A subset of systemic sclerosis but not of systemic lupus erythematosus is defined by isolated anti-Ku autoantibodies

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ABSTRACT

Objective. *We aimed to analyse the annual incidence of anti-Ku antibodies and to study their clinical associations in patients mainly affected by systemic sclerosis (SSc) and systemic lupus erythematosus (SLE) overlap diseases.*

Methods. *Anti-Ku were detected by counterimmunoelectrophoresis in a total of 203 sera during 14 years of anti-ENA detection. Anti-Ku+ sera belong to 46 patients, mostly affected by UCTD (12 cases), SSc spectrum diseases (13 cases), including SSc/PM, SSc/DM and SSc; and SLE spectrum diseases (9 cases), including SLE, SLE/APS, SLE/SS, SLE/PM.*

Results. *Anti-Ku antibodies represent 2% of all anti-ENA positive sera, and are found in 1.3-3% of anti-ENA positive sera per year. Anti-Ku+ SSc represents 2% of all SSc patients. All anti-Ku+ SSc spectrum diseases showed a limited cutaneous disease; myositis, dysphagia and isolated anti-Ku in more than 70% of cases. Interstitial lung disease (ILD) was found in 54% of SSc patients, frequently with mild functional impairment. When compared with other limited SSc cases, anti-Ku+ SSc showed a higher rate of male patients, arthritis, myositis and ILD. A lower rate of digital ulcers was found, although both groups showed the same rate of SSc pattern at nailfold capillaroscopy.*

Anti-Ku+ SLE patients (representing 1.5% of all SLE) showed cutaneous features, Raynaud's phenomenon, multiple autoantibodies, without major organ manifestations.

Isolated anti-Ku+ patients significantly show a diagnosis within SSc spectrum diseases, with arthralgias, Raynaud's phenomenon, dysphagia, ILD, myositis and sclerodactyly

Conclusion. *In SLE spectrum diseases, anti-Ku are found associated with other autoantibodies, without a peculiar clinical profile, except for Raynaud's phenomenon and severe alterations of capillary bed. In SSc anti-Ku are frequently associated with myositis and ILD, mostly found as isolated autoantibodies.*

Introduction

Anti-Ku antibodies are a quite rare anti-nuclear specificity, originally described as a marker of systemic sclerosis-polymyositis (SSc/PM) overlap syndrome (1); subsequently found also in other connective tissue diseases, such as systemic lupus erythematosus (SLE) and SSc (2, 3).

Anti-Ku antibodies are usually associated with Raynaud's phenomenon, arthritis and myositis, independently from the underlying autoimmune disease (2, 4). Within SSc, anti-Ku are found in about 2–5% of cases (2,5), frequently with a limited cutaneous extent, articular features and rare severe organ involvement. By contrast, the prevalence of anti-Ku within SLE does not seem to be associated to a specific clinical subset, but to a peculiar ethnical distribution (3).

The aim of this study is to analyse the incidence of anti-Ku antibodies detected in a diagnostic laboratory within a single Rheumatology Unit and to define any clinical associations in SSc overlap or SLE overlap diseases.

Patients and methods

Patients

Among sera analysed for anti-ENA characterisation from 1995 to 2012, 203 resulted positive for anti-Ku antibodies, corresponding to 46 patients. Clinical data of anti-Ku+ subjects were retrospectively obtained from clinical charts. The clinical diagnoses were achieved basing on classification criteria previously used (2). Active intersti-

tial lung disease (ILD) was defined on the basis of ground glass opacities documented by high resolution computed tomography and/or broncho-alveolar lavage analysis and scored according to Medsger criteria (6). Myositis was diagnosed by elevation of muscle enzymes, specific EMG features and muscle histological findings.

The evaluation of hand microcirculation was made by nailfold capillaroscopy (NC) using videocapillaroscopy (DS Medigroup, Italy). The patterns of capillary changes were defined according to Cutolo *et al*. (7).

