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Cystatin C as a Predictor of Renal Function and Methotrexate-Associated Toxicities in Patients With Rheumatoid Arthritis

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ABSTRACT. ***Objective***. Methotrexate (MTX) is an anchor drug for most patients with rheumatoid arthritis (RA); however, its use may be limited depending on renal function. Therefore, this study aimed to examine the discrepancy in the estimated glomerular filtration rate (eGFR) using conventional serum creatinine (SCr)-, cystatin C-, and MTX-associated toxicities in patients with RA.

Methods. In total, 436 patients were enrolled, and eGFR was evaluated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on both cystatin C and SCr levels. The CKD and MTX dosing stages were classified according to eGFR. MTX-associated toxicities were also evaluated.

Results. The mean eGFR using CKD-EPI cystatin C ($CKD\text{-}EPI_{cys}$) was 89.44 mL/min/1.73 m^2, lower than the eGFR using CKD-EPI SCr ($CKD\text{-}EPI_{SCr}$) of 95.55 mL/min/1.73 m^2. After converting eGFR to $CKD\text{-}EPI_{cys}$ by $CKD\text{-}EPI_{SCr}$, 29.8% of patients were reclassified to a higher stage according to the Kidney Disease: Improving Global Outcomes CKD stage. Also, according to the MTX guidelines, 6.4% of the group with an eGFR > 50 mL/min/1.73 m^2 were reclassified to eGFR 10-50 mL/1.73 m^2, requiring dose adjustment. The incidence of MTX-associated toxicities, such as anemia, leukopenia, and nephrotoxicity, was significantly higher in the CKD stage-changed group than in the nonstage-changed group.

Conclusion. Our results showed that eGFR based on SCr was overestimated compared with eGFR based on cystatin C. In addition, we demonstrated that MTX-associated toxicities were significantly increased in the group with a changed stage when the eGFR was converted from $CKD\text{-}EPI_{SCr}$ to $CKD\text{-}EPI_{cys}$.

Key Indexing Terms: cystatin C, glomerular filtration rate, methotrexate, rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease mainly affecting joints.[1] Although great progress has been made in the treatment of RA in the last few decades, the basis of treatment remains disease-modifying antirheumatic drugs. Methotrexate (MTX) is an anchor drug used as the first-line treatment for most patients with RA.[2] RA is pathophysiologically associated with glomerulonephritis or tubular disease, and therapeutic agents for RA may cause secondary renal damage.[3,4] Conversely, renal function decline may also limit the use of drugs such as MTX for RA treatment.[5] Therefore, accurate measurement of renal function is important for the treatment and prognosis of patients with RA considering these reciprocal adverse effects.

The glomerular filtration rate (GFR) is an indicator of renal function. It is traditionally calculated using the Cockroft-Gault equation or the Modification of Diet in Renal Disease Study equation (MDRD) based on serum creatinine (SCr). Recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation has been recommended for Asian patients with mild renal impairment. However, the accurate assessment of renal function is limited even if sex, age, and race are corrected because SCr used in the equation is derived from muscle.[6-9] Cystatin C, on the other hand, is a reversible cysteine protease inhibitor. It is constantly produced in all nucleated cells, filtered by the glomeruli, and metabolized by the proximal convoluted tubules. Thus, it has been spotlighted as a potentially ideal marker to evaluate renal function. The CKD-EPI equation using cystatin C ($CKD\text{-}EPI_{cys}$) has been developed. Several studies have reported a higher accuracy of cystatin C than SCr in measuring renal function in patients with muscle wasting, such as liver disease, malignancy, obesity, and acute renal failure[10-12]; however, limited information is available regarding patients with RA. Therefore, this study aimed to examine the discrepancy in the estimated GFR (eGFR) using SCr-, cystatin C-, and MTX-associated toxicities in patients with RA.

