Anti-Ku Antibodies in Connective Tissue Diseases: Clinical and Serological Evaluation of 14 Patients

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ABSTRACT. Objective. To assess the clinical and serological associations of anti-Ku antibodies.

Methods. Fourteen patients with anti-Ku antibody detected by counterimmunoelectrophoresis (CIE) and immunoblot (IB) were retrospectively evaluated.

Results. Patients (13 women, one man) had a mean age of 60.3 years (range 19–83). Seven patients had overlap syndromes: 5 polymyositis/scleroderma (PM/SSc), one systemic lupus erythematous (SLE)/SSc/PM, and one SLE/PM. Three additional patients had undifferentiated connective tissue disease, 2 primary Sjögren’s syndrome (SS), one psoriatic arthritis, and one SSc. The clinical manifestations most frequently recorded were arthralgias (86%), myositis (50%) and Raynaud’s phenomenon (78.6%). Five patients had esophageal dysmotility, while 6 showed interstitial pulmonary fibrosis (4 of them with reduced DLCO). No case of pulmonary hypertension was observed. All patients had very high titer of ANA with speckled and nucleolar pattern. All the sera were positive for anti-Ku antibodies by CIE: all but one were confirmed by IB. Eight sera contained isolated antibodies to Ku proteins: both subunits were recognized in 7 cases, while isolate reactivity to the 70 kDa protein was detected in one case. Five sera contained additional antibody specificities: anti-Ro 60 kDa in 4 cases, and anti-La/SSB, anti-SL, and anti-PM-Scl in one case each.

Conclusion. Anti-Ku antibody is found in a wide spectrum of connective tissue diseases including overlap syndromes with SSc and myositis. Raynaud’s phenomenon and muscular and joint involvement are the most frequent clinical features associated with anti-Ku antibodies, which are frequently detected in association with anti-Ro/SSA and/or other antinuclear specificities. (J Rheumatol 2002;29:1393–7)

Key Indexing Terms:
ANTIKU ANTIBODIES OVERLAP SYNDROMES ANTI-ENA ANTIBODIES COUNTERIMMUNOELECTROPHORESIS IMMUNOBLOTTING

The Ku antigen is a DNA-binding nuclear protein complex composed of 2 polypeptides of 86 and 70 kDa in 1:1 ratio1–4. Autoantibodies to Ku were originally described in sera from patients with a polymyositis-scleroderma (PM/SSc) overlap syndrome1. Although originally thought to be relatively specific for this rare autoimmune disease, autoantibodies to Ku are also found in sera of some patients with systemic lupus erythematous (SLE), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), and other clinical conditions2,5,8.

Anti-Ku reactivity was investigated with different methodological approaches in different studies. While Mimori, et al1 originally used immunodiffusion assay to detect anti-Ku antibodies, other investigators have used ELISA, immunoblot (IB), or immunoprecipitation assays9–11. Moreover, differences in prevalence of the anti-Ku antibodies have been reported in African-American SLE patients compared to Caucasians12. Therefore, case selection and clinical and racial differences in the cohort of patients studied and methods employed to detect anti-Ku antibodies may account for some of the disagreements reported in the literature. Currently, the prevalence of antibodies to Ku protein in various autoimmune diseases varies widely, ranging from 3% with IB analysis to 55% using a capture ELISA1,2,5,11,13.

We described 14 patients with anti-Ku antibodies and their clinical and serological features. Antibodies were screened with counterimmunoelectrophoresis (CIE) and then tested with IB to correlate the fine specificity of the antibody with the clinical picture and immunological features.

