SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AS A BIOMARKER OF LUPUS NEPHRITIS

BY

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACR	American College of Rheumatology
ANA	Antinuclear antibody
ARA	American Rheumatism Association
ARB	Angiotensin receptor blocker
BILAG	British Isles Lupus Assessment Group
CKD	Chronic kidney disease
CNS	Central nervous system
CYC	Cyclophosphamide
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
eGFR	Estimated Glomerular Filtratition Rate
ELISA	Enzyme-linked Immunosorbent Assay
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
GFR	Glomerular filtration rate
GN	Glomerulonephritis
HCQ	Hydroxychloroquine
HUSM	Hospital Universiti Sains Malaysia
ISN/RPS	International Society of Nephrology/ Renal Pathology Society
IqR	Interquartile range
LN	Lupus nephritis
MMF	Mycophenolate mofetil
mRNA	Messenger Ribonucleic Acid
RBC	Red blood cell

rBILAG	Renal British Isles Lupus Assessment Group
SBP	Systolic blood pressure
SLE	Systemic Lupus Erythematosus
UFEME	Urine full examination and microscopy examination
UPCR	Urine protein-creatinine ratio
USM	Universiti Sains Malaysia
VEGF	Vascular endothelial growth factor
WBC	White blood cell
WHO	World Health Organisation

ABSTRACT

RESEARCH TITLE:

Serum Vascular Endothelial Growth Factor (VEGF) as a biomarker of lupus nephritis.

INTRODUCTION

Previous studies had shown that serum VEGF levels were elevated in patients with active systemic lupus erythematosus (SLE) particularly in lupus nephritis (LN) patients. The abnormality of VEGF production is postulated to play an important role in the pathogenesis of LN.

OBJECTIVE

Our study aimed to assess the levels of serum VEGF in patients with lupus nephritis and to determine its association with LN activity.

METHODOLOGY

We studied 46 lupus nephritis and 46 non-LN SLE patients with 26 healthy female controls. Laboratory investigations included urinalysis, urine protein-creatinine ratio, serum creatinine, albumin and VEGF level were examined. Blood pressure, renal biopsy result and treatment were recorded. Lupus nephritis activity was evaluated by renal British Isles Lupus Assessment Group (BILAG) score.

RESULTS

Serum levels of VEGF were significantly increased in LN patients (median level 533.65pg/mL) when compared to healthy controls (343.00pg/mL) ($p<0.001$), but not significant when compared to non-LN SLE (436.14pg/mL) ($p=0.93$). Furthermore, serum

VEGF levels were found significantly higher in the sera of patients with active nephritis compared to quiescent nephritis (p=0.024). However, there were no correlations between serum VEGF with individual clinical and laboratory indicator of renal disease activity: BP, urine protein-creatinine ratio, renal function and serum albumin. The study also did not find statistically significant relationship between serum VEGF levels and histological classes of LN and the presence of active urinary sediments.

CONCLUSION

Our finding of increased levels of serum VEGF in lupus nephritis group especially in active nephritis underlines its role in the pathogenesis of lupus nephritis but the clinical potential of this biomarker need to be further studied.

ABSTRAK

TAJUK KAJIAN

Paras Vascular Endothelial Growth Factor (VEGF) di dalam serum sebagai biopenanda penyakit lupus nephritis.

PENGENALAN

Beberapa kajian mengenai VEGF sebelum ini telah menunjukkan bahawa terdapat peningkatan paras VEGF di dalam serum pesakit SLE yang mempunyai tahap penyakit yang aktif terutamanya dalam pesakit lupus nefritis (LN). Teori penghasilan paras VEGF yang tidak normal ini mungkin memainkan peranan penting dalam patogenesis penyakit lupus nephritis.

OBJEKTIF

Objektif kajian ini adalah untuk menentukan paras VEGF di dalam serum pesakit lupus nefritis serta mengkaji kaitannya terhadap tahap aktiviti penyakit lupus nefritis.

METODOLOGI

Dalam kajian ini, kami telah mengkaji 46 orang pesakit lupus nefritis, 46 orang pesakit SLE tanpa lupus nephritis serta 26 orang sukarelawan wanita yang sihat. Siasatan makmal termasuklah analisis urin, nisbah paras protin-kreatinin dalam urin, serta paras kretinin, albumin dan VEGF dalam serum pesakit ditentukan. Bacaan tekanan darah, keputusan biopsi buah pinggang serta ubatan yang diambil pesakit turut direkodkan. Tahap aktiviti lupus nefritis ditentukan oleh suatu sistem pemarkahan khas yang dikenali sebagai 'renal British Isles Lupus Assessment Group' (rBILAG).

KEPUTUSAN

Kami mendapati paras VEGF yang tinggi di dalam serum pesakit lupus nefritis (paras median 533.65pg/mL). Paras ini adalah ketara apabila dibandingkan dengan kontrol sihat (343.00pg/mL) ($p<0.001$) tetapi tiada perbezaan ketara jika dibandingkan dengan pesakit SLE yang tidak mempunyai lupus nefritis (436.14pg/mL) ($p=0.93$). Paras VEGF juga didapati tinggi di dalam serum pesakit yang mempunyai tahap nefritis yang aktif berbanding pesakit yang mempunyai tahap nefritis yang tidak aktif ($p=0.024$). Walaubagaimanapun, kajian kami mendapati tiada perhubungan di antara paras VEGF dalam serum dengan tahap tekanan darah, nisbah paras protin-kretinin dalam urin, fungsi ginjal dan tahap albumin dalam serum pesakit. Kami juga mendapati tiada perbezaan paras VEGF di antara kelas-kelas histologi penyakit lupus nefritis.

KESIMPULAN

Kajian kami mendapati peningkatan paras VEGF dalam serum pesakit lupus nefritis terutamanya pada tahap nefritis yang aktif. Keputusan ini mencadangkan bahawa wujudnya kaitan di antara paras VEGF dalam serum dalam patogenesis penyakit lupus nefritis. Walaubagaimanapun, kajian lanjut diperlukan bagi menentukan peranan biopenanda ini dari aspek klinikal.

