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Clinical associations of the positive anti Ro52 without Ro60 autoantibodies: undifferentiated connective tissue diseases

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ABSTRACT

Aims Autoantibodies targeting Ro52 and Ro60 antigens are historically reported as anti SSA/Ro. In general anti SSA/Ro results are either anti Ro52+Ro60+ or anti Ro52−Ro60+ antibodies. Anti Ro52 without anti Ro60 (Ro52+ Ro60−) antibodies are often not reported routinely. This study intends to review the potential significance of these autoantibodies in the management of connective tissue diseases.

Method A retrospective survey of Ro52+Ro60− was carried out as part of the service evaluation of extractable nuclear antigen antibodies (ENA) reporting from the immunology laboratory, the NHS Greater Glasgow and Clyde (GGC), UK. The clinical documents and laboratory results of 97 patients with Ro52+Ro60− and 100 patients with Ro52+Ro60+ were reviewed.

Results Seventy-one patients (73%) with anti Ro52+Ro60− antibodies have been diagnosed with autoimmune conditions including undifferentiated connective tissue diseases (n=14, 14%), systemic lupus erythematosus (n=10, 10%), Sjögren's syndrome (n=10, 10%) and rheumatoid arthritis (n=13, 13%). Twenty-three patients (24%) with anti Ro52+Ro60− antibodies have no autoimmune features but were found to have significant clinical conditions including malignancies. In contrast, 87 patients (87%) with anti Ro52+Ro60+ antibodies have autoimmune conditions including Sjögren's syndrome (n=34, 34%), systemic lupus erythematosus (SLE; n=23, 23%), undifferentiated connective tissue diseases (n=12, 12%) and rheumatoid arthritis (n=6, 6%).

Conclusion Anti Ro52 without anti Ro60 (Ro52+Ro60−) antibodies should be reported. In the majority of patients these autoantibodies were associated with various autoimmune diseases. Anti Ro52+Ro60− antibodies were also found in patients with significant clinical conditions including malignancies even though there was no suggestion of autoimmunity at the time of testing.

INTRODUCTION

Autoantibodies are widely accepted for screening for (eg, anti nuclear antibodies, ANA) or characterising (eg, anti mitochondrial antibodies) autoimmune diseases.[1 2] Some are useful as biomarkers (eg, IgA anti tissue transglutaminase antibodies,[3–5] anti proteinase 3[6 7]). Anti Ro52 antibody (Ro52+) is one of the autoantibodies directed against extractable nuclear antigens (ENA). Positive ANA samples with relevant patterns are checked for their anti ENA antibody identities.

It is not uncommon that some patients have features of connective tissue disease (CTD) but the diagnostic or classification criteria for any of the defined CTDs (eg, systemic lupus erythematosus, Sjögren's syndrome, etc) are not fulfilled.[8–10] The term 'undifferentiated connective tissue disease' (UCTD) was introduced in 1999[11] to describe this group. Anti SSA/Ro immunoglobulin G (IgG) antibodies have been reported in a proportion of patients with UCTD.[10 12] However in a proportion of patients with UCTD, the anti ENA screen was positive but ENA identities were reported negative. This suggests the possibility of variation in reporting ENA antibodies. A variation in the sensitivity of assays used for the detection of anti Ro antibodies has also been reported.[13 14]

Many laboratories in the UK do not routinely report positive anti Ro52+Ro60− (email survey among UK immunology laboratories, unpublished data). Anti Ro52 antibodies have been reported in Sjögren's syndrome (SS),[15–17] systemic lupus erythematosus (SLE),[17–19] systemic sclerosis (SSC),[20–22] diffuse cutaneous systemic sclerosis,[23] primary biliary cirrhosis (PBC),[19 24] polymyositis/dermatomyositis (PM/DM),[19 20] interstitial lung disease (ILD)[20 22] and malignancies.[16 23 25–27] This article provides data on the clinical conditions of the patients found to have positive anti Ro52 without Ro60 antibodies (Ro52+Ro60−) based on a survey carried out in the NHS Greater Glasgow and Clyde (GGC) immunology laboratory. These are regional data representing all patients reviewed in many hospitals within the GGC area.

