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ORIGINAL ARTICLE

Primary membranous nephropathy in the Italian region of Emilia Romagna: results of a multicenter study with extended follow-up

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Abstract

Background Since primary membranous nephropathy is a heterogeneous disease with variable outcomes and multiple possible therapeutic approaches, all 13 Nephrology Units of the Italian region Emilia Romagna decided to analyze their experience in the management of this challenging glomerular disease.

Methods We retrospectively studied 205 consecutive adult patients affected by biopsy-proven primary membranous nephropathy, recruited from January 2010 through December 2017. The primary outcome was patient and renal survival. The secondary outcome was the rate of complete remission and partial remission of proteinuria. Relapse incidence, treatment patterns and adverse events were also assessed.

Results Median (IQR) follow-up was 36 (24–60) months. Overall patient and renal survival were 87.4% after 5 years. At the end of follow-up, 83 patients (40%) had complete remission and 72 patients (35%) had partial remission. Among responders, less than a quarter (23%) relapsed. Most patients (83%) underwent immunosuppressive therapy within 6 months of biopsy. A cyclic regimen of corticosteroid and cytotoxic agents was the most commonly used treatment schedule (63%), followed by rituximab (28%). Multivariable analysis showed that the cyclic regimen significantly correlates with complete remission (odds ratio 0.26; 95% CI 0.08–0.79) when compared to rituximab ($p < 0.05$).

Conclusions In our large study, both short- and long-term outcomes were positive and consistent with those published in the literature. Our data suggest that the use of immunosuppressive therapy within the first 6 months after biopsy appears

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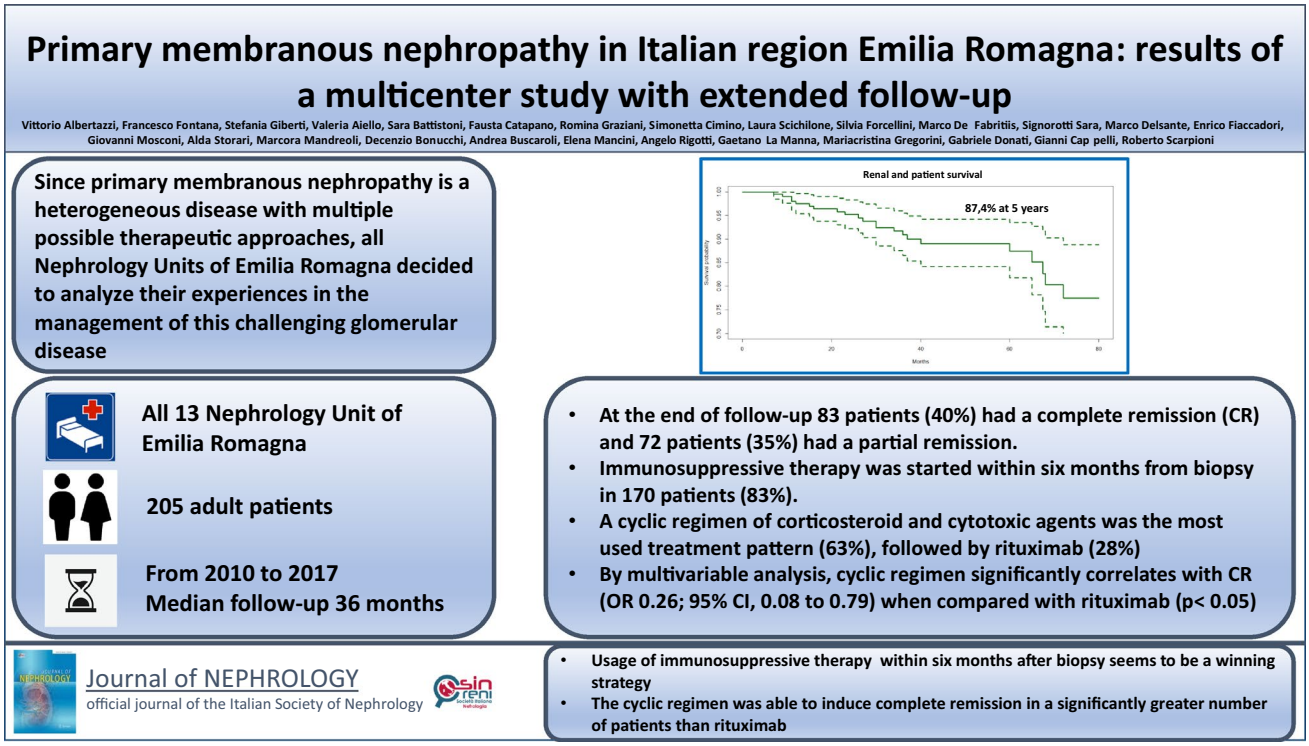
to be a winning strategy, and that the cyclic regimen also warrants a prominent role in primary membranous nephropathy treatment, since definitive proof of rituximab superiority is lacking.

Graphic Abstract



Keywords Primary membranous nephropathy · Patient and renal survival · Remission · Cyclic regimen · Rituximab

Introduction

Membranous nephropathy is the most common cause of nephrotic syndrome in the adult population. Primary membranous nephropathy accounts for about three-quarters of all cases of membranous nephropathy [1]. The natural history of the disease tells us that approximately one-third of untreated patients experience spontaneous remission within the first 2 years after onset; on the other hand, the remaining subjects continue to show persistent proteinuria with slow progression to end-stage kidney disease (ESKD) in 30–40% of cases in a period of 5–15 years [1–5].

Regarding the management of patients affected by primary membranous nephropathy, supportive care is a benchmark of treatment that is based on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, lipid-lowering agents and adequate blood pressure control [6]. Instead, the use of immunosuppressive therapy for primary membranous nephropathy and its timing is still debated, taking into account the benign course in a relevant percentage of patients and the important side effects of immunosuppression, namely infections and cancer onset. Therefore the 2012, and the most recent 2021 "KDIGO clinical practice guidelines for glomerulonephritis" recommend starting immunosuppressive treatment after 6 months of observation and, in any case, only in patients with nephrotic syndrome presenting risk factors of progressive loss of kidney function: eGFR < 60 ml/min/1.73 m^2, decrease in eGFR > 20% within 12 months of diagnosis, proteinuria > 8 g/24 h for more than 6 months, severe or life-threatening symptoms related to nephrotic syndrome [7, 8].