1. INTRODUCTION

1.1 SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder with a broad spectrum of clinical presentations (Tan EM et al, 1982). SLE is a chronic illness that may be life-threatening when major organs are affected but more commonly results in chronic debilitating ill health. It is characterised by pathogenic auto antibodies production and tissue deposition of immune complexes. No single cause for SLE has been identified though factors such as sunlight and drugs may precipitate the condition and there is a complex genetic basis.

1.2 INCIDENCE AND PREVALENCE

The incidence of SLE in the general population varies according to the characteristics of the population studied, i.e. age, gender, race, ethnic/national origin or period of time studied, but also depending on changes in classification criteria. SLE is more common among women in their reproductive years (Cross and Benton, 1999), with highest incidence seen in premenopausal period between the ages of 20 to 50 years (Siegle et al, 1970; Hochberg, 1985) and female to male ratio range from 6 to 14:1 (McCarty et al, 1995; Voulgari et al, 2002; Soto ME et al, 2004). The prevalence of SLE in United States is 1 in 250 in black females and 1 in 1000 in white females (Manolios and Schrieber, 1997). Estimated incidence rates in North America, South America and Europe range from 2 to 8 per 100 000 per year (Vilar and Sato, 2002; Jeminez et al, 2003), tripling in the last 40 years due to improved detection of mild disease.

Both geography and race effect affect SLE prevalence. SLE is more common in the urban than rural areas; and there is higher prevalence among Asians, Afro- American, Afro-Carribbeans, Hipanic Americans (Serdula and Rhoads, 1979; Hochberg, 1985; Lawrence et al, 1989) but lower prevalence among blacks in Africa (Symmons, 1995). A study by Hopskinson et al (1994) revealed that the highest prevalence rate was seen in Afro- Carribbeans (207/100 000), followed by Asians (48.8/100 000). Racial differences in the clinical features of SLE have also been noted, with blacks developing SLE at younger age, discoid lupus being more common, and higher prevalence of antibodies to Smith (Sm) and Ribonucleoprotein (RNP) antigen, compared to whites (Ward and Studenski, 1990). Previous study in Malaysia reported a ratio of 12:1 was seen in all major ethnic group; Malays, Chinese and Indian (Wang et al, 1997).

1.3 DIAGNOSTIC CRITERIA OF SLE

A person is having SLE if she or he fulfils 4 out of 11 criteria based on 1997 American Rheumatism Association (ARA) revised criteria, which confers 96% sensitivity and specificity (Hochberg, 1997). The eleven criteria are described in Table 1.1.

Criteria	Comments
Discoid rash	Erythematous raised patches with adherent scaling and follicular plugging; atrophic scarring can be seen in older lesions
Photosensitivity	By patient history or physician observation
Oral ulcers	Oral or nasopharyngeal, usually painless, observed by physician
Arthritis	Nonerosive; 2 or more peripheral joints affected by tenderness, swelling, or an effusion
Serositis	Pleuritis defined as history of pleuritic pain or rub heard by a physician or a pleural effusion; pericarditis documented by ECG, rub, or pericardial effusion
Renal disorder	Cellular casts (red cell, hemoglobin, granular, tubular, or mixed) or persistent proteinuria > 0.5 g/d or > 3+ if quantification not performed
Neurological disorder	Seizures or psychosis (without other offending drug or metabolic derangement)
Hematological disorder	Hemolytic anemia (with reticulocytosis) or leukopenia (leukocyte count < 4000/μL on 2 or more occasions) or lymphopenia (lymphocyte count < 1500/μL) on 2 or more occasions or thrombocytopenia (platelet count < 100,000/μL) in the absence of drugs
Immunological disorder	Anti-DNA: antibody to native DNA or anti-Smith; or positive finding of antiphospholipid antibodies based on (1) abnormal level of IgG or IgM anticardiolipin antibodies, (2) a positive test result for lupus anticoagulant using a standard method, or (3) false positive serological test for syphilis known to be positive for 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
Antinuclear antibody test	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point and in the absence of drugs known to be associated with "drug-induced" lupus
Malar rash	Fixed erythema, flat or raised, over the malar eminences, sparing the nasolabial folds

Adapted from Klippel JH, ed. *Primer on the Rheumatic Diseases*. 2001.[3]

Table 1.1 1997 American Rheumatism Association (ARA) revised criteria for SLE. Adapted from Klippel JH, Primer on the Rheumatic Diseases, 2001.

1.4 EVALUATION OF SLE ACTIVITY

There are two major scoring systems to evaluate the activity of lupus in clinical studies. These systems are not used in routine medical practice, but for quantification of lupus disease activity primarily for the purpose of determining whether a new drug evaluated for the disease is effective.

The most commonly used study of lupus activity is called the SLE Disease Activity Index, and the acronym for it is SLEDAI. It is a list of 24 items, 16 of which are clinical items such as seizure, psychosis, organic brain syndrome, visual disturbance, other neurological problems, hair loss, new rash, muscle weakness, arthritis, blood vessel inflammation, mouth sores, chest pain worse with deep breathing and manifestations of pleurisy and/or pericarditis and fever. Eight of the 24 items are laboratory results such as urinalysis testing, blood complement levels, increased anti-DNA antibody levels, low platelets, and low white blood cell count. These items are scored based on whether these manifestations are present or absent in the previous 10 days.

The other major study instrument is called the BILAG, which stands for the British Isles Lupus Activity Group. The BILAG is an organ-specific 86-question assessment based on the principle of the doctor's intent to treat, which requires an assessment of improved (1), the same (2), worse (3), or new (4) over the last month. Within each organ system, multiple manifestations and laboratory tests are combined into a single score for that organ. The resulting scores for each organ can be A through E, where A is very active disease, B is moderate activity, C is mild stable disease, D is resolved activity, and E indicates the organ was never involved. There are eight general headings; General, Mouth and Skin, Neurological, Joints and Muscles, Cardiovascular and Pulmonary, Blood Vessel Inflammation (Vasculitis), Kidney, and Blood.

