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Systemic lupus erythematosus and its laboratory diagnosis

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is one of the 'mesenchymal' collagen diseases which have several common characteristics.[1] These diseases have an unknown pathogenesis and all have similarity of manifestations. SLE and all the collagen diseases are characterized by fibrinoid degeneration of the collagen fibers, elastic fibers, ground substance and fibroblasts, the exact nature of which is not understood.[2]

The classic cases of SLE are a combination of systemic and skin changes, and are quire easily recognized.. However as more came to be known about this "curious disease and its diagnosis"[3] the more difficult it became to diagnose a patient in the earlier stages of the disease when the patient does not, and indeed may never, show all of the classic manifestations. The development of new diagnostic tests and heightened awareness of physicians have now established that the disease is more common than formerly believed and that it exists in very mild to rapidly fatal forms.[4] The laboratory plays an important role in the diagnosis of this disease and short of a biopsy, not many of the other collagen diseases can be diagnosed as well in the laboratory.[5] Diagnosis is very important, as treatment for each of the collagen diseases varies at the present time.

The purpose of this paper is to examine the history, etiology, and diagnosis of SLE and to summarize the laboratory studies useful in the diagnosis.

HISTORY

1305 - Corrosive ulcers of the skin were termed lupus (wolf) because the disease ate away the part with rapidity or devoured it like a wolf.[6]

1827 - The erythematous variety was termed fluxus sebaceus by Rayer.[6]

1851 - Cazenave named the disease lupus erythematosus which still persists today.[6]

1872 - Kaposi described the acute disease (skin form with fever and systemic "toxic" manifestations).[7]

1895 - Osler described the widespread viseral manifestations.[6]

1917 - Libman described vasdular changes.[6]

1932 - Gross described the hematoxylin bodies.[8]

1941 - Klemperer described the connective tissue changes.[6]

1948 - Hargraves described the LE cell[9] and set into motion tremendous investigation into the mechanism of production and the meaning of the cell.[5]

1950's - Serum abnormalities and other laboratory tests became more important in establishing the diagnosis.[4]

1958 - Dameshek introduced the consideration of autoantibodies and the immunologic aspects.[5]

1960 - Isolation and identification of other antinuclear factors by chemical and immuno-fluorescent techniques.[7]

1964 - Skin test for SLE.[10]

ETIOLOGY

As stated in the introduction, the etiology is unknown; however a number of etiologies have been proposed.

Before the 19th century most of the etiologies for the disorder were superstitious. For the fifty years preceding 1917 when Libman described the vascular changes, the etiology had been assumed to be tuberculous.

Libman's anatomical descriptions began the "collagen" era when the etiology was believed to be "a generalized disturbance of collagen."[11] The discovery of the LE cell phenomenon (1948) provided a break from the morphologic and histopathologic approach to the predominantly immunologic approach to the etiology of the disease which exists today.

The hypothesis was advanced that SLE is a complex autoimmune disturbance "characterized on the one hand by various hematologic disturbances involving red cells, platelets, leukocytes, syphilitic antigen, and blood coagulants; and on the other hand by a more or less generalized involvement of small blood vessels."[11] The various combinations of abnormalities were seen because of the different autoantibodies formed.

Basic to the autoimmune theories is the precept that a homeostatic mechanism develops in early life whereby the developing embryo is exposed to nearly all of its own antigens, and consequently a tolerance to these antigens is developed which persists to adult life.[12]

Dameshek[11] postulated that a few cells or clones are present which may develop one or several antibodies which cause the immunological disturbances which become manifest in the clinical diagnosis. He states these cells arise from mutation, viral or chemical, or have been present from birth (self or maternally derived) with immunologic tolerance for

years. He further postulates a series of gradual piling up of 'multiple immunologic abnormalities' over a one to twenty year period.

Hill[12] considered the syndrome of SLE might arise and self perpetuate itself in three ways:

1) Cells of the R-E system become modified so that they fail to recognize the normal breakdown products of connective tissue as 'self'.

2) Normal breakdown products become changed as a result of action by or attachment to a prosthetic group, and are not recognized as 'self' by the R-E system.

3) An abnormality in the quantity or nature of breakdown products due to some unknown cause might lead to their non-recognition as 'self'.

In recent years, objective evidence of immunological abnormalities in patients has been obtained:

1) identification of many types of autoantibodies

2) a skin reactivity to the patient's own tissue material

3) low levels of circulating complement

4) the presence of immunological abnormalities in relatives of patients with SLE.

