centers (the Japanese Pediatric IgA Nephropathy Treatment Study Group) (5). The study protocol was in accordance with the standards of the ethics committee at each center, and all of the patients' parents gave informed consent. The follow-up study protocol was approved by the regional research ethics vetting boards.

The entry and exclusion criteria of this trial have been reported in detail elsewhere (5). Briefly, between 1990 and 1993, 78 eligible children less than 15 years old with biopsy-proven IgA nephropathy showing diffuse mesangial proliferation were randomized to either the 2-year combination therapy ($n = 40$) or the control therapy ($n = 38$). The patients given the combination therapy received prednisolone, azathioprine, heparin-warfarin, and dipyridamole for 24 months. Prednisolone was given orally at a dose of 2 mg/kg per day (maximum, 80 mg/d) in three divided doses for 4 weeks, followed by 2 mg/kg given as a single dose in the morning of every other day for 4 weeks, 1.5 mg/kg every other day for 4 weeks, and 1 mg/kg every other day for 21 months. Azathioprine was given orally at a dose of 2 mg/kg per day as a single morning dose for 24 months. Heparin was given by continuous intravenous infusion in sufficient doses to keep the partial thromboplastin time at 60 seconds for 28 days. This was followed by oral warfarin given as a single morning dose to maintain the thrombotest at 30 to 50% for 23 months. Dipyridamole was given orally at a dose of 5 mg/kg per day (400 mg/d) in three divided doses for 24 months. The patients given the control therapy received only heparin-warfarin and dipyridamole for 24 months, and the treatment protocols for these medications were the same as those for the combination therapy. Diffuse mesangial proliferation was defined as >80% of glomeruli showing mesangial proliferation (more than three cells per peripheral mesangial area) on the basis of the World Health Organization criteria (6). Therapies used after the completion of the 2-year treatment periods were not restricted. We investigated the long-term outcome of children by reviewing hospital medical records or by telephone contact with the patients or their family members. The renal outcome at the last observation was graded as no proteinuria, mild proteinuria, heavy proteinuria, renal insufficiency, or ESRF.

Outcome Definitions

The primary endpoint was the development of ESRF that required renal replacement therapy. Renal insufficiency was defined as an estimated GFR (eGFR) of <60 ml/min per 1.73 m^2. eGFR was calculated using the Schwartz formula (7) for less than 18 years of age and the Cockroft-Gault formula (8) for more than 18 years of age. Proteinuria was evaluated using the urinary protein amount per day (g/1.73 m^2 per day) during the initial 2-year study period; after that, the early morning urinary protein/creatinine ratio (uP/Cr) was used. Heavy, mild, and no proteinuria were defined as ≥1.0, 0.2 to 1.0 and <0.1 g/1.73 m^2 per day, or ≥1.0, 0.2 to 1.0 and <0.2 g/g, respectively.

Statistical Analyses

The data were analyzed with JMP version 8.0 (SAS Institute Japan Ltd., Tokyo, Japan). We used the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. Renal survival curves were calculated using the Kaplan-Meier method, and comparisons were made with a log-rank test. For multivariate analysis, we utilized the Cox proportional hazard model in a stepwise fashion, entering and eliminating variables with a P value of 0.20 in the model. Statistical significance was established at $P < 0.05$.

Results

Baseline Characteristics

The baseline characteristics of each group are shown in Table 1. The clinical and pathologic findings in the two groups were similar with the exception of urinary protein excretion, which was higher in the combination therapy group than in the heparin-warfarin and dipyridamole therapy group.

Clinical and pathologic data at the end of the initial 2-year treatments are shown in Table 2. Four patients in the heparin-warfarin and dipyridamole therapy group were lost to follow-up during the 2-year study period. The mean 24-hour urinary protein excretion and the ratio of patients with heavy proteinuria (≥1.0 g/1.73 m^2 per day) were significantly lower in the combination therapy group than in the heparin-warfarin and dipyridamole therapy group. On the other hand, the ratio of patients with no proteinuria (<0.2 g/1.73 m^2 per day) was significantly higher in the combination therapy group. The value of serum IgA and the intensity of hematuria were significantly lower in the combination therapy group. In terms of pathologic findings, the mean percentages of glomeruli showing global sclerosis and mesangial proliferation were lower in the combination therapy group than in the heparin-warfarin and dipyridamole therapy group, although this was not statistically significant. The intensity of mesangial IgA deposits was significantly lower in the combination therapy group than in the control group.

The treatments used after the initial 2-year study period are shown in Table 3. Because they were not controlled, there were some differences between the two groups. On the basis of the outcome of this RCT, more patients received the combination therapy in the heparin-warfarin and dipyridamole therapy group than in the combination therapy group. On the other hand, angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), which were restricted in the initial 2-year study period, were more often used in the combination therapy group than in the control group.

Renal Survival

The median duration of observation was 10 years (range, 0.5 to 18) for the total study population, 11.5 years (range, 4 to 18) for the combination therapy group, and 7 years (range, 0.5 to 17) in the heparin-warfarin and dipyridamole therapy group. The outcome in each group at the last observation is shown in Table 4. Two of 40 patients (5%) who received combination therapy and five of 34 patients (14.7%) who received heparin-warfarin and dipyridamole therapy developed ESRF. Figure 1 shows a comparison of probabilities for renal survival

Table 1. Clinical and pathological findings at the start of initial treatments

	Combination Therapy (n = 40)	Heparin-Warfarin and Dipyridamole Therapy (n = 38)	P
Gender (Men/Women)	22/18	29/9	0.06
Age at diagnosis (years)	12.2 ± 3.0	11.6 ± 2.3	0.10
Initial presentation (Chance/Gross hematuria)	34/6	30/8	0.56
Creatinine clearance (ml/min per 1.73 m^2)	144 ± 52	152 ± 47	0.35
Serum albumin (g/dl)	3.73 ± 0.65	3.94 ± 0.59	0.36
Serum cholesterol (mg/dl)	210 ± 116	194 ± 47	0.13
Serum IgA (mg/dl)	290 ± 115	276 ± 98	0.75
Urinary protein excretion (g/1.73 m^2/day)	2.09 ± 1.78	1.35 ± 1.26	0.02
Hematuria[a]	2.9 ± 0.8	2.6 ± 0.9	0.16
Glomeruli			
showing sclerosis (%)	5.7 ± 8.6	4.1 ± 5.8	0.65
showing crescents (%)	23.6 ± 20.7	21.2 ± 17.9	0.68
showing capsular adhesions (%)	9.6 ± 10.0	7.0 ± 7.1	0.40
showing mesangial proliferations (%)	91.7 ± 7.4	90.2 ± 7.0	0.11
Intensity of mesangial IgA deposits[b]	2.2 ± 0.6	2.3 ± 0.5	0.72

The plus-minus values are the means ± SD.
[a]Hematuria was quantified using dipsticks, and macrohematuria was quantified as 4.
[b]The intensity of deposits on immunofluorescence microscopy was graded semiquantitatively on a scale from 0 to 3+: 0, no; 1+, slight; 2+, moderate; 3+, intense.

Table 2. Clinical and pathological findings of at the end of initial 2-year treatments

	Combination Therapy (n = 40)	Heparin-Warfarin and Dipyridamole Therapy (n = 34)	P
Creatinine clearance (ml/min per 1.73 m^2)	147 ± 33	145 ± 44	0.89
Serum IgA (mg/dl)	229 ± 87	281 ± 92	0.03
Urinary protein excretion (g/1.73 m^2 per day)	0.28 ± 0.36	1.07 ± 1.57	0.01
Patients with heavy proteinuria (≧1.0 g/1.73 m^2 per day)	2 (5.0%)	10 (23.5%)	0.009
Patient without proteinuria (<0.2 g/1.73 m^2 per day)	20 (50.0%)	6 (17.6%)	0.007
Hematuria[a]	0.5 ± 1.0	1.5 ± 1.1	0.0002
Glomeruli			
showing sclerosis (%)	5.0 ± 6.9	16.4 ± 23.0	0.07
showing crescents (%)	0.4 ± 1.1	4.4 ± 10.3	0.16
showing capsular adhesions (%)	8.1 ± 11.2	6.4 ± 10.4	0.86
showing mesangial proliferations (%)	30.7 ± 26.7	57.5 ± 36.4	0.07
Intensity of mesangial IgA deposits[b]	1.3 ± 1.1	2.2 ± 0.6	0.02

The plus-minus values are the means ± SD.
[a]Hematuria was quantified using dipsticks, and macrohematuria was quantified as 4.
[b]The intensity of deposits on immunofluorescence microscopy was graded semiquantitatively on a scale from 0 to 3+: 0, no; 1+, slight; 2+, moderate; 3+, intense.

between the two groups. The probability for renal survival in the combination therapy group was significantly better than that of the dipyridamole and heparin-warfarin therapy group (P = 0.03); the 10-year renal survival probability of each group was 97.1% (95% confidence interval [CI], 81.4 to 99.6%) and 84.8% (95% CI, 55.4 to 95.5%), respectively. We also analyzed baseline (at diagnosis) eGFR 50% reduction-free survival (Figure 2). The results were similar to the renal survival probability.