METHODS

Patients. Patients with RA who received MTX-based treatment at Dankook University Hospital between March 2018 and August 2021 were retrospec-

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tively included in the study. The exclusion criteria were as follows: (1) MTX use < 3 months; (2) age < 18 years; (3) missed SCr or cystatin C measurement; (4) dialysis at diagnosis; and (5) malignancy at diagnosis. Based on these criteria, 436 patients with RA were enrolled in this study. Data on demographic and clinical information, including age, sex, BMI (calculated as weight in kilograms divided by height in meters squared), body surface area (BSA), disease activity, and medication history, were obtained.[13] BSA was calculated using the Mosteller formula:

$$\sqrt{\frac{\text{height (cm)} \times \text{weight (km)}}{3600}}.$$ [4]

This study was conducted in strict accordance with the ethical principles embodied in the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Dankook University Hospital (IRB approval no. 2022-10-043).

Assessment of renal function.

• *Measurement of eGFR.* The eGFR was calculated using the CKD-EPI equations based on SCr and cystatin C levels. SCr was determined using an enzymatic technique (cobas 8000 modular analyzer series; Roche Diagnostics System), and cystatin C was measured using a turbidimetric immunoassay (TBA-C8000; Toshiba Medical Systems). When using the CKD-EPI equations, the race value was selected as White or other.[6,15] Each equation is as follows:

1. CKD-EPI serum creatinine ($CKD\text{-}EPI_{SCr}$) equation:

Male with SCr ≤ 0.9: $141 \times (0.993)^{age} \times \left(\frac{SCr}{0.9}\right)^{-0.411}$

Male with SCr > 0.9: $141 \times (0.993)^{age} \times \left(\frac{SCr}{0.9}\right)^{-1.209}$

Female with SCr ≤ 0.7: $144 \times (0.993)^{age} \times \left(\frac{SCr}{0.7}\right)^{-0.329}$

Female with SCr > 0.7: $144 \times (0.993)^{age} \times \left(\frac{SCr}{0.7}\right)^{-1.209}$

2. CKD-EPI cystatin C ($CKD\text{-}EPI_{cys}$) equation:

Female or male with serum cystatin C (S_{cys}) ≤ 0.8:

$133 \times \left(\frac{S_{cys}}{0.8}\right)^{-0.499} \times (0.996)^{age}$ [× 0.932 if female]

Female or male with cystatin C > 0.8:

$133 \times \left(\frac{S_{cys}}{0.8}\right)^{-1.328} \times (0.996)^{age}$ [× 0.932 if female]

• *Classification of CKD stage.* CKD stage was classified according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines: stage 1 (eGFR ≥ 90 mL/min/1.73 m^2), stage 2 (eGFR = 60-89 mL/min/1.73 m^2), stage 3 (eGFR = 30-59 mL/min/1.73 m^2), stage 4 (eGFR = 15-29 mL/min/1.73 m^2), and stage 5 (eGFR < 15 mL/min/1.73 m^2). Additionally, CKD stage 3 was subdivided into stages 3A and stage 3B based on whether eGFR was above or below 45 mL/min/1.73 m^2.[16]

• *Evaluation of MTX-associated toxicities.* MTX-related toxicities were investigated at each outpatient clinic visit for hematologic abnormalities (leukopenia, anemia, and thrombocytopenia), pulmonary, gastrointestinal (GI) symptoms (nausea, vomiting, and diarrhea), hepatotoxicity, nephrotoxicity, dermatological (alopecia and flushing), nervous system symptoms (headache), severe infection, and malignancy. Each related toxicity was defined as follows: (1) leukopenia: white blood cell (WBC) < 4000/μL; (2) anemia: hemoglobin < 9 g/L and platelet < 100,000/μL; (3) hepatoxicity: any increase in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels above the upper laboratory limit of normal; (4) nephrotoxicity: a 1.5-fold increase in basal SCr level or a ≥ 25% decrease in GFR by $CKD\text{-}EPI_{SCr}$; (5) severe infection: infection requiring hospitalization and intravenous antibiotics; (6) all respiratory events assumed to be related to MTX: radiological findings suggestive of ground-glass opacities or interstitial lung abnormalities without a better clinical explanation, which necessitated the discontinuation of MTX therapy; and (7) malignancy: new tumor onset after MTX treatment.[17-20]

Statistical analyses. A paired *t* test was used to compare continuous variables between the 2 groups. Fisher exact, chi-square, or McNemar tests were used to determine associations of categorical variables between the groups. Multivariate logistic regression analysis was used to evaluate the associated factors in the stage-change groups. All analyses were performed using SPSS software (version 25 for Windows; IBM Corp.). Statistical significance was set at $P < 0.05$.