The mechanism of cell damage in SLE remains puzzling. Holman[4] states the minimal visible change in cells strongly suggests that influences are at work which alter cell function without altering cell morphology. Identification of these influences would assist in an understanding of the pathogenesis of SLE.

Despite the abundance of autoimmune reactions in SLE often involving crucial cell constituents such as chromosonal and genetic materials, not a single type of tissue damage has been identified which can be blamed

onto these reactions. Indeed, preliminary evidence suggests that in certain circumstances antitissue antibodies, whether autoantibodies or not, may be protective rather than harmful.[13] Until the biological role of autoantibodies and their pathogenic mechanism is established, the term "autoimmune disease" should either be used with qualification or be avoided. The presence of autoantibodies, even if they are regarded as by-products of the pathogenic mechanism, suggests that immunological phenomena might play a role in pathogenesis, but our inadequate understanding of human immunological responses makes it unwise to change the suggestion into an assumption.

Other theories are based on endocrine factors, anaphylactic hypersensitivity, and disturbed protein metabolism. Some investigators also feel that certain drugs, solar radiation, focal infection, or stress may act as trigger or aggravating factors.[2]

Recent genetic studies have added still another important dimension to the complex problem of etiology.[7] It is uncommon for more than one member of a family to be affected; however, more than forty families have been reported in which the disease has been familial, usually with more than one member of the immediate family affected.[14] However the varied relationships makes definition of any genetic pattern impossible at this time and no genetic basis exists as a proven entity.

DEFINITION

SLE is a self-perpetuating, remitting, febrile, inflammatory, noninfectious disease of unknown etiology affecting predominantly the vascular system.[7] It occurs chiefly among younger females. A genetic factor appears to be important in the disease. Certain organs appear

to be more frequently affected, but every organ can be involved. Clinical manifestations vary from patient to patient. The following symptoms predominate: intermittent fever, arthritis, and skin eruptions. Immunologically the disease is characterized by multiplicity of autoimmune phenomena. Antinuclear antibodies, if looked for, have always been found at least once in the course of the disease. Histologic findings consist of vascular changes with fibrinoid deposits and hematoxylin bodies (nuclear material altered by corresponding autoantibodies). These hematoxylin bodies are the most specific substrate of the disease.

DIAGNOSIS

Clinical

The above definition is sufficiently complex to make diagnosis a difficult problem. The essential feature of SLE is a wide variation in clinical expression due to involvement of many different tissues.

The typical patient coming to a physician is a young woman, less than thirty years of age. If the disease is acute in nature she is bedridden, with a high fever, and may have an erythema which extends across the nose and cheeks in a butterfly pattern. The patient appears to be utterly exhausted. She is unable to move without fatigue, and without pain in muscles and joints. She may have oral ulcerations and even without this, a distaste for food. She is unaccountably and severely ill. Despite these findings and probable moderate weight loss the physical examination reveals very little.[15]

Instead of the sudden, explosive onset, there may have been a long period of chronic ill health during which time the patient was not well

enough for normal activities but not sick enough to be bedridden. For months or years the disease may be diagnosed as another disease. The clinical course is characterized by spontaneous remissions and exacerbations. After a remission SLE may reappear in another organ system. Occasionally the clinical characteristics change so dramatically that another disease is diagnosed.

Two-thirds of cases diagnosed at present are considered to evolve gradually, making early diagnosis more difficult. The physician must learn to recognize the variable course of the disease and its more subtle manifestations so that he can make an earlier diagnosis. As long as there is a question as to whether SLE is a distinct disease or whether it is a spectrum of diseases, there will always be 'borderline cases'.

Various manifestations present clinically. The more common will be presented first. The approximate percentages of cases showing each of the manifestations is included.

Arthritis and Arthralgia (50-90%)—Of all the systems involved in SLE, the musculoskeletal is implicated most frequently. This is usually joint involvement and one may obtain a history of vague aches and pains around the joints for many years.[15] Physical examination is usually negative and deformity or severe involvement is rare. Since arthritis is not specifically characteristic of lupus, other disease entities are frequently diagnosed before the SLE is recognized.[16]

Mucocutaneous (50-66%)—The well known 'butterfly rash' is seen in 33-40% of cases.[7] The scarring lesions of discoidal L.E. are sometimes seen in early cases. Temporary diffuse alopecia is reported in some acute cases.[2] Other cutaneous manifestations include Raynaud's phenomonen, purpura, mucous membrane ulcerations, subcutaneous nodules, urticaria, and bullous or other nondescript eruptions.

