

WHAT TO DO WITH A POSITIVE ANA TEST?

Authors: Peter N. Malleson MBBS, MRCPUK, FRCPC¹, Murray J Mackinnon MSc, MSc², Michaela Sailer-Hoeck MD³.

Affiliations:

¹Peter Malleson: Professor, Department of Pediatrics, Division of Rheumatology, University of British Columbia, Canada

²Murray Mackinnon Statistician B.C. Cancer Agency

³Michaela Sailer-Hoeck, Pediatric Rheumatologist, Clinical Department of Pediatrics, Clinical Division of General Pediatrics, Medical University of Innsbruck, Austria

Correspondence to: Peter Malleson

Email address: malleson@interchange.ubc.ca

Introduction

Since the introduction of the indirect immunofluorescence (IF) test for antinuclear antibodies (ANA) by Friou in 1957 (1), ordering tests for ANA have become almost a knee-jerk response to the question “could this patient have a rheumatic disease?” What is the evidence that ordering such tests is of any value, and what should be done with a positive test?

The ANA test in health and disease

Ideally a test should always be positive in those with a disease, and always negative in those without a disease. Of course this situation rarely if ever occurs, but to be useful a test generally needs to have a sensitivity and specificity of at least 90%. That is at least 9 out of 10 individuals with the disease will have a positive test and 9 out of 10 individuals without the disease will have a negative

test. Unfortunately the ANA test, whether performed by IF or by an enzyme linked immunosorbent method, fails to demonstrate these test characteristics.

Part of the problem is that the test is being used indiscriminately as part of “the rheumatologic work-up” beloved of family physicians and residents. No test can sensibly be expected to be accurate in the diagnoses of diseases as different as juvenile idiopathic arthritis (JIA), rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and the vasculitides. Yet, in practice, this is what often seems to be asked of the ANA test. However, even if the test is used more sensibly to address specific questions such as: “does this child with a rash and fever have lupus?” or “does this child with a swollen knee have JIA?”, or “is this child with JIA going to develop uveitis?”, we would argue that the ANA test is simply not accurate enough to answer these questions.

ANA in Healthy populations

A number of studies have looked at the frequency of positive ANA tests in “healthy” individuals. A study by Arroyave et al. in 1988 (2) screened sera from 241 “normal” children, testing for only IgG ANA, using both mouse kidney and human epithelial cells (HEp-2 cells). The study found a maximum positivity rate of only 2.0% at the lowest dilutions. However, data from adult studies have found much higher rates. In an adult study from 15 international laboratories using HEp-2 cells as substrate (3), ANA positive tests occurred in 31.7% of a putatively normal population at a serum dilution of 1:40, and even at a dilution of 1:320, 3.3% of the sera were positive. Interestingly the ANA frequency did not differ significantly across the age range of 20-60 years. The rate of ANA positivity among blood donors in Holland was also quite high at 12.7%, with titers greater than 1:80 occurring in over 4% (4).

It is not clear why there is such a low frequency of ANA positivity found in the only childhood study of which we are aware, compared to the much higher frequency found in most adult studies (of which only two of many are referenced here). However, the frequency of ANA among clinic populations is more pertinent to our discussion than the situation in the normal population as ANA tests are

usually performed on children or adults with musculoskeletal or rheumatologic symptoms or signs.

ANA in Clinic Populations

ANA titers

Chudwin et al. in 1983 (5) evaluated the clinical and laboratory findings in 138 children with a positive ANA test. Although the authors interpreted the fact that two thirds of the patients had a specific connective tissue disease as being indicative that the ANA test is useful, the fact that one third did not have a definitive inflammatory disease, indicates that the ANA test has a very high false positivity rate.

