Relationship between cellular communication network factor 1 (CCN1) and carotid atherosclerosis in patients with rheumatoid arthritis

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ABSTRACT

Background: The cellular communication network factor 1 (CCN1) is one of the matricellular proteins of the CCN family involved in chronic inflammatory disorders like rheumatoid arthritis (RA) and involved in human atherosclerotic lesions. This study was aimed to assess the levels of serum CCN1 in patients with rheumatoid arthritis (RA), evaluating its relation to carotid intima-media thickness (CIMT) and predisposition to subclinical carotid atherosclerosis and its impact on activity of RA disease.

Materials and Methods: This is a case-control study that included 105 RA patients classified into active and inactive groups according to disease activity score (DAS28) with 50 healthy matched controls. Clinical and laboratory assessments were done including enzyme-linked immunosorbent assay (ELISA) measurement of CCN1 with a bilateral assessment of CIMT using high resolution-ultrasonography. Comparison of CCN1 between RA patients and controls, a correlation between CCN1, DAS28, swollen joint count (SJC), tender joint count (TJC), and CIMT were analyzed.

Results: There was significant elevation of CCN1 in RA patients compared to controls (235.62±62.5 vs. 73.11±18.2, respectively). The cut off value of CCN1 was 99.25 pg/ml, with an area under the curve (AUC) =0.995, p<0.001, 98 % sensitivity and 95% specificity. CCN1 was inversely correlated with DAS28 and its components in both active and inactive RA patients (r=- 0.92, r=- 0.94, p<0.001). CCN1 was inversely correlated with SJC (r= -0.64, r= - 0.67, p<0.001), TJC (r=- 0.56, r= - 0.63, p<0.001), and with Larsen x-ray score (r=- 0.68, r= - 0.78, p<0.001) in both active and inactive RA patients, respectively. The CCN1 levels in active RA patients were significantly lower than that in patients with low disease activity. A significant positive correlation between CCN1 levels and CIMT in RA patient groups (r=0.88, r=0.47, p<0.001, respectively) was found.

Conclusion: Serum CCN1 could be a helpful biomarker in the diagnosis of RA, associated with RA remission. Disruption of serum CCN1 is engaged in the pathogenesis of atherosclerosis in RA patients which could be a clue for a future treatment strategy of atherosclerosis in RA by controlling CCN1 disruption. Regular follow-up of RA patients is recommended for early detection of subclinical atherosclerosis. New research ideas for controlling CCN1 disruption as new aspects of atherosclerosis treatment in RA patients are needed.

KEYWORDS:
Atherosclerosis; the cellular communication network factor 1 (CCN1); disease activity; rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune chronic inflammatory disorder of unknown cause with a female to male ratio of 3:1 manifested with articular damage and disability in addition to extra-articular manifestations affecting multiple organs like the heart, lungs, eyes, and mouth.[1-4] Atherosclerosis is an important complication of RA mostly due to chronic inflammation, which requires continuous follow up of those patients.[5,6]

RA involves symmetric small and large synovial joints causing pain, swelling, and stiffness.[1] Gradual onset polyarthralgia with symmetrical, intermittent, and migratory joint involvement, especially in the hands and feet are the most typical clinical presentations of RA.[3,4] The chronic pain leads to joint destruction and disability that usually progresses from peripheral to more proximal joints.[3]

Clinical symptoms in combination with an erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), Anti-cyclic citrullinated peptide (Anti-CCP), and X-ray are the main lines of RA diagnosis and follow up.[4,7,8] For the detection of RA, combined RF and anti-CCP have sensitivity and specificity of 90.2% and 83.3% respectively. However, they cannot differentiate patients with the active disease from those in remission.[8]

Thus, there is a need to establish an accurate diagnostic biomarker for RA.[7] The matricellular protein cellular communication network factor 1 (CCN1) is a novel extracellular matrix protein of the CCN family that consists

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of six distinct members (CCN1–6) encoded by immediate early gene due to growth factor response.[9] Specific integrins and heparin cell surface sulfate proteoglycans co-receptors mediate the function of CCN1.[10] Several studies reported high expression of CCN1 protein in synovial fluid, fibroblast-like synoviocytes (FLS), and peripheral blood mononuclear cells of RA patients.[11] So, it can be used as a diagnostic marker to distinguish RA patients from healthy controls and patients with other autoimmune diseases.[11]

CCN1 which is also called Cysteine-rich angiogenic inducer 61 (Cyr61) has multiple well-established functions including the ability to regulate a wide range of cell functions like cell growth and adhesion and participates in inflammation, neovascularization, and thrombosis. Disruption of CCN1 leads to several disorders and leads to the bad prognosis of vascular diseases, cancers, and chronic inflammatory diseases like RA.[10,11] At the same time, CCN1 could strongly inhibit the migration of immune cells, having anti-osteoclastogenic and anti-inflammatory properties.[11]

