Outcome of Positive Antinuclear Antibodies in Individuals Without Connective Tissue Disease

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ABSTRACT. ***Objective.*** To determine if individuals with high titer antinuclear antibodies (ANA) but without clinical evidence of connective tissue disease (CTD) subsequently develop CTD or experience a change in ANA positivity.

Methods. We included patients from an initial study database as well as those reviewed in an outpatient rheumatology clinic at the University of Alberta Hospital over the past 8 years. A telephone survey targeting signs and symptoms of CTD was conducted. Serum samples from consenting patients were then assayed for ANA, antibodies to extractable nuclear antigens (ENA), and anti-dsDNA by the Rheumatic Disease Unit at the University of Alberta Hospital.

Results. Sixty-two patients completed the telephone survey and 53 completed both the telephone survey and repeat serological blood investigations. Mean length of followup was 5.4 years, with an age range from 19 to 87 years. Forty-eight of 53 patients (91%) remained ANA positive on repeat testing, and 5 patients were also ENA positive. Three patients had been diagnosed with CTD since the previous study. The most common clinical features on telephone survey included joint pain (34 patients) followed by Raynaud's phenomenon (11 patients).

Conclusion. Patients tended to remain ANA positive on repeat testing. Three out of 53 patients had developed CTD, reflecting the more sensitive but less specific nature of ANA testing. Another common condition associated with ANA positivity was hypothyroidism. Continued longterm followup with larger cohorts is needed. (J Rheumatol 2003;30:736–9)

Key Indexing Terms:
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Primary care and specialist physicians often order fluorescent antinuclear antibody (ANA) tests as initial screening for connective tissue diseases (CTD)[1]. The ultimate clinical utility of this highly sensitive test depends on the pretest diagnostic probability. ANA positivity is detected with a significantly increased frequency in most autoimmune diseases including scleroderma, Sjögren's syndrome, rheumatoid arthritis, mixed connective disease, and systemic lupus erythematosus (SLE). ANA positivity can also be found in organ autoimmune disease including the thyroid, liver, and lung as well as other disorders including chronic infections (mononucleosis, tuberculosis, and subacute bacterial endocarditis). ANA positivity in the general population varies according to titer, and it has been shown that up to 32% of normal individuals can have a positive result at a 1/40 titer[2]. Further, the prevalence of autoantibodies has been shown to be higher in the elderly population, with a trend reflecting greater prevalence in women than men[1,3,4].

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The usefulness of a positive ANA test for diagnosing CTD is limited without clinical correlation. Unnecessary further laboratory studies, physician referrals, and greater anxiety will often result from the positive ANA label in apparently healthy individuals. There are currently no longterm followup studies of patients without CTD who are ANA positive. The purpose of this followup study was to review a sample of patients with high titer ANA but no evidence of CTD at the time of testing.

MATERIALS AND METHODS

Patient selection. We reviewed patients from a previous study of individuals with high titer ANA as well as patients reviewed in an outpatient rheumatology clinic at the University of Alberta since 1990. Most patients were identified by Vaile, *et al*[5] and were ANA positive with a high titer (in our laboratory, a high titer is defined as 1:640, 0.5% prevalence of positives in normal controls). The sample of patients from the previous study were initially identified from the records of 2 laboratories in the city of Edmonton, University of Alberta Hospital and Dynacare Kaspar Medical Laboratories, both affiliated with the Capital Health Authority, who perform all the ANA testing for northern Alberta.

These records originated from the period of January to June 1996 and included patients with a positive ANA at 4 dilutions above the screening titer and previously identified as having no detectable CTD. The remainder of the sample was identified by a rheumatologist at the University of Alberta Hospital as being individuals with no CTD who had been seen in the past 7 years. Each patient's presentation was variable, and included such features as a positive ANA result identified by the family physician, nonspecific joint pains, or family history of autoimmunity. The Health Research Ethics Board at the University of Alberta approved this project.

Survey. A telephone survey of 120 potential patients was conducted over a 7 month period from November 2000 to May 2001. Once consent was obtained, each patient answered a survey targeting signs and symptoms of CTD including sicca symptoms, rash, arthritis, sun sensitivity, oral ulceration, Raynaud's phenomenon, medical comorbidities, and medications. The survey questions were the same as those previously posed to the patients identified from the initial study. A laboratory requisition was then sent to each patient to repeat the ANA, anti-ENA, and native DNA tests. Any patient with questionable complaints or an unclear diagnosis was formally reviewed at the University of Alberta Hospital to determine if there was clinical evidence of a rheumatological disease.

The post-survey diagnoses included no CTD, CTD, or other (for disorders where positive ANA may be expected).

