Peer Review File

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Reviewer A

Comment 1: *General Comments*

- Page 2, line 39. Should be "1%-7%"

- Page 2, line 41; page 6, line 6, line 9; page 3, line 33, line 46; Page 6, line 37, line 41, line 42; Page 7, line 11, line 21, line 24, line 30, line 39, line 44: add space between word and reference number.

- Page 3, line 31: clearly state what the "standard techniques" are.

- All Tables, including those in supplements, should be formatted consistently.

Reply 1: We appreciated the careful review and detailed reminders on revision. We have carefully and thoroughly proofread the manuscript to correct all the incorrect formats. Connection symbols and required spaces have been revised as mentioned. "Standard techniques" on Method section has also been described in more detail as suggested. The format of tables and supplemental tables seemed to be disordered during upload. We have corrected the error and unify the layout of all tables.

Changes in the text:

1.1 Connection symbols have been revised. (See Page 5, line 6) "It accounts for approximately 1%-7% of all cases of biopsy-confirmed glomerulonephritis among all ages."

1.2 We have added all spaces between the previous word and reference.

1.3 We have added the detailed contents of "standard techniques" as suggested. The relevant texts are provided below as a screen dump for your quick reference. (See page 7, line 11-15) "All biopsies were prepared by standard techniques for light microscopy (LM), immunofluorescence (IF), and electron microscopy (EM), where available. Hematoxylin and eosin (HE) stain and periodic acid-silver methenamine (PASM) stain were applied for LM. The intensity of immunofluorescent staining for IgG, IgA, IgM, C3, C4, C1q, C3d, C9 and Fb was graded from negative to 3+ on IF. Distribution, type and extent of deposits were recorded on EM."

1.4 All tables and supplemental tables have been formatted unitedly and provided below for your quick reference.

Table 1 Definition of clinical remission, manifestations and diagnosis groups

Term	Interpretation
Current Criterion for C3G	Immunostaining reveals C3 dominance and ≥2 orders of intensity greater than any combination of IgG, IgM, IgA, and C1q.
Complete remission	eGFR≥90ml/min·1.73m^2 24-hour urinary protein<0.2g or negative/trace in dipstick hematuria<5/Hp or negative/trace in dipstick
Partial remission	eGFR≥90ml/min·1.73m^2 Reduction of proteinuria of more than 50% compared to highest value (reduction more than 50% of 24 hour urinary protein, or decrease at least 2 order in dipstick) with or without hematuria
Non-remission	Lack of complete or partial response
Early diagnosis	Renal biopsy was performed within 6 months after disease onset
Delayed diagnosis	Renal biopsy was performed over 6 months after disease onset
Nephrotic syndrome	Edema, 24-hour urinary protein>50mg/kg, hypoalbuminemia (< 30 g/L)
Nephritic syndrome	macroscopic hematuria, edema, hypertension, abnormal function (eGFR < 90 ml/min ·1.73 m^2), proteinuria (24-hour urinary protein>50mg/kg)
Nephritic-nephrotic syndrome	Joint occurrence of nephrotic and nephritic syndrome

C3G, C3 glomerulopathy; eGFR was calculated by the Schwartz formula using a local k-factor of 0.49 in CKD1-2, 36.5 in CKD3-5.

Table 2 Clinical manifestations at onset and response for treatments at last follow-up

