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Efficacy of combination therapy for childhood complicated focal IgA nephropathy

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Abstract

Background: Patients with immunoglobulin A nephropathy who present with focal mesangial proliferation (focal IgAN) can have a relatively good prognosis, and renin–angiotensin system inhibitor (RAS-i) is commonly used as the initial treatment. However, there are some complicated focal IgAN cases with resistance to RAS-i treatment or nephrotic-range proteinuria. Thus, combination therapy including corticosteroids is often used. This study aimed to evaluate the efficacy of combination therapy for complicated focal IgAN cases by comparing to diffuse mesangial proliferation (diffuse IgAN).

Methods: We conducted a multicenter retrospective study on 88 children who received 2-year combination therapy. The participants were classified based on pathological severity: focal IgAN (n=26) and diffuse IgAN (n=62).

Results: In total, 26 patients with focal IgAN and 52 with diffuse IgAN achieved proteinuria disappearance within 2 years (100% vs. 83.9%, P=0.03). Moreover, the time to proteinuria disappearance was significantly shorter in the focal IgAN group than in the diffuse IgAN group (2.9 vs. 4.2 months, P<0.01) and all patients with focal IgAN achieved proteinuria disappearance within 8 months. At the last observation (8.6 vs. 10.4 years, P=0.13), only patients with diffuse IgAN (n=12) had greater than stage 2 chronic kidney disease. In terms of irreversible adverse events, one patient exhibited cataracts.

Conclusion: Combination therapy was significantly effective in patients with complicated focal IgAN. Moreover, the long-term prognosis was good, and the duration of combination therapy for complicated focal IgAN can be decreased to reduce adverse events.

Keywords: complicated focal mesangial proliferative IgA nephropathy; resistance; renin–angiotensin system inhibitor; combination therapy; relapse

Introduction

Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis among children. In a study of long-term follow-up without treatment, 11% of pediatric patients with IgAN developed end-stage kidney disease (ESKD) within 15 years.[1 nn] Widely accepted consensus treatment approaches for IgAN are lacking owing to heterogeneous disease severity. According to Japanese evidence-based guidelines, pediatric patients with IgAN can be treated using different strategies based on pathological severity (focal mesangial proliferation observed in focal IgAN versus diffuse mesangial proliferation observed in diffuse IgAN).[2] In patients with focal IgAN, renin–angiotensin system inhibitor (RAS-i) treatment can be initially used and patients with diffuse IgAN can receive combination therapy with corticosteroids and immunosuppressants for 2 years.[3–5]

It is possible for patients with focal IgAN to have a relatively good prognosis after RAS-i treatment alone.[4,6,7] However, some patients with focal IgAN exhibit nephrotic-range proteinuria[8] or resistance to RAS-i treatment.[4,6] Given that nephrotic-range proteinuria and persistent proteinuria are poor renal prognostic factors,[8,9] combination therapy is commonly used in complicated cases, including those with focal IgAN. However, no study to date has evaluated the efficacy of combination therapy for complicated focal IgAN. Therefore, in the current study, we aimed to validate the efficacy of combination therapy in pediatric patients with complicated focal IgAN compared to those with diffuse IgAN.

Patients and methods

Study design

We conducted a multicenter retrospective study at six medical hospitals in Japan, which were as follows: Kobe University Hospital, Wakayama Medical University Hospital, Hyogo Prefectural Children's Hospital, Takatsuki General Hospital, Kakogawa City Hospital, and Japanese Red Cross Society Himeji Hospital. The study was performed in accordance with the Declaration of Helsinki and approved by the regional research ethics boards of each institution. This study enrolled children with IgAN who were recently treated with 2-year combination therapy between January 2000 and December 2018. The participants were classified into two groups based on pathological severity, and the efficacy of 2-year combination therapy was compared between pediatric patients with complicated focal IgAN and those with diffuse IgAN.

Patients

The inclusion criteria were as follows: (1) patients aged under 18 years at the start of combination therapy; (2) absence of secondary IgAN such as IgA vasculitis, systemic lupus erythematosus, and chronic liver disease; (3) those who did not receive previous treatment with corticosteroids or immunosuppressants; (4) those with proteinuria at the beginning of combination therapy; and (5) those with sufficient kidney biopsy specimens available for pathological evaluation (minimum of 10 glomeruli).

The pathological diagnosis of IgAN was based on the presence of IgA in the mesangial region of the glomerulus, either as sole or predominant immunoglobulin deposits. All patients with IgAN were diagnosed by a single pathologist (NY) using the same criteria. World Health Organization criteria define focal IgAN as the presence of moderate or severe mesangial cell proliferation, crescent formation, adhesions, or sclerotic lesions in <80% of the glomeruli and diffuse IgAN was defined as the presence of these changes in ≥80% of the glomeruli. Kidney

105 pathological findings were re-evaluated using the following Oxford classification system for
106 IgAN: mesangial hypercellularity (M1/M0), segmental glomerulosclerosis or adhesion (S0/S1),
107 endocapillary hypercellularity (E1/E2), tubular atrophy/interstitial fibrosis (T0/T1+T2), and
108 crescents (C0/C1+C2).[10–13]
109
110 **Treatments**
111 All participants received combination therapy with prednisone (PSL) and mizoribine (MZR)
112 (with and without warfarin and dipyridamole or with and without RAS-i). The treatment dose
113 and duration were similar to those used in previous randomized controlled trials (RCTs) and
114 were as follows:[5,9,14,15] PSL was orally administered at a dose of 2 mg/kg/day (maximum 80
115 mg/day) for 4 weeks, followed by doses of 2 mg/kg once every other day for 4 weeks, 1.5 mg/kg
116 once every other day for 4 weeks, and 1 mg/kg once every other day for 21 months. MZR was
117 orally administered at a dose of 4 mg/kg/day (maximum 150 mg/day) for 24 months. Warfarin
118 was administered to maintain a thrombotest value of 30%–80% for 24 months. Dipyridamole
119 was orally administered at a dose of 6 mg/kg/day (maximum 300 mg/day) for 24 months. RAS-i
120 treatment was orally administered for more than 24 months at a dose of 0.1–0.4 mg/kg/day
121 (maximum 20 mg/day) for angiotensin-converting enzyme inhibitors and/or at a dose of 0.07–1.0
122 mg/kg/day (maximum 8 mg/day) for angiotensin II receptor blockers.
123
124 **Clinical data**
125 Anonymized data were collected using a standardized electronic form. Data on birth, sex,
126 opportunity of diagnosis, date of onset and kidney biopsy, and history of gross hematuria were
127 recorded. Clinical information on height, weight, blood pressure, serum creatinine, serum
128 albumin, urinary erythrocyte count, and urine protein-to-creatinine ratio [uP/Cr (g/g)] were

collected. In particular, at 0, 6, 12, and 24 months after the beginning of combination therapy, data on uP/Cr were recorded. Moreover, information on side effects and duration from the initiation of combination therapy to proteinuria and hematuria disappearance was documented.

