Association of anti-Ro52 antibody with depression and anxiety in patients with connective tissue diseases: an observational, single-centre, cross-sectional study

L. Yang[1], X. Wang[1], H. Kang[2], B. Gu[1], Q. Ren[1], M. Shen[1], D. Su[1]

[1]Department of Rheumatology and Immunology, and [2]Department of Rehabilitation Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu Province, China.

Abstract

Objective

To explore the risk factors of anxiety and depression, especially their association with serum autoantibodies, in patients with connective tissue diseases (CTDs).

Methods

Three hundred and fifty-two inpatients with CTDs were recruited and their demographic, serological and imaging data were collected through the medical record system. Depression and anxiety were assessed by the Patient Health Questionnaire-9 (PHQ-9) and the Generalized Anxiety Disorder-7 Scale (GAD-7) respectively. Analysis of variance (ANOVA), rank sum test, chi-square test and logistic regression were performed to investigate risk factors for depression and anxiety.

Results

The prevalence of depression (PHQ-9 ≥ 5) and anxiety (GAD-7 ≥5) in CTD patients was significantly higher than that in the Chinese general population (depression: 44.3% vs. 32.2%, anxiety: 39.5% vs. 22.2%). Sleep time was a protective factor for both depression and anxiety (OR=0.734, 95% CI: 0.616~0.874, p<0.001 and OR=0.684, 95% CI: 0.559~0.835, P<0.001, respectively) while anti-Ro52 antibody was a risk factor for them (OR=5.466, 95% CI: 2.978~10.032, p<0.001 and OR=4.075, 95% CI: 2.073~8.010, p<0.001, respectively). Further analysis showed that anti-Ro52 antibody was a risk factor for depression and anxiety in all four subgroups, namely SLE, SS, RA, and other CTDs.

Conclusion

Anti-Ro52 antibody is probably a risk factor for depression and anxiety in patients with connective tissue diseases. CTD patients with the presence of anti-Ro52 antibody are more prone to depression and anxiety than those without it.

Key words

anti-Ro52 antibody, connective tissue disease, depression, anxiety

*Leilei Yang, MSc**
*Xiaoqin Wang, MSc**
*Haiyan Kang, MSc**
Bingjie Gu, MD
Qijie Ren, MSc
Minning Shen, MSc
Dinglei Su, MD

**Contributed equally.*

Please address correspondence to:
Dinglei Su
Department of Rheumatology and Immunology,
Nanjing First Hospital,
Nanjing Medical University,
no. 68 Changle Road,
210006 Nanjing (Jiangsu), China.
E-mail: sudinglei@163.com

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Introduction

Mental disorders are highly prevalent and largely undertreated in all countries. In 2019, before the COVID-19 pandemic, it is estimated that one eighth of the world's population (about 970 million people) suffer from mental disorders. The prevalence of overall mental disorders was 13%, among which depression and anxiety are the most common, accounting for 28.9% and 31% respectively (1). The number of people living with depression and anxiety rose significantly due to the COVID-19 pandemic (2), which caused serious consequences and were the main causes of disability for many years (3). Currently, suicide is still the main cause of death worldwide (4). A recent study in China also revealed significantly higher prevalence of mild anxiety and mild depression (15.8% and 22.19%, respectively) during the early outbreak of COVID-19 compared with the prevalence in the past year (3.6% and 5.0%, respectively) (5).

Risk factors for depression include family history of depression, early life abuse and neglect, female sex, fatigue (6) and medical illness, especially metabolic and autoimmune disorders (7). There is a close bidirectional relationship between depression and autoimmunity. On the one hand, patients with depression have various immune abnormalities, including elevated levels of inflammatory cytokines, upregulated ratio of Th1/Th2 subsets, increased number of Th17, and over-production of certain autoantibodies such as antiphospholipid antibodies (7-8), which increases their risk of autoimmune diseases (9). On the other hand, the risk of depression and anxiety in patients with autoimmune diseases also increased significantly (10-12).

Connective tissue diseases (CTDs) are heterogeneous systemic autoimmune disorders characterised by excessive production of various autoantibodies that target the connective tissues of the body, including systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), rheumatoid arthritis (RA), idiopathic inflammatory myopathies (IIM) such as dermatomyositis (DM), polymyositis (PM), antisynthetase syndrome (ASS), systemic sclerosis (SSc), and mixed connective tissue disease (MCTD) (13). Neuropsychiatric problems are highly prevalent in patients with SLE, with the prevalence of depression and anxiety in SLE ranging from 17–75% and 6–52% respectively (11). The disease-related factors associated with both depression and anxiety are lupus disease activity and glucocorticoid therapy, while the presence of anti-P ribosomal autoantibody is associated with depression occurrence and severity (11, 14). The prevalence estimates for depression in primary SS range between 8.33 and 75.56% (15), and disease activity and symptoms of dry eye and dry mouth are factors that contribute to both depression and anxiety (16). Immunologically mediated small vascular lesions in the brain and anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis might be the potential pathogenesis of pSS comorbid with depression (17).

