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Randomised, Controlled Trial on the Efficacy of Reduced-Dose Cyclical Corticosteroids and Cyclophosphamide in Primary Membranous Nephropathy

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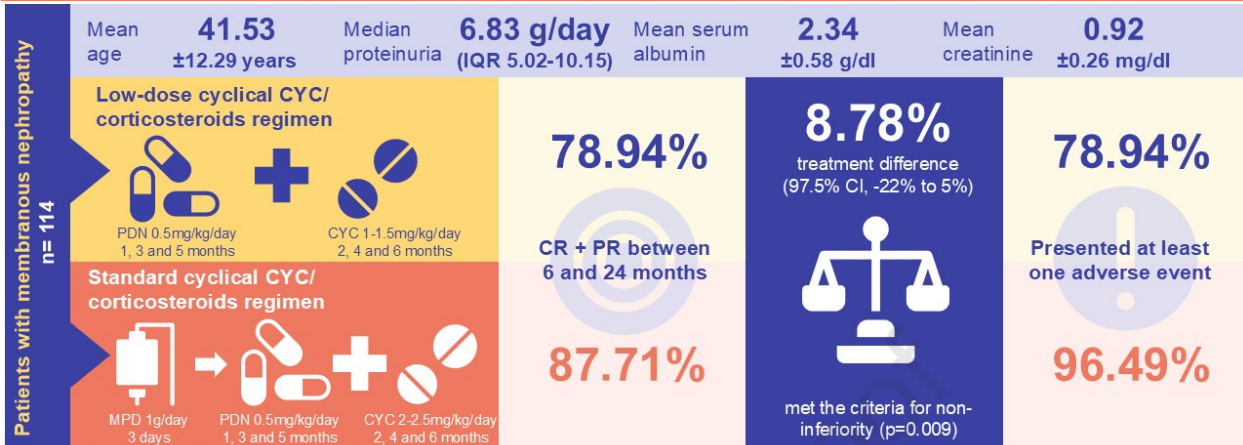
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Randomised, Controlled Trial on the Efficacy of Reduced-Dose Cyclical Corticosteroids and Cyclophosphamide in Primary Membranous Nephropathy



CYC, cyclophosphamide; MPD, methylprednisolone; PDN, prednisolone; CR, complete remission; PR, partial remission

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Conclusion Low-dose cyclophosphamide/corticosteroids is non-inferior to the standard-dose of cyclical cyclophosphamide/corticosteroids in achieving proteinuria remission in MN.

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Abstract

membranous nephropathy (MN) is associated with significant safety concerns. Several retrospective studies suggest that a lower dose may effectively induce remission in MN participants. This randomized trial assessed whether the lower dose of cyclical cyclophosphamide/corticosteroids was non-inferior to the standard-dose in patients with MN.

Methods: We randomly assigned 114 participants (32 women and 82 men) to receive either the low-dose (prednisolone-0.50 mg/kg/day during months-1,3, and 5, along with oral CYC-1.0-1.5 mg/kg/day during months-2,4 and 6) or the standard-dose cyclophosphamide/corticosteroids (Methylprednisolone-1 g/day for 3 days, followed by prednisolone-0.50 mg/kg/day during months-1,3, and 5, along with oral CYC-2.0-2.5 mg/kg/day during months-2,4 and 6). The primary outcome- complete (CR) or partial remission (PR) of proteinuria between months 6 and 24.

Results: A total of 57 participants were randomized into each group. The mean age was 41.53±12.29 years, median proteinuria, mean serum albumin, and creatinine levels were 6.83 g/day (IQR 5.02-10.15), 2.34±0.59 g/dl and 0.92±0.33 mg/dl were comparable between groups. In the intention-to-treat analysis, 45 participants (78.94%) in the low-dose group and 50 participants (87.71%) in the standard-dose group achieved the primary outcome (treatment difference-8.78 %, confidence interval(CI), -0.24 to 0.067), and met the criteria for non-inferiority (p=0.009). Forty-five (78.94%) and 55 (96.49%) participants receiving the low-dose and standard-dose cyclophosphamide/corticosteroids experienced at least one adverse event (difference -17.54 % CI- 1.52% to 33.45%).

Conclusion: The results suggest that low-dose cyclophosphamide/corticosteroids may be as effective as the standard-dose of cyclical cyclophosphamide/corticosteroids in achieving proteinuria remission in MN.

Keywords: Membranous nephropathy, nephrotic syndrome, modified Ponticelli regimen, cyclophosphamide, prednisolone, corticosteroids.

Introduction

diabetic adults. Up to one-third of patients experience spontaneous remission, while a significant majority require immunosuppressive medications to achieve clinical remission (1, 2). The Kidney Disease Improving Global Outcomes (KDIGO) categorizes the risk of MN progression from “low” to “very high” based on factors such as glomerular filtration rate (GFR), proteinuria, urinary biomarkers, and anti-M-type phospholipase A2 receptor (PLA2R) antibody levels (3). KDIGO guidelines advocate using lifestyle measures, blood pressure control, and renin-angiotensin-aldosterone system inhibition for all, restricting the use of immunosuppressive therapy in patients at moderate to very high risk of deterioration of kidney function (3). The immunosuppressive agents recommended for MN include cyclic corticosteroids plus cyclophosphamide, Rituximab, and calcineurin inhibitors (CNI) (2-4).

