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(54) **DIAGNOSIS AND MONITORING OF SYSTEMIC LUPUS ERYTHEMATOSUS AND OF SCLERODERMA**

DIAGNOSE UND AUFZEICHNUNG VON SYSTEMISCHEM LUPUS ERYTHEMATOSUS UND VON SKLERODERM

DIAGNOSTIC ET CONTROLE DU LUPUS ERYTHEMATEUX SYSTEMATIQUE ET DE LA SCLERODERMIE

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Description

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] Certain work described herein was supported by Grant No. N01AR92239 between the NIH and University of Pittsburgh. The Government may have certain rights in this invention.

FIELD OF THE INVENTION

[0002] This invention relates to the diagnosis and/or monitoring of patients with systemic lupus erythematosus or scleroderma, including methods for carrying out this activity.

BACKGROUND OF THE INVENTION

[0003] This invention relates to the diagnosis and/or monitoring of patients with systemic lupus erythematosus (SLE) and with systemic sclerosis (scleroderma). The invention also provides means for distinguishing between the two diseases and helps doctors distinguish SLE and scleroderma from other diseases.

[0004] Systemic lupus erythematosus (SLE) or lupus is the prototypic autoimmune disease resulting in multiorgan involvement. This anti-self response is characterized by autoantibodies directed against a variety of nuclear and cytoplasmic cellular components. These autoantibodies bind to their respective antigens, forming immune complexes which circulate and eventually deposit in tissues. This immune complex deposition causes chronic inflammation and tissue damage.

[0005] Diagnosing and monitoring disease activity are both problematic in patients with SLE. Diagnosis is problematic because the spectrum of disease is broad and ranges from subtle or vague symptoms to life threatening multi-organ failure. There are other diseases with multi-system involvement that can be mistaken for systemic lupus, or vice versa. Criteria were developed for the purpose of disease classification in 1971 (Cohen, AS, et al., 1971, Preliminary criteria for the classification of systemic lupus erythematosus. Bull Rheum Dis 21:643-648) and revised in 1982 (Tan, EM, et al., 1982. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arth Rheum 25:1271-1277.) and 1997 (Hochberg, MC. 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arth Rheum 40:1725). These criteria are meant to ensure that patients from different geographic locations are comparable. Of the eleven criteria, the presence of four or more, either serially or simultaneously, is sufficient for classification of a patient as having SLE. Although the criteria serve as useful reminders of those features that distinguish lupus from other related autoimmune diseases, they are unavoidably fallible. Determining the presence or absence of the criteria often requires interpretation. If liberal standards are applied for determining the presence or absence of a sign or symptom, one could easily diagnose a patient as having lupus when in fact they do not. Similarly, the range of clinical manifestations in SLE is much greater than that described by the eleven criteria and each manifestation can vary in the level of activity and severity from one patient to another. To further complicate a difficult diagnosis, symptoms of SLE continually evolve over the course of the disease. New symptoms in previously unaffected organs can develop over time. There is no definitive test for lupus and, thus, it is often misdiagnosed.

[0006] Monitoring disease activity is also problematic in caring for patients with lupus. Lupus progresses in a series of flares, or periods of acute illness, followed by remissions. The symptoms of a flare, which vary considerably between patients and even within the same patient, include malaise, fever, symmetric joint pain, and photosensitivity (development of rashes after brief sun exposure). Other symptoms of lupus include hair loss, ulcers of mucous membranes and inflammation of the lining of the heart and lungs which leads to chest pain. Red blood cells, platelets and white blood cells can be targeted in lupus, resulting in anemia and bleeding problems. More seriously, immune complex deposition and chronic inflammation in the blood vessels can lead to kidney involvement and occasionally failure requiring dialysis or kidney transplantation. Since the blood vessel is a major target of the autoimmune response in lupus, premature strokes and heart disease are not uncommon. Over time, however, these flares can lead to irreversible organ damage. In order to minimize such damage, earlier and more accurate detection of disease flares would not only expedite appropriate treatment, but would reduce the frequency of unnecessary interventions. From an investigative standpoint, the ability to uniformly describe the "extent of inflammation" or activity of disease in individual organ systems or as a general measure is an invaluable research tool. Furthermore, a measure of disease activity can be used as a response variable in a therapeutic trial.

[0007] Two of the most commonly used instruments are the Systemic Lupus Disease Activity Index (SLEDAI) (Bombardier, C., D. D. Gladman, et al. (1992). Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arth Rheum 35: 630-40), and the Systemic Lupus Activity Measure (SLAM) (Liang, M. H., S. A. Socher, et al. (1989). Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. Arth Rheum 32: 1107-18). The SLEDAI includes 24 items representing 9 organ

systems. The variables are obtained by history, physical examination and laboratory assessment. Each item is weighted from 1 to 8 based on the significance of the organ involved. For example, mouth ulcers are scored as 2, while seizures are scored as 8. The laboratory parameters that are included in the SLEDAI include white blood cell count, platelet count, urinalysis, serum C3, C4 and anti-dsDNA. The total maximum score is 105. The SLAM includes 32 items representing 11 organ systems. The items are scored not only as present/absent, but graded on a scale of 1 to 3 based on severity. The total possible score for the SLAM is 86. Both the SLEDAI and the SLAM have been shown to be valid, reliable, and sensitive to change over time (Liang, M. H., S. A. Socher, et al. (1989). Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. Arth Rheum 32:1107-18), and are widely used in research protocols and clinical trials. These indices are particularly useful for examining the value of newly proposed serologic or inflammatory markers of disease activity in SLE.

[0008] Despite the obvious utility of these instruments, there are some drawbacks. First, there is not always complete agreement between the SLAM and the SLEDAI in the same set of patients. There are several possible reasons for these discrepancies. Unlike the SLEDAI, the SLAM includes constitutional symptoms such as fatigue and fever, which may or may not be considered attributable to active SLE; this activity index relies on physician interpretation. In addition, the SLEDAI does not capture mild degrees of activity in some organ systems and does not have descriptors for several types of activity such as hemolytic anemia. For these and other reasons, most studies incorporate more than one measure of disease activity.

[0009] A general review of the state of the art can be found in Ramsey-Goldman, R. and Manzi, S. Systemic Lupus Erythematosus . In: Goldman and Hatch, Ed. Women and Health. Academic Press, San Diego, CA 2000: 704-723.

[0010] Systemic sclerosis (scleroderma) is a chronic disorder of connective tissue characterized by inflammation and fibrosis and by degenerative changes of the blood vessels, skin, gastrointestinal tract, lung, heart and kidney. Scleroderma is a disabling and life-threatening disease. Criteria have been developed for the classification of patients with scleroderma (Masi AT, Rodnan GP, Medsger TA Jr, et al. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arth Rheum 1980; 23:581-590). These criteria are intended for description of large series of patients in research studies and not for diagnosis of individual patients. The major criterion is sclerodermatosus skin changes (thickening of the skin) in any location proximal to the digits. With the addition of any two or three minor criteria [sclerodactyly (skin thickening involving the digits), digital pitting scars, bibasilar pulmonary interstitial fibrosis] the sensitivity for the diagnosis increases. However, nearly 10% of individuals with definite scleroderma do not satisfy these criteria (Medsger TA Jr. Comment on scleroderma criteria cooperative study. In: Black CM, Myers AR, eds. Current Topics in Rheumatology: Systemic Sclerosis. New York : Gower Medical Publishing, 1985:16-17).

[0011] The status of a scleroderma patient or "severity" of his/her disease at a given time represents some combination of irreversible changes or "damage" and potentially reversible changes or "activity." Inflammation, early in the course of disease, leads to fibrosis and scarring later. If one could accurately detect the inflammatory activity, early intervention may prevent future irreversible damage. However, it is often difficult for clinicians to distinguish disease damage from disease activity. In part, this may be because clinical evidence of activity can be extremely subtle. In addition, there is no reliable laboratory marker of inflammation. Cross-sectional and longitudinal assessment of disease damage and activity are essential in evaluating the natural history of disease and in measuring the effectiveness of interventions, both in individual patients and in clinical trials. A review of this disorder can be found in Medsger TA Jr. Systemic sclerosis (scleroderma): clinical aspects. In: Koopman WJ, ed. Arthritis and Allied Conditions. 13th ed. Philadelphia: Lea and Febiger, 1997: 1433-1464.

[0012] The complement system consists of a complex network of more than 30 functionally linked proteins that interact in a highly regulated manner to provide many of the effector functions of humoral immunity and inflammation, thereby serving as the major defense mechanism against bacterial and fungal infections. This system of proteins acts against invasion by foreign organisms via three distinct pathways: the classical pathway (in the presence of antibody) or the alternative pathway (in the absence of antibody) and the lectin pathway. Once activated, the proteins within each pathway form a cascade involving sequential self-assembly into multimolecular complexes that perform various functions intended to eradicate the foreign antigens that initiated the response.

[0013] The classical pathway is usually triggered by an antibody bound to a foreign particle. It consists of several components that are specific to the classical pathway and designated C1, C4, C2, (in that order in the pathway).

[0014] In the classical pathway, the first component Clq is bound to an antigen-antibody complex, activating the pathway. This event is followed by sequential activation of the two serine proteases C1r and C1s. Activated C1s has two substrates, the final two proteins of the classical pathway, namely C4 and C2. Protein C4 is cleaved into C4a and C4b. Protein C2 is cleaved to form C2a and C2b. Fragments C4b and C2a assemble to form C4b2a, which cleaves protein C3 into C3a and C3b, which completes activation of the classical pathway.

[0015] Fragments C4b and C3b are subject to further degradation by Factor I. This factor cleaves C4b to generate C4d and also cleaves C3b, to generate iC3b followed by C3d. Thus, activation of the classical pathway of complement can lead to deposition of a number of fragments, including C4d and iC3b on immune complexes or other activating surfaces. These fragments are ligands for complement receptor type 1 (CR1) on erythrocytes or red blood cells.

[0016] There have been inconsistent reports regarding complement proteins and SLE. One manifestation that has been reported in patients having SLE is a diminished expression of the complement receptor CR1 on erythrocytes [E-CR_1] as compared to normal individuals. This has been reported, for example, by Ross et al., J. Immunol., Vol. 135, p. 2005 (1985), Corvetta et al., J. Rheumatol., Vol. 18, 1021 (1991) and by others. Other studies seem to show there is no correlation. Iida et al., J. Exp. Med. 155,1427 (1982) noted that the CR1 number on erythrocytes varied inversely with disease activity, with lower numbers occurring during periods of most severe manifestations of SLE, and higher numbers being observed during periods of remission in the same patients.

[0017] Tausk et al. (Arthritis and Rheumatism, 1990, vol. 33, No. 6, pages 888-892), Jouvin et al. (Complement, 1986, vol. 3, pages 88-96) and Hammond et al. (Arthritis and Rheumatism, 1989, vol. 32, No. 3, pages 259-264 report that the level of CR1 is decreased in patients with SLE compared to that of patients not having SLE.

[0018] Senaldi et al. disclose in Annals of the Rheumatic Diseases, 1988, vol. 47, pages 913-917, that indices of C4 activation, C4d and C4d/ C4 provide a laboratory measure of disease activity in SLE patients.

[0019] Manzi et al. report in Arthritis and Rheumatism, 1996, vol. 39, No. 7, pages 1178-1188, that serum C4d levels are an indicator for SLE.

[0020] Senaldi et al. report in Arthritis and Rheumatism, 1989, vol. 32, No. 10, pages 1262-1267 that the levels of soluble C4d are higher in patients with systemic sclerosis than in controls groups.

[0021] Marquart et al. disclose in Clin Exp Immunol, 1995, vol. 101, pages 60-65, a flow cytrometic assay to measure expression of complement receptors and regulatory proteins on B cells from SLE patients.

BRIEF SUMMARY OF THE INVENTION

[0022] The invention involves the use of determinations of complement component C4d, and of complement receptor CR1, a receptor present on the surfaces of erythrocytes that acts as a receptor for proteolytic fragments of C4 and C3.

[0023] In one aspect, this invention relates to a method of diagnosing systemic lupus erythematosus or scleroderma in an individual, comprising (a) determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of red blood cells in the sample, and (b) comparing the determination with the quantity of component C4d deposited on surfaces of red blood cells of individuals not having systemic lupus erythematosus or with the quantity of the component known to be present on the surface of red blood cells of individuals not having scleroderma.