Autoantibodies are crucial tools in the diagnosis and treatment of CTDs, some of which are specific diagnostic markers while some can also be involved in tissue damage and reflect the disease activity (*e.g.* anti-dsDNA antibodies in SLE). In recent years, it has been realised that anti-Ro52 (or anti-TRIM21) antibody is one of the most widespread autoantibodies detected in sera of patients with CTDs, usually in association with other specific autoantibodies such as anti-SSA/SSB in SLE and SS. According to different studies, anti-Ro52 antibodies were found in primary SS (37–75%), SLE (42–50%), SSc (8%-38%), IIM (17%-58%) and MCTD (29%) (18). In addition, many studies showed that anti-Ro52 was associated with interstitial lung disease, correlating with poor outcome and worse survival, and probably played a direct pathogenic role in congenital heart block in neonatal lupus erythematosus (NLE) as well as in the QT interval prolongation in some adults (19). Whether anti-Ro52 is associated with depression and anxiety in patients with CTDs has not been reported. The aim of this observational, single-centre, cross-sectional study was to investigate the risk factors for depression and anxiety in CTD patients,

especially the role of autoantibodies including anti-Ro52.

Materials and methods

Study population

This cross-sectional study was conducted from August 1, 2019 to June 30, 2022 in the inpatient department of the Rheumatology and Immunology Department of Nanjing First Hospital. All subjects in this study were definitely diagnosed as CTDs, including SLE, SS, RA, SSc, DM, PM and MCTD. The inclusion criteria were: (1) Chinese citizens aged ≥18 years, (2) voluntary participation in the survey and (3) being able to complete the GAD-7 and PHQ-9 questionnaires independently. The exclusion criteria were: (1) prior medical history with severe mental illnesses, (2) prior use of medical drugs to treat mental disorders, (3) pregnant and lactating women, (4) medical history of malignant tumor and (5) disability caused by diseases other than CTDs. This study was carried out in accordance with the Declaration of Helsinki and was approved by the ethics committee of Nanjing First Hospital.

Data collection

The data of each enrolled patient can be divided into four parts. The first part is the demographic data of the subject, including gender, age, marital status, education level, course of disease and sleep time. The second part is about the main diagnosis of the patient, comorbid diseases, and major complications, including other autoimmune diseases, fibromyalgia, thyroid diseases (such as hyperthyroidism, hypothyroidism and Hashimoto's thyroiditis), diabetes, infection, other chronic diseases (such as hypertension, coronary heart disease, stroke), interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH). In addition, pain assessment based on the Visual Analogue Scale (VAS) score, disease activity evaluation for SLE with SLE Disease Activity Index (SLEDAI)-2000 and RA with Disease Activity Score (DAS)28-CRP, as well as the medications (including corticosteroids and immunosuppressants) used by patients were recorded. In the third part, results of laboratory

Table I. Univariate analysis of demographic, clinical and laboratory parameters between CTD patients with or without depression.

