Factors Influencing Choice of Treatment in Idiopathic Membranous Nephropathy

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Table of Contents

Abstract

Background

The treatment of patients with IMN may include immunomodulation in patients resistant to conservative therapy alone, or perceived at high risk of progression. This study assesses the factors influencing the decision to treat with immunomodulators and the choice of treatment between alkylating agents and calcineurin inhibitors (CNI).

Methods

An inception cohort of 720 adults with biopsy-proven IMN (from 1976-2005) was derived from the Glomerular Disease Collaborative Network (N=328) and the Toronto Glomerulonephritis Registry (N=392). Subjects were allocated to groups based on initial treatment: no immunotherapy, alkylating agents, CNI, glucocorticoids (GC) only, or other immunosuppressant. The second treatment was considered in patients who initially received GC alone. Factors influencing decision to treat were analyzed using logistic regression, and those influencing choice of treatment by multinomial logit.

Results

Serum albumin and proteinuria were significantly different between those treated (66.3%) and those not treated (33.7%). Patients with a serum albumin of <2.5 mg/dL were more likely to be treated (OR 1.88, p=0.007, 95% CI [1.19,2.98]), as were those with greater 24 hour proteinuria (OR 1.12 for each 1g/day increase,p=<0.001, 95% CI [1.06,1.18]). Patients in the TGNR were less likely to be treated than those in the GDCN (OR 0.45, p=0.001, 95% CI [0.28,0.72]). Of the factors analyzed with regards to choice of treatment, only sex was significant. Females were 3.49 times more likely to receive a calcineurin inhibitor than an alkylating agent (p=0.011, 95% CI [1.33,9.20]). Changes in choice of first therapy were observed over time. GC alone was the predominant approach until about 1994, at which time alkylating agents became the predominant choice. CNI were used in a minority of patients.

Discussion

Serum albumin, proteinuria, and registry were the only independent determinants of the decision to treat with immunomodulators. Sex was the only factor associated with the specific choice of therapy between alkylating agents and CNI; however, the sample size of those treated may have been too small to detect differences.

Factors Influencing Choice of Treatment in Idiopathic Membranous Nephropathy

Introduction

Idiopathic membranous nephropathy (IMN) is the most common cause of nephrotic syndrome in adults. The course of the disease is variable, with approximately equal numbers of patients experiencing spontaneous remission, having persistent proteinuria but stable renal function, or progressing to end-stage renal disease (ESRD). A recent study found that 32% of patients receiving only conservative therapy (no immunosuppression) achieved spontaneous remission over a mean follow-up time of 6.2 years.[1] Though some prognostic factors have been identified that can help predict the likelihood of progression and stratify risk[1,2] - thus identifying some patients as low-risk and not likely to benefit from treatment- there are still a number of patients at moderate to high risk of progressive disease. Immunomodulatory treatment can significantly decrease the rate of progression to ESRD,[3-6] and those at increased risk for progression may derive substantial benefit from immunomodulatory therapy. However, the potential adverse effects associated with immunomodulatory treatment are significant, and thus the decision of whether to treat an individual patient and with what regimen is an important one.

Initially, glucocorticoids (steroids) were commonly used for treatment of IMN, but more recent data has suggested that they are not effective when used alone. In 1989, Cattran et al. conducted an RCT comparing a 6 month course of prednisone to conservative treatment; after a mean follow-up of 48 months, they found no significant difference in change in creatinine clearance, remission rate, or progression to renal survival.[7] In 1990, Healy et al. conducted another relatively large RCT comparing a short course of high-dose prednisone to controls and found that after 3 years, there was no significant

difference between groups in creatinine clearance or proteinuria.[8] Thus, other agents have replaced steroids as first line treatment for IMN.

The combination of an alkylating agent (cyclophosphamide or chlorambucil) and glucocorticoids given alternately for 6 months has been shown to be effective in increasing the remission rate and renal survival in patients with IMN. Also known as the Ponticelli protocol, this regimen is commonly used as first line choice for patients with IMN receiving immunosuppressive therapy. Two randomized controlled trials demonstrated the efficacy of a 6-month regimen alternating an alkylating agent and steroids.[3,4] The first, by Ponticelli et al. in 1995, found that after 10 years patients who received the immunosuppressive treatment had significantly lower rates of ESRD (60% with chlorambucil/steroids vs. 92% for conservative treatment).[3] The second study also followed patients for 10 years and found a similar reduction in the proportion who progressed to ESRD: 65% of those taking cyclophosphamide/steroids vs. 89% of those receiving conservative treatment.[4] In 1998, another randomized trial by Ponticelli et al. demonstrated that cyclophosphamide plus steroids had equivalent efficacy to chlorambucil plus steroids.[9]

Cyclosporine (a calcineurin inhibitor) is another immunosuppressive drug that has been used to treat IMN; several studies point toward its efficacy in treatment of IMN.[5,6] Cattran et al. in 1995 randomized patients with progressive disease to cyclosporine or placebo. They found that treatment slowed the decline in renal function and decreased proteinuria in these patients.[5] In 2001, Cattran at al. randomized patients with normal renal function to cyclosporine plus steroids or steroids alone. Patients in the cyclosporine group had lower levels of proteinuria and a greater rate of remission (compared with

the steroids-only group), though there was no difference in renal function between the two groups.[6] However, these studies lack long-term follow-up and did not compare renal survival.

Although both are commonly used as immunomodulatory treatment for IMN, there is little high quality evidence to guide the choice between cyclophosphamide- and cyclosporine-based regimens. A single head-to-head RCT comparing the two was done by Chen et al. in 2010.[10] They found no significant difference in remission rates or change in eGFR between groups; however, their follow-up period was relatively short (1 year), and quality of the study was poor. Thus, the question remains unresolved and more studies will need to be done before it can be answered.

The choice of treatment depends upon a number of factors, yet which of these factors predicts the decision to treat or the choice of treatment is unknown and likely varies significantly between clinicians. Further, the relative efficacy of each treatment is unknown; though both provide a significant potential benefit, they also have substantial side effects and adverse events associated with their use. Comparing outcomes retrospectively between patients receiving different treatments would be valuable. Importantly, this would be the only feasible way to compare long term outcomes, such as renal survival and mortality, not just short term rates of remission (as is usually studied in prospective comparisons). However, because the risks associated with each treatment regimen are different, it is most plausible that the choice of therapy is not random. Therefore, understanding which factors predict choice of treatment is necessary if an unbiased comparison of these agents is to be conducted. The purpose of this current study is therefore to identify what factors are associated with the decision to treat and with choice of treatment.

Methods

We conducted a retrospective analysis of a pooled cohort of patients with IMN drawn from two large glomerular disease registries: GDCN and TGNR. Patients were assigned to one of four groups based on initial treatment: no treatment, alkylating agents (cyclophosphamide or chlorambucil), calcineurin inhibitors (cyclosporine or tacrolimus), or steroids alone. A number of patient factors thought to be related to the decision to treat and/or choice of treatment were identified a priori and analyzed using logistic regression techniques. We first analyzed the decision of whether to treat with an immunomodulatory agent (treated vs. untreated). Next, among patients who were treated, we determined the likelihood of receiving each agent based on various patient factors using multinomial logistic regression (Figure 1). We felt that the 2-step analysis was appropriate for two reasons. First, this is the logical sequence of decision-making in a clinical setting. Second, the factors influencing the decision of whether to treat may be different than those influencing the choice of treatment. We suspect the decision to treat depends upon prognostic factors, whereas the choice of treatment might be related more to side effect profile (e.g. sex, comorbidities, BMI). All patients enrolled in their respective registries did so with IRB approved informed consent. The current analysis study was approved by the University of North Carolina institutional review board.