[0024] In a second aspect, this invention relates to a method of monitoring disease activity of systemic lupus erythematosus in an individual or of monitoring scleroderma in an individual, comprising (a) determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of the red blood cells in the sample, and (b) comparing the determination with the quantity of component C4d deposited on surfaces of red blood cells previously obtained from the individual.

[0025] In preferred aspects of this invention, the method also includes determining complement receptor CR1 on the surfaces of red blood cells in the sample and comparing that determination with the quantity of CR1 present on the surfaces of red blood cells of patients not having SLE or scleroderma, respectively.

[0026] In other preferred aspects of this invention, the ratio of C4d to CR1 on surfaces of red blood cells is determined and compared with that of patients not having SLE or scleroderma, respectively, or if monitoring of a patient is being conducted, that ratio is compared with such a ratio previously determined for the patient (or for cells previously obtained from the patient).

[0027] In another preferred aspect of this invention, the method also includes determining complement receptor CR1 on the surfaces of red blood cells in the sample, and comparing said determinations with the quantity of CR1 deposited on the surfaces of red blood cells previously obtained from individuals with systemic lupus erythematosus and scleroderma respectively as a method of monitoring disease activity.

[0028] According to a further aspect, the present invention relates to the use of a computer readable medium comprising:

(a) code for receiving data corresponding to a determination of complement component C4d deposited on surfaces of red blood cells;
(b) code for retrieving a reference value for complement component C4d deposited on surfaces of red blood cells of individuals;
(c) code for comparing the data in (a) with the reference value in (b),

wherein the comparison of the data in (a) with the reference value in (b) provides information regarding whether a patient has systemic lupus erythematosus or scleroderma, in a method for diagnosing or monitoring systemic lupus erythematosus or scleroderma in an individual comprising (a) automatically determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of red blood cells in the sample, and (b)

automatically comparing the determination with a reference value for component C4d deposited on surfaces of red blood cells, wherein the codes (a) through (c) are executed on a digital computer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] Figure 1 is a graphical depiction of levels of complement component C4d and complement receptor CR1 on the red blood cells of healthy individuals, i.e. those not having systemic lupus erythematosus, scleroderma or other known diseases. Figure 1a and 1b show the same data depicted using two different Y-axes. C4d and CR1 cutpoints, used to differentiate these healthy controls from patients with SLE, are shown.

[0030] Figure 2 is a graphical depiction of levels of complement component C4d and complement receptor CR1 on the red blood cells of patients diagnosed as having systemic lupus erythematosus. Figure 2a and 2b show the same data depicted using two different Y-axes. C4d and CR1 cutpoints, used to differentiate these SLE patients from healthy controls, are shown.

[0031] Figure 3 is a graphical depiction of levels of complement component C4d and complement receptor CR1 on the red blood cells of patients with diseases other than SLE and scleroderma.

[0032] Figure 4 is a graphical depiction of levels of complement component C4d and complement receptor CR1 on the red blood cells of healthy individuals, i.e. those not having systemic lupus erythematosus, scleroderma or other known diseases. These are the same healthy controls represented in Figure 1. In this Figure, the C4d cutpoint used to distinguish these healthy controls from patients with scleroderma, is shown. A CR1 cutpoint is not required to distinguish these two groups.

[0033] Figure 5 is a graphical depiction of levels of complement component C4d and complement receptor CR1 on the red blood cells of patients diagnosed as having scleroderma. The C4d cutpoint, used to distinguish these scleroderma patients from healthy controls, is shown.

DETAILED DESCRIPTION OF THE INVENTION

General discussion

[0034] The methods of this invention enable the diagnosis and/or monitoring of SLE and scleroderma. Because these two conditions are serious health problems, there is a need for relatively accurate and early diagnosis of these conditions. Likewise, the ability to monitor the activity of these diseases is of great importance.

[0035] The invention involves the use of determinations of complement component C4d and optionally also of complement receptor CR1, a receptor present on the surfaces of erythrocytes that acts as a receptor for proteolytic fragments of C3 and C4.

[0036] In the most general sense, the methods of this invention are based on the discovery by the inventors that a determination of C4d deposited on surfaces of red blood cells of a patient can serve as a diagnostic marker for either SLE or scleroderma. As will be discussed below, a combination of this determination with a determination of CR1 expressed on surfaces of red blood cells of the same patient can aid in distinguishing between SLE and scleroderma as well as helping doctors distinguish between the two diseases and other diseases having similar manifestations.

[0037] In diagnosing the occurrence, or previous occurrence, of either disease, complement component C4d deposited on surfaces of red blood cells in a sample is determined. Complement receptor CR1 on the surfaces of the same red blood cells is preferably also determined. One or both of these determinations is then compared with the quantities of C4d and CR1, respectively, usually found on the surfaces of red blood cells of individuals not having SLE or scleroderma.

[0038] In monitoring disease activity of a patient with either disorder, the same determinations are made in the patient's blood sample, and are then compared with determinations of the quantities of C4d and CR1 present on surfaces of red blood cells in a sample obtained from the same patient in the past.

[0039] In both instances, when speaking of "determination" and "quantity," we mean to include both an absolute amount or quantity of material, as well as (in addition, or alternatively), a ratio of C4d to CR1. As will be discussed below, either or both of these measurements can be used, particularly in diagnosing either SLE or scleroderma in a patient.

General procedures

[0040] The invention involves conducting assays on blood samples obtained from patients to determine C4d and preferably also CR1.

[0041] Samples of blood are obtained from the patient and are treated with EDTA (ethylenediaminetetraacetate) to inhibit complement activation. The samples are maintained at room temperature or under cold conditions. Assays are run preferably within 48 hours.

[0042] The determination of C4d and CR1 may be done by a number of methods including flow cytometry, ELISA

using red blood cell lysates, and radioimmunoassay. In one embodiment of this invention, the determination of the level of C4d and CR1 is made using flow cytometric methods, with measurements taken by direct or indirect immunofluorescence using polyclonal or monoclonal antibodies specific for each of the two molecules. Each of these two molecules can be measured with a separate sample or using a single sample. The mean fluorescence channel (MFC) for erythrocyte CR1 and C4d is determined individually. The same type of assay may be used for diagnosis or for monitoring disease activity in patients known to have SLE or scleroderma.

[0043] Development of an assay of this type for CR1 and for C4d is known in the art and described in Freysdottir, et al., J. Immunol. Meth. vol. 135, 2005 (1991). That assay was a flow cytometric assay for CR1 and for protein fragments C4d and C3d on erythrocytes, and was described as enabling the identification of individuals having comparatively high or comparatively low levels of CR1. However, erythrocyte C4d and C3d were low and often not detectable above background (limits of detection). These authors suggest a possible use of their assay in providing general information regarding immune complex load or clearing in patients. However, their work was limited to developing the assay for general use. They did not investigate the use or application of their work for diagnosing or monitoring the activity of particular diseases.

Kits

[0044] Kits for conducting the assays for both the diagnosing of disease and monitoring of disease activity will use any of the various reagents needed to perform the methods described herein. For example using the immunofluorescence assays, the kits will generally comprise a conjugate of a monoclonal antibody specific for complement component C4d with a fluorescent moiety, and preferably also a conjugate of a monoclonal antibody specific for complement receptor CR1 with a different fluorescent moiety. Additionally, the kits will comprise such other material as may be needed in carrying out assays of this type, for example, buffers, radiolabelled antibodies, colorimeter reagents etc.

[0045] The antibodies for use in these methods and kits are known. Hybridomas secreting Anti-CR1 antibodies are available from the American Type Culture Collection in Maryland (ATCC #HB 8592). A general reference is U.S. Pat 4,672,044. Scripps Clinic and Research Foundation, La Jolla, CA. Anti-C4d antibodies are available from Quidel Corp. in San Diego, California (#A213) and are generally described in Rogers, J., N. Cooper, et al. Complement activation by beta-amyloid in Alzheimer disease. PNAS 89:10016-10020, 1992; Schwab, C. et al.. Neurofibrillary tangles of Guam Parkinson-dementia are associated with reactive microglia and complement proteins. Brain Res 707(2):196 1996; Gemmell, C. A flow cytometric immunoassay to quantify adsorption of complement activation products on artificial surfaces. J Biomed Mater Res 37:474-480, 1997; and, Stoltzner, S.E., et al. Temporal accrual of complement proteins in amyloid plaques in patients with Down's syndrome with Alzheimer's disease. Am J Path 156:489-499, 2000.

[0046] The determination of the C4d and CR1 values may alternatively be conducted using a number of standard measurement techniques such as ELISA. Instead of fluorescent labels, there may be used labels of other types, such as radioactive and colorimetric labels. If such other types of assays are to be used, the kits will comprise monoclonal antibodies specific for C4d and CR1 conjugated with appropriate labels such as radioactive iodine, avidin, biotin or enzymes such as peroxidase.

Diagnostic methods

[0047] Diagnosis of a patient with SLE or scleroderma is carried out by comparing the determination of C4d and preferably also of CR1 with a base value or range of values for the quantities of these entities typically present on the surfaces of red blood cells in normal individuals. In normal individuals, C4d is present in relatively low levels on surfaces of red blood cells. When using flow cytometric measurement with indirect immunofluorescence, the MFC of C4d on red blood cells in healthy individuals ranged from 1.06 to 16.12 (mean 5.7). (Table II and Table VII). The MFC of erythrocyte C4d in patients having SLE was higher than that of healthy individuals and ranged from 2.66 to 155.03 (mean 23.9). (Table III and Table VII). Patients with scleroderma also had elevated levels of C4d as compared to healthy individuals. In patients with scleroderma, the MFC of erythrocyte C4d ranged from 2.86 to 28.89 (mean 11.6). (Table VI and Table V).

[0048] Conversely, as is generally known in the art, the level of CR1 on surfaces of erythrocytes of individuals having SLE was usually lower than in healthy individuals. In the latter, the value of the MFC for erythrocyte CR1 ranged from 10.53 to 50.83 (mean 25.4) (Table II and Table VII), whereas the MFC for erythrocyte CR1 from patients having SLE ranged from 1.41 to 40.89 (mean 12.4). (Table will and Table VII). Patients with scleroderma had MFC values for erythrocyte CR1 (range 4.69 to 38.26, mean 18.4) (Table VI and Table VII) that were lower than healthy individuals but higher than those with SLE.

[0049] A further indication of a diagnosis of SLE is based on the ratio of erythrocyte CR1 to erythrocyte C4d in these assays. More than 93% of patients with SLE had a CR1:C4d ratio less than 3.00, whereas more than 77% of healthy individuals had a ratio of greater than 3.00. Thus, a ratio of CR1:C4d less than 3.00 in an individual is an indication of SLE.

[0050] A further method to distinguish between a diagnosis of SLE and a diagnosis of scleroderma is to compare the

erythrocyte CR1:C4d ratios. Greater than 47% of patients with SLE had a CR1:C4d ratio less than 0.69, whereas only one of 30 patients with scleroderma had a CR1:C4d ratio less than 0.69. Thus, a ratio of CR1:C4d less than 0.69 distinguishes SLE from scleroderma.

Monitoring of patients

[0051] A particular feature of the methods of this invention is the ability to monitor the activity of a patient's disease. The life span of a red blood cell is approximately 120 days. Therefore, a particular feature of this assay or method is to indicate or reflect SLE or scleroderma activity that has occurred in the patient during the preceding several weeks or even several months. It is possible, using this procedure, to identify the occurrence of a flare-up of SLE or scleroderma during the previous few weeks or possibly even the previous several months due to persistence of C4d deposited on the surface of red blood cells. The timing of a previous occurrence may be approximated by separating from the sample the C4d-bearing erythrocytes and ascertaining their age by conventional techniques such as density gradient centrifugation (Rennie, C. M., S. Thompson, et al. (1979). Human erythrocyte fractionation in Percoll density gradients. Clinica Chimica Acta 98: 119-125).

[0052] Also of importance is that the method of this invention is capable of detecting evidence of complement activation in scleroderma, which is a relatively non-inflammatory disease. At the present time, there are no useful circulating markers, or, for that matter, markers of any kind, for measuring disease activity of scleroderma.

Automation and computer software

[0053] The determinations of C4d and CR1 and the diagnostic and disease activity monitoring methods described above can be carried out manually, but often are conveniently carried out using an automated system and/or equipment, in which the blood sample is analyzed automatically to make the necessary determination or determinations, and the comparison with the base or reference value is carried out automatically, using computer software appropriate to that purpose.

