Pediatric mesangial proliferative glomerulonephritis has increased the platelet thrombus formation potentials under high-shear flow condition

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Short Title: The platelet thrombus formation of mesangial proliferative glomerulonephritis

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Abstract

Introduction: Blood coagulation is associated with glomerulonephritis (GN) pathophysiology. Using whole-blood-based rotational thromboelastometry, we recently reported that the degree of hypercoagulability in pediatric patients with immunoglobulin A nephropathy (IgAN), a GN, might be associated with pathological severity. To further clarify the coagulation status of mesangial proliferative glomerulonephritis (MesPGN), we assessed the platelet thrombus formation (PTF) under high-shear flow using a microchip-based flow chamber system (T-TAS®).

Methods: Thirty-four pediatric patients definitively diagnosed with MesPGN by renal biopsy at Nara Medical University Hospital between 2015 and 2022 were enrolled, and 29 patients (case group; median age, 8.0 years) were assessed. Microchips coated with collagen (PL-chip) were used to assess PTF at high-shear in whole blood. The times to increase by 10 and 30 kPa (T_{10} and T_{30}) from baseline were calculated and compared with those of the pediatric controls. Changes in the parameters during the treatment course and the relationship between pathological severity and the parameters were evaluated.

Results: T_{10} and T_{30} parameters in the PL-chip were significantly shorter and the area under the curves were greater in the case group than those in the control group (both p <0.05). Each parameter was enhanced during the 3-week treatment but improved after the end of treatment. No significant relationship was observed between pathological severity and these parameters. Little PTF difference was observed between IgAN and Henoch-Schönlein purpura nephritis.

Conclusions: Pediatric MesPGN increased the potential for PTF under high-shear flow conditions.

Introduction

Mesangial proliferative glomerulonephritis (MesPGN), represented by immunoglobulin A nephropathy (IgAN), is the most common form of chronic glomerulonephritis (GN). The pathogenesis of chronic GN is complex and involves several pathways [1]. Immune complexes stimulate the mesangial cells to proliferate and secrete various mediators, resulting in glomerular injury [2,3]. Endothelial cell damage activates the blood coagulation pathway, likely resulting in the collapse of glomerular capillaries and damage to and repair of the extracellular matrix, leading to glomerulosclerosis. Many coagulative mediators associated with these mechanisms, such as coagulation factors, platelets, platelet-derived growth factor, platelet-activating factor, fibrinogen, and the von Willebrand factor (VWF), have been evaluated and reported, individually [4-10]. One report indicated that coagulation factors lead to fibrin deposition and crescent formation in the glomeruli [4]. Another report suggested that platelets may be associated with the development of GN through these complex mechanisms [5]. Platelet-secreted products may enhance immune complex localization, stimulate glomerular remodeling after injury, and ultimately distort the normal glomerular architecture and function [5]. Thus, several reports studied the individual factors involved in the progression of GN. However, few studies have evaluated whole blood samples, especially in children, and the precise underlying mechanism(s) remains unclear.

We recently evaluated the coagulation status of pediatric patients with IgAN using whole blood-based rotational thromboelastometry (ROTEM) and reported that the coagulant state of this disease was hypercoagulable, which may be associated with the pathological severity of the disease [11]. Although ROTEM using whole blood samples enables the evaluation of the

interactive mechanisms that govern hemostasis, including platelets, coagulation factors, and other blood cell components, evaluating the coagulation status under high-shear stress conditions is difficult.

The total thrombus formation system (T-TAS®; Fujimori Kogyo; Kanagawa, Japan) is a flow chamber system using a microchip for analyzing platelet thrombus formation (PTF) under variable flow conditions [12]. Two types of microchips were used for this assessment: platelet (PL) (collagen-coated) and atherome (AR) (collagen/thromboplastin-coated). This approach has the advantage that data can be obtained under high-shear flow (1,000-2,000 s^{-1}) reflecting the arterial environment and under low-shear flow (240 s^{-1}) reflecting the venous environment [12]. T-TAS has already been used to evaluate drugs, such as antithrombotic or antiplatelet agents [12-14] and coagulation bleeding disorders, such as von Willebrand disease (VWD) [15,16], hemodilution during cardiopulmonary bypass [17], and acute-phase Kawasaki disease [18]. In the present study, we focused on PTF under high-shear flow conditions and attempted to evaluate the coagulable state using this modern approach in pediatric patients with MesPGN.

Materials and Methods

Patients and controls

Between January 1, 2015, and December 31, 2022, 34 patients with MesPGN aged 15 years or younger, who had been definitively diagnosed by renal biopsy at Nara Medical University Hospital, were enrolled. Sufficient renal tissue was obtained from all patients. Patients who were treated at the time of diagnosis were excluded, and 29 patients were analyzed (case group). Fifteen children who visited our hospital and met the following criteria during the same period

were enrolled as control individuals (control group): (1) aged 15 years or younger, (2) no proteinuria or hematuria, (3) no renal dysfunction, (4) no medication, and (5) 2 pediatric nephrologists and 2 thrombologists determined no association with coagulation pathology. **Figure 1** summarizes the selection of the participants. The case group included 19 patients with IgAN and 10 with Henoch-Schönlein purpura nephritis (HSPN).

Clinical data and pathological findings

Clinical data included age, sex, ABO blood type, systolic and diastolic blood pressure, and laboratory data immediately before the renal biopsy. The laboratory data were as follows: serum creatinine, estimated glomerular filtration ratio (eGFR) assessed by the Schwartz formula [19], serum total protein, serum albumin, serum total cholesterol, hemoglobin level, platelet counts, urinary protein-to-creatinine ratio, prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, fibrinogen, VWF ristocetin cofactor (VWF: RCo) activity, and VWF antigen (VWF: Ag). All kidney tissues were examined by light and immunofluorescence microscopy and reviewed by the same two pediatric nephrologists. MesPGN was diagnosed by the proliferation of mesangial cells with an increase in the mesangial matrix and was based on the World Health Organization pathological classification [20]. We used the proportion of glomeruli with mesangial hypercellularity or cellular/fibrocellular crescents in the biopsy samples as indicators of pathological severity.

Treatments

Depending on the severity, one of the following three treatments was implemented: (**a**) intravenous methylprednisolone pulse (IVMP) and combination therapy (prednisolone and a

combination of mizoribine, dipyridamole, warfarin, and angiotensin-converting enzyme inhibitors), (**b**) combination therapy alone, and (**c**) angiotensin-converting enzyme inhibitors. These treatments were given to the patients, based on the previous reports with some modifications [21-24].

In group (**a**), methylprednisolone was administered intravenously at a dose of 30 mg/kg body weight (maximum, 1 g) for three consecutive days weekly for two weeks. Prednisolone was administered orally at a dose of 1 mg/kg body weight per day (maximum, 60 mg) in three divided doses for 2 weeks after IVMP therapy. Thereafter, prednisolone was tapered and discontinued while monitoring urinary protein levels. Mizoribine was administered at a dose of 4 mg/kg body weight per day (maximum 150 mg) for approximately 2 years. Dipyridamole was administered at a dose of 6 mg/kg body weight/day (maximum 300 mg) for approximately 2 years. Warfarin was administered in a single morning dose to maintain the prothrombin time-international normalized ratio (PT-INR) at approximately 1.5 for 3 months. Dipyridamole and warfarin doses were reduced or discontinued in case of adverse events. Angiotensin-converting enzyme inhibitors were administered for approximately two years. In group (**b**), prednisolone was administered orally at a dose of 2 mg/kg body weight per day (maximum 60 mg) in three divided doses for 4 weeks. Thereafter, the prednisolone dose was tapered and discontinued while monitoring the urinary protein levels. Mizoribine, dipyridamole, warfarin, and angiotensin-converting enzyme inhibitors were administered as described in group (a). In group (**c**), an angiotensin-converting enzyme inhibitor was administered for approximately 2 years.