Methods. Antibodies to ENA were determined by counterimmunoelectrophoresis; antinuclear antibodies (ANA) were detected by indirect immunofluorescence (IIF) on HEp2 cells as substrate (Kallestad, Chaska, MN, USA) and considered positive at titre >1:160. Anti-dsDNA antibodies were studied by FARR assay (Kodak Clinical Diagnostics LTD, Amersham, UK). Anti-cardiolipin and anti-β2 glycoprotein I were detected by ELISA, as previously cited (2).

Statistical analysis

All the parameters were evaluated by χ^2 or Fisher's exact test. Student's *t* or Mann-Whitney tests were used to perform comparison between two groups. Multivariate analysis was performed using logistic regression model (StatView). Statistical significance was accepted at $p<0.05$.

Results

Between 1995 and 2012, anti-Ku antibodies were found in a total of 203 sera among 9642 anti-ENA positive sera, representing the 2.1% of all anti-ENA positivities detected in 16 years (range: 1.3–3% per year). Anti-Ku+ sera belonged to a total of 46 patients, retrospectively analysed: they showed a female to male ratio of 4:1, with a mean age at onset of 55.6 years (SD: 17.9 years). The clinical diagnoses were summarised in Table I. Thirteen patients showed a diagnosis within SSc spectrum diseases (including SSc/PM, SSc/DM and SSc); while 9 patients showed a SLE spectrum disease (including SLE, SLE/APS, SLE/SS, SLE/PM).

Table I. Diagnoses of 46 patients with anti-Ku antibodies.

	n. 46	%
UCTD	12	26
SSc/PM	10	21.7
SSc/DM	1	2.2
PM	5	10.8
SSc	2	4.3
SLE	3	6.5
SLE/APS	2	4.3
SLE/PM	1	2.2
SLE/SS	2	4.3
Primary SS	4	8.7
APS	1	2.2
RA/SS	1	2.2
RA	1	2.2
MCTD	1	2.2

UCTD: undifferentiated connective tissue disease; SSc: systemic sclerosis, PM: polymyositis; DM: dermatomyositis; SLE: systemic lupus erythematosus; APS: anti-phospholipid syndrome, RA: rheumatoid arthritis, SS: Sjögren's syndrome, MCTD: mixed connective tissue disease.

Clinical features

The main clinical features recorded were represented by arthralgias (86.9%), with arthritis in 52%, Raynaud's phenomenon (76%), sicca (63%) and myositis (36.9%).

Anti-Ku+ UCTD showed a stable disease with mild features, namely arthralgias (91.7%), Raynaud's phenomenon (58%) and sicca (50%).

SSc spectrum diseases patients represent 2.3% of the total 560 SSc cases followed in our Unit. They showed a clinical picture characterised by limited cutaneous involvement (100%), myositis (84.6%), dysphagia (79%), sicca (61%), ILD (53.8%). SLE spectrum diseases patients (9 cases) represent 1.5% of 532 SLE cases followed by our Unit. No difference was found between anti-Ku+ and anti-Ku- SLE patients. Anti-Ku+ SLE did not show major organ involvement, such as renal or neurologic involvement. Photosensitivity and Raynaud's phenomenon were found 6 patients each (67%). Oral ulcers, malar rash, subacute cutaneous lupus were found in 2 cases, each. Anti-phospholipid syndrome was diagnosed in two cases.

Disease associations

SLE spectrum diseases showed a lower age at disease onset ($p=0.0002$) and a higher frequency of persistent fever (p. 0.03), while SSc spectrum diseases had a higher rate of myositis ($p=0.003$) and isolated anti-Ku antibodies ($p=0.0031$) (Table II).

All anti-Ku+ SSc cases show a limited cutaneous disease: they were compared with anti-centromere (ACA)+ SSc cases, followed by the same Rheumatology Unit. ACA+ cases were Caucasians of Italian ancestry as well as anti-Ku+ ones. Clinical data were shown in Table III. In multivariate analysis, the occurrence of myositis, arthritis and ILD are significantly associated with anti-Ku ($p<0.0001$, $p=0.02$ and $p<0.0001$, respectively).

