CONTINUING MEDICAL EDUCATION

ADVANCES IN LABORATORY DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology characterised by involvement of multiple organ systems and manifesting with protean clinical features. [1-3] The clinical presentation and course of SLE are extremely variable. Some patients have spontaneous remissions, others have mild skin and joint involvement that responds favourably to conservative measures, whereas in a small percentage it is fatal - death resulting from progressive multisystem disease unresponsive to high dose corticosteroids and cytotoxic drugs. The systemic manifestations in SLE overshadow the cutaneous lesions and usually precede the cutaneous lesions and only a quarter of SLE patients present with prominent cutaneous lesions at the onset of their disease; however, approximately 80% of patients eventually develop cutaneous lesions during the course of their disease. [4]

Pathogenesis

Immunopathogenesis[5]

There is compelling evidence to suggest that the immune system plays a pivotal role in the etiopathogenesis of SLE. Immune dysregulation involving stem cells, T and B cells, natural killer (NK) cells and the reticuloendothelial (RES) system as well as the cytokine abnormalities, can explain many of the findings in SLE. [6]

Stem cells In lupus-prone mice, there is a suggestion that either the stem cells are phenotypically abnormal or have a genetic ability to induce autoimmunity. The bone marrow stem cells also have a greater propensity to form B cell colonies and the B cells in turn produce more antibodies. In humans, stem cells abnormalities have not yet been delineated.

T cell The main abnormality in T cells is their failure to suppress antibody production. They also provide excess help to auto-antibody producing B cells.

B cells There is an increase in the number and / or activity of B cells at all stages of lymphocyte maturation. These autoreactive B cells are quite similar to conventional B cells.

Natural Killer cells In SLE patients, NK cells are reduced not only in numbers but are abnormal in their function. This impairment may be due to their insensitivity to cytokines or alteration of their function due to binding of their Fc receptors to excessive circulating immune complexes. The dysregulation of NK cell function allows for an uncontrolled expansion of autoantibody producing B cell clones, amplifying inflammation.

Reticuloendothelial system The clearance of circulatory immune complexes in SLE is limited due

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to defective splenic Fc receptor mediated and hepatic C3b receptor mediated clearance of immune complexes. Whether these defects are secondary to saturation of the RES by the excess of immune complexes or due to primary RES is not clear .

Cytokines

A number of cytokine abnormalities have been noted in SLE. These include decreased levels of IL-1, lower production and response to several cytokines(IL-6, IL-4, B-cell growth factor, IL-5) and also formation of autoantibodies during active disease. Serum -souble IL-2 rceptors are elevated and are a sensitive predictor of disease activity.

Role of antibodies in SLE Antibodies mediate inflammation and injury in SLE through two mechanisms.

a) ***Direct pathogenic effect*** They may be responsible for the cutaneous and cardiac lesions.

b) Immune complexes Deposition of immune complexes in various tissues results in inflammation through complement activation, leucocyte chemotaxis and the release of tissue mediators of inflammation leading to vascular injury. However, the reason for different patients have differences in visceral involvement is uncertain.

Genetic predisposition [7]

There is enough evidence to suggest that patients with SLE may have a genetic predisposition. This contention is supported by twin studies and familial aggregation of SLE cases. SLE is commoner in some ethnic races and with the C4AQO allele.[8,9]

Other factors

Ultraviolet rays (UVR) may precipitate the onset or exacerbate the course of SLE in upto 60% of patient.[10] The mechanism of action of UVR in SLE remains unknown, though antibodies to UVR-denatured DNA have been demonstrated. The precipitation of SLE by drugs especially hydrallazine is well documented.[11] The features of drug induced SLE, however, differ from the spontaneous disease: it is uncommon in Blacks, occurs at an older age group, renal and neurological involvement is rare, anti-histone antibodies are frequent, anti-DNA antibodies are absent and serum complement levels are normal.[11]Other factors implicated in precipitation of SLE are bacterial and viral infections and mental and physical stress. At present, SLE is thought to be a disorder in which genetic defects are present but yet not delineated. These defects lead to defective homeostasis between B and T cells, so that when the individual is challenged by ultraviolet rays, or infection or by an unknown stimulus, the B cells become hyperactive leading to a variety of autoantibodies.

Laboratory investigations

Laboratory investigations are necessary to confirrn the diagnosis of SLE although occasionally even after extensive investigations one may not reach a final diagnosis. Laboratory findings should always be correlated with clinical findings in order to come to a final diagnosis (Table-1).[12]

Extensive investigations are also required to find the extent of systemic involvement. Finally some tests are required in the follow-up to monitor response to therapy.