Anti Ro52 antibodies

The SSA/Ro antigen system contains two major isoforms with molecular weights of 60 kDa and 52 kDa.[12 28] Historically, autoantibodies to Ro52/Ro60 antigens could not be identified separately[29] and were known simply as anti SSA/Ro. Anti Ro52+ autoantibodies (Ro52+) recognise a 52 kDa protein complexed with Y1−Y5 RNA. Ro52 is a predominantly cytoplasmic protein that contains a RING, a B-box motif, a coiled-coil domain and a B30.2 (or PRYSPRY) region in the C-terminal end. Based on this molecular structure, Ro52 belongs to the family of tripartite motif proteins (TRIM) and it is also denoted TRIM21. Ro52 has E3 ligase activity and functions in the process of ubiquitination.[30 31] It can be up-regulated and translocated into the nucleus in a pro-inflammatory environment and regulates type 1 interferon and cytokine production.[32]

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[33] This suggests Ro52+ (not only the antibody) may have an important role in autoimmunity.[34 35] The expression of Ro52 protein correlates with inflammation in autoimmune diseases, for example, salivary glands in primary SS.[36] Although these antibodies have been reported in many autoimmune diseases,[37] they are not specific given that they have been detected in other systemic conditions without autoimmune features.[27 38 39] This survey provides data indicating that Ro52+Ro60− can be detected in many significant disease conditions although they may not be directly related to the non-autoimmune conditions. The presence of Ro52+Ro60− may increase the probability of autoimmune diseases in patients with a chronic inflammatory presentation.

METHOD

The study is a retrospective review of laboratory data (Telepath) and clinical documents from the GGC hospital computer system (Clinical Portal).

Study population

The study cohort was selected by extracting all samples with a positive anti Ro52 antibody result from the laboratory computer system (Telepath) between September 2005 and January 2014. These data included the sample identification number and Community Health Index (CHI) and were analysed using Microsoft Office Excel. There was no patient contact or review in person. Therefore no specific ethics approval was required for this survey as per the local guidance. The following inclusion and exclusion criteria were applied in selecting patients:

Inclusion criteria

1. Positive ENA screening and ENA identity results with positive anti Ro52 (Ro52+)
2. Patients attending primary or secondary care facilities within the GGC health board
3. The clinical relevance of the ENA results had been reviewed by rheumatology consultants.

Exclusion criteria

1. Samples submitted from surrounding health boards via external referral laboratories
2. ENA identity results with negative anti Ro52 (Ro52−)
3. No available documentation regarding the clinical relevance of the ENA results.

The study population (figure 1) represented a small proportion of patients with positive Ro52+Ro60− identified from Telepath. The GGC Immunology Department provides the immunology service for the West of Scotland. Approximately 200 ANA and 35 ENA identity tests are carried out every week. Our immunology laboratory receives samples requesting nuclear antibodies from both primary care and secondary care clinicians from the local GGC catchment areas. Samples from referral laboratories from outlying health boards are also received for ENA identity testing but were not included in the study as relevant