The optimal immunosuppressive treatment of primary membranous nephropathy is still far from being determined [9, 10].

A 6-month cyclic regimen of alternating intravenous and oral glucocorticoids with a cytotoxic drug (chlorambucil or cyclophosphamide) every other month, known as the "Ponticelli regimen", has proven to be effective in preventing ESKD and death [4, 11, 12]. In addition, this therapeutic option significantly increases the rate of remission. Due to potential adverse effects linked to the toxicity of alkylating agents, KDIGO clinical practice guidelines suggest

administering this protocol to patients with nephrotic syndrome and at high risk of progression to ESKD.

Calcineurin inhibitors (cyclosporin and tacrolimus) are considered a valid alternative to cytotoxic-based regimens. Their administration results in a considerably high remission rate, but at the same time their use is associated with a high incidence of relapses after discontinuation; nephrotoxicity is a major concern [13, 14].

Recently, a meaningful breakthrough was the discovery of two types of circulating antibodies against intrinsic podocyte antigens: phospholipase A2 receptor (PLA2R) and thrombospondin-like domain 7A (THSD7A). Antibodies directed to PLA2R and THSD7A are estimated to be present in 50–80% and 2–4% of patients with primary membranous nephropathy, respectively [15, 16]. Some studies clearly showed a direct correlation between anti-PLA2R levels and disease activity; therefore, these autoantibodies are gaining consensus as a useful tool for the diagnosis, prognosis and monitoring of treatment response of primary membranous nephropathy [17, 18].

The discovery of anti-PLA2R antibodies provided proof that B-cell alteration plays a key role in primary membranous nephropathy pathogenesis. Therefore, the use of rituximab received a strong boost due to its more selective action against B lymphocytes compared to other immunosuppressive agents [19]. The first retrospective studies indicated that rituximab can induce remission in 60–80% of treated patients [20, 21]; two further randomized controlled trials (RCTs) established that rituximab performed better than supportive therapy alone and cyclosporine with regard to remission rate [22, 23]. Conversely, in the last years, two RCTs failed to demonstrate the superiority of rituximab use against cyclic regimens in terms of remission rates and safety profile [24, 25].

Nowadays the scenario of the management and treatment of primary membranous nephropathy is more than ever complex and varied. Therefore, the thirteen Nephrology Units in the Italian region of Emilia Romagna decided to collect and share data regarding patients affected by primary membranous nephropathy with the aim of verifying how the management of this glomerular disease is being handled in everyday clinical practice.

Here, we report the results of a multicenter retrospective cohort study with seven years of follow-up, involving 205 patients with biopsy-proven primary membranous nephropathy, who were followed up in Emilia Romagna from 1st January, 2010, to 31st December, 2018.

Patients and methods

Study design and participants

The Scientific Committee of the Gruppo di Studio della Glomerulonefrite Membranosa in Emilia Romagna (GLOMER) planned an observational, longitudinal retrospective cohort study of patients with biopsy-proven primary membranous nephropathy. All thirteen Nephrology Units in Emilia Romagna agreed to participate in the study. From 1st January, 2010 to 31st December, 2018 participating centers collected data of all consecutive patients affected by primary membranous nephropathy. Patients with a diagnosis

Table 1 Baseline characteristics

	All patients ($n=205$)
Age at biopsy (yrs), mean (SD)	61.8 (15.3)
Male sex, *n* (%)	127 (62)
Body weight (kg), mean (SD)	76.9 (15.4)
Body mass index (kg/m^2), mean (SD)	26.5 (4.4)
Blood pressure (mmHg), mean (SD)	
Systolic	133.6 (19.6)
Diastolic	77.1 (9.9)
Cardiovascular diseases, *n* (%)	85 (41.5)
Diabetes, *n* (%)	34 (16.6)
Hypertension, *n* (%)	145 (70.7)
Smoking, *n* (%)	45 (22)
Serum creatinine (mg/dl), mean (SD)	1.1 (0.8)
eGFR[a] (ml/min per 1.73 m^2), mean (SD)	76.9 (29.8)
Urinary protein (g/24 h), median (IQR)	6.1 (3.8–9)
Serum albumin (g/dl), median (IQR)	2.6 (2.3–3.1)
Patients positive for anti-PLA2R[b,c], *n* (%)	43 (66)
Anti-PLA2R levels (RU/ml)[c], median (IQR)	118 (37.5–342)
Nephrotic syndrome, *n* (%)	97 (47.3)
Histologic classification[d], *n* (%)	
Stage I	47 (31)
Stage II	69 (46)
Stage III	28 (18)
Stage IV	6 (4)
Interstitial fibrosis, tubular atrophy[d], *n* (%)	
< 25%	160 (88)
26–50%	19 (10)
> 50%	4 (2)

Data are presented as mean (SD) and median (IQR) for continuous measures, and *n* (%) for categorical measures

