Clinico-Pathological features of repeat renal biopsies in patients with lupus nephritis at Groote Schuur Hospital, Cape Town

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DECLARATION

I, Shepherd Kajawo, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, or is to be submitted for another degree in this or any other university.

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DEDICATION

For my lovely wife, Marian and beautiful daughter Anesu

TABLE OF CONTENTS **Page**

PREAMBLE

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FIGURES AND TABLES **Page**

Abbreviations

ACE-Angiotensin-converting enzyme inhibitor

ARB-Angiotensin II receptor blocker

ANA-Anti Nuclear Antibodies

Anti-dsDNA-Anti double stranded DNA antibodies.

CKD-Chronic Kidney Disease

CR-Complete Remission

ESKD-End Stage Kidney Disease

EGFR-Estimated Glomerular Filtration Rate

Glomerular basement membrane-GBM

GSH-Groote Schuur Hospital

ISN-International Society of Nephrology

KDIGO-Kidney Disease Improving Global Outcomes

LN-Lupus Nephritis

MMF-Mycophenolate Mofetil

PR-Partial Remission

SLE-Systemic Lupus Erythematosus

SLICC-Systemic lupus international collaborating clinics

SA-South Africa

RPS-Renal Pathology Service

UPCR-Urine Protein creatinine ratio

WHO-World Health Organisation

Abstract

Background: Repeat renal biopsies in patients with lupus nephritis (LN) are usually performed to guide treatment or to establish disease chronicity. Their value is not clear from available literature. There is also no available data in Africa to guide clinicians.

Methods: This was a retrospective study of patients undergoing a repeat renal biopsy between January 2003 and December 2014 from a single centre in Cape Town, South Africa. Relevant demographic, clinical and histological records of patients with repeat renal biopsies were documented. Comparison of data from 1st and 2nd renal biopsy was performed.

Results: 44 patients had at least 2 biopsies performed during the study period. Most patients were females (81. 8%). The mean biopsy interval was 2.8±1.8 years. Proteinuria was the main indication for repeat biopsy (36.1%). The glomerular filtration rate and proteinuria worsened between the two biopsies (p=0.001 and 0.019) respectively suggesting disease progression. Most patients (65.4%) with a non-proliferative class of LN at first biopsy progressed into a proliferative class whereas patients with initial proliferative LN at first biopsy (77.8%) remained as proliferative at repeat biopsy. Treatment was changed in 85% of patients at second biopsy.

Conclusion: Repeat renal biopsies in patients with LN presents a useful means of assessing disease progression and provides guidance regarding modification of treatment. More studies are however required to evaluate the value of repeat biopsies and perhaps the need for protocol renal biopsies in patients with LN.

Chapter 1: Structured Literature review

(Word count 3154)

1.0 Objectives of the Literature review

1.To give a broad overview of what is known about Systemic Lupus Erythematosus (SLE) and Lupus Nephritis (LN) from the published literature and International society guidelines.

2.To put into context what is already known about LN globally in an African perspective.

3.To identify important studies on repeat renal biopsies performed to date.

4.To Identify clinical relevance of repeat renal biopsies, if any, from the published literature

2.0 Broad Overview of SLE

2.1 Epidemiology

Systemic lupus erythematosus (SLE) is a chronic multi system autoimmune disease characterised by frequent remissions and relapses. The pattern of disease that dominates during the first few years of illness normally prevails throughout the course of disease.[1]

Women are nine times more affected than males.[2] The reported prevalence of SLE is 20 to 150 cases per 100,000 in United States of America.[3] However the disease is said to be rare in West Africa with increasing frequency in Central and Southern Africa.[4] Highest prevalence of the disease is reported in people of African descent living in America, the Caribbean and Europe.[5]

This prevalence gradient is thought to be related to:

a) Racial admixture.[4] In Cape Town, South Africa for example SLE is reported more frequently in patients of mixed ancestry.[6]
b) Increased exposure to environmental factors like tobacco products and viral infections.[7]
c) Genetic polymorphisms like in the Fc gamma RIIb that offer protection to malaria may increase susceptibility to SLE in Africans and Asians.[8]

The reported incidence and Prevalence of SLE in Africa is thought to be largely underestimated because of under diagnosis due to poor access to health care, low disease recognition within primary health care settings, limited access to diagnostic tools and inadequate numbers of specialist physicians.[9,10]

2.2 Diagnosis of SLE

Patients are classified as having SLE if they meet the new classification criteria, Systemic Lupus International Collaborating Clinics (SLICC) criteria which has improved sensitivity compared to the old ACR criteria.[11] (Table 1) The presence of clinical features of nephritis raises suspicions of LN which should be confirmed on histology.

Table 1: SLICC Classification Criteria for SLE[11]

Requires 4 or more criteria (at least 1 clinical and 1 laboratory criteria)

Clinical Criteria	**Immunological Criteria**
1.Acute cutaneous Lupus	1.ANA
2.Chronic cutaneous Lupus	2.Anti-DNA
3.Oral or Nasal ulcers	3.Anti-Sm
4.Non-Scarring alopecia	4.Antiphospholipid Antibody
5.Arthritis	5.Low complement (C3, C4, CH50)
6.Serositis	6.Direct Coombs test
7.Renal involvement	
8.Neurologicinvolvement	
9.Haemolytic Anaemia	
10.Leukopaenia	
11.Thrombocytopaenia(<100 000/mm3)	

2.3 Clinical Presentation

SLE may go on for many months and possibly years undetected due to the presence of vague constitutional symptoms such as fever and malaise. This can result in delayed diagnosis therefore constituting an unmet need in patients with SLE.[12] A high index of suspicion is therefore needed in patients without typical features to avoid late presentation with irreversible end-organ damage.

Table 2 shows the various clinical presentations based on a study of 1000 European SLE Patients and another study from South Africa with 226 patients with SLE.[13,14] The table show a higher number of patients with nephropathy in SA.

Table 2: Prevalence of clinical features in patients with LN

SLE Manifestation	Euro Lupus cohort (%) Cervera et al [13] **N=1000**	SA Cohort (%) Wadee et al[14] **N=226**
Arthritis	84	70.4
Malar Rash	58	58.4
Fever	52	*nr
Photosensitivity	45	38.9
Nephropathy	39	43.8
Serositis	36	18.1
Raynaud's Phenomenon	34	nr
Neurologic Involvement	27	15.9
Oral ulcers	24	38.5
Thrombocytopenia	22	12.8
Sicca Syndrome	16	nr
Livedo Reticularis	14	nr
Thrombosis	14	nr
Lymphadenopathy	12	nr
Discoid Lesions	10	41.5
Myositis	9	nr
Haemolytic Anaemia	8	nr
Lung Involvement	7	Nr
Subcutaneous Lesions	6	nr
Chorea	2	nr

*nr=not reported

3.0 Kidney involvement in SLE

3.1 - Defining kidney disease in SLE

The presence of Lupus Nephritis(LN) should be considered in any lupus patient with impaired kidney function, proteinuria, hypertension, or an active urine sediment according to KDIGO guidelines.[15]

An active sediment include presence of haematuria, leukocytes, red blood cell casts and white blood cell casts. Kidney involvement is specifically defined as persistent proteinuria (>0.5 g/24 h) or presence of cellular red cell casts by the SLICC criteria.[11]

However LN must be confirmed by kidney biopsy because the histologic findings provide the basis for treatment recommendations for LN.[15] A large study in Cape Town actually found out that most patients with LN will present with the proliferative form of the disease (Class III and IV) making it imperative to make an early diagnosis.[6]

The clinical presentation of LN varies from asymptomatic haematuria and/or proteinuria to nephrotic syndrome and rapidly progressive glomerulonephritis with loss of renal function. In South Africa, Nephrotic range proteinuria is a very common initial presentation.[16] In a study of 251 patients with LN, proteinuria was reported to be positively correlated with proliferative LN in SA.[6] Urinalysis therefore presents an early opportunity for early diagnosis, for example LN must be strongly suspected in any SLE patient with high titres of dsDNA and a positive dipstick for blood.[6,10]

3.2 LN a challenging problem in Africa and Globally

Lupus nephritis is the commonest secondary glomerular disease reported in South Africa and other parts of the world.[16-18] This is further supported by a recent systematic review from Africa which found LN and Hepatitis B to be commonest causes of secondary glomerular diseases.[19] An estimated 25–50% of SLE patients will have urinary abnormalities or decline in renal function in the early course of the disease with up to 60% of adults and 80% of children with SLE developing renal abnormalities in the latter course of the disease.[20] There is good evidence that renal involvement in SLE is associated with reduced quality of life and increased morbidity and mortality.[21] In the Euro Lupus Project, which had 1000 patients with SLE from several European countries that were followed prospectively from 1991,renal involvement was reported to be 39%.[13] In South Africa the reported prevalence is slightly higher (43%).[14] However much higher prevalence of LN have been reported in other parts of Africa, 49,5% in North Africa in a study of 749 patients and up to 70% in Zimbabwe.[22,23]

3.3 Clinical Course of LN

The presence of the following factors on initial presentation has been shown in many studies to predict poor outcomes in LN.[24-27]:

- Elevated serum creatinine
- Hypertension
- Nephrotic range proteinuria
- Anaemia
- Black and Hispanic race.

In South Africa, Ayodele et al reported that baseline serum creatinine and failure of remission in the first year were associated with poor renal outcomes in patients with biopsy proven LN.[28] In another study from South Africa, 50% of patients with proliferative LN reached the composite end point of doubling of creatinine, ESRD or death.[29] This is possibly explained by a high prevalence of APOL1 gene risk alleles in LN patients of African ancestry, which has been found to be a risk for faster CKD progression and ESRD.[30]

Once SLE patients develop LN their outcomes are worse than those with no kidney involvement suggesting that LN is a manifestation of a more severe form of SLE. The reported mortality of SLE in Africa is very high, in a study from South Africa for example about 72% of patients were either dead or lost to follow up after 55 months and nephritis was the only predictor of mortality on multivariate analysis.[14]

3.4 Classification of kidney disease in SLE

LN is classified using a new classification system (ISN/RPS,Table 3) which is an improvement of the old modified WHO system.[31,32] It allows standardisation of definitions and emphasise clinically relevant lesions making comparability easier between centers.[33]The ISN classification system divides glomerular disorders into six different patterns (or classes) based upon kidney biopsy histopathology.[32] It is recommended that each biopsy specimen should contain at least 10 glomeruli.[34]

Table 3 ISN/RPS Classification of LN[31]

Class I	**Minimal mesangial lupus nephritis**
	Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
Class II	**Mesangial proliferative lupus nephritis**
	Purely mesangial hyper cellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits
	A few isolated sub-epithelial or sub endothelial deposits may be visible by immunofluorescence or electron microscopy, but not by light microscopy
Class III	**Focal lupus nephritis** Active or inactive focal, segmental or global endo- or extra capillary glomerulonephritis involving <50% of all glomeruli, typically with focal sub endothelial immune deposits, with or without mesangial alterations
III (A)	Active lesions: focal proliferative lupus nephritis
III (A/C)	Active and chronic lesions: focal proliferative and sclerosing lupus nephritis
III (C)	Chronic inactive lesions with glomerular scars: focal sclerosing lupus nephritis
Class IV	**Diffuse lupus nephritis** Active or inactive diffuse, segmental or global endo- or extra capillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse sub endothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation.
IV-S (A)	Active lesions: diffuse segmental proliferative lupus nephritis
IV-G (A)	Active lesions: diffuse global proliferative lupus nephritis
IV-S (A/C)	Active and chronic lesions: diffuse segmental proliferative and sclerosing lupus nephritis
IV-G (A/C)	Active and chronic lesions: diffuse global proliferative and sclerosing lupus nephritis
IV-S (C)	Chronic inactive lesions with scars: diffuse segmental sclerosing lupus nephritis
IV-G (C)	Chronic inactive lesions with scars: diffuse global sclerosing lupus nephritis
Class V	**Membranous lupus nephritis** Global or segmental sub epithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed Class V lupus nephritis may show advanced sclerosis
Class VI	**Advanced sclerotic lupus nephritis** ≥90% of glomeruli globally sclerosed without residual activity

5.0 - Treatment of lupus nephritis

The 2003 ISN/RPS classification of LN allows for standardization of treatment. The aim of treatment is to achieve a complete remission (CR) or partial remission(PR) because this is associated with improved outcomes compared with no response.[35] The recommended treatment regimens are summarized in Table 4 and 5 and briefly discussed below:

5.1 General measures-For all patients

a) Aggressive antihypertensive control with the goal blood pressure less than 140/90 mmHg if no albuminuria but 130/80 in the presence of albuminuria.[36,37]

b) In patients with proteinuria, antiproteinuric therapy with an ACEI or ARB and goal protein excretion is less than 500 mg per day or at least 60 percent below baseline.[38]

c) All patients with SLE regardless of disease activity should be treated with hydroxychloroquine or chloroquine unless contraindicated.[10] Hydroxychloroquine and chloroquine has been shown to reduce constitutional symptoms, musculoskeletal manifestations, flare rates, thrombotic events, organ damage accrual and mortality.[39,40]

5.2 Immunosuppressive Treatment

Patients with proliferative LN should be pulsed with steroids and started on cyclophosphamide or mycophenolate mofetil (MMF) as part of induction treatment[10,15] This is then followed by maintenance treatment with MMF or Azathioprine with low doses of steroids.[10,15] There is no consensus on the duration of maintenance though at least 1 year is generally advised.[15] In South Africa the practise is to give maintenance treatment for at least 2 years.[10]

A modified National Institute of Health Study (NIH) protocol is used in South Africa instead.[10] Cyclophosphamide cheaper than MMF, hence MMF is reserved to those intolerant or have failed induction with cyclophosphamide. However, this may change as generics become easily available.