MATERIALS AND METHODS

Patients. Fourteen patients with anti-Ku antibodies detected by CIE were evaluated. All were attending the Clinical Immunology Unit of Brescia.
The mean followup was 7.03 years (range 1–14; median 5 yrs). Seven patients were affected by overlap syndromes, 3 patients had undifferentiated connective tissue disease (UCTD), one systemic sclerosis (SSc) with mild muscular involvement, 2 primary Sjögren’s syndrome (primary SS), and one psoriatic arthritis. The diagnoses were based on American College of Rheumatology (ACR) criteria for SLE21, preliminary ACR criteria for diffuse SSc21, Bohan and Peter’s criteria for myositis23, and Vitali’s criteria for primary SS23. Four patients developing an overlap syndrome had presented the first overlap associated symptom 2 to 24 months before our first observation, the other 3 patients after 9 to 30 months of followup. The patients were seen at least every 6 months in our outpatient clinics and were examined by the same senior medical staff.

Clinical and laboratory data were identified from medical records. Immunological testing. The occurrence of positive tests for rheumatoid factor (RF; Bonty Diagnostici, Milan, Italy), antinuclear antibodies (ANA; indirect immunofluorescence on HEp-2 cells; Kallestad, Chaska, MN, USA), anti-dsDNA antibodies (Farr assay, Kodak Clinical Diagnostics, Amershams, UK), anticardiolipin antibodies (aCL; enzyme immunoassay), and anti-extractable nuclear antigen (ENA; detected by CIE) was considered at all times during the patient’s followup. aCL were measured following the method suggested by the International Standardization Workshop14 and levels ≥ 1:80, ≥ 1:40 and ≥ 1:20 were considered positive. A value ≥ 4.2 UI/ml of dsDNA binding is considered positive in our laboratory. RF and ANA were considered positive at a titer ≥ 1:40 and ≥ 1:80, respectively. Total hemolytic complement activity was determined by an automated device (Behring Chromotimer; Dade Behring SpA, Milano, Italy); the complement fractions C3 (normal range 80–160 mg/dl) and C4 (normal range 10–40 mg/dl) were measured by laser nephelometry (Nephelometer Analyzer II; Dade Behring SpA) using standard reagents.

Antibodies to ENA were determined by CIE, according to Bernstein, et al30, using a rabbit thymus extract (Peel-Freeze, Rogers, AR, USA); antibodies to Ro/SSA were determined by CIE, using human spleen extract as substrate. Human spleen extract was prepared according to Clark, et al29 and Venables, et al31. The 14 anti-Ku sera were tested by IB, using nitrocellulose strips with electrophoretically separated antigen extracts of HEp-2 cells (Euroimmun, Lubeck, Germany). Every strip was incubated with 1.5 ml of sera, diluted 1:50, for 60 min; the antigen-antibody complexes were detected by adding 1.5 ml of 1:10 diluted anti-human IgG, conjugated with alkaline phosphatase, in each strip channel. After 60 min, 1.5 ml of sera, diluted 1:50, for 60 min; the antigen-antibody complexes were detected by adding 1.5 ml of 1:10 diluted anti-human IgG, conjugated with alkaline phosphatase, in each strip channel. After 60 min, 1.5 ml of substrate (nitroblue tetrazolium chloride/5 bromo-4-chloro-3-indolylphosphate) was added to each strip for 10 min. The reaction was stopped with distilled water.

RESULTS
Clinical data. We evaluated 14 patients with anti-Ku antibodies identified on CIE over a 5 year period (1995–2000; about 30,000 ENA analyses were performed): 13 were female and one male, the mean age at the onset of symptoms was 56.5 years (SD 28.99) and the mean followup was 7.03 years (SD 7.7). Five patients (35.7%) were diagnosed as PM/SSc, one patient as PM/SLE, and one as SSc/PM/SLE overlap syndromes. Another patient had diffuse SSc with myalgias, mild elevation in muscular enzymes, and normal electromyogram; 2 other patients had primary SS. One patient had cutaneous psoriasis associated with chronic asymmetric arthritis of the hands diagnosed as psoriatic arthritis, according to Wright and Moll’s classification22. Three additional patients had UCTD according to the preliminary classification criteria proposed by Mosca, et al23.

