PROGNOSTIC VALUE OF NEOPTERIN AND IMMUNE COMPLEXES AS A PARAMETERS REFLECTING THE IMMUNE SYSTEM IN RHEUMATOID ARTHRITIS

THESIS
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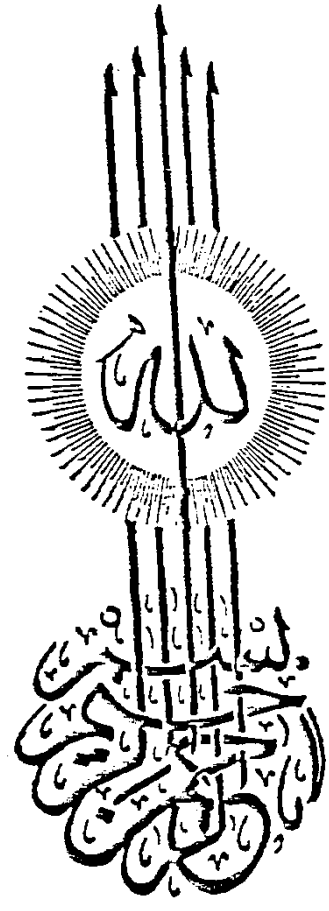


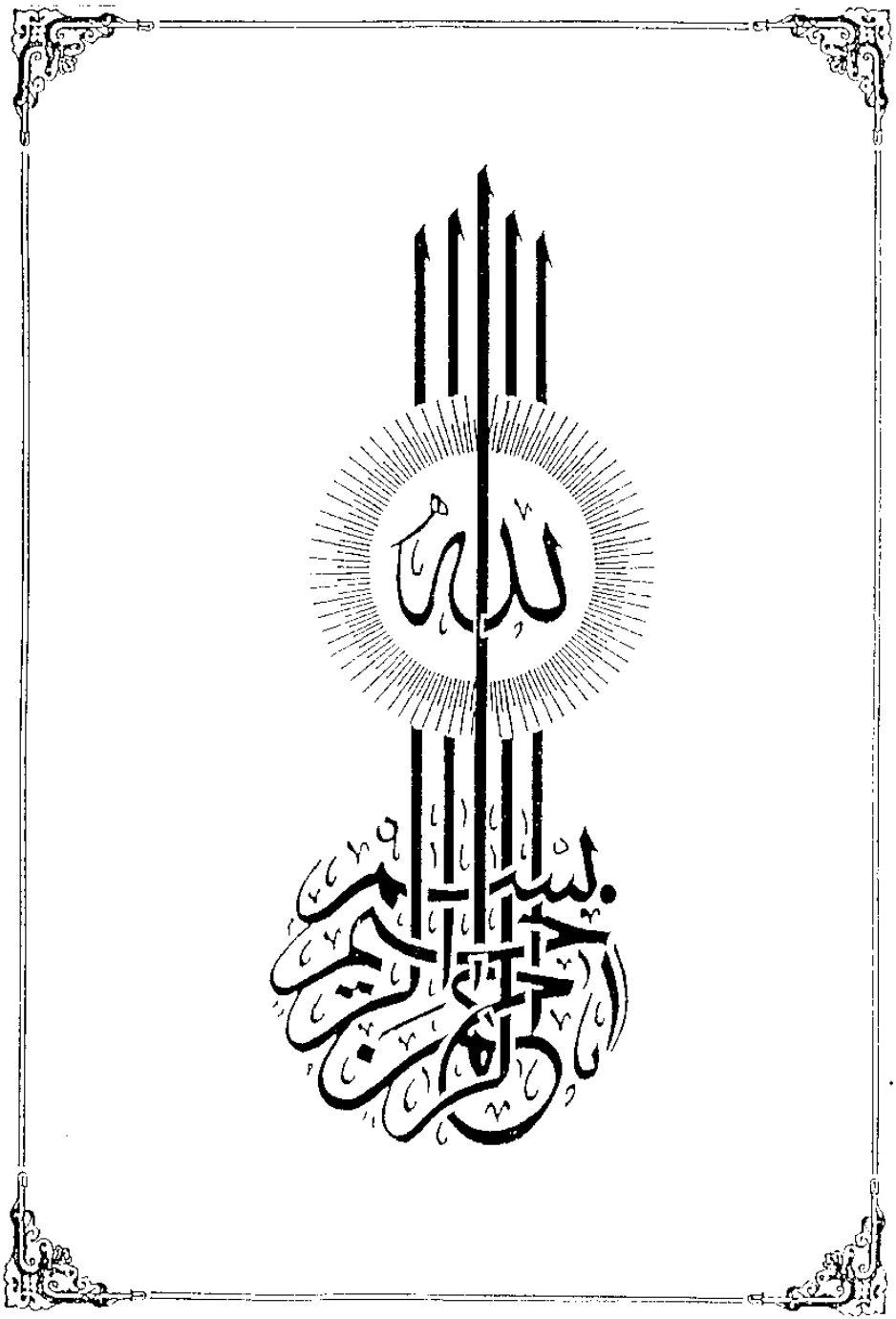
قالوا

سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا

إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

(سورة البقرة آية ٣٢)



