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Original Paper

Chlormethine Hydrochloride is Not Inferior to Tacrolimus in Treating Steroid-Resistant Nephrotic Syndrome

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Key Words
Steroid-resistant nephrotic syndrome • Chlormethine hydrochloride • Tacrolimus

Abstract
Background/Aims: The present study aimed to explore the equivalence of CHL and tacrolimus (TAC), despite reports regarding the efficacy and safety of TAC in treating SRNS patients. ***Methods:*** A retrospective cohort study of CHL or TAC treatment was performed by collecting the medical records of SRNS patients with a pathological classification of focal segmental glomurular sclerosis (FSGS) or membranous nephropathy (MN) from December 2008 to December 2014 in a 3A grade hospital in southern China. The treatment regimen includes 6 months of induction therapy and a subsequent 6 to 30 months of maintenance therapy, which were evaluated by the scheduled follow-up and the detection of proteinuria and serum creatinine levels. The treatment outcomes were classified as complete remission, partial remission or no remission. ***Results:*** In a total of 146 SRNS patients, CHL treatment showed a higher proportion of complete remission (27.8% vs 14.9%) or partial remission (52.8% vs 37.8%) compared to TAC treatment ($P < 0.10$) at the stage of induction therapy. The CHL treatment of SRNS patients with FSGS showed better efficacy than treatment of the TAC group, but the difference of efficacy in the pathological type of MN between CHL and TAC group was not significant ($P > 0.10$). During maintenance therapy, the difference between the CHL and TAC groups was not significant in the SRNS patients with FSGS or MN ($P > 0.10$). In addition, the difference of adverse effects between CHL and TAC group was not significant ($P > 0.10$), although there was a slightly higher proportion of nausea and vomiting in the CHL group. ***Conclusion:*** The non-inferior efficacy of CHL treatment on the SRNS patients with

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FSGS or MN compared to TAC treatment, which highlighted CHL can be considered to be alternative treatment for SRNS patients in the clinical setting.

Introduction

Nitrogen mustard was developed as a derivative of sulphur mustard that includes chlormethine hydrochloride (CHL), chlorambucil, melphalan, cyclophosphamide and estramustine, which are widely used in humans and have progressively lower toxicity in normal cells [1]. It acts as a DNA alkylating agent and shows cytotoxic effects on proliferating cells, particularly in the depletion of B- and T- lymphocytes [1, 2], which were applied as an immunosuppressant used to treat renal disease since 1950s, especially for those patients who are resistant or dependent on steroid therapy due to the ability to inhibit autoimmune injury in renal diseases [3, 4]. Additionally, the appropriate treatment of nitrogen mustard did not affect the function of liver, making it safer for patients with liver dysfunction [5]. Here, in our hospital located in the south of China, physicians favor nitrogen mustard such as CHL in clinical setting for SRNS treatment because it cost less than the expensive tacrolimus (TAC) or Mycophenolate mofetil (MMF) drugs and presents equal efficacy.

Steroid-resistant nephrotic syndrome (SRNS) is characterized as having unsatisfactory outcomes with steroid treatment and develops mainly during the treatment of idiopathic nephrotic syndrome (INS). It can progress gradually into end-stage renal disease (ESRD), accounting for approximately 10-20% of INS patients [6-9]. Of the adult onset nephrotic syndrome patients, membranous nephropathy (MN) and focal segmental glomurular sclerosis (FSGS) are the common clinicopathological types, accounting for 12.3% and 11.0% of the total cases, respectively [10]. For the pathological type of FSGS, the focal and segmental location of the sclerotic lesions is considered to be the fundamental basis, and its glomerular features are a prototype of podocyte loss-driven glomerulosclerosis [11], accompanied with an increased matrix and associated closely with podocyte injury and parietal epithelial cell (PEC) migration [12].

MN is characterized by glomerular lesions that occur in immune complexes at the base of podocytes, with the consequent activation of complement and inflammation triggered by the membrane attack complex C5b-9 [13]. Recently, studies have demonstrated the significant efficacy of nitrogen mustard or TAC in treating patients with idiopathic membranous nephropathy (IMN) [14-16], which showed a significant remission with a reduction in proteinuria or plasma creatinine in IMN patients and a reduced probability of developing end-stage renal disease [17, 18]. Additionally, existing evidence supports the use of a combination of steroid and alkylating agents, such as nitrogen mustard [19]. Here, derivatives of nitrogen mustard, such as chlorambucil, which is considered a cost-effective therapy, and combined treatment with steroids can prevent clinical SRNS patients from prolonged heavy proteinuria and potential complications. However, the data addressing the independent evaluation of CHL treatment has been poorly reported in the literature [20, 21]. For this reason, our study aims to evaluate the equivalent efficiency between affordable CHL treatment and relatively expensive TAC treatment. It is our hope that it provides a clinical reference for CHL as an alternative treatment strategy for SRNS patients.

