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# Two-Year Follow-up Study of Membranous Nephropathy Treated With Tacrolimus and Corticosteroids Versus Cyclical Corticosteroids and Cyclophosphamide

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**Introduction:** Both cCTX/GCs and CNIs are recommended as first-line agents in the management of PMN. The present study is an extended report of patients randomized to receive TAC/GCs or cCTX/GCs at 2 years post randomization.

**Methods:** Seventy patients enrolled in the clinical trial Tacrolimus Combined With Corticosteroids Versus Modified Ponticelli Regimen in Treatment of Idiopathic Membranous Nephropathy: Randomized Control Trial were followed quarterly between 12 and 24 months. At the end of 24 months, 3 patients were lost to follow-up.

**Results:** At 18 months, 66% and 89% ($P = 0.04$) were in remission in TAC/GCs and cCTX/GCs groups, respectively. At 18 and 24 months, 60% and 86% ($P = 0.03$) of cases were in remission in the TAC/GCs and cCTX/GCs groups, respectively. At 18 months, 57% and 83% ($P = 0.03$) of the patients in TAC/GCs and cCTX/GCs groups were in remission without need of any additional immunosuppression (persistent remission) and, at 24 months, 43% and 80% ($P = 0.002$) were in persistent remission in TAC/GCs and cCTX/GCs groups, respectively. Relapse rate after any remission was 40% and 6.7% in TAC/GCs and cCTX/GCs groups, respectively ($P = 0.007$). There was an association of aPLA2R titers with remission or resistance ($P = 0.006$) in relapsing PMN. The significant decrease in eGFR after 12 months of TAC/GCs therapy normalized at 18 and 24 months.

**Discussion:** At 2 years after randomization, relapse rates are higher for TAC/GCs compared with cCTX/GCs in PMN patients. Thus, cCTX/GCs are better than TAC/GCs in the longer term in PMN patients.

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Both calcineurin inhibitors (CNIs) and cyclical cyclophosphamide (cCTX) with glucocorticoids (GCs) (CTX/GCs) are recommended in the management of primary membranous nephropathy (PMN).[1] The 2 regimes are equally efficacious, but have different adverse event profiles.[1,2]

We reported a remission rate at the end of 1 year of 77% and 71% with cCTX/GCs and tacrolimus (TAC) with steroids, respectively.[3] At least 3 other randomized controlled trials have reported the 12-month remission rates of 65% to 89% and 44% to 69%,[4–6] with TAC/GCs and cCTX/GCs, respectively.

Cattran *et al.*[7] reported a remission rate of 75% after 6-month treatment with cyclosporine and steroids. The remission rates fell to 46% and 39% at 12 and 24 months once cyclosporine was stopped. In other studies, the relapse rates after stopping TAC/cyclosporine and cCTX/GCs range from 47% to 50% and 20% to 31%, respectively.[7–10] The high rates of relapse compounded with nephrotoxicity of long-term use of CNIs, makes it worthwhile to study the remission rates

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**Table 1.** Clinical outcome at various time points

| | 18 mo | | 24 mo | |
|---|---|---|---|---|
| | TAC/GCs ($n$ = 35) | cCTX/GCs ($n$ = 35) | TAC/GCs ($n$ = 35) | cCTX/GCs ($n$ = 35) |
| Remission (ITT)[a] | 23 (65.7)[b] | 31 (88.6)[c] | 21 (60)[d] | 30 (85.7)[e] |
| CR | 15 (42.8) | 20 (57.2) | 12 (34.3) | 20 (57.2) |
| PR | 08 (22.8) | 11 (31.4) | 09 (25.7) | 10 (28.5) |
| Resistant | 07 (20.0) | 04 (11.4) | 07 (20.0) | 03 (8.6) |
| Relapse | 05 (14.3)[f] | NA[g] | 07 (20.0)[h] | 02 (5.7)[i] |
| Remission[j] | 20 (57.1)[k] | 29 (82.8)[l] | 15 (42.8)[m] | 28 (80)[n] |