Hay et al, 1993, demonstrated good between-rater reliability for the BILAG index for each organ-based system. There was no evidence of bias between observers. A study demonstrated that BILAG 2004 index had good overall sensitivity (87%) and specificity (99%) when compared with the 'gold standard' criterion (starting or increasing disease-modifying therapy). There were high positive predictive values overall (80%), and for each organ-based system, with the exception of the neurological system. It is suitable for use in longitudinal study in SLE (Yee CS et al, 2009). It appears that SLEDAI is

less able at detecting disease activity requiring increase in therapy when compared to BILAG, which is not surprising as it has far fewer items than BILAG (24 versus 97) (Chee et al, 2007). In this study, we used renal BILAG scoring system as an objective evaluation of renal disease activity. The detail of renal BILAG scoring system will be outlined in Chapter 3 and APPENDIX D.

1.5 PROGNOSIS

SLE carries a highly variable prognosis for individual patients. The natural history of SLE ranges from relatively benign disease to rapidly progressive and even fatal disease. SLE often waxes and wanes in affected individuals throughout life, and features of the disease vary greatly between individuals. The disease course is milder and survival rate higher among persons with isolated skin and musculoskeletal involvement than in those with renal (Faurschou et al, 2010) and CNS disease (Kasitanon et al, 2006). A recent consortium report of 298 SLE patients followed for 5.5 years noted falls in SLE Disease Activity Index 2000 (SLEDAI-2K) scores after the first year of clinical follow up and gradual increases in cumulative mean Systemic Lupus International Collaborating Clinics (SLICC) damage index scores (Urowitz et al, 2012).

Mortality in patients with SLE has decreased over the past 20 years (Ruiz-Irastorva et al 2001). Prior to 1955, the 5-year survival rate in SLE was less than 50%; currently, the average 10-year survival rate exceeds 90% (Trager and Ward, 2001; Urowitz et al, 2012), and the 15-year survival rate is approximately 80% (Abu-Shakra et al, 1995). Ten-year survival rates in other countries within Asia and Africa are significantly lower,

ranging from 60-70% (Murali et al, 1997; Wang et al, 2007), but may reflect detection bias of severe cases only.

Decreased mortality rates associated with SLE can be attributed to earlier diagnosis (including milder cases), improvement in disease-specific treatments, and advances in general medical care. Yet, according to the Centers for Disease Control and Prevention, however, one third of SLE-related deaths in the United States occur in patients younger than 45 years, making this a serious issue despite declining overall mortality rates.

In 1976, Urowitz first reported bimodal mortality in early versus late SLE, noting that SLE-related deaths usually occur within the first 5-10 years of symptom onset. Mortality in the first few years of illness is typically from severe SLE disease (eg, CNS, renal, or cardiovascular involvement) or infection related to immunosuppressive treatment. Infections account for 29% of all deaths in these patients (Cervera et al, 1999).

Late deaths (after age 35 years) are generally from myocardial infarction or stroke secondary to accelerated atherosclerosis (Manzi et al, 1997; Ruiz-Irastorva et al, 2001; Trager and Ward, 2001; Gladman and Urowitz, 2007). Manzi et al, 1997, reported that women aged 35-44 years with SLE were 50 times more likely to develop myocardial ischemia than healthy Framingham control women. The presence of lupus nephritis may increase these risks (Faurschou et al, 2011). Causes of accelerated coronary artery disease in persons with SLE are likely multifactorial. They include endothelial dysfunction, inflammatory mediators, corticosteroid-induced atherogenesis, and dyslipidemia.

The influence of race on prognosis has been widely debated. The LUMINA study group examined SLE among black, white, and Hispanic patients in the United States (including Puerto Rico) and reported that both disease activity and poverty predicted higher mortality among racial and ethnic minorities (Alarcon et al, 2001).

1.6 LUPUS NEPHRITIS

1.6.1 OVERVIEW OF LUPUS NEPHRITIS

Lupus nephritis (LN) is a common manifestation of systemic lupus erythematosus (SLE) and is associated with substantial morbidity and mortality. The term lupus nephritis encompasses diverse patterns of renal disease encountered in SLE, including glomerular, tubulointerstitial, and vascular pathology. Specifically, the term lupus nephritis should be reserved for immune complex-mediated renal injury and its sequelae (Baldwin et al, 1970; Pollak et al, 1964). Approximately 35% of adults with SLE have clinical evidence of nephritis at the time of diagnosis, with an estimated total of 50-60% developing nephritis during the first 10 years of disease (Dooley et al, 2004; Kasitanon et al, 2006). In those over 50 years of age at onset, less than 5% have LN (Cameron, 1999). The prevalence of nephritis is significantly higher in African and Hispanics than in whites, and is higher in men than in women. Renal damage is more likely to develope in non-white groups (Alarcon et al, 2002).

Data extracted from Malaysian Registry of Renal Biopsy (MRRB) from 2005 to 2006 have showed the peak age of incidence of LN was in the second and third decade of life and female to male ratio was 6.0 to 1 (Yahya R, 2008). There was no racial predilection: 54% of patients are Malays, 30% Chinese, 6% Indian and 10% others. Class IV is the predominant histopathological finding (54%), followed by class III (13%),

class V (9%) and class II (9%). In terms of clinical presentation, there was 45% with asymptomatic urine abnormalities, 35% with nephrotic syndrome 13% with nephritic syndrome, and 7% with nephritic-nephritic syndrome. Forty-one percent had impaired renal function (eGFR <60 ml/min) and 13% were hypertensive at presentation. There was no correlation between clinical presentation and age, gender or histopathological finding (Yahya R, 2008).