[0054] Thus, in one aspect, the invention comprises a method for diagnosing or monitoring systemic lupus erythematosus in an individual comprising (a) automatically determining, in a blood sample from the individual containing red blood cells, complement component C4d and complement receptor CR1 deposited on surfaces of red blood cells in the sample, and (b) automatically comparing said determinations with reference values for component C4d and receptor CR1, respectively, on surfaces of red blood cells. In another aspect this automated method includes one in which the reference values comprise a ratio of C4d:CR1.

[0055] The invention also comprises a method for diagnosing or monitoring scleroderma in an individual comprising (a) automatically determining, in a blood sample from the individual containing red blood cells, complement component C4d and complement receptor CR1 deposited on surfaces of red blood cells in the sample, and (b) automatically comparing said determinations with reference values for component C4d and receptor CR1, respectively, on surfaces of red blood cells. In another aspect this automated method also includes one in which the reference values comprise a ratio of C4d:CR1.

[0056] Another aspect of the invention comprises a method for diagnosing or monitoring systemic lupus erythematosus in an individual comprising (a) automatically determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of the blood cells in the sample, and (b) automatically comparing said determination with a reference value for component C4d deposited on surfaces of red blood cells.

[0057] A further aspect of the invention comprises a method for diagnosing or monitoring scleroderma in an individual comprising (a) automatically determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of red blood cells in the sample, and (b) automatically comparing said determination with a reference value for component C4d deposited on surfaces of red blood cells.

[0058] A further aspect of the invention comprises the use computer-readable media, the computer-readable media including:

(a) code for receiving data corresponding to a determination of complement component C4d deposited on surfaces of red blood cells;

(b) code for retrieving a reference value for complement component C4d deposited on surfaces of red blood cells of individuals; and

(c) code for comparing the data in (a) with the reference value of (b)

wherein the comparison of the data in (a) with the reference value in (b) provides information regarding whether a patient has systemic lupus erythematosus or scleroderma, in a method for diagnosing or monitoring systemic lupus erythematosus or scleroderma in an individual comprising (a) automatically determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of red blood cells in the

sample, and (b) automatically comparing the determination with a reference value for component C4d deposited on surfaces of red blood cells, wherein the codes (a) through (c) are executed on a digital computer;

According to a preferred embodiment of the aforementioned use, the computer-readable media may further comprise:

(d) code for receiving data corresponding to a determination of complement receptor CR1 deposited on surfaces of red blood cells;

(e) code for retrieving a range of reference values for complement receptor CR1 deposited on surfaces of red blood cells of individuals; and

(f) code for comparing the data in (d) with the reference values of (e).

[0059] In embodiments of the invention, one or more reference values may be stored in a memory associated with a digital computer. After data corresponding to a determination of complement C4d is obtained (e.g., from an appropriate analytical instrument), the digital computer may compare the C4d data with one or more appropriate reference values. After this comparison takes place, the digital computer can automatically determine if the data corresponding to the determination of complement C4d is associated with SLE.

[0060] Accordingly, the embodiments of the invention are embodied by computer code that is executed by a digital computer. The digital computer may be a micro, mini or large frame computer using any standard or specialized operating system such as a Windows™ based operating system. The code may be stored on any suitable computer readable media. Examples of computer readable media include magnetic, electronic, or optical disks, tapes, sticks, chips, etc. The code may also be written by those of ordinary skill in the art and in any suitable computer programming language including, C, C++, etc.

[0061] The following examples are provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical parameters which could be changed or modified to yield essentially similar results.

EXAMPLES AND EXPERIMENTAL DATA

[0062] The following examples are provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

Example 1: Assays of C4d and CR1 in Healthy Controls: Minimal Fluctuation Over Time

[0063] Initially, six healthy individuals were studied. Each of these normal controls was studied on at least two separate days, and one individual was studied on six different days over a span of three months. As shown in Table I, C4d was detected at low levels on erythrocytes of each of the six healthy individuals, and the level of C4d detected was remarkably constant over days, weeks, and even months within a given individual. These data demonstrated that C4d could be easily and reliably detected on erythrocytes of any individual, and the consistent levels observed in healthy individuals indicated that even slight fluctuations reflect disease activity. As expected, CR1 was present at much higher levels than C4d on erythrocytes of all normal individuals, yet CR1 levels also demonstrated minimal fluctuation over days, weeks, and even months.

[0064] Samples of 1 mL of EDTA-anticoagulated peripheral blood were taken from each individual and used as a source of red blood cells. The cells were washed and resuspended in FACS buffer. Levels of C4d and CR1 were measured by indirect immunofluorescence using monoclonal antibodies specific for C4d and CR1, respectively, with C4d and CR1 each being measured with a separate sample. Levels of C4d and CR1 are quantitated by flow cytometry using a FACSCalibur cytometer (Becton Dickinson). The red blood cells were identified by forward and side scatter and the mean fluorescence channel (MFC) was determined individually for C4d and CR1 respectively.

[0065] More particularly, blood was collected in 5cc lavender-topped tubes containing EDTA as an anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Whole blood was diluted in phosphate buffered saline containing 1 % bovine calf serum (PBSB). Erythrocytes were pelleted, washed with PBSB, and aliquotted for antibody staining. Monoclonal antibodies (mAb) were added to erythrocytes at a concentration of 10 μg/ml. The cells were incubated for 20 min at 4° C, and washed with cold PBSB + 0.2% sodium azide. A secondary antibody, goat anti-mouse IgG conjugated to fluorescein isothyocyanate (FITC) from Jackson Immunoresearch Laboratories (# 115-096-062) was added to cells at a concentration of 10 μg/ml. Cells were incubated and washed as described above, resuspended in PBSB + 0.2% sodium azide, and analyzed by flow cytometry using a FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA). Nonspecific binding of immunoglobulins to erythrocytes was determined by performing identical assays in parallel using the isotype control antibody MOPC21 (obtained from ATCC). Specific binding of anti-C4d and anti-CR1 were determined by subtracting the MFC obtained with MOPC21 from the MFC obtained with anti-C4d and anti-CR1 respectively. Sibinovic, K.H., M. Potter, L. D'Hoostelaere, B. Rode, J. Wax. 1976, Catalogue of Plasmacytomas and Other Tumors of the Lymphoreticular System, Third Edition, Litton Bionetics, Inc. p33.

Table I. C4d and CR1 on Erythrocytes of Healthy Individuals

Healthy Individual	Date	C4d	CR1
001	071200	9.06	55.12
001	071300	9.47	54.35
001	071400	9.66	53.54
001	072000	9.88	51.61
002	071300	6.91	40.50
002	072000	6.13	47.07
003	071400	8.94	23.06
003	072000	8.68	22.45
004	071400	10.77	53.82
004	072000	10.82	50.91
004	100900	10.06	45.24
005	083000	8.36	32.42
005	090700	10.31	30.49
005	092500	10.06	32.99
005	092700	8.77	28.51
005	100500	8.63	31.02
005	110800	8.88	25.83
006	100900	7.04	36.47
006	102500	6.84	34.20
006	112800	6.85	35.24

Example 2: Assays of CR1 and C4d to Distinguish Patients with SLE from Healthy Controls

[0066] This example describes conducting assays on patients to diagnose systemic lupus erythematosus, and to establish reference values or ranges of values for complement component C4d and complement receptor CR1.

[0067] Pilot studies of serial determinations in normal individuals were followed by studies of patients with SLE. For this purpose, we recruited 86 patients with lupus from our outpatient office. A single determination of erythrocyte CR1 and C4d was made, using the same assay, in 86 individuals who met ACR criteria for the diagnosis of SLE (Table III) and in 35 healthy controls (Table II). The mean and median values of CR1 and C4d for patients with SLE and healthy controls are shown in Table VII. Whereas the mean value for C4d in healthy individuals was 5.7, the mean value for C4d among patients with SLE was 23.9 (p= 0.0001). The mean MFC for CR1 among the 35 healthy individuals was 25.4, whereas the mean MFC for CR1 in the 86 patients with SLE was 12.4 (p=0.0001). Figures 1(a,b) and 2(a,b) represent graphs of single specimen determinations of C4d vs. CR1 in the 35 healthy individuals and in the 86 patients with SLE, respectively.

Table II. Healthy Controls (n=35)

ID	RBC CR1	RBC C4
2001	48.11	8.66
2002	36.85	3.36
2003	19.55	5.43
2004	50.83	5.95
2005	38.39	5.74
2006	28.98	4.92

(continued)

ID	RBC CR1	RBC C4
2007	41.82	6.65
2008	32.86	3.43
2009	22.26	3.11
2010	32.62	5.53
2011	20.28	16.12
2012	18.08	7.86
2013	24.91	5.90
2014	19.99	6.13
2015	29.34	4.46
2016	16.00	4.73
2017	34.83	2.05
2018	10.53	5.49
2019	23.47	4.93
2020	16.57	4.09
2021	25.76	8.52
2022	14.19	3.50
2025	10.86	12.62
2026	16.55	5.89
2027	25.56	4.39
2028	23.81	5.69
2029	21.27	2.81
2030	10.78	4.32
2031	17.89	10.84
2032	28.70	4.94
2033	26.27	1.06
2034	32.08	4.30
2035	12.46	5.64
2036	35.16	5.45
2037	23.31	5.06

Table III. SLE Patients (n=86)

Patient ID	RBC CR1	RBC C4
1001	5.81	25.68
1002	6.98	12.87
1003	7.87	55.28
1004	14.27	155.03
1005	30.60	45.00
1006	9.22	43.76
1007	40.89	13.45
1008	7.18	27.00
1009	14.32	13.66
1010	12.23	33.17
1011	23.93	18.65
1012	12.41	6.16
1013	12.26	20.64
1014	19.18	17.74
1015	1.41	11.61
1016	4.77	16.52
1017	15.87	8.92

(continued)

Patient ID	RBC CR1	RBC C4
1018	14.48	9.78
1019	15.44	10.53
1021	17.11	11.13
1022	12.19	70.62
1023	12.56	22.68
1024	13.75	13.02
1025	5.20	13.65
1026	7.07	6.95
1027	6.40	6.85
1029	11.27	4.14
1030	18.39	5.10
1031	6.29	25.40
1032	5.67	6.53
1033	9.24	9.24
1034	12.45	8.53
1035	35.40	22.65
1036	7.37	31.18
1037	19.09	11.56
1038	7.10	83.70
1039	23.19	22.98
1040	20.26	7.70
1041	18.82	12.02
1042	9.32	16.86
1043	19.68	9.49
1044	23.86	5.46
1045	10.32	12.91
1046	17.47	8.49
1047	8.84	17.76
1048	5.27	19.44
1049	13.53	10.24
1050	19.54	17.51
1051	2.99	13.29
1052	10.59	12.35
1053	13.94	93.05
1054	3.43	88.92
1055	19.19	30.64
1056	26.33	13.39
1057	17.56	9.41
1058	6.43	14.22
1059	5.62	10.26
1060	17.36	5.03
1061	11.79	21.94
1062	12.83	25.85
1063	9.41	12.92
1064	5.59	27.63
1065	11.07	2.73
1066	1.92	31.26
1067	18.96	10.75
1068	7.65	89.64
1071	10.32	3.54

(continued)

Patient ID	RBC CR1	RBC C4
1072	5.57	4.63
1073	6.82	22.05
1074	16.60	2.66
1075	11.66	11.61
1076	5.87	146.43
1077	12.53	14.51
1078	4.22	9.72
1079	10.50	11.85
1080	9.38	27.02
1081	14.03	27.93
1082	6.57	19.94
1083	8.92	6.64
1084	21.93	9.50
1085	7.91	32.65
1086	12.70	13.90
1087	5.43	67.68
1089	5.12	10.63
1090	8.16	34.54
1091	11.96	6.51

[0068] These observations indicated that the combined determinations of erythrocyte CR1 and C4d levels would be a useful diagnostic assay for SLE. Using a CART statistical analysis, we determined the sensitivity and specificity of erythrocyte C4d and CR1 in the diagnosis of SLE using computer-generated cutpoints. Sensitivity is the probability that the assay is positive in a person who has the disease and specificity is the probability that the assay is negative in a person who does not have the disease. These cutpoints are demonstrated in Figures 1 and 2 by the solid lines that divide the graphs into four quadrants. These graphs demonstrate that the "lupus profile" that was most commonly observed was a low CR1 and an elevated C4d. In SLE patients compared to healthy controls, the sensitivity and specificity of these measures were 87% and 91%, respectively (Table VIII). The positive predictive values and negative predictive values were also determined. Positive predictive value (PPV) is the likelihood that a person has the disease if the test is positive. The PPV for SLE vs Healthy Controls was 96%. Negative predictive value (NPV) is the likelihood that a person does not have the disease if the test is negative. The NPV for SLE vs Healthy Controls was 74%. No other currently available assay has such high combined PPV and NPV. These data indicate the utility of erythrocyte CR1 and C4 determinations in the diagnosis of SLE

