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Autoantibodies in systemic lupus erythematosus (SLE)

Autoimmune diseases are characterized by cellular and humoral immune response against self antigens, which causes that in most cases autoreactive B and T lymphocytes are present. They can be divided into two general groups: diseases with organ-specific autoimmunity and those with organ-nonspecific autoimmunity (1–3).

This review will be focused only on SLE, one of the most frequent (beside RA) and severe connective tissue diseases (CTD). SLE belongs to the group of organ nonspecific group diseases of unknown etiology. In SLE the immune response abnormalities, hyperreactivity of B lymphocytes are present. That is why SLE patients' sera include autoantibodies to cellular autoantigens, mostly autoantibodies directed against nuclear antigens (ANA) such as: anti ds and ssDNA antibodies, anti-histones and anti-nucleosome antibodies and anti-ribonucleoprotein (anti-Sm) and also autoantibodies against other nuclear antigens like U_{1sn}RNP, SSA (Ro), SSB (La). Beside all these above mentioned antibodies in SLE we have got a variety of connective tissue protein and non- protein components like e.g. antibodies to different type of collagens, elastin, proteoglycans and other components (3, 4).

Autoantibodies are considered as the main diagnostic tool for CTDs. A characteristic profile of autoantibodies (called "marker autoantibodies") is found in each of the diseases above. Determination of involved markers specificities can be useful in establishing the correct diagnosis and prognosis and enables clinicians to settle the proper treatment as well as observe results of that treatment (2, 5). In case of SLE the presence of positive (elevated) titers of ANA and assessment of two main antibody specificities – anti-dsDNA and anti-Sm are important items during diagnostic process (minor diagnostic criteria) (6–8).

At present time we have at disposal many methods of autoantibody assessment and because of that some insights will also be given into two of the most common techniques used for determination of autoantibodies in the clinical and research laboratory setting: the indirect immunoflurescence (IIF) assay and immunoenzymatic methods (ELISA) and the Western blot (WB) procedure (9). They are useful prognostic markers in some situations and facilitate clinical and treatment follow-up.

It seems to us that the indirect immunofluorescence method (IIF) has been the most powerful, sensitive and comprehensive test for screening of autoantibodies, until immunoenzymatic (EIA) methods (ELISA, Western-blotting) in the late 60s were worked out. The immunoenzymatic tests are very useful because of their simplicity and reliability. But there is one more excellent test named "Colorzyme" (presented by Immuno-Concept Corporation from USA) worked out by combining the EIA and IIF tests.

More and more newly found resources of marker autoantibodies and methods lead to standardization methods and specimens on which they are marked. During properly executed

strategy of ANA assessment usually a multi-stage ("cascade") methodology is used. In the first stage a screening test, usually IIF-ANA or immunoperoxidase "Colorzyme" method based in Hep-2 cells and ELISA-screen are used.

If ANA are positive, the next step is to establish ANA specificity. Eventually in the final step of ANA assessment fine-specificities are assessed if there is a clinical demand. The fourth stage is needed only in case, when we are unable to confirm in the tested serum any typical specificity of ANA and it is usually done by combining biochemical and immunochemical methodology. Strict following of these procedures guarantees that in most of the tested sera specificities are assessed properly. Reproducibility of results lets us avoid mistakes and what is even more important – generates reasonable costs of diagnostics (4, 10).

AUTOANTIBODIES INCLUDED IN THE SLE DIAGNOSTIC CRITERIA – ANTI-DNA AND ANTI-SM ANTIBODIES

Anti-DNA antibodies are a common epiphenomenon in the sera of patients with connective tissue diseases (CTD-s), because they occur not only in most of the SLE sera, but also in the sera of patients with necrotizing processes (11).