	Non-depression (n=196)	Depression (n=156)	Total	*p*-value
Age (year)	56.5 (16.7)	57.7 (13.9)	57.0 (15.5)	0.651
Disease duration (year)	7.8 (10.8)	7.6 (10.0)	7.7 (10.4)	0.730
Education time (year)	8.5 (4.5)	8.8 (4.3)	8.6 (4.4)	0.665
Sleep (hour)	6.4 (1.3)	5.7 (1.6)	6.1 (1.5)	**<0.0001**
Gender				**0.034**
Male	39 (19.9)	18 (11.5)	57 (16.2)	
Female	157 (80.1)	138 (88.5)	295 (83.8)	
Marital status				0.486
Unmarried	35 (17.9)	25 (16)	60 (17)	
Married/divorced or widowed	161 (82.1)	130 (83.3)	291 (82.7)	
Comorbid illness				
Other CTDs				**0.007**
Yes	24 (12.2)	36 (23.1)	60 (17)	
No	172 (87.8)	120 (76.9)	292 (83)	
Thyroid disease				0.578
Yes	38 (19.4)	34 (21.8)	72 (20.5)	
No	158 (80.6)	122 (78.2)	280 (79.5)	
Diabetes				0.494
Yes	24 (12.2)	23 (14.7)	47 (13.4)	
No	172 (87.8)	133 (85.3)	305 (86.6)	
Other chronic diseases				0.663
Yes	81 (42.2)	69 (44.5)	150 (43.2)	
No	111 (57.8)	86 (55.5)	197 (56.8)	
Infection				0.513
Yes	36 (18.4)	33 (21.2)	69 (19.6)	
No	160 (81.6)	123 (78.8)	283 (80.4)	
Fibromyalgia				**0.025**
Yes	5 (2.6)	12 (7.7)	17 (4.8)	
No	191 (97.4)	144 (92.3)	335 (95.2)	
Pain (VAS score)	2.7 (2.6)	1.8 (2.5)	2.3 (2.6)	**0.001**
Prednisone (mg/d)	13.0 (18.0)	21.7 (58.9)	16.8 (41.6)	0.270
Anti-Ro52				**<0.0001**
(+)	42 (21.4)	100 (64.1)	142 (40.3)	
(-)	154 (78.6)	56 (35.9)	210 (59.7)	
aPLs				0.845
(+)	14 (7.1)	12 (7.7)	26 (7.4)	
(-)	182 (92.9)	144 (92.3)	326 (92.6)	
Anti-Rib-P				**0.013**
(+)	14 (7.1)	24 (15.4)	38 (10.8)	
(-)	182 (92.9)	132 (84.6)	314 (89.2)	
Anti-SSA				**0.007**
(+)	46 (23.5)	57 (36.5)	103 (29.3)	
(-)	150 (76.5)	99 (63.5)	249 (70.7)	
Anti-SSB				**0.001**
(+)	8 (4.1)	22 (14.1)	30 (8.5)	
(-)	188 (95.9)	134 (85.9)	322 (91.5)	
Anti-RNP/Sm				0.417
(+)	22 (11.2)	22 (14.1)	44 (12.5)	.
(-)	174 (88.8)	134 (85.9)	308 (87.5)	
Anti-CENP-B				0.760
(+)	17 (8.7)	15 (9.6)	32 (9.1)	
(-)	179 (91.3)	141 (90.4)	320 (90.9)	
ILD				**0.011**
Yes	36 (18.5)	47 (30.1)	83 (23.6)	
No	159 (81.5)	109 (69.9)	268 (76.4)	
PAH				**0.010**
Yes	18 (9.2)	29 (18.6)	47 (13.4)	
No	178 (90.8)	127 (81.4)	305 (86.6)	
ESR (mm/h)	54.18 (42.55)	60.76 (42.13)	57.03 (42.43)	0.095
CRP (mg/L)	20.56 (33.50)	20.55 (34.63)	20.56 (33.96)	0.457
C3 (g/L)	0.82 (0.23)	0.75 (0.21)	0.79 (0.221)	**0.007**
C4 (g/L)	0.38 (2.45)	0.28 (1.15)	0.33 (1.98)	0.427
IgG (g/L)	14.71 (8.31)	15.34 (6.50)	14.99 (7.55)	0.182

Data are expressed as number of participants (%) or mean (SD). Sleep time, gender, presence of comorbid CTDs, fibromyalgia, pain, presence of anti-Ro52, anti-ribosomal P protein, anti-SSA and anti-SSB antibodies, serum level of complement C3, and presence of ILD or PAH were significantly different between the two groups ($p<0.05$).

CTD: connective tissue disease; VAS: visual analogue scale; aPLs: antiphospholipid antibodies; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

tests of the subjects during hospitalisation were recorded, including (1) erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP); (2) antinuclear antibodies (ANA) and anti-Extractable Nuclear Antigen (ENA) autoantibody profiles, which include autoantibodies against SSA, SSB, RNP/Sm, Sm, dsDNA, Scl-70, Jo-1, nucleosome, histone, ribosome P protein, and centromeric protein B; (3) antiphospholipid antibodies (aPLs) including anti-cardiolipin antibody and anti-β2-glycoprotein 1 antibody; (4) immunoglobulin (Ig) G and complement C3 and C4. The detection methods utilised for ANA, anti-ENA profiles, and antiphospholipid antibodies were immunofluorescence, immunoblotting, and enzyme-linked immunosorbent assay (ELISA), respectively. The fourth part is the assessment of depression and anxiety, which was completed through a questionnaire by each patient. It takes about 3–5 minutes for a subject to complete the questionnaire.

Assessment of depression and anxiety

Depression was measured by the Patient Health Questionnaire 9 (PHQ-9) which has been identified as the most reliable screening tool and widely validated in primary care (20). It consists of nine items measuring depressive symptoms corresponding to the diagnostic criteria for major depressive disorder. Each item is scored on a four-point Likert scale (0–3) with higher total scores reflecting greater depression severity. The following cut-offs correlate with level of depression severity: score 0–4 means minimal depression, score 5–9 means mild depression, score 10–14 means moderate depression, and score 15 or greater means severe depression (21).

Anxiety was measured by the Generalized Anxiety Disorders Scale-7 (GAD-7). GAD-7 is a seven-item diagnostic tool with great psychometric properties and has been validated in both the primary care setting and the general population. Each item is scored on a four-point Likert scale (0–3) with higher total scores indicating greater anxiety severity. The following cutoffs correlate with level of anxiety severity: score 0–4 means minimal anxiety, score 5–9 means mild anxiety, score 10–14 means moderate anxiety, and score 15 or greater means severe anxiety (22).