Table I: The 1982 ARA criteria for diagnosis of systemic lupus erythematosus

1.Malar rash	Fixed erythema, flat or raised, over the malar eminences
2.Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur
3.Photosensitivity	
4.Oral ulcers	Includes oral and nasopharyngeal, lesions observed by physicians.
5.Arthritis	Nonerosive arthritis involving two or more peripheral joints characterized by tenderness, swelling or effusion.
6.Serositis	Pleuritis or pericarditis documented by ECG or rub or evidence of pericardial effusion.
7.Renal disorder	Proteinuria greater than 0.5 g/d or greater than 3+or cellular casts
8.Neurologic disorder	Seizures without other cause or psychosis without other cause
9.Haematologic disorder	Haemolytic anemia or leukopenia less than 4000/ul or thrombocytopenia (less than 100,000ul) in the absence of offending drugs.
10. Immunologic disorder	Positive LE cell preparation or anti-dsDNA or antiSm antibodies or false-positive VDRL.
11. Antinuclear antibodies	An abnormal titer or ANAs by immunofluorescence or an equivalent assay at point in time in the absence of drugs known to induce ANAs.

Laboratory findings in SLE can be broadly categorised into,

A) Haematologic alterations

B) Coagulation parameter alterations

C) Serologic findings

D) Immunologic findings

E) System specific findings

Haematologic alterations

Haematologic changes in the form of anemia, leukopenia and thrombocytopenia are frequently encountered in patients with SLE.

a) Anemia Anemia is seen in about 80% of patients with active disease and could be due to deficiency of iron, haemolysis or renal dysfunction. The serum iron levels are usually low and rise after corticosteroid therapy.[13]

Haemolytic anemia occurs due to binding of the autoantibodies to proteins on the membranes of erythrocytes, This protein, which may also be present on membranes of lymphocytes, platelets, glomerular cells and neural cells is called lupus associated membrane protein (LAMP).[14,15] Reaction of the autoantibody with antigen is associated with complement fixation and haemolysis. The presentation and course of immuno-haemolytic anemia is variable. In its mildest form, which occurs in 15% of patients with SLE, the only evidence of auto-haemolysis is a positive direct Coombs test.[16] Anemia secondary to renal failure is seen in SLE patients with concomitant severe renal involvement and is due to erythropoietin deficiency. Pure red cell aplasia has also been reported in SLE.[17]

b) Leukopenia Leukopenia is present in about 33% of the patients though occasionally leucocytosis has been reported. The leucopenia is due to the presence of anti-phospholipids and also due to the presence of antibodies to the lymphocytes and polymorphs.[18] The absolute lymphocyte count is reduced to less than 1500/ml due to the presence of cold reactive lymphocytotoxic autoantibodies which in combination with complement produce lymphopenia (predominantly of T cells).[19]

c) Thrombocytopenia Platelet count is less than 100,000/ml in about 20% of patients and is due to immune complex mediated destruction of platelets. Thrombocytopenia may appear only during exacerbation of SLE or be a mild persisting feature.[20] In patients presenting with thrombocytopenic purpura the platelet count is usually less than 40,000.

d) Erythrocyte sedimentation rate (ESR) ESR is raised at some time in nearly 90% of patients, though some patients have a normal ESR throughout the course of the illness.

Serological findings

Presence of anticardiolipin antibodies is responsible for the false positive serological tests for syphilis (VDRL, RPR and FTA-Abs) in 25% of patients.[21] An atypical beaded pattern of fluorescence of the *Treponema pallidum* antigen is seen with sera from certain patients of SLE in the FTA-Abs test.[22]

Immunological findings

The presence of high titres of the autoantibodies accompanied by evidence of complement activation is the most striking abnormality identified in patients with SLE.[3] Several antibodies have been identified in SLE, of which antinuclear antibodies are the best screening tests.