Cyclical alkylating agents (cyclophosphamide or chlorambucil)/corticosteroids is the only treatment regimen shown to prevent the development of end-stage kidney disease (ESKD) or reduce mortality (5, 6). The other advantage of this regime is that it is inexpensive. Yet its practice is restricted owing to concerns about toxicities like increased risk of infections, gonadal (7) and bone marrow toxicity, and malignancies (8). The cumulative dosage of corticosteroids administered for treating MN exceeds that used for managing conditions such as vasculitis (9) or kidney allograft rejection (10), making it among the highest in kidney diseases. Rituximab has emerged as a potential alternative (11-14). Despite the availability of biosimilars leading to cost reductions, it remains unaffordable for a significant proportion of the global population, as treatment expenses are often not covered by public healthcare systems in lower-income countries, pushing patients toward catastrophic health expenditures(15). Also, the uncertainties regarding its effectiveness in individuals with high anti-PLA2R titers (16) limit its adoption as first-line therapy over cyclical cyclophosphamide/corticosteroids.

Given that a quarter of the global population lives below the poverty threshold (17), the cheaper cyclophosphamide/corticosteroids treatment is likely to remain the preferred option for managing MN in resource-limited settings in the foreseeable future. The toxicities associated with cyclophosphamide/corticosteroids are often dose-related, and in real-world experience, the dosing of cyclical cyclophosphamide/corticosteroids is quite variable (18). This includes using a reduced methylprednisolone dose, 250 to 500 mg per infusion. Multiple observational studies on intravenous cyclophosphamide/corticosteroids indicate that proteinuric remission occurs in approximately two-thirds to three-fourths of patients (19-24). Notably, the doses of both cyclophosphamide/corticosteroids were significantly lower than those used in the standard cyclical cyclophosphamide/corticosteroids regimen. Factoring in the available evidence, it is crucial to determine whether a lower-intensity regimen of cyclical cyclophosphamide/corticosteroids is as effective as the standard therapy in inducing clinical

remission in patients with MN. Therefore, we designed a randomised clinical trial to compare the efficacy of low-dose cyclical cyclophosphamide/corticosteroids regimen with the conventional cyclic regimen in patients with MN with a high risk of disease progression.

Material Methods

Trial design and participants

The multicentric, randomised, parallel-group, open-label, active-controlled trial was conducted at the Department of Nephrology, Postgraduate Institute of Medical Education and Research, Chandigarh, Osmania Hospital in Hyderabad, Command Hospital, Lucknow and Army Hospital Research and Referral New Delhi, India. Individuals between the ages of 18 and 70 with biopsy-proven MN and experiencing persistent nephrotic syndrome (defined below), despite treatment with non-immunosuppressive therapies such as maximally tolerated doses of ACE inhibitors or angiotensin II receptor blockers (ARBs) for at least three months, were considered eligible for inclusion. Additionally, individuals meeting one of the following criteria were also eligible: those with MN and thrombotic complications, those requiring hospitalization for complications of nephrotic syndrome, persistent severe or disabling symptoms or severe hypoalbuminemia (<2 g/dL) that can be included before the completion of 3-month observation period at the discretion of the investigator independent of the proteinuria value. Participants with an estimated glomerular filtration rate (eGFR) below 45 ml/min/ 1.73m^2 , a history of or ongoing infections including hepatitis B/C and HIV, presence of anti-nuclear factor positivity, malignancy-related MN, evidence of significant tubular atrophy and interstitial fibrosis, pre-existing diabetes mellitus, abnormal liver function tests, secondary MN, and those who had received any immunosuppressive therapy within the preceding 12 months were excluded (Supplemental File). Approval was obtained from authorized ethics committee at each participating center, and the trial was registered at the Clinical Trials Registry India (CTRI/2019/09/021287).

Intervention

All eligible participants were assigned randomly to receive either the low-dose cyclical cyclophosphamide/corticosteroids regimen, which consisted of oral prednisolone at a dose of 0.5 mg/kg per day for 30 days in the first, third, and fifth months, and oral cyclophosphamide at a dose of 1.0 - 1.5 mg/kg per day in the second, fourth, and sixth months or the standard cyclical cyclophosphamide/corticosteroids regimen (methylprednisolone 1 g/day in 100 mL normal saline administered over 45 minutes for three consecutive days, followed by oral prednisolone at a dose of 0.5 mg/kg per day for 27 days in the first, third, and fifth months, along with oral cyclophosphamide at a dose of 2.0 - 2.5 mg/kg per day in the second, fourth, and sixth months.

Randomisation

Using computer-based random numbers, participants were randomly assigned to one of the labelled sealed envelopes containing the specified intervention. The author who performed the randomization did not participate in the enrolment and allocation of treatment to the participants. Neither the participants, the treating physicians, nor the individuals assessing clinical outcomes and analysing data were blinded to the treatment assignments.

Follow-up and outcomes

Participants were monitored every four weeks (± 1 week) for the first six months, followed by assessments every 12 weeks until the 24 months. Data collected at baseline included blood counts, serum creatinine, serum albumin, urine analysis, 24-hour urine protein levels, lipid profile, and anti-PLA2R titers. Complete blood counts, serum creatinine and albumin, urine routine examination and proteinuria were performed at each follow-up. Serum anti-PLA2R titers were repeated at the end of the 3rd, 6th and 12th months. Participants received symptomatic treatment as required including diuretics for oedema, calcium supplements, cotrimoxazole prophylaxis for *Pneumocystis jirovecii* and statins.