Example 3: Assays of CR1 and C4d for Distinguishing Patients with SLE from Patients with Other Diseases

[0069] These studies of patients with SLE vs. healthy controls were followed by studies to compare patients with SLE with patients diagnosed with diseases other than SLE (n=111). For these comparison, we studied patients with rheumatoid arthritis (n=15), osteoarthritis (n=2), hepatitis C virus infection (n=17), polymyositis/dermatomyositis (n=33), Wegener's granulomatosis (n=1), Sjogren's syndrome (n=3), sarcoidosis (n=1), urticarial vasculitis (n=1), sickle cell anemia (n=8), overlap syndrome/undifferentiated connective tissue disease (n=15), leukemia/lymphoma (n=9), primary Raynaud's syndrome (n=3), hemophilia (n=2), and psoriatic arthritis (n=1). A single determination of erythrocyte CR1 and C4d was made, using the same assay. The mean and median values of CR1 and C4d for patients with SLE, as compared with patients with other diseases are shown in Table VII. Whereas the mean value for C4d in patients with other diseases was 9.3, the mean value for C4d among patients with SLE was 23.9 (p= 0.0001). The mean MFC for CR1 among the 111 patients with other diseases was 18.3, whereas the mean MFC for CR1 in the 86 patients with SLE was 12.4 q) =0.0001). Figure 3 is a graph of single specimen determinations of C4d vs. CR1 in the 111 patients with other diseases. In SLE patients compared to patients with other diseases, the sensitivity and specificity of these measures were 87% and 89%, respectively (Table VIII). The PPV and NPV were 86% and 90%, respectively. No other currently available test has such high combined probability.

Example 4: Assays of CR1 and C4d for Measuring Disease Activity in Patients with SLE

[0070] We then examined the utility of erythrocyte C4d and CR1 levels in measuring disease activity as defined by the Systemic Lupus Activity measure (SLAM). We present the results of the first 86 lupus patients entered into the study. Using a univariate linear regression model, erythrocyte CR1 (p=0.0007) was the only significant predictor of SLAM (Table IV). Serum C3, C4 and anti-dsDNA antibody were not significantly associated with disease activity. In multivariate models, controlling for all variables, erythrocyte CR1 was independently associated with SLAM (p=0.001) (Table V). Association of anti-ds DNA antibodies with disease activity did not reach statistical significance in this multivariate analysis. These data show that erythrocyte C4d and CR1 determinations provide valuable clinically useful information that is not obtained with traditional "gold standard" measurements of serum C3, serum C4, and anti-dsDNA antibody.

Table IV. Association with SLE DiseaseActivity Univariate Analysis

Variable	P-value
E-CR1	0.0007
E-C4	0.6
Serum C3	0.93
Serum C4	0.81
anti-dsDNA Ab	0.15

Table V. Association with SLE Disease Activity Multivariate Analysis

Variable	P-value
E-CR1	0.001
anti-dsDNA Ab	0.07

Example 5: Use of C4d Determination for the Diagnosis of Scleroderma.

[0071] The same methodology was used to test a sample of 30 scleroderma patients (Table VI) in comparison with the same 35 healthy individuals (Table II). Whereas the mean value for C4d in healthy individuals was 5.7, the mean value for C4d among patients with scleroderma was 11.6 (p= 0.0001, Table VII). The mean MFC for CR1 among the 35 normal controls was 25.4, whereas the mean MFC for CR1 in the 30 patients with scleroderma was 18.4 (p = 0.0001, Table VII). Figure 4 is a graph of single specimen determinations of C4d vs. CR1 in 35 healthy individuals. Figure 5 is a graph of single specimen determinations of C4d vs. CR1 in the 30 patients with scleroderma.

Table VI. Scleroderma Patients (n=30)

Patient ID	**RBC CRI**	**RBC C4**
3001	38.26	16.77
3002	10.97	9.71
3003	28.77	15.33
3004	17.85	7.96
3006	23.95	22.12
3008	16.26	8.41
3009	18.92	19.95
3010	15.12	10.80
3011	27.05	6.37
3012	32.42	10.74
3013	9.62	8.46
3014	10.03	12.87
3017	19.69	8.51
3019	13.93	5.38
3020	13.79	8.25
3021	26.40	7.82
3022	13.67	5.26

(continued)

Patient ID	RBC CRI	RBC C4
3023	10.06	2.86
3024	20.02	28.89
3025	14.32	19.30
3026	4.69	12.73
3027	20.06	9.98
3028	25.37	6.15
3029	15.3	6.66
3030	19.2	8.00
3031	21.28	26.12
3032	11.80	13.70
3034	11.13	5.78
3035	24.19	17.00
3036	19.3	5.84

Table VII. Analysis of RBC-CR1 and RBC-C4

		RBC-CRI			RBC-C4d		
		Mean	Standard Deviation	Median	Mean	Standard Deviation	Median
SLE	(n=86)	12.4	7.2	11.5	23.9	27.9	13.6
Scleroderma	(n=30)	18.4	7.5	18.4	11.6	6.5	9.1
Healthy Controls	(n=35)	25.4	10.2	23.8	5.7	2.9	5.4
Other Diseases	(n=111)	18.3	7.3	17.3	9.3	6.8	8.0

RBC CR1 and C4 are not normally distributed in any of the comparisons. Therefore, Wilcoxon rank sum test was used.

Comparison -	CR1 (p values)	C4 (p values)
SLE vs Healthy Controls	0.0001	0.0001
SLE vs Other Diseases	0.0001	0.0001
SLE vs Scleroderma	0.0001	0.004
Scleroderma vs Healthy Controls	0.004	0.0001

[0072] Using a CART statistical analysis, we determined the sensitivity and specificity of erythrocyte C4d and CR1 in the diagnosis of scleroderma using computer-generated cutpoints. In scleroderma patients compared to healthy controls, the sensitivity and specificity of these measures was 83% and 80%, respectively (Table VIII) The positive predictive values and negative predictive values were also determined. The PPV for distinguishing patients with scleroderma from healthy controls was 78%. The NPV for distinguishing patients with scleroderma from healthy controls was 85%. There is no single laboratory test currently used with equivalent sensitivity and specificity, PPV, and NPV for scleroderma.

Table VIII. CART Analysis of RBC-CRI and RBC-C4d

Comparison	Sensitivity	Specificity	PPV	NPV
SLE vs. Healthy Control	.87	.91	.96	.74
SLE vs. Other Diseases	.87	.89	.86	.90
Scleroderma vs. Healthy Controls	.83	.80	.78	.85

Claims

1. A method of diagnosing systemic lupus erythematosus or scleroderma in an individual, comprising (a) determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of red blood cells in the sample, and (b) comparing said determination with the quantity of component C4d deposited on surfaces of red blood cells of individuals not having systemic lupus erythematosus or with the quantity of said component known to be present on the surface of red blood cells of individuals not having scleroderma.

2. A method according to claim 1 of diagnosing systemic lupus erythematosus, wherein step a) further comprises determining, in a blood sample from the individual containing red blood cells, complement receptor CR1 deposited on surfaces of red blood cells in the sample, and wherein step b) further comprises comparing said determination with the quantity of receptor CR1 deposited on surfaces of red blood cells of individuals not having systemic lupus erythematosus.

3. A method according to claim 1 of diagnosing scleroderma, further comprising determining in the blood sample complement receptor CR1 deposited on surfaces of the red blood cells, and in which step (b) comprises comparing the ratio of C4d:CR1 deposited on surfaces of red blood cells in the sample with the ratio of C4d:CR1 on surfaces of red blood cells of individuals not having scleroderma.

4. A method according to claim 1 of diagnosing systemic lupus erythematosus, wherein the complement component C4d is determined in step (a) automatically, and wherein in step (b) the determination is compared automatically with a reference value for component C4d deposited on surfaces of red blood cells.

5. A method according to claim 2, wherein the complement component C4d and the complement receptor CR1 are determined in step (a) automatically, and wherein in step (b) the determinations are compared automatically with reference values for component C4d and receptor CR1, respectively, on surfaces of red blood cells.

6. A method according to claim 1 of diagnosing scleroderma, wherein the complement component C4d is determined in step (a) automatically, and wherein in step (b) the determination is compared automatically with a reference value for component C4d deposited on surfaces of red blood cells.

7. A method according to claim 1 of diagnosing scleroderma, wherein step (a) comprises automatically determining, in a blood sample from the individual containing red blood cells, complement component C4d and complement receptor CR1 deposited on surfaces of the red blood cells in the sample, and wherein step (b) comprises automatically comparing said determinations with reference values for component C4d and receptor CR1, respectively, on surfaces of red blood cells.

8. A method according to claim 1 in which the determination of C4d is conducted by a method comprising binding the C4d to a conjugate of a monoclonal antibody specific for C4d with a labeled moiety, and determining the labeled moiety.

9. A method according to Claim 8 in which the labeled moiety is a fluorescent moiety.

10. A method according to claim 9 in which the fluorescent moiety is determined by determining the mean fluorescence channel using flow cytometric analysis.

11. A method according to claim 2 in which step (b) comprises comparing the ratio of C4d:CRI deposited on surfaces of red blood cells in the sample with the ratio of C4d:CRI on surfaces of red blood cells of individuals not having systemic lupus erythematosus.

12. A method according to claim 2 in which the determinations of C4d and CR1 are conducted by a method comprising binding the C4d to a conjugate of a monoclonal antibody specific for component C4d with a first labeled moiety, binding the CR1 to a conjugate of a monoclonal antibody specific for CR1 with a second labeled moiety, and determining the first and second labeled moieties.

13. A method according to Claim 12 in which the labeled moieties are fluorescent moieties.

14. A method according to claim 13 in which the fluorescent moieties are determined by determining the mean fluores-

cence channel using flow cytometric analysis.

15. A method according to claim 5 or 7 in which the reference values comprise a ratio of C4d:CRl.

16. A method of monitoring disease activity of systemic lupus erythematosus in an individual or of monitoring scleroderma in an individual, comprising (a) determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of the red blood cells in the sample, and (b) comparing said determination with the quantity of component C4d deposited on surfaces of red blood cells previously obtained from the individual.

17. A method according to claim 16 in which the determination of C4d is conducted by a method comprising binding the C4d to a conjugate of a monoclonal antibody specific for C4d with a labeled moiety, and determining the first labeled moiety.

18. A method according to Claim 17 in which the labeled moiety is a fluorescent moiety.

19. A method according to claim 18 in which the fluorescent moiety is determined by determining the mean fluorescence channel using flow cytometric analysis.

20. A method according to claim 16, wherein the complement component C4d is determined automatically in step (a), and wherein said determination is automatically compared with a reference value for component C4d deposited on surfaces of red blood cells in step (b).

21. A method according to claim 16 of monitoring disease activity of systemic lupus erythematosus, wherein step a) further comprises determining, in a blood sample from the individual containing red blood cells, complement receptor CR1 deposited on surfaces of red blood cells in the sample, and wherein step b) further comprises comparing said determination with the quantity of receptor CR1 deposited on surfaces of red blood cells previously obtained from the individual.

22. A method according to claim 21 in which step (b) comprises comparing the ratio of C4d:CR1 deposited on surfaces of red blood cells in the sample with the ratio of C4d:CR1 deposited on surfaces of red blood cells previously obtained from the individual.

23. A method according to claim 21 in which the determination of C4d and CR1 is conducted by a method comprising binding the C4d to a conjugate of a monoclonal antibody specific for C4d with a first labeled moiety, binding the CR1 to a conjugate of a monoclonal antibody specific for CR1 with a second labeled moiety, and determining the first and second labeled moieties.

24. A method according to Claim 23 in which the labeled moieties are fluorescent moieties.

25. A method according to claim 24 in which the fluorescent moieties are determined by determining the mean fluorescence channel using flow cytometric analysis.