Respiratory manifestations (10%)--Dyspnea is a frequent complaint and may be a manifestation of any one of the many respiratory processes of this disease, including congestive failure, pulmonary edema, and pleural or pericardial effusion.[6] Pleural effusion is the most common effusion seen.[7]

Renal involvement (60%)--Involvement of the kidneys in the patient with is the most serious complication of the disease. Nephritis and glomerulonephritis are the two most common diagnoses.[15] Proteinuria is less significant than microhematuria or the presence of cellular casts which indicate more severe glomerular damage.[1]

Cardiac changes (50%)--The blood pressure is usually hypotensive. The pulse rate may be 120 per minute or higher. A persistent tachycardia should not be overlooked as it may reflect heart involvement.[3] A gallop rhythm is sometimes present.[2]

Neurological (less than 5%)--Seizures and psychosis without apparent cause are the most common in this group. Idiopathic epilepsy may be present for several years before other manifestations of SLE appear.[3] Peripheral neuritis has also been noted. A significant number of patients are under Psychiatric care long before organic manifestations are noted.[15]

Histopathology

Pathologic changes are seen primarily in the connective tissues[2,16] with the essential changes being:

1) vasculitis of the smaller vessels, the typical arterial lesion being subendothelial fibrinoid necrosis with proliferation of fibroblasts but only slight inflammatory reaction.

2) increase in the deep metachromatic staining of the intracellular ground substance.

3) fibrinoid degeneration of collagen fibers forming homogenous, eosinophilic masses.

4) hematoxylin bodies arising from altered nuclei of mesenchymal origin.

The pathological features of the disease are surprisingly meager for a disease that involves so many organ systems, and at autopsy there may be almost no gross anatomic lesions.

Typical lesions are 'wire-loop' lesions in the kidney, verrucous changes in the endocardium, focal lesions in the myocardium and pericardium and periarterial fibrosis in the spleen. Other findings are not as specifi for SLE (ie pneumonitis).

The lesion of the glomerulus of the kidney consists of thickening of the basement membrane shown to contain gamma globulin on immuno-fluorescent microscopy. This lesion occurs in 60% of patients.[7]

The endocardial change is seen in 40% of patients and is a nonbacterial verrucous endocarditis of the valves and/or endocardium. The myocardial lesions reveal fibrinoid degeneration of interstitial collagen fibers.[17]

The periarterial fibrosis of the spleen is not seen as often but is distinctive when present. It consists of concentric rings of thick collagen fibers with few interstitial fibroblasts.[17]

Laboratory

This section, as outlined in the introduction, will present the laboratory findings that can or might be useful as an adjunct to diagnosis. One author stated that the diagnosis consists of prolonged observation and a detailed laboratory survey.[2] It must, however, always be remembered that the laboratory diagnosis must be supported by the clinical diagnosis.

Despite the multiplicity of signs, sumptoms, and pathologic features found in disseminated lupus, there is yet no single finding which is completely diagnostic. The hematoxylin body and the LE cell rank highest on the scale of near-pathognomonic signs and although they have been reported separately in other diseases, they have been reported in combination only in lupus.[8]

Hemotoxylin bodies--Gross described the hematoxylin bodies in 1932.[8] They are found in 90% of cases of SLE coming to autopsy. They have been reported in a single case of angitis and one case of sclerderma. The bodies appear extracellularly as smudges and amorphous masses staining avidly with purple hematoxylin of H & E stain. The hematoxylin bodies are swollen cell nuclei devoid of cytoplasm and coated with gamma globulin.

LE Cells--The LE cell phenomenon remains of the greatest importance, and is highly significant if properly evaluated.[7] Altered, phagocytized nuclear material, derived from leukocytes forms the basis of the characteristic LE cell, which is a polymorphonuclear leukocyte largely filled with this homogeneous material, appearing much like ground glass and staining red-purple with Wright's stain. The phenomenon depends on affinity of a specific component of serum gamma globulin (LE factor) for cell nuclei and nucleoprotein.[18] The LE factor causes nucleolysis and homogenization of nuclei of some of the white cells. This material then is phagocytized by viable leukocytes. The total loss of chromatic structure and the Feulgen reaction of the inclusion body serve to differentiate it from other similar substances. There are a number of standardized techniques for doing the test on peripheral blood.

