# Is high titre ANA specific for connective tissue disease?

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## Abstract

### Objective

*A positive antinuclear antibody (ANA), while sensitive, is not specific for systemic lupus erythematosus or connective tissue diseases (CTD). The purpose of the present study was to review those sera with a high titre (≥ 4 dilutions above screening) ANA and determine from a review of the charts if these higher titres offered a satisfactory specificity for CTD.*

### Methods

*All FANA testing in this region is carried out in one of two related laboratories. We reviewed the medical records of patients who had a positive ANA at a titre 4 dilutions above screenmg at this city-wide laboratory over a 6-month period to determine whether this titre ("high titre") may offer relative diagnostic certainty. Antibodies to extractable nuclear antigens (ENA) and native DNA were also obtained.*

### Results

*422 ANA results were positive at high titre. The medical record was available for review in 320 patients, of whom 238 (75%) were seen by a specialist physician, almost always including a rheumatologist. Our review determined that 35% had a diagnosis of connective tissue disease, 21% had a diagnosis of a possible/ probable inflammatory disease, 16% had an alternative specific diagnosis provided, and in 29% no final disease specific diagnosis was recorded but CTD was not suggested to us or the specialist by the data available. One or more anti-ENA antibodies and/or anti-DNA were positive in 69 (22%) and 8% of the sera tested respectively.*

### Conclusion

*While long term follow-up is still required, a significant proportion of patients with high titre ANA have no CTD at the time of testing. Setting a higher cutoff for reporting of ANA may not increase specificity sufficiently to make it a useful alternative or addition to reporting a positive or negative value at screening titre alone.*

**Key words**

ANA, titre, specificity.

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## Introduction

Tests for antibodies reactive with nuclear components (ANA) provide a useful technique in the diagnosis and assessment of patients with possible systemic lupus erythematosus (SLE) and are also of diagnostic and, depending upon antigenic specificities, sometimes of prognostic, significance in a variety of other connective tissue diseases (CTD). The usual initial test in the assessment of possible SLE remains the fluorescent antinuclear antibody test (FANA). Depending on the question asked, other more antigen specific tests may also be appropriate at this stage, although they are often introduced only if the FANA is positive. FANA are traditionally reported as a titre, but whether the actual titre serves any benefit in addition to a dichotomous response - positive or negative - is not clear, and it does add to the cost. Following the ANA titre is not useful in monitoring disease activity in patients with systemic lupus erythematosus (SLE) (1) and this method of reporting may contribute to a perception that high titre reflects a high likelihood of a significant connective tissue disease or of a more severe disease.

Standardization is difficult for a variety of reasons (2) and the National Committee for Clinical Laboratory Standards (US) guidelines recommend that each laboratory should establish its own reference levels (2) so that the test sensitivities and specificities are known. Traditionally this screening dilution has been 1 in 40, but a recent study has demonstrated that with more sensitive assays, up to 32% of normals may have positive results at this titre, with 5% positive at a titre 1 in 160, and 3.3% positive at 1 in 320 (3). Studies were not done to determine if an even higher specificity could be achieved nor were the false positive individuals assessed clinically to ensure that no features of connective tissue disease existed. Our laboratory reports ANA both at a screening titre, shown to have about 95% specificity in normal individuals (1:40) - reported as positive or negative - and also at a single further titre 4 dilutions higher (1:640), reported as high titre, a practice previously described by others (4). Prior to this, the actual titre was determined and reported as such. Other laboratories currently reporting their results seem to have adopted the same pragmatic approach to determining what is a positive result (5-7). Inappropriate referral and investigations generate unnecessary cost. Historically up to 65% of ANA testing performed in our tertiary referral institution laboratory are positive, as compared to about 20% of those referred to the community laboratory. In practice we have found a number of patients are referred for subsequent specialist assessment primarily because of a positive ANA, initially done as a “screening” test despite the absence of symptoms appropriate to suggest a connective tissue disease (8). Unfortunately, a positive ANA in this context can confuse rather than clarify the clinical picture, particularly for the family doctor, but also for the patient. Although practitioners may readily discount a low positive result, a result expressed as a “high titre” can cause concern. We questioned whether those higher titres do warrant the presumption of clinical significance. A recent review of 153 sera with positive ANA tests showed only 17 to be from patients with SLE, and only a further 22 related to other rheumatic diseases. The test thus had a rather poor positive predictive value for SLE of 11% (9). The purpose of the present study was to review those sera specifically with a high titre (≥4 dilutions above screening) and to determine from a review of the charts if these higher titres were indeed truly suggestive for connective tissue disease and warranted further analysis, as has been suggested (4).