Materials and Methods

Study Design

The medical records of 146 SRNS patients in a 3A grade hospital in southern China were reviewed, and the efficacy were evaluated by the scheduled follow-up and detection of proteinuria and serum creatinine levels once every three months. All SRNS patients were diagnosed pathologically as FSGS or MN in the period of December 2008 to December 2014, and histologic variant subtypes of FSGS and the evaluation of

the interstitial fibrosis and tubular atrophy (IFTA) were based on the Columbia classification and the Banff criteria [22, 23]. This retrospective cohort study was approved by the Human Research Ethics Committees in the Second Xiangya Hospital of Central South University, China. Research cohort and control were defined as the groups of CHL treatment and TAC treatment, respectively.

Definitions

Standard definitions of SRNS, remission or no response referred to the guidelines from Improving Global Outcomes (KDIGO) [24]. In detail, SRNS was defined as nephrotic range proteinuria (3-4+ proteinuria by dipstick and spot urine protein to creatinine, with a urine protein-to-creatinine ratio (Up/Uc) ≥ 2.0 mg/mg) in spite of therapy with prednisolone for 8 weeks (2 mg/kg daily followed by 1.5 mg/kg on alternate days for 4 weeks each). Complete remission was defined as proteinuria < 0.3 g/d, with normal SCr levels (< 133 μmol/L or < 1.5 mg/dL) that were analyzed at 6 months. Partial remission was defined as a reduction of proteinuria ranging from 0.3 to 3.5 g/d or a decrease in the baseline proteinuria of more than 50%, with a less than 25% increase in the SCr levels. No response was defined as proteinuria > 3.5 g/d or a decrease of baseline proteinuria less than 50%, with more than a 25% increase in the SCr levels.

Inclusion and Exclusion criteria

The inclusion criteria of the study subjects were as follows: ① age ≥ 18 years, and ② SRNS patients with biopsy-proven diagnosis of FSGS or MN. Exclusion criteria were as follows: ① The presence of secondary causes of nephrotic syndrome (e.g., lupus erythematosus, immunoglobulin A nephropathy, amyloidosis), ② An estimated Glomerular Filtration Rate (eGFR) < 40 mL/min per 1.73 m^2 (eGFR was calculated based on a modified Chinese diet in renal disease (MDRD) formula: c-aGFR = $186 \times [Cr]^{-0.203} \times [age]^{-1.154} \times [female \times 0.742] \times 1.233$) [25], ③ A diagnosis of active infectious disease (e.g., HIV, hepatitis B or C, or known malignancy), and ④ A plan to become pregnant within 12 months following the randomization procedure.

Therapeutic strategy

The treatment procedure was designed as follows: (1) All patients were given oral prednisone at 1 mg/kg/d (up to a maximum dose of 60 mg) for 8 weeks. They were then given a dose that was reduced by 5 mg/d every 2 weeks to 0.5 mg/kg/d or reaching 30 mg/d at 8 weeks, after which they were given a tapered dose every 2 weeks from 2.5 mg to 10 mg/d. This was sustained for half a year to one year. (2) The treatment protocol included 6 months of induction therapy and subsequent 6 month to 30 month of maintenance therapy. At the stage of induction therapy, CHL treatment was performed by injecting an initial dose of 1 mg on the first day, 3 mg on the third day, and 5 mg on the fifth day. It was then injected two times every week until a maximum dose of 1 mg/kg was reached. TAC treatment was performed by the administration of an oral dose of 0.1 mg/kg/day, once every 12 hours, which was maintained for 6 months. Subsequently, at the stage of maintenance therapy, the protocol of CHL treatment was repeated, with 1 mg, 3 mg and 5 mg being given. The TAC treatment protocol was performed by decreasing the frequency of oral TAC by 0.1 mg/kg/day once every 24 hours if patients achieved complete remission after 6 months, or continuing oral TAC treatment if only partial remission or no remission was achieved. (3) During CHL or TAC treatment, all patients were given the same doses of angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker. Anticoagulants, statins, and antihypertensive drugs were added temporarily for the purpose of controlling blood pressure. (4) The follow-up protocols were conducted once a week during induction therapy and once a month during maintenance therapy among the patients receiving CHL or TAC treatment. Additionally, the liver and kidney function and routine blood tests were conducted once every 2 or 4 weeks during the induction period and during maintenance therapy. If serious adverse effects, such as the events of CKD stage 4/5, death, myelosuppression, infections, nausea and vomiting, headache, phlebitis, elevated liver transaminase, occurred or if the patient achieved complete remission, we considered this to be the end point of observation. The corresponding measures such as discontinuing the drug or taking a half dose were thus performed.

Statistical analysis

The clinical or pathological characteristics of the patients were analyzed by the T test or Chi-square (χ^2) test, and the difference of treatment outcomes or adverse effects was evaluated by the χ^2 test. A *P* value less than 0.10 was considered significant.

