biomnis
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Focus
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Anti-DNA antibodies

Anti-DNA antibodies

Double-stranded anti-DNA antibodies are excellent diagnostic markers and follow-up markers for systemic lupus erythematosus (SLE). Less specific than anti-Sm antibodies, anti-PCNA antibodies or anti-ribosomal P antibodies, they are nevertheless much more common. Their sensitivity and specificity for SLE gives them diagnostic value as they are part of the criteria used by the American Rheumatism Association for the diagnosis of this disease

Terminology used: DNA and anti-DNA antibodies

In the nucleus of eukaryote cells, double-stranded DNA (DNAds) is bound to histones. Native DNA is therefore double-stranded DNA (DNAds), surrounding the histones and forming nucleosomes. Anti-DNAds antibodies, anti-nucleosome antibodies and anti-histone antibodies can be screened for.

Diagnostic significance

Anti-DNAds antibodies and anti-nucleosome are markers for SLE. However, anti-histone antibodies are of little clinical utility, especially with regards to the diagnosis of drug-induced lupus (as previously mentioned), as they are found in a very large number of pathologies.

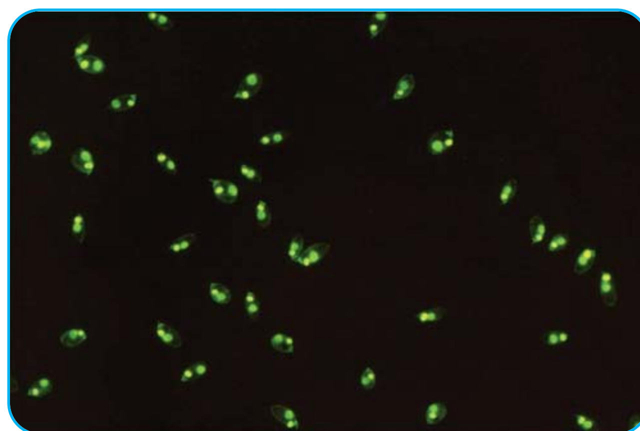
Anti-nucleosome antibodies: SLE marker

They are screened for using the Elisa EIA method or the fluorescence method. These are SLE markers, in combination with (most commonly) or without anti-DNAds antibodies. In cases where SLE is strongly suspected, with a supporting fluorescence image (intense staining of the chromatin) and absence of anti-DNAds antibodies, it is advisable to screen for anti-nucleosome antibodies, notably because they can appear earlier.

Anti-DNAds antibodies

They are screened for using three types of methods (caution, these methods do not detect exactly the same antibodies).

■ Indirect immunofluorescence (IF) on *Crithidia luciliae*:
With this method the anti-DNA antibodies are more strongly revealed from the kinetoplast (kDNA) antibodies, i.e. both circular and double-stranded DNA (although human DNA is double-stranded and not circular). Actually, C. luciliae is a flagellated trypanosome, a fly parasite (not pathogenic in humans). They have a large nucleus and a kinetoplast that contains DNAds. Screening for anti-DNAds is carried out via IIF on a flagellated culture and the result is positive when the kinetoplast is detected through fluorescence (green after staining with ethidium bromide; orange if negative) regardless of whether there is fluorescence signal from the nucleus.
Reading is difficult and subjective; in addition, the screen detects anti-kDNA antibodies and not anti-DNAds (sometimes, anti-kDNA antibodies are positive whereas the anti-DNAds remain negative).