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Abbreviations:

ACD	Anaemia of chronic disease
ADCC	Antibody dependent cellular cytotoxicity
ADL	Activities of daily living
AI	Articular index
ARA	American Rheumatoid Association
AS	Ankylosing spondylitis
AZA	Azathioprine
C	Complement
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CIC	Circulating immune complexes
ConA	Concanavalin A
CRP	C reactive protein
CSFs	Colony stimulating factors
DMARDs	Disease modifying antirheumatic drugs
EPO	Erythropoietin
ESR	Erythrocyte sedimentation rate
Fc fragment	crystalizable fragment
Fd	Further disruption
GS	Grip strength
GTP	Guanosine triphosphate
H_2O_2	Hydrogen peroxide
IC	Immune complexes
HPLC	High performance liquid chromatography
IDA	Iron deficiency anaemia
IDO	Indolamine dioxygenase
IFN	Interferon
IL	Interleukin
LGL	Large granular lymphocyte
LPS	Lipopolysaccharide
MAF	Macrophage activating factor
MDGA	Mean disease grading activity
MP	Mercaptopurine
MS	Morning stiffness
MTX	Methotrexate
MVA	Multivariate analysis
MADP	Nicotinamide dinucleotide phosphate

NK	Natural killer
NSAIDs	Non steroidal anti-inflammatory drugs
NSBT	Non specific binding tubes
PAF	Platelet activating factor
PBL	Peripheral blood lymphocyte
PBMC	Peripheral blood mononuclear cell
PEG	Polyethylene glycol
PG	Prostaglandin
PHA	Phytohemagglutinin
PMNL	Polymorphonuclear leucocyte
PS	Pain scale
PTS	Pyruvyl tetrahydropterin synthase
PV	Plasma viscosity
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RHuEPa	Recombinant human erythropoietin
RIA	Radioimmunoassay
RIFN	Recombinant interferon
ROM	Range of joint motion
SAP	Serum amyloid protein
SF	Synovial fluid
SFN	Synovial fluid neopterin
SLE	Systemic lupus erythematosus
SIRs	Soluble immunosupressor factors
SN	Serum neopterin
SR	Sepiapterin reductase
TNF	Tumour necrosis factor

CONTENTS:

INTRODUCTION
AND AIM OF THE WORK

Introduction:

Rheumatoid arthritis "RA" is the disease most identified the discipline of rheumatology. Both the humoral and cellular arms of the immune response are involved in the pathogenesis of the disease ***(Firestein et al., 1987)***. The precise cause of which is unknown. It is characterized by an erosive, proliferative synovitis. A significant proportion of patients have a chronic course with progressive joint destruction and disability. The concept of RA is rather complex, involving many aspect, the unknown pathogenesis, the chronicity of the disease process, its variability and tendency to exacerbate and remit, as well as its biochemical and immunologic complexity and finally its variable response to therapy, all combine together to complicate the process of assessment of disease activity, prognosis and its therapeutic approach ***(Fries, 1989)***.

Despite many years of intensive investigative efforts the etiology of RA remain obscure. ***Claude et al. (1985)*** when discussing the etiology of RA, assumed that an initiating agent activates an immune response in a host which results in tissue inflammation. Rheumatoid activity and severity can be evaluated by clinical examination and laboratory investigations. Although it is not possible to

predict with certainty the outcome in any single patient, a number of clinical and laboratory features may offer a guide to prognosis. However, the interpretation of studies evaluating the prognosis of RA is hindered by the use of different outcome measures. The relief of pain of a greater priority to patients than prevention of disability ***(Mckenna and Wright, 1985)***, but it is more difficult to evaluate ***(Bird and Dixon, 1987)***. As with other chronic disease, both physicians and patients are eager to know the chances for remission in RA and are anxious about the treatment of severe morbidity or death. Moreover, the fluctuating clinical course of rheumatoid arthritis makes the accurate clinical assessment puzzling and laborious.

The evidence of involvement of immune complexes in the pathogenesis of RA is based on direct demonstration of such complexes in the synovial fluid "SF" ***(Winchester et al.,1971a)*** and on observation of the different biological changes associated with the formation of immune complexes such as increased vascular permeability, accumulation of cellular blood elements and reduction of complement levels in SF. Polymorphonuclear leukocytes attracted by complement derived chemotactic factors, ingest the immune complexes.

