VALUE OF NEOPTERIN ESTIMATION AS A MARKER OF SLE DISEASE ACTIVITY

Thesis

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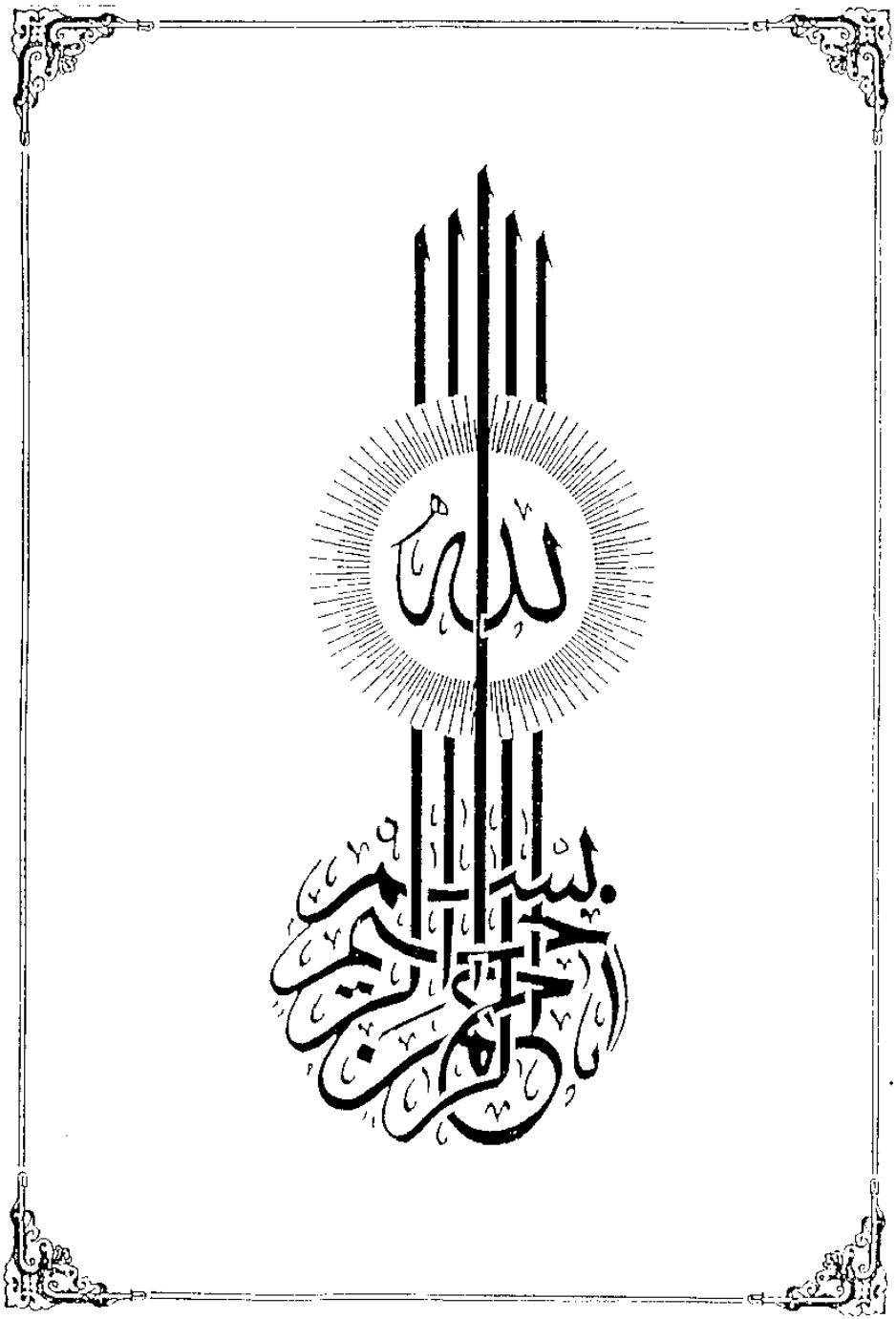
LIST OF ABBREVIATIONS