Predicted Risk for ESRF

We evaluated predicted risk factors using univariate and multivariate analyses using the Cox proportional hazard model for ESRF. The initial 2-year treatments (combination or control) and ratios of glomeruli showing global sclerosis at diagnosis were included as significant risk factors in both the univariate and multivariate analyses for ESRF (Table 5). The urinary protein excretion at diagnosis was significantly included as a risk factor in

Table 3. Treatments used after the initial 2-year study period

	Combination Therapy (n = 40)	Heparin-Warfarin and Dipyridamole Therapy (n = 34)
Combination		
with ACEI or ARB	0 (0.0%)	2 (5.9%)
without ACEI or ARB	0 (0.0%)	5 (14.7%)
Steroid and immunosuppressant		
with ACEI or ARB	3 (7.5%)	0 (0.0%)
without ACEI or ARB	0 (0.0%)	1 (2.9%)
Steroid		
with ACEI or ARB	6 (15.0%)	1 (2.9%)
without ACEI or ARB	1 (2.5%)	0 (0.0%)
ACEI or ARB (partly with anti-platelets and/or warfarin and/or Sairei-to)	11 (27.5%)	3 (8.8%)
Anti-platelets and/or warfarin and/or Sairei-to	6 (15.0%)	4 (11.8%)
Warfarin alone	0 (0.0%)	1 (2.9%)
Sairei-to alone	6 (15.0%)	4 (11.8%)
No treatment	7 (17.5%)	9 (26.5%)
Unknown	0 (0.0%)	4 (11.8%)

Combination treatment included prednisolone, azathioprine, heparin-warfarin, and dipyridamole. The immunosuppressant used was azathioprine or mizoribine. Sairei-to is a Chinese herbal medicine.

Table 4. Renal outcome at the last observation

	Combination Therapy (n = 40)	Heparin-Warfarin and Dipyridamole Therapy (n = 34)
No proteinuria (uP/Cr <0.2 g/g)	24 (60.0%)	18 (52.9%)
Mild proteinuria (≤0.2 uP/Cr <1.0 g/g)	12 (30.0%)	6 (17.6%)
Heavy proteinuria (uP/Cr ≥1.0 g/g)	1 (2.5%)	4 (11.8%)
Renal insufficiency (eGFR <60 ml/min per 1.73 m^2)	1 (2.5%)	1 (2.9%)
ESRF (requiring renal replacement therapy)	2 (5.0%)	5 (14.7%)

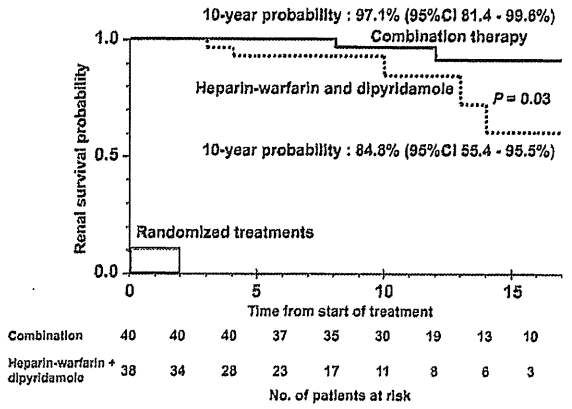

Figure 1. | Kaplan-Meier plot of renal survival stratified by the groups of initial 2-year treatments in children with IgA nephropathy showing diffuse mesangial proliferation.

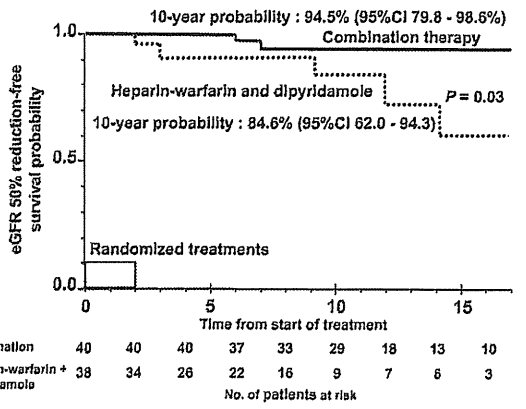

Figure 2. | Kaplan-Meier plot of eGFR 50% reduction-free survival stratified by the groups of initial 2-year treatments in children with IgA nephropathy showing diffuse mesangial proliferation.

the multivariate analysis for ESRF (Table 5). The ratios of glomeruli showing crescents and mesangial proliferations at diagnosis were not selected as significant risk factors for ESRF with a multivariate Cox model in a stepwise fashion.

Proteinuria at the End of the Initial 2-Year Treatments and Outcome

Because the importance of a change in proteinuria with treatment has been emphasized in IgA nephropathy, we compared the long-term outcomes of patients with pro-

Table 5. Predicted risk factors for ESRF

	Univariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*
Initial treatment (combination or control)	0.2	0.0 to 0.9	0.03	0.03	0.0 to 0.3	0.002
Urinary protein excretion at diagnosis (g/1.73 m^2 per day)	2.3	1.0 to 5.0	0.06	2.7	1.1 to 6.5	0.03
Glomeruli showing global sclerosis at diagnosis (%)	1.1	1.0 to 1.1	0.04	1.1	1.0 to 1.2	0.02

HR, hazard ratio.

teinuria at the end of the initial 2-year treatment with those without proteinuria. The probability of renal survival in patients with proteinuria was significantly worse than in those without proteinuria ($P = 0.04$). The 10-year renal survival probability after the start of initial treatment in each group was 88.9% (95% CI, 72.4 to 95.8%) and 100%, respectively (Figure 3).

Discussion

Our previous two RCTs and clinical trial showed the short-term effectiveness of combination therapy in childhood IgA nephropathy (5,9,10). To the best of our knowledge, no well-designed RCTs of childhood IgA nephropathy have been performed with the exception of those that we conducted. However, the long-term effectiveness of combination therapy has not been determined. This study is the first long-term follow-up study of such a RCT. This prolonged follow-up study of a previous RCT for children with IgA nephropathy showing diffuse mesangial proliferation demonstrated a significant superiority in renal survival in patients who received combination therapy compared with those who received control therapy. Because of the variable rate of progression to renal failure and the probable multifactorial pathogenesis of IgA nephropathy (11), it is desirable to evaluate the effectiveness of any IgA nephropathy treatment by a prospective controlled trial. However, although the ultimate endpoint in any clinical trial of progressive IgA nephropathy is the development of chronic renal insufficiency, most pediatric patients do not develop chronic renal insufficiency during the study period (12). Therefore, the data in this study provide us with unique and valuable information about IgA nephropathy in children.

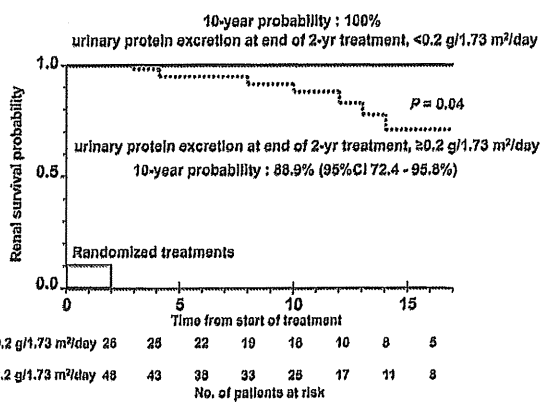

Figure 3. | Kaplan-Meier plot of renal survival stratified by urinary protein excretion at the end of initial 2-year treatments in children with IgA nephropathy showing diffuse mesangial proliferation. Normal urinary protein excretion is defined as less than 0.2 g/1.73 m^2 per day.

Kaplan-Meier analysis, the log-rank test, and the multivariate Cox model clearly indicated the significant long-term effectiveness of the combination therapy in this study. Even though this was a prospective observational study, factors after the initial RCT period were difficult to control between the two groups. Therefore, proper analyses of long-term follow-up data are important to evaluate disease outcome and treatment effectiveness (13,14). Valid statistical evaluation using a multivariate Cox model was thought to work effectively in this study.

