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Original Article

ORIGINAL ARTICLE

Obinutuzumab treatment for membranous nephropathy: effectiveness and safety concerns during the COVID-19 pandemic

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ABSTRACT

Background. Obinutuzumab is a humanized and glycoengineered anti-CD20 monoclonal antibody that has been shown to induce more profound B-cell depletion than rituximab. The effectiveness and safety of obinutuzumab in the treatment of membranous nephropathy remain unclear.
Methods. This was a retrospective study conducted in Huashan Hospital, Fudan University between 1 December 2021 and 30 November 2023. Patients with membranous nephropathy were included to assess the effectiveness and safety of obinutuzumab and prevalence of severe pneumonia during the outbreak of COVID-19 in China.
Results. Eighteen patients were included in the study assessing the effectiveness of obinutuzumab. After a 12-month follow-up, 14 patients (78%) achieved remission, with six (33%) achieving complete remission and eight (44%) achieving partial remission. Among the 18 obinutuzumab-treated patients contracting COVID-19 for the first time, six (33%) developed severe pneumonia, and one died. By contrast, two of the 37 patients receiving glucocorticoids combined with cyclophosphamide, and none of the 44 patients on calcineurin inhibitors or the 46 patients on rituximab developed severe pneumonia. However, compared to patients receiving rituximab or glucocorticoids plus cyclophosphamide, the obinutuzumab-treated patients had a longer duration of membranous nephropathy and immunosuppressive therapy. Therefore, cardinal matching was employed to balance these baseline characteristics. Owing to small sample size for each regimen, patients receiving all the three non-obinutuzumab immunosuppressive regimens were grouped as a control cohort. After matching for age, gender, remission status, duration of membranous nephropathy, duration of immunosuppressive therapy, and ongoing immunosuppression, the obinutuzumab-treated patients still had a significantly higher incidence of severe pneumonia compared to those on other regimens ($P = .019$).
Conclusion. Obinutuzumab was an effective treatment option for patients with membranous nephropathy. On the other hand, it was associated with a higher incidence of severe pneumonia following COVID-19 infection compared to other immunosuppressive regimens.

Keywords: COVID-19, membranous nephropathy, obinutuzumab, severe pneumonia

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KEY LEARNING POINTS

What was known:

- Obinutuzumab is a humanized and glycoengineered type II anti-CD20 monoclonal antibody that has been shown to induce deeper and longer-lasting B-cell depletion than rituximab.
- Obinutuzumab could induce remission in rituximab-resistant membranous nephropathy.

This study adds:

- Patients with membranous nephropathy have shown successful treatment outcomes with obinutuzumab. However, obinutuzumab was associated with a higher incidence of severe pneumonia following COVID-19 infection compared to other immunosuppressive treatment for membranous nephropathy.

Potential impact:

- Our results may prompt further studies to examine the effect of obinutuzumab on infections in patients with membranous nephropathy. Caution should be exercised when using obinutuzumab regimen in the treatment of membranous nephropathy for the occurrence of severe COVID-19 pneumonia.

INTRODUCTION

Membranous nephropathy is the leading cause of primary nephrotic syndrome in adults. Without immunosuppressive therapy, ~20% of patients will progress to end-stage kidney disease within 10 years [1]. Evidence supports the necessity of immunosuppressive treatment for those at high risk of progression [2]. Traditional first-line immunosuppressive treatments include glucocorticoids plus cyclophosphamide (CTX) and calcineurin inhibitors (CNIs) [3]. Over the past decade, rituximab, a chimeric mouse-human anti-CD20 monoclonal antibody, has been widely utilized, but ~40% of patients do not respond to it [4].

Obinutuzumab is a humanized and glycoengineered type II anti-CD20 monoclonal antibody that has been shown to induce more profound and longer-lasting B-cell depletion than rituximab [5, 6]. A randomized controlled trial showed that obinutuzumab was more effective than rituximab when used in combination with standard chemotherapy for patients with chronic lymphocytic leukemia [7]. In patients with systemic lupus erythematosus, obinutuzumab showed greater B-cell cytotoxicity and activation of natural killer cells, and was more effective than rituximab in treating lupus nephritis [8]. The efficacy and safety of obinutuzumab in patients with membranous nephropathy remain unclear, although several case reports have shown successful use of obinutuzumab in individuals resistant to rituximab [9–11]. A phase III, global, multicenter clinical trial (the MAJESTY study) is ongoing to investigate the use of obinutuzumab in the treatment of membranous nephropathy.

Coronavirus disease 2019 (COVID-19) is a highly contagious respiratory illness, which first emerged in December 2019, and has rapidly spread worldwide since then [12]. In China, we experienced a widespread outbreak of COVID-19 between December 2022 and August 2023 when the strict control measures were no longer implemented. This outbreak resulted in >90% of the population being infected with the virus within 8 months [13]. This provided us with a good opportunity to investigate how this COVID-19 infection could affect patients with membranous nephropathy who are immunocompromised due to hypogammaglobulinemia and/or immunosuppressive therapy.

This study aims to evaluate the effectiveness of obinutuzumab in treating membranous nephropathy, and to investigate whether obinutuzumab treatment is associated with a greater risk of severe COVID-19 compared to other standard immunosuppressive regimens. This study will provide valuable insights into the role of obinutuzumab in the management of patients with membranous nephropathy.

