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Pattern of repeat kidney biopsy findings in quiescent proliferative lupus nephritis

[1]Dr. Ravi N Sangapur, [2]Dr. GN Ravikumar Patel
[1]Senior Resident, Departmentof General Medicine, S Nijalingappa Medical College,Bagalkote, Karnataka, India
[2]Consultant Nephrologist, Nanjappa Hospital, Shivamogga, Karnataka, India

Corresponding Author: Dr. GN Ravikumar Patel (gnrpatel@gmail.com)

Abstract

Background: The optimal duration of maintenance therapy is controversial in proliferative lupus nephritis. Discordance between clinical parameters of renal remission and histological findings in immediate post induction phase as well as maintenance phase has made repeat biopsy a compulsory tool to confirm the histological remission. But timing of repeat kidney biopsy is debatable. Aim of this study was to find the pattern of repeat kidney biopsy findings proliferative lupus nephritis.

Methods: Repeat biopsy (biopsy 2) performed on patients of biopsy proven (biopsy 1) proliferative lupus nephritis (ISN/RPS 2004 class III/IV±V) between November 2011 to September 2017 on maintenance therapy and in complete clinical remission for at least 2-years. Clinical and histologic findings at biopsy 1 and biopsy 2 of 29 patients were compared

Results: Average time taken achieve complete remission was 9(2-24) months. Average duration of follow up, maintenance therapy and complete clinical remission the cohort was 68±17.8, 62.5±14.2 and 58.9±17.3 months respectively. Histological remission was observed in 93.1%. Other than duration of complete remission on maintenance therapy none of the variables failed to predict the histological remission.

Conclusion: Majority of patients were in complete histological remission in repeat kidney biopsy proliferative lupus nephritis following sustained clinical remission on maintenance immunosuppressive therapy.

Introduction

Lupus Nephritis(LN) is one of the severe complication of Systemic Lupus Erythematosus(SLE) characterised by relapses and remissions. Kidney biopsy has important role in the initial diagnosis, assessing severity, staging and choosing appropriate therapy in LN management[1].

Class III and IV (International Society of Nephrology/Renal Pathology Society [ISN/RPS]) were considered as severe form of LN and which needs treatment in two phases. Induction phase is aggressive treatment for first six months and maintenance phase for prolonged duration[2].Maintenance immunosuppressive therapy in severe LN aims to keep persistent autoimmunity under control and to prevent flares of lupus nephritis.Optimum duration of maintenance phase is unknown though study by Boumpas*et al.* [2] suggested continuation of maintenance therapy for extra two years after achieving complete remission. As these patients will be on long term immunosuppression, they are at risk of severe side effects. Few published data available on protocol repeat biopsies done after induction phase has shown persistence of histological activity[3-6].The reason may be due to early timing of protocol repeat kidney biopsy. The cost effectiveness and invasiveness of early timed biopsies are questionable. Stopping maintenance therapy in complete clinical remission without

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considering the histological remission poses risk of flare [7] and each flare was a predictor of progressive kidney damage[8].Sustained clinical remission in LN has better predicted favourable long term outcome, lower mortality and decreased progression to ESKD[9].Appropriate timing of repeat kidney biopsy to see histological remission was based on expert opinion and there are no proper guidelines. Reliable predictors for correlation between clinical and histological remission on repeat kidney biopsy are lacking and studies done till date has shown discordance between clinical and renal response[5, 7].A recent prospective study of repeat kidney biopsy after one year after complete clinical remission has shown that 44.4% persisting histological activity and stopping immunosuppression in such patients resulted in lupus flare[7].

We undertook this study to find correlation between the clinical remission and histological remission in repeat kidney biopsy findings of quiescent proliferative lupus nephritis and appropriate duration of complete clinical remission.

Materials and Methods

This study was single centre retrospective cohort with prospective observational study, conducted in the department of nephrology, a tertiary care centre located in southern part of the India between September 2011 to December 2019, The study protocol was approved by the Institutional Ethics Committee.

Study population

Study subjects were SLE patients who had biopsy proven proliferative LN (class III/IV±V) followed up in department of nephrology of our hospital. Adult patients (aged 18-60) with initial biopsy proven class III/IV ± V LN, minimum 36 months' azathioprine maintenance therapy and at least 24 months of complete clinical remission were included in this study. Exclusion Criteria's wereage less than 18 years or more than 60 years, eGFR less the 60ml/min/1/73m^2 at the time inclusion, presence of extra-renal lupus activity features where immunosuppressive could not be tapered, presence of life-threatening complications such as severe infection, poor compliance t therapy. Study design and patient flow are shown in figure 1.