Study population

The cohort (N=720) was comprised of patients from the Glomerular Disease Colloborative Network (GDCN, n=328) enrolled between 1969 and 2010 and from the Toronto Glomerulonephritis Registry (TGNR, n=392) enrolled between 1974 and 2010. Records were collected for patients in the registries

from time of initial biopsy diagnosis or from time of clinical presentation. Inclusion criteria were age ≥16 years old, biopsy-proven IMN, and follow up after biopsy. Patients were excluded if they had evidence of a secondary cause of membranous nephropathy (MN), including hepatitis B, hepatitis C, human immunodeficiency virus (HIV), syphilis, lupus (SLE), or if they had other autoimmune disease requiring immunosuppression.

Of an original 470 patients with MN identified from the GDCN and 520 from the TGNR, 118 were excluded for secondary causes or incomplete data, 130 for inadequate follow-up, 8 for inadequate biopsy, and 14 for age <16 years old (see Figure 2). This yielded a final cohort of 720 subjects.

Variables

All registry data to be reviewed and analyzed were specified prior to collection. A comprehensive list of variables thought to influence treatment choice was considered; these included age, sex, body mass index (BMI), estimated glomerular filtration rate (eGFR), blood pressure, time from biopsy to treatment initiation, pathologic stage, degree of interstitial fibrosis, baseline proteinuria, baseline serum albumin, insurance status, race, and education level. The presence of hypertension, diabetes, hyperlipidemia, as well as any history of cardiovascular disease, malignancy, venous thromboembolism (VTE) or pulmonary embolus (PE), or psychiatric disease were also considered. Site (GDCN or TGNR) was analyzed to determine whether there were systematic differences in choice of treatment. Factors were chosen if they were known or suspected to influence the clinical decision to treat with an immunomodulatory agent and/or the choice of which type of agent to use. Variables of interest included those that predict prognosis, as well as patient factors that might influence the choice of one agent over another, particularly as they related to the side effect profiles of the different agents. For example, fertility (age and sex) is of concern when using cyclophosphamide, BMI and insulin resistance are important

considerations when using steroids, and low GFR or hypertension are relevant when choosing to use a calcineurin inhibitor.

Variables were later excluded if no data was available in the registry, if there was a large proportion of missing data (see Table 1), or if there was an insignificant relationship with outcome of interest. The final analysis of decision to treat included the covariates site, sex, age, race, serum albumin, 24-hour proteinuria, eGFR, and mean arterial pressure (MAP); the analysis of choice of treatment included site, sex, age, serum albumin, eGFR, and MAP. When including eGFR in the model, we tried analyzing it in two different ways- as a continuous variable and as a categorical variable, grouped into clinically meaningful ranges. The latter corresponded to levels at which decision making was predicted/expected to change - i.e. very low such that there might be a relative contraindication for calcineurin inhibitors or that treatment might be considered futile, intermediate such that the patient would be more likely to merit and benefit from treatment, and higher where the patient has a better prognosis and is less likely to be treated (since they are more likely have a benign course even if untreated).

Statistical analysis

We first compared all patients receiving immunomodulatory treatment (alkylating agent, calcineurin inhibitor, steroids, or other immunomodulators) to those receiving no immunomodulatory treatment (Figure 1). Group determination was made by initial immunosuppressive agent or regimen used. The second treatment was considered in patients who initially received steroids alone. The reason for doing this was that steroids are often given first either empirically before a definitive diagnosis is made (prior to biopsy), or possibly without recognition or awareness that this is no longer a recommended option for this disease. Our objective in this study was to compare calcineurin inhibitors to alkylating agents,

and felt that this method of classification would more appropriately categorize patients like this. Patients were included in a treatment group if treatment was initiated, regardless of whether it was discontinued early and regardless of outcome, as our objective was to analyze the variables affecting choice to treat. Logistic regression was used to calculate odds ratios for the likelihood of receiving treatment for each of the patient factors listed above.

The second step of the analysis investigated choice of treatment among those patients who received immunosuppressive therapy. The three treatment groups were alkylating agents (chlorambucil and cyclophosphamide), calcineurin inhibitors (cyclosporine and tacrolimus), and steroids only. Patients were included in the alkylating agent or calcineurin inhibitor group if they received either type of drug, regardless of concomitant steroids use. For patients who initially received steroids alone, the second treatment was considered. Patients receiving other immunosuppressant therapy (azathioprine, rituximab, mycophenolate mofetil, or methotrexate) were excluded from this analysis. Although some of these treatments have begun to be used more frequently (e.g. rituximab, mycophenolate mofetil), they remain relatively new and have not been well-studied yet; we had few subjects who received these medications as first-line therapy (n= 41). As in the first step of the analysis, group allocation was made if treatment was initiated, regardless of duration. We used multinomial logistic regression to model the relationship between the previously-specified patient factors and choice of immunosuppressive agent.

Results were expressed as adjusted odds ratios with 95% CIs, and were considered significant if associated with a p-value of 0.05 or less. All analyses were performed with STATA version 11 (StataCorp, College Station, Texas).

Results

Characteristics of Sample

The cohort was comprised of 720 adults with biopsy-proven membranous nephropathy, drawn from 2 glomerular disease registries. Approximately 62% of subjects were male and 78% were white, with a mean age at biopsy of 49.1 years (Table 2). The mean eGFR at biopsy was 75.8 mL/min, mean serum albumin was 2.6 mg/dL, and mean 24-hour proteinuria was 8.1 g/day.

Factors Associated with Decision to Treat

The logistic regression model compared the 477 patients (66.3%) who received treatment to the 243 who did not (33.7%). Serum albumin, 24 hour proteinuria, and site (registry) were found to be significantly different between treated and untreated patients (Table 3). Patients with a serum albumin of <2.5 mg/dL had 1.88 times the odds of being treated than those with a serum albumin $\geq$2.5mg/dL (p=0.007, 95% CI [1.19,2.98]). For each 1 g/day increase in 24 hour proteinuria, patients had 1.12 times the odds of being treated (p=<0.001, 95% CI [1.06,1.18]).

Patients in the TGNR were 0.45 times as likely to be treated as those in the GDCN (p=0.001, 95% CI [0.28,0.72]). Approximately 77% of GDCN subjects received some type of immunomodulatory treatment, compared to 57% of TGNR subjects (Table 4). This difference can be accounted for by the difference in number of patients receiving steroids alone: 56.7% of GDCN patients compared with 36.5%

of TGNR patients. The proportion of patients receiving alkylating agents, calcineurin inhibitors, and other immunosuppressants was comparable between sites (Table 4).

Time from biopsy to treatment initiation was calculated for treated patients. Among treated patients, 56.3% received treatment within 1 month, 68.9% within 3 months, 77.1% within 6 months, and 84.0% within 1 year.

Factors Associated with Choice of Treatment

The multinomial logit model compared patients receiving alkylating agents, calcineurin inhibitors, and steroids. Patients receiving other immunomodulators (mycophenolate mofetil, azathioprine, rituximab, or methotrexate) were included in the analysis of decision to treat but excluded from this comparison as the group was small and comprised of patients taking different types of agents. Of the 477 patients who were treated with immunomodulators, 159 (33.3%) received alkylating agents, 41 (8.6%) received calcineurin inhibitors, and 255 (53.5%) received steroids only (Table 3). For the comparison of factors affecting choice of treatment, we only included patients who were treated in 1983 or later, as this was when use of alkylating agents and calcineurin inhibitors began (Figure 3).

Gender was the only factor found to be significant in determining choice of treatment. Females were 3.49 times more likely to receive a calcineurin inhibitor than an alkylating agent (p=0.011, 95% CI [1.33,9.20]), and 0.38 times as likely to receive an alkylating agent compared to steroids alone (p=0.001, 95% CI [0.21,0.69]). The results of the multinomial logit analysis of choice of treatment are summarized in Table 5.

We also compared the choice of treatment by year to assess what trends existed in the use of different agents over time (Figure 3). Years of treatment initiation for this cohort ranged from 1963 to 2006. Prior to 1983 there was no use of calcineurin inhibitors and almost no use of alkylating agents; treatment was almost entirely with steroids alone. Use of calcineurin inhibitors and alkylating agents increased beginning in 1983, and by 1994 alkylating agents surpassed steroids alone as the predominant choice of treatment.