ACL : Anticardiolipin antibodies.
ACR : American college of Rheumatology.
AMLR : Autologus mixed lymphocyte reaction.
ANA : Antinuclear antibodies.
BBB : Blood brain barrier.
BCGF : B-cell growth factor.
BRA : Bone resorbing activity.
BSF-1 : B-cell stimulating factor-1.
C : Complement.
$CD4^+$: T-helpr cells.
$CD8^+$: T-Suppressor cells.
C-GMP : Cyclic guanosine monophosphate.
CIC_s : Circulating immune complexes.
CL : Crithidia Luciliae.
Conc A : Concanavalin A.
CPK : Creatinine phosphokinase.
CRP : C-reactive protein.
DEJ : Dermoepidermal junction.
DNA : Deoxyribonucleic acid.
ds-DNA : Double stranded - DNA.
ELIZA : Enzyme linked immunosorbent assay.
GTP : Guanosine triphosphate.
HIV : Human insufficiency Virus.
HPLC : High performance liquid chromatography.
hur : human recombinant.
IF : Immunofluorescence.
IFN : Interferon.



Ig	: Immunoglobulin.
IL	: Interleukin.
LAC	: Lupus anticoagulant.
LBT	: Lupus Band Test.
LBH	: Lactate dehydrogenase.
LPS	: Lipopolysaccharide.
NAA	: Neutrophil aggregation activity.
NK	: Natural killer.
PBMNC	: peripheral blood mononuclear cells.
PG	: Prostaglandins.
PHA	: Phytohaemagglutinines.
PMN	: Polymorphnuclear.
NP-SLE	: Neuropsychiatric - SLE.
NSAID	: Non steroidal anti-inflammatory drugs.
RIA	: Radioimmunoassay.
SGOT	: Serum Glutamate oxaloacetate Transferase.
SGPT	: Serum Glutamate pyruvate transferase.
SLE	: Systemic lupus Erythematosus.
SS-A	: Streptolysin S-A.
SS-B	: Streptolysin S-B.
TCRF	: T-cell replacing factor (IL-5).
TNF	: Tumour necrosis factor.
UCH	: University College Hospital.
VDRL	: Veneral disease Reference laboratory.

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INTRODUCTION

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Systemic Lupus Erythematosus (SLE), the prototype autoimmune rheumatic disease, is a chronic inflammatory disease characterised by protean clinical manifestations and occurrence of a variety of autoantibodies (**BORG et al., 1990b**). This chronic inflammatory disease appears to result from an immunoregulatory disturbance brought about by the interplay of genetic, hormonal and environmental factors (**Segovia, 1989**).

In 1987 lahita stated that the original description of SLE disease is attributed to Biett (1822). He added that, in 1851 Cazanave was the first one to use the name Lupus erythematosus to describe the disease's cutaneous manifestations. The term lupus meaning literally "wolf" in latin, had been used to describe the lesion's destructive nature. Kaposi, and later Osler, focused attention on the systemic nature of the disease with descriptions of cerebral, respiratory, cardiac, gastrointestinal, renal as well as the articular and haematologic manifestations (**Osler,1904**).

The concept of SLE is rather complex, involving many aspects. The unknown pathogenesis, the chronicity of the disease process, its variability, its tendency to exacerbate and remit, its biochemical and immunologic complexity and its variable response to therapy combine to complicate the process of assessment of disease activity and its therapeutic approach (**Fries, 1989**). Since the etiology of SLE remains obscure the disease activity can only be evaluated by clinical examination and indirect laboratory tests. Inspite of the intensive measures to make various systems for disease evaluation as objective as possible, there is little published informations about which of the many indices available is the most sensitive one to changes with disease activity (**Liang et al.,1989**).

The assessment of SLE disease activity is important in planning appropriate therapy and, when determined serially, in judging the success or failure of therapy. In general, major disease activity warrants more intensive and aggressive approaches to management, whereas

whereas minor disease activity can typically be controlled with more conservative and less toxic forms of therapy (**Klippel, 1988**).