[a]eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation

[b]Anti-PLA2R positivity defined as a value > 20 RU/ml

[c]Data are available for 65 patients

[d]Data are available for 150 patients

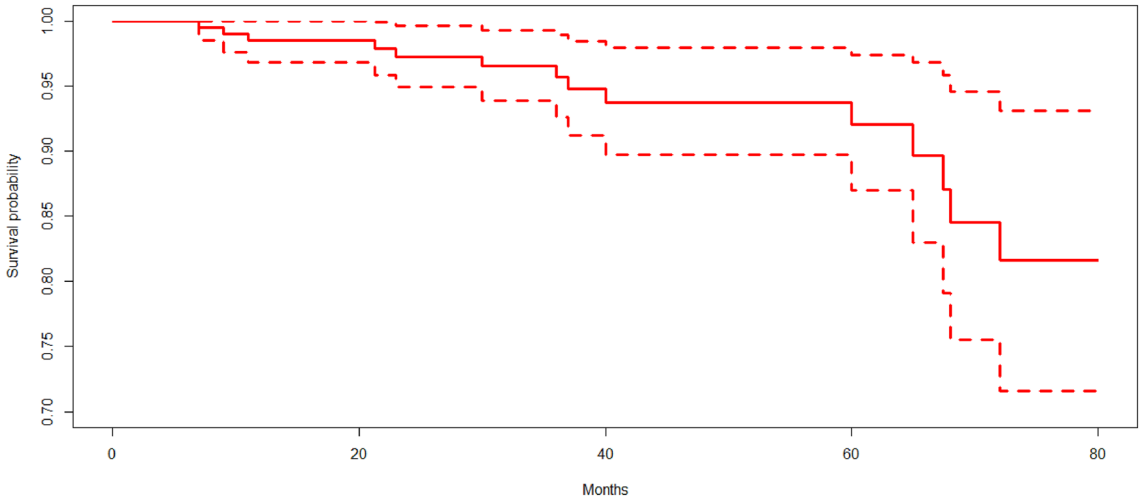


Fig. 1 Patient survival. Kaplan–Meier estimates of patient survival; dotted lines show 95% CI

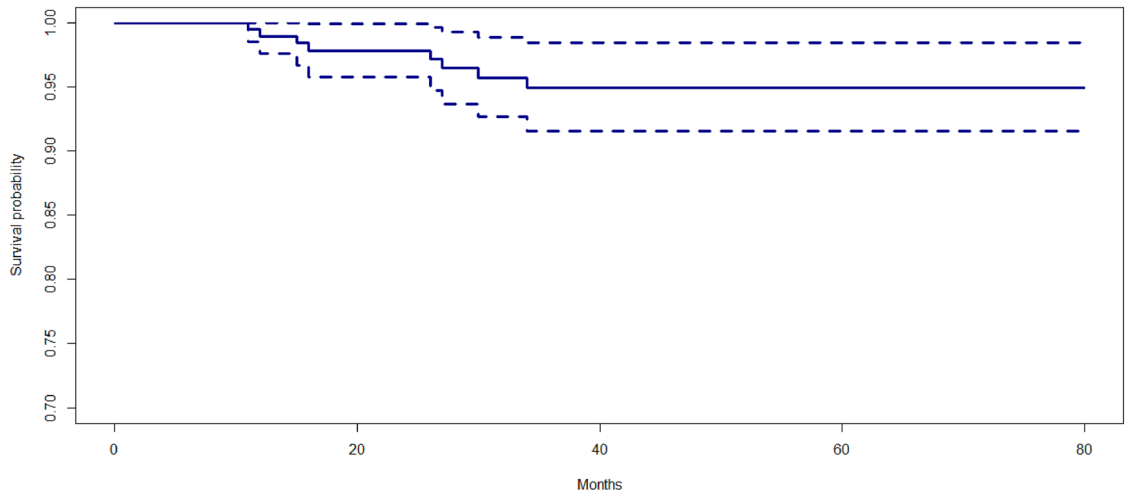


Fig. 2 Renal survival. Kaplan–Meier estimates of renal survival; dotted lines show 95% CI

of secondary membranous nephropathy were excluded. All patients had to be adults (age > 18 years), and a minimum of 12 months of follow-up was required.

The local Ethical Committee of Area Vasta Nord of Emilia Romagna and all other local Ethics Committees in the region approved the study. Written informed consent was obtained from all patients.

Two hundred and five patients were included in the study.

Follow-up

For each patient, the date of biopsy was considered as baseline. Follow-up was censored on December 31st, 2018, otherwise, follow-up ended at the time of the last outpatient visit or patient death or the onset of end-stage kidney disease. Clinical and analytical data were collected from the patients' electronic medical records at baseline, 6 months after diagnosis and then yearly from the initial baseline measurement. A total of eight time points were evaluated, and the following laboratory and clinical data were collected at each time point: blood pressure, estimated glomerular filtration rate (eGFR) calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation, 24 h proteinuria, serum albumin, positivity for anti-PLA2R, anti-PLA2R serum levels, complete remission of proteinuria partial remission of proteinuria relapse of nephrotic syndrome, loss to follow-up, death, ESKD, ongoing immunosuppressive treatment, ongoing supportive care, treatment-related adverse events.

A uniform, shared protocol was completed by participating centers.

The median (IQR) follow-up was 36 (24–60) months.

Outcomes and definitions

The primary outcome was patient survival, renal survival and the composite outcome formed by patient and renal survival. Renal survival was defined as the time to the first occurrence of any of the following events: starting dialysis, receiving a kidney transplant, or eGFR falling to < 15 ml/min at any point during follow-up.

The secondary outcome was the presence of complete remission or partial remission of proteinuria at the end of follow-up. Nephrotic syndrome, complete remission, partial remission and relapses were defined according to KDIGO definitions [7].

In the biopsy study, the Churg-Ehrenreich histological classification was used [26].

Statistical methods

In descriptive statistics, the distribution of continuous variables was summarized with mean and SD or median plus 25th and 75th percentile (interquartile range) when appropriate. For categorical variables, the description was based on frequencies or percentages.

The primary outcome probability as the time to event was evaluated by univariate analysis with Kaplan–Meier curves and log-rank test, and by multivariate Cox regression.

As primary outcome variables were given by survival times exposed to right censoring, their description was complemented by Kaplan–Meier functions. Confidence intervals around the Kaplan–Meier curves displayed in plots were computed according to the Greenwood method. When curves pertained to two groups a log-rank test was used. Concerning multivariate survival analyses, the Cox proportional hazard regression model was used. For multivariate analyses related to categorical responses (e.g., remission), we used a multinomial regression model.

All computations were performed using the software R (R Core Team, 2018). Specifically, Kaplan–Meier curves and Cox proportional hazard regression models were estimated using the survival package (quote), while multinomial regression models were estimated using the nnet package.