Discussion

There are a variety of factors that clinicians consider when deciding whether to use immunomodulatory treatment for a patient with IMN and if so, what type of regimen should be used. Though existing studies and recommendations describe prognostic factors and guidelines on who should be treated,[1,2,11] the decision is not clear-cut in many instances, and often depends on numerous patient factors and clinical data. We analyzed a cohort of patients with IMN drawn from 2 glomerular disease registries to determine which factors were associated with an increased likelihood of being treated. The goal of this analysis was to determine whether there were differences between patients receiving different types of treatment (specifically, alkylating agents and calcineurin inhibitors) and if so, what they were. This is necessary before outcomes can be compared between the two groups. A retrospective comparison like this would provide valuable information on long-term efficacy and outcomes, but selection bias must be assessed first.

Serum albumin level, proteinuria, and site were independent predictors of the decision to treat with immunomodulators; an albumin level of <2.5 mg/dL, higher 24 hour proteinuria levels, and being in the GDCN (vs. TGNR) were associated with an increased likelihood of being treated. The association of albumin and proteinuria with decision to treat is consistent with current evidence and recommendations, as these correlate with prognosis and likelihood of progression, and the decision to treat is based largely upon expected risk of progression.

The reason for the significant difference in likelihood of treatment between sites is not clear. However, the difference in treatment rates between sites is accounted for by a substantial difference in the use of steroids; both sites had similar rates of use of alkylating agents, calcineurin inhibitors, and other immunosuppressants. One potential explanation for the difference in rates of treatment is a difference in composition of the two registries, in terms of proportion of clinicians or subjects in an academic setting versus a private practice setting, and the relationship of the individual nephrologists and the university program. We did not pursue this question here, but it remains an interesting issue for future investigation. Sex, age at biopsy, race, eGFR, and blood pressure were not found have a significant independent association with the decision to treat.

For patients receiving treatment, we also analyzed factors thought to influence the choice of therapy-alkylating agent-based, calcineurin inhibitor-based, or steroids alone. In this analysis, females were significantly less likely to receive an alkylating agent as compared to a calcineurin inhibitor or steroids alone. This makes sense clinically, as alkylating agents are generally avoided in women of childbearing potential. Site, age, albumin level, proteinuria, eGFR, and blood pressure were not found to be independent predictors of one treatment over another. Some of these were surprising, as we expected

there to be differences in more factors between groups (e.g. eGFR and BP relevant to choice to use calcineurin inhibitor, as described earlier). However, our sample size was likely too small to detect significant differences that may exist. Though the cohort was quite large, the number of patients who received alkylating agents and especially calcineurin inhibitors was surprisingly small. The limited numbers in these groups, as well as missing data, decreased the number of observations in the multinomial logit model and thus its power to detect differences.

In addition to identifying the factors predictive of treatment decision, we also characterized some of the temporal patterns of treatment in this cohort. Specifically, we looked at the choice of treatment over time (by year of treatment) as well as the time from biopsy to treatment. The trends in choice of treatment during the observation period were interesting. Use of alkylating agents and calcineurin inhibitors did not emerge until 1983; prior to this almost all immunomodulatory treatment was with steroids alone. We expected to find these different 'eras' of treatment, given that calcineurin inhibitors were not available earlier and data supporting the use of alkylating agents did not begin to accumulate until the 1980s. Use of alkylating agents increased over time, and in 1994 they became the most common choice of treatment, surpassing steroids alone. However, the choice of steroids alone as a first line agent continued to be common, which was surprising, given the available evidence that this is not an effective treatment option for IMN.[7,8]

The distribution of time from biopsy to treatment was also surprising. The majority of patients who received immunomodulatory treatment were treated within the first few months of biopsy. For patients diagnosed with IMN, the literature suggests in most cases that patients be followed for about 6-12 months after biopsy or presentation before deciding whether to treat. This allows the clinician to

understand the clinical course and obtain more prognostic information, which is important, as the natural history of IMN can vary, from stable and benign in some to progression to ESRD in others. Though numerous prognostic factors have been identified, many of these are not very strong predictors of progression, and often are unable to predict patients' course or progression with much certainty. It was surprising to find that most patients received treatment early in their course, rather than after an observation period to better assess their course and risk of progression. The reasons for early treatment in a majority of patients are unclear, but the finding highlights a discrepancy between perceived or recommended practice and actual practice which would be worth pursuing.

Our combined cohort, the product of a collaboration that utilized 2 major glomerular disease registries, provided a sample far larger than prior studies of MN have been able to achieve, yet was still limited in its power to identify and analyze differences between treatment groups. In order to begin investigating and answering some of the important questions about treatment of this disease, such as the comparative effectiveness of different treatment choices, more data and even greater collaboration will be needed. Though the design of retrospective studies is limited and on their own are generally not adequate to answer clinical questions with certainty, they are a very important part of clinical outcomes research. Good quality retrospective analyses can identify important issues for further investigation and can prompt further prospective research, and are therefore a necessary first step in pursuing better clinical information and evidence to guide decision-making.

The results of this study address an important question that must be answered before a retrospective comparison of outcomes can be undertaken. Specifically, whether there are significant differences between patients receiving different treatments (alkylating agents versus calcineurin inhibitors), what

those differences are, and how they affect the choice of treatment. As there is good reason to believe that patients receiving different treatments may differ in meaningful ways from each other, they cannot be compared without controlling for these factors. Thus, we sought to understand and quantify any selection bias present between these groups. Having identified the factors associated with choice of treatment, these can be controlled for in a comparison of outcomes between groups. It is important to note that there may be factors that differ between groups and would contribute to potential selection bias, but which did not achieve statistical significance in this analysis (due to small sample size. To address this, other factors thought to be meaningful could also be controlled for, or other statistical methods could be used to account for these differences, such as propensity scores. Though there are limitations, this analysis provides important and relevant information and makes an outcomes analysis between these groups feasible.

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Table 1. Number of subjects with missing values for covariates considered

Variable	Number of subjects with missing data (%)
Sex	0 (0)
Age at biopsy	0 (0)
BMI	356 (49.4)
Serum albumin	112 (15.6)
24 hr proteinuria	83 (11.5)
eGFR	74 (10.3)
MAP	71 (9.9)
Smoking status	282 (39.2)

Table 2. Cohort Characteristics

	Combined Cohort (n=720)	GDCN (n=328)	TGNR (n=392)
Gender			
Female	272 (37.8%)	138	134
Male	448 (62.2%)	190	258
Mean age at biopsy (SD)	49.1 (15.6)	51.0 (15.3)	47.5 (15.6)
Race			
White	490 (77.5%)	219	271
Black	80 (12.7%)	61	19
Other	62 (9.8%)	6	56
Smoking			
Never	162 (37.0%)	56	106
Ex	131 (29.9%)	43	88
Current	145 (33.1%)	52	93
Mean BMI at biopsy (SD)	27.3 (5.5)	29.7 (6.9)	26.8 (5.0)
Mean serum albumin at biopsy (SD), mg/dL	2.6 (0.7)	2.5 (0.8)	2.7 (0.7)
Mean 24 hour proteinuria at biopsy (SD), g/day	8.1 (7.2)	9.3 (9.0)	7.0 (4.5)
Mean eGFR at biopsy (SD), mL/min	75.8 (34.4)	78.4 (37.6)	73.6 (31.3)
Blood pressure (MAP)	102.2 (14.0)	102.4 (14.6)	102.1 (13.5)

Values given as n (%) for categorical data and mean (SD) for continuous data. BMI=body mass index, eGFR=estimated glomerular filtration rate, MAP=mean arterial pressure, GDCN=Glomerular Disease Collaborative Network, TGNR=Toronto Glomerulonephritis Registry.

Table 3. Factors influencing decision to treat.