The Japanese Society for Pediatrics Endocrinology calculation software (jspe.umin.jp/jspe_test/medical/files/taikakushisuv2.xlsx) was used to calculate length/height-for-age and obesity index.

Definitions

Patients with complicated focal IgA were defined as those who received combination therapy despite the initial diagnosis of focal IgAN because of nephrotic-range proteinuria, resistance to RAS-i treatment, or histological results resembling diffuse IgAN. Proteinuria and hematuria were defined as a uP/Cr of ≥0.2 g/g and ≥5 red blood cells per high-power field, respectively. Date of the disappearance of proteinuria and hematuria was defined as the first day of two consecutive negative examination results. Remission was defined as the disappearance of proteinuria and hematuria. Relapse of proteinuria was defined as the appearance of proteinuria after its disappearance at an earlier date. Nephrotic syndrome was defined as hypoalbuminemia (serum albumin ≤2.5 mg/dL) and nephrotic-range proteinuria (uP/Cr >2.0 g/g). Estimated glomerular filtration rate (eGFR) was calculated using creatinine-based equation for Japanese children, as reported previously.[16,17] ESKD was defined as stage 5 chronic kidney disease (CKD) (eGFR <15 mL/min/1.73 m^2 or need for kidney replacement therapy).

Statistical analyses

The JMP software version 14 (SAS Institute Inc., Raleigh, NC) was used for data analysis. Data with normally distribution were expressed as mean ± standard deviation (SD) and nonparametric data as median and interquartile range. Continuous and categorical data were compared using Pearson's chi-square test and Fisher's exact test, respectively. Cumulative event rates were calculated using the Kaplan–Meier method. Two-tailed P values of <0.05 were considered statistically significant.

Results

Patients

In total, 99 children received combination therapy during the study period (Figure 1). Among these, 27 and 72 patients were diagnosed with focal and diffuse IgAN, respectively. In the focal IgAN group, one patient with missing data was excluded. In the diffuse IgAN group, three patients without proteinuria at the beginning of treatment, two patients without sufficient biopsy samples, and five patients with missing data were excluded. The final analysis groups included 26 patients with focal IgAN and 62 patients with diffuse IgAN. In focal IgAN group, the indications for combination therapy were resistance to initial RAS-i therapy (n=6), nephrotic-range proteinuria at diagnosis (n=12), and histological results close to diffuse IgAN (n=8). The retrospective analysis of cases with histological results close to diffuse IgAN revealed that these cases exhibited ≥60% of glomeruli with mesangial proliferation, crescent formation, adhesions, or sclerotic lesions (n=7) and/or ≥20% of glomeruli with crescent formation (n=6).

Second biopsy was performed on five patients who were initially diagnosed with focal IgAN but were refractory to >2-year RAS-i treatment. All patients pathologically diagnosed with diffuse IgAN were classified under the diffuse IgAN group. By contrast, 2-year combination therapy was started without re-evaluation of pathological findings in 6 patients with resistance to

<1-year RAS-i treatment (n=5) or 2.3-year dipyridamole treatment (n=1), and these patients were classified under the focal IgAN group (Supplemental Table 1).

Clinical and pathological findings

In both groups, the most common opportunity for detecting urine abnormalities was during the annual school urine screening (Table 1). According to the Oxford classification system, histological analyses revealed significant differences in M0/M1 ratios (P<0.01) because this element was associated with the group classification. There was no statistically significant difference in terms of previous treatment (23.1% vs. 8.1%, P=0.05) and combination therapy, which included anticoagulants, antiplatelet agents, or RAS-i. Severe adverse events were observed in one patient with cataract in the focal IgAN group and six patients in the diffuse IgAN group (n=2, hyperuricemia requiring discontinuation of mizoribine; n=1, pancytopenia; n=1, drug-induced pancreatitis; n=1, psychosis; and n=1, drug-induced kidney dysfunction). In the diffuse IgAN group, mizoribine was discontinued in two patients and was changed to azathioprine treatment in one patient. RAS-i treatment was discontinued in two patients.

In addition, we evaluated growth disturbance and weight gain, important side effects of PSL. The typical timing of height growth spurt in adolescence is 11−15 years in boys and 9−13 years in girls. Thus, we investigated height gain in patients who received 2-year combination therapy during these ages and identified 37 patients, including 18 boys and 19 girls, with a follow-up period of 7.9±4.8 years. The length/height-for-age (Standard deviation: SD) significantly decreased (−0.35±0.12 SD, P<0.01) after 2-year combination therapy. However, the length/height-for-age had caught up (0.0±0.15 SD, P=0.96) at the last follow-up (age, 20.4±4.7

197 years). Regarding weight gain, there was no significant change in obesity index before and at the
198 end of the 2-year combination therapy (−0.90±2.58 vs. 1.82±2.60 kg, P=0.33).
199
200 **Outcomes**
201 *Time to proteinuria or hematuria disappearance or to achieve remission*
202 Based on the Kaplan–Meier analysis, the time to proteinuria disappearance was significantly
203 shorter in the focal IgAN group than in the diffuse IgAN group (2.9 vs. 4.2 months, P<0.01)
204 (Figure 2). In addition, all patients in the focal IgAN achieved proteinuria disappearance within 8
205 months. However, there was no significant difference in terms of duration from treatment to
206 hematuria disappearance or remission between the two groups (Supplemental Figures 1,2).
207
208 *Relapsing proteinuria during treatment*
209 At 6 and 12 months after the start of combination therapy, the proportion of patients with
210 proteinuria negative significantly differed between the two groups (focal vs. diffuse IgAN:
211 92.3% vs. 64.5% at 6 months, P<0.01; 92.3% vs. 72.6% at 12 months, P=0.04) (Table 2, Figure
212 3). Proteinuria was treated in all children in the focal IgAN group and 52 (83.9%) in the diffuse
213 IgAN group. In addition, 22 (84.6%) patients in the focal IgAN group and 38 (61.3%) patients in
214 the diffuse IgAN group, respectively, did not experience relapse of proteinuria during the 2-year
215 combination therapy. Four (15.4%) patients in the focal IgAN group relapsed without any
216 triggers, with one patient each at 9, 12, 20, and 23 months, respectively. Meanwhile, 14 (22.6%)
217 patients in the diffuse IgAN group experienced relapse of proteinuria. Although combination
218 therapy in terms of proteinuria disappearance was significantly more effective in patients with
219 focal IgAN than in those with diffuse IgAN (100% vs. 83.9%, P=0.03), there was no significant

difference in the relapse rate after proteinuria disappearance between the two groups (15.4% vs. 22.6%, P=0.45).