In 1997 we evaluated the results of all the ANA tests performed at British Columbia Children's Hospital between 1991 and 1995 (6). We found that the ANA test was positive at a titer of 1:20 or greater in 41% of all sera tested, and in 65% of all patients in whom a diagnosis could be obtained from the ordering physician. The frequency was the same for those with or without a diagnosis of a rheumatic disease. At a screening serum dilution of 1:40 a positive test had a sensitivity of only 63% and a positive predictive value (the frequency that a positive test is indicative of disease) of only **33%** for any rheumatic disease.

For lupus, Mixed Connective Tissue Disease (MCTD) or overlap syndrome the ANA had a very high sensitivity of 98%, but a very low positive predictive rate of only 10%. Positive and negative predictive values are affected by the prevalence of the disease being tested. Therefore one might expect somewhat better predictive values from a pediatric rheumatology clinic than from a wide population of unwell children. We concluded from this study that although a negative ANA test made the diagnosis of lupus or MCTD extremely unlikely, a positive test at even moderately high titers of 1:160 has little or no diagnostic value.

ANA immunofluorescent staining

As our laboratory also provided information on the patterns of immunofluorescent staining, we have also been able to evaluate what further use this information might provide (previously unpublished data). Of 1369 individual patients sera tested, 445 were ANA positive in children with a known diagnosis; 135 children having a rheumatic disease (JRA, lupus, MCTD, or juvenile dermatomyositis (JDM)) and 310 without a rheumatic disease. Homogeneous, mitotic staining patterns were seen much more commonly in children with a rheumatic disease than those without ($p=0.001$). Interestingly, a nucleolar pattern of staining was seen more commonly in children without a rheumatic disease ($p=0.03$). This lack of association of the nucleolar pattern in children with scleroderma specifically, and rheumatic disease in general, has been commented on previously (7, 8).

No combination of ANA titer, or staining pattern was specific for any particular rheumatic disease. The test combinations with the best positive predictive values for a rheumatic disease were: i. a titer $\geq 1:640$ with mitotic positive staining or ii. a titer $\geq 1:640$ with a homogenous and mitotic staining pattern; these tests had positive predictive values of 77% and 72% respectively, but these results were only slightly higher than the positive predictive value of 69% obtained with a titer of $\geq 1:640$ alone, ignoring the pattern of staining.

Although the addition of patterns somewhat increases the specificity and positive predictive value of the ANA test for a rheumatic disease, it does so at the expense of both the sensitivity and negative predictive value of the test using titers alone. The pattern of staining also does not appear to be helpful in distinguishing between rheumatic diseases. For example, although high titer homogenous, mitotic positive staining was the most common combination seen in children with lupus, it was actually found in only 27.3% of ANA positive lupus patients. This combination was also found in 12.5% of ANA positive JRA and 15.4% of ANA positive JDM patients. This lack of specificity of the ANA immunofluorescent pattern has been recognized previously, both for adults and children (7, 9, 10).

A study by Parker (10) evaluated the usefulness of combining ANA titer and pattern; although they felt that knowledge of both titer and pattern was helpful, they did not calculate specific test characteristics, and in fact no combination was restricted to any single rheumatic disease. Our assessment of these data is that the addition of information about patterns of immunofluorescence does not appear to significantly improve the utility of the ANA test.

Referrals for positive ANA titers

As part of a study exploring what precipitated a referral to a pediatric rheumatologist, McGhee et al. (11) found that children referred, at least in part, because of a positive ANA test were no more likely to have a chronic inflammatory disease than children with a negative test.

In another study from a pediatric rheumatology clinic (12), only 55% of all of the children with a positive ANA test had an inflammatory rheumatic disease. Positive antibodies to dsDNA or to extractable nuclear antigens (Sm, RNP) indicating lupus or MCTD were strongly correlated with an ANA titer $\geq 1:640$. The authors recommended therefore, that these more specific tests be performed only if the child had a positive ANA test at high titer.

All these studies demonstrate that a positive ANA test is found frequently in a pediatric hospital population, and even in high titer has only a poor ability to determine whether a child has an inflammatory rheumatic disease.