Blood samples

Whole blood samples were collected from 29 patients with MesPGN and 15 pediatric controls. In all cases, the samples were obtained under good general conditions. Whole blood samples were collected in commercially available tubes (Roche Diagnostics, Rotkreuz, Switzerland) containing hirudin (final concentration 25 g/mL). Samples from the case group were collected before treatment and at 1, 2, and 3 weeks after the start of treatment. In addition, for post-treatment analysis, samples were collected approximately 2 years after the initiation of treatment, when the cases had no proteinuria and were off all medications.

Microchip-based flow chamber system (T-TAS® assay)

The T-TAS assays (Fujimori Kogyo) were performed with minor protocol modifications [13,15,16]. We focused on PTF in the glomerular vessels related to glomerular damage, and evaluated samples mainly using PL-chip. **Figure 2** shows the flow pressure waveform and video microscopy images in a representative pattern. **Supplementary Material 1** shows video movies using the PL-chip. Briefly, a PL microchip coated with type I collagen was used to evaluate the PTF, which reflects the arterial environment. Hirudin-anticoagulated whole blood (350 μL) was perfused through a collagen-coated capillary at 24 μL/min, corresponding to initial wall shear rates of 2,000 s^{-1}. Thrombus formation led to gradual occlusion within the tracts, which was assessed by monitoring changes in flow pressure, and the process of thrombus formation was recorded using a video microscope. The pressure was recorded for 10 min or until occlusion (60 kPa increase in flow pressure from the baseline). The following parameters were calculated to analyze the PTF inside the PL-chip: T_{10} or T_{30} (time to 10 or 30 kPa), the time required for the flow pressure to increase 10 or 30 kPa from baseline due to partial occlusion of the capillary, which indicated the start of thrombus formation or growth of thrombi, respectively; and area

under the curve (AUC), the area under the flow pressure curve (below 60 kPa) for 5 min after the start of perfusion, which quantified thrombus stability.

In addition, an AR microchip coated with collagen and thromboplastin was used to evaluate the fibrin-rich thrombus formation, which reflects the venous environment. Citrated whole blood (416 μL) with corn trypsin inhibitor (14 μL; final concentration of 30 μg/mL) was mixed with 0.2 M $CaCl_2$ (30 μL), and perfused through a collagen and thromboplastin-coated capillary at 8 μL/min, corresponding to initial wall shear rates of 240 s^{-1}. T_{10}, T_{30}, and AUC, the area under the flow pressure curve (below 80 kPa) for 30 min after the start of perfusion, were calculated.

Statistical analysis

We analyzed the data using JMP®10 (SAS Institute Inc., Cary, NC, USA). Missing data were excluded from the analysis. The Wilcoxon rank sum test was used for analyzing the continuous variables, and the χ-square test was used for analyzing the binary variables. A Steel test was used to analyze the changes in the parameters. Statistical significance was set at a *p*-value of <0.05.

Results

Clinical characteristics of pediatric MesPGN patients

Table 1 summarizes the clinical data. The MesPGN group (n=29) showed lower serum total protein and albumin levels ($p = 0.005$ and <0.001, respectively) and higher serum total cholesterol, urinary protein-to-creatinine ratio, and D-dimer and fibrinogen levels ($p = 0.03$, 0.02, 0.007, and <0.001, respectively) than those of the control group. Other clinical characteristics were comparable between the two groups. Pathological findings revealed mesangial

hypercellularity in greater than 50% of the glomeruli in 20 patients with MesPGN (69 %), and cellular/fibrocellular crescents in 19 patients (66 %). All patients with MesPGN received this treatment regimen. Four patients (14%) were treated with IVMP and combination therapy, 20 (69%) were treated with combination therapy, and 5 (17%) were treated with angiotensin-converting enzyme inhibitor monotherapy.

Evaluation of T-TAS parameters in the pediatric MesPGN patients

First, we measured the PL-chip parameters to assess PTF in pediatric patients with MesPGN before treatment. Both the T_{10} and T_{30} values were significantly shorter in the case group than in the control group (both p <0.05), and the AUC was also significantly greater (p <0.05) (shown in **Fig. 3**). These findings suggest that pediatric patients with MesPGN exhibit increased functional potential of the PTF, particularly early initiation of thrombus formation, the rapid growth of thrombi, and strong thrombus stability. In addition, we measured the AR-chip parameters to assess the fibrin-rich thrombus formation. All parameters were not significantly different (**Supplementary Material 2.a**).

Next, we assessed the changes in the PL-chip parameters in the case group during treatment (shown in **Fig. 4**). All patients evaluated until post-treatment were in remission. The T_{10} and T_{30} values at 1 and 2 weeks after the start of treatment were significantly lower than those at pretreatment, and the AUC was also significantly greater (shown in **Fig. 4.a**). T_{10} and T_{30} at post-treatment tended to be prolonged compared with pre-treatment, but not significantly. The AUC at post-treatment also tended to be lower than that at pre-treatment; however, the difference was not significant. All parameters at 1, 2, and 3 weeks after the start of treatment were significantly

different from those in the control group, whereas those after treatment were not significantly different.

To clarify the effect of antiplatelet agent therapy, we evaluated the changes in parameters during treatment in patients receiving combination therapy with or without antiplatelet agents. All the parameters showed similar patterns of change between the two groups, as shown in **Figure 4.b** and **4.c**. These findings suggest that the addition of antiplatelet agents did not affect PTF under high-shear flow in the present study.

We further measured the changes in the AR-chip parameters in the case group during treatment (shown in **Supplementary Material 2.b**). The T_{10} and T_{30} values at 1 and 2 weeks after the start of treatment were significantly lower than those at pretreatment, and the AUC was also significantly greater, respectively. The T_{10} and AUC at 1, 2, and 3 weeks after the start of treatment, and the T_{30} values at 1 and 2 weeks after the start of treatment were significantly different from those in the control group, respectively. These changes in the case group were similar to those of the PL-chip parameters.

Changes in other parameters (platelet count, fibrinogen level, and VWF: RCo) in the case group during treatment are shown in **Supplementary Material 3**. Platelet counts at 1 and 2 weeks after treatment significantly increased relative to pre-treatment levels. Fibrinogen levels at 1, 2, and 3 weeks after treatment and post-treatment were significantly lower than those before treatment. VWF: RCo levels at 2 and 3 weeks after treatment were significantly higher than those in the controls, but at any phase, they were not significantly different from the pre-treatment levels.

