Publication of the Month

Phadia
Setting the Standard

August 08/11: ANA detection by indirect immunofluorescence and EliA CTD Screen

Key messages:

- *At equal specificity, the sensitivity of indirect immunofluorescence was lower than the sensitivity of EliA CTD Screen.*
- *A positive result by EliA CTD Screen had a higher likelihood ratio than a positive result by indirect immunofluorescence.*
- *On the other hand, as expected, the negative likelihood of IIF on HEp-2 is lower than that of EliA CTD Screen.*

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Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay

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Background: For the detection of antinuclear antibodies the indirect immunofluorescence (IIF) method is more and more replaced by solid phase assays. This study compares IIF on HEp-2000 cells (overexpressing SSA/Ro) with the EliA CTD Screen.

Summary: The study cohort consisted of 236 patients with different connective tissue diseases, 149 healthy blood donors, 139 patients with chronic fatigue syndrome, and 134 diseased controls.

The sensitivity of EliA CTD Screen for systemic lupus erythematosus, systemic sclerosis, primary Sjögren's syndrome, mixed connective tissue disease, and inflammatory myopathy was 74%, 72%, 89%, 100%, and 39%, respectively. The positivity in blood donors, in patients with chronic fatigue syndrome, and in diseased controls was <4%. However, among these controls a substantial portion was positive for antinuclear antibodies measured by IIF on HEp-2. 18 % of diseased controls tested positive at a cutoff titer of 1:160, while it was still 6% at a dilution of 1:640

Negative likelihood ratios at a screening cutoff of 1:40 were sufficient (<0.1) for SLE, scleroderma and Sjögren's syndrome. Positive likelihood ratios were sufficient (>10) for SLE, scleroderma and MCTD, however, only at a cutoff of >1:640. In EliA CTD Screen, apart from scleroderma the other connective tissue diseases showed positive likelihood ratios exceeding a ratio of 10. Highest likelihood ratios were detected for SLE and Sjögren's syndrome patients; lowest for patients with inflammatory myopathies. At a cutoff titer which gives IIF the same specificity as EliA CTD Screen the sensitivity of IIF was considerably lower. While EliA CTD Screen detects a proportion of CTD patients who are missed by IIF the solid phase method also misses some patients detected by IIF, who show antibodies other than those included in the CTD Screen.

Generally, a positive test result by EliA CTD Screen had a higher likelihood ratio for systemic rheumatic disease than a positive test result by indirect immunofluorescence. A negative test result by indirect immunofluorescence, however, had a lower likelihood ratio than a negative test result by EliA CTD Screen, indicating that the negative predictive value was higher for indirect immunofluorescence than for EliA CTD Screen.

The examination of the individual antigens contained in the EliA CTD Screen assay confirmed the classical disease associations of specific antibodies.

Conclusions: EliA CTD Screen is superior in specificity, sensitivity at equal specificity, positive likelihood ratio and positive predictive value compared to indirect immunofluorescence. On the other hand, indirect immunofluorescence shows better negative likelihood ratio and negative predictive value than EliA CTD Screen.

Comment: There is a tendency in Europe and particularly in the US to go back to IIF on HEp-2 for the first step of ANA screening. The low specificity and low standardisation of this method is accepted with the argument that it is most important to find as many patients as possible. The result is an alarmingly high number of patients with false positive results. In this study it was shown that a positive result on HEp-2 with a titer of less than 1:640 is not a strong indication for connective tissue disease as the positive likelihood ratio is too low. Therefore, IIF is not very useful as indicative test in diagnosis but for *exclusion of* SLE, Sjögren's syndrome or scleroderma, as a patient is very unlikely to have one of these diseases when IIF is negative.