Development of Connective Tissue Diseases in ANA positive individuals

It could be argued that the finding of a positive ANA test indicates that the child has an occult disease that will become manifest later. Is there any evidence to support or refute this?

There is some evidence that ANA may sometimes precede the development of lupus by several years. Using Finland's Social Security Institution's population registry, Aho et al. (13) were able to trace 16 serum samples from apparently healthy subjects who later developed SLE or MCTD.

Ten of the 16 (62.5%) samples were positive for ANA. Eight of the 11 (72.7%) were positive when the interval from sampling to onset of first symptoms was ≤ 2 years, and 2 of 5 cases (40%) were positive when the interval was > 3 years. Based on an incidence rate of lupus of 5/100,000/year, the authors calculated that lupus would develop in less than one percent of the ANA positive individuals. Cabral et al. (14) followed the course of 24 children who were considered clinically not to have an inflammatory disease despite being ANA positive and found that no patient developed an overt inflammatory disease during a follow-up period of 61 months (range 13 to 138 months).

Some studies have evaluated the outcome in patients with fibromyalgia (a musculoskeletal pain condition not thought to be an autoimmune disease) who were also ANA positive, and have not found that the occurrence of a positive ANA influences outcome. Al-Allaf et al. (15) found the ANA positivity rate (titers not given, a positive result was simply defined as “plus”) in their adult patients with fibromyalgia was 8.8% (almost identical to the 8.9% ANA positivity rate in their control patients with osteoarthritis). The 12 individuals who had fibromyalgia and were ANA positive were matched for age and sex with 12 ANA negative patients. Over a 2-4 year follow-up period one patient in the ANA positive group fulfilled criteria for lupus, and one in the ANA negative group fulfilled criteria for Sjögren’s syndrome. The authors concluded that the ANA test (at least in low titer) was not a good predictor of future connective tissue disease. In a study of 59 pediatric patients with fibromyalgia (16), 17 (28.8%) were ANA positive (mean titer 1:160). Fifty patients were followed for a mean of 18.3 months and during that time no patient developed a connective tissue disease.

We would conclude from these findings that only rarely is the presence of ANA positivity the harbinger of undeclared lupus or another connective tissue disease.

ANA and other diseases

It should also be remembered that ANA are associated not only with the classical autoimmune diseases, but also with infection (17), malignancy (18), and

drugs (19). Environmental toxins may also predispose to ANA production. Although hopefully not relevant in pediatrics, there is evidence of an increased frequency of ANA in individuals with silicone breast implants (20), and at least two studies have suggested that rural populations have a higher frequency of ANA positivity than urban populations, perhaps due to toxin exposure (21,22). Therefore the finding of a positive ANA test should not blind the physician to the possibility of a non-autoimmune diagnosis.

A situation where a positive ANA test may be of some value is in children diagnosed with idiopathic thrombocytopenic purpura (ITP). In a study of 87 children with ITP, it was found that 36% of those with a positive ANA (titer $\geq 1:40$) developed further “autoimmune symptoms”, five children developing lupus, compared to none of those who were ANA negative ($p < 0.001$) (23).

ANA positivity as a risk factor for uveitis in children with JIA

There is little doubt that in children with JIA the ANA test is more frequently positive in those with uveitis than in children without uveitis. The American Academy of Pediatrics recommends performing the ANA test as part of the screen for uveitis (24). However, although there is a *statistically* significant difference between children with and without uveitis, we would argue that this is of little *clinical* significance. In a recent study from Finland (25), for example, uveitis was found in 104 of 426 new cases of juvenile chronic arthritis.

Antinuclear antibodies were found in 66% of those with uveitis compared to 37% of those without uveitis, a statistically significant difference. However if the presence or absence of a positive ANA test was used in determining the frequency of ophthalmologic examinations, it is possible that some of the 46/104 children with uveitis and negative ANAs might well have had a delayed diagnosis due to the partial reliance on the ANA positivity to determine frequency of eye checkups.