Values are n (%).
cCTX, cyclical cyclophosphamide; CI, confidence interval; CR, proteinuria <500 mg/d with normal serum albumin (≥3.5 g/dl) and serum creatinine; GCs, glucocorticoids; ITT, intention to treat; NA not applicable; PR, proteinuria ≥500 mg/d, but <2 g/d or <50% of baseline with normal serum albumin (≥3.5 g/dl) and serum creatinine; TAC, tacrolimus.
[a]Included all remission irrespective of the use of second line agents, TAC/GCs, and cCTX/GCs.
[b*c]0.24 (95% CI: 0.07–0.86; $P = 0.04$).
[d*e]0.25 (95% CI: 0.07–0.80; $P = 0.03$).
[f+h versus g+i]$P = 0.007$.
[j]Cases with remission without any need of any further immunosuppression.
[k*l]0.27 (95% CI: 0.08–0.87; $P = 0.03$).
[m*n]0.18 (95% CI: 0.06–0.54; $P = 0.002$).

after stopping the drug. The present study reports the remission rates at 18 and 24 months.

## METHODS

The present report is an extended (2-year) follow-up of patients enrolled in a randomized controlled trial that compared tacrolimus combined with corticosteroids and cCTX/GCs (Tacrolimus Combined With Corticosteroids Versus Modified Ponticelli Regimen in Treatment of Idiopathic Membranous Nephropathy: Randomized Control Trial; CTRI/2013/10/004061) carried at the Department of Nephrology, PGIMER, Chandigarh, India.[3] Briefly, the study included adults with biopsy-proven PMN and persistent nephrotic syndrome in spite of 6 months of nonimmunosuppressive symptomatic treatment with full angiotensin-blockade.

### Therapy

Patients received either oral prednisolone (0.5 mg/kg/d for 6 months and subsequent taper by 0.1 mg/kg/wk) and *TAC* (trough level = 7.46 ± 1.28 ng/ml for the first 6 months and 4.83 ± 0.59 ng/ml for next 6 months)[3] for 12 months, followed by taper by 50% every 2 weeks to stop or cCTX/GCs (6-month course of alternate months of steroid and cyclophosphamide).[3,11]

### Endpoint

A total of 70 patients were enrolled in the study starting from September 21, 2011 to December 2, 2013. Two-year follow-up was completed by December 2015. The primary endpoint of the trial was remission rate at the end of 6 and 12 months.[3] At the end of 12 months, 71% ($n$ = 25) and 77% ($n$ = 27) of the study participants achieved remission.[3] During the study it was decided to continue the follow-up of the enrolled patients without any prespecified interventions. Patients were followed quarterly from months 12 to 24 with proteinuria, serum creatinine, and albumin. The Institute Ethics Committee approved the study, and all subjects provided written informed consent.

### Definitions

Nephrotic syndrome: proteinuria of >4 g/d or ≥2.0 g/d along with serum albumin <2.5 g/dl.[3,11] Complete remission: proteinuria <500 mg/d with normal serum albumin (≥3.5 g/dl) and serum creatinine. Partial

