THE CYTOPENIAS
IN
SYSTEMIC LUPUS ERYTHEMATOSUS

INGRID ARONSON
BSc (Hons) MBChB

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Department of Haematology
University of Cape Town
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CHAPTER ONE
OBJECTIVES

OBJECTIVES

Systemic lupus erythematosus (SLE) is a disease of unknown aetiology in which a wide variety of tissues and cells are damaged by deposition of pathogenic auto-antibodies and/or immune complexes.

It has been observed that the majority of these patients manifest some abnormality of the haematopoietic system. Cytopenias affecting one or more cell lines are particularly common, their reported incidences varying widely in different published series. The mechanisms underlying these abnormalities have not as yet been clearly defined. This is in part due to uncertainty about the role of auto-immunity in their pathogenesis, and in further part due to the simultaneous occurrence of various complicating factors that include drugs and renal failure.

To document the incidence of the cytopenias, as well as to define as far as possible the mechanisms underlying these abnormalities, haematological studies were conducted on 70 selected SLE patients attending Groote Schuur Hospital. A further objective was the presentation of guidelines for the management of cytopenias in these patients.

CHAPTER TWO
LITERATURE REVIEW -
SYSTEMIC LUPUS ERYTHEMATOSUS

LITERATURE REVIEW - SYSTEMIC LUPUS ERYTHEMATOSUS

DEFINITION

Systemic lupus erythematosus (SLE) is a clinical syndrome of unknown cause or causes characterized by inflammation and multisystem involvement. It displays a widely variable presentation and course, being subject to multiple remissions and exacerbations in one or more systems. In approximately 30% of cases, the disease is induced by known drugs.

CLASSIFICATION

SLE has been classified in the group of disorders labeled collagen vascular diseases or connective tissue disorders. Although the former is a misnomer, the term applies to a category of distinct illnesses of differing aetiology but having similar clinical manifestation that affect connective tissue either primarily or secondarily. The syndromes usually included are serum sickness, rheumatoid arthritis, SLE, polyarteritis, dermatomyositis, and scleroderma (1).

A wide variety of clinical and laboratory abnormalities are associated with SLE. Because considerable overlap exists with other rheumatic disorders, attempts have been made to classify SLE in a manner that would allow for the greatest degree of sensitivity and specificity. The first international classification system was devised in Geneva in 1965-1966. Subsequently, the International Reference Centre established criteria based on two dozen centres (2). This was superseded by the American Rheumatism Association (ARA) preliminary criteria, published in 1971 (3). In 1979 an ARA subcommittee was created to re-evaluate these criteria, and in 1982 published revised criteria (4). These are the presently accepted criteria, and are detailed in Table 2.1.

GEOGRAPHY, RACE AND SEX

SLE has a worldwide distribution. Conflicting reports have documented apparent increases in incidence and prevalence among certain racial, religious, or geographical groupings. The incidence and prevalence of SLE in Jamaica, Uganda, Great Britain, Malaysia, New Zealand, Australia, Iraq, Norway, China, Zimbabwe, Finland and Sweden are probably within close ranges of each other (1). Despite some studies showing an increased incidence of lupus in black Americans (5,6), socioeconomic factors may be operative here. Claims that SLE is rare in black Africans have been recently challenged by Dessein, Gledhill and Rossouw (7). In Cape Town, SLE is most frequently encountered in females of mixed ancestry (8).

Ninety percent of cases occur in women, usually of child-bearing age, but children, men, and the elderly can be affected (9). After age 50 the preponderance of females is much smaller (1).

1982 Revised Criteria for Classification of Systemic Lupus Erythematosus*

Criterion	*Definition*
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
5. Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	a) Pleuritis—convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion OR b) Pericarditis—documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	a) Persistent proteinuria greater than 0.5 g per day or greater than 3+ if quantitation not performed OR b) Cellular casts—may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	a) Seizures—in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance OR b) Psychosis—in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	a) Hemolytic anemia—with reticulocytosis OR b) Leukopenia—less than 4,000/mm^3 total on 2 or more occasions OR c) Lymphopenia—less than 1,500/mm^3 on 2 or more occasions OR d) Thrombocytopenia—less than 100,000/mm^3 in the absence of offending drugs
10. Immunologic disorder	a) Positive LE cell preparation OR b) Anti-DNA: antibody to native DNA in abnormal titer OR c) Anti-Sm: presence of antibody to Sm nuclear antigen OR d) False-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced lupus" syndrome

*The proposed classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person shall be said to have systemic lupus erythematosus if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.

Table 2.1. The 1982 revised criteria for the classification of systemic lupus erythematosus.
Source: Wallace DJ, Dubois EL (1).

PATHOGENESIS

The aetiology of SLE is multifactorial with at least four major components playing a crucial role: genetics, viruses, sex hormones and stress. In a given patient, one, two, three, or all four factors may be required for the disease to become clinically manifest, with the actual contribution from each factor varying in different patients.

Genetic Predisposition

This is indicated by a high concordance for clinical disease in monozygotic but not dizygotic twins, and a 10 percent frequency of patients with more than one affected individual in the family (9).

Normal individuals who have HLA-B8/DR3 antigens have evidence of immunologic hyperresponsiveness as evidenced by studies of humoral and cellular immunity (10,11). In SLE, there is only a weak genetic association with HLA DR3 and DR2 (12), suggesting that other genes might also contribute to disease susceptibility. A significant proportion of SLE patients possess null alleles for the complement genes C4A, C4B and C2, resulting in a complement deficiency. This may cause inefficient clearing of immune complexes from the circulation with deposition in the tissues, leading to inflammatory reactions (13). Furthermore, HLA-DR3 is in strong linkage disequilibrium with the C4A and C4B null alleles (14), so that it is unclear whether the complement deficiency itself, the HLA gene, or another gene closely linked with HLA is responsible for the association. An additional abnormality in these patients is a deficiency of erythrocyte CR1 (15), which would further diminish host clearance of immune complexes.

Viruses

Viruses have been strongly suspected as aetiologic agents, but this is not proven. Every attempt to isolate a virus or other agent from lupus patients has failed (16).

Sex Hormones

SLE is 10 times more common in females than in males because normal females are more immunologically reactive than normal males and therefore more likely to develop autoimmune disorders (17). The basis for these observations lies in the ability of sex steroid hormones to modulate immune reactivity, with androgens suppressing and oestrogens augmenting antibody responses. Men and women with SLE have increased hydroxylation of oestrogen and oestrone to 16-hydroxyoestrone, producing prolonged oestrogenic stimulation (18). There is also evidence for decreased total androgens in SLE (19).

Stress

The existence of important physiologic connections between the neuroendocrine, classical endocrine and immune systems is well established (20). Stress, mood and emotions can act directly or indirectly on all three systems. Coupled with a strong biologic predisposition from the other three factors mentioned above, a stressful event, through immune or other mechanisms, might be enough to trigger the onset of SLE (1).

IMMUNOLOGY

Patients with SLE have numerous immune abnormalities. Among the earliest manifestations of active SLE are hypergammaglobulinaemia and impaired T-cell function. When the disease is quiescent, many of the immune defects return toward normal. Therefore, they may not be the cause but rather the result of other factors which underlie disease.

B-cell abnormalities

Central to the development of SLE is the overproduction of antibodies reactive with cell membrane molecules, cytoplasmic proteins, and nuclear determinants (most notably DNA). These autoantibodies are responsible, in whole or in part, for a variety of pathogenic processes, including abnormalities in T-cell function, glomerulonephritis, and vasculitis. In active lupus, patients have hypergammaglobulinaemia (21) and increased numbers of activated B cells. (22,23). There is a large increase in numbers of immunoglobulin-secreting cells in patients with active disease (22,23), and a strong correlation between this increase and disease activity (22). Although serum immunoglobulin levels rise during periods of active disease, the concentration of certain antibodies (such as anti-DNA) may rise at a disproportionately rapid rate in some patients. Thus autoantibody production in SLE may be induced and perpetuated by immunization with autoantigens or antigens cross-reactive with self. Furthermore, it is possible that hypergammaglobulinaemia results directly from increased autoantibody production. Thus it is postulated that the B-cell hyper-proliferation results from their exposure to both self and foreign antigens in the context of a polyclonal activator (24).

The nature of autoantibodies

The precise nature of autoantibody-secreting B-cells in SLE is uncertain. There is also considerable controversy concerning the nature of autoantibodies themselves. While similar to conventional antibodies in general structure, it has nevertheless been proposed that autoantibodies are unusually cross-reactive. Thus, the reactivity of lupus serum with a variety of different autoantigens has been attributed to the presence of autoantibodies capable of binding to a wide variety of self-determinants (25,26). The precise epitopes recognized by such antibodies have not been determined.

T-cell function

During active disease, T-cell function is much impaired. This includes impaired T-cell both helper and suppressor function, impaired IL-2 production, impaired T-cell-mediated cytotoxicity, and impaired skin test responses to standard challenge. These T-cell defects may be secondary to disease rather than primary defects, and may serve as mechanisms for perpetuation of disease processes. Thus, they may not contribute importantly to induction of disease. After the active disease recedes, T-cell function begins to return toward normal (24).

Immune regulation

Abnormal immune regulation appears to play a critical role in the pathogenesis and perpetuation of SLE. Available data suggests that polyclonal immune activity precedes impaired T-cell responses to antigens and mitogens. It is only after such stimulatory activities have led to substantial immune hyperactivity that T-cell functions are reduced. That sequence suggests that many of the impaired T-cell responses observed in lupus are secondary to the immune hyperactivity and not the cause of it. The secondary immune defects most likely result from hypergammaglobulinaemia, increased prostaglandin production

secondary to immune activation, and imbalance between interferon and IL-4 production (increased) and IL-2 production (decreased) (24).

SEROLOGY

Autoantibodies

The disturbance in immunologic homeostasis in SLE is manifested primarily by the presence of many types of autoantibodies in the serum, as listed in Table 2.2.

Autoantibodies in patients with SLE

	Incidence, %	Antigen detected	Clinical importance
Antinuclear antibodies	95	Multiple nuclear and cytoplasmic antigens	Human cell line substrates are more sensitive than standard murine tissues. A repeatedly negative test on both makes SLE diagnosis unlikely. Multiple antibodies are detected.
Anti-DNA	70	DNA	Anti-dsDNA is relatively disease-specific; anti-ssDNA is not. Associated with nephritis and clinical activity.
Anti-Sm	30	Polypeptides complexed to 6 species of small nuclear RNA	Specific for SLE.
Anti-RNP	40	Polypeptides complexed to UIRNA	High titer seen in syndromes with features of polymyositis, scleroderma, lupus and mixed connective tissue disease. If present in SLE without anti-DNA, risk for nephritis is low.
Anti-Ro (SSA)	30	RNA polymerase	Associated with Sjögren's syndrome, DR3 haplotype, subacute cutaneous lupus, complement deficiencies, ANA-negative lupus, lupus in the elderly, neonatal lupus, congenital heart block in infants. Can cause nephritis.
Anti-La (SSB)	10	Protein complexed to RNAs	When associated with anti-Ro, risk for nephritis is low.
Antihistone	70	Histones	More frequent in drug-induced LE (95 percent) than in spontaneous SLE.
Anticardiolipin	50	Phospholipid	Increases risk for venous or arterial thrombosis and for spontaneous abortion. Associated with prolonged PTT (lupus anticoagulant) and false-positive VDRL.
Antierythrocyte	60	Erythrocyte surface antigens	A small proportion of these patients develop overt hemolysis.
Antiplatelet	–	Platelet surface	Associated with thrombocytopenia.
Antilymphocyte	70	Lymphocyte surface antigens	Probably associated with leukopenia and abnormal T-cell function.
Antineuronal	60	Neuronal surface antigens	In CSF, high IgG titers correlate with diffuse but not focal CNS lupus.

Table 2.2 Autoantibodies in patients with systemic lupus erythematosus.
Source: Harrison's Principles of Internal Medicine (9).

Those of greatest clinical significance are directed against nuclear antigens, namely antinuclear antibodies (ANA). These are invariably present in SLE, and can be detected in as many as 99% of patients with this disease. However ANA can be demonstrated in 5% of normal individuals, and occasionally in certain diseases not considered to be autoimmune, for example infectious mononucleosis and chronic active hepatitis.

ANA immunologic specificities can be divided into three general categories: antibody directed against DNA, those directed against histone (a family of basic nuclear proteins), and those against nonhistone nuclear antigens (1).

Anti-DNA antibodies can be classified according to their reactivities with double-stranded or single-stranded DNA. Clinically, antibodies to double-stranded DNA are highly specific for SLE and rarely seen in a significant titer in other systemic rheumatic diseases. The prevalence of these antibodies in SLE depends mainly on the disease activity and on the organ systems involved when the antibodies are measured. They are increased during phases of increased disease activity and are decreased when

patients are in remission. Thus, quantitation of antibody to double-stranded DNA is recommended as a useful tool in assessing disease activity. Antibodies to single-stranded DNA are commonly found in SLE, but are frequently observed in other systemic rheumatic diseases, and occasionally in normal individuals. Thus this antibody is usually not a useful diagnostic marker (1).