This study also reveals the importance of a change in proteinuria in IgA nephropathy with the 2-year treatment. We observed an 87% reduction in proteinuria from baseline in the combination therapy group compared with only a 21% reduction in the dipyridamole and heparin-warfarin group at the end of the initial 2-year treatments. The proportion of patients without proteinuria was significantly high, whereas the proportion with heavy proteinuria was significantly low at the end of the initial 2-year treatment in the combination group. The probability of renal survival in patients without proteinuria at the end of the initial 2-year treatment was 100% during the entire long-term follow-up period. These findings are likely to be pathophysiological grounds for the long-term outcome in this study. Severe proteinuria at diagnosis and/or during follow-up is a strong predictor in adult IgA nephropathy according to the most accurate studies of the literature (13). Our previous study also supported this finding, even in child IgA nephropathy (4). Interestingly, a recent review reported that mean proteinuria during follow-up was a powerful independent prognostic predictor, but proteinuria at baseline was not significantly related to renal progression (15). Actually, in a substantial number of reports, presenting proteinuria has not been found to be a significant long-term predictor (14,16–19). Coppo *et al.* (17) found that presenting proteinuria was NS as an independent prognostic value in their trial when examined by a multivariate Cox model. Our recent analysis in 500 children with IgA nephropathy supported this result (14). There is a possibility that the proteinuria level at

diagnosis can be improved by appropriate treatments; thus, it may not be an absolute prognostic factor.

Corticosteroids have been widely used to treat moderate to severe IgA nephropathy. The current cumulative evidence suggests that corticosteroids have statistically significant effects on protecting renal function and reducing proteinuria in adult patients with IgA nephropathy (20–28). In a notable study, an Italian prospective RCT demonstrated that a 6-month course of steroid treatment protected against deterioration of renal function during follow-up (20). The long-term follow-up data of the trial showed that corticosteroids significantly reduced proteinuria and protected against renal function deterioration (21). This study has provided further evidence for the use of corticosteroids in childhood IgA nephropathy.

The role of immunosuppressive drugs in the treatment of IgA nephropathy remains controversial. Goumenos *et al.* (29) retrospectively reported that treatment with prednisolone and azathioprine appeared to be beneficial in slowing the progression of severe adult IgA nephropathy. We conducted another multicenter prospective RCT comparing combination therapy with prednisolone monotherapy for severe childhood IgA nephropathy (9). The results of this study showed that patients who received prednisolone monotherapy had more sclerosed glomeruli than did those who received combination therapy, although proteinuria was reduced in both groups. We believe that immunosuppressants play an important role in combination therapy, as do corticosteroids, especially in preventing the progression of glomerular sclerosis, which was a significant outcome factor in the present follow-up study.

The natural course of childhood IgA nephropathy showing diffuse mesangial proliferation was reported to be 83% of renal survival at 5 years (4). The disease course of the dipyridamole and heparin-warfarin therapy group was almost identical to the natural course of childhood IgA nephropathy showing diffuse mesangial proliferation. We believe that the 2-year combination therapy decreased protein excretion, suppressed acute inflammation of glomeruli, reduced IgA production, and minimized the abnormal immune response after IgA deposition, which contributed to the prevention of glomerular sclerosis and the preservation of long-term renal survival (5,30).

A limitation of this study was the impossibility of treatment control after the initial 2-year study period. On the basis of the short-term outcome of this RCT, more patients received the combination therapy in the control therapy group than in the combination therapy group. On the other hand, because the use of ACEIs, which was restricted in the initial 2-year study period, has become clearly effective even in childhood IgA nephropathy (31), there was a tendency toward using it more often in the combination therapy group than in the control group. This may have affected the long-term outcome in this study. In particular, the former may have reduced the difference in long-term outcome between the two groups. However, such a situation seems to be ethically justifiable.

In our experience renal function is reversible in eGFR of 89 to 60 ml/min per 1.73 m^2, whereas it is irreversible in eGFR of <60 ml/min per 1.73 m^2 in children with IgA nephropathy (4). Therefore, we have used eGFR of <60 ml/min per 1.73 m^2 as a definition for renal insufficiency.

Conclusions

In conclusion, the 2-year combination therapy consisting of prednisolone, azathioprine, dipyridamole, and heparin-warfarin not only ameliorated the acute phase of nephritis, it also improved the long-term prognosis of childhood IgA nephropathy with diffuse mesangial proliferation. The long-term effectiveness of the combination therapy seems to be based on the reduction of urinary protein excretion and the suppression of glomerular sclerosis progression.

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Disclosures

None.

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EDUCATIONAL REVIEW

Posterior reversible encephalopathy syndrome in children with kidney diseases

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Abstract Posterior reversible encephalopathy syndrome (PRES) was originally used to describe a reversible, predominantly posterior leukoencephalopathy in patients who had renal insufficiency, hypertension, or who received immunosuppressive therapy. Since PRES is prevalent in children with kidney diseases, awareness and understanding of it is important for practicing pediatric nephrologists. A comprehensive approach to the diagnosis of PRES includes thorough determination of predisposing factors, clinical symptoms, and mandatory appropriate imaging. Unfortunately, the pathophysiology of PRES is still obscure and specificity of radiological examination has not yet been established. Two major predisposing factors, namely hypertension and calcineurin inhibitors, are well recognized. In addition, nephrotic syndrome is a common underlying condition for development of PRES. Frequent symptoms include altered consciousness (coma, stupor, lethargy, confusion), seizure, headache, and visual disturbance. Most of these symptoms usually develop abruptly and resolve within a few weeks after proper management. Cranial magnetic resonance (MR) imaging is the first-line modality of imaging studies for detecting PRES. Diffusion-weighted imaging with quantification of apparent diffusion coefficient (ADC) values by ADC mapping may provide more accurate and specific images in the future.

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Introduction

Posterior reversible encephalopathy syndrome (PRES) is a relatively new disease entity first described by Hinchey et al. in 1996 [1]. Since then, major advances have been made in the understanding of this condition. PRES has frequently been reported in pediatric patients, particularly in children with kidney diseases needing critical care [2–8].

Cerebral hyperperfusion and consequent vasogenic edema have been considered the major pathological events [1, 9], but the underlying pathophysiology leading to vasogenic edema is still rather obscure [10]. Furthermore, difficulty in diagnosing PRES can be frustrating for physicians. Thus, the significance of early and accurate diagnosis of PRES cannot be over-emphasized. In this review, PRES is considered in the particular context of children with kidney diseases.

Disease entity and nomenclature

Posterior reversible encephalopathy syndrome (PRES) was first reported as reversible posterior leukoencephalopathy syndrome (RPLS) in 1996 [1]. Originally, it was described as a reversible, predominantly posterior leukoencephalopathy in patients who had renal insufficiency or hypertension or who received immunosuppressive therapy. This concept encompassed several independent conditions, including hypertensive encephalopathy, preeclampsia/eclampsia, hemolytic uremic syndrome/thrombotic thrombocytopenia (HUS/TTP), uremic encephalopathy, and a severe type of

neurotoxicity of immunosuppressants into one entity. It has attracted interest and stimulated research from clinicians with different backgrounds and specialties who take care of these conditions. In this context, the establishment of this disease concept has made a big impact on the advance in the understanding in this field.

Recently, it has become clear that the terminology describing this condition should be modified. Hinchey et al. proposed the term "reversible posterior leukoencephalopathy syndrome" (RPLS) to emphasize the reversible nature and relatively restricted distribution of the lesions. However, RPLS itself appears to be a misnomer [11], as this condition is not necessarily reversible [11–16] and is not confined to the white matter and the posterior cerebral areas [3, 17, 18]. Instead, several alternative terms have been advocated, such as PRES [17], hyperperfusion encephalopathy [19], reversible posterior cerebral edema syndrome [20], and reversible occipitoparietal encephalopathy [21]. Although there is still some debate about its accuracy, "PRES" has been the most widely accepted so far, and will therefore be used in the present review.

An important differential diagnosis is hypertensive encephalopathy, which has been historically recognized as a neurological dysfunction induced by malignant hypertension regardless of imaging abnormalities. No precise definition of hypertensive encephalopathy has been established, either clinically or pathologically. Furthermore, PRES can occur without significant hypertension. Hypertensive emergency, defined as a situation that requires immediate blood-pressure reduction to prevent or limit target organ damage [22], is also used to describe a similar condition, but may include symptoms other than neurological conditions.