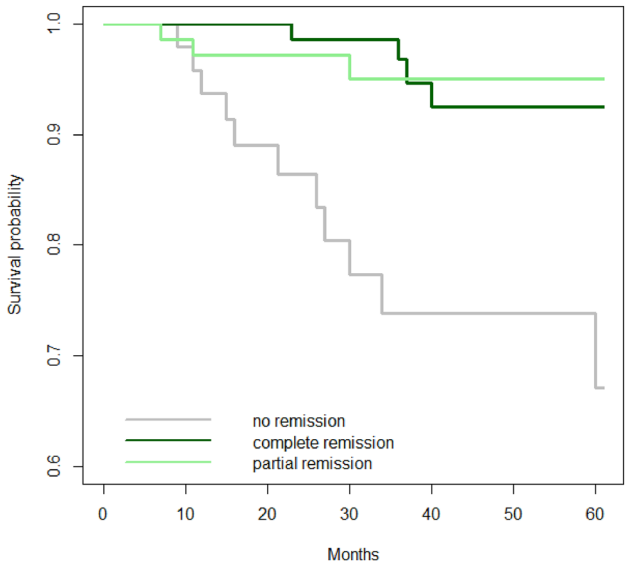


Fig. 4 Patient and renal survival according to remission at the end of follow-up. Kaplan–Meier estimates of patient and renal survival according to remission status at the end of the follow-up period

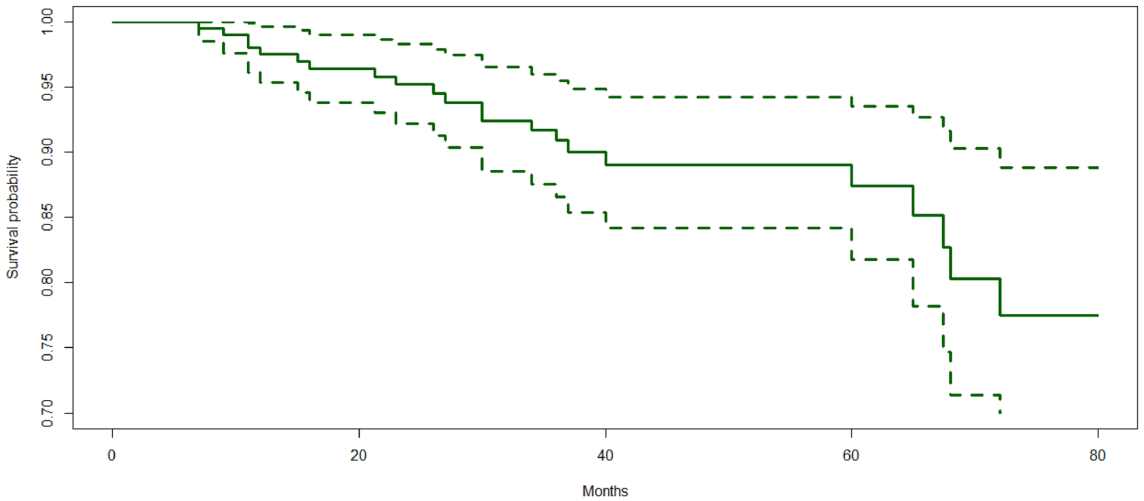


Fig. 3 Patient and renal survival. Kaplan–Meier estimates of patient and renal survival; dotted lines show 95% CI

Results

Study participants

Across all 13 renal centers in Emilia Romagna there were two hundred and five patients with a diagnosis of primary membranous nephropathy.

The baseline demographic and clinical characteristics of the 205 patients included in the study are summarized in Table 1.

The mean age of the patients was 62 years, 62% were male. In our cohort, participants showed quite well preserved renal function, with a mean (SD) eGFR of 77 (30) ml/min per 1.73 m^2, a medium grade of proteinuria with a median (IQR) value of 6.1 (3.8–9) g/24 h, while median (IQR) serum albumin was 2.6 (2.3–3.1) g/dl; nephrotic syndrome was present in less than half of the patients (47%).

Anti-PLA2R status was assessed at baseline in 65 patients, 43 (66%) of whom resulted positive. Median (IQR) anti-PLA2R levels in serum was 118 (37–342) RU/ml.

Renal biopsies revealed stage I disease in 47 patients (31%), stage II in 69 (46%), stage III in 28 (18%) and stage IV in 6 (4%). One hundred sixty (88%) patients had interstitial fibrosis and tubular atrophy in less than 25% of the specimen, 19 patients (10%) had interstitial fibrosis and tubular atrophy in 25 to 50% of the specimen, and 4 (2%) subjects had interstitial fibrosis and tubular atrophy in over 50% of the specimen. Histological description of the kidney biopsy was available for 105 patients.

Primary outcome

During the follow-up, we had lost 34 patients (17% of the total); 8 patients reached ESKD (all started hemodialysis) and 15 patients died. In particular, five patients died of cardiovascular disease, one died due to sepsis, seven patients had neoplasia and the remaining two patients died of other causes.

In our study, patient survival was 92% (95% CI 87–97.4%) at 5 years and renal survival was 95% (95% CI 91.6–98.5%) at 5 years. Considering the composite event made up of death and ESKD, by way of Kaplan–Meier curves, we estimated patient and renal survival at 5 years of 87.4% (95% CI 81.8–93.5) (Figs. 1,2,3).

By multivariate analysis, using a Cox proportional hazard model, we analyzed the impact of the following clinical baseline characteristics on composite patient and renal survival outcome: gender, age, proteinuria at biopsy and eGFR at baseline. The first three clinical features did not correlate with the outcome; instead, eGFR at baseline was a good, statistically significant ($p < 0.001$) prognostic factor of patient and renal survival (HR 0.96; 95% CI 0.94–0.98).

On the other hand, concerning histological features, such as stage of the disease and grade of interstitial fibrosis and tubular atrophy, none had a significant impact on the probability of survival.