## Methods

This project was approved by the Health Research Ethics Board of the Faculty of Medicine of the University of Alberta. Two laboratories in the City of Edmonton, the University of Alberta Hospital (UAH) and Dynacare Kasper Medical Laboratories (DKML), both under the aegis of the Capital Health Authority, perform all the ANA testing for northern Alberta, Canada. The technique is indirect immunofluorescence using a Hep-2 cell substrate, and has been previously reported (10). It was adjusted to give approximately a 5% false positive rate in normal controls, by using de-

scribed techniques of conjugate dilution (11), i.e. checkerboard titration, and rejecting weak positive staining as a negative result (as recommended) (2). Standard sera including normal controls are included on a regular basis, and sera are exchanged between the laboratories as part of quality control maintenance.

We reviewed the records of both laboratories for the period of January to June 1996 to extract the names of patients who had a positive antinuclear antibody at 4 dilutions above the screening titre. The attending physician of these patients was identified and contacted where possible and, with physician and patient consent, the medical records of the family physician and any specialist were reviewed by a physician or medical student according to a standardized proforma. In cases where the medical record was geographically remote, the local physician was mailed a shorter questionnaire. Data extracted from the medical record included demographic data, initial and final diagnosis where available. Symptoms or signs of connective tissue diseases (Raynaud's phenomenon, rash, arthritis, sun sensitivity, oral ulcers, sicca symptoms and dysphagia), were sought according to a proforma, as were comorbidities and medications. Results of concurrent tests for antibodies to extractable nuclear antigens (ENA) (12) and native DNA (13) (nDNA) were also noted or were assayed if not previously tested.

*Terminology*

"False" positive was used not in the sense of a laboratory error, but in the usual sense of a background positive in normals, or a positive apparently unrelated to the symptoms or disease in question. We were particularly interested in whether the attending family physician or the other physicians considered the patient to have a connective tissue disease (CTD) of any type. Diagnoses were recorded individually and then recoded into four main diagnostic categories. The distinctions between categories 1 and 2 have been maintained in the results, but these categories are combined in the discussion.

1. Autoimmune disease, i.e. SLE and other CTD where a positive ANA would usually be anticipated, and may prove of particular diagnostic benefit including lupus variants, drug induced disease, and scleroderma.
2. Other disorders where a positive FANA, although not a "false" positive, rarely provided useful additional information, e.g. rheumatoid arthritis (RA), endocrine disease, active hepatitis, etc. Although arguable, myositis and juvenile arthritis were also included in this heading. This category included additional patients with a diagnosis of, at most, probable or possible forms of connective tissue disease. Sjögren's was included here as diagnostic confirmation, either by biopsy or ophthalmologic assessment, was not present.
3. A specific diagnosis other than inflammatory connective tissue disease.
4. No elements of connective tissue disease clearly noted, but no clear cut specific alternative diagnosis established - generally forms of soft tissue rheumatism and/or psychogenic disorders, e.g. chronic fatigue.

We erred on the side of inclusion to try and ensure that no patients in groups 3 or 4 could possibly be regarded as having a symptomatic or diagnosable ANA associated disorder. Thus, the diagnosis, if made by a specialist, was accepted; that by the family physician was also, if any supportive elements in the clinical chart could be found.

We re-analysed specimens from a randomly selected subgroup of these high titre sera of 10 patients known to have active SLE and from a group of 20 in which connective tissue disease had been ruled out on clinical grounds. These specimens were titred out to the endpoint to compare the resulting titres.

As part of regular quality control, we also assessed 200 new serum samples taken from blood donors and supplied by the Canadian Red Cross.