Long-term outcomes

Fifteen patients in the focal IgAN group and 42 in the diffuse IgAN group were followed for >5 years. There were no significant differences in clinical and pathological characteristics (Supplemental Table 2). In the focal IgAN group, six patients received RAS-i treatment and two patients had proteinuria at last observation. Of these, five patients continued RAS-i treatment after 2-year combination therapy and one patient resumed RAS-i treatment because of proteinuria recurrence after discontinuing RAS-i treatment. The kidney function of all patients in the focal IgAN group was normal (Table 3). Greater than stage 2 CKD was only observed in the diffuse IgAN group (n=12), and two patients experienced progression to greater than stage 3 CKD. One of these two patients was initially diagnosed with IgAN with nephrotic syndrome and developed stage 3b CKD after 11 years of treatment. In this patient, proteinuria, which had disappeared initially, relapsed during the 2-year combination therapy and continued. The other patient who presented with ESKD 4 years after treatment continued to exhibit heavy proteinuria despite treatment with combination therapy and cyclophosphamide and experienced rapidly deteriorating kidney dysfunction. The proportion of patients with negative proteinuria and normal kidney function (eGFR ≥90 mL/min/1.73 m^2) was significantly better in the focal IgAN group than in the diffuse IgAN group (86.7% vs. 57.1%, P=0.04; 100% vs. 73.8%, P=0.03, respectively).

Discussion

IgA nephropathy is the most common chronic glomerulonephritis among children. However, determining a unified treatment strategy is challenging due to varying severity. The present study aimed to evaluate the efficacy of 2-year combination therapy in patients with complicated focal IgAN, including those resistant to RAS-i treatment, those with nephrotic-range proteinuria at diagnosis, and those with histological results close to diffuse IgAN. We found that the patients with complicated focal IgAN exhibited relatively severe pathological findings, such as segmental sclerosis and crescents, compared to those with common focal IgAN; these findings were relatively similar to those observed in patients with diffuse IgAN. However, despite these pathological findings, combination therapy was significantly effective and the prognosis during the last assessment was excellent in these patients.

There are no studies evaluating the efficacy of immunosuppressant therapy including steroids for pediatric patients with focal IgAN. Some pediatric patients with clinically and pathologically mild disease achieve spontaneous remission without treatment[18]; RAS-i treatment was reported to result in the disappearance of proteinuria within 24 months in approximately 90% of pediatric patients with focal IgAN.[4] Furthermore, minimal proteinuria (<0.5 g/day/1.73m^2) after supportive therapy is a good renal prognostic factor.[7] Accumulating data suggest that RAS-i treatment should be initiated in patients with focal IgAN.

However, persistent or heavy proteinuria is a poor renal prognostic factor in IgAN.[8,19,20] Therefore, patients with focal IgAN resistant to RAS-i treatment should be administered combination therapy; however, although the long-term outcome of RAS-i treatment alone is unknown. In an RCT of patients with focal IgAN who received RAS-i treatment, proteinuria disappeared in 60%, 80%, and 90% of the patients at 6, 12, and 24 months following treatment initiation, respectively.[4] Additionally, Coppo et al. recommended both PSL and

immunosuppressive therapy for pediatric patients with resistance to RAS-i treatment for at least 6 months.[21] Therefore, we recommend switching from RAS-i treatment to combination therapy for focal IgAN in patients with deteriorating proteinuria despite RAS-i treatment and those with persistent proteinuria for more than 6 months after treatment initiation.

Transient adverse effects such as moon face, hypertension, glaucoma, and hyperuricemia are commonly observed in patients receiving 2-year combination therapy. Irreversible side effects of PSL, such as osteoporosis, aseptic necrosis of the femur, cataract, and growth disturbance, should also be recognized. Approximately 20%–25% of patients with IgAN who receive 2-year combination therapy present with side effects, and approximately 0%–5% of patients experience irreversible adverse events.[3,5,22] In the present study, 7 of the 88 patients (8.0%) had severe side effects and 1 (1.1%) patient developed cataract. Patients who received the 2-year combination therapy during the expected adolescent growth spurt showed altered length/height-for-age; however, this subsequently recovered after treatment discontinuation. Different from a previous report,[3] the present study indicated no significant change in obesity index associated with the 2-year combination therapy, which might be attributable to several reasons. For example, exercise restriction, which was recommended for pediatric patients with hematuria or proteinuria in the 1990s, might have contributed to the changes in obesity index observed in previous studies. In fact, even patients without steroid treatment exhibited significantly increased obesity index in a previous report.[3] Albeit relatively rare, irreversible side effects should be considered as a risk of combination therapy and the routine use of corticosteroids should not be recommended for all pediatric patients with IgAN, particularly for those with pathologically mild conditions.[7,18]

The disappearance of proteinuria is correlated with favorable prognosis.[7,23] We found that routine 2-year combination therapy was quite effective in eliminating proteinuria in complicated focal IgAN cases. In addition, all patients achieved proteinuria disappearance within 8 months. However, 4 patients experienced relapse in proteinuria under combination therapy while 22 patients maintained proteinuria disappearance. As the risk factors for proteinuria relapse are unknown, further investigation is needed. Nevertheless, these results indicate that a treatment period shorter than 2 years might be considered for patients with complicated focal IgAN without any risk factors for the relapse of IgAN.