Table 2 Immunosuppressive drugs

	No./total no (%)
CORTICOSTEROIDS	168/205 (82)
CYCLIC REGIMEN	129/205 (63)
CYCLIC REGIMEN with CYCLOPHOSPHAMIDE	106/205 (52)
CYCLIC REGIMEN with CHLORAMBUCIL	26/205(13)
RITUXIMAB	57/205 (28)
CYCLOSPORINE	22/205 (11)
TACROLIMUS	2/205 (1)
MYCOPHENOLATE	16/205 (8)
ACTH	0
None	17/205 (8)

ACTH adrenocorticotropic hormone

Secondary outcome

At the end of follow-up 155 patients (75%) showed either complete or partial remission of nephrotic syndrome. Complete remission was present in 83 patients (40%) and partial remission was found in 72 patients (35%).

Multivariable models were constructed to test the association between clinical baseline characteristics (gender, age, proteinuria and eGFR at baseline) and complete or partial remission; a multinomial regression model showed that the estimated glomerular filtration rate at baseline was a statistically significant ($p < 0.05$), independent positive predictive factor of complete remission (OR 1.02; 95% CI 1–1.03). The same clinical data did not correlate with the probability of partial remission. In a similar model, histological features (disease stage and grade of interstitial fibrosis and tubular atrophy) proved to have no statistically significant impact on complete or partial remission.

By multivariable analysis, using a Cox proportional hazard model, we found that patients with complete and partial remission had a better probability of patient and renal survival when compared to patients with persisting nephrotic syndrome. In particular, complete remission was associated with a 5-year survival of 92.5% (95% CI 85–99%) versus 67.1% (95% CI 51–88%) in absence of remission ($p < 0.04$); partial remission was associated with a 5-year survival of 95.1% (95% CI 89–100%) resulting in a significantly better prognosis in comparison to patients without complete or partial remission ($p < 0.02$) (Fig. 4).

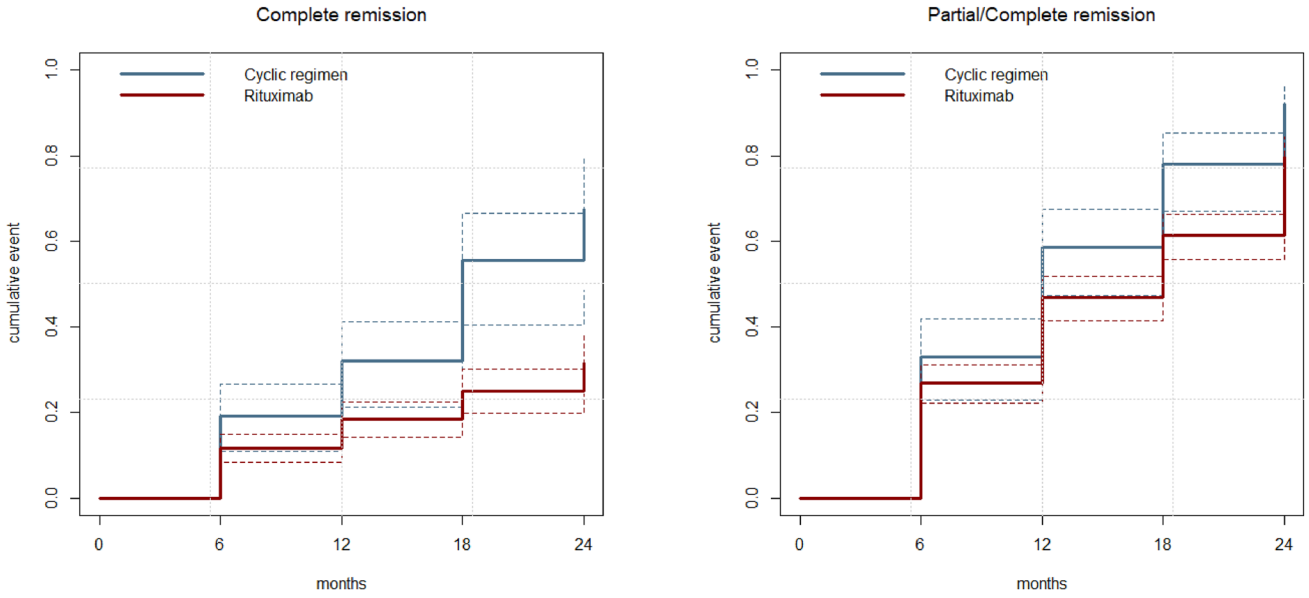


Fig. 5 Complete and composite (complete or partial) remission in cyclic regimen and rituximab groups. Kaplan–Meier estimates of complete and composite (complete or partial) remission in the cyclic regimen and rituximab groups

Table 3 Complete and composite (complete or partial) remission at 6 to 24 months in the cyclic regimen and rituximab groups

Time points	RTX	CYT	0R (95% CI)
No. of patients with remission/total no. (%)			
Complete remission			
6 mo	0/31 (0)	5/103 (5)	
12 mo	7/31 (23)	31/103 (30)	1.47 (0.57–3.78)
18 mo	6/21 (29)	46/90 (51)	2.61 (0.93–7.34)
24 mo	4/11 (36)	37/70 (53)	1.96 (0.52–7.30)
Complete or partial remission			
6 mo	13/31 (42)	60/103 (58)	1.93 (0.86–4.43)
12 mo	21/31 (68)	83/103 (81)	1.97 (0.8–4.84)
18 mo	10/21 (48)	77/90 (86)	6.51 (2.3–18.4)
24 mo	8/11 (73)	64/70 (91)	4.00 (0.83–19.2)

RTX rituximab alone, *CYT* cyclic regimen alone

During follow-up, nephrotic syndrome relapse occurred in 36/155 patients (23%) with a mean (SD) time from remission to relapse of 17.8 (17.4) months. In our population, five patients had two episodes of relapse.

Treatment options

In our study, we found widespread use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. At least one of these drugs was used in 180/205 patients (88%). Both drugs were administered to 38/205 patients (18%). One hundred forty-five patients were treated with angiotensin-converting enzyme inhibitors and 78 patients were treated with angiotensin receptor blockers.