Nailfold capillaroscopy (NC) analysis

NC was performed in 30 patients: abnormal NC was found in 22 cases (73.3%), as shown in Table IV. NC scleroderma pattern was significantly found in SSc spectrum diseases (80%; $p=0.002$). Nevertheless, NC SSc pattern was detected in 50% of SLE spectrum diseas-

Table II. Clinical features of anti-Ku+ patients with SLE and SSc spectrum diseases.

	SLE spectrum diseases n=9 (%)	SSc spectrum diseases n=13 (%)	*p*-value
Age at onset, mean±SD (years)	31 ± 17.9	64.1 ± 9.8	<0.00001
F/M	9/0	10/3	ns
Raynaud's phenomenon	6 (66.7)	13 (100)	ns
fever	3 (33.4)	0 0.03	
arthritis	6 (66.7)	5 (38.4)	ns
myositis	0	10 (76.9)	0.0031
sicca	5 (55.6)	8 (61.5)	ns
ILD	1 (11)	7 (53.8)	ns
cranial neuropathies	0	3 (23)	ns
isolated anti-Ku	0	10 (76.9)	0.0031

ILD: interstitial lung disease.

Table III. Comparison between anti-Ku+SSc and anti-centromere (ACA)+ SSc.

	Anti-Ku+ SSc or overlap: 13 (%)	ACA+ SSc: 67 (%)	*p*-value
F/M	10/3	66/1	0.009
Limited SSc	13 (100)	66 (98.5)	ns
Digital ulcers	1 (7.7)	37 (55)	0.004
Myositis	10 (77)	0	<0.00001
Arthritis	5 (38.4)	8 (12)	0.049
ILD	7 (53.8)	3 (4.7)	<0.00001
Disphagia	9 (69)	48 (71.6)	ns
Sicca	8 (61.5)	41 (61.2)	ns
NC SSc pattern	8/10 (80)	45/57 (79)	ns

Table IV. NC features in most frequent anti-Ku positive autoimmune diseases.

Total NC (30)	SSc spectrum disease n=10	PM n=4	UCTD n=8	SLE spectrum disease n=4
SSc pattern (10)	8 (80%)	0	0	2 (50%)
Non specific alterations (12)	2 (20%)	2 (50%)	5 (62.5%)	2 (50%)
Normal (8)	0	2 (50%)	3 (37.5%)	1 (25%)

NC: nailfold capillaroscopy.

es, 50% of PM and in 62.5% of UCTD cases. NC alterations were equally found in isolated and non-isolated anti-Ku+ patients. NC was performed in 11 among 15 cases of ILD: SSc pattern and non-specific alterations were detected in 4 cases, each; a normal NC was evident in 3 cases of ILD (37.5%).

Serological features

Anti-Ku antibodies were detected as isolated specificity in 24 cases (52%), with a speckled (17 sera) or speckled and nucleolar IIF staining (7 sera). In 22 sera anti-Ku were associated with other anti-ENA antibodies, frequently anti-Ro (11 cases), anti-La (3 cases), anti-Ki (3 cases). Anti-dsDNA antibodies were globally found in 11 cases; mostly affected by SLE spectrum diseases (8 cases). Anti-cardiolipin or anti-beta2GPI antibodies were globally detected in 7 cases (27%).

Isolated anti-Ku+ cases

Patients with isolated anti-Ku antibodies showed a diagnosis within the SSc spectrum diseases or PM (globally 15 cases: 62.5%) comparing with patients with anti-Ku and other ANA specificities (3 cases: 13.6%. p=0.002). Isolated anti-Ku+ patients did not include cases affected by SLE spectrum diseases (p<0.00001).