RESULTS

Baseline characteristics. The baseline characteristics of the patients with RA are summarized in Table 1. Our study included 109 male and 327 female patients. The mean age and RA duration were 58.5 and 7.7 years, respectively. The mean BMI and BSA were 23.5 and 1.6 m^2, respectively. Among the comor-

Table 1. Baseline characteristics of the enrolled patients with RA.

	Patients, N = 436	*P*
Age, yrs	58.5 ± 13.4	
Sex, male	109 (25)	
Disease duration, yrs	7.7 ± 5.3	
BMI, kg/m^2	23.5 ± 3.8	
BSA, m^2	1.6 ± 0.2	
Comorbidities		
DM	62 (14.2)	
HTN	146 (33.5)	
Dyslipidemia	62 (14.2)	
Smoking	32 (7.3)	
Medications administered		
MTX / mean dose per wk, mg	436 (100) / 11.8 ± 3.3	
LEF	212 (48.6)	
SSZ	179 (41)	
HCQ	172 (39.4)	
Tacrolimus	107 (24.5)	
GC	331 (75.9)	
NSAID	182 (41.7)	
bDMARD	47 (10.8)	
tsDMARD	18 (4.1)	
ACEi or ARB	52 (11.9)	
Gastric acid secretion inhibitor or prokinetic agent	143 (32.7)	
RF positivity	334 (76.6)	
ACPA positivity	349 (80)	
Initial SCr, mg/dL	0.68 ± 0.16	
Cystatin C, mg/L	0.98 ± 0.36	
eGFR equation		**< 0.01**
$CKD\text{-}EPI_{SCr}$, mL/min/1.73 m^2	95.55 ± 18.09	
$CKD\text{-}EPI_{cys}$, mL/min/1.73 m^2	89.44 ± 23.91	

Data are presented as mean ± SD or n (%). Values in bold are statistically significant. ACEi: angiotensin-converting enzyme inhibitor; ACPA: anticitrullinated protein antibody; ARB: angiotensin receptor blocker; bDMARD: biologic disease-modifying antirheumatic drug; BSA: body surface area; CKD-EPI: chronic kidney disease epidemiology collaboration; cys: cystatin C; DM: diabetes mellitus; DMARD: disease-modifying antirheumatic drug; eGFR: estimated glomerular filtration rate; GC: glucocorticoid; HCQ: hydroxychloroquine; HTN: hypertension; LEF: leflunomide; MTX: methotrexate; NSAID: nonsteroidal antiinflammatory drug; RA: rheumatoid arthritis; RF: rheumatoid factor; SCr: serum creatinine; SSZ: sulfasalazine; tsDMARD: targeted synthetic disease-modifying antirheumatic drug.

bidities, hypertension (HTN) was found in 33.5% of patients, diabetes mellitus (DM) in 14.2%, and dyslipidemia in 14.2%. All patients were administered MTX at a mean dose of 11.8 mg/week. Rheumatoid factor and anticitrullinated protein antibodies were present in 76.6% and 80% of patients, respectively. The initial mean SCr level was 0.68 mg/dL, and the cystatin C level was 0.98 mg/L. Moreover, the mean eGFR by CKD-EPI$_{cys}$ was 89.44 mL/min/1.73 m^2, which was statistically significantly lower than the mean eGFR by CKD-EPI$_{SCr}$ of 95.55 mL/min/1.73 m^2.

Comparison of CKD stage using CKD-EPI$_{SCr}$ and CKD-EPI$_{cys}$ equations. Patients were classified into 5 CKD stages using eGFR calculated through CKD-EPI equation based on SCr and cystatin C according to the KDIGO CKD staging guideline. The distribution of CKD stages using eGFR based on SCr and cystatin C was as follows: 71.5% vs 57.1% for CKD stage 1; 23.4% vs 30.7% for stage 2; 3.7% vs 5% for stage 3A; 1.1% vs 4.4% for stage 3B, 0.02% vs 2.5% for CKD stage 4; and 0% vs 0.02% for stage 5. Significant differences between the 2 groups were observed at all stages except stage 3A and stage 5 (Table 2).