Immunosuppressive therapy was started within 6 months of biopsy in 170 of the 205 patients (83%), while it was not administered in 17 patients (8%).

Immunosuppressive drug use is summarized in Table 2.

Focusing on immunosuppressive therapy, in our study 168 participants (82%) were administered oral or intravenous steroids, either alone or in combination with other immunosuppressive drugs.

A 6-month cyclic regimen of alternating intravenous and oral glucocorticoids with a cytotoxic drug was used in 129 patients (63%). Among these patients, 106 (52%) were treated with cyclophosphamide, whereas chlorambucil was administered to 26 patients (13%). The mean (SD) cumulative dose of cyclophosphamide was 7 (4.6) g per patient.

Besides steroids and a cyclic regimen, rituximab was the most frequently prescribed drug, and indeed a rituximab-based regimen was utilized in 57 patients (28%). Three different administration patterns of rituximab were identified: rituximab was given at a dose of 375 mg/m^2 every 4 weeks in 44 patients (77%), 1 g of rituximab on days 1 and 15 in 11 patients (20%) and lastly, in two patients (3%) the rituximab dosage was 375 mg/m^2 followed by a second dose depending on CD19 + B-cell count.

Calcineurin inhibitors were less often used, in particular, cyclosporin was employed in 22 patients (11%) and tacrolimus in 2 patients (1%). Mycophenolate mofetil was used in 16 patients (8%), while adrenocorticotropic hormones were not used at all.

Table 4 Subgroup analysis of composite outcome (complete or partial remission) at the end of follow up by non pre-specified baseline patient characteristics

Group	RTX			CYT			OR (CI)
	Remission	Total	%	Remission	Total	%	
Sex M	11	18	61%	55	64	86%	3.88 (1.17; 12.83)
Sex F	11	13	85%	34	39	87%	1.24 (0.16; 6.69)
Age ≥ 55 years	16	21	76%	67	79	85%	1.74 (0.49; 5.48)
Age < 55 years	6	10	60%	22	24	92%	7.33 (1.15; 63.30)
eGFR ≥ 85 ml/min/1.73 m^2	11	13	85%	43	48	90%	1.56 (0.20; 8.41)
eGFR < 85 ml/min/1.73 m^2	11	18	61%	46	55	84%	3.25 (0.97; 10.80)
Albumin > 2.5 g/dl	15	22	68%	54	59	92%	5.04 (1.41; 19.29)
Albumin ≤ 2.5 g/dl	7	9	78%	35	44	80%	1.11 (0.14; 5.62)
History of hypertension	16	23	70%	58	69	84%	2.31 (0.74; 6.88)
No hypertension	6	8	75%	31	34	91%	3.44 (0.39; 25.64)
Proteinuria ≥ 6 g/24 h	12	17	71%	49	58	84%	2.26 (0.61; 7.91)
Proteinuria < 6 g/24 h	10	14	71%	40	45	89%	3.20 (0.68; 14.41)
BMI ≥ 25 kg/m^2	10	14	71%	42	48	88%	2.80 (0.62; 11.82)
BMI < 25 kg/m^2	7	9	78%	25	29	86%	1.78 (0.22; 11.37)
Stage 1, 2	8	13	62%	58	65	89%	5.18 (1.28; 20.62)
Stage 3, 4	3	5	60%	12	14	86%	4.00 (0.36; 47.90)

RTX rituximab alone, *CYT* cyclic regimen alone

Table 5 Adverse events

	No./total no (%)	%
Any adverse events	92/205 (45)	45
Hospitalization[a]	111/205 (46)	46
Mild-moderate infections	30/205 (15)	15
Severe leukopenia[b]	8/205 (4)	4
Drug infusion reaction	11/205 (5)	5
Cardiovascular disease	33/205 (16)	16
Cancer	13/205 (6)	6
Steroid diabetes	12/205 (6)	6
Steroid psychosis	4/205 (2)	2

[a]Hospitalization for IV drug infusion or adverse events treatment

[b]White Blood cells < 3500/mm^3

Cyclic regimen versus rituximab

To better investigate the role of different immunosuppressive therapies with regard to remission probability, we divided our population into four groups according to the specific immunosuppressive drugs that were used. The first group of 103 patients was made up of subjects treated with a cyclic regimen alone, the second group included 31 patients who underwent therapy with rituximab alone, the third group had 26 patients who underwent treatment with both a cyclic regimen and rituximab, and the last group, which was made up of 45 subjects, was treated with other drugs.

Taking into account patients in complete and partial remission at the end of the follow-up period, the cyclic regimen alone compared to rituximab alone showed better performance in the induction of nephrotic syndrome remission: 89 of 103 patients (86%) in the cyclic regimen alone group and 22 of 31 patients (71%) in the rituximab alone group had complete or partial remission; 53 patients (51%) in the cyclic regimen alone group and 10 patients (32%) in the rituximab alone group had complete remission. As shown in Fig. 5, the occurrence of remission was also faster in the cyclic regimen alone group, with a significant difference in the number of complete or partial remissions at 18 months (Table 3).

In post hoc analysis, the cyclic regimen alone showed significantly greater efficacy across different non-prespecified subgroups: male sex, age < 55 years, albumin > 2.5 g/dl and stage 1,2 (Table 4).

By multivariable analysis, a multinomial regression model showed that the cyclic regimen alone was associated with complete remission (OR 0.26; 95% CI 0.08–0.79) in a statistically significant way ($p < 0.05$) when compared to rituximab alone. On the contrary, there was no significant difference between the cyclic regimen alone and rituximab alone in terms of partial remission induction.

Considering the composite outcome formed by death and ESKD we found that in the cyclic regimen group, 12/103 patients (11.6%) reached the event, whereas in the rituximab group, 3/31 patients (9.7%) did. This difference was not statistically significant.

Adverse events

During the study, adverse events occurred in 92 participants (45%) (Table 5).