Autoantibodies to histones have multiple specificities and are reactive with all of the five major classes of histones: Hl, H2A, H2B, H3 and H4. A close association exists between antihistone antibodies and drug-induced LE, particularly in patients taking procainamide. However, these antibodies have been reported in 80% of patients with idiopathic SLE (1).

The third major class of ANA in SLE is characterized by reactivities with soluble nonhistone nuclear protein and RNA-protein complexes. Antibody to Sm was the first such antibody reported, and it is present almost exclusively in SLE. It is, in fact, considered a diagnostic marker for the disease. Although it is present in only 30% of patients with SLE, it is highly specific in that it has not been detected in normal sera or in the sera of patients with other systemic rheumatic diseases. Antibodies to U1-RNP (nuclear RNP) antigen are found in high titer in patients with mixed connective tissue disease, but also in SLE patients and those with a variety of systemic rheumatic diseases. ENA (originally extractable nuclear antigen) complex refers to the association of U1-RNP and Sm in complexes (1).

Two other types of ANA also included in this group occur in patients with SLE and patients with Sjogren's syndrome - anti-SS-A/Ro and anti-SS-B/La antibodies. The former is present in 30-40% of patients with SLE, approximately 70% of patients with Sjogren's syndrome, and about 20% of patients with rheumatoid arthritis. Several clinical associations exist between the presence of anti-SS-A/Ro and certain subtypes of SLE, for example subacute cutaneous lupus and neonatal lupus. Antibody to SS-B/La is detected in the sera of SLE patients less frequently than anti-SS-A/Ro, of the order of 15%. However, it can be detected in 45-60% of patients with Sjogren's syndrome. Except for the overlapping presence of Sjogren's syndrome in patients with SLE, no distinguishing clinical features appear to be associated with the presence of anti-SS-B/La (1).

There are a number of lesser important antibodies found in some SLE patients, for example antibodies to proliferating cell nuclear antigen (PCNA), MA and Ki antigen and to high mobility group (HMG) proteins (1).

Other serologic abnormalities found in SLE include the presence of rheumatoid factors in about 30%, anti-neuron antibodies in patients with neuropsychiatric manifestations, and anti-erythrocyte, anti-leucocyte and anti-platelet antibodies. Anticardiolipin antibodies (about 60% of patients, but not specific for SLE) belong to a heterogeneous group of autoantibodies found in SLE sera reactive with phospholipids. Other antiphospholipid antibodies include lupus anticoagulant and antibodies responsible for a biologic false positive VDRL test. High serum titer of anticardiolipin antibodies in SLE is significantly associated with venous or arterial thrombosis, thrombocytopenia, and/or spontaneous multiple abortions (1).

Immune Complexes

Considerable evidence shows that much of the pathology in patients with SLE can be attributed to the presence of immune complexes. These consist of combinations of antigens and antibodies and may either form in the circulation and later deposit in tissues, or arise in situ. They do not appear to cause tissue inflammation directly, but rather through activation of the complement system. This causes release of various mediators, promotes cell interaction, and ultimately results in inflammation. In SLE, there is defective reticuloendothelial clearance of these immune complexes due to a number of factors, some inherited, and some acquired. Measurement of immune complexes or evaluation of the complement

system (which can serve as an indirect measure of the presence of immune complexes) often correlates with the clinical aspects of SLE (1).

Complement

Complement deficiencies in SLE patients may be inherited or acquired, and involve virtually any of the complement components. Assessment of the complement system is by measurement of total haemolytic complement (CH50) or haemolytic measurement of individual components, with C3 and C4 being estimated most frequently. Low levels of complement usually reflect disease activity, especially in patients with nephritis, with CH50 being the most sensitive measure of complement activation. Associations have been noted between low levels of individual components and various facets of SLE (1).

CLINICAL SPECTRUM

Clinical manifestations of the disease are determined by which antibody subpopulations and immune complexes are present in the patient's repertoire, which organs, cells, or cell products are their targets, and which patients have the ability to correct these abnormalities.

At its onset, SLE may involve only one organ system, with additional manifestations occurring later, or may be multi-systemic (9). The relative incidences of the commonest clinical manifestations are shown in Figure 2.1.

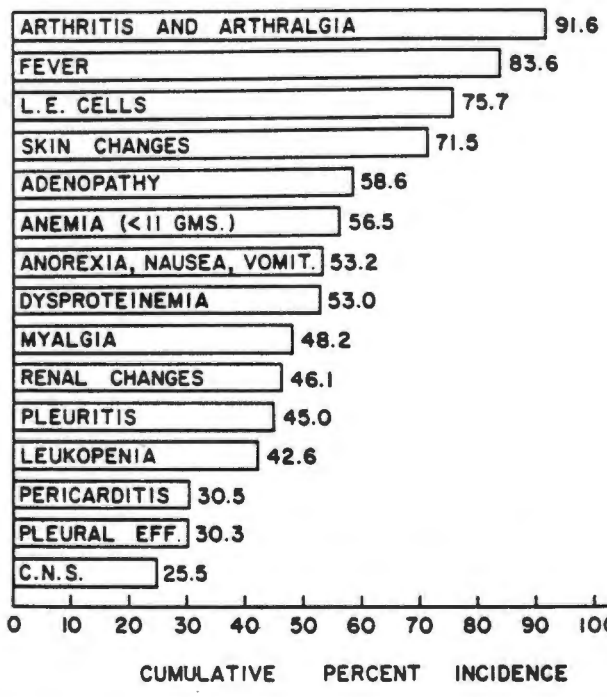


Fig 2.1 Cumulative percentage incidence of the commonest clinical manifestations of 520 cases of SLE. Source: Wallace DJ, Dubois EL (1).

Disease severity varies from mild and intermittent to persistent and ultimately fatal. Most patients experience exacerbations interspersed with periods of relative quiescence, with fewer than 10 percent having long-lasting symptom-free remissions.

Systemic symptoms are usually prominent and include fatigue, malaise, fever, anorexia, weight loss, and nausea (9).

Musculoskeletal

Almost all SLE patients experience arthralgias and myalgias; most develop arthritis. Pain is often out of proportion to physical findings, which include symmetric fusiform swelling of joints (most frequently proximal interphalangeal and metacarpophalangeal joints of the hands, wrists and knees), diffuse puffiness of hands and feet, and tenosynovitis. Joint deformities are unusual. Erosions are rare, but subcutaneous nodules over the elbows and fingers may occur. Myopathy can be inflammatory and related to active disease, or iatrogenically drug induced. Ischaemic necrosis of bone is a common cause of hip and shoulder pain (9).

Cutaneous

The malar or butterfly rash is a fixed erythematous eruption, flat or raised, over the cheeks and bridge of the nose, often involving the chin and ears. A more diffuse maculopapular rash, predominant in sun-exposed areas, is also common, and usually indicates disease flare-up. Loss of scalp hair is usually patchy but can be extensive. Vasculitic skin lesions include subcutaneous nodules, ulcers (usually on the legs), purpura, and infarcts of skin or digits. Discoid lesions occur in some patients with SLE and can be disfiguring. They are circular with an erythematous rim, raised, and scaly, with follicular plugging and telangiectasia. Central scarring produces depigmentation and permanent loss of appendages. They occur over the scalp, external ears, face and sun-exposed areas of the arms, back and chest. Only 5 percent of individuals with discoid LE progress to SLE; however 20 percent of SLE patients have DLE lesions. Less frequent SLE skin lesions include urticaria, periorbital oedema, bullae, erythema multiforme, lichen-planus-like lesions, and panniculitis or lupus profundus.

Patients with subacute cutaneous lupus (SCLE) are a distinct subset with recurring extensive skin lesions. Arthritis and fatigue are frequent; central nervous system and renal involvement are rare. Some are ANA negative, but the majority have antibodies to Ro (SS-A) or to single-stranded DNA and carry the HLA-DR3 phenotype. The skin lesions are photosensitive polycyclic annular or papulosquamous psoriasiform over the arm, trunk and face; they become hypopigmented but not scarred.

Mucous membrane lesions are usually small, shallow, painless ulcers in the mouth (usually over the palate) and nose (9).

Renal Manifestations

Although almost all SLE patients have deposits of immunoglobulin in glomeruli, only one-half have clinical nephritis, defined by persistent proteinuria. At presentation, most patients are asymptomatic (unless already uraemic), except for those with oedema of the nephrotic syndrome. Urinalysis shows haematuria, cylindruria, and proteinuria. Most patients with mesangial or mild focal glomerulonephritis do not develop deterioration of renal function. In those with more severe, active, or chronic lesions, renal failure is a major cause of death. Renal biopsy is an important investigation to aid subsequent therapeutic decisions, with modalities including glucocorticoid and immunosuppressive therapy, dialysis and renal transplantation (9).

Nervous System

Any region of the brain can be involved in SLE, as can the meninges, spinal cord, and cranial and peripheral nerves. Central nervous system events may be isolated, single, or multiple, but usually occur in the setting of active disease in other systems. Mild mental dysfunction is the most frequent manifestation. Seizures are common and may be grand mal, petit mal, or focal. Other manifestations include psychosis, organic brain syndromes, headache (including migraine), focal infarcts with resultant deficits, extrapyramidal disorders, cerebellar dysfunction, hypothalamic dysfunction with inappropriate ADH secretion, pseudotumour cerebri, subarachnoid haemorrhage, aseptic meningitis, transverse myelitis with paraplegia or quadriplegia, optic neuritis, cranial nerve palsies, and peripheral sensorimotor neuropathy resulting either in mononeuritis multiplex or glove-and-stocking deficit. Depression and anxiety are frequent. Neurologic problems usually improve (with the exception of deficits related to infarcts) with therapy and/or time, but recurrences are common (9).

Vascular

Thrombosis in capillaries, in small vessels, and in medium-sized veins and arteries can be a major problem. Although vasculitis may play a role, there is increasing evidence that anticardiolipin antibodies may initiate clotting. In addition, degenerative vascular changes associated with years of immune-complex deposition in vessel walls may predispose to symptomatic coronary artery disease in relatively young individuals with SLE (9).

Cardiopulmonary

Pericardial pain is the most frequent symptom of cardiac lupus; pericardial effusions also occur. Myocarditis can cause arrhythmias and/or cardiac failure. Endocarditis of the Libman-Sacks verrucous type, a diagnosis made at autopsy, is usually not clinically significant; however it can cause aortic or mitral regurgitation. Rarely myocardial infarcts result from vasculitis of the coronary arteries.

Pleurisy and pleural effusions are common manifestations of SLE. Lupus pneumonitis causes recurrent episodes of fever, dyspnoea and cough; x-rays show infiltrates and/or areas of platelike atelectasis. Interstitial pneumonitis leading to fibrosis occurs in a small proportion of patients. Occasionally, patients may develop pulmonary hypertension. Infrequent but often fatal pulmonary manifestations include adult respiratory distress syndrome (ARDS) and massive intraalveolar haemorrhage (9).

Gastrointestinal

Nonspecific gastrointestinal symptoms are common, but vasculitis of the intestine is the most dangerous manifestation. It causes acute or subacute cramping pain, vomiting and diarrhoea, and leads to intestinal perforation and death in almost one-half of the affected patients. Vasculitis is usually present simultaneously in other systems. Another gastrointestinal manifestation of SLE is a pseudo-obstruction picture in which patients present with acute cramping abdominal pain. Acute pancreatitis may occur and can be severe; it may result from steroid therapy or from active SLE. Elevated serum levels of liver enzymes, especially transaminases, are common in patients with active SLE, but are not associated with significant hepatic damage; they return to normal as the disease is treated (9).

Ocular

The most important ocular manifestation of SLE is retinal vasculitis with infarcts; blindness can develop over a period of days. Other ocular abnormalities include conjunctivitis, episcleritis, and optic neuritis. The sicca syndrome is frequent (9).

Drug-induced lupus

Several drugs can cause a syndrome resembling SLE in individuals without any obvious predisposition to the disease. The most common offender is procainamide, which induces ANA in 50-75 percent of individuals within a few months; 20 percent of patients receiving the drug develop clinical drug-induced LE. Hydralazine induces ANA in 25-30 percent of individuals, and lupus-like symptoms in 10 percent. Both procainamide and hydralazine-induced lupus occur more commonly in women, are uncommon in blacks, and are more likely to occur in individuals who acetylate the drug slowly; this is especially true for hydralazine. The clinical syndrome consists of polyarthralgias and systemic symptoms in most patients. Polyarthritis occurs in 25-50 percent, and pleuropericarditis in 30 percent, of patients with hydralazine- and 50 percent of patients with procainamide-induced lupus. Other manifestations typical of idiopathic SLE are unusual, including nephritis and CNS involvement. All patients with drug-induced lupus are ANA-positive; most have antibodies to histones. Antibodies to double-stranded DNA and hypocomplementaemia are rarely present, which is helpful in distinguishing drug-induced from idiopathic lupus. Anaemia, leucopenia, lupus anticoagulant, thrombocytopenia, cryoglobulins, rheumatoid factors, false-positive VDRL and positive direct Coombs' tests can occur.