			at disease onset				Treatment				Follow-up	
ID	Causes	Pathological Diagnosis	Presentation	C3 (g/L)	C3NeF	eGFR	Steroids	MMF	CTX	ACEI	Response for steroids	Remission or not
1	HBV infection	IC-MPGN	Nephritic nephrotic syndrome	1.45	NA	37.2	+		+		Resistant	Non-response, ESRD, deceased
2		IC-MPGN	Nephrotic syndrome with hematuria	0.91	No abnormal	111.6	+			+	Resistant	Partial remission
3		IC-MPGN	Nephrotic syndrome with hematuria	0.28	Abnormal	79.8	+	+	+	+	Resistant	Complete remission
4	HBV infection	IC-MPGN	Nephritic nephrotic syndrome	0.06	No abnormal	109.5	+			+	Sensitive	Complete remission
5	Aymé-Gripp Syndrome *MAF*: p.Ser54Leu (*de novo*)	U-MPGN	Asymptomatic hemoglobinuria	0.88	NA	138.9				+	/	Non-response
6	MMA *MMACHC*: p.Trp203Ter(het;p,wt;m,het); p.Gln27Arg(het;p,het;m,wt)	IC-MPGN	Nephritic syndrome	1.29	NA	78.6	+	+		+	Resistant	Non-response
7		IC-MPGN	Nephritic syndrome	1.54	NA	131.4	+			+	Resistant	Partial remission
8		IC-MPGN	Nephrotic syndrome with hematuria	0.15	NA	194.7	+	+			Resistant	Non-response
9		IC-MPGN	Nephrotic syndrome with hematuria	0.74	NA	96.2	+	+		+	Resistant	Complete remission
10	HBV infection	IC-MPGN	Nephrotic syndrome with hematuria	1.48	NA	104				+	/	Complete remission
11	HBV infection	U-MPGN	Asymptomatic hemoglobinuria	NA	NA	NA	+				Resistant	Non-response
12		IC-MPGN	Nephritic nephrotic syndrome	1.74	NA	103.9	+		+		Resistant	Complete remission
13	MMA *MMACHC*: p.Trp203Ter(het;p.het;m,wt)	C3G	Nephritic nephrotic syndrome	1.21	No abnormal	21.4	+	+			Resistant	Non-response, ESRD Transplantation without relapse
14		IC-MPGN	Nephritic nephrotic syndrome	0.13	Abnormal	102.6	+	+		+	Resistant	Complete remission
15		U-MPGN	Nephrotic syndrome with CKD3	0.87	NA	47	+		+		Resistant	Non-response
16		IC-MPGN	Nephritic nephrotic syndrome	2.15	NA	79.3	+		+		Resistant	Non-response
17	RA	U-MPGN	Nephritic syndrome	1.40	NA	47	+	+	+	+	Resistant	Non-response

ACEI, angiotensin converting enzyme inhibitor; C3G, C3 glomerulopathy; C3NeF, C3 nephritic factor; CTX, Cyclophosphamide; ESRD, end stage of renal disease; HBV, hepatitis B virus; IC-MPGN, immune-complex mediated membranoproliferative glomerulonephritis; *MAF*, MAF BZIP Transcription Factor; MMA, methylmalonic acidemia; *MMACHC*, Metabolism Of Cobalamin Associated C; MMF, mycophenolate mofetil; NA, no data; RA, rheumatoid arthritis, U-MPGN, unclassifiable membranoproliferative glomerulonephritis; eGFR was calculated by the Schwartz formula using a local k-factor of 0.49 in CKD1-2, 36.5 in CKD3-5.

Supplementary table 1 Base line clinical characterizations of the patients with MPGN