Pathophysiology of PRES

Vasogenic edema has been established as the pathognomonic change in the brain in patients with PRES [1, 9]. There are two major contradicting theories regarding the pathophysiology leading to onset of vasogenic edema: hyperperfusion due to autoregulatory failure of the cerebral vasculature and hypoperfusion due to vasoconstriction of the cerebral artery. These may co-exist or occur alternatively [23].

Hyperperfusion has predominantly been considered the major process for inducing vasogenic edema [9, 22]. Cerebral blood flow is controlled by the sympathetic nervous system, and is autoregulated to maintain constant flow within a systemic blood pressure range [22]. When blood pressure exceeds a certain set point in cases of significant systemic hypertension, autoregulation failure may occur. This results in cerebral hyperperfusion and breakdown of the blood–brain barrier, which in turn leads to vasogenic edema.

Although the "hyperperfusion theory" is widely accepted, vasoconstriction leading to cerebral hypoperfusion has also been shown to play a role in the development of vasogenic edema [10, 24]. In this case, hypoperfusion is followed by transient brain ischemia, and finally results in vasogenic edema through activation of vascular endothelial growth factor, which was also previously known as the vascular permeability factor [10].

In both theories, vasogenic edema is the principal mechanism. This is supported in most cases by findings from cranial magnetic resonance (MR) imaging, particularly diffusion-weighted imaging (DWI) and apparent diffusion coefficient (ADC) mapping [9, 14]. Rare reports of brain biopsy also confirm this pathology [25].

Endothelial cell dysfunction may also play a role in the development of vasogenic edema. Calcineurin inhibitors are known to injure the vascular endothelium [26–28], damage endothelial cells of the cerebral arteries, and subsequently release vasoactive agents that may induce vasogenic edema. In patients with preeclampsia/eclampsia and HUS/TTP, endothelial cell damage plays a pivotal role in the pathogenesis of these conditions and may also contribute to the development of PRES.

Taken together, vasogenic edema is the common underlying mechanism of PRES that could be caused by several predisposing factors and divergent processes. These processes can be important clues to prevention and treatment of PRES, and warrant further investigations.

Experience in children

Following the initial description by Hinchey et al., [1] PRES has also been reported in children with kidney [2–4, 6–8, 29, 30], rheumatological [6, 30, 31], and hematological disorders [13, 32, 33]; after kidney [3, 5], liver [15], and bone-marrow and stem cell transplantation [13, 33, 34], as well as with critical conditions [13]. PRES has become a well-recognized condition in children with a wide variety of diseases.

A summary of the case series in children [3, 13] and adults [1] is listed in Table 1. There are no major differences in clinical presentation between children and adults. Although the precise overall frequency of PRES in children is unknown, the reported frequency of PRES is 3.5% [35] and 5.6% [3] in children after kidney transplantation.

Diagnosis

Early and accurate diagnosis is of paramount significance. Early diagnosis can lead to removal of causative factors and

Table 1 Summary of posterior reversible encephalopathy syndrome (PRES) case series

	Ishikura et al. [3]	Kwon et al. [13]	Hinchey et al. [1]
Number of patients	20	12	15
Median age (years)/male: female	8.7 /13:7	9.8 (mean) /8:4	39 /2:13
Underlying conditions	Kidney transplantation, 10	Kidney transplantation, 2	Liver transplantation, 4
	Nephrotic syndrome, 7	Nephrotic syndrome, 2	Eclampsia, 3
	APSGN, 2	Bone marrow transplantation, 1	Systemic lupus erythematosus, 2
	DMS, 1	Neurogenic tumor, 3	Kidney transplantation, 1
		Hemolytic uremic syndrome, 2	Bone marrow transplantation, 1
		Cardiac procedure, 1	Acute nephritis, 1
		Leukemia,1	Others, 3
Number of patients with hypertension	19	12	12
Number of patients administered calcineurin inhibitors	Cyclosporine, 11 Tacrolimus, 4	Cyclosporine, 4	Cyclosporine, 4 Tacrolimus, 3
Symptoms (repeatedly counted)	Altered consciousness (coma, stupor, lethargy, confusion), 19	Seizures, 5 Visual disturbance, 4 Headache, 2	Altered consciousness (coma, stupor, lethargy, confusion), 11
	Seizures, 17	Altered mental status, 1	Seizures, 10
	Headache, 11		Visual disturbance, 10
	Visual disturbance, 9		Headache, 8
Lesions on cranial images (lobes) (repeatedly counted)	Occipital, 16 Anterior, 13 Parietal, 8	(Parieto-occipital lobes in the majority of patients) Cerebellum, 4	Occipital, 14 Parietal, 13 Temporal, 9
	Temporal, 5	Basal ganglia, 2	Anterior, 8
	Cerebellum, 4	Anterior parietal, 1	Pons, 2
		Frontal, 1	Cerebellum, 1
			Thalamus, 1
			Caudate body, 1
Lesions on cranial images (depth) (repeatedly counted)	White and gray matter, 15 White matter alone, 3 Gray matter alone, 2	(Both white matter and cortical gray matter in the majority of patients Cortical gray matter alone, 2	White and Gray matter, 4 White matter alone, 11
Outcome	Complete recovery, 18	Complete recovery, 11	Complete recovery, 15
	Mental retardation, 2	Neurological sequelae, 1	

APSGN, acute poststreptococcal glomerulonephritis; DMS, diffuse mesangial sclerosis

avoidance of potentially harmful investigations and treatment [15]. On the other hand, delay in diagnosis and prompt treatment may result in permanent neurological damage [6]. PRES is diagnosed based on characteristic clinical and radiographic patterns in the setting of predisposing factors [23].

Clinical symptoms

Table 1 summarizes common clinical symptoms of PRES described in children [3, 13] and adults [1]. Frequent symptoms include altered consciousness (coma, stupor, lethargy, confusion), seizure, headache, and visual disturbance. Most of these symptoms usually develop abruptly and resolve within a few weeks with proper management. Ophthalmological symptoms are relatively specific [23]. These include visual blurring, scotomas, visual hallucinations, and cortical blindness. Development of Anton's syndrome (denial of visual loss) has also been reported [1]. Although the reversible nature of the symptoms is one of the most essential clues to diagnosing PRES, permanent neurological damage is known to occur without early recognition and prompt treatment.

Predisposing factors

There are several predisposing factors in the development of PRES (Table 2). The clinical symptoms and neuroradio-

logical findings of PRES are typically indistinguishable, regardless of etiology and underlying factors.

Two major risk factors are well known: hypertension and calcineurin inhibitor therapy. Table 1 shows a high frequency of hypertension in the patients with PRES. Many other reports also support this [6, 11, 13, 19, 21, 30, 36]. Abrupt or intermittent increase in blood pressure may pose a greater risk than chronic hypertension [11, 19]. On the other hand, reported cases with hypertension may not necessarily exceed the threshold of autoregulation failure. Furthermore, PRES can develop in the absence of hypertension [10, 21, 24, 32]. Discrepancies between the severity of the lesions and hypertension have been reported [37]. Thus, the exact role of hypertension in the pathogenesis of PRES, whether it is the cause or effect, has not been fully elucidated.

The use of the calcineurin inhibitors, cyclosporine and tacrolimus, is also an important predisposing factor in the development of PRES [5, 13, 26–28, 34, 36–38]. This may explain the prevalence of PRES in the recipients of kidney transplants and children with immunological disorders.

Although the close relation between calcineurin inhibitor therapy and PRES is obvious, the precise underlying mechanism remains unknown. Although high blood concentration of calcineurin inhibitors may increase the risk, some patients develop PRES within the therapeutic range [3]. The clinical course of PRES associated with cyclosporine may be different from that associated with tacrolimus; the former is more frequently associated with hypertension and normal blood concentration than the latter [26]. However, this difference is not uniformly reported [28]. Discontinuation and re-introduction of calcineurin inhibitors after an episode of PRES are also important issues and will be discussed later.

wNephrotic syndrome is a condition that may predispose to the development of PRES [2, 4, 8]. Patients with nephrotic syndrome are at risk of PRES since they often receive calcineurin inhibitors and are often hypertensive. Furthermore, in case of nephrotic state with massive edema, fluid retention and increased vascular permeability may represent additional risk factors [2, 39]. Recently, T cell activation and inflammatory cytokine production have been suggested as additional predisposing factors for PRES [10] in other conditions, such as solid organ transplantation and autoimmune diseases. These same factors may indeed exist in children with nephrotic syndrome, particularly during relapses. Meticulous control of hypertension and blood concentration of calcineurin inhibitors is an important strategy in managing children with nephrotic syndrome in order to prevent the development of PRES.