Overall survival in SLE is approximately 95% at 5 years of diagnosis and 92% at 10 years of diagnosis. The presence of LN significantly reduces survival to approximately 88% at 10 years, with even lower survival in African Americans (Bernatsky et al, 2006; Cervera et al, 2003). LN varies from mild subclinical disease to severe diffuse proliferative lupus nephritis which may progress to end-stage renal disease (ESRD) without appropriate treatment. Like SLE itself, LN showed a marked variability in its presentation, ranging from mild proteinuria to rapidly progressing glomerular nephritis causing renal insufficiency within weeks. Of the different pathological classes, diffuse proliferative glomerulonephritis (Class IV) has the worst prognosis, resulting in 11-48% of patients with end stage renal disease at 5 years.

1.6.2 DIAGNOSTIC CRITERIA

LN is defined as clinical and laboratory manifestations that meet ACR criteria (persistent proteinuria >0.5 gm per day or greater than 3+ by dipstick, and/or cellular casts including red blood cells (RBCs), hemoglobin, granular, tubular, or mixed) (Tan EM et al, 1982). A review of the ACR criteria has recommended that a spot urine protein/creatinine ratio of >0.5 can be substituted for the 24-hour protein measurement, and "active urinary sediment" (>5 RBCs/high-power field (hpf), >5 white blood cells

(WBCs)/hpf in the absence of infection, or cellular casts limited to RBC or WBC casts) can be substituted for cellular casts (Dooley MA et al, 2004). An additional, perhaps optimal, criterion is a renal biopsy sample demonstrating immune complex–mediated glomerulonephritis compatible with LN. Finally, the Core Executive Panel of ACR agreed that the diagnosis of LN should also be considered valid if based on the opinion of a rheumatologist or nephrologist.

1.6.3 RENAL BIOPSY AND HISTOLOGY

All patients with clinical evidence of active lupus nephritis, previously untreated, are recommended to undergo renal biopsy (unless strongly contraindicated) so that glomerular disease can be classified by current ISN/RPS classification (Table 1.4). In addition, disease can be evaluated for activity and chronicity and for tubular and vascular changes (Markowitz and D'Agati, 2007). Finally, biopsies may identify additional or alternative causes of renal disease, such as tubular necrosis related to medications, hypovolemia, or hypotension. Biopsy is most highly recommended in patients with the characteristics indicated in Table 1.2.

	Level of evidence
Increasing serum creatinine without compelling alternative causes (such as sepsis, hypovolemia, or medication)	C
Confirmed proteinuria of ≥1.0 gm per 24 hours (either 24-hour urine specimens or spot protein/creatinine ratios are acceptable)	C
Combinations of the following, assuming the findings are confirmed in at least 2 tests done within a short period of time and in the absence of alternative causes: a. Proteinuria ≥0.5 gm per 24 hours plus hematuria, defined as ≥5 RBCs per hpf b. Proteinuria ≥0.5 gm per 24 hours plus cellular casts	C
* RBCs = red blood cells; hpf = high-power field.	

Table 1.2 Indication for renal biopsy in patients with systemic lupus erythematosus. Adapted from Bevra et al, ACR Guidelines for Lupus Nephritis, 2012.

Classifications of lupus nephritis have been based entirely on glomerular lesions, beginning with the original 1974 WHO Classification and subsequent revisions. The World Health Organisation (WHO) classification for lupus nephritis (Table 1.3) has been updated to allow more accurate descriptions of renal histopathology specimens by the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) (Table 1.4). The ISN/RPS 2003 Classification of lupus nephritis includes multiple changes from its predecessors, most notably removal of the subclasses of lupus nephritis class V (i.e.Va–Vd), elimination of the normal category (WHO class I), clearer definitions for all classes, a standard approach to sclerotic glomeruli, and the division of lupus nephritis class IV into subcategories based on whether the endocapillary

involvement is predominantly segmental (lupus nephritis IV-S) or global (lupus nephritis IV-G).

CLASS I	Normal a. Nil by all techniques b. Normal by LM but deposit by EM or IF
CLASS II	Pure Mesangial Alteration (Mesangiopathy) a. Mesangial widening mild hypercellularity b. Moderate hypercellularity
CLASS III	Focal Segmental Glomerulonephritis a. Active necrotising lesion b. Active and sclerosing lesions c. Sclerosing lesions
CLASS IV	Diffuse Glomerulonephritis a. Without segment lesions b. Active necrotising lesion c. Active and sclerosing lesions d. Sclerosing lesions
CLASS V	Diffuse Membranous Glomerulonephritis a. Pure b. Associated with Class III or IV
CLASSVI	Advanced Sclerosing Glomerulonephritis

Table 1.3 WHO morphologic classification of lupus nephritis (modified) Jacob Churg 1995. Adapted from Churg et al, Classification and Atlas of Glomerular Diseases 2nd Edition 1995.

Class I	Minimal mesangial lupus nephritis
Class II	Mesangial proliferative lupus nephritis
Class III	Focal lupus nephritis* (<50% of glomeruli) III (A): active lesions III (A/C): active and chronic lesions III (C): chronic lesions
Class IV	Diffuse lupus nephritis* (≥50% of glomeruli) Diffuse segmental (IV-S) or global (IV-G) lupus nephritis IV (A): active lesions IV (A/C): active and chronic lesions IV (C): chronic lesions
Class V	Membranous lupus nephritis
Class VI	Advanced sclerosing lupus nephritis (≥90% globally sclerosed glomeruli without residual activity)

*Indicate the proportion of glomeruli with active and with sclerotic lesions. *Indicate the proportion of glomeruli with fibrinoid necrosis and with cellular crescents. *Indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, severity of arterio-sclerosis or other vascular lesions. *Class V may occur in combination with III or IV in which case both will be diagnosed.

Table 1.4 The International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) 2003 classification of lupus nephritis. Adapted from Markowitz and D' Agati, 2007.

Two studies have found a higher level of interobserver reproducibility with the ISN/RPS 2003 classification (Fumess and Taub, 2006; Yokohama et al, 2004), which was attributed to clearer distinctions between classes and elimination of the class V subgroups. Importantly, in one study the percentage of cases of class IV doubled from 23% by the WHO classification to 46% by the ISN/RPS 2003. The marked increase in lupus nephritis class IV resulted from the elimination of class Vd and the inclusion of sclerotic glomeruli in the assessment of total glomeruli affected by lupus nephritis, and was associated with a reciprocal decline in lupus nephritis class III and lupus nephritis class V (Fumess and Taub, 2006).