Lessared and his coworkers (1983) found that with the use of Cl_q binding assay, a positive correlation was found with the joint count, CRP, ESR and

presence of rheumatoid factor"s" "RFS". No correlation was noted with the disease duration. It has been suggested that the Cl_c binding assay was a useful parameter in monitoring progress in RA patients. In another provocative study, more erosions of the hands and feet were found after 1 year follow-up in patients who initially had raised levels of immune complexes ***(Withrington et al., 1984)***.

Neopterin is a pyrazino pyrimidine derivative, which increases in autoimmune diseases, it reflects the state of activation of T-lymphocyte macrophage axis ***(Reibnegger et al., 1986)***. They also stated that we can depend on its level as a new excellent biochemical marker which can reflect the state of rheumatoid activity, as they found that there is a good link between its level and the radiological stage of RA. This observation was emphasized by ***Hausen and his coworkers in 1989***.

Determination of neopterin levels is an excellent marker for the in vivo activation state of cell mediated immunity ***(Broadbent et al., 1991 and Schiller et al., 1991)***. In ***1991, Allebes and his associates*** believed that neopterin titre is an important parameter in the follow-up studies, which showed that neopterin level decreases if therapy effectively lowered the disease activity. So, repetitive neopterin evaluation as a prognostic parameter in rheumatoid arthritis seems worth while in our work.

Anti-rheumatoid drug therapy is potentially toxic and therefore needs to be directed at those most likely to benefit. An ability to predict the outcome of rheumatoid arthritis is also important when offering advice on prognosis, so that consideration may be given to alteration in lifestyle or employment.

A wide range of drugs are available for treatment of RA, the physician should select appropriate drugs to help and control the disease activity. A balancing potential benefit against potential toxicity is necessary, the best method for obtaining such information is through the use of well designed randomized trials, especially those with follow-up studies. In the absence of knowledge about the mechanisms of the disease and the mode of action of drugs, the only way forward is by clinical trials of different combination therapy ***(Huskisson, 1987)***. The disease modifying antirheumatic drugs "DMARDs" or slow acting antirheumatic drugs are so named because it is thought that they may favorably modify the progression of RA and its disability. Many of currently used second line drugs in the treatment of RA are supposed to act on the T-cell/macrophage axis. Thus, the need for a sensitive indicator reflecting changes in this part of immune system seems to be urgent. Moreover, the future therapeutic approach for the treatment of RA will increase the need for more specific markers to monitor the biological response of related therapies ***(Fauci and Young, 1989)***.

It is becoming increasingly clear that the so-called pyramid approach to treating RA patients is a failed concept. Patients who are treated in this manner often have suboptimal outcomes in terms of functional status and mortality ***(Kenneth et al., 1990, Golbus, 1993 and Wilske, 1993).*** Rheumatologists now favour a much more aggressive approach to treat RA. At present, with the demonstrated long term failure of incremental single drug therapy in the pyramid, the complexities of the inflammatory reaction, and the short two years therapeutic window before the appearance of erosions, the early use of combination of drugs seems a reasonable approach ***(Wilske and Healey, 1989).*** In ***1990, Palules*** described the rational for combined drugs therapy, combining drugs with presumably different site of action will act together in an attempt to eradicate RA, with the use of drugs with different toxicities or lower doses of toxic drugs should minimize risk.

Azathioprine "AZA" and Levamisole are examples of this group of DMARDs, Azathioprine which is immunodepressant, it is a cell cycle specific antimetabolite. It undergoes rapid hepatic metabolism to 6 mercaptopurine and then further to a false purine antagonist, it has been given American Food and Drug Administration "FDA" approval for treatment of RA. Levamisole has an immunorestorative effect, i.e., normalized subnormal immune responses "immunostimulant". It is a three ring

molecule with extremely low molecular weight, it enhances lymphoid cell function and corrects the chemotactic defect of neutrophils "polymorphonuclear leucocyte" ***(Rabson et al., 1977)*** and monocytes ***(Snyderman and Pike, 1978)***. Levamisole has been shown to be effective in treatment of RA patient, but because of its high incidence of toxic effect, it may be difficult to be considered for routine treatment ***(Kinsella et al., 1980 and Veys et al., 1981)***. The exact combination of medication is not as important as the concept of early control of inflammation before joint damage occurs ***(Wilske, 1993)***. Thus, a trial of the efficacy of a combination of Azathioprine and Levamisole therapy seems worth while.

Aim of the work:

The aim of this work is:

- To evaluate neopterin as a prognostic criterion and as a parameter of disease severity in Egyptian rheumatoid patients, through its quantitative measurement in serum and synovial fluid and to test the efficacy of this new biochemical marker.
- To correlate the level of neopterin to immune complexes as a follow-up and prognostic parameter.
- To evaluate the disease modifying effect of Azathioprine and Levamisole singly and in combination.