Commentary

Clinical utility of antinuclear antibodies: titrating the serum first

In most populations, up to 75 per cent of individuals with positive antinuclear antibody (ANA) tests do not have identifiable rheumatic disease. However, ANAs in healthy people are usually present in low concentrations, frequently polyreactive, have low affinity and often of the immunoglobulin M (IgM) class. In contrast, patients with systemic lupus erythematosus (SLE) usually have high titres of high-affinity IgG ANA against specific disease-associated nuclear antigens. These differences have been exploited for diagnostic purposes by creating a border zone between positive and negative sera.

It is generally accepted that tests like ANA detection should give negative results in at least 95 per cent of healthy normal controls. This can easily be achieved by increasing the dilution of the sera to be considered as the border between positive and negative. On the other hand, we want nearly all sera from SLE patients to give positive results. If the diagnosis of SLE was based on the presence of ANA, like that of the mixed connective tissue disease on the presence of anti-U1-ribonucleoprotein (U1-RNP), this would never be a problem. That, however, is not the case. The border dilution between positive and negative has to be chosen at the dilution at which at least 95 per cent of the patients with definite SLE are ANA positive.

In this issue, Ghosh et al. have attempted to determine the optimum screening dilution of sera for testing ANA for diagnosis of SLE, using indirect immunofluorescence (IIF) for their laboratory. They tested sera of patients with SLE, idiopathic inflammatory polymyositis/dermatomyositis and rheumatoid arthritis by indirect IIF using a commercial kit at five standard dilutions. Receiver operator characteristics (ROC) curve were constructed to define the optimum dilution that distinguished healthy sera from the diseased ones. Their results showed that at 1:40 dilution, sera of 95.3 per cent of SLE and 13.8 per cent of normal individuals were ANA positive, whereas at 1:80 dilution it was 95.3 per cent for SLE and 4.3 per cent for healthy individuals. The best discrimination between healthy individuals and the SLE patients was at screening dilution of 1:80 in their laboratory. In other words, if the laboratory were to use a dilution of 1:40 then 13.8 per cent normal individuals would test positive, while the latter figure would decline to 4.3 per cent at a dilution of 1:80. In either case the ANA prevalence among patients with SLE would remain at 93.5 per cent.

This important study highlights the need for following several principles and emphasizes the need for tests to be standardized for different populations and laboratories. The first of these is to optimize diagnostic “specificity” (per cent negative controls) and “sensitivity” (per cent positive patients). Both these figures should be as high as possible. In addressing the issues of sensitivity and specificity it is pertinent to remember that ANAs are far more frequent in healthy females than in healthy males and in elderly females than in young females. The border dilution should not accept positive healthy young boys and be less stringent on healthy old women. The border dilution settled in one laboratory referring to a certain population cannot be copied for another laboratory which not only serves another population but also does not perform the test and readings in exactly the same way.

The choice of the optimal cut-off level is a trade-off between optimizing sensitivity and specificity. A rational cut-off requires consideration of the clinical situation where the test is to be used and the knowledge of auxiliary characteristics of the target and differential diagnostic diseases. Since only <95 per cent of patients with SLE develop ANA, testing for ANA can never achieve 100 per cent sensitivity. False-negative ANA results occur by necessity, making ANA a
probabilistically useful but not obligate diagnostic marker for SLE and Sjogren's syndrome (SS). The clinical risk of a false negative ANA result is predictable, manageable and within acceptable limits because SLE is a clinically defined syndrome. In contrast, false-positive ANA results may be of considerable clinical concern. When performing ANA determinations in a routine setting, it is important to remember that the test is primarily a screening method before other more defined serological tests are performed. Patients with definite SLE who are ANA-negative exist, but if they constitute over 0.5 per cent it is recommended that the method is studied once more even though, statistically 5 per cent is acceptable. On the other hand, factors like age and sex should be taken into consideration when interpreting the results of a test. Thus, a positive ANA test in a woman over 60 yr is not alarming, in contrast to positive result in a boy.

Each laboratory is a unique setting and together with the referring clinicians, should have its own policy on the performance of the test and the choice of the border between positive and negative results. In that context the study by Ghosh et al will be an important one and should encourage other laboratories to generate their own controls. Participation in quality control can only strengthen the process.

It is evident that the laboratory should be well equipped and that the rules for good laboratory practice (GLP) are respected. A protocol should indicate that each major change in the performance of the method, that is, a new cell line as substrate, another conjugate, another microscope, but also a new technician, should lead to intensification of quality control activities. Of the greatest importance is regular participation (at a minimum once in the six months) in quality control rounds organized by external organizations. Such organizations will ask the laboratory to examine some known positive sera (sometimes representing hidden dilution series) and negative controls. Sera known to be difficult to interpret should also be included. Reference sera are also available from organizations like the CDC and the WHO.

In the midst of all the efforts to standardize laboratory tests, attempts must be made to familiarize the clinicians regarding the correct clinical interpretation of the test. Use of a term like antinuclear factor for ANA indicates a lack of awareness of the appropriate use and interpretation of the test. Finally, with appropriate dilution there would be a reduction in what Calin termed the ‘iatrogenic antinuclear antibody neurosis’ of referred patients with nonspecific complaints and a positive ANA.

Ajay Wanchu
Department of Internal Medicine
Postgraduate Institute of Medical Education & Research
Chandigarh 160012, India
e-mail: awanchu@yahoo.com

References