Primary outcome was first complete (CR) or partial remission (PR) between months 6 and 24. *Secondary outcomes* were CR and PR rates at 6, 12, and 18 months; anti-PLA2R titers at various intervals; and safety profile.

Sample size

The sample size is based on both clinical outcomes and safety profiles. In determining the sample size, based on our and previous experience, we assumed that 80% (11, 13, 25) of the patients in both groups would achieve clinical remission at the end of 24 months. We set a non-inferiority margin of 25 percentage points for the relative difference in remission rates, using a one-sided alpha level of 0.025. The margin was set by considerations of convenience (elimination of intravenous injections), reduced adverse effects (lowered immunosuppressive dosing), and cost savings (avoidance of pulse methylprednisolone injections) and efficacy in response to other first-line therapy (rituximab, 60%) (11, 12). Considering a dropout rate of 5% (based on our experience with MN) and a statistical power of 90%, it was necessary to enrol 57 participants per group to establish non-inferiority.

Definitions

Nephrotic syndrome was defined as proteinuria (24-hour quantification) > 3.5 g/day, or ≥ 2.5 g/day with a serum albumin < 2.5 g/dl (5, 26). CR was defined as proteinuria less than 500 mg/day, with normal serum albumin (≥ 3.5 g/dL) and stable serum creatinine (26) and PR as proteinuria levels ranging from 500 mg/day to less than 3.5 g/day and less than 50% of baseline, alongside serum albumin levels of ≥ 3.0 g/dL and stable serum creatinine levels. Those

who didn't achieve either a CR or PR by 24 months, died or required additional treatment were
(Medizinische Labordiagnostika AG, Lübeck, Germany) exceeding 14 RU/mL. Immunological
remission: Anti-PLA2R titers <14 RU/ml. Recurrence of nephrotic syndrome following any form
of remission, occurring after a period of at least 6 months, was classified as relapse.

Statistical analysis

Continuous data are presented as either mean $\pm$ standard deviation (SD) or median with
interquartile range (IQR), depending on the distribution. Qualitative or categorical variables are
described using frequencies and proportions. The primary analysis was conducted based on the
intention-to-treat (ITT) principle, regardless of therapy adherence to assess the difference in
the percentage points with 97.5% confidence interval (CI). Primary outcome was compared
between the low-dose cyclical cyclophosphamide/corticosteroids regimen and standard-dose
cyclical cyclophosphamide/corticosteroids regimen using Wald test with a non-inferiority
margin (delta) of 0.25, as stated above. For missing data, the ITT analysis was performed with
the last value carried forward. An additional non-inferiority analysis was conducted as a
secondary assessment of the primary outcome specifically for the per-protocol population, and
of the secondary outcome, which was remission achieved without the need for further
immunosuppressive treatment in the intention-to-treat group.

For the remaining secondary analyses, participant characteristics were assessed using the Chi-
square test or Fisher's exact test for categorical variables, and the T-test, Mann-Whitney U test,
or Wilcoxon rank-sum test for continuous variables, as appropriate. Subgroup analyses of the
primary outcome were conducted to assess whether the treatment effects differed across
subgroups defined by baseline characteristics, including age, albumin levels, proteinuria,
creatinine, and anti-PLA2R status. All statistical tests were two-tailed, and p-values < 0.05 were
regarded as statistically significant. Statistical analysis was performed using GraphPad Prism 10
(San Diego, California, USA) and R Statistical Software (R version 4.2.3).

Results

Between September 2019 and September 2021, a total of 140 participants were enrolled in the
study. Of these, 114 who fully met both the inclusion and exclusion criteria were randomly
assigned to either the low-dose or standard-dose cyclical cyclophosphamide/corticosteroids
groups in a 1:1 ratio (**Figure 1**). The mean age of the participants was 41.53 ± 12.29 years. The
study included 32 females (28.07%) and 82 males (71.93%). The median proteinuria was 6.83
gm/day (IQR 5.02, 10.15). The mean albumin and serum creatinine were 2.34 ± 0.59 g/dl and
 0.92 ± 0.33 mg/dl, respectively. A total of 86 (75.43%) participants had PLA2R-related MN, with a
median titer of 161 (78, 305) RU/ml. Forty-five participants (52.32%) exhibited an anti-PLA2R
antibody titer exceeding 150 RU/ml.

participants (7.02%) had relapsing disease (all within last 24 months). All relapsed cases had undergone immunosuppressive therapy over a year before their current treatment (median 29 months, IQR 13.25,58.50). Twenty-seven (23.7%) participants were hypertensive. One hundred twelve (98.24%) were on ACEi/ARBs. One participant in the low-dose group developed diabetes mellitus two months into the therapy. Consequently, the treatment was discontinued. However, the participant continued follow-up. Six participants were lost to follow-up.

The average prednisolone dose was 3.11 ± 0.60 gm in the low-dose group and 13.43 ± 1.31 gm in the standard-dose group (steroid equivalent 1 mg methylprednisolone = 1.2 mg of prednisolone). The mean dose of cyclophosphamide was 7.767 ± 2.423 gm in the low-dose group and 12.19 ± 2.24 gm in the standard-dose group. The eGFR at baseline was 89.34 ± 27.24 ml/min/1.73m². Baseline parameters were comparable between the groups (**Table 1**).