Approximately 15%–50% of children with diffuse IgAN continue to present with proteinuria after receiving 2-year combination therapy[9,14,24] and may develop ESKD in the future.[20,23,25,26] In the present study, all patients in the focal IgAN group and 83.9% of the patients in the diffuse IgAN group achieved proteinuria disappearance during combination therapy. Only two patients with diffuse IgAN developed greater than stage 3 CKD during the long-term follow-up. The outcomes of patients with diffuse IgAN observed in the current cohort were similar to those reported in previous studies (Table 4).[24,25,27] However, some patients in the present study received RAS-i treatment and the amount of proteinuria might have been underestimated. The proportion of patients without proteinuria who preserved normal kidney function during the last assessment was significantly better in the focal IgAN group than in the diffuse IgAN group, and none of the patients developed CKD. Therefore, the long-term kidney outcomes were better in the focal IgAN group and combination therapy was strongly effective in patients with complicated focal IgAN.

Currently, combination therapy is the only evidence-based treatment supported by RCTs for pediatric IgAN patients. The present study revealed that 10% and 20% of the patients with

311 diffuse IgAN exhibited continued and relapsed proteinuria, respectively, after the 2-year
312 combination therapy. Previously described cases had different backgrounds compared with the
313 patients included in the present study; however, pulse methylprednisolone followed by short-
314 term prednisolone and tonsillectomy for clinically or pathologically severe pediatric IgAN
315 resulted in the disappearance of proteinuria in all patients with no irreversible adverse effects.[28]
316 Another RCT found that steroid pulse therapy and tonsillectomy had the same efficacy as
317 combination therapy for pediatric IgAN.[27]

318 In the present study, the same pathologist diagnosed all patients, addressing potential
319 concerns regarding subjective differences in pathological evaluation and indicating the use of
320 consistent standards for pathological diagnosis throughout the study. In previous studies, the
321 lower limit of mesangial proliferation for IgAN diagnosis was 50%,[24,27] in contrast to that used in
322 the current study (80%). Therefore, the number of patients with more severe conditions was
323 higher in the focal IgAN group in the current study; however, the patients with complicated focal
324 IgAN had excellent prognosis and therapy response.

325 The current study has several limitations. First, only a small number of patients were included,
326 and this research was retrospective in nature. Second, 6 patients were excluded due to lack of
327 data. Third, 6 patients who were resistant to <1-year RAS-i treatment were classified under the
328 focal IgAN group without pathological re-evaluation. Fourth, patients with negative proteinuria
329 were considered to be in remission; however, all patients receiving RAS-i treatment continued
330 the same treatment after the discontinuation of combination therapy and some patients were
331 receiving combination therapy under remission. It is possible that they will experience
332 proteinuria after discontinuing these treatments. Finally, subgroup analysis of patients with
333 complicated focal IgAN was not conducted due to the small number of participants.

Conclusion

Combination therapy with PSL and MZR was strongly effective in patients with complicated focal IgAN. Moreover, the prognosis during the last follow-up was excellent, and the adverse effects were acceptable. Since the time from treatment to proteinuria disappearance was significantly short, modification in treatment duration, and dose should be reconsidered in future investigations.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

Consultations: Kazumoto Iijima (Takeda Pharmaceutical Co., Ltd., and Sanofi K.K.), Kandai Nozu (Eisai Co., Ltd.), Honoraria: Koichi Nakanishi (Sanofi K.K., AstraZeneca K.K., DiichiSankyo Co., Ltd., Asahi Kasei Pharma Co., and Sumitomo Dainippon Pharma Co., Ltd.), Kazumoto Iijima (Shionogi & Co., Ltd.), Kandai Nozu (Sumitomo Dainippon Pharma Co., Ltd. and Mochida Pharmaceutical Co., Ltd.), Grants received: Koichi Nakanishi (DiichiSankyo Co., Ltd., Asahi Kasei Pharma Co., Pfizer Inc., and Shionogi & Co., Ltd), Kazumoto Iijima (Shionogi & Co., Ltd., Eisai Co., Ltd., Mochida Pharmaceutical Co., Ltd., and Nippon Pharmaceutical Co., Ltd.).

Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki and the ethical guidelines issued by the Ministry of Health, Labour and Welfare of Japan (2017). The study was

approved by the institutional research committee of the institutions at which the studies were conducted.

Informed Consent

Because data from patient medical records were used, informed consent was not obtained, and this is in accordance with the aforementioned guidelines. The study protocol was displayed publicly in a poster at each institution, and this is also in accordance with the guidelines for patients' benefit. Therefore, patients could refuse to attend this study. Personal data were masked as stipulated in the ethical guidelines.

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Figure legends

Fig. 1 Flow diagram of patient selection

In total, 99 children with IgAN, including 27 and 72 patients with focal and diffuse IgAN, respectively, were recently treated with combination therapy. In the focal IgAN group, one patient with missing data was excluded. In the diffuse IgAN group, three patients without proteinuria at the beginning of treatment, two patients without sufficient biopsy samples, and five patients with missing data were excluded. The final analysis included 26 patients with focal IgAN and 62 patients with diffuse IgAN. In the focal IgAN group, the indications for combination therapy were resistance to initial therapy (n=6), nephrotic-range proteinuria at diagnosis (n=12), and histological results close to diffuse IgAN (n=8)

Fig. 2 Disappearance of proteinuria

Proteinuria in all children in the focal IgAN group was eliminated within 8 months. However, 10 (16.1%) patients in the IgAN group still presented with proteinuria. The achievement of proteinuria disappearance was significantly earlier in the focal IgAN group than in the diffuse IgAN group ($P<0.01$).

Clinical numerical data were presented as mean ± standard deviation

uP/Cr: urinary creatine protein ratio

Fig. 3 Time course Changes in uP/Cr

Four (15.4%) patients in the focal IgAN group at 9, 12, 20, and 23 months and fourteen (22.6%) patients in the diffuse IgAN group relapsed during combination therapy without trigger, and ten (16.1%) patients in the diffuse IgAN group still presented with proteinuria.

uP/Cr: urinary protein creatinine ratio

Supplemental Fig. 1 Cumulative disappearance rate of hematuria

There were no significant differences between the two groups in terms of time to hematuria disappearance (P=0.59).

Supplemental Fig. 2 Cumulative rate of remission

There were no significant differences between the two groups in terms of time to remission (P=0.14).