Other kidney diseases in children who are predisposed to the development of PRES include acute glomerulonephritis [7, 40], HUS [13, 41], lupus nephritis [31, 42], Wegener's granulomatosis [29], and Henoch–Schönlein purpura nephritis [43]. Malignant tumors and hematological diseases are also frequent predisposing factors in children [33, 34, 44]. Several drugs other than calcineurin inhibitors and steroids, including monoclonal antibodies, such as rituximab [45], have been reported as risk factors (Table 2). To date, there is no evidence supporting genetic susceptibility to PRES.

Table 2 Predisposing factors for PRES

Underlying conditions	Medications
Hypertension	Calcineurin inhibitors
Preeclampsia/eclampsia, HELLP syndrome	Cyclosporine
Post-transplant	Tacrolimus
Bone marrow transplantation	Monoclonal antibodies
Solid organ transplantation	Rituximab
Kidney diseases	Bevacizumab
Nephrotic syndrome	Cancer chemotherapy
Acute poststreptococcal glomerulonephritis	Cisplatin
Hemolytic uremic syndrome/ thrombotic thrombocytopenia	Cytarabine
Acute kidney injury	Interferon-α
Chronic kidney disease	Steroids
Autoimmune diseases	Blood transfusion
Systemic lupus erythematosus	
Systemic sclerosis	
Wegener's granulomatosis	
Polyarteritis nodosa	
Sepsis and multiple organ failure	
Acute intermittent porphyria	
Surgery	

Imaging studies

Although there are some limitations, cranial MR imaging is the gold standard of imaging studies for detecting the lesions of PRES [11, 14]. Typical cranial images show focal regions of relatively symmetric hemispheric edema (Fig. 1). The parietal and occipital lobes are most commonly affected. T2-weighted images and fluid-attenuated inversion recovery (FLAIR) images have been frequently employed [11], with both of the sequences showing lesions as hyperintensities. For detection of subtle peripheral lesions, FLAIR is superior to conventional MR techniques, as this sequence suppresses the signal of the adjacent cerebrospinal fluid and can therefore render the lesions of PRES more conspicuous [17, 46]. The majority of the cases

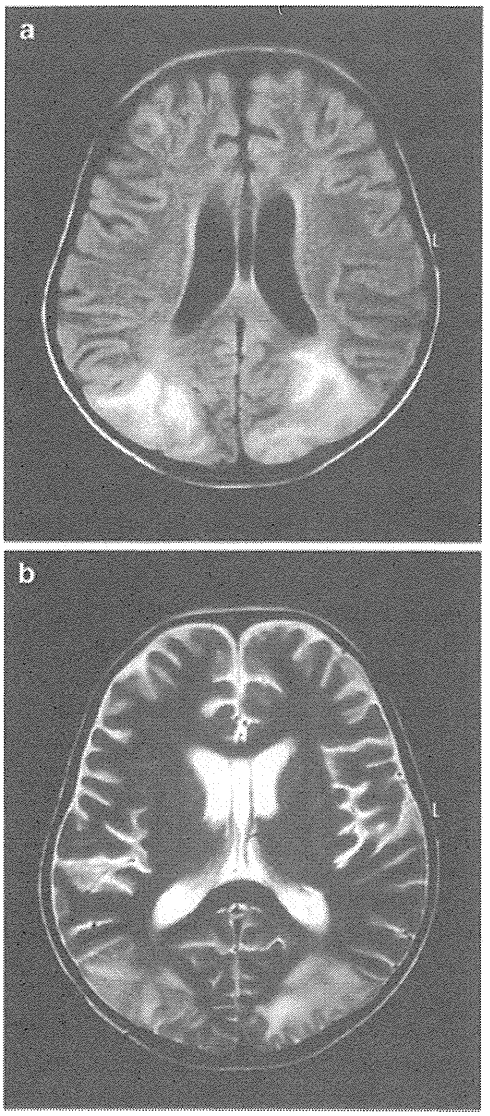

Fig. 1 Conventional cranial magnetic resonance (MR) images in children with posterior reversible encephalopathy syndrome (PRES). Imaging findings of a 5-year-old Japanese boy with hypertension and renal failure who developed PRES. MR images on day 2 are shown (**a** fluid-attenuated inversion recovery; **b** T2-weighted images). Bilateral, relatively symmetrical high-intensity areas are depicted in the white and gray matter of the occipital region

show complete recovery within a few weeks, but lesions are not always reversible.

Recently developed DWI with quantification by ADC mapping may provide more accurate and specific images [9, 11, 14, 15]. "Diffusion" in MR imaging denotes small movements of water molecules known as Brownian motion, and DWI and ADC mapping evaluate this small motion. In the state of vasogenic edema, the principal pathophysiological component of PRES, water molecules outside the cells can move freely, resulting in an increase in diffusion. This is the mechanism underlying the increased ADC values usually observed in the lesions of PRES. On the other hand, in the state of cytotoxic edema, water is locked in the cells with impaired Na/K-ATPase, and movement of water molecules is restricted, which results in a decrease in diffusion [47]. The typical condition of cytotoxic edema includes infarction. Taken together, DWI and ADC mapping are expected to enable differentiation between PRES and infarction, or to predict outcome of PRES by detecting the severe or advanced lesions of PRES that can result in irreversible consequences [12].

To evaluate the diffusion state of the lesions, clinicians are required to understand the characteristic and somewhat complicated patterns of DWI and ADC mapping (Fig. 2; Table 3). As a rule, when the diffusion state increases in the lesion, DWI reveals low intensity while ADC mapping shows high intensity. In DWI, pictures are clear and lesions can be identified easily, but they are occasionally affected by a result of T2-weighted imaging known as the "T2 shine-through" effect [11, 14]. With ADC mapping on the other hand, images are rather indistinguishable, but reflect diffusion state directly. Therefore, a useful approach is to first detect the lesions by DWI, then evaluate the diffusion state by ADC mapping. The settings of MRI vary, but we use the following in our institution: all patients undergo MR imaging with a 1.5-Tesla imaging system (Intra-Achieva, Philips, The Netherlands). T2-weighted MR imaging and isotropic DW imaging are performed using diffusion gradients in three orthogonal directions, with a maximal b value of 1,000 s/mm^2. DW imaging is performed using a single-shot, multisection, spin-echo echo-planar imaging sequence with the following parameters: 3000/65 (TR/TE); flip angle, 90°; and matrix size, 128*128 mm. For each patient, ADC maps are generated from the DW images. In the case of vasogenic edema, the DWI lesions occasionally show high intensity due to the T2 shine-through effect, and ADC mapping shows lesions with high intensity, indicating increased diffusion of water molecules. On the other hand, complete recovery of both clinical and radiological abnormalities has also been reported in adults [48] and children [49] who developed PRES with restricted ADC values in various cerebral areas. Further advances with MR imaging, including DWI with ADC mapping, is required to improve diagnostic accuracy and the ability to predict outcomes in patients with early-stage PRES, and to understand the complex pathophysiology.

Magnetic resonance angiography, MR spectroscopy, and MR perfusion scans are infrequently performed in patients with PRES [13, 19, 50], and the experience in the pediatric population is limited. MR angiography has shown moderate to severe vessel irregularity consistent with vasoconstriction and vasodilation in the majority of the patients, and MR