To simplify statistics, patients were categorised into two groups based on their depression and anxiety score respectively: depression group with the PHQ-9 score≥5, and non-depression group with the PHQ-9 score<5; anxiety group with the GAD-7 score≥5, and non-anxiety group with the GAD-7 score<5. Patients with the score of PHQ-9 ≥10 or GAD-7≥10 were defined as with moderate/severe anxiety or depression, respectively.

Statistical analysis

Data were analysed using SAS, version 9.4 (SAS Institute Inc.). Descriptive statistics were used for presentation of patient characteristics. Normality was checked using the Shapiro-Wilk test. Categorical data were summarised as counts and percentages, and continuous variables in normal distribution were presented as mean ± standard deviation (SD) while continuous variables in non-normal distribution were presented as median (interquartile range (IQR)). For continuous variables with normal distribution and homogeneity of variance, one way analysis of variance (ANOVA) was performed to evaluate the discrepancies between two groups, while for continuous variables without normal distribution or homogeneity of variance, rank sum test was performed instead. For categorical variables, the chi-square test was used. If the *p*-value of the univariate test was less than 0.05, which was regarded as statistically significant, further multivariate analysis would be carried out.

Table II. Multivariate logistic regression analysis of parameters with statistical significance in the univariate analysis for CTD patients with depression.

	S.E	*p*-value	OR	95%CI	
				Lower limit	Upper limit
Sleep time	0.090	**<0.001**	0.734	0.616	0.874
Gender	0.183	0.182	0.614	0.300	1.257
Comorbid with other CTDs	0.344	0.135	1.672	0.852	3.282
Fibromyalgia	0.615	**0.048**	3.370	1.010	11.242
Pain (VAS)	0.054	0.463	0.961	0.865	1.068
Anti-Ro52	0.310	**<0.001**	5.466	2.978	10.032
Anti-Rib-P	0.462	0.136	1.991	0.806	4.923
Anti-SSA	0.360	0.070	0.521	0.257	1.055
Anti-SSB	0.540	0.079	2.582	0.896	7.437
ILD	0.314	0.593	1.183	0.639	2.189
PAH	0.396	0.215	1.633	0.752	3.547
C3	0.668	0.772	0.824	0.222	3.053

Sleep time was a protective factor while anti-Ro52 antibody and fibromyalgia were risk factors for depression in CTD patients.
CTD: connective tissue disease; VAS: visual analogue scale; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension.

Results

The overall prevalence of depression and anxiety in CTD patients

Among the 352 CTD patients enrolled in this study, the prevalence of depression (PHQ-9 score ≥5) and anxiety (GAD-7 score ≥5) was higher than that of the general population in China both during and before the COVID-19 pandemic, with the prevalence of depression was 44.3%, 32.2%, and 3.6%, respectively, and the prevalence of anxiety was 39.5%, 22.3%, and 5.0%, respectively. Furthermore, the prevalence of moderate and severe depression (PHQ-9 score ≥10) and moderate and severe anxiety (GAD-7 score ≥10) also increased significantly in CTD patients compared with that of the general population in China during the COVID-19 pandemic (17.0% *vs*. 10.5% and 13.4% *vs*. 6.4%, respectively) (Supplementary Table S1).

Risk factors of depression in overall CTD patients

Firstly, univariate analysis was conducted for all the variables measured in

Table III. Univariate analysis of demographic, clinical and laboratory parameters between CTD patients with or without anxiety.

