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ORIGINAL ARTICLE



Predicting factors for disappearance of anti-mutated citrullinated vimentin antibodies in sera of patients with rheumatoid arthritis

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ABSTRACT

Objectives: We aimed to determine the predicting factors for disappearance of anti-mutated citrullinated vimentin antibody (anti-MCV Ab) in sera from rheumatoid arthritis (RA) patients.

Methods: In 2013, 95 RA patients whose Disease Activity Score with erythrocyte sedimentation rate were moderate to severe (DAS28-ESR ≥ 3.2) at baseline were enrolled. Titers of anti-MCV Ab and anti-cyclic citrullinated peptide (anti-CCP) Ab for 2013 and 2014 were measured. The association of anti-MCV disappearance with disease activity, treatment, interstitial lung disease (ILD), and serum markers of ILD were retrospectively examined. Predicting factors of anti-MCV disappearance were determined by multivariable analysis.

Results: While anti-CCP positivity rate did not change during the year, anti-MCV Ab changed from positive to negative in 18 patients (=19.0%). Continuous biological disease-modifying anti-rheumatic drug use, prednisolone dose (≥ 5.0 mg daily), and low KL-6 level (< 191 U/mL) were determined as predicting factors of anti-MCV disappearance by multivariable analysis. In our cohort, anti-MCV Ab disappearance was not linked to clinical and radiological improvement.

Conclusion: Different from anti-CCP Ab, anti-MCV Ab in sera from RA patients can disappear in a year. Some predicting factors for such negative seroconversion were found, whereas clinical significance of anti-MCV Ab disappearance was undetermined.

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anti-citrullinated protein antibodies; biological disease-modifying antirheumatic drug; interstitial lung disease; rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that injures cartilage and bone by chronic inflammation. Since anti-citrullinated protein/peptide antibodies (ACPA) are specifically observed in RA [1,2], anti-cyclic citrullinated peptide antibody (anti-CCP Ab) is clinically important for diagnosing RA [3]. Additionally, anti-CCP-positive RA patients showed more radiographic progressions than anti-CCP-negative patients [4,5]. Citrullinated vimentin, fibrinogen, type II collagen, and α -enolase are well-known target antigens of ACPA [6]. The association of an individual Ab against each citrullinated autoantigen associated with the pathogenesis of RA has recently been studied. Mutated citrullinated vimentin (MCV), an isoform of citrullinated vimentin, in which glycine residues are replaced by arginine, was found in synovial fluid of RA patients, and ELISA for anti-MCV Ab was developed by Bang et al. [7]. While both anti-MCV and anti-CCP Abs are specific for RA [8], the anti-MCV titer may be more correlated with disease activity and radiographic progression than the anti-CCP titer [9,10]. Recently, anti-MCV Ab was reported to react with

osteoclasts and lead to bone loss [11]. In early RA patients treated with initial therapy, anti-(non-mutated) citrullinated vimentin Ab was the most frequent to disappear among anti-CCP, anti-citrullinated fibrinogen, and anti-citrullinated α -enolase Ab. Moreover, anti-MCV Ab disappearance was correlated with better radiographic outcome [12]. Several studies have reported that ACPA positivity is closely associated with interstitial lung disease (ILD) in RA [13,14]. Moreover, citrullinated proteins are observed in lung tissues with RA-ILD [15]; hence, ACPA genesis in focal organ has been discussed recently. Krebs von de Lungen-6 (KL-6) [16–18] and surfactant protein D (SP-D) [19] were both reported as serum markers of ILD including RA-ILD and demonstrated that its serum levels could be useful for detecting ILD and evaluating ILD activity.

It is believed that immunological remission, which is the seroconversion from positive to negative for autoantibodies (auto-Abs) and is a deeper remission than clinical or imaging remission, may be required for drug-free remission [20]. However, it is rare that anti-CCP Ab titer is decreased to negative range in clinical practice. The purpose of the

present study is to examine the frequency, clinical significance, and predicting factors of negative seroconversion of anti-MCV Ab, which is known to be more pathogenic [11] and be more changeable than anti-CCP Ab [12].