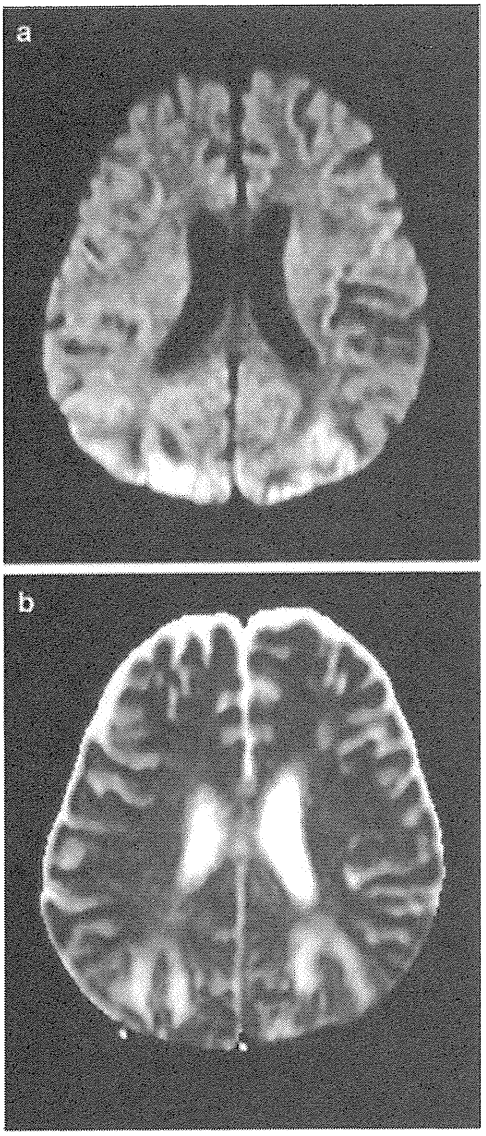

Fig. 2 **a** Diffusion-weighted image (DWI) and **b** apparent diffusion coefficient (ADC) mapping of the same patient as in Fig. 1. High-intensity areas are shown in both images. On DWI this results from the T2 shine-through effect, and on ADC mapping it indicates an increase in diffusion, suggesting vasogenic edema

perfusion imaging has shown significantly reduced relative cerebral blood volume in one case series [51]. These might be attractive approaches to exploring the mechanism of PRES, but are currently not practical in the clinical setting.

Conventional cranial computed tomography (CT) has been widely used [1]. In this technique, the lesions appear as low-density areas (Fig. 3). The main merits of CT are that it is convenient to perform in the acute phase and is a sensitive test for ruling out intracranial hemorrhage [36]. However, it has limited sensitivity for the detection of PRES, particularly in the acute phase [26].

Cranial imaging abnormalities of PRES are quite variable with regard to lesions and severity. Distribution of the cranial lesions of PRES is more widely spread than one might imagine from the terms "PRES" or "RPLS." The occipital lobes are most commonly affected, but the parietal, temporal, and frontal lobes and the cerebellum are also frequently involved [3, 23, 24]. In rare cases, the lesions are detected in the pontine or basal ganglia [18, 52]. In addition, the lesions are not confined to the subcortical white matter in most cases, but also involve the cortex or the deep white matter [11, 52]. Irreversible lesions may complicate; restricted diffusion and hemorrhage were present in 26% and 9% of patients respectively in one large case series [53].

Other investigations

Other investigations do not usually provide specific and pathognomonic information. Electroencephalography occasionally shows nonspecific slow waves and spikes [30], and is only valuable for evaluating seizure activity and for ruling out subclinical status epilepticus [50]. Cerebrospinal fluid examination is only useful for ruling out infective or inflammatory diseases.

Differential diagnosis

Ruling out cerebral infarction and venous thrombosis is important in several conditions in the field of nephrology. Progressive multiple leukoencephalopathy (PML), opportunistic infection of the brain caused by the JC virus with a lethal outcome, has become an emerging condition in this field. The increased use of strong immunosuppressive medication in kidney transplantation and in the treatment of auto-immune diseases, including calcineurin inhibitors, mycophenolate mofetil, and rituximab [54–56], is likely to result in increased incidence of PML. Sometimes T2-weighted and FLAIR images of PML mimic those of PRES, and DWI findings of patients with PML differ in asynchronous lesions and are dependent on the stage [57].

Table 3 Imaging patterns of MRI

	Vasogenic edema	Cytotoxic edema
T2	High intensity	High intensity
FLAIR	High intensity	High intensity
DWI	Low intensity (occasionally high[a])	Strongly high intensity
ADC mapping	High intensity	Low intensity

FLAIR, fluid-attenuated inversion recovery; DWI, diffusion-weighted imaging; ADC, apparent diffusion coefficient

[a] Due to T2 shine-through effect

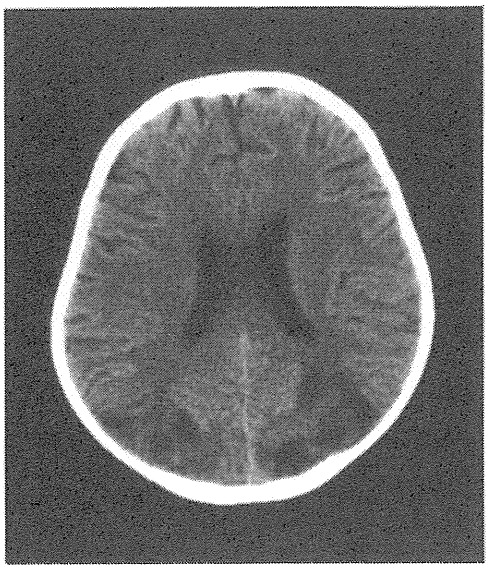

Fig. 3 Cranial CT images of the same patient. Distinct low-density areas are shown in the corresponding areas of Figs. 1 and 2.

Also, although the condition is irreversible and lethal, the clinical presentation is very pleiomorphic [54]. Therefore, to confirm diagnosis, detection of JC virus DNA from the cerebrospinal fluid is required.

Pseudotumor cerebri, an idiopathic condition of an elevated intracranial pressure lesion, shows similar symptoms, including headache, nausea, and visual disturbance [58]. This condition may occur at any age in childhood. Pseudotumor cerebri can be distinguished from PRES using normal cranial MRI. Furthermore, PRES has not been associated with elevated intracranial pressure. Other differential diagnoses of PRES include acute disseminated encephalomyelitis, infectious encephalitis, and X-linked adrenoleukodystrophy.

Treatment

The mainstays of the management of PRES are control of blood pressure, treatment of seizures, and removal or reduction of other causative factors. Patients are best managed in the ICU setting [50].

Although the precise role of hypertension is still unclear, the control of hypertension has been an essential component of the management of PRES [19, 22, 30]. After ruling out cerebral infarction, blood pressure should be lowered to near the 99th percentile level of the corresponding age and sex, or the mean arterial pressure should be reduced by 25% within the first hour followed by much slower reduction thereafter [50]. Intravenous therapy is preferred, and various parenteral drugs have been used, including nicardipine, labetalol, sodium nitroprusside, and hydralazine. Among these, in our experience with pediatric patients, nicardipine is most effective in lowering blood pressure promptly to within the appropriate range [3]. It is infused with an initial dose of 0.5 μg/kg/min and can be increased up to 5 μg/kg/min.

Seizures may progress to status epilepticus, and the importance of early treatment must be stressed [50]. Seizures should be treated with intravenous anticonvulsants. Diazepam and lorazepam are often used as the first-line agents, with phenytoin and phenobarbital as the second line. Midazolam is also a useful treatment.

Elimination of other predisposing factors should be considered. As already stated, discontinuation of calcineurin inhibitors or their re-administration after the episode of PRES are issues of controversy. One particularly controversial issue concerns recipients of organ transplants, as the withdrawal can cause acute rejection of the graft. Dose reduction without discontinuance of calcineurin inhibitors has resulted in good outcomes in some case series [5, 36], while others prefer their discontinuation at least for a certain period [27, 38, 50]. In one case series in patients with solid organ transplantation, the original immunosuppressive agent was resumed in 47% and an alternative immunosuppressive agent (cyclosporine to tacrolimus and vice versa) was used in 32% [26]. Continued administration [8] as well as re-administration [2] of cyclosporine after the episode of PRES in children with nephrotic syndrome have been reported as well.

Correction of fluid retention and electrolyte disturbance may be required if they exist. Management for increased intracranial pressure in patients with PRES is rarely reported.

Long-term prognosis

The long-term prognosis is an important issue in children with PRES, but little evidence exists. In our case series in 20 children, 2 children had prolonged neurological consequences, namely developmental delay [3]. Other case series showed several neurological consequences or imaging abnormalities in 8–17% of the children with PRES [6, 13, 34]. However, this information could be biased since reversibility is an important diagnostic criterion for PRES.

Another important neurological consequence is the development of epilepsy at a later period. Some cases with development of secondary epilepsy have been reported [44]. These may suggest the necessity of following the patients for the long-term, including those who show complete clinical and radiological recovery in the short term.

Subtle neurological consequences, including subclinical developmental delay and learning disabilities, are of particular significance in children. However, little informa-

tion has been reported and long-term follow-up studies are needed.

Conclusion

Posterior reversible encephalopathy syndrome is prevalent in children with kidney diseases and further understanding is required for adequate management. Accurate and immediate diagnosis is of particular importance. To accomplish this, a comprehensive approach through detecting suggestive clinical symptoms, discovering predisposing factors, and early assessment of imaging studies is needed, particularly as the pathophysiology of PRES is still not fully understood and a specific diagnostic examination has yet to be established.