TABLE 1 Backgrounds of Patient characteristics in the two groups

	Focal IgAN (n = 26)	**Diffuse IgAN (n = 62)**	***P* values**
Demographic			
Age at onset (years)	9.9 ± 3.1	11.0 ± 2.9	0.10
Male (%)	15 (57.7%)	30 (48.4%)	0.43
Age at renal biopsy (years)	10.5 ± 3.7	12.1 ± 3.5	0.05
Duration from onset to renal biopsy (month)	10.3 ± 18.7	13.3 ± 17.5	0.08
Detection presentation			
Annual urinary screening program	16 (61.5%)	41 (66.1%)	0.68
Gross hematuria	5 (19.2%)	13 (21.0%)	0.85
Edema	2 (7.7%)	6 (9.7%)	0.77
Incidental	3 (11.5%)	2 (3.2%)	0.12
Clinical findings			
Gross hematuria episode	12 (46.2%)	24 (38.7%)	0.52
Presence of hypertension	2 (7.7%)	10 (16.1%)	0.29
Presence of nephrotic syndrome	7 (26.9%)	8 (12.9%)	0.11
Serum albumin revel (mg/dL)	3.2 ± 0.83	3.5 ± 0.76	0.14
eGFR (mL/min/1.73 m2) at biopsy	110.0 ± 21.4	112.3 ± 27.5	0.79
eGFR < 60 (mL/min/1.73 m2)	2 (7.7%)	3 (4.8%)	0.60
eGFR < 90 (mL/min/1.73 m2)	3 (11.5%)	12 (19.4%)	0.37
Proteinuria at biopsy (g/g)	3.1 ± 2.9	2.5 ± 3.2	0.15
presence of microhematuria	26 (100%)	60 (96.8%)	0.35
Pathological finding (Oxford criteria)			
Mesangial score (M0/M1)	21/5	22/38*1)	<0.01
Endocapillary hypercellularity (E0/E1)	15/11	40/20*1)	0.43
Segmental sclerosis and adhesion (S0/S1)	19/7	31/29*1)	0.06
Tubular atrophy and interstitial fibrosis (T0/T1+T2)	26/0+0	58/1+1*1)	0.35
Crescents (C0/C1+C2)	7/16+3	12/25+23*1)	0.48
Treatment			
Age of start the combination therapy (year)	10.5 ± 4.0	11.8 ± 3.5	0.11
Presence of previous treatment	6 (23.1%)	5 (8.1%)	0.05
Combination therapy			
PSL + MZR + Anticoagulant/Antiplatelet	15 (57.7%)	35 (56.5%)	0.91
PSL + MZR	11 (42.3%)	27 (43.6%)	0.91
ACE-I/ARB	19 (73.1%)	35 (56.5%)	0.14
Supportive therapy (Antihypertensive drug)	0 (0%)	3 (4.8%)	0.25
Severe Adverse effects	1 (3.8%)	6 (9.7%)	0.47

Data are presented as the mean ± standard deviation (SD), and as a number with the percentage in parenthesis.

*1) In diffuse IgAN, the details of pathological findings in 2 cases were no data and we could know only the severity.

PSL: prednisone, MZR: mizoribine, RAS inhibitors: renin-angiotensin system inhibitors

TABLE 2 Time course of Urine findings

The timing after beginning of combination therapy	6 months			12 months			24 months		
	Focal IgAN	Diffuse IgAN	P value	Focal IgAN	Diffuse IgAN	P value	Focal IgAN	Diffuse IgAN	P value
Proteinuria disappearance	24 (92.3%)	40 (64.5%)	<0.01	24 (92.3%)	45 (72.6%)	0.04	22 (84.6%)	42 (67.7%)	0.15
Hematuria disappearance	7 (26.9%)	17 (27.4%)	0.96	16 (61.5%)	35 (56.5%)	0.66	20 (76.9%)	40 (64.5%)	0.25
Remission	6 (23.1%)	12 (19.4%)	0.69	16 (61.5%)	30 (48.4%)	0.26	20 (76.9%)	32 (51.6%)	0.03

TABLE 3: Long-term prognosis of patients followed over 5 years

	Focal IgAN (n = 15)	**Diffuse IgAN (n = 42)**	**P value**
Age at the last observation (year)	20.0 ± 5.3	22.1 ± 5.4	0.20
Observation period (year)	8.6 ± 3.1	10.4 ± 4.0	0.13
The rate of negative proteinuria	13 (86.7%)	24 (57.1%)	0.04
Kidney function (90 ≦ eGFR)	15 (100%)	31 (73.8%)	0.03
(60 ≦ eGFR < 90)	0 (0%)	9 (21.4%)	0.05
(eGFR < 60)	0 (0%)	2 (4.8%)[*1)]	0.39

[*1)] The level of eGFR was 37.3 mL/min/1.73 m^2: 11 years after starting treatment (n = 1)
under peritoneal dialysis: from 5 years after starting treatment (n = 1)