The changes in stage according to KDIGO and MTX dosing guidelines. The changes in the CKD stage when eGFR by CKD-EPI$_{SCr}$ was converted to eGFR by CKD-EPI$_{cys}$ are shown in Table 3A. The changes in CKD stage from eGFR by SCr to eGFR by cystatin C were as follows: (1) CKD stage 1 by SCr: 25% (78/312) to CKD stage 2 by cystatin C and 0.3% (1/312) to CKD stage 3A by cystatin C; (2) CKD stage 2 by SCr: 15.7% (16/102) to stage 1 by cystatin C, 20.5% (21/102) to CKD stage 3A by cystatin C, 8.8% (9/102) to CKD stage 3B by cystatin C, and 1% (1/102) to stage 4 by cystatin C; (3) CKD stage 3A by SCr: 6.2% (1/16) to stage 2 by cystatin C, 56.2% (9/16) to CKD stage 3B by cystatin C, and 37.5% (6/16) to CKD stage 4 by cystatin C; (4) CKD stage 3B by SCr: 80% (4/5) to stage 4 by cystatin C; and (5) CKD stage 4 by SCr: 100% (1/1) to stage 5 by cystatin C. In 29.8% of all patients, conversion of eGFR by SCr to eGFR by cystatin C led to reclassification to a higher stage, but 16 patients (3.7%) at stage 2 and 1 patient (0.2%) at stage 3A were reclassified as a lower stage.

According to the MTX dosing guidelines, the patients were classified into 3 categories: (1) eGFR < 10 mL/min/1.73 m^2, (2) eGFR 10-50 mL/min/1.73 m^2, and (3) eGFR > 50 mL/min/1.73 m^2. The changes in the category when eGFR by SCr was converted to eGFR by cystatin C are shown in Table 3B. None of the patients had an eGFR < 10 mL/min/1.73 m^2. In the eGFR 10-50 mL/min/1.73 m^2 category, all 11 patients showed no change in the category when eGFR was calculated using SCr or cystatin C. However, in the eGFR > 50 mL/min/1.73 m^2 category, 6.4% (27/425) patients were converted to eGFR 10-50 mL/min/1.73 m^2, requiring MTX dose reduction.

The associated factors of reclassification of CKD stages when converting eGFR by SCr to eGFR by cystatin C. When converting eGFR based on SCr to eGFR based on cystatin C, 130 patients were reclassified into the upward CKD stages. Logistic regression analysis showed that several factors, including age (odds ratio [OR] 1.05, 95% CI 1.02-1.07), presence of DM (OR 1.95, 95% CI 1.03-3.69), HTN (OR 1.84, 95% CI 1.08-3.10), and use of leflunomide (LEF; OR 2.24, 95% CI 1.34-3.74) were significantly associated with the change of CKD stages (Table 4).

Comparison of MTX-associated toxicities between the stage-changed group and nonstage-changed group. The overall incidence of MTX-associated toxicities was 30.7% in the stage-changed group and 20.6% in the nonstage-changed group, indicating a significant difference between the groups (P = 0.02). MTX-associated toxicities in all patients were the most common, with 7.1% of patients exhibiting GI symptoms, followed by 5.7% hepatotoxicity, 2.1% leukopenia, 1.4% anemia, and 1.4% alopecia. Among the MTX-associated toxicities, anemia, leukopenia, and nephrotoxicity were statistically significantly higher in the stage-changed group than in the nonstage-changed group (anemia: 3.1% vs 0.6%, P = 0.04; leukopenia: 4.6% vs 0.9%, P = 0.02; nephrotoxicity: 3.1% vs 0.3%, P = 0.01; Table 5).

Table 2. Comparison of KDIGO stage using CKD-EPI$_{SCr}$ and CKD-EPI$_{cys}$ equations.