Methods

Patients

From 2013 to 2014, 280 RA patients fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria in Kyoto University RA Management Alliance (KURAMA) cohort. Actually, many patients achieved sustained clinical remission and low disease activity in both 2013 and 2014. In such patients, no major modification of DMARD treatment was observed because treatment target is already achieved. It would be impossible for such patients to examine clinical significance of anti-MCV Ab. Inclusion criteria in this retrospective study, therefore, is to have moderate or high Disease Activity Score-28 (DAS-28) activity with erythrocyte sedimentation rate (ESR) (DAS28-ESR ≥ 3.2) in 2013. Because patients who newly started ($n=5$), stopped ($n=2$), and switched ($n=2$) biological disease-modifying anti-rheumatic drugs (bDMARDs) during the year were excluded for accurate treatment analysis, 95 patients were enrolled in the present study.

The study was designed in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee of Kyoto University Graduate School and Faculty of Medicine (approval number: E458). Before enrollment, written informed consent was obtained from all patients.

ACPA measurement

ELISA kit was used for anti-MCV (Orgentec Diagnostika, Mainz, Germany) and anti-CCP (Medical & Biological Laboratories Co., Nagoya, Japan). According to the manufacturers' instructions, positive values were set to ≥ 20 U/mL for anti-MCV and ≥ 4.5 U/mL for anti-CCP, respectively.

Assessment of ILD

We examined ILD, including serum markers in this study. From chest CT scans, each attending rheumatologist and radiologist determined whether an enrolled patient had ILD. In 2013, KL-6 and SP-D were both measured at baseline. KL-6 was measured by electrochemiluminescence immunoassay (Sekisui Medical Corp., Tokyo, Japan) and SP-D by ELISA (Yamasa Shoyu Corp., Choshi, Japan).

Radiographic evaluation of arthritis

Eighty-four patients were available for scoring radiographs of hands and feet during both years. All radiographs were dually evaluated by two readers and scored using the van der Heijde modified Total Sharp Score (mTSS), which is

based on the sum of joint erosion score (Δ JES) and joint space narrowing (Δ JSN). Each average of the progression score (Δ mTSS) during a year was used for analysis.

Statistical analysis

Univariate analysis was performed using Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables. Logistic regression analysis was used for multivariate analysis. Multivariable analysis was performed using variables with $p < .05$ in the univariate analysis. ILD existence is correlated with both KL-6 and SP-D. Thereafter, multivariate logistic regression analysis was performed separately in three models, which included three correlated variables one by one. Since none of the patients had ILD in the anti-MCV disappearance group, we used Firth's method for ILD variables [21]. To compare anti-MCV disappearance rate in the bDMARD use group with that in the non-use group and in ILD group with that in the non-ILD group, Fisher's exact test was used. The receiver operating characteristic (ROC) curves were conducted to determine the cutoff level of the serum marker of ILD and the prednisolone (PSL) dose for anti-MCV disappearance. The Spearman correlation coefficient was used to determine the correlation of anti-MCV and anti-CCP titers. All analyses were performed using JMP Pro version 12 (SAS Institute Inc., Cary, NC).

Results

Characteristics of the enrolled patients

The profiles and treatments for 95 patients in 2013 and 2014 are shown in Table 1. The mean age was 64.9 years and disease duration was 16.3 years in 2013. The mean of

Table 1. Profiles of enrolled RA patients.

	2013	2014	<i>p</i> Value
Age (years)	64.9 $\pm$ 11.0	65.9 $\pm$ 11.2	–
Female (%)	85.2	–	–
Disease duration (years)	16.3 $\pm$ 12.3	17.3 $\pm$ 12.2	–
Current smoker (%)	21.8	15.0	n.s.
DAS28 (ESR)	4.23 $\pm$ 0.87	3.59 $\pm$ 1.01	<.001
HAQ	1.07 $\pm$ 0.71	1.06 $\pm$ 0.79	n.s.
Δ mTSS (2014–2013)	–	1.00 $\pm$ 2.34	–
Δ JES	–	0.39 $\pm$ 1.22	–
Δ JSN	–	0.60 $\pm$ 1.39	–
ILD (%)	16.8	16.8	–
PSL user (%)	47.3	44.0	n.s.
Dose* (mg/day)	2.4 $\pm$ 3.3	2.5 $\pm$ 3.6	n.s.
csDMARD user (%)	64.2	64.2	–
MTX user (%)	69.4	67.0	n.s.
Dose* (mg/week)	5.0 $\pm$ 4.2	4.4 $\pm$ 3.9	n.s.
bDMARD user (%)	35.8	35.8	–
TNF inhibitor (%)	24.2	24.2	–
Tocilizumab (%)	6.3	6.3	–
Abatacept (%)	5.3	5.3	–