At the cellular level, purified CCN1 supports cell adhesion, stimulates cell migration, enhances mitogenesis, promotes cell survival, and induces chondrogenic differentiation in limb mesenchyme.[12] Also, the expression of genes involved in angiogenesis and matrix remodeling is induced by CCN1, So, the control of these processes might underlie the biological roles of CCN1 in several disorders, such as vessel morphogenesis, skeletal development, wound repair, and tumor growth.[11]

In RA, it was reported that CCN1 mRNA was strongly increased in lymphoblastoid B cell lines derived from RA discordant monozygotic twins, being one of the three most overexpressed genes.[11] Also, it was found that CCN1 could not only stimulate IL-6 production by FLS via the CCN1/$\alpha v\beta 5$/Akt/NF-κB signaling pathway but also promote neutrophil infiltration via upregulation of IL-8 production in RA-FLS.[13] A recent study demonstrated that CCN1 promoted vascular endothelial growth factor expression in osteoblasts through negative regulation of miR-126 via the PKC-α signaling pathway and increased endothelial progenitor cell angiogenesis in RA.[14]

Recently the relationship between CCN1 and vascular diseases has been reported. CCN1 immunoreactivity was significantly associated with myocardial ischemia, interstitial edema, and coronary arteries atherosclerosis.[11,15] Few studies have explored the relationship between RA disease activity and serum CCN1 levels demonstrating that CCN1 is inversely correlated with DAS28 in RA patients and all disease activity indices including swollen joint counts (SJC) and tender joint counts (TJC), ESR, and CRP. The CCN1 levels were observed highest in the low TJC/SJC group and decreased in patients with a high number of TJC/SJC.[11] So, this study aimed to assess the levels of serum CCN1 in patients with RA, evaluating its impact on disease activity and its relation to carotid intima-media thickness (CIMT) and predisposition to subclinical carotid atherosclerosis.

The *in vitro* effect of CCN1 on cell cultures was explored previously in several studies, where it was founded that IL-6 production was decreased by CCN1 knockdown in fibroblast-like synoviocytes (FLS). Also, these studies showed that IL-6 production is activated by CCN1 via the avb5/Akt/NF-kB signaling pathway. A co-culture system was used consisting of purified CD4+ T cells and RA FLS and it was founded that RA FLS stimulated Th17 differentiation, and the pro-Th17 differentiation effect of RA FLS can be attenuated or stimulated by CCN1 RNA interference or addition of exogenous CCN1, respectively.[16-18]

The *in vitro* effect of CCN1 in atherosclerosis was also reported in previous studies where it was founded that CCN1 had an in vitro effect on smooth muscle rich tissues demonstrating that mechanical strain-dependent induction of the CCN1 gene involves signaling cascades through RhoA-mediated actin remodeling and the p38 stress-activated protein kinase (SAPK).[19,20]

MATERIALS AND METHODS

Study design and patient population

This case-control study included 105 RA patients according to Fan et al., 2019 with at least 80% power at two-sided 95% significance level and the ratio of case/control 2:1. Recruited from the rheumatology clinic in Menoufia University (MU), Egypt, from December 2018 to December 2019 with 50 healthy age and gender-matched controls.

Study participants

RA patients fulfilled the 2010 American College of Rheumatology (ACR) classification criteria for RA and their age was > 18 years.[21] 50 healthy subjects with matched age and gender were recruited as a control group.

Subjects with peripheral vascular disease, familial dyslipidemia, and subjects with conditions known to affect serum CCN1 levels including cancer, infection except after 3 to 6 months, liver diseases, coronary heart diseases, hypothyroidism, renal disorder (serum creatinine: ≥3.0 mg/dl or creatinine clearance: ≤30 ml/min), and other autoimmune diseases were excluded from this study.

Ethical approval and informed consent

This study was approved by the Institutional Review Board of MU, Egypt (approval IRB no.19102018INTPH1) and was carried following the Declaration of Helsinki ethics. Informed consent was taken from all subjects included in this study.

Clinical assessment

All patients underwent history taking including disease duration, special habits, cumulative steroid dose in the previous year, and clinical atherosclerosis symptoms like intermittent claudication, chest pain, fatigue, or confusion.

Clinical and physical assessment including morning stiffness, TJC, SJC and visual analogue scale during the last week on a scale between 0 and 10 mm, where 0 is no pain and 10 is the highest level of pain was done for RA patients.[22] Disease activity was assessed by disease activity score including 28 joint counts (DAS28) categorising the disease activity into high, moderate, low disease activity, and remission.

