RESEARCH ARTICLE

Retrospective analysis of the clinical significance of Ro52/TRIM21 antibody and specific antinuclear antibody patterns by indirect immunofluorescence

Kaifang Liu[1], Yunfeng Liao[2], Pu Li[3], Jing Shi[4]

Abstract

Objective: To determine the clinical significance of Ro52 protein/tripartite motif-containing 21 antibody and specific antinuclear antibody patterns using indirect immunofluorescence technique.

Method: The retrospective study was conducted at the clinical laboratory of the First Affiliated Hospital of Chongqing Medical University, China, and comprised data from January 2017 to December 2021 of patients who underwent antinuclear antibody and anti-extractable nuclear antigen antibody detection. Inpatients with Ro52 antibody-positive status were taken as the cases, while anti-Ro52 negative patients with clear clinical diagnosis were taken as the controls. Data was analysed using SPSS 19.

Results: There were 1802 cases and 1211 controls. Positive Ro52 showed significantly greater frequency in patients with primary Sjogren's syndrome, systemic lupus erythematosus, inflammatory myositis, dry eyes and interstitial lung disease ($p<0.05$). Ro52 antibody showed high positive predictive value for primary Sjogren's syndrome 25(96.15%), systemic lupus erythematosus 259(91.20%), connective tissue disease-associated interstitial lung disease 45(86.67%) and inflammatory myositis 60(86.67%). Antinuclear antibody indirect immunofluorescence patterns most frequently detected were nuclear speckled 128(40.89%) and cytoplasmic speckled 126(40.26%) ($p<0.05$). Interstitial lung disease was associated with the presence of cytoplasmic speckled antinuclear antibody indirect immunofluorescence pattern 24(19.2%), while tumours 47(36.5%) and hepatitis B 26(20.3%) seemed to be more frequent with nuclear speckled pattern ($p<0.05$). The simultaneous reactivity extractable nuclear antigen antibodies most frequently detected were antinuclear antibody+Ro52+anti-Sjogren's syndrome A+ 558(33.96%).

Conclusion: Ro52 antibody positivity was found to be associated with Sjogren's syndrome, systemic lupus erythematosus, inflammatory myositis, dry eye and interstitial lung disease. The antinuclear antibody immunofluorescence pattern of Ro52 positive was single and primarily granular cytoplasm type. Antinuclear antibody negative and Ro52 positive in the serum of patients also had certain significance in auxiliary disease diagnosis.

Key Words: Anti-Ro52 autoantibody, Autoimmune disease, Sjogren's syndrome, Xerophthalmia, Tripartite motif-containing 21 protein.

(JPMA 74: 236; 2024) **DOI: https://doi.org/10.47391/JPMA.8027**

Introduction

Ro52 protein/tripartite motif-containing 21 (TRIM21) belongs to the TRIM family of proteins and contains a RING (RING Finger Domain) and a B-box (B-Box zinc finger gene containing protein) motif, followed by a coiled-coil (CC) domain and a B30.2 (or PRYSPRY) region at the C-terminal end.[1] The anti-Ro52 antibody is one of the most widely distributed and common anti-nuclear antibodies (ANAs), which can be found in the sera of patients with various autoimmune diseases. Although its specificity is low, it is closely associated with certain specific clinical manifestations of autoimmune diseases, such as systemic lupus erythematosus (SLE), Primary Sjogren's syndrome (pSS), autoimmune hepatitis, primary biliary cholangitis, myositis, neonatal atrioventricular block and pulmonary interstitial fibrosis[2,3]. In addition, the Ro52 antibody is also detected in some non-autoimmune diseases, such as viral infections and neoplastic diseases[1,3,4]. Regarding the clinical significance of the Ro52 antibody, there have been more studies in European and American populations than in Asian populations[5]. As the clinical significance and diagnostic value of Ro52 antibody differ greatly from those reported in the literature, some immunological laboratories have stopped using Ro52 antibody detection[6].

As an important autoimmune disease-related antigen, the biological function of Ro52 has been a concern for

[1,2,4]Department of Laboratory Medicine, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China, [3]Department of Laboratory Medicine, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China.

Correspondence: Jing Shi. Email: jingyuns@hospital.cqmu.edu.cn

ORCID ID. 0000-0002-0857-1794

Submission complete: 15-02-2023 **Review began**: 06-03-2023

Acceptance: 14-10-2023 **Review end:** 09-09-2023

researchers. Many studies have found that Ro52 plays various roles in cells, including the regulation of cell proliferation and apoptosis, signal transduction, and interaction with nuclear deoxyribonucleic acid (DNA)[1,4,7].

Ro52/TRIM21 is among the most common autoantibodies in systemic autoimmune rheumatic diseases, but the clinical association and specific ANA patterns by indirect immunofluorescence (IIF) remain poorly understood. The current study was planned to describe the clinical significance, serological associations and specific ANA-IIF patterns of Ro52/TRIM21 antibody in a large number of patients in a tertiary care setting.