Questions

(Answers appear following the reference list)

1. Symptoms of PRES include:
   a) Seizures
   b) Headache
   c) Visual disturbance
   d) Altered consciousness
   e) All the of the above
2. Which of the following agents is used for management of PRES?
   a) Steroid
   b) Antihypertensive agents
   c) Immunoglobulin
   d) Antibiotics
   e) All of the above
3. The most sensitive modality for the diagnosis of PRES is:
   a) Cranial CT
   b) Cranial MRI
   c) Electroencephalography
   d) Funduscopic examination
   e) Cerebrospinal fluid study
4. PRES can develop in children with:
   a) Hemolytic uremic syndrome
   b) Nephrotic syndrome
   c) Kidney transplant
   d) Acute poststreptococcal glomerulonephritis
   e) All of the above
5. The chief pathophysiological component of PRES is:
   a) Cytotoxic edema
   b) Hemorrhage
   c) Vasogenic edema
   d) Infection
   e) Degeneration of neurons

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Answers

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ORIGINAL ARTICLE

Maintenance therapy with mycophenolate mofetil after rituximab in pediatric patients with steroid-dependent nephrotic syndrome

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Abstract Rituximab (RTX) has a significant steroid-sparing effect in children with steroid-dependent nephrotic syndrome (SDNS). However, patients are likely to relapse with the recovery of CD20+ cells. We conducted a small prospective cohort study with a historical control to evaluate the effect of RTX infusion followed by mycophenolate mofetil (MMF) as a maintenance therapy. Nine patients with SDNS who stopped their steroid treatment but were treated with MMF after RTX infusion were prospectively observed (group A). Seven patients with SDNS who discontinued steroid and immunosuppressive agents after RTX administration served as a control (group B). During the first year after the administration of RTX, six patients in group A and one patient in group B did not suffer a relapse ($p<0.05$). The number of patients who relapsed during the 1 year preceding RTX treatment did not differ between the two groups [4.1 (A) vs. 5.7 (B)], but it was significantly lower in the MMF-treated group 1 year after the RTX treatment [0.4 (A) vs. 2.3 (B), $p<0.005$]. The daily amount of prednisolone after the RTX treatment was lower in group A than in group B (0.11 vs. 0.46 mg/kg/day, respectively; $p<0.05$). Three patients in group A and five patients in group B relapsed to SDNS and needed additional RTX treatment(s) within 1 year (odds ratio 5.0). Based on these results, we conclude that maintenance therapy with MMF after RTX is a good clinical option.

Keywords Nephrotic syndrome · Steroid-dependent · Rituximab · Mycophenolate mofetil · Children

Abbreviations

CPA	Cyclophosphamide
MMF	Mycophenolate mofetil
MPA	Mycophenolic acid
MZR	Mizoribine
PSL	Prednisolone
RTX	Rituximab
SDNS	Steroid-dependent nephrotic syndrome

Introduction

Since 2004, there have been many reports of the efficacy of rituximab (RTX) against childhood steroid-dependent nephrotic syndrome (SDNS) [1]. The incidental discovery of this drug's effect has improved the prognosis of childhood SDNS [1, 2]. In an earlier prospective study carried out by our group, we reported the effect of single-dose therapy with RTX on 12 children with refractory SDNS [3]. Following the administration of RTX, all of these patients were able to discontinue steroids, and there was a significant decrease in the frequency of relapse, period of steroid use, and mean steroid dosages. However, the efficacy of the treatment was transient in most patients, with nine of the 12 patients (75%) having relapses within 1 year. Most of the relapses developed simultaneously with the recovery of B-cells. Seven of the patients required

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additional RTX treatment because of steroid dependency. The results of this study agreed with those reported earlier which also suggested that relapse occurs after the re-emergence of B cells [4, 5]. Interestingly, in our previous study, two of the three patients who did not relapse continued on mycophenolate mofetil (MMF) therapy after RTX [3]. One of the patients who did not suffer relapse until day 353 also continued on MMF therapy. This observation led us to hypothesize that maintenance with MMF may be more effective in preventing relapse than RTX monotherapy. We therefore conducted a prospective pilot trial to evaluate the efficacy of maintenance with MMF after RTX.

Patients and methods

The patients included in this study met the following criteria: (1) age <20 years, (2) idiopathic nephrotic syndrome, (3) steroid dependency (two sequential relapses during tapering or within 2 weeks of discontinuation of steroid therapy [6]) on conventional immunosuppressants, such as cyclosporine (CsA), cyclophosphamide (CPA), mizoribine (MZR), or MMF, (4) no history of RTX treatment, and (5) no signs of infection, including human immunodeficiency virus (HIV) or hepatitis B or C. Patients with nephrotic syndrome due to primary and secondary glomerulonephritis were excluded from the study based on the finding of renal biopsy. A summary of the patients' characteristics is given in Table 1.

A total of 19 patients met the inclusion criteria. The clinical course of nine patients with SDNS who stopped their steroid treatment but were treated with MMF as maintenance therapy after the administration of RTX was prospectively observed; these patients were designated group A. Steroid and immunosuppressive agents, except for MMF, were discontinued after a single dose of RTX. In group A, three patients had been treated with MMF until the administration of RTX, but they sustained MMF therapy after RTX. The remaining seven patients discontinued steroid use and all immunosuppressants within 3 months after a single dose of RTX (group B). These seven patients, who had been treated in our institute, were extracted from our previous multicenter pilot study as a historical control against group A in the prospective study [3]. There was no significant difference in onset age and the age for RTX use between the two groups (Table 1).

Table 1 Summary of patients' characteristics

Patient no.	Sex[a]	Onset age (months)	Age at RTX treatment (months)	Renal histology[b]	History of previous immunosuppressant[c]	Treatment at RTX use[c]
Group A: RTX/MMF						
1	M	31	215	MGA	CsA, CPA, MZR, MMF	PSL, MMF
2	F	108	172	MGA	CsA, CPA, MZR, MMF	PSL, CsA, MMF
3	M	102	217	MGA	CsA, MZR	PSL, MZR
4	M	58	121	MGA	CsA, CPA, MZR	PSL, CsA, MZR
5	M	104	165	MGA	CsA, CPA, MZR	PSL, MZR
6	M	53	91	MGA	CsA, MZR	PSL, MZR
7	M	62	82	MGA	CsA, CPA, MZR	PSL, CsA, MZR
8	F	144	189	MGA	CsA, CPA, MZR	PSL, CsA, MZR
9	M	36	198	MGA	CsA, CPA, MZR, MMF	PSL, CsA, MMF
Group B: RTX						
1	M	60	172	FSGS	CsA, CPA, MZR	PSL, CsA, MZR
2	F	98	121	MGA	CsA, CPA, MZR	PSL, CsA
3	M	102	196	MGA	CsA, MZR	PSL, MZR
4	M	185	225	MGA	CsA,	PSL, CsA
5	M	53	153	MGA	CsA, CPA, CHL, MZR	PSL, CsA, MZR
6	M	20	60	MGA	CsA, CPA, MZR	PSL, MZR
7	M	121	239	MGA	CsA, MZR	PSL, MZR

RTX, Rituximab; MMF, mycophenolate mofetil

[a] M, Male; F, female

[b] MGA, Minor glomerular abnormalities; FSGS, focal segmental glomerulosclerosis

[c] CsA, cyclosporine A; CPA, cyclophosphamide; CHL, chlorambucil; MZR, mizoribine; PSL, prednisolone

Between the two groups, we compared: (1) frequency of relapse during the first year following the RTX treatment, (2) daily dosage of steroid for 1 year before and after the RTX treatment, (3) duration of depletion of CD20+ cells in peripheral blood, and (4) adverse events. We administered RTX intravenously in a single dose of 375 mg/m^2 body surface area (BSA) (maximum 500 mg) after obtaining remission with prednisolone (PSL). Steroid dosages were tapered and discontinued in both groups, although the detailed protocol for tapering steroid dosage was not restricted. The median discontinuation of PSL in groups A and B was 77 (range 53–120) and 73 (58–98) days after RTX treatment (not significant), respectively. CsA and MZR were tapered and discontinued by 3 months in both groups. The dose of MMF was 27.7±6.6 mg/kg (1000–1200 mg/m^2, which was determined from previous reports [10–12]. However, the serum concentrations of mycophenolic acid (MPA) could not be measured because of its limited availability in Japan. Profiles of the patients are summarized in Table 1. Approval of the off-label use of RTX and MMF for this study protocol was obtained from the Institutional Review Boards of the National Center for Child Health and Development. Informed consent from parents and patients were obtained before the administration of RTX and MMF.