The management of SLE today is rather more of an art than a science (**Liang et al., 1988a**). Indeed in much of the literature on SLE, disease activity is rarely defined,and there has not been agreement on what disease activity means (**Albert et al.,1978**). The improved survival rate for SLE patients makes it important to evaluate patients' clinical state in finer distinctions than dead and alive (**Fries, 1989**). Moreover, the fluctuating course of SLE makes the accurate clinical assessment puzzling and laborious. The patients require proper medication during periods of high clinical activity which again requires proper adjustment all through this fluctuating course and unpredictable drug response.

Immunoregulating drugs used to treat SLE are potentially toxic and therefore need to be directed at those most likely to benefit (**Pisetsky, 1986**).

With no attributable cause on which to base etiology of autoimmune diseases, diagnosis rests on grouping criteria which individually are non specific, but collectively give (the best fit) such in the case of (SLE), the prototype autoimmune rheumatic disease. The American College of Rheumatology (ACR) criteria for classification of SLE are now well accepted and generally used (**Tan et al., 1982**)., However, they do not address the issue of disease activity. According to these criteria, one patient may accumulate four criteria to be diagnosed as a lupus patient, over a period of a few years, and have no criteria present at the time of evaluation for a study of disease activity. Such a patient could be deemed to have SLE, but this says nothing of the state of the disease activity at the time. So in evaluating the clinical-laboratory responses to various modes of therapy and when following the natural course of the disease, the criteria of disease activity are more important to consider.

In a disease like SLE where transient symptoms and signs are common, it is important to know if a new clinical feature indicates worsening of the underlying disease. The currently available tests used in routine screening provide only a barely adequate assessment of disease activity. Therefore the need to develop new laboratory tests remains of paramount importance (**Morrow et al., 1982**).

The role of any individual serologic marker in the pathogenesis of the clinical disease activity of SLE is not well defined, and no serologic parameter has been found to correlate perfectly with the disease activity (**Steinberg, 1981**). Each of the molecules measured as a serologic marker is produced independently and is propably removed at a different rate by different clearance mechanisms. Therefore, the time course of each marker during the disease process must be different and independent serologic parameters would be expected to correlate better with disease activity rather than with each other (**Ytterberg and Schnitzer., 1982**). The most commonly employed parameters for disease activity include ESR, anti-DNA-antibody titre, Complement assessment,and circulating immune complex levels. All these are objective and useful measures in assessing the disease activity particularly when serial determinations are available in a single patient. However, it is probably true to say that these tests are most useful when they agree with the clinical observation of disease activity (**Valentijn et al., 1985; Herbert et al., 1990**).

Clinical assessment in SLE is largely directed at noting the presence of organ involvement, particularly fresh development in a flare of disease. There is still a great need for simple measures to quantitate, even on a crude scale, the degree of involvment of individual organs. Thus renal disease is one of the serious manifestations of lupus and the degree of renal damage can be assessed accurately using the usual tests of renal functions. However, most of these give little idea of the amount of active inflammation currently occurring in the kidney, which has to be monitored by more invasive methods like renal biopsy. It also has to be remembered that the degree of renal disease reflects only renal involvement and may bear little or no relation to activity in other organs. The importance of monitoring renal function should not obscure the necessity to assess each facet of lupus activity separately (**Bacon., 1982**).

Attempts have been made to use a combined clinical score reflecting the activity in all the different organs. However there may actually be less sensitivity if all the data is put in a single figure. From the individual patient's point of view the most serious point is the loss of relevant detail in a single overall index. It is becoming clear that there are different clinical patterns of disease expression in SLE, perhaps different subsets of disease, recognized by different serologic tests. It may be that these require different therapies. More effort needs to be directed towards linking laboratory assessments of disease activity with specific and at least semiquantitative assessments of specific clinical manifestations, rather than trying to correlate them with an overall assessment of disease activity. More recent attempts have been performed allover the world to improve the methods of evaluating disease activity and to find new activity markers that are more specific, sensitive, reproducible and make sound biological sense (**Bacon., 1982**).