The initial therapeutic approach should be discontinuance of the suspect drug; most patients improve in days or a few weeks. In patients with severe symptoms, a short course (2-10 weeks) of steroids is indicated. Clinical symptoms rarely persist more than 6 months, but ANA may remain positive for years. Other drugs which infrequently induce lupus-like illnesses include isoniazid, chlorpromazine, penicillamine and methyldopa. Most lupus-inducing drugs can be used safely in patients with idiopathic lupus if there are no suitable alternatives (9).

Prognosis

The overall survival in patients with SLE is approximately 71 percent over 10 years. Patients with severe involvement of the brain, lungs, heart, or kidney have the worst outcomes in terms of survival and disability. Infections and renal failure are the leading causes of death (9).

TREATMENT

Lupus is a chronic disease that often has a relapsing course. The primary therapeutic strategy, therefore, is to achieve and maintain adequate suppression of the disease without incurring unacceptable drug side-effects.

Salicylates are useful in treating fever, fatigue, joint pain and inflammation and serositis. They can be used as an adjunct to corticosteroids or immunosuppressive agents in patients with internal organ involvement. Nonsteroidal anti-inflammatory drugs (NSAID's) are used as substitutes for salicylates in treating fever, arthralgia and serositis, or they may be used synergistically with salicylates. However, some NSAID toxicities are especially frequent in SLE patients, including hepatitis, aseptic meningitis and renal impairment.

The dermitides of SLE, including discoid LE, and occasionally lupus arthritis, may respond to antimalarials. Side effects include retinal toxicity, rash, myopathy and neuropathy. Other therapies for skin rash include use of prophylactic sunscreens and topical corticosteroids (9).

Some disease manifestations require the use of systemic corticosteroids for effective management. Autoimmune haematologic manifestations, including anaemia and thrombocytopenia, pulmonary parenchymal disease, lupus nephritis and organic central nervous system disease are usually treated first with corticosteroids. In adult patients, high dose glucocorticoids approximately equivalent to prednisone 1mg/kg/day are given for severe disease manifestations. This dose is tapered as disease activity is controlled. Some acutely ill patients, including those with nephritis, are treated with a short course of high dose intravenous pulse methylprednisolone (27). The range of adverse reactions experienced with corticosteroids is broad and includes suppression of the hypothalamic-pituitary axis, hypertension, increased susceptibility to infection, glucose intolerance, osteoporosis and psychosis.

Failure to control severe systemic manifestations with corticosteroids requires the introduction of immunosuppressive agents. These drugs may also be used for their steroid-sparing properties or when intolerable corticosteroid toxicity occurs.

The immunosuppressive drugs most commonly used in SLE are the alkylating agents cyclophosphamide, chlorambucil and mechlorethamine (nitrogen mustard), and the antimetabolite azathioprine. The antimetabolite methotrexate has been used sparingly.

Cyclophosphamide has been the most extensively studied of the alkylating agents in SLE. Its immunologic effects include depletion of B-cells at low doses, CD8 T-cells (T8, suppressor/cytotoxic cells) at slightly higher doses, and CD4 T-cells (T4, helper/inducer cells) at high doses. It has been widely used in the treatment of lupus nephritis. Although there have been no controlled trials on the use of cyclophosphamide in the treatment of life-threatening and/or active major organ disease except for the kidney, there are many reports that indicate a role for its use in these settings, which include complications involving the lungs, nervous system and haematologic system (28). Both daily oral and bolus intravenous regimes have been used. The most important toxicities in the doses used for treatment of rheumatic diseases are major infection, gonadal failure, bladder toxicity and malignancy. Minor toxicities include nausea and vomiting and alopecia.

Azathioprine, whose major immunosuppressive effects are probably mediated by a preferential reduction of natural killer cells, may be useful as a steroid-sparing agent in some patients. More specifically, this drug may be effective in the treatment of some patients with antimalarial-resistant discoid lupus, and a subset of patients with mild to moderate proliferative glomerulonephritis. Azathioprine should probably not be the first immunosuppressive drug used for life-threatening disease or active pulmonary, haematologic, or central nervous system disease (28). There are few major side effects associated with this drug as compared with cyclophosphamide. They include major infection, leucopenia, rarely hepatotoxicity and malignancy.

Chlorambucil has been used to treat lupus nephritis, but is less effective than cyclophosphamide. It may have a place in steroid-resistant nephrotic syndrome (29).

Nitrogen mustard is used with some success in the treatment of renal lupus.

Preliminary small scale studies of Cyclosporin A, either as a sole agent or in combination with steroids, suggest that a subset of severely affected patients may benefit from this drug (30). However, definitive evaluation awaits reports of further large scale trials. Side effects are significant and include nephrotoxicity, hypertension, hyperkalaemia, hyperuricaemia and hypertrichosis.

HAEMATOLOGIC MANIFESTATIONS

THE CYTOPENIAS

Haematologic abnormalities occur in almost every patient with SLE, and may be presenting manifestations of the disease. Amongst these abnormalities, the cytopenias are particularly common.

As the focus of this study has been the investigation of cytopenias in SLE, aspects relating to anaemia, leucopenia and thrombocytopenia will be covered in depth in this literature review.

Anaemia

The commonest haematologic abnormality is anaemia, defined as a haemoglobin level below 110g/L, with a reported incidence varying from 38-98% in different series (31,32,33,34). The patients in these series were not stratified into treated and untreated groups, nor was it specified at what stage of the disease the blood specimens were taken.

The causes of anaemia are multiple, and both non-immune and immune mechanisms have been incriminated (35). Mechanisms are more logically discussed under these two broad groups, rather than adopting a physiological approach.

Anaemia - non-immune mechanisms

These will be dealt with first, and they include mainly anaemia of chronic disease, iron deficiency, renal disease and drugs.

Anaemic SLE patients most commonly have the anaemia of chronic disease (ACD) (35). It is usually of moderate severity, with the haemoglobin rarely falling below 80g/l unless additional factors are present (36). Most often, ACD takes the form of a normochromic, normocytic anaemia, but is not infrequently hypochromic, and sometimes microcytic. The reticulocyte count is normal, being low for the degree of anaemia. The bone marrow shows no specific diagnostic features. Iron stores are normal but the percentage of sideroblasts is reduced, suggesting a failure of iron incorporation. The abnormality in iron metabolism can be established by finding subnormal values for serum iron concentration and transferrin saturation in association with evidence of normal or increased iron stores. Serum ferritin concentration usually is increased in ACD, a finding that is often the most convenient means of distinguishing it from iron deficiency. Decreased serum transferrin levels are found frequently, but this is neither sensitive nor specific enough to be useful in excluding iron deficiency. Ferrokinetic abnormalities include a rapid disappearance of Fe from the plasma and a subsequent fast uptake in the macrophage/monocyte system (37).

A number of other biochemical changes occur in association with the anaemia of chronic disease. Most of these are manifestations of the acute phase response. New proteins appear in plasma, including C-reactive protein and amyloid A protein. Synthesis of other proteins is considerably augmented, amongst which are fibrinogen, caeruloplasmin and haptoglobin. The levels of certain other plasma proteins, for example albumin and transferrin are reduced in plasma (37).

Several factors have been implicated in the pathogenesis of ACD, with varying contributions in different disease states.

Many of the findings can be accounted for by release of a monokine interleukin-1 (IL-1), also known as leucocyte endogenous mediator or endogenous pyrogen. This is released from activated monocytes, with bacterial endotoxins, certain lymphokines and phagocytic challenges being among the factors stimulating

its biosynthesis. IL-1 induces fever, leucocytosis, and a variety of biochemical changes, including hypoferraemia and alterations in plasma protein synthesis, collectively known as the acute phase response. It is proposed that ACD results from the long-term elaboration of IL-1 and that release of this substance is the common pathogenetic factor found in the illnesses that are associated with ACD (37). The hypoferraemia is probably caused by defective release of iron from cells - particularly from macrophages, but also from hepatocytes and intestinal epithelium. This may be due to liberation of lactoferrin from neutrophils, which could either remove iron from transferrin, or compete with transferrin for the iron released by macrophages. This iron is then returned to the macrophage, where it becomes incorporated into stores (38). A second mechanism which may contribute to the hypoferraemia is induction of apoferritin synthesis by inflammation. This would bind a larger-than-normal proportion of the iron entering the cell, effectively diverting the metal from the rapid-release pathway (39).

Erythrokinetic studies in ACD have detected a modest reduction in erythrocyte survival without an adequate compensatory increase in the rate of red cell production (40,41,42,43). The reduced erythrocyte survival is probably related to an increase in phagocytic activity by activated macrophages (43).

In patients with ACD, levels of erythropoietin are inappropriately low for the degree of anaemia (44,45,46). However, the fact that erythropoiesis in ACD can increase when stimulated by hypoxia (47,48), indicates that the erythropoietin secreting mechanism retains the capacity to respond to this physiological stimulus. The response, however, appears to be blunted (49).

The relative contributions of these factors to the pathophysiology of ACD vary in different disease states. In the collagen disease group, most studies have been conducted on rheumatoid arthritis patients, but their applicability to SLE has not been tested.

Occult gastrointestinal blood loss possibly on the basis of vasculitis or anti-inflammatory or steroid drug ingestion, may result in iron deficiency anaemia, while uraemic patients have anaemia of chronic disease with superimposed erythropoietin deficiency.

Aplastic anaemia may occur in SLE secondary to the use of nitrogen mustard derivatives, antimalarials, or other agents such as chloramphenicol (1).

A few cases of sideroblastic anaemia in SLE have been reported (50,51), and there have been several isolated cases of concurrent SLE and sickle cell anaemia (52,53,54,55,56,57). Pure red cell aplasia has been seen in SLE (58,58,60). These are all rare occurrences and contribute only very minimally to the causation of anaemia.

Anaemia - immune mechanisms

These include autoimmune haemolytic anaemia and the occurrence of humoral inhibitors of erythropoiesis.

Autoimmune haemolytic anaemias occur with some frequency in SLE, and may predate the appearance of diagnosable SLE (61,62). The anaemia may be severe, with haemoglobin values below 70g/L (31,32).

Immunoglobulin, complement, or both, may be found on the surface of red cells in between 18% and 65% of patients; however, fewer than 10 percent of patients manifest autoimmune haemolytic anaemia during the course of their illness (32,61,63,64). Thus a positive direct Coombs' test in the absence of haemolysis should be regarded as one of the multiple abnormal serologic findings in SLE, which in part is a reflection of the hyperreactivity of these patients to auto-and hetero-antigens. Autoantibodies to red cells in SLE patients are warm antibodies, most commonly belonging to the IgG class, with IgG1 being the predominant subclass.

The Coombs' test pattern of reactivity in these patients is usually either Type II, involving both immunoglobulin and complement components on the red cell surface, or Type III, in which red cells are coated with complement components alone (65,66,67). The specificity of warm antibodies in SLE is not completely known; however, antibody eluted from Coombs' test-positive SLE red cells usually shows no known Rh specificity (68). These antibodies often show a strong reaction with Rh null cells (69), thus suggesting that the specificity of the immunoglobulin is directed against (a) non-Rh determinants on the red cells (70,71).

A few cases of SLE patients with haemolytic anaemia associated with cold agglutinins have been reported (61,64,72,73). However, the Coombs' test in these patients has been described as variable, and most have had low titer cold agglutinins. Thus a confirmed association between SLE haemolytic anaemia and cold agglutinins as the cause of haemolysis is tenuous (73).

Addition of large amounts of erythropoietin to bone marrow cultured in vitro results in the formation of committed stem cell colonies, namely BFU-E, or burst forming unit-erythroid. BFU-E's are primitive erythroid elements that synthesize embryonal haemoglobin and are unable to differentiate to the anucleate mature red cell stage. If grown in cultures to which homologous serum enriched with erythropoietin has been added, the BFU-E's mature into CFU-E's, which are colony forming unit-erythroid. These then produce normal anucleate red cells containing adult haemoglobin.

A number of examples of humoral inhibitors of erythropoiesis have been found in SLE patients (74,75,76,77,78,79). Five anaemic patients were studied by Kallen et al (75); their sera suppressed CFU-E growth of normal human bone marrow and all were steroid responsive. Dainiak et al (76) demonstrated that serums from 7 severely anaemic patients with SLE contained a factor which inhibited both BFU-E and CFU-E proliferation and/or differentiation with both autologous and allogeneic bone marrow in vitro. This inhibitor had immunoglobulin properties and was removed by plasmapheresis or steroids. Meyer et al (77) suggested that a circulating autoantibody in an SLE patient causing autoimmune haemolytic anaemia could also influence the proliferation or maturation of erythroid progenitor cells. There may be a role played by T-cell mediated suppression of erythroid progenitor cells (78), or by an IgG complement-dependant antibody (79).

Treatment

The anaemia of chronic disease in SLE is rarely life-threatening, and treatment should be directed at the underlying disease process. Modest doses of corticosteroids are frequently efficacious in the treatment of the systemic manifestations of the disease, and thus secondarily, the associated anaemia. There is no response to oral iron therapy (36), unless concomitant absolute deficiency exists (80). In experimental animals, the anaemia can be corrected by giving erythropoietin (47,48). The role of replacement therapy with this recombinant hormone in correcting the anaemia and improving the quality of life in patients with SLE remains to be determined.

