STUDY PROTOCOL:

A RANDOMIZED CONTROL TRIAL OF RITUXIMAB VERSUS MODIFIED PONTICELLI REGIMEN IN THE TREATMENT OF PRIMARY MEMBRANOUS NEPHROPATHY – A PILOT STUDY

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AIMS:

To compare the efficacy and safety of rituximab with modified Ponticelli regimen in the treatment of Primary Membranous Nephropathy (PMN)

OBJECTIVES:

- To assess non-inferiority of Rituximab over modified Ponticelli regimen in inducing remission (complete or partial) at 6 months in patients with primary membranous nephropathy (PMN).
- To compare the adverse events in these two regimens used for the treatment of primary MN

STUDY DESIGN:

This study was designed as an open-label non inferiority Randomized controlled trial between two known regimens for the treatment of PMN.

PLACE OF THE STUDY:

This study was conducted in Muljibhai Patel Urological Hospital (MPUH), Nadiad located in the state of Gujarat in the Western part of India. Approval for the conduct of the study was

obtained from the Institutional Review Board (IRB) of MPUH, Nadiad. (IRB clearance Reference: EC/525/2018 enclosed)

DURATION OF THE STUDY:

Enrolment period for the study was from August 2018 to December 2020 (29 months). Patients enrolled and randomized in the study were followed up till June 2021 for a total study period of 35 months. This study will be continued till at least 12 months follow-up of all patients is completed. Enrollment and randomization in the study will be ongoing till sample size is achieved.

PATIENTS:

Inclusion criteria:

The following patients were considered eligible for inclusion in the study

1) Patients older than 18 years who provide written informed consent.

2) Biopsy-proven primary MN within 2 years of enrolment with nephrotic range proteinuria denoted by 24-hour urine protein ≥ 3.5g or UPCR (urine protein:creatinine ratio) ≥ 3500mg/g as well as the following:

a. Serology or biopsy positive for AntiPLA2R

b. Serology or biopsy negative for AntiPLA2R and secondary causes ruled out

c. Evaluation for secondary causes was done in all patients, even in patients who were positive for Anti-PLA2R antibodies on serology or PLA2R antigen on biopsy because these have also been found in some cases of secondary MN.

3) Estimated GFR ≥30 mL/min/1.73m2. The CKD-EPI creatinine equation was used for calculation of the eGFR.

4) Treatment with an ACEI or ARB for at least 3 months before enrolment [unless intolerance to ACEI/ARB, contraindications to their use or a low BP that could induce side effects, at the treating nephrologist's discretion] with a controlled BP for at least last 3 months (target ≤ 140/80 mmHg). Patients showing severe or disabling symptoms related to nephrotic syndrome or severe hypoalbuminemia (<2 g/dL) were included for initiation of immunosuppression protocol before completion of 3-month observation period, at the discretion of the treating nephrologist and investigator.

Exclusion criteria:

The following were considered among the exclusion criteria for the study

1) Secondary MN

2) Active serious infections

3) Pregnant women

4) Suspected or known hypersensitivity to either interventional drug

5) Patients with persistently low estimated GFR < 30ml/min/1.73m2 in the absence of acute causes such as acute tubular injury, renal vein thrombosis and others.

OUTCOMES:

Primary end point:

• Proportion of patients reaching complete or partial remission at 6 months defined according to the 2012 KDIGO guidelines as follows 6:

– Response – Complete or partial remission

• Complete remission: A reduction of proteinuria to <0.3g/24h (uPCR < 300mg/g) with normal serum albumin concentration and normal serum creatinine

• Partial remission: A reduction of proteinuria to < 3.5g/day (uPCR < 3500mg/g) and a 50% or greater reduction from peak values accompanied by an improvement or normalisation of serum albumin concentration and stable serum creatinine

– Limited response – Proteinuria reduced from baseline level >50% but >3.5 g/24 h

– No response – Reduction of proteinuria <50% from baseline level

Secondary end points:

1) The proportion of patients reaching either complete or partial remission at 12 months after therapy

2) The proportion of patients with relapsing nephrotic syndrome among patients who previously underwent partial remission or complete remission, which was defined as follows:

• Reappearance of proteinuria >3.5 g/24 h and at least 50% higher than the lowest post-treatment value in those who previously had partial or complete remission.

3) The time to relapse of nephrotic syndrome after initial remission.

4) Serum anti-PLA2R levels before treatment and at 6 and 12 months post-therapy.