	Non-anxiety (n=213)	Anxiety (n=139)	Total	*p*-value
Age (year)	56.5 (16.7)	57.8 (13.5)	57 (15.5)	0.809
Disease duration (year)	8.1 (11.2)	7.1 (9.0)	7.7 (10.4)	0.995
Education time (year)	8.6 (4.5)	8.7 (4.2)	8.6 (4.4)	0.851
Sleep time (hour)	6.5 (1.4)	5.5 (1.4)	6.1 (1.5)	**<0.0001**
Gender				0.456
Male	37 (17.4)	20 (14.4)	57 (16.2)	
Female	176 (82.6)	119 (85.6)	295 (83.8)	
Marital status		0.457		
Unmarried	37 (17.4)	23 (16.5)	60 (17)	
Married/divorced or widowed	176 (82.6)	115 (82.7)	291 (82.7)	
Comorbid illness				
Other CTDs				**0.016**
Yes	28 (13.1)	32 (23)	60 (17)	
No	185 (86.9)	107 (77)	292 (83)	
Thyroid disease				0.132
Yes	38 (17.8)	34 (24.5)	72 (20.5)	
No	175 (82.2)	105 (75.5)	280 (79.5)	
Diabetes				0.254
Yes	32 (15)	15 (10.8)	47 (13.4)	
No	181 (85)	124 (89.2)	305 (86.6)	
Other chronic diseases				0.787
Yes	92 (43.8)	58 (42.3)	150 (43.2)	
No	118 (56.2)	79 (57.7)	197 (56.8)	
Infection				0.946
Yes	42 (19.7)	27 (19.4)	69 (19.6)	
No	171 (80.3)	112 (80.6)	283 (80.4)	
Fibromyalgia				**0.001**
Yes	4 (1.9)	13 (9.4)	17 (4.8)	
No	209 (98.1)	126 (90.6)	335 (95.2)	
Fatigue				**<0.0001**
Yes	79 (37.1)	119 (85.6)	198 (56.3)	
No	134 (62.9)	20 (14.4)	154 (43.8)	
Pain (VAS score)	2.6 (2.6)	1.8 (2.4)	2.3 (2.6)	**0.006**
Prednisone (mg/d)	13.0 (18.0)	22.7 (62.0)	16.8 (41.6)	0.137
Anti-Ro52				**<0.0001**
(+)	48 (22.5)	94 (67.6)	142 (40.3)	
(-)	165 (77.5)	45 (32.4)	210 (59.7)	
aPLs				0.911
(+)	16 (7.5)	10 (7.2)	26 (7.4)	
(-)	197 (92.5)	129 (92.8)	326 (92.6)	
Anti-Rib-P				0.293
(+)	20 (9.4)	18 (12.9)	38 (10.8)	
(-)	193 (90.6)	121 (87.1)	314 (89.2)	
Anti-SSA				**0.001**
(+)	48 (22.5)	55 (39.6)	103 (29.3)	
(-)	165 (77.5)	84 (60.4)	249 (70.7)	
Anti-SSB				**0.0052**
(+)	11 (5.2)	19 (13.7)	30 (8.5)	
(-)	202 (94.8)	120 (86.3)	322 (91.5)	
Anti-RNP/Sm				0.592
(+)	25 (11.7)	19 (13.7)	44 (12.5)	
(-)	188 (88.3)	120 (86.3)	308 (87.5)	
Anti-CENP-B				0.605
(+)	18 (8.5)	14 (10.1)	32 (9.1)	
(-)	195 (91.5)	125 (89.9)	320 (90.9)	
ILD				**0.019**
Yes	41 (19.3)	42 (30.2)	83 (23.6)	
No	171 (80.7)	97 (69.8)	268 (76.4)	
PAH				**0.017**
Yes	21 (9.9)	26 (18.7)	47 (13.4)	
No	192 (90.1)	113 (81.3)	305 (86.6)	
ESR (mm/h)	57.07 (42.89)	56.97 (41.83)	57.03 (42.43)	0.868
CRP (mg/L)	18.07 (29.19)	20.56 (33.96)	20.56 (33.96)	0.693
C3 (g/L)	0.82 (0.23)	0.75 (0.19)	0.79 (0.22)	**0.005**
C4 (g/L)	0.36 (2.35)	0.29 (1.22)	0.33 (1.98)	0.276
IgG (g/L)	14.08 (5.25)	16.39 (9.94)	14.99 (7.55)	**0.005**

Data are expressed as number of participants (%) or mean (SD). Sleep time, comorbidity with other CTDs, fibromyalgia, fatigue, pain, presence of anti-Ro52, anti-SSA and anti-SSB antibodies, serum level of complement C3 and IgG, and presence of ILD or PAH were significantly different between the two groups ($p<0.05$). CTD: connective tissue disease; VAS: visual analogue scale; aPLs: antiphospholipid antibodies; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

this study between CTD patients with depression and those without depression (non-depression). As presented in Table I, sleep time, gender, presence of comorbid CTDs, fibromyalgia, pain intensity (VAS score), presence of anti-Ro52, anti-ribosomal P protein, anti-SSA and anti-SSB antibodies, serum level of complement C3, and presence of ILD or PAH were significantly different between the two groups (P $p<0.05$), while age, disease duration, comorbidity with thyroid disease, diabetes and other chronic diseases, infection, dosage of corticosteroid (equivalent of prednisone), presence of other autoantibodies including APS, anti-RNP/Sm and anti-CENP-B, ESR, CRP, and serum levels of complement C4 and IgG showed no significant difference ($p>0.05$).

Then, in order to determine the independent risk factors of depression, the variables found to be statistically significant in the univariate analysis were included in the multivariate logistic regression analysis. The results showed that sleep time was a protective factor (OR=0.734, 95% CI: 0.616~0.874, $p<0.001$) while anti-Ro52 antibody was a risk factor (OR=5.466, 95% CI: 2.978~10.032, $p<0.001$) (Table II).