Materials and Methods

The retrospective study was conducted at the clinical laboratory of the First Affiliated Hospital of Chongqing Medical University, China, and comprised data from January 2017 to December 2021 of patients who underwent ANA and anti-extractable nuclear antigen (ENA) antibody detection. All the participants were Han Chinese. Data of patients who had inconsistent repeated results, duplicate detections, data with missing elements and data with unclear information was excluded. Data included related to antibody-positive inpatients, outpatients and patients undergoing regular physical examination and screening at the institutional health management centre.

The diagnosis was confirmed by reviewing the patient's medical records and laboratory/histopathology results, according to the classification criteria present at the time of analysis[8-18]. Autoimmune disease was defined when a patient presented with one of the following: SLE, rheumatoid arthritis (RA), pSS, interstitial lung disease (ILD), mixed connective tissue disease (MCTD) and inflammatory myositis (IM). Other non-autoimmune pathologies included various non-autoimmune diseases, such as dry eyes, tumours, infections or tuberculosis (TB). The first-time result of Ro52 test was selected from the same patient with multiple admissions to the hospital. The fluorescence pattern and titre were analysed retrospectively. In addition, Ro52 antibody-negative patients were set as the control group for ANA and anti-ENA tests.

Approval was obtained from the institutional ethics review committee.

ANA screening and titration had been performed via IIF on human epithelial-2 (HEp-2) cell-coated slides (Euroimmun, Lübeck, Germany). The animal pathogenic haemoflagellate Crithidia luciliae was used to detect autoantibodies against double-stranded DNA (dsDNA) by IIF. The slides were incubated with sequential dilutions of serum from 1/100 to 1/1600 and revealed with fluorescein isothiocyanate (FITC)-bound anti-human immunoglobulin G (IgG) antibodies for ANA detection. The International Consensus on ANA Patterns (ICAP) classification was introduced in the institutional laboratory only in 2016[19]. ANA titre ≥1/100 was considered positive, and anti-dsDNA titre ≥1/10 was considered positive, too. Each serum was also simultaneously screened for anti-ENAs. Anti-ENA spectrum was detected by immunoblotting (Euroimmun), including 15 items: anti-Sjogren's Syndrome A (anti-SSA), anti-SSB, anti-Ro52, anti-u1-nRNP (antibodies to U1 ribonucleoproteins), anti-Histone, AMA-M2 (anti-mitochondrial antibody m2 subtype), anti-Sm (anti-Smith antibodies), AnuA (anti-nucleosome antibody), anti-CENP (anti-centromere proteins antibody), anti-Jo-1(anti-Jo-1 antibody), anti-Scl-70 (anti Scl-70 antibody), anti-PM-Scl(anti- polymyositis-sclerosis antibodies), anti-PCNA (anti-proliferating cell nuclear antigen antibody), anti-RNP (antibodies to ribonucleoproteins) and anti-dsDNA. The SSA antigen on ENA membrane strip was extracted from bovine spleen and thymus, and purified by affinity chromatography to natural SSA, which binds only to Ro60. The Ro52 antigen on ENA membrane strip was a recombinant Ro52 protein (52 KDa), which had no cross-reactivity with Ro60. Quality control products for ANA testing were used (Bio-Rad, United States). Detection was performed in line with the instructions provided by the manufacturer for validation.

Euroimmun IF Sprinter (Euroimmun AG, Lübeck, Germany) was used for fully automated processing of IIF tests. EUROBlotMaster (Euroimmun AG, Lübeck, Germany) was used for fully automated processing of immunoblots. The incubated strips were digitized by EUROBlotCamera and used EUROLineScan to evaluate (EUROIMMUN, Lübeck, Germany).

Data was analysed using SPSS 19. Qualitative data was expressed as frequencies and percentages, while quantitative data was expressed as mean and standard deviation. Analysis of variance (ANOVA) was used to compare the mean values of continuous variables, and chi-squared test was used for comparisons between qualitative variables. In addition, comparisons between independent groups were carried out by using chi-squared or Fisher's exact tests for categorical variables, and the student's t-test or Mann-Whitney test for quantitative parameters. Data normality and assumption of homoscedasticity were evaluated using the Fisher-Snedecor test. Logistic regression analysis was applied to calculate the odds ratio (OR) and 95% confidence

intervals (Cis). Two-tailed p<0.05 was considered statistically significant. Positive predictive value (PPV) and negative predictive value (NPV) for Ro52 antibody as screening tests were also worked out.