26. A method according to claim 21, wherein the complement component C4d and the complement receptor CR1 are automatically determined in step (a), and wherein said determinations are automatically compared in step (b) with reference values for component C4d and receptor CR1, respectively, on surfaces of red blood cells.

27. A method according to claim 26 in which the reference values comprise a ratio of C4d:CRl.

28. Use of a computer readable medium, said computer readable medium comprising:

(a) code for receiving data corresponding to a determination of complement component C4d deposited on surfaces of red blood cells;
(b) code for retrieving a reference value for complement component C4d deposited on surfaces of red blood cells of individuals;
(c) code for comparing the data in (a) with the reference value in (b), wherein said comparison of the data in (a) with the reference value in (b) provides information regarding whether a patient has systemic lupus erythematosus or scleroderma,

in a method for diagnosing or monitoring systemic lupus erythematosus or scleroderma in an individual comprising (a) automatically determining, in a blood sample from the individual containing red blood cells, complement component C4d deposited on surfaces of red blood cells in the sample, and (b) automatically comparing said determination with a reference value for component C4d deposited on surfaces of red blood cells, wherein said codes (a) through (c) are executed on a digital computer.

29. Use according to claim 28, wherein the computer readable medium further comprises:

(d) code for receiving data corresponding to a determination of complement receptor CR1 deposited on surfaces of red blood cells;
(e) code for retrieving a range of reference values for complement receptor CR1 deposited on surfaces of red blood cells of individuals; and
(f) code for comparing the data in (d) with the reference values of (e),
wherein said codes (d) through (f) are also executed on said digital computer.

Patentansprüche

1. Verfahren zum Diagnostizieren von systemischem Lupus erythematodes oder von Scleroderma in einem Individuum, welches umfasst: (a) das Bestimmen, in einer Blutprobe von dem Individuum, welche rote Blutzellen enthält, der Komplementkomponente C4d, welche auf der Oberfläche von roten Blutzellen in der Probe abgeschieden ist, und (b) das Vergleichen der Bestimmung mit der Menge der Komponente C4d, welche auf der Oberfläche von roten Blutzellen von Individuen abgeschieden sind, welche keinem systemischen Lupus erythematodes aufweisen, oder mit der Menge der Komponente, von der bekannt ist, dass diese auf der Oberfläche von roten Blutzellen von Individuen vorliegt, welche kein Scleroderma aufweisen.

2. Verfahren nach Anspruch 1 zum Diagnostizieren von systemischem Lupus erythematodes, wobei der Schritt (a) des Weiteren das Bestimmen, in einer Blutprobe von dem Individuum, welche rote Blutzellen enthält, von Komplementrezeptor CR1, welcher auf der Oberfläche von roten Blutzellen in der Probe abgeschieden ist, umfasst, und, wobei der Schritt (b) des Weiteren das Vergleichen der Bestimmung mit der Menge von Rezeptor CR1 umfasst, welcher auf der Oberfläche von roten Blutzellen von Individuen abgeschieden ist, welche keinen systemischen Lupus erythematodes aufweisen.

3. Verfahren nach Anspruch 1 zum Diagnostizieren von Scleroderma, welches des Weiteren das Bestimmen, in der Blutprobe, von Komplementrezeptor CR1 umfasst, welcher auf der Oberfläche der roten Blutzellen abgeschieden ist, und, bei dem der Schritt (b) das Vergleich des Verhältnisses von auf der Oberfläche von roten Blutzellen in der Probe abgeschiedenen C4d:CR1 mit dem Verhältnis von C4d:CR1 auf der Oberfläche von roten Blutzellen von Individuen, welche kein Scleroderma aufweisen, umfasst.

4. Verfahren nach Anspruch 1 zum Diagnostizieren von systemischem Lupus erythematodes, bei dem die Komplementkomponente C4d in dem Schritt (a) automatisch bestimmt wird, und, bei dem in dem Schritt (b) die Bestimmung automatisch mit einem Referenzwert für die auf der Oberfläche von roten Blutzellen abgeschiedene Komponente C4d verglichen wird.

5. Verfahren nach Anspruch 2, bei dem die Komplementkomponente C4d und der Komplementrezeptor CR1 in dem Schritt (a) automatisch bestimmt werden, und, bei dem in dem Schritt (b) die Bestimmungen automatisch mit Referenzwerten für die Komponente C4d bzw. den Rezeptor RC1 auf der Oberfläche von roten Blutzellen verglichen werden.

6. Verfahren nach Anspruch 1 zum Diagnostizieren von Scleroderma, bei dem die Komplementkomponente C4d in dem Schritt (a) automatisch bestimmt wird, und, bei dem in dem Schritt (b) die Bestimmung automatisch mit einem Referenzwert für die auf der Oberfläche von roten Blutzellen abgeschiedene Komponente C4d verglichen wird.

7. Verfahren nach Anspruch 1 zum Diagnostizieren von Scleroderma, bei dem der Schritt (a) das automatische Bestimmen, in einer rote Blutzellen enthaltenden Blutprobe von dem Individuum, der Komplementkomponente C4d und des Komplementrezeptors CR1, welche auf der Oberfläche der roten Blutzellen in der Probe abgeschieden sind, umfasst, und, wobei der Schritt (b) das automatische Vergleichen der Bestimmungen mit Referenzwerten für die Komponente C4d bzw. dem Rezeptor CRI auf der Oberfläche der roten Blutzellen umfasst.

8. Verfahren nach Anspruch 1, bei dem die Bestimmung von C4d durch ein Verfahren durchgeführt wird, welches das Binden des C4d an ein Konjugat eines für C4d spezifischen monoklonalen Antikörpers mit einem markierten Rest umfasst und das Bestimmen des markierten Restes umfasst.

9. Verfahren nach Anspruch 8, bei dem der markierte Rest ein fluoreszierender Rest ist.

10. Verfahren nach Anspruch 9, bei dem der fluoreszierende Rest durch Bestimmen des durchschnittlichen Fluoreszenzkanals unter Verwenden von flusszytometrischer Analyse bestimmt wird.

11. Verfahren nach Anspruch 2, bei dem der Schritt (b) das Vergleichen des Verhältnisses von C4d:CRI, welche auf der Oberfläche von roten Blutzellen in der Probe abgeschieden sind, mit dem Verhältnis von C4d:CR1 auf der Oberfläche von roten Blutzellen von Individuen, welche keinen systemischen Lupus erythematodes aufweisen, umfasst.

12. Verfahren nach Anspruch 2, bei dem die Bestimmungen von C4d und von CR1 durch ein Verfahren durchgeführt werden, welches das Binden des C4d an ein Konjugat eines für die Komponente C4d spezifischen monoklonalen Antikörpers mit einem ersten markierten Rest, das Binden des CR1 an ein Konjugat eines für CR1 spezifischen monoklonalen Antikörpers mit einem zweiten markierten Rest und das Bestimmen der ersten und zweiten markierten Reste umfasst.

13. Verfahren nach Anspruch 12, bei dem die markierten Reste fluoreszierende Reste sind.

14. Verfahren nach Anspruch 13, bei dem die fluoreszierenden Reste durch Bestimmen des durchschnittlichen Fluoreszenzkanals unter Verwendung von flusszytometrischer Analyse bestimmt werden.

15. Verfahren nach Anspruch 5 oder 7, bei dem die Referenzwerte ein Verhältnis von C4d:CR1 aufweisen.

16. Verfahren zum Überwachen der Krankheitsaktivität von systemischem Lupus erythematodes in einem Individuum oder zum Überwachen von Scleroderma in einem Individuum, welches umfasst: (a) das Bestimmen, in einer rote Blutzellen enthaltenden Blutprobe von dem Individuum, der Komplementkomponente C4d, welche auf der Oberfläche der roten Blutzellen in der Probe abgeschieden ist, und (b) das Vergleichen der Bestimmung mit der Menge der Komponente C4d, welche auf der Oberfläche von roten Blutzellen abgeschieden ist, welche vorher aus dem Individuum erhalten worden sind.

17. Verfahren nach Anspruch 16, bei dem die Bestimmung von C4d durch ein Verfahren durchgeführt wird, welches das Binden des C4d an ein Konjugat eines für C4d spezifischen monoklonalen Antikörpers mit einem markierten Rest und das Bestimmen des ersten markierten Restes umfasst.

18. Verfahren nach Anspruch 17, bei dem der markierte Rest ein fluoreszierender Rest ist.

19. Verfahren nach Anspruch 18, bei dem der fluoreszierende Rest durch Bestimmen des durchschnittlichen Fluoreszenzkanals unter Verwendung von flusszytometrischer Analyse bestimmt wird.

20. Verfahren nach Anspruch 16, bei dem die Komplementkomponente C4d in dem Schritt (a) automatisch bestimmt wird, und, bei dem die Bestimmung in dem Schritt (b) automatisch mit einem Referenzwert für die Komponente C4d, welche auf der Oberfläche von roten Blutzellen abgeschieden ist, verglichen wird.

21. Verfahren nach Anspruch 16 zum Überwachen der Krankheitsaktivität von systemischem Lupus erythematodes, bei dem der Schritt (a) des Weiteren das Bestimmen, in einer rote Blutzellen enthaltenden Probe von dem Individuum, des Komplementrezeptors CR1, welcher auf der Oberfläche von roten Blutzellen in der Probe abgeschieden ist, umfasst, und, wobei der Schritt (b) des Weiteren das Vergleichen der Bestimmung mit der Menge von Rezeptor CR1 umfasst, welcher auf der Oberfläche von roten Blutzellen abgeschieden ist, welche zuvor aus dem Individuum erhalten worden sind.

22. Verfahren nach Anspruch 21, bei dem der Schritt (b) das Vergleichen des Verhältnisses von auf der Oberfläche von roten Blutzellen in der Probe abgeschiedenen C4d:CR1 mit dem Verhältnis von auf der Oberfläche von roten Blutzellen, welche zuvor aus dem Individuum erhalten worden sind, abgeschiedenen C4d:CR1 umfasst.

23. Verfahren nach Anspruch 21, wobei die Bestimmung von C4d und von CR1 durch ein Verfahren durchgeführt wird, welches das Binden des C4d an ein Konjugat eines für C4d spezifischen monoklonalen Antikörper mit einem ersten markierten Rest umfasst, das Binden des CR1 an ein Konjugat eines für CR1 spezifischen monoklonalen Antikörper mit einem zweiten markierten Rest umfasst und das Bestimmen der ersten und zweiten markierten Reste umfasst.

24. Verfahren nach Anspruch 23, bei dem die markierten Reste fluoreszierende Reste sind.

25. Verfahren nach Anspruch 24, bei dem die fluoreszierenden Reste durch Bestimmen des durchschnittlichen Fluoreszenzkanals unter Verwendung von flusszytometrischer Analyse bestimmt werden.

26. Verfahren nach Anspruch 21, bei dem die Komplementkomponente C4d und der Komplementrezeptor CR1 in dem Schritt (a) automatisch bestimmt werden, und, bei dem die Bestimmungen in dem Schritt (b) automatisch mit Referenzwerten für die Komponente C4d bzw. den Rezeptor CR1 auf der Oberfläche von roten Blutzellen verglichen werden.

27. Verfahren nach Anspruch 26, bei dem die Referenzwerte ein Verhältnis von C4d:CR1 umfassen.

28. Verwendung eines computerlesbaren Mediums, wobei das computerlesbare Medium umfasst:

(a) einen Code zum Erhalten von Daten entsprechend einer Bestimmung von Komplementkomponente C4d, welche auf der Oberfläche von roten Blutzellen abgeschieden ist,
(b) einen Code zum Erhalten eines Referenzwertes für Komplementkomponente C4d, welche auf der Oberfläche von roten Blutzellen von Individuen abgeschieden ist,
(c) einen Code zum Vergleichen der Daten in (a) mit dem Referenzwert in (b), wobei der Vergleich der Daten in (a) mit dem Referenzwert in (b) Informationen bezogen darauf liefert, ob ein Patient systemischen Lupus erythematodes oder Scleroderma aufweist,
in einem Verfahren zum Diagnostizieren oder zum Überwachen von systemischem Lupus erythematodes oder von Scleroderma in einem Individuum, welches umfasst: (a) das automatische Bestimmen, in einer rote Blutzellen enthaltenen Blutprobe von dem Individuum, von Komplementkomponente C4d, welche auf der Oberfläche von roten Blutzellen in der Probe abgeschieden ist, und (b) das automatische Vergleichen der Bestimmung mit einem Referenzwert für die Komponente C4d, welche auf der Oberfläche von roten Blutzellen abgeschieden ist, wobei die Codes (a) bis (c) auf einem Digitalcomputer durchgeführt werden.