Risk factors of anxiety in overall CTD patients

Univariate analysis was made for all the variables measured in this study between CTD patients with anxiety and those without (non-anxiety). Sleep time, comorbidity with other CTDs, fibromyalgia, pain intensity (VAS score), presence of anti-Ro52, anti-SSA and anti-SSB antibodies, serum level of complement C3 and IgG, and presence of ILD or PAH were significantly different between the two groups ($p<0.05$) (Table III).

To determine the independent risk factors of anxiety, the variables found to be statistically significant in the univariate analysis were included in the multivariate logistic regression analysis. As a result, sleep time was a protective factor (OR=0.684, 95% CI: 0.559~0.835, $p<0.001$) while anti-Ro52 antibody was a risk factor (OR=4.075, 95% CI: 2.073~8.010, $p<0.001$) (Table IV).

Table IV. Multivariate logistic regression analysis of parameters with statistical significance in the univariate analysis for CTD patients with anxiety.

	S.E	*p*-value	OR	95%CI	
				Lower limit	Upper limit
Sleep time	0.102	**<0.001**	**0.684**	0.559	0.835
Comorbid with other CTDs	0.382	0.685	1.167	0.553	2.466
Fibromyalgia	0.749	**0.042**	**4.578**	1.054	19.879
Fatigue	0.339	**<0.001**	**7.958**	4.098	15.452
Pain	0.063	0.996	1.000	0.884	1.131
Anti-Ro52	0.345	**<0.001**	**4.075**	2.073	8.010
Anti-SSA	0.386	0.619	1.211	0.568	2.582
Anti-SSB	0.562	0.511	0.692	0.230	2.079
ILD	0.355	0.752	1.119	0.558	2.244
PAH	0.427	0.624	1.233	0.534	2.846
C3	0.692	0.649	0.730	0.188	2.831
IgG	0.020	**0.028**	**1.045**	1.005	1.087

Sleep time was a protective factor while fibromyalgia, fatigue anti-Ro52 antibody and IgG were risk factors for anxiety in CTD patients.
CTD: connective tissue disease; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension.

Table V. Prevalence of depression in overall CTDs patients and different subgroups with or without anti-Ro52 antibody.

	Anti-Ro52	Depression (n, %)	Non-depression (n, %)	Prevalence	*p*
Overall CTDs	(+)	100 (64.1)	42 (21.4)	70.4%	**<0.000**
	(-)	56 (35.9)	154 (78.6)	26.7%	
SLE	(+)	30 (65.2)	16 (34.8)	65.2%	**0.004**
	(-)	16 (34.8)	30 (65.2)	34.8%	
SS	(+)	47 (75.8)	16 (35.6)	74.6%	**<0.000**
	(-)	15 (24.2)	29 (64.4)	34.1%	
RA	(+)	8 (36.4)	3 (7.1)	72.7%	**0.003**
	(-)	14 (63.6)	39 (92.9)	26.4%	
Other CTDs	(+)	15 (57.7)	7 (11.1)	68.2%	**<0.000**
	(-)	11 (42.3)	56 (88.9)	16.4%	

Data are expressed as number of participants (%). In both overall CTD patients and different disease subgroups, the prevalence of depression in anti-Ro52 positive subjects increased significantly compared with that in anti-Ro52 negative ones ($p<0.05$).

Table VI. Prevalence of anxiety in overall CTDs patients and different subgroups with or without anti-Ro52 antibody.

	Anti-Ro52	Anxiety (n, %)	Non-anxiety (n, %)	Prevalence	*p*
Overall CTDs	(+)	94 (67.6)	48 (22.5)	66.20%	<0.000
	(-)	45 (32.4)	165 (77.5)	21.4%	
SLE	(+)	29 (69)	17 (34)	63.0%	0.001
	(-)	13 (31)	33 (66)	28.3%	
SS	(+)	44 (75.9)	19 (38.8)	69.8%	0.000
	(-)	14 (24.1)	30 (61.2)	31.8%	
RA	(+)	7 (53.8)	4 (7.8)	63.6%	<0.000
	(-)	6 (46.2)	47 (92.2)	11.3%	
Other CTDs	(+)	14 (53.8)	8 (12.7)	63.6%	<0.000
	(-)	12 (46.2)	55 (87.3)	17.9%	

Data are expressed as number of participants (%). In both overall CTD patients and different disease subgroups, the prevalence of anxiety in anti-Ro52 positive subjects increased significantly compared with that in anti-Ro52 negative ones ($p<0.05$).