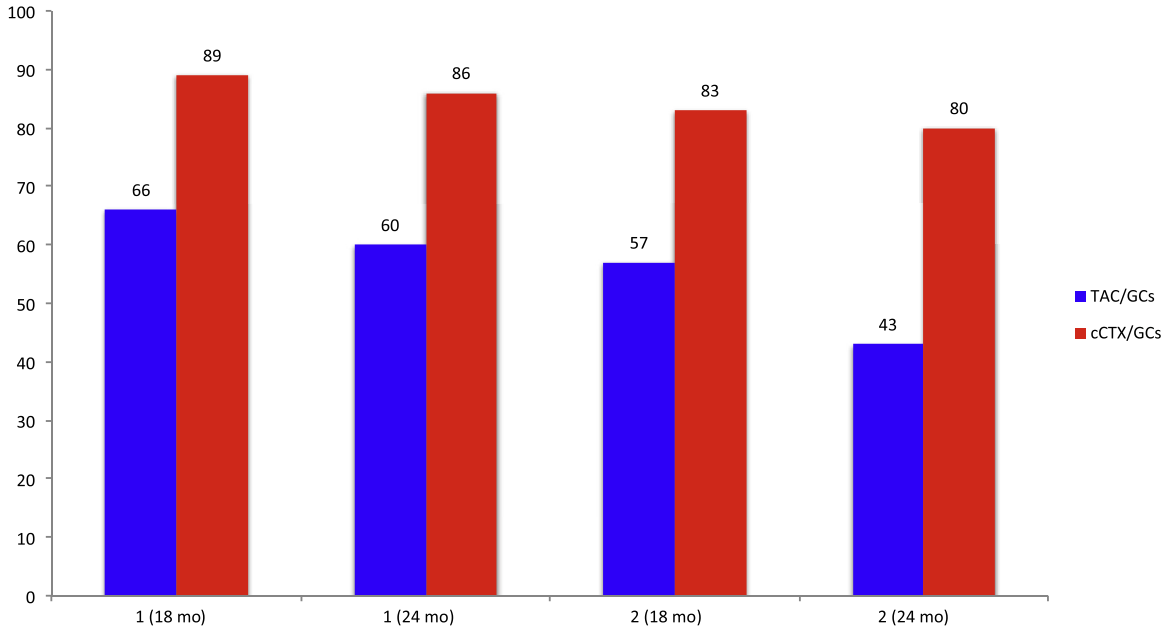


**Figure 1.** Remission rate at various time points (1—intention to treat; 2—remission without any secondary immunosuppression). cCTX, cyclical cyclophosphamide; GCs, glucocorticoids; TAC, tacrolimus.

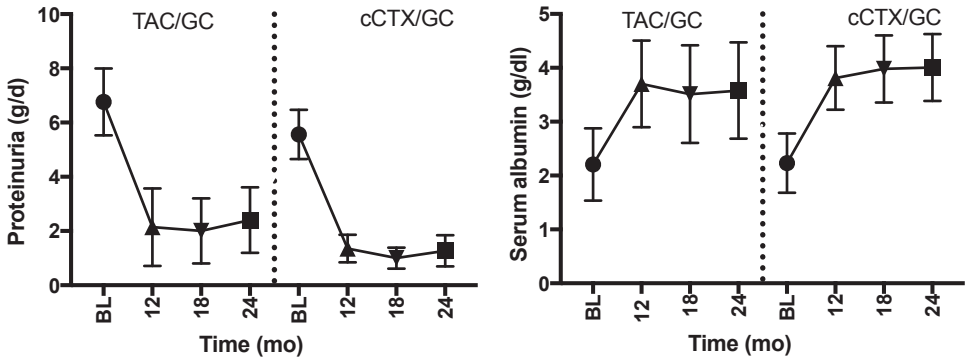


**Figure 2.** Graphical representation of proteinuria (right) and serum albumin (left) at various time intervals. The values are expressed as means and SDs (vertical lines). BL, baseline; cCTX, cyclical cyclophosphamide; GCs, glucocorticoids; TAC, tacrolimus.

remission: proteinuria ≥500 mg/d, but <2 g/d or <50% of baseline with normal serum albumin (≥3.5 g/dl) and serum creatinine. Relapse: nephrotic range proteinuria after any remission (complete remission or partial remission).

### Statistical Analysis

As described elsewhere,[3] the study was designed as a noninferiority trial (10%), with a sample size of 70, equally divided to receive either TAC/GCs or cCTX/GCs.[3] The analysis was based on intention to treat. Intention to treat was performed with last value carried forward. In addition, we have also analyzed the number of patients who persisted to have remission without need of any secondary immunosuppression. Continuous data are reported as mean ± SD. Categorical values are represented as absolute values and percentages. The primary analysis was done using a $\chi 2$ or Fisher exact test. For secondary outcomes both *t* test and $\chi 2$ test were used to compare the difference between the 2 groups. Mann-Whitney *U* test was used to compare nonparametric data. For association of antibodies to M-type phospholipase A2 receptor (aPLA2R) to relapse, a simple visual impression was performed, for treatment outcome with secondary immunosuppression a $\chi 2$ or Fisher exact test was performed (comparing clinical remission or resistance with aPLA2R presence or absence) and analysis of variance for estimated glomerular filtration rate (eGFR) comparison. Statistical analyses were done using Graph Pad Prism 6 (San Diego, CA). $P < 0.05$ was considered significant and was 2-sided.

## RESULTS

A total of 84 cases were screened, of which 70 were enrolled in the study (35 randomized to receive either cCTX/GCs or TAC/GCs). Sixty-seven cases completed 24 months of follow-up. There were no differences in the baseline parameters between the study groups.[3]

The outcome at the end of 6 and 12 months is mentioned elsewhere.[3] At the end of 18 and 24 months, 23 (65.7%) versus 31 (88.6%) (difference of 22.86%; 95% confidence interval [CI]: 3.30–44.7; $P = 0.04$) cases and 21 (60%) versus 30 (85.7%) (difference of 25.71%; 95%CI 5.81, 48.64; $P = 0.03$) patients were in remission in TAC/GCs and cCTX/GCs groups, respectively. The number of patients in complete and partial remission at 18 and 24 months after starting therapy is shown in Table 1 (Figure 1).