*Average of dose is calculated, including patients who do not take PSL or MTX. bDMARD: biological disease-modifying antirheumatic drug; CCP: cyclic citrullinated peptide; csDMARD: conventional synthetic disease-modifying antirheumatic disease; DAS: disease activity score; ESR: erythrocyte sedimentation rate; HAQ: health assessment questionnaire; ILD: interstitial lung disease; JES: joint erosion score; JSN: joint space narrowing; MCV: mutated citrullinated vimentin; mTSS: van der Heijde modified total sharp score; MTX: methotrexate; PSL: prednisolone; n.s.: not significant.

DAS28-ESR decreased from 4.23 to 3.59 during a year, whereas the health assessment questionnaire (HAQ) remained almost unchanged. Radiographic change evaluated by Δ mTSS was 1.00, change in joint erosion score (Δ JES) was 0.39, and change in joint space narrowing (Δ JSN) was 0.60. In 2013, methotrexate (MTX), PSL use, and bDMARD use was 69.4%, 47.3%, and 35.8%, respectively. Although mean DAS28 at 2014 was 3.59 in the present cohort, 64.8% of the enrolled patients had SDAI remission or low disease activity in 2014 and 47.1% of patients had EULAR good or moderate response.

Correlation between anti-MCV and anti-CCP titers

In the 2013 cohort, anti-MCV and anti-CCP positivity rates were 71.5% ($n = 68$) and 89.5% ($n = 85$), respectively (Table 2). Most (97.0%) of anti-MCV-positive patients had anti-CCP Ab, and there was significant correlation between anti-MCV and anti-CCP titers ($r = 0.67$, $p < .001$). In contrast, there was no correlation between the change in anti-MCV titer and anti-CCP during a year ($r = 0.20$, $p = .04$) (Figure 1). There were no differences in patients' profile and treatment between anti-MCV positive and negative groups in 2013 (Supplementary Table 1).

Table 2. Change in ACPA in each year.

	2013	2014	<i>p</i> Value
Anti-CCP positive (%)	89.5	89.5	–
Anti-MCV positive (%)	71.5	61.0	n.s.
Remained positive	–	52.6	–
Remained negative	–	20.0	–
Changed from negative to positive (appeared)	–	8.4	–
Changed from positive to negative (disappeared)	–	19.0	–
Anti-CCP titer (U/mL)	215 $\pm$ 398	363 $\pm$ 398	n.s.
Anti-MCV titer (U/mL)	718 $\pm$ 1772	382 $\pm$ 833	n.s.

($n = 95$).

CCP: cyclic citrullinated peptide; MCV: mutated citrullinated vimentin; n.s.: not significant.

Change in anti-MCV positive rates between 2013 and 2014

While the anti-CCP positivity rate did not change (89.5%) from 2013 to 2014, the anti-MCV positivity rate changed from 71.5% to 61.0% (Table 2). Specifically, anti-MCV titers changed from positive to negative in 19.0% of the patients (disappeared group) and from negative to positive in 8.4% (appeared group). The 2014 titers remained positive in 52.6% of the patients (remained positive group) and 20% were still negative (remained negative group).