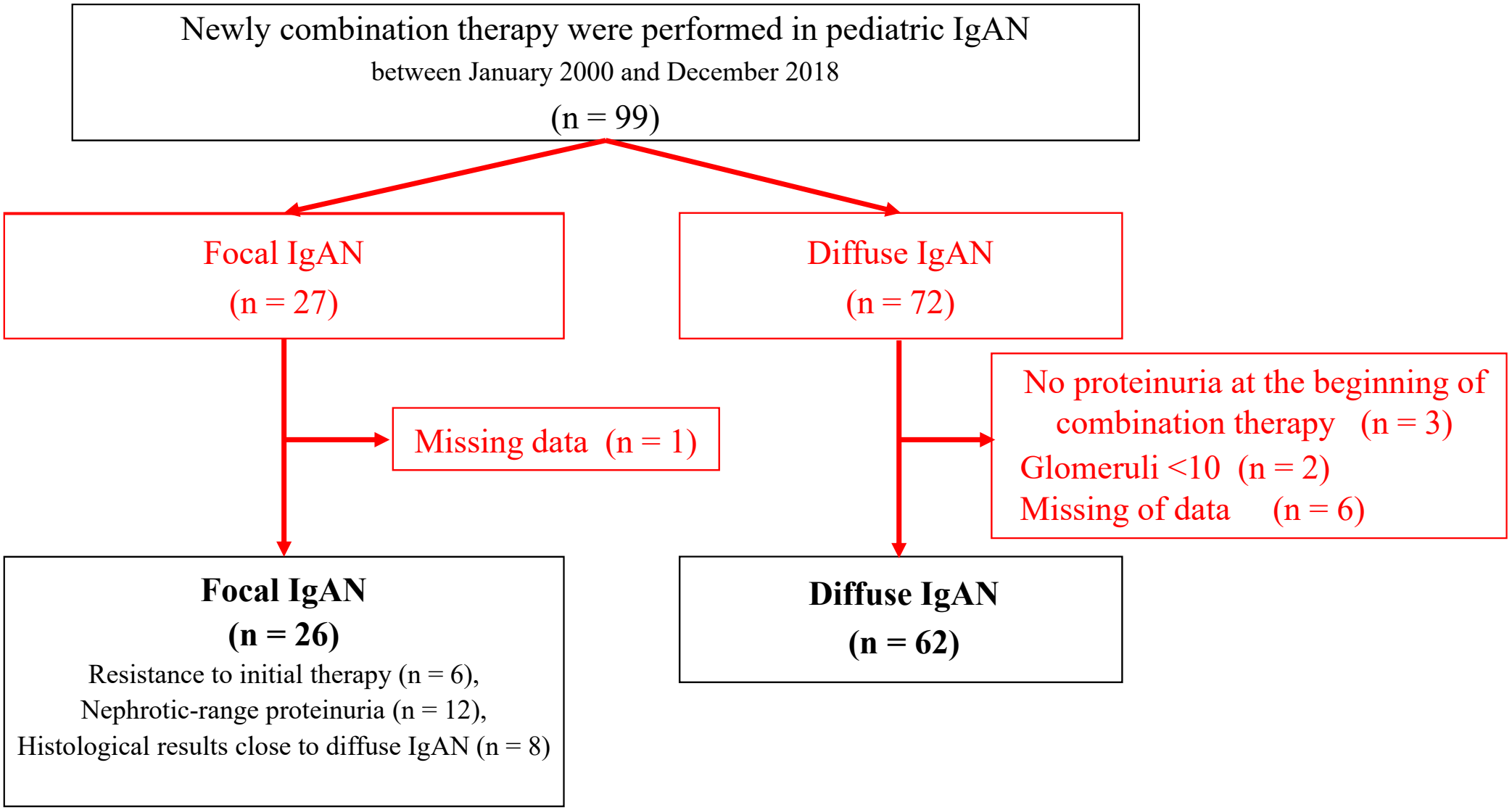
eGFR: estimated glomerular filtration rate.

TABLE 4: Efficacy of the combination therapy in childhood IgAN

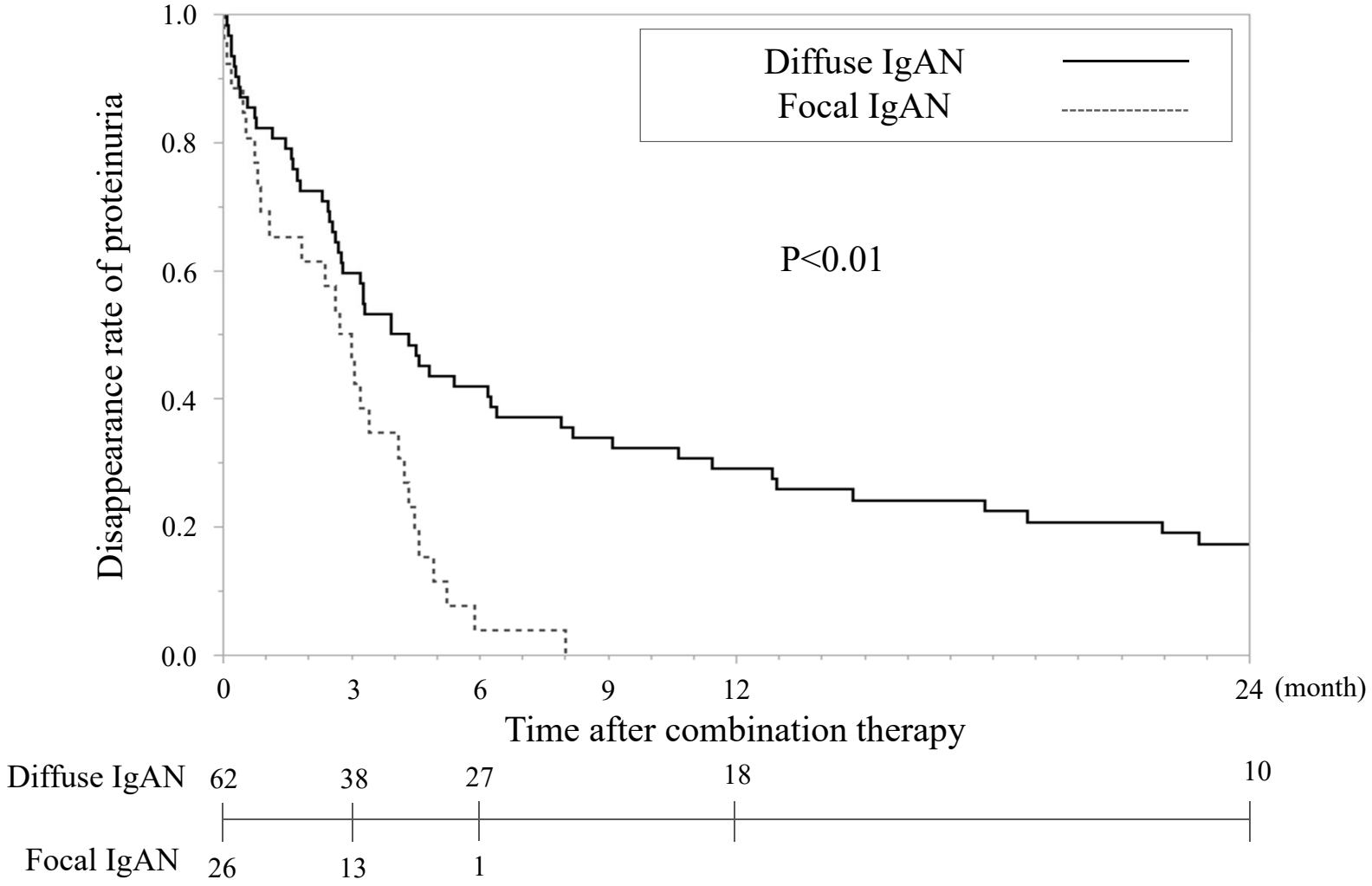
	Kamei Diffuse IgAN	**Matsushita Diffuse IgAN**	**Kawasaki Diffuse IgAN**	**This study Diffuse IgAN**	**This study Focal IgAN**
study period	1990-1998	1994-2007	1997-2009	2000-2018	2000-2018
24-month after starting treatment					
rate of negative proteinuria	50% (20/40)	54.9% (19/32)	ND	67.7% (42/62)	84.6% (22/26)
last observation					
rate of negative proteinuria	60.0% (24/40)	65.6% (21/32)	61% (27/44)	57.1% (24/42)	86.7% (13/15)
eGFR <60	7.5% (3/40)	0% (0/32)	11.5% (5/44)	4.8% (2/42)	0% (0/15)

eGFR: estimated glomerular filtration rate.