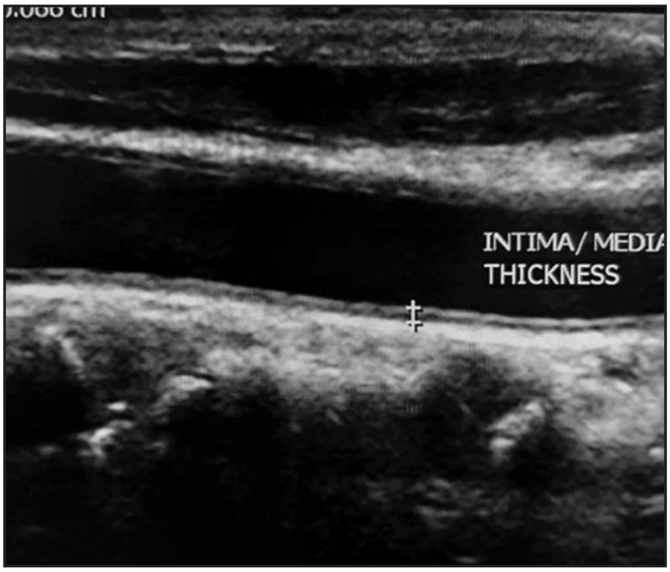

Fig. 1: Longitudinal grayscale ultrasound image at the level of common carotid artery showing intima/ media thickness = 0.66 mm.

RA patients were divided into two groups according to DAS28: patients with moderate to high disease activity (DAS28 ≥3.2 defined as active RA patients) and patients with low disease activity to remission (DAS28< 3.2 defined as inactive RA patients).[11]

Laboratory assessment and immunoassays
Complete blood picture, blood urea, serum creatinine, and ESR (by Westergren pipette)[23] were done. Lipid profile was done including total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG).[24] Serum samples of the patients were analyzed for Anti-CCP antibodies by enzyme-linked immunosorbent assay (ELISA) (Immunoscan RA CCP2, Euro-Diagnostica, Arnhem, the Netherlands) according to the manufacturer's instructions. The results for the Anti-CCP antibody were reported qualitatively where it is considered positive at serum levels ≥20.0U/ml.[25]

RF titer was obtained using the latex agglutination method (RF Direct Latex; EDALAB, France) and the normal range for RF is less than 14 IU/ml.[25] Laboratory assessment for CRP titer (SPINREACT, S.A/S.A.U Ctra. Santa Coloma.7 E-17176, and Spain) was done.

Undiluted serum samples were obtained to assess serum CCN1 by ELISA kit [ELISA Kit for Cysteine Rich Protein, Angiogenic Inducer 61(CYR61) – Cloud –Clone Corp, Katy, Texas, USA] following all internal manufacture procedure. The absorbance was measured at 450 nm and a standard curve was used to calculate serum CCN1 concentration.[26]
Radiological assessment:

Plain x-rays on both hands, wrists, and feet was done and graded by Larsen score from 0 where the joints are normal to 5 where there are mutilating abnormalities.[27] The radiological findings were graded by the same radiologist who was blinded to DAS28.

CIMT assessment:
Bilateral assessment of CIMT was done using high-resolution ultrasonography (Philips-HD11XE with multi-frequency linear 7-12 MHz transducer) after 15 minutes rest and the participants were examined in a supine position with neck extension and the chin turned contralateral to the side being examined. All patients and controls underwent the same scanning technique (Figure. 1). An average of CIMT of right and left common carotid arteries were used. CIMT ranged from 0.59-0.95mm is considered abnormal and 1.0 mm or more is considered high risk.[1,28]

Statistical analysis
SPSS version 20 with IBM compatible computer was used for statistical analysis. Number and percent for qualitative data and mean, standard deviation, and range for quantitative data were used. For comparison between groups having quantitative variables and comparison between two groups not normally distributed Student's t-test and Mann-Whitney test (U) were used, respectively. A one-way ANOVA test was used to compare between more than two groups having quantitative variables. A comparison between more than two groups with unequal distribution having quantitative variables was done using the Kruskal Wallis test. To study the association between two qualitative variables Chi-squared test (χ^2) was used. To correlate between two quantitative variables, the Pearson correlation coefficient test was used. Spearman correlation was used to correlate between not normally distributed quantitative variables. For all statistics, a p-value of ≤0.05 was statistically significant and ≤0.001 was highly significant. The receiver-operating characteristic (ROC) curve was used to determine the cutoff point of CCN1 in terms of sensitivity and specificity.[29]

RESULTS

A total of 105 RA patients classified into active and inactive groups according to DAS28 with fifty age and gender-matched controls were included.

Demographic and clinical characteristics of the studied groups
Active RA patients included 14 males (31.1%) and 31 females (68.9%) with a mean age of 48.62±13.33 years. Inactive RA patients included 18 males (30 %) and 42 females (70 %) with a mean age of 43.55±12.19 years. Controls were 17 males (34%) and 33 females (66%) with a mean age of 46.72±11.63 years with no statistically significant difference (p>0.05) between them regarding demographic characteristics, ensuring homogeneity of both groups (Table I).