MATERIALS AND METHODS

Study population

This study was conducted at the Membranous Nephropathy Clinic in Huashan Hospital, Fudan University, China. Membranous nephropathy was diagnosed by renal biopsy and/or serum anti-phospholipase A2 receptor (PLA_2R) antibodies with a titer higher than 14 RU/ml. Between 1 December 2021 and 30 November 2022, which was prior to the outbreak of COVID-19 in China, a total of 19 patients with primary membranous nephropathy received obinutuzumab at this center. Among them, eight were resistant to prior immunosuppressive therapy, four were dependent on CNIs, and five experienced relapses before obinutuzumab treatment. Among the remaining two patients, one received obinutuzumab as his initial immunosuppressive therapy, while the other received obinutuzumab 8 months after rituximab although he had achieved partial remission. Excluding the patient with partial remission, 18 patients were included to assess the effectiveness of obinutuzumab. During the outbreak of COVID-19 between December 2022 and August 2023 in China, patients with primary and secondary membranous nephropathy, who were followed up at this center were included to investigate the incidence of severe pneumonia. Patients were excluded if they declined to participate in the study or had developed end-stage kidney disease before COVID-19 infection. This study has been approved by the ethics review board at Huashan Hospital, Fudan University, and informed consent was obtained from included patients.

Treatment

Obinutuzumab was administered intravenously at a dose of 1 g for 1 to 2 doses with a minimum 2-week interval between two doses. Premedication including 80 mg of intravenous methylprednisolone, 10 mg of oral ebastine, and 600 mg of oral acetaminophen was given 30 minutes before obinutuzumab infusion. Among the 19 patients receiving obinutuzumab, 18 had prior immunosuppressive therapies. The time lapse between the cessation of the last immunosuppressive therapy and the administration of obinutuzumab varied depending on the specific immunosuppressive regimens. For patients previously treated

Table 1: Patient characteristics at the initiation of obinutuzumab.

	Obinutuzumab-treated patients $n = 18$
Age, years (SD)	55.7 (12.2)
Male, *n* (%)	16 (88.9)
Duration of MN, months (IQR)	52.5 (28.0, 92.3)
Duration of IST[a], months (IQR)	38.0 (20.5, 55.3)
PLA2R associated-MN, *n* (%)	18 (100)
Anti-PLA2R antibody, RU/ml (IQR)	27.7 (12.4, 59.2)
Serum albumin, g/dl (IQR)	2.8 (2.2, 3.2)
Urine protein, g/day (IQR)	5.4 (4.1, 8.7)
eGFR, ml/min/1.73 m^2 (IQR)	80.8 (58.9, 113.5)
A history of IST, *n* (%)	17 (94.4)
Rituxiamb, *n* (%)	12 (66.7)
GCs + CTX, *n* (%)	7 (38.9)
CNIs ± GCs, *n* (%)	14 (77.8)

MN, membranous nephropathy; IST, immunosuppressive therapy; GCs, glucocorticoids.
[a]The cumulative time of immunosuppression before obinutuzumab treatment.

Table 2: Response after obinutuzumab therapy[a].

	6 months	12 months
Anti-PLA2R antibody titer ≤14 RU/ml	18/18 (100%)	17/18 (94%)
Anti-PLA2R antibody titer <2 RU/ml	14/18 (78%)	16/18 (89%)
Complete remission	2/18 (11%)	6/18 (33%)
Partial remission	14/18 (78%)	9/18 (50%)
Overall remission	16/18 (89%)	15/18 (83%)

[a]A total of 19 patients were enrolled, and one patient died 3 months after the treatment.

with rituximab, the time lapse was >6 months, and the peripheral B cells had recovered to normal level before obinutuzumab treatment. If the last immunosuppressive regimen was glucocorticoids combined with CTX, the time lapse was also >6 months. In cases where patients receiving CNIs and/or glucocorticoids, withdrawal was not mandatory prior to obinutuzumab infusion, but these medications would be tapered off after that.

Data collection

Patient information, including age, gender, duration of membranous nephropathy, immunosuppressive regimens, duration of immunosuppressive therapy, serum albumin, serum creatinine, urine protein quantification, serum anti-PLA2R antibodies level, peripheral B-cell counts, and comorbidities, was collected. To evaluate the effectiveness of obinutuzumab, data at the initiation of obinutuzumab treatment were documented as baseline characteristics. To explore the incidence of severe COVID-19, the information was collected at the time of COVID-19 infection, along with patients' symptoms, results from chest CT scans, pneumonia diagnosis, requirements for intravenous medications, and details regarding hospitalization. The radiology reports of patients who underwent chest CT scans were carefully reviewed for any signs of pneumonia. For patients requiring hospitalization due to pneumonia, their medical details and clinical outcomes were validated and documented by two experienced nephrologists through comprehensive interviews and a thorough examination of their inpatient medical records.

Definitions

Complete remission was defined as proteinuria <0.3 g/24 h or urine protein creatinine ratio <0.3 g/g, along with a serum albumin level of ≥3.5 g/dl and stable or improved renal function. Partial remission was defined as proteinuria <3.5 g/24 h and ≥ 0.3 g/24 h (or 0.3 g/g ≤ urine protein creatinine ratio < 3.0 g/g), with a ≥50% reduction from baseline, improved serum albumin, and stable or improved renal function. Treatment resistance was defined by the persistence of nephrotic syndrome, or persistence of serum PLA2R-Ab at high or unchanged levels, or PCR decreasing to values between 2 and 3.5 g/d but without an increase of serum albumin to normal, and subsequent rise in PCR, 1 year after initiation of glucocorticoids combined with CTX or at least 6 months after receiving rituximab or CNIs regimens. CNIs dependence was defined as a relapse occurring during the CNIs tapering or within 6 months after CNIs withdrawal.