The first modality of treatment for SLE acquired haemolytic anaemia is corticosteroids at a dose of 1-1.5mg/kg/day. Approximately 75% of patients are expected to respond (81,82). Splenectomy is the second option in patients unresponsive to corticosteroids or in whom unacceptably high doses are required to suppress disease activity. However, this procedure rarely produces any long-term favourable results in the majority of instances of SLE-induced haemolytic anaemia (1). Refractory cases of immune haemolytic anaemia may respond to immunosuppressive therapy, and uncontrolled trials have claimed that these agents are valuable in this setting (81,82,83,84). The drugs most frequently used have been azathioprine and cyclophosphamide. Their use should be limited to resistant disease, as side effects, as discussed above, may be serious, particularly with long term therapy.

Early studies evaluating the effectiveness of Cyclosporin-A in autoimmune diseases suggest that this agent may have a role in treating refractory immune cytopenias, including haemolytic anaemia (30).

Leucopenia

Leucopenia, defined as a white cell count below $4x10^9$/L, is reported to occur in between 35-80% of SLE patients (4,31,33,34). This may be the result of a depression in circulating granulocytes or lymphocytes or both; however the magnitude of the decrease in granulocytes is usually much greater than that of lymphocytes (72).

A number of mechanisms for the granulocytopenia have been suggested, but convincing proof is lacking. Those incriminated here include decreased mobilization and drugs, accounting for the non-immune group, and immune peripheral destruction and central humoral inhibition.

Leucopenia - non-immune mechanisms

Investigation of bone marrow granulocyte reserves may be performed by intramuscular injection of etiocholanolone, a naturally occurring hormone metabolite which is a potent stimulus for the mobilization of bone marrow granulocytes. In this in vivo test, granulocyte responses to etiocholanolone were found to be abnormal in 62% of SLE patients (85). There was no correlation between baseline granulocyte count and the magnitude of response to etiocholanolone. Most of the patients showing an abnormal response had normal bone marrow granulocyte reserve. However, the validity of this study has been challenged, as corticosteroids in dosages greater than 20mg of prednisone per day maximally challenge the bone marrow with regard to mobilization of granulocyte reserve (86). The average prednisone dose in the patients studied here was 34mg per day, which suggests that they were already maximally stimulated and would thus not be expected to show a normal granulocyte response to etiocholanolone. A further study also showed a depressed response to etiocholanolone in a patient, with improvement after azathioprine and/or prednisone (87).

Drug induced granulocytopenia, for example due to phenylbutazone or azathioprine, should be borne in mind, and other factors such as hypersplenism and infection may be contributory.

Leucopenia - immune mechanisms

Antibodies to granulocytes represent an important mechanism, with peripheral destruction of the antibody-coated cells. Patients with SLE have increased granulocyte-associated IgG, which results from binding of both circulating immune complexes and granulocyte autoantibodies. However, the level of granulocyte-associated IgG does not correlate with the granulocyte count (88). Of the many issues that remain unresolved, four are relevant. These are whether immune complexes alone cause granulocytopenia or whether granulocyte autoantibodies are also involved; why immune complexes might cause only granulocytopenia since there are many other target tissues, and why many patients with circulating immune complexes are not granulocytopenic.

The problem of the relevance of granulocyte antibodies is further compounded by the multitude of tests available for measuring anti-neutrophil antibodies, which include granulocytotoxic assays, staphylococcal protein A (SPA) assays, leucoagglutination assays, antiglobulin immunofluorescence techniques, antiglobulin consumption assays, radio-labelled antiglobulin tests and solid-phase immunoassay (89,90,91,92,93,94). Differences in specificity and sensitivity make it difficult to compare results of the various tests. Furthermore, the presence of isoantibodies against leucocytes, which may be a consequence

of multiple pregnancies and/or blood transfusions, must be differentiated from true leucocyte autoantibodies in the interpretation of the results.

Killman (95) performed leucocyte agglutination tests on patients with connective tissue diseases, and found that the prevalence of these agglutinins was higher in the SLE group than in the other connective tissue diseases. Two independent studies have demonstrated the high frequency of positive direct antiglobulin consumption tests on leucocytes in SLE (96,97) . Dausset et al reported positive results in 22 of 24 SLE patients tested (96), while Engelfriet et al found 9 positives of 23 SLE patients. Circulating antibodies reactive with normal human leucocytes were detected with the indirect antiglobulin consumption test in over 90% of SLE patients (97).

A simple in vitro approach to elucidating the cause of granulocytopenia in patients with SLE is not presently available (98). However, efforts to determine whether there is increased granulocyte-associated IgG are probably valuable because such patients are said to respond well to corticosteroids (99).

Colony forming units in culture, or CFU-C, are haematopoietic colonies which are grown in vitro and represent committed stem cells. Addition of colony stimulating factors to the culture medium results in enhanced production of GM-CFUc's, which are colony forming unit-granulocyte macrophage. These then generate granulocytes and macrophages.

Central bone marrow depression of granulocyte synthesis has been demonstrated using the colony-forming unit assay. Mouse bone marrow colony-forming units were incubated in the presence of human colony-stimulating factor as a model for in vivo human marrow production. Forty-three percent of SLE sera retarded bone marrow colony formation, whereas none of the control sera showed this effect. The degree of marrow colony retardation was directly correlated with amounts of test serum added (100). Further support for bone marrow depression of granulopoiesis in SLE patients was seen in a study of 16 patients. Here the number of CFU-C in the bone marrow of these patients was significantly decreased in comparison with the control group, and furthermore these numbers correlated strongly with the peripheral granulocyte/monocyte counts. In addition, peripheral blood and bone marrow T-lymphocytes suppressed the colony formation of autologous or allogeneic bone marrow CFU-C, suggesting a suppressor role of T-lymphocytes on marrow CFU-C (101). Serum from a patient with SLE and aplastic anaemia was shown to contain an IgG complement-dependant antibody which suppressed CFU-GM growth from normal donor bone marrow. The antibody was removed by plasma exchange (102).

Treatment

Management of clinically significant granulocytopenia has conventionally relied on various forms of immunosuppression, including corticosteroids (99), and cyclophosphamide (31,32,99,103), with variable results. The role of haematopoietic growth factors has not yet been evaluated in this setting.

Lymphocytopenia is common in SLE and is usually due to lymphocytotoxic antibodies. It will not be discussed further in the present study.

Thrombocytopenia

Thrombocytopenia, when defined as less than about $150x10^{9}/L$, occurs in 7-41% of patients with SLE (3,4,31,32,33,72,104,105) with a minority (5%) having thrombocytopenia of a severe degree. Purpuric lesions are the main manifestations of the low platelet counts. Thrombocytopenia of mild to moderate degree ($50\text{-}100x10^{9}/L$), often appears during exacerbations of the disease without any bleeding tendency (1).

Thrombocytopenia - non-immune mechanisms

Depression of thrombocyte production as shown by a markedly reduced number of megakaryocytes in the marrow is rare (106,107).

Thrombocytopenia - immune mechanisms

In contrast to the above, peripheral platelet destruction is an established mechanism and autoimmune thrombocytopenia can be a prodrome for SLE (108). Some patients with thrombocytopenic purpura labeled as idiopathic at the onset later develop a classical clinical course of SLE (109,110). Thus thrombocytopenia in SLE, as in chronic immune thrombocytopenic purpura (ITP), has been considered to be due to increased peripheral destruction of platelets brought about by autoimmune mechanisms. Platelet survival studies in SLE with chromium 51-labeled platelets have demonstrated shortened life span (111).

Many in vitro tests have been devised to demonstrate anti-platelet antibodies in SLE, but a test of universally accepted specificity, sensitivity and reproducibility is not available. Anti-platelet antibody assays which have been employed in SLE include platelet agglutination (112,113), direct antiglobulin consumption test (96,114), dextran agglutination test (115), platelet factor 3 method (116), and indirect immunofluorescence test (117,118). These tests are frequently positive in thrombocytopenic SLE patients, but have limited clinical application because of technical deficiencies. The latter include measurement of circulating immune complexes with the anti-platelet antibodies, as well as lack of sensitivity and specificity. Karpatkin et al (116) detected platelet antibodies in 78% of SLE patients, of whom only 14% were thrombocytopenic.

Tests that measure platelet-associated IgG are widely used (119). Platelet-associated IgG has been measured and found to be increased in practically all SLE patients with thrombocytopenia (120,121,122,123,124). Kelton and co-workers (122) reported an inverse correlation between platelet count and platelet-associated IgG in 10 thrombocytopenic SLE patients. Mulshine et al (125) confirmed this inverse correlation, further observing that SLE patients with normal platelet counts had platelet-associated IgG even lower than that of normal controls. On the other hand, Bonacossa and associates (126) found elevated platelet-bound IgG in nonthrombocytopenic SLE patients, and this too has been supported by other workers (127). The IgG antiplatelet antibody in SLE involves all 4 subclasses of IgG (128).

The nature of the platelet-associated IgG is not completely understood. It may represent IgG antibody bound to platelet specific antigens, known as autoantibodies, IgG antibody bound to HLA or blood group antigens or to exogenous antigens adsorbed on the surface of platelets, IgG non-specifically bound to damaged platelets, or circulating immune complexes bound to platelet surface Fc receptors (119,129). Kaplan et al (130) showed that three out of nine SLE patients with thrombocytopenia possessed a true autoantibody to platelet-specific antigens.

In those patients with no detectable increase in platelet associated IgG and thrombocytopenia, cellular immune destruction may be causative, as in the case of idiopathic thrombocytopenic purpura without detectable surface antibody (131,132).

An increase in bone marrow megakaryocyte numbers is paralleled by an increase in megathrombocytes, which are large platelets, in the peripheral blood (133). Two-thirds of non-thrombocytopenic patients with SLE were found to have an increased megathrombocyte count and number of megakaryocytes (133). Five of these eight patients studied had shortened platelet survival. This suggested the presence of a

compensated thrombocytolytic state, a theory supported by Karpatkin et al (134). These authors showed a similar increase in the percentage of megathrombocytes in non-thrombocytopenic patients with SLE, and detected platelet antibody in 85 per cent of SLE patients.

Treatment

Corticosteroid therapy in doses of 1.5mg/kg/day of prednisone equivalent is the treatment of choice for the autoimmune thrombocytopenia of SLE. Responses often occur only after 4-6 weeks. Steroid therapy failure occurs in 10-20% of these patients, who may require immunosuppressive agents or splenectomy (1). Azathioprine and cyclophosphamide are beneficial in thrombocytopenia associated with SLE (135,136,137). A 4-5 week therapeutic trial of the immunosuppressive agent is recommended before considering the thrombocytopenic patient a treatment failure (138). Vincristine may be of use in life-threatening thrombocytopenia unresponsive to other treatment mobilities (139). Danazol (140,141,142) and high-dose intravenous gammaglobulin (143) have also been used for refractory disease. Plasma exchange is reserved for life-threatening situations (28).

OTHER ABNORMALITIES

A number of other haematological problems, mainly of the coagulation system, may occur in SLE patients.

Mild qualitative platelet defects, which are usually not associated with significant bleeding problems, are sometimes found (144).

Patients may rarely develop antibodies to certain haemostatic factors, including the Von Willebrand factor and factors VIII, IX, and XI, with resultant haemorrhage (1).

The lupus anticoagulant is an immunoglobulin reacting with procoagulant phospholipids occurring in approximately 10 percent of SLE patients. It is thought to act by inhibiting the activation of prothrombin by prothrombinase. Clinical associations include venous and arterial thrombosis and recurrent spontaneous abortions, but not haemorrhage. A subset of patients with the lupus anticoagulant have hypoprothrombinaemia, and they may suffer from severe bleeding (1).

In summary, cytopenias appear to be common findings in SLE patients. However, the mechanisms responsible are not well defined. This study was thus undertaken to document the incidences of the individual cytopenias in our local SLE patient population, as well as to attempt to contribute to the understanding of underlying mechanisms. This information could aid in future therapeutic decisions.

CHAPTER THREE
MATERIALS AND METHODS

MATERIALS AND METHODS

PATIENT SELECTION

Seventy patients fulfilling the presently accepted criteria for the diagnosis of systemic lupus erythematosus (4) were studied (Table 2.1). Except for two patients who were hospitalized with newly diagnosed lupus, the remainder were clinically stable and attended the Groote Schuur Hospital Lupus outpatients clinic. None of the patients were receiving steroid or immunosuppressive therapy at the time of the study, and splenectomized subjects were excluded. Clinical status, lupus serology and therapy was documented. Bone marrow studies were conducted on sixteen patients, six of whom belonged to the original group of seventy, the remainder being SLE patients who required this procedure for therapeutic reasons. The marrow examinations in the former six subjects were all performed at a later stage than the peripheral blood studies.