29. Verwendung nach Anspruch 28, wobei das computerlesbare Medium des Weiteren umfasst:

(d) einen Code für das Erhalten von Daten entsprechend einer Bestimmung von Komplementrezeptor CR1, welcher auf der Oberfläche von roten Blutzellen abgeschieden ist,
(e) einen Code zum Erhalten eines Bereichs von Referenzwerten für den Komplementrezeptor CR1, welcher auf der Oberfläche von roten Blutzellen von Individuen abgeschieden ist, sowie
(f) einen Code zum Vergleichen der Daten in (d) mit den Referenzwerten von (e),
wobei die Codes (d) bis (f) ebenfalls auf dem Digitalcomputer durchgeführt werden.

Revendications

1. Méthode de diagnostic du lupus érythémateux disséminé ou de la sclérodermie chez un individu, comprenant

(a) la détermination, dans un échantillon de sang provenant de l'individu, contenant des globules rouges, du constituant C4d du complément déposé sur les surfaces des globules rouges dans l'échantillon, et (b) la comparaison de ladite détermination avec la quantité de constituant C4d déposée sur les surfaces des globules rouges d'individus ne présentant pas de lupus érythémateux disséminé ou bien avec la quantité dudit constituant connu en tant que quantité présente sur la surface des globules rouges d'individus ne présentant pas de sclérodermie.

2. Méthode suivant la revendication 1 de diagnostic du lupus érythémateux disséminé, dans laquelle l'étape (a) comprend en outre la détermination, dans un échantillon de sang provenant de l'individu, contenant des globules rouges, du récepteur CR1 du complément déposé sur la surface des globules rouges dans l'échantillon, et dans laquelle l'étape (b) comprend en outre la comparaison de ladite détermination avec la quantité de récepteurs CR1 déposée

sur les surfaces des globules rouges d'individus ne présentant pas de lupus érythémateux disséminé.

3. Méthode suivant la revendication 1 de diagnostic de la sclérodermie, comprenant en outre la détermination dans l'échantillon de sang du récepteur du complément CR1 déposé sur les surfaces des globules rouges, et dans laquelle l'étape (b) comprend la comparaison du rapport de C4d:CR1 déposés sur les surfaces des globules rouges dans l'échantillon, avec le rapport de C4d:CR1 sur les surfaces des globules rouges d'individus ne présentant pas de sclérodermie.

4. Méthode suivant la revendication 1 de diagnostic du lupus érythémateux disséminé, dans laquelle le constituant C4d du complément est déterminé dans l'étape (a) automatiquement, et dans laquelle, dans l'étape (b), la détermination est comparée automatiquement à une valeur de référence pour le constituant C4d déposé sur les surfaces des globules rouges.

5. Méthode suivant la revendication 2, dans laquelle le constituant C4d du complément et le récepteur du complément CR1 sont déterminés dans l'étape (a) automatiquement, et dans laquelle, dans l'étape (b), les déterminations sont comparées automatiquement à des valeurs de référence pour le constituant C4d et le récepteur CR1, respectivement, sur les surfaces des globules rouges.

6. Méthode suivant la revendication 1 de diagnostic de la sclérodermie, dans laquelle le constituant C4d du complément est déterminé dans l'étape (a) automatiquement, et dans laquelle, dans l'étape (b), la détermination est comparée automatiquement à une valeur de référence pour le constituant C4d déposé sur les surfaces des globules rouges.

7. Méthode suivant la revendication 1 de diagnostic de la sclérodermie, dans laquelle l'étape (a) comprend la détermination automatique, dans un échantillon de sang provenant de l'individu, contenant des globules rouges, du constituant C4d du complément et du récepteur du complément CR1 déposés sur les surfaces des globules rouges dans l'échantillon, et dans laquelle l'étape (b) comprend la comparaison automatique desdites déterminations à des valeurs de référence pour le constituant C4d et le récepteur CR1, respectivement, sur les surfaces des globules rouges.

8. Méthode suivant la revendication 1, dans laquelle la détermination de C4d est effectuée par une méthode comprenant la liaison du C4d à un conjugué d'un anticorps monoclonal spécifique de C4d avec un groupement marqué, et la détermination du groupement marqué.

9. Méthode suivant la revendication 8, dans laquelle le groupement marqué est un groupement fluorescent.

10. Méthode suivant la revendication 9, dans laquelle le groupement fluorescent est déterminé en déterminant le canal de fluorescence moyenne par analyse de cytométrie de flux.

11. Méthode suivant la revendication 2, dans laquelle l'étape (b) comprend la comparaison du rapport de C4d:CR1 déposés sur les surfaces des globules rouges dans l'échantillon avec le rapport de C4d:CR1 sur les surfaces des globules rouges d'individus ne présentant pas de lupus érythémateux disséminé.

12. Méthode suivant la revendication 2, dans laquelle les déterminations de C4d et CR1 sont effectuées par une méthode comprenant la liaison de C4d à un conjugué d'un anticorps monoclonal spécifique du constituant C4d avec un premier groupement marqué, la liaison de CR1 à un conjugué d'un anticorps monoclonal spécifique de CR1 avec un second groupement marqué, et la détermination des premier et second groupements marqués.

13. Méthode suivant la revendication 12, dans laquelle les groupements marqués sont des groupements fluorescents.

14. Méthode suivant la revendication 13, dans laquelle les groupements fluorescents sont déterminés en déterminant le canal de fluorescence moyenne par analyse de cytométrie de flux.

15. Méthode suivant la revendication 5 ou 7, dans laquelle les valeurs de référence comprennent un rapport C4d:CR1.

16. Méthode pour contrôler l'activité pathologique du lupus érythémateux disséminé chez un individu ou pour contrôler la sclérodermie chez un individu, comprenant (a) la détermination, dans un échantillon de sang provenant de l'individu, contenant des globules rouges, du constituant C4d du complément déposé sur les surfaces des globules rouges dans l'échantillon, et (b) la comparaison de ladite détermination avec la quantité de constituant C4d déposée

sur les surfaces des globules rouges obtenus préalablement chez l'individu.

17. Méthode suivant la revendication 16, dans laquelle la détermination de C4d est effectuée par une méthode comprenant la liaison de C4d à un conjugué d'un anticorps monoclonal spécifique de C4d avec un groupement marqué, et la détermination du premier groupement marqué.

18. Méthode suivant la revendication 17, dans laquelle le groupement marqué est un groupement fluorescent.

19. Méthode suivant la revendication 18, dans laquelle le groupement fluorescent est déterminé en déterminant le canal de fluorescence moyenne par analyse de cytométrie de flux.

20. Méthode suivant la revendication 16, dans laquelle le constituant C4d du complément est déterminé automatiquement dans l'étape (a), et dans laquelle ladite détermination est comparée automatiquement à une valeur de référence pour le constituant C4d déposé sur les surfaces des globules rouges dans l'étape (b).

21. Méthode suivant la revendication 16 pour contrôler l'activité pathologique du lupus érythémateux disséminé, dans laquelle l'étape (a) comprend en outre la détermination, dans un échantillon de sang provenant de l'individu, contenant des globules rouges, du récepteur CR1 du complément déposé sur la surface des globules rouges dans l'échantillon, et dans laquelle l'étape (b) comprend en outre la comparaison de ladite détermination avec la quantité de récepteur CR1 déposée sur la surface des globules rouges obtenus préalablement chez l'individu.

22. Méthode suivant la revendication 21, dans laquelle l'étape (b) comprend la comparaison du rapport de C4d:CR1 déposés sur les surfaces des globules rouges dans l'échantillon avec le rapport de C4d:CR1 déposés sur les surfaces des globules rouges obtenus préalablement chez l'individu.

23. Méthode suivant la revendication 21, dans laquelle la détermination de C4d et CR1 est effectuée par une méthode comprenant la liaison de C4d à un conjugué d'un anticorps monoclonal spécifique de C4d avec un premier groupement marqué, la liaison de CR1 à un conjugué d'un anticorps monoclonal spécifique de CR1 avec un second groupement marqué, et la détermination des premier et second groupements marqués.

24. Méthode suivant la revendication 23, dans laquelle les groupements marqués sont des groupements fluorescents.

25. Méthode suivant la revendication 24, dans laquelle les groupements fluorescents sont déterminés en déterminant le canal de fluorescence moyenne par analyse de cytométrie de flux.

26. Méthode suivant la revendication 21, dans laquelle le constituant C4d du complément et le récepteur du complément CR1 sont déterminés automatiquement dans l'étape (a), et dans laquelle lesdites déterminations sont comparées automatiquement dans l'étape (b) à des valeurs de référence pour le constituant C4d et le récepteur CR1, respectivement, sur les surfaces des globules rouges.

27. Méthode suivant la revendication 26, dans laquelle les valeurs de référence comprennent un rapport C4d:CR1.

28. Utilisation d'un support apte à la lecteure par ordinateur, ledit support apte à la lecture par ordinateur comprenant :

(a) un code pour recevoir des données correspondant à une détermination du constituant C4d du complément déposé sur les surfaces des globules rouges ;
(b) un code pour extraire une valeur de référence pour le constituant C4d du complément déposé sur les surface des globules rouges d'individus ;
(c) un code pour comparer les données en (a) à la valeur de référence en (b), ladite comparaison des données en (a) à la valeur de référence en (b) fournissant une information concernant la présence d'un lupus érythémateux disséminé ou d'une sclérodermie chez un patient,
dans une méthode de diagnostic ou de contrôle du lupus érythémateux disséminé ou de la sclérodermie chez un individu, comprenant (a) la détermination automatique, dans un échantillon de sang provenant de l'individu, contenant des globules rouges, du constituant C4d du complément déposé sur les surfaces des globules rouges dans l'échantillon, et (b) la comparaison automatique de ladite détermination à une valeur de référence pour le constituant C4d déposé sur les surfaces des globules rouges, où lesdits (a) à (c) sont exécutés sur un calculateur numérique.

29. Utilisation suivant la revendication 28, dans laquelle le support apte à la lecture par ordinateur comprend en outre :

(d) un code pour recevoir des données correspondant à une détermination du récepteur CR1 du complément déposé sur les surfaces des globules rouges ;
(e) un code pour extraire un ensemble de valeurs de référence pour le récepteur du complément CR1 déposé sur les surfaces des globules rouges d'individus ; et
(f) un code pour la comparaison des données en (d) aux valeurs de référence de (e),
lesdits codes (d) à (f) étant également exécutés sur ledit calculateur numérique.

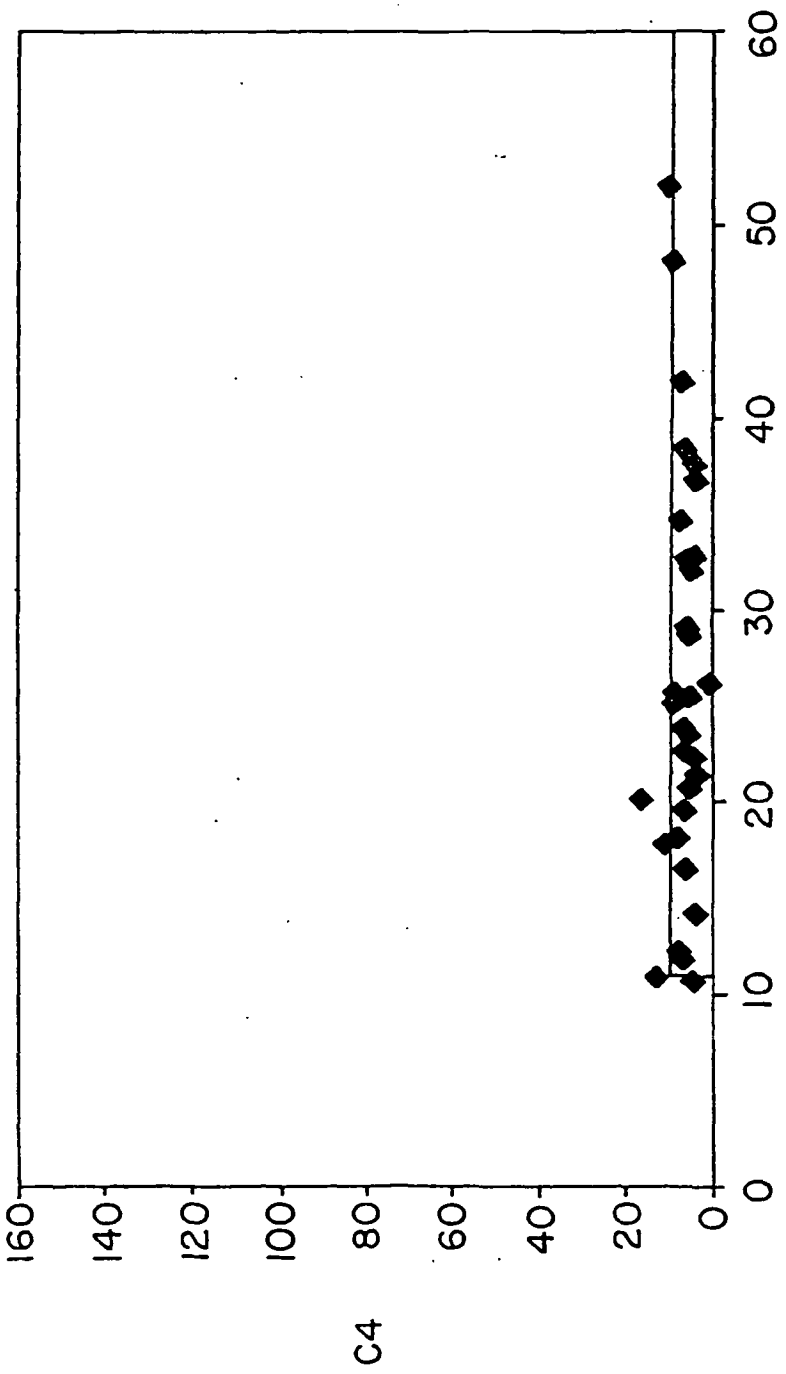

Fig. 1a.