A significant increase in the ESR, VAS, cholesterol, LDL, CCN1, and CIMT in RA patients compared to controls was found with significant differences regarding DAS28, SJC, TJC, Larsen x-ray score, and disease duration between both RA groups (active & inactive). The mean disease duration for RA patients was 96.26±56.76 months for the active group and 65.70±47.93 months for the inactive group. RF was positive in 77 RA patients (73.3%) and 78 RA patients had positive Anti-CCP antibodies (74.3%) (Table I).

Table I: Demographic and clinical characteristics of the studied groups

Demographic characteristics	Studied groups			Test of significance	P-value	Post Hoc test
	Active RA (n=45) Mean±SD	Inactive RA (n=60) Mean±SD	Controls (n=50) Mean±SD			
Age (years)	48.6±13.33	43.5±12.19	46.7±11.63	F=2.27	0.11	___
Sex: No. (%)						
female	31 (68.9%)	42 (70%)	33 (66%)	χ^2 = 0.2	0.90	___
male	14 (31.1%)	18 (30%)	17 (34%)			
Disease duration (months)	96.26±56.76	65.70±47.93	__	U=2.83	0.005	___
DAS 28	4.59±0.79	2.36±0.55	___	t=16.91	<0.001	___
SJC 2.42±0.81	1.50±0.56	___	U = 6.85	<0.001	___	
TJC 4.40±0.91	2.38±0.86	___	U = 11.53	<0.001	___	
X-RAY SCORE (Larsen score)	3.31±0.70	1.20±0.73	___	U = 14.88	<0.001	
RF positive No. (%)	35 (77.8%)	42 (70 %)	___	χ^2 = 0.45	0.51	
Anti-ccp Positive No. (%)	36 (80%)	42 (70%)	___	χ^2 = 0.87	0.34	
ESR (mm/hour)	62.75±21.96	14.61±2.86	10.42±1.65	K= 113.82	<0.001	P1< 0.001 P2 < 0.001 P3= 0.002
VAS	6.42±1.55	3.45±1.36	1.60±0.74	K= 98.15	<0.001	P1 < 0.001 P2 < 0.001 P3 < 0.001
CCN1 (pg/ml)	200.82±37.21	261.73±65.14	73.11±18.24	F=188.34	<0.001	P1 < 0.001 P2 < 0.001 P3 < 0.001
Cholesterol (mg/dl)	172.75±31.98	163.17±21.09	142.15±20.53	F=16.71	<0.001	P1= 0.05 P2 < 0.001 P3 < 0.001
HDL (mg/dl)	41.62±2.46	41.55±3.42	42.15±2.38	F=0.57	0.56	___
LDL (mg/dl)	112.51±17.44	120.58±17.45	107.28±17.59	F=7.33	0.002	P1= 0.02 P2 = 0.17 P3 < 0.001
Triglycerides (mg/dl)	83.95±20.53	88.71±21.79	87.7±21.78	F= 0.66	0.51	___
Mean CIMT (mm2)	0.79±0.16	0.72±0.19	0.35±0.03	F=113.16	<0.001	P1= 0.04 P2 < 0.001 P3 < 0.001

t= t-test, U= Mann-Whitney test, F: Anova test, K: Kruskal Wallis test, χ2: chi-square test, P1: between active RA group and inactive RA, P2: between active RA group and control, P3: between inactive RA group and control group. HDL= high-density lipoproteins, LDL= low-density lipoproteins, CIMT= carotid intima-media thickness, ESR= erythrocyte sedimentation rate, VAS= visual analogue scale, RF= rheumatoid factor, DAS28= disease activity score, SJC= swollen joint count, TJC= tender joint count.

Table II: Comparison between RA patients and controls regarding Cyr61, lipid profile, and CIMT

Studied parameters	Studied groups		Test of significance	P-value
	RA (n=105) Mean±SD	Controls (n=50) Mean±SD		
CCN1 (pg/ml)	235.62±62.53	73.11±18.24	24.07	<0.001
Cholesterol (mg/dl)	167.28±26.60	142.15±20.53	5.38	<0.001
H.DL(mg/dl)	41.58±3.03	42.15±2.38	1.06	0.28
L.D.L(mg/dl)	117.12±17.82	107.28±17.59	2.98	0.003
Triglycerides (mg/dl)	86.67±21.29	87.67±21.78	0.25	0.80
mean CIMT (mm2)	0.75±0.24	0.35±0.03	U=10.49	<0.001

U= Mann-Whitney test, HDL= high-density lipoproteins, LDL= low-density lipoproteins, CIMT= carotid intima-media thickness.