Relationship between the pathological findings and T-TAS parameters in pediatric MesPGN patients

We recently demonstrated that the degree of hypercoagulability in pediatric patients with IgAN might be associated with pathological severity using whole-blood-based rotational thromboelastometry [11]. Therefore, we focused on the relationship between pathological severity and PL-chip parameters. As a previous report [25] suggested that the severity of mesangial hypercellularity and crescents were among the predictors of renal outcomes in pediatric patients with IgAN, these pathological parameters were used in this analysis (**Table 2**). The presence of mesangial hypercellularity ≥50% and ≥80% in the observed glomeruli was not significantly associated with the shortened T_{10} and T_{30}, or the increased AUC. Similarly, the presence of cellular/fibrocellular crescents was not significantly associated with any of the parameters. These results suggest that the increased functional potential of the PTF in pediatric patients with MesPGN is unlikely to be associated with pathological severity.

Comparison of PL-chip parameters between the pediatric IgAN and HSPN patients

Morphological and immunohistochemical manifestations of IgAN and HSPN are similar. Further, whether these two diseases are identical is debatable for a long time [26,27]. Under high-shear conditions, T_{10}, T_{30}, and AUC were not significantly different between pediatric IgAN and HSPN (**Table 3**). These findings demonstrate that pediatric patients with IgAN and HSPN show similar PTF potentials under these conditions.

Discussion

Here, we demonstrated that pediatric patients with MesPGN exhibit increased PTF potential under high-shear flow conditions. Previous studies have suggested a relationship between platelets and GN development [5,28], which is consistent with our results. One study has suggested that platelets are associated with GN development via complex mechanisms [5]. Platelet-derived products may enhance immune complex localization, stimulate glomerular remodeling after injury, and ultimately distort the normal glomerular architecture and function [5]. Another report has described platelets as relevant mediators of renal injury caused by primary endothelial lesions [28]. Our findings also supported that patients with MesPGN had no significant increase in platelet counts, and platelet activation or platelet-derived products might be associated with glomerular injury.

Fibrinogen and VWF levels are associated with mural thrombus growth under high-shear flow, and these parameters may be associated with those obtained using a PL-chip [29]. Previous reports have suggested that fibrinogen is linked to podocyte injury and renal tubular atrophy in glomerular diseases [9,30]. Our report also supported that fibrinogen levels in pediatric patients with MesPGN were significantly higher than those in controls and might be associated with increased PTF potential. VWF levels are also associated with renal diseases such as primary GN, IgAN, lupus nephritis, and focal segmental glomerulosclerosis, indicating the importance of endothelial damage [10,31,32]. In the present study, the MesPGN group tended to have higher VWF: RCo levels than that of the control group; however, this difference was not statistically significant. Both fibrinogen and VWF levels, especially fibrinogen levels, may be associated with an increased potential for PTF in MesPGN. It is important to confirm the increased potential of PTF in pediatric MesPGN using whole blood samples under high-shear flow.

T-TAS parameters in pediatric MesPGN patients receiving treatment indicated an increased potential of PTF, especially 1-2 weeks after the start of treatment compared to pre-treatment, and improved post-treatment (shown in **Fig. 4**). This tendency was similar in the cases of combination therapy, irrespective of the presence of antiplatelet agents. The reason(s) for these results may be raised as the influence of steroid therapy. Steroid treatment induces hypercoagulability through various mechanisms, such as an increase in factors VII, VIII, and XI and platelet counts [33,34]. Other report indicated that steroid enhanced VWF immunoreactivity in vascular endothelial cells [35]. Our data demonstrated that platelet counts increased after 1-2 weeks of treatment, which may be associated with the effects of steroid therapy (shown in **Supplementary Material 3**). This increase in platelets may be responsible for the increased potential of PTF 1-2 weeks after the start of treatment.

Furthermore, the antiplatelet agent used in this study, dipyridamole, has the effect of increasing cGMP by inhibiting PDE-5, and the effect of antiplatelet is weaker than other antiplatelet agents [36]. This weak effect may be one of the reason(s) why no clear effect could be detected in this ex-vivo study. And the reason for the improvement 3 weeks after the start of treatment, especially in patients receiving combination therapy without dipyridamole, may be the effect of treatment and the weakness of the effect of dipyridamole. However, it is difficult to determine the reason from this study alone, and we need to evaluate the larger number of patients. In some groups, antiplatelet agents are used to treat GN, particularly IgAN. Antiplatelet agents had been reported to result in reduced proteinuria and protect renal function [37, 38]. In contrast, the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines do not suggest the use of

antiplatelet agents to treat IgAN [39]. The changes in T-TAS parameters in patients receiving combination therapy without antiplatelet agents suggested that the effect of antiplatelet agents was not sufficient to affect the parameters and appeared unlikely to support the need for agents, similar to the KDIGO guidelines.

The analysis of T-TAS parameters in the pathological severity of the diseases (**Table 2)** suggested that the increased functional potential of PTF in pediatric patients with MesPGN was not associated with their pathological severity. We previously reported the hypercoagulable state of pediatric patients with IgAN using whole-blood-based ROTEM, which may be associated with the pathological severity of the disease [11], which is unlikely to support the present results. One reason for this discrepancy is that mesangial cell proliferation or crescent formation is the result of GN [40], and PTF, the first stage of coagulation, may not have a significant association with disease severity. Another reason is that the present study was performed under high-shear flow, which is different from the low-shear flow condition (~50 s^{-1}) of ROTEM, and additionally included diseases other than IgAN. Regarding pediatric MesPGN, we may not need to consider the use of antiplatelet agents even in the presence of severe pathological findings, supporting the KDIGO guidelines [39].

Finally, we evaluated pediatric patients with IgAN and HSPN. Both have similar characteristics, including pathogenesis, and the pathological findings are identical and cannot be differentiated from each other [41]. Therefore, the identity of these diseases has been debated for a long time. Sugiyama *et al.* [26] reported that HSPN had considerable glomerular capillaries with subendothelial IgA deposition and factors associated with galactose-deficient IgA1, an important

factor in the development of nephritis, were inconsistent between the two diseases. Kamei *et al.* [27] presented examples of patients who were first diagnosed with IgAN and later developed purpura; IgAN and HSPN were different manifestations of a single origin. The T-TAS parameters did not differ significantly between pediatric patients with IgAN and HSPN, and no significant difference was observed in terms of PTF. Few reports have described the indications of the differences between the two diseases from the viewpoint of platelet-associated thrombus formation, making this study highly valuable.

The present study had several limitations. First, because of the small number of enrolled patients and their various clinical conditions and treatments, a multivariate analysis could not be performed. Second, healthy children could not be evaluated as controls, and the number of enrolled cases as a control was small. Third, laboratory markers associated with platelet activation, such as platelet-derived growth factor, platelet factor 4, and soluble P-selectin were not evaluated. Fourth, we could not assess T-TAS parameters under hypoalbuminemic conditions. Hypoalbuminemia may affect the PTF. Finally, changes in T-TAS parameters were not compared with those of patients with other diseases receiving steroid therapy. Our group has been evaluating pediatric cases of other disease using steroids, and will publish the data. Nevertheless, we conclude that pediatric patients with MesPGN could exhibit the increased functional potential of PTF and platelet activation and fibrinogen levels may be associated with glomerular injury.

