A Randomized Control Trial of Rituximab+ Cyclophosphamide + Steroid Therapy vs Cyclophosphamide + Steroid Therapy in Progressive Idiopathic Membranous Nephropathy

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A) Study Background and Rationale

Membranous nephropathy (MN) is among the most common causes of the nephrotic syndrome in nondiabetic adults. About 75% of these cases are idiopathic; the remaining 25% are secondary to a wide variety of causes, such as infections, drugs, and autoimmune disease. It is generally accepted that approximately 1/3 of MN patients will achieve spontaneous remission, 1/3 will remain proteinuric with stable renal function, and 1/3 will progress to end stage renal disease (ESRD) within 5-15 years (Glassock). The burden of ESRD remains large, both in terms of healthcare cost and life expectancy. For example, the five year survival rate of non-diabetic patients on dialysis is only 30-50%.

All patients with MN are treated with medications which target the renin-angiotensin system, namely ACE inhibitors or Angiotensin receptor blockers, as well as lipid lowering therapy, a low salt and low protein diet, and diuretics for edema. This is considered symptomatic management of nephropathy, designed to preserve renal function, decrease edema, and decrease risk of thrombosis. Those with moderate or highly progressive disease (determined by proteinuria and GFR) are selected for immunosuppressive therapy to induce complete or partial remission of the disease. Risk factors for progressive disease include male sex, older age of onset, nephrotic range proteinuria (especially if greater than 8-10 g/day), and increased serum creatinine at presentation. Patients are risk stratified based on these factors as well as their estimated GFR to determine whether immunosuppressive therapy would be beneficial.

First-line immunosuppressive therapy of MN usually consists of alkylating agents (usually cyclophosphamide) or calcineurin inhibitors (usually tacrolimus) in combination with low dose glucocorticoids. Some patients are started on steroids alone, although studies have shown that this approach is no more effective than placebo (McQuarrie). The goal for these therapies is either complete remission (defined as proteinuria ≤0.3 g/24h with estimated GFR≥60 ml/min/1.73m^2) or partial remission (defined as ≥50% reduction in proteinuria with proteinuria >0.3 g/24 h and <3.5 g/24h at last follow-up). Both complete and partial remission have been associated with decreased risk of ESRD and death (Cattran). Due to spontaneous remission, it is hard to determine true efficacy of these medications as some patients would have achieved spontaneous remission without treatment. Therefore estimates must be based on comparisons to expected remissions/remissions in the placebo group. Several studies suggest that the rate of complete or partial remission with these medication regimens is about 40%. For example, a randomized control trial of 93 patients comparing cyclophosphamide with steroids to symptomatic treatment in patients with moderate disease showed a partial or complete remission rate 40% higher than symptomatic treatment alone (Jha). Another RCT of 51 patients resistant to steroids alone were treated with prednisone plus cyclophosphamide or placebo for 6 months; although the intervention group had a very high rate of remission at 6 months, at one year it only maintained 26% more remissions than the placebo group (Cattran). Even despite remission at 1 year follow up, the progression to ESRD remains high. A recent meta-analysis has shown that despite the use of corticosteroids and alkylating agents, up to 40% of patients on these regimens still progress to end-stage renal failure (Perna). It also showed that a statistically significant number of patients discontinued the regimen due to adverse events, mainly leukopenia and cushingoid features (Perna).

The adverse effects of cyclophosphamide include leucopenia, alopecia, GI symptoms, and more harmful effects such as hemorrhagic cystitis, interstitial pneumonitis, pulmonary fibrosis, and Stevens Johnson Syndrome. The adverse effects of steroids include Cushing's syndrome (weight gain, diabetes mellitus, muscle loss, fatigue), psychological changes, osteoporosis, and easy bruising. Because some patients will have to have multiple rounds of immunosuppressive therapy, regimens with better tolerability and fewer side effects would benefit them greatly.