Results

General characteristics

A total of 146 SRNS patients were included in the present analysis, of which 72 patients with 37 FSGS or 35 MN were treated by CHL, and 74 patients with 39 FSGS or 35 MN were treated by TAC according to the scheduled protocol. The equilibrium test was performed by evaluating the distribution of baseline characteristics, such as the age at onset of disease or at enrollment, the male/female ratio, the duration of disease, routine blood tests, renal function examination, and the pathological feature such as ratio of FSGS vs MN, the distribution of FSGS subtypes, the average IFTA score, which were not significant between the CHL and TAC group ($P > 0.05$), indicating that the distributions of the baseline or pathological types in patients between the CHL and TAC groups were comparable. In addition, in the CHL or TAC group, the results of detecting daily proteinuria and eGFR during treatment showed a slight decrease at the stage of induction therapy. This rebounded slightly during maintenance therapy, and more details are shown in Table 1, Table 2 & Fig. 1.

Table 1. Clinical characteristics of the patients at the initiation of treatment. Notes: n, the number of sample; M, median; IQR, interquartile range; the data of table was showed by M (IQR); IQR are represented as the range of upper quartile (75%) and lower quartile (25%); NOS, not otherwise specified in FSGS; Tip, tip variant of FSGS; Perihilar, perihilar variant of FSGS; a, Histological subtypes of FSGS refers to Columbia classification, the variants of cellular and collapsing were not considered according to the exclusion criteria; b, IFTA score refers to Banff criteria (0, no abnormalities; 1, abnormalities affecting < 1/3 of the sample; 2, 1/3 ~ 2/3; 3, > 2/3 of the sample)

Variables	CHL group M (IQR)	TAC group M (IQR)	P value of χ^2 or t test
Ratio of male vs female	1.18	1.24	0.88
Age at onset of disease	21.5 (18.3~26)	22.5 (20.0~28.5)	0.10
Age at enrollment	24 (20.0~29.0)	25 (20.0~30.3)	0.25
Duration of disease (months)	9 (3.0~33.5)	9 (3.0~24.5)	0.57
Frequency of pathological types (FSGS: MN)	37: 35	39: 35	0.87
[a] Distribution of FSGS subtypes (NOS: Tip: Perihilar)	17: 15: 5	19: 14: 6	0.92
[b] Average IFTA score	1.6	1.5	0.78
Systolic blood pressure (mmHg)	121 (112.5~136.5)	127.5 (113.8~136.0)	0.99
Diastolic blood pressure (mmHg)	80 (75~89)	79.5 (75~86)	0.39
Blood glucose (mmol/L)	4.3 (4.0~5.1)	4.2 (4.0~5.0)	0.18
Haemoglobin (g/dL)	143 (127.3~156)	146.5 (133.8~155.0)	0.97
Serum albumin (g/L)	18.1 (15.0~22.6)	19.4 (17.3~22.0)	0.41
Triglyceride (mmol/L)	3.0 (2.4~3.8)	3.2 (2.1~4.1)	0.96
Total cholesterol (mmol/L)	10.1 (8.6~12.4)	10.1 (9.2~12.5)	0.27
C3 complement (g/L)	1.2 (1~1.3)	1.1 (1.0~1.2)	0.89
Daily proteinuria (g)	6.6 (5.0~8.8)	6.3 (5.1~8.6)	0.28
Serum creatinine (mg/dL)	1.1 (1.0~1.2)	1.1 (0.9~1.3)	0.64
eGFR (mL/min/1.73 m^2)	94.8 (75.4~118.1)	93.3 (73.0~121.1)	0.91

Table 2. Dynamic characteristics of proteinuria and eGFR during treatment. Notes: m, month; eGFR, estimated Glomerular Filtration Rate; 0 m indicates the results of IQR at the initiation of treatment; The values of IQR at 0 m refer to the data of table 1

Group	Indices of detection	Results of different time points, M (IQR)				
		0 m	3 m	6 m	18 m	30 m
CHL group	Daily proteinuria (g)	6.6	4.2 (3.3~6.7)	3.8 (2.1~5.8)	2.7 (1.5~4.7)	3.1 (2.2~5.6)
	eGFR (mL/min/1.73 m^2)	94.8	76.5 (61.8~98.7)	73.9 (57.4~101.6)	64.6 (54.5~82.6)	82.5 (73.7~105.5)
TAC group	Daily proteinuria	6.3	4.6 (3.2~7.1)	4.8 (3.4~7.5)	3.7 (2.8~5.5)	4.0 (3.4~5.9)
	eGFR	93.3	82.1 (68.3~102.1)	84.9 (72.2~103.7)	79.3 (64.6~102.5)	71.8 (61.4~95.5)