Anti-DNA antibodies are composed of two general populations, first directed against really native DNA (double stranded DNA – anti-dsDNA) and the other (second) targeting single-strand DNA (ssDNA, synonym denatured DNA). The epitopes for both anti-dsDNA and ssDNA pools are located in different parts of the DNA molecule, because anti-ssDNA antibodies recognize purine and pyrimidine bases (and nearest to them area), originally not exposed in the native DNA. On the contrary, antibodies to dsDNA recognize rather sugar-phosphate backbone and because of that are able to react with both types of DNA (native and denaturated) and also cross-react with different kinds of phospholipids (e.g. cardiolipin) (4, 12). The nativity of DNA used for test, should be exercised with special caution, especially when contaminating anti-ssDNA antibodies are present in the tested sera, otherwise the possibility of false positive results may alter the clinical status of diagnosis (4, 12).

Another diagnostic obstacle may be generated under occurrence of circulating immune complexes (CIC) composed of DNA/anti-DNA. It is a common problem because in the SLE patients' sera exist DNA released from nonapoptotically dying cells and this can interfere with final result, giving false positive or false negative results – depending on the CIC composition (13).

Anti-dsDNA antibodies occur in about 70–90% and possess high specificity for positive results ranging from 70 to 95% (4). Another important property of the anti-dsDNA antibodies is that they tend to fluctuate (correlate) with disease activity (2, 3). Also, of major importance is the fact that only this autoantibody pool is involved in the pathogenetic processes occurring in SLE, by deposition in kidney of formalin fluid phase CIC-containing extracellular DNA and anti-DNA antibodies (13). Finally, we should pay attention to the fact, that in the CTD-s sera a pool of antibodies includes atypical forms of DNA-Z-DNA (14, 15), but their clinical relevance is not definitely established and still constitutes a topic in the area of anti-DNA antibodies researches (14).

Antibodies to Sm antigen in the SLE patients were identified by prof. Tan and his co-workers in 1966 (4, 8). Anti Sm antibodies occur in about 15–30% of the SLE cases and are considered as highly specific (higher than 95%) for SLE. They were shown to exhibit a relationship with disease activity independent of fluctuations in dsDNA autoantibody titers (8, 16).

Major targets of Sm autoantibodies are the B (28 kD), B' (29 kD), and D (16 kD) polypeptides, although all core proteins of snRNPs, from A to G, may be recognized (2, 7). Sera positive for anti-Sm also immunoprecipitate U1, U2, U4, U5 and U6 snRNAs (17, 18).

The possibility that anti-Sm positive sera also react and immunoprecipitate with other snRNPs molecules, like U_{1-6} snRNPs, may create problems with the assessment of specificities to particular antigens belonging to this group – especially in case of differentiation of U_1snRNP (solid marker for MCTD) from other snRNPs (2). In the B polypeptide, three different epitopes were described (3), but the D antigen is targeted by two distinct populations of anti-Sm D antibodies, one recognizing only the full length antigen, the other reacting with the carboxyl terminus containing a supercharged structure with homology to Epstein-Barr virus nuclear antigen type 1 (EBNA-1) (4). Clinical correlations are not strong, but involve the central nervous system, kidney disease, lung fibrosis, and pericarditis (4).

ANTIBODIES NOT INCLUDED IN SLE DIAGNOSTIC CRITERIA

Anti-nucleosome antibodies (anti nucleohiston – NuHi) are the most promising group of autoantibodies detected in about 60% of the SLE patients sera (19). Anti-NuHi antibodies are of a special importance especially that in about 10–25% of the SLE patients' sera we cannot assess "marker" anti dsDNA or anti-Sm autoantibodies – which are included in SLE diagnostic criteria (20).

The nucleosome consists of the core particle that contains a tetramer of histones (H2A, H2B, H_3 and H_4) around which the helical DNA is wrapped and histone H1, which is located at the point where DNA enters and exits the nucleosome (19, 21). Some studies have demonstrated the ability of anti-dsDNA and antihistone lupus antibodies to react with certain target epitopes of the nucleosome (22).