Isolated anti-Ku+ patients frequently showed arthralgias (83%), Raynaud's phenomenon (83%), dysphagia (42%), ILD (42%). Myositis and sclerodactyly were significantly more frequent in isolated anti-Ku+ (50% and 42%, respectively) when compared with other patients (p=0.02 and p=0.0016, respectively).

Discussion

Anti-Ku antibodies represent about 2% of all anti-ENA positivities detected in 16 years of routine analyses performed by a single Rheumatology Unit laboratory. A rate of 1.3–3% per year of anti-Ku positivity was found from 2006 to 2012, without differences with the rate of positivity previously found between 1995 and 2005 (2). The prevalence of anti-Ku in systemic autoimmune diseases shows significant variations according to the method employed for detection: highly sensitive assays (such as ELISA, dot blots with recombinant antigens or immunoprecipitation) can detect also low titre antibodies frequently associated to different non-autoimmune disorders (4). Relatively insensitive methods such as CIE or immunodiffusion can detect anti-Ku at high titre, probably almost exclusively within systemic autoimmune diseases (1, 2).

Anti-Ku antibodies do not show a peculiar IIF pattern, usually characterised as a reticular or diffuse nuclear staining with or without nucleolar positivity (1, 2). Recently, a dense speckled nuclear staining with homogeneous nucleoli was considered a specific IIF pattern for anti-Ku (4). In our series, this pattern was found only in 29% of isolated anti-Ku+ sera, while most of them show a dense speckled ANA without nucleolar staining. The mixed IIF pattern could be due to the co-existence of multiple autoantibodies associated with anti-Ku (4).

When found as isolated antibody, anti-Ku represents a serological marker of SSc overlap diseases, confirming previous papers (1, 2), while all patients affected by SLE spectrum diseases showed anti-Ku associated with other autoantibodies (namely anti-DNA, anti-Ro/SSA or anti-Ki). The absence of anti-Sm could be due to their low prevalence in Caucasian SLE patients (8), but also to the low number of SLE cases here considered. In fact, anti-Ku represent 1.5% of all SLE patients, followed by our Unit, confirming the low prevalence of anti-Ku in Caucasian SLE patients (3, 9). They showed a prevalent cutaneous and articular involvement without major organ complications, but with a higher rate of Raynaud's phenomenon (67%) compared with anti-Ku negative SLE patients (42.8%; submitted personal data).

By contrast, within SSc spectrum diseases anti-Ku did not associate with other specific markers (namely anti-Jo1, PM/Scl, anti-centromere or anti-topoisomerase I), confirming the well-known rare coexistence of multiple autoantibodies in SSc (10)

Among SSc, anti-Ku antibodies define a peculiar limited cutaneous subset, with frequent muscular-skeletal involvement and a higher rate of ILD that what found in ACA+ group or what described in anti-PM/Scl+ cases (11). We found ILD in about 1/3 of anti-Ku+ patients with a mild functional impairment in most cases, according to other authors (4).

In addition, anti-Ku+SSc patients rarely showed digital ulcers compar-

ing with ACA+SSc ones, although the same rate of nailfold capillaroscopy alterations was found in both groups.

Abnormal NC features were detected in about two thirds of anti-Ku+ patients. SSc pattern (active and late type) was recorded mostly within SSc spectrum disease, but also in SLE. This data could indicate a deep alteration of microcirculation bed associated with anti-Ku antibodies, regardless of the associated disease. NC scleroderma patterns have been associated in SSc with disease severity, disease subset (12) and future onset of digital ulcers (13). In addition, prognostic associations have been made between the quantitative assessment of capillaroscopic SSc parameters and SSc-related clinical associations (14). In our cases, SSc patterns at NC did not correlate with the onset of digital ulcers or ILD except for two cases of extensive ILD with severe Medsger's score.

In conclusion, isolated anti-Ku defines a subset of limited SSc in overlap with myositis, frequent ILD and arthritis. By contrast in SLE, anti-Ku are found associated with other autoantibodies, without a peculiar clinical profile.

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