Twenty subjects (57.1%) and 29 (82.8%) maintained clinical remission without need of any further immunosuppressive agents in the TAC/GCs and cCTX/GCs groups, respectively, at 18 months (difference of 25.71%; 95% CI: 5.46–49.24; $P = 0.03$), and 15 (42.8%) and 28 (80%) maintained remission at 24 months in TAC/GCs and cCTX/GCs groups, respectively (difference of 37.14; 95% CI: 17.68–61.96; $P = 0.002$) (Figure 1). There was no difference in proteinuria

**Table 2.** Outcome parameters at various time points

| | 15 mo | | | 18 mo | | |
|---|---|---|---|---|---|---|
| | TAC/GCs ($n = 35$) | cCTX/GCs ($n = 35$) | *P* value | TAC/GCs ($n = 35$) | cCTX/GCs ($n = 35$) | *P* value |
| Proteinuria (g/d) | 2.23 ± 3.59 (1.00–3.47) | 1.10 ± 1.18 (0.70–1.51) | 0.08 | 2.00 ± 3.50 (0.80–3.20) | 1.00 ± 1.12 (0.61–1.38) | 0.11 |
| Serum albumin (g/dl) | 3.48 ± 0.97 (3.15–3.81) | 3.93 ± 0.54 (3.74–4.12) | 0.02 | 3.51 ± 0.90 (3.20–3.82) | 3.97 ± 0.62 (3.76–4.19) | 0.01 |
| Serum creatinine (mg/dl) | 0.96 ± 0.18 (0.89–1.02) | 0.93 ± 0.26 (0.84–1.02) | 0.62 | 0.95 ± 0.17 (0.89–1.01) | 0.94 ± 0.23 (0.86–1.02) | 0.78 |

Values are means ± SDs and 95% confidence intervals.
cCTX, cyclical cyclophosphamide; GCs, glucocorticoids; TAC, tacrolimus.

between groups at different follow-up points (Figure 2). However, serum albumin was significantly lower in the TAC/GCs compared with the cCTX/GCs group at 15 ($P = 0.02$), 18 ($P = 0.01$), 21 ($P = 0.005$), and 24 months ($P = 0.02$) (Figure 2). There was no difference in serum creatinine and eGFR between both the groups at various above-mentioned time points. The details of the proteinuria, serum albumin, and serum creatinine are mentioned in Table 2. Among cases achieving any remission, 10 subjects (40%) and 2 (6.7%) had relapsed at 24 months in TAC/GCs and cCTX/GCs (3 subjects had remission after 12 months of starting cCTX/GCs, of which 1 relapsed) groups, respectively ($P = 0.0069$).

## Second Line Agents

Thirteen cases in the TAC/GCs group and 4 cases in the cCTX/GCs group received immunosuppressive treatment for relapsing or resistant disease. Nine cases of the TAC/GCs group received cCTX/GCs (Table 3). At the end of 6 months of cCTX/GCs therapy, clinical and serological remission was achieved in 7 subjects (77.8%). The therapy was well tolerated, but for 2 cases, who developed upper respiratory tract infection. Three cases on cCTX/GCs received TAC therapy. Of the 3 cases, 1 patient (33.3%) achieved clinical remission at 12 months; however, the proteinuria relapsed on tapering TAC. Five cases (4 on TAC/GCs and 1 on cCTX/GCs) received a single dose of rituximab (375 mg/m$^2$) with monthly monitoring of CD-19 (target <1% CD-19 cells with absolute count of <5 cells/μl). Rituximab was repeated on increase in CD-19 count (CD-19 >1% or ≥CD cells/μl). Remission was achieved in 3 cases (60%). The details are mentioned in Table 4. Among 17 subjects requiring a second course of immunosuppression, 16 (94.1%) were related to aPLA$_2$R. There was a significant association of aPLA$_2$R to treatment outcome at the end of therapy ($P = 0.007$).