Results

Of the 24,263 cases who underwent ANA IIF and Ro52 antibody test, 15,071(62%) were females and 9192(38%) were males. The positivity rate of Ro52 antibody was 2563(10.61%, accounting for 31.12% of ANA-positive cases; 2227(86.9%) females with median age 48 years (interquartile range [IQR] 38-59 years), and 336(13.1%) males with median age 57 years (IQR 42-68 years). The male-to-female ratio was 1:6.3 (p<0.05). The overall median age of ANA and Ro52 antibody-positive patients was 59 years (IQR 47-70 years), which was significantly higher than that of the 1211 negative controls (p<0.05).

The positive rate of Ro52 antibody in 1802 autoimmune diseases cases was 627(77.40%) compared 1175(53.30%) of 2203 non-autoimmune disease cases (p<0.05).

Positive Ro52 antibody showed a significantly greater frequency in patients with pSS, SLE, IM, xerophthalmia and ILD than the control group (p<0.05) (Table 1). SLE, IM, ILD and xerophthalmia in the anti-Ro52 positive group were 7, 6 and 2 times more prevalent than in the anti-Ro52 negative group, respectively. Ro52 antibody showed PPV 96.15% and NPV 40.51% for pSS, followed by SLE 91.20% and 43.46%, connective tissue disease-associated interstitial lung disease (CTD-ILD) 86.67% and 40.60%, IM 86.67% and 40.74%, xerophthalmia 75.86% and 43.46% respectively.

A total of 1935 Ro52 antibody-positive cases with clear ANA fluorescence patterns and titres as well as ENA results were analysed. Among 1643 ENA antibodies positivity was noted for ANA+Ro52+SSA + in 558(33.96%), followed by ANA+Ro52+ 313(19.05%), ANA+Ro52+SSA+SSB 232(14.12%) and ANA+Ro52 + SSA+u1-nRNP 184(11.20%).

Among the ANA IIF patterns of 313 patients with Ro52 antibody-positive alone, 279(89.1%) were single pattern and 34(10.8%) were complex patterns. Cytoplasmic speckled pattern 126(40.26%) showed a higher prevalence in the Ro52 antibody-positive alone cases than in 985(19.29%) of 5107 Ro52 antibody-negative

Table-1: Main diseases or clinical manifestations associated with Ro52 antibody.

Associated clinical diagnosis	Whole group (N =3013)		Anti-Ro52 positive (n =1802)		Anti-Ro52 negative *(n =1211)*		*P valus*	Odds ratio (95% CI)		
	n	%	n	%	n	%				
Systemic lupus erythematosus	284	9.43	259	14.37	25	2.06	<0.001*	7.96	5.25	12.09
Interstitial lung disease	71	2.36	53	2.94	18	1.49	0.006*	2.01	1.17	3.45
isolated ILD	26	0.86	14	0.78	12	0.99	0.533			
CTD-ILD	45	1.49	39	2.16	6	0.50	<0.001*	4.44	1.88	10.53
Inflammatory myositis	60	1.99	52	2.89	8	0.66	<0.001*	4.47	2.12	9.44
PM	26	0.86	23	1.28	3	0.25	0.002*	5.21	1.56	17.34
DM	29	0.96	25	1.39	4	0.33	0.004*	4.25	1.47	12.23
other IM	5	0.17	4	0.22	1	0.08	0.654			
Primary Sjogren's syndrome	26	0.86	25	1.39	1	0.08	<0.001*	17.02	2.3	125.8
Rheumatoid arthritis	147	4.88	96	5.33	51	4.21	NS			
Mixed connective tissue disease	192	6.37	127	7.05	65	5.37	0.037*	1.34	0.98	1.82
Dry eye	87	2.89	66	3.66	21	1.73	0.001*	2.15	1.31	3.54
Tumour	143	4.75	86	4.77	57	4.71	NS			
Infection	140	4.65	77	4.27	63	5.2	NS			
Tuberculosis	51	1.69	35	1.94	16	1.32	NS			
Other systemic diseases										
Respiratory system disease	448	14.87	251	13.93	197	16.27	NS			
Urinary system diseases	386	12.81	183	10.16	203	16.76	NS			
Digestive system disease	241	8	138	7.66	103	8.51	NS			
Circulation system disease	196	6.51	116	6.44	80	6.61	NS			
Blood disease	153	5.08	86	4.77	67	5.53	NS			
Peripheral neuropathy	154	5.11	52	2.89	102	8.42	NS			
Endocrine disease	32	1.06	20	1.11	12	0.99	NS			

Qualitative data were compared with chi-square test or, when not possible, the Fischer exact test. * Anti-Ro52 positive vs negative group, p<0.05. NS: Not significant, SLE: Systemic lupus erythematosus, CTD-ILD: Connective tissue disease-associated interstitial lung disease, IM: Inflammatory myositis, RA: Rheumatoid arthritis, MCTD: Mixed connective tissue disease: pSS: Primary Sjogren's syndrome. Other diseases were not included in the analysis owing to few and scattered cases.