Antinuclear antibodies (ANA)[24]. ANA are autoantibodies reactive with nuclear antigens and are found in over 95% of patients with SLE. Although ANA is a very sensitive test, it has a low specificity making it a useful screening test. Titres upto 1 in 32,00 have been reported but the titres may not bear any relation to the activity, progress or duration of the disease. A high titre (>1 in 64) of ANA in a patient with signs and symptoms of a multi-system disorder suggests the possibility of SLE or systemic sclerosis and most certainly excludes polyarteritis nodosa or cutaneous vasculitis. Patients apparently in good health found to have a high titre of ANA should be followed up carefully for several years as there is a considerable likelihood of them developing LE or systemic sclerosis. A low titre (<1 in 16) in the absence of any clinical symptoms and signs can be ignored.[23]

ANAs are best detected by indirect immunofluorescence technique using a variety of substrates (rat liver, mouse kidney, HEp 2 cells, *Crithidia lucilia).* The test is performed by reacting the serum of the patient with the frozen sections of animal tissue and staining with fluorescein conjugated antihuman 1g G. ANA titres, which provide a semi-quantitative measure of the level of antibody in the serum, are usually lower if an animal organ (rat liver, mouse kidney) is used as a substrate compared with cell culture lines (HEp 2- a human laryngeal cell line). Using rat liver, four staining patterns can be demonstrated; these may be helpful in defining clinical subsets of SLE [24] though more than one pattern can be demonstrated in a single individual.

Table II : Pattern of antinuclear antibodies

Pattern	Antigen	Clinical significance
Homogenous	Ribonucleoprotein Histones DNA	Commonest SLE, other connective tissue diseases.
Speckled	Extractable nuclear protein RNA polymerase	SLE, Rowel's syndrome, systemic sclerosis
Peripheral	DNA	Specific for active SLE Association with nephritis
Nucleolar	DNA topisomerase RNA Polymerase Fibrillin	Systemic sclerosis

a) ***Homogenous pattern*** In this pattern the nuclei stain uniformally. The autoantibodies specifically associated with this pattern are antibodies to histones and deoxy-ribonueleoprotein and sometimes to the native ds DNA. The homogenous ANA (which is the same as the LE cell factor) is the commonest form of ANA found. It is more sensitive than the LE cell test.

b) Speckled pattern

This shows minute points of fluorescence scattered all over the nucleus; the antigens asso-

ciated with this pattern are the extractable nuclear antigens - Sm, U, RNP, Scl 70, SS-A/Ro, SS-B/La and some unspecified antigens,

c) Peripheral pattern or membranous pattern In this, the staining occurs at the periphery of the nucleus. Sera containing anti-DNA antibodies give rise to this pattern. The peripheral pattern, in high titre, is present in 50% of patients with active phase of the disease and is infrequent in other diseases.[26] The so-called shrunken peripheral pattern is regarded as a poor prognostic sign because of high incidence of associated renal disease. [26] It may appear 10-15 days before an exacerbation of the disease and is associated with a fall in serum complement.

d) Nuelcolar pattern Shows uniform staining of each nucleolus. It is often associated with less intense staining of the rest of the nucleus. This pattern is commonly seen in patients with systemic sclerosis,

e) Other patterns[27] If HEp 2 cells are used as a substrate further patterns have been identified; these include the centromere pattern (associated with CREST syndrome and in 6% of patients with SLE) fine and coarse speckled pattern and a ground glass appearance (produced by Scl 70 antibody in systemic sclerosis). There are several patterns of nucleolar staining homogenous, speckled and clumpy. The full clinical implications of these patterns have not been delineated.

Antibodies to DNA

Circulating antibodies to DNA (ss-DNA and ds DNA)are almost always present in active disease, [28] and may occur in the absence of antinuclear factors.[29] Elevated levels of antibodies to ds DNA are highly specific for SLE (98%) and may fluctuate with disease activity. However, they are present in only 60% of patients with SLE. These antibodies may have a pathogenic role and are frequently associated with nephritis. They are useful in monitoring disease activity (though this should not be the only criterion) and response to therapy.[30] Antibodies to ssDNA are found frequently in a variety of inflammatory and autoimmune diseases and have little diagnostic specificity.

The currently used methods for detecting and quantifying anti ssDNA and anti dsDNA are radioimmunoassay, Elisa and immunofluorescence. Immunoflurosence with *Crithidia luciliae*- is used for detecting anti dsDNA antibodies only.[5] The demonstration of anti dsDNA by Farr technique is the most specific aid to diagnosis. Radio-labelled DNA is incubated with test serum and the DNA-anti DNA complexes are precipitated by 50% ammonium sulphate. Comparison of the radioactivity in the supernatant and precipitate gives the DNA-binding activity. Values above 30% are abnormal. The level is raised in 83% of patients with SLE and in 100% of those with active disease. A rise in the index may precede an exacerbation of the disease and levels fall with remission.[31] A high binding capacity is associated with poor prognosis and renal disorder. [24] Normal indices are found in drug induced LE and in other connective tissue disorders with ANA positivity. The peripheral staining pattern of anti nuclear antibody (which is due to anti DNA antibodies) does not correlate with anti-DNA antibodies or with disease activity. [32]