The designations "A" and "C" indicate whether active or chronic changes are present; the higher the chronicity the less likely that the nephritis will respond to immunosuppression (Markowitz and D'Agati, 2007; Hiramitsu, 2008). However, A or C

classifications were not included in the entry criteria for clinical trials in LN published to date, and therefore they are not considered in the recommendations.

1.6.4 TREATMENT

1.6.4.1 Adjunctive treatment

The ACR recommended that all SLE patients with nephritis be treated with a background of hydroxychloroquine (HCQ), unless there is a contraindication. This opinion was based on a prospective controlled trial (Canadian Hydroxychloroquine Study Group, 1991) showing that flare rates of lupus are lower in SLE patients continuing HCQ compared to those who switched to placebo, and on recent cross-sectional and prospective data (Fessler et al, 2005; Pons-Estel, 2009) showing significantly lower damage accrual, including renal damage, in SLE patients receiving HCQ. In addition, HCQ treatment may reduce the risk of clotting events in SLE (Wallace, 1987; Jung et al, 2010).

All LN patients with proteinuria ≥0.5 gm per 24 hours (or equivalent by protein/creatinine ratios on spot urine samples) should have blockade of the renin–angiotensin system, which drives intraglomerular pressure. Treatment with either angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) reduces proteinuria by approximately 30%, and significantly delays doubling of serum creatinine and progression to end-stage renal disease in patients with non-diabetic chronic renal disease (Mann and Bakris, 2011). These classes of medications are contraindicated in pregnancy. The use of combination ACE inhibitors/ ARB therapies is controversial (Kunz et al, 2008). ACE inhibitors or ARB treatments are

superior to calcium-channel blockers and diuretics alone in preserving renal function in chronic kidney disease (Agodoa et al, 2001).

The hypertension should be control with the target ≤ 130/80 mmHg. The recommendation is based on prospective trials and meta-analyses showing that observing this target is associated with a significant delay in progression of renal disease, compared to higher target or inadequate blood pressure control.

1.6.4.2 Specific treatment

Treatment will be based in large part on the classification of type of LN by WHO or ISN/RPS criteria (Markowitz and D'Agati, 2007; Weening et al, 2004). As a result, the recommendations are presented according to the histologic classification of nephritis. *Class I* (minimal mesangial immune deposits on immunofluorescence with normal light microscopy) and class II (mesangial hypercellularity or matrix expansion on light microscopy with immune deposits confined to mesangium on immunofluorescence) generally do not require immunosuppressive treatment. In general, patients with class III (subendothelial immune deposits and proliferative changes in <50% of glomeruli) and class IV (subendothelial deposits and proliferative glomerular changes involving >50% of glomeruli) require aggressive therapy with glucocorticoids and immunosuppressive agents. Class V (subepithelial immune deposits and membranous thickening of glomerular capillaries) when combined with class III or IV should be treated in the same manner as class III or IV. Class V alone ("pure membranous LN") may be approached somewhat differently, and will be discussed later in this chapter. Histologic class VI (sclerosis of >90% of glomeruli) generally requires preparation for renal replacement therapy rather than immunosuppression.

1.6.4.3 Induction therapy for proliferative LN (Class III and IV)

The ACR guideline recommended MMF (2–3 gm total daily orally) or intravenous (IV) CYC along with glucocorticoids (Figure 1.1). MMF and CYC are considered equivalent based on recent high-quality studies, a meta-analysis, and expert opinion (Appel et al, 2009; Chan et al, 2005; Ginzler et al, 2005; Ong et al, Touma et al, 2011). Long term studies with MMF are not as abundant as those with CYC; data show good results for induction therapy with MMF of 3 gm total dose daily for 6 months, followed by maintenance with lower doses of MMF for 3 years (Dooley et al, 2011).

MMF has been similar in efficacy in all races studied to date (whites, Asians, African Americans, and Latin/Hispanic Americans). The Aspreva Lupus Management Study (ALMS) trial comparing response rates of LN to MMF plus glucocorticoids showed similar improvement in whites, Asians, and other races (primarily African Americans and Hispanics). However, the ACR Panel voted that Asians compared to non-Asians might require lower doses of MMF for similar efficacy. Therefore, the physician might aim for 3 gm per day total daily highest dose in non-Asians and 2 gm per day in Asians. A recent study (Weng et al, 2010) reported good responses in Taiwanese treated with these lower doses. There is evidence that African Americans and Hispanics with LN respond less well to IV CYC than do patients of white or Asian races (Appel et al, 2009; Dooley et al, 1997; Isenberg et al, 2010). MMF/mycophenolic acid (MPA) may be an initial choice more likely to induce improvement in patients who are African American or Hispanic (Isenberg et al, 2010).