Comparison between the profiles of the anti-MCV remained positive and disappeared groups

To explore the predicting factor for anti-MCV disappearance, we compared the anti-MCV remained positive and disappeared groups by univariate analysis (Table 3). Age and disease duration were similar in both groups, while female patients tended to be frequent in the disappeared group than in the remained positive group (100.0% vs. 80.0%, $p = .052$). While there were no differences in DAS28 and HAQ in 2013 baseline between both groups, Δ HAQ from 2013 to 2014 in the disappeared group was more apparent than that in the remained positive group (-0.18 vs. 0.035 , $p = .038$). Between the remained positive and disappeared groups, no difference was seen in Δ mTSS (1.43 vs. 1.03 , $p = .43$), Δ JES (0.57 vs. 0.27 , $p = .86$), and Δ JSN (0.85 vs. 0.75 , $p = .77$) (Supplementary Figure 1).

At baseline, there was no correlation between anti-MCV Ab titer and DAS28 ($r = 0.17$). Also, correlation between Δ anti-MCV and Δ DAS was not found ($r = 0.08$) (Supplementary Figure 2). Little correlation between Δ anti-MCV Ab titer and Δ HAQ ($r = 0.26$, $p = .01$) was observed (Data not shown).

There were no ILD patients in the disappeared group, while 22.0% of patients had ILD in the remained positive group ($p = .029$). Additionally, KL-6 and SP-D [14–19] were lower in the disappeared group than in the remained positive group at baseline (KL-6 191 vs. 314 U/mL, $p = .0004$;

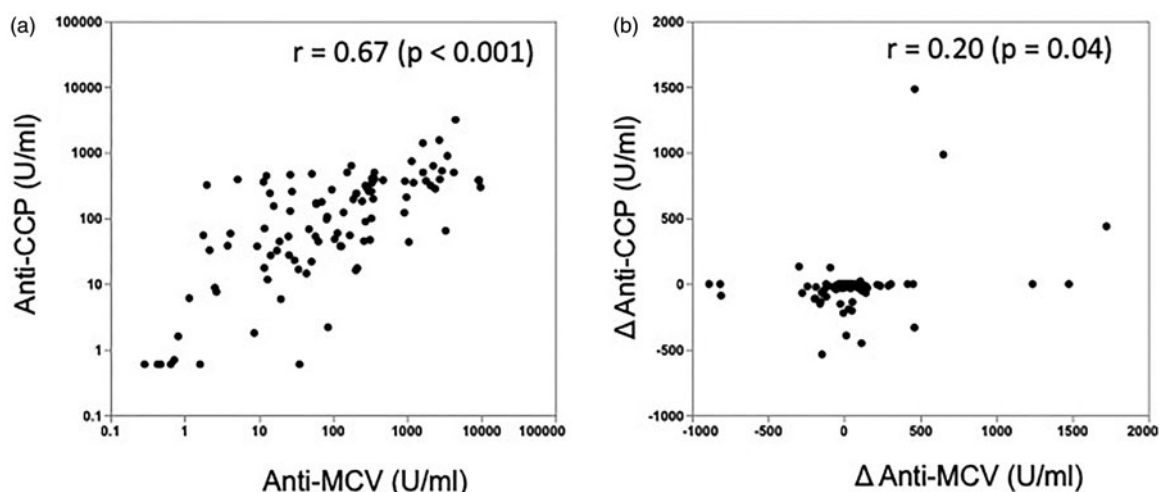


Figure 1. Correlation of anti-CCP with anti-MCV titers. (a) The anti-MCV titers correlated significant with the anti-CCP titers ($r = 0.67$, $p < .001$). (b) There was no correlation between the change of anti-MCV titer (Δ anti-MCV) and anti-CCP (Δ anti-CCP) during a year ($r = 0.20$, $p = .04$).

Table 3. Univariate analysis of the anti-MCV remained positive and disappeared groups.