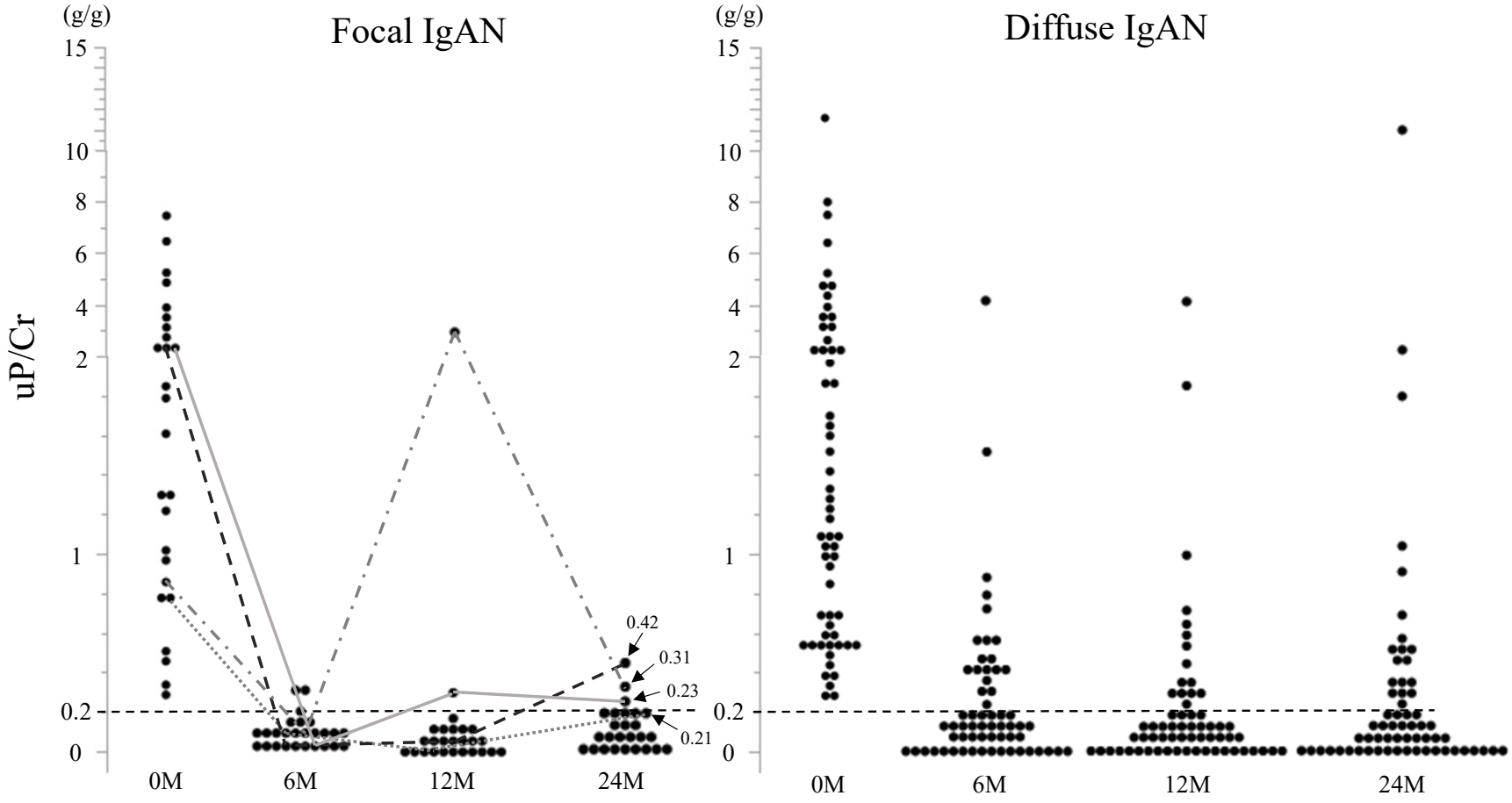
Aoto et al. Figure1



Aoto et al. Figure2



Aoto et al. Figure3



SUPPLEMENTAL TABLE 1: Previous treatment duration and clinical data at initiation of combination therapy in patients received pretreatment

Patient characteristics	**Focal IgAN** **n = 6**	**Diffuse IgAN** **n = 5**	***P* values**
Duration of previous treatment (year)	0.71 (0.3.8－12.8)	3.10 (2.42－3.92)	0.01
At the start of combination therapy			
Serum albumin revel (mg/dL)	3.9 (3.7－4.2)	3.5 (3.0－4.2)	0.12
Proteinuria (g/g)	0.86 (0.49－1.55)	0.66 (0.52－4.2)	0.65

Numerical data are shown as median and interquartile range.