Stage According to the KDIGO Guideline	CKD-EPI$_{SCr}$, N = 436	CKD-EPI$_{cys}$, N = 436	*P*
1	312 (71.5)	249 (57.1)	**< 0.01**
2	102 (23.4)	134 (30.7)	**0.02**
3A	16 (3.7)	22 (5)	0.32
3B	5 (1.1)	19 (4.4)	**< 0.01**
4	1 (0.02)	11 (2.5)	**< 0.01**
5	0 (0)	1 (0.02)	> 0.99

Data are presented as n (%). Values in bold are statistically significant. CKD-EPI: chronic kidney disease epidemiology collaboration; cys: cystatin C; KDIGO: Kidney Disease: Improving Global Outcomes; SCr: serum creatinine.

DISCUSSION

To our knowledge, this study is the first to report the usefulness of cystatin C in measuring renal function and its association with toxicity after treatment in patients with RA. A previous study has reported the usefulness of cystatin C compared to SCr in patients with RA; however, it focused only on comparing eGFR, and the association with prognosis after treatment has been overlooked.[21] Therefore, we analyzed the clinical effects by applying CKD stage and MTX dosing guidelines rather than merely comparing eGFR.

In our study, eGFR using the CKD-EPI equation was compared based on SCr and cystatin C and showed that eGFR using CKD-EPI$_{cys}$ was significantly lower than that using the CKD-EPI$_{SCr}$ equation. A previous study by Nakashima et al reported that the eGFR by MDRD$_{SCr}$ was higher than that by MDRD$_{cys}$ in patients with RA, and was associated with lower BMI, lower hemoglobin, nonuse of nonsteroidal antiinflammatory drugs, DM, and Steinbrocker radiological stage 4. Although this study differs in that the eGFR was calculated using the MDRD equation, the results were consistent with ours.[21] These results suggest that the traditional eGFR based on SCr may overestimate renal function in patients with RA. It can also lead to inappropriate drug choices and dose adjustments, leading to acute kidney injury and drug toxicity.

Table 3. Changes of KDIGO and MTX dosing guideline stage using CKD-EPI$_{SCr}$ and CKD-EPI$_{cys}$ equations.

A. Change of KDIGO stage using CKD-EPI$_{SCr}$ and CKD-EPI$_{cys}$ equations.

		CKD-EPI$_{cys}$					
	Stage	1, n = 249	2, n = 134	3A, n = 22	3B, n = 19	4, n = 11	5, n = 1
CKD-EPI$_{SCr}$	1, n = 312	233	78	1	0	0	0
	2, n = 102	16	55	21	9	1	0
	3A, n = 16	0	1	0	9	6	0
	3B, n = 5	0	0	0	1	4	0
	4, n = 1	0	0	0	0	0	1
	5, n = 0	0	0	0	0	0	0

B. Change of MTX dosing guideline stage using CKD-EPI$_{SCr}$ and CKD-EPI$_{cys}$ equations.

		CKD-EPI$_{cys}$		
	eGFR, mL/min/1.73 m^2	< 10, n = 0	10-50, n = 38	> 50, n = 398
CKD-EPI$_{SCr}$	< 10, n = 0	0	0	0
	10-50, n = 11	0	11	0
	> 50, n = 425	0	27	398

Values are n. Light gray and dark gray cells indicate change of classification to upward and downward stages, respectively. CKD-EPI: chronic kidney disease epidemiology collaboration; cys: cystatin C; eGFR: estimated glomerular filtration rate; KDIGO: Kidney Disease: Improving Global Outcomes; MTX: methotrexate; SCr: serum creatinine.

Our study analyzed the change in categories according to eGFR in the CKD and MTX dosing guideline stages. As for eGFR by CKD-EPI$_{SCr}$, 5% of patients with RA had true CKD with eGFR < 60 mL/min/1.73m^2, but when converted to eGFR by CKD-EPI$_{cys}$, it increased to 12.1%. This result indicates that patients with RA who are assumed to have normal renal function may have renal impairment. Moreover, even in categories in the MTX dosing guidelines, the proportion of patients with RA requiring MTX dose adjustment for low eGFR increased more than 3-fold (2.5% vs 8.7%) when converting from CKD-EPI$_{SCr}$ to CKD-EPI$_{cys}$.