	Remained positive (n = 50)	Disappeared (n = 18)	p Value
Age	66.6 ± 10.3	60.5 ± 14.5	n.s.
Female (%)	80.0	100.0	n.s.
Disease duration (years)	17.1 ± 14.0	14.8 ± 8.2	n.s.
Current smoker (%)	20.6	30.7	n.s.
Anti-MCV titer in 2013	1205.6 ± 2259.3	428.7 ± 1031.3	.0057
DAS28 in 2013	4.29 ± 0.76	4.29 ± 0.98	n.s.
ΔDAS28 (2014–2013)	−0.74 ± 1.12	−0.87 ± 1.03	n.s.
HAQ in 2013	1.00 ± 0.70	1.29 ± 0.79	n.s.
ΔHAQ (2014–2013)	0.035 ± 0.46	−0.18 ± 0.36	.038
ΔmTSS (2014–2013)	1.43 ± 3.01	1.03 ± 1.91	n.s.
ΔJES	0.57 ± 1.67	0.27 ± 0.51	n.s.
ΔJSN	0.85 ± 1.60	0.75 ± 1.67	n.s.
ILD (%)	22.0	0.0	.029
KL-6 in 2013 (U/mL)	314 ± 213	191 ± 33	.0004
SP-D in 2013 (ng/mL)	67.0 ± 82.2	40.1 ± 29.3	.02
PSL user (%)	40.0	55.5	n.s.
PSL dose* (mg/day)	1.5 ± 2.3	4.4 ± 4.9	.03
csDMARD user (%)	70.0	27.8	.0043
MTX monotherapy (%)	56.0	27.7	n.s.
MTX dose* (mg/week)	5.7 ± 4.4	4.5 ± 4.1	n.s.
bDMARD user (%)	30.0	72.2	.0043
bDMARD use duration* (years)	0.66 ± 1.61	1.77 ± 2.55	.0063
TNF inhibitor	18.0	38.9	–
Abatacept	4.0	11.1	–
Tocilizumab	2.0	22.2	–

*Average of dose and duration is calculated including patients who do not take PSL or MTX or bDMARD.

Duration of bDMARD use is calculated from start to until 2013 and duration of using other bDMARD is excluded.

bDMARD: biological disease-modifying antirheumatic drug; csDMARD: conventional synthetic disease-modifying antirheumatic drug; DAS: disease activity score; HAQ: health assessment questionnaire; ILD: interstitial lung disease; JES: joint erosion score; JSN: joint space narrowing; KL-6: Krebs von de lungen-6; mTSS: van der Heijde modified total sharp score; MCV: mutated citrullinated vimentin; MTX: methotrexate; PSL: prednisolone; SP-D: surfactant protein D; n.s.: not significant.

Table 4. Multivariate analysis of predicting factors for anti-MCV disappearance.

	Model A		Model B		Model C	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Anti-MCV titer in 2013 (U/mL)	1.00 (0.99–1.00)	.63	0.99 (0.99–1.00)	.21	0.99 (0.99–1.00)	.19
ΔHAQ	0.46 (0.10–2.11)	.31	0.48 (0.07–2.68)	.41	0.37 (0.06–1.88)	.23
bDMARD use	5.35 (1.45–19.8)	.01	4.65 (1.19–20.2)	.027	5.95 (1.59–25.7)	.01
PSL dose (by 1 mg/day)	1.22 (1.00–1.48)	.04	1.24 (1.02–1.59)	.027	1.25 (1.04–1.06)	.02
ILD	0.11 (0.006–2.15)	.14	–	–	–	–
KL-6 (by 100 U/mL)	–	–	0.51 (0.17–0.98)	.043	–	–
SP-D (by 10 ng/mL)	–	–	–	–	0.84 (0.66–1.01)	.11

bDMARD: biological disease-modifying antirheumatic drug; HAQ: health assessment questionnaire; ILD: interstitial lung disease; KL-6: Krebs von de lungen-6; MCV: mutated citrullinated vimentin; PSL: prednisolone; SP-D: surfactant protein D.

SP-D 40.1 vs. 67.0 ng/mL, $p = .02$) in 2013. While there was no difference in PSL users between both groups, the PSL dose in the disappeared group was higher than that in the remained positive group (4.4 vs. 1.5 mg/day, $p = .03$). There were no differences in MTX monotherapy and dosage of MTX between both groups. bDMARD were more frequently and used longer in the disappeared group than in the remained positive group (72.2% vs. 30.0%, $p = .0043$; 1.77 ± 2.55 vs. 0.66 ± 1.61 years, $p = .0063$). Also, there was no difference of bDMARD use between in patients with ILD and without ILD (45.4% vs. 40.3%, $p = .75$) in our cohort.