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Statement of Ethics

This study was approved by the Medical Research Ethics Committee of Nara Medical University (approval no. 2365). Blood samples from MesPGN and control patients were collected after obtaining written informed consent according to the university ethical guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Takashi Omae, **Tomoaki Ishikawa**, **Kenichi Ogiwara**, and **Keiji Nogami** contributed to the study's conception and design. Material preparation and data collection were mainly performed using **Takashi Omae** and **Tomoaki Ishikawa**. Data analysis and interpretation were mainly performed using **Takashi Omae**, **Kenichi Ogiwara**, and **Keiji Nogami**. **Takashi Omae** and **Keiji Nogami** wrote the first draft of the manuscript, and **Tomoaki Ishikawa** and **Kenichi Ogiwara** commented on the manuscript. **Takashi Omae**, **Tomoaki Ishikawa**, **Kenichi Ogiwara**, and **Keiji Nogami** have read and approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Figure Legends

Fig. 1. Flow chart of study participants' selection

HSPN, Henoch-Schönlein purpura nephritis; IgAN, immunoglobulin A nephropathy; MesPGN, mesangial proliferative glomerulonephritis.

Fig. 2. Flow pressure waveform and video microscopy images by T-TAS, and representative pattern

Panel (**a**) shows a typical flow pressure waveform by T-TAS. *Panels* (**b**) show the video images by T-TAS for the PL-chip. PL, platelet; T-TAS, Total Thrombus-formation Analysis System.

Fig. 3. Comparison of the PL-chip parameters by T-TAS between pediatric MesPGN patients in pre-treatment and controls

Each box plot indicates median and interquartile ranges. T_{10} or T_{30} is the time required for the flow pressure to increase by 10 or 30 kPa from the baseline owing to partial occlusion of the capillary. The AUC is the area under the flow pressure curve (below 60 kPa) for 5 min after the start of perfusion. Wilcoxon rank-sum test was used for comparisons. Statistical significance was set at $p < 0.05$. $*p < 0.05$ compared with controls. AUC, area under the curve; MesPGN, mesangial proliferative glomerulonephritis; PL, platelet; T-TAS, total thrombus formation system.

Fig. 4. Changes in PL-chip parameters by T-TAS in pediatric MesPGN patients

Panel (**a**) shows the PL-chip parameters for all patients. *Panel* (**b**) shows the PL-chip parameters

of patients receiving combination therapy alone, including dipyridamole. *Panel* (**c**) shows the PL-chip parameters of patients receiving combination therapy without dipyridamole. The *gray* zone shows the interquartile range of the PL-chip parameters in the controls. Steel tests were used for comparison with the pre-treatment results. Wilcoxon rank-sum tests were used for comparison with controls. Statistical significance was set at $p < 0.05$. $*p < 0.05$, $**p < 0.01$ compared with pre-treatment. $\dagger p < 0.05$, $\dagger\dagger p < 0.01$ compared with controls. AUC, area under the curve; MesPGN, mesangial proliferative glomerulonephritis; PL, platelet; T-TAS, total thrombus formation system; w, weeks.

Table 1. Clinical characteristics profiles of pediatric MesPGN patients

	Normal range	Case (n = 29)	Control (n = 15)	*p*-Value
Age (years)	-	8.0 [7.0-12.0]	6.0 [3.0-11.0]	0.06
Sex (M/F)	-	12 / 17	6 / 9	0.93
ABO blood type (A/B/AB/O/Unknown)	-	13/5/3/8/0	4/2/1/1/7	0.92
Systolic blood pressure (mmHg)	-	107 [100-112]	104 [98.0-111]	0.70
Diastolic blood pressure (mmHg)	-	60 [58-67]	57 [52-64]	0.15
Serum creatinine (mg/dL)	0.16-0.95 (M) [b] 0.16-0.75 (F) [b]	0.40 [0.36-0.52]	0.37 [0.25-0.50]	0.18
eGFR (mL/min/1.73 m^2)	≥60	128 [118-143]	126 [119-165]	0.64
Serum total protein (g/dL)	6.3-7.8	6.4 [5.8-6.7]	6.7 [6.6-7.1]	**0.005**
Serum albumin (g/dL)	3.7-4.9	3.8 [3.6-4.0]	4.5 [4.4-4.6]	**< 0.001**
Serum total cholesterol (mg/dL)	130-220	200 [172-233]	178 [163-184]	**0.03**
Hemoglobin (g/dL)	13.5-17.6 (M) 11.3-15.2 (F)	13.0 [12.0-13.6]	12.7 [12.0-13.5]	0.4
Platelet ($\times 10^3/\mu L$)	150-350	284 [264-332]	288 [263-344]	0.79
Urinary protein-to-creatinine ratio (g/g · cre)	<0.15	1.1 [0.67-2.7]	0.02 [0.02-0.02]	**0.02**
PT (s)	11-13	12.3 [11.4-13.0]	12.1 [11.4-12.8]	0.87
APTT (s)	25-40	28.7 [27.5-31.2]	28.4 [26.2-31.3]	0.94
D-dimer (μg/mL)	<1.0	0.7 [0.6-1.1]	0.6 [0.5-0.7]	**0.007**
Fibrinogen (mg/dL)	200-400	334 [275-423]	247 [229-270]	**< 0.001**
VWF: RCo (%)	65-135	113 [90.2-140]	101 [72.5-111]	0.06
VWF:Ag (%)	65-135	121 [99.5-156]	110 [89.3-134]	0.28
Pathological findings				
Proportion of observed glomeruli with mesangial hypercellularity (< 50% / ≥ 50%)	-	9 / 20	-	-
Cellular/fibrocellular crescent (Absent / Present)	-	10 / 19	-	-
Medications				
IVMP and combination therapy [a]	-	4	-	-
Combination therapy [a]	-	20	-	-
Angiotensin-converting enzyme inhibitors	-	5	-	-
No medication	-	0	-	-

Abbreviations: Ag, antigen; APTT, activated partial thromboplastin time; eGFR, estimated glomerular filtration rate; F, female; IVMP, intravenous methylprednisolone pulse; M, male; MesPGN, mesangial proliferative glomerulonephritis; PT, prothrombin time; RCo, ristocetin cofactor activity; VWF, von Willebrand factor.

Note: Data are presented as medians, with interquartile ranges enclosed in brackets. Sex, ABO blood type, pathological findings and medications are presented as the number of cases. Wilcoxon rank sum tests were used for continuous variables and χ-square tests for binary variables. All significant *p*-values ($p < 0.05$) of comparison between control group were highlighted bold.

[a] Combination therapy: prednisolone, mizoribine, and some combination of dipyridamole, warfarin, and angiotensin-converting enzyme inhibitors.

[b] Values vary by age.

Table 2. Relationship between the pathological findings and PL-chip parameters in pediatric MesPGN patients by T-TAS

	T_{10} (min)	T_{30} (min)	AUC
Mesangial hypercellularity (n)			
< 50% (9)	2.2 [1.8-2.2]	3.0 [2.8-3.4]	136 [119-150]
≥ 50% (20)	2.3 [1.6-2.8]	3.4 [2.4-3.9]	135 [101-164]
***p*-Value**	0.65	0.51	0.45
< 80% (22)	2.1 [1.6-2.7]	3.0 [2.3-3.6]	141 [105-165]
≥ 80% (7)	2.6 [1.9-2.9]	3.9 [3.0-4.5]	123 [87.8-153]
***p*-Value**	0.33	0.31	0.36
Cellular/fibrocellular crescents (n)			
Absent (10)	1.9 [1.5-2.6]	2.9 [2.2-4.0]	148 [107-179]
Present (19)	2.2 [1.8-2.7]	3.0 [2.8–3.9]	129 [102-155]
***p*-Value**	0.43	0.66	0.46

Abbreviations: AUC, area under the curve; MesPGN, mesangial proliferative glomerulonephritis; PL, platelet; T-TAS, total thrombus formation system.