Risk factors of depression in different CTD subgroups

We divided the 352 CTD patients into four subgroups according to their main diagnosis: SLE, SS, RA, and other CTDs (including SSc, IIM and MCTD). Multivariate logistic regression analysis was performed for each group with the variables found to be statistically significant in the univariate analysis for overall CTD patients with depression as the independent variables and the presence of depression as the dependent variable. As a result, anti-Ro52 antibody was significantly different in all four subgroups and was a risk factor for depression in these CTD patients (OR=3.785, 6.233, 10.686, and 8.945, respectively). Besides, sleep time was shown to be a protective factor in SS and RA groups (OR=0.570 and 0.373, respectively) while PAH was a risk factor in RA group (OR=14.527) and comorbidity with other CTDs was a risk factor in SLE group (OR=3.853). In contrast, anti-SSA was not statistically significant in either subgroup (Suppl. Tables S2.1-S2.4).

Risk factors of anxiety in different CTD subgroups

Similarly, multivariate logistic regression analysis was performed for each group with the variables found to be statistically significant in the univariate analysis for overall CTD patients with anxiety as the independent variables and the presence of anxiety as the dependent variable. The results showed that anti-Ro52 antibody was significantly different in the subgroups of SS, RA and other CTDs, and was a risk factor for anxiety in these patients (OR=6.885, 43.043, and 6.229, respectively). Meanwhile, Sleep time was shown to be a protective factor in SLE and SS groups (OR=0.495 and OR=0.381, respectively), while fatigue seemed to be a risk factor in the subgroups of SLE, SS and other CTDs (OR=15.539, OR=13.796, OR=7.559, respectively). Due to the low incidence of SSB positivity and fibromyalgia in some subgroups, they were not included in the multivariable analysis. In contrast, anti-SSA was not statistically significant in either subgroup (Suppl. Tables S3.1-S3.4).

To eliminate possible deviation caused by the difference of demographic characteristics between each subgroup and overall CTD subjects, we also carried out univariate analysis of depression and anxiety for patients in each subgroup, and then took the parameters with statistical significance as independent variables and the presence of depression

Table VII. Comparison of PHQ-9 and GAD-7 scores between CTD patients with or without anti-Ro52 antibody.

	Number	PHQ-9	t	P	GAD-7	t	*p*
Anti-Ro52			6.24	**<0.000**		8.09	**<0.000**
(+)	142	6.7 (4.6)			6.4 (4.9)		
(-)	210	3.5 (4.8)			2.4 (3.8)		
Total	352	4.8 (4.9)			4.0 (4.7)		

Data are expressed as number of participants or mean (SD). The PHQ-9 and GAD-7 scores were markedly higher in patients with anti-Ro52 in comparison with those without ($p<0.05$).

or anxiety as dependent variables for multivariate logistic regression analysis. Very similar results were obtained which showed that anti-Ro52 antibody, but not anti-SSA, was significantly different in each subgroup and was a risk factor for depression (OR=3.128-7.215) and anxiety (OR=2.970-9.098) (Suppl. Tables S4.1–11.2). Furthermore, anti-SSA was not significantly related with depression and anxiety in overall CTD patients either (Tables II and IV).

Prevalence and severity of depression and anxiety in CTD patients with anti-Ro52 antibody

Finally, we analysed the prevalence and severity of depression and anxiety in patients with anti-Ro52 antibody. The results showed that in both overall CTD patients and different disease subgroups, the prevalence of depression and anxiety in anti-Ro52 positive subjects increased significantly compared with that in anti-Ro52 negative ones (Tables V and VI). Furthermore, the PHQ-9 and GAD-7 scores were markedly higher in patients with anti-Ro52 in comparison with those without, indicating a more severe condition in anti-Ro52 positive CTD patients (Table VII).

Discussion

Previous studies have shown that the risk of depression, anxiety and other mental disorders in patients with autoimmune diseases is significantly higher than that in healthy population (10-12). As the time we recruited subjects coincided with the COVID-19 pandemic, in the first year of which the prevalence of anxiety and depression increased more than 25% according to the 2020 Global Disease, Injury and Risk Factor Burden Study (GBD 2020) (2), we selected the general population of China during the prevalence of COVID-19 as control. This study demonstrated that the risk of depression and anxiety of CTD patients is significantly increased, and anti-Ro52 antibody is a risk factor for both.

The mechanism underlying the development of mental illness in CTD patients remains unclear. Previous studies have indicated that depression and anxiety may have similar pathological mechanisms with CTDs based on an interaction between the central nervous system and the immune system. In short, CTDs may cause dysfunction of the central nervous system and eventually lead to depression and anxiety, whereas patients with depression and anxiety are prone to immune disorders and more likely to develop CTDs (23, 24). The bidirectional relationship between anxiety/depression and CTDs indicates that they are likely to share common pathogenic factors.