RESULTS

Demographics. Sixty-two of 116 potential patients completed the telephone survey and 53 completed both the survey and serological blood investigations. Of the 9 patients who completed only the survey, one patient refused blood tests, and the remaining 8 patients did not complete the blood tests despite followup telephone calls. Of the 54 patients who did not complete the survey, 4 refused, one had died, and the remainder (49 patients) could not be contacted (e.g., number not in service, wrong number, etc.). The ages ranged from 19 to 87 years with a mean of 47 years. The sex distribution included 53 (85%) women and 9 (15%) men. Mean length of followup was 5.4 years.

Serology. Forty-eight of 53 patients remained ANA positive, while 4 were ANA negative on repeat testing and one patient's ANA result was lost. Anticentromere positivity was detected in one of the 48 positive ANA patients. Anti-ENA positivity was found in 5 patients (Table 1). Two patients were anti-SSA positive, one was anti-SSB positive, and 2 patients were anti-SSA/SSB positive. Three anti-ENA positive patients remained anti-ENA positive from the previous study, whereas 2 of these 5 patients had no previous anti-ENA measurement. Native DNA autoantibodies were negative in all patients.

Clinical features. The most common clinical features from the telephone survey included joint pains, Raynaud's phenomenon, and rash. Thirty-four patients complained of arthritis. Twelve patients reported a diagnosis of osteoarthritis, but none of the 34 patients reported significant morning stiffness. Raynaud's phenomenon was the second most common clinical feature reported by 11 patients. Three patients who had Raynaud's were prescribed adalat (calcium channel blocker). Ten patients complained of rash including one with vitiligo, one with a previously identified photodermatitis, one with darker pigmentation attributed to her ethnic background, one with psoriasis, and 3 with rosacea. Five patients described oral ulceration, 3 of 5 describing classic canker sores. Five patients described sicca symptoms, 2 of whom were diagnosed with scleroderma and CREST, respectively, and one patient had been recently (within one year) diagnosed with Sjögren's syndrome after a formal ophthalmologic and rheumatologic assessment (Table 1). The remaining 2 patients were not diagnosed clinically with CTD. Seven patients were hypothyroid and required thyroid supplementation. Three patients had developed features of CTD since the previous assessment (Table 2). One patient had been newly diagnosed with CREST and one with scleroderma, having 4 and 3 features of CTD, respectively. The third patient had developed Sjögren's syndrome with 3 features of CTD, as described previously. This patient was anti-SSA positive whereas the 4 other anti-ENA positive patients did not have a CTD diagnosis. A fourth patient described psoriasis with associated arthritis. Diseases identified in the previous study included 7 patients with fibromyalgia, 12 with osteoarthritis, one with uveitis (without evidence of CTD or inflammatory bowel disease), and 4 with hypothyroidism. Other diagnoses made since the previous study included one patient with sarcoidosis and 3 patients with hypothyroidism.

DISCUSSION

Since the initial description of the LE cell phenomenon over 40 years ago, ANA has been detected with immunofluorescence on tissue sections and subsequently Hep-2 cell lines[6]. Certain factors must be considered when interpreting positive ANA. These include (1) differences in assay method between laboratories resulting in the significant intra- and inter-laboratory variability within the immunofluorescence technique[2], (2) debate about the ELISA kits and their sensitivities, and (3) variance in cutoff points for negative and positive values depending on the assay kit[7]. The issue of titer was further investigated by Vaile, *et al*[5], who determined that at least 44% of high titer (1:640) ANA patients did not appear to have CTD after assessment by a specialist physician. That study questioned the utility of setting higher cutoff points for ANA versus reporting results as either positive and negative at a screening titer (1:40) that had a 7% positive rate in normal sera.

While the physician may seek clinical correlation more aggressively for a patient with a high titer ANA, the question still remains what to do with any positive ANA result whether or not the local laboratory reports the titer. A high

Table 1. Antibody profile of patients completing serology and telephone survey.

Diagnostic Category	No. of Patients	ANA Positive	ds-DNA	SSA Positive	SSB Positive	SSA + SSB Positive
CTD	3	3	0	1	0	0
No CTD	50	45	0	1	1	2

Table 2. Profile of patients who developed CTD.

Age	Sex	CTD Features	Serology	Diagnosis
49	F	Joint pain, sicca, RP, alopecia	ANA + (centromere positive)	CREST
48	F	Arthritis, sicca, RP	ANA +	Scleroderma
51	M	Joint pain, sicca, RP	ANA + SSA +	Sjögren's syndrome

RP: Raynaud's phenomenon.

titer cutoff may reduce the prevalence of positive results in normal sera, but in practice this is not sufficient to affect the post-test probability of CTD[5].