	Adjusted Odds Ratio	P-value	95% CI
Site/registry			
GDCN	1		
TGN	0.45	0.001	[0.28,0.72]
Gender			
Male	1		
Female	0.73	0.18	[0.46,1.16]
Age at biopsy	0.99	0.11	[0.97,1.00]
Race			
White	1		
Other	0.86	0.57	[0.51,1.46]
Serum albumin <2.5 mg/dL	1.88	0.007	[1.19,2.98]
24 hour proteinuria (g/day)	1.12	<0.001	[1.06,1.18]
eGFR (per 10 mL/min)	0.97	0.45	[0.89,1.05]
Blood pressure (MAP)	1	0.72	[0.98,1.01]

GDCN=Glomerular Disease Collaborative Network, TGNR=Toronto Glomerulonephritis Registry, eGFR=estimated glomerular filtration rate, MAP=mean arterial pressure.

Table 4. Distribution of different treatments among cohort.

	Combined Cohort **n=720**	**GDCN** **n=328**	**TGN** **n=392**
Treated	477 (66.3%)	253 (77.1%)	224 (57.1%)
Alkylating agent	106 (14.7%)	50 (15.2%)	56 (14.3%)
With steroids	76	45	31
Without steroids	30	5	25
Calcineurin inhibitor	30 (4.2%)	11 (3.4%)	19 (4.8%)
With steroids	8	5	3
Without steroids	22	6	16
Other immunosuppressant	12 (1.7%)	5 (1.5%)	7 (1.8%)
With steroids	8	2	6
Without steroids	4	3	1
Steroids only	329 (45.7%)	186 (56.7%)	143 (36.5%)
2nd treatment- alkylating agent	53	31	22
2nd treatment- calcineurin inhibitor	11	3	8
2nd treatment- other immunosuppressant	8	5	3
No other treatment	255	147	110

Values given as n (%).

Table 5. Factors influencing choice of treatment.

	Alkylating agent vs. Steroids only			Calcineurin inhibitor vs. Steroids only			Calcineurin inhibitor vs. Alkylating agent		
	RRR	p-value	95% CI	RRR	p-value	95% CI	RRR	p-value	95% CI
site= TGNR	0.51	0.359	[0.123,2.14]	0.235	0.262	[0.019,2.94]	0.46	0.561	[0.034,6.31]
sex= female	0.38	**0.001**	[0.207,0.685]	1.32	0.551	[0.534,3.24]	3.49	**0.011**	[1.33,9.20]
age at biopsy	1.01	0.416	[0.988,1.03]	0.991	0.606	[0.959,1.02]	0.983	0.329	[0.949,1.02]
albumin	0.78	0.282	[0.500,1.22]	0.487	0.147	[0.184,1.29]	0.622	0.358	[0.226,1.71]
24 hr proteinuria	1.05	0.284	[0.960,1.15]	1.13	0.039	[1.01,1.27]	1.08	0.193	[0.963,1.21]
eGFR, per 10 mL/min	0.98	0.608	[0.898,1.07]	0.985	0.852	[0.843,1.15]	1.01	0.926	[0.859,1.18]
MAP	1.01	0.222	[0.993,1.03]	0.984	0.382	[0.950,1.02]	0.973	0.14	[0.939,1.01]

TGNR=Toronto Glomerulonephritis Registry, eGFR=estimated glomerular filtration rate, MAP=mean arterial pressure.

Figure 1. Treatment group allocation and approach to analysis.

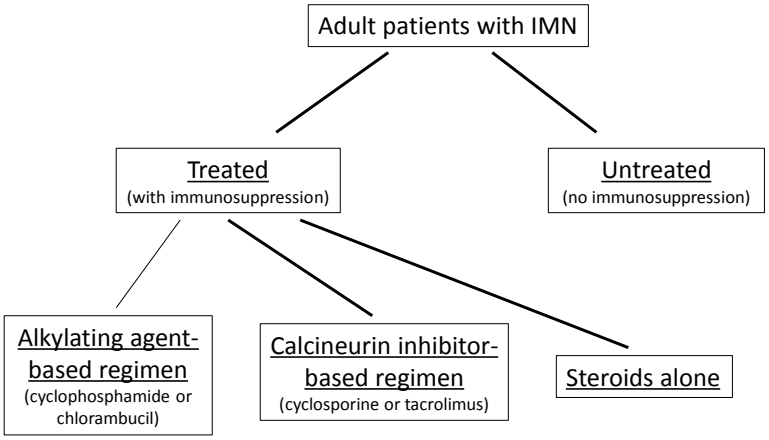


Figure 2. Cohort derivation.

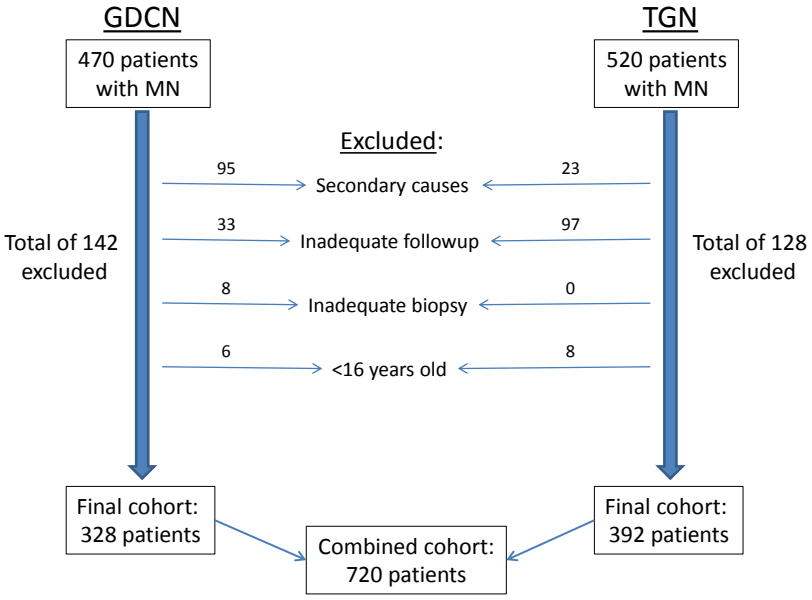
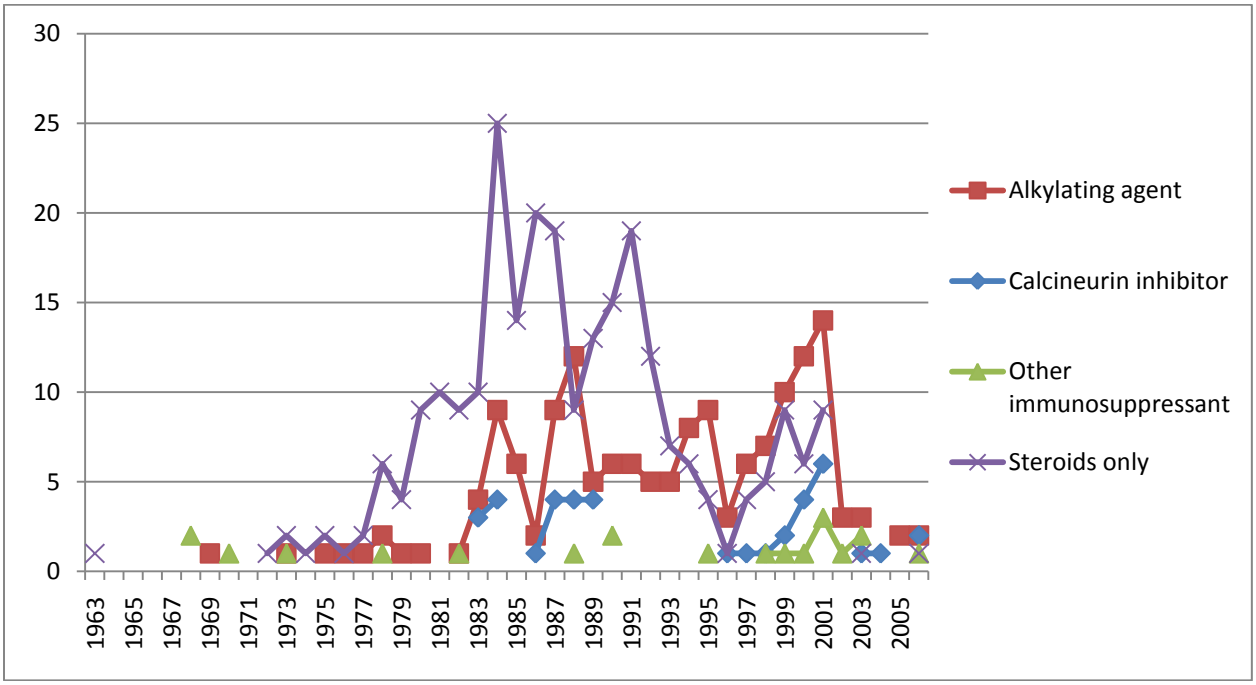


Figure 3. Choice of treatment over time.