To assess the effectiveness of obinutuzumab, the index date referred to the date of the first obinutuzumab infusion. In the study of severe COVID-19 pneumonia, the index date referred to the occurrence of individual infection. The duration of

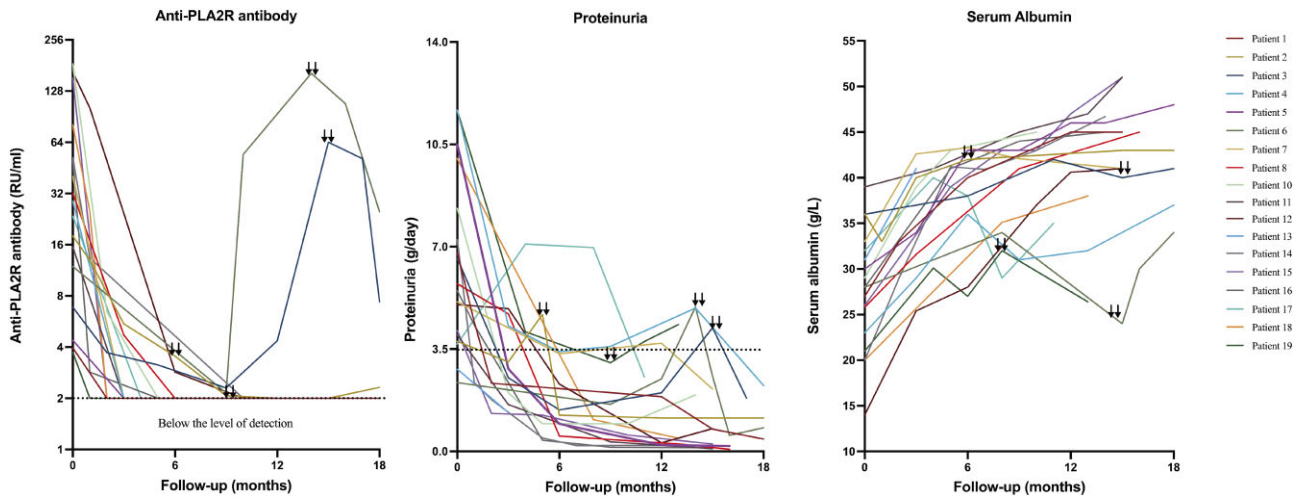

Figure 1: Trends of anti-PLA2R antibody, proteinuria, and serum albumin after obinutuzumab.

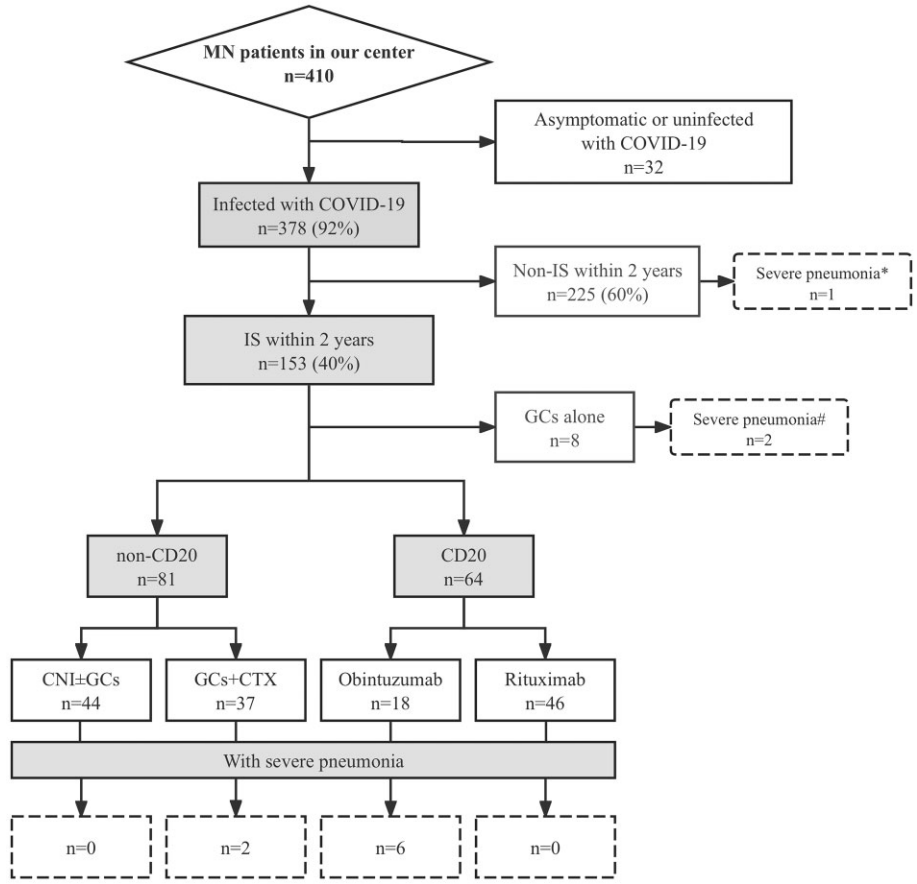

Figure 2: Study flow chart. GCs, glucocorticoids. *The patient experiencing severe pneumonia had Waldenstrom macroglobulinemia. #One of the patients experiencing severe pneumonia was complicated by cryoglobulinemia, and the other had rheumatoid arthritis and pancreatic carcinoma.

membranous nephropathy was calculated as the time interval between the initial diagnosis and the index date. Immunosuppression was defined as being on immunosuppressants, in peripheral B-cell depletion (<5/μl) after CD20-mAb infusion, or within 6 months after CTX discontinuation. The duration of immunosuppressive therapy was defined as the cumulative time of immunosuppression. If the data of B-cell counts was unavailable, the time was calculated by 6 months after rituximab or obinutuzumab infusion. COVID-19 infection was defined as follows: (i) a confirmed diagnosis by a SARS-CoV-2 PCR test; or (ii) patients showing symptoms related to COVID-19 and having close contact with individuals diagnosed with or highly suspected of having COVID-19 during the 2022 outbreak in China. Severe pneumonia was defined through lung CT scans and the need for hospitalization due to pneumonia.