LABORATORY PROCEDURES

PERIPHERAL BLOOD

These specimens were obtained from all the seventy patients on a single occasion after an overnight fast and the following investigations performed:

- A full blood count (FBC) on the Coulter S Plus 11.
- Reticulocyte count, expressed as a percentage with correction for red cell count (145).
- Monospecific direct Coombs' test, utilizing specific anti-IgG and anti-complement antisera (145).
- Serum iron, percentage saturation, total iron binding capacity (TIBC) and serum ferritin by radioimmunoassay (145).
- Serum and red cell folate and serum vitamin B12 levels by radioimmunoassay (145).
- Measurement of serum anti-neutrophil antibody levels were kindly performed by the laboratory of Professor J.P. Crowley, of Rhode Island Hospital, Providence. A cellular enzyme linked immunosorbent assay (CELISA) technique was employed, and the assay was performed twice on each patient, utilizing normal human polymorphonuclear neutrophils (PMN's) and an HL-60 line as target cells respectively (146). Briefly, the assay features a solid phase microtitre method in which the enzyme urease is fixed to the antihuman globulin conjugate reagent and uses either PMN's or HL-60 line cells as targets. The assay detects autoimmune and isoimmune antineutrophil antibodies and IgG containing circulating immune complexes. An attempt was made to establish this assay in our laboratory, based on the method of Crowley et al (146). This proved unsuccessful, however, due to failure to obtain significantly different absorbance readings between heated (positive control) serum and unheated (negative control) serum. This problem remains unsolved at the present time.
- Platelet-associated immunoglobulin levels were quantitated by direct ELISA technique, using a modification of the method of Nel and Stevens (147). The assay measures platelet-associated IgG by measuring the competition for binding of anti-IgG between the platelet-associated IgG and IgG on the surface of the wells on microtitre plates. Polyvinyl microtitre plates (Nunc Immunoplate Cat. No.

439454) are coated with IgG from pooled human sera. Platelets are prepared from 10-15mls EDTA-anticoagulated blood by differential centrifugation, and the count is adjusted to $50x10^9$/L. The platelets are then incubated in the microtitre wells, together with antihuman IgG which is conjugated to horseradish peroxidase enzyme (Dako Code No. P214). The anti-human IgG binds to Ig on the wells and to IgG on the platelet surface, if this is present. The greater the amount of antiglobulin consumed by the reaction with platelets, the less is available to bind to the immune adsorbent in the microtitre well. Platelets are then washed out of the wells and the unbound fraction of antiserum is quantitated by allowing it to react with a chromogenic substrate (o-phenylenediamine/hydrogen peroxide) for the enzyme and measuring the intensity spectrophotometrically. The colour produced is inversely proportional to the amount of platelet membrane-bound IgG. This is calculated by subtraction and expressed as a percentage binding. A series of standards of IgG (Behring Code No. ORDT 06/07) ranging from 25-800ng IgG are assayed simultaneously, and the amount of platelet-associated IgG is obtained from the sigmoid-shaped standard curve using the calculated percentage binding. Platelets from normal donors gave values of 10-100ng IgG/10^7 platelets.

BONE MARROW - MORPHOLOGY

Sixteen SLE patients, of whom six belonged to the original group of seventy who had earlier had peripheral blood studies, required bone marrow aspiration and trephine biopsy in the course of their disease. At the time of marrow sampling, seven were receiving steroid and/or immunosuppressive (cyclophosphamide) therapy.

The marrow samples were obtained from the posterior iliac crest and were prepared and stained in the usual manner. They were examined morphologically to assess cellularity and the individual cell lines. Iron status and megaloblastic features were noted and the anatomy of the blood vessels examined.

BONE MARROW - IN VITRO CULTURE

In vitro bone marrow culture studies were performed on 10 of the patients (five of the peripheral blood study group), from whom sufficient marrow aspirate could be obtained, as follows:

Erythroid Colony Culture

10 ml of bone marrow aspirate and 10 ml of clotted blood was obtained from each patient and processed according to a modified method of Iscove (148) and Johnson (149). The aspirate was collected into 0.3ml preservative free heparin (Pularin), diluted 1:3 in 20% AB serum in McCoy's tissue culture medium (Gibco Cat. No. 074-1550) and separated on Ficoll Hypaque (1.077g/ml). The mononuclear cells were washed in 20% serum in McCoy's tissue culture medium, and $2.0x10^5$cells/ml were added to wells of tissue culture plates. 0.5ml of the respective serum type - AB, patient's serum (room temperature), and patient's serum (heat inactivated at 56° C for 15 minutes) was added to each of three tubes, together with 0.125ml erythropoietin (Connaught) of 0.25units/ml final concentration for CFU-E and 2.0units/ml final concentration for BFU-E, 2-mercapto-ethanol (Merck), with a final concentration of 10^{-4} , 0.125ml asparagine of 0.02g/ml final concentration, the appropriate volume of cells, which were equal to $2.0x10^5$cells/ml, and agar (Difco Bacto-agar 0140-01) at a final concentration of 0.3%. The mixtures were then plated into wells of a culture dish. The plates were incubated in humidified 5% CO_2 at 37° C for 7 days (CFU-E) and 14 days (immature BFU-E). Agar gels were harvested by fixing with formal acetone buffer, washed with distilled water, allowed to dry on microscopy slides and stained with haematoxylin-tetra-aminobiphenyl hydrochloride (BDH). The number of CFU-E, defined as fewer than

32 and 32-64 cells per group, and BFU-E, greater than 64 cells per group, were recorded for each of the three different serum groups. The numbers obtained in the control (AB) serum group were compared with those in the patient groups (plain and decomplemented serum).

Granulocyte:Macrophage Colony (GM-CFUc) Culture

10ml of bone marrow aspirate was obtained per patient and processed by a technique adapted from a micro agar culture method for cloning erythropoietic progenitors (150). The aspirate was collected into 0.3ml of preservative free heparin (Pularin), diluted 1:1 with 20% AB serum in McCoy's tissue culture medium (Gibco Cat. No. 074-1550), and separated on Ficoll Hypaque (1.077g/ml). The mononuclear cells were washed in McCoy's tissue culture medium. A suspension of mononuclear cells at a concentration of $2.0x10^5$cells/ml was made in McCoy's tissue culture medium with each of 15% foetal calf serum (FCS) (State Health Laboratories), patient's serum and patient's serum heated to 56° C for 15 minutes. The final concentration of agar (Difco Bacto-agar 0140-01) added was 0.3%. 0.25ml of this suspension was pipetted into multiwell tissue culture plates. An overlayer of 0.25ml of colony stimulating activity (CSA), derived from leucocyte conditioned medium, (CSA-LCM) diluted in the appropriate serum was added to the wells. A control well overlayered with the appropriate 15% serum in McCoy's tissue culture medium without CSA was set up for each patient. The plates were incubated in 5%CO_2 at 37°C for 7 and 12 days respectively. Agar gels were harvested by fixing with 2.5% glutaraldehyde, washed with distilled water, allowed to dry on microscope slides and stained with a Romanovsky stain (May-Grunwald-Giemsa). The number of colonies, defined as a group of more than 40 cells, and clusters, a group of 4-40 cells, was recorded for each of the three serum groups. The numbers obtained in the control (FCS) group were contrasted with the patient groups (plain and decomplemented serum).

STATISTICAL ANALYSIS

Correlations were analyzed by linear regression and determination of correlation coefficients. Differences between the mean values in the in vitro bone marrow culture studies were evaluated using the Student's t test.

CHAPTER FOUR
RESULTS

RESULTS

Seventy patients had peripheral blood studies over a period spanning 20 months. In the same period, sixteen bone marrow examinations were performed. Of these, six were done on the original peripheral blood group, all at a time subsequent to the blood studies. The remaining ten marrows were from other SLE patients.

PATIENT DATA

In the group who had peripheral blood studied, the age of the patients ranged from 15 to 65 years with a mean of 39 years. There were 5 males and 65 females. There was a predominance of mixed ancestry (Coloured) (151) patients in this study, which reflects the true incidence as seen in the Western Cape. The sex and race distribution is shown in Figure 4.1.

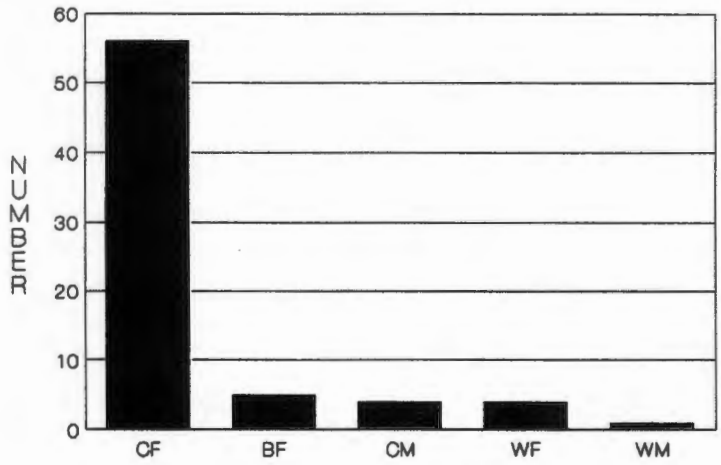


Fig. 4.1 Race and sex distribution of SLE patient population. (C = coloured (mixed ancestory); B = black; W = white; M = male; F = female).

The mean duration of diagnosed SLE at the time of the study was 4.3 years, ranging from newly diagnosed patients, of which there were two, to 18 years.

Thirty-six of the seventy patients were assessed as having active disease on the basis of clinical and/or serological findings. Serological tests were not undertaken on those patients thought to have only mildly active or inactive lupus. Besides two newly diagnosed patients, the remainder were chronic and clinically stable, being regularly seen at the Lupus outpatients clinic. Three patients had chronic renal failure. None were taking steroid or immunosuppressive medication at the time of study, and splenectomized subjects were excluded. Patient profiles are given in the Appendix. Subjects having bone marrow studies all had active disease, based on the above criteria. They included seven on steroid and/or immunosuppressive therapy and three with chronic renal failure.

LABORATORY DATA

CYTOPENIAS

In the peripheral blood study group, a total of 29 patients exhibited cytopenia(s) of one or more cell lines. Nineteen patients were anaemic, nine were both anaemic and leucopenic, and only a single patient was pancytopenic, defined as haemoglobin less than 110g/L, white cell count below $4x10^9$/L, and platelets below $100x10^9$/L. This is diagrammatically represented in Figure 4.2.

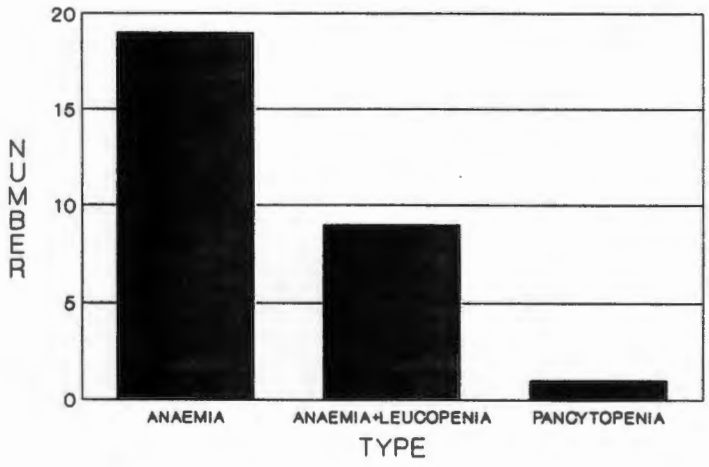


Fig. 4.2 Frequency of the cytopenias (n = 29)

ANAEMIA

Peripheral Blood Data

The mean haemoglobin value was 113 g/L, with a range of 63-169 (males) and 60-152 (females). Twenty-nine patients (41%) were anaemic, with a haemoglobin level below 110 g/L. The majority of these, 12 (17%), were shown on iron studies to have an anaemia of chronic disease. They were closely followed by 10(14%) patients with iron deficiency. Two patients were folate deficient, one was Vitamin B12 deficient, and the remaining four had anaemias which could not be characterized. All three chronic renal failure patients were anaemic (range 63-108g/L). One belonged to the chronic disorders anaemia group, one was iron deficient, and one was in the non-characterized group. These features are shown in the diagram in Figure 4.3.

Coombs' test was positive in 17 (24%) patients. The specificity was as follows: IgG positive - 11, complement positive - 4, and both IgG and complement positive - 2 patients. This is diagrammatically represented in Figure 4.4. None of these patients exhibited haemolytic anaemia (defined here as anaemia and Coombs' positivity with raised reticulocyte count in the presence of normal iron, folate and vitamin B12 status) at the time of the study; however, four had either had an autoimmune haemolytic anaemia previously or were observed to develop this complication with time. There was no correlation between anaemia and Coombs' positivity. .

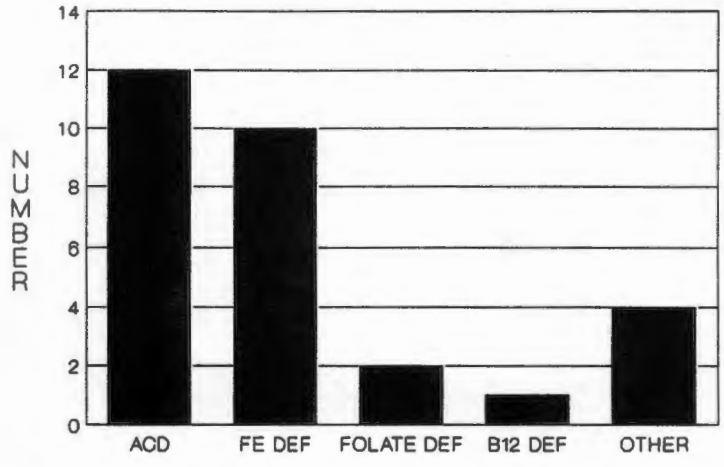


Fig. 4.3 Characterisation of anaemia (n = 29). (ACD = anaemia of chronic disease; FE DEF = iron deficiency; FOLATE DEF = folate deficiency; B12 DEF = vitamin B12 deficiency.)

