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LABORATORY STANDARDS IN THE DIAGNOSIS AND THERAPY MONITORING OF SYSTEMIC LUPUS ERYTHEMATOSUS

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9.1 Introduction

Systemic lupus erythematosus is the classic type of polysystemic autoimmune diseases. Lupus arises when the immune system mistakenly produces antibodies that attack the body's own tissues, including the kidneys, skin and brain. The causes of the attack are complex, and the impairment of all the main cell types of immunoregulation, T, B, dendritic cells and phagocytes are involved in this process.

The current criteria of the diagnosis are consisting of both clinical and laboratory parameters, as follows:

- Malarrash (a rash, often butterfly-shaped, over the cheeks)
- Discoid rash (a type involving red raised patches)
- Photosensitivity (reaction to sunlight in which a skin rash arises or worsens)
- Nose or mouth ulcers, typically painless
- Nonerosive arthritis (which does not involve damage to the bones around the joints) in two or more joints
- Inflammation of the lining in the lung or heart (also known as pleuritis or pericarditis)
- Kidney disorder marked by high levels in the urine of protein or of abnormal substances derived from red or white blood cells or kidney tubule cells
- Neurological disorder marked by seizures or psychosis not explained by drugs or metabolic disturbances (such as an electrolyte imbalance)
- Blood disorder characterized by abnormally low concentrations of red or white blood cells or platelets (specifically, hemolytic anemia, leukopenia, lymphopenia or thrombocytopenia) and not caused by medications
- Positive test for antinuclear antibodies not explained by drugs known to trigger their appearance
- Positive test for antibodies against double-stranded DNA, Sm or certain phospholipids or a false positive result on a syphilis test.

9.2 The pathogenesis of SLE

SLE is a multifactorial disease induced by environmental factors in patients with certain

type of genetic and hormonal backgrounds. HLA-A1, HLA-B8, HLA-DR3 genotype and C4A*Q0 complotype are the main factors of genetic predisposition for SLE. It is a fundamental clinical observation that in the induction of SLE the role of one or more provoking factors can be recognized in every case, for example, sunlight, infections (mostly viral infections like Epstein Barr virus), hormonal, mainly oestrogen effects (the gender dependent occurrence of disease: female/male = 9/1), in addition some drugs (hydralazine and procainamid).

Though a great variety of the defects in the various signal transduction and apoptosis pathways has been described in the immunocompetents cells of SLE patients, the main problem is the loss of tolerance toward the autoantigens (the impairment of immunoregulatory T cells) and the abundant production of pathologic autoantibodies leading to tissue and organ destructions all over the body. These antibodies are of IgG type with high affinity to the autoantigens, in contrast to the "physiological" autoantibodies of IgM character and with low affinity to autoantigens and being harmless toward the tissues.

From pathological point of view all autoimmune diseases - included SLE- are special types of inflammations induced by the direct tissue damage elicited by autoreactive cells or antibodies, or by the release of various mediator substances from the white cells accumulated on the damaged area. Therefore, the results of some common laboratory tests can already give information to orient toward the diagnosis of SLE.

9.3. The common laboratory tests of inflammation used in the diagnosis of SLE

Table1. *Changes in the results of common laboratory tests*

Blood cells	
White cell count	☒
Number of neutrophils	☒
Number of lymphocytes	☒
Number of monocytes	☒
Number of erythrocytes (hemoglobin)	☒
Sedimentation of erythrocytes (We)	accelerated
Serology	
IgG, IgA, IgM	
Complement factor 3 (C3)	☒

Complement factor 4 (C4)	☒
Complement activity (CH50)	☒
C reactive protein	
Transferrin	☒
Immunocomplex	
Cryoglobulin	appears in association with vasculitis

9.4 Measurement of circulating cytokines in patients with SLE

The increased activity of CD3+ CD4+ (ic. IL-2, IL-12, IFNγ positive) Th1 "helper" cells results in elevated serum levels of IL-1β, IL-2, IL-12, TNFα and IFN α and IFNγ. On the other hand, IL-4, IL-6, IL-8, IL-10 and IL-13 are the products of CD3+ CD4+ (ic. IL-4, IL-8, IL-13 positive) Th2 "helper" cells. Whereas SLE is a disease with Th1 dominance, the measurement of IL-2 and INFγ may have a diagnostic importance. Cytokine changes measured in a patient with acute SLE treated with a high dose of glucocorticosteroid are presented in the following observation gained in our laboratory:

Table 2.*Changes in serum levels of circulating cytokines in a patient with acute SLE after glycocorticosteroid treatment*

Type of cytokine	**Serum levels (pg/ml)**	
	before steroid	**after steroid**
Interleukin 2	8.15	50.2
Interleukin 4	0.75	9.42
Interleukin 10	30.42	63.55
Interferong	69.8	51.4

9.5 Changes in the peripheral distribution of lymphocytes in SLE

Flow cytometry gives the chance to determine the various subsets of peripheral lymphocytes. In addition to the above mentioned two subsets of T "helper" cells (Th1 and Th2), it is worth measuring the CD8+ "cytotoxic" , CD19+ "antibody producing" B and CD56+ "natural killer" cells. CD3+HLA-DR+ lymphocytes represent the "late" type,

whereas the CD3+CD69+ cells the "early" types of "activated" T cells. The three forms of regulatory T lymphocytes (Treg cells) are the following:

a) CD3+CD4+(ic. TGFβ positive) Th3 cells,
b) CD3+CD4+ (ic. IL-10 positive) Tr1 cells,
c) CD3+CD25+ (Foxp3 positive) suppressor T cells.

Table 3. *Changes in lymphocyte subsets*

Lymphocyte subsets	**Change**
$CD3^+$ T cell	☒
$CD4^+$ T cell (helper cell)	☒
$CD8^+$ T cell (cytotoxic cell)	
$CD56^+$ (natural killer cell)	
$CD3^+$ $HLADR^+$ ("late" activated T cell)	
$CD3^+$ $CD69^+$ ("early" activated T cell)	
$CD3^+$ $CD4^+$ (ic. IL-2, IFN g^+) Th1 T cell	
$CD3^+$ $CD4^+$ (ic. IL-4, IL-6^+) Th2 T cell	☒
$CD3^+$ $CD4^+$ (ic. TGF b^+) Th3 T cell	(in fibrosis)
$CD3^+$ $CD4^+$ (ic. IL-10^+) Tr1 T cell	
$CD4^+$ $CD25^+$ suppressor regulative T cell	☒

9.6 The types and occurrence of pathological autoantibodies in SLE

The serological verification of the various "marker" autoantibodies of SLE is the most important task of the laboratory for the diagnosis of SLE. This is a fundamental part of work proving the "autoimmune" background of the disease, furthermore, giving the chance to monitor the efficacy of therapy and to demonstrate the potential coexistence of an other autoimmune disease in a form of "overlap syndrome". We use to say that for the laboratory diagnosis of SLE it is crucial to verify one of the "marker" antibodies in the patient at least once.

The "antinuclear autoantibodies (ANA)" and "antiphospholipid autoantibodies" are forming the majority of "marker" antibodies of SLE.

Antinuclear autoantibodies (ANA or "antinuclear factor" ANF)

Antigens: double stranded (ds) DNA,
extractable nuclear antigens, Sm (U1 RNP)
nucleosome (chromatine): mixture of DNA + histone (H2A, H2B, H3, H4) molecules
nucleoplasma and nucleomatrix antigens : SS-A, SS-B.

Laboratory detection:
IF, ELISA, immunoblotting.

Antiphospholipid autoantibodies

Antigens: cardiolipin
β2 -glycoprotein I
lupus anticoagulans

Laboratory detection:
ELISA: RIA for cardiolipin and β2 glycoprotein I (IgG and IgM antibodies)
APTI measurement for the detection of lupus anticoagulans (prolongation).

9.7 Occurrence (per cent) of the most important autoantibodies in the sera of SLE patients

1.	**Anti-double stranded DNA (anti-dsDNA)*	60-70 %
2.	Anti-histone (H1, H2A, H2B, H3, H4)	40-60
3.	**Anti- nucleosome (anti- chromatine)*	85-90
4.	**Anti-Sm*	30-35
6.	Anti-U1RNP	20-25
7.	Anti-SS-A	40-50
8.	Anti-SS-B	20-25
9.	PCNA	1-5
10.	Anti-ribosomal P protein	12-16
11.	Rf	1-5
12.	**Antiphospholipid (anti-cardiolipin and anti-b2 glycoprotein I)*	40-50
13.	ANCA	