Antibodies to non histones [5]

Several antibodies have been demonstrated to non- histone moieties of the cell

Antibodies to Smith (Sm) and ribonucleo protein antigen. The term extractable nuclear (ENA) in-

cludes antibodies to Sm and nuclear ribonucleoprotein (nRNP) antigens - two non- histone antigens. These antigens are a heterogeneous group, comprising of 5 distinct RNPs that associate with proteins; these particles are recognised by specific autoantibodies in some SLE patients. Autoantibodies to the UI RNP particle are termed as anti UI RNP antibodies. These heterogenous antibodies can be detected by immuno-diffusion methods (Ouchterlony double diffusion gels using the appropriate antigen extracts and reference serum counter-immunofluorescence).

Antibodies to UI RNP are found in 40% of SLE patients especially in those with Raynauds disease and myositis, or in SLE patients with overlapping features of scleroderma and polymyositis. Typically patients with anti UI RNP antibodies do not have anti dsDNA antibodies and have mild disease with infrequent renal and CNS involvement. The titres may correlate with disease activity.[33,34] Antibodies to Sm antigen are found in 10-30% of SLE patients and are highly specific for SLE and occur particularly in patients with renal, and CNS disease and vasculitis.

SSA/ Ro and SSB La autoantibodies Another pair of small nRNPs (SSA/R, SSB/La) cooperate with RNA polymerase III during transcription. These antigens were initially defined as cytoplasmic in orign in patients with SLE and Sjogren's syndrome, but later it was noted that these antigens vary in expression at different stages of the cell cycle and can be localised either in the cytoplasm or the nucleus. Ro and La antigens are identical to SSA and SSB antigens respectively. Production of auto-antibodies to these antigens is linked to HLA_4 - B_8 and DRW3 loci.

Anti SSA/Ro and anti SSB/La are found in 25-40% and 10% of SLE patients respectively. Antibodies to SSA/Ro can occur without antibodies to SSB/La but when antibodies occur to SSB/La, they are always associated with antibodies to SSA/Ro. These patients, (with combination of antibodies) have less frequent and less severe renal disease, than those with anti SSA/Ro alone. The increased renal involvement in patients with anti SS-A /Ro alone may be a reflection of the concomitant presence of anti-DNA antibodies associated with this autoantibody profile. Anti SSA/Ro positive SLE patients often have photosensitivity, prominent sicca symptoms, a positive rheumatoid factor and hyper gammaglobulinemia.[25,35] Anti SSA/Ro antibodies are also found in those patients who have Sjogren's syndrome alone, in about 3% of normal individuals, relatives of patients with autoimmune diseases and patients with SLE but who are ANA negative. Anti SSA/Ro antibodies are markers for neonatal lupus[36] and are also found in subacute cutaneous LE[37] A very characteristic appearance of Anti SSB/La is in patients with erythema multiforme and LE (Rowell's syndrome). In addi-

Table III : Antibodies in patients with SLE

Antibody	Incidence (%)	Antigen detected	Clinical significance
Anti-DNA	70	ds DNA	Specific for SLE. High titres associated with nephritis and activity.
Anti-Sm	10-30	UI-5RNA	Specific for SLE especially nephritis, CNS disease and vasculitis;
Anti RNP	40	UI RNA	With polymyositis, lupus, scleroderma and mixed connective tissue disease
Anti Ro(SS-A)	30	YI-y5 RNA	Sjogrens syndrome, Subacute LE, ANA Negative lupus, neonatal SLE
Anti La(SS-B)	10	Phosphoprotein	Always associated with anti Ro; associated with Shogren's syndrome; mild SLE, less risk of nephritis.
Antihistones	70%	Histones	Drug induced LE (95%) Less in spontaneous LE
Antiribosomal P	20%	Ribsomal protein	?Cerebral lupus

tion these patients of Rowell's syndrome have a speckled pattern of ANA and are positive for rheumatoid factor[38].

Antibodies to ribosomal P-protein Autoantibodies that react with the P-protein of ribosomes are largely limited to patients with lupus (5-10%). The previously emphasised clinical association of these antibodies with severe depression or lupus psychosis is now being questioned[39].

Other antibodies Antibodies to the various components of blood have been found in lupus patients. The commonest manifestations are leukopenia, Coombs' positive haemolytic anemia and idiopathic thrombocytopenia.