Statistical analysis

Continuous variables were presented as means ± standard deviation for a normal distribution and as median (IQR) for a non-normal distribution. Comparisons between two groups were conducted using an independent *t*-test for normal distribution data and the Mann–Whitney *U*-test for non-normal data. Categorical variables were expressed as frequencies and percentages. Comparisons between two groups were made using Pearson's chi-square test or Fisher's exact test for categorical variables. Statistical analysis was carried out using the SPSS v.23 statistical software.

Matching for covariates balance was performed to establish a well-balanced dataset for comparison using the MatchIt package (v.4.5.0) in R. Patients receiving non-obinutuzumab standard immunosuppressive regimens, including glucocorticoids combined with CTX, CNIs with/without glucocorticoids, and rituximab, were used as a control cohort. Six variables, including age, gender, remission status, duration of membranous nephropathy, duration of immunosuppressive therapy, and ongoing immunosuppression, were used for matching. Various matching methods such as cardinality, nearest, optimal, full, genetic, coarsened exact matching, and subclass were attempted. Ultimately, the cardinality method with a tolerance parameter of 0.05 was chosen due to its optimal trade-off in balancing performance and effective sample size. Cardinality matching is a subset selection method that prioritizes the identification of the largest subset of units meeting user-defined balance constraints on standardized mean differences, known as tolerance. Statistical analysis was conducted after matching. All statistical analyses were two-sided and a *P* value <.05 was considered significant.

Table 3: Differences between patients treated with obinutuzumab and other immunosuppressive regimens.

	Obinutuzumab[a] $n = 18$	Rituximab[b] $n = 46$	P[a,b]	GCs + CTX[c] $n = 37$	P[a,c]	CNIs ± GCs[c] $n = 44$	P[a,c]
Age, years (SD)	55 (10.7)	56.3 (14.0)	.781	56.6 (14.2)	.847	57.5 (16.5)	.781
Male, *n* (%)	16 (88.9)	24 (52.2)	.006	29 (78.4)	.565	29 (63.0)	.042
Duration of MN, months (IQR)	57.5 (35.5, 97.5)	31.5 (16.5, 68.3)	.01	26.0 (17.0, 56.0)	.004	61.0 (33.3, 90.8)	.914
Duration of IST[d], months (IQR)	46.5 (26.8, 61.3)	14.0 (6.0, 29.8)	<.001	17.0 (11.0, 23.0)	<.001	48.5 (31.5, 65.8)	.475
Immunosuppression, n (%)	18 (100)	14 (30.4)	<.001	28 (75.7)	.023	37 (84.1)	.096
Combined IST within 6 months, n (%)	10 (55.6)	9 (19.6)	.005	——	——	——	——
Time from the last dose to infection, days (IQR)	97.0 (55.3, 135)	293.5 (135.5, 377.3)	<.001	——	——	——	——
B-cell depletion[e], n (%)	18 (100)	14 (30.4)	<.001	——	——	——	——
Remission							
Partial remission, n (%)	10 (55.6)	27 (58.7)	.819	14 (37.8)	.214	26 (59.1)	.819
Complete remission, n (%)	0	7 (15.2)	.191	8 (14.5)	.084	16 (34.8)	.01
Overall remission, n (%)	10 (55.6)	34 (73.9)	.154	22 (59.5)	.783	42 (95.5)	<.001
Vaccination, n(%)	7 (38.9)	16 (34.8)	.758	17 (45.9)	.620	14 (31.8)	.593
Comorbidity							
Hypertension, n (%)	8 (44.4)	18 (39.1)	.697	18 (48.6)	.769	21 (45.7)	.93
Diabetes, *n* (%)	4 (22.2)	7 (15.2)	.765	9 (24.3)	1	7 (15.2)	.765
Coronary artery disease, n (%)	0	2 (4.3)	1	4 (10.8)	.371	2 (4.3)	1
Respiratory disease, n (%)	2 (11.1)	3 (6.5)	.923	3 (8.1)	1	1 (2.3)	.189
Chronic liver disease, n (%)	0	3 (6.5)	.553	4 (10.8)	.371	3 (6.5)	.553
Cancer, *n* (%)	0	0	——	2 (5.4)	1	2 (4.3)	1
Without comorbidity, n (%)	9 (50.0)	23 (50.0)	1	17 (45.9)	.778	21 (45.7)	.754
Severe pneumonia, n (%)	6 (33.3)	0	<.001	2 (5.4)	.019	0	.002

[a]Obinutuzumab alone, $n = 7$; Obinutuzumab + CNIs, $n = 10$; Obinutuzumab + GCs, $n = 1$
[b]Rituximab alone, $n = 35$; Rituximab + CNIs, $n = 4$; Rituximab + GCs, $n = 5$
[c]CNIs alone, $n = 34$; CNIs + GCs, $n = 10$
[d]The cumulative time of immunosuppression before COVID-19 infection.
[e]The count of B cells $<5/\mu$l.
MN, membranous nephropathy; IST, immunosuppressive therapy; GCs, glucocorticoids.

RESULTS

Patients with membranous nephropathy were successfully treated with obinutuzumab

Among the 18 participants included in the study to assess the effectiveness of obinutuzumab, 16 (89%) were male. The average age was 56 ± 12 years old, and the duration of membranous nephropathy was 53 (IQR, 28–92) months. At the initiation of obinutuzumab treatment, estimated glomerular filtration rate (eGFR) was 81 (IQR, 59–114) ml/min/1.73 m^2. All patients had their serum PLA2R antibodies >2 RU/ml, with a median titer of 27.7 (IQR, 12.4–59.2). Sixteen patients had proteinuria >3.5 g/day, and 16 patients had serum albumin levels <3.5 g/dl. The two patients with proteinuria <3.5 g/day were dependent on tacrolimus. They received obinutuzumab due to disease worsening even before tacrolimus discontinuation (Table 1, Supplementary Table 1 and Supplementary Fig. 1).