Table-2: ANA-IIF patterns and titers associated with anti-Ro52 positive alone.

ANA-IIF patterns	Prevalence (N =7413)		anti-Ro52 positive alone (n =313)		Anti-Ro52 negative (*n* =5107)		*P* values	
	n	%	n	%	n	%	Comparison of three groups	Comparison of groups 2 and 3
nuclear speckled	3809	51.38	128	40.89	2288	44.80	<0.001	0.116
cytoplasmic speckled	1473	19.87	126	40.26	985	19.29	<0.001	<0.001*
nuclear homogeneous	1420	19.16	22	7.03	1166	22.83	<0.001	<0.001*
nucleolar	523	7.06	22	7.03	417	8.17	<0.001	0.474
nucleolar envelope	93	1.25	17	5.43	63	1.23	<0.001	<0.001
centromere	454	6.12	2	0.64	344	6.74	<0.001	<0.001
spindle fibres	31	0.42	2	0.64	27	0.53	0.45	0.795
centrosome	12	0.16	2	0.64	8	0.16	0.111	0.054
Golgi	3	0.04	2	0.64	9	0.18	<0.001	0.077
lysosome	16	0.22	1	0.32	2	0.04	<0.001	0.041
cytoplasmic linear	15	0.20	0	0	9	0.18	0.739	0.457
discrete nuclear	64	0.86	0	0	60	1.17	0.22	0.054
ANA-IIF titers								
1:100	4660	62.9	241	77.0	3627	70.2	<0.001	0.24
1:320	1883	25.4	59	18.8	1038	20.1	<0.001	0.563
≥1:1000	870	11.7	13	4.2	442	8.5	<0.001	0.04

chi-square test. ANA-IIF: Antinuclear antibody-indirect immunofluorescence.

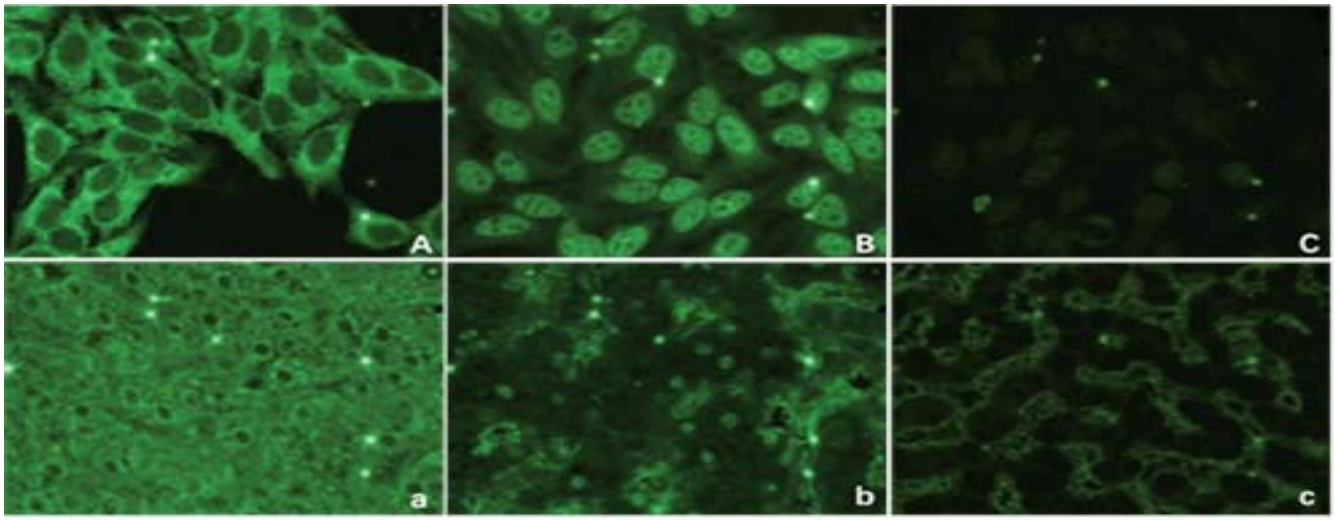

Figure: Typical ANA IIF pattern of Ro52 antibody. Panel A: ANA IIF pattern of Ro52 positive sample on human epithelial cell2 (HEp2) (Cytoplasmic speckled); Panel B: ANA IIF pattern of Ro52 positive sample on HEp2 cells (Nuclear speckled); Panel a, b: ANA IIF pattern of Ro52 positive sample on monkey liver tissue; and Panel C, c: Negative control (healthy individuals). ANA-IIF: Antinuclear antibody-indirect immunofluorescence.

controls (p<0.001), while nuclear homogeneous showed a lower prevalence (7.03% vs 22.83%). ANA IIF titters were mainly low-to-moderate in Ro52 antibody-positive alone cases (Table 2). Typical nuclear-speckled pattern 128(40.89%) and cytoplasmic speckled pattern 126(40.26%) were found in samples with anti-Ro52 antibody as the sole antibody (Figure).