Patient No.	Age (y) /Sex	Clinical findings at disease onset										
		Prior infection	Edema	Hypertension	Hematuria (/HPF)	24-hour urinary protein (g)	P/C	Lipstick of proteinuria	Serum albumin (g/L)	Other renal/extrarenal manifestations	Positive family history	LM/EM
1	9/F	(-)	(+)	(-)	(-)	8.53	*NA*	3+	↓[a]			Glomeruli hyalinized, mesangial and endocapillary proliferation, crescents, tubular atrophy/NA
2	4/M	Dermal	(+)	(-)	71.3	2.57	8.92	3+	27.2			Mesangial and endocapillary proliferation, individual crescents, 10% glomeruli hyalinized, tubular atrophy/Mild mesangial electron-dense deposits
3	5/M	Dermal	(-)	(-)	135	1.53	2	2+	30.8			Mesangial and endocapillary proliferation, mild interstitial fibrosis, segmental sclerosis, individual crescents, double contours of GBM/Mesangial, subendothelial and rare subepithelial electron-dense deposits
4	11/M	(-)	(+)	(-)	112	2.86	0.62	2+	38		Consanguineous marriage; A cousin had nephrotic syndrome	Mesangial and endocapillary proliferation/Mild mesangial electron-dense deposits
5	6/M	(-)	(-)	(-)	12.32	0.26	0.81	2+	43.8	Cataract; intellectual disability; hearing impairment		Mesangial and endocapillary proliferation, interstitial fibrosis/Renal afferent arteriolar dense deposits
6	4/M	(-)	(-)	(+)	67	0.54	3.95	3+	40.5	Hearing impairment	The elder sister: diagnosed kidney disease at 4 years old and deceased at 6; Mother: SLE	Mesangial and endocapillary proliferation, individual crescents/Subepithelial and rare subendothelial electron-dense deposits
7	11/F	Respiratory	(-)	(-)	195	0.8	1.06	3+	18.4			Mesangial proliferation/NA
8	10/M	Dermal	(-)	(-)	35	2.54	1.42	3+	26.4		Parents: recurrent urticaria; Cousins: history of NS or hematuria	Mesangial and endocapillary proliferation, mild interstitial fibrosis, double contours of GBM /No observed electron-dense deposits
9	12/M	Respiratory	(+)	(+)	25	7.098	*NA*	3+	↓[b]			Mesangial and endocapillary proliferation, tubular atrophy /No observed electron-dense deposits
10	10/F	(-)	(+)	(-)	50	2.6	*NA*	2+	↓[c]			Mesangial and endocapillary proliferation, interstitial fibrosis /Mesangial and subepithelial electron-dense deposits
11	4/M	Dermal	(-)	(-)	6	5.9	*NA*	3+	Normal[d]	Renal malrotation (Right)	Aunt and uncle: history of edema and proteinuria; Uncle: diabetes insipidus	Mesangial and endocapillary proliferation, double contours of GBM, glomeruli hyalinized, interstitial fibrosis/NA
12	11/F	(-)	(+)	(-)	70	2.78	*NA*	2+	↓[e]			Mesangial and endocapillary proliferation, double contours of GBM, tubular atrophy/NA
13	12/F	(-)	(-)	(+)	7	2.53	2.18	3+	35.5			Segmental sclerosis, mesangial and endocapillary proliferation, crescents, tubular atrophy, interstitial fibrosis, glomeruli hyalinized/No observed electron-dense deposits
14	12/F	Respiratory	(+)	(+)	8	4.17	4.34	3+	23.9			Mesangial and endocapillary proliferation/Mild mesangial and subendothelial electron-dense deposits
15	9/F	Respiratory	(+)	(-)	(-)	14.9	*NA*	3+	↓[f]			Mesangial and endocapillary proliferation, tubular atrophy/NA
16	12/M	(-)	(+)	(-)	20	4.88	*NA*	3+	31			Crescents, mesangial and endocapillary proliferation, segmental sclerosis, tubular atrophy, interstitial fibrosis/Severe subendothelial and subepithelial electron-dense deposits
17	7/M	(-)	(-)	(-)	∞	1.5	*NA*	2+	NA			Mesangial and endocapillary proliferation, crescents, tubular atrophy, /Mild mesangial electron-dense deposits

F, female; LM, light microscopy; EM, electron microscopy; GBM, glomerular basement membrane; HPF, High power field; SLE, systemic lupus erythematosus; M, male; NS, nephrotic syndrome; y, years; NA, not available; P/C, urinary protein/creatine. Serum album of patient 1, 9, 10, 11,12 and 15 were evaluated by serum protein electrophoresis. 33% (a), 59.9% (b), 55.1% (c), 66.7% (d), 52.8% (e) and 58.4% (f).