During the follow-up period, one patient died because of COVID-19 infection 3 months after receiving obinutuzumab. The remaining 17 patients were all followed up for at least 12 months, with a median duration of 15 (IQR, 14–16) months. At the sixth month, serum anti-PLA2R antibodies were <14 RU/ml in 17 (94%) patients and <2 RU/ml in 13 (72%) patients. Remission was achieved in 15 (83%) patients, with complete remission in two and partial remission in 14. After 12 months, serum anti-PLA2R antibodies were <14 RU/ml in 16 (89%) patients and <2 RU/ml in 15 (83%). Remission was achieved in 14 (78%) patients, with complete remission in six (33%) and partial remission in eight (44%). The six patients with complete remission and seven out of the nine patients with partial remission maintained their remission status until the last follow-up. Two patients with partial remission experienced relapse, and they achieved partial remission again after a second course of obinutuzumab infusion. Among the three patients without remission at 12 months, one achieved partial remission at 16 months while the other two had their anti-PLA2R antibodies persistently <2 RU/ml 3 months since obinutuzumab treatment but without clinical remission after 20 and 25 months of follow-up, respectively. Kidney function maintained stable in all patients throughout the follow-up period (Table 2 and Fig. 1).

During the outbreak of COVID-19, 17 out of 18 patients were infected with COVID-19. Among them, six developed severe pneumonia and one patient died. There were no other severe adverse events reported during the study period. One patient reported generalized itching during obinutuzumab infusion, which resolved spontaneously.

Patients treated with obinutuzumab had a high incidence of severe pneumonia following COVID-19 infection

To investigate whether obinutuzumab induced a high incidence of severe pneumonia following COVID-19 infection, data on non-obinutuzumab treated membranous patients were analyzed. During the outbreak of COVID-19, a total of 410 adult membranous nephropathy patients were followed up at our center, and 378 patients (92%) contracted COVID-19 for the first time. Among the infected patients, 225 (60%) had not received immunosuppressants during the past 2 years. Only one of the 225 patients, who also had Waldenstrom macroglobulinemia,

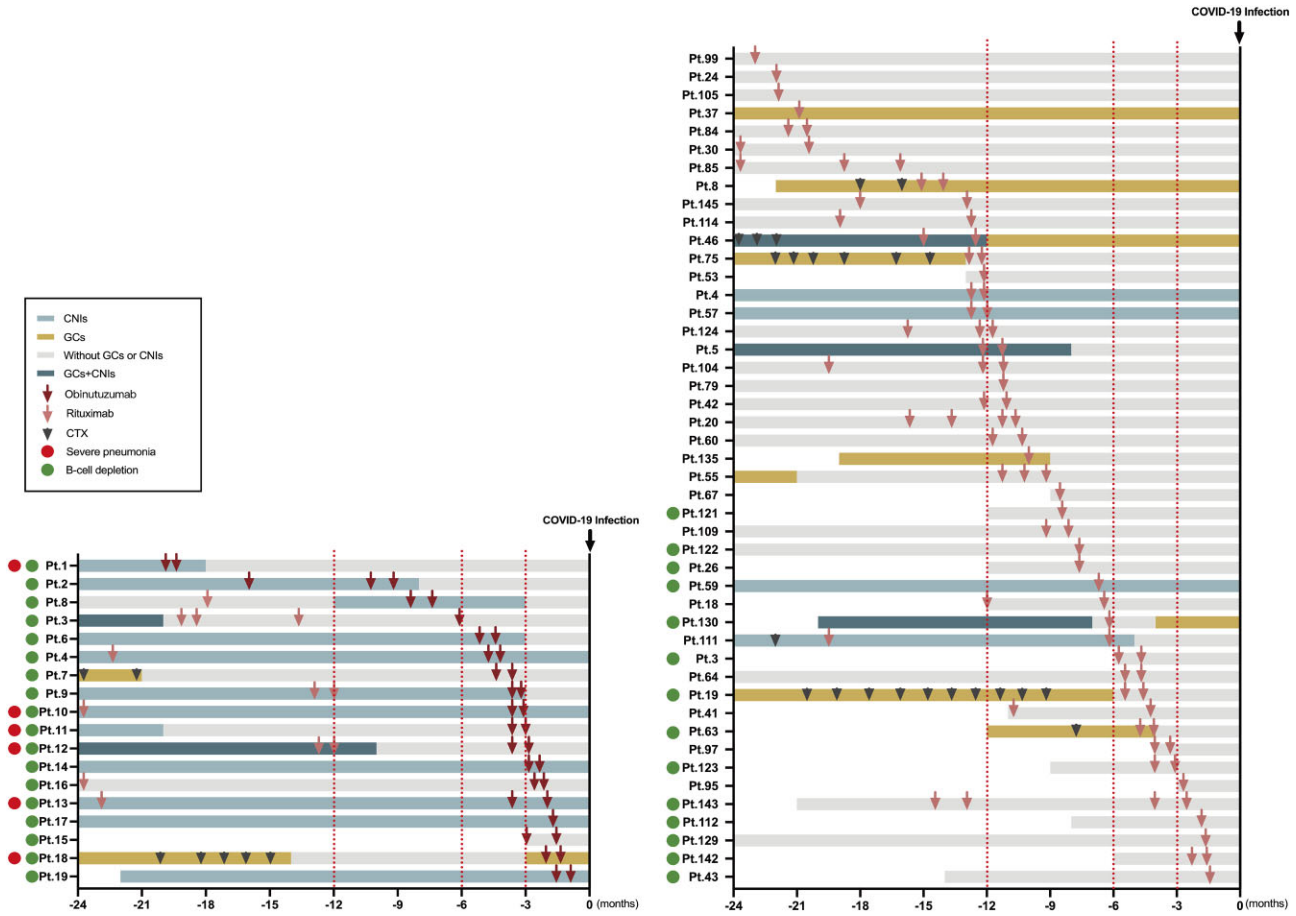

Figure 3: Clinical medication history of patients treated with obinituzumab and rituximab in the 2 years preceding the COVID-19 infection. GCs, glucocorticoids.