The frequency of associated clinical diagnosis and the difference of gray value (IB, Immunoblotting method) in isolated anti-Ro52 patients' group with cytoplasmic speckled ANA IIF pattern versus nuclear speckled pattern showed ILD to be more frequent in the isolated anti-Ro52 group with cytoplasmic speckled than the nuclear speckled pattern (19.2% versus 9.5%), and PM/DM (polymyositis and dermatomyositis) (12.8% versus 2.7%). Tumours, especially lung cancer, seemed to be more frequent in the isolated anti-Ro52 group with nuclear speckled patterns than with cytoplasmic speckled patterns (36.5% versus 16.7%) and hepatitis B (20.3% versus 9.0%).

Discussion

The study found that in the Han ethnicity of Chongqing in southwest China, the rate of positivity Ro52 antibody in 24,263 patients was 10.61%, and the Ro52 antibody-positive rate of females (86.9%) was higher than that of males (13.1%), and the age of ANA+Ro52 antibody-positive patients was higher than that of the controls. These findings are consistent with the characteristics of autoimmune diseases[20, 21]. This survey involved almost all departments and physical systems, and non-immune diseases accounted for a certain proportion of cases. The percentage of Ro52 antibody positive in autoimmune diseases was higher (77.40%) than in non-autoimmune diseases (53.30%) which agreed with literature[2].

Among the autoimmune diseases associated with Ro52 antibody positivity in serum samples from western China, pSS, SLE, IM, CTD-ILD and MCTD were the most common ($p<0.05$), which is in accordance with the relevant studies in European and American populations[3,22,23]. Ro52 antibody showed high PPV (96.15%), but modest NPV (40.51%) for pSS, followed by SLE (91.20% PPV, 43.46% NPV), CTD-ILD (86.67% PPV, 40.60% NPV), and IM (86.67% PPV, 40.74% NPV). Ro52 antibody was reported as the most common specific autoantibody in pSS (66.7%)[6,24]. In SLE, systemic sclerosis (SSc) and autoimmune myositis, approximately one-third of the patients showed Ro52 antibody, photosensitivity was closely related to Ro/SSA antibody and approximately two-thirds of skin specimens of patients with LE (Lupus Erythematosus) showed Ro52 antibody[25-27]. Among patients with autoimmune myositis, the Ro52 antibody is often co-expressed with anti-synthetase autoantibodies (Jo-1 antibody, Ku antibody and U1RNP), but, among patients with immune myositis, up to 14% express the Ro52 antibody alone, while some studies have reported that the presence of the Ro52 antibody, either alone or in conjunction with other autoantibodies, is associated with ILD[28,29]. According to several studies, the CC (coiled-coil) domain is the most commonly targeted region of the Ro52 protein in patients with SLE and autoimmune myositis[30]. Interestingly, patients with SS appeared to have specificities against several different Ro52 epitopes, including the RING, B-box (B-Box zinc finger gene containing protein) and CC domains[31].

Among non-autoimmune diseases, the percentage of Ro52 antibody positive in xerophthalmia (75.9%) was higher than in the control group (24.1%) in the current study. Ro52 antibody showed 75.86% PPV and 43.46% NPV for xerophthalmia. However, there is no relevant report in China or elsewhere in this regard. Many factors are involved in the pathogenesis of xerophthalmia/dry eye, including environmental factors, infection, endogenous load, antigens and genetic factors, which collectively induce stress to the ocular surface[32,33]. Recent studies have shown that dry eye is an inflammatory disease that shares many characteristics with autoimmune diseases. The results of immunohistological evaluations confirmed that the characteristics of combined inflammation in SS and non-SS patients with dry eye were identical. The primary manifestations were T-cell infiltration and up-regulation of cluster of differentiation 3 (CD3), CD4 and lymphocyte activation markers[33], suggesting that the clinical symptoms of dry eye can be determined by T-cell activation and autoimmune inflammation caused by this activation, and this might be associated with the increased Ro52 antibody expression in dry eye. On the other hand, dry eye is one of the symptoms of SS, and Ro52 expression was statistically different in SS compared to the control group. Some of the dry eye patients in the current study may not have met the criteria for SS or could be early SS pending diagnosis, so they showed positive Ro52. The difference in Ro52 expression between patients with dry eye and patients with syndromes will be further investigated.

Furthermore, Ro52 antibody positivity has been reported in some blood diseases, chronic nephritis, chronic renal insufficiency and chronic renal failure[34-35]. Such patients with non-autoimmune diseases might show autoimmune organ damage, including SLE, which is most likely to involve the kidney.