5) Efficacy outcome variables during the study period including trend of magnitude of proteinuria, serum albumin, serum proteins, serum creatinine and estimated GFR (measured by the CKD-EPI formula)101

6) Proportion of patients developing adverse events during the study period

PROCEDURE AND FOLLOW-UP:

All patients with nephrotic syndrome with biopsy suggestive of membranous nephropathy presenting to MPUH, Nadiad were evaluated for inclusion in the study. If the patients met the above inclusion criteria and exclusion criteria, they were proposed to participate in this trial after providing complete information about their disease, options of treatment, potential outcomes, risk and benefits of both therapies, and trial process, including the number of visits, clinical and laboratory determinations, and time of follow-up. Once the patient consented to inclusion in the study and confirmed participation by signing an informed consent form, they were randomised into one of the 2 groups according to the protocol given below. (Patient information sheet and consent form attached in annexures 3-8)

Randomisation:

The patients were randomized with an equal allocation ratio (1:1) to intervention with Rituximab or the modified Ponticelli regimen (steroids plus cyclophosphamide). We used a random number-producing algorithm for block randomisation using sealed envelope online software. Randomisation and treatment allocation were then done by the primary investigator using this randomisation list in concealed manner using sealed envelopes. This was unblinded study and both principal investigator and patient were aware of the treatment allocation group and drug/treatment being administered after randomisation. Statistical analysis was also not blinded in this trial.

Data and sample collection – Evaluation of baseline characteristics

1) Demographics (date of birth, gender, race, place of residence and occupation)

2) Medical history (any history of prior medical diseases like diabetes mellitus or hypertension or surgical interventions)

3) Concomitant medications (all over-the-counter or prescription medication, native medications, previous therapy given for MN including ACEI/ARB therapy and all forms of prior immunosuppression)

4) Prior complications of MN including AKI and thrombosis

5) Physical examination (height, weight, oral temperature, resting pulse and BP measurements measured after the participant has been sitting for at least 5 min)

6) Chest X ray

7) Laboratory tests:

• Blood tests: Haemoglobin, white blood cell, platelet count, glucose, urea, creatinine, ALT, total and indirect bilirubin, alkaline phosphatase, total proteins, albumin, calcium, phosphorus, sodium, potassium and total cholesterol. HbA1c were done in diabetics and those with impaired glucose tolerance. Beta-human chorionic gonadotropin was done in case of doubt of urine pregnancy test.

- Urine tests: Urine protein:creatinine ratio (UPCR), 24 hours urine protein excretion and urine pregnancy test when appropriate. UPCR was used to monitor proteinuria according to the 2012 KDIGO guidelines on management of MN.6

- Serum anti-PLA2R measurement at baseline – by EUROIMMUN AntiPLA2R (IgG) ELISA (quantitative method)

Subsequent assessments:

For each visit, we dispensed the study medications and recorded any concomitant medications taken by the patient. All end points/outcome measures were noted and adverse events were recorded. Laboratory tests were done to assess response to therapy at 1, 3, 6 and 12 months of follow-up after start of protocol therapy. These tests included the tests described above at baseline with some mandatory tests such as serum creatinine, serum proteins, serum albumin and UPCR. Serum antiPLA2R was measured at 6 months and 12 months of follow-up. Response to rituximab was assessed by measurement of CD19+ve B-cells at 1 and 6 months after administration of first dose of rituximab. CD19+ve cells are measured by flowcytometry. All subjects who were lost to follow-up in our centre were contacted over telephone and necessary reports were obtained for determination of efficacy and safety outcomes at primary end-point.

Treatment protocol:

All patients who met the inclusion criteria underwent an observation period of 3 months to watch for spontaneous remission. All patients were started on ACE inhibitors or ARBs with an attempt to increase to the maximum tolerated dose. Those with severe manifestations of nephrotic syndrome and complications of the disease were started earlier on the treatment protocol without this waiting period. 8 patients in the rituximab group (30.8%) and 15 patients in the modified Ponticelli regimen group (57.7%) were started on protocol therapy without prior ACEI or ARB therapy in view of indications for starting immunosuppression.

Study arm: Rituximab injection 500mg IV given on days 1 and 15. Each patient received premedication with injection methylprednisolone 125 mg or injection hydrocortisone 100mg intravenously along with injection Pheniramine 45.5mg and tablet Paracetamol 500mg. Comparison Arm: Modified Ponticelli regimen – Cyclical steroids/cyclophosphamide for 6 months

Months 1, 3 and 5: 1 g IV methylprednisolone daily (Days 1–3), then oral prednisolone (0.5 mg/kg/day) for 27 days (Days 4–30).

Months 2, 4 and 6: Oral cyclophosphamide (2.0 mg/kg/day) for 30 days.

At 6 months, both groups of patients were assessed for primary end point. Further immunosuppression was decided based on the response including remission and the level of antiPLA2R. In the rituximab group, CD19+ve B-cell count at 6 months was reviewed. In case of non-response at 6 months in either group, decision to change immunosuppression protocol was made by the primary treating nephrologist. In the rituximab group, in case of non-response with B-cell recovery at 6 months, 2 more doses of rituximab of 500mg each were

given. Any further changes in treatment were done according to the primary treating nephrologist. Both treatment groups received prophylaxis with cotrimoxazole (trimethoprim/sulfamethoxazole 80/400 mg, orally) daily during the period of treatment.