Method of data collection

The patients will be selected to participate in the study based on the eligibility criteria. Written and informed consent was taken from all subjects to participate in the study. All data of patients of SLE with initial biopsy (biopsy 1) proven proliferative lupus nephritis collected. Short term as well as long term treatment outcome and every 3 monthly follow up investigations analysed. Response evaluation data include time to attain complete remission and relapse events were studied. Patients with initial biopsy proven class III/IV ± V LN to assess correlation between clinical parameters with histological findings on repeat kidney biopsy done as per inclusion criteria. Any adverse drug reactions reported will be recorded in case record form. All patients were understood that the repeat biopsy findings would not impact the further treatment decisions. Primary end point of the study was histologic findings in repeat biopsy of long term clinical remitted proliferative LN and correlating them with clinical parameters.

Kidney biopsy

Kidney biopsy was performed through 16 or18-gauge percutaneous needle with standard precautions. All the kidney biopsy was adequate (≥10glomeruli) and examined by light microscopy and direct Immunofluorescence (IF). For light microscopy, specimens were fixed with 10% buffered formalin. Consecutive 3μm sections were used for histological staining. Sections were stained with Haematoxylin-eosin, periodic acid schiff stain, masson Trichrome and methenamine silver stains. All initial (Biopsy1) and repeat (Biopsy2) biopsies were read by a single renal pathologist who was blinded to the patient's clinical data and remitted status.

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All kidney biopsies were classified as per ISN/RPS system(R).Modified National Institute Health activity index(AI) chronicity index(CI) were calculated for each biopsy [38]. Activity Index(AI) of zero in repeat kidney biopsy is considered as histological remission.

Treatment protocol and follow up

All patients were diagnosed clinically as SLE and active LN. Severe proliferative class III/IV±V confirmed by biopsy 1 initially were started on 3 pulses intravenous methylprednisolone 1g per day for three consecutive days followed by 0.5mg/kg/day oral prednisolone. All the patients were given induction with intravenous cyclophosphamide at dosage of 0.7g-1g/m^2 body surface area for every monthly for six-month duration. Oral prednisolone was slowly tapered off at rate of 5mg/month until dose of 5mg at sixth month. All the patients after induction therapy were started on maintenance immunosuppression with azathioprine at dosage 2-2.5mg/kg body weight and dose was adjusted as per total leukocyte count (TLC). Those who relapsed on maintenance therapy or drug noncompliance were either treated with increasing dosage of azathioprine or repeating induction with cyclophosphamide as given at the time of diagnosis respectively. At the time repeat biopsy, all the patients were on azathioprine maintenance therapy at dosage of 1-2mg/kg body weight and median oral prednisolone dosage of 4 mg per day (range 0-5mg/day). Additionally, all patients were administered a low to normal protein diet, low-sodium diet, hydroxychloroquine (HCQ) 200 mg/d and renin angiotensin system (RAS) blockers titrated to a target of 130/80 mm Hg or lowest tolerated blood pressure.

Patient follow up visits was done at monthly interval for six months till induction therapy got over and every 3-6 monthly there after till the time of biopsy 2. Each visit was analysed for response by set of laboratory investigations and clinical evaluation. Medication adherence was assessed by talking to patients and relatives as well as checking empty medicine packs.

Definition of renal response

1. **Complete clinical remission(CR):** Reduction in proteinuria to <0.5g/day or its equivalent on urine protein creatinine ration, inactive urinary sediments, normal kidney function, 50% reduction serum creatinine from baseline and serum albumin more than 3.5g/dl. Urine sediments was considered inactive in the absence of red blood cells casts, white blood casts and glomerular hematuria (< 5% dysmorphic RBCs per hpf).
2. **Partial clinical remission:** Reduction in proteinuria of 50% from baseline, with absolute values between 500mg and 3.5g/day and stable kidney function.
3. **Relapse or lupus nephritis flare:** Increasing disease activity that requiring restarting immunosuppression. This included new glomerular hematuria, an increase serum creatinine level ≥ 0.3mg/dl and/or increase proteinuria to over 500mg/day.

4. **Histological remission:** It is defined as modified NIH activity index score of 0 [7].

Statistical analysis: Statistical software SPSS 22.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Quantitative data were expressed as mean ± SD for normally distributed data or median with 25% and 75% interquartile range for non-normally distributed data. Categorical data were expressed as percentages. For comparison of clinical and pathological features of patients, the Student's t-test, one-way ANOVA analysis of variance, Chi-square test and Mann-Whitney tests were used. Kaplan-Meier curves were used to analyse the patients' prognoses. Survival analysis was performed using the log-rank test. Statistical significance was considered as P value is less than 0.05.

