Publication of the Month

Excellence in Autoimmunity

July 07/09: Detection of anti DNA antibodies by IIF on HEp2 cells

Detection of antinuclear antibodies is useful for the diagnosis of connective tissue diseases such as systemic lupus erythematosus (SLE), Sjögren's syndrome, scleroderma or polymyositis, dermatomyositis. In 96% of SLE patients circulating ANA can be detected. They are also found in other diseases such as liver cirrhosis or cancer and even in healthy individuals. In most labs, detection of antinuclear autoantibodies is done by indirect immunofluorescence (IIF) on HEp2 cells. In this method, autoantibodies against nuclear proteins produce specific patterns – the most common are homogeneous, speckled and nucleolar. Antibodies against double stranded DNA (dsDNA) react with the DNA in the cell nucleus of the HEp2 cell and, thus, produce a fluorescent pattern.

Many labs do not test dsDNA antibodies in parallel with IIF on HEp2 because they rely on the sensitivity of the IIF method. This laboratory practice should be questioned as a specific identification of anti-dsDNA antibodies is crucial for a proper diagnosis of SLE. An interpretation of a homogeneous pattern is not sufficient. On the other hand, not all anti-dsDNA antibodies produce homogeneous patterns but can also occur with other patterns, which was shown in the following study:

Servais G, Karmali R, Guillaume MP, Badot V, Duchateau J, Corazza F
Anti DNA antibodies are not restricted to a specific pattern of fluorescence on HEp2 cells
Clin Chem Lab Med 2009; 47: 543-549

Retrospective data from 1823 routinely tested sera from 405 patients were evaluated. The data came from 58 SLE patients, 236 patients with other diseases (52 RA, 9 Still's disease, 19 juvenile idiopathic arthritis, 54 systemic autoimmune diseases but not SLE, 24 organ specific autoimmune diseases, 21 hepatitis C, 23 HIV, 34 cancer) and as controls 111 patients with inflammation. Sera were tested at a 1:80 dilution on HEp2 slides and all positive sera were retested using serial dilutions. Anti dsDNA and anti nucleosomes were measured by ELISA.

Table 1 shows the frequency of antibodies against dsDNA, membrane associated DNA (mDNA), nucleosomes, histones and anti-ENA.

	Homogeneous	Speckled	Nucleolar	Other
dsDNA	37 %	37 %	16 %	11 %
mDNA	13 %	78 %	13 %	0 %
Nucleosomes	33 %	50 %	2 %	13 %
Histones	66 %	48 %	1 %	0 %
Anti-ENA	17 %	38 %	1 %	3 %

Homogeneous patterns were identified in all patients except those with HIV. Patients with rheumatoid arthritis showed the highest frequency of a homogeneous pattern (67% vs 33% of SLE). However, the highest titers with homogeneous patterns were found in SLE. If a restricted algorithm that was limited to a homogeneous pattern only had been applied, 67 % of the SLE serum samples would not have been explored further and the diagnosis would possibly have been missed.

The authors conclude that the nuclear pattern is not related to a peculiar diagnosis and so is the homogeneous pattern not a specific pattern for SLE.
The authors encourage pursuing identification of anti DNA, even in the presence of a non-homogeneous pattern when the HEp2 titer reaches the conventional level of 1:80.