The oral Cyclophosphamide regimen is not favoured because it is associated with longer treatment duration, greater cumulative doses and more severe leucopenia.[41]

- The Euro Lupus regimen which uses less doses of cyclophosphamide is not favoured in Africa where the disease is much more severe compared to the population in the Euro Lupus study.[10,29,42]

Table 4: Recommended Induction treatment for Proliferative LN.[15]

Class	**III & IV**
NIH Regimen	IV cyclophosphamide 0.5-1 g/m^2 monthly for 6 months
Euro-Lupus Regimen	IV Cyclophosphamide 500mg every 2 weeks for 3 months
Oral Cyclophosphamide Regimen	1 -1.5mg/kg per day 150mg max per day for 2-4 months
MMF regimen	MMF up to 3g/d for 6 months
All regimens	IV Methylprednisolone in severe cases followed by oral prednisolone 0.5-1mg/kg tapered over 6 to 12 months according to clinical response

Table 5: Treatment of Non-Proliferative Lupus Nephritis.[15]

Class	Recommendation
I	Treatment as dictated by the extra renal clinical manifestations of lupus.
II	<1g proteinuria: Treatment as dictated by the extra renal clinical manifestations of lupus >3g proteinuria: Corticosteroids or Calcineurin Inhibitors
V	Normal kidney function and non–nephrotic range Proteinuria: antiproteinuric and antihypertensive medications, and only receive corticosteroids and immunosuppressive as dictated by the extra renal manifestations lupus. Persistent nephrotic proteinuria: Corticosteroids plus an additional immunosuppressive agent: cyclophosphamide or Calcineurin inhibitor or MMF
VI	Treatment as dictated by the extra renal clinical manifestations of lupus.

6.0 - Flare of lupus nephritis

6.1- Definition of a lupus flare

A flare or relapse is a measurable increase in disease activity in one or more organ systems involving new or worse clinical findings, laboratory measurements or changes in activity of daily living.[15] It must be considered clinically significant and usually there would be consideration of an increase in treatment. Appendix 9.

6.2 - Predicting Flares in Lupus Nephritis

As high as 20% of patients with LN can have a new kidney flare per year of follow-up.[43] Even in patients who achieve CR , as much as 40% will experience a kidney relapse within a median of 41 months after remission.[44]

Increased serum anti-dsDNA antibodies and low complement especially CH50, C3, and C4 can be useful in predicting lupus flares.[43,45] Recently anti-C1q was found to have higher correlation with flares of LN than other serum markers.[46] Increased proteinuria, an active urine sediment, a rise in creatinine or a fall in eGFR can also be used to identify a flare.[15]

6.3 Limitation of Clinical and Serological Markers in Predicting flares

The challenge in managing lupus flares is that none of these biomarkers can replace renal histology and indeed it is quite possible to have significant renal activity on biopsy in the absence of heavy proteinuria or an active urine sediment.[47]

Standard clinical and laboratory parameters have limited predictive values for discriminating between active LN and chronic disease.[48] Thus, serological markers of disease activity do not adequately reflect the amount of renal inflammation in LN and cannot replace renal biopsy as a diagnostic and predictive tool for active disease. Therefore clinical and laboratory features alone are often not sufficient to guide treatment hence the need for renal biopsies.[49-51] Therefore a renal biopsy is considered as the gold standard for assessing renal activity and hence defining the need for a change in treatment.[15]

7.0 - Renal biopsies in Lupus Nephritis

7.1- Diagnostic Role

In patients with SLE a renal biopsy is recommended in the following circumstances.[15,52]:

1.Protein excretion greater than 500 mg/day.
2.An active urinary sediment (haematuria, cellular casts).
3.A rising serum creatinine that is not clearly attributable to another mechanism.

In those patients with an indication for renal biopsy it is important for the procedure to be performed as soon as possible as delays have been associated with poor long term prognosis.[53,54]

7.2 Repeat renal biopsy strategy: Protocol Vs Repeat biopsy after a flare

There is often need to repeat renal biopsies in patients treated for LN due to the limitations of clinical and laboratory measures described earlier. There are two strategies employed in performing repeat kidney biopsies:

- Protocol biopsies at end of induction or maintenance treatment to assess treatment efficacy and need for more immunosuppression.[48,55-60]
- Repeat renal biopsies only after a clinically suspected flare to assist in making treatment choices.[15,61-65]

There is no consensus on which practice is superior and clinical practice varies from centre to centre with most clinicians and international guidelines preferring clinically driven repeat kidney biopsies.[15,66]

8.0-Repeat Renal biopsies and Treatment decisions

8.1 Protocol Kidney Biopsies at end of induction or maintenance treatment

The challenge in managing LN is that even after achieving CR, patients can surprisingly still have active disease in their kidneys which makes protocol biopsies at the end of maintenance treatment useful is such cases.[48]

In a prospective study of 77 patients in Saudi Arabia by Alsuwaida, only 40% of patients with CR had no histological evidence of active disease.[48] Similarly Zickert et al in Sweden found that 29% of patients with CR had active lesions when protocol biopsies were done 8 months after induction immunosuppression.[67] Recently an Argentinian study has also shown that LN can still be active after several years of immunosuppression and clinical quiescence, and that ESKD in LN can occur rapidly.[57] Therefore, remission status lacks sensitivity and specificity for differentiating renal activity and damage in LN.[48] This makes it difficult to decide when to stop treatment in such cases with clinically quiescent disease after maintenance treatment without repeating a kidney biopsy. These active lesions may lead to rapid renal decline when treatment is discontinued.[57,68]

A recent multicentre study in Italy with 142 patients reported important histological changes at repeat biopsy and recommended use of protocol biopsies[69]. In this study the disease was more severe in both first and repeat biopsy in Class IV-G compared with Class III and IV-S patients and repeat renal biopsy predicted better trend of serum creatinine and proteinuria than the first biopsy.[69,70]. This important study strongly suggests that even in subclasses of proliferative LN important clinical and histological features occur which may even point towards differences in pathogenesis.[70]

More so, it has also been observed that patients with old renal scarring may continue to have low-grade proteinuria despite absence of any activity at repeat biopsy resulting in maintenance of unnecessary immunosuppression. Alvarado et al in a study of 25 LN patients in Argentina illustrates this well when they found that 60% of those with ongoing proteinuria did not have any activity in their kidneys at the repeat biopsy.[57]

8.2 Clinically driven repeat renal biopsies after a flare

This mainly stems from a number of studies that have demonstrated changes in histological class when LN patients relapse.[62,71,72] This has treatment implications since therapy for LN is now based on the histological class of the disease.[48] In membranous nephritis (Class V) for example, it has been shown that as much as 35% can transform into proliferative classes which usually warrant treatment change.[73] Greloni et al in a retrospective study of 45 patients found re-biopsies to result in treatment changes in 87.3% of their cases.[65] Similar findings have also been reported from other studies.[49,59,61,62,74]

It has also been shown that the fibrosis score or degree of inflammation seen in repeat biopsies help in assessing prognosis, predicting flares and response to treatment.[61,65,69,75] In a prospective study of 71 patients for example, the outcome of renal relapse was determined by the initial response of inflammatory and chronicity elements to therapy, with those with prior partial reversal of interstitial and glomerular scarring having a good outcome, whereas those in whom fibrotic lesions have continued to increase having a poor outcome.[75]

8.3 The Controversy

On the contrary, there is a scholarly view that there is no role for repeat biopsy either in the form of protocol biopsies or after a flare.

The argument here is that if one was treated for diffuse proliferative disease in the first biopsy for example, the relapse is more likely to represent recurrent proliferative disease on second biopsy.[63,76,77] Therefore, the repeat renal biopsy is unlikely to provide any additional data that would affect treatment in such patients.[63,70] As much as 84% of patients with a proliferative histology in their reference biopsy were found by Daleboudt et al in Netherlands to remain in a proliferative class at the repeat renal biopsy.[63] Since the recommended treatment of class III and IV by guidelines is the same, treatment is unlikely to be changed in such cases thus making repeat biopsies unnecessary[15]. The small risk of bleeding and the cost associated with the procedure makes it imperative that the re-biopsy is performed when the benefit to the patient is clear.[49,63]

9.0 Clinical Equipoise in Repeat Renal biopsies

9.1-Comparative studies in Repeat Renal biopsies

Table 6 below summarises some of the major findings from the published literature on studies of patients with repeat renal biopsies. The largest study to date is that of Lu et al from China which had 156 biopsies and had the largest histological class transformation (75%). There are no published reports from Africa. There is a general trend for CI to increase between the biopsies and lower AI at the repeat biopsy as shown by the table.

Table 6: Review of some of the major studies published on repeat renal biopsies

	Pagni et al[69]	Daleboudt et al[63]	Wang et al[64]	Greloni et al[65]	Alvarado et al[4]	Lu et al[78]	Esdaile et al[59]	Bajaj et al[62]
Country	Italy	Netherlands	China	Argentina	Argentina	China	US	Canada
Sample size	142	35	44	45	25	156	42	57
Female (%)	88.8	74.2	95	40	88	91	NR	84.2
Publication year	2013	2009	2012	2014	2014	2011	1993	2000
Study design	Retrospective	Retrospective	Retrospective	Retrospective	Prospective	Retrospective	Prospective	Retrospective
Study duration (type)	37yrs (multi-centre)	NR(single)	16yrs(single)	8.7yrs(single)	6yrs (single)	20yrs(single)	17yrs(single)	24yrs(Single)
Biopsy interval in years (SD)	4.9 ± 4.9	4.1 ± 3.6	NR	3.4(4.4) *	6	NR	2.1(1.8-2.5) **	4.2yrs
AI-1/AI-2	4.5 / 3.3	6.18 / 5.27	5.8 / 4.7	NR	8.9/4.3/0.96	6.8	7/2	5.09/3.96
CI-1 / CI-2	1.5 / 3.6	2.6 / 4.2	1.8/3.4	2.9/6.6	2.8/4.2/4.3	2.6	2/2	1.30/3.37
Change in chronicity score	+2.1	+1.6	+1.6	NR	+1.5	NR	0	+2.07
Proliferative class at biopsy 1 and 2(%)	70.3/70.4	87.8/93.9	NR	60/66.7	NR	71.7/59.4	83.3/50	58/44
Proliferative to Proliferative switch (%)	82	95.3	NR	75.6	NR	60	54.3	75
Non-Proliferative to Proliferative switch (%)	42.9	83.3	NR	58.3	NR	58	28.6	22.2
Overall Pathological transition (%)	40.8	55.7	64	54.9	NR	75	46	40.4
Proteinuria biopsy g/24h 1/2 (%)	3.5/3.1	NR	NR	NR	3.3/1.13/0.32	3.28/#	0.99/0.50	2.48/1.35
Changes in treatment (%)	NR	77.5	34	87.3	64	NR	NR	77

9.2 Gaps in Literature

- No studies in South Africa and the whole of Africa looking at repeat biopsies in patients with lupus nephritis. This is an important area to study given that LN is not rare in Africa[9] and in fact it is probably more aggressive and thus limiting the generalisability of data from other parts of the world. There is strong evidence for the genetic heterogeneity of SLE and reported differences in severity in different population groups making it imperative to have local data to guide clinicians. (Appendix 7)
- Evidence available is from very small studies which are mainly retrospective in nature looking at the utility of re-biopsies hence larger prospective trials, using protocol biopsies, are needed to investigate more deeply the relationship between histology and clinical data in re-biopsies.

9.3 - Rationale of the present study

Due to paucity and absence of quality evidence for re-biopsy of patients with LN, the KDIGO recommendation is to either repeat kidney biopsies or change to an alternative recommended initial therapy in patients with rising creatinine or proteinuria during the first 3 months of treatment.[15] KDIGO also recommends that if CR has not been achieved after 12 months of maintenance therapy, to consider performing a repeat kidney biopsy before change in therapy.[15]

At Groote Schuur Hospital (GSH), we do not routinely perform kidney biopsies after starting immunosuppressive treatment (Protocol biopsies). Rather, patients are routinely monitored and followed up using clinical measures such as serum creatinine, urine protein excretion, and urine microscopy. Persistent abnormalities of these measures are utilized for deciding timing of repeat renal biopsies in patients with LN. We therefore repeat renal biopsies in our patients at GSH if there is evidence of worsening disease or refractory to treatment, evidence of relapse (to show transformation or progression in histological class or change in activity and chronicity scores) and to demonstrate other pathologies.[10] This is in line with international best practice.[61,62] It therefore remains unclear whether repeat biopsies result in clinically

meaningful histological changes and thus significant treatment changes due to unavailable local data.

10.0 Study Objectives

10.1 Main Objective

To evaluate the clinical relevance of repeat renal biopsy in patient with LN as practised in the renal unit at GSH.

10.2 Specific objectives

1.To compare the histological class before and after the biopsy.

2.To determine the proportion of patients whose treatment was altered after the repeat biopsy.

3.To compare the activity and chronicity indices between the repeat and reference biopsy.

4 To compare clinical and laboratory features (proteinuria, serum creatinine) before and after biopsy.

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Chapter 2:Journal Ready Manuscript

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Title

Clinico-pathological features of repeat renal biopsies in patients with lupus nephritis at Groote Schuur Hospital, Cape Town.

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Abstract

Background: Repeat renal biopsies in patients with lupus nephritis (LN) are usually done to guide treatment or to establish disease chronicity. Their value is not clear from available literature. There is also no available data in Africa to guide clinicians.

Methods: This was a retrospective study of patients undergoing a repeat renal biopsy between January 2003 and December 2014 from a single centre in Cape Town, South Africa. Relevant demographic, clinical and histological records of patients with repeat renal biopsies were documented. Comparison of data from 1st and 2nd renal biopsy was performed.

Results: 44 patients had at least 2 biopsies done during the study period. Most patients were females (81.8%). The mean biopsy interval was 2.8± 1.8 (range 0.38 – 9.4) years. Proteinuria was the main indication for the repeat biopsy (36.1%). The glomerular filtration rate and proteinuria worsened between the two biopsies (p=0.001 and 0.019) respectively suggesting disease progression. Most patients (65.4%) with a non-proliferative class of LN at first biopsy progressed into a proliferative class whereas patients with initial proliferative LN at first biopsy (77.8%) remained as proliferative at repeat biopsy. Treatment was changed in 85% of patients at second biopsy.