Altogether, 111/205 participants (46%) were hospitalized either for the treatment of adverse events or to undergo intravenous therapy such as IV corticosteroid or rituximab administration. Eleven participants (5%) had an infusion-related reaction with rituximab. Severe leukopenia (< 3500/mm^3) was observed in eight participants (4%). Mild-moderate infections requiring hospitalization occurred in 30 participants (15%). During the follow-up period, 33 subjects (16%) suffered a serious cardiovascular event, while 13 subjects (6%) developed cancer. Steroid-related diabetes and psychosis were found respectively in 12 (6%) and 4 (2%) participants.

Discussion

The baseline clinical characteristics of patients in our GLOMER study portray a population with a low or, at most, moderate risk of progressive loss of kidney function, according to KDIGO clinical practice guidelines for glomerulonephritis [7, 8]: a mean (SD) serum creatinine of 1.1 (0.8) mg/dl, a median (IQR) proteinuria of 6.1 (3.8–9) g/24 h and nephrotic syndrome were observed in less than half of the patients (47%). At the same time, if we compare our study population to those of the three most recent randomized controlled trials, we find lower baseline proteinuria compared to MENTOR (8.9 g/24 h) and STARMEN (7.4 g/24 h), and similar compared to RI-CYCLO (6 g/24 h). On the contrary, patients in our GLOMER study show worse renal function with a mean eGFR of 76.9 ml/min per 1.73 m^2 versus 84.9 ml/min per 1.73 m^2 and 87.4 ml/min per 1.73 m^2 in MENTOR, 79.8 ml/min per 1.73 m^2 in STARMEN and 84 ml/min per 1.73 m^2 in RI-CYCLO [23–25].

The GLOMER study shows excellent results in long-term survival: patient survival at 5 years is 92%, 5-year renal survival is 95% and overall patient and renal survival at 5 years is 84.7%. These findings are consistent with those of the few available studies in which ESKD is the primary outcome, and that have at least 5–7 years of follow-up. An Italian RCT by Ponticelli with a 10-year follow-up of patients treated with methylprednisolone plus chlorambucil reported patient and renal survival of 92% after 10 years [4]. In an Indian RCT, 10-year renal survival was 89% for patients assigned to a cyclic regimen with glucocorticoids and cyclophosphamide [12]. A Dutch prospective study of 65 patients with primary membranous nephropathy, treated with a cyclic regimen of steroids and cyclophosphamide, reports renal survival of 86% after 5 years and 74% after 7 years [27].

In our opinion, because of the sample size (205 subjects) and length of follow-up (7 years), the primary outcome of this study warrants particular relevance since there are only a few studies available reporting long-term results in patients treated for primary membranous nephropathy that are comparable with regard to size and length of observation.

The GLOMER study shows high rates of combined complete and partial remission of proteinuria at the end of follow up. Overall, of 205 patients, 155 (75%) showed either complete or partial remission of nephrotic syndrome,. This result is in line with data reported in some RCTs and retrospective studies concerning the three main therapeutic options in primary membranous nephropathy: a cyclic regimen with corticosteroid and cytotoxic drugs has shown efficacy in inducing remission of nephrotic syndrome in about 60–80% of patients, calcineurin inhibitors in about 65–70% of patients and rituximab in about 60–70% of patients [4, 13, 28].

In our study, complete remission is present at the end of follow-up in 83/205 patients (40%), while partial remission is detected in 72/205 (35%); thus, among the 155 responders, complete remission is slightly predominant over partial remission. This rather unusual ratio between complete and partial remission that is observed in our study could be explained by the intensive use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, administered to 88% of patients, together with the length of follow-up. These two factors may have allowed a greater number of patients to achieve a reduction in proteinuria to below the cut-off value of 0.3 g/24 h compared to the results in shorter studies such as RCTs.

The relatively low rate of nephrotic syndrome relapse (23%) in our series likely reflects the low use of calcineurin inhibitors for immunosuppressive therapy since the higher risk of relapse with calcineurin inhibitors is well known; indeed just 24 patients (13%) were treated with cyclosporin or tacrolimus [13, 14, 29].

Our study confirms that achieving complete and partial remission provides patients with a statistically significant better probability of being alive with preserved kidney function as compared to patients with persisting nephrotic syndrome. In our population, 5-year patient and renal survival in case of complete and partial remission is respectively 92.5% and 95.1%. This finding is clinically relevant since it might allow us to consider not only complete remission but also partial remission as a valid surrogate of a stronger outcome such as death or ESKD [30].

Traditionally, clinical features associated with a higher risk of developing ESKD in primary membranous nephropathy include male sex, older age at onset (mostly if above 60 years), nephrotic range proteinuria (particularly if protein

excretion exceeds 8–10 g/24 h) and a decreased eGFR at presentation [1]. In our study, multivariate analysis showed that eGFR at baseline alone is a good, statistically significant ($p < 0.001$) prognostic factor of patient and renal survival. Furthermore, a multinomial regression model shows that the eGFR at baseline is an independent, statistically significant ($p < 0.05$), positive predictive factor for complete remission, but not for partial remission. Regarding our data, male sex, age at onset and degree of proteinuria do not seem to be correlated to short-term (remission) and long-term (survival) outcomes of primary membranous nephropathy.

In our study, histological features such as disease stage and grade of interstitial fibrosis and tubular atrophy do not correlate with remission probability. Unlike what has been reported in a recent Greek study [31], in our experience, the grade of interstitial fibrosis and tubular atrophy was not able to predict renal and patient survival; however, it should be pointed out that this finding might have been influenced by the fact that in our series only 2% of patients had interstitial fibrosis and tubular atrophy in over 50% of the specimen.

As suggested by KDIGO clinical practice guidelines, supportive therapy with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers has been widely utilized in our series. In fact, at least one of these drugs was used in 88% of patients. Both drugs were administered to 18% of subjects [6].