Multivariate analysis of the predicting factors for anti-MCV disappearance

A multivariate analysis was performed to identify the predicting factors for anti-MCV disappearance. We selected anti-MCV titer in base line, ΔHAQ, PSL dose, bDMARD use, ILD complications, and serum markers of ILD, which appeared to be the predicting factors ($p < .05$) in the

univariate analysis. Since ILD (model A) and serum markers of ILD (model B: SP-D and C: KL-6) were correlated, the multivariable analysis was performed separately in three models (Table 4). All models indicated that bDMARD use (model A: OR 5.35, $p = .01$; model B: OR 4.65, $p = .027$; model C: OR 5.95, $p = .01$) and PSL dose (≥ 5.0 mg daily) (model A: OR 1.22, $p = .04$; model B: OR 1.24, $p = .027$; model C: OR 1.25, $p = .02$) were the predicting factors for anti-MCV disappearance. While there was no relation between ILD and anti-MCV disappearance in model A (OR 0.11, $p = .14$) and between SP-D and anti-MCV disappearance in model C (OR 0.84, $p = .11$), lower levels of KL-6 were associated with anti-MCV disappearance in model B (OR 0.51, $p = .043$).

Association between anti-MCV disappearance and bDMARD use

Since the multivariable analysis indicated bDMARD use as an independent predicting factor for anti-MCV

disappearance, we examined anti-MCV disappearance rate in bDMARD users (continuous use between 2013 and 2014) and non-users (Figure 2). Anti-MCV disappearance was more frequently observed in bDMARD users than in non-users (42.3% vs. 10.2%, $p = .005$). Also, decrease of anti-MCV Ab titer was significant in bDMARD users than in non-users (-493 ± 1727 vs. -247 ± 1785 U/mL, $p = .005$).

Association between anti-MCV disappearance and ILD or serum marker of ILD

To consider the possible association between ILD and anti-MCV disappearance, we examined the anti-MCV disappearance rate in ILD and non-ILD patients (Figure 2). While anti-MCV disappearance was not recognized in ILD patients, the disappearance rate was 30.5% in non-ILD patients ($p = .02$). The decreased titer of anti-MCV Ab was not statistically different between in ILD and non-ILD patients (-186 ± 1296 vs. -1075 ± 3156 U/mL, $p = .94$).

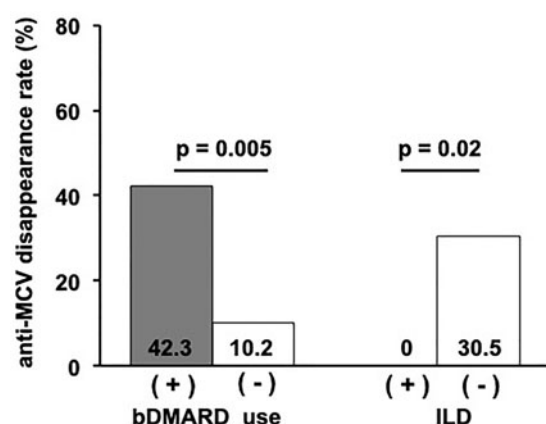


Figure 2. Association of anti-MCV disappearance with bDMARD use and ILD. In 42.3% of bDMARD users, serum anti-MCV Abs disappeared. In RA patients with ILD, anti-MCV disappearance was not recognized. bDMARD: biological disease modifying antirheumatic drug; ILD: interstitial lung disease; MCV: mutated citrullinated vimentin.

By performing ROC analysis, the cutoff KL-6 level for anti-MCV disappearance was estimated to be 191 U/mL with 66.6% sensitivity, 82.7% specificity, and 0.78 AUC (Figure 3(a)). Using this cutoff value, anti-MCV Abs disappeared in the low KL-6 level group (< 191 U/mL) compared to the high KL-6 level group (≥ 191 U/mL) (55.0% vs. 14.0%, $p = .0006$).