Primary outcome

In the ITT analysis, 45 (78.94%) participants in the low-dose group and 50 (87.72%) in the standard-dose group achieved the primary outcome; risk difference 8.78 percentage points (1-sided 97.5% CI, -22% to 5%) between groups, and the lower limit of the 1-sided 97.5% confidence interval of the difference met the criterion for noninferiority ($P=0.009$) (**Figure 2**).

In the per-protocol analysis (a total of 104 participants were analyzed, with 52 in each group). Six participants were lost to follow-up before completing six months of therapy, three participants died before completing six months, and treatment was discontinued for one participant due to diabetes mellitus. A total of 43 participants (82.69%) in the low-dose group and 49 (94.23%) in the standard-dose group achieved the primary outcome (risk difference 11.54%, 97.5% CI -14% to 8%, p for non-inferiority = 0.01).

Secondary outcomes

At 24 months, 38 (66.67%) and 40 (70.17%) participants in the low-dose and standard-dose group achieved relapse-free remission (risk difference, 3.50% 97.5% CI -5% to -2%, p-value for non-inferiority = 0.007) (**Supplemental Figure 1**). In the per-protocol analysis, 32 participants (61.53%) in the low-dose group and 36 participants (69.23%) in the standard-dose group achieved and maintained remission without the need for additional immunosuppressive treatment (risk difference, 7.69% 97.5% CI -11% to -4%).

At 6 months, 37 (64.91%) in the low-dose group and 42 (73.68%) in the standard-dose achieved any remission (risk difference 8.77%, 97.5% CI -11.30 to 20.00%). At 12 months, 41 (71.92%) in

the low-dose group and 49 (85.96%) in the standard-dose achieved any remission between 1
2
3
4 The CR and PR between both groups at different time points are shown in **Supplemental Figure**
5 **2a-d/ Supplemental Figure 3**. At 12 months, 13 patients (22.80%) in the low-dose group and 15
6 (26.31%) in the standard-dose group were in CR, with a difference of 3.51 percentage points
7 (97.5% CI -5.6% to -1.4%) (**Supplemental Figure 2b**). At 24 months, 21 individuals (36.84%) in
8 the low-dose group and 24 individuals (42.10%) in the standard-dose group were in CR, with a
9 difference of 5.26% (97.5% CI -24% to 13%) (**Supplemental Figure 2d**). At 12 months, 28
10 (49.1%) participants in the low-dose group and 34 (59.6%) participants in the standard-dose
11 group were in PR, respectively, with a difference of 10.5% (97.5% CI: -28.7% to 7.7%). At 24
12 months, 24 (42.1%) participants in the low-dose group and 26 (45.6%) participants in the
13 standard-dose group were in PR, respectively, with a difference of 3.5% (97.5% CI: -21.7% to
14 14.7%).

15
16 The median proteinuria declined from 6.77 (5.14,9.25) to 0.75 (0.19,3.11) g/day in the low-dose
17 group (difference -5, CI -6.82 to -3.9) and from 6.93 (4.68,11.00) to 0.85 (0.18,1.71) g/day in the
18 standard-dose group (difference -5.1 CI -6.97 to -3.83) at 24 months (**Figure 3a**). The mean
19 serum albumin increased from 2.39±0.56 to 3.73±0.62 g/day in the low-dose group (difference
20 1.34, CI 1.11 to 1.57) and from 2.28±0.60 to 3.82±0.83 g/day in the standard-dose group
21 (difference 1.50, 1.17 to 1.83) at 24 months (**Figure 3a**). Compared to baseline, there was no
22 change in the eGFR at 24 months in either group (**Figure 3b**). The median anti-PLA2R decreased
23 from 162 (68 to 251) to 2 (2 to 35) RU/ml in the low-dose group (difference 102, CI 47 to 196)
24 and from 161 (87 to 397) to 6 (2 to 15) RU/ml in the standard-dose group (difference 129, CI 48
25 to 199) at 24 months (**Figure 3c**). There was no significant difference in serological remission
26 rates between the groups at 3, 6, or 12 months (**Supplemental Table 1**). There was no
27 significant difference between the groups based on the prespecified variables: age, proteinuria,
28 serum albumin, serum creatinine, and anti-PLA2R levels (**Supplemental Figure 4**).
29

30 *Relapse*

31 By the end of two years, 24 (11 in low-dose and 13 in standard-dose group) participants
32 (25.26%) who initially achieved remission experienced a relapse of nephrotic syndrome.
33

34 After 9 months, 13 (11.40%) participants were administered rituximab due to either treatment
35 failure or a relapse of nephrotic syndrome. All the participants who received rituximab for
36 treatment failure were considered as non-responders. Two participants in the low-dose group
37 had a decline in eGFR to <30 ml/min/1.73 m², both participants were non-responders. Details of
38 participants who experienced relapse of nephrotic syndrome persisting until month 24 or who
39 required second-line therapy for relapse are provided in **Supplemental Table 2**.

A total of 45 (78.94%) and 55 (96.49%) participants receiving the low-dose and standard-dose regimens experienced at least one adverse event (percentage difference 17.54% CI 1.52% to 33.45%) (**Table 2**). Participants receiving the standard-dose regimen experienced a higher incidence of adverse effects than those on the low-dose schedule (186 events versus 156 events). The majority of these events were non-serious. The occurrence of drug-related adverse effects was numerically lower in the low-dose group compared to the standard-dose group (**Supplemental Table 3**).