The history of 2 patients with overlap diseases, including SLE, deserves a brief description. At the onset, one patient had clinical features of SSc with PM; then she developed arterial hypertension and proteinuria. Renal biopsy revealed a membranous glomerulonephritis; echocardiography detected mild pericardial effusion. These data supported a diagnosis of SSc/PM/SLE overlap syndrome.

Another patient was referred to our clinic with severe muscle weakness, myalgias, arthritis, dysphagia, exertional dyspnea, Raynaud’s phenomenon, oral ulcers, xerostomia, and photosensitivity. Laboratory findings showed increase of serum CPK, polycythaemia rubra vera, high titer anti-dsDNA antibodies and ANA. Muscle biopsy and electromyogram confirmed a diagnosis of PM/SLE. No other investigations were undertaken because the patient was pregnant. Treatment with prednisolone (30 mg qid), cyclosporin A (3 mg/kg qid), and hydroxychloroquine (200 mg qid) was started and a complete clinical remission was promptly achieved. She had premature delivery at 34 weeks’ gestation.

Table 1 shows the clinical features observed at onset and during followup in 14 patients with anti-Ku antibodies. Over time the clinical picture tended to become more consistent and to better define clearcut diseases or syndromes. Arthralgias and Raynaud’s phenomenon were the most frequently reported manifestations at onset and at the end of the observation period. Mucocutaneous features were represented by malar rash, heliotropic rash, psoriasis, alopecia, oral ulcers, and photosensitivity in one patient each. Rheumatoid nodules were observed in one patient with erosive arthritis. Seven patients (50%) had myalgias with

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>At Onset, n (%)</th>
<th>During Followup, n (%)</th>
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<tbody>
<tr>
<td>Arthralgias</td>
<td>6 (43)</td>
<td>12 (86)</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>6 (43)</td>
<td>11 (79)</td>
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<tr>
<td>Fatigue</td>
<td>3 (21)</td>
<td>8 (57)</td>
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<tr>
<td>Fever</td>
<td>1 (7)</td>
<td>1 (7)</td>
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<tr>
<td>Sclerodactyly</td>
<td>3 (21)</td>
<td>6 (43)</td>
</tr>
<tr>
<td>Myositis</td>
<td>3 (21)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>1 (7)</td>
<td>4 (28)</td>
</tr>
<tr>
<td>Myalgias</td>
<td>3 (21)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>Xerostomia</td>
<td>3 (21)</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>0</td>
<td>5 (36)</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>3 (21)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>Xerophthalmia</td>
<td>2 (14)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>Serositis</td>
<td>1 (7)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>1 (7)</td>
<td>1 (7)</td>
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<tr>
<td>Calcinosis</td>
<td>0</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Microstomia</td>
<td>0</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Jaccoud’s arthropathy</td>
<td>0</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Parotid gland enlargement</td>
<td>2 (14)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Telangectasias</td>
<td>2 (14)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>1 (7)</td>
<td>1 (7)</td>
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<tr>
<td>Photosensitivity</td>
<td>1 (7)</td>
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muscle weakness: all had myositis with increase of CPK, abnormal electromyogram, and in 5 cases, inflammatory infiltrates at muscle biopsy.

A nailfold capillaroscopy was performed in 10 patients: in 5 cases a scleroderma pattern was detected, in 2 only microhemorrhages, and in 3 cases a normal pattern was recorded. Five patients reported dysphagia with esophageal reflux: all of them underwent manometry, revealing absent peristalsis of distal esophagus and low lower esophageal sphincter pressure. In 6 patients, chest radiography revealed interstitial fibrosis; 4 patients had a mild reduction of diffusing capacity for carbon monoxide (DLCO) that was stable during followup in all but one individual. No patient had pulmonary hypertension.