Note: Data are presented as medians, with interquartile ranges enclosed in brackets. T_{10} or T_{30} is the time required for the flow pressure to increase 10 or 30 kPa from baseline due to partial occlusion of the capillary. AUC is area under the flow pressure curve (below 60 kPa) for 5min after the start of perfusion. Wilcoxon rank sum tests were used for comparison. Statistical significance is set at $p < 0.05$.

Table 3. PL-chip parameters of pediatric IgAN and HSPN patients by T-TAS

	T_{10} (min)	T_{30} (min)	AUC
IgAN (n = 19)	2.4 [1.7–2.7]	3.4 [2.6–4.0]	128 [102-159]
HSPN (n = 10)	2.0 [1.6-2.2]	2.9 [2.1–3.7]	148 [126–169]
***p*-Value**	0.60	0.51	0.45

Abbreviations: AUC, area under the curve; HSPN, Henoch-Schönlein purpura nephritis; IgAN, immunoglobulin A nephropathy; PL, platelet; T-TAS, total thrombus formation system.
Note: Data are presented as medians, with interquartile ranges enclosed in brackets. T_{10} or T_{30} is the time required for the flow pressure to increase 10 or 30 kPa from baseline due to partial occlusion of the capillary. AUC is area under the flow pressure curve (below 60 kPa) for 5 min after the start of perfusion. Wilcoxon rank sum tests were used for comparison with controls. Statistical significance is set at $p < 0.05$.

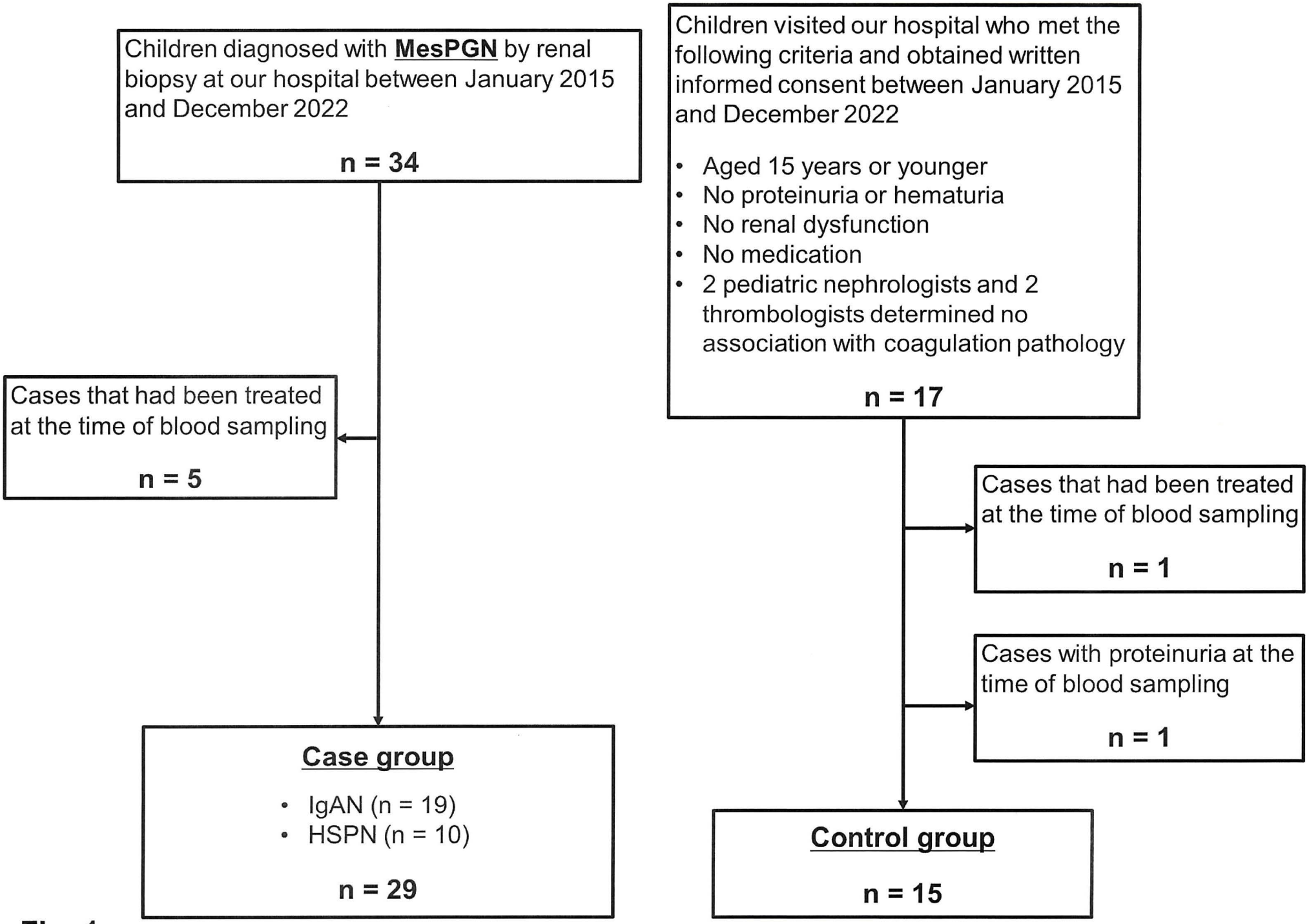
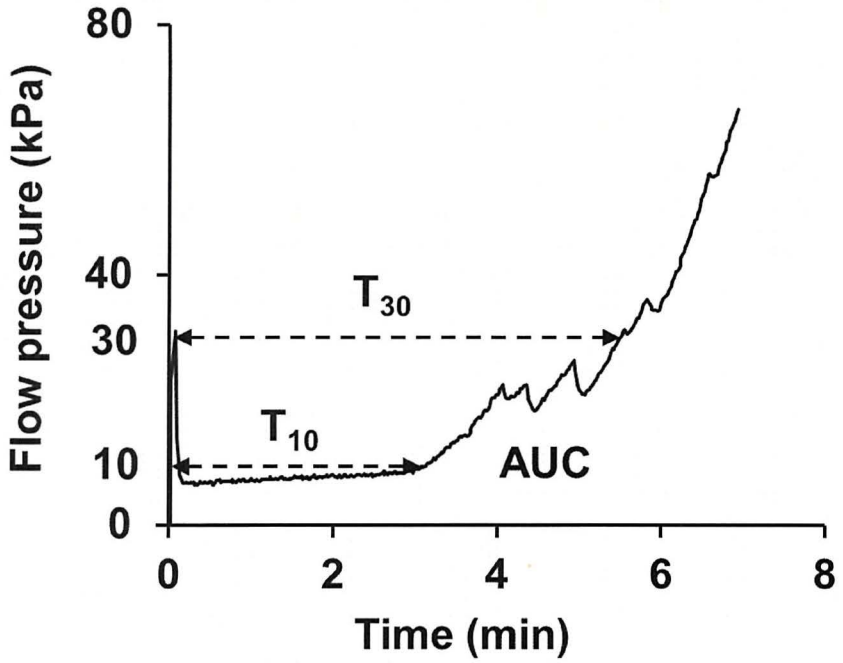