The induction of immune response depends on the recognition by T cells of antigenic determinants in association with major histocompatibility complex (MHC) class II molecules (**Schwartz, 1985**). An aberrant expression of HLA class II might lead to chronic stimulation of auto-reactive T cells in the autoimmune disease (**Londei et al., 1985**).

There is considerable evidence of T-lymphocyte hyperactivity in SLE patients. Interferon gamma(IFN-gamma), a product of activated T-lymphocytes, is a major activator of human monocytes. Tumour necrosis factor was suggested to share IFN-gamma in some aspects of its monocyte activation. Patients with active lupus have increased number of circulating T-lymphocyte bearing Ia antigen, a phenotypic feature associated with T-lymphocyte activation (**Cohen et al., 1982**).

Moreover, a central event associated with SLE is the activation of resident tissue macrophages and recruited circulating monocytes which infiltrate the site of inflammation.Patients with SLE had been found to have more monocytes and mononuclear cells that made more immunoglobulins, but, with impaired phagocytic function. The

increased numbers of monocytes in SLE patients together with some subtle functional differences in these cells, may be important in the progression of this disease (**Boumpas et al., 1986 and Boswell and Schur ., 1989**).

Monocyte mononuclear cells especially from active lupus patients have an impaired accessory cell function as regards antibody response with no stimulating effect on suppressor cell activity (**Muryoi et al ., 1989 and Sawada and Takaei ., 1990**).

In a study conducted by Sasaki et al.(1989) they found that lupus monocytes actively participate in the spontaneously occurring anti-DNA- antibody synthesis by lupus lymphocytes. Meanwhile, anti-DNA- antibody suppressed IL-6 production by monocytes / macrophages and increased the immunoglobulin synthesis by SLE lymphocytes.

In 1991 Uetrecht suggested that procainamide, which causes the highest incidence of drug - induced lupus, may be oxidised to reactive metabolites by the myeloperoxidase system of monocytes. He postulated that the initial step in drug - induced lupus could be haptenization of a protein on the surface of monocytes by these reactive metabolites.

Many of the currently used drugs in the treatment of SLE such as non steroidal anti-inflammatory drugs, glucocorticoids, chloroquine and azathioprine are supposed to act on the T cell/macrophage axis. Thus, the need for a sensitive indicator reflecting changes in this part of the immune system seems to be urgent. Moreover, the future therapeutic approachs for the treatment of SLE will increase the need for more specific markers to monitor the biological response of related therapies (**Fauci and Young, 1989**). Activated macrophages liberate a number of inflammatory mediators including reactive oxygen metabolites and cytokines as interleukin 1(IL-1), alpha and beta, tumour necrosis factor (TNF), interferon alpha and colony stimulating factors (CSF). Among the secretory products of activated monocytes are the purine and pyrimidine products (**Roska and Lipsky, 1989**).

Neopterin, a pyrazino-pyrimidine derivative from guanosine triphosphate, was found to be an excellent biochemichal marker for the in vivo activation state of cell-mediated immunity (**Hausen et al., 1989; Allebes et al., 1991; Broadbent et al., 1991; Hengster et al., 1991 and Schiller et al., 1991**). In vitro, major amounts of neopterin are released exclusively by macrophages, particularly when stimulated with IFN gamma derived from activated T-cells (**Huber et al., 1984; Goldstein et al., 1989; Fuchs et al., 1990; and Broadbent et al., 1991**). Recognition of human leucocytic antigen (HLA) differences is a prerequisite for release of neopterin in allogenic mixed lymphocyte cultures (**Huber et al., 1983 a & b**). Similarly, an allogenic-induced in vitro neopterin release was proved by (**Troppmair et al., 1988**). The release of neopterin was inhibited in mixed lymphocytic culture by adding antibodies to IFN gamma (**Troppmair et al., 1988**).