In our GLOMER study, although patients had a low-moderate risk of progressive loss of kidney function, the therapeutic approach was rather aggressive with early immunosuppressive therapy administration. Immunosuppressive therapy was given to the majority of patients (92%), and a particularly interesting fact is that immunosuppressive therapy had been started within 6 months of biopsy in 170 of the 205 patients (83%). Even though early use of immunosuppressive therapy in primary membranous nephropathy treatment is a breach of KDIGO recommendations, it seems to be a common clinical practice worldwide, as suggested by the international cohort study CureGN, which reports the start of immunosuppressive therapy within 6 months after biopsy in 178 of 324 patients overall (55%) [32].

In our GLOMER study, first-line immunosuppressive therapy involved a 6-month cyclic regimen of alternating intravenous and oral glucocorticoids with a cytotoxic drug, since this therapeutic schedule has been used in 129 patients altogether (63%), with a prevalence of cyclophosphamide (106 patients) over chlorambucil (26 patients).

Overall, 57 patients (28%) were treated with a rituximab-based regimen, making this drug the second most highly used therapeutic option in our series. It must be highlighted that rituximab use increased progressively during the observation period, and indeed the number of patients treated with rituximab increased annually from just 2 in 2010 to 11 in 2017. This is possibly a consequence of the increasing confidence in rituximab management acquired by clinicians over time.

Lastly, calcineurin inhibitors and mycophenolate mofetil were not used much in immunosuppressive therapy; they were used in 24 patients (12%) and 16 patients (8%), respectively.

In our series, the cyclic regimen shows a better remission rate as compared to rituximab, and a multinomial regression model reveals that the cyclic regimen is associated with complete remission in a statistically significant way ($p < 0.05$) when compared to rituximab. In contrast, the difference in partial remission rate does not reach statistical significance. Furthermore, the cyclic regimen appears to induce remission of proteinuria in a shorter time.

As already reported in the literature, and also seen in our study, treatment of patients affected by primary membranous nephropathy was burdened by a significant incidence of adverse events likely connected to immunosuppressive therapy. Adverse events occurred in 92 participants (45%), and altogether there were 111 hospitalizations (46%). Previous studies reported a 10% prevalence of cancer in patients with membranous nephropathy [33]; we had just 13 cases of neoplasia (6% of patients), despite the prevalent use of a regimen based on alkylating agents, which are traditionally considered drugs with a greater risk of malignancy than rituximab or calcineurin inhibitors.

We did not set out to establish a correlation between an adverse event and a specific therapy, hence our decision not to investigate single drug safety profiles.

Our study has all the strengths inherent to a large multicenter study with lengthy follow-up. Although GLOMER is a regionwide and not a nationwide study, it is one of the largest studies present in the literature, involving 205 patients with primary membranous nephropathy. A Spanish nationwide retrospective study (GLOSEN) reported 122 patients with primary membranous nephropathy treated with tacrolimus, while the international longitudinal cohort study Cure Glomerulonephropathy Network (CureGN) only reported treatment patterns and not clinical results among 324 patients [32, 34]. Furthermore, thanks to an observation period of seven years and a median (IQR) follow-up of 36 (24–60) months, GLOMER is one of the longest studies on primary membranous nephropathy treatment, together with two RCTs with a 10-year follow-up published in 1995 and 2007, respectively [4, 12].

We acknowledge all the general limitations inherent to an observational, retrospective study, in addition to the lack of a control group and the following specific limitations**.** We lost a significant number of participants (17%) to follow-up which can be explained by the duration of the observation period and by the fact that some patients did not live in the Emilia Romagna region.

Unfortunately, in Emilia Romagna the serum test for circulating anti-PLA2R antibodies became routinely available some years after the beginning of the study, which is why anti-PLA2R status at baseline was available for only 65 of 205 patients. Due to the lack of data about anti-PLA2R levels in our patients, we were not able to investigate the immunologic response to immunosuppressive therapy and therefore to confirm the clinical usefulness of anti-PLA2R monitoring in the management of primary membranous nephropathy [17, 18].

In our opinion, the GLOMER study reveals and confirms that in clinical practice, clinicians tend to start immunosuppressive therapy within the first 6 months following primary membranous nephropathy diagnosis, even in patients that are not at high or very high risk of progressive loss of kidney function [8, 32]. The favorable short- and long-term results of our series suggest that this pragmatic therapeutic approach could be a winning strategy, regardless of the chosen drug regimen. Considering that our study population has a low or, at most, moderate risk of progression, this finding could give rise to discussion about a recommendation of the current KDIGO guidelines for primary membranous nephropathy that recommends the use of immunosuppression within 6 months of diagnosis only in patients at high or very high risk of progressive loss of kidney function [7, 8]. In our opinion, RCTs are needed to compare outcomes in patients affected by primary membranous nephropathy presenting low and moderate risk of progression, treated with immunosuppression before or after 6 months of biopsy.

Lastly, the GLOMER study, as well as two recent RCTs (RI-CYCLO and STARMEN), show that a cyclic regimen is able to induce complete remission in a significantly greater number of patients than rituximab. Compared to RI-CYCLO and STARMEN, our study has a larger sample size (134 patients versus 74 in RI-CYCLO and 86 in STARMEN), equal or longer follow-up (36 months versus 36 in RI-CYCLO and 24 in STARMEN), and, most importantly, remission of proteinuria is detected not only 1 or 2 years later, but also at the end of a long observation period (36 months) [24, 25].

In conclusion, a cyclic regimen with corticosteroid and cytotoxic drugs seems to warrant a prominent role in primary membranous nephropathy treatment, while currently, full proof of rituximab superiority in efficacy and safety over the cyclic regimen is still lacking.

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Author contributions All authors were involved in conception and study design; Drs. Albertazzi, Fontana, Giberti, Aiello, Battistoni, Catapano, Graziani, Cimino, Schichilone, Forcellini, De Fabritiis, Signorotti, and Del Sante participated in data collection; Dr. Scarpioni and Dr. Albertazzi analyzed the data and drafted the manuscript. All authors revised and approved the final version of the manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The local Ethical Committee of Area Vasta Nord of Emilia Romagna and all other local Ethics Committees in the region approved the study.

Informed consent Written informed consent was obtained from all patients.

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