Children diagnosed with MesPGN by renal biopsy at our hospital between January 2015 and December 2022
n = 34
Cases that had been treated at the time of blood sampling
n = 5
Case group
IgAN (n = 19)
HSPN (n = 10)
n = 29
Children visited our hospital who met the following criteria and obtained written informed consent between January 2015 and December 2022
Aged 15 years or younger
No proteinuria or hematuria
No renal dysfunction
No medication
2 pediatric nephrologists and 2 thrombologists determined no association with coagulation pathology
n = 17
Cases that had been treated at the time of blood sampling
n = 1
Cases with proteinuria at the time of blood sampling
n = 1
Control group
n = 15

Fig. 1

(a)


Flow pressure (kPa)
80
40
30
10
0
T_{30}
T_{10}
AUC
0
2
4
6
8
Time (min)

(b)

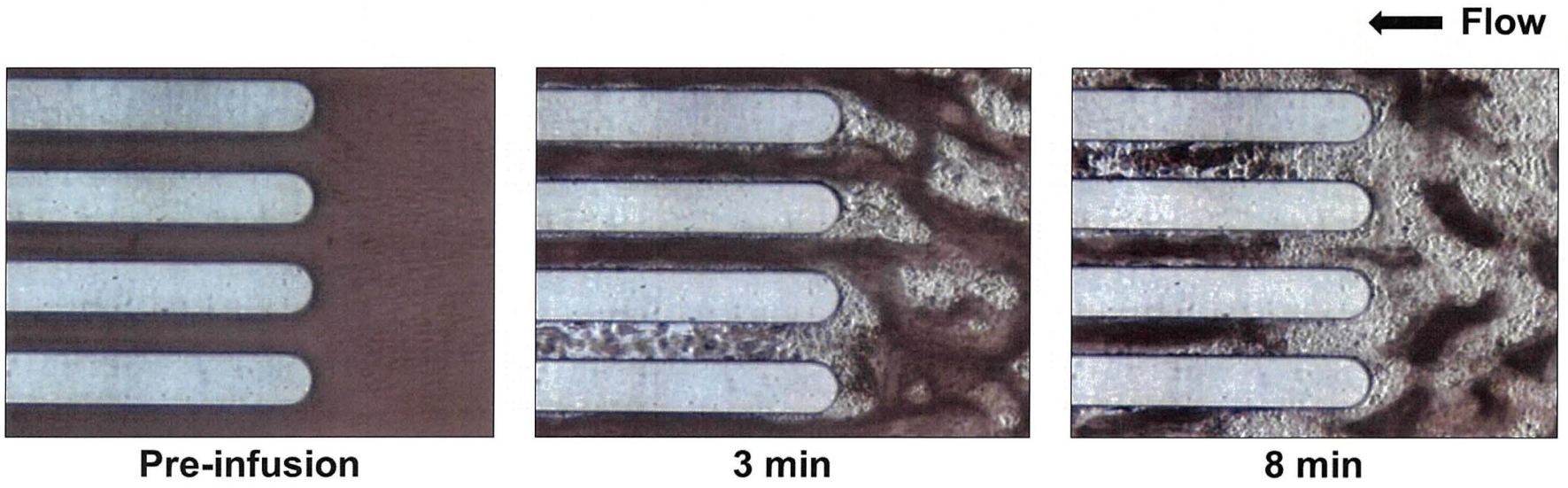

Flow
Pre-infusion
3 min
8 min

Fig. 2

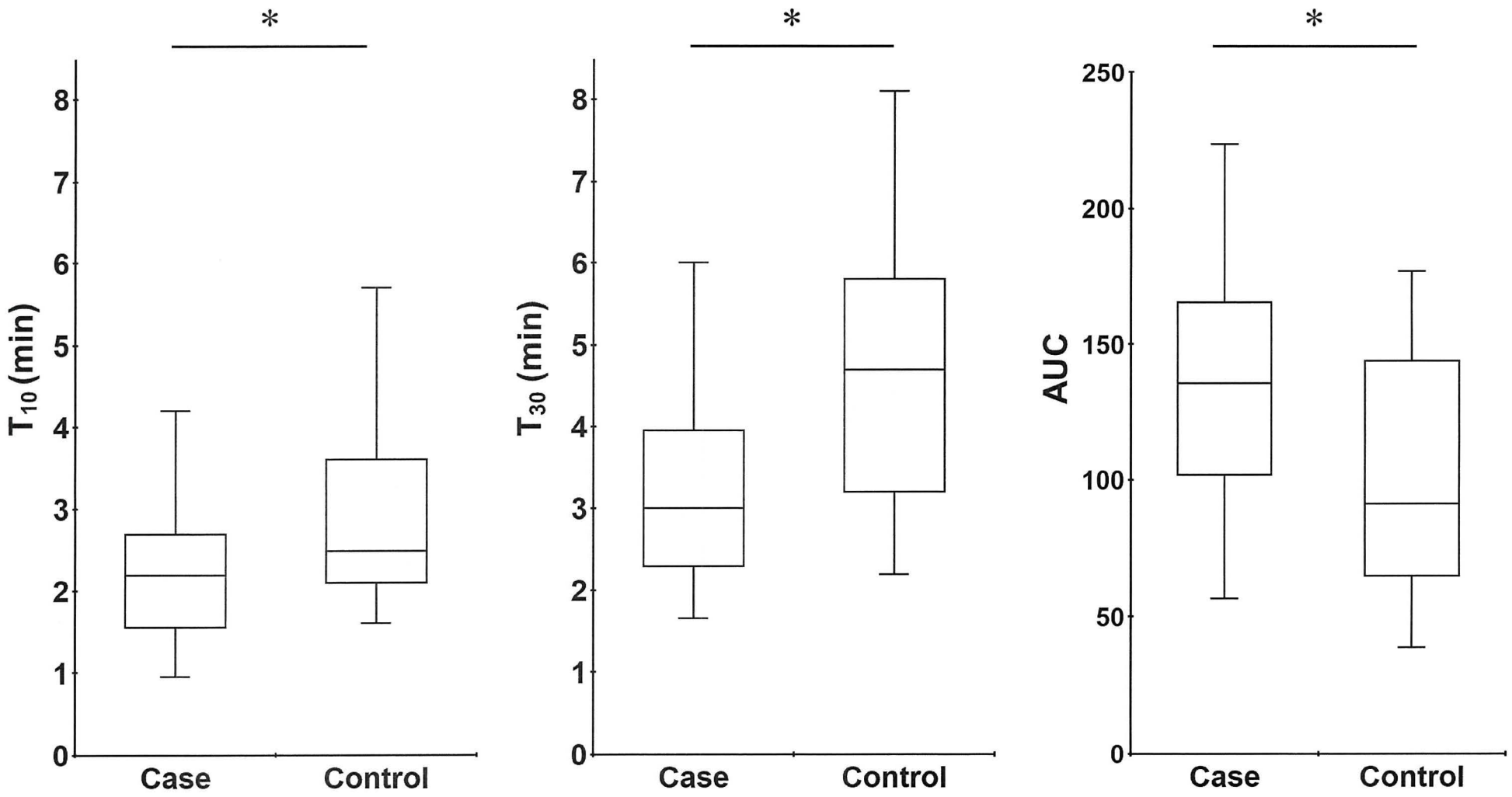

*
*
*
T_{10} (min)
T_{30} (min)
AUC
0
1
2
3
4
5
6
7
8
50
100
150
200
250
Case
Control
Case
Control
Case
Control