The test represents a method of demonstrating the LE factor in serum; the LE cell (final product) acting as an indicator of the reaction.

LE cell formation is largely an in vitro, and not an in vivo, phenomenon. 'Extracellular material' may be found surrounded by clusters of PMN's called 'rosettes', or as free globules. This extracellular material in itself has no diagnostic significance and may occur in preparations in the absence of LE cells. The test is positive only if two or more typical LE cells are seen.[8] The correlation between the number of LE cells seen and the activity of the disease is poor.[5] One must also remember that a negative result does not exclude the disease.

A positive test is found in 70-100% (80%) in various series. LE cells have been reported in cases of polyarteritis nodosa, rheumatoid arthritis, dermatomyositis, leukemia, pernicious anemia, Hodgkin's disease, miliary tuberculosis, dermatitis herpetiformis, pemphigus, hepatitis, liver cirrhosis, hemolytic anemia, peripheral vascular disease, cardiac failure, and following the ingestion of various drugs (eg. penicillin, hydralazine, phenylbutazone, hydantoin).[12] However, impressive as this list is, the actual incidence of false positives is very low, and indeed, rare. Some authors have postulated that some of the methods used to prepare LE cells are at fault in that there is more trauma to the leukocytes, making more nuclei available and causing an increased incidence of false positives.[8] Others state that some other substance, resembling the LE cell substance, is phagocytized.[16] These cells are the 'tart' cells.

Antinuclear antibodies—There would appear to be a group of circulating antibodies directed against various constituents of the nucleus of patients with SLE and, to a lesser degree, in patients with other connective tissue disorders.[4]

Several methods have been devised for the detection of antinuclear antibodies, and several antinuclear antibodies are known to exist. The

methods used are:

1) direct observation of structural alteration of nuclei as in the LE cell test.

2) identification of gamma globulin on cell nuclei by Coon's fluorescent antibody technique or Coomb's gamma globulin consumption method.

3) agglutination by serum of inert particles (latex or tanned rbc's) coated with various nuclear constituents, such as nucleoprotein or DNA.

4) direct identification of an antigen-antibody reaction by immunologic techniques of complement fixation, precipitation, and passive cutaneous anaphylaxis.[19]

The Fluorescent Antinuclear Test (FAT) is now used quite extensively in the diagnosis of SLE and varies in sensitivity and specificity depending on the laboratory and technical details.

The LE cell indicates the first of these antinuclear antibodies which is a serum factor against nucleoprotein (DNA and histone). This factor reacts with all cell nuclei irrespective of organ or species, the only requirement is that it contain DNA and histone.[7] When the fluorescent antibody technique is used a <u>homogeneous</u> staining of nuclei is seen.[21] With this technique a few cases of other connective tissue disorders have demonstrated this antibody but almost all the cases are so advanced as to be easily differentiated clinically.

A second antinuclear antibody reacts with soluble proteins of the nucleus and is detected by complement fixation. In the FAT the nuclei have a <u>speckled</u> staining.[21] This antibody is also found in other connective tissue disorders, but differs from the LE serum factor in that it is not reactive with all nuclei (eg. frog nuclei).

A third is an anti-DNA antibody and can be identified by various techniques. It is usually not identified if the LE cell antibody is present, and therefore becomes useful in identifying some of the cases in which the typical antibody is not found. In the FAT the nuclei are seen to have a 'shaggy nuclear staining.[21] This antibody is found in only a minority of patients with SLE.

A fourth antinuclear antibody is one against nuclear histone, and is detected in about 10% of SLE cases by complement fixation. It too is usually not identified if the typical LE cell antibody is present.[20]

A fifth appears possibly to be an anti-RNA antibody. In the FAT there is staining only of the nucleoli.[21]

Most investigators believe these antinuclear antibodies do not play a primary pathogenic role, but that they arise from a disturbed immunologic mechanism, the nature of which is not clearly understood. Available evidence suggests that these autoantibodies are not directly harmful to normal tissues in vivo. They pass through the placenta but have not been shown to harm the infant. In tissue culture they do not appear to be damaging to cells. Despite their presence before death they are not found localized on or within most cells at postmortem.[4]