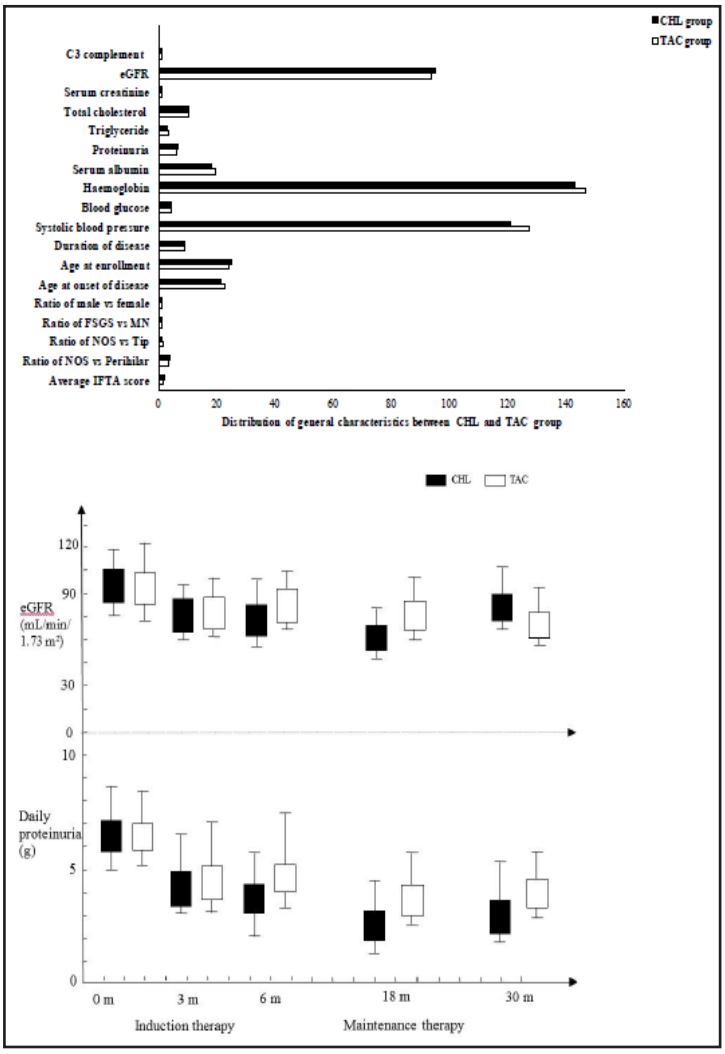

Fig. 1. General characteristics of the patients across the course of treatment. Notes: CHL, Chlormethine hydrochloride; TAC, tacrolimus; eGFR, estimated Glomerular Filtration Rate; FSGS, focal segmental glomurular sclerosis; MN, membranous nephropathy; IFTA, interstitial fibrosis and tubular atrophy; Scale marks of each grid were represented as 15 mL/min/1.73 m^2 for eGFR, 1 g for proteinuria, respectively.

Outcomes of induction therapy

At the end of 6 months of induction therapy in FSGS patients, the treatment of CHL group showed a better outcome than the TAC group, which were represented as the higher proportion of complete remission and partial remission, and the lower proportion of no remission compared to TAC group (*P1* < 0.10). However, the difference in the outcomes between the CHL group and the TAC group was not significant in the MN patients (*P1* > 0.10). Furthermore, to compare the pathological types of FSGS vs. MN, the difference between the FSGS type and MN type was not significant for either CHL treatment or TAC treatment (*P2* > 0.10), although a slight elevation in the proportion of patients who did not go into remission was observed in the TAC group with FSGS (*P2* = 0.09). In addition, in the FSGS and MN patients, CHL treatment produced a better outcome than did TAC treatment, as represented by the higher proportion of complete remission and partial remission and the lower proportion of no remission compared to the TAC group (*P1* < 0.10). For more details, see Table 3 and Fig. 2.

Effects of maintenance therapy

When comparing CHL and TAC treatments during maintenance therapy, the results showed no significant difference in the proportion of complete remission and partial remission (*P1* > 0.10), although there was a lower proportion of no remission in the CHL group (*P1* = 0.03). Additionally, across all FSGS and MN patients, similar results were observed between the CHL and TAC groups, although there was a decrease in the proportion of no remission patients in the CHL group (*P1* = 0.07). For the comparison of the pathological type of FSGS vs. MN, the difference in the outcomes was not significant after CHL or TAC treatment (*P2* > 0.10). More details are shown in Table 4 and Fig. 3.