Supplementary table 2 Clinical characteristics and laboratory parameters of patients with MPGN at last follow-up

Patient No.	Comlications	Clinical findings at last follow-up						
		Hematuria (/HPF)	eGFR (ml/min 1.73m^2)	24 hours urinary protein (g)	Lipstick of proteinuria	C3 (g/d)	C3 NeF	Time from disease onset to renal biopsy (months)
1	Hypertension dental ulcers	(-)	5.9	4.8	2+	NA	NA	19.6
2	dental ulcers	72.18	148.7	NA	1+	NA	NA	0.6
3	Hypertension	63.39	109.5	0.11	(-)	0.83	(-)	1.3
4	Hypertension	21.55	110.3	NA	micro	1.1	NA	1.8
5	(-)	(-)	190.9	NA	1+	NA	NA	1.7
6	(-)	(-)	66.1	NA	2+	NA	NA	1.2
7	(-)	(-)	148.0	NA	+	1.65	NA	0.3
8	(-)	90	115.1	1.08	3+	0.1	NA	13.1
9	(-)	(-)	134.9	0.49	(-)	NA	NA	4.4
10	(-)	(-)	104.0	NA	(-)	NA	NA	2.0
11	(-)	(-)	33.9	NA	2+	NA	NA	84.4
12	(-)	(-)	110.0	NA	(-)	NA	NA	1.6
13	(-)	(-)	5.6	NA	NA	NA	NA	7.3
14	High intraocular pressure Binocular cataract Urinary tract infection	(-)	135.2	0.11	(-)	0.64	(-)	2.6
15	Urinary tract infection	(-)	70.8	(-)	(-)	NA	NA	15.4
16	Urinary tract infection	(-)	NA	NA	3+	NA	NA	7.1
17	blurred vision	(-)	75.4	NA	(-)	NA	NA	63.7

C3NeF, C3 Nephritis Factor; HPF, High power field; NA, not available; eGFR was calculated by the Schwartz formula using a local k-factor of 0.49 in CKD1-2, 36.5 in CKD3-5.

Comment 2: *Introduction:*

- Authors should include more background literature in the introduction to better support the rational behind this case study.

Reply 2: Thank you for the instructive suggestion on improving the accessibility of our manuscript. We have modified the Introduction section extensively and further summarized the representative progress of current studies about MPGN. Modifications are shown below.

Changes in the text: We have added more background literature on the Introduction section. (See page 5, line 6-9, line 19-25; page 6, line 4-12. The "Introduction" section is provided below as a screen dump for your quick reference.

"Membranoproliferative glomerulonephritis (MPGN) has been recognized as a rare pathological pattern of glomerulopathy clinically characterized by proteinuria, hematuria, hypertension and often impaired renal function at disease onset. It accounts for approximately 1%-7% of all cases of biopsy-confirmed glomerulonephritis among all ages (1-4). Recent studies had revealed the incidence of this pattern was significantly declined in the 21st century, however, rates of progression to ESRD and death remained unimproved (4,5). In up to 50% of the affected children, MPGN leads to end-stage renal disease (ESRD) within ten years (6).

The typical features of MPGN on light microscopy include mesangial cellularity, endocapillary proliferation, and capillary-wall remodeling (with the formation of double contours), and lobular accentuation of glomerular tufts. Based on the electron-microscopical findings, MPGN is traditionally classified as primary (idiopathic) MPGN type I (MPGN I, with subendothelial deposits), type II (MPGN II, with dense deposits in the glomerular basement membrane), or type III (MPGN III both subepithelial and subendothelial deposits,) or secondary MPGN (7). However, this kind of assortment neither indicates the etiology of MPGN, nor provides competent evidence for subsequent treatments. Lately, abnormal activation of complement via alternative pathway was found to mediate the formation of MPGN pattern (8). In 2013, an expert consensus was established and proposed a practical approach to view MPGN as immune-complex-mediated MPGN (IC-MPGN) and C3 glomerulopathy (C3G) based on immunofluorescence of renal biopsies (9). C3G was recognized by the new classification as a distinct type from MPGN pattern and further subdivided into dense deposit disease (DDD) and C3 glomerulonephritis (C3GN), depending on the position of electron-microscopical deposits. Although IC-MPGN and C3G were distinguished in histopathology, the essential borderline and interrelation between these two entities are still ambiguous.