Neopterin levels are easily detected by high performance liquid chromatography (HPLC) in urine (**Hausen et al., 1982a**) and in serum, cerebrospinal fluid and synovial fluid by radioimmunoassay (**Rokos et al., 1985**). Barak and his colleagues (1989a) developed an enzyme-linked immunosorbent assay for neopterin determination. This achievement would probably facilitate the use of neopterin estimation in the assessment of the clinical activity as well as the response to therapy in different diseases in which neopterin determination seems to be valuable without the need of more equipped laboratories containing gamma counters and HPLC apparatus.

In a broad spectrum of diseases which are recognized or supposed to involve activation of monocytes\macrophages by IFN gamma derived from activated T-lymphocytes, the applicability and utility of neopterin profile as a technichally simple test for a variety of specific clinical problems have been demonstrated by many authors. These diseases include;

1) acute viral infection (**Wachter et al ., 1979**);
2) intracellular bacterial infection (**Fuchs et al ., 1982**);
3) parasitic infection (**Reibnegger et al ., 1984**);
4) schizophrenia (**Sperner et al ., 1989**);

5) infection with human insufficiency virus (HIV) (**Plettenberg et al., 1990; Hengster et al., 1991; Paloczi et al., 1991; Ranki et al., 1991 and Sheppard et al., 1991**);

6) malignant tumours (**Liberati et al., 1990 and Autlitzky et al., 1991**);

7) hepatitis (**Leonardi et al ., 1991**);

8) allograft rejection (**Reibnegger and Aichberger, 1991**);

9) autoimmune diseases (**Clements et al., 1990; Hagihara et al., 1990 and Leohirun et al., 1991**).

Clinical studies have shown that, in these diseases, the neopterin test provides appropriate information regarding the extent, and particularly the activity, of the pathological processes (**Wachter et al., 1989**).

Only one disease is known to cause elevation of neopterin without activation of cell mediated immunity, namely dihydrobiopterin deficiency, a variant of atypical phenylketonuria. This is an extremely rare disease (estimated frequency less than one in million of live born infants). The disease is not known in adults (**Hausen et al., 1989**).

Autoimmune diseases show a promising application for neopterin in clinical medicine, because, neopterin levels reflect accurately the clinical activity and the extent of disease. Serum neopterin of patients with active juvenile dermatomyositis was found to be increased four to eight times over age matched controls. Levels from inactive patients were in the normal range. Follow up of patients revealed decreased neopterin levels, which correlated with improvement in the clinical picture (improved muscle power and decreased rash) as well as decreased levels of creatinine phosphokinase (CPK), aldolase, lactate dehydrogenase (LDH), and factor VIII related antigen. Thus measurements of neopterin may provide an additional parameter by which disease activity may be monitored with juvenile dermatomyositis (**Myones et al., 1989**).

Clements et al. (1990) have measured the levels of serum neopterin in patients with both limited scleroderma and diffuse scleroderma. They found that neopterin levels were higher in patients with diffuse scleroderma and limited scleroderma as compared to healthy controls.

According to **Mansour (1990)**, serum neopterin is significantly high in rheumatoid arthritis patients with active disease than in normal controls. Also synovial fluid neopterin was very useful for discriminating between inflammatory and non inflammatory arthritis. The study also showed that serum levels of neopterin increased significantly as the disease becomes more active. Synovial fluid neopterin was significantly elevated than serum levels in patients with severe disease activity but not in patients with moderate disease, or non inflammatory effusion.

SLE is another rheumatic disease in which application of neopterin as a marker of disease activity seems to be promising. Neopterin, as a recently introduced biochemical marker for the activation of cell mediated immunity,might usefully complement the typically employed clinical and laboratory variables to assess the clinical activity of SLE, particularly during treatment.

Recent studies demonstrated that serum and urinary neopterin levels were significantly elevated in patients with SLE (**Hagihara et al., 1990 and Leohirun et al., 1991**). There are no reports on the efficacy of serum neopterin levels in reflecting the activity of SLE.

AIM OF THE WORK