Conclusion: Repeat renal biopsies in patients with LN presents a useful means of assessing disease progression and provides guidance regarding modification of treatment. More studies are however required to evaluate the value of repeat biopsies and perhaps the need for protocol renal biopsies in patients with LN.

Keywords

activity score, chronicity score, renal biopsy, repeat biopsies, proliferative lupus nephritis

Introduction

Patients with lupus nephritis (LN) may experience frequent flares over the course of their disease.[1] Clinical and laboratory features are often not sufficient to guide the treatment of these patients hence the need for repeat renal biopsies. [2-4] As there is no consensus about repeat renal biopsies in patients with LN, they are either performed as protocol biopsies at the end of induction or maintenance treatment or only when a flare is suspected, but this is centre specific. [5, 6]

LN patients with chronic renal scarring may continue to have low-grade proteinuria even without any disease activity in their kidneys resulting in unnecessary immunosuppression with potential for complications. [7] Alvarado et al in a study of 25 LN patients in Argentina illustrates this well when they found that 60% of those with ongoing proteinuria did not have any activity in their kidneys at the repeat biopsy.[7] Thus, clinically quiescent LN during ongoing maintenance treatment presents a challenge regarding timing of reduction or discontinuation of immunosuppression without repeating a renal biopsy as there may be histologically active disease. [7, 8] One of the important reason for repeat biopsies in patients with LN is to identify significant class transformations requiring change of therapy.[2, 9-15] However, there are questions regarding the value of these repeat biopsies if the first biopsy showed proliferative LN given that the same is likely to be seen on repeat biopsy.[15, 16] In one study, 84% of patients with proliferative histology in their reference biopsy remained in a proliferative class of LN at repeat biopsy [17]. Currently, there is insufficient evidence for major guidelines to make strong recommendations on repeat renal biopsy. [18] At our centre in Cape Town, repeat renal biopsies are performed in patients with LN to assess disease activity during a flare or to determine degree of chronic changes, both criteria driven by the need to understand if treatment change will be required.

The utility of a repeat renal biopsy in patients with biopsy proven LN has not been thoroughly investigated in Africa. The aim of this study is therefore to assess the clinical relevance of repeat renal biopsy in patients with LN at a single centre.

Methods

This study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC Ref 732/2014) and was designed as a retrospective analysis of repeat renal biopsies performed for patients known with LN from 1st January 2003 to 31st December 2014. We identified eligible patients through our local renal biopsy database. Patients who have had only one renal biopsy, transplant biopsies and all other cases of non-lupus biopsies were excluded. Although a few patients had more than 2 biopsies performed during the period of assessment, our focus was to compare the clinical, biochemical, histological and treatment features observed between 1st and 2nd biopsies. Paper and electronic records were searched to obtain relevant demographic, clinical, histological and treatment information on all patients who were included. The indication for repeat renal biopsy was categorized as: (i) active urinary sediment, (ii) elevated serum creatinine, (iii) elevated serum creatinine with a urinary abnormality (active sediment, presence of red blood cells or casts, proteinuria), and (iv) elevated proteinuria.A second Pathologist different from the one who reported the initial biopsies again reviewed all histological reports and entered the ISN/RPS final class .[19] Activity and chronicity scores for each patient at the time of first and second biopsies were assessed and recorded using standard criteria.[20]

The average time between 1st and 2nd biopsies was calculated and recorded. Clinical features, histological findings and differences in frequency of LN class between biopsies were recorded. The estimated Glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation.[21]

Treatment received for induction and maintenance therapies at 1st and 2nd renal biopsies were also documented. Changes to treatment that occurred due to the histologic findings of the 2nd renal biopsy was recorded as: (i) treatment escalation – if there was need to increase dose of corticosteroids or other alkylating agents or introduce a new immunosuppression; (ii) treatment reduction – if there was need to stop or reduce the dose of ongoing immunosuppression; and (iii) no change to treatment if there was no change in dose or type of immunosuppression given after the repeat biopsy.

Statistical analysis:

The data was analysed using SPSS Version 23.0 for Windows (SPSS Chicago IL). The results were presented as percentages or as mean ± SD. The students t-test (for continuous variables) and the chi-square test (for categorical variables) were used to test for differences between variables at the 1st and 2nd biopsies. P-values less than 0.05 was considered statistically significant.

Results

A total of 2581 native renal biopsies were performed during the study period. Of these, 369 (14.3%) were in patients with LN of which 44 patients (11.9%) had at least one repeat biopsy. The baseline and demographic characteristics of the patients at biopsy 1 and 2 are shown in Table 1. The average age at first biopsy was 25.7 ±10.3 years and 28.4 ±10.2 years at 2nd biopsy with mean biopsy interval being 2.8±1.8 years. Most patients were females (81. 8%) and there was a predominance of patients of mixed ancestry (75%) while black Africans and white patients made up 22.7% and 1.8% respectively.

Elevated proteinuria was the main indication for renal biopsy at 1st and 2nd biopsy (48.7% vs 36.1%; p=0.489) respectively. A significantly increased number of patients had a repeat biopsy due to elevated serum creatinine, compared to the first biopsy (5.1% vs 13.9%; p=0.016). Similarly, there was an increase in the proportion of patients who had a re-biopsy due to elevated serum creatinine with abnormal urine sediment at second biopsy than at first biopsy (12.8% vs 27.8%, p=0.895) (Table 1).

There was a significant reduction in the proportion of patients with positive double stranded DNA at 2nd biopsy (69.9% vs 63.6%; p=0.022). There was also significant worsening of eGFR between 1st and 2nd biopsies (88.9±49.2ml/min vs 54.7±59.0ml/min; p=0.001) as well as significant worsening of proteinuria (3.0 g/24hrs±3.0 vs 5.0 g/24hrs±4.0, p=0.019) (Figure 1).

The mean number of glomeruli obtained at both biopsies were similar (14.1± 8.7 and 14.3 ± 7.5; p=0.890) and there were no significant differences in the average number of cellular crescents (p=0.195), number of fibrous crescents (p=0.173) and

percentage of interstitial fibrosis (p=0.309) seen. However, there were significantly more patients with crescents at the second biopsy (p=0.005). The mean activity index (3.9±4.4 vs 7.0±5.7; p=0.005), chronicity index (1.0±1.5 vs 3.5±2.7; p=0.000) and number of sclerosed glomeruli (0.5±1.2 vs 4.3±7.8; p=0.004) were significantly raised at the repeat biopsy time point, respectively. In addition, we frequently observed transformation in histological class between initial and follow-up biopsies; 40.9% had proliferative LN at 1st biopsy compared to 70.4% at 2nd biopsy (Figure 2). At 2nd biopsy, class transformation was 100% for patients with class I LN at 1st biopsy, 80% for those with class II LN, and 86% for those with class V LN, respectively (Table 2). Most of the transformation of class at 2nd biopsy was to class IV. Majority of patients with proliferative LN at 1st biopsy (77.8%) remained in one of the proliferative classes whereas 65.4% with an initial non-proliferative LN class transformed into a proliferative class at 2nd biopsy.

Treatment given for induction and maintenance therapies as well as other adjunctive therapies given to patients after the 1st and 2nd biopsies are shown in (Table 3). The repeat biopsy resulted in immunosuppressive treatment escalation in 72.5%, reduction in 12.5% and no changes to treatment in 15.0%. Hence, there was an overall treatment change in 85.0% as a result of the second biopsy. (Table 3.

Discussion

This study shows that there is a significant class transformation in patients with LN undergoing a repeat renal biopsy at our center. The observed change in LN class between the initial and follow-up biopsies led to significant change in treatment following repeat biopsy. This therefore points to the need for protocols for repeat biopsies in our setting given the implications for disease outcome. One large International cohort study of repeat biopsies in patients with LN also found significant class switch at repeat biopsy and concluded that a repeat biopsy strategy could provide additional information on long-term renal outcomes.[22] The same study also suggested that a strategy of protocol biopsies could be useful in clinical trials to better understand the therapeutic response and the natural history of LN.[22]

Although the KDIGO guideline does not specifically provide information regarding the timing of a repeat biopsy in patients with LN, repeat biopsies have often been performed during a disease flare [10, 12, 13, 17] or as protocol biopsies at the end of induction therapy[7, 23-25] or at 1 – 2 years to assess the efficacy of maintenance therapy.[6, 15, 26, 27] In a study, comparing the histologic and clinical responses of proliferative LN to standard-of-care induction therapies, Malvar et al found that 7 patients showed persistent cellular crescents and sub endothelial immune deposits and/or glomerular capillary necrosis at repeat biopsy and that 2 of these patients had achieved complete clinical renal response, one patient had a partial renal response and four had no renal response.[25] The patients were treated for another 6 months of MMF (3 g/day), before being placed on maintenance immunosuppression. They concluded that early clinical and histologic outcomes are discordant in proliferative LN, and neither correlates with long-term renal outcome.[25] However, use of protocol biopsies, rather than biopsies performed only during a clinical disease flare, may be more likely to provide better evidence for disease progression, patient outcome and need for early treatment change. More data is still needed from prospective studies and clinical trials to define the role of protocol biopsies in understanding disease pathogenesis and outcomes.

Our study also showed that most patients who on initial biopsy had a proliferative LN, especially patients with class IV LN, often remained in the same class at follow up biopsy. This is in agreement with Daleboudt et al who reported that 84% of patients in their study with proliferative LN at 1st biopsy continued to have proliferative disease at repeat biopsy.[17] This finding questions the value of repeating a kidney biopsy in such patients if the initial biopsy was of a proliferative type. On the other hand some may justify the need for repeat biopsies in patients with initial proliferative LN given that it often leads to change in current treatment[12]. However, the change of treatment as reported in this study is that of change from ongoing maintenance treatment (minimal immunosuppression) to a new induction phase treatment (increased dose or new immunosuppression) Table 3. As shown in the table the proportion of patients getting induction treatment after second biopsy appear fewer than at 1st biopsy because the change in treatment is from

maintenance treatment before 2nd biopsy not comparison between induction therapies at the 2 biopsies. Thus, whether a repeat biopsy in patients with initial proliferative LN is only needed to guide therapeutic changes will remain a matter of debate. This is because in many patients, treatment escalation would still have occurred from a clinical perspective due to an ongoing flare.

Change in chronicity score at repeat biopsy may remain a strong reason to advocate for repeat biopsy in patients with LN and should be reported in all biopsies of patients with LN.[28] In the current study, there was a significant increase in both activity and chronicity indices between biopsies (Figure 3) translating to significantly reduced eGFR and increased 24-hour proteinuria between biopsies (Figure 1). Several other studies have shown significant increases in chronicity scores at repeat biopsy even after initial successful treatment.[27, 29] Bao et al reported a significantly ameliorated activity index ($p<0.0001$) at repeat biopsy whereas chronicity index had significantly increased ($p=0.028$) in patients at complete remission after induction therapy in a study that investigated the value of multi-targeted therapy in patients with proliferative LN.[29] They found no difference in AI ($p=0.346$) and significant increase in chronicity index ($p=0.032$) for patients who did not achieve complete remission after induction therapy.[29] The average biopsy interval in many studies that report increased chronicity score at repeat biopsy is often between 2.1 to 6 years (Table 4).[7, 9, 12, 17, 22, 24, 30] The average biopsy interval from our center was 2.8±1.8 years which could be the reason for the significantly increased chronicity score obtained at repeat biopsy.

There are some limitations we encountered in conducting this research, for instance, although the pathologists at our center use the ISN/RPS classification for LN, subclasses that reports segmental or global lesions in the biopsy are not routinely reported. Such information may have helped us to understand how those patients with a predominantly proliferative class of disease change classes (e.g. from IV[S] to IV[G]) even though they still remained within the proliferative type of LN. Other limitations are the retrospective design of the study and the low sample size available for analysis. However, the strength of this study is that it has been reported from a developing country setting where renal biopsies are usually not available in

the evaluation of patients and therefore may provide assurance for the clinical treatment of patients with suspected proliferative disease in the absence of facilities to perform a kidney biopsy. We however urge that a biopsy be considered to guide therapy in such patients where possible.

Conclusion:

Repeat renal biopsies in patients with LN presents a useful means of assessing disease progression and provides guidance regarding modification of treatment. More studies are however required to evaluate the value of repeat biopsies and perhaps the need for protocol renal biopsies in patients with LN.