Ro52 antibody usually coexisted with other ENA antibodies in the serum of patients. The current study confirmed that the autoantibodies most frequently detected as co-positive were ANA+Ro52+SSA+ (33.96%), followed by ANA+Ro52+ (19.05%), ANA+Ro52+SSA+SSB (14.12%) and ANA+Ro52+SSA+u1-nRNP (11.20%). These autoantibodies played a key role in the diagnosis of autoimmune diseases and were useful and important complementary tools for the diagnosis and follow-up of patients with several autoimmune diseases.

Concerning the clinical diagnosis of patients with ANA-negative and Ro52-positive alone cases, there was no significant difference in most diseases between Ro52+ANA- and Ro52+ANA+ group. This may be related to the fact that ANA-positive had not yet appeared in the early stage of related diseases, while Ro52 had begun to show differential expression. This area needs to be further explored.

Specific data based on a large case series involving ANA IIF patterns in Ro52 antibody-positive alone serum are still

lacking. The current analyses confirmed that the percentage of single ANA IIF patterns was 89.1% versus 10.8% of complex patterns in serum samples with Ro52 antibody-positive alone. The ANA IIF patterns most frequently detected were typical nuclear-speckled patterns (40.89%) and cytoplasmic speckled patterns (40.26%). Particularly, it was found that the cytoplasmic speckled pattern showed a higher prevalence in the Ro52 antibody-positive alone cases than in the controls (40.26% vs 19.29%), while the nuclear homogeneous pattern showed a lower prevalence (7.03% vs 22.83%). The pattern distribution was consistent with the cytoplasmic localisation of the Ro52 protein. This might be helpful in the research and analysis of Ro52 antibody therapy and its clinical significance. Some studies have reported a more homogeneous pattern in Ro52 antibody-positive serum, as that might not exclude the simultaneous positivity of other anti-ENAs.

The present study found that ILD was associated with the presence of cytoplasmic speckled ANA IIF pattern (19.2% vs 9.5%) in isolated anti-Ro52 antibody-positive patients, while lung cancer and hepatitis B seemed to be more frequent with nuclear speckled pattern (36.5% versus 16.7%, 20.3% vs 9.0%). It might in part lie with the fact that Ro52 can interact with both cytoplasmic (UBE2D1, Ubiquitin-conjugating enzyme E2 D1) and nuclear (UBE2E1 Ubiquitin-conjugating enzyme E2 D1) E2s (ubiquitin conjugating enzyme)[1].

Ro52 may therefore regulate both cytoplasmic and nuclear substrates via ubiquitination. Anti-Ro52 demonstrated a number of associations with clinical features of diseases, which may be useful in identifying a subgroup of patients at risk of developing certain symptoms, biochemical profiles or prognoses. The Ro52/TRIM21 protein plays an important role in innate and adaptive immunity. Additionally, it plays an important role in regulating inflammation and is involved in the pathogenesis of autoimmune diseases. The Ro52 protein and its regulatory molecules and pathways are important targets for further research and targetted therapies.

The current study has certain limitations. Some diagnoses were based on the referring physician's decision and the classification and clinical stage of diseases remain to be further refined. Even though the results seem coherent with earlier results, these limitations should be kept in mind when interpreting the current findings. Prospective studies are needed to determine whether or not anti-Ro52 antibodies analysis contributes to the diagnosis of the patient's related diseases.

Conclusion

The findings confirmed some known association of Ro52/TRIM21 antibody with gender, age and AID (autoimmune disease)/non-AID status, and added some new details for diagnostic purposes that may lay the foundation for further studies on the clinical significance and molecular mechanisms of Ro52 in specific diseases and provide a basis for further classification of autoantibody fluorescent patterns.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: Chongqing Science and Technology Commission, China.