Results

A total of 29 patients of SLE with quiescent proliferative lupus nephritis underwent repeat kidney biopsy were studied.Clinical, serologic and histological findings at biopsy 1 are shown in table 1. Most common clinical presentation was Nephrotic syndrome (65%) followed by rapidly progressive glomerulonephritis (21%) and nephritic syndrome was 14%. Most

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common histological class 18(62%) has class IV active(A) lesions, 8(27.6%) has class IV active/chronic (A/C) and 3(10.34%) were in class III active(A) lesions.

Table 1: Demographic, clinical and histologic characteristics at biopsy 1

Variables	Cohort(n=29)
Age(years), mean(SD)	31.6 (9.5)
Gender (female: male)	27:2
Duration of disease (months), mean(range)	16(1-120)
Extra renal symptoms, n(%)	20(69%)
Hypertension, n(%) Mean SBP(mmhg), mean(SD) Mean DBP(mmhg), mean(SD)	20(69%) 140(15.3) 86(11.1)
Bad obstetric history, n(%)	5(14.5%)
Hypothyroidism, n(%)	7(24.1%)
Hemoglobin (g/dl), mean(SD)	9.94(1.65)
Urinary active sediments, n(SD)	25(86.2%)
24 hour proteinuria(g/d), mean(SD)	3.9(2.1)
Serum creatinine(mg/dl), mean(SD)	1.37(0.83)
eGFR(mg/min per 1.73m^2), mean(SD)	66.6(34.1)
Serum albumin (g/dl), mean(SD)	2.4(0.590
Total cholesterol, mean(SD)	240.1(65.5)
C3 (mg/dl), mean(SD)	35.2(18.6)
C4 (mg/dl), mean(SD)	7.8(5.2)
Low C3, n(%)	25(86.2)
Low C4, n(%)	20(68.9)
Anti-dsDNA positive, n(%)	22(75.9%)
Class III LN, n(%)	3(10.3)
Class IV LN, n(%)	25(86.2)
Mixed(class IV+V), n(%)	1(3.4%)
Activity Index(AI), median(range)	8(3-20)
Chronicity Index(CI), median(range)	1(0-3)

Clinical, serologic and histologic findings at biopsy 2 are shown in table 2.

Table 2: Clinical and histologic characteristics at biopsy 2

Variables	Cohort(n=29)
Time to CR(months), mean(range)	9(2-24)
Cumulative CYC dose(grams), mean(range)	6.24(3-9.5)
Duration of maintenance therapy(months), mean(range)	62.5(36-90)
Duration of CR(months), mean(SD)	58.9(17.3)
Duration of between biopsy 1 and biopsy 2, mean(SD)	61.5(18.6)
Duration of follow up(months), mean(SD)	68(17.8)
History of prior renal relapse, n(%)	4(13.7%)
24 hour proteinuria(g/d), mean(SD)	0.24(0.1)
Serum creatinine(mg/dl), mean(SD)	0.63(0.15)

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eGFR(mg/min per 1.73m2), mean(SD)	118.5(17)
Serum albumin(gm/dl), mean(SD)	4.1(0.3)
Anti-dsDNA positive, n(%)	2(6.9)
C3 (mg/dl), mean(SD)	113(18.3)
C4 (mg/dl), mean(SD)	37(12.8)
Activity Index=0, n(%)	27(93.1)
Chronicity Index(CI), median(range)	2(0-3)
Findings in biopsy 2 (transformation) Normal, n(%) Class V, n(%) Class II, n(%) Class III(from class IV)	 20(58) 5(17.2) 2(6.8) 2(6.8)
Follow up SBP(mmhg), mean(SD)	122.2(12)
Follow up DBP(mmhg), mean(SD)	77.7(7.9)

Of all patients underwent repeat kidney biopsy, 27(93.1%) had complete histological remission(AI=0). Rest 2(6.8%) patients with no histological remission and both were less than 48 months of complete clinical remission and maintenance therapy. AI of these two was 4 and 2 respectively.

In our study, adverse events related repeat biopsy were very minimal and no serious adverse events (death, organ loss, and requiring intervention to control bleeding). Only 2 patients (6.8%) had developed microscopic hematuria but were self-limited and hemodynamically stable. None of them developed hematoma and no patient required a blood transfusion.

Discussion

Lupus nephritis is the severe manifestation of SLE with relapsing and remitting in its disease course if not managed properly can lead ESRD. Proliferative LN (ISN/RPS class III/IV±V) is more serious histological class needs extra attention in its management. Treatment of proliferative LN is a gladiatorial approach to achieve clinical remission by induction treatment and long term maintenance therapy to prevent future flares. Optimal duration of maintenance therapy to prevent flares is controversial. Bertsias GK *etal*., in EULAR/ERA-EDTA recommendations suggested the 36 months of maintenance therapy but this is based on expert's opinion and limited to some ethnicity of population[10].Exposure of long termimmunosuppressive has serious adverse events like infections, metabolic side effects. Discontinuation of maintenance therapy based only on clinical data (reduction of proteinuria,normal renal function and negative SLE serology) resulted flares and these recurrent episodes will cause further progression of renal damage leads to ESKD.