The next line of immunosuppressive drugs includes rituximab and mycophenylate mofetil. Rituximab is a monoclonal antibody directed against the B cell antigen CD20. Experimental models have shown that B cell activation is a key step in the pathogenesis of membranous nephropathy, therefore rituximab could be a targeted treatment for the disease. The drug is also used to treat leukemias, lymphomas, and autoimmune diseases such as rheumatoid arthritis, SLE, and MS. Because of its specific mechanism of action, it is generally well tolerated. The vast majority of glomerular disease centers use rituximab as a second line therapy in patients who have failed prior immunosuppressive therapy with steroids or a combination of steroids with either alkylating agents or calcineurin inhibitors. Only one center, the Remuzzi group in Italy, uses rituximab as a first line immunosuppressant after a trial of ACE inhibitors alone (Bomback). Several case reports and case series have examined the use of rituximab in inducing remission of primary MN and secondary MN. In contrast to cyclophosphamide, major adverse effects of the drug seen in patients taking it for MN are infusion related reactions (such as itching, flushing, and rash) and infection. A recent meta-analysis of the literature suggests that rituximab achieves a 15 to 20% rate of complete remission and a 35 to 40% rate of partial remission (Bomback). This data is based entirely on case series and case reports; no randomized trials examining the efficacy of rituximab, as either a second line or first line agent, have been conducted. The data on rituximab as a first line agent is expectedly limited, thus a trial of combination therapy will allow an examination of the drug's efficacy during an earlier point of disease progression without putting participants in undue harm (from failure to give adequate treatment). It is anticipated that the combination of rituximab with other therapies will increase remissions without significant increases in side effects due to its targeted mechanism of action.

The purpose of this study is to examine the efficacy of treatment with combined immunosuppressive regimen of cyclophosphamide, rituximab, and steroids in patients with idiopathic membranous nephropathy who have failed conservative management with steroids and ACE inhibitors/ARBS.

B) Study Design

This will be a prospective, randomized, controlled clinical trial to evaluate the efficacy of a regimen of rituximab, cyclophosphamide, and steroids compared to cyclophosphamide and steroid therapy in the treatment of biopsy proven membranous nephropathy that has been resistant to symptomatic treatment and steroids alone. This will allow the investigators to compare an accepted first line regimen with a new combination therapy that is likely to reach higher rates of remission. Patients with secondary MN will be excluded to reduce confounding data from other systemic illnesses. The participant pool will consist of patients who present to New York Presbysterian/Columbia University Medical Center, as well as 2-3 other medical centers, for primary evaluation or as referrals from other physicians. Given the low prevalence of progressive MN, enrollment is anticipated to take 1-2 years. Written consent will be obtained from all participants prior to study enrollment. The study will undergo IRB review and approval at each institution.

Patients will be randomized either to a control group (cyclophosphamide + steroid) or to the treatment group (rituximab + cyclophosphamide + steroid). Randomization will be stratified according to the following clinical prognostic factors: baseline urinary protein excretion and estimated GFR. Baseline proteinuria <8 g/d and GFR > 60 ml/min/1.73m^2 at baseline have both been associated with an increased probability of remission from proteinuria and lower risk of developing ESRD.

In order to reduce confounding factors and maintain the generalizability of the study, all patients will continue symptomatic therapy, which is given to all patients with MN regardless of prognosis. Due to the anti-proteinuric, renal sparing effects of renin-angiotensin system blocking drugs, patients will continue the same doses of ACE inhibitors or angiotensin receptor blockers that they were taking prior to study initiation. In addition, other anti-hypertensive drugs will be prescribed as needed to achieve target BP of < 135/85. Patients will also be treated with dietary modification and/or statin therapy to achieve a goal LDL <160 mg/dL. Statin therapy may be initiated during the study and adjusted during the study to achieve this goal. In addition, both groups will be encouraged to maintain a low salt, low protein diet, and to continue any diuretics prescribed before the initiation of the study.

The primary outcome will be a composite of achieving complete or partial remission in 1 year of treatment. Complete remission is defined as <0.3 g/d proteinuria plus stable renal function (eGFR within 15% of baseline). Partial remission is defined as >0.3 but <3 g/d proteinuria with a >50% reduction in proteinuria from baseline plus stable renal function. Both complete and partial remission will be used because both are associated with decreased likelihood of progression to ESRD (Troyanov). The following secondary outcomes will be collected and recorded: serum creatinine, progression to ESRD, mortality, treatment withdrawal, and adverse events/effects.