Nucleosomes prepared by conventional methodology (i.e. digestion of chromatin only with nuclease S7) give us preparations also reacting with 10–68% sera of patients with scleroderma, because it also contains H1 histone, Scl-70 antigen (degradation product of Topoisomerase I) and other chromatin antigens. In the newly developed ELISA, nucleosomes prepared in the same way were additionally treated with NaCl solution (end concentration 0.55 M) and further purified by sucrose density gradient centrifugation called second generation nucleosomes. This method yielded mononucleosomes which were free of H1, Scl-70, other nonhistone proteins and chromatin fragments free, as verified by gel electrophoresis (SDS-PAGE).

This above mentioned treatment give us the NuHi (second generation nucleosomes) preparations which do not react with sera from scleroderma, polymyositis patients and of course, with healthy blood donors sera (21, 23). This second generation NuHi based ELISA demonstrated high specificity (> 95%) for SLE and were comparable with anti-dsDNA antibodies/ sensitivity in about 60% (23).

So far, the number of publications concerning the clinical importance of anti-NuHi has not been impressive, especially in Polish-language publications (22), but it rises systematically. The measurement of anti-chromatin antibodies appears to be a useful additional test that can help in the diagnosis and treatment of SLE. These antibodies are both highly sensitive and specific for SLE, and are a useful marker for an increased risk of *lupus nephritis*.

Recently it was suggested that the nucleosome is the principal antigen in the pathophysiology of SLE, and that anti-nucleosome antibodies are associated with pathomechanisms which cause an organic damage (2, 4).

Beside the above mentioned, nucleosomes autoantibodies have to be taken under consideration because this group of autoantibodies gives us the most common ANA pattern in SLE – homogeneous-speckled, especially in not so rare cases, when basic "marker" autoantibody is absent. A list of these autoantibodies is also presented in Table 1.

In this group of autoantibodies we included antibodies to: ribosomal protein P, proliferating cell nuclear antigen (PCNA), Ku antigen, high mobility group protein (HMG) and also ubiquitin (4, 24).

The average frequencies of appearance of these antibodies are: 10–15% for anti-rib-P antibodies, > 5% for anti-PCNA, 1–16% for anti-Ku, above 30% (up to 70%) for anti-HMG and 20–30% for anti-ubiquitin antibodies (2, 3). Unfortunately, with exception of anti-rib-P and anti-PCNA antibodies, which hold relatively high specificity for SLE, other antibodies also appear in other CTDs and possess significantly lower specificity for SLE (10). From the newly published data (10, 20, 25), we know that these specificities are responsible for the substantial part of ANA positive sera, in which the search for particular specificity was unsuccessful.

Table 1. Autoantibodies groups in SLE and their clinical correlations (4)

Autoantibody		ANA Hep-2 IIF "pattern"	Sensitivity	Specificity*	Clinical correlations and other comments
Marker antibodies	anti-dsDNA	homogeneous	> 60–90%	> 95%	disease activity, *lupus nephritis*
	anti-Sm	speckled	15–30%	> 95%	kidney and CNS involvement
Auxiliary antibodies	anti nucleosomes /anti-NuHi/	homogeneous	~ 60%	> 95%	disease activity, *lupus nephritis*
	anti-ribosomal protein P	cytoplasmic	10%	> 95%	kidney involvement neuro-psychiatric syndromes
	anti-PCNA	speckled (mitotic cells)	3%	> 95%	kidney and CNS involvement, TCP
	anti-Ku	homogeneous	1–16%	low	idiopathic pulmonary hypertension
	anti-HMG	homogeneous	4–11%	low	unknown
	anti-ubiquitin	unknown	20–30%	low	unknown
Other antibodies	anti-ssDNA	ANA-negative	~ 70%	low	necrotisation marker
	anti-U_1sn-RNP	speckled	~ 30%	low	MCTD – marker
	anti-SSA/SSB /anti-Ro/La/	fine speckled	30–60%	low	Sjögren syndrome markers
	anti-histones	homogeneous	~ 50%	low	DIL marker
	anti-phospholipids**	unknown	30–40%	low	APS marker
	anti-neuronal antigens	unknown	unknown (rather low)	low	SLE with CNS involvement

* For SLE cases

** Together anti-phospholipids, LAC and anti-cofactors antibodies

A separate and a largely complex group are forming anti-phospholipid antibodies (anti-PLs) composed of anti-particular phospholipid antibodies (e.g. anti-cardiolipin antibodies), so-called lupus anti-coagulant (LAC) and anti-cofactor antibodies, which are present in about 30–40% of the SLE patients' sera, and have low specificity in SLE cases (12).