References

1. Oke V, Wahren-Herlenius M. The immunobiology of Ro52 (TRIM21) in autoimmunity: a critical review. J Autoimmun. 2012; 39:77-82. doi: 10.1016/j.jaut.2012.01.014.
2. Dugar M, Cox S, Limaye V, Gordon TP, Roberts-Thomson PJ. Diagnostic utility of anti-Ro52 detection in systemic autoimmunity. Postgrad Med J. 2010; 86:79-82. doi: 10.1136/pgmj.2009.089656.
3. Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G, et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. Autoimmun Rev. 2011; 10:150-4. doi: 10.1016/j.autrev.2010.09.005.
4. Lee AYS. A review of the role and clinical utility of anti-Ro52/TRIM21 in systemic autoimmunity. Rheumatol Int. 2017; 37:1323-33. doi: 10.1007/s00296-017-3718-1.
5. Wodkowski M, Hudson M, Proudman S, Walker J, Stevens W, Nikpour M, et al. Monospecific anti-Ro52/TRIM21 antibodies in a tri-nation cohort of 1574 systemic sclerosis subjects: evidence of an association with interstitial lung disease and worse survival. Clin Exp Rheumatol. 2015; 33:S131-5.
6. Robbins A, Hentzien M, Toquet S, Didier K, Servettaz A, Pham BN, et al. Diagnostic Utility of Separate Anti-Ro60 and Anti-Ro52/TRIM21 Antibody Detection in Autoimmune Diseases. Front Immunol. 2019; 10:444. doi: 10.3389/fimmu.2019.00444.
7. Yang B, Wang J, Sun B. Trim21: a novel negative regulator in DNA sensor signaling. Cell Mol Immunol. 2013; 10:190-2. doi: 10.1038/cmi.2013.12.
8. Chinese Rheumatology Association; Guidelines for diagnosis and treatment of systemic lupus erythematosus. Zhonghua Feng Shi Bing Xue Za Zhi.2010; 14:342-6. DOI:10.3760/cma.j.issn.1007-7480.2010.05.016.
9. Chinese Rheumatology Association; National Clinical Research Center for Dermatologic and Immunologic Diseases; Chinese Systemic Lupus Erythematosus Treatment and Research Group. [2020 Chinese guidelines for the diagnosis and treatment of systemic lupus erythematosus]. Zhonghua Nei Ke Za Zhi. 2020; 59:172-85. doi: 10.3760/cma.j.issn.0578-1426.2020.03.002.
10. Chinese Rheumatology Association; Guidelines for diagnosis and treatment of mixed connective tissue diseases. Zhonghua Feng Shi Bing Xue Za Zhi. 2011; 15:42-5. DOI:10.3760/cma.j.issn.1007-7480.2011.01.011.
11. Chinese Rheumatology Association; Guidelines for diagnosis and treatment of rheumatoid arthritis. Zhonghua Feng Shi Bing Xue Za Zhi. 2010; 14:265-70. DOI:10.3760/cma.j.issn.1007-7480.2010.04.014.