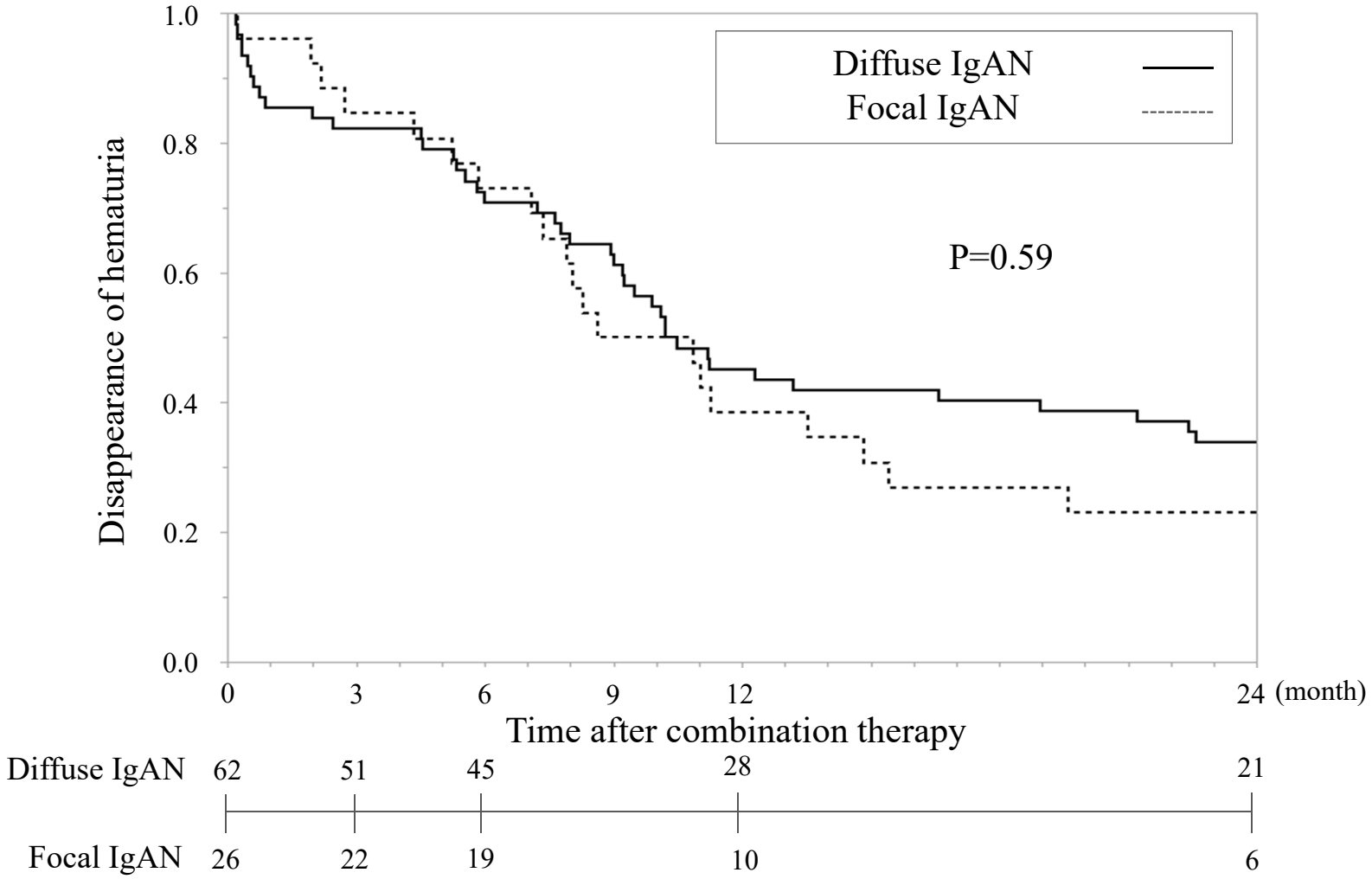
SUPPLEMENTAL TABLE 2: Background of clinical and pathological characteristics of patients followed over 5 years

Patient characteristics	**Focal IgAN n = 15**	**Diffuse IgAN n = 42**	***P* values**
Demographic			
Age at onset (years)	10.0 ± 3.2	10.7 ± 2.9	0.54
Male (%)	8 (53.3%)	19 (45.2%)	0.59
Age at renal biopsy (years)	11.0 ± 4.0	11.7 ± 3.3	0.51
Detection presentation			
Annual urinary screening program	9 (60.0%)	26 (61.9%)	0.90
Gross hematuria	4 (26.7%)	10 (23.8%)	0.83
Edema	1 (6.7%)	6 (14.3%)	0.44
Incidental	1 (6.7%)	0 (0.0%)	0.09
Clinical characteristics at biopsy			
Gross hematuria episode	6 (40.0%)	16 (38.1%)	0.90
Presence of hypertension	0 (0.0%)	9 (21.4%)	0.05
Presence of nephrotic syndrome	4 (26.7%)	7 (16.7%)	0.40
Serum albumin revel (mg/dL)	3.3 ± 0.85	3.5 ± 0.80	0.58
eGFR (mL/min/1.73 m2)	111.2 ± 26.4	116.0 ± 27.5	0.88
eGFR < 60 (mL/min/1.73 m2)	2 (13.3%)	1 (2.4%)	0.10
Proteinuria (g/g)	2.6 ± 2.4	2.8 ± 3.6	0.66
presence of microhematuria	15 (100%)	40 (95.4%)	0.39
Pathological finding (Oxford criteria)			
Mesangial score (M0/M1)	11/4	12/28 *1)	<0.01
Endocapillary hypercellularity (E0/E1)	14/1	26/14 *1)	0.04
Segmental sclerosis and adhesion (S0/S1)	11/4	21/19 *1)	0.16
Tubular atrophy and interstitial fibrosis (T0/T1+T2)	15/0+0	38/1+1 *1)	0.38
Crescents (C0/C1+C2)	6/7+2	9/19+12 *1)	0.30
Treatment			
Age of start the combination therapy (year)	11.0 ± 4.2	11.3 ± 3.3	0.79
Presence of previous treatment	4 (26.7%)	2 (4.8%)	0.02
PSL + MZR + Anticoagulant/Antiplatelet	10 (66.7%)	28 (66.7%)	1.00
PSL + MZR	3 (33.3%)	14 (33.3%)	1.00
ACE-I/ARB	11 (73.3%)	18 (42.9%)	0.04
Supportive therapy (Antihypertensive drug)	0 (0.0%)	2 (4.8%)	0.39
Severe Adverse effects	1 (6.7%)	4 (9.5%)	0.74

[1)] In diffuse IgAN, the details of pathological findings in 2 cases were no data and we could know only the severity. Clinical numerical data are shown as mean ± standard deviation (SD), and as a number with the percentage in parenthesis.

eGFR: estimated glomerular filtration rate, PSL: prednisone, MZR: mizoribine, RAS inhibitors: renin-angiotensin system inhibitors

Aoto et al. Supplemental Figure 1



Aoto et al. Supplemental Figure 2