Several studies were done to evaluate the causes, clinical presentations, effects of various treatments and prognosis of adult MPGN (10-14), yet studies of children MPGN remain to be small-scale with narrow cases (15-17). The rareness of the disease as well the terminology shift with the revolution of diagnostic classification conceal the authentic characteristics and outcomes from being concluded. Consequently, effective therapies of MPGN pattern and ameliorated prognosis have not been made further. The current therapies for MPGN including steroids and immunosuppressants suggested by KDIGO guidelines (18) have not shown consistent benefits and the evidence for

therapeutic efficacy in children was extremely limited. Moreover, the latest classification of IC-MPGN and C3G remained to be evaluated on its utility in children with MPGN. Therefore, we aim to retrospectively analyze the clinical, pathological and pathogenic diagnosis of MPGN in children to provide an optimized strategy for early diagnosis of MPGN. We present the retrospective case study in accordance with the STROBE reporting checklist."

Comment 3: *Methods:*
- Page 4, line 6: authors should state why a Mann-Whitney test was used in place of Student's T-Test.
Reply 3: Thanks for the reviewer pointing out it. The quantitative data of children such as ages of onset, follow-up, initial eGFR and initial proteinuria (24-hour urine protein quantification) did not accord with normal distribution and homoscedasticity, therefore, the Mann-Whitney test was preferred to the Student's T-test.
Changes in the text: We have modified the sentence to state clearer for why we used Mann-Whitney U test as follows. (See Page 8, line 11-12)
"Mann-Whitney U test (for continuous variables that do not conform to the normal distribution and homoscedasticity) and the Fisher exact probability test (for categorical variables) were used to analyze the differences among participants with different clinical features stratified into early or delayed diagnosis groups."

Comment 4: *Results:*
- Page 4, line 19, line 20; page 5, line 31: In text reporting of IQR should be "(IQR = 5.6-11.9 years)"
- Page 3, Line 28-29; Page 5, line 32-40: reporting of these results (see highlighted text) should be consistent across the entire Results section.
- Page 5, line 20: Is "mental retardation" the currently accepted medical term for this?
- Page 6, line 4: Check the formatting of "(p<0.05, Table 3)". It appears to be different to the rest of the text.
Reply 4: We appreciate your careful review and exact advice. All data have been proofread carefully to make consistence. Format problems have been revised as mentioned. We have checked the acceptance of the term "mental retardation", and found the previous term, while capable of expressing this meaning, had been used less frequently in recent years. We have replaced it with "intellectual disability" that have been used more frequently.
Changes in the text: We have modified our text and table format as advised.
4.1 We have revised the format of reporting IQR on page 8, line 25; page 9, line 1-2 "The median age at onset was 9.9 years (IQR= 5.6-11.9 years) with a median eGFR of 102.6 mL/min·1.73 m2 (IQR= 47.0-121.5 mL/min·1.73 m2) at the time of admission."
4.2 All results have been checked carefully to make a consistence.
4.3 We have replaced "mental retardation" with "intellectual disability" as an accepted medical term in describing mental abnormality. See Page, line; "One patient (patient #6) was diagnosed with deafness, bilateral cataracts and intellectual disability within one year old who present non-nephrotic range proteinuria at age of eight years old."

4.4 P value has been formatted as a capitalized letter unitedly.

Comment 5: *Discussion:*
- Page 6, line 38, line 40, line 42: "(12/17)", "(12/17)", "(7/17, 41.2%)", "23.5%" really isn't needed in the Discussion section as it should have been described in the Results section.
- Page 7, line 30-33, line 44-47: Sentence syntax is confusing, authors should rewrite to make the intended message clear.
Reply 5: Thank you for the instructive suggestion on "Discussion" section to make the manuscript more logical. We have deleted the text mentioned on the Discussion section and describe the clinical characteristics in Result section in a precise way. Sentence syntax has been revised to make a clearer statement as mentioned. We enclose the revised sentences and the sentences before or after them for your quick review.
Changes in the text:
5.1 We have deleted the text mentioned on the Discussion section and describe the clinical characteristics in Result section in a precise way. (See page 9, line 4-6; line 13-24)
5.2 We have rearranged the logic between sentences as mentioned. (See page 14, line 22-25; page 15, line 1-5;
"A new proposed diagnostic standard of C3G was C3 dominant at least two orders of magnitude more intense than any other immune reactant, which requires validation by alternative pathway evaluation (20,21). (Delete the previous sentence) We applied this standard in our pediatric patients and found two patients with obvious alternative pathway evaluation would be failed to identify the diagnosis of C3G in this condition. Similar situations were also observed in other pediatric MPGN studies (15,16). Accordingly, there do exist several pediatric MPGN patients with AP dysregulation who do not conform to the "C3 dominant at least two orders of magnitude more intense than any other immune reactant" criterion."
"We discovered most patients who received early diagnosis reached complete or partial remission at the last follow-up, whereas patients who received delayed diagnosis all turned out non-response for usual treatments. It suggests that early pathological diagnosis in children may help to optimize the treatment and prevent the decline of renal function. Although there are controversial results, it has recently been shown that the treatment of MPGN with corticosteroids plus MMF in adults caused better kidney survival as compared with patients treated with other immunosuppressants and untreated patients (28)."