12. Chinese Rheumatology Association; 2018 Chinese rheumatoid arthritis Diagnosis and Treatment Guidelines. Zhonghua Nei Ke Za Zhi. 2018; 57:242-51. DOI:10.3760/cma.j.issn.0578-1426.2018.04.004.
13. Chinese Rheumatology Association; Guidelines for diagnosis and treatment of Sjogren's syndrome. Zhonghua Feng Shi Bing Xue Za Zhi. 2010; 14:766-8. doi:10.3760/cma.j.issn.1007-7480.2010.11.011.
14. Chinese Thoracic Society; Guidelines for the diagnosis and Treatment of idiopathic pulmonary (interstitial) fibrosis (Draft). Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25:387-9.
15. Group of Pulmonary Vascular and Interstitial Diseases Associated with Rheumatic Diseases, Chinese Association of Rheumatology and Immunology Physicians; Chinese Rheumatic Disease Data Center. [2018 Chinese expert-based consensus statement regarding the diagnosis and treatment of interstitial lung disease associated with connective tissue diseases]. Zhonghua Nei Ke Za Zhi. 2018; 57:558-65. doi: 10.3760/cma.j.issn.0578-1426.2018.08.005.
16. Chinese Rheumatology Association; Guidelines for diagnosis and treatment of polymyositis and dermatomyositis. Zhonghua Feng Shi Bing Xue Za Zhi. 2010; 14:828-31. DOI:10.3760/cma.j.issn.1007-7480.2010.12.008.
17. Chinese Branch of the Asian Dry Eye Society; Ocular Surface and Tear Film Diseases Group of Ophthalmology Committee of Cross-Straits Medicine Exchange Association; Expert Consensus on Diagnosis and Treatment of meibomian gland dysfunction in China. Zhonghua Yan Ke Za Zhi. 2017; 5:657-61. doi:10.3760/cma.j.issn.0412-4081.2017.09.005.
18. Chinese Branch of the Asian Dry Eye Society; Ocular Surface and Tear Film Diseases Group of Ophthalmology Committee of Cross-Straits Medicine Exchange Association; Ocular Surface and Dry Eye Group of Chinese Ophthalmologist Association. [Chinese expert consensus on dry eye: dry eye related to immunologic diseases (2021)]. Zhonghua Yan Ke Za Zhi. 2021; 57:898-907. doi: 10.3760/cma.j.cn112142-20210929-00466.
19. Damoiseaux J, Andrade LEC, Carballo OG, Conrad K, Francescantonio PLC, Fritzler MJ, et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective. Ann Rheum Dis. 2019; 78:879-89. doi: 10.1136/annrheumdis-2018-214436.
20. Roved J, Westerdahl H, Hasselquist D. Sex differences in immune responses: Hormonal effects, antagonistic selection, and evolutionary consequences. Horm Behav. 2017; 88:95-105. doi: 10.1016/j.yhbeh.2016.11.017.
21. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016; 16:626-38. doi: 10.1038/nri.2016.90.
22. Casal-Dominguez M, Pinal-Fernandez I, Corse AM, Paik J, Albayda J, Casciola-Rosen L, et al. Muscular and extramuscular features of myositis patients with anti-U1-RNP autoantibodies. Neurology. 2019; 92:e1416-e26. doi: 10.1212/WNL.0000000000007188.
23. Infantino M, Manfredi M, Grossi V, Benucci M, Morozzi G, Tonutti E, et al. An effective algorithm for the serological diagnosis of idiopathic inflammatory myopathies: The key role of anti-Ro52 antibodies. Clin Chim Acta. 2017; 475:15-9. doi: 10.1016/j.cca.2017.10.002.
24. Mariette X, Criswell LA. Primary Sjögren's Syndrome. N Engl J Med. 2018; 378:931-9. doi: 10.1056/NEJMcp1702514.
25. Kamiyama R, Yoshimi R, Takeno M, Iribe Y, Tsukahara T, Kishimoto D, et al. Dysfunction of TRIM21 in interferon signature of systemic lupus erythematosus. Mod Rheumatol. 2018; 28:993-1003. doi: 10.1080/14397595.2018.1436028.
26. Kvarnström M, Dzikaite-Ottosson V, Ottosson L, Gustafsson JT, Gunnarsson I, Svenungsson E, et al. Autoantibodies to the functionally active RING-domain of Ro52/SSA are associated with disease activity in patients with lupus. Lupus. 2013; 22:477-85. doi: 10.1177/0961203313479420.
27. Hanly JG, Su L, Farewell V, Fritzler MJ. Comparison between multiplex assays for autoantibody detection in systemic lupus erythematosus. J Immunol Methods. 2010; 358:75-80. doi: 10.1016/j.jim.2010.04.005.
28. Ferreira JP, Almeida I, Marinho A, Cerveira C, Vasconcelos C. Anti-ro52 antibodies and interstitial lung disease in connective tissue diseases excluding scleroderma. ISRN Rheumatol. 2012; 2012:415272. doi: 10.5402/2012/415272.
29. Sabbagh S, Pinal-Fernandez I, Kishi T, Targoff IN, Miller FW, Rider LG, et al. Childhood Myositis Heterogeneity Collaborative Study Group. Anti-Ro52 autoantibodies are associated with interstitial lung disease and more severe disease in patients with juvenile myositis. Ann Rheum Dis. 2019; 78:988-95. doi: 10.1136/annrheumdis-2018-215004.
30. Rutjes SA, Vree Egberts WT, Jongen P, Van Den Hoogen F, Pruijn GJ, Van Venrooij WJ. Anti-Ro52 antibodies frequently co-occur with anti-Jo-1 antibodies in sera from patients with idiopathic inflammatory myopathy. Clin Exp Immunol. 1997; 109:32-40. doi: 10.1046/j.1365-2249.1997.4081308.x.
31. Al Kindi MA, Colella AD, Chataway TK, Jackson MW, Wang JJ, Gordon TP. Secreted autoantibody repertoires in Sjögren's syndrome and systemic lupus erythematosus: A proteomic approach. Autoimmun Rev. 2016; 15:405-10. doi: 10.1016/j.autrev.2016.01.008.
32. Baudouin C, Messmer EM, Aragona P, Geerling G, Akova YA, Benítez-del-Castillo J, et al. Revisiting the vicious circle of dry eye disease: a focus on the pathophysiology of meibomian gland dysfunction. Br J Ophthalmol. 2016; 100:300-6. doi: 10.1136/bjophthalmol-2015-307415.
33. Pflugfelder SC, de Paiva CS. The Pathophysiology of Dry Eye Disease: What We Know and Future Directions for Research. Ophthalmology. 2017; 124:S4-S13. doi: 10.1016/j.ophtha.2017.07.010.
34. Alonso-Larruga A, Bustabad S, Navarro-Gonzálvez JA, Rodríguez-Lozano B, Franco A, Barrios Y. Isolated Ro52 Antibodies as Immunological Marker of a Mild Phenotype of Undifferentiated Connective Tissue Diseases. Int J Rheumatol. 2017; 2017:3076017. doi: 10.1155/2017/3076017.
35. Frodlund M, Reid S, Wetterö J, Dahlström Ö, Sjöwall C, Leonard D. The majority of Swedish systemic lupus erythematosus patients are still affected by irreversible organ impairment: factors related to damage accrual in two regional cohorts. Lupus. 2019; 28:1261-72. doi: 10.1177/0961203319860198.

Author's Contributions

KL: Design and acquisition.
YL: Analysis and interpretation of the work.
PL: Drafting, critical revising.
JS: Conception and design of the work, final approval, accountable for all aspects of the work.