Systematic Review

Introduction

Idiopathic membranous nephropathy (IMN) is the most common cause of nephrotic syndrome in adults. The course of the disease is variable, with approximately equal numbers of patients experiencing spontaneous remission, having persistent proteinuria but stable renal function, or progressing to end-stage renal disease (ESRD). Untreated, some patients will do well, whereas a significant number will progress to ESRD. Some prognostic factors can help predict the likelihood of progression and stratify risk- thus identifying some low-risk patients who are not likely to benefit from treatment- a significant number are at moderate to high risk of progressive disease. In these patients, there is a significant potential to benefit from treatment, but this must be weighed against the risks of treatment.

Alkylating agents and calcineurin inhibitors are both accepted options for first-line treatment of IMN. Unfortunately, these treatment options have a number of associated side effects and risks. Examples of important risks include potential infertility in women with cyclophosphamide, increased risk of malignancy with long-term use of cyclophosphamide, risk of renal failure with calcineurin inhibitors, and risks of weight gain, glucose intolerance or diabetes, among many others, with glucocorticoid (steroid) use.

These agents can be used alone or in combination with steroids. Steroids are sometimes used alone for treatment; however, evidence has demonstrated that this is not an effective treatment option.[1,2] Thus this review will not address steroid treatment of IMN, but rather address treatments that are less well tested and understood. Given the variable course of IMN as well as the risks and potential benefits associated with immunomodulatory treatment, it is important to understand the evidence supporting

the efficacy of these agents. Further, it would be valuable to determine what is known about the comparative effectiveness of these two agents and what work remains to be done in this area.

The purpose of this systematic review was to synthesize and describe the existing evidence regarding the effectiveness of calcineurin inhibitors and alkylating agents for the treatment of IMN, and to determine whether one is more effective than the other for initial treatment.

Methods

Data sources and Searches

The search strategy included a search of MEDLINE. PubMed was searched using the MeSH terms ("membranous glomerulonephritis") AND [("cyclosporine" or "tacrolimus) OR ("cyclophosphamide" OR "chlorambucil")], with results limited to those in English, as well as those in humans and adults (ages 19+), published in any year. Related articles and bibliographies of relevant articles were also reviewed.

Study Selection

A single author (SM) performed the above searches as well as the study selection. This review sought to include studies that addressed the efficacy of immunomodulatory treatment with either an alkylating agent or a calcineurin inhibitor as an intervention in adults with IMN. Study designs to be included were randomized controlled trials (RCTs), prospective and retrospective cohort studies, and case control studies. Case reports and case series would be excluded, as would studies without a control or comparison group. Comparison groups include any of the following: a control group receiving conservative treatment (i.e. no immunosuppression), placebo or no treatment group, or a group receiving the other agent of interest (e.g. if the intervention was a calcineurin inhibitor, the comparison might be an alkylating agent). The outcome of interest is efficacy or effectiveness of the treatment; this

could be measured or defined in several ways: mortality, renal survival (or time to ESRD/renal replacement/transplant), remission rate (partial and complete), and renal function (eGFR). Other inclusion criteria are that articles be available through the University of North Carolina Health Sciences Library and be in English.

Data Extraction and Quality Assessment

Articles were reviewed and their methodology appraised using predetermined quality criteria. To evaluate the methodologic rigor and quality of the studies a single author (SM) assessed the potential for selection bias, measurement bias, magnitude of effect, and overall internal validity. External validity, or generalizability, was also evaluated. Articles were assigned an overall grade based on design and quality. The grade was based on the overall assessment of the internal validity of the study, taking into account potential bias and how significantly it might affect the results found in the study. Data to be compared between studies was extracted and summarized in a table (Table 1).

Results

Search Results

The initial MeSH search in PubMed returned 140 results- the titles were reviewed first to determine relevance to the study question and selection criteria; if studies could not be confidently excluded based on title, the abstract was reviewed. Of the 140 search results, 108 citations were excluded based on the title and/or abstract. Of those remaining, 14 could not be retrieved and four were not in English, leaving 14 articles to undergo full-text review (Figure). Of these, four of the full-text articles were excluded: two for wrong population, one for wrong intervention, and one for wrong control/comparison group. Ultimately, 10 studies were included in the review.

Study Characteristics

Table 1 summarizes the characteristics of included studies. There were six RCTs,[3-8] two prospective cohort studies,[9,10] one non-randomized controlled trial,[11] and one retrospective cohort study.[12] The sample size of included studies ranged from 17 to 93 subjects. The study population in all cases included patients with IMN, and most studies required that subjects also have nephrotic range proteinuria. Three were directed at patients with progressive disease or renal insufficiency.

Study Quality

Table 2 summarizes the appraisal and grading of the studies.

Three studies received a grade of 'good,' three were 'fair,' and four studies were 'poor.' The major limitation to quality was selection bias, as well as some measurement bias. Problems with selection bias included significant levels of dropout or loss to follow-up and baseline differences between groups. External validity was generally good- most of the RCTs were not too limiting in their exclusion criteria- but many of the studies were limited by short follow-up time, as well as use of intermediate outcomes.

Findings

Alkylating agents vs. calcineurin inhibitors

Only one study directly compared alkylating agents to calcineurin inhibitors.[3] This study found no difference in remission rates at one year between patients treated with one agent or the other. However, this study was of poor quality overall and had a relatively short followup of one year. Additionally, the study was conducted in China, which may limit the generalizability of the findings when considering North American or Western populations. Thus, there is currently no good quality evidence

available to address the question of whether alkylating agents or calcineurin inhibitors are more effective in treating IMN.

Alkylating agents vs. control

Five studies compared the efficacy of alkylating agent-based regimens to control- conservative treatment or steroids.[6-8,10,12] There were three RCTs,[8-10] one prospective cohort study,[10] and one retrospective cohort study.[12] Four of the five studies found that treatment with an alkylating agent improved outcomes, though primary outcomes and endpoints varied between studies.[6,7,10,12] The 1992 study by Falk et al. failed to find a difference in renal survival between patients treated with cyclophosphamide + steroids and those treated with steroids alone.[8] This study was relatively small (n=26) and of fair quality. Taken together, the bulk of the evidence regarding alkylating agent-based regimens supports an improvement in outcomes with their use. The most convincing evidence comes from the two large RCTs- by Jha et al. in 2007 and Ponticelli et al. in 1995- both of which have 10 years of follow-up.[6,7]

Ponticelli et al. evaluated a 6-month regimen of alternating chlorambucil and steroids that became known as the Ponticelli protocol; this was compared with conservative treatment in a group of 81 subjects from Italy.[7] This study found that at 10 years, the proportion of subjects reaching ESRD was significantly smaller in the treated group than in the control group. Strengths of this study include the long duration of follow-up (compared with other studies) as well as their choice of ESRD as an endpoint, which provides more meaningful evidence than intermediate outcomes (such as renal function and remission rate) alone. The internal validity of this study is limited somewhat by the number of patients lost to follow-up, though the magnitude of the effect found was fairly large and the potential selection bias would likely not be able to account for the differences found.