Table 3. Comparison of treatment outcomes at 6 months of induction therapy. Notes: P1 value indicates the comparison of chi-square test between CHL and TAC group, *P < 0.10, indicating a significant difference between CHL and TAC group in FSGS patients; ▲P < 0.10, indicating a significant difference between CHL and TAC group in patients of FSGS and MN; P2 value indicates the difference of treatment outcome by chi-square test between FSGS and MN group, △P < 0.10, indicating a significant difference between FSGS and MN group

Pathological types	Outcomes of treatment	CHL group n1 (%)	TAC group n2 (%)	P1 value	P2 value
FSGS / MN		37/35	39/35		
	Complete remission	11 (29.7)/ 9 (25.7)	5 (12.8)/ 6 (17.1)	0.07*/ 0.38	0.70/ 0.60
FSGS / MN	Partial remission	20 (54.1)/ 18 (51.4)	12 (30.8)/ 16 (45.7)	0.04*/ 0.63	0.82/ 0.19
	No response	6 (16.2)/ 8 (22.9)	22 (56.4)/ 13 (37.1)	<<0.01*/ 0.19	0.48/ 0.09△
	Complete remission	20 (27.8)	11 (14.9)	0.06▲	
FSGS + MN	Partial remission	38 (52.8)	28 (37.8)	0.07▲	
	No response	14 (19.4)	35 (47.3)	<<0.00▲	

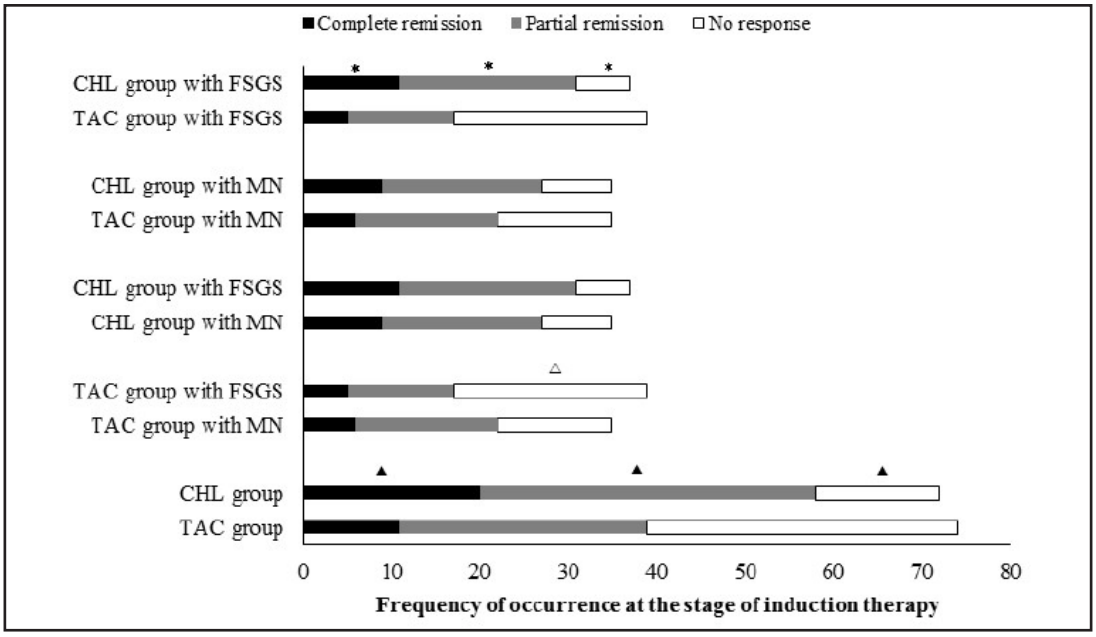

Fig. 2. Comparison of treatment outcomes at 6 months of induction therapy. Notes: *P < 0.10, indicating a significant difference between CHL and TAC group in FSGS patients; ▲P < 0.10, indicating a significant difference between CHL and TAC group in patients of FSGS and MN; △P < 0.10, indicating a significant difference between FSGS and MN group.

Table 4. Comparison of treatment outcomes at the stage of maintenance therapy. Notes: *P < 0.10, indicating a significant difference between CHL and TAC group in FSGS patients; ▲P < 0.10, indicating a significant difference between CHL and TAC group in patients of FSGS and MN

Pathological types	Outcomes of treatment	CHL group n1 (%)	TAC group n2 (%)	P1 value	P2 value
	Complete remission	4 (15.4)/ 2 (7.7)	2 (5.9)/ 1 (3.4)	0.43/ 0.92	0.66/ 1.00
FSGS / MN	Partial remission	11 (42.3)/ 10 (38.5)	8 (23.5)/ 11 (37.9)	0.12/ 0.97	0.78/ 0.21
	No response	11 (42.3)/ 14 (53.8)	24 (70.6)/ 17 (58.6)	0.03*/ 0.72	0.40/ 0.32
	Complete remission	6 (11.5)	3 (4.8)	0.32	
FSGS + MN	Partial remission	21 (40.4)	19 (30.1)	0.25	
	No response	25 (48.1)	41 (65.1)	0.07▲	