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Figure legends:

Figure 1: Differences in estimated glomerular filtration rate and protein excretion between1st and 2nd biopsy.
1(a): shows change in estimated GFR between first and second renal biopsy; eGFR-BX1 – estimated glomerular filtration rate at first biopsy; eGFR-BX-2 – estimated glomerular filtration rate at 2ND biopsy; 1(b): shows changes in urine protein creatinine ratio between biopsy 1 and biopsy 2; UPCR – urine protein-to-creatinine ratio
Figure 2: Histological class switches between first and second biopsy
Proliferative classes: class 3,4,3+5 and 4+5
Non-Proliferative classes: class 1,2,5 and 6
Figure 3: Shows changes in Activity and Chronicity index between biopsy 1 and biopsy 2.
3(a): Shows changes in Activity index between biopsy 1 and 2; 3(b): Shows changes in Chronicity index between biopsy 1 and 2

Figure 1 n=44

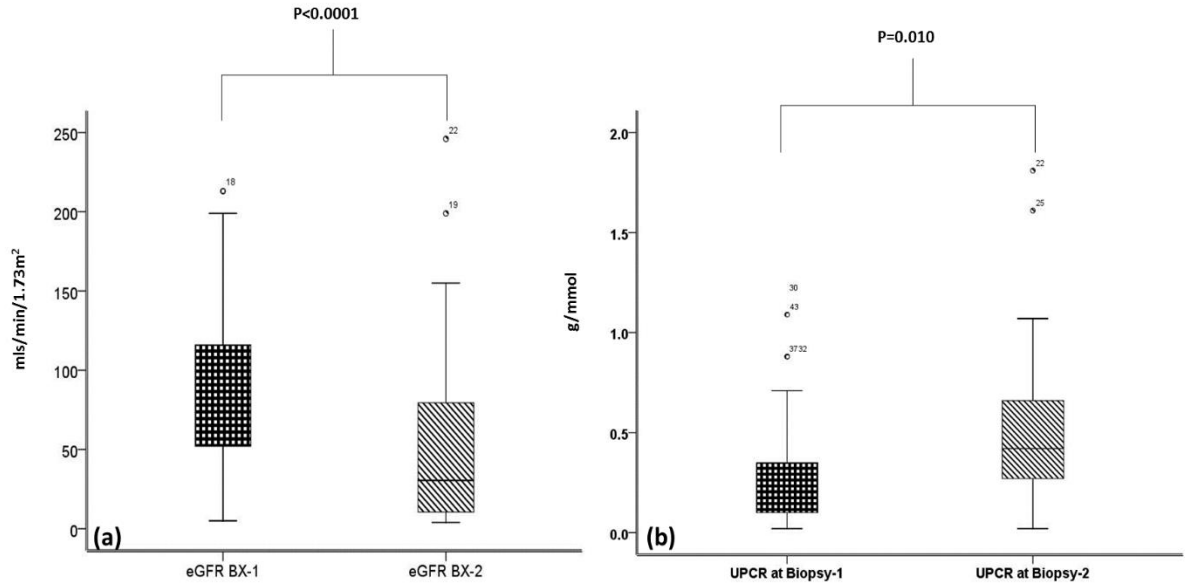

Figure 1: Differences in estimated glomerular filtration rate and protein excretion between reference and 2nd biopsy.1(a): shows change in estimated GFR between first and second renal biopsy; eGFR-BX1 – estimated glomerular filtration rate at first biopsy; eGFR-BX-2 – estimated glomerular filtration rate at 2ND biopsy; 1(b): shows changes in urine protein creatinine ratio between biopsy 1 and biopsy 2; UPCR – urine protein-to-creatinine ratio

Figure 2 n=44

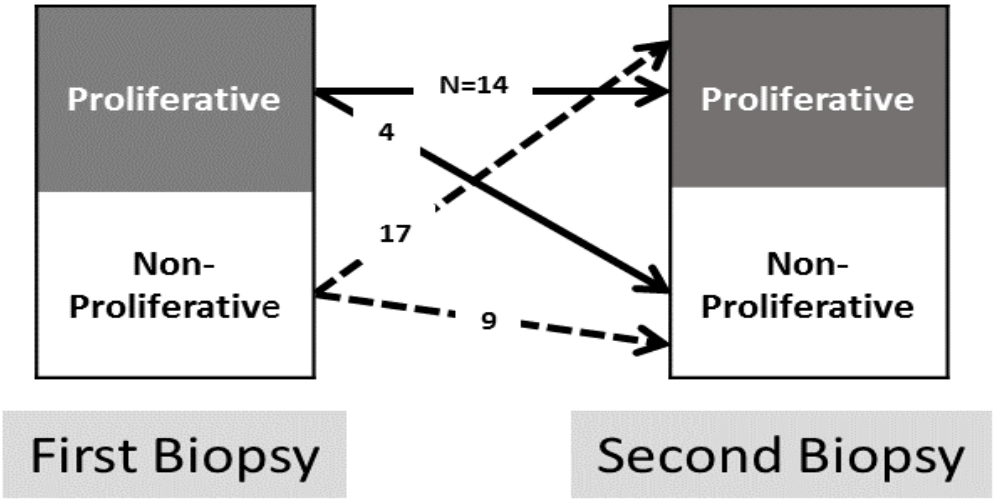

Figure 2: Histological class switches between first and repeat biopsy

Proliferative classes: class 3,4,3+5 and 4+5

Non-Proliferative classes: class 1,2,5 and 6

Figure 3 n=44

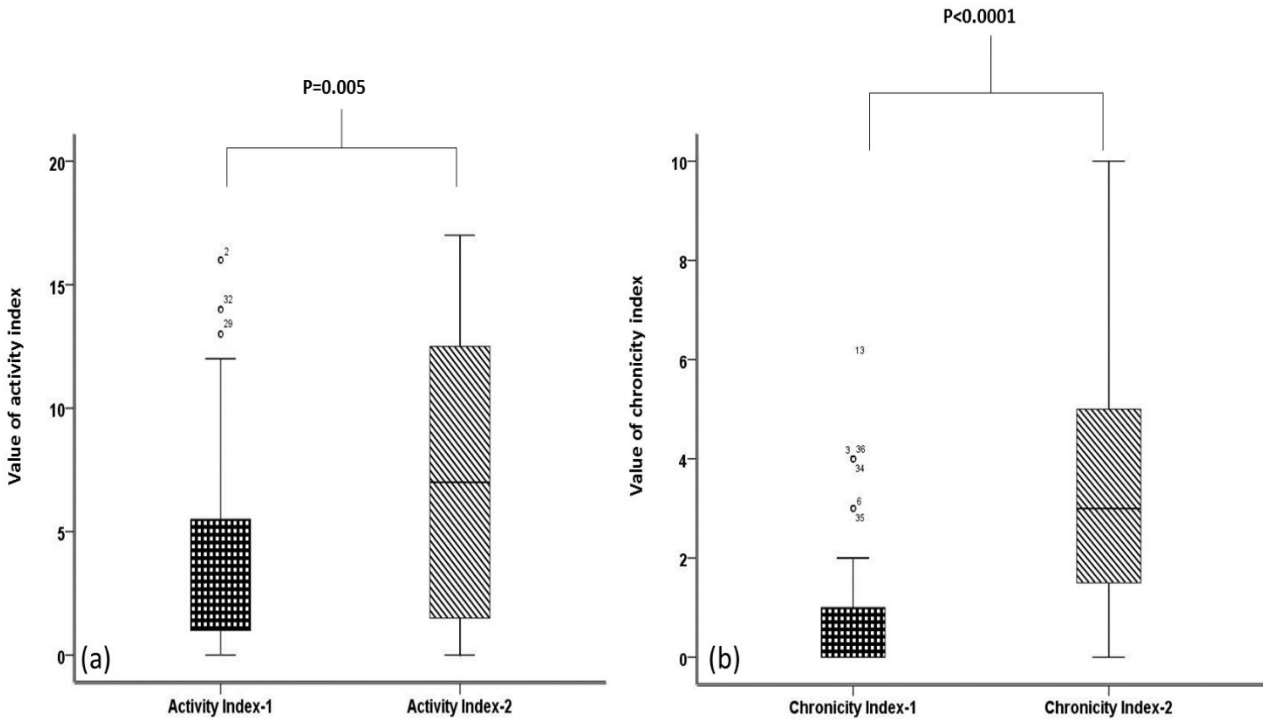

Figure 3: Shows changes in Activity and Chronicity index between biopsy 1 and biopsy 2.3(a): Shows changes in Activity index between biopsy 1 and 2; 3(b): Shows changes in Chronicity index between biopsy 1 and 2.

Table 1: Clinical and Demographic Features at First and Second Biopsy

Variable	1st Biopsy n=44	2nd Biopsy n=44	P-value
Demographics			
Age (Range) (Years)	25.7 ±10.3	28.4±10.2	<0.0001
Gender (Female) (%)	81.8	81.8	
Race (%)-black	22.7	22.7	
-coloured	75.0	75.0	
-white	1.8	1.8	
Biopsy interval (years)	2.8±1.8		
Biopsy indications (%)			
- Active urine	33.3	22.2	0.777
- Elevated Scr*	5.1	13.9	0.016
-Elevated Scr + urine abnormality	12.8	27.8	0.895
- Elevated proteinuria	48.7	36.1	0.489
Clinical and Laboratory variable			
SBP(mmHg)	128.2±26.7	141.0±27.5	0.015
DBP(mmHg)	77.0±19.0	88.0±18.7	0.006
eGFR(ml/min)	88.9±49.2	54.7±59.0	0.001
UPCR(mg/mmol)	0.3±0.3	0.5±0.4	0.019
Albumin(g/l)	27.9±8.5	27.5±6.7	0.799
C3	0.46±0.40	0.62±0.35	0.742
Low C3 (%)	52.3	54.5	
C4	0.13±0.11	0.19±0.16	0.194
Low C4 (%)	25.0	20.5	
Anti dsDNA level	507.9±323.9	306.2±282.1	0.022
Positive ds-DNA (%)	69.9	63.6	
Histology			
Number of glomeruli	14.1±8.7	14.3±7.5	0.890
Interstitial fibrosis present (%)	30.8	46.2	0.309
Activity index	3.9±4.4	7.0±5.7	0.005
Chronicity index	1.0±1.5	3.5±2.7	0.000
Number of patients with cellular crescents (%)	8.0(18.2)	18.0(40.9)	0.005
Average number of cellular crescents per biopsy	1.2±3.4	2.0±3.4	0.195
Number of patients with sclerosed gloms (%)	10.0(22.7)	23.0(52.3)	0.724
Average number sclerosed gloms per biopsy	0.5±1.2	4.3±7.8	0.004
Number of patients with fibrous crescents (%)	3.0(6.8)	4.0(9.1)	0.254
Average number of fibrous crescents per biopsy	0.1±0.3	0.5±1.9	0.173
Number of patients with necrotizing lesions (%)	9.0(20.5)	14.0(31.8)	1.000
Average number of necrotizing lesions per biopsy	0.2±0.4	0.9±1.9	0.025

Normal Ranges C3: 0.90 - 1.8 C4: 0.10 - 0.40
Anti dsDNA: <10 IU: Negative 10-15 IU: Equivocal >15 IU: Positive
*Serum creatinine.

Table 2: Histological Changes between First and Second Biopsy

First Biopsy (n=44)	Second Biopsy (n=44)							Change of class (%)
	II (n=3)	III (n=4)	IV (n=19)	V (n=5)	V+III (n=2)	V+IV (n=6)	VI (n=5)	
I(n=2)	0	0	2	0	0	0	0	100
II(n=10)	2	1	3	1	0	2	1	80
III(n=7)	1	2	2	1	0	1	0	71
IV(n=8)	0	1	6	0	0	0	1	25
V(n=14)	0	0	4	2	2	3	3	86
V+IV(n=3)	0	0	2	1	0	0	0	100

Table 3: Description of treatment received at biopsy 1 and biopsy 2

Treatment Regimens	Biopsy 1 (%) n=44	Biopsy 2 (%) n=44
General		
Chloroquine	64.1	45.9
ACEI	88.9	84.2
Induction		
Pulse Methyl Prednisolone	36.8	47.4
Cyclophophamide	62.5	59.0
Mycophenolate mofetil	7.7	5.3.0
Maintenance		
Prednisolone	97.4	89.7
Azathioprine	50.0	43.6
Mycophenolate mofetil	20.5	15.9
Cyclosporine	2.6	2.3
Treatment Escalation	72.5	
Treatment Reduction	12.5	
No Change	15.0	
Overall Treatment change	85.0	

Table 4: Features from selected studies of repeat renal biopsies in patients with lupus nephritis from various centres

	Pagni et al[22]	Daleboudt et al[17]	Wang et al[9]	Greloni et al[12]	Alvarado et al[7]	Lu et al[30]	Esdaile et al[15]	Bajaj et al[10]	Kajawo et al Current study
Country	Italy	Netherlands	China	Argentina	Argentina	China	US	Canada	South Africa
Sample size	142	35	44	45	25	156	42	57	44
Female (%)	88.8	74.2	95	40	88	91	NR	84.2	81.8
Publication year	2013	2009	2012	2014	2014	2011	1993	2000	2016
Study design	Retrospective	Retrospective	Retrospective	Retrospective	Prospective	Retrospective	Prospective	Retrospective	Retrospective
Study duration (type)	37yrs (multi-centre)	NR(single)	16yrs(single)	8.7yrs(single)	6yrs (single)	20yrs(single)	17yrs(single)	24yrs(Single)	12yrs(single)
Biopsy interval in years (SD)	4.9 ± 4.9	4.1 ± 3.6	NR	3.4(4.4) *	6	NR	2.1(1.8-2.5)**	4.2yrs	2.8±1.8
AI-1/AI-2	4.5 / 3.3	6.18 / 5.27	5.8 / 4.7	NR	8.9/4.3/0.96	6.8	7/2	5.09/3.96	3.92/7.03
CI-1 / CI-2	1.5 / 3.6	2.6 / 4.2	1.8/3.4	2.9/6.6	2.8/4.2/4.3	2.6	2/2	1.30/3.37	0.97/3.53
Change in chronicity score	+2.1	+1.6	+1.6	NR	+1.5	NR	0	+2.07	+2.56
Proliferative class at biopsy 1 and 2(%)	70.3/70.4	87.8/93.9	NR	60/66.7	NR	71.7/59.4	83.3/50	58/44	40.9/70.4
Proliferative to Proliferative switch (%)	82	84	NR	75.6	NR	60	54.3	75	77.8
Non-Proliferative to Proliferative switch (%)	42.9	83.3	NR	58.3	NR	58	28.6	22.2	65.4
Overall Pathological transition (%)	40.8	55.7	64	54.9	NR	75	46	40.4	72.7
Proteinuria biopsy g/24h 1/2 (%)	3.5/3.1	NR	NR	NR	3.3/1.13/0.32	3.28/#	0.99/0.50	2.48/1.35	3/5
Changes in treatment (%)	NR	77.5	34	87.3	64	NR	NR	77	85

*Median Inter quartile range **Interquartile range # Information missing.