MTX is the main drug used to treat RA. Since it is mainly

Table 4. Logistic regression analysis of factors in the stage-changed group.

	Univariable Analysis			Multivariable Analysis[a]		
	OR	95% CI	*P*	OR	95% CI	*P*
Age, yrs	1.05	1.02-1.07	**< 0.01**	1.05	1.03-1.08	**< 0.01**
Male sex	1.31	0.73-2.36	0.36			
Disease duration, yrs	1.03	0.98-1.08	0.22			
BMI, kg/m^2	1.05	0.98-1.12	0.16			
DM	1.95	1.03-3.69	**0.04**	2.03	1.12-3.67	**0.02**
HTN	1.84	1.08-3.10	**0.03**	1.94	1.19-3.16	**0.01**
Dyslipidemia	0.73	0.37-1.42	0.35			
Smoking	2.02	0.83-4.89	0.12			
MTX dose	0.97	0.90-1.04	0.39			
LEF	2.24	1.34-3.74	**< 0.01**	1.92	1.22-3.04	**0.01**
SSZ	0.91	0.54-1.52	0.71			
HCQ	0.80	0.48-1.35	0.41			
Tacrolimus	1.23	0.71-2.16	0.46			
NSAID	0.67	0.40-1.11	0.12			
tsDMARD	0.74	0.18-3.02	0.67			
ACEi or ARB	1.29	0.70-2.37	0.42			
RF positivity	1.14	0.61-2.12	0.69			
ACPA positivity	0.78	0.41-1.51	0.47			
ESR	1.00	0.99-1.02	0.97			

Values in bold are statistically significant. [a] Only statistically significant variables from the univariable analysis were used in the multivariable analysis. ACEi: angiotensin-converting enzyme inhibitor; ACPA: anticitrullinated peptide antibody; ARB: angiotensin receptor blocker; DM: diabetes mellitus; ESR: erythrocyte sedimentation rate; HCQ: hydroxychloroquine; HTN: hypertension; LEF: leflunomide; MTX: methotrexate; NSAID: nonsteroidal antiinflammatory drug; OR: odds ratio; RF: rheumatoid factor; SSZ: sulfasalazine; tsDMARD: targeted synthetic disease-modifying antirheumatic drug.

Table 5. Comparison of MTX-associated toxicities between the stage-changed and nonstage-changed groups.

MTX-associated Toxicity, n	Stage-changed Group, n = 130	Nonstage-changed Group, n = 306	*P*
Total events, n = 103	40 (30.7)	63 (20.6)	**0.02**
Alopecia, n = 6	1 (0.8)	5 (1.6)	0.48
Anemia, n = 6	4 (3.1)	2 (0.6)	**0.04**
Flushing, n = 1	1 (0.8)	0 (0)	0.13
Headache, n = 1	1 (0.8)	0 (0)	0.13
Hepatotoxicity, n = 25	11 (8.5)	14 (4.6)	0.11
All respiratory events assumed to be related to MTX, n = 5	2 (1.5)	3 (1)	0.62
Serious Infection, n = 3	1 (0.8)	2 (0.6)	0.89
Leukopenia, n = 9	6 (4.6)	3 (0.9)	**0.02**
Malignancy, n = 3	0 (0)	3 (0.9)	0.26
GI symptoms, n = 31	5 (3.8)	26 (8.5)	0.08
Nephrotoxicity n = 5	4 (3.1)	1 (0.3)	**0.01**
Stomatitis, n = 4	2 (1.5)	2 (0.6)	0.38
Thrombocytopenia, n = 4	2 (1.5)	2 (0.6)	0.38

Data are presented as n (%). Values in bold are statistically significant. GI: gastrointestinal; MTX: methotrexate.

excreted by the kidneys, it is recommended to reduce its dose below a GFR of 50 mL/min/1.73 m^2; it is contraindicated below a GFR of 10 mL/min/1.73 m^2. Therefore, it should be noted that a 3-fold increase from GFR > 50 mL/min/1.73 m^2 to 10-50 mL/min/1.73 m^2 may lead to severe toxicities due to MTX overdose.[22]