Antibody to proliferating cell nuclear antigen is specific for SLE and not found in other connective tissue disorders. It is, however, present only in a minority of SLE patients (5%). Patients with this autoantibody may have a higher incidence of diffuse proliferative glomerulonephritis.[40]

LE cell Test[41], now superseded by more sensitive and specific tests for LE, is positive in over 80% of patients of SLE. When present in large numbers are highly suggestive of SLE; a positive LE cell is also a feature of drug induced LE[41]. LE cells are also sometimes demonstrated in chronic DLE, systemic sclerosis and rheumatoid arthritis.[42]

LE cells are granulocytes which have ingested nuclear material from degenerative cells - this nuclear material stains basophilic. Sometimes there are extracellular masses of nuclear material with a rosette of leucocytes. This phenomenon occurs due to the presence of LE factor which is an antibody ot deoxyribonucleoprotein.

Cryoglobulins are found in 11% of patients. Cryoglobulinemia may precede the manifestation of SLE by many years[43]. Cold agglutinins are seen in about 6% of the patients.

Complement It has been observed that estimation of CH50, C3, C4 and circulating immune complexes are rarely helpful in assessing disease activity[44] though in some cases a decrease in complement levels may precede clinical evidence of exacerbation with return to normal levels on remission. This is especially true for levels of classical pathway proteins C1q, C2 and C4; levels of C3 are less frequently abnormal and a reduction of C3 is often an indication of severe disease especially of renal disease.[45]

Newer complement assays, which better reflect the disease activity, have been devised. Measurement of levels of C3d and Ba[46], iC3b[47], C1 inh-C1s, C1r complexes[48], C3a[50]and neoantigens of the membrane attack complex[51] reflect disease activity.[52] Increased expression of complement receptor, CR3, on neutrophils has been reported to correlate with disease activity. However, even with these newer approaches no single assay of complement activity provides anything beyond an approximate correlation with disease activity.

Inherited deficiencies of the major complement components occur with SLE.[46] These include deficiencies of C1, C2, C4 as well as C5-C9, the commonest being a homozygous C2 deficiency. One third of the patients with C2 deficiency manifest with SLE but these patients show a low incidence of renal disease, low titres of anti-DNA antibodies and infrequent lupus band test.[53] Deficiency of C1 esterase inhibitor and C1q have also been reported to manifest with features of SLE.[53]

Coagulation parameters

Lupus anticoagulant (LA)[54] is an acquired immunoglobulin which reacts with the phospholipid fraction of platelets resulting in thrombosis in 14% of SLE patients. Presence of LA is recognised by prolongation of partial thromboplastin time and failure of added normal plasma to correct this defect. More sensitive tests include the Russel viper venom time and the rabbit brain neutral phospholipid tests. Antibodies to cardiolipin are detected by Elisa. Infrequently antibodies to clotting factors (VIII and IX) can be seen.

Organ specific findings

1. Skin. [3,35] The pathological changes of SLE have been well described. There is no single diagnostic pathological feature in the skin, but a combination of features aid the diagnosis. Early lesions of SLE may show only non-specific changes. In well developed lesions there may be hyperkeratosis without parakeratosis and keratotic plugging of the hair follicles. Liquefaction degeneration of the basal-cell layer is common. The dermal tissues are edematous and sometimes vesicle formation occurs at the dermoepidermal junction with dilation of superficial vessels and perivascular lymphocytic infiltration but changes in blood vessels are infrequent. Fibrinoid deposits are frequently seen. These consist of precipitation of fibrin in the ground substance and appear as granular strongly eosinophilic, PAS-positive diastase resistant deposits between collagen bundles, in the walls of dermal vessels, in the papillary dermis or beneath the epidermis in the basement membrane zone. Subcutaneous fat is frequently involved; there may be focal mucin deposition associated with a predominantly lymphocytic infiltrate. Adipocytes may be separated by edema and fibrinoid deposits.

Using monoclonal antibodies the infiltrate is shown to consist of abundant T-cells and Ia positive cells with rather fewer B-cells and macrophages. Helper/inducer T cells and suppressor/cytotoxic T cells occur in equal numbers.[56]

Immunohistology [3,55] In about 80% patients of SLE patients immunoglobulins, especially IgG but less frequently IgM and IgA, together with complement (C1,C3) have been demonstrated at the dermo-epidermal junction in a continuous granular band. Deposits are invariably seen in the lesional skin but are less so in early and late lesions. If IgG, IgM and IgA are all present the dignosis is SLE; if IG and IgM are present diagnosis of SLE is likely. In three quarters of the cases the lupus band (usually IgG or IgM) test is also positive from non-lesional exposed skin and in half the patients from the unexposed normal skin. The presence of IgG lupus band (but not IgM) in unexposed normal skin rarely occurs in other diseases, being diagnostic of SLE and indicates a poor prognosis.