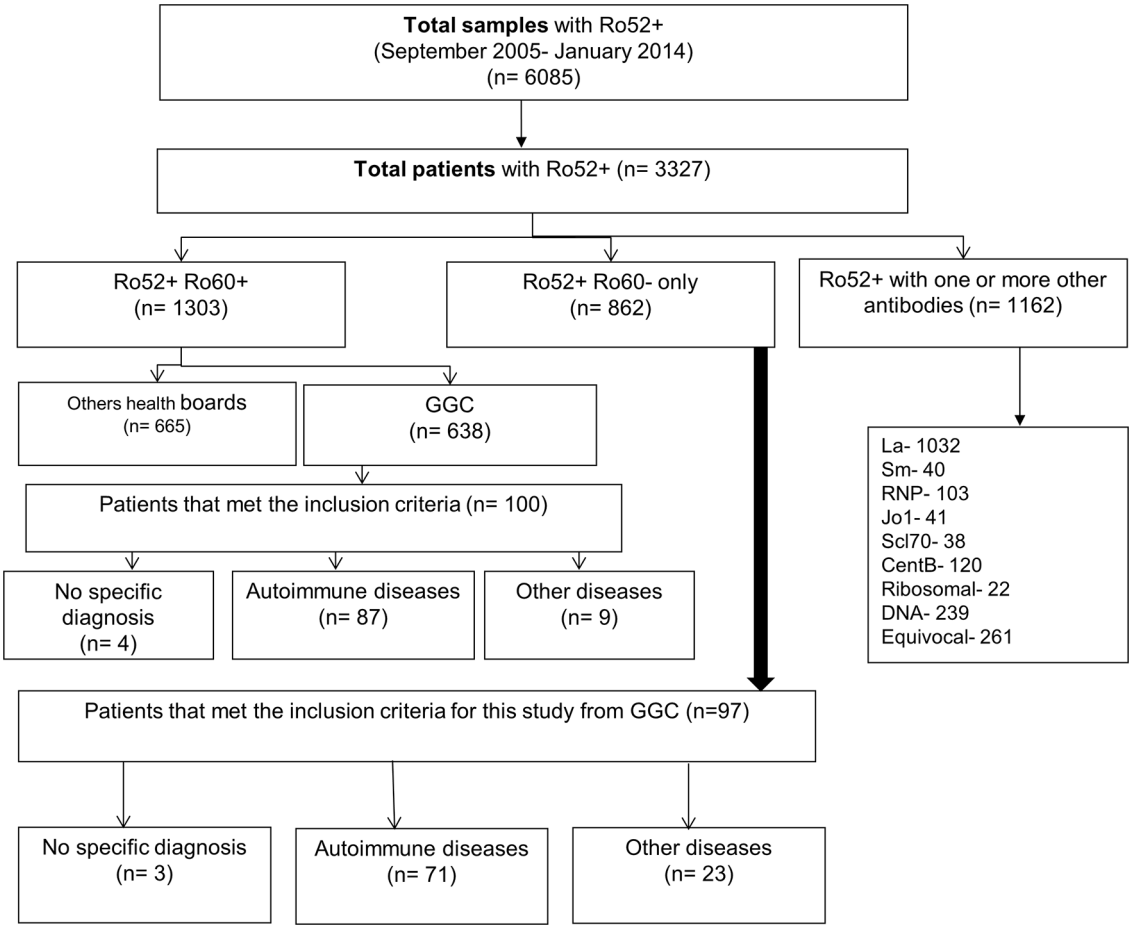

Figure 1 NHS Greater Glasgow and Clyde (GGC) patients with positive anti Ro52+ antibodies on ENA identity testing (n=3327).

information such as ANA results and clinical details were not accessible (figure 1).

Assay methods

The following assay methods were carried out according to the assay manufacturers' instruction:

1. ANA: indirect immunofluorescence (IIF); Hep2000: Zenit, A. Menarini, Berkshire, UK
2. Anti DNA screening: enzyme immunoassay (EIA); Phadia VarelisA, Freiburg, Germany
3. Anti DNA confirmation: Crithidia lucillae indirect immunofluorescence test (CLIFT); Zenit, A. Menarini, Berkshire, UK
4. Anti ENA screening: Phadia 250 EliA Symphony, Freiburg, Germany
5. Anti ENA identity: FIDIS Connective 10 Multiplex, Luminex technology; Theradiag (BMD), Marne La Vallee, France.

The following strategy is applied to all samples requiring nuclear antibodies testing:

1. The ANA screening is carried out at a sample dilution of 1 in 40.
2. Repeat requests for ANA (within 30 days) are usually rejected.
3. Strong positive samples with the following patterns (homogeneous, speckled, nucleolar) are titrated at 1 in 160, 1 in 640 and 1 in 2560 dilutions.
4. Strong homogeneous patterns (titre 1 in 160 or more) are screened for anti DNA and the positive samples are confirmed by CLIFT.
5. Other strong positive patterns (titre 1 in 160 or more) including speckled and nucleolar patterns have anti ENA screening checked and then screen positive samples have their ENA identity checked.
6. Specific requests for anti DNA and ENA identity from specialist clinics (eg, rheumatology) are also allowed outside the above protocol.

Quality control rules were applied to all assays. All assays were carried out and routinely maintained according to the assay manufacturer's instruction. No non-conformity or misclassification in the performance of these assays was found in the external quality assessment (EQA) during the study period that would affect the results of this study.

The clinical details of patients with Ro52+Ro60− (n=97) and Ro52+Ro60+ (n=100) were reviewed. The clinical diagnoses and ENA results of selected patients (n=30) who had their ENA identity checked more than once over the 8-year study were also reviewed to assess the stability of their ENA identity results (see online supplementary table 1).

RESULTS

A total of 6085 samples (3327 patients) were found to be positive for anti Ro52 antibodies (Ro52+) on ENA identity testing over the 8-years study (figure 1).

Isolated positive anti Ro52 antibodies (Ro52+Ro60–) are relatively common

Eight hundred and sixty-two patients (26%) were Ro52+Ro60− positive, 1303 patients (39%) were Ro52+Ro60+ positive and 1162 patients (35%) were Ro52+Ro60− positive with one or more of the other ENA antibodies. This study is a retrospective data and document survey, and no sample was available for rechecking ENA/Ro antibodies using another assay method (eg, Phadia EliA) for comparison. However our ENA screening uses the Phadia 250 EliA Symphony. EliA Symphony wells are coated with human recombinant antigens U1RNP (RNP70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, centromere B, Scl-70, Jo-1 proteins, and native purified Sm proteins. Our ENA identity assay (FIDIS™ Connective 10 Multiplex) allows the detection of 10 autoantibodies including recombinant dsDNA, SS-B, TRIM21 (Ro52), CENP-B, Jo-1, and native purified Scl-70, SmRNP, Sm, Ribosomal and SS-A (Ro60). Therefore positive screening on the EliA Symphony assay was highly likely due to the Ro52 when all other specificities except Ro52 were negative on the FIDIS™ Connective 10 Multiplex.

