American Society of Rheumatology Criteria for RA [11]. Patients' records were obtained from their medical records. None of these patients were treated with operation of gastrectomy. All 70 patients had not received MTX. Thirty-three cases were treated with DMARDs except for MTX and 37 cases were treated without DMARDs. DMARDs treatment was continued at least 4 months in all the patients. We estimated these patients' disease activity to be stable and, therefore, were included in this study. Additionally, patients showing anemia (hemoglobin (Hb) <10.8 g/dl) were excluded from this study.

Laboratory procedure and vitamin B12, folic acid assay

Hb, MCV, serum creatinine, CRP, and RF were assessed by routine laboratory methods. Erythrocyte sedimentation rate (ESR) was measured by Westergren method. Folic acid and vitamin B12 were each measured using folic acid and vitamin B12 assay kits with chemiluminescent Immunoassay (CLIA) technique (Beckman Coulter Corp, Tokyo, Japan). All of these samples were measured in duplicate.

Statistical analysis

Correlation between CRP and its possible modifiers (sex, age, MCV, Hb, folic acid, and vitamin B12) was assessed by Spearman rank-order correlation coefficients. Dose dependent association between CRP quintile rank and each possible modifier was tested by ordinal logistic regression model adjusted for all other possible modifiers. The statistical significance of these associations was expressed as P for trend. All statistical analyses were performed by using SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA), and $P < 0.05$ was considered statistically significant.

The study protocol was approved by the institutional review board of Niigata University Medical and Dental Hospital, and the subjects gave informed consent to participate in this study.

Results

Clinical features

Seventy patients with RA treated without MTX were evaluated in this study. Twelve patients were male and 58 were female. Table 1 shows the clinical characteristics and laboratory findings of these patients at the time MCV measurement was assessed. The mean duration was about 10 years and many of these patients were treated with a few csDMARDs. Inflammatory measures such as ESR and CRP were elevated. Rheumatoid factor (RF) was also frequently elevated due to disease activity of RA. About 5% of these patients presented renal insufficiency, but their serum creatinine levels were minimally elevated. The abnormal elevation of MCV was detected only in one patient and the level of MCV was slightly elevated with upper limit. The incidence of anemia such as gastroduodenal ulcer was not detected Low level of serum folic acid was about 5% and low level of vitamin B12 deficiency was about 8%. However, both of their serum folic acid and vitamin B12 depression were not so severe.

csDMARDs treatment of these patients

All 70 patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs) and 33 patients were treated with csDMARDs without MTX. The details of csDMARDs are shown in Table 2. SASP and bucillamine (BCL) were frequently used in these patients. About half of these patients were not treated with csDMARDs. Oral prednisolone therapy was given to 27 patients and their dose was 3.2 ± 5.0mg daily. MTX therapy is well known to act by inhibiting the metabolism of folic acid and the effect of MCV was easy to be thought of. Thus we exclud-

Table 1. Patients characteristics.

Subjects	Number or Mean	SD	Maximum	Minimum
Age (years)	61	12.7	84	19
Sex (Male/Female)	12/58			
Disease duration (years)	10.0	9.2	27	0.5
Cre (mg/dL)	0.8	0.5	2.9	0.4
CRP (mg/dL)	1.7	12.7	9.2	0.05
ESR (mm/h)	32.0	22.3	92.0	7.0
RF (IU/mL)	252.4	509.2	3.0	269.0
MCV (fL)	92.7	4.6	101.5	79.8
Hb (g/dL)	13.0	1.3	16.2	10.8
Folic acid (ng/mL)	5.4	2.8	14.1	1.6
Vitamin B12 (pg/mL)	516.0	502.0	3000.0	85.0
	Number (%)			
Elevation of Cre (>1.2 mg/dL)	4 (5.7)			
Elevation of MCV (>100 fL)	1 (1.4)			
Depression of Hb (<10.7 g/dL)	0 (0)			
Depression of folic acid (<2.5 ng/mL)	4 (5.7)			
Depression of vitamin B_{12} (<180 pg/mL)	6 (8.6)			

SD: standard deviation; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; Cre: serum creatinine. MCV: mean corpuscular volume = hematocrit (%) / red blood cell count (104/mm3) × 10.

Table 2. Details of DMARDs treated with RA patients.

csDMARDs	Number of patients
SASP	13
SASP + AZ	2
SASP + CY	1
DPC	2
DPC + AZ	1
GST	2
AF	2
BCL	1
GST + BCL	8
PSL	27
ND	31

csDMARDs: Conventional synthetic disease modifying anti-rheumatic drugs; SSZ: sulfasalazine; AZ: azthopurine; CY: cyclophosphamide; DPC:D-penicillamine; GST: gold sodium thiomalate; AF: auranofin; ACT: actarit; BCL:bucillamine; PSL: prednisolone; ND: No csDMARDs

excluded patients treated with MTX in this study.

Correlation between CRP and other variants

The correlation between CRP and other variants were tested by Spearman rank- order correlation coefficient. The results were shown in Table 3. Significant negative correlations were present between CRP and age, MCV, and Hb. However, serum levels of folic acid and vitamin B12 were not significantly correlated respectively.

Correlation between CRP quintile and other variants

The correlation between CRP quintile and other variants were tested by Spearman rank-order correlation coefficient. All variants were adjusted to each other in the table. The results were presented in Table 4. Significant inverse correlations were present between CRP and MCV. As for MCV, the fluctuation was within normal range, but the more the CRP was increasing, the more MCV was decreasing. In contrast, the other variants of sex, age, Hb, folic acid and vitamin B12 were not significant, respectively.