**Results**

A total of 7,744 ANA tests were performed for the period, 2,344 in the tertiary referral hospital laboratory and 5,400 in the private laboratory (DKML), whose referral base is predominantly primary care physicians. 1,453 (62%) of the UAH tests were positive at screening and 328 (14%) at 4 dilutions above screening (a titre of 1 in 640 or greater); 1,134 (21%) of the DKML tests were positive at screening and 94 (2%) at 4 dilutions above screening. Of the 422 patients with positive high titre results, the medical record was available for review in 320 (75%). In the remaining cases, the attending medical practitioner could not be identified in 77, and in the other cases the local doctor or patient declined permission. Of the 320 patients for whom data was available, we reviewed the record ourselves in 92% of cases and the attending practitioner returned the questionnaire in 8% of cases.

The median and mean ages were 44 and 44.7 years (16 to 81 years). 266 (84%) were female. 231 (70%) were seen by a rheumatologist. 87 (26%) were seen by other specialist physicians including nephrologists, dermatologists, internal medicine physicians, neurologists, paediatricians and ophthalmologists, almost always as well as by a rheumatologist, with 78 patients not seen by any specialist physician. The diagnoses used were either the final diagnosis of the specialist, or, if the patient had not been referred, a diagnosis based on the charts of the family physician. These are shown in Table I. In four patients SLE was sus-

**Table I.** Antibody profile in 4 categories of patients, all of whom had high titre positive FANA.

| Diagnostic category | No. of pts. | No. tested for ANA subtypes | Pos. DNA Abs. | Anti-SM | Anti-RNP | Anti-SSA | Anti-SSB | Anti-SSA/B |
|---|---|---|---|---|---|---|---|---|
| 1 (CTD) | 112 | 108 | 21 | 8 | 26 | 24 | 10 | 3 |
| 2 (Possible/probable CTD) | 65 | 57 | 0 | 0 | 4 | 9 | 4 | 4 |
| 3 (Non-CTD diagnosis) | 52 | 44 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 (No diagnosis) | 91 | 58 | 1 | 0 | 2 | 3 | 4 | 2 |

**Table II.** A comparison of endpoint titres in sera from 10 patients with active SLE and in 20 non-CTD patients.

| | Highest dilution at which ANA remained positive* | | | | |
|---|---|---|---|---|---|
| | 640 | 1280 | 2560 | 5120 | 10240 |
| SLE | 4 (40%) | 2 (20%) | 3 (30%) | 1 (10%) | 0 (0%) |
| No CTD | 8 (40%) | 6 (30%) | 3 (15%) | 2 (10%) | 1 (5%) |

* All sera were selected because they had a FANA titre of at least 1:640

pected by the local doctor but no referral was made to a specialist physician, and in one, another connective tissue disease was suspected with no referral.
112 patients had a diagnosis of CTD usually made by a specialist, but not always fulfilling standard criteria, e.g. the ACR criteria for SLE. Our review concurred at least with this possible diagnosis in all cases. 65 had a diagnosis of another inflammatory disease or a possible/probable CTD. In 52, a connective tissue disease diagnosis was not supported and another specific diagnosis made. It is of note that in 224 patients from the whole group (71%) no initial diagnosis had been recorded by the family physician in their chart, i.e. prior to referral, although reference was usually made to the positive FANA in the referral. It was often ordered as part of what was termed in the chart a "rheumatology screen". The percentage group allocations were markedly similar for the two laboratories involved.
In relation to SLE we searched the records for mention of arthritis or arthralgia, Raynaud's phenomenon, rash, nephropathy, serositis, sicca symptoms, sun sensitivity and oral ulcers. Specific documentation of the presence or absence of these features of connective tissue disease was present in a minority of records reviewed. Arthritis or arthralgia, Raynaud's phenomenon and rash were the most frequent clinical features. 22% had only one clinical feature present and 17% had two or more. 61% had none of these specific features recorded as present. Features of other diseases, e.g. muscle pain and dry mouth, were commonly recorded.
The frequencies of anti-ENA and -DNA antibodies are shown in Table I in relation to diagnostic category. These tests were done on all the high titre sera where sufficient stored serum could be located. ENA antibodies were measured in 83% of these positive sera. Anti-SSA was positive in 1 individual considered to have no connective tissue disease. Antibodies to nDNA were measured in 64% of sera and positive in 8%; all of these patients were in category 1, i.e. lupus-like disease. The physicians' diagnostic decisions may of course have been based on the previous demonstration that these antibodies appear specific for SLE.
Fifteen of 200 (7.5%) normal sera had a positive screening test for ANA, 1 was positive at 1: 640 titre. None had a positive test for either anti-ENA or anti-DNA antibodies.
We re-analysed the stored specimens of 10 patients definitely considered to have SLE and 20 patients definitely considered not to have CTD, according to the specialist (OA or fibromyalgia), to assess whether the endpoints of ANA titres in these two groups differed (Table II); they did not.