Diseases found in patients with Ro52+Ro60– antibodies include autoimmune conditions and systemic conditions without obvious autoimmune features

The vast majority of patients with Ro52+ Ro60− were excluded from this study as per the criteria stated previously. Only 97 patients fulfilled the inclusion criteria for this survey. The majority of these patients (71 patients with Ro52+Ro60−) presented with one or more autoimmune conditions (figure 1 and table 1).

ENA identities are usually stable over many years

The ENA identities were stable over many years based on the limited data from 30 patients collected for this retrospective study (see online supplementary table 1). Of 30 patients with repeat tests, 24 (80%) had identical specificities when tests were repeated between 1 and 6 years later (see online supplementary table 1). Twenty-seven patients with Ro52+Ro60− had their anti DNA levels checked together with the ENA identity. All these were negative (table 2). Eleven patients with Ro52+Ro60+ also had their anti DNA levels checked together with the ENA identity and three of them had negative results (see online supplementary table 2). Therefore, out of these 38 patients, only three were positive for the anti DNA (7.8%). ENA identities support the clinical phenotype but may not vary with disease activity or the anti DNA level. Further comprehensive studies would improve our knowledge.

Autoimmune diseases found in patients with anti Ro52+Ro60– antibodies

Autoimmune diseases were found to be the predominant conditions in patients with anti Ro52+Ro60−table 2. All these patients were ENA screening positive but had no other detectable autoantibodies included in the FIDIS™ Connective 10 Multiplex.

Seventy-one (73%) patients (male:female 7:64) from this cohort (table 2) (figure 2) were diagnosed with autoimmune diseases. Some 85% (n=57) of patients with autoimmune conditions were aged 50 and over. The majority (n=60) of these patients had more than one autoimmune disease or had other systemic diseases. The autoimmune diseases found in this cohort included SLE, discoid lupus erythematosus (DLE), PBC, SS, systemic sclerosis (SSC), rheumatoid arthritis (RA), interstitial lung diseases (ILD), autoimmune hepatitis (AIH), pernicious anaemia (PA), microscopic polyangiitis, Wegener's granulomatosis, polymyalgia rheumatica (PMR) etc. RA, UCTD, SS and SLE were the most common autoimmune diseases in this cohort. Fifty-eight patients were under the care of rheumatologists.

Fourteen (14%) patients were treated for UCTD. The majority of these patients presented with inflammatory polyarthritis (IP). A strong positive ANA (titre 1:160–1:2560) was found in 11 patients, with the majority having a speckled or nucleolar

Table 1 Summary of patients with isolated anti Ro52 positive antibodies (Ro52+Ro60–) in this study population (n=97)

Category	Number	Age range	Sex (M:F)	Clinical conditions (number)
Autoimmune	71	27–86	7:64	1. UCTD (14) 2. RA (13) 3. SS (10) 4. SLE (8) 5. DLE (2) 6. SSC (3) 7. Vasculitis (2) 8. AIH (4) 9. PBC (3) 10. ILD (2) 11. Myasthenia gravis, hypothyroidism (2) 12. Lichen planus (1) 13. Bullous pemphigoid (1) 14. Hypothyroidism (1) 15. Miscellaneous a. Granulomatous CVID, PA (1) b. Raynaud's (1) c. Psoriatic arthritis (1) d. PMR (1) e. Raynaud's, myopathy (1)
Other systemic diseases	23	31–91	6:17	1. Musculoskeletal: osteoarthritis, gout 2. Cardiovascular: antiphospholipid syndrome, cardiac arrhythmia 3. Neoplastic: paraproteinaemia, neoplasms 4. Dermatological: asteatotic dermatitis, Jessner's benign lymphocytic infiltrate 5. Infections 6. Miscellaneous: neuropathy, acute generalised exanthematous pustulosis
No specific diagnosis	3	23–47	0:3	Severe hair loss (1) Arthralgia (1) Ganglion, bronchial hyperactivity (1)
Total	97	21–91		

AIH, autoimmune hepatitis; CVID, common variable immunodeficiency disorders; DLE, discoid lupus erythematosus; ILD, Interstitial lung disease; PA, pernicious anaemia; PBC, primary biliary cirrhosis; PMR, polymyalgia rheumatica; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; SSC, systemic sclerosis; UCTD, undifferentiated connective diseases/inflammatory polyarthritis.

pattern. One patient with UCTD had a weak ANA (titre 1:40). Forty-three patients including eight with UCTD (one declined) received disease modifying anti-rheumatic drugs (DMARDs) or biologic therapy: corticosteroid, hydroxychloroquine, mycophenolate mofetil, methotrexate, azathioprine, cyclophosphamide, sulphasalazine, leflunomide or rituximab. Some patients were treated with more than one DMARD either sequentially or in combination.