Electron microscopy (EM) EM examination of the cutaneous lesions of SLE shows marked changes in the basal cells and lamina densa. The basal cells show vacuolisation of their cytoplasm progressing to necrosis. The Civatte bodies show finely filamentous to amorphous granular appearance.

The antigen-antibody complexes have been localised by immunoelectron microscopy to be beneath the lamina densa. They appear as irregular aggregates in the uppermost portion of the dermis, in the ground substance and occasionally on the collagen fibrils. A small amount of the immunoreactants may also be seen within the lamina lucida and lamina densa.

2 Kidneys [57,58] With active nephritis, urinalysis shows

proteinuria, haematuria and cellular and granular casts. Urinary protein excretion measured over 24 hours increases during periods of activity. Many patients of SLE eventually develop nephrotic syndrome which is reflected as persistent massive proteinuria. Renal function tests (blood urea, serum creatinine)also require careful monitoring.

Renal biopsy should be considered when results of renal histopathology would affect therapy because recent prospective studies have cast a doubt on the value of routine renal biopsy. There is also evidence that renal biopsy is less reliable as an indicator of renal involvement than is proteinuria, though histological evidence of nephritis can occur in the absence of proteinuria or microscopic changes and with normal renal functions. Even with diffuse proliferative glomerulonephritis 25% of the patients have a normal renal function and urinalysis. Glomerulonephritis in SLE is caused by deposition of circulating immune complexes or *in situ* immune complex formation in the mesangium and glomerular basement membrane. Information regarding location of immune deposits, histologic pattern of renal damage and activity and chronicity of lesions are all useful in predicting prognosis and selecting appropriate treatment. In mild GN, unlikely to lead to renal failure, immunoglobulin deposits are confined to the mesangium and histologically there is no mesangial proliferation. If the immune complexes are deposited in the capillary basement membrane, prognosis worsens.

3. Cardiopulmonary system Electrocardiography and x-ray would be able to detect most of the complications of cardiac lupus. Valvular insufficiency, aortic or mitral, which is a rare complication of Libman Sach endocarditis,however, need to be confirmed by an echocardiogram.[59]

Radiological examination of the lungs is an essential part of the work up of an SLE patient and the findings are dependent on the stage of disease. [60] The commonest manifestations are pleural thickening and pleural effusion. Involvement of lungs is less frequent and is shown mainly as fleeting infiltrates, and/or areas of plate-like atelactasis. However, the most common cause of pulmonary infiltrates in patients with SLE is infection - this should be ruled out. Interstitial fibrosis can follow pneumonitis. [61] There is a high incidence of anti-Ro antibodies in patients with lupus pneumonitis.[62] Pulmonary function tests may be abnormal even in those patients showing no radiological abnormality. Impairment of pulmonary diffusion is more common than reduction in lung volumes. [63]

4. Gastrointestinal system Motility studies have shown impaired contractions of esophagus in one-third of patients especially in patients with Raynaud's phenomenon. The impaired motility can occur in any part of the eosphagus but is particularly common in the upper third. [64]

5. Central nervous system(CNS)[3] Cerebrospinal fluid (CSF) abnormalities occur in about 32% of the patients with neuropsychiatric symptoms. Protein elevations occur frequently while increased cellular counts are occasionally seen. Depressed levels of C4 complement in CSF are found in patients with active CNS involvement.[65] The electroencephalogram [66] and conventional brain scanning and oxygen- 15 brain scanning may be helpful in diagnosis of cerebral LE and abnormalities relate to the clinical progress of the disease. [67] Cranial computerised tomography may reveal areas of infarction and cerebral atrophy while magnetic resonance imaging may show early abnormalities not detected by tomography. [68] Autoantibodies to neuronal antigens are detected in about 20%

of the patients with SLE especially in those with neuropsychiatric symptoms. [69]

6.Musculoskeletal system Electromyographic abnormalities are seen to correlate with motor weakness. Though serum aldolase is frequently raised, serum creatine phosphokinase is usually normal.[70]

If four of these criteria are present at any time during the course of disease, a diagnosis of systemic lupus can be made with 98% specificity and 97% sensitivity.

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