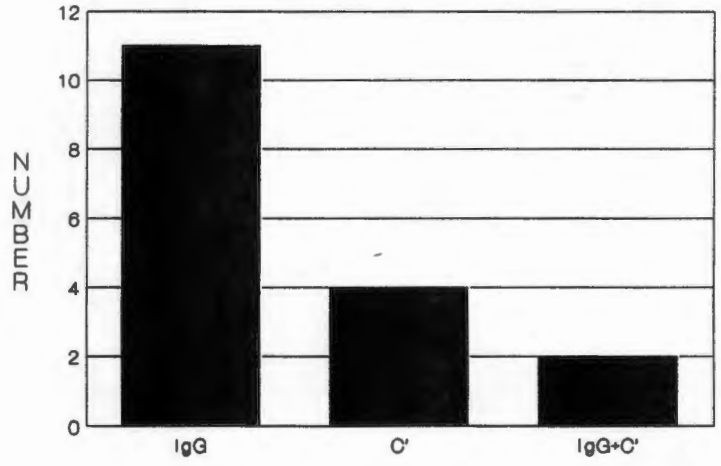


Fig. 4.4 Specificity of Coombs' positivity (n = 17). (C' = complement.)

In vitro erythroid bone marrow cultures

In vitro erythroid bone marrow cultures were performed in 10 patients. Table 4.1 shows the results obtained. At the time of sampling, nine of the ten patients were anaemic, with haemoglobin levels between 36-108 g/L and seven were receiving steroid therapy, one of whom was also on cyclophosphamide. Four patients had raised anti-DNA titers. There was no significant difference in BFU-E or CFU-E growth on addition of autologous serum (plain or decomplemented) to the patient's marrow, compared to control serum. Plate 4.1 illustrates the in vitro BFU-E growth in one of the patients' bone marrow on addition of autologous serum at room temperature to the cultures.

Table 4.1. In vitro erythroid bone marrow cultures.

BFU-E	colonies/2x10^{5}cells	p value
control (AB serum)	32 ± 77	
autologous serum	2 ± 5	NS
control (AB serum)	32 ± 77	
autologous serum 56°C	26 ± 63	NS

CFU-E	colonies/2x10^{5}cells	p value
control (AB serum)	349 ± 326	
autologous serum	291 ± 167	NS
control (AB serum)	349 ± 326	
autologous serum 56°C	318 ± 121	NS

NS = not significant

LEUCOPENIA

Peripheral Blood Data

The mean white cell count was 5.7x10^9/L, with a range of 2.4-11x10^9/L. Sixteen patients (23%) were leucopenic with a white cell count below 4x10^9/L and 8 (11%) were absolutely neutropenic, defined as a neutrophil count less than 2.0x10^9/L. Lymphopenia, with an absolute count less than 1.5x10^9/L, was present in 34(49%) subjects.

Assay of anti-neutrophil antibody activity showed 41 (59%) positivity in the CELISA assay with HL-60 cells, 6 (9%) positive with the normal human polymorphonuclear neutrophils (PMN's) and 4 (6%) positive with both assays. These results are shown in Figure 4.5.

No correlation existed between absolute neutrophil count and anti-neutrophil antibody level with either of the assays (HL-60: p = 0.69, r = -0.048; PMN's: p = 0.02, r = 0.27).

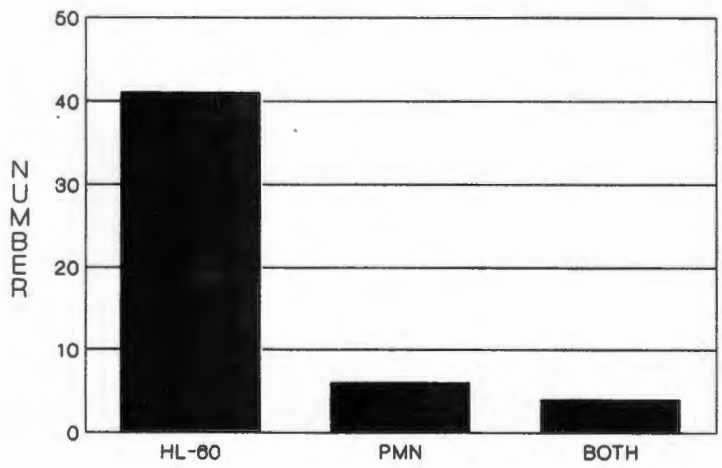


Fig. 4.5 Anti-neutrophil antibody positivity by CELISA assay (PMN = polymorphonuclear neutrophils).

In vitro GM-CFUc bone marrow cultures

In vitro GM-CFUc bone marrow cultures were performed on 10 patients. The results are shown in Table 4.2. At the time of sampling, only one patient was leucopenic, and none were absolutely neutropenic. Seven were receiving steroid therapy, two of whom were also on cyclophosphamide. Four patients had raised anti-DNA titers. Both on days 7 and 12 addition of autologous serum (plain or decomplemented) to the patient's marrow gave rise to significantly enhanced GM-CFUc growth. However, as the control serum here was foetal calf and not normal human serum, the difference in growth may be accounted for by this species difference, with possible inhibition of growth occurring with the foetal calf serum. Plate 4.2 illustrates the comparison of GM-CFUc growth in one patient on day 7 with addition to the cultures of control versus autologous serum.

Table 4.2. In vitro GM-CFUc bone marrow cultures.

Day 7		
	colonies/2x10^{5}cells	p value
control (FCS) autologous serum	173 ± 118 237 ± 138	p<0.05 (CL 64 ± 50)
control (FCS) autologous serum 56°C	173 ± 118 213 ± 125	p<0.05 (CL 40 ± 32)
Day 12		
	colonies/2x10^{5}cells	p value
control (FCS) autologous serum	64 ± 51 133 ± 81	p<0.05 (CL 69 ± 56)
control (FCS) autologous serum 56°C	64 ± 51 113 ± 72	p<0.05 (CL 49 ± 46)

FCS = foetal calf serum. CL represents confidence limits at 95% level.

THROMBOCYTOPENIA

The mean platelet count of the seventy patients was 267x10^9/L, with a range of 88-593x10^9/L. Only 2 patients (3%) were thrombocytopenic, with a platelet count below 100x10^9/L.

Platelet associated immunoglobulin levels were raised in 21 (30%) patients, with values ranging from 110-1143ng IgG/10^7 platelets. However, there was no correlation between platelet count and platelet antibody level ($p = 0.099$, $r = -0.199$). Furthermore, no correlation existed between platelet count and mean platelet volume (MPV) ($p = 0.058$, $r = -0.28$).

BONE MARROW FINDINGS

Sixteen bone marrow samples were examined by aspiration and trephine biopsy. Fifteen patients were anaemic at the time of sampling, with haemoglobin ranging from 36-108g/L, mean 78g/L; five were leucopenic with white cell counts ranging from 1.3-3.9x10^9/L and a mean of 3x10^9/L, and four had platelet counts below 100x10^9/L, ranging from 36-88x10^9/L, with a mean of 63x10^9/L. Seven subjects were receiving steroid and/or immunosuppressive (cyclophosphamide) therapy.

The bone marrow was assessed as normocellular in 5 patients, mild to moderately hypocellular in 7 and slightly hypercellular in 4. Erythroid line was deficient in 5 patients with 2 patients having mild granulocytic hyperplasia. Megakaryocytes were prominent in 3 cases. Iron stains revealed 8 subjects with an anaemia of chronic disease, while 3 were absolutely iron deficient. There was evidence of megaloblastosis in 5 patients. Features of renal osteodystrophy were seen in 3 specimens, while none of the biopsies revealed evidence of vasculitis.

IMMUNOLOGICAL DATA

A positive correlation existed between anti-neutrophil antibody level with HL-60 cells and anti-platelet antibody level (p.05, r = 0.31), but not with the PMN assay (p = 0.05, r = 0.23).

Attempts to show a correlation between either haemoglobin, white cell count or platelet count and anti-DNA level as a marker of disease activity were negative. Values obtained for p and r were: haemoglobin: p = 0.027, r = -0.28; white cell count: p = 0.05, r = -0.25; platelet count: p = 0.42, r = 0.104.

Consideration of subjects with antibody titers raised against the respective haematological cells revealed that 8 were both Coombs' positive and anti-neutrophil antibody positive (by either assay); 3 were Coombs' positive and platelet antibody positive; 16 had antibodies to both neutrophils and platelets, and 3 patients were positive for all 3 antibody types. Of 21 subjects having one or more of these antibodies, 9 had anti-DNA titers above the normal range. None of the 3 patients having antibodies against all cells had raised anti-DNA levels. These figures are summarized in Table 4.3.

Table 4.3. Immunological data.

Test	Number Positive	
Coombs'; NAb	8	
Coombs'; Plt Ab	3	
NAb; Plt Ab	16	(positive correlation)
Coombs'; NAb; Plt Ab	3	
Anti-DNA Ab	9/21	