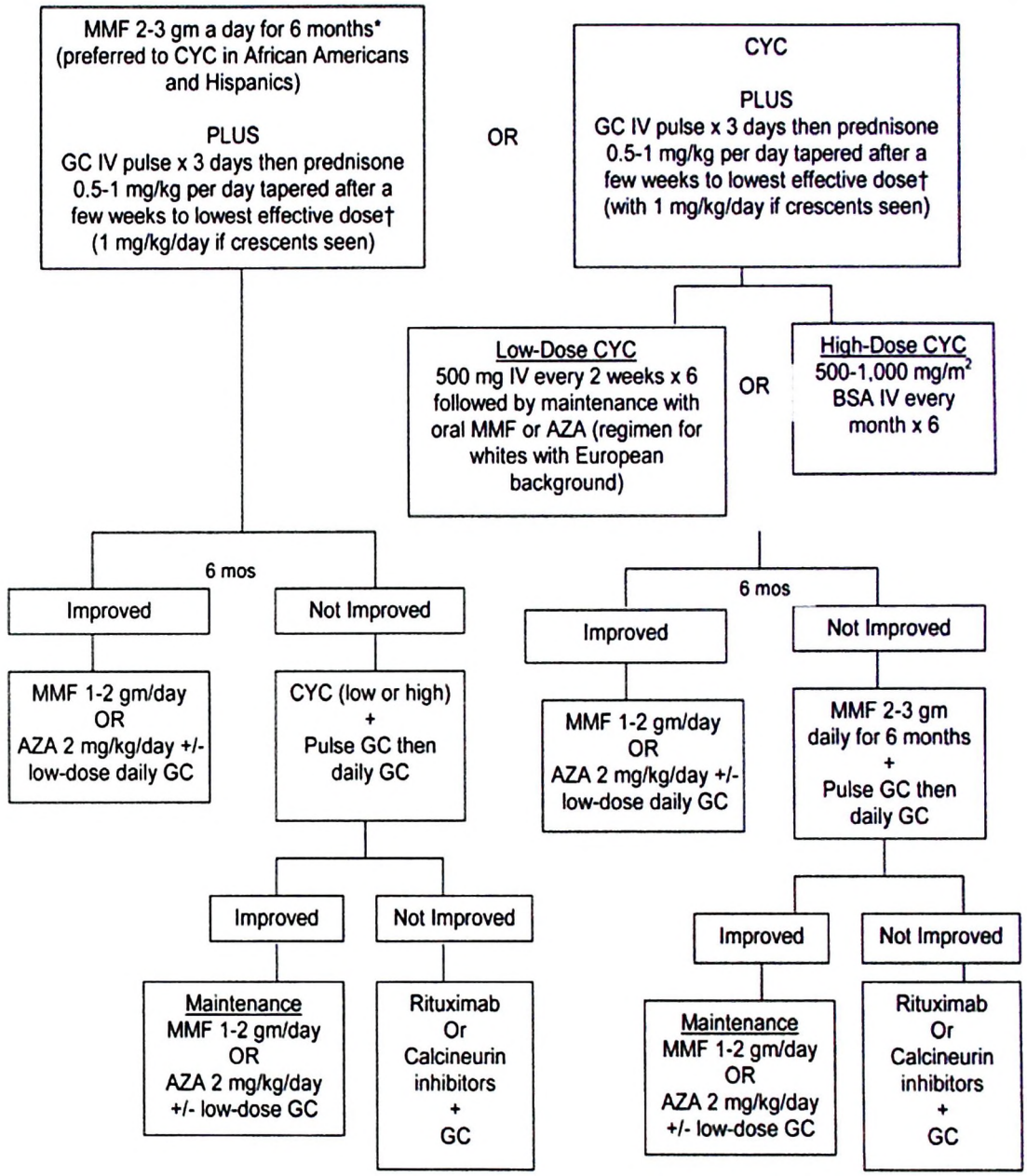

Figure 1.1 Class III/IV induction therapy. MMF _ mycophenolate mofetil; * = the Task Force Panel discussed their preference of MMF over cyclophosphamide (CYC) in patients who desire to preserve fertility; GC =glucocorticoids; IV = intravenous; † = recommended background therapies for most patients are discussed in section III in the text; AZA = azathioprine; BSA = body surface area. Adapted from Bevra et al, ACR Guidelines for Lupus Nephritis 2012.

1.6.4.4 Induction therapy for pure Class V LN

The ACR Guidelines for LN recommends that patients with pure class V LN and with nephrotic range proteinuria be started on prednisolone (0.5 mg/kg/day) plus MMF 2–3 gm total daily dose (Figure 1.2). In a retrospective analysis of patients with class V nephritis, MMF 2–3 gm total daily dose orally plus daily prednisolone (mean 27 mg daily) for 6 months resulted in improvement similar to that with IV CYC (0.5–1.0 mg/kg IV monthly for 6 months) plus prednisolone, with 0–30% of patients having nephrotic range proteinuria after 6 months.

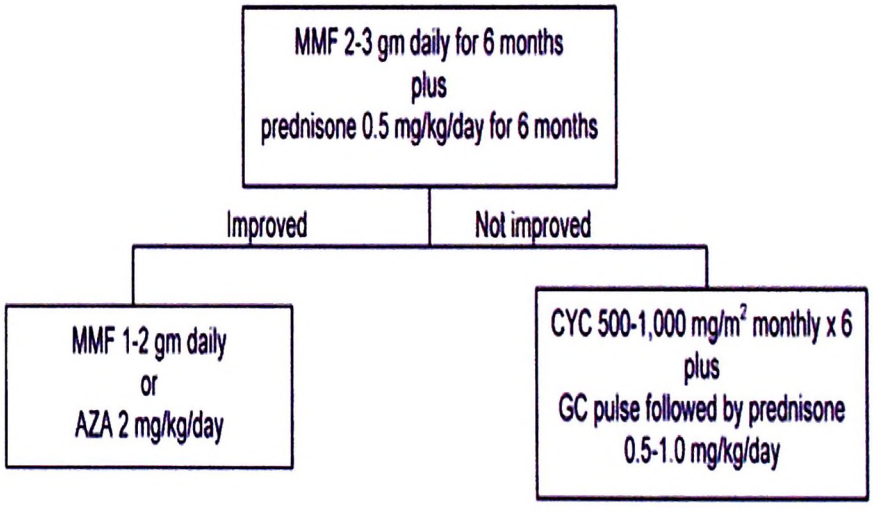

Figure1.2 Treatment of class V without proliferative changes and with nephrotic range proteinuria (≥3 gm/24 hours). Recommended background therapies for most patients are discussed in section III in the text. MMF= mycophenolate mofetil; AZA = azathioprine; CYC = cyclophosphamide; GC = glucocorticoids.
Adapted from Bevra et al, ACR Guidelines of Lupus Nephritis 2012.