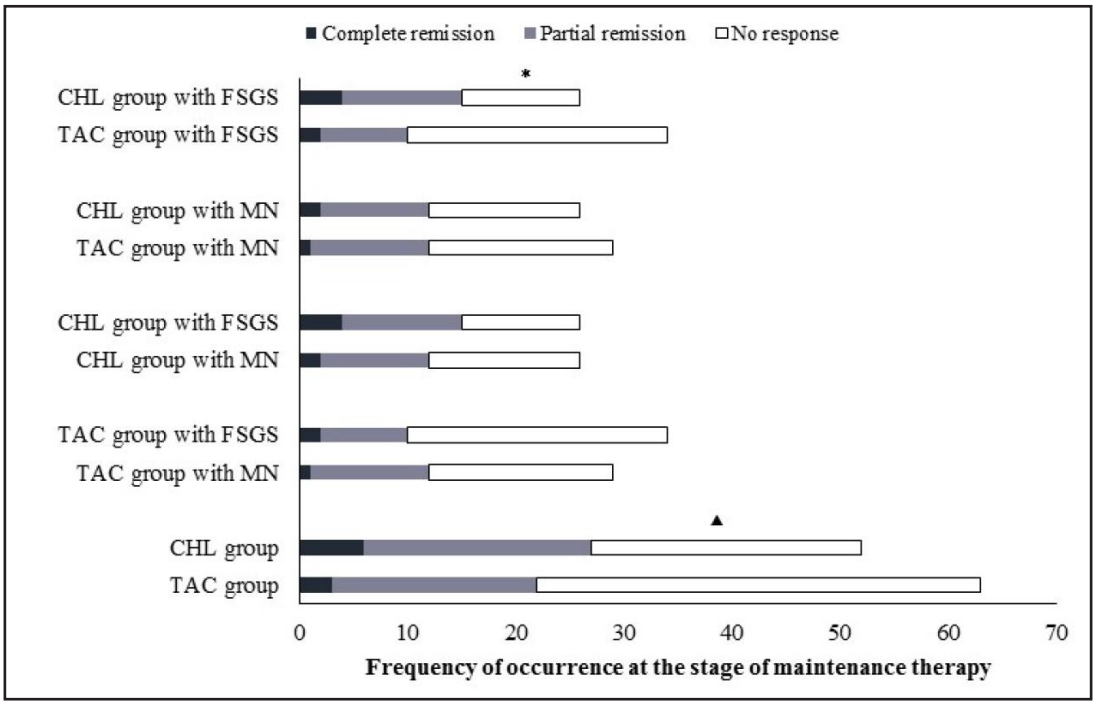

Fig. 3. Comparison of treatment outcomes at the stage of maintenance therapy.

Adverse effects of induction or maintenance therapy

Generally, in the period of induction or maintenance therapy, none of the patients had developed stage 4/5 CKD and no deaths occurred after either CHL or TAC treatment. Nevertheless, a total of 20 patients with FSGS (27.8%) in the CHL group and 10 patients with FSGS (13.5%) in the TAC group had different adverse effects ($P < 0.10$), but the difference in adverse effects in patients with MN between the CHL and TAC group was not significant ($P > 0.10$). In the CHL group, an incidence of 7 (9.7%) in prolonged leucopenia (> 3 weeks) and an incidence of 2 (2.8%) in prolonged thrombocytopenia (> 3 weeks) were recorded. However, no patients developed myelosuppression in the TAC group. The difference in the infection incidence between the CHL group and the TAC group was not significant ($P > 0.10$). In addition, a slightly higher proportion of nausea and vomiting was found in the CHL group (11, 15.3%) than found in the TAC group (5, 6.8%), with a P value of the χ^2 test of 0.099. More details are shown in Table 5 and Fig. 4.

Discussion

The present study evaluated the equivalent efficacy of CHL vs. TAC treatment in SRNS patients with FSGS or MN. Our results showed higher proportions of complete remission and partial remission at the stage of induction therapy in all patients and in patients with FSGS in the CHL group compared to the TAC treatment group. During maintenance therapy, no significant difference in the proportion of complete remission and partial remission was observed between the CHL and TAC groups, and the difference in the outcomes between FSGS and MN was not significant in the CHL or TAC group. In addition, during induction or maintenance therapy, the difference in the incidence of adverse effects such as myelosuppression or infection was not significant, even though there was a slight increase in nausea and vomiting in the CHL group relative to the TAC group.