developed severe pneumonia. Eight patients received glucocorticoids within the past 2 years for various reasons, and two of them developed severe pneumonia: one was complicated by cryoglobulinemia, while the other had rheumatoid arthritis and pancreatic carcinoma. The remaining 145 patients received immunosuppressive regimens including glucocorticoids combined with CTX, CNIs with/without glucocorticoids, rituximab, and obinutuzumab (Fig. 2). Among these 145 patients, 53 had been vaccinated against COVID-19. Of these, 39 were vaccinated >12 months ago, and 50 were vaccinated >6 months ago.

Among 37 patients receiving glucocorticoids combined with CTX, two developed severe pneumonia, and none of the 44 patients on calcineurin inhibitors nor the 46 patients on rituximab experienced severe pneumonia. Among the 18 obinutuzumab-treated patients, six developed severe pneumonia and one died. The rate of severe pneumonia in the obinutuzumab group was significantly higher than those in the other three groups. However, when compared to patients receiving rituximab, the obinutuzumab-treated patients receiving obinutuzumab were more likely to be male and in B-cell depletion. They also had a longer duration of membranous nephropathy and immunosuppressive therapy than those on rituximab or CTX, and were less likely to be male and in remission than those on CNIs regimen (Table 3 and Fig. 3).

To further evaluate whether obinutuzumab treatment was associated with an increased risk of severe pneumonia, patients on non-obinutuzumab immunosuppressive regimens for membranous nephropathy were used as a control group. After matching for age, gender, remission status, duration of membranous nephropathy, duration of immunosuppressive therapy, and ongoing immunosuppression, 18 patients were identified. They included seven patients receiving glucocorticoids combined with CTX, seven patients receiving CNIs with/without glucocorticoids, and four patients receiving rituximab. As shown in Table 4, the obinutuzumab-treated patients experienced a significantly higher incidence of severe pneumonia compared to those on other treatment regimens.

At the time of COVID-19 infection, all 18 patients receiving obinutuzumab were B-cell depleted. Twelve patients were in partial remissions. Six patients had concurrent use of CNIs, with no significant difference in the occurrence of severe pneumonia between those with and without concurrent use of CNIs (2/6 vs. 4/12). Among the six patients with severe pneumonia, all had a history of other immunosuppressive therapies. Five developed severe pneumonia within 4 months and one patient died 20 months after the last dose of obinutuzumab (Table 5). Compared to the patients without severe pneumonia, those with severe pneumonia tended to be older, have lower eGFR, longer durations of membranous nephropathy and immunosuppressive therapy, and a shorter time interval between the last dose of obinutuzumab and infection. However, the differences between them were not statistically significant (Table 6).

Table 4: Differences between patients receiving obinutuzumab and other IST after matching.

	Obinutuzumab $n = 18$	Other IST $n = 18$	SMD
[a]Age, years (SD)	58.0 (48.3,63.8)	59.5 (40.0,67.8)	0.004
[a]Male, *n* (%)	16 (88.9)	16 (88.9)	<0.001
[a]Duration of MN, months (IQR)	57.5 (36.8,89.8)	64.5 (23.3,100.3)	0.02
[a]Duration of IST[b] , months (IQR)	46.5 (29.8,60.5)	36.5 (11.3,86.0)	<0.001
[a]Immunosuppression[c], *n* (%)	18 (100)	18 (100)	<0.001
[a]Overall remission, *n* (%)	10 (55.6)	10 (55.6)	<0.001
Without comorbidity, *n* (%)	9 (50.0)	9 (50.0)	<0.001
Comorbidity			
Hypertension, *n* (%)	8 (44.4)	7 (38.9)	0.113
Diabetes, *n* (%)	4 (22.2)	5 (27.8)	0.129
Coronary artery disease, *n* (%)	2 (11.1)	2 (11.1)	0.5
Respiratory disease, *n* (%)	2 (11.1)	1 (5.6)	0.202
Severe pneumonia, *n* (%)	6 (33.3)	0	0.972

[a]Variables for matching.
MN, membranous nephropathy; IST, immunosuppressive therapy.
[b]Cumulative time of immunosuppression before COVID-19 infection.
[c]Defined as being on immunosuppressants, in B-cell depletion (<5/μl) after CD20-mAb infusion, or within 6 months after CTX discontinuation.

DISCUSSION

In our study, we observed that obinutuzumab showed a high efficacy for membranous nephropathy, with an overall remission rate of 83% at 12 months. However, during the outbreak of COVID-19 in China, six out of 18 patients infected with COVID-19 developed severe pneumonia. Compared to patients treated with non-obinutuzumab immunosuppressive regimens during the same period, those treated with obinutuzumab exhibited a significantly higher incidence of severe pneumonia after matching for baseline factors. This study highlights the importance of considering COVID-19 infection in patients undergoing obinutuzumab treatment, particularly those with refractory membranous nephropathy.

Recent studies also suggested that obinutuzumab is effective in treating refractory membranous nephropathy, particularly in patients resistant to rituximab [9–11]. In our study, nine out of 10 CNIs-dependent patients, previously treated with rituximab to taper off CNIs but unsuccessful, achieved remission following obinutuzumab treatment. These findings collectively suggest that obinutuzumab shows promise as a treatment strategy for patients with membranous nephropathy who do not respond to rituximab. Although both obinutuzumab and rituximab are anti-CD20 monoclonal antibodies, obinutuzumab's effectiveness in membranous nephropathy is likely significantly better than rituximab, possibly due to its stronger CD20 depletion capacity. Reports indicate that increasing rituximab doses could be effective for some patients who did not respond well initially, supporting the notion that stronger B-cell clearance may enhance effectiveness [15]. As obinutuzumab has a profounder B-cell clearance, its safety, particularly the incidence of infection and severe disease after infection, remains unclear due to limited clinical data.