At the conclusion of the 1 year study protocol, patients who experienced remission (complete or partial) will be followed for an additional 1 year to determine whether remission is sustained and to monitor for any treatment-related long-term toxicities. Patients with persistence or relapse of nephrotic range proteinuria will be offered alternative therapies at their physician's discretion.

Categorical data will be analyzed using the chi-square test for proportions. Using multivariate analysis, we will look for variables that significantly correlate with partial or complete remission, including age, sex, race/ethnicity, baseline level of proteinuria, baseline level of albumin, baseline serum creatinine, and baseline eGFR.

Statistical Analysis:

The determination of sample size is based on a composite of studies which show the complete/partial remission rate of cyclophosphamide + steroid therapy to be approximately 40%. Assuming an effect size of 20%, and in order to achieve 80% power with a 5% Type I error rate, a sample size of 214 patients (107 in each arm) was calculated using a chi square test.

C) Study Procedure

Patients in the control and intervention group will receive the following 6 month regimen: Intravenous methylprednisolone 1 g/d for 3 consecutive days followed by oral prednisolone 0.5 mg/kg per day for 27 days in the first, third, and fifth months and oral cyclophosphamide at 2 mg/kg per d in the second, fourth, and sixth months. This regimen, known as the Ponticelli regimen has been used in several RCT with proven efficacy (Qualglia).

Patients assigned to the intervention group will receive the following additional regimen: Rituximab 375 mg/m(2) once weekly for 4 weeks. This regimen has been used in multiple case series and observational studies on rituximab in glomerular diseases (Bomback).

Patients will undergo detailed clinical assessments at study entry and monthly for the first 6 months, and then every three months for the duration of the 1 year study. Patients who achieve remission at 1 year will be re-assessed for remission in another year, to monitor the relapse rate. The assessments will include a 24-hour urine collection for protein and creatinine, serum creatinine, and estimated creatinine clearance. Estimated GFR will be calculated according to the 4-variable Modification of Diet in Renal Disease Study equation, in concordance with other trials. Patients will be monitored for nephrotic symptoms (such as edema) as well as for side effects of the medications (see Study Drugs). Patients will be seen more frequently as indicated by their clinical course. Withdrawal from the study will be based on side effects. Based on meta-analysis of rituximab use, we do not anticipate a significant increased burden of side effects in the intervention group, however we will monitor patients in the intervention group closely for infection and will draw complete blood counts at follow up visits to monitor for cytopenias. Patients will be allowed to withdraw from the study at any time, and if patients develop significant side effects from the treatment, we will withdraw them from the study.

D) Study Drugs

Rituximab: Rituximab is a monoclonal antibody to the CD20 receptor on B cells. The following side effect profile is based on rituximab's use in hematologic malignancies, which require longer therapeutic regimens. Studies have shown decreased incidence of these side effects in RA patients who take the drug at lower doses than cancer patients. It is anticipated that incidence of side effect for patients in this study will resemble RA patients due to similar low dosing regimens. Its side effects include:

- Central nervous system: Fever, chills, headache, pain
- Dermatologic/Allergic: Rash, pruritus, angioedema, bronchospasm
- Gastrointestinal: Nausea, abdominal pain
- Hematologic: Cytopenias, lymphopenia, leucopenia, neutropenia, neutropenic fever, thrombocytopenia
- Neuromuscular & skeletal: Weakness
- Respiratory: Cough, rhinitis

Cyclophosphamide: Cyclophosphamide is an alkylating agent that prevents cell division by cross-linking DNA strands and decreasing DNA synthesis. It is a cell cycle phase nonspecific agent. Cyclophosphamide is a prodrug that must be metabolized to active metabolites in the liver. Side effects include:

- Dermatologic: Alopecia
- Endocrine & metabolic: Infertility, amenorrhea
- Gastrointestinal: Nausea, vomiting, anorexia, diarrhea, mucositis, stomatitis
- Genitourinary: Severe, potentially fatal acute hemorrhagic cystitis (7% to 40%)
- Hematologic: Leukopenia, thrombocytopenia
- Rare but life threatening: cardiac necrosis, interstitial pneumonitis, pulmonary fibrosis (with high doses), anaphylaxis, Stevens Johnson syndrome

Prednisolone/Methylprednisolone: Steroids up-regulate the expression of anti-inflammatory proteins and down-regulate the expression of pro-inflammatory proteins by binding intracellular receptors that act as transcription factors in the body. Side effects include:

- Cardiovascular: CHF, edema, hypertension
- Central nervous system: insomnia, malaise, pseudotumor cerebri, psychic disorders
- Dermatologic: Bruising, facial erythema, hirsutism, petechiae, thin fragile skin, urticaria
- Endocrine & metabolic: Carbohydrate tolerance decreased, Cushing's syndrome, diabetes mellitus, growth suppression, hyperglycemia, hypernatremia, hypokalemia, hypokalemic alkalosis, menstrual irregularities, negative nitrogen balance, pituitary adrenal axis suppression
- Gastrointestinal: Abdominal distention, increased appetite, indigestion, nausea, pancreatitis, peptic ulcer, ulcerative esophagitis, weight gain
- Hepatic: LFTs increased (usually reversible)
- Neuromuscular & skeletal: Arthralgia, aseptic necrosis (humeral/femoral heads), fractures, muscle mass decreased, muscle weakness, osteoporosis,
- Ocular: Cataracts, exophthalmus, eyelid edema, glaucoma, intraocular pressure increased, irritation

E) Medical Devices

N/A

F) Study Questionnaires

N/A

G) Study Subjects

Inclusion criteria:
Age > 18 years
Patients with biopsy-proven membranous nephropathy and nephrotic syndrome (24-hour protein excretion of >3 g/day and hypoalbuminemia with albumin <3 g/dL)
Renal biopsy within three years of study entry
GFR >60 mL/min per 1.73 m2 body surface area at study entry
ACEI or ARB therapy for at least 6 months before study entry

Exclusion criteria:
Secondary MN or any systemic disease known to be associated with secondary MN
Previous treatment with cyclophosphamide, calcineurin inhibitors, MMF, or rituximab
Active or chronic infection (including Hep B, Hep C, or HIV infection)
Preexisting malignancy
Diabetes mellitus
Pregnancy in female patients
White blood cell count below 2.5 × 109/L, a platelet count below 100 × 109/L, hemoglobin concentration less than 7 g/dL

H) Recruitment of Subjects

Patients will be recruited from New York-Presbyterian Hospital / Columbia University Medical Center, as well as from 2-3 other medical centers. Nephrologists at these centers will be informed of the study protocol and encouraged to consider whether their patients would be appropriate for the study.

I) Confidentiality of Study Data

All data will be de-identified and stored securely.

J) Potential Conflict of Interest

There are no potential conflicts of interest to disclose.

K) Location of Study

This study will be conducted in the outpatient nephrology clinics (both the fellow clinic and faculty clinics) at New York-Presbyterian Hospital / Columbia University Medical Center, as well as at the outpatient nephrology clinics (fellow and faculty) of co-centers.

L) Potential Risks

The risks associated with the study are related to the potential side effects of the three drugs involved in the study. The list of potential adverse effects can be found in section D, “Study Drugs”.

M) Potential Benefits

The potential benefits of this study include attaining a complete or partial remission from nephrotic-range proteinuria and potentially decreasing the risk of progressing to end-stage renal disease.

N) Alternative Therapies

Alternative therapies include symptomatic management (ACE/ARB therapy in combination with diet modification, BP control, lipid control, and diuretics), steroid treatment, and other immunosuppressive drugs. Additional immunosuppressive drugs include calcineurin inhibitors and mycophenylate mofetil.

O) Compensation to Subjects

Participants will not be compensated for participation in this study.

P) Costs to Subjects

Participants will not incur costs associated with participation in this study.

Q) Minors as Research Subjects

N/A

R) Radiation or Radioactive Substances

N/A

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