## Adverse Events

The adverse events at the end of 12 months were mentioned elsewhere.[3] The eGFR calculated by the Modification of Diet in Renal Disease formula showed a reduction from baseline through 24 months in the TAC/GCs group ($P = 0.001$), whereas no change was noted in the cCTX/GCs group ($P = 0.23$) (Figure 3) (intention to treat). The mixed model showed no significant differences in eGFR levels between the 2 study groups at various time points. However, in within group comparisons of eGFR levels over time, the fixed-effect mixed model showed significant reduction ($P = 0.003$) in eGFR in TAC/GCs group compared with baseline, whereas no significant reductions ($P = 0.108$) in eGFR were observed for cCTX/GCs group. There were no significant differences between both groups at various time points. Between 12 and 24 months, there were 3 episodes (8.57%) and 2 (5.71%) of upper respiratory tract infections in the TAC/GCs and cCTX/GCs groups, respectively, of which 1 patient (who also received TAC for resistant disease) in the cCTX/GCs group died due to tuberculosis. This patient was off any immunosuppression 6 months prior to his death.

**Table 3.** Antibodies to PLA2R in patients with relapse

| Case no. | Age /sex | Primary therapy | aPLA2R (RU/ml) (baseline) | aPLA2R (RU/ml) (6 mo) | aPLA2R (RU/ml) (12 mo) | aPLA2R (RU/ml) (relapse) |
|---|---|---|---|---|---|---|
| 1 | 48/F | TAC/GCs | 337.16 | 3.32 | 4.51 | 221.71 |
| 2 | 60/F | TAC/GCs | 214.13 | 1.25 | 1.41 | 322.37 |
| 3 | 41/M | TAC/GCs | 203.46 | 4.24 | 4.28 | 186.67 |
| 4 | 42/M | TAC/GCs | 159.50 | 1.30 | 1.25 | 294.73 |
| 5 | 40/M | TAC/GCs | 117.47 | 6.26 | 0.45 | NA |
| 6 | 38/M | TAC/GCs | 89.15 | 2.06 | 0.45 | 56.68 |
| 7 | 36/M | TAC/GCs | 84.22 | 15.04 | 6.20 | 23.26 |
| 8 | 48/M | TAC/GCs | 50.55 | 36.30 | 0.45 | 135.90 |
| 9 | 45/F | TAC/GCs | 21.41 | 15.04 | 2.04 | 32.52 |
| 10 | 53/M | cCTX/GCs | 1016.78 | 4.51 | 5.18 | 0.01 (negative) |
| 11 | 24/M | cCTX/GCs | 525.25 | 10.79 | 9.21 | 46.58 |
| 12 | 45/M | TAC/GCs | 221.81 | 1.36 | 0.45 | 82.92 |

aPLA2R > 20 RU/ml were considered positive.
aPLA2R, Antibodies to m-type phospholipase A2 receptor; cCTX, cyclical cyclophosphamide; F, female; GCs, glucocorticoids; M, male; NA, not available; RU, resonance unit; TAC, tacrolimus.

# DISCUSSION

This is the first study to show the comparative efficacy of TAC/GCs versus cCTX/GCs, and it shows that 2 years after therapy initiation for nonimmunosuppressive

**Table 2.** (Continued) Outcome parameters at various time points

| | 21 mo | | | 24 mo | | |
|---|---|---|---|---|---|---|
| | TAC/GCs ($n = 35$) | cCTX/GCs ($n = 35$) | *P* value | TAC/GCs ($n = 35$) | cCTX/GCs ($n = 35$) | *P* value |
| Proteinuria (g/d) | 2.30 ± 3.53 (1.08–3.51) | 1.04 ± 1.44 (0.54–1.54) | 0.05 | 2.40 ± 3.51 (1.19–3.61) | 1.27 ± 1.67 (0.69–1.84) | 0.08 |
| Serum albumin (g/dl) | 3.48 ± 0.84 (3.18–3.77) | 3.97 ± 0.56 (3.78–4.17) | 0.005 | 3.57 ± 0.89 (3.27–3.88) | 4.00 ± 0.62 (3.79–4.22) | 0.02 |
| Serum creatinine (mg/dl) | 0.91 ± 0.20 (0.84–0.98) | 0.93 ± 0.21 (0.86–1.00) | 0.64 | 0.98 ± 0.25 (0.90–1.07) | 0.92 ± 0.18 (0.86–0.98) | 0.24 |