NR: Not reported. AI: Activity Index. CI= Chronicity Inde

APPENDICES

Appendix 1: Data Capture Sheet

Hospital No	Sex	DOB	Gender	Race											
BIOPSY 1	Bx Date	**age-1**	**indication-1**	**Dipstix Prot-1**	**Dipstix RBC-1**	**SBP-1**	**DBP-1**	**ACE / ARB-1**	**Chloroquine-1**	**Medrol-1**	**Cyc - Induction-1**	**MMF-induction-1**	**Pred-M-1**	**AZA-M -1**	**CYC-M-1**
	MMF-M-1	**CyA-M-1**	**HB Bx-1**	**WBC Bx-1**	**C3 Bx-1**	**C4 Bx-1**	**C4 Titre**	**ANA Bx-1**	**ANACAT1**	**dS-DNA Bx-1**	**ds-DNACAT1**	**Chol Bx-1**	**ALB Bx-1**	**Scr Bx-1**	**eGFR BX-1**
	UPCR Bx1	**Final Histo class Bx 1**	**Original Histo class Bx 1**	**Histologycat1**	**No gloms-1**	**# crescents-1**	**# sclerosed gloms-1**	**Fibrous crescents-1**	**Necrotizing Lesions-1**	**Int Fibrosis-1**	**Activity-i-1**	**Chronicity-i-1**	**BX year-1**		

BIOPSY 2	Bx Date	**age-2**	**indication -2**	**Dipstix Prot-2**	**Dipstix RBC-2**	**SBP-2**	**DBP-2**	**ACE / ARB-2**	**Chloroquine-2**	**Medrol-2**	**Cyc - Induction-2**	**MMF-induction-2**	**Pred-M -2**	**AZA-M -2**	**CYC-M-2**
	MMF-M-2	**CyA-M-2**	**HB Bx-2**	**WBC Bx-**	**C3 Bx-2**	**C4 Bx-2**	**C4 Titre**	**ANA Bx-2**	**ANACAT1**	**dS-DNA Bx-2**	**ds-DNACAT 2**	**Chol Bx-2**	**ALB Bx-2**	**Scr Bx-1**	**eGFR BX-2**
	UPCR Bx2	**Final Histo class Bx 2**	**Original Histo class Bx 2**	**Histologycat2**	**No gloms-2**	**# crescents-2**	**# sclerosed gloms-2**	**Fibrous crescents-2**	**Necrotizing Lesions-2**	**Int Fibrosis-2**	**Activity-i-2**	**Chronicity-i-2**	**BX year-2**	**Biopsy 1-2 interval (mths**	

Appendix 2: Ethics Approval Letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

28 October 2014

HREC REF: 732/2014

Dr I Okpechi
Nephrology & Hypertension
E13
NGSH

Dear Dr Okpechi

PROJECT TITLE: CLINICO-PATHOLOGICAL FEATURES OF THE RE-BIOPSY IN PATIENTS WITH PREVIOUS BIOPSY PROVEN LUPUS NEPHRITIS AT GROOTE SCHUUR HOSPITAL, CAPE TOWN (MMED Candidate - Dr S Kajawo)

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 27 October 2014.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th October 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Dr Shepherd Kajawo will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

HREC /32/2014

Appendix 3: Acceptance of Abstract for Poster Presentation at South African Renal Congress

Dear Shepherd Kajawo

Abstract: Clinico-pathological features of the re-biopsy in patients with lupus nephritis at Groote Schuur Hospital, Cape Town

We are pleased to inform you that your abstract has been accepted as a poster at the Congress.

Please note the following:

1. Please confirm by return email that you will present your work at the Congress.
2. If you have not yet registered, please do so as soon as possible. If you are not registered by 17 August your presentation will be removed from the programme.
3. You may be required to be at your poster during the lunch and tea breaks on Saturday.
4. Posters should be a maximum size of A0 = 841 mm in width and 1090 mm in height.
5. Materials for fixing your poster to the poster boards will be provided.
6. All accepted abstracts will be published as received as part of the Congress Proceedings in the African Journal of Nephrology. Please re-check your abstract for errors, remove any figures, tables or references, and ensure that you remain within the limit of 300 words. Your updated abstract should reach us by 17 August.

   See also http://www.sa-renalsociety.org/SARenalCongress/2016/abstracts.asp.

Best regards

The Organising Committee

www.sa-renalsociety.org/SARSCongress/2016

Appendix 4: Acceptance of Abstract for Poster Presentation at Medicine Research day

DEPARTMENT OF MEDICINE

ACTIVE IN CLINICAL SERVICE, HEALTH EDUCATION AND RESEARCH

ACTING HEAD: PROFESSOR GARY MAARTENS

J floor, Old Main Building, Groote Schuur Hospital, Observatory, 7925, CapeTown, South Africa
Phone: +27 21 4066200 Fax: +27 21 4486815 URL: http://web.uct.ac.za/depts/medicine/

15 September 2016

Dear Dr Kajawo

LETTER OF ACCEPTANCE OF SUBMITTED ABSTRACT FOR 2016 MEDICINE RESEARCH DAY

Thank you for submitting your abstract entitled **"Clinico-pathological features of the re-biopsy in patients with lupus nephritis at Groote Schuur Hospital, Cape Town"** for consideration for presentation at the 42nd Annual Department of Medicine Research Day which is to be held in Lecture Theatre II, New Groote Schuur Hospital on Thursday, 6TH October 2016. We are delighted to inform you that your abstract has been accepted for **POSTER** presentation. For the first time, Medicine Research Day will take place over a 2-day period (Wednesday 5th October and Thursday 6th October 2016). We hope you will be able to attend the exiting lectures and presentations scheduled for Thursday.

You should prepare your POSTER in a portrait format (height 109cm and width 84cm). Your poster will be presented on **Wednesday 5th October 2016** at Klein Schuur (beside the New GSH lecture theatre 2) and you will receive notification about the time of your presentation. You should leave your poster hanging up until the end of Research Day Presentations the following day (Thursday 6th October).

If you have any questions, kindly contact Zam Ndzotyana (zam.ndzotyana@uct.ac.za).

We look forward to your participation at this year's research day program.

Yours sincerely

GARY MAARTENS

Appendix 5: Acceptance for Publication in Lupus

Decision Letter (LUP-16-608.R1)

From: editorial@lupusjournal.co.uk

To: skajawo@gmail.com, skajawo@yahoo.co.uk

CC:

Subject: Lupus - Decision on Manuscript ID LUP-16-608.R1

Body: 01-Feb-2017

Dear Dr. Kajawo:

Thank you very much for revising your manuscript entitled "Clinico-pathological features of repeat renal biopsies in patients with lupus nephritis at Groote Schuur Hospital, Cape Town.". I am very pleased to let you know that this has now been accepted for publication in Lupus and you should receive proofs from the publishers within the next few weeks.

You will shortly receive a second email requesting you submit a contributor agreement online without which we cannot commence with publication.

If you would like your article to be freely available online immediately upon publication (as some funding bodies now require), you can opt for it to be published under the SAGE Choice Scheme on payment of a publication fee. Please simply follow the link to the Contributor Agreement form in the next email and you will be able to access instructions and further information about this option within the online form.

Thank you very much for your contribution to Lupus. We look forward to your continued support.

With best regards.

Yours sincerely,

Maria Laura Bertolaccini
Managing Editor

Date Sent: 01-Feb-2017

Lupus (2017) 0, 1–8
journals.sagepub.com/home/lup

LUPUS

Clinico-pathological features of repeat renal biopsies in patients with lupus nephritis at Groote Schuur Hospital, Cape Town

S Kajawo[1,2], FCJ Botha[3] and IG Okpechi[1]
[1]Division of Nephrology and Hypertension, University of Cape Town, South Africa; [2]Division of Clinical Practice and Patient Care, National University of Science and Technology, Bulawayo, Zimbabwe; and [3]Division of Anatomical Pathology, University of Cape Town; and National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa

Background: Repeat renal biopsies in patients with lupus nephritis are usually done to guide treatment or to establish disease chronicity. Their value is not clear from available literature. There are also no available data in Africa to guide clinicians. **Methods:** This was a retrospective study of patients undergoing a repeat renal biopsy between January 2003 and December 2014 from a single centre in Cape Town, South Africa. Relevant demographic, clinical and histological records of patients with repeat renal biopsies were documented. Comparison of data from first and second renal biopsy was performed. **Results:** Forty-four patients had at least two biopsies done during the study period. Most patients were females (81.8%). The mean biopsy interval was 2.8 ± 1.8 (range 0.38–9.4) years. Proteinuria was the main indication for the repeat biopsy (36.1%). The glomerular filtration rate and proteinuria worsened between the two biopsies ($p = 0.001$ and 0.019, respectively) suggesting disease progression. Most patients (65.4%) with a non-proliferative class of lupus nephritis at first biopsy progressed into a proliferative class, whereas patients with initial proliferative lupus nephritis at first biopsy (77.8%) remained as proliferative at repeat biopsy. Treatment was changed in 85% of patients at second biopsy. **Conclusion:** Repeat renal biopsies in patients with lupus nephritis presents a useful means of assessing disease progression and provides guidance regarding modification of treatment. More studies are, however, required to evaluate the value of repeat biopsies and perhaps the need for protocol renal biopsies in patients with lupus nephritis. *Lupus* (2017) **0,** 1–8.

Key words: Activity score; chronicity score; renal biopsy; repeat biopsies; proliferative lupus nephritis

Appendix 6: Instructions to Authors Lupus Journal-SAGE

The manuscript has been accepted by LUPUS for publication

"Manuscript Submission Guidelines

Pre-submission: helping readers find your article
Before you submit your manuscript, go back and review your title, keywords and abstract. These elements are key to ensuring that readers will be able to find your article online through online search engines such as Google. More information and guidance on how best to title your article, write your abstract and select your keywords can be found here: How to Help Readers Find Your Article Online.

One simple thing you can do to improve your article's visibility and ensure proper indexing and cross-linking is to provide **full names for all authors**. Please refer to our guidelines for author names, prepared in consultation with Google Scholar, for more information.

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SAGE is a member of the Committee on Publication Ethics (COPE) and follows their best practice guidelines.
For authors submitting to medical journals, SAGE recommends that authors follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals formulated by the International Committee of Medical Journal Editors (ICMJE).

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Please see the submission guidelines of the journal you wish to submit to. View the complete list of SAGE Journals.

Please note that as part of the submission process you may be asked to provide the names of a number of peers who could be called upon to review your manuscript. Recommended reviewers should be experts in their fields and should be able to provide an objective assessment of the manuscript. Please be aware of any conflicts of interest when recommending reviewers. Examples of conflicts of interest include (but are not limited to) the below:

- The reviewer should have no prior knowledge of your submission
- The reviewer should not have recently collaborated with any of the authors
- Reviewer nominees from the same institution as any of the authors are not permitted

Please note that the journal's editors are not obliged to invite any recommended/opposed reviewers to assess your manuscript.

Authorship

For authors submitting to technical or medical journals, papers should only be submitted for consideration once consent is given by all contributing authors. Those submitting papers should carefully check that all those whose work contributed to the paper are acknowledged as contributing authors.
The list of authors should include all those who can legitimately claim authorship. This is all those who:

1. Made a substantial contribution to the concept and design, acquisition of data or analysis and interpretation of data,
2. Drafted the article or revised it critically for important intellectual content,
3. Approved the version to be published.

Authors should meet the conditions of all of the points above. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.
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Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship, although all contributors who do not meet the criteria for authorship should be listed in the Acknowledgments section. Please refer to the International Committee of Medical Journal Editors (ICMJE) authorship guidelines for more information on authorship.

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General guidance for authors submitting to medical journals (please view the journal's submission guidelines for specific requirements)

Research ethics and patient consent
Medical research involving human subjects must be conducted according to the World Medical Association Declaration of Helsinki

Submitted manuscripts should conform to the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, and all papers reporting animal and/or human studies must state in the methods section that the relevant Ethics Committee or Institutional Review Board provided (or waived) approval. Please ensure that you have provided the full name and institution of the review committee, in addition to the approval number.

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Please also refer to the ICMJE Recommendations for the Protection of Research Participants

All research involving animals submitted for publication must be approved by an ethics committee with oversight of the facility in which the studies were conducted.

Clinical trials
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Reporting guidelines
The relevant EQUATOR Network reporting guidelines should be followed depending on the type of study. For example, all randomized controlled trials submitted for publication should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow chart as a cited figure, and a completed CONSORT checklist as a supplementary file.

Other resources can be found at NLM's Research Reporting Guidelines and Initiatives

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SAGE UK Style Guide

Version UK3/August 2011

6.2 SAGE Vancouver

1. General

1. Reference numbers have full points in the reference list.
2. Please ensure that publications are referenced in the order in which they appear in the text.
3. Journal titles should be abbreviated according to the standard in the Index Medicus. If unsure, please check for any inconsistencies within reference lists. For STM journals, please refer also to the following: http://scieng.library.ubc.ca/coden/.
4. Do not separate initials with spaces or full points, but add a full point after last initial before the title.
5. Up to three authors may be listed. If more, then list the first three authors and represent the rest by et al. Fewer author names followed by et al. is also acceptable. Where et al. is used, it should always be upright, not italic in both references and textual citations.
6. Last Names containing de, van, von, De, Van, Von, de la, etc. should be listed under D and V respectively. List them as: De Roux DP and not Roux DP, de. When cited in the main text without the first name, use capitals for De, Van, Von, De la, etc. (Van Dijk, year)
7. Names containing Jr or II should be listed as follows:
    - Author Last Name Initial Jr (year)
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2. Text citations

Please use superscript numerals *after* the punctuation (STM) or numbers in square brackets (HSS), and check that it corresponds to the correct number in the reference list.

3. Reference styles

Book

1. Huff D. *How to lie with statistics*. 4th ed. London: Penguin, 1991, p.51.

Chapter in book

1. Huff D and Black TL. Comprehensive statistics. In: Miller C and Smith H (eds) *How to lie with statistics*. 4th ed. London: Penguin, 1991, pp.51–55.