Table III: Clinical performance of Cysteine-rich 61 (Cyr61), RF, & Anti-CCP in RA patients

Studied parameters	Optimal cutoff point	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Diagnostic accuracy (95%CI)	DOR (95%CI)
CCN1 (pg/ml)	99.25	19.62 (5.08-75.76)	97% (93–99)	95% (82–99)	98% (93–100)	95% (82–99)	98% (93–100)
RF (mg/dl)	8.11	73% (64–81)	82% (67–92)	92% (83–96)	54% (41–67)	76% (68–82)	12.96 (5.15–32.64)
Anti-CCP (u/ml)	17.08	74% (65–82)	78% (61–89)	90% (81–95)	53% (40–66)	75% (67–82)	9.95 (4.20–23.55)

95% CI= 95% confidence interval, PPV=Positive predictive value, NPV= Negative predictive value, DOR= diagnostic odds ratio.

Table IV: Correlation between CCN1, disease duration, DAS 28, ESR, VAS, and CIMT in active and inactive RA patients

Studied parameters	CCN1 (pg/ml)			
	Inactive RA (n=60)		Active RA (n=45)	
	P value	r	P value	r
Disease duration (months)	- 0.07**	0.66	- 0.27**	0.03
DAS 28	- 0.92*	<0.001	- 0.94*	<0.001
SJC	- 0.67*	<0.001	- 0.64	<0.001
TJC	- 0.63	<0.001	- 0.56	<0.001
Larsen score	- 0.78	<0.001	- 0.68	<0.001
ESR (mm/hour)	- 0.44*	0.002	- 0.82*	<0.001
VAS	- 0.65*	<0.001	- 0.49*	<0.001
CIMT (mm2)	0.88*	<0.001	0.47*	<0.001

*Pearson correlation coefficient, **Spearman correlation coefficient, VAS= visual analogue scale, DAS28= disease activity score, SJC= swollen joint count, TJC= tender joint count, ESR= erythrocyte sedimentation rate, CIMT= carotid intima-media thickness, RA= rheumatoid arthritis.

According to DAS 28, there were 45 (42.85%) active RA patients and 60 (57.14%) inactive RA patients. 32 active RA patients (71.1%, $p<0.001$) and only 2 inactive RA patients (3.3%, $p<0.001$) were on corticosteroid treatment (Table I).

CCN1 levels, lipid profile, and CIMT measurements in RA patients and controls

There were significant differences regarding Cholesterol and LDL between RA patients and controls ($p<0.001$ and $p=0.003$, respectively) with no significant differences regarding HDL ($p=0.28$) and triglycerides ($p=0.80$). Serum levels of CCN1 were significantly higher in RA patients compared to healthy controls (235.62±62.53 vs. 73.11±18.24, $p<0.001$, respectively). CIMT was increased in RA patients compared to controls (0.75±0.24 vs. 0.35±0.03, respectively). This means that the CCN1 could differentiate RA patients from controls and is associated with atherosclerosis development risk (Table II).

Clinical performance of CCN1, RF & Anti- CCP in RA

Table III illustrates the clinical performance of CCN1, RF, & Anti-CCP in RA patients. Serum CCN1 can discriminate RA patients from healthy controls with an area under the curve of 0.995 (95% CI 0.98 to 0.100, $p<0.001$). The optimal cutoff point of CCN1 equals 99.25 pg/ml with a sensitivity of 98% and specificity of 95% with positive predictive value (PPV) of 98% and negative predictive value (NPV) of 95 % (Table III).

CCN1and CIMT in active and inactive RA patients

Serum levels of CCN1were significantly higher in inactive RA patients compared to active RA patients (261.73±65.14 vs. 200.82±37.21, $p<0.001$, respectively). Pearson correlation showed that CCN1 serum levels were inversely correlated with DAS28 (r= - 0.94, r= - 0.92, $p<0.001$), SJC (r= - 0.64, r= - 0.67, $p<0.001$), TJC (r= - 0.56, r=- 0.63, $p<0.001$), Larsen score (r= - 0.68, r= - 0.78, $p<0.001$), ESR (r=- 0.82, $p<0.001$), and VAS (r=- 0.49, $p<0.001$), in active and inactive RA patient groups respectively (Table IV).

CIMT was significantly high in RA patients compared to controls (0.75±0.24 vs. 0.35±0.03, respectively) and Pearson correlation showed that CCN1 serum levels were positively correlated with CIMT in active and inactive RA patient groups (r=0.47, $p<0.001$, r=0.88, $p<0.001$), respectively (Table IV).