**Table 4.** Outcomes of subjects treated with secondary immunosuppression

| | | Prior to 1st (TAC/GCs) | | Prior to cCTX/GCs | | 2 mo | | 4 mo | | 6 mo | | aPLA2R (RU/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Age*/sex | Indication | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/d) | 1 | 2 | 3 |
| 24/M | Resistant | 2.30 | 1.62 | 4.70 | 2.90 | 2.50 | 2.32 | 1.20 | 3.59 | 0.6 | 4.32 | 0.45[a] | 27.27 | 0.10 |
| 45/M | Resistant | 10.30 | 1.42 | 3.00 | 2.16 | 2.40 | 1.80 | 2.70 | 2.07 | 3.00 | 2.19 | 938.21 | 201.95 | 182.39 |
| 26/M | Resistant | 6.60 | 1.80 | 7.50 | 2.47 | 1.30 | 3.05 | 0.76 | 4.10 | 0.71 | 3.68 | 217.09 | 186.67 | 52.23 |
| 41/F | Relapse | 5.00 | 2.20 | 4.20 | 2.91 | 3.76 | 2.64 | 0.86 | 3.65 | 0.88 | 4.01 | 203.46 | 364.23 | 0.81 |
| 18/F | Resistant | 5.70 | 1.50 | 3.90 | 2.59 | 4.50 | 1.10 | 1.50 | 2.16 | 2.50 | 2.48 | 1468.54 | 440.92 | 359.30 |
| 22/M | Relapse | 7.38 | 1.90 | 11.50 | 2.62 | 2.40 | 3.21 | 1.40 | 3.57 | 0.80 | 4.10 | 118.80 | 235.18 | 0.37 |
| 55/M | Resistant | 6.23 | 4.40 | 14.70 | 3.19 | 3.20 | 3.70 | 2.01 | 3.80 | 0.28 | 3.96 | 0.45[a] | 156.51 | 0.29 |
| 45/F | Relapse | 7.40 | 2.40 | 5.00 | 2.83 | 1.00 | 3.42 | 0.15 | 4.50 | 0.20 | 4.13 | 21.41 | 32.52 | 0.31 |
| 48/M | Relapse | 6.00 | 1.85 | 4.00 | 2.77 | 1.50 | 2.20 | 1.00 | 3.90 | 0.96 | 3.90 | 50.55 | 135.90 | 0.59 |

| | | Prior to 1st (CTX/GCs) | | Prior to TAC | | 6 mo | | 12 mo | | 18 mo | | aPLA2R (RU/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Age*/sex | Indication | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | 1 | 2 | 3 |
| 40/M | Resistant | 7.50 | 1.58 | 4.60 | 3.80 | 1.90 | 4.00 | 2.80 | 3.72 | 7.92 | 2.85 | 350.87 | 71.83 | 142.34 |
| 58/F | Resistant | 4.40 | 2.95 | 3.80 | 2.70 | 2.50 | 3.42 | 4.86 | 3.10 | 5.18 | 3.35 | 393.01 | 76.72 | 55.19 |
| 54/M | Resistant | 4.00 | 2.20 | 4.63 | 2.60 | 4.30 | 2.90 | 4.60 | 2.20 | Expired | Expired | 0.45 | 0.45 | NA |

| | | Prior to primary IST | | Prior to rituximab | | 2 mo | | 4 mo | | 6 mo | | aPLA2R (RU/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Age*/sex | Indication | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | Proteinuria (g/d) | Serum albumin (g/dl) | 1 | 2 | 3 |
| 45/M[b] | Resistant | 6.50 | 2.42 | 3.46 | 2.59 | 1.2 | 2.48 | 4.5 | 3.13 | 4.31 | 3.15 | 326.73 | 578.30 | 138.87 |
| 48/F[b] | Relapse | 4.40 | 2.50 | 3.50 | 2.60 | 3.23 | 3.46 | 0.92 | 3.48 | 0.53 | 3.60 | 337.16 | 221.71 | 0.85 |
| 18/M[b] | Resistant | 6.29 | 1.90 | 4.00 | 1.90 | 1.80 | 2.12 | 2.00 | 2.02 | 3.67 | 2.72 | 841.25 | 144.89 | 16.18 |
| 34/M[c] | Resistant | 14.80 | 2.40 | 4.20 | 3.25 | 2.40 | 3.54 | 0.08 | 4.04 | 1.62 | 4.07 | 61.32 | 101.85 | 3.14 |
| 60/F[b] | Relapse | 3.10 | 2.40 | 5.16 | 3.30 | 2.44 | 3.50 | 1.08 | 3.70 | 1.83 | 3.80 | 214.13 | 322.37 | 7.70 |