Journal article

1. Ludbrook J, Miller T and Russel A. Musculovenous pumps in the human lower limb. *Am Heart J* 1966; 71: 635–641.

2. Araki C, Black TL, Patberg FT, et al. Significance of calf muscle pump function in venous ulceration. *J Vasc Surg* 1994; 20: 872–879.

Journal article published ahead of print

1. Ludbrook J. Musculovenous pumps in the human lower limb. *Am Heart J*. Epub ahead of print 12 June 2011. DOI: 10.1177/09544327167940.

Website

1. Smith JR. Choosing your reference style. *Online Referencing 2(3)*, http://orj.sagepub.com (2003, accessed 12 October 2008).

2. National Center for Professional Certification. Factors affecting organizational climate and retention, www.cwla.org./programmes/triechmann/2002fbwfiles (2002, accessed 10 July 2010).

Conference paper

1. Peters J. Musculovenous pumps in the human lower limb. In: *ASME conference on automatic transmissions* (ed A O'Brien), Pisa, Italy, 29 May–2 June 2003, paper no. GE1234, pp.4–10. New York: ASME.

6.2 SAGE Vancouver

1. General

1. Reference numbers have full points in the reference list.
2. Please ensure that publications are referenced in the order in which they appear in the text.
3. Journal titles should be abbreviated according to the standard in the Index Medicus. If unsure, please check for any inconsistencies within reference lists. For STM journals, please refer also to the following: http://scieng.library.ubc.ca/coden/.
4. Do not separate initials with spaces or full points, but add a full point after last initial before the title.
5. Up to three authors may be listed. If more, then list the first three authors and represent the rest by et al. Fewer author names followed by et al. is also acceptable. Where et al. is used, it should always be upright, not italic in both references and textual citations.
6. Last Names containing de, van, von, De, Van, Von, de la, etc. should be listed under D and V respectively. List them as: De Roux DP and not Roux DP, de. When cited in the main text without the first name, use capitals for De, Van, Von, De la, etc. (Van Dijk, year)
7. Names containing Jr or II should be listed as follows:
    - Author Last Name Initial Jr (year)
    - Author Last Name Initial II (year)

2. Text citations

Please use superscript numerals *after* the punctuation (STM) or numbers in square brackets (HSS), and check that it corresponds to the correct number in the reference list.

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2. National Center for Professional Certification. Factors affecting organizational climate and retention, www.cwla.org./programmes/triechmann/2002fbwfiles (2002, accessed 10 July 2010).

Conference paper

1. Peters J. Musculovenous pumps in the human lower limb. In: *ASME conference on automatic transmissions* (ed A O'Brien), Pisa, Italy, 29 May–2 June 2003, paper no. GE1234, pp.4–10. New York: ASME.

2. Article opening material

2.1 Headings

1. Headings should have an initial capital with everything else lowercase, unless proper names.
2. Italics can be included in A heads (H1) if needed, e.g. mathematical symbol or genus name.
3. Headings are unnumbered and formatted as below.
4. Where headings are referred to in the text use section names, as headings are not numbered.

A head (H1) (bold with initial cap, all the rest lowercase)

Introduction

The mucosa of the small and large intestines is the largest reservoir of tissue macrophages (Mϕ) in both humans and mice.[1] Although Mϕ possess various

B head (H2) (italic with initial cap, all the rest lowercase)

Human samples

Human specimens of normal large intestine were obtained from normal tissues of three patients with colon cancer who had their large intestine resected for

C head (H3) (same as B head, but set as first line of paragraph, full out; italic with initial cap, all the rest lowercase, followed by a full stop. Following text runs on)

Single nucleotide primer extension. The PCR product from bisulfite-treated genomic DNA was cleaned with ExoSAP (USB) prior to SNuPE reaction. For calibra-

Headings for Abstract, Keywords, Funding, Acknowledgements, Conflict of interest (in that order), References, Appendices are same as A head but smaller font size

Acknowledgements

We thank Dr van Lookeren Campagne (Genentech) for providing blocking mAb against CR1g (clone 14G8) and isotype control mAb (anti-ragweed).

(CEs: where a template is being used there is no need to format these. Where no template is being used, please format as bold/italic, but there is no need to mark the font sizes, TS will format.)

2.2 Article types

Where a journal displays article types, these should appear on the first page of each article, left aligned above the horizontal rule, and in italics.

General technical or research papers should be classified as *Original Article* (with uppercase initial caps) for STM, and *Article* for HSS. (Check with the PE, as there is some variation between journals.)

Other usual paper types are as follows: *Review Article, Case Study, Technical Note, Case Report*. Individual journals may also have other paper types, as agreed with the Editor. Where no particular convention has been agreed, *Original Article* should be followed for STM, and *Article* for HSS.

2.3 Article title

Please format with an initial capital only and remaining words lower case, unless proper names. Italics can be included where necessary (e.g. genus name). Run on subtitle after colon, with initial capital after colon.

2.4 Author names, affiliations, and corresponding address

Authors

List authors in the order that they appear on the manuscript. Authors' first name should be in full, middle names should be initials *without* full stops (e.g. Simon PS Sharma) and no spaces between multiple initials. No series comma before the 'and' before the final author name.

Affiliations

Affiliations should contain only the following: department *or* faculty, institution, country. Some HSS journals may have institution and country only. Do not include titles, positions, qualifications, street names, or postcodes/zip codes. Affiliations should *not* end in a full stop.

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Mark A Creager[1], Reena L Pande[1] and William R Hiatt[2,3]

HSS: affiliations should directly follow each author name, as follows:

Mark A Creager

(Department of Engineering,) Southampton University, UK

Reena L Pande

(Department of Engineering,) Southampton University, UK

William R Hiatt

County Hospital, CA, USA; Harvard Medical School, USA

Multiple affiliations are separated by a semi-colon.

Corresponding author

The affiliations and corresponding author information is positioned as follows:
Bottom of the right column on the first page of each paper, separated from the text with a horizontal rule (some exceptions apply for specific journals).

Corresponding author:
John Smith, Department of Social Studies, South Bank University, 4 Sample Road, London SE17 9OP, UK
Email: john.smith@sbu.ac.uk

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[1]Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Borstel, Germany
[2]Microbiology Department, Chemical Faculty, Gdańsk University of Technology, Gdańsk, Poland
[3]Novartis, Basel, Switzerland

Corresponding author:
Sven Müller-Loennies, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Parkallee 22, D-23845 Borstel, Germany.
Email: sml@fz-borstel.de

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Please note: 'Email' with cap E and without hyphen. Email should start a new line. There *should* be a full stop after the country in the corresponding address.

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2.5 Abstract and keywords

Abstract should appear in bold without a colon, text should start on the next line, with no indent.

Keywords (all one word) should appear in bold without a colon. The keywords should start on the next line, separated by commas only, not semi-colons. The first keyword should have an initial cap.

Abstract
Anaphylaxis related to drug therapy with 5-HT3 antagonists, in particular, palonosetron has not been reported frequently in the literature. Here a case is presented where the patient possibly had an anaphylactic reaction to palonosetron. In this case report, a 40-year-old female with ovarian cancer developed shortness of breath and hypotension after receiving her palonosetron as part of her premedication for chemotherapy. The patient recovered successfully with fluids and supportive care. This case demonstrates that even after successful treatment in the past with palonosetron a patient may later develop a hypersensitivity to the agent.

Keywords
Palonosetron, anaphylaxis, hypersensitivity, 5-HT3 receptor antagonist

In some journals, Abstracts have sub-headings, e.g. Methods, Conclusion etc. These should be formatted in bold with a colon in bold and each sub-heading should start a new paragraph. The text should run on after each heading with an initial capital.

Submitted/accepted dates

For journals that publish received/revised/accepted dates (applies to specific journals, if unsure please check with the PE), this should appear after the Keywords and be formatted thus:

Date received 29 July 2010; reviewed 30 August 2010; accepted 5 November 2010

Keywords
H5N1, apoptosis, TRAIL, caspase-10

Date received: 30 March 2011; revised: 19 April 2011; accepted: 28 April 2011

2.6 Running heads

Recto: should be author surname(s), e.g. *Smith*, or *Smith and Jones*, or *Smith et al.* (for three or more authors, and et al. is also in italic).
Verso: full journal title in italic, followed by 0(0).
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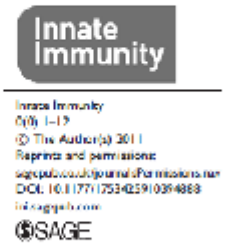
All papers in the standard SAGE design will have a journal logo in the top right with an imprint box underneath (although the logo may be missing on journals that are new to the SAGE design). The imprint box will contain the following information: journal name, vol/issue/page numbers (for papers in production, vol/issue are represented by 0(0), page numbers are the number of pages in the PDF, e.g. 1–9), copyright line, link to permissions web page, DOI, journal URL, SAGE logo:



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4. Source: in cases where permission is required and has been obtained, this should appear below the caption in the following form: Source: reproduced with permission from publisher, year, reference number (Vancouver), author, date (Harvard).
5. Any abbreviations needing to be spelled out should be listed after the caption, starting on the next line, in the following format: IC: internal combustion; PID: proportional–integral–derivative).
6. Captions are positioned below the figures and left aligned.
7. Captions should start, for example, **Figure 1.** (with a full point also in bold) and have a full point at the end. Where the text runs onto multiple lines, the captions need not be justified but should be aligned left.
8. Where figures have multiple parts, these should be labelled as (a), (b), (c), etc. (not A, B, C). Captions should contain subheadings for all parts if not present in the figure itself.
9. All figures should be numbered consecutively and cited in the text as Figure 1, Figure 2 etc. (Figure should be spelled out in full, not abbreviated).
10. Text citations: figures should be referenced in the text as follows: Figure 1, or Figures 1 and 2, or Figures 2 to 4, or Figure 1(a) and (b), or Figure 2(a) to (c). Where the figure citation is not part of the sentence it should be placed in parentheses.

Examples:
Please see Figure 2 for an illustration of the model used
The model used was an X3G standard type, exported from Germany (Figure 2 or see Figure 2).

3.3 Tables

1. Tables do not need to be a full column width or page width, but should be the appropriate width for the content. They will be laid out by the TS so no work is required by CEs on table layout, only on content.
2. Table headings should be left aligned, even when they relate to multiple columns, unless this creates confusion.

3. Tables should only have minimal horizontal rules for clarity, and no vertical rules (done by TS, no need for CE to format).
4. All tables should be numbered consecutively and cited in the text as Table 1, Table 2 etc. (Table should be spelled out in full, not abbreviated).
5. Table permissions: any tables reproduced from another publication need permission. In cases where those publishers listed on the STM permission Guidelines page (http://www.stm-assoc.org/permissions-guidelines/), permission is not required and only the reference number need by present in the caption. Some publishers ask for certain text, e.g. Elsevier.
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7. Any abbreviations needing to be spelled out should be listed under the table (smaller font, TS will format), in the following format: IC: internal combustion; PID: proportional–integral–derivative.
8. General notes to the Table should be positioned below the Table, typeset in a smaller font and should start 'Note:', and end in a full stop. Do *not* add the word 'Note:' unless needed for clarity.
9. Footnotes should be represented in the table by superscript letters [a], [b], [c], etc., and appear below the Table (smaller font, TS will format). Each footnote should start a new line and end with a full stop. These notes should precede the source for the table, if included.
10. Captions are positioned above the table and left aligned.
11. Captions should start, for example, **Table 1.** (with a full point also in bold) and have a full point at the end. Where the text runs onto multiple lines, the captions need not be justified but aligned left.
12. Dates in Tables can be shortened to, for example, 4 Dec 10, if space is lacking. Do not use the form 04/12/10, as this could be confused as 12 April in US.
13. Normal text in columns should always be left aligned. Data in tables should be aligned on units if all the data in that column take the same units. Otherwise, the data should be left aligned. Units in table headings should be enclosed by parentheses, not square brackets (if any brackets are required at all).

3.4 Lists

1. For lists where items are not full sentences, use (a), (b), (c) etc. or bullet points (whichever is more appropriate) and separate items with semi-colons. Start list with a preceding colon and end list with a full stop.
2. For lists where items are full sentences or multiple sentences, use 1. 2. 3. Start list with a preceding full stop or semi-colon (whichever is more appropriate), and end list with a full stop.
3. List numbering/bullets should be full out and left aligned, with text indented and aligned. Lists should be separated from preceding/following text with a line space.
4. Where list items include headings, that heading should be italic, same size as text and end in a full stop. The following text should run on.

3.5 Maths/equations (see section 5, p. 14 for more details)

1. Equations should be left aligned with a 3 mm indent, *not* centred.
2. Equations can be broken at operator symbols (x, -, +, etc.), and continue on the next line, starting with the operator itself.
3. Equations should be separated from text above and below by at least one line space.
4. Any equation numbers should be enclosed in parentheses and right aligned, and aligned horizontally with the bottom line of the equation or equations, where multiple terms are covered by one equation number. (Not all equations need be numbered, see section 5).

General note: text following Figures, Tables, equations does not need to be full out with no indent. If the next block of text after any of these items is a new paragraph, then this may be indented.

3.6 Appendices

Maths notation list

1. Where present, notation should appear as Appendix 1, following the references. The heading *Notation* should be a B-head (not Notations; it is not plural).
2. Abbreviations list should be separated from mathematical notation under a separate B-head *Abbreviations*.

3. Notation should be listed in alphabetical order, English letters first, followed by Greek, followed by numbers, followed by symbols.
4. Subscripts and superscript should come under a separate C-head (italic and smaller font), and symbols should follow the same order as in point 2 above.
5. The Notation section does not need to be cited in the text, like other Appendices.
6. Notation list should be left aligned. Text in the notation section should be left aligned in general, not justified.
7. Please note that a notation list is not compulsory in mathematical papers, as long as all symbols are defined in the text.

Other appendices

1. Numbering of figures/tables/equations in Appendices should follow on from the numbering in the text.
2. All tables/figures should have captions.
3. All appendices should be cited in the text, e.g. (see Appendix 1). If they are not cited, authors need to be queried for a citation position.