Comment 6: *Figure 1:*
- "immune- complex" should be "immune-complex"
Reply 6: Thank you for your careful reading of the manuscript. We have revised the problem as advised.
Changes in the text: We have modified our text and table format as advised. (See legend of Figure 1) "IC-MPGN, immune-complex mediated membranoproliferative glomerulonephritis;"

Comment 7: *Table 1:*
- The formatting text in the "Definition" column creates confusion. Authors should reformate text to make the intended message clear.
Reply 7: Thanks for the reviewer pointing out it to help us modify the diction. We have replaced the term “Definition” with “Interpretation” to make our text more understandable.
Changes in the text: We have edited the text in Table 1 from “Definition” to “Interpretation”. (See Table 1, headline).

Reviewer B
Comment 1: *This study is limited to a single facility study. In addition to that, although it is discussed in the limitation, it is difficult to grasp as a result that the prognosis of MPGN can be referred to because the observation period is short. Therefore, I judged this manuscript is not suitable for this journal.*
Reply 1: We appreciate your time for reading our manuscript and we are sorry for the shortages of single facility and relatively short-term observation as you mentioned.
MPGN is a rare histopathologic manifestation and presents worse prognosis compared to other patterns of glomerulonephritis. Decreasing incidence of MPGN has been found in 21st centry in most countries with the improved sanitary condition. Current incidence has been estimated as 1-2 per million population (1), therefore, several studies of MPGN with single facility have less than 20 cases (2, 3, 4). Although our patients analyzed in this study were come from the same medical facility, the cases were recruited from all over the country because our hospital is one of the National Medical Centers of Children. The characteristics of MPGN in our patients were representative to some extent.
In addition, the criterions of histopathological diagnosis, clinical presentations, serological markers and principles of treatment were consistent and stable as a single facility study. Considered that the intensity of immunoglobins and C3 deposits were somewhat subjectively evaluated by pathologist, our data provided reliable data that are compared under the same criteria. Despite our data is insufficient to obtain some statistically significant results, our cases would provide certain evidences for finding the underlying etiology and pathological diagnosis of MPGN in children.
Finally, thank you very much for your time involved in reviewing this manuscript and response. We hope our data would make a small but worthwhile effect to progres in studies of pediatric MPGN. We would appreciate your further suggestions and another opportunity for us to revise this manuscript.

(1) McGrogan A, Franssen CF, de Vries CS. The incidence of primary glomerulonephritis worldwide: a systematic review of the literature. Nephrol Dial Transplant 2011;26:414-30.
(2) Holle J, Berenberg-Gossler L, Wu K, et al. Outcome of membranoproliferative glomerulonephritis and C3-glomerulopathy in children and adolescents. Pediatr Nephrol 2018;33:2289-98.

(3) Spartà G, Gaspert A, Neuhaus TJ, et al. Membranoproliferative glomerulonephritis and C3 glomerulopathy in children: change in treatment modality? A report of a case series. Clin Kidney J 2018;11:479-90.
(4) Okuda Y, Ishikura K, Hamada R, et al. Membranoproliferative glomerulonephritis and C3 glomerulonephritis: frequency, clinical features, and outcome in children. Nephrology (Carlton) 2015;20:286-92.