Time frames are as follows: 1—before starting primary immunosuppression, 2—before starting second line agent, 3—at completion of second line agent (6 months for patients who received CTX/GCs or rituximab as second line agent and 12 months for those who received tacrolimus as second line agent).
aPLA2R, antibodies to m-type phospholipase A2 receptor; cCTX, cyclical cyclophosphamide; F, female; GCs, glucocorticoids; IST, immunosuppressive therapy; M, male; NA, not available; RU, resonance unit; TAC, tacrolimus.
[a]Cases with baseline aPLA2R negative, but serial aPLA2R positive (also had enhanced staining for PLA2R in glomeruli positive).
[b]Primary immunosuppression was TAC/GCs.
[c]Primary immunosuppression was cCTX/GCs.

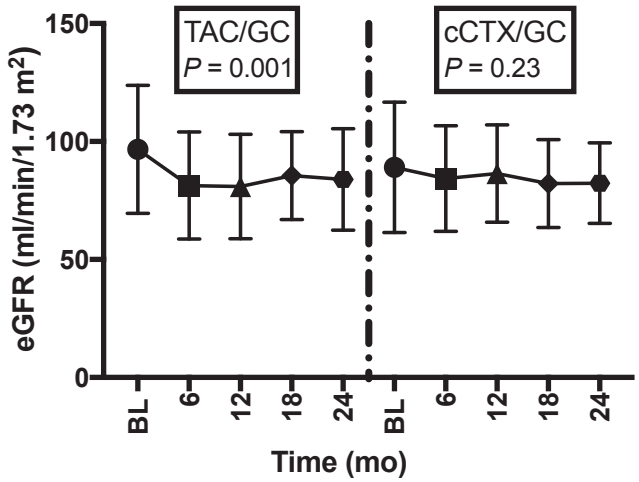


**Figure 3.** Graphical representation of estimated glomerular filtration rate (eGFR) over various time points. The values are expressed as means and SDs (vertical lines). BL, baseline; cCTX, cyclical cyclophosphamide; GCs, glucocorticoids; TAC, tacrolimus.

symptomatic treatment refractory PMN, TAC/GCs was inferior to cCTX/GCs.

Praga *et al.*,[10] enrolled 48 subjects of which 25 received TAC for a period of 18 months and 23 received conservative therapy. At the end of 6, 12, and 18 months of therapy, 56%, 72%, and 76% of the subjects were in clinical remission, respectively. At all the time points, the remission rates in the TAC group was significantly better than in the conservative therapy group. Twenty-six percent of the subjects in the conservative treatment group had 50% increases in serum creatinine compared with 4% on TAC ($P = 0.03$). The benefits of TAC therapy were marred by the 50% relapse rate on stopping the drug.

There are at least 3 randomized controlled trials from China that have compared TAC/GCs with CTX/GCs for management of PMN,[4–6] but they failed to find a difference in remission rates. However, none of the studies have reported their outcomes after stopping TAC. Praga[12] advocated long-term treatment with the lowest effective doses to avoid the relapse of nephrotic syndrome. However, the data from transplant literature suggest that over three-fourths of the subjects on CNI therapy developed biopsy-proven nephrotoxicity in 1 year, and this number was over 90% by 5 years.[13] In a small observational study of TAC in steroid-dependent minimal change disease, we observed that 36% of the subjects had relapse of nephrotic syndrome and needed continuation of TAC for maintenance of remission. All 4 subjects underwent screening biopsy at 96 months, of which, 75% had, evidence of chronic nephrotoxicity.[14] Compared with patients on cCTX/GCs, those on TAC/GCs had significant reduction in eGFR at various time points compared with baseline. This may be partly explained by nephrotoxic potential of TAC.[13,14]

The relapse rate was also higher in the TAC group than in subjects on cCTX/GCs. On critical analysis, it is apparent that higher relapse rate with TAC therapy affected the final outcome. Although, the study was designed as a noninferiority study based on percentage remission at 12 months rather than at 24 months, extended follow-up suggests that CTX/GCs patients have better rates of remission maintenance than do those on TAC/GCs.