The present results demonstrate that CHL treatment is not inferior to TAC treatment in the period of induction or maintenance therapy, especially for SRNS patients with FSGS,

Table 5. Comparison of adverse events in the course of CHL or TAC treatment. Notes: One patient may have more than one type of adverse effects; *P < 0.10, indicating a significant difference between CHL and TAC group

Adverse events	CHL group n1 (%)	TAC group n2 (%)	χ^2 test (P value)
FSGS	20 (27.8)	10 (13.5)	0.03*
MN	18 (25.0)	12 (16.2)	0.19
Myelosuppression	9 (12.5)	0	
Prolonged leukopenia (> 3 weeks)	7 (9.7)	0	
Prolonged thrombocytopenia (> 3 weeks)	2 (2.8)	0	
Infections	19 (26.4)	13 (17.6)	0.19
Upper respiratory tract	9 (12.5)	5 (6.8)	0.24
Urinary tract	4 (5.6)	2 (2.7)	0.38
Gastrointestinal	4 (5.6)	4 (5.4)	0.97
Herpes zoster	2 (2.8)	0	
Herpes simplex	0	2 (2.7)	
Other adverse effects	28 (38.9)	18 (24.3)	
Nausea and vomiting	11 (15.3)	5 (6.8)	0.09*
Headache	9 (12.5)	8 (10.8)	0.75
Phlebitis	4 (5.6)	0	
Elevated liver transaminase	4 (5.6)	5 (6.8)	0.76

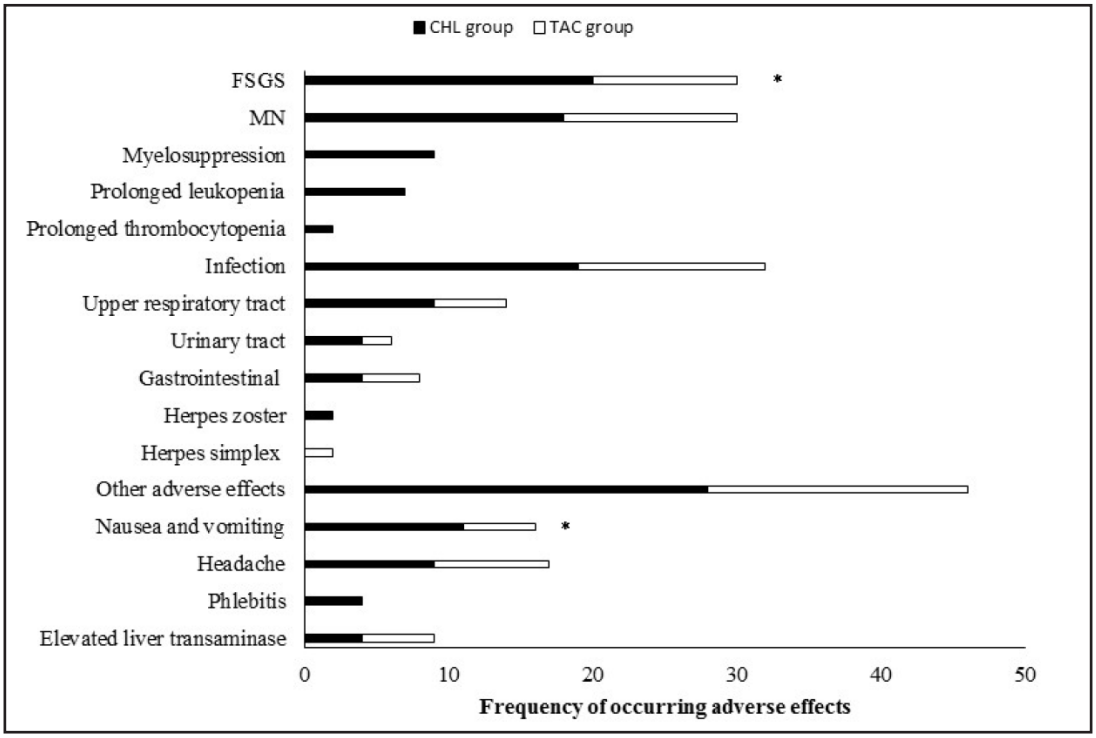

Fig. 4. Comparison of adverse effects between CHL and TAC group. Notes: * P < 0.10, indicting a significant difference between CHL group and TAC group.

which is represented as reducing proteinuria and slight fluctuations of the SCr levels within the normal range. The results were similar to those of prednisolone and chlorambucil treatment by Howman's study, which showed a lower risk of a further 20% decline in the renal function relative to the supportive care group (with an incidence of 58% vs. 84%) [26]. Mechanically, one possible reason is the depletion of B- and T-lymphocytes or the inhibition of podocyte injury induced by macrolide immunosuppressants or alkylating agents. This was demonstrated in a previous study [1, 27, 28]. The activation of B- or T- lymphocytes triggers antibody-independent autoimmune damage, which is considered to be the most