Table 3. Spearman rank-order correlation coefficient (ρ) between CRP and each of the possible modifiers.

Variants	ρ	P-value
Sex (Female 1, Male 0)	-0.03	0.822
Age	-0.24	0.046
MCV	-0.35	0.003
Hb	-0.30	0.011
Folic acid	-0.12	0.332
Vitamin B12	0.06	0.648

Table 4. Adjusted dose dependent association between CRP quintile and each of the possible modifiers.

quintile [range of CRP]	1st (n=17) [0.05]	2nd (n=15) [0.1-0.3]	3rd (n=12) [0.4-0.7]	4th (n=13) [0.8-1.6]	5th (n=13) [1.8-9.2]	P for trend*
Sex (% of female)	88.2%	80.0%	83.3%	69.2%	92.3%	0.271
Age	64.9 (10.9)	64.6 (14.3)	63.2 (8.8)	58.6 (10.9)	54.2 (15.8)	0.083
MCV	94.7 (3.7)	94.0 (3.3)	92.7 (3.6)	91.0 (6.1)	90.3 (4.7)	0.035
Hb	13.3 (1.1)	13.3 (1.2)	13.0 (1.1)	12.4 (1.4)	12.8 (1.5)	0.201
Folic acid	5.2 (2.9)	6.3 (3.0)	6.1 (2.6)	4.6 (2.4)	5.0 (2.7)	0.900
Vitamin B12	385.6 (214.0)	467.3 (212.9)	664.5 (805.2)	506.9 (374.9)	615.4 (735.0)	0.148

mean (SD), * Adjusted for all variables in the table each other using ordinal logistic regression model.

Discussion

Anemia is often present in patients with RA. Many types of anemia are associated with RA [12,13]. Vitamin B12 and folic acid deficiency are reported to be more prevalent among patients with RA than controls [14,15]. The current treatment paradigm of RA entails the early use of csDMARDs [16]. The various csDMARDs available include MTX, SASP, BCL, actarit (ACT), leflunomide, azathioprine, chloroquine, cyclosporine, gold and D-penicillamine (D-PC). MTX is the most popular drug due to its proven clinical efficacy and low discontinuation rates demonstrated in several long-term observational studies [17]. Hematological toxicity of MTX is well known, because of its pharmacological action [18]. However, in clinical practice, there are many patients who cannot use MTX, because of interstitial lung disease, chronic kidney disease, and infections. Thus we examined our RA patients treated with DMARDs but not with MTX. SASP has been demonstrated to inhibit both folate transport across intestine and various folate-metabolizing enzymes [19]. The effect of SASP on folate-dependent enzymes is particularly similar to the anti-rheumatoid action of MTX. A low level of folate is often seen in patients with RA and it might be expected that treatment with SASP may cause folate deficiency. However, no major effect on serum and red cell folate levels was seen in SASP treated patients [20]. Among our patients, one patient treated with SASP presented a depression of folic acid under 2.5 pg/ml and two additional patients treated with SASP revealed a relatively low level of folic acid below 3.0 pg/ml. In Japan, BCL was frequently used for the treatment of RA. BCL has 2 sulfhydryl moieties and its chemical structure is similar to D-PC. And BCL was shown to possess similar pharmacokinetic actions to D-PC [21]. However, the hematological effect of BCL remains to be validated. None of our patients showed low levels of folic acid below 3 pg/ml. Renal insufficiency was a consequence of anemia due to a lack of erythropoietin. The presence of anemia detected by a lack of erythropoietin was usually shown in normocytic and normochromic anemia [22] and usually did not affect MCV.

Vitamin B12 is associated with enlargement of MCV in patients with anemia. Pernicious anemia is a condition in which the body cannot make enough healthy red blood cells because it does not have enough vitamin B12 [23]. Vitamin B12 is a nutrient found in certain foods. Patients who have pernicious anemia cannot absorb enough vitamin B12 from foods due to a lack of intrinsic factors [24]. This leads to vitamin B12 deficiency. It is also well known that a deficiency of vitamin B12 or folic acid results in megaloblastic anemia in post-gastrectomized patients. Our patients had no history of gastrectomy. The number of patients with depression of vitamin B12 was 6/70 (8.6%). In these patients, their average MCV was 97.2±2.0. The level of MCV in these six patients was relatively high compared to the rest of our patients. A vitamin B12 deficiency may have contributed to MCV enlargement in our RA patients. Anemia of chronic disease is a form of anemia seen in chronic illnesses, chronic infection, chronic immune activation or malignancy. Anemia of chronic disease is often a mild normocytic anemia, but can sometimes be more severe, or can sometimes be microcytic anemia [25-30]. In our study, multivariate analysis revealed that the CRP level and MCV were inversely correlated. Our patients were not correlated with Hb level in Table 4. The degree of MCV

depression was depended on the level of the inflammation of RA and the level of Hb was not correlated with the level of CRP.

Conclusion

Our data suggested that change of MCV was depend on the inflammation and was not related to the level of folic acid and vitamin B12. Moreover, chronic inflammation may be contributing factor. The doses of csDMARDs, such as SASP, prescribed for RA in Japan are less than those prescribe abroad. Additional studies are warranted in which RA patients are carefully monitored for several years after the onset of the disease to determine changes in MCV and CRP.

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Conflict of Interest Statement

None of the authors has a conflict of interest to declare.

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