A later study by the same group demonstrated that using cyclophosphamide in place of chlorambucil was equally efficacious but resulted in fewer side effects;[13] chlorambucil is no longer used in alkylating agent-based regimens.

In 2007, Jha et al. published the results of a similar RCT comparing cyclophosphamide and steroids to conservative therapy.[6] Their study was slightly larger (n=93), and also followed patients for 10 years. They compared the percentage of patients achieving remission, the percentage with ESRD-free survival, and the percentage not reaching any endpoints (death, ESRD, or doubling of serum creatinine) at 10 years, and found significant differences between experimental and control groups for each. This study had some loss to followup, though not as much as in the Ponticelli study[7] described above. Further, there was some crossover from control to treatment (control subjects had the option to be treated with an alkylating agent 24 months or more after randomization if they wished). With an intention-to-treat (ITT) analysis, such crossover would bias toward the null and underestimate the effect size.

Torres et al. conducted a retrospective comparison of 2 historical cohorts.[12] They compared the outcomes of patients with progressive IMN treated during 2 consecutive time periods at their hospital. During the first, patients did not receive any immunosuppressive treatment, and during the second, all patients with progressive disease received chlorambucil and steroids. This study was limited by the potential selection bias and confounding inherent in the retrospective design, though the groups seemed similar at baseline with respect to a number of variables. This study found large differences between groups in change in creatinine clearance over time as well as percentage with renal survival, though no difference was found in patient survival at any time.

In 1992, Jindal et al. published the findings of a small prospective cohort of patients (n=9) with IMN that had been treated with cyclophosphamide.[10] They compared the cohort to a matched control (n=17) that had never been treated with cyclophosphamide, drawn from a glomerular disease registry in Toronto. The controls were matched on five factors, and appeared similar for these variables, but there were a number of other potentially confounding factors that were not matched. As with the above study by Torres et al.,[12] this potential bias limits internal validity, though the longer follow-up and minimal exclusion criteria make this observational study a valuable complement to RCTs.

Calcineurin inhibitors vs. control

Four studies compared the efficacy of calcineurin inhibitor-based regimens to control- conservative treatment, placebo, or steroids.[4,5,9,11] The quality of the studies varied, as did the endpoints measured, but the evidence pointed toward an overall benefit associated with use of calcineurin inhibitors. The 1995 study by Cattran et al. found that treatment of IMN with cyclosporine slowed the rate of decline in creatinine clearance significantly, as compared with a placebo group.[11] This study was relatively small and the endpoint was an intermediate outcome, but demonstrated a clinically important effect and served as a starting point for other investigations of cyclosporine's efficacy. This study included only patients with worsening renal function (progressive disease).

A later RCT by Cattran et al. in 2001 enrolled patients with IMN who had failed to achieve remission with at least 2 months of steroid treatment (steroid-resistant).[5] This RCT was of good quality and found a significant difference in rates of remission at all measured time points (26, 52, and 78 weeks). The outcome of interest was different (remission rate vs. change in creatinine clearance), and though still an intermediate outcome (as compared to renal survival or mortality), both studies demonstrated a benefit with cyclosporine treatment.

In 2007, Praga et al. conducted an RCT evaluating a different calcineurin inhibitor, tacrolimus, as treatment for IMN.[4] Their study was of fair quality, limited by higher dropout rates and some baseline differences between tacrolimus and control groups. The outcome of interest in this study was remission rate after treatment, at 12 and 18 months. They also found a significant magnitude of effect, which would likely outweigh the effect of any selection bias.

The 2001 study by Yao et al. found similar results, with significantly different rates of remission following treatment with cyclosporine (versus conservative treatment with captopril).[9] However, the internal validity of their study wasn't as good, and its applicability to North American populations may be limited.

Discussion

A number of studies have addressed the efficacy of calcineurin inhibitors and alkylating agents in treatment of IMN. These range from small, uncontrolled observational studies (excluded from this review) to larger, controlled and/or randomized ones. There is some good quality evidence demonstrating an association between each agent and intermediate outcomes or indicators of effectiveness. The existing evidence for calcineurin inhibitors demonstrates their role in increasing the likelihood of remission (both partial and complete) as well as decreasing the rate of decline in renal function (as measured by creatinine clearance). Both cyclosporine and tacrolimus have been evaluated in prospective trials and appear to be effective. The evidence for alkylating agents is somewhat stronger, as there have been larger studies with a longer duration of follow-up. A number of these have used renal survival (survival free of end-stage renal disease, or ESRD) as an endpoint, which is a more patient-oriented and clinically significant outcome. Several studies, of varying quality and size, have

found significant differences in renal survival between patients treated with alkylating agents and controls. To date, just one study has addressed the comparative effectiveness of alkylating agents and calcineurin inhibitors. Alone, it does not constitute good evidence for clinical decision-making or recommendations; more studies are needed to compare these agents head-to-head.

The four studies comparing calcineurin inhibitors to a control all had similar findings that pointed toward the efficacy of calcineurin inhibitor-based regimens for treatment of IMN. The consistency of these findings supports this hypothesis, and it is important to note that the results were found in different populations of patients with IMN- some with progressive disease, some steroid-resistant, and some with persistent (though not necessarily progressive) disease. One important limitation of these studies is the relatively short follow-up. The longest follow-up was in the prospective study by Yao et al.,[9] but the RCTs were limited in the duration of their follow-up. Also, these studies evaluated intermediate outcomes, such as creatinine clearance and remission rates (partial and complete), but did not measure more meaningful and patient-centered outcomes such as renal survival or mortality. Doing so would likely require significantly greater followup time, which is difficult with an RCT.

Implications for research

This review summarizes the existing evidence regarding treatment of IMN with alkylating agents and calcineurin inhibitors as of early 2011. A number of RCTs of varying quality have been performed, as well as some controlled observational studies, to assess this. When taken together, the majority of these studies are consistent with each other in the direction of the effect found, and provide more reliable evidence of the efficacy of these agents as measured by the outcomes identified. One limitation of the evidence is the lack of data on outcomes such as mortality, and for calcineurin inhibitors, on renal

survival. This is partly due to the small number of good-quality studies with long-term follow-up, since these outcomes take longer to develop than remission or changes in renal function.

The studies summarized above used varying doses of medications and durations of treatment, and consequently, there is not any clear evidence regarding the lowest effective dose or shortest effective duration of treatment. Some studies have tested low-dose regimens (particularly of calcineurin inhibitors) in an attempt to address this, but further studies are needed.

Finally, more research is needed to directly compare the effectiveness of calcineurin inhibitors and alkylating agents. A large RCT with long-term follow-up would be expensive and time consuming, and is impractical, especially as a first step. Efforts should be directed at designing good quality observational studies, which are an important complement to RCTs. Observational studies are far more inclusive and generalizable, and so can help expand the more rigorous findings of RCTs to a broader patient population.

Figure. Results of literature search and article selection.