Patients undergoing immunosuppressive therapy may experience compromised immune system, potentially facilitating the unchecked spread of SARS-CoV-2, the virus causing COVID-19 [14]. Reports have indicated that immunosuppressive therapy is linked to increased risks of hospitalization and mortality following COVID-19 [16–19]. However, studies have yielded inconsistent results. Some studies have shown that individuals taking immunosuppressants for organ transplant rejection prevention or the treatment of inflammatory or autoimmune diseases did not have a worse outcome when hospitalized with COVID-19 [20–22]. Additionally different immunosuppressive regimens for the same disease may have varying affects on the severity of COVID-19 [23]. A prospective study revealed worse COVID-19 outcomes in inflammatory arthritis patients receiving glucocorticoids, but not in those on maintenance anti-cytokine therapy [24]. A meta-analysis demonstrated that autoimmune disease patients had an increased risk of COVID-19, primarily attributed to glucocorticoid use, while biologic or targeted synthetic disease-modifying antirheumatic drugs (DMARDs) monotherapy was associated with a lower risk of severe COVID-19 [25].

It remains unclear whether different immunosuppressive regimens for membranous nephropathy affect the severity of COVID-19 infection. It should be noted that the development of severe COVID-19 may be associated with not only immunosuppressive medications, but also patients' conditions such as ages, duration of membranous nephropathy and immunosuppressive therapy, ongoing immunosuppression, and immunities to the microorganism, the virulence of the virus strain, and the season of the year. The outbreak of the COVID-19 in China, with >90% of the population being infected within several months, allowed us to analyze the data with fewer interfering factors such as the strain of the virus. In our study, we found that the rates of COVID-19 infection between patients treated with obinutuzumab and non-obinutuzumab immunosuppressants during the outbreak were similar, but patients receiving obinutuzumab have more severe cases. This finding is consistent with a recent study suggesting that obinutuzumab treatment in hematological patients infected with the Omicron variant COVID-19 was associated with a worse clinical outcome [26]. This higher risk may be related to the potent CD20 depletion capacity of obinutuzumab.

Since rituximab and obinutuzumab both target CD20 and work by depleting B cells, we are very interested in the differences between them. B-cell depletion is a sign of immunosuppression. Among the patients with B-cell depletion at the time of infection, patients receiving obinutuzumab still had a higher risk of severe pneumonia than those receiving rituximab (6/18 vs. 0/14, $P = .024$). However, we did not conclude obinutuzumab contributed a higher risk compared to rituximab,

Table 5: Characteristics of obinutuzumab-treated patients at the time of COVID-19 infection.

Patient no.	Age (years)	Gender	Duration of MN (months)	Duration of IST[c] (months)	Doses of OBI (doses)	OBI time[d] (days)	Combined IST	B cells (/μl)	PLA2R-Ab (RU/ml)	Urine protein (g/day)	Serum albumin (g/dl)	eGFR (ml/min/ 1.73 m^2)	Comorbidity
1[#]	62	Male	138	61	2	373	None	0	<2	0.4	4.0	62.3	Hypertension
2	45	Male	50	47	3	109	None	0	2.1	1.4	4.2	133.7	——
3	69	Male	74	49	1	185	None	1	3.1	1.4	3.8	79	Diabetes
4	39	Male	46	46	2	144	CNIs	3	<2	4.3	2.9	123	——
6	64	Male	66	63	2	145	None	0	<2	1.6	3.6	98.2	——
7	51	Male	36	45	2	129	None	0.5	<2	6.7	4.3	52.9	Hypertension; Diabetes
8	58	Female	39	39	2	115	None	0.9	<2	1.9	3.5	78.7	——
9	53	Male	60	60	2	114	None	1	<2	1.1	4.0	81.5	——
10[a]	63	Male	126	123	2	99	CNIs	2	6.4	2	3.2	47	Hypertension; Diabetes; Chronic respiratory disease
11[a]	48	Male	55	48	2	95	None	0.2	<2	1.4	3.5	117.5	——
12[a]	45	Male	34	18	2	93	None	0	2.9	2	2.9	89.8	Hypertension
13[b]	64	Male	105	89	2	58	CNIs	0.3	4.7	1.3	4.1	42.1	——
14	57	Female	62	61	2	71	CNIs	0.4	13.4	1.8	3.3	100.9	——
15	39	Male	38	2	2	45	None	0.6	<2	1.3	3.5	136.9	Hypertension; Diabetes; Chronic respiratory disease
16	61	Male	28	26	2	62	None	0	2.9	4.3	2.5	87.3	Hypertension
17	33	Male	95	92	1	68	CNIs	0.7	<2	7	4.0	135.2	Hypertension
18[a]	65	Male	23	23	2	40	GCs	0	<2	10	2.1	47.5	Hypertension
19	59	Male	141	38	2	33	CNIs	NA	<2	4	3.0	69.8	——

[a]Severe pneumonia;
[b]Severe pneumonia, death
MN, membranous nephropathy; IST, immunosuppressive therapy; OBI, obinutuzumab; GCs, glucocorticoids.
[c]Cumulative time of immunosuppression before COVID-19 infection.
[d]Time from the last dose of obinutuzumab to infection.

Table 6: Differences between the obinutuzumab-treated patients with and without severe pneumonia.