Two participants in the low-dose group and four participants in the standard-dose group experienced serious adverse events (SAEs). In the low-dose group, one participant required hospitalization for severe anaemia associated with sickle cell disease and was subsequently lost to follow-up. The other participant needed admission for glycaemic control, was initiated on insulin, and had further therapy withheld. In the standard-dose group, four participants experienced SAEs: two developed bacterial pneumonia, which required intravenous antibiotics; another participant developed cellulitis, which was also successfully treated with intravenous antibiotics; and one participant was diagnosed with pulmonary tuberculosis. Specifically, more participants in the standard-dose group experienced leukopenia, upper respiratory tract infections (URTI)/pneumonia, other infections, gastritis, and bone pain. In contrast, the incidence of diabetes mellitus and headaches was higher in the low-dose group. In female participants aged ≤ 45 years, four (50%) and two (40%) participants attained premature menopause in the low-dose and standard-dose, respectively. Three participants died during the study. One participant, a 21-year-old with no prior history of cardiac conditions, died suddenly at home, likely due to a suspected cardiac event (low-dose group), potentially pulmonary thromboembolism or stroke. The participant had been on prophylactic anticoagulation. The other two participants (standard-group) succumbed to severe infections: one developed right-sided bronchopneumonia, resulting in septic shock, while the other suffered from cellulitis, which also progressed to septic shock. The majority of adverse events occurred within the first 6 months.

Discussion

The study suggests that low-dose cyclical cyclophosphamide/corticosteroids is non-inferior to standard-dose cyclophosphamide/corticosteroids in terms of clinical remission in patients with MN at a moderate-high risk of disease progression. Participants in the low-dose group experienced fewer adverse effects. However, there was no difference in the incidence of serious adverse effects between the two groups.

Even though the seminal RCTs of cyclophosphamide/Prednisolone used a specific dose not based on any specific rationale (27). In practice, the dosage and frequency of administration of cyclophosphamide/corticosteroids have varied around the world (18). In a global survey, *Bose et al.* found that *only* one-third of patients were prescribed cyclophosphamide/corticosteroids in accordance with the modified Ponticelli regimen(18). This finding suggests that nearly two-thirds of patients receive cyclophosphamide/corticosteroids at doses that deviate from the standard cyclical regimen. It is possible, therefore, that an optimal clinical response may be achieved with a lower dose at a lower adverse event risk, which could result in greater confidence in using it safely. We eliminated the methylprednisolone pulse and reduced the cyclophosphamide dose, thereby achieving a 73% reduction in corticosteroid dosage and a one-third reduction in cyclophosphamide dosage compared to the standard protocol.

The present study represents the first systematic effort to evaluate the cyclical use of low-dose oral cyclophosphamide/corticosteroids in patients with membranous nephropathy. At least six studies have reported favourable outcomes with reduced doses of cyclophosphamide and corticosteroids in patients with primary MN. Three-fourths of the patients treated with the low-dose regimen responded by the end of one year after initiating therapy. Most low-dose strategies involving cyclophosphamide utilize intravenous preparations and are retrospective in nature and they are summarised in Table 3 (19-24). The remission rates in the low-dose group of the present study are comparable to those of previous studies, despite a lower cumulative dose of corticosteroids. The response rates in the standard-dose group align with our previous observation(25), the RI-CYCLO(13) (multicenter randomized, open-label study comparing rituximab with a modified Ponticelli regimen in adults with primary MN) and STARMEN (11) (multicenter, open-label, randomized controlled study comparing sequential tacrolimus–rituximab therapy with a modified Ponticelli regimen in adults with biopsy-proven primary membranous nephropathy) trials. Although the CR rates are lower than those observed in our previous study and the STARMEN trial, they are comparable to those in the RI-CYCLO trial and other intravenous cyclophosphamide or low-dose steroid regimens(13, 19, 21, 22, 24). Notably, a substantial proportion of participants achieved proteinuria levels below 1 gram. One needs to interpret these results with caution as cross-trial comparisons may be inappropriate, as the patients had different racial and ethnic profiles, varying risks of infection, and different standards of care in their management.

About one-fifth of participants in the low-dose and standard-dose group relapsed. In the RI-CYCLO trial(13), 22% of participants relapsed after achieving remission between months 6 and 24, whereas the STARMEN trial reported a relapse rate of only 2.7%(11).

Over 50% of the participants had anti-PLA2R > 150 RU/ml(16), often associated with the study was comparable to the STARMEN trial(11), and better than that observed in the RI-CYCLO trial(13) (50-60%). The temporal trends in anti-PLA2R levels were similar between the two groups. Immunological remission was achieved more rapidly in participants treated with the standard dose than the low dose. This may be attributed to the higher corticosteroid dose (rather than cyclophosphamide) used in the standard regimen, which could have led to more rapid remission. This finding *may* encourage us to use an adaptive approach, where one would initiate treatment at a higher dose and tapered rapidly once immunological remission is achieved. Non-responders exhibited higher baseline anti-PLA2R titers, consistent with existing evidence.