DISCUSSION

The matricellular protein, CCN1 is encoded by an immediate-early gene induced by growth factor and it is transcriptionally activated within minutes of stimulation by injury stimuli especially inflammation. However, it is expressed at low levels in quiescent cells.[11]

CCN1 controls the cell cycle, stimulates chemostasis, and augments the growth factor effects.[30] It also has an important role in angiogenesis by promoting the survival of the endothelial cells and stimulating pro-angiogenic factors.[13] The expression of CCN1 was found to be high in peripheral blood mononuclear cells fibroblast-like synoviocytes (FLS) and synovial fluid of RA patients.[13,31]

In this study, a high expression of CCN1 in RA patients was reported compared to the healthy control group exploring its value in discriminating RA patients from healthy controls. This was consistent with previous preclinical studies showing overexpression of CCN1 in the synovial fluids and peripheral blood mononuclear cells of RA patients.[10,31]
RA patients are twice likely to develop sudden cardiac death attributed mostly (50%) to cardiovascular disease. The histochemical analysis in individuals who died of sudden cardiac death revealed that CCN1 was significantly elevated (80%) and associated with myocardial ischemia and atherosclerosis of coronary arteries.[6,32] Rawla et al., supported this hypothesis reporting that the prevalence of cardiovascular diseases in patients with RA is high and multifactorial.[6]

CCN1 was significantly high in RA patients compared to controls with a statistically significant positive correlation with CIMT. Studies by Rawla et al., and Deng et al., are consistent with our results reporting the important role of CCN1 in atherosclerosis pathogenesis.[6,32]

Several studies have demonstrated the association between CCN1 and various aspects of atherosclerosis demonstrating that it is highly expressed in atherosclerotic plaques, contributing to the development of cardiovascular and cerebrovascular diseases and peripheral arterial diseases.[14,33] Besides, CCN1 levels were associated with rapid mortality in acute heart failure (AHF) patients and coronary heart disease (CAD) and could be a potential marker of myocardial ischemic injury and prognosis in patients with the acute coronary syndrome (ACS).[34,35]

Furthermore, CCN1 expression in human atherosclerotic lesions was significantly elevated.[32] This comes in agreement with our study reporting that CCN1 is a predisposing factor for atherosclerosis in RA patients in combination with hyperlipidemia and other factors including the chronic inflammatory nature of the disease.

Interestingly, serum CCN1 was more elevated in inactive RA patients than those with active disease. Spearman correlation analysis revealed that CCN1 levels were negatively correlated with almost all disease activity indices in statistics [Tables I, IV]. When RA patients were stratified by numbers of TJC and SJC, the CCN1 levels were the highest in patients with a low number of TJC and SJC and decreased in active patients with an increasing number of TJC and SJC. These results were supported by Fan et.al., and Woo et al.,[11,36] who reported significantly high levels of CCN1 in RA patients compared to controls (211.57 vs. 37.24, respectively) with negative correlation with DAS28 ($r = -0.174$, $p = 0.010$).

There was also a negative correlation between CCN1 and DAS28 which is complicated in its explanation. In this study, the negative correlation of CCN1 with disease activity is attributed to the strong anti-inflammatory protective activities of CCN1 promoting tissue repair which is accompanied by inflammation resolution.

To explore the role of CCN1 in pulmonary hypertension associated with systemic lupus erythematosus, a multi-center study revealed that patients with higher CCN1 levels had better survival than those with lower levels.[12] However, the significant-up regulation of CCN1 expression in the development and progression of arthritis in RA was reported.[31,37]

CCN1 has a critical role in promoting recovery and mucosal healing in colitis.[38] Exogenous administration of CCN1 accelerated mucosal restitution of colitis in wild type, suggesting a therapeutic potential for CCN1 in inflammatory bowel disease (IBD).[38] IBD and RA share important pathogenesis mechanisms, especially the contribution of the Th1/Th2 cytokine balance.[39]

Regarding both sensitivity and specificity, ROC analysis revealed that CCN1 had higher sensitivity and specificity (98 % and 95%, respectively) compared to both RF and Anti-CCP with a cutoff point of 99.25 pg/ml (AUC of 0.995, 95% CI 0.98 - 0.100, p-value <0.001) exploring the ability of CCN1 to discriminate RA patients from healthy controls and supporting our hypothesis that CCN1 could be used as a diagnostic tool of RA (Table III). These results are consistent with Fan et al., who reported CCN1 sensitivity of 92.09% and specificity of 98.00 in RA patients.[11]

This study explored that serum CCN1 levels had a positive correlation with CIMT predisposing to atherosclerosis as a RA comorbidity. Serum CCN1 levels were significantly elevated in RA patients compared to healthy controls with a negative correlation with RA disease activity. To the best of our knowledge, this study is one of the early studies exploring the effect of CCN1 on CIMT in RA patients. However, further research suggestions for controlling CCN1 disruption as new aspects of treatment of atherosclerosis in RA are needed.