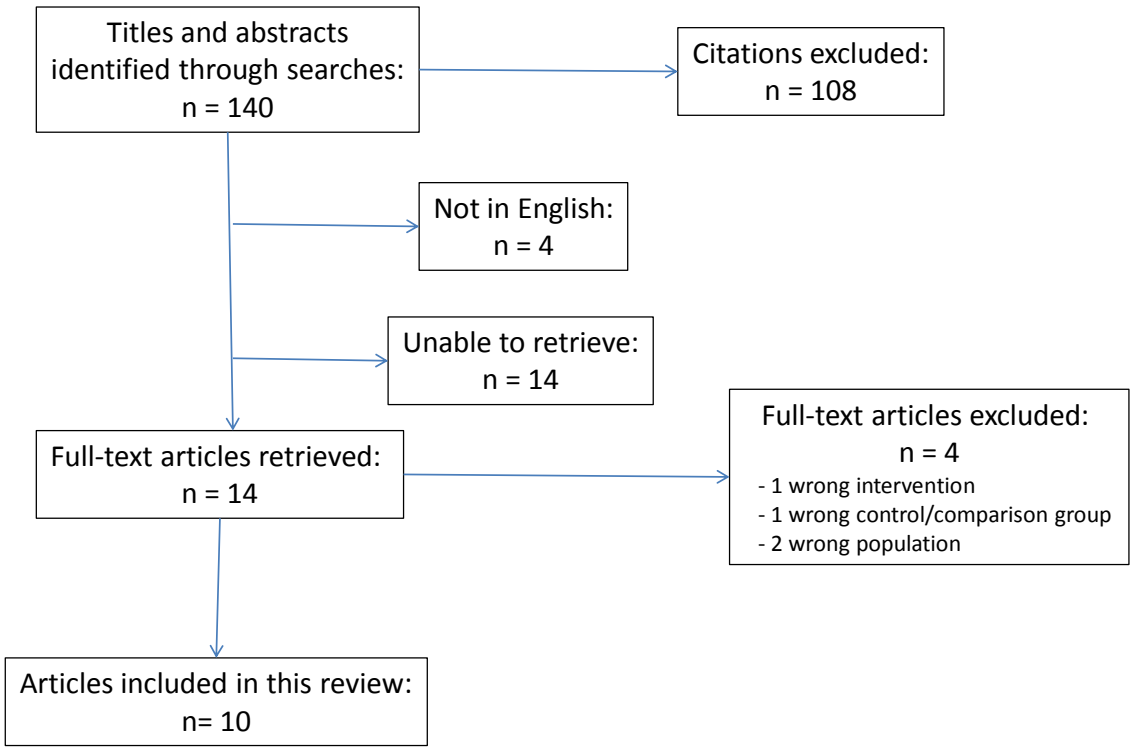


Table 1

	Study	Study design	Treatment studied	Comparison/control	# of subjects
Calcineurin inhibitor vs. alkylating agent	**Chen et al. 2010**	RCT	Tacrolimus + steroid	Cyclophosphamide + steroid	73
Calcineurin inhibitor vs. conservative/placebo	**Praga et al. 2007**	RCT	Tacrolimus	Conservative	48
	Yao et al. 2001	Prospective cohort with control	Cyclosporine	Conservative (Captopril)	30
	Cattran et al. 2001	RCT	Cyclosporine + steroid	Steroid	51
	Cattran et al. 1995	Controlled trial (non-randomized)	Cyclosporine	Placebo	17
Alkylating agent vs. conservative/placebo	**Jha et al. 2007**	RCT	Cyclophosphamide + steroid	Conservative	93
	Torres et al. 2002	Retrospective cohort (comparison of 2 historical cohorts)	Chlorambucil + steroid	Conservative	39
	Ponticelli et al. 1995	RCT	Chlorambucil + steroid	Conservative	81
	Falk et al. 1992	RCT	Cyclophosphamide + steroid	Steroid	26
	Jindal et al. 1992	Prospective cohort with control	Cyclophosphamide +/- steroid	Steroid (some)	26

Table 1, continued

	Study	Population characteristics	Regimen/dose	Followup duration
Calcineurin inhibitor vs. alkylating agent	**Chen et al. 2010**	IMN + nephrotic syndrome; Chinese, drawn from 6 centers	Tacrolimus 0.1mg/kg/day adjusted to trough level of 5-10ng/mL for 6 months, then decreased to trough level of 2-5ng/mL for 3 months	12 months
Calcineurin inhibitor vs. conservative/placebo	**Praga et al. 2007**	IMN + nephrotic syndrome lasting ≥9 months; conducted from 13 centers in Spain	Tacrolimus 0.05mg/kg/day divided BID, adjusted to trough level of 3-5ng/mL, increased to trough level of 5-8ng/mL if no remission at 2 months, continued for 12 mos; then tapered for 6 months (months 12-18)	18 months
	Yao et al. 2001	IMN	Cyclosporin A 5mg/kg/day, tapered over first 3 months then maintained at 2mg/kg/day for 12 months (total 15 months treatment)	Average followup was 44.4 +/- 9.6 months in cyclosporine group, 45.1 +/- 5.6 months in control group
	Cattran et al. 2001	IMN + nephrotic syndrome, steroid resistant (failed to achieve remission after ≥8 weeks of prednisone treatment)	Cyclosporine 3.5mg/kg/day divided, adjusted to trough levels of 125-225 micrograms/L for 26 weeks, then tapered for 4 weeks; Prednisone 0.15mg/kg/day for 26 weeks, then tapered for 8 weeks	78 weeks
	Cattran et al. 1995	IMN + nephrotic syndrome, worsening creatinine clearance over 12 months	Cyclosporine 3.5 mg/kg/day divided BID, adjusted to achieve trough level of 110-170 micrograms/L, continued for 12 months	Average followup was 49 months in cyclosporine group and 48 months in control group
Alkylating agent vs. conservative/placebo	**Jha et al. 2007**	IMN + nephrotic syndrome; excluded if systemic illness, DM, cancer, RVT, or immunosuppressants for ≥2months	IV methylprednisolone 1g/day x3 days followed by 27 days PO prednisolone 0.5mg/kg/day in months 1,3,5; PO cyclophosphamide 2mg/kg/day in months 2,4,6	10 years
	Torres et al. 2002	IMN + renal insufficiency, from single hospital in Spain	Prednisone 1 mg/kg/day for 1st month, 0.5mg/kg/day for 2nd month, 0.5mg/kg QOD for 3rd-6th month; 0.15 mg/kg day Chlorambucil PO for 1st 14 weeks	Average followup was 51.8 +/- 36.5 months in chlorambucil group, 46.8 +/- 37.5 months in control group
	Ponticelli et al. 1995	IMN + nephrotic syndrome	1g IV methylprednisone daily x3days followed by 0.5mg/kg/day PO prednisone for 27 days in months 1,3,5; chlorambucil 0.2mg/kg/day in months 2,4,6	10 years
	Falk et al. 1992	IMN + progressive disease (deteriorating renal function or persistent nephrotic syndrome with morbid complications)	IV cyclophosphamide 0.5g/m^2 with 3 days of pulse methylprednisolone, followed by 1 mg/kg prednisone	Average followup was 29.2 +/- 17.1 months
	Jindal et al. 1992	IMN + nephrotic syndrome	Cyclophosphamide given ≥2 months	Average followup was 83 +/- 13 months in treated group and 64 +/- 7 months in control group

Table 2

	Authors, year	Comparability of groups	Dropouts, adherence, crossovers	**Selection bias**	**Measurement bias**
Calcineurin inhibitor vs. alkylating agent	**Chen et al. 2010**	Good randomization strategy; groups comparable in table 1	Relatively high dropout rate: in tacrolimus group 6/39 withdrew, in control group 3/34 withdrew and 4/34 lost to followup; ITT analysis done	Dropouts concerning- 15% of tacrolimus group, 21% of control group	Patients and physicians aware of assignment, but endpoints judged at central location by masked investigators, histopathologists blinded
Calcineurin inhibitor vs. conservative/placebo	**Praga et al. 2007**	Appropriate randomization strategy; baseline differences between groups in age, proteinuria, eGFR, BP- would bias away from null	Relatively high dropout rate: 2/25 withrew from tacrolimus group and 1/23 from control; 2/25 from tacrolimus group and 3/23 from control group lost to followup; ITT analysis done	Dropouts concerning- 16% of tacrolimus group, 17% of control group	Patients and physicians aware of assignment, not specified whether investigators measuring outcomes blinded
	Yao et al. 2001	Don't describe how group assignment was made; groups mostly comparable in Table 1 (younger in cyclosporine group, but serum creatinine higher in control group)	Not described	Non-randomized; generally comparable on listed characteristics, but could be differences in unmeasured characteristics	Objective definitions of endpoints provided, but not clear how measured or whether blinded
	Cattran et al. 2001	Good randomization strategy; groups similar in Table 1- greater proportion of males in cyclosporine group, but this would bias toward null	Monitored adherence and found to be >90%; 4 dropped out prior to final endpoint (78 weeks)- data from last followup assessment used	Dropout rates acceptable, no significant source of selection bias apparent	Patients masked but physicians were not, endpoints measured by central lab blinded to patient assignment
	Cattran et al. 1995	Appropriate randomization strategy; groups generally similar- some differences in age, gender, and creatinine clearance, but bias toward null	None	No significant source of selection bias apparent	Patients masked but physicians were not