The low-dose regimen demonstrated a numerically lower incidence of adverse effects compared to the standard-dose regimen. The most common side effect was URTI, which was numerically higher in the standard-dose group compared to the low-dose group. Overall, the rates of SAEs were low, with no differences between groups. However, the standard-dose group experienced more severe SAEs, predominantly involving hospitalizations due to serious infections. Similar observations have been reported in the STARMEN and RI-CYCLO trials, where no differences in SAEs were found between the groups. Another crucial aspect to address is the three deaths observed in the study: two were infection-related, and one was attributed to suspected sudden cardiac death. The infection-related fatalities were likely due to the combined effect of the nephrotic state and immunosuppressive therapy. In the recently completed clinical trials, the RI-CYCLO trial reported one death due to lung cancer. We did not encounter any malignancies in contrast to the RI-CYCLO and STARMEN trial, the difference could be related to the relatively younger population in our study (41 year versus 55 years). Our previous study also showed that the incidence of SAEs with cyclical cyclophosphamide/corticosteroids was comparable to tacrolimus-based therapy (26). Over 40% of participants under 45 years of age experienced menopause with either therapy. Nearly all participants over 35 years attained menopause, suggesting that even a lower dose of cyclophosphamide is gonadotoxic, which remains a significant concern, as MN predominantly affects middle-aged individuals. Hence, gonadotoxicity will continue to be an important issue. The incidence of leucopenia is similar to the STARMEN trial (11). The standard dosing regimen is frequently criticised for its potential long-term toxicity, particularly affecting rapidly dividing cells in the body. Having stated the above, we need to be mindful of the fact that the SAE were not different between both the groups and patients with

Given the variability in cyclophosphamide/corticosteroids dosing in patients with MN, this randomized study offers valuable insights. Considering the comparable efficacy and potential long-term safety (relative to standard-doses), the low-dose cyclical

cyclophosphamide/corticosteroids regimen presents a viable option for patients with predominantly treatment-naïve patients—rather than those with relapsing disease—and the exclusion of non-responders to rituximab or calcineurin inhibitors (CNIs) are notable strengths. Despite its strengths, the study has some limitations, including the limited generalizability, lack of patient-reported outcomes, core outcome measures, lack of blinding for patients, treating nephrologists, and data analysts, as well as a relatively short period of follow-up of only two years and the unavailability of anti-PLA2R in a few patients. There are a few additional questions that remain unanswered. The first relates to interpreting non-inferiority clinical trials—many such trials in the past have failed to demonstrate the same effect in real-world settings. Therefore, although this trial shows non-inferiority of the low-dose therapy compared to the standard-dose regimen, its findings will require validation in real-life scenarios.

Although prescribing high-dose immunosuppressive therapy is generally avoided across various clinical conditions, it is worth noting that the incidence of SAEs was not significantly different between the two groups. Furthermore, we are uncertain whether these results can be extrapolated to patients at very high risk of progression. These patients may benefit more from standard-dose therapy, as the rapidity of response could impact long-term kidney function.

In conclusion, the study results signal that low-dose cyclophosphamide/corticosteroids *may* be comparable to standard cyclophosphamide/corticosteroids in achieving proteinuria remission in patients with membranous nephropathy.

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Data statement: The individual participant data (anonymised) will be shared on request 3
months following article publication. Data will be provided to researchers whose proposed use
has been approved by an independent review committee and after obtaining a no-objection
certificate from the PGIMER ethics committee. Data requests should be submitted to the
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statistical inputs and manuscript preparation, Ritambhra Nada: Pathology expert and
manuscript preparation, Manish Rathi/Harbir Kohli: Clinical follow-up and expert inputs.

Supplementary Material

Supplemental File: Protocol

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Supplemental Table 2: Details of the patients who had a relapse of nephrotic syndrome

Supplemental Table 3: Adverse events based on the association with immunosuppressive therapy

Supplemental Figure 1: Kaplan-Meier curves for relapse-free remission between both the groups

Supplemental Figure 2: Complete remission (CR), partial remission (PR), non-responders and relapse at various time points

Supplemental Figure 3: Kaplan-Meier curves for Complete (left) and Partial remission (right)

Supplemental Figure 4: Pre-specified subgroup analysis of the composite outcome (complete or partial remission) at 24-month follow-up

CONSORT CHECKLIST
(PDF)

Supplementary information is available at KI Report's website.

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1 **Table 1: Demographic and clinical characteristics at baseline**

	Total	Low-dose	Standard-dose
	(n=114)	(n=57)	(n=57)
Age (years)	41.53 ± 12.29	41.39 ± 13.08	41.67 ± 11.57
Gender (F:M)	32:82	17:40	15:42
Proteinuria (g/day)	6.83(5.02-10.15)	6.77(5.14-9.25)	6.93(4.68-11)
Serum albumin (g/dl)	2.34 ± 0.58	2.39 ± 0.56	2.28 ± 0.60
Serum creatinine (mg/dl)	0.92 ± 0.26	0.90 ± 0.25	0.94 ± 0.28
Total cholesterol (mg/dl)	250.0 (208,307.5)	267.9 (218.7, 305.9)	247.0 (192.3, 308.5)
Systolic BP (mm Hg)	125.60 ± 13.44	126.90 ± 13.70	124.2 ± 13.15
Diastolic BP (mm Hg)	79.31 ± 10.46	80.53 ± 7.74	78.0± 12.5
Weight (kg)	69.99 ± 13.96	69.31 ±14.60	70.68 ± 13.3
PLA2R positive	86 (75.43%)	44 (77.19%)	42 (73.68%)
Anti-PLA2R (RU/ml)	161 (78,305)	162 (68,251)	161 (87,397)
Anti-PLA2R (RU/ml)			
<14	28 (24.56%)	13 (22.80%)	15 (26.31%)
14-50	14 (12.28%)	08 (14.03%)	06 (10.52%)
50-100	13 (11.40%)	07 (12.28%)	06 (10.52%)
100-150	14 (12.28%)	06 (10.52%)	08 (14.03%)
>150	45 (39.47%)	23 (40.35%)	22 (38.59%)

