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EDITORIAL

# Antinuclear antibodies: The medium is the message

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Marshall McLuhan's famous one-liner referred to television as the medium and its mass communication potential as the message in the then emerging global village. In current medical parlance, signs and symptoms are phenotypes, i.e. the visible medium by which the message, i.e. the resulting interaction between genotypes and environmental factors, is expressed. For the sake of argument, I propose that ANA testing is the medium that is also a key genetic message on connective tissue diseases (CTDs) and autoimmunity. Whether obtaining that information is useful is an evolving proposition paralleling medical progress.

Wakeland *et al*. recently reviewed their work on the component phenotypes of congenic strains carrying at least three lupus susceptibility genes on a normal mouse background (1). The Sle1 gene mediates the loss of tolerance to nuclear antigens, i.e. it allows the appearance of ANAs (2). Sle2 lowers the activation threshold of B cells (3). Sle3 mediates a dysregulation of CD4 (+) T cells (4). In those studies, Sle1-ANA is always the key: necessary but not sufficient for the development of lupus. The combination of Sle1-ANA with other autoimmune accelerating or suppressing genes results in systemic autoimmunity with variable penetrance and organ expression (5, 6). Those data are " ... the fulfillment of the genetic equivalent of Koch's postulates ..." (5).

How can a clinician estimate the genetic contribution in a given patient? In human autoimmune diseases, "polygenic" is the buzzword that corresponds to 20-40 genes in lupus-prone families (7). One marginally informative but reliable way is to use the clinical history to explore the immediate and the extended family tree of the patient looking for multiple autoimmune features or diseases. As those occur in the same or different members of the family, finding some supports the notion of terrain. Several HLA haplotypes are statistically associated with terrain but never strongly enough to be useful to clinical decision-making. On the other hand, clinicians know that ANAs are invariably associated with a number of individuals presenting any one of the many autoimmune features or diseases. It would thus be logical to assume that humans also develop ANAs in the context of a putative Sle1 equivalent and ANAs would be a critical disease susceptibility gene. That gene/ANA could either be clinically silent or result in disease if associated with one or more disease modulating genes or environmental triggers. We submit that in testing for ANAs, the clinician is identifying in a remarkably inexpensive way a genetic marker of susceptibility to autoimmunity. The exact implication of that message for the individual patient is still not readily quantifiable, but that is likely to change with the expansion of genomics.

If ANAs mean autoimmunity, shouldn't we systematically screen for them? Screening means testing asymptomatic people or testing for no good clinical reasons and to be capable of acting on the basis of the result. Clearly, the answer is currently "no". On the other hand, testing more or less symptomatic individuals to establish if they have a genetic predisposition to autoimmune features or diseases may be acceptable depending on how the results are interpreted by both doctors and patients. At this point, no risk calculation is possible, but simple post-test reassurance goes a long way.

If ANAs cannot be precisely interpreted, is ANA testing worthwhile at all? Except for the centromeric pattern, ANAs have no specific positive diagnostic value, i.e. they cannot rule in any single disease. While most SLE patients are ANA positive, they are not the only ones and that is consistent with ANAs being a generic genetic "autoimmune message." Of greater importance is the fact that non-SLE patients are usually ANA negative. Paradoxically, ANAs are thus most useful if negative as they rule out a disease. The epidemiological notions of sensitivity with true or false positive rates, specificity with true or false negative rates, positive and negative predictive values, and receiver operator characteristic (ROC) curve are familiar to most medical students when they graduate, but practicing physicians must be reminded periodically of these notions (8). As a diagnostic or screening laboratory test, ANA testing is not doing well using any one of those calculations.

In spite of that, if we were to accept ANA

testing as valuable, which titres would constitute clinically worthwhile negative and positive tests ? There is minor confusion on this issue and that is the propos of Vaile *et al.* (9). ROC curves are graphic plots of true versus false positive test results obtained at various cut-off points. Although, Vaile *et al*. do not use strictly that robust tool, their sero-clinical analysis concludes that there is no clinical benefit in reporting positive using a higher cut-off point. That is controversial (10). In Vaile *et al*., "high titre positive" is defined as > 1/640 and the screening dilution on Hep2 cells is 1/40. At this time, it is worth recalling one of the fundamental principles of serology: a significant difference between two titres is at least a two-log dilution. In practice, when one uses a screening dilution of 1/40, that sets the real cut-off at least at 1/160, a titre that is serologically different from 1/40 on the downside and 1/640 on the upside. There are three consequences for the clinical laboratory. First, reporting as positive anything > 1/40 is not realistic as it is equivalent to screening "healthy" people (11). It produces much patient and doctor anxiety and will waste money. Second, reporting as positive an ANA titre > 1/160 gives the same information as > 1/640. Third, anything equal to or below 1/160 should be reported as negative unless clinically correlated.

Given those considerations, do family doctors order too many ANA tests ? In the data of Vaile *et al*., it is clear that there is a selection bias for positive sera in the University Hospital laboratory used by specialists and another for negative sera in the Community Laboratory used by family doctors. Both biases are normal and expected considering the different patient populations, the different clinical presentations, and the intent of the respective doctors in ordering the test. In our view, the data reflect an excellent use of the ANA test by both groups in their respective arenas. In general, family doctors show good clinical sense when they look for ANAs based on very non-specific clinical complaints. At least they rule out disease. They also show good clinical sense not to feel obligated to make a diagnosis on a patient with a positive ANA when they are confronted with complex but incomplete phenotypic information. For good measure, they should be taught that there is no such thing as a false positive ANA: it means either a terrain or a disease. In both cases, that should constitute useful clinical information to be sorted, weighed as well as possible, and shared with the patient. Good preventive medicine means well-informed, reassured patients and doctors.

In conclusion, we believe that a positive ANA test should not necessarily be related to frank autoimmune or chronic inflammatory diseases or features. ANAs are a good example of nature-nurture influences. On the nurture side, a population may be composed of people with difficult economic and social conditions that may lead to a strong environmental assault on their adaptive immune system and result in more acute and chronic infectious/inflammatory diseases with positive ANAs but without clearly defined or frank autoimmune features. On the nature side, recent work dissecting the genes in lupus prone mice clearly show that a predisposition to develop ANAs can exist in the absence of the full complement of genes producing diseases (1). Given the heterogeneity of CTDs and the dilution of the gene pool in the human species, the probability of having one ANA susceptibility gene is much higher than the probability of having that same gene with the 30-40 others combining to produce a disease phenotype (7). That is why ANAs without disease are much more frequent in absolute terms than ANAs with disease. Restricting the use of the ANA test and the understanding of the ANA phenomenon to satisfy short-term economic and strictly diagnostic considerations, respectively, is counterproductive. Doctors should give due consideration to interpreting ANAs as markers for a predisposing autoimmune terrain, either in an apparently normal individual, in a patient, or in somebody living under harsh environmental conditions capable of inducing ANAs.

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