LIMITATIONS OF THE STUDY

This study has some limitations, firstly, the protective role of CCN1 in RA needs to be further assessed by using more precise animal experiments and clinical studies with a larger sample size. Secondly, a long-term follow-up duration in order to evaluate the CCN1 level and its correlation with CIMT in RA patients is needed. Lastly, patients with hyperlipidemia should have been excluded from this study to explore the effect of CNN1 on CIMT.

CONCLUSIONS

Serum CCN1 can be a helpful biomarker in RA diagnosis, associated with RA remission. Disruption of serum CCN1 is involved in the pathogenesis of atherosclerosis in RA patients which could be a clue for a future treatment strategy of atherosclerosis in RA by controlling CCN1 disruption. Regular follow-up of RA patients is recommended for early detection of subclinical atherosclerosis.

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AVAILABILITY OF DATA AND MATERIALS

The data sets during and/or analyzed during the current study available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Karami M, Mohammadzadeh Z, Ebrahimian S, Moradi M, Zahra S. Ultrasonography of articular and periarticular structures as a relapse predictor in patients with clinically remitted rheumatoid arthritis. Indian J Rheumatol 2020; 15: 17-22.
2. Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, et al. Rheumatoid arthritis. Nat Rev Dis Primers 2018; 4: 18001.
3. Pelechas E, Kaltsonoudis E, Voulgari PV, and Drosos AA. Rheumatoid Arthritis. In: Pelechas E, Kaltsonoudis E, Voulgari PV, and Drosos AA, (eds.). Illustrated Handbook of Rheumatic and musculoskeletal Diseases. Cham: Springer International Publishing, 2019, p. 45-76.
4. Sparks JA. Rheumatoid Arthritis. Ann Intern Med 2019; 170: ITC1-ITC16.
5. Abd El-Monem S, Ali A, Hashaad N, Bendary A, and Abd El-Aziz H. Association of rheumatoid arthritis disease activity, the severity with electrocardiographic findings, and carotid artery atherosclerosis. EgyRAR 2019; 46: 11-20.
6. Rawla P. Cardiac and vascular complications in rheumatoid arthritis. Reum 2019; 57: 27-36.
7. de Brito Rocha S, Baldo DC, and Andrade LEC. Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. Adv Rheumatol 2019; 59: 2.
8. Shen R, Ren X, Jing R, et al. Rheumatoid Factor, Anti-Cyclic Citrullinated Peptide Antibody, C-Reactive Protein, and Erythrocyte Sedimentation Rate for the Clinical Diagnosis of Rheumatoid Arthritis. Lab Med 2015; 46: 226-9.