2 F- Female, M- Male, PLA2R- M-Type Phospholipase A2 receptor, BP- blood pressure

3

1 **Table 2: Safety profile**

	Low-dose	Standard-dose
Serious adverse events		
<i>Fatal</i>	01 (1.75)	02 (3.50)
<i>Non-Fatal</i>	05 (8.77)	04 (7.01)
Diabetes Mellitus	07 (12.28)	03 (5.26)
Anaemia	09 (15.78)	11 (19.29)
Leucopenia	06 (10.52)	14 (24.56)
Respiratory tract infection		
<i>URTI</i>	17* (29.82)	22 [§] (38.59)
<i>LRTI</i>	05 (8.77)	03 [^] (5.26)
<i>Tuberculosis</i>	None	01 (1.75)
Anxiety	09 (15.78)	11 (19.29)
Weakness	09 (15.78)	13 (22.80)
Headache	18 (31.57)	15 (26.31)
Reversible kidney injury	01 (1.75)	01 (1.75)
Body pain	09 (15.78)	17 (29.82)
Bone pain	20 (35.08)	28 (49.12)
Hypotension	07 (12.28)	07 (12.28)
Hypertension	08 (14.03)	06 (10.52)
Gastritis	12 (21.05)	16 (28.07)
Menopause (age ≤45 years)	4 out of 8 (50)	2 out of 5 (40)
Other infections		
<i>Gastroenteritis</i>	03 (5.26)	05 (8.77)
<i>Tinea</i>	02 (3.50)	01 (1.75)
<i>UTI</i>	03 [#] (5.26)	04 [#] (7.01)
<i>Urogenital</i>	01 (1.75)	None

2 Serious adverse events: Any adverse effect resulting in death, being life-threatening, or necessitating inpatient
3 hospitalization.
4 The value in bracket suggest percentage, URTI- upper respiratory tract infection, LRTI- lower respiratory tract
5 infection, UTI+- urinary tract infection.
6 * 3 patients had URI twice, # 1 patient had UTI twice, § 1 patient had URI twice, ^ 3 patients had both URI and LRTI
7 UTI- urinary tract infections, URI- upper respiratory tract infection, LRTI- lower respiratory tract infection.
8 Other infections: *Gastroenteritis 03, Tinea- 02, Urogenital infections- 01. **Gastroenteritis- 05, Cellulitis- 01,
9 Tinea- 01.
10 Leukopenia: Defined as a leukocyte count of less than 4,000/μL. For patients with an absolute neutrophil count
11 above 1,500/μL, a 25% dose reduction was required in the subsequent month. Premature menopause: Defined as
12 the cessation of menstruation for a period of 6 months or longer in a patient with a previously regular menstrual
13 cycle. Gastritis: Characterized by abdominal pain that responded to treatment with proton pump inhibitors, with
14 normal findings on ultrasonography. Hypertension: blood pressure of >140/90 mm Hg, which is new onset or
15 worsening of previous hypertension. Hypotension: blood pressure of <100 mm Hg (systolic) with symptoms. Bone
16 pain was defined as diffuse, dull, aching pain centred around bones such as the hips, pelvis, spine, and ribs.
17 Weakness was defined as a lack of strength in performing daily activities.

Table 3: Summary of studies on lower doses of cyclophosphamide and corticosteroids

Reference	Numbers	Regimen	Follow-up (months)	CR	PR	Total	Relapse
Rao et al. 2019 (24)	41	IV MP 500 mg X3 (month 1,3, and 5) followed by 0.5 mg/kg of oral prednisolone + oral cyclophosphamide 2 mg/kg/day (month 2, 4 and 6)	06	25.8%	45.2%	71%	NA
Stefan et al. 2022 (19)	41	IV cyclophosphamide 600 mg 4 weekly for 6 months+ oral Prednisolone 0.75 mg/kg with tapering to 0.5 mg/kg at 3 months and 0.1 mg/kg by 6 months	06 12 24	-	-	24% 57% 77%	11%
Mathirani et al. 2017 (20)	41	3 weekly IV cyclophosphamide for 6 months+ Oral Prednisolone 1 mg/kg reduced every two weeks and stopped at 6 months	24	-	-	70%	-
Luzardo et al. 2022 (21)	55	6-month course of alternating steroids (month 1, 3 and 5) and IV cyclophosphamide single dose 15 mg/kg at month 2, 4 and 6	24	42%	29%	71%	27%
Kanigicherla et al. 2016 (23)	17	IV cyclophosphamide at a dose of 600 mg/m ² every 4 weeks, for up to 6 months, in conjunction with oral Prednisolone at a dose of 0.75 mg/kg daily (up to 60 mg/day) – with gradual tapering to 0.5 mg/kg per day by 3 months and 0.1 mg/kg per day by 6 months	12	41%	53%	94%	6%
Yuan et al. 2011 (22)	32	Monthly i.v. cyclophosphamide (500-750 mg/m(2)) and oral prednisone for at least 6 months	15 30*	40.6% 40.6%	40.6% 40.6%	81.2% 81.2%	15.6%