For all patients, clinical and laboratory parameters, including complete blood counts, biochemical parameters, serum immunoglobulin levels, and CD20+ cell counts by flow cytometry, were monitored once a month for 12 months. B-cell depletion was defined as a CD20+ cell count of fewer than five cells per cubic millimeter at any time, and B-cell recovery was defined as a CD20+ cell count of more than 15 cells per cubic millimeter.

Statistical analysis

The Kaplan–Meier method and Log-rank test were used for the analysis of relapse-free survival. To compare the number of relapses before and after RTX infusion, we used the Wilcoxon signed-rank test. The Mann–Whitney test was used to analyze the duration of B-cell depletion, onset age, and the age at RTX therapy. The Kruskal–Wallis test was used to investigate the PSL dosages. Fisher's exact probability test was used to assess if the re-administration of RTX was necessary. Statistical significance was established at $p<0.05$.

Results

Relapse rate at 1 year after RTX infusion

During the first year following the RTX treatment, relapse occurred in three patients in group A and six patients in group B ($p<0.05$) (Fig. 1). In both groups, the number of patients who relapsed during this time was statistically lower than that before the RTX infusion ($p<0.05$) (Fig. 2). RTX remarkably reduced the number of relapses in patients with SDNS. The average number of relapses in group A and B patients during the year preceding the RTX treatment was 4.1 versus 5.7, respectively (not significant), and number of relapses during the year following RTX treatment was 0.4 versus 2.3, respectively ($p<0.005$ for group A versus B) (Fig. 3).

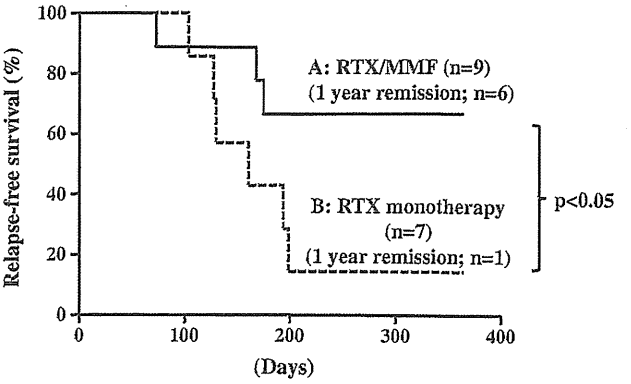

Fig. 1 Relapse-free survival during the first year after rituximab (*RTX*) treatment *MMF* Mycophenolate mofetil

Total steroid dose

All patients in both groups were able to discontinue PSL. Average daily doses (mg/kg/day) of PSL before and after RTX treatment were 0.52 and 0.29 in group B (not significant) and 0.46 and 0.11 in group A ($p<0.05$), respectively. The average daily dose of PSL after RTX treatment was significantly lower in group A than in group B ($p<0.05$) (Fig. 4). Thus, maintenance therapy with MMF after the RTX infusion has a significant steroid-sparing effect that was greater than that of RTX monotherapy.

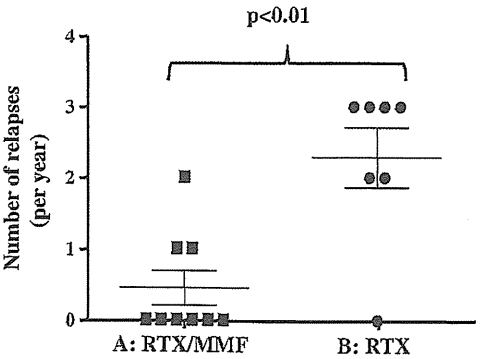

Fig. 2 Number of relapses during the first year following RTX treatment

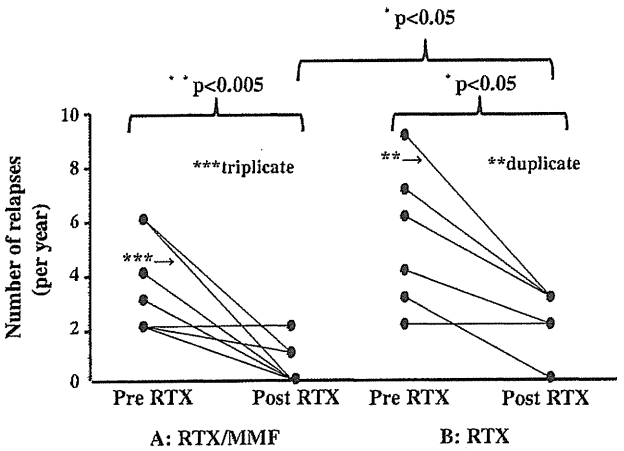

Fig. 3 Comparison of the number of relapses during the year preceding RTX treatment with that in the 1-year period following RTX treatment

Rate of rituximab re-use

Five patients in group B and three patients in group A relapsed to SDNS and required additional courses of RTX within 1 year following the initial RTX treatment. The risk of needing additional RTX treatments was higher for group B patients than those in group A (odds ratio 5.0; 95% confidence interval 0.58–42.8). All patients successfully discontinued steroid use after the additional RTX treatment. However, within 1 year from the initial RTX dose, one patient in group A and one patient in group B needed one more additional course of RTX because each relapsed during steroid tapering.

Duration of CD20+ cell depletion

Maintenance therapy with MMF may prolong the period of depletion of CD20+ cells, which may in turn reduce the relapse rate. However, re-emergence of CD20+ cells after RTX did occur, at 149±33 and 131±72 days, respectively, in group A and group B (not significant) (Fig. 5).

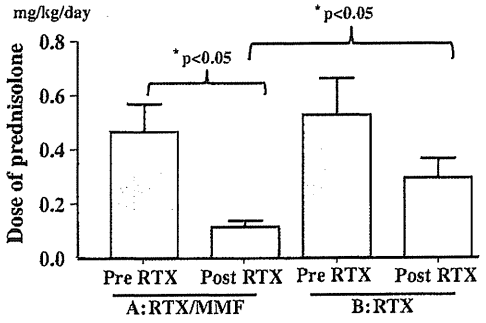

Fig. 4 Comparison of the dose of prednisolone for 1 year before and after RTX treatment

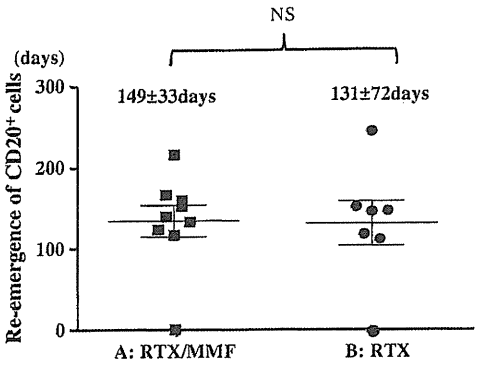

Fig. 5 Re-emergence of CD20+ cells in peripheral blood after RTX therapy. *NS* Not significant

Adverse events

Mild infusion reactions, such as cough, sore throat, skin rash, and discomfort, were observed in nine patients in groups A and B after the RTX infusion, but these did not need specific treatment. Two patients in group A experienced transient diarrhea due to MMF. No severe adverse effects occurred in either group.

Discussion

In this pilot study, maintenance therapy with MMF after a single dose of RTX against refractory SDNS significantly prolonged the relapse-free period and reduced the relapse rate and the daily dose of steroids compared to RTX monotherapy.

There have been many reports of the excellent steroid-sparing effect of RTX against childhood SDNS. However, most patients are likely to relapse with the recovery of B cells [3–5]. Because of these issues, the administration of a single dose at regular intervals, consecutive multiple administrations within a short period of time, or maintenance therapy with various immunosuppressive agents after RTX are feasible solutions. A single infusion of RTX can deplete B cells for about 5 months [3, 4]. The efficacy of additional RTX administrations just after the re-emergence of B cells has been reported in children with SDNS [7]. The effect that persistent B cell depletion has on the developing immune system in children is unknown. However, one consequence of B cell depletion is the lack of the ability to produce antibodies, thereby rendering vaccination is impossible, which is a critical issue in children. Therefore, the administration of a single dose of RTX at regular intervals is currently not recommended for children. The consecutive multiple administration of RTX within a short period, such as two or four consecutive shots every week, has already been reported. Most of these patients are