Association between anti-MCV disappearance and PSL dose

To examine the amount of PSL that affects anti-MCV disappearance, the cutoff value was estimated by ROC analysis (Figure 3(b)). The cutoff PSL dose was 5.0 mg with 55.5% sensitivity, 84.0% specificity, and 0.65 AUC. Additionally, the anti-MCV disappearance rate was higher in patients with high PSL dose (≥ 5.0 mg daily) than in patients with low PSL dose (< 5 mg daily) (55.8% vs. 16.0%, $p = .0017$).

Predicting factor of anti-MCV appearance

Comparing the anti-MCV appeared group ($n = 8$) with the remained negative group ($n = 19$), no differences were recognized in their background profiles, disease activity, and treatments (Supplementary Table 2). Therefore, the predicting factor for anti-MCV development was not determined.

Discussion

Since anti-CCP disappearance is rare, there are very few reports regarding anti-CCP disappearance [22]. In contrast, anti-Sa Ab, which is identical to anti-citrullinated vimentin Ab, was previously reported to frequently disappear [23]. This is the first report to determine factors predicting anti-MCV disappearance.

Kastbom et al. reported that anti-(non-mutated) citrullinated vimentin Ab may most frequently disappear among

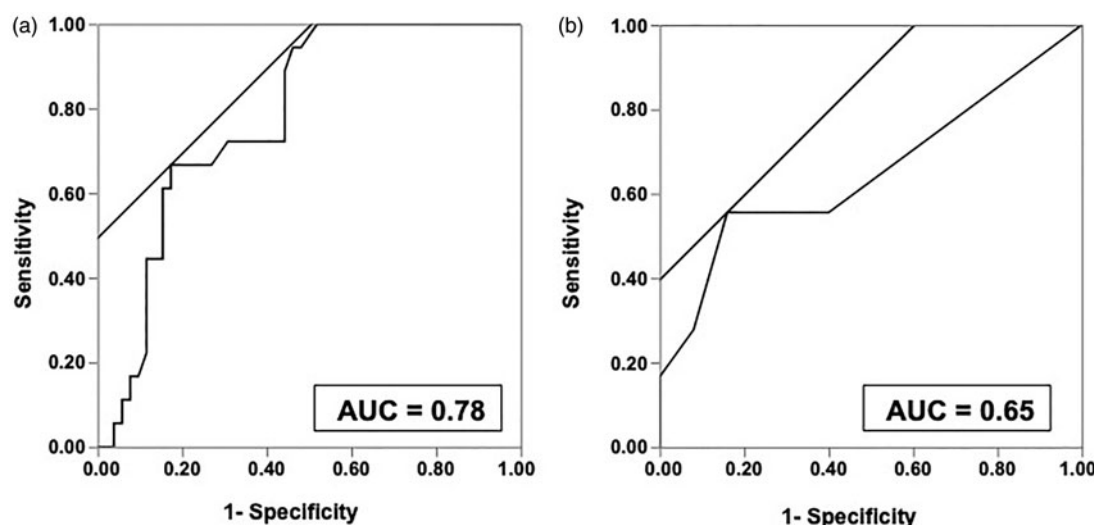


Figure 3. Cutoff values estimated by ROC analysis. (a) KL-6 level for anti-MCV disappearance (< 191 U/mL). In our cohort, 66.6% of sensitivity, 82.7% of specificity, and 0.78 of AUC were recognized. (b) PSL dose for anti-MCV disappearance (≥ 5.0 mg daily). In our cohort, 55.5% of sensitivity, 84.0% of specificity, and 0.65 of AUC were recognized. KL-6: Krebs von de lungen-6; MCV: mutated citrullinated vimentin; PSL: prednisolone.

ACPAs, including anti-CCP, anti-citrullinated fibrinogen, and α -enolase Abs, after intensive RA treatment in the Swedish FarmacoTherapy (SWEFOT) trial [12]. In addition, anti-(non-mutated) citrullinated vimentin Ab is statistically associated with better radiographic outcome [12]. In our cohort, however, there appeared to be no correlations of anti-MCV titers with disease activity and radiographic progression. These results suggested that anti-MCV titer is not always associated with RA disease activity change in our cohort. This discrepancy may be caused by the difference in the observation period as the radiographic evaluation in the SWEFOT trial was performed for 2 years, which is longer than our study (1 year). The enrolled patients were also different; the majority of the SWEFOT trial included early RA patients, while our study included established patients. Additionally, non-mutated citrullinated vimentin was used in the SWEFOT trial, but 'mutated' citrullinated vimentin is used in our study. Serum Abs in RA patients react more actively with 'mutated citrullinated vimentin' than with non-mutated citrullinated vimentin [7].