14. Anti-erythrocytes

15. Anti-thrombocytes

16. Anti-endothelium

17. Anti-C1q

**Marker antibodies of the disease*

9.8 Additional useful laboratory measurements for the diagnosis of SLE

Serological tests:

a. Complement measurements (C3, C4, CH50 - decrease)
b. C3a and C5a (increase)
b. Acut phase proteins (CRP - increases)
c. Circulating immunocomplexes (increase)
d. DNase activity (decreases)

9.9 Interpretation of pathological laboratory data in SLE

a) Pathological laboratory results supporting the diagnosis:

Serological tests:
- Anti-ds - DNA
- Anti- Sm
- Anti-nucleosome (anti-chromatine)
- Anti- phospholipid IgG (mainly anti-β2 glycoporotein I)
- Decrease in C3, C4, CH50
- Increase in C3a and C5a
- Increase in CRP level and We (erythorocyte sedimentation)

Cellular tests:
- Leukopenia
- Lymphopenia
- Thrombocytopenia
- Decrease in the number of CD4+CD25+ suppressor T cells
- Decreased activity and production of superoxide anions by phagocytes

LE cell positivity (atypic granulocytes phagocytosing apoptotic nucleic fragments)

Urinary tests:
- Proteinuria
- Cylindruria

b) Pathologic laboratory results reflecting the activity of SLE

Serological tests:
- High level of anti-ds-DNA
- Hypocomlementaemia
- Increase in the level of C5a
- Increase in the level of circulating immunocomplexes
- Increased erythrocyte sedimentation

Cellular tests:
- High degree of leuko/lymphopenia
- Great decrease in the number of CD4+CD25+ suppressor T cells

c) Laboratory results reflecting successful therapy of SLE

Serological tests:
- Decrease in the level of anti-ds-DNA
- Increase in the level of C3, C4 and in the value of CH50
- Decrease in the level of C5a
- Decrease in the level of circulating immunocomplexes
- Decrease in erythrocyte sedimentation

Cellular tests:
- Increase in the number of leukocytes/lymphocytes
- Increase in the number of CD4+ CD25+ suppressor T cells

d) Antibodies reflecting subtypes of SLE or associations with other autoimmune diseases (overlaps)

- anti-SS-A: association with Sj□gren syndrome (or cardiac block in neonates)
- anti-histone: drug induced SLE
- anti-phospholipid: antiphospholipid syndrome
- p-ANCA/ anti-C1q: lupus nephritis
- anti-ribosome P protein: SLE with psychiatric syndromes

cryoglobulin: SLE with vasculitis

9.10 Autoantibodies in "healthy" subjects

The specificity and sensitivity of laboratory tests applied for autoantibody determinations is strongly depend on the dilution of sera used for the measurements. It is recommended that all antibody determinations (especially the immunofluorescence measurements) should be concurrently carried out in the serum dilutions of 1:40 and 1:160. It also has to be mentioned that after infections some antimicrobial antibodies can crossreact with the autoantigens of nuclear type used in the in vitro assays of autoantibody determinations. These are positive reactions from laboratory point of

view, but totally irrelevant from the clinical aspect of autoimmune diseases. Therefore, the close contact between the clinicians and laboratory specialists is very important in the common interpretation of clinical and laboratorial data.

9.11 The current principles of successful therapy of SLE

- Diagnosis in the earliest time (optimal co-operation between clinics and laboratory).
- Early starting of the most effective immunosuppression in order to prevent the occurrence of irreversible damages in the various organs.

List of abbreviations

ANA = antinuclear antibodies; ANCA = antineutrophil cytoplasmic antigen; ANF = antinuclear factor = ANA; APTI = activated partial thromboplastin time; CD = cluster defined; CRP = C reactive protein; DNA = deoxyribonucleic acid; DNase = deoxyribonuclease; ELISA = enzyme linked immunusorbent assay; HLA = human leukocyte antigen; ic = intracytoplasmic; IF = indirect immunofluorescence; IFNγ = interferon γ; IL = interleukin; PCNA = proliferating cell nuclear antigen; RIA = radioimmunoassay; Rf = rheumatoid factor; SLE = systemic lupus erythematosus; TGFβ = transforming growth factor β

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