3.7 Notes and footnotes

Textual notes

HSS

References: Vancouver style reference citations are represented as textual notes, as a numeral enclosed in a square bracket. Harvard style references are as follows (Smith, 1999).
Any other textual notes: are indicated by a superscript Arabic numeral placed *after* the punctuation. All textual notes should be collected and placed after the text and before the reference section with the heading **Notes**.

STM

References: Vancouver style reference citations are represented as textual notes, as a superscript Arabic numeral. Harvard style references are as follows (Smith, 1999).
Any other textual notes (whether references are Harvard or Vancouver) are indicated by a superscript Arabic letter and the corresponding footnote appears at the bottom of the relevant column.
In STM journals, footnotes should be edited into the text if appropriately and easily incorporated. However, please leave footnotes if this is not possible.

Authors' biographical notes

These should appear at the end of the paper with the heading **Author biography** (or **biographies**), in same font size as References/Funding etc. heading. Follow journal style.

3.8 Book reviews

Please check that the book details are given in this format at the top of each review.

Author, *title*, publisher: place, date of publication; 000 pp.: ISBN, price (hbk), ISBN, price (pbk)
Editor(s) (ed[s].), *title*, publisher: place, date of publication; 000 pp.: ISBN , price (hbk), ISBN, price (pbk)

4. Spelling, punctuation and formatting

4.1 Author style/voice

We will endeavour to keep the author's voice as much as possible:

1. Some authors write in the first person. CEs please note that we will *not* be taking articles out of the first person into the third person.
2. Where American authors have used American spellings, we should also endeavour to keep the author's grammar/punctuation, e.g. closed em-dashes instead of spaced en-dashes, single quotation marks within double, series comma etc.
3. Where UK authors have used –ise spellings throughout their papers in a consistent fashion, please do not change. Where there is inconsistency, use -ize.

4.2 General spelling rules

The general rules are as follows:

- UK spellings should be followed for European articles (-ise is acceptable)
- US spellings should be followed for North American articles
- Rest of the world – follow author style but make it consistent
- Canadian spellings should be standardized to UK or US, depending on author preference
- The following list shows some common exceptions to the '-ize' rule:

Samples							
advertise	arise	devise	enfranchise	expertise	merchandise	promise	surmise
advise	chastise	disenfranchise	enterprise	franchise	misadvise	reprise	surprise
affranchise	circumcise	disguise	exercise	improvise	premise	revise	televise
apprise	comprise	emprise	excise	incise	prise	supervise	treatise

Note also: analyse (for UK), catalyse, dialyse, paralyse.

Do not mix English and US spellings. Some common US variations in spelling:							
analyze	color	favor	fulfill	labor	license (noun)	program	traveler/traveling
behavior	counseling	fetus	gray	mold	pediatrics	practice (verb)	willful

Follow author style regarding use of the possessive's for proper names ending in s. However, 's is not used for classical names, e.g. Socrates' philosophy.

The following books are recommended: *Hart's Rules*; *Fowler's Modern Usage*.

4.3 Punctuation and formatting

Commas

- Follow author style but make consistent
- Oxford or series comma are not generally used; only use an Oxford/series comma if essential for clarity

Parentheses

These can be used throughout. Double sets of parentheses are acceptable, e.g. (see Figure 2(a)). Do not use square brackets in the text, except in the following circumstances.

Square brackets are used only to enclose an author's comment within a quote, e.g. [sic], [emphasis added]. Square brackets are also used for equations and mathematical expressions within the text.

Quotes

Use single quotes, with double quotes within quoted material. (See section 4.1 for exceptions for articles written by US authors.)

Hyphenation

The basic rule is to follow author style but be consistent.

Use of upper and lower case

Money

For currency use the common symbol or abbreviation: £, US$, AUD$, etc. – where the quantity is stated, but not when the unit of currency is being referred to in general terms, examples follow:

- The price of oil rose to US$25 per barrel.
- The US dollar was at an all-time low.
- £150m, *not* millions or mlns.

Units in the text

1. Where units are referred to in the text in general terms, they should be written out in full.
2. Where a specific quantity is used, the abbreviated form of the unit must be used; e.g. the nails were several centimetres long; the nails were each 2 cm in length.
3. Always use numerals with the abbreviated unit and use abbreviated units wherever possible – in lists of statistics, in tables and line artwork.
4. Numeral and units should be separated by a thin space, i.e. 100 km, not 100km (this does not need to be indicated by the CE, the TS will format, PR/PE to check). NOTE: exception to the thin space rule applies for percent and degree symbols, i.e. 90% and 35.7°
5. Abbreviations of units are the same for singular and plural (do not add an s); they do not take a full point. E.g. 25 min, 55 s
6. Use SI units wherever possible (see specific Journal webpages for more specific notes).

Numbers

1. Spell out numbers one to nine; for numbers 10 and over use numerals, except at the beginning of a sentence. Re-work the sentence if necessary.
2. Use numerals with percentages (use the **%** symbol, not per cent or percent), with units, in statistical passages, in tables, etc.
3. Spell out and hyphenate one-half, two-thirds, etc.
4. Do not use a comma in 4-digit numbers (thousands) but do use one in 5-digit numbers (tens of thousands) and above, e.g. 5643; 1298; 14,600; 342,885; 1,000,001. Do *not* use a thin space.
5. Do not contract number ranges, e.g. page ranges and dates; i.e. use pp. 24–29, 13–15 October, 1981–1999 etc.
6. Decimal points are never raised off the line.
7. Do not mix spelled-out numerals and units: 6 cm not six cm.

Dates

1. Write out dates in text and refs as follows: 30 September 2003, except in Tables if space is short, then a shortened version may be used, e.g. 11 Sep 08 (do not use 11/9/08, as this could be confused in the US as 9th November).
2. Do not use an inverted comma in decades, e.g. 1960s, mid-1930s. Avoid 80s, etc.
3. Use numerals for centuries (except in history journals where it is spelled out), e.g. a 21st-century dilemma.

4.4 Abbreviations

General

1. Do not use abbreviations in the title of a paper, in the abstract, or keywords, unless the full version is very long and clumsy or the abbreviation is better known than the full term (e.g. DNA). Abbreviations may be used in headings and subheadings if they has already been defined previously in the paper at first usage. If in doubt, spell out.
2. Define an abbreviation the first time that it is used (except in the Abstract): write the term out in full followed by the abbreviation in parentheses. Use the abbreviation consistently thereafter, including at the start of sentences.
3. For plural terms, use plural abbreviations, e.g. low-density lipoprotein, LDL; low-density lipoproteins, LDLs.
4. If you need to abbreviate months or days of the week (for example, in a crowded table), use the first three letters without a full-stop (Mon, Tue; Jan, Feb).

5. If abbreviations are used in a figure or table, they must all be defined in the caption or in a Table note/footnote even if they are also defined in the text.
6. Do not use abbreviations invented by the author of a paper for that paper – ideally, only conventional, generally accepted abbreviations should be used.
7. Do not abbreviate single words (exceptions apply) or use two-letter abbreviations other than those listed below. (Two-letter engineering abbreviations are available in the IMechE Style Guide supplement).
8. Abbreviations consisting of capital letters, and acronyms and contractions, should not take full points, e.g. USA, UK, MA, UN, WHO, PhD, NATO (or Nato), UNESCO (or Unesco), AD, BC
9. Unfamiliar (but generally accepted) abbreviations should always be written out in full when first mentioned, with the abbreviated form following in parentheses, e.g. "The Confederación Española de Derechas Autónomas (CEDA) was formed". Thereafter use the abbreviation.
10. Contractions do *not* take a full point, e.g. Mr, St, Ltd, edn, Dr, neither do contracting degrees (Dr, DPhil, PhD, MSc). The following abbreviations take full points: no., Co., p., pp., vol., ch. (but use vols and chs), e.g., ed. (but use eds), et al., etc., i.e., cf., (note that this means 'compare' and not 'see'), n.d.
11. No comma after e.g., i.e. or cf. Etc. has a full stop and is usually preceded by a comma in a list. They may be used in lists or figure or table legends, and within parentheses.
12. In reference lists, notes, footnotes, corresponding author address (if required) and authors' biographical notes, please use the standard abbreviated form for American states (and Canadian/Australian territories). Please spell out in full in the text (see section 7.3 for full list of US state abbreviations).

Some journals use abbreviations that do not need to be spelled out, even at first usage. For a full list of abbreviations that do not need to spelled out for each individual journal, please visit the journal webpage.

STM abbreviations: some abbreviations of terms that we do not define in full are listed here (follow style given):

- SD = standard deviation
- SEM = standard error of the mean
- NS = not significant
- a.m. in the morning (but use 24-hour clock if possible)
- p.m. in the afternoon
- N/A = not applicable
- Chemical symbols (H_2O, H_2SO_4) may be used without definition. However, write in full unless this is inappropriate (e.g. 'Water consists of hydrogen and oxygen'; 'Nitric oxide is also found in peripheral nerves'). Refer to *Scientific terminology* notes for further guidance.

See the Appendix (pp. 26 and 27) for a full list of accepted general two-letter STM abbreviations and engineering abbreviations.

5. Technical content: maths, equations, etc.

5.1 Maths notation convention

There is no specific convention for mathematical notation in terms of matrices, vectors, variables, operators, functions, subscripts, superscripts and scalars. CE please follow the author's symbols and notation conventions, ensuring that these are consistent throughout the paper.

Please query the author if any symbols are unclear, duplicated with more than one definition, or undefined.

5.2 Equations

Layout of equations

1. Equations should be left aligned on a 3 mm indent, *not* centred.
2. Equations should be numbered in sequence throughout the text, with the numbering continuing through all appendices. However, equations only need to be numbered if cited in the text, and not all equations necessarily need to be numbered.
3. Equation numbers should be set flush right and in sequence. Each numbered equation should have its own line.
4. No punctuation is used before or after an equation (i.e. no commas, colons, hyphens etc.)
5. The equation number should align with the *bottom line of equation*. Where the equation number covers multiple equations, it should align with the bottom line of the last equation.
6. When referred to in text, equations take the form 'equation (1)'. When a range of equation numbers is referred to, use the form: equations (1) and (2); equations (1) to (3); equations, (1), (2), and (5) to (7).

With the assumptions outlined previously, conservation of momentum and the definition of velocity change gives

$$m_1 u_1 + m_2 u_2 = m_1 v_1 + m_2 v_2 \quad (1)$$

$$\Delta v = v \quad u \quad (2)$$

Equations (1) and (2) lead to

$$\Delta v_1 = \quad \Delta v_2 \frac{m_2}{m_1} \quad (3)$$

A diagram showing a generalized impact configuration

7. If two or more small equations or conditions can fit on one line, then they should be well separated with a 2-em space. Commas and words, set upright not italic, may be used to enhance clarity.
8. Equations in text must be reduced to one line depth. Display equations are built up to two line depth. For instance, the equation $(x - y)/(x^2 + 2y - 3)$ runs on in the text but for display becomes
$$\frac{x - y}{x^2 + 2y - 3}$$
9. CEs: Spaces between + and – and other operators need not be marked. TS will format.
10. Unless separating small equations and conditions, as shown above, odd words between equations such as 'where', 'and', 'thus', 'therefore' should be on a separate line from the equations and flush left. Only use initial capitals for these if they start a new sentence.
11. When a single equation has been presented with a label/header (e.g. 'momentum conservation equation', 'blade element momentum theory', etc.), present the label before the equation, full left, half-line above, and in roman.
12. Where an equation is too long to fit on one line, take over whole terms starting if possible with a + or – or = symbol, and indent.
13. Where a bracketed term has to be split over lines move the second part to the right to show it is still part of the same term (align to the right of the bracket).
14. Pairs of opening and closing brackets should be the same size, even when they are on different lines.
15. Where an equation breaks at an equals sign indent a further em in from the first line.
16. Where equations are split over 2 lines, the break should occur before the operator:

$$m_2(1+e_2)(U_{2p}-U_{1p})$$
$$=(m_1+m_2)\Delta v_1 \quad m_1 h_1 \Delta\omega_1 \quad m_2 h_2 \Delta\omega_2 \qquad (9)$$

5.3 Units

SI preferred. Expressions such as rpm, psi, cfm, gpm, mph, kph, tsi, revs should be avoided. Use instead r/min, lbf/in^2, gal/min, mile/h, km/h, ton/in^2, rotational speed, etc.

Notes: Greek μ in μm should always be roman; MPa and GPa should always have a capital P.

5.4 Symbols and operators

A thin non-breaking space should separate symbols and operators from numerals, and be present either side of multiplication dots and all operators, e.g. +, -, =, x, <, >, etc. (this does not need to be indicated by the CE, the TS will format)

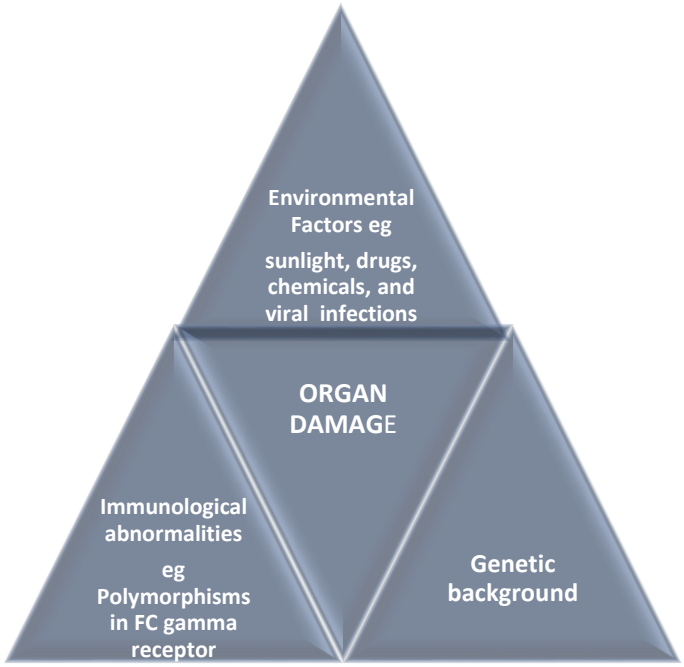
Appendices and notation (see section 2.6, p. 7)

Appendix 7

Pathogenesis of SLE and LN

A complex interplay between immunological, genetic, hormonal and environmental factors is thought to account for the development of SLE.[1]

Figure below show the interaction of environmental,immunological and genetic factors in SLE



Self-nuclear antigen exposure from defective dead cell clearance result in loss of immune tolerance and lifelong persistence of ANA antibodies, indicating persistently active autoreactive T and B cell clones[1, 2].