Diverse pathogenic autoantibodies have been identified in CTDs, and multitudes of studies also hinted that autoantibodies may play an important role in the pathogenesis of depression and anxiety (25-29). It was even hypothesised that depression is a kind of autoimmune disease from the perspective of autoantibodies (30). Therefore, we speculate that the increased risk of depression and anxiety in CTD patients may be related to autoantibodies. Previous studies reported that some autoantibodies may be related to depression and anxiety, such as anti-thyroid peroxidase antibodies (anti-TPOs), antiphospholipid antibodies (aPLs), and anti-ribosomal P antibodies (anti-Rib-Ps). However, our results showed that aPLs and anti-Rib-Ps have no statistical significance in the risk of depression and anxiety in overall CTD patients. In addition, the correlation of aPLs and anti-RibPs with depression and anxiety in the RA, SS and other CTD subgroups cannot be analysed due to extremely low positive rate of the two autoantibodies in these patients.

As a widespread autoantibody detected in various CTDs and other autoimmune diseases, anti-Ro52 antibody has attracted great attention in recent years (19). Ro52 is a 52-kDa protein that contains a RING finger domain, B-box motifs and a coiled-coil domain. This structural feature places Ro52 within the tripartite motif proteins (TRIM) family and is designated as TRIM21 protein. Ro52 mediates the ubiquitination of interferon regulatory factors (IRF) through its E3 ubiquitin ligase activity, thus inhibiting the excessive production of type 1 interferons in the anti-viral immunity and the subsequent prolonged immune system activation, thus avoiding the development of autoimmune diseases (31). Therefore, anti-Ro52 antibodies may play a potential role in the pathogenesis of various CTDs, though they are historical markers of SS. It is now well established that anti-Ro52 was associated with disease activity and leukopenia in SS and SLE, ILD in SSc, ASS, DM, and MCTD, especially rapidly progressive ILD in ASS and anti-MDA5 positive DM (18). In this study, we found that in overall CTD patients and different disease subgroups, the prevalence of depression and anxiety was significantly higher in patients with anti-Ro52 antibody than those without. As ILD was reported to be related to depression and anxiety (30), and anti-Ro52 was associated with ILD (32), we conducted logistic regression analysis after adjusting for ILD and other known confounding factors that may affect depression and anxiety. Not surprisingly, the results showed that anti-Ro52 antibody was a risk factor for depression and anxiety in CTD patients. More importantly, the subgroup analysis also revealed that anti-Ro52 was a risk factor for depression and anxiety in SLE, SS, RA and other CTDs. Therefore, anti-Ro52 might play a role in the development of depression and anxiety in CTD patients.

The role of anti-Ro52 antibody in the pathogenesis of depression and anxiety

is still unclear. A recent study revealed the presence of anti-Ro52 antibodies in the CSF of an SS patient with cerebellar degeneration, and animal experiments showed that Ro52/TRIM21 expression was present throughout murine brains, including the hippocampus, cerebral cortex and cerebellum, indicating that anti-Ro/SSA antibodies were likely responsible for cerebellar degeneration in patients with SS (33). Another study reported that intrathecal production of anti-52-kD SSA antibodies was observed in patients with SS with CNS involvement, suggesting that CSF anti-52-kD SSA might serve as a biomarker for SS-related CNS disease (34), including mental disorders such as depression and anxiety. We hypothesised that anti-Ro52 might directly affect neurotransmitter systems in the brain, such as the serotonin or dopamine systems, which are known to be involved in the regulation of mood and anxiety. In addition, anti-Ro52 might bind to cell surface receptors on neurons or glial cells, disrupting normal cell signaling and function. This could lead to changes in brain structure and function that are associated with depression and anxiety. These hypotheses require rigorous research for confirmation.

There are some limitations to this study. Firstly, this is a cross-sectional study that lacks longitudinal observation of the subjects, and there may also be deviations in the selection of subjects. Secondly, some cofounding factors such as family income, living alone, concomitant drugs other than corticosteroids, etc. (35, 36), which are related to the risk of depression and anxiety, have not been included in statistical analysis due to the absence of data. Besides, there was a lack of activity assessment data for SS and other CTD patients. Thirdly, we used the PHQ-9 and GAD-7 to evaluate depression and anxiety, respectively, among numerous validated screening tools, and different results might be obtained with different tools (37). Fourthly, this is only a single-centre study and our findings may not reflect the characteristics of the CTD patients in the whole China. A larger, multicentre, longitudinal study is needed to corroborate and comprehensively understand the impact of anti-Ro52 antibody on the risk of depression and anxiety in CTD patients. Last but not least, although our results showed a high correlation between anti-Ro52 and depression and anxiety from a statistical perspective, it does not necessarily indicate a causal relationship between anti-Ro52 and depression and anxiety due to the above-mentioned limitations and lack of quantitative testing of anti-Ro52.

In conclusion, we demonstrated that anti-Ro52 was highly associated with depression and anxiety in CTD patients, and the prevalence and severity of depression and anxiety in CTD patients were higher in those with anti-Ro52 autoantibodies than those without. Our findings may provide clues and inspiration for future research in this area.

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