	Total $n = 18$	No severe pneumonia $n = 12$	Severe pneumonia $n = 6$	P
Age, years (SD)	55 (10.7)	52.9 (11.4)	59.2 (8.3)	.317
Male, *n* (%)	16 (88.9)	10 (83.3)	6 (100)	.529
Duration of MN, months (IQR)	57.5 (35.5, 97.5)	54.0 (35.6, 71.8)	79.3 (31.1, 128.3)	1
Overall remission, *n* (%)	10 (55.6)	6 (50)	4 (66.7)	.867
PLA2R-Ab <2 RU/ml, *n* (%)	11 (61.1)	8 (66.7)	3 (50)	1
Serum albumin, g/dl (SD)	3.6 (0.6)	3.8 (0.4)	3.2 (0.8)	.046
Urine protein, g/day (IQR)	1.7 (1.1,3.4)	1.3 (0.7, 3.4)	2.0 (1.8, 4.6)	.046
eGFR, ml/min/1.73 m^2 (IQR)	87.4 (67.2, 113.5)	96.7 (76.9, 123.1)	61.1 (39.0, 98.2)	.405
Duration of IST[a], months (IQR)	47.5 (35.0, 61.5)	46.5 (38.3, 60.8)	54.5 (22.0, 97.5)	.317
OBI time[b], days (IQR)	97 (61, 133)	111.5 (63.5, 140.3)	94 (53.5, 167.5)	.317
Combination with other immunosuppressants, n (%)	7 (38.9)	4 (33.3)	3 (50)	.627
B-cell depletion[c], *n* (%)	18 (100)	12 (100)	6 (100)	1
Vaccination, *n* (%)	7 (38.9)	5 (41.7)	2 (33.3)	1
Without comorbidity, *n* (%)	9 (50)	7 (58.3)	2 (33.3)	.617
Comorbidity				
Hypertension, *n* (%)	8 (44.4)	4 (33.3)	4 (66.7)	.402
Diabetes, *n* (%)	4 (22.2)	3 (25)	1 (16.7)	1
Respiratory disease, *n* (%)	2 (11.1)	1 (8.3)	1 (16.7)	1

MN, membranous nephropathy; IST, immunosuppressive therapy; OBI, obinutuzumab.
[a]Cumulative time of immunosuppression before COVID-19 infection.
[b]Time from the last dose of obinutuzumab to infection.
[c]The count of B cells <5/μl.

because patients receiving obinutuzumab had a longer duration of membranous nephropathy and immunosuppressive therapy. After matching for these factors, we could only identify only four patients from each group. Further, we grouped patients receiving the three non-obinutuzumab immunosuppressive regimens as a control cohort. After balancing the baseline characteristics including age, gender, remission status, duration of membranous nephropathy, duration of immunosuppressive therapies, and ongoing immunosupression, obinutuzumab still showed a higher risk of severe disease. This result may prompt further studies to examine whether obinutuzumab is associated with a higher risk of severe COVID-19 or other infections.

The present study also analyzed the potential risk factors associated with the development of severe pneumonia. Compared to the obinutuzumab-treated patients without severe pneumonia, those with severe pneumonia had higher levels of proteinuria, and lower levels of serum albumin. They tended to be older, and have a longer duration of immunosuppressive therapy and a shorter time interval between the last dose of obinutuzumab and infection. However, these differences have not reached statistically significant. It is important to note that the sample size was small. Additionally, the combination of obinutuzumab and CNIs, which suppresses T-cell immunity, may exacerbate the severity of COVID-19 infections, although more studies are needed to validate this hypothesis. Therefore, we discontinued CNIs on the detection of a COVID-19 infection in our clinical practice. However, there was no significant difference in severe pneumonia between patients with and without a history of concurrent use (3/11 vs. 3/7), and between patients with and without concurrent use at the time of infection (2/6 vs. 4/12). Since the sample size was small, it is advisable to remain vigilant for the occurrence of severe infections within 6 months following obinutuzumab treatment or using CNIs concurrently.

There were several limitations in the study. First, this was a case series, and we did not compare the effectiveness of obinutuzumab on membranous nephropathy with other immunosuppressive regimens. Second, most patients had a history of other immunosuppressive treatments, and some were still using or had recently used other immunosuppressants at the time of obinutuzumab infusion. Therefore, the effectiveness and safety could not be solely attributed to obinutuzumab. Third, this was not a randomized controlled study, and bias may exist when investigating the difference in severe COVID-19 prevalence between obinutuzumab and other regimens. Fourth, all patients included in the study contracted COVID-19 infection for the first time. Given that subsequent infections may be milder than the initial episode, it remains unclear whether patients receiving obinutuzumab have a higher risk of severe disease when contracting a subsequent COVID-19. Fifth, the sample size was small, which limited the definitive analysis of effectiveness, mortality, and risk factors for severe pneumonia. Larger studies are required to address these issues.

In conclusion, patients with membranous nephropathy have shown successful treatment response to obinutuzumab. However, they experienced a higher incidence of severe pneumonia following COVID-19 infection compared to those receiving other immunosuppressive regimens. Our results may prompt further studies to examine the effect of obinutuzumab on infections. Until further and stronger evidence emerges, it is crucial to maintain vigilant for the occurrence of severe COVID-19 pneumonia when utilizing this treatment regimen.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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AUTHORS' CONTRIBUTIONS

X.Q. conceived the idea and designed the study. X.M., W.Y., C.R., Z.H., and Z.M., contributed to the data collection. X.M., H.X., and X.Q. analyzed the data. X.M. and X.Q. were the main contributors in writing the manuscript. X.Q. and H.C. critically reviewed and revised the manuscript. All authors were involved in the design, interpretation of data, and final approval of the manuscript.

All authors had access to the data and played a role in writing this article.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in its online supplementary material.

CONFLICT OF INTEREST

None declared.

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