Fig. 3

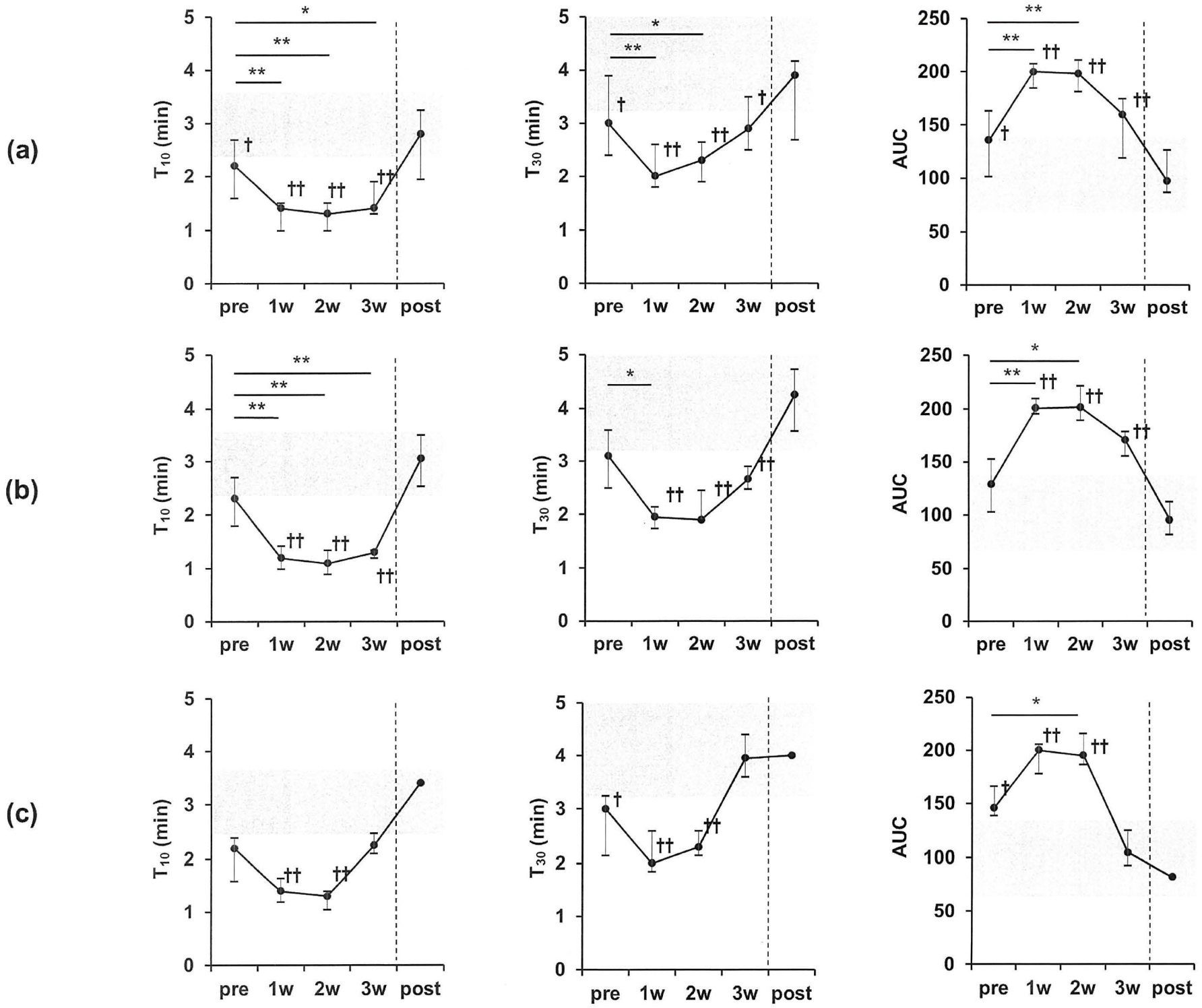
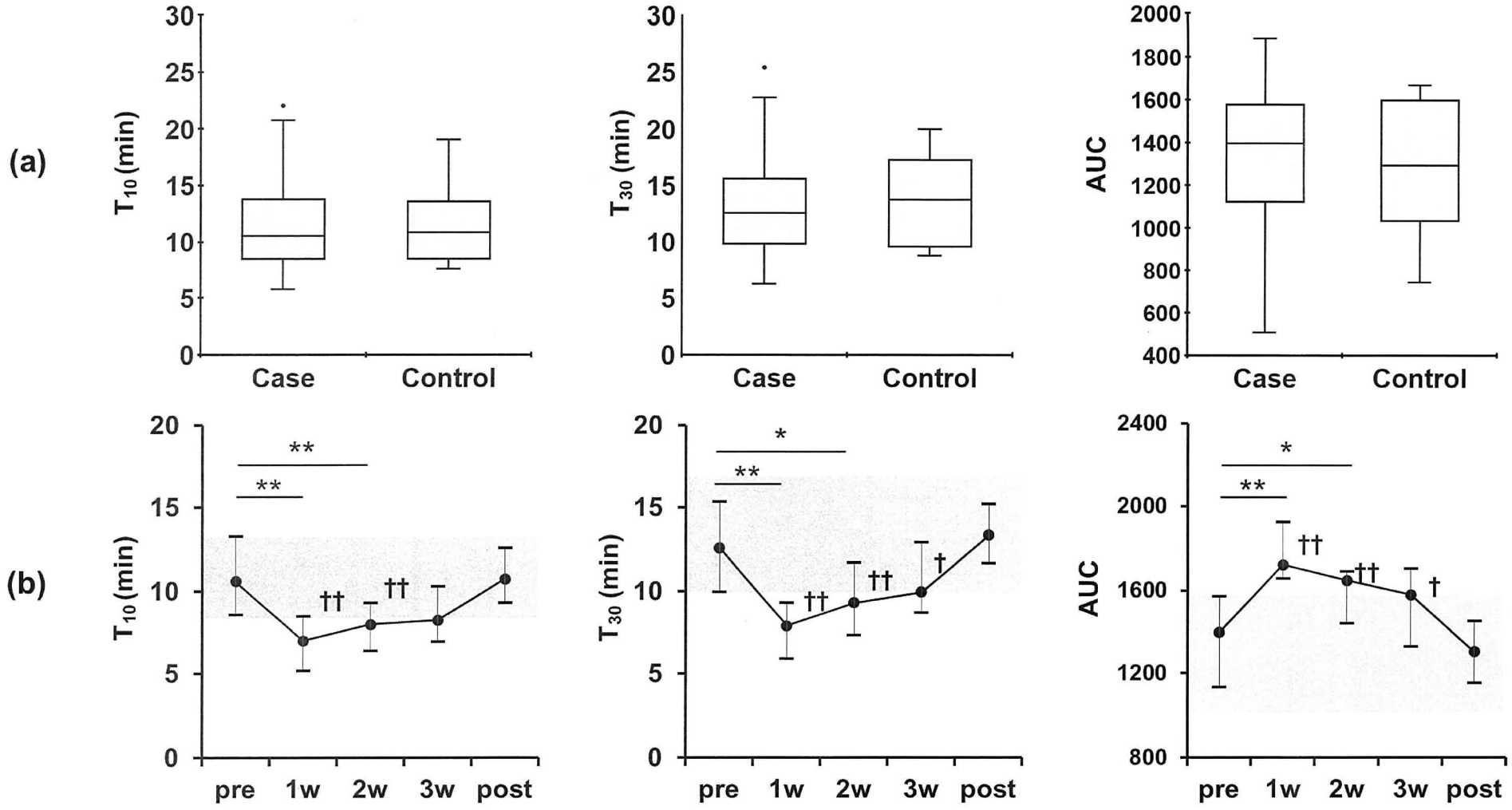
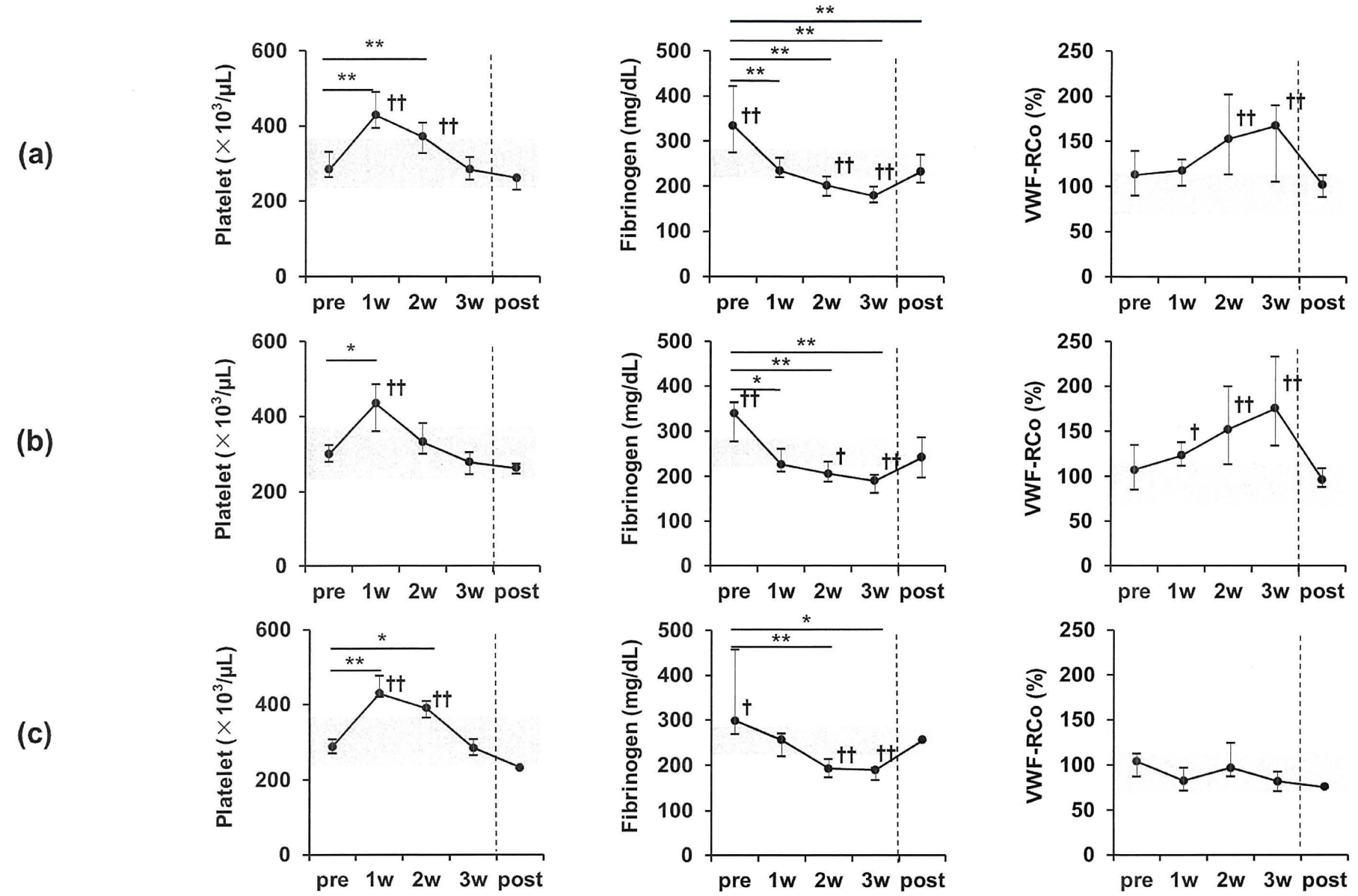