The development of CTD in ANA positive individuals over time remains uncertain. We reviewed a cohort of patients previously shown to have positive ANA tests without CTD and identified any changes in their clinical status or serology over a mean of 5 years. While there have been comparison studies of clinico-serological features between matched control and SLE patients[8], there are no good longterm followup studies of such selected positive ANA individuals. ANA positivity alone in healthy individuals is not a good predictor of evolving CTD[9].

We have confirmed that a positive ANA persists over time, as evidenced by 91% positivity on repeat testing. The demographics revealed a female predominance that is commonly reported in previous studies describing ANA positivity and the increased association of autoimmunity with females[10-12]. Anti-ENA positivity persisted for 3 of 5 patients and the other 2 patients had no previous anti-ENA testing. The patient with newly diagnosed Sjögren's syndrome from December 2000 was one of the 2 patients with no previous anti-ENA measurement. Classically, anti-ENA are detected in various combinations in Sjögren's syndrome and SLE, as well as a variety of other disorders including neonatal lupus and mixed and undifferentiated CTD. Given that these patients had been selected from a large number with positive ANA, but at that time no clinical evidence of CTD, it is consistent with previous reports that we should find some false positive anti-ENA antibodies[13]. That 4 out of 5 anti-ENA positive patients had no evidence of CTD is consistent with the existence of false positive anti-ENA results as documented previously[13] and does not warrant any different clinical monitoring.

The new diagnosis of CTD in 3 patients occurred over the course of 5 years of followup after the previous study. The patient with CREST (anticentromere positive) was diagnosed in 1998 by a rheumatologist, while the patient with Sjögren's syndrome was diagnosed in December 2000. The patient with CREST had been experiencing Raynaud's phenomenon and sicca symptoms from the beginning of the followup period, which suggests early CREST not identified or overlooked from the previous study. The other scleroderma patient had no identified sicca, Raynaud's phenomenon, or arthritis in the previous study.

The most common clinical symptom identified during the telephone survey was joint pain, one-third of which was reported by patients diagnosed with osteoarthritis. A screen of patients with joint pain for morning stiffness or joint distribution revealed no suggestive features of an inflammatory process.

Raynaud's phenomenon was the second most common complaint and perhaps may be the strongest feature in the face of a positive ANA to suggest a future CTD. The rash feature yielded no positive description for a malar rash, although the vitiligo and psoriasis cases both suggest an underlying autoimmune process. The 5 patients with sicca symptoms included the 3 patients who had manifested CTD symptoms in the past 5 years, including CREST, scleroderma, and Sjögren's syndrome. The patients had 3 or more features of CTD on telephone survey and had been formally assessed by a rheumatologist in the past 3 years.

ANA positivity reflecting organ autoimmunity within the general population was well demonstrated with the 7 hypothyroid patients. None of these patients had any CTD diagnoses. The progression of 3 patients to hypothyroidism since the previous study might suggest that patients with no clinical evidence of CTD and ANA positivity should have additional screening for autoimmune hypothyroid disease as this is an easily treated and monitored disease. This suggestion is tempered by the finding that hypothyroidism only develops in 55% of women after a 20 year followup of ANA positive individuals[14].

While we acknowledge the limitations of a telephone survey in identifying early, subtle features of CTD, we believe that the screening questions would identify patients with clinically important CTD. The questionnaire was not validated, but was the same question set used for the previous study, in which the positive ANA patients without CTD were identified. Consequently, any changes in clinical features for each patient would be identified and compared to previous features. The identification of CTD in the 3 patients was therefore based on the clinical survey, correlating serology, and subsequent rheumatological assessment versus the survey data alone. The questionnaire served merely as an effective screening tool to identify patients requiring a closer look. Any patient with positive anti-ENA but no features on telephone survey were therefore not assessed in the office. Changes in ANA titer between the previous and current study were not assessed due to changes

in our laboratory and ANA reporting. As noted, ANA is currently reported as positive at a screening titer of 1:40.

The strength of association between ANA and disease varies tremendously, leading to the ultimate question of what came first, the disease or the antibody? This question is extremely relevant to this study because the answer could ultimately adjust the clinician's perspective of ANA positivity in the healthy patient. No simple answer may exist, the final clinical outcome depending on more complicated factors including genetics and environmental exposure. The complex interplay of multiple variables may result in clinical scenarios that could never be fully anticipated by the ANA laboratory test alone. The ANA test remains most useful in the context of a strong pretest probability of CTD and a formal clinical assessment. Longterm followup with larger cohorts is needed.

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