1.6.4.5 Maintenance therapy for LN

The ACR guidelines recommended that either AZA or MMF be used for maintenance therapy (Figure 1.1). Two recent prospective trials studied maintenance treatment of patients with LN following induction treatments (Dooley et al, 2011; Houssiau et al, 2010). In the larger study (Dooley et al, 2011), which had sites in the US, Western Europe, China, Argentina, and Mexico, patients who improved after 6 months of either high-dose CYC or MMF were randomized to be maintained on either AZA 2 mg/kg/day or MMF 2 gm total daily dose. Prednisolone up to 10 mg daily was permitted. Over 3 years of follow up, MMF was statistically better than AZA in time to treatment failure (a composite including death, end stage renal disease, doubling of serum creatinine, and renal flare), and in each element of the composite score. Severe adverse events occurred in significantly more patients receiving AZA than receiving MMF.

In the smaller study (Houssiau et al, 2010), with sites in Western and Southern Europe, all patients receiving low-dose CYC, regardless of initial response, were randomized for maintenance therapy with either AZA, with a goal of 2 mg/kg/day, or MMF, with a goal of 2 gm/day. Over a period of 4 years there were no statistically significant differences in any outcome measures, including death, renal flares, end-stage renal disease, or doubling of serum creatinine.

1.7 NOVEL BIOMARKER FOR LUPUS NEPHRITIS

Current laboratory markers for lupus nephritis such as proteinuria, urine protein-to-creatinine ratio, creatinine clearance, anti-dsDNA, and complement levels are unsatisfactory (Chi Chiu Mok, 2010). They lack sensitivity and specificity for differentiating renal activity and damage in lupus nephritis (Saisoong et al, 2006).

Significant kidney damage can occur before renal function is impaired and first detection by laboratory parameters. Persistent proteinuria may not necessarily indicate ongoing inflammation in the kidneys and may be contributed by pre-existing chronic lesions or recent damage in the kidneys during the course of the disease. Flares of nephritis can occur without any observable and recent increase in the degree of proteinuria. Renal biopsy is the gold standard for providing information on the histological classes of lupus nephritis and the relative degree of activity and chronicity in the glomeruli. However, it is invasive and serial biopsies that are impractical in the monitoring of lupus nephritis. Thus, novel biomarkers that are able to discriminate lupus renal activity and its severity, predict renal flares, and monitor treatment response and disease progress are clearly necessary.

A biomarker refers to a biologic, biochemical, or molecular event that can be assayed qualitatively and quantitatively by laboratory techniques. The levels of biomarkers should correlate with disease pathogenesis or activity in different organ systems. An ideal biomarker for lupus nephritis should possess the following properties: (1) good correlation with renal activity as reflected by the degree of proteinuria and urine sediments, (2) sensitive to change so that it can be used for serial monitoring of disease activity in the kidneys and defining treatment response and clinical remission, (3) ability to predict renal activity/flares before an obvious change in conventional clinical parameters occurs so that early treatment/ preventive strategies can be considered, (4) specific to nephritis among patients with SLE, and (5) specific to SLE for aiding early diagnosis of lupus nephritis. In addition, a useful biomarker should be easy to assay, simple to interpret, and readily available in most laboratories with a reasonable cost (Chi Chiu Mok, 2010).

1.7.1 ROLE OF CYTOKINES AS BIOMARKER IN SLE AND LN

Cytokines have emerged as important players in the pathogenesis of autoimmune diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Anticytokine therapies have revolutionized the treatment of RA and Crohn's disease, though have yet to have a major impact in SLE. Knowledge of cytokine biology is gradually increasing, with new cytokines being identified, and more information about cytokine interactions. Increasing knowledge of cytokines is contributing to further understanding about the pathogenesis of SLE. This in turn may lead to the potential use of cytokine measurements as part of a monitoring strategy for SLE and lupus nephritis, as well as identification of possible therapeutic targets.

Routine methods of assessing disease activity in SLE are of limited sensitivity and specificity. Current testing includes acute phase markers, erythrocyte sedimentation rate and C-reactive protein and autoimmune serology, including anti-double-stranded DNA (dsDNA) antibodies, anti-C1q antibodies and complement C3 and C4 levels. Although a relationship between dsDNA antibodies has been shown in several studies (Borg et al, 1990), in a meta-analysis, the mean positive likelihood ratio of anti-dsDNA antibodies as a marker of lupus activity was 4.14, indicative of low overall predictive value (Kavanaugh et al, 2002). Both complement components, C3 and C4 had high false positive and negative rates (Birmingham et al, 2008). Sensitivity, specificity, and positive predictive values were correspondingly low; being C3 (sensitivity/specificity 75%/71%) and C4 (sensitivity/specificity 48%/71%) (Birmingham et al, 2008). Thus, improved markers are required to assist clinicians looking after lupus patients.

Such markers may help in a variety of ways, including early detection of flare, distinction between flare and chronic damage, distinction between flare and infection

and monitoring response to therapy. Organ involvement in SLE cannot be accurately predicted, and it is interesting to speculate if newer tests can help predict disease course. Thus, cytokine measures have been studied for associations with organ involvement as well as their potential ability to monitor disease. For suspected kidney involvement, renal biopsy is the gold standard for diagnosis. However, as it is invasive and has risks of haemorrhage and infection, it presents a less satisfactory method for monitoring renal involvement. Lupus nephritis requires long-term monitoring over several years, as flares may occur, as well as progressive deterioration of function. Serum cytokine markers may be useful, and there is potential for studying urine as a window into the kidney. It also has to be borne in mind that urinary measurements may also reflect to some extent the degree of tubular dysfunction, rather than purely reflecting underlying glomerular pathology.

Current research focuses on whether cytokine measurements can assist in early detection of renal flare in known lupus nephritis, in distinguishing between flare and chronic damage, detecting renal remission in lupus nephritis, assessing the likely duration of immunosuppression and reducing the need for invasive renal biopsy. A large number of investigators have explored the potential and organ involvement. Many cytokines have been investigated, and studies have differed in their assessment, by testing different cytokines individually or in groups/panels, different sizes of population and different lupus populations. Studies have also differed by testing different samples, including blood, urine and tissue biopsies. Results from the various studies have identified some common cytokine markers, though some conflicting results have also been produced. Biomarkers that correlate with lupus renal activity in cross-sectional studies were listed in Table 1.5 and Table 1.6.