Fig. 4



Supplementary Material 2 Comparison of the AR-chip parameters by T-TAS between pediatric MesPGN patients in pre-treatment and controls, and changes in AR-chip parameters in pediatric MesPGN patients

Panel (a) shows the comparison of the AR-chip parameters between pediatric MesPGN patients in pre-treatment and controls. Each box plot indicates median and interquartile ranges. *Panel* (b) shows the changes in AR-chip parameters in MesPGN patients. The *gray* zone shows the interquartile range of the AR-chip parameters in the controls. AR-chip parameters T_{10} or T_{30} is the time required for the flow pressure to increase by 10 or 30 kPa from the baseline owing to partial occlusion of the capillary. The AUC is the area under the flow pressure curve (below 60 kPa) for 30 min after the start of perfusion. Wilcoxon rank-sum test was used for comparisons. Steel tests were used for comparison with the pre-treatment results. Statistical significance was set at $p < 0.05$. *$p < 0.05$, **$p < 0.01$ compared with pre-treatment. †$p < 0.05$, ††$p < 0.01$ compared with controls. AR, atherome; AUC, area under the curve; MesPGN, mesangial proliferative glomerulonephritis; T-TAS, total thrombus formation system.



Supplementary Material 3 Changes in laboratory data in pediatric MesPGN patients

Panel (a) shows the changes in platelet count, fibrinogen levels, and VWF: RCo. *Panel* (b) shows the changes in platelet count, fibrinogen levels, and VWF: RCo of patients receiving combination therapy alone, including dipyridamole. *Panel* (c) shows the parameters of patients receiving combination therapy without dipyridamole. The *gray* zone shows the interquartile range of the parameters in the controls. Steel tests were used for comparison with the pre-treatment results. Wilcoxon rank-sum tests were used for comparison with controls. Statistical significance was set at $p < 0.05$. *$p < 0.05$, **$p < 0.01$ compared with pre-treatment. †$p < 0.05$, ††$p < 0.01$ compared with controls. MesPGN, mesangial proliferative glomerulonephritis; RCo, ristocetin cofactor activity; VWF, von Willebrand factor; w, weeks.