Table 2, continued

	Authors, year	Comparability of groups	Dropouts, adherence, crossovers	**Selection bias**	**Measurement bias**
Alkylating agent vs. conservative/placebo					
	Jha et al. 2007	Appropriate randomization strategy, comparable in table 1	7/53 from control and 4/51 from experimental group lost to followup (excluded from analysis); 15 of 46 control patients were treated (2-4.5yrs later)- crossover; ITT analysis done	Some dropout/loss to followup; only crossover was from control to treatment, which would bias toward null	Clearly defined endpoints but don't describe how/who measured, no blinding
	Torres et al. 2002	Not randomized (2 historical cohorts); generally similar in Table 1- difference in gender would bias away from null and difference in proteinuria would bias toward null	None	Non-randomized; generally comparable groups at baseline but different treatment periods/'eras' may be associated with other difference(s) in treatment or outcomes	Use of medical records- effect of missing data could be non-differential; investigators not blinded to patient group
	Ponticelli et al. 1995	Described in Ponticelli et al., N Engl J Med, 1989;320:8-13- unavailable from UNC. Patients reported to be similar on a number of important variables/prognostic factors.	10/39 from control and 9/42 from chlorambucil group lost to followup, 3/39 from control and 1/42 from chlorambucil group died; for these patients, data from last observation used in analysis; ITT analysis done	Randomized, groups reported to be comparable; significant loss to followup creates significant selection bias	Clearly defined endpoints, but unclear how/who measured, whether blinded
	Falk et al. 1992	Appropriate randomization strategy; groups comparable in Table 1 except for race	1 patient in cyclophosphamide group lost to followup after 1 yr, 1 patient in control group dropped out	Randomization and comparability generally ok, unclear whether race would have differential effect	Not described
	Jindal et al. 1992	17 control patients (never received cyclophosphamide) matched for time period of biopsy, age at biopsy, nephrotic syndrome, and 2 peak serum creatinine concentrations to 9 treated patients in cohort; comparable in Table 1 (more thromboembolic events in treated group, but bias toward null)	1 patient in cyclophosphamide group dropped out after 13 months	Non-randomized; controlled for some important variables/prognostic factors, but may be others that are different between treated subjects and controls	Clearly defined outcomes, but not how or who measures not clear. No blinding. Control data from medical records (registry).

Table 2, continued

Authors, year	Potential confounders	**Results- magnitude and direction**	**Overall internal validity, quality grade**	External validity	Other
Chen et al. 2010	Possibly differential dropout/loss to followup	After 12 mos, remission rates similar- 79% in tacrolimus group and 69% in control group; eGFR without significant change in either group and without significant difference between groups	**poor** No significant differences between groups at 1 year, but relatively high dropout rate limits validity of findings	Done in China, limits applicability to North American or Western populations	Relatively short followup period (1 yr)
Praga et al. 2007	Some baseline differences in prognostic factors (age, proteinuria, eGFR, BP), favoring better outcome in tacrolimus group	After 12 mos (end of full dose course), 72% of tacrolimus group and 22% of control group in remission (p<0.001); after 18 mos (end of taper), 76% of tacrolimus group and 30% of control group in remission (p=0.003)	**fair** Baseline differences between groups and dropouts may confound findings, but large magnitude of effect found	Requirement of ≥9 mos of nephrotic syndrome and eGFR ≥50mL/min limits some of the population to whom this might be applied	Relatively short followup (especially after stopping tacrolimus)- only followed patients through end of taper (18 mos)
Yao et al. 2001	Indication for choosing whether to treat- may be differences between those treated and not	After 15 mos (end of treatment course), 80% of cyclosporine group had achieved remission and 13% of control group had achieved remission (p<0.05). At time of last visit, there was no significant difference in remission between groups- 60% of cyclosporine group and 33% of control group were in remission (p=NS).	**poor** Non-randomized and group assignment not described, measurement not described	Inclusion criteria not too limiting. Performed in China, limits applicability to North American or Western populations.	Longer followup than some other studies
Cattran et al. 2001		After 26 wks, 75% of cyclosporine group and 22% of control group in remission (p<0.001); after 52 wks, 46% of cyclosporine group and 13% of control group in remission (p=0.004); after 78 wks, 39% of cyclosporine group and 13% of control group in remission (p=0.007)	**good** Good study design intended to reduce potential sources of bias, clearly described, no major areas of concern; large magnitude of effect	Inclusion criteria make findings applicable to smaller population- failed 8 weeks of prednisone treatment, creatinine clearance ≥42mL/min, BP ≤135/85	
Cattran et al. 1995		12 mos after treatment initiation, rate of decline of Cr clearance in treated group improved from -2.43 to -0.73 mL/min/month (p<0.02), and in control group remained stable at -2.18 to -2.05 mL/min/month	**good** Overall good design, no bias apparent that would threaten findings	Inclusion criteria not too limiting- include creatinine clearance >30, no serious co-morbid medical condition	

Table 2, continued

Authors, year	Potential confounders	**Results- magnitude and direction**	**Overall internal validity, quality grade**	External validity	Other
Jha et al. 2007		At 10 years' followup, 16 control and 34 experimental pts achieved remission (p<0.0001); 65% of control and 89% of experimental pts achieved dialysis-free survival (p=0.016); 44% of control and 79% of experimental did not reach any of endpoints (death, ESRD, doubling of Cr) (p=0.0006).	**good** Some loss to followup is potential source of selection bias, but crossovers bias toward null and thus may result in underestimation of magnitude of effect	Performed in India, may limit applicability of findings to North American or Western populations, though consistent with other large RCT	Long followup of RCT provides valuable data not available in other studies; evaluation of ESRD, death, renal failure, and quality of life as endpoints is more meaningful
Torres et al. 2002	Differences between historical cohorts- different overall treatment/care due to historical effect	At last followup, no significant change in serum creatinine in treated group, significant increase in control group (p<0.001). Probability of renal survival after 7 yrs was 90% in treated group, 20% in control group (p<0.01); no significant difference between groups in patient survival at 4 or 7 years of followup.	**poor** Limited by retrospective design and use of 2 historical cohorts from different time periods, though relatively large magnitude of effect may reflect true differences		Longer followup is a strength, but small study. Measured more meaningful/patient-centered outcomes (renal survival, patient survival)
Ponticelli et al. 1995	Possibly differential loss to followup	92% of treated did not progress to ESRD vs. 60% of controls did not progress to ESRD, p=0.0038; by 10 years 88% of treated had a remission as first event vs. 47% of controls (p=0.0000)	**fair** Significant selection bias due to relatively high dropout/loss to followup. Large magnitude of effect indicates real findings in spite of any biases (though magnitude could be smaller)		Long followup of RCT provides valuable data not available in other studies; evaluation of ESRD as an endpoint is more meaningful
Falk et al. 1992		No significant difference in renal survival between groups	**fair** Measurement not clear or well-described, selection bias appears limited		Measured a more meaningful outcome- renal survival.
Jindal et al. 1992	Possibly sex, proteinuria, location/site of treatment (e.g. university hospital versus private nephrologist), other factors not controlled for	At final followup, 12.5% of cyclophosphamide group had reached ESRD, compared to 58.8% of controls (p<0.05).	**poor** Significant potential for selection bias, measurement bias		

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