NAb = anti-neutrophil antibody
Plt Ab = platelet antibody

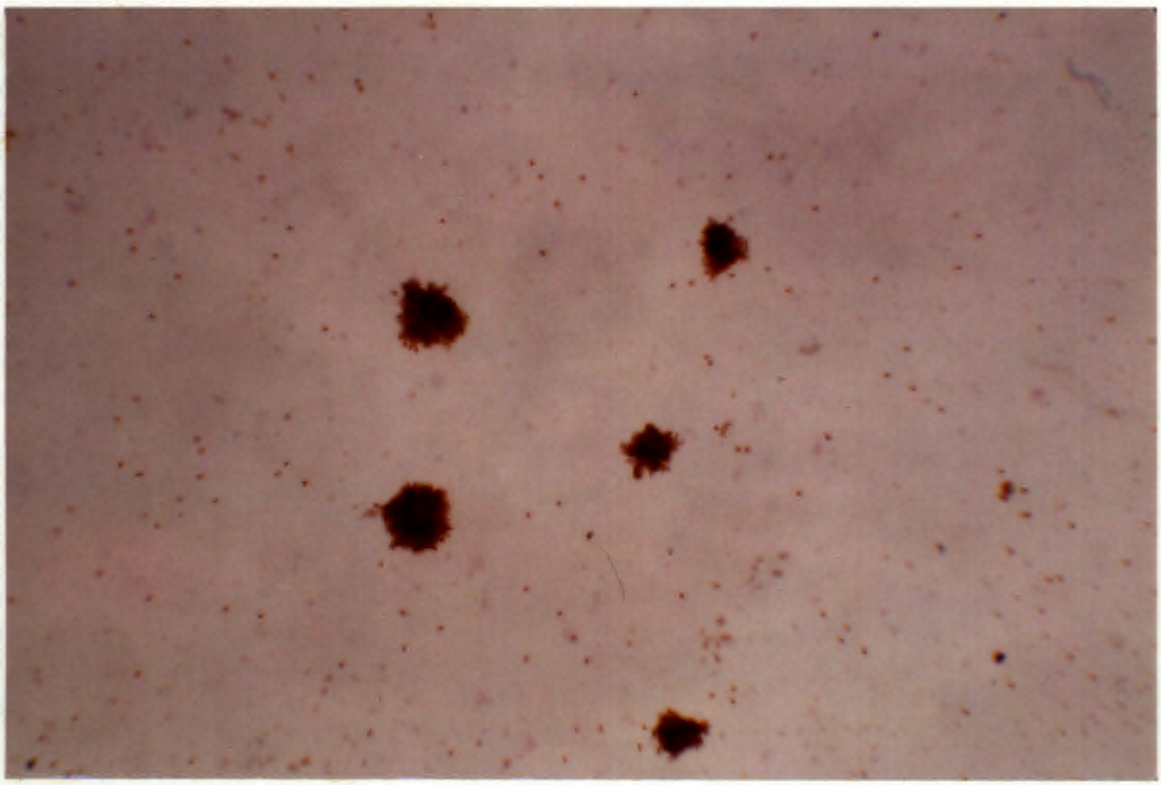
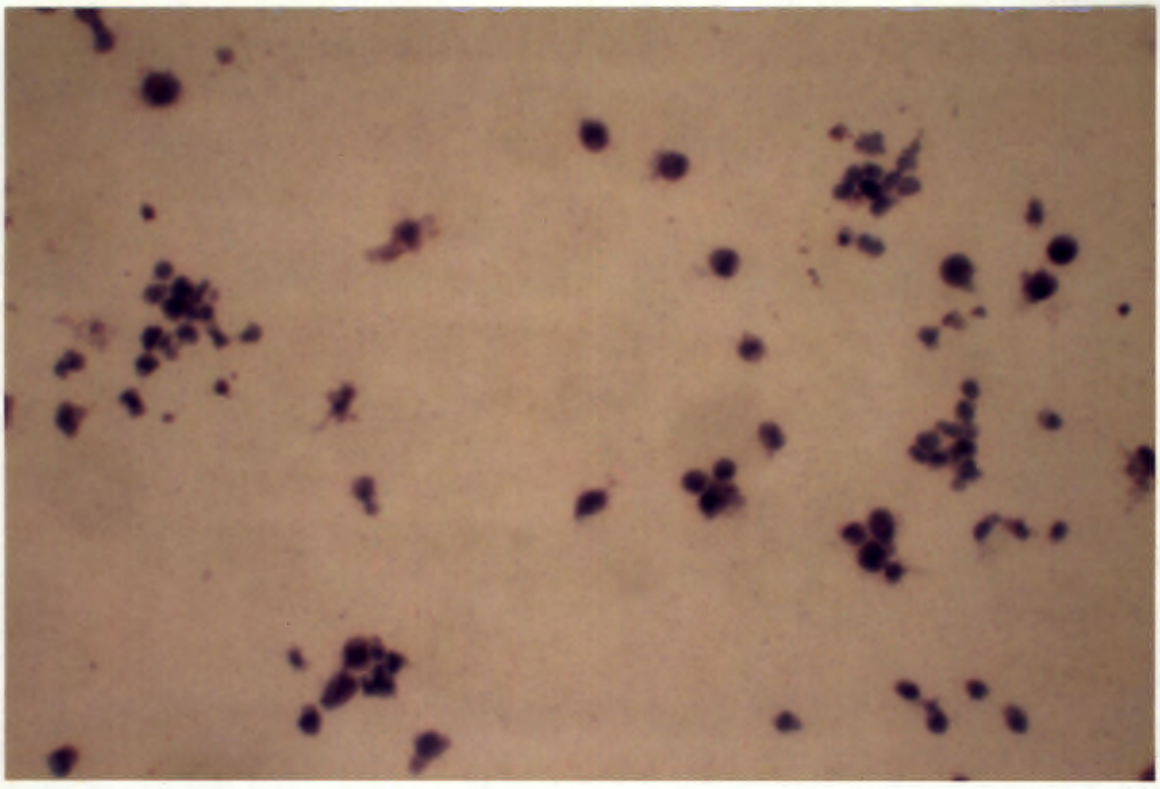
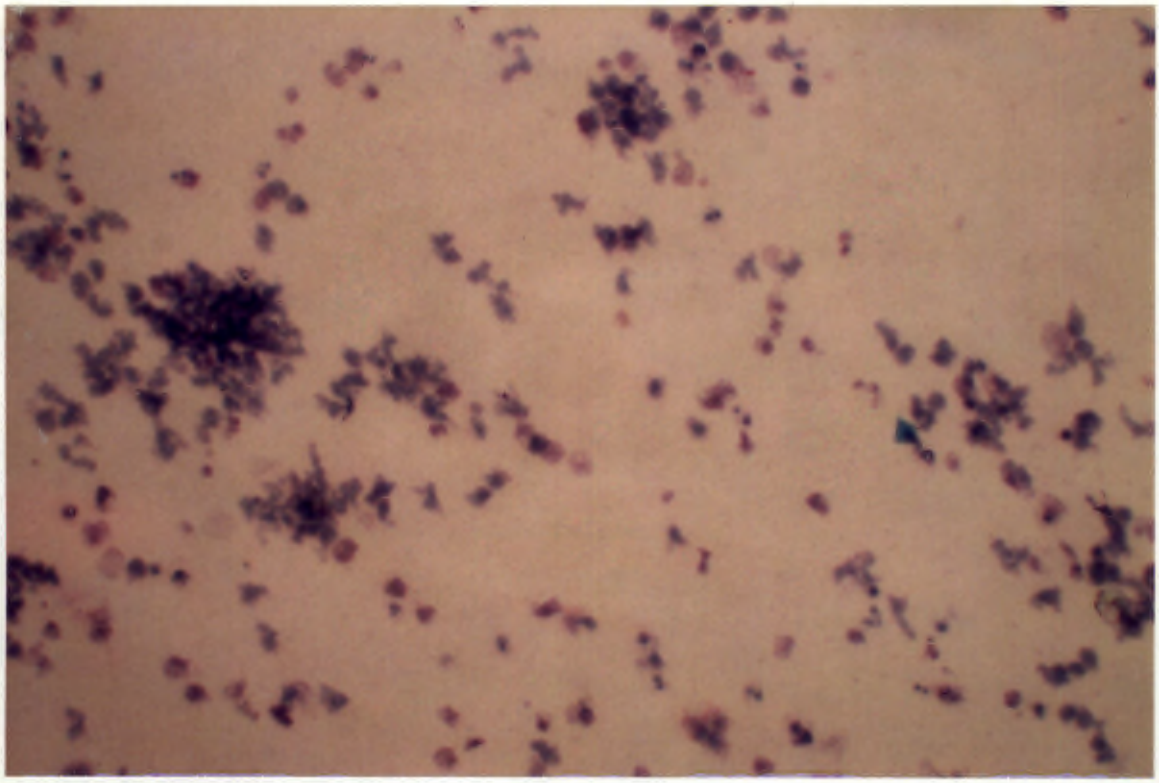


Plate 4.1. In vitro BFU-E in an SLE patient on addition of autologous serum at room temperature.



(a)



(b)

Plate 4.2 In vitro GM-CFUc on day 7 showing colonies and clusters on addition of control serum (a) and autologous serum (b).

CHAPTER FIVE
DISCUSSION

DISCUSSION

Systemic lupus erythematosus (SLE) is a multisystem disease characterized by the production of pathogenic autoantibodies and immune complexes causing tissue and cellular damage.

One of the main targets is the haematologic system, and the majority of SLE patients will demonstrate some abnormality involving it during the course of their disease.

This study has focused on the cytopenias encountered in SLE patients. In order to minimize the effects of drugs on haematological parameters, subjects were chosen who had either never been exposed to, or were not taking, steroids or immunosuppressive agents at the time of the study. Of seventy patients studied, thirty-six had active disease. However, except for two subjects who were hospitalized with newly diagnosed lupus, the remainder were chronic sufferers, being followed as outpatients.

Although SLE is characterized by recurrent exacerbations and remissions which may also be reflected in changing haematological status, patients were studied at a single time point. This enables one to establish a baseline measure of haematological function, upon which further fluctuations may be observed to be superimposed.

A number of studies have been conducted on large groups of SLE patients, in which the incidences of the cytopenias have been documented (31,32,33,34). However, these figures include patients at different stages of the disease, as well as those taking steroids and/or immunosuppressives. These factors may have a substantial effect on the figures obtained.

Elucidation of the mechanisms responsible for the cytopenias has been bedevilled by the very nature of SLE, being regarded as an autoimmune disease of unknown aetiology, together with the presence of various complicating factors, for example infection, drug therapy and concomitant renal failure. At present these mechanisms remain largely undefined, despite a large body of studies, many of which are of an immunological nature.

This study has documented the incidence of the individual cytopenias in 70 selected SLE patients and has attempted to define the mechanisms underlying these abnormalities.

ANAEMIA

The incidence of anaemia, defined as a haemoglobin of less than 110g/L, was 41% in this series, which is in keeping with the reported range of 38-98% (31,32,33,34). Consistent with previous studies (35), the majority of these patients (17%) had an anaemia of chronic disease. Fourteen percent of patients were anaemic on the basis of iron deficiency. Although this may be due to occult gastrointestinal blood loss (35) presumably on the basis of vasculitis or anti-inflammatory or steroid drug ingestion, the majority of the patients were females of childbearing age, in whom iron deficiency may be a common occurrence. The reason for folate deficiency in 2 patients, and vitamin B12 deficiency in 1 patient, may not be specifically related to SLE, and 4 subjects were anaemic due to an undetermined cause. Chronic renal failure was contributory in three cases.

Bone marrow examination was helpful in showing an anaemia of chronic disease in eight of sixteen patients studied, and also demonstrated mild to moderate erythroid hypoplasia in five, some of the latter possibly being on the basis of renal failure. None of the patients had red cell aplasia.

Coombs' positivity in 17(24%) of patients is consistent with the reported incidence of 18-65% (32,61,63,64). However, the pattern of Coombs' positivity here was most frequently IgG alone in 11 patients, with complement alone in 4, and both IgG and complement being positive in only 2 patients. This is at variance with other series, in which Type II, where both immunoglobulin and complement are found, or Type III reactions, with complement alone, are most frequently encountered (65,66,67). The reason for this difference in pattern of reactivity is not immediately obvious. Although none of the patients had autoimmune haemolytic anaemia at the time of study, four previously had, or subsequently developed, this complication in the course of their disease.

Erythroid cultures (CFU-E and BFU-E) were performed on 10 patients, but failed to show an inhibitory influence of autologous serum (either plain or decomplemented) on erythroid colony growth compared to control serum. Although CFU-E and BFU-E growth was decreased on addition of autologous serum (both plain and decomplemented) compared to control serum, these differences were not statistically significant. Although 9 of the patients were anaemic, the majority were on steroid therapy, which may conceivably mask serum immune activity. Furthermore, the numbers studied here were small and studies of larger groups of patients are necessary before definitive statements can be made. These patients should preferably not be receiving any form of immunosuppressive therapy.

A number of in vitro studies have demonstrated humoral inhibition of erythropoiesis in SLE patients. Yamasaki et al (78) demonstrated a decreased incidence of CFU-E in 9 SLE patients, and further showed that addition of allogeneic and autologous T-lymphocytes from SLE patients not receiving corticosteroid therapy suppressed CFU-E colony formation. Dainiak et al (76) demonstrated inhibitory activity in sera from 7 anaemic patients on both autologous and allogeneic BFU-E and CFU-E growth, and this was abolished with steroids and plasma exchange. Five cases of serum suppression of CFU-E growth in normal human bone marrow were described by Kallen et al (75), all of whom responded to steroids. Red cell hypoplasia in an SLE patient was shown to be associated with inhibition of erythroid colony growth by the IgG fraction of the patient's serum, suggesting the presence of an autoantibody to erythroid precursors (74). The serum of an SLE patient with aplastic anaemia was shown to contain a complement-dependant IgG antibody that inhibited erythroid and granulocytic colony formation of normal human bone marrow (79). This improved after plasma exchange with haematologic recovery. Meyer et al (77) demonstrated a serum inhibitor of erythropoiesis in an SLE patient with autoimmune haemolytic anaemia and periodic episodes of red cell hypoplasia.

The results of these studies suggest that humoral inhibition of erythropoiesis may be operative in a subset of patients, but demonstration that this is a widespread phenomenon in the pathogenesis of anaemia awaits larger scale in vitro bone marrow culture studies.

LEUCOPENIA

A white count below $4x10^9/L$ was found in 23% of patients. This is somewhat lower than the reported incidence of 35-80% (4,31,33,34). The observed difference may be due to the fact that almost half of the patients in this study had inactive disease.

The present study was concerned with granulocytopenia, although it is recognized that lymphocytopenia is a common occurrence in SLE. Eight (11%) patients were absolutely neutropenic.

The mechanisms underlying granulocytopenia in SLE are complex, and despite extensive investigations, few firm conclusions have been made. Contributing factors include impaired bone marrow mobilization

of granulocyte reserves (85,86,87); peripheral immune-mediated granulocyte destruction (98); and central marrow suppression of granulocyte synthesis (100,101,102). In some cases multiple mechanisms may be operative.

None of the bone marrows examined demonstrated granulocyte hypoproduction.

Studies assessing mobilization of marrow granulocyte reserves were not undertaken.

The difficulties in interpreting results of anti-neutrophil antibody assays have been discussed above. These include elucidation of the relative roles of granulocyte autoantibodies and immune complexes in the granulocytopenia, and evaluation of multiple different assays with varying sensitivities and specificities.

However, a number of studies have demonstrated the presence of anti-neutrophil antibodies in SLE patients (88,95,96,97,99), although the level of granulocyte-associated IgG does not correlate with the granulocyte count (88).

In the present study an ELISA assay utilizing both HL-60 cells and human polymorphonuclear neutrophils (PMN's) was employed for measuring serum anti-neutrophil antibody levels. Assay with the HL-60 cells was shown to be more sensitive, being positive in 41 (59%) patients, with 9% positivity with PMN's. No correlation existed between the antibody level and the absolute granulocyte count. Thus although direct comparisons are not possible between these and other assay results, granulocyte-associated immunoglobulin probably does play a role in peripheral destruction of these cells; however the magnitude of its contribution to granulocytopenia has not been determined. Demonstration of an anti-neutrophil antibody in a granulocytopenic patient may be still be useful, in that such a patient may be expected to respond to steroid or other immunosuppressive therapy.

In vitro GM-CFUc studies conducted in 10 patients showed a significant enhancement of colony growth when autologous serum (plain or decomplemented) was added to bone marrow compared to control serum. While the possibility exists that this difference may be due to growth inhibition by foetal calf serum in the control cultures, these results would appear to be in direct contradiction to previous studies, in which inhibition of CFU-C growth on addition of sera was demonstrated in SLE patients, not all of whom were neutropenic (100,101,102). However, these may not be directly comparable due to fundamental differences in study design. One study used mouse bone marrow cells and patient's serum compared to control serum (100), the other reported the effect of patient's peripheral blood and marrow T-lymphocytes on CFU-C's (101), and the third detailed the effect of disease-phase versus remission-phase serum on normal marrow CFU-GM in one patient (102). In the present study, only a single patient was leucopenic and none of the 10 were absolutely neutropenic. It is conceivable that these sera contained stimulatory growth factors which enhanced central granulocyte synthesis and whose absence may contribute to granulocytopenia. This hypothesis requires confirmation with investigation of larger groups of both neutropenic and non-neutropenic patients. Furthermore, most of the patients studied here were receiving immunosuppressive therapy (mainly corticosteroids), which may also modify immune function at the central level.