CR- complete remission, PR- partial remission, *median follow-up

Figure legend

Figure 1: Study flow chart

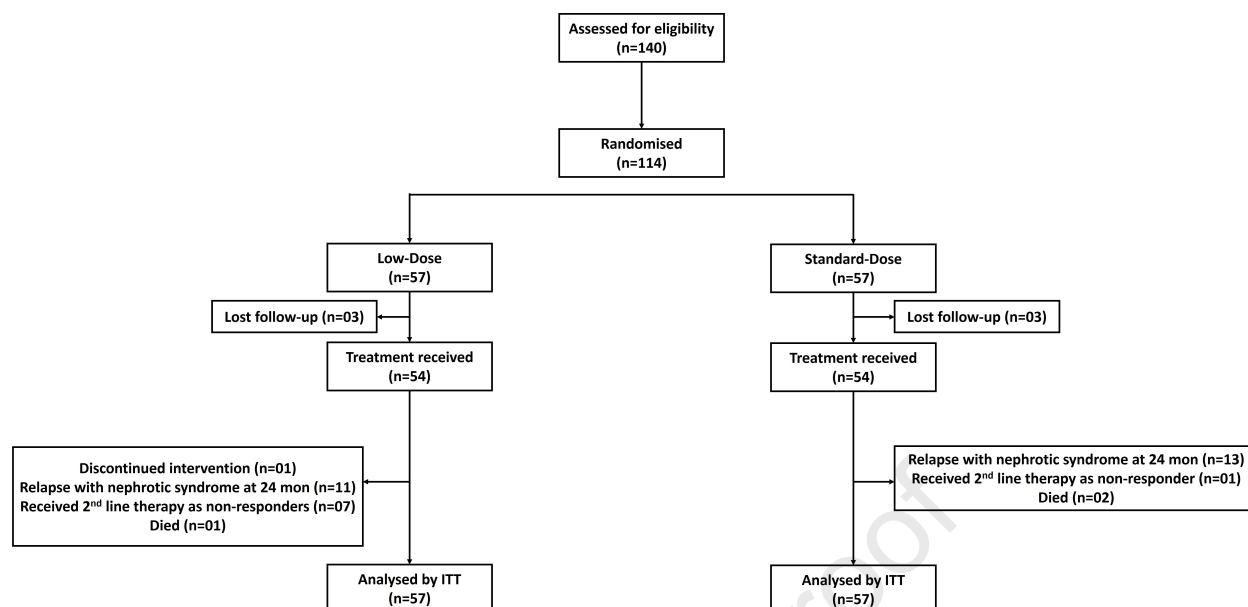
Figure 2: Remission rates at various time points

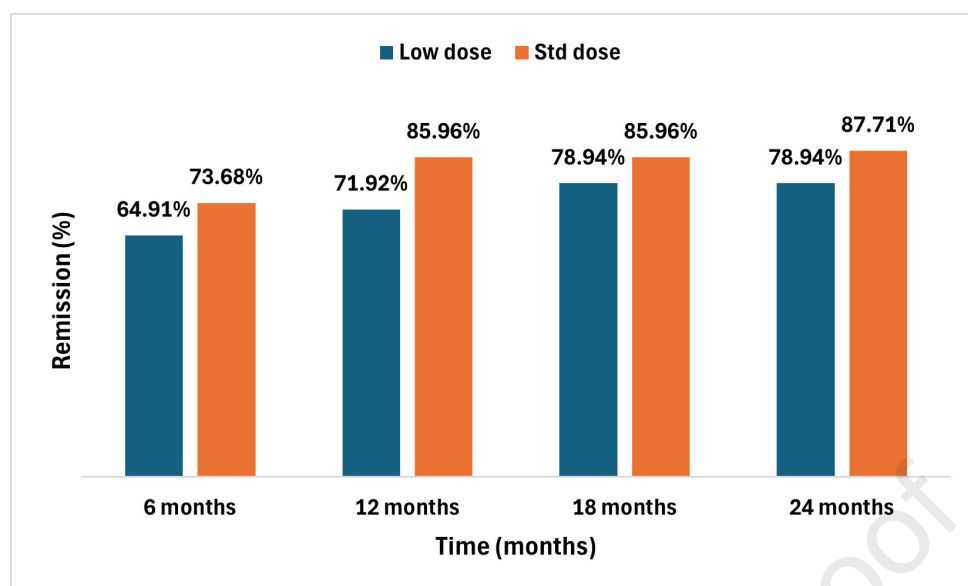
Footnote: 6 mon- risk difference 8.77%, 97.5% CI -11.30 to 20.00%, 12 mon- risk difference 14.04%, 97.5% CI -4.07% to 33.22%, 18 mon- risk difference 7.01%, 97.5% -9.58, 23.97 and 24 mon- risk difference 8.78%, 97.5% CI, -22% to 5%.

Figure 3: a. Proteinuria and serum albumin over time, by assigned treatment (top) b. estimated glomerular filtration rate, by assigned treatment (middle) c. Anti-PLA2R, by assigned treatment (below). Blue: low-dose, and Red: standard-dose.

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