## Discussion

The role of an ANA test is most commonly in helping with the positive or negative diagnosis of SLE, or one of its variants. It may also be of value in other situations, e.g. assessing the clinical significance of Raynaud's, or, for example, in categorizing subtypes of juvenile idiopathic arthritis, etc. Although it is often positive in patients with RA and other CTDs, hepatitis, and many other disorders, it is not known to have any diagnostic or prognostic significance there. Even though there is no evidence that it is helpful, we have included these as category 2 to distinguish it from categories 3 and 4 where we believe the result is actually unhelpful and indeed the test should not have been ordered, based on the clinical information available in the charts. We have deliberately included patients into categories 1 and 2 even where the diagnosis may not have been definitive, in order to exclude as far as possible from category 3 or 4 any patients with possible connective tissue disease manifestations. We have also included patients with a diagnosis of Sjögren's syndrome even though the eventual diagnosis may well have been influenced primarily by the results of the laboratory investigations.
Whether a given serum dilution is appropriate to use as a screening test for ANA depends on the reason for the test. It is clear that there is no dilution that can reliably distinguish between normal and diseased populations (3). While it seems particularly important as a screening test to avoid missing true positives, i.e. to have a high sensitivity, if there are too many false positives this may result in increased costs of further testing as well as patient anxiety while awaiting specialist consultation, etc. We (and others 6, 7, l4) have, for several years, screened at a titre that produces a range of from 3 to 8% of false positives. While a titre 4 dilutions above this has been seen to be positive in patients without connective tissue diseases, we wondered whether it provided diagnostic certainty to a sufficient degree to designate it - or any other titre - as "high titre".
Our data show that even using a higher titre ANA as a cutoff, a substantial proportion of subjects (44%) do not appear to have any connective tissue disease, mostly after review by a specialist physician, with CTD ruled out and a specific alternative diagnosis made in 16%. In a further 28% no specific diagnosis was reached, but CTD was not suggested. The higher titre cutoff may raise specificity somewhat, with 34% of our group having a diagnosis of lupus and a total of 57% with lupus or another possible/probable disease associated with positive ANA (i.e., categories 1 and 2). From the opposite perspective, almost one half of the patients with high titre ANA (44%) still had no discernable CTD.
In some cases where no firm diagnosis was obtained, this is likely to have been due to nonspecific symptoms, the diagnostic conclusions referring to possible myofascial pain, muscle pains, tendonitis and psychogenic diagnoses. The re-

ferral to a rheumatologist seemingly was often prompted or legitimized by the positive ANA result. The high proportion of family physician records (71%) with no diagnosis recorded other than the FANA result suggests that referral may have been on the basis of the test result alone.