Anticardiolipin antibodies (ACA), lupus anticoagulant (LAC) and anti-cofactor antibodies are part of a larger group of antibodies that strongly associates with thrombosis, recurrent fetal loss and thrombocytopenia. The antiphospholipid antibody syndrome (APS) may occur in the presence of systemic *lupus erythematosus* (SLE) and related autoimmune disease; alternatively the so-called primary antiphospholipids syndrome (PAPS) also occurs in the absence of an autoimmune disorder.

In connection with problems concerning the direct pathogenic rule of aPLs mechanisms involved in generation of neuro-psychiatric syndromes in SLE, a new group of antibodies is taken under consideration – the group of anti-neuronal antibodies, but the clinical significance of anti-neuronal antibodies needs further studies.

Finally, it is our duty to admit that in the sera of SLE patients there may also exist "marker" antibodies to other CTDs, like e.g. antibodies to U_1snRNP (up to 30%), histons (50%), Ro (La) (40–60%) and to other antigens belonging to ENA group (like Scl-70, Jo-I) (2–4), but this situation may be only a manifestation of coexistence of more than one CTD (overlap syndrome) in a single patient. These antibodies should be taken carefully under consideration whenever characteristic clinical manifestations occur (2–4).

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SUMMARY

In the presented review the authors discuss the diagnostic and clinical importance of the "marker" autoantibodies included in the SLE diagnostic criteria (ANA, anti-dsDNA and anti-Sm antibodies) and the so-called "auxiliary" autoantibodies (for example antibodies to nucleosomes ribosomal protein P, PCNA, Ku, HMG and ubiquitin) – not included in the diagnostic criteria, but possessing a relatively high "specificity" (but not necessarily high sensitivity). Additionally their correlation with clinical manifestation, disease activity, treatment and also prognostic value were discussed. Because we usually observe very characteristic profiles of these antibodies in certain diseases (immune response is of a "mosaic" type), assessment of these autoantibodies can be a very useful tool in differential diagnosis of the CTDs, especially in not so rare cases, when basic "marker" autoantibody is absent. We take into consideration only those above-mentioned autoantibodies when kits for assessment are available as a commercial package and for other autoantibodies, when the technology of preparation of antigens and autoantibody assessment is so simple that the whole procedure can be done in a routine way.

Autoprzeciwciała występujące w SLE (TRU)

W pracy przeglądowej autorzy omawiają kliniczne i diagnostyczne znaczenie autoprzeciwciał „markerowych", występujących w SLE (toczeń rumieniowaty układowy), włączonych do kryteriów diagnostycznych TRU oraz tzw. przeciwciał „pomocniczych" (anty-nukleosomy anty-rib P, anty-PCNA, anty-Ku, anty-HMG, anty-ubikwityna), niewłączonych do kryteriów diagnostycznych TRU, ale posiadających względnie wysoką swoistość (choć niekoniecznie wysoką czułość), a także ich korelację z objawami klinicznymi. Ponieważ zwykle obserwujemy bardzo charakterystyczne profile (odpowiedź przeciwciałowa ma charakter mozaikowy) tych przeciwciał w poszczególnych jednostkach chorobowych, dlatego oznaczanie tych autoprzeciwciał może być użytecznym narzędziem

w diagnostyce różnicowej układowych chorób tkanki łącznej, szczególnie w tych przypadkach, gdy brak jest w surowicy podstawowych przeciwciał „markerowych". Przy omawianiu autoprzeciwciał wzięto pod uwagę dostępność na rynku gotowych zestawów do ich oznaczania i/lub stosunkową prostotę preparatyki poszczególnych antygenów, stosowanej w trybie oznaczeń rutynowych.