Clinical conditions without autoimmune features found in patients with Ro52+Ro60– antibodies (n=23)

Twenty-three patients (male:female 7:16) from this cohort (see online supplementary table 3) (figure 3) had no confirmed autoimmune diseases, with 83% of these patients (n=19) aged 60 and above. These patients also had strong positive ANA titres (1:160–2560) with speckled or nucleolar or homogeneous patterns or mixed patterns. The clinical conditions seen in these patients included gout, paraproteinaemia, infections (eg, viral hepatitis), neuropathies, neoplasms, sarcoidosis, dermatitis and Jessner's benign lymphocytic infiltrate.

Clinical conditions found in patients with Ro52+Ro60+ antibodies (n=100)

A hundred patients with anti Ro52+Ro60+ were selected as per the criteria stated in the methodology section. The clinical conditions of these patients (see online supplementary table 2) were compared to the anti Ro52+Ro60− cohort. Patients in this cohort have been reviewed by at least one rheumatology consultant regarding their ENA antibodies. Eighty-seven patients (male:female ratio 7:80; age 20–83) presented with clinical conditions including autoimmune diseases. These autoimmune conditions included 34 (34%) SS, 23 (23%) SLE, 12 (12%) UCTD, 6 (6%) DLE and 5 (5%) RA. Other autoimmune diseases included AIH, vasculitis, and giant cell arteritis. Six patients were diagnosed with significant clinical conditions with no obvious autoimmune features: cancers (lung, breast, colon), gout, retroperitoneal fibrosis, neuropathy, inflammatory bowel diseases, cerebrovascular disease, etc. Four patients with anti Ro52+Ro60+ antibodies had no significant clinical conditions at the time of their clinical assessment.

DISCUSSION

Variation in the detection of anti ENA antibodies by routine immunoassays

The method of ANA/Ro screening has improved since the time rodent tissue was used as a substrate. Expression of Ro antigens is affected by differences in the manufacturing process[40 41] and also subject to variability between species: there is less expression in rodent cells than in human and primate cells.[42] Hep2000 cells are used as the standard substrate in our laboratory.

Hep2000 cells are transfected with Ro60 antigens to over-express this antigen. IIF using Hep2000 has increased sensitivity for detecting anti Ro60 antibodies but a negative result does not exclude the presence of isolated anti Ro52 antibodies. In addition, a strongly positive ANA may mask the characteristic Ro pattern seen with IIF on Hep2000 cells.[43] The sensitivity

Table 2 Summary of autoimmune diseases in patients with Ro52+Ro60– antibodies (n=71)