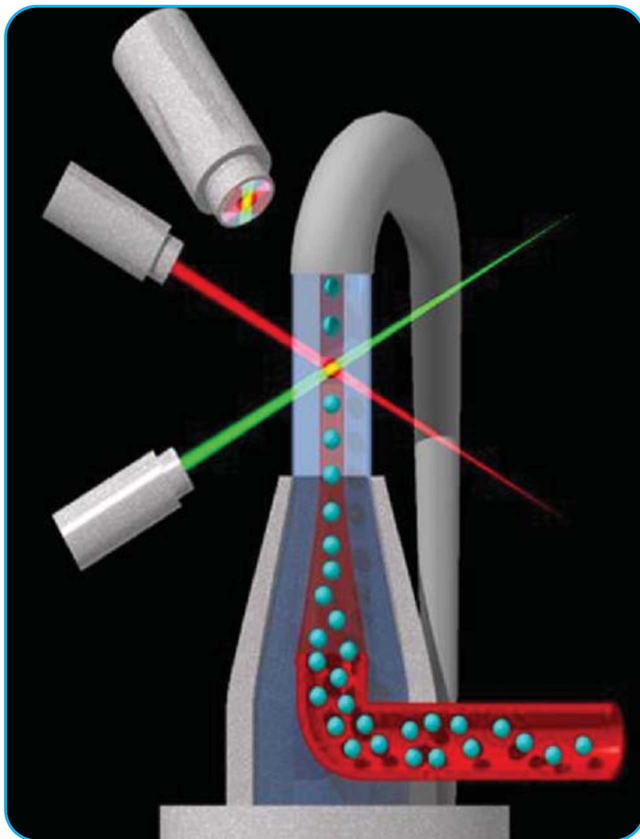


Positive anti-DNA antibodies *Crithidia luciliae*

■ Farr test: This remains the reference method, however, this is only available in a handful of laboratories, as it requires dedicated equipment and certification for radioimmunology. This test detects high-avidity anti-DNAds antibodies and mainly isotype IgG; these antibodies are significantly aggressive towards the kidneys and are associated with severe forms of SLE.
The Farr test is not as insensitive as previously indicated to IgM and gives false positive in patients undergoing biotherapy (anti-TNFα). The main downside is related to the constraints of using radioelements.
d'utilisation des radioéléments.

The Elisa immunoenzymatic methods, FEIA (fluorescence) or flow cytometry (Luminex®)

They highlight antibodies of both low and high avidity. Automatable and computable, they allow large series to be run. However, as with any immunological method, the problem lies in the choice of antigenic source. The three commercialised methods do not use the same antigens (different thresholds), some are more specific and other more sensitive. The choice depends on the organisation and the recruitment used within the laboratory: small or large series? Urgent or not? Targeted recruitment (rheumatology) or a larger scale (internal medicine with infectious diseases: caution, possible interference), which contributes to favouring either sensitivity or specificity.



Luminex® technology

Prognostic interest

Anti-DNAds antibodies are used to monitor lupus patients. An increase in their titre causes concern over a relapse or a flare up of the disease; in contrast, a decrease in the titre indicates successful treatment. Just like anti-nucleosome antibodies, anti-DNAds antibodies are associated with lupus-related nephropathy.

Also the choice of the test to be used is very important: in this context, certain authors prefer the Farr test (prognostic value for relapse and greater sensitivity 80%).

In practice

At Biomnis, since 15 September 2012, we have stopped performing screens for anti-DNAds antibodies by indirect immunofluorescence on C. luciliae. This test, which is specific but not sensitive enough (see Table 1), can be replaced by the FARR test.

Therefore, for first-line testing we recommend screening for native anti-DNA antibodies using the "classic" methods (ELISA, immunofluorescence, EIA etc.), which are robust, quick and sensitive methods, and reserve the Farr test for second-line testing (clinically difficult cases, anti-nuclear antibody/anti-DNAds mismatches). The Farr test (historical reference test for anti-DNAds antibodies) detects high-affinity antibodies, which is very specific for SLE.

Table 1: details on the research methods for anti-DNAds antibodies

	ELISA	*C. luciliae*	Farr test
Sensitivity	+++	+	+++
Specificity	± to +++	+++	+++
Interferences	Numerous (virosis etc.)	Zone phenomenon Anti-histone antibodies	Heparin, Dextran, Polyanions, anti-TNF
Restriction	(-)	(±)	++ (RIA)

Conclusion

Anti-DNAds antibodies are significant markers for SLE: the IgG isotype is of diagnostic utility, is associated with lupus-related nephropathy and have prognostic value. Caution: check the antigenic source used in the tests.

Carole Emile, from a communication made by Pascale Chrétien during the 7th GEAI conference, Paris, June 2012.