What should be done?

So what should be done with a positive ANA test? Our answer would be exemplified by the answer a local inhabitant gave when asked directions from

place A to B by a foreign tourist: “I wouldn’t be leaving from here!”. In other words, it would be best if the ANA test had not been done in the first place!

We would suggest that a positive ANA test can safely be ignored unless there are other suggestive clinical signs, and simple laboratory tests (such as a raised ESR or cytopenias) that point towards a diagnosis of lupus or similar connective tissue disease. Given the high false positive rate of ANA tests, a positive test cannot be used as confirmatory evidence that the child with a swollen joint has JIA, rather than some other serious condition such as septic arthritis, leukemia, or hemophilia. Similarly, symptoms such as fatigue and aches and pains in a child, should not be ascribed to lupus simply because of a positive ANA test. It is much more likely that she has an idiopathic pain syndrome such as fibromyalgia. A negative ANA test is more useful than a positive one, as it does, for all practical purposes, exclude the diagnosis of lupus in a child.

What is needed is a cost-effectiveness study to evaluate whether the screening ANA test should be replaced by testing initially for anti-dsDNA and anti-ENA (anti-Sm, RNP, SSA and SSB) antibodies. Until that study is done, we would recommend that non-rheumatologists only do an ANA test if there is a fairly high probability (perhaps a 10+% chance) that a child’s symptoms could be due to lupus or Mixed Connective Tissue Disease. If the test is positive at a titer of $\geq 1:160$ then it would be appropriate to order antibodies to dsDNA and ENA, with lower titers being ignored. We would strongly recommend that the ANA test is not ordered indiscriminately as part of “a rheumatologic work-up”.

Conclusion

The question why the ANA test is so frequently positive in populations without an overt autoimmune disease is a fascinating one. It suggests that the breaking of immunological tolerance is really quite common, but that this tolerance breakdown only relatively rarely leads to disease. However, from a practical, clinical point of view, the ANA test has such a high false-positivity rate that a positive test is of little, if any, clinical utility.

Acknowledgements:

We would like to acknowledge the help of Louis Wadsworth MBBS, FRCP, Director, Hematopathology Program British Columbia's Children's Hospital who provided generous help with acquiring the ANA data presented here.

References

1. Friou GJ. Fluorescent spot test for anti-nuclear antibodies. *Arthritis Rheum* 1962; 5:407-410.
2. Arroyave CM, Giambrone MJ, Rich KC, Walaszek M. The frequency of antinuclear antibody (ANA) in children by use of mouse kidney (MK) and human epithelial cells (HEp-2) as substrates. *J Allergy Clin Immunol* 1988; 82:741-744.
3. Tan EM, Feltkamp TEW, Smolen JS, et al. Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum* 1997; 40:1601-1611.
4. de Vlam K, De Keyser F, Verbruggen G, Vandebossche M, Vanneuville B, D'Haese D et al. Detection and identification of antinuclear autoantibodies in the serum of normal blood donors. *Clin Exp Rheumatol* 1993; 11:393-397.
5. Chudwin DS, Ammann AJ, Cowan MJ, Wara DW. Significance of a positive antinuclear antibody test in a pediatric population. *Am J Dis Child* 1983; 137:1103-1106.
6. Malleson PN, Sailer M, Mackinnon MJ. Usefulness of antinuclear antibody testing to screen for rheumatic diseases. *Arch Dis Child* 1997; 77:299-304.
7. Osborn TG, Patel NJ, Moore TL, Zuckner J. Use of the HEp-2 cell substrate in the detection of antinuclear antibodies in juvenile rheumatoid arthritis. *Arthritis Rheum* 1984; 27:1286-1289.