Infliximab [24] and rituximab [25] are bDMARDs which reportedly decrease the Ab titers against citrullinated vimentin. Similarly, our data indicated that negative seroconversion of anti-MCV Ab can occur with bDMARD treatment. It should be interesting to determine the mode of action that favors anti-MCV disappearance. However, the number of patients using bDMARD in our cohort was too few to answer this important point. Previously, anti-CCP titers were reported to be decreased by rituximab [25], abatacept [26], and tumor necrosis factor (TNF) inhibitors [26]. In addition to rituximab which affects B cells, abatacept is reported to suppress B cells through IL-17 inhibition [27], and TNF inhibitors are also reported to decrease memory B cells [25,28,29] in RA. These mechanisms of B cell downregulation by bDMARD might lead to anti-MCV disappearance. On the other hand, bDMARD treatment can suppress citrulline-reactive Th1 cells in patients with RA [30]. It is possible that bDMARD treatment decreases MCV-reactive T cells by bDMARD use. Whereas it is required to determine the detailed mechanism of bDMARD-mediated anti-MCV Ab disappearance, the sustained use of bDMARD may be preferable for negative seroconversion of anti-MCV Ab.

Harre et al. reported that MCV antigen can be expressed on osteoclast precursors and recognized by anti-MCV Ab [11]. Since bDMARDs can suppress osteoclast formation regardless of their mode of action, MCV antigen on osteoclasts may be downregulated. As the appearance of high titers of ACPA production is antigen-driven [31,32] and T cell-dependent [33–35], another possible mechanism of anti-MCV disappearance in bDMARD users can be attributed to decreased autoantigens.

Our data suggested possible correlation between ILD and anti-MCV disappearance, whereas sustained bDMARD use might be more dominant factor than ILD. The generation of anti-CCP Ab is closely associated with RA-ILD [14]. ACPAs have been detected in sputum from seronegative patients at risk of RA due to family history [36]. In another previous study, ACPA levels were higher in bronchoalveolar

lavage fluid than in serum from ACPA-positive RA patients [37]. These reports suggest that ACPA production may be stimulated by pulmonary tissue modification followed by active vimentin citrullination. Considering these data together with our results, continuous activation of vimentin citrullination might stimulate anti-MCV Ab production. In association with ILD, note that low KL-6 level (< 191 IU/mL) was recognized as the predicting factor for anti-MCV disappearance. Whereas SP-D is a useful marker in RA-ILD, as well as KL-6 [14], SP-D appeared not to be associated with anti-MCV disappearance in our cohort. Both markers are derived from alveolar epithelial type II cells (AECIIs). Although KL-6 is expressed in all phases of AECIIs, SP-D is expressed only in matured AECIIs [38–40]. Our data suggested that the immature phase of AECIIs may also be important for MCV expression.

The PSL dose (≥ 5.0 mg daily), but not PSL use, was one of the predicting factors for anti-MCV disappearance in multivariate analysis. Since PSL is the most effective agent for extra-articular manifestation, vimentin citrullination in other tissues, apart from lungs, may be suppressed by daily dosage of PSL at 5 mg or higher.

In conclusion, we strongly suggest that bDMARD use, ≥ 5.0 mg daily dose of PSL, and low KL-6 level are the independent predicting factors for anti-MCV disappearance. Additionally, anti-MCV disappearance occurs particularly in RA patients without ILD. There are, however, some limitations in the present study. Our data is retrospective observation of small number of patients in a single center. Also, most patients had established RA. To determine clinical significance of anti-MCV disappearance in RA patients, a prospective study using larger size of patient cohort should be conducted.

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Conflict of interest

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