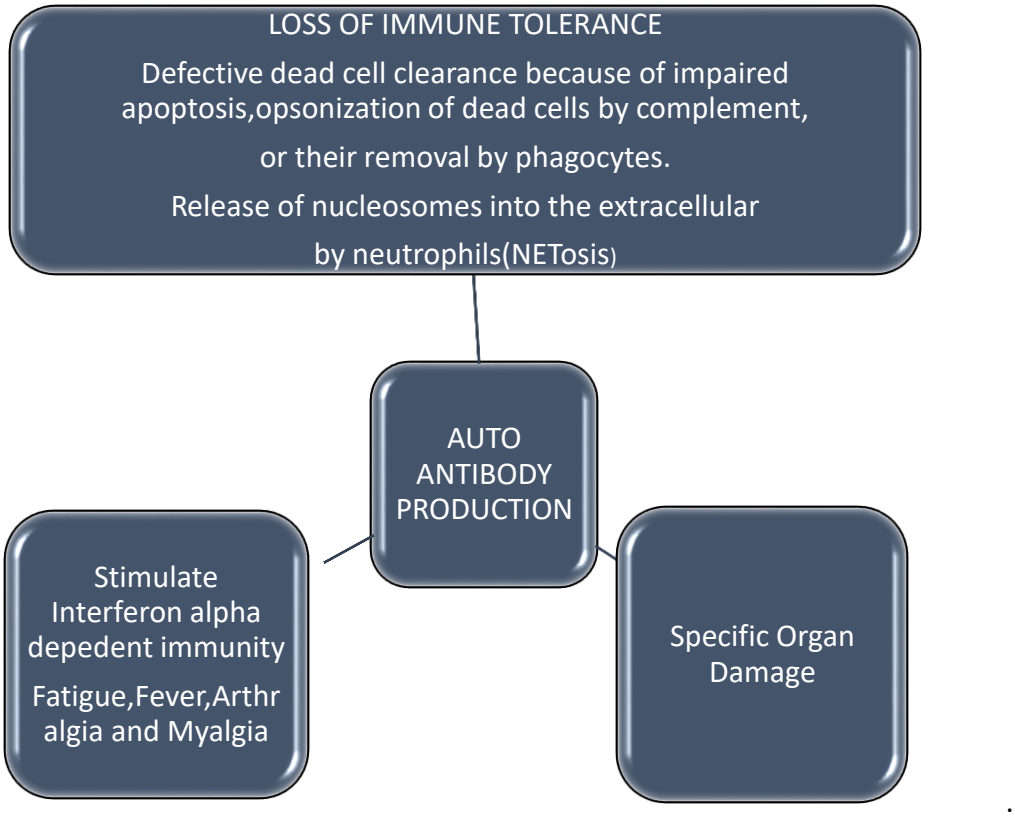
3.5 Role of Genetic factors in Pathogenesis

Evidence for genetic component in SLE is provided for by:

- High heritability in twin studies, siblings and first and second-degree relatives with SLE.
- Emerging data from Genome Wide Association Studies.[3]
- Some polymorphisms in immunoglobulin receptor alleles present on macrophages, have been associated with susceptibility to lupus nephritis in some studies.[4, 5]
- HLA-regulating genes and genes that regulate the complement system.[6]

There appears to be an understanding that there may be a background genetic abnormality to predispose an individual to developing SLE.[3]

Figure below summarise the cellular mechanism leading to autoantibody production



1. Anders HJ and Rovin B. A pathophysiology-based approach to the diagnosis and treatment of lupus nephritis. *Kidney international*. 2016.

2. Bosch X. Systemic lupus erythematosus and the neutrophil. *The New England journal of medicine*. 2011; 365: 758-60.

3. Tiffin N, Adeyemo A and Okpechi I. A diverse array of genetic factors contribute to the pathogenesis of systemic lupus erythematosus. *Orphanet journal of rare diseases*. 2013; 8: 2.

4. Karassa FB, Trikalinos TA and Ioannidis JP. Role of the Fcgamma receptor IIa polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. *Arthritis and rheumatism*. 2002; 46: 1563-71.

5. Salmon JE, Millard S, Schachter LA, et al. Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. *The Journal of clinical investigation*. 1996; 97: 1348-54.

6. Sterner RM, Hartono SP and Grande JP. The Pathogenesis of Lupus Nephritis. *Journal of clinical & cellular immunology*. 2014; 5: 205.

Appendix 8

Mechanisms of Kidney Damage in Lupus

Immune complex deposition in the kidneys plays a pivotal role in LN. These immune deposits occur in the mesangium, sub endothelium, or sub epithelium depending on the characteristics of both the antigen and antibody. The intrarenal aetiology of lupus nephritis involves antibody binding to multiple intrarenal autoantigens rather than the deposition of circulating immune complexes.[1]

The site of binding or deposition is determined by size or charge of the immune complexes. Large intact immune complexes or anionic antigens which cannot cross the negative charge barrier in the glomerular capillary wall are deposited in the mesangium and sub endothelial space.[2, 3]

On the other hand, sub epithelial deposits may form when a cationic antigen crosses the GBM or an autoantibody directed against epithelial cell antigens.

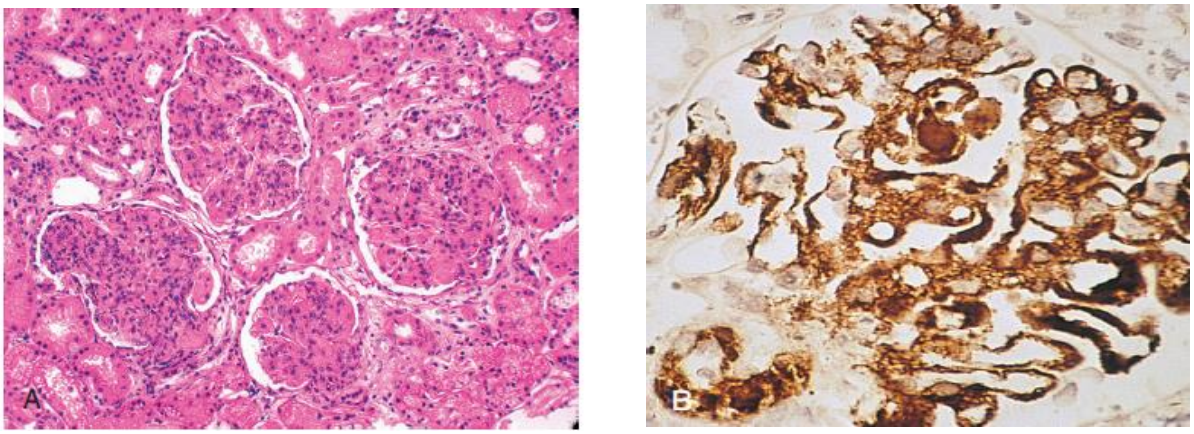
These immune complexes are mainly due to anti-double-stranded DNA antibodies directed against nucleosomes.[4]These immune complexes may also contain chromatin, C1q, laminin, Sm, La (SS-B), Ro (SS-A), ubiquitin, and ribosomes.[5-7]

3.7 Sub endothelial or Mesangial deposits

Deposits that occur in the mesangium and sub endothelium are in communication with the vascular space since they are proximal to the GBM.

Thus, complement is activated generating chemo attractants C3a and C5a resulting in the influx of neutrophils and mononuclear cells. These changes manifest histologically by a mesangial, focal or diffuse proliferative glomerulonephritis. This manifests clinically as an active urine sediment, proteinuria, and in some cases acute decline in renal function.

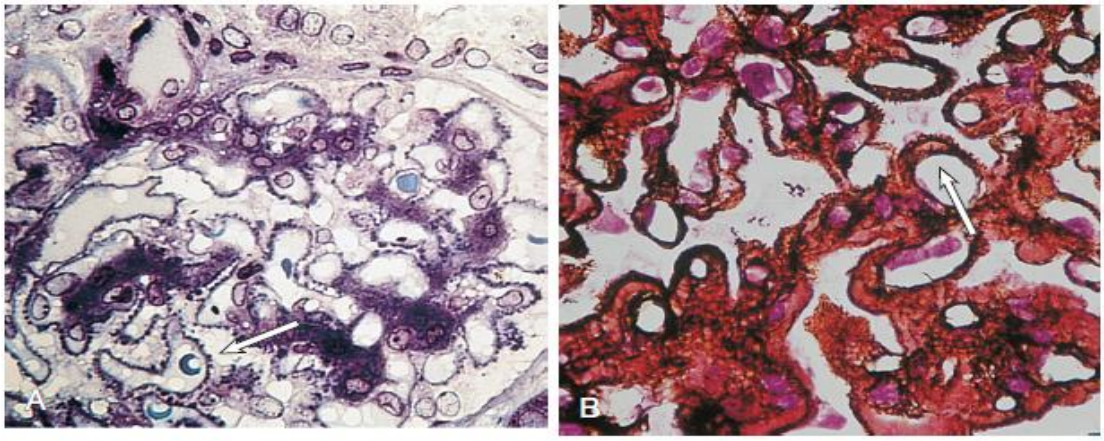
Figure 1.[8]



3.8 Sub Epithelial deposits

Sub epithelial deposits are separated from the circulation by the GBM hence the inflammatory response is minimal and injury is limited[3]. The injury is limited to the glomerular epithelial cells and the primary clinical manifestation is proteinuria, which is often in the nephrotic range. Histologically, these patients most commonly have membranous nephropathy.

Figure 2.[8]



3.9 IgG subclass in Sub Epithelial or Sub endothelial deposits

Further evidence to support this delineation can be found by analysing the distribution of the IgG subclasses in the immune complexes. Sub class IgG1 and IgG3 fix complement, while IgG2 does so less avidly and IgG4 does not fix complement.[9] Consistent with this hypothesis are the observations that anti-DNA antibodies associated with diffuse proliferative glomerulonephritis hence sub endothelial tend to be IgG1 and IgG3.[10] On the other hand the immune deposits in membranous nephropathy thus sub epithelial are more likely to be IgG2 and IgG4.[11]

1. **Lech M and Anders HJ. The pathogenesis of lupus nephritis. *Journal of the American Society of Nephrology : JASN*. 2013; 24: 1357-66.**
2. **Kashgarian M. Lupus nephritis: lessons from the path lab. *Kidney international*. 1994; 45: 928-38.**
3. **Fries JW, Mendrick DL and Rennke HG. Determinants of immune complex-mediated glomerulonephritis. *Kidney international*. 1988; 34: 333-45.**
4. **O'Flynn J, Flierman R, van der Pol P, et al. Nucleosomes and C1q bound to glomerular endothelial cells serve as targets for autoantibodies and determine complement activation. *Molecular immunology*. 2011; 49: 75-83.**
5. **Mannik M, Merrill CE, Stamps LD and Wener MH. Multiple autoantibodies form the glomerular immune deposits in patients with systemic lupus erythematosus. *The Journal of rheumatology*. 2003; 30: 1495-504.**
6. **Yin Y, Wu X, Shan G and Zhang X. Diagnostic value of serum anti-C1q antibodies in patients with lupus nephritis: a meta-analysis. *Lupus*. 2012; 21: 1088-97.**
7. **Ben-Yehuda A, Rasooly L, Bar-Tana R, et al. The urine of SLE patients contains antibodies that bind to the laminin component of the extracellular matrix. *Journal of autoimmunity*. 1995; 8: 279-91.**
8. **Richard J. Johnson JFaJF and .*Chapter 26 :Lupus Nehritis*. 5 ed. 2015, p.1288.**
9. **Schur PH. IgG subclasses--a review. *Annals of allergy*. 1987; 58: 89-96, 9.**
10. **Schur PH, Monroe M and Rothfield N. The gammaG subclass of antinuclear and antinucleic acid antibodies. *Arthritis and rheumatism*. 1972; 15: 174-82.**
11. **Lewis EJ, Busch GJ and Schur PH. Gamma G globulin subgroup composition of the glomerular deposits in human renal diseases. *The Journal of clinical investigation*. 1970; 49: 1103-13.**

Appendix 9:Definition of relapses/Flare of LN.[1]

Mild kidney relapse	Moderate kidney relapse	Severe kidney relapse
Increase in glomerular haematuria from <5 to >15 RBC/hpf*, with ≥2 acanthocytes/hpf AND/OR recurrence of ≥1 RBC cast, WBC cast (no infection), or both	If baseline creatinine is: <177 umol/l, an increase of 17.7–88.4 umol/l >177 umol/l], an increase of 35.4–132.6 umol/l AND/OR If baseline uPCR is: < 500 mg/g an increase to >1000 mg/g 500–1000 mg/g, an increase to ≥2000 mg/g, but less than absolute increase of < 5000 mg/g >1000 mg/g, an increase of ≥2-fold with absolute uPCR < 5000 mg/	If baseline creatinine is: < 177 umol/l, an increase of >88.4 mmol/ ≥ 177 mmol/l, an increase of > 132.6 mmol/l AND/OR an absolute increase of uPCR > 5000 mg/g

*high power field

1. KDIGO. KDIGO Clinical Practice Guideline for Glomerulonephritis. ***Kidney International Supplements*****. 2012; 2: 209**.