Rational Interpretation of Autoantibodies

VR Joshi

Director Research, Consultant Physician & Rheumatologist, P. D. Hinduja National Hospital, & Medical Research Centre, Mahim, Mumbai 400 016.

160

ABSTRACT

Laboratory tests are becoming an important component of clinical practice. There is a tendency to over rely on these tests to make rather than to confirm a clinical diagnosis. Laboratory tests carry inherent limitations of sensitivity, specificity and positive and negative predictive values. This apart, technical aspects and the meaning of ranges defined as normal need to be clearly understood. In this article, an attempt is made to highlight these areas.

"A fool with a tool is still a fool"

- Lars Leksell

INTRODUCTION

Laboratory tests are an integral part of today's clinical practice. Increasingly, clinicians tend to rely on the test results to arrive at a diagnosis. The number of tests is ever increasing. More often than not these tests are costly. For correct practice of medicine it is not only important to select the tests judiciously but also to have a clear understanding of their strengths, and weaknesses, their validity and reliability (validity = ability of a test to detect what it is supposed to detect; reliability = consistency of result).

SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUE

Four indices of validity, namely sensitivity, specificity, positive and negative predictive value are important.

Sensitivity addresses the question – "If 100 patients with a disease are tested, how many will test +ve with the test under consideration?" It is in fact a measure of false -ve test result i.e. a negative result in the presence of the disease. If a test is 100% sensitive, no patient with the disease would test –ve. i.e. their would be no false –ve and a –ve result would reject the diagnosis. But if sensitivity is less, say 80%, the test will carry 20% false negativity i.e. one out of every five patients with the disease will be missed out.

A highly sensitive test is useful when one does not want to miss a diagnosis. Examples of such a situation are (i) the disease is serious and should not be missed, (ii) disease is treatable / curable. (iii) highly sensitive tests are, good for screening purposes (epidemiological studies).

Specificity addresses the question "How many individuals who are free of the disease would correctly test –ve with the test under consideration?" It is a measure of false +ve result. A test with 100% specificity, when +ve, excludes any other diagnostic consideration as there would or can be no false +ve results. On the other hand with lesser specificity, say 80%, 20% patients without the disease will test +ve i.e. 1 in 5 will wrongly test +ve. A highly specific test is useful to confirm a diagnosis and exclude other possibilities e.g. anti-Sm antibody test carries near 100% specificity and hence when +ve is diagnostic of SLE.

But in clinical practice merely knowing sensitivity and specificity does not suffice. One needs to know the positive and negative predictive values of the test.

Positive predictive value (PPV) defines the chances (odds) of having a disease when the test gives a +ve result. It is calculated by dividing the number of true +ves by the sum of true positive + false positives. It is obviously desirable to have a test with high PPV.

Negative predictive value (NPV) defines the chances (odds) of absence of a disease with a –ve test result. It is calculated by dividing the number of true –ves by the sum of true and false –ves. Again a test with high a NPV is desirable.

Effect of pretest probability on PPV and NPV

Both PPV and NPV are affected by pretest disease probability. PPV diminishes and NPV increases with low disease prevalence or probability and PPV increases with higher pretest disease probability e. g. ANA is a highly sensitive and specific test for SLE (both approximately 95%). But, SLE is an uncommon disease (prevalence < 0.001%). In this scenario, there would be, in a given population, more (false) ANA +ve individuals (5%), than patients of SLE (< 0.001%) and therefore even with a +ve

Table 1: Results of RF test in normal persons and patients (partly hypothetical)

Titer	Healthy Individuals		RA patients			
Titer	+ve	-ve	+ve	-ve	PPV	NPV
Neat	5	95	90	10	95	90
1 : 10	3	97	80	20	96	83
1 : 20	1	99	75	25	99	80
1 : 40 and above	0	100	60	40	100	71

test result odds are that the person does not have SLE. The PPV in this example works out to < 1% with a NPV of > 99%. On the other hand, if there is a patient with a high (> 50%) pretest probability of SLE (young female with fever, rash, oral ulcers, arthralgia, leucopenia, and abnormal urinary findings) the PPV reaches > 90%.

In essence, a diagnostic test is of value only when applied to appropriate clinical situation. A randomly applied test is more likely to mislead than be of any help.