Previous studies reported that 10% to 37% of patients experience MTX-associated toxicities, and some reported that up to 72.9% of patients have MTX-associated toxicities at least once. GI symptoms are the most common, and hepatotoxicity and bone marrow suppression are also common.[23] Our study analyzed MTX-associated toxicities in a group with a changed stage after reclassification from CKD-EPI_{SCr} to CKD-EPI_{cys}. The study outcomes showed a statistically significant increase in overall MTX-associated toxicities incidence, particularly anemia and nephrotoxicity, in the group with changed stages. Interestingly, among MTX-associated toxicities, GI symptoms had the highest overall prevalence in our study, as in other studies. Nevertheless, unlike other MTX-associated toxicities, the prevalence was higher in the nonstage-changed group than in the stage-changed group. This difference is likely attributable to a higher rate of a history of GI-protective drug use in the stage-changed group (46.1% vs 27.1%).

Nephrotoxicity was not listed among the toxicities of low-dose MTX reported by Ridker et al in their large-scale study conducted with patients with coronary artery disease.[24] Nevertheless, several previous studies reported a higher incidence of MTX-associated toxicities in patients with RA accompanied by renal impairment, and showed that renal impairment increased the risk of MTX-induced pancytopenia.[25-27] The results of our study are consistent with the findings of these previous studies and, interestingly, suggest that MTX-associated toxicities can also result from an overdose due to inappropriate renal function measurement. In particular, the trend for a higher incidence of MTX-associated toxicities observed in the stage-changed group highlights the importance of accurate renal function measurement, not only for nephrotoxicity but also for all potential MTX-associated toxicities in patients with RA. In our study, the factors associated with reclassification to a higher stage when eGFR was calculated from SCr to cystatin C were age, DM, HTN, and LEF use. This is partly consistent with previous studies, reminding us that in vulnerable patients with RA who have comorbidities, such as old age, DM, and HTN, the assessment of renal function using cystatin C is a critical issue from the perspective of prognosis.[28,29]

Our study had several limitations. First, although the most accurate measurement of the GFR uses inulin or 51SCr-EDTA, we did not use these methods because they cannot be easily measured in clinical practice. Therefore, we attempted to increase accuracy using the CKD-EPI equations, which are known to be the most accurate in Asian patients with mild renal insufficiency.[8,9] Second, since this is a retrospective study, the medical records for patients' MTX-associated toxicities and adverse events may have contained limited information. Third, individual circumstances such as comorbidities and concomitant medications may be confounding factors in the outcome; however, they were not completely excluded. Finally, the small number of enrolled patients imposed a limitation on the acquisition of statistically significant results. Nevertheless, we used our best efforts to include a large number of patients, as compared to other similar studies on MTX-associated toxicities in patients with RA.[12,21] Larger-scale studies should be conducted in the future.

In this study, our results showed that eGFR based on SCr was overestimated compared with eGFR based on cystatin C. In addition, we demonstrated that MTX-associated toxicities were significantly increased in the group with a changed stage when the eGFR was converted from CKD-EPI_{SCr} to CKD-EPI_{cys}. Therefore, when evaluating renal function in patients with RA, measurement using cystatin C will be a useful

method for determining the MTX dose and predicting safety from MTX-associated toxicities. Future validation studies for the accurate measurement of renal function in patients will be needed.