Thus although the present study has failed to add support for a role of direct humoral inhibition of central granulocyte synthesis, it has raised the possibility of a role for stimulatory factors in the pathogenesis of granulocytopenia in a subset of patients.

THROMBOCYTOPENIA

Only 2(3%) patients in this study had platelet counts below $100x10^9$/L, while the numbers reported below $150x10^9$/L range from 7-41% (3,4,31,32,33,72,104,105). However, only about 5% are reportedly severely thrombocytopenic with counts below $50x10^9$/L. Once again, the fact that almost half the patients studied here had inactive disease may have been contributory. Some of the subjects were in fact observed to decrease their platelet counts at a later stage.

Megakaryocyte hypoplasia was not demonstrated in any of the bone marrows.

Peripheral immune destruction of platelets secondary to deposition of autoantibodies and/or immune complexes remains the most likely mechanism of thrombocytopenia in these patients.

Platelet-associated immunoglobulin levels were increased in 21(30%) patients by direct ELISA technique in this study; however no correlation was shown between platelet count and platelet antibody level. Many studies have demonstrated frequent anti-platelet antibody positivity in SLE patients (120,121,122,123,124). Kelton et al (122) and Mulshine et al (125) demonstrated an inverse correlation between platelet count and platelet-associated IgG in thrombocytopenic SLE patients; however, Bonacossa et al (126) and Karpatkin et al (127) found elevated platelet-bound IgG in nonthrombocytopenic SLE patients. Thus a uniform set of conditions with respect to platelet count and platelet antibody does not appear to exist.

Some investigators favour the presence of a compensated thrombocytolytic state in SLE (127,133,134). The present study did not show any correlation between platelet count and mean platelet volume to support this theory. In addition, two of the three patients with megakaryocytic hyperplasia were thrombocytopenic, suggesting peripheral platelet consumption.

IMMUNOLOGICAL DATA

A number of patients were found to exhibit immunological activity against one or more circulating haematological cells, demonstrated by 16 patients with both anti-neutrophil and anti-platelet antibodies, 8 with Coombs' and anti-neutrophil antibody positivity, 3 with Coombs' and anti-platelet antibody positivity, and 3 had antibodies to all the cell types. However, none of the latter 3 patients had raised anti-DNA titers, and only 9 out of 21 subjects in the antibody positive groups were anti-DNA positive. Bearing in mind that about half of the patients had active but stable disease at the time of study, very few appeared to have broad range immunological activity against many different target cells or cellular constituents. This may be more convincingly shown in patients with more florid disease. Interestingly, a positive correlation existed between antibody levels against platelets and neutrophils (by the more sensitive HL-60 assay). This possibly suggests a tendency for patients to simultaneously form antibodies to more than one cell type, demonstrating enhanced immune reactivity.

TREATMENT

Management of cytopenic SLE patients is difficult and documentation of their incidence, and a clearer understanding of their pathogenesis, would aid in future therapeutic decisions.

Based on studies thus far, immunosuppressive drugs, by damping down immune responses, and splenectomy, by removing the organ responsible for sequestering antibody-coated cells, would appear to be the most logical modalities of treatment.

Anaemia, with chronic disease being the commonest cause, would benefit from treatment of the underlying condition, initially with steroids, followed by immunosuppressive agents in more severe cases. The value of employing recombinant erythropoietin in this setting remains to be established. Careful documentation of iron deficiency would precede appropriate replacement therapy. Autoimmune haemolytic anaemia refractory to steroids may require splenectomy or immunosuppressive drugs, namely cyclophosphamide or azathioprine. The role of Cyclosporin A is still being evaluated. Clinically significant granulocytopenia may benefit from a trial of cyclophosphamide, while haematopoietic growth factors and Cyclosporin A could be future options. Some cases of immune mediated thrombocytopenia, if unresponsive to corticosteroids, may respond to splenectomy, immunosuppressives being reserved for refractory patients. Once again, Cyclosporin A may in future prove to be of value in this setting.

In summary, this study has confirmed the high prevalence of cytopenias in our select group of SLE patients, with anaemia being the commonest abnormality, followed by neutropenia and thrombocytopenia. Most patients had chronic disease anaemia; iron deficiency was also relatively frequent. Autoimmune haemolytic anaemia was not a feature of our patient group. Chronic renal failure contributed to anaemia in three cases. Humoral inhibition of erythropoiesis was not demonstrated. Peripheral immune granulocyte destruction was presumably the main mechanism underlying the observed neutropenia. The possibility of absence of stimulatory growth factors in the pathogenesis of neutopenia was suggested. Thrombocytopenia, which was observed in two patients, was thought to be on the basis of peripheral platelet destruction, with many more non-thrombocytopenic subjects having elevated levels of platelet-associated immunoglobulin.

CHAPTER SIX
CONCLUSIONS

CONCLUSIONS

This study has confirmed the frequent occurrence of cytopenias in patients with systemic lupus erythematosus. Precise definition of underlying mechanisms is still not possible, but we have added support to that thought to account for the majority of cases of anaemia, namely chronic disease. Immune mediated destruction of granulocytes and platelets is the most likely cause of granulocytopenia and thrombocytopenia, although this could not be conclusively shown here. Documentation of these mechanisms is important to guide therapeutic decisions, as management is complex and at present relies mainly on the use of potentially harmful drugs. Future efforts should be directed at greater understanding of haematopoietic changes that occur in chronic disease, as well as standardization of immune-based assays to improve detection of peripheral destructive mechanisms.

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APPENDIX: PATIENT PROFILES

KEY

W = White

C = Coloured (mixed ancestry)

B = Black

M = Male

F = Female

Hb = haemoglobin (g/L)

Wcc = white cell count ($x10^9$/L)

Platelets = platelet count ($x10^9$/L)

NSAIDS = non-steroidal anti-inflammatory drugs

AM = anti-malarial drugs

Abnormal lupus serology: N/D = not done

Disease status: A = active; I = inactive; new = newly diagnosed

AIHA = auto-immune haemolytic anaemia; CRF = chronic renal failure.

Patient Number	Age	Race/ Sex	Clinical Features	Haematology			Abnormal Lupus Serology	Therapy		Disease Status
				Hb	Wcc	Platelets		NSAIDS	AM	
1	34	CF	panniculitis	133	6.1	166	N/D	–	–	A
2	53	CF	vitiligo	151	5.7	158	–	–	–	I
3	59	CF	well; previous AIHA	126	5.2	209	–	–	+	I
4	54	CF	well	129	3.8	225	N/D	–	–	I
5	37	CF	chronic discoid LE	137	8.1	250	N/D	–	–	I
6	41	CF	rash	105	4.6	402	+	–	–	A
7	49	CF	discharging sinuses tibia (osteitis)	111	9.5	311	N/D	–	–	I
8	32	CF	fatigue, weight loss, arthralgia, Raynauds; subsequent AIHA	91	3.7	317	+	+	–	A
9	29	CF	arthralgia, mild flexor tenosynovitis	126	5	415	N/D	+	+	A
10	32	CF	alopecia, active discoid LE, arthralgia	94	4.6	210	N/D	+	–	A
11	23	CF	fever, rash, polyarthritis, knee effusions, pleural effusions	74	3.4	360	+	+	–	A (new)
12	48	CF	flexor tenosynovitis	111	2.8	277	N/D	+	–	I
13	31	CF	arthralgia (hands, shoulders, knees)	113	4.7	298	+	+	+	A
14	65	BF	flexor tenosynovitis	95	2.7	327	N/D	+	–	I
15	26	CF	painful stiff joints, Raynauds	128	5.8	286	N/D	+	–	A
16	39	BF	synovitis both wrists	117	6.9	383	N/D	+	+	A
17	41	CF	well	91	3.3	159	N/D	–	–	I
18	28	CF	synovitis both wrists	127	9.6	352	N/D	+	–	A
19	54	CF	arthritis both hands	108	3.9	298	N/D	+	–	A

Patient Number	Age	Race/ Sex	Clinical Features	Haematology			Abnormal Lupus Serology	Therapy		Disease Status
				Hb	Wcc	Platelets		NSAIDS	AM	
20	26	CF	arthritis (elbow, shoulder)	121	5.3	230	N/D	+	+	A
21	24	CF	well; CRF	108	4.3	330	N/D	+	–	I
22	26	WF	flexor tenosynovitis	134	3.8	217	N/D	+	+	I
23	62	WF	thickened skin (hands, shins, feet)	108	3.9	183	N/D	–	–	I
24	32	CM	generalized myalgia, vasculitis, CRF	63	2.4	88	+	–	–	A
25	41	CF	cheilitis ? cause	83	4.4	236	N/D	–	–	I
26	59	WF	active discoid LE, alopecia	141	3.9	152	N/D	–	+	A
27	49	CF	well	104	4.2	285	N/D	+	–	I
28	21	CF	rash, alopecia, nephrotic syndrome	82	5.2	187	N/D	–	+	A
29	38	CF	nephrotic syndrome	111	5.3	99	+	–	–	A
30	42	CF	discoid LE; previous AIHA	111	4	207	N/D	–	–	I
31	45	CF	well	80	6.1	390	N/D	–	–	I
32	27	CF	well	100	4.6	224	N/D	–	–	I
33	25	CF	arthritis (hands, knees)	89	3.3	303	N/D	+	+	A
34	31	CF	erythema multiforme	117	8.3	319	N/D	–	+	A
35	15	CF	anorexia, rash, arthralgia (hands, feet)	76	9.7	593	N/D	+	+	A
36	50	CF	arthralgia (hands, feet)	135	10	188	N/D	–	–	A
37	52	CF	active discoid LE, arthritis	95	5.1	294	N/D	–	–	A
38	30	CF	neck stiffness ? cause; previous skin involvement	96	6.2	158	N/D	+	+	I

Patient Number	Age	Race/ Sex	Clinical Features	Haematology			Abnormal Lupus Serology	Therapy		Disease Status
				Hb	Wcc	Platelets		NSAIDS	AM	
39	37	CF	generalized arthralgia, swollen wrist, discoid skin lesions	106	3.5	108	N/D	+	–	A
40	60	WM	well; previous discoid LE	151	7.7	307	N/D	–	+	I
41	60	WF	well	112	5.2	464	N/D	+	–	I
42	37	CF	arthralgia (hands)	114	4.8	217	N/D	+	–	A
43	47	CF	well	130	4.8	175	N/D	–	–	I
44	46	CF	synovitis	124	3.7	401	N/D	–	–	A
45	24	CF	well	130	4.6	304	N/D	–	–	I
46	27	CF	painful stiff joints, eczema, Raynauds	107	5.6	288	N/D	+	–	A
47	38	CM	arthralgia	116	3.2	195	N/D	–	–	A
48	33	CF	well	134	6.9	348	N/D	+	–	I
49	24	CF	malar rash, arthralgia	64	4.8	261	+	+	–	A
50	49	BM	active discoid LE (face, scalp)	94	5.8	109	+	–	–	A
51	36	CM	rash (face, hands, legs)	169	6.1	194	N/D	–	+	A
52	29	CF	well	120	11	501	N/D	–	–	I
53	34	CM	arthralgia	152	7.6	309	N/D	+	–	A
54	34	CF	well	126	6.1	288	N/D	–	–	I
55	34	CF	well; CRF	91	5.8	254	N/D	–	–	I
56	17	CF	malar rash, vasculitic skin lesions, photosensitivity, alopecia, mucosal ulcers, pleural effusions, hepatomegaly	60	8.3	410	+	–	–	A (new)

Patient Number	Age	Race/ Sex	Clinical Features	Haematology			Abnormal Lupus Serology	Therapy		Disease Status
				Hb	Wcc	Platelets		NSAIDS	AM	
57	20	CF	well	143	5.4	172	N/D	–	–	I
58	48	CF	well	109	6.1	350	N/D	+	–	I
59	31	CF	Raynauds; previous arthralgia	113	8.1	297	N/D	+	–	I
60	44	CF	well; previous and subsequent AIHA	132	6.9	202	N/D	–	–	I
61	40	CF	well	116	6.9	267	N/D	–	–	I
62	53	CF	painful shoulder ? cause	101	7.9	397	N/D	–	–	I
63	52	BF	well	133	9.6	300	N/D	+	–	I
64	31	BF	arthralgia, fixed drug eruption	104	3.7	300	N/D	+	–	A
65	51	CF	weight loss, arthralgia	150	7.8	179	N/D	+	+	A
66	28	CF	arthralgia	113	5	254	N/D	+	–	A
67	26	CF	well	116	5.5	271	N/D	–	–	I
68	54	CF	well	143	7.4	226	N/D	–	–	I
69	33	CF	synovitis, flexor tenosynovitis	118	5.6	202	+	+	–	A
70	28	CF	synovitis, knee effusion	88	5.5	380	N/D	+	+	A