8. Deane PMG, Liard G, Siegel DM, Baum J. The outcome of children referred to a pediatric rheumatology clinic with a positive antinuclear antibody test but without an autoimmune disease. *Pediatrics* 1995; 95:892-895.
9. Wangel AG, Teppo A-M, Pollard A, Howarth S. Antibody profiles of sera giving different nuclear staining patterns. *Scand J Rheumatol* 1984; 13:303-309.
10. Parker MD, Kerby GP. Combined titre and fluorescent pattern of IgG antinuclear antibodies using cultured cell monolayers in evaluating connective tissue diseases. *Ann Rheum Dis* 1974; 33:465-472.
11. McGhee JL, Burks FN, Sheckels JL, Jarvis JN. Identifying children with chronic arthritis based on chief complaints: absence of predictive value for musculoskeletal pain as an indicator of rheumatic disease in children. *Pediatrics* 2002; 110:354-359.
12. Perilloux BC, Shetty AK, Leiva LE, Gedalia A. Antinuclear antibody (ANA) and ANA profile tests in children with autoimmune disorders: a retrospective study. *Clin Rheumatol* 2000; 19:200-203.
13. Aho K, Koskela P, Makitalo R, Heliovaara M, Palosuo T. Antinuclear antibodies heralding the onset of systemic lupus erythematosus. *J Rheumatol* 1992; 19:1377-1379.
14. Cabral DA, Petty RE, Fung M, Malleson PN. Persistent antinuclear antibodies in children without identifiable inflammatory rheumatic or autoimmune disease. *Pediatrics* 1992; 89:441-444.
15. Al Allaf AW, Ottewell L, Pullar T. The prevalence and significance of positive antinuclear antibodies in patients with fibromyalgia syndrome: 2-4 years' follow-up. *Clin Rheumatol* 2002; 21:472-477.
16. Gedalia A, Garcia CO, Molina JF, Bradford NJ, Espinoza LR. Fibromyalgia syndrome: experience in a pediatric rheumatology clinic. *Clin Exp Rheumatol* 2000; 18:415-419.

17. Allen RC, Dewez P, Stuart L, Gatenby PA, Sturgess A. Antinuclear antibodies using HEp-2 cells in normal children and in children with common infections. *J Paediatr Child Health* 1991; 27:39-42.
18. Swissa M, Amital-Teplizki H, Haim N, Cohen Y, Shoenfeld Y. Autoantibodies in neoplasia. An unresolved enigma. *Cancer* 1990; 65:2554-2558.
19. Byrne PA, Williams BD, Pritchard MH. Minocycline-related lupus. *Br J Rheumatol* 1994; 33:674-676.
20. Cuellar ML, Scopelitis E, Tenenbaum SA, Garry RF, Silveira LH, Cabrera G et al. Serum antinuclear antibodies in women with silicone breast implants. *J Rheumatol* 1995; 22:236-240.
21. Rosenberg AM, Semchuk KM, McDuffie HH, Ledingham DL, Cordeiro DM, Cessna AJ et al. Prevalence of antinuclear antibodies in a rural population. *J Toxicol Environ Health A* 1999; 57:225-236.
22. Spiewak R, Stojek N. Antinuclear antibodies among eastern-Polish rural inhabitants. *Ann Agric Environ Med* 2003; 10:207-209.
23. Zimmerman SA, Ware RE. Clinical significance of the antinuclear antibody test in selected children with idiopathic thrombocytopenic purpura. *J Pediatr Hematol Oncol* 1997; 19:297-303.
24. American Academy of Pediatrics Section on Rheumatology and Section on Ophthalmology: Guidelines for ophthalmologic examinations in children with juvenile rheumatoid arthritis. *Pediatrics* 1993; 92:295-296.
25. Kotaniemi K, Kautiainen H, Karma A, Aho K. Occurrence of uveitis in recently diagnosed juvenile chronic arthritis: a prospective study. *Ophthalmol* 2001; 108:2071-2075.