Laboratory findings. Eleven patients (78.6%) had elevated erythrocyte sedimentation rate, in 6 patients (43%) with concomitant increase of C-reactive protein. Elevations of CPK and hepatic cytolytic enzymes were detected in 64 and 36% of patients, respectively. Only 2 patients had hematoctysis.

| Table 2. Laboratory findings in 14 patients positive for anti-Ku antibodies. |
|-------------------------|------------------|
| Antinuclear antibodies  | 14 (100)         |
| Elevated ESR           | 11 (78)          |
| Abnormal C-reactive protein | 6 (43) |
| Hypergammaglobulinemia  | 5 (36)          |
| Elevated CPK           | 9 (64)          |
| Low C4                  | 3 (21)          |
| Low C3                  | 3 (21)          |
| Reduced CH50            | 3 (21)          |
| Elevated hepatic enzymes | 5 (36) |
| Anti-dsDNA antibodies   | 4 (28)          |
| Rheumatoid factor       | 1 (7)           |
| Anti-β2-GPI antibodies  | 1 (7)           |
| Anticardiolipin antibodies | 1 (7) |
| Cryoglobulinemia        | 2 (14)          |
| Leukopenia              | 2 (14)          |
| Anemia                  | 1 (7)           |
| Thrombocytopenia        | 1 (7)           |

PL-2 antigens. Anti-70 kDa protein of Ku was recognized by 10 sera (isolated by one serum), while anti-80 kDa was detected by 12 sera (isolated by 2 sera).

DISCUSSION

Although detected in a wide spectrum of autoimmune conditions, anti-Ku antibody has been proposed by Mimori, et al to be one of the serologic markers for the overlap syndromes, especially those including PM or dermatomyositis. This clinical and serological association has also been confirmed by other authors examining single or small numbers of anti-Ku positive patients. Nevertheless, anti-Ku antibodies have been found in the serum of patients with many autoimmune conditions including, among others, SLE, SSc, and mixed connective tissue disease. In addition, Chan, et al detected this autoantibody in sera of patients with Graves’ disease, and Isern, et al found it in patients with primary pulmonary hypertension. These observations have not been confirmed by other studies.

In our study, half of the anti-Ku positive patients are affected by PM overlapped with SSc, SLE, or both. They represent all of the patients with PM/SSc overlap syndrome attending our clinic as assessed from clinical records. Therefore, according to Mimori, et al, in spite of its rather low specificity, it should be appropriate to consider anti-Ku as a marker of such overlap disease, at least when detected by relatively insensitive techniques such as Ouchterlony’s immunodiffusion or CIE. Indeed, the prevalence of anti-Ku antibodies in various autoimmune diseases is greatly influenced by the detection method employed. The highly sensitive ELISA or immunoprecipitation methods afford the detection of low titer antibodies widely represented in autoimmune sera and even detected in cancer, while immunodiffusion or CIE detects only high titer antibodies. This can be of clinical relevance, as in the case of ANA...
losing some of their value as diagnostic markers when detected by ELISA\textsuperscript{29,30}. Therefore, we hypothesize, based on results from our study and that of Mimori, et al\textsuperscript{1} using immunodiffusion techniques, that only high titer anti-Ku are related to some autoimmune diseases. This is in contrast to results reported by Cooley, et al\textsuperscript{8}, who also detected anti-Ku by means of CIE. Nevertheless, overlap syndromes were diagnosed in about one-third of the patients with anti-Ku reported in the literature (Table 4), while SLE and SS were reported in 28 and 14%, respectively, and inflammatory muscle disease is reported in 79% of the patients with overlap syndromes.

Raynaud’s phenomenon and muscular/articular manifestations were prominent in our cohort of patients independent of their final diagnosis. These findings are in keeping with those of other authors\textsuperscript{8}.