TRADE-OFF BETWEEN SENSITIVITY AND SPECIFICITY

The results of laboratory tests (expressed in units) are a continuum between totally normal values to absolutely abnormal values. In the middle lie values which could be present in both healthy and affected individuals. Choosing values which totally exclude any false +ve result would mean missing many cases and keeping it low would mean misdiagnosis of normals as diseased individuals (Table 1).

In the above example if we take neat titer as a cut-off point to diagnose maximum number of RA patients (make it maximally sensitive) we will "mislabel" five normal healthy individuals. On the other hand if we use a titer of 1 : 40 or above (to make it highly specific) though no normal person will be mislabeled, 40% of RA patients will be "left out". The cut-off points given by the laboratories are therefore a trade-off or a compromise between the highest sensitivity and the highest specificity. The cut-off point used for RF test is 1:20 titer which will 'misdiagnose' 1% and correctly 'diagnose' 75% RA patients – a reasonable compromise.

PRESENCE OF AUTOANTIBODY IS NOT SYNONYM TO PRESENCE OF AUTOIMMUNE DISEASE

Autoimmunity (i.e. presence of autoantibody) is common but autoimmune diseases are uncommon. Autoantibodies can be present in otherwise perfectly healthy individuals. Their prevalence increases with age e.g. rheumatoid factor may be present in over 20% of the elderly while in very young it is present in less than 1%. *It is only when an autoantibody is associated with clinical manifestations compatible with an autoimmune disorder that its presence becomes relevant and of diagnostic significance.*

TECHNIQUE

There are many methods of testing autoantibodies and this can be an important source of error or confusion. e.g. anti-nuclear antibody test can be performed with fluorescent (FANA) technique, or ELISA. The latter is popular because of its ease to perform and relatively low cost. However, as things stand today, ELISA test for ANA is not favoured as it can give both false +ve (being highly sensitive) and false –ve results (it tests only an 'x' number antigens as compared to FANA). Even for FANA, the substrate used makes a difference. Rabbit liver is not rich in all the nuclear antigens. HEP-2 cells or cultured fibroblasts are better substrates as they express many nuclear antigens. As per today's paradigm (i) for diagnostic purposes only FANA using HEP-2 or cultured fibroblasts is preferred. FANA has the added advantage of pattern recognition, which helps to choose antibody subsets (anti dsDNA, antibody to ENA etc.) (see Table 2); and (ii) antibodies to ENAs and other antigens are better tested with ELISA because of its higher sensitivity – provided the test i.e. antigen preparation has been standardised.

Table 2: Prognostic value of autoantibodies

Clinical situation	Antibody	Implication
Early arthritis (presumed RA)	Rheumatoid factor (RF)	PPV 0.82; NPV 0.76
- " -	Antibodies to cyclic citrullinated peptide (CCP)	Specificity 0.94; Sensitivity 0.47; PPV 0.82; NPV 0.676
- " -	Combined anti CCP and RF	Specificity 0.99; Sensitivity 0.34 Persistent, erosive arthritis
SSc	Anti-topoisomerase	New or worsening organ involvement Higher odds of developing right heart failure
SLE	Rising titers of anti ds-DNA;	Lupus nephritis
	anti-Ro; Anti-La	Neonatal lupus, complete heart block
Dermatomyositis – polymyositis	Anti – Jo–1;	ILD
	Anti – SRP	Severe myositis

Anti-ds DNA antibody is tested with FANA or Farr assay, though ELISA is becoming popular and acceptable. For anti-ds DNA antibodies, ELISA is more sensitive but less specific, than FANA or Farr assay. Further, results vary with different commercial preparations of ELISA making comparisons difficult. Latex and similar techniques to detect ANAs should not be accepted. For RF, latex test serves well though nephelometry is better. SCAT, though highly specific is technically difficult and less sensitive. It is clear that a clinician needs to know more about the test system used to perform a test for proper interpretation. Inter-test kit variability is a practical problem. Reference standards are important.

MONITORING

Some autoantibodies can be used to monitor disease activity. e.g. anti-dsDNA (best done with Farr assay), ANCA, and anticardiolipin antibodies.