common cause of nephrotic syndrome [29]. Indeed, the pleased outcome that CHL treatment is not inferior to TAC treatment is significant for lower income SRNS patients located in the rural area in southern China. Moreover, in both the induction stage and the maintenance stage, the treatment showed no significantly different outcomes between the FSGS and MN groups, which indicates the non-specific nature of the pathology in terms of the therapeutic effects of CHL or TAC treatment. The result was similar to that reported by Senthil et al, who showed no difference in the proportion of patients achieving remission in the MN and FSGS groups following Mycophenolate mofetil treatment [30]. One possible reason for this is the similar pathogenesis characteristic of FSGS vs MN, such that the FSGS lesion might be secondary to primary MN [31]. However, other studies, such as that by Ponticelli et al, showed that the treatment of chlorambucil and prednisolone increased the remission rate and improved the renal survival of IMN patients [17]. Similarly, Li et al. showed that oral tacrolimus led to a higher proportion of complete remission in minimal change nephropathy or mesangioproliferative glomerulonephritis than that of FSGS [32]. Thus, the similar or different therapeutic effects among different pathological types such as FSGS or MN indicate the treatment effects of pathotype-specific is controversial and thus requires further study.

Noticeably, CHL treatment showed a lower incidence of adverse effects than the previous studies. For example, Susan [4] reported that 17.2% of patients developed prolonged leukopenia with the lower dose of chlorambucil (< 0.3 mg/kg) and 30.6% of patients developed leukopenia with the higher dose (≥ 0.3 mg/kg). Grupe [33] reported that 24% of patients developed prolonged leukopenia and 8% developed herpes zoster when treated with chlorambucil (0.32 mg/kg). This is similar to acute leukemia, which accounts for approximately 85% of cases of neoplasia associated with nitrogen mustard exposure [34]. Interestingly, in our study, only 9.7% of the patients had prolonged leukopenia, and only 2.8% had prolonged thrombocytopenia. No patients developed seizures, azoospermia, or neoplasia or died, although 15.3% of the patients experienced nausea or vomiting. In our opinion, the possible reasons for this are as follows: First, the doses of CHL had not exceeded their cytotoxic dosage and we established a personalized treatment protocol. CHL was given intravenously at an initial dosage of 1 mg on the first day, 3 mg on the third day, and 5 mg on the fifth day. It was then given two times every week for one month until reaching a maximum dose of 1 mg/kg. Assessing step-by-step tolerance and controlling the level of CHL in the blood over a small range can minimize the adverse side effects. Second, reasonable care was taken in the course of treatment SRNS patients with FSGS, and antimetic drugs were added. Finally, large and different vessels were selected in case of phlebitis before CHL injection. However, side effects, such as prolonged leukopenia or prolonged thrombocytopenia in the SRNS patients following CHL treatment cannot be neglected, even though the no significant difference between the CHL and the TAC group were observed. Many measures, such as reducing the dose of CHL by 50% when the leukocyte count < 5×10^9 / L or discontinuing the use of CHL when the leukocyte count was less than 4000 per cubic millimeter should be performed in a timely manner in case of prolonged leukopenia or thrombocytopenia. Furthermore, oral drugs for promoting leukocyte proliferation, such as Leucongen, or the subcutaneous injection of recombinant human granulocyte colony-stimulating factor can be performed in order to take special care to prevent infection and improve the side effects of leukopenia or thrombocytopenia.

In summary, the equivalent efficacy and safety of CHL vs. TAC in SRNS patients has helped patients in developing countries. The 2012 KDIGO recommended mustard nitrogen (cyclophosphamide, chlorambucil) as a corticosteroid-sparing agent for calcineurin inhibitors (CNIs). MMFs are much more expensive than other drugs, and this may limit access to them in many countries. Some retrospective studies have suggested that steroid and alkylating agents, such as chlorambucil, can favor remission of FSGS [34, 35]. Alkylating agents are associated with higher remission rates and a longer time of remission than calcinuurin inhibitors [36], and the high costs of tacrolimus treatment may be less effective in patients with FSGS than other types, such as minimal change nephropathy [32].

In addition, some limitations should be discussed regarding the present study. First, the lack of detailed genetic evaluation in our study produced limitations in further analysis. Indeed, evidence has shown that SRNS is associated with multiple genetic mutations and is associated with several pathways affecting the function of podocytes [9, 37], which may become potential targets for future therapy with fewer side effects, leading to a long-lasting remission. Therefore, the factors of patients with genetic mutations should be considered and be balanced for future study. Second, we should determine whether intravenous chlormethine is superior to oral chlorambucil, in order to determine the uniformity in the route and dosage for the treatment of SRNS patients with FSGS.

Conclusion

Our study concluded that CHL shows equivalent or improved efficacy as an agent for the treatment of SRNS patients with FSGS compared to TAC treatment. The side effects of CHL treatment were well tolerated, and no serious adverse outcomes (such as stage 4/5 CKD or death) occurred during the inducing period or maintenance therapy. The present results highlighted the fact that CHL is an effective alternative treatment strategy for the management of SRNS patients with FSGS who cannot afford expensive immunosuppressive agents such as TAC in the population of southern China.

Disclosure Statement

The authors declare that they have no competing interests and nothing to disclose.

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