Antibodies to cellular soluble antigens detected in addition to anti-Ku were found in 5 of the 14 patients reported here (35.7%) with either CIE or IB. The most frequently detected antibody specificity was directed to Ro/SSA (3 sera by CIE and 4 by IB); other antibodies were against La/SSB, SL, and PM-Scl. Neither anti-Sm nor other autoantibodies generally considered markers of disease (antitopoisomerase I, anticentromere, anti-U1 RNP, anti-Jo-1) were found in our patients, in contrast to other reports\textsuperscript{1,5,8,25,28}. The high rate of overlap syndromes and

\begin{table*}[h]
\centering
\caption{Correlation between immunoblot and CIE in 14 anti-Ku positive sera.}
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Diagnosis} & \textbf{CIE} & \textbf{Anti-ENA Specificity} & \textbf{IB} \\
\hline
PM/SSc & Ku & Ku 70 + 80 kDa & \\
PM/SSc & Ku & Ku 70 + 80 kDa & \\
PM/SSc & Ku & Ku 70 kDa & \\
PM/SSc & Ku + UDA & Ku 80 kDa + Ro/SSA 60 kDa & \\
PM/SSc & Ku & Ku 70 + 80 kDa + Ro/SSA 60 kDa & \\
PM/SSc/SLE & Ku & Ku 70 + 80 kDa & \\
SSc & Ku & Ku 70 + 80 kDa & Negative \\
UCTD & Ku + UDA & Ku 70 + 80 kDa & \\
UCTD & Ku + Ro/SSA & Ku 70 + 80 kDa & \\
UCTD & Ku & Ku 80 kDa + PM/Scl & \\
Psoriatic arthritis & Ku & Ku 70 + 80 kDa & & \\
Primary SS & Ku + Ro/SSA + La/SSB & Ku 80 kDa + Ro/SSA 52 + 60 kDa + La/SSB & \\
Primary SS & Ku + SL + Ro/SSA & Ku 70 + 80 kDa + Ro/SSA 60 kDa + SL & \\
PM/SLE & Ku & Ku 70 + 80 kDa & \\
\hline
\end{tabular}
\end{table*}

\begin{table*}[h]
\centering
\caption{Number of patients with diseases related to anti-Ku antibodies.}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Overlap Syndromes*} & \textbf{SLE} & \textbf{DM/PM} & \textbf{SSc} & \textbf{Other Diseases **} & \textbf{Total} & \textbf{Reference} \\
\hline
2 & 3 & 1 & 2 & 2 & 10 & 31 \\
5 & 10 & 2 & 7 & 2 & 26 & 5 \\
4 & 0 & 1 & 0 & 3 & 8 & 32 \\
0 & 10 & 0 & 4 & 11 & 25 & 8 \\
2 & 6 & 0 & 1 & 1 & 10 & 10 \\
8 & 0 & 0 & 2 & 0 & 10 & 4 \\
1 & — & — & — & — & 1 & 26 \\
2 & — & 1 & — & — & 3 & 25 \\
— & — & — & 2 & — & 2 & 27 \\
— & 4 & — & 1 & — & 5 & 2 \\
6 & 1 & — & 1 & — & 8 & 1 \\
16 & 10 & 1 & 1 & 0 & 28 & 11 \\
1 & 1 & 0 & 0 & 6 & 8 & 28 \\
7 & 0 & 0 & 1 & 6 & 14 & Present study \\
\hline
Total 54 & 45 & 6 & 22 & & & 159 \\
\hline
\end{tabular}
\end{table*}


\* Overlap syndromes: 2 MCTD, 1 SSc/pSS, 3 SLE/PM, 7 SLE/SSc/PM, 5 SLE/pSS, 21 SSc/PM. Overlap syndromes including PM: 31/39 (79%). \** Other diseases: 14 UCTD, 1 discoid lupus, 4 rheumatoid arthritis, 6 primary SS, 1 psoriatic arthritis, 1 Wegener granulomatosis.
primary SS might probably explain the autoantibody profile detected in our study, while other diseases such as SLE or SSc were prevalent in other studies.\textsuperscript{2,5,8,10,11}

Antibodies to Ku protein should be considered a serologic marker for overlap syndromes, especially when myositis is present; anti-Ku are frequently detected in association with other antinuclear specificities including other markers of overlap syndromes.

REFERENCES