No.	Age (sex)	Main clinical diagnoses	Associated clinical conditions	ANA titre	Anti DNA (VarelisA, IU/mL)	Treatment received for CTD
1	81 (f)	UCTD	Bowen's disease lower legs, pulmonary amyloidosis, bronchiectasis	160 HS	2.9	ND
2	52 (f)	UCTD	Hypothyroidism	2560 NS	5.9	HCQ
3	48 (f)	UCTD	Arthritis	Negative	ND	MTX, SLZ
4	31 (f)	UCTD	Inflammatory arthropathy, Raynaud's, serositis	2560 HS	7	Declined HCQ
5	51 (f)	UCTD	Myasthenia gravis, hypothyroidism, pre-PBC	160 H	4.4	MMF, PRED
6	36 (f)	UCTD	Degenerative spondylosis	160 S	ND	ND
7	70 (m)	UCTD	Neutrophilic dermatoses, muscle weakness, previous Lyme's disease	640 H	7.1	ND
8	27 (f)	UCTD	Acne vulgaris, chronic diarrhoea	160 S	10.2	ND
9	52 (f)	UCTD	Hypothyroidism, pernicious anaemia, IHD, polycystic ovary syndrome	2560S	ND	HCQ
10	52 (f)	UCTD	Systemic granulomatous disorder, MELAS syndrome, IHD, DM, anaemia	40 S	ND	MMF, PRED
11	78 (m)	UCTD	Seborrheic keratosis	2560 S	ND	ND
12	65 (m)	UCTD	ILD, previous myositis	Ribo	ND	SLZ, AZA
13	50 (m)	UCTD	Neuropathy, tendinitis	640 S	ND	
14	56 (f)	UCTD	Inflammatory arthropathy	160 H	9.7	CQ
15	52 (f)	RA		160 S	5.3	MTX
16	73 (f)	RA	Chronic iron deficiency anaemia	640 S	ND	
17	54 (f)	RA	OA	Cyto	ND	MTX, HCQ
18	47 (f)	RA	Chronic active hepatitis	160 HS	8.1	MTX
19	51 (f)	RA	Peripheral neuropathy	40 S	ND	SLZ, HCQ
20	54 (f)	RA		640 S	12.4	MTX, SLZ, HCQ
21	22 (f)	RA	AIH, DM	640 S		AZA
22	56 (f)	RA	MELAS syndrome	Negative	ND	HCQ
23	84 (f)	RA	Possible SS, OA, bronchiectasis	640 HS	9.6	MTX
24	54 (f)	RA	Incidental pulmonary nodule	2560 NS	ND	SLZ, MTX, LEF, rituximab
25	63 (m)	RA	ILD	160 Hcyto	3.4	HCQ, AZA
26	74 (f)	RA		2560 S	ND	
27	75 (f)	RA	OA	2560 S	ND	MTX
28	52 (f)	SLE	Hypothyroidism	2560 S	ND	HCQ
29	67 (f)	SLE	Osteoporosis	160 S	ND	HCQ
30	52 (f)	SLE		40 S	ND	HCQ
31	51 (f)	SLE		2560 S	ND	HCQ
32	63 (f)	SLE	SS, elbow capsulitis	160 S	59.4	HCQ
33	65 (f)	SLE	Raynaud's, osteopenia	160 H	4	HCQ
34	73 (f)	SLE	RA, hypertension, chronic otitis media	2560 SMS	ND	HCQ
35	79 (f)	SLE	ILD, MND, hypertension	160 H	14.3	HCQ
36	60 (f)	DLE	Trochanteric bursitis	640 HN	13	HCQ
37	42 (f)	DLE		160 H	4.2	HCQ
38	72 (f)	SS	OA	ND	ND	HCQ
39	86 (f)	SS	Hashimoto's thyroiditis	2560 S	ND	ND
40	69 (f)	SS	OA, stage three melanoma	2560 S	4.2	ND
41	70 (f)	SS		640 S	2.8	HCQ
42	51 (f)	SS	Myositis, ILD, alopecia, DM	2560 S	ND	MMF, MTX, PRED, AZA
43	77 (f)	SS		Cyto	10.7	MTX
44	78 (f)	SS	OA, dermatitis, asbestosis	2560 HN	6.6	HCQ
45	59 (f)	SS		2560 NS	ND	HCQ
46	76 (f)	SS	Hypertension, diabetes, bilateral mastectomy for breast cancer	2560 S	ND	ND
47	63 (f)	SS	Severe alopecia, OA	160N S	6.8	HCQ
48	38 (f)	AIH	Granulomatous rosacea	2560 N	ND	ND
49	49 (f)	AIH	Portal hypertension	160 HS	ND	ND

Continued

Table 2 Continued

No.	Age (sex)	Main clinical diagnoses	Associated clinical conditions	ANA titre	Anti DNA (VarelisA, IU/mL)	Treatment received for CTD
50	71 (f)	AIH	Hashimoto's thyroiditis, OA, squamous cell carcinoma	2560HNM	38.3	MMF
51	33 (f)	AIH	Myasthenia gravis, liver transplant, pulmonary hypertension	640 HS	14.2	MMF, PRED, CPM, CSP
52	48 (f)	PBC		40 S	ND	ND
53	80 (f)	PBC	Portal hypertension	2560 NS	ND	ND
54	77 (f)	PBC	Hypertension	2560 N	ND	ND
55	54 (f)	SSC	ILD, Raynaud's	640 S	ND	ND
56	57 (f)	SSC		40 S	ND	MMF
57	55 (f)	Limited scleroderma	ILD, Raynaud's	Ribo	ND	MMF, CPM, PRED
58	76 (f)	ILD	Adrenal ulceration, previous bowel resection for diverticulitis, HH, IHD	2560 NS	ND	ND
59	79 (f)	ILD		Ribo	ND	ND
60	78 (f)	Microscopic polyangiitis	Sigmoid tumour, IHD, COPD	40 S	ND	ND
61	69 (f)	Wegener's granulomatosis		C	ND	AZA
62	84 (m)	Bullous pemphigoid	Acute interstitial nephritis, psoriasis	ND	ND	PRED
63	75 (f)	Lichen planus		2560 S	3.2	ND
64	64 (f)	Raynaud's	CVA	2560 S	6	ND
65	63 (f)	Raynaud's	Myofibrillary myopathy, hypergammaglobulinaemia	160 S	ND	ND
66	57 (f)	Myasthenia gravis	Hypothyroidism, venous sinus thrombosis	Negative	ND	ND
67	64 (f)	Myasthenia gravis	Bilateral frozen shoulders, calcinosis cutis, DM	2560 S	ND	AZA
68	74 (f)	Pernicious anaemia	CVID, granulomatous liver disease, hypertension, COPD, osteoporosis	160 H	ND	ND
69	78 (f)	Hypothyroidism	Cirrhosis due to non-alcoholic steatohepatitis, portal hypertension, DM	640 HS	9.4	ND
70	51 (f)	PMR	Achilles tendinopathy	2560 NS	ND	ND
71	69 (m)	Psoriatic arthritis	Colon cancer	2560 N	ND	ND