Serum protein abnormalities--Serum protein changes are usually present in SLE. Hyperglobulinemia with tendency toward reversal of the A/G ratio is the usual abnormality seen.[12] Electrophoresis shows a decrease in the albumin (less than 3.5 gms. in one-half of cases), a slight increase in the alpha$_2$ globulins, and a quite large increase in the gamma globulin fraction (greater than 3.0 gms. in one-half of cases). An increase in fibrinogen has been reported in a few cases.[7] These protein changes are not specific and are frequently seen in rheumatoid arthritis. The hypergammaglobulinemia, however, is useful in overall laboratory appraisal.[16]

Protein changes are dependant on the severity of SLE, and whether or not protein-losing nephropathy is present. Thus, if nephritis is present, one may find a lower TSP and albumin, and a higher alpha$_2$ globulin with a smaller increase of the gamma globulins. This may be partially accountable by the proteinuria itself.[2]

There are several other non-specific abnormalities involving the serum proteins.[3,16,19]

1) The sedimentation rate is increased in 98% of patients and is believed secondary to the presence of abnormal serum globulins and increased fibrinogen.

2) A positive cephalin-cholesterol flocculation and thymol turbidity test is seen in 75% of cases, which reflects serum protein abnormalities in a non-specific fashion.

3) A precipitate formation with the addition of p-toluene sulfonic acid to undiluted serum, which any patient with disordered serum proteins or hypergammaglobulinemia may show.

4) False-positive biologic reaction for syphilis occurs in SLE in the absence of clinical evidence of syphilis and negative reaction to the newer tests which employ the specific antigen rather than the non-specific cardiolipin.

5) Cryoproteins are found in the plasma of over one-half of patients studied and seem to be related to hypergammaglobulinemia. They are found more frequently in patients with renal disease.

6) The C-reactive proteins may be reduced in SLE and makes this disease different from periarteritis nodosa, rheumatic fever, and rheumatoid artheritis which show an increased C-reactive protein during periods of disease activity.

Hematologic abnormalities--Almost all SLE patients have one or more hematologic abnormalities at some time in the course of the disease. Several are associated with abnormal circulating substances, such as the gamma globulin which causes a positive Coombs reaction, and another gamma globulin which reacts with thromboplastin or thrombin to prolong clotting time.[4]

Anemia. Eighty percent have a normocytic, normochromic anemia of moderate degree. This anemia improves with therapy for SLE. Its etiology is obscure. An acquired hemolytic anemia with a positive direct Coomb's is symptomatic in 3-10%. This is not due to hypersplenism and the LE factor has not been proven to cause the hemolysis.[12]

Leukopenia. A depressed white count is seen in over one-half of patients, usually in the low normal to subnormal category (4000). This is a leukopenia with relative lymphocytosis. A striking lymphopenia may also occur. The strange, rare phenomenon of 1% lymphocytes in leukopenia makes SLE virtually unique among diseases uncomplicated by drug reactions. In severe cases there is usually an eosinopenia. After steroid therapy is started, the eosinophils may increase, a paradox which seems to be exclusive to SLE.[15]

Thrombocytopenia. The number of blood platelets is diminished below 150,000 in about one-half of all patients, but is below 100,000 in only 5%. A circulating substance against platelets has been proven by experiments. On this basis it is assumed that thrombocytopenia is one of the autoimmune reactions in SLE. However the plasma anti-platelet antibody has not been identified with certainty. The reduced numbers of platelets is usually thought responsible in the cases of purpuric bleeding seen. Splenectomy provides improvement of the

thrombocytopenia in most cases, but without effect on the eventual course of the SLE.[7]

Red cell antibodies. Antibodies against red cell antigens are made readily by SLE patients (sometimes five or six kinds are found in one patient after multiple transfusions.) Transfusion reactions are said to occur more frequently in SLE patients but this is questionable.[19]

Circulating anticoagulant. A circulating anticoagulant, characterized by immediate gross clumping of rbc's, exceedingly rapid ESR, and slow clotting of the supernatant plasma after obtaining the specimen, has been identified. It inhibits the second stage of coagulation (prothrombin to thrombin) and therefore both clotting time and prothrombin time are prolonged.[23] A false positive serologic test for syphilis is more common in SLE patients with an anticoagulant.