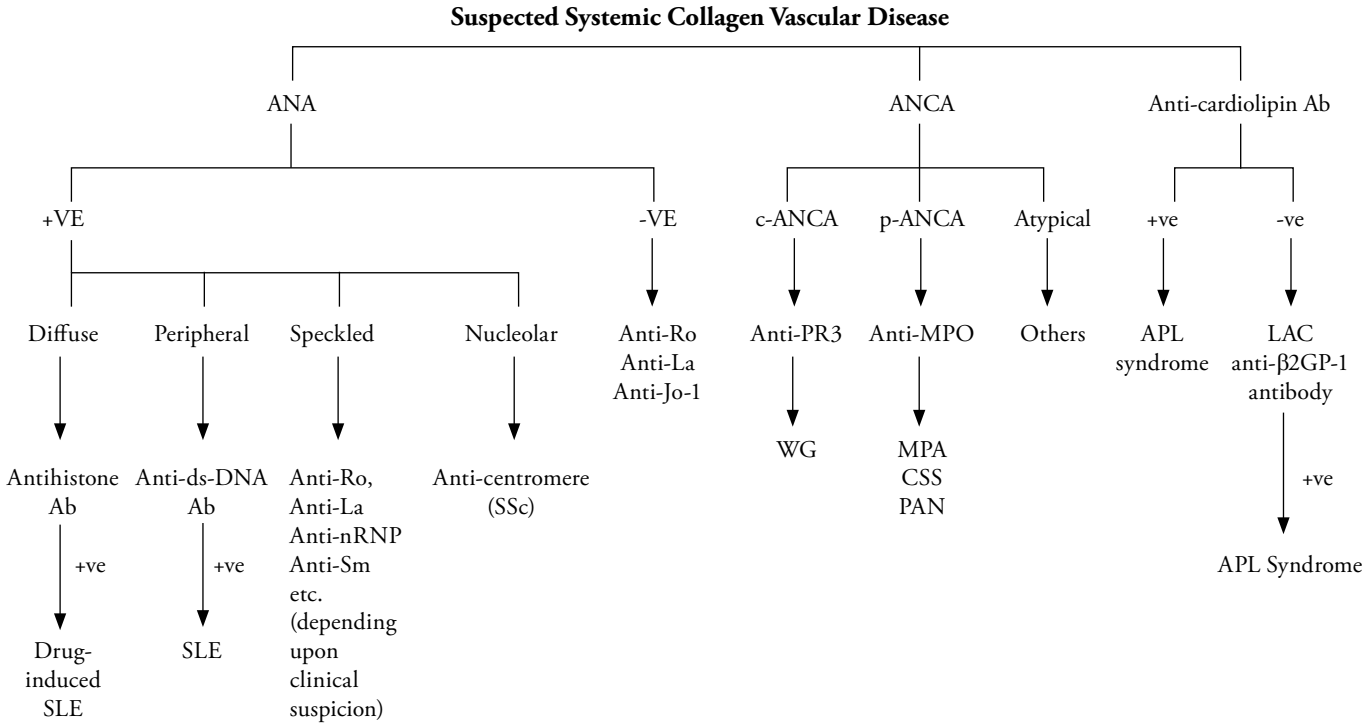

Fig. 1 : Algorithm for antibody testing ina suspected case of collagen vascular disease

Repetition of test is indicated in some situations to confirm the diagnosis. It is especially advised for anticardiolipin antibodies as infections and drugs produce these antibodies transiently.

PROGNOSTIC IMPLICATIONS

Apart from diagnostic use, some autoantibodies may have prognostic value. Some examples are given in Table 2:

ALGORITHMS

It is not uncommon to see anti ds-DNA antibody test being ordered without checking ANA or even if ANA is negative. This is not correct. ANA should be the first test ordered. If +ve, then alone, other antinuclear antibodies should be tested (an exception could be anti-Ro, anti-Jo–1 antibodies which can give –ve ANA result. This is because these antigens are mainly cytoplasmic). An logarithm for tests in a suspected case of systemic collagen vascular disease is shown in Fig. 1.

REFERENCES

1. Tze-wey Loong. Understanding sensitivity and specificity with the right side of the brain. *BMJ* 2003;327:716-19.
2. Wener MH. Autoantibodies and other laboratory tests In Rheumatoid Arthritis. E. Williams St. Clair, David S, Pisetsky, Barton F Haynes Ed. Lippincott Williams and Wilkins 1st Ed 2004 pg 64-79.
3. Peter J. Maddison, Pearl Huey. Serological profile in Oxford Text Book of Rheumatology. David A. Isenberg, Peter J. Maddision, Patricia Woo, David Glass and Fordinand, C. Breedveld Eds. Oxford university press, Oxford. 3rd Ed. Pg. 491-499.
4. Guidelines for use of immunologic tests. *Arthritis Rheumatism* 2002;47: 434-44.