Reference ranges of anti DNA: VarelisA (negative, <35; equivocal, 35–55; positive, >55).
AIH, autoimmune hepatitis; AZA, azathioprine; C, centromere pattern; COPD, chronic obstructive pulmonary disease; CPM, cyclophosphamide; CSP, cyclosporine; CVA, cerebrovascular accident; cyto, cytoplasmic pattern; DLE, discoid lupus erythematosus; DM, diabetes mellitus; H, homogeneous pattern; HCQ, hydroxychloroquine; HH, hiatus hernia; HS, homogeneous speckled; IHD, Ischaemic heart disease; ILD, interstitial lung disease; LEF, leflunomide; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MMF, mycophenolate mofetil; MND, motor neuron disease; MTX, methotrexate; N, nucleolar pattern; ND, no document available; NM, nuclear membrane pattern; NS, nucleolar speckled; OA, osteoarthritis; PBC, primary biliary cirrhosis; PMR, polymyalgia rheumatica; PRED, prednisolone; ribo, ribosomal pattern; RA, rheumatoid arthritis; S, speckled pattern;SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; SSC, systemic sclerosis; SLZ, sulphasalazine; UCTD, undifferentiated connective diseases/ inflammatory polyarthritis.

of Ro60 and Ro52 in Hep2000 cells has been reported to be 77% and 9.7%, respectively, in correlation with the double immunodiffusion (thymus/spleen natural antigen) and line immunoassay (recombinant antigen).[44] The true prevalence of Ro52+Ro60− is unknown given that negative ANA samples are not routinely checked for ENA. In addition, the sensitivity of assays for detecting ENA varies.[14] Therefore, if anti Ro52

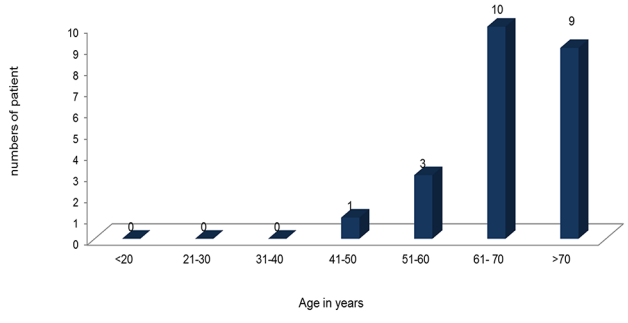

Figure 2 Age distribution of Ro52+ Ro60− patients with autoimmune diseases (n= 71; male:female ratio 6:65).

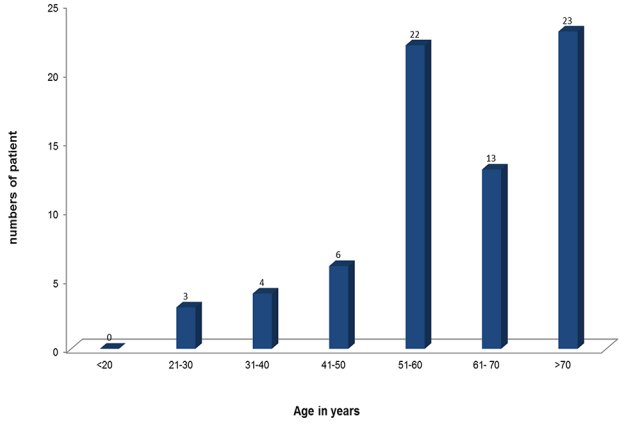

Figure 3 Age distribution of Ro52+ Ro60− patients with systemic diseases (n=23; male:female ratio 6:17).