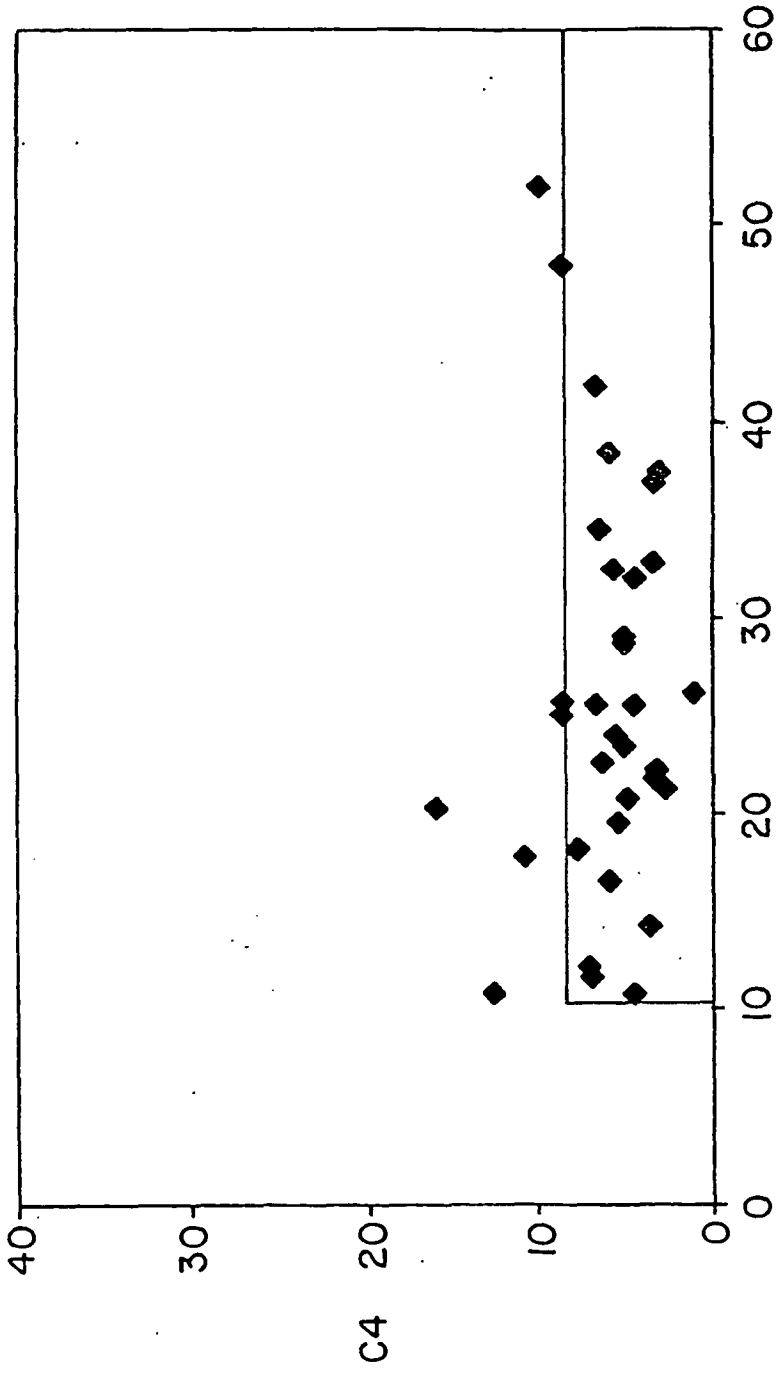

Fig. 1b.

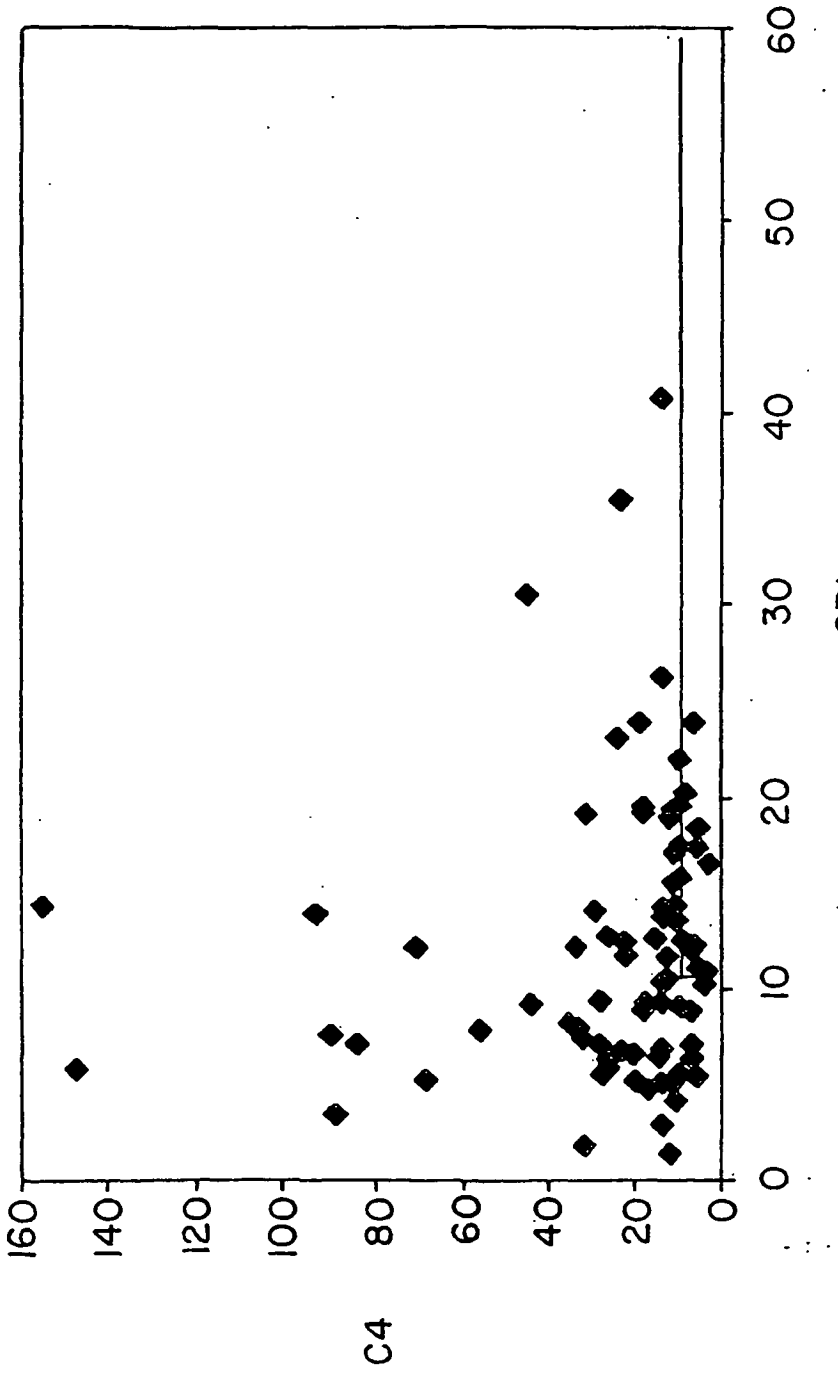

Fig. 2a.