Discordance between complete clinical remission and histological remission in both induction and maintenance phase has made repeat kidney biopsy a compulsory tool to confirm histological remission[3-6].Timing of repeat biopsy in maintenance phase still a debatable. De Rosa et al in their prospective observational study, repeat biopsy done after 36 months of maintenance therapy and at least 1-year of complete remission and immunosuppression was stopped irrespective of histological remission status; this has resulted a flare in histological activity group[7].In recent study by Malvar A *et al*., second kidney biopsy performed in all after 42 months from initiation of therapy to look for histological remission and there after biopsy repeated every second year in those patients with persistent histological activity in previous biopsy[11].We assumed that, 1-year complete clinical remission was too early to do repeat biopsy to see histological remission and invasiveness as well as cost-effectiveness of repeated biopsy based management of maintenance therapy was controversial. Pakchotanon R *et al*. showed prolonged complete clinical remission in LN was predictor reduced mortality, CKD and ESKD[9].To overcome the lacunae of this knowledge, we performed repeat kidney

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biopsy in quiescent proliferative lupus nephritis after 36 months of maintenance therapy and 24 months of complete clinical remission to find the correlation between the sustained clinical remission and histological findings.

In present study, we performed 32 repeat kidney biopsies in patients with quiescent proliferative LN patients. After excluding 3 inadequate biopsies, we analysed demographic, clinical and histologic characteristics of the remaining 29 patients. Demographic, clinical and histologic characteristics at biopsy 1 were similar to previous study done in our center[83] as well as other studies[7, 12].

Mean duration of follow up and mean duration between biopsy 1 and biopsy 2 was 68±17.8 and 61.5±18.6 months respectively. Average duration of follow up in similar studies done previously was varying due to different methodology used for inclusion criteria[4-7, 11].Average time to achieve complete remission and cumulative dose of CYC was consistent with previous studies[4, 12, 14].

Biochemical and serological markers at biopsy 2 were comparable with similar studies published[6, 7, 11].We observed only 6.9% of patients with positive anti ds-DNA antibody and difference was attributed to sustained clinical remission on maintenance after initiation of treatment.

Histological remission at biopsy 2 was 93.1% in a present study.In a study done by Alvarado AS *et al*. [6] on Argentinian proliferative LN population of 25 patients, repeat biopsy done after 42 months initial treatment and at least 24 months clinical inactivity, it was found that 52% patients were in histological remission. Similar two studies, repeat kidney biopsy done on proliferative LN Argentinian population by De Rosa *et al*. [7] and Malvar A *et al*. [11] showed a histological remission in 55.6% and 96.05% respectively. It was observed that, increase in duration of follow up and complete clinical remission on maintenance therapy increases the chance of histological remission. Higher proportions of histological remission in our cohort was attributed to longer duration of maintenance therapy and complete clinical remission and study done by MalvarA *et al*.[11] also showed similar observations.

Histological transformation from ISN/RPS one class to other is common in repeat kidney biopsy of proliferative LN, but it depends on timing of repeat biopsy. In our study, it was found that 58% became completely normal (class ILN) on light microscopy. In recent studies done to see histological remission did not take in to consideration of histological transformation [5,7,8].Repeat kidney biopsy studies done for other indications shown that varying rates histological transformation from proliferative to non-proliferative LN and these were not consistent with our cohort [3,6,11]. This discordance could be due to repeat biopsyperformed after varying duration of follow up and different indication for biopsy after initial treatment. The more proportion of histologically benign transformation in present cohort was due to longer duration of complete clinical remission before repeat kidney biopsy.

Our cohort demonstrated that LN patients followed on maintenance therapy and sustained CR were important prerequisites to achieve histologic quiescence. From retrospective data by Moroni *et al*. suggested prolonged course of maintenance immunosuppression was more important to achieving sustained clinical remission[15].

In conclusion, our study has demonstrated that sustained clinical remission on maintenance therapy for more than 48-months duration has correlated with histological remission on repeat kidney biopsy in quiescent proliferative lupus nephritis.

The study has limitations. Our study was small cohort and restricted to specific population and ethnicity. In our study histological resolution was based light microscopy and IF microscopy and did not take into consideration of electron microscopy. It is a cross-sectional study, follow up is required to study the role of repeat kidney biopsy in withdrawal of immunosuppression.

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