These results support and extend the findings of Cattran *et al.*[7] that suggest that CNI-induced remission is usually temporary. In contrast, 2 randomized controlled trials have documented the long-term efficacy of cCTX/GCs in the management of PMN. Ponticelli *et al.*[15] and Jha *et al.*[11] reported an initial remission rate of 82% and 72%, respectively, with a relapse rate of about 25%. The 10-year renal survival in the cyclical alkylating agents/GCs and placebo was 89% to 92% and 60% to 65%, respectively.[9,15] Use of CTX therapy has been clouded by 2 side effects—gonadal dysfunction and risk of malignancy. Most of the patients receiving cCTX/GCs receive a cumulative CTX dose in excess of 150 mg/kg, which can compromise gonadal function and would be one of the limiting factors for use of the drug.[16] Van den Brand *et al.*[2] reported a 3-fold increase in the risk of malignancy in CTX-treated PMN subjects. Most of their patients received oral CTX (1.5 mg/kg) therapy for 12 months.[2] None of the subjects on cCTX/GCs therapy in the study by Jha *et al.*[11] and Ponticelli *et al.*[15] developed any malignancy after 10 years of follow-up.

With respect to the treatment of patients with refractory or dependent PMN, cCTX/GCs were able to induce a response in 77% of TAC/GCs resistant or dependent cases, whereas TAC alone therapy was effective in only one-third of cCTX/GCs resistant subjects. Rituximab was effective in 60% of TAC/GCs or cCTX/GCs resistant or dependent PMN cases. However, most of the responses reported are with short-term follow-up. The report clearly suggests that cCTX/GCs could be potentially used in CNI-resistant PMN.

The study is limited by a lack of follow-up of significant duration to gauge long-term complications. To conclude, at 24 months after therapy initiation for nonimmunosuppressive symptomatic treatment refractory PMN, cCTX/GCs are better than TAC/GCs.

## DISCLOSURE

All the authors declared no competing interests.

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## REFERENCES

1. Chapter 7: Idiopathic membranous nephropathy. *Kidney Int Suppl*. 2012;2:186–197.
2. van den Brand JA, van Dijk PR, Hofstra JM, Wetzels JF. Cancer risk after cyclophosphamide treatment in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol*. 2014;9:1066–1073.
3. Ramachandran R, Hn HK, Kumar V, et al. Tacrolimus combined with corticosteroids versus Modified Ponticelli regimen in treatment of idiopathic membranous nephropathy: randomized control trial. *Nephrology (Carlton)*. 2016;21:139–146.
4. He L, Peng Y, Liu H, et al. Treatment of idiopathic membranous nephropathy with combination of low-dose tacrolimus and corticosteroids. *J Nephrol*. 2013;26:564–571.
5. Chen M, Li H, Li XY, et al. Tacrolimus combined with corticosteroids in treatment of nephrotic idiopathic membranous nephropathy: a multicenter randomized controlled trial. *Am J Med Sci*. 2010;339:233–238.
6. Xu J, Zhang W, Xu Y, et al. Tacrolimus combined with corticosteroids in idiopathic membranous nephropathy: a randomized, prospective, controlled trial. *Contrib Nephrol*. 2013;181:152–162.
7. Cattran DC, Appel GB, Hebert LA, et al. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int*. 2001;59:1484–1490.
8. du Buf-Vereijken PW, Branten AJ, Wetzels JF, Membranous Nephropathy Study Group. Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate. *Nephrol Dial Transplant*. 2004;19:1142–1148.
9. Ponticelli C, Altieri P, Scolari F, et al. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 1998;9:444–450.
10. Praga M, Barrio V, Juarez GF, et al. Tacrolimus monotherapy in membranous nephropathy: a randomized controlled trial. *Kidney Int*. 2007;71:924–930.
11. Jha V, Ganguli A, Saha TK, et al. A randomized, controlled trial of steroids and cyclophosphamide in adults with nephrotic syndrome caused by idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2007;18:1899–1904.
12. Praga M. Response to "Tacrolimus in membranous nephropathy". *Kidney Int*. 2008;74:824.
13. Nankivell BJ, Borrows RJ, Fung CL, et al. The natural history of chronic allograft nephropathy. *N Engl J Med*. 2003;349:2326–2333.
14. Ramachandran R, Kumar DA, Nada R, et al. Chronic nephrotoxicity limits successful use of Tacrolimus in the management of adult steroid-dependent minimal change disease. *Nephrology (Carlton)*. 2015;20:384–385.
15. Ponticelli C, Zucchelli P, Passerini P, et al. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int*. 1995;48:1600–1604.
16. Jarrell J, Lai EV, Barr R, et al. Ovarian toxicity of cyclophosphamide alone and in combination with ovarian irradiation in the rat. *Cancer Res*. 1987;47:2340–2343.