CTD was clinically excluded in 52 patients (16%). In a further 91 we could not definitively exclude CTD because no acceptable final diagnosis had been reached, yet there was no evidence to suggest or support CTD in the chart, and the test was often designated by the family practitioner, inappropriately, as a "screen". Furthermore, the diagnosis of SLE, etc. was not even suggested in the chart as a possibility by either the family physician or a specialist, where seen. In 58 of these patients, the family physician ordering the test had not thought a referral was warranted despite the positive result. We understood this to suggest that the possibility of a significant CTD did not seem high even to that physician, despite the positive ANA. However, in 4 other patients a diagnosis of SLE was listed by the family physician, yet no referral was made. It seems likely that these patients had this diagnosis of SLE based largely on the results of serology, with minor associated symptoms, and, from our observations, had accepted diagnostic criteria been used, they would certainly not have been so diagnosed (15). Category 2 contained patients with RA, adult onset Raynaud's, and undiagnosed polyarthritis, where a positive ANA test may be commonly seen, although rarely of diagnostic value. However, it seemed that some patients were diagnosed for example as Sjögren's on the basis of minimal symptoms, e.g. of dry mouth, but positive serology for SSA or SSB. This may reflect assumptions of uncertain validity about the specificity and positive predictive value of these tests. This was also the most frequent antibody subtype(s) seen in category 4.

When further analysing subgroups of patients with positive tests and with either definite lupus or definite absence of CTD, there was little difference in the endpoint titre. It appears that even very high titre results, although alarming, may be of little immediate clinical consequence (Table II).

Perhaps education of physicians to think in terms of pre-test probabilities and the effect of this on a positive test may reduce the inappropriate tendency, especially of family physicians, to use ANA as a screening test for connective tissue diseases, in the absence of an important clinical likelihood before testing. In other words, it may be better thought of as a test to confirm a clinical suspicion of lupus, and in this context the titre itself appears unimportant. Thus, any titre deemed positive by the laboratory can be used as one of the (ARA) diagnostic criteria. In one of the two arms of the laboratory (DKML), only 20% of the specimens referred had a positive test, even at the screening dilution. This percentage is only 12.5% above our results in normal controls, suggesting a major inappropriate use of the test, at least in this community, and we believe elsewhere too.

If it is used as a screening test, then with the conventional test and a false positive rate of 7%, one can calculate that, given a pre-test probability of SLE in the background population of 0.1%, the post-test probability remains low at 1.4% (16). With the high titre test and a false positive rate in normal, healthy controls of 0.5%, the post-test probability still remains remains relatively low at 17%, well below a level acceptable for a clinical diagnosis. The false positive rate in healthy blood donors may be artificially low, for, in context, a physician wants to be able to distinguish SLE from other causes of ill health. Our high titre result had a positive rate of 44%, in individuals with no discernable connective tissue disease at that time. Many others were patients with RA, etc., where the positive test was not "false", but where the result would not have been clinically helpful. Furthermore, in category 1, of 68 patients with possible or probable SLE, the diagnosis was already suspected and a positive ANA had previously been obtained in 58, suggesting that these current tests may have contributed little to clinical management.

There are no good prospective studies of cohorts of individuals with a positive FANA. The Finnish retrospective data suggest they may have an increased likelihood to develop SLE in the future (17), but as the risks remain unknown it seems valid to categorize these individuals as non-CTD at this time.

Our patients were unselected, representing all high titre results obtained in this region over the period, but the results are almost exactly the same as those described from a hospital based series of patients where 55% of high titre results were not associated with any connestive tissue disease (9).

Thus, while less than 1% of our normal healthy controls were positive at high titre, our results suggest that in practice the use of a designated high titre cutoff is of limited benefit, as a substantial proportion of patients with this result still clearly do not have a connective tissue disease. Indeed, measurement and reporting of high titre as opposed to the usual screening titre may be counter productive by encouraging further investigation in the absence of clinical features of a CTD, without conferring a sufficient increase in specificity to justify either. We suggest that reporting of ANA positivity at the screening dilution only, may remain a reasonable and cost effective way to report ANA, particularly if individual laboratories maintain a false positive rate of the order of 5%.

Our results are likely to be influenced by the seemingly frequent inappropriateness of the test request in our setting. Even if this could be reduced to a minimum, it was not clear that any clinically useful information was conveyed by designating a result as "high titre" for categories 1 and 2. In this laboratory 25% of the sera with a positive test for antibodies to nDNA have a FANA of less than 1: 640, and would therefore have been missed if a high titre designation were required as a screen for further testing. Further studies are needed here.

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