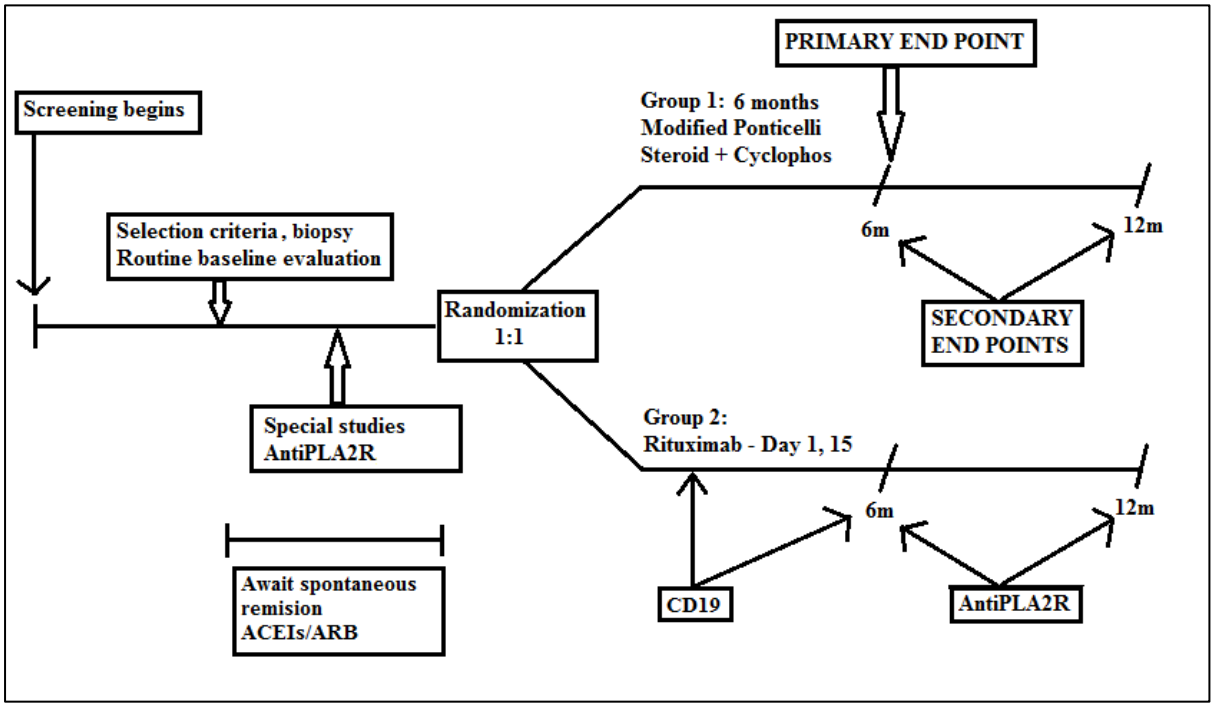

Figure 1: Study design

STATISTICAL ANALYSIS:

This study was designed as a non-inferiority study to compare two known effective treatment regimens for primary MN and their effect on partial and complete remission at 6 months. On the basis of a study in India, a short-term remission rate of 60% in the comparison group of modified Ponticelli regimen was considered.94 Rituximab has been shown to be associated with remission rate of 50-60% over a period of 6-12 months after administration.65,68,103 A non-inferiority margin of 15% was assumed, indicating that rituximab will be considered non-inferior if the response rate in the rituximab arm is at most 15% worse than the modified Ponticelli regimen. Assuming a remission rate of 60% in both groups and a one-sided alpha of 0.05, enrolment of 132 patients would be required in each study arm to achieve a power of 80% to show that rituximab is non-inferior for the given margin. Intention-to-treat analysis was done in this study. Considering the time period of the study and the annual number of membranous nephropathy patients treated in our hospital, it was decided to conduct this study as short-term pilot study comparing these 2 regimens in patients presenting to our centre with primary MN in the enrolment period. However, the study will be continued by the department of nephrology till the expected sample size is reached.

Differences in the baseline characteristics were analyzed by the independent t-test for quantitative continuous variables and normal approximation by the 2-sample proportions test

was used for qualitative categorical variables. Primary outcome of complete or partial remission rate at 6 months was analyzed by the 2-sample proportions test. The risk difference between the 2 groups that was generated was then assessed for lower limit of confidence interval meeting criteria for non-inferiority. Other outcomes such as remission rates and non-response rates at 6 and 12 months were also assessed similarly. Efficacy outcome variables such as mean creatinine, eGFR, serum proteins and albumin and proteinuria were analyzed by the independent t-test. Adverse events were analyzed by both overall event rate for every 100 patients and incidence of adverse events in each groups. Subgroup analysis was done by analysing the odds ratios for remission in each subgroup.