Authors, year	Biomarkers studied	Main Findings
Tseng et al., 2007	Antiendothelial cell antibody	Antiendothelial cell antibody titer higher in active lupus nephritis than nonrenal SLE and correlates with disease activity score
Sabry et al., 2007	Serum ICAM-1	Intracellular adhesion molecule (ICAM)-1 level significantly higher in lupus nephritis than nonrenal SLE and correlates with disease activity score.
Morgan et al., 2007	Serum apoCIII	Total apolipoprotein (apo) CIII levels significantly elevated in lupus nephritis than nonrenal SLE and controls.
Fu et al., 2008	Peripheral blood leukocyte chemokine transcriptional levels	Interferon-inducible chemokines in peripheral blood leucocytes higher in active than inactive lupus nephritis and associated with SLE disease activity score.
Hoftman et al., 2008	MAGE-B2 antibodies	Positive melanoma-associated antigen gene B2 (MAGE-B2) antibody associated with higher SLE disease activity score and active lupus nephritis.
Tucci et al., 2008	Serum and urine IL-12	Glomerular expression of IL-12 predominantly occurs in class IV and V lupus nephritis, serum and urine IL-12 higher in lupus nephritis than nonrenal SLE.
Tan et al., 2008	Anti-CRP antibody	IgG autoantibody against monomeric CRP prevalent in patients with lupus nephritis and associated with SLE disease activity score and renal tubulointerstitial lesions.

Table 1.5 Serum biomarkers that correlate with lupus nephritis activity in cross-sectional studies. Adapted from Chi Chiu Mok, Biomarkers of Lupus Nephritis: A Critical Appraisal, 2010.

Author, years	Biomarkers studied	Main Findings
Kiani et al., 2009	Urine osteoprotegerin (OPG)	OPG strongly associated with renal activity descriptors of the SELENA SLEDAI; medium/high levels of OPG predictive of a urine protein/creatinine ratio of ≥0.5
Wang et al., 2009	FOXP3 mRNA expression in urinary sediments	FOXP3 mRNA expression significantly higher in active than inactive lupus nephritis, and in proliferative than non proliferative nephritis, FOXP3 mRNA level correlated with proteinuria and histological activity index; persistent elevation associated with poor treatment response.
Dhaun et al., 2009	Urine endothelin-1	Fractional excretion of endothelin-1 and urinary endothelin-1/creatinine ratio higher in lupus nephritis than other chronic inflammatory renal diseases when renal function is normal.
Enghard et al., 2009	Urine CXCR3+CD4+T cells	Urinary CXCR3+CD4+ T cells are enriched in lupus nephritis and correlated with SLE disease activity; higher concentration of urinary CXCR3+CD4+ T cells is found in active than inactive nephritis.
Tucci et al., 2008	Serum and urine IL-12	Glomerular expression of IL-12 predominantly occurs in class IV and V lupus nephritis; serum and urine IL-12 higher in lupus nephritis than nonrenal SLE.
Wu et al., 2007	Urine VCAM-1, P-selectin, TNFR-1, and CXCL16	Urinary VCAM-1, P-selectin, TNFR-1, and CXCL16 elevated in lupus nephritis, correlating with proteinuria and SLE disease activity scores; urinary VCAM-1 and CXCL16 showed superior specificity and sensitivity in distinguishing subjects with active renal disease from the other SLE patients.
Hammad et al., 2006	Urine TGFβ-1	Urinary TGFβ-1 level significantly higher in active than silent lupus nephritis, correlated with anti-dsDNA and C3 levels.
Chan et al., 2004	TGFβ and MCP-1 mRNA expression in urine sediments.	Urinary TGFβ and MCP-1 mRNA expression correlated with histological activity index on renal biopsy.

Table 1.5 Urine biomarkers that correlate with lupus nephritis activity in cross-sectional studies. Adapted from Chi Chiu Mok, Biomarkers of Lupus Nephritis: A Critical Appraisal, 2010.

1.8 VASCULAR ENDOTHELIAL GROWTH FACTOR

1.9.1 OVERVIEW OF VEGF

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is a potent mediator of both angiogenesis and vasculogenesis in the fetus and adult (Leung et al, 1989). It is a member of the PDGF family that is characterized by the presence of eight conserved cysteine residues in a cystine knot structure and the formation of antiparallel disulfide-linked dimers. Humans express alternately spliced isoforms of 121, 145, 165, 183, 189, and 206 amino acids (aa) in length (Robinson, C.J and S.E. Stringer, 2001). VEGF165 appears to be the most abundant and potent isoform, followed by VEGF121 and VEGF189 (Byrne et al, 2005). Isoforms other than VEGF121 contain basic heparin-binding regions and are not freely diffusible. Human VEGF165 shares 88% amino acid sequence identity with corresponding regions of mouse and rat VEGF. VEGF is expressed in multiple cells and tissues including skeletal and cardiac muscle (Sugishita, Y. et al, 2000), hepatocytes (Yamane et al, 1994), osteoblasts (Goad et al, 1996), neutrophils (Gaudry et al, 1997), macrophages (Mclaren J et al, 1996), keratinocytes (Diaz et al, 2000), brown adipose tissue (Asano et al, 1997), CD34+ stem cells (Bautz F et al, 2000), endothelial cells (Namiki et al, 1995), fibroblasts, and vascular smooth muscle cells (Nauck et al, 1997). VEGF expression is induced by hypoxia and cytokines such as IL-1, IL-6, IL-8, oncostatin M and TNF-α. VEGF isoforms are differentially expressed during development and in the adult (Byrne et al, 2005).

VEGF dimers bind to two related receptor tyrosine kinases, VEGF R1 (also called Flt-1) and VEGF R2 (Flk-1/KDR), and induce their homodimerization and autophosphorylation. These receptors have seven extracellular immunoglobulin-like