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REFERENCES

1. Karstila K, Korpela M, Sihvonen S, Mustonen J. Prognosis of clinical renal disease and incidence of new renal findings in patients with rheumatoid arthritis: follow-up of a population-based study. Clin Rheumatol 2007;26:2089-95.
2. Demoruelle MK, Deane KD. Treatment strategies in early rheumatoid arthritis and prevention of rheumatoid arthritis. Curr Rheumatol Rep 2012;14:472-80.
3. Karie S, Gandjbakhch F, Janus N, et al. Kidney disease in RA patients: prevalence and implication on RA-related drugs management: the Matrix study. Rheumatology 2008;47:350-4.
4. Adu D, Berisa F, Howie AJ, et al. Glomerulonephritis in rheumatoid arthritis. Br J Rheumatol 1993;32:1008-11.
5. Rheumatoid Arthritis Clinical Trial Archive Group. The effect of age and renal function on the efficacy and toxicity of methotrexate in rheumatoid arthritis. J Rheumatol 1995;22:218-23.
6. Levey AS, Coresh J, Greene T, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 2006;145:247-54.
7. Palacio-Lacambra ME, Comas-Reixach I, Blanco-Grau A, Suñé-Negre JM, Segarra-Medrano A, Montoro-Ronsano JB. Comparison of the Cockcroft–Gault, MDRD and CKD-EPI equations for estimating ganciclovir clearance. Br J Clin Pharmacol 2018;84:2120-8.
8. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150:604-12.
9. Ix JH, Wassel CL, Stevens LA, et al. Equations to estimate creatinine excretion rate: the CKD epidemiology collaboration. Clin J Am Soc Nephrol 2011;6:184-91.
10. Bökenkamp A, Herget-Rosenthal S, Bökenkamp R. Cystatin C, kidney function and cardiovascular disease. Pediatr Nephrol 2006;21:1223-30.
11. Chew JS, Saleem M, Florkowski CM, George PM. Cystatin C—a paradigm of evidence based laboratory medicine. Clin Biochem Rev 2008;29:47-62.
12. Yun HW, Kim CJ, Kim JW, Kim HA, Suh CH, Jung JY. The assessment of muscle mass and function in patients with long-standing rheumatoid arthritis. J Clin Med 2021;10:3458.
13. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894:1-253.
14. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med 1987;317:1098.
15. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 2012;367:20-9.
16. Kidney Disease: Improving Global Outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013;3:1-50.
17. Yokuş O, Gedik H. Etiological causes of pancytopenia: a report of 137 cases. Avicenna J Med 2016;6:109-12.
18. Sparks JA, Dellaripa PF, Glynn RJ, et al. Pulmonary adverse events in patients receiving low-dose methotrexate in the randomized, double-blind, placebo-controlled cardiovascular inflammation reduction trial. Arthritis Rheumatol 2020;72:2065-71.
19. Regev A, Björnsson ES. Drug-induced liver injury: morbidity, mortality, and Hy's law. Gastroenterology 2014;147:20-4.
20. Silva-Fernández L, De Cock D, Lunt M, et al. Serious infection risk after 1 year between patients with rheumatoid arthritis treated with rituximab or with a second TNFi after initial TNFi failure: results from the British Society for Rheumatology Biologics Register for rheumatoid arthritis. Rheumatology 2018;57:1533-40.
21. Nakashima A, Horita S, Matsunaga T, et al. Factors contributing to discrepant estimated glomerular filtration values measured by creatinine and cystatin C in patients with rheumatoid arthritis. Sci Rep 2021;11:9884.
22. Aronoff GR, Berns JS, Brier ME, et al. Drug prescribing in renal failure. 5th ed. Philadelphia: American College of Physicians; 2007.
23. 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.
24. Ridker PM, Everett BM, Pradhan A, et al. Low-dose methotrexate for the prevention of atherosclerotic events. N Engl J Med 2019;380:752-62.
25. Mori S, Hidaka M, Kawakita T, et al. Factors associated with myelosuppression related to low-dose methotrexate therapy for inflammatory rheumatic diseases. PLOS ONE 2016;11:e0154744.
26. Lee JS, Oh JS, Kim YG, Lee CK, Yoo B, Hong S. Methotrexate-related toxicity in patients with rheumatoid arthritis and renal dysfunction. Rheumatol Int 2020;40:765-70.
27. Park GT, Jeon DW, Roh KH, et al. A case of pancytopenia secondary to low-dose pulse methotrexate therapy in a patient with rheumatoid arthritis and renal insufficiency. Korean J Intern Med 1999;14:85-7.
28. Benoit SW, Ciccia EA, Devarajan P. Cystatin C as a biomarker of chronic kidney disease: latest developments. Expert Rev Mol Diagn 2020;20:1019-26.
29. Targońska-Stepniak B, Majdan M. Cystatin C concentration is correlated with disease activity in rheumatoid arthritis patients. Scand J Rheumatol 2011;40:341-6.