antibodies are suspected, another assay method containing Ro52 antigens should be used for counter checking. A combination of assay methods is more sensitive for screening for autoimmune diseases.[45]

Anti ENA identities were found to be unchanged for up to 4 years in this cohort (see online supplementary table 1). This suggests rechecking ENA identity is unnecessary within 4 years unless the clinical phenotype has changed. Anti Ro52+Ro60− antibodies may be an independent marker with low specificity for a specific autoimmune disease, so results must be interpreted in light of other parameters. Even the anti dsDNA levels based on the routine assays are of limited utility in monitoring SLE unless other parameters are also considered.[46 47] Further prospective studies would provide more understanding of anti Ro52+Ro60− antibodies.

Clinical significance of anti Ro52 antibodies

Many laboratories do not report isolated positive anti Ro52 (Ro52+Ro60−) antibodies routinely as previous studies reported that specific testing for Ro52 provided no additional value.[14 48] It seems that the Ro52+Ro60− results have not been generally accepted as being as clinically significant as Ro52+Ro60+. However our data suggest Ro52+Ro60− antibodies can be associated with a broader autoimmune phenotype than previously reported. Patients with well-defined autoimmune disorders (SLE, SS, DLE, RA) account for 69 of 87 (79%) of the Ro52+Ro60+ group, whereas the same conditions represent 33 of 71 (46%) in the Ro52+Ro60− group. However, similar proportions of patients with UCTD were seen in both the Ro52+Ro60+ group (12 of 87; 14%) and the Ro52+Ro60− group (14 of 71; 20%). Based on previous reports, anti Ro52+Ro60− antibodies are likely to be the most common autoantibodies detected in autoimmune diseases.[37 49] Our findings reflect this view. Approximately 23% of this cohort (Ro52+Ro60−) presented with no autoimmune features but with significant systemic diseases including malignancy. These may not be directly related to the presence of these autoantibodies. Therefore even if there is no feature of any autoimmune disease, the presence of Ro52+Ro60− warrants further clinical consideration.

Our data may have a potential selection bias due to the following reasons. Previously, Ro52+Ro60− results were reported as 'no significant ENA antibody was detected on the ENA identity checking'. Ro52+Ro60− results started being reported only after this study was carried out. Therefore, patients with less specific autoimmune symptoms may not have been followed up or referred to the rheumatology clinics. A significant proportion of patients were outside the GGC area. Therefore, the outcome of further assessment of patients with Ro52+Ro60− outside the GGC area was unknown. Similarly, the outcome of further assessment or clinical details of patients with Ro52+Ro60+ were also unknown.

This study highlights the potential significance of anti Ro52+Ro60− antibodies. We found a high probability of autoimmune disease with a broader spectrum of associated conditions than previously reported. Based on the testing strategy and clinical criteria used in their diagnoses, the positive predictive value (PPV) for autoimmune disease was 73% (71/97) and 87% (87/100) for Ro52+Ro60− and Ro52+Ro60+, respectively.

A significant proportion of autoimmune diseases in this cohort included UCTD and many of these patients were being treated with DMARDs. A proportion of patients with UCTD have been reported to progress into well-defined CTDs such as SLE and SS.[10] However, more data would be required to clarify the role of Ro52+Ro60− when considering DMARDs. The true proportions of UCTD with Ro52+Ro60− and UCTD with Ro52+Ro60+ are unknown. So far data are insufficient for these cohorts given that there are variations in Ro52+Ro60− reporting and also the limited prospective data regarding the autoantibody profiles of UCTDs that progress to well-defined CTDs.

It would be interesting to observe whether our findings are repeated in other centres with more detailed review and a larger patient cohort. Further evaluation/prospective studies of UCTD with specific clinical details such as the age of onset, the severity of the associated conditions, progress of the disease and response to specific treatment, would be valuable in further characterising the disease phenotype of Ro52+Ro60− results. Moreover, Ro52+Ro60− should be included in the EQA scheme to improve the quality of reporting and consistency within assay methods.

Take home messages

- Anti Ro52+Ro60− antibodies should be reported if included in the panel or specifically requested by the clinician.
- In a proportion of undifferentiated connective tissue diseases, Ro52+Ro60− may be the only positive ENA antibody detected by the routine assays provided by many diagnostic laboratories.
- Patients with anti Ro52+Ro60− antibodies warrant further review for early detection and management of potentially significant diseases.

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Clinical associations of the positive anti Ro52 without Ro60 autoantibodies: undifferentiated connective tissue diseases

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