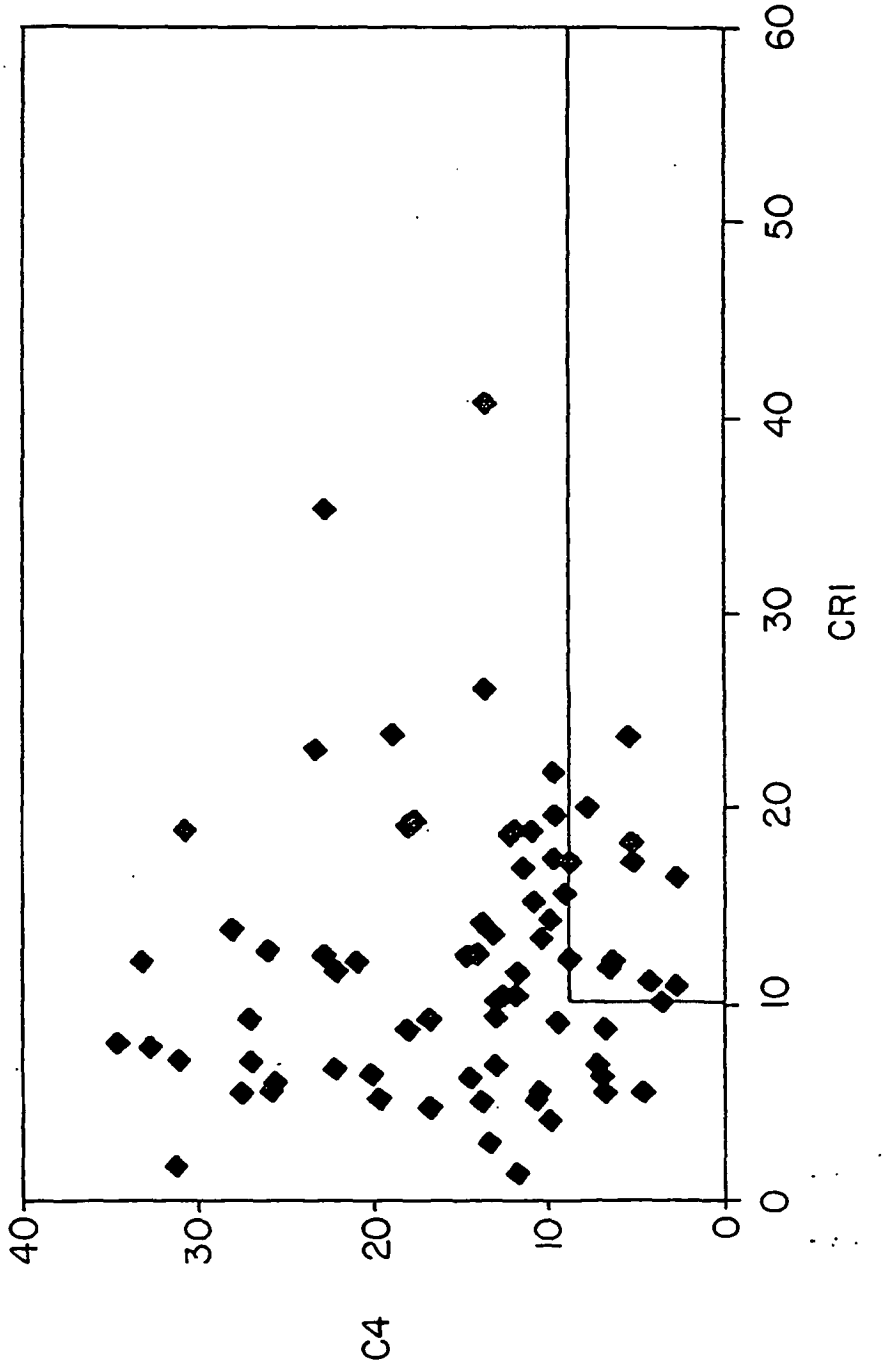

Fig. 2b

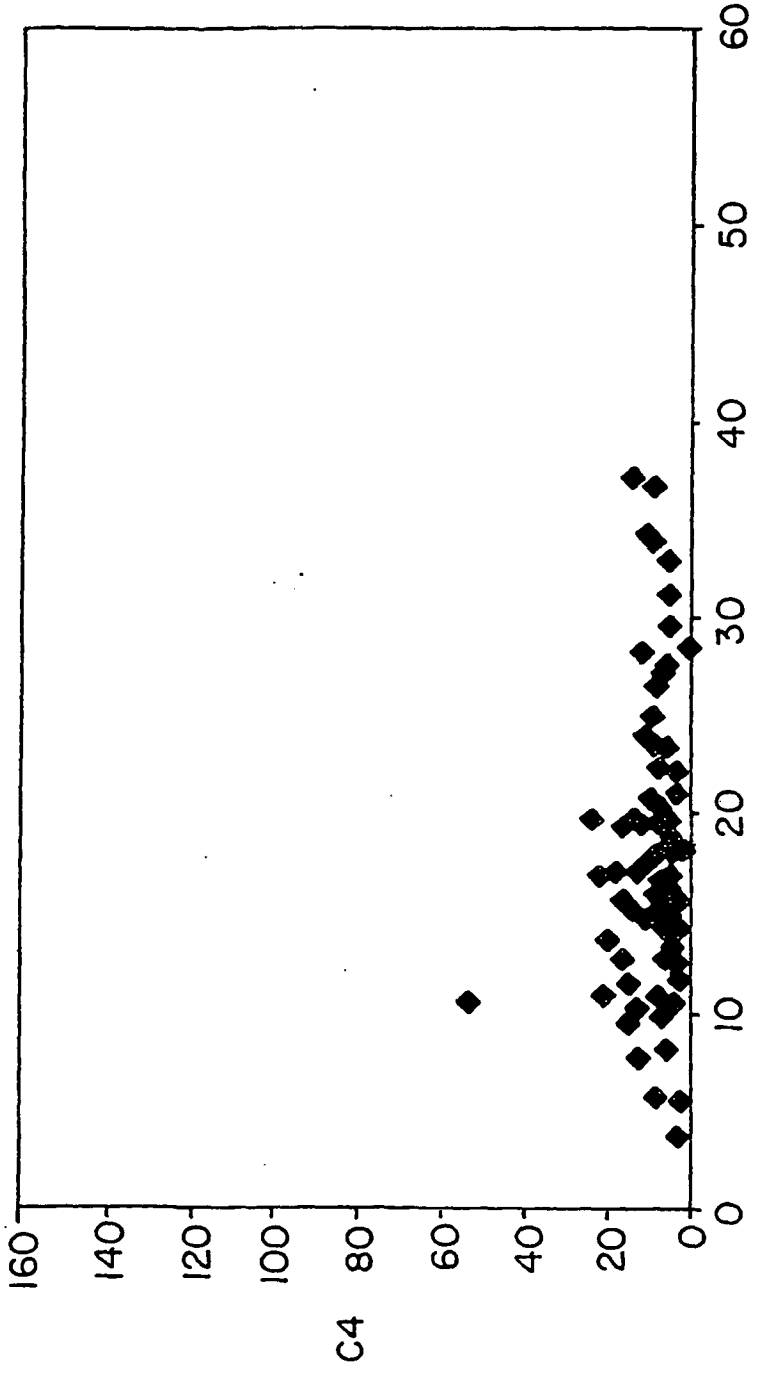

Fig. 3.

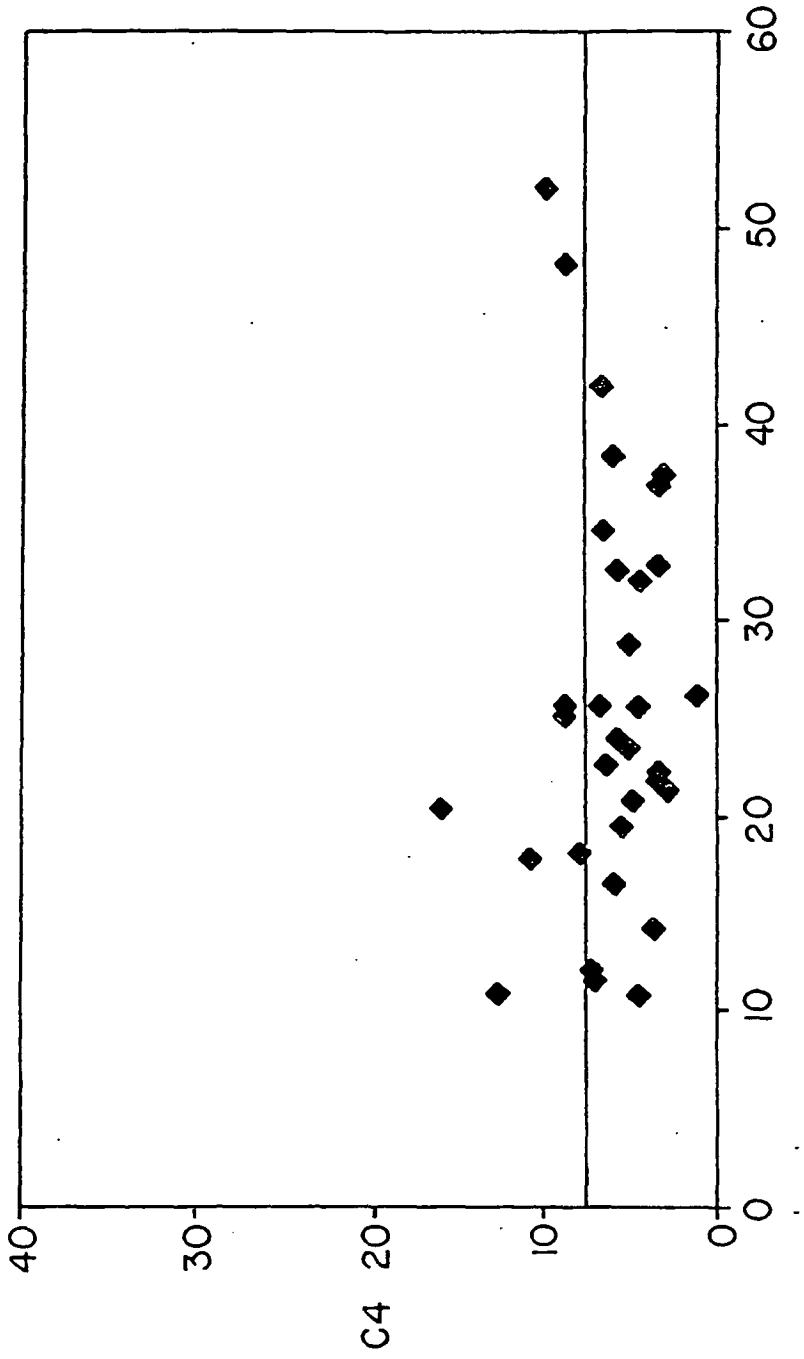

Fig. 4.

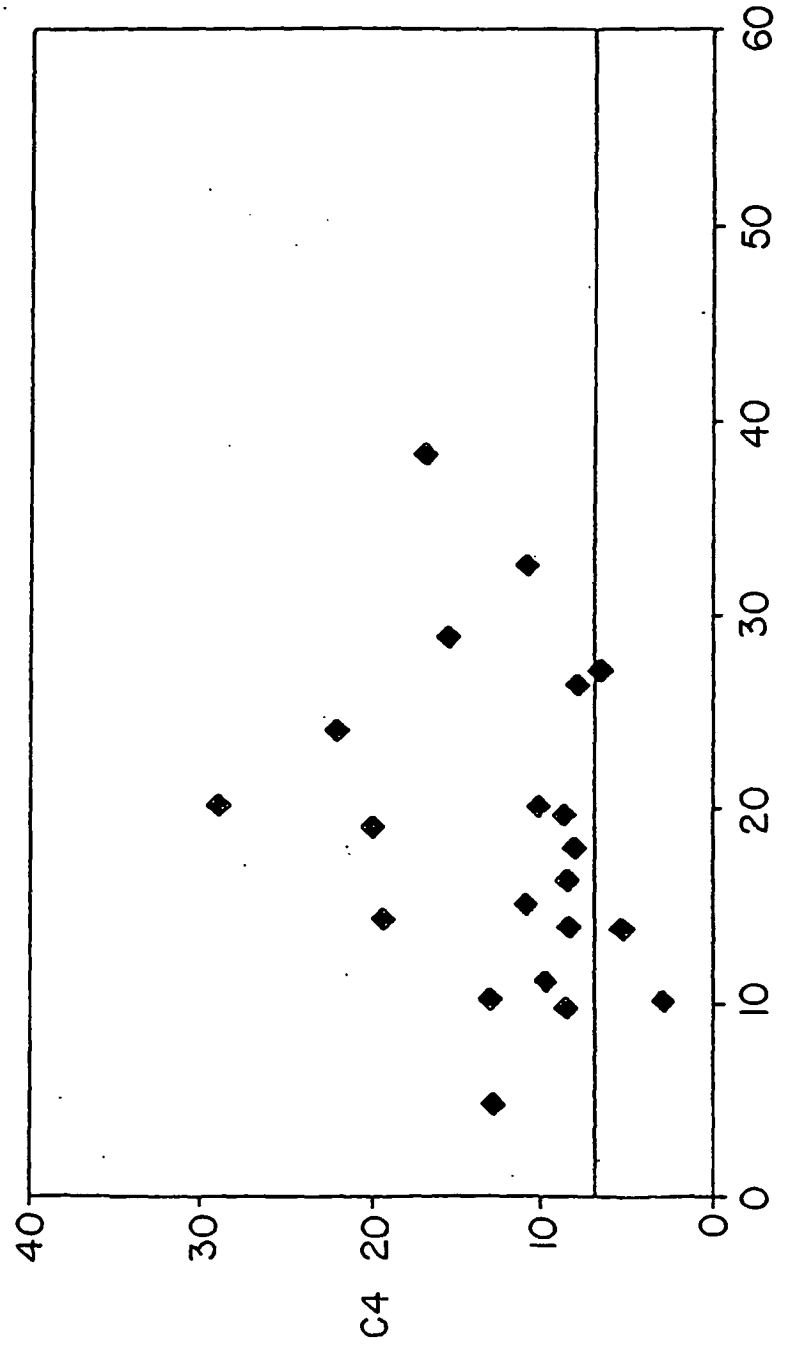

Fig. 5.

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	系统性红斑狼疮和硬皮病的诊断和监测		
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摘要(译)

诊断和监测系统性红斑狼疮（SLE）或硬皮病的方法，方法是在被诊断或监测的个体的血液样本中确定沉积在样本中红细胞表面的补体成分C4d，还可以确定沉积的补体受体CR1 在红细胞表面。 为了进行诊断，将其与正常个体红细胞上存在的C4d（和可选的CR1）的数量进行比较。 为了监测，将其与先前从单个患者获得的一个或多个样品中的值进行比较。 可以利用C4d和CR1的个体值和/或正常个体中两者的比率进行比较。

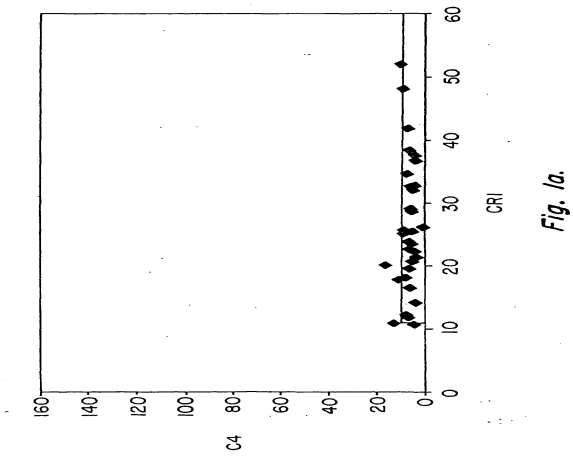

Fig. 1a.