9. Wei Y, Peng L, Li Y, et al. Higher Serum CCN3 Is Associated with Disease Activity and Inflammatory Markers in Rheumatoid Arthritis. J. Immunol Res 2020; 2020: 3891425.
10. Zhang H, Lian M, Zhang J, et al. A functional characteristic of cysteine-rich protein 61: Modulation of myeloid-derived suppressor cells in liver inflammation. J Hepatol (Baltimore, Md) 2018; 67: 232-46.
11. Fan Y, Yang X, Zhao J, et al. Cysteine-rich 61 (Cyr61): a biomarker reflecting disease activity in rheumatoid arthritis. Arthritis Res Ther 2019; 21: 123-.
12. Fan Y, Zhao J, Qian J, et al. Cysteine-rich protein 61 as a novel biomarker in systemic lupus erythematosus-associated pulmonary arterial hypertension. Clin. Exp.Rheumatol 2019; 37: 623-32.
13. Chaqour B. Caught between a "Rho" and a hard place: are CCN1/CYR61 and CCN2/CTGF the arbiters of microvascular stiffness? Cell Commun. Signal 2020; 14: 21-9.
14. Feng B, Xu G, Sun K, Duan K, Shi B, and Zhang N. Association of serum Cyr61 levels with peripheral arterial disease in subjects with type 2 diabetes. Cardiovasc. Diabetol 2020; 19: 194.
15. Zhao J, Zhang C, Liu J, et al. Prognostic Significance of Serum Cysteine-Rich Protein 61 in Patients with Acute Heart Failure. Cell. Physiol. Biochem 2018; 48: 1177-87.
16. Lin J, Zhou Z, Huo R, et al. Cyr61 Induces IL-6 Production by Fibroblast-like Synoviocytes Promoting Th17 Differentiation in Rheumatoid Arthritis. J. Immunol 2012; 188: 5776.
17. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, and Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat. Immunol 2007; 8: 942-9.
18. Chen C-Y, Fuh L-J, Huang C-C, et al. Enhancement of CCL2 expression and monocyte migration by CCN1 in osteoblasts through inhibiting miR-518a-5p: implication of rheumatoid arthritis therapy. Sci Rep 2017; 7: 421.
19. Hanna M, Liu H, Amir J, et al. Mechanical regulation of the proangiogenic factor CCN1/CYR61 gene requires the combined activities of MRTF-A and CREB-binding protein histone acetyltransferase. J Biol Chem 2009; 284: 23125-36.
20. Ziegler T, Abdel Rahman F, Jurisch V, and Kupatt C. Atherosclerosis and the Capillary Network; Pathophysiology and Potential Therapeutic Strategies. Cell J 2019; 9: 50.
21. Aletaha D, Neogi T, Silman AJ, 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.
22. Klimek L, Bergmann K-C, Biedermann T, et al. Visual analogue scales (VAS): Measuring instruments for the documentation of symptoms and therapy monitoring in cases of allergic rhinitis in everyday health care: Position Paper of the German Society of Allergology (AeDA) and the German Society of Allergy and Clinical Immunology (DGAKI), ENT Section, in collaboration with the working group on Clinical Immunology, Allergology and Environmental Medicine of the German Society of Otorhinolaryngology, Head and Neck Surgery (DGHNOKHC). Allergo J Int 2017; 26: 16-24.
23. Popov N and Stanisheva S. Erythrocyte sedimentation rate, C-reactive protein determination, serum protein fractions, fibrinogen level and antistreptolysin reaction in 52 rheumatic children. Arch Dis Child 1958; 33: 529-31.
24. Glasziou PP, Irwig L, Kirby AC, Tonkin AM, and Simes RJ. Which lipid measurement should we monitor? An analysis of the LIPID study. BMJ Open 2014; 4: e003512.
25. Taylor P, Gartemann J, Hsieh J, and Creeden J. A systematic review of serum biomarkers anti-cyclic citrullinated Peptide and rheumatoid factor as tests for rheumatoid arthritis. Autoimmune Dis 2011; 2011: 815038.
26. Schutze N, Kunzi-Rapp K, Wagemanns R, Noth U, Jatzke S, and Jakob F. Expression, purification, and functional testing of recombinant CYR61/CCN1. Protein Expr. Purif 2005; 42: 219-25.
27. Larsen A, Dale K, and Eek M. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol.: diagnosis 1977; 18: 481-91.
28. Rueda-Gotor J, Llorca J, Corrales A, et al. Cardiovascular risk stratification in axial spondyloarthritis: carotid ultrasound is more sensitive than coronary artery calcification score to detect high-cardiovascular risk axial spondyloarthritis patients. Clin Exp Rheumatol 2018; 36: 73-80.
29. Levesque R and Inc S. SPSS Programming and Data Management: A Guide for SPSS and SAS Users. SPSS Incorporated, 2007.
30. Zhu Y, Almuntashiri S, Han Y, Wang X, P RS, and Zhang D. The Roles of CCN1/CYR61 in Pulmonary Diseases. Int J Mol Sci 2020; 21.
31. Sulaiman FN, Wong KK, Ahmad WAW, and Ghazali WSW. Anti-cyclic citrullinated peptide antibody is highly associated with rheumatoid factor and radiological defects in rheumatoid arthritis patients. Med 2019; 98: e14945.
32. Deng J, Qian X, Li J, Li Y, Li Y, and Luo Y. Evaluation of serum cysteine-rich protein 61 levels in patients with coronary artery disease. Biomark. Med 2018; 12: 329-39.
33. Carbone F, Bonaventura A, Liberale L, et al. Atherosclerosis in Rheumatoid Arthritis: Promoters and Opponents. Clin Rev Allergy Immunol 2020; 58: 1-14.
34. Kim I, Park CS, and Lee HY. Angiotensin II Type 1 Receptor Blocker, Fimasartan, Reduces Vascular Smooth Muscle Cell Senescence by Inhibiting the CYR61 Signaling Pathway. Korean Circ J 2019; 49: 615-26.
35. Winzap P, Davies A, Klingenberg R, et al. Diabetes and baseline glucose are associated with inflammation, left ventricular function and short- and long-term outcome in acute coronary syndromes: role of the novel biomarker Cyr 61. Cardiovasc. Diabetol 2019; 18: 142.
36. Woo SJ, Noh HS, Lee NY, et al. Myeloid sirtuin 6 deficiency accelerates experimental rheumatoid arthritis by enhancing macrophage activation and infiltration into synovium. EBioMedicine 2018; 38: 228-37.
37. Humphreys J, Hyrich K, and Symmons D. What is the impact of biologic therapies on common co-morbidities in patients with rheumatoid arthritis? Arthritis Res Ther 2016; 18: 282.
38. Choi JS, Kim KH, and Lau LF. The matricellular protein CCN1 promotes mucosal healing in murine colitis through IL-6. Mucosal Immunol 2015; 8: 1285-96.
39. Li P, Zheng Y, and Chen X. Drugs for Autoimmune Inflammatory Diseases: From Small Molecule Compounds to Anti-TNF Biologics. Front Pharmacol 2017; 8: 460.