Bone marrow. Early in the disease the bone marrow is rich and exhibits a myeloid hyperplasia. An increase in the number of megakaryocytes is also frequent. Late in the disease the marrow may become hypoplastic probably as a consequence of the vascular changes. When the vascular system becomes greatly altered by deposits of fibrinoid material the hematopoietic function of the marrow is impaired.[24] Increased numbers of plasma cells are seen, as well as occasional hematoxylin bodies, and the LE cell phenomenon.

Serum complement--The serum complement is low in most patients with SLE and may provide useful index of the activity of SLE especially the renal component, and thus, a guide to treatment. All four components of complement are reduced.[19]

Rheumatoid factor--More than one-third of SLE patients reveal a positive RF test.[5] If positive these patients usually have more arthritis

and/or peripheral vascular lesions. In one study when only the cold precipitable fraction of the serum was used in patients with a positive test the repeat test with this cold fraction revealed only one positive in thirty cases. This cold fraction test may then offer a way of reducing the number of positive RF tests seen in SLE patients.[19] In another study the serum antinuclear activity in patients with rheumatoid arthritis usually was in the 19S macroglobulins; whereas, this activity in SLE patients was usually in the 7S gamma globulin group. A patient with both the rheumatoid factor and the LE factor has been reported and separation of the factors supported the above data.[25]

Skin test--Recently a skin test for the diagnosis of SLE has been suggested and worked out.[10] The test involves the injection of 0.1 cc intracutaneously of DNA solution (from salmon sperm and calf thymus) with grading of the reaction of the erythema and induration. Most of the patients had positives at eight hours that were gone by 48 hours. in appearance, this response is essentially the same as with tuberculin except for difference in timing. The reaction is not dependant on disease activity or the circulating autoantibodies. In these respects it resembles a delayed hypersensitivity reaction. All patients studied who had SLE had positive reactions. This type of test could make the diagnosis of SLE easier, less expensive, and less time consuming both to the patient and his physician.

Neurological--A peripheral neuropathy with a decreased nerve conduction time is sometimes seen. Elevated CSF protein is usually found in patients with SLE. Electromyography may reveal evidence of myositis.[4]

SUMMARY

This paper presents brief historical considerations of SLE followed by a discussion of possible etiologies of the disease, emphasizing current autoimmune theories.

The clinical diagnosis and the histopathology are then presented to emphasize that the clinician must recognize the multiple ways the disease may present itself and the changing patterns which may evolve.

A synopsis of laboratory abnormalities found in SLE follows, presenting those laboratory tests most helpful to the clinician in the diagnosis. Tests which are still primarily research tools are not discussed.

The L.E. cell phenomenon, if found, remains the standard of diagnosis, and is highly significant if properly evaluated. The LE factor, responsible for this phenomenon, is an antinuclear antibody of the 7S gamma globulin group and can also be identified by fluorescent technique which is becoming a more widely used procedure in the clinical laboratory. Several other antinuclear antibodies are discussed with a brief presentation of their identification and specificity.

Serum protein changes are invariably present in SLE patients. This is usually a hypergammaglobulinemia with reversal of the A/G ratio. Other non-specific abnormalities involving the serum proteins are: an increased sed rate, positive flocculation and turbidity tests, a false positive biologic reaction for syphilis, a cryoprotein, and reduced C-reactive protein.

The more common hematologic abnormalities are a moderate anemia, leukopenia, and/or thrombocytopenia, seen in the majority of cases. A

red cell antibody or a circulating anticoagulant may be identified. The bone marrow picture is variable but may assist diagnosis.

Skin tests appear to have a promising future with many clinical studies underway.

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BIBLIOGRAPHY

1. Holman, H.R.: SLE, J Pediat 56:1099119 (Jan) 1960.

2. Pascher, R.: Lupus Erythematosus, Med Clin N Amer 43:917-928 (May) 1959.

3. Mitchell, J.H.: A Curious Disease and its Diagnosis, Practitioner 194:522-524 (April) 1965.

4. Holman, H.R.: Systemic Lupus Erythematosus, in Immunological Diseases, M. Sampter (ed.), Little, Brown, Co., 1965, pp 737-748.

5. Barton, E.M.: Abnormal Serum Proteins as Aids in Diagnosis of RA and SLE, Med Clin N Amer 43:607-643 (March) 1959.

6. Gold, S.: Progress in the Understanding of LE, Brit J Derm 72:231-239 (June) 1960.

7. Miescher, P.A.; and Riethmuller, D.: Diagnosis and Treatment of SLE, Seminars Hemat 2:1-28 (Jan) 1965.

8. Altrocchi, P.: SLE, J Chron Dis 11:34-49 (Jan) 1960.

9. Hargraves, M.M.; Richmond, H.; and Morton, R.: Presentation of Two Bone Marrow Elements; 'Tart' Cell and 'L.E.' Cell, Proc Staff Meet Mayo Clin 23:25-28 (Jan 21) 1948.

10. Ores, R.O.; and Lange, K.: Skin Test for the Diagnosis of SLE, Amer J Med Sci 248:562-566 (Nov) 1964.

11. Dameshek, W.: What is Systemic Lupus?, AMA Arch Intern Med 106:162-167 (Aug) 1960.

12. Hill, L.C.: SLE, Brit Med J 5046:655-660 (Sept 21), and 6047: 726-732 (Sept 28) 1957.

13. Snell, G.D., et al: Depression by Antibody of the Immune Response to Homografts and its Role in Immunological Enhancement, J Exp Med 112:293-298 (March) 1960.

14. Peterson, R.D.A.; and Good, R.A.: Genetics in Mesenchymal Diseases, Ann Rev Med 14:1-40, 1963.

15. Haserick, J.R.: Modern Concepts of SLE, J Chron Dis 1:317-334 (March) 1955.

16. Shulman, L.E.; and Harvey, A.M.: SLE, DM, May 1956.

17. Baggenstoss, A.H.: Viseral Lesions in SLE, Proc Staff Meet Mayo Clin 27:419-423 (Oct 22) 1952.

18. Holman, H.R.; and Kunkel, H.G.: Affinity Between the LE Serum Factor and Cell Nuclei and Nucleoprotein, Science 126:162-163 (July 26) 1957.

19. Shulman, L.E.: Serologic Abnormalities in SLE, J Chron Dis 16:889-964 (Aug) 1963.

20. Robbins, W.C., et al: Complement Fixation with Cell Nuclei and DNA in LE, Proc Soc Exper Biol Med 96:575-579 (Dec) 1957.

21. Friou, G.F.: Clinical Application of a Test for Lupus Globulin Nucleohistone Interaction Using Fluorescent Antibody, Yale J Biol Med 31:40-47, (Sept) 1958.

22. Fallet, G.H.; Lospalluto, J.; and Ziff, M.: Chromatographic and Electrophoretic Studies of LE Factor, Arth Rheum 1:419-434 (Oct) 1958.

23. Schwartz, S.O.: Systemic Lupus Erythematosus, in Hematology in Practice, McGraw-Hill, 1961, pp 237-243.

24. Burkhardt, R.: The Bone Marrow in SLE, Seminars Hemat 2:29-46 (Jan) 1965.

25. Friedman, I.A., et al: The L.E. Phenomenon in Rheumatoid Arthritis, Ann Intern Med 46:1113-1136 (June) 1957.

Supplementary References

26. Christian, C.C.; Mendez-Brajan, R.; and Larsen, D.L.: Latex Agglutination Test for SLE, Proc Soc Exper Biol Med 98:820-823 (Aug-Sept) 1958.

27. Friou, G.F.: Significance of Lupus-Globulin Nucleoprotein Reaction, Ann Intern Med 49:866-875 (Oct) 1958.

28. Haserick, J.R.; and Bortz, D.W.: Normal Bone Marrow Inclusion Phenomena Induced by Lupus Erythematosus, Plasma J Invest Dermat 13:47-49 (Jan)1949.

29. Kayhoe, D.E.; Nason, J.P.; and Bozicevich, M.A.: Clinical Evaluation of the DNA Bentonite Flocculation Test for SLE, NEJM 263:5-10 (July 7) 1960.

30. Miyasoto, F.; Pollak, V.E.; and Barcelo, R.: Urinary Gamma Globulin Excretion in SLE, Amer J Clin Path 45:541-543 (May) 1966.

31. Moore, J.E.; and Lutz, W.B.: Natural History of SLE: Approach to Study Through Chronic Biological False Positive Reactors, J Chron Dis 1:297-316 (March) 1955.

32. Pollak, V.E.: Antinuclear Antibodies in Families of Patients with SLE, NEJM 271:165-171 (July 23) 1964.

33. Thompson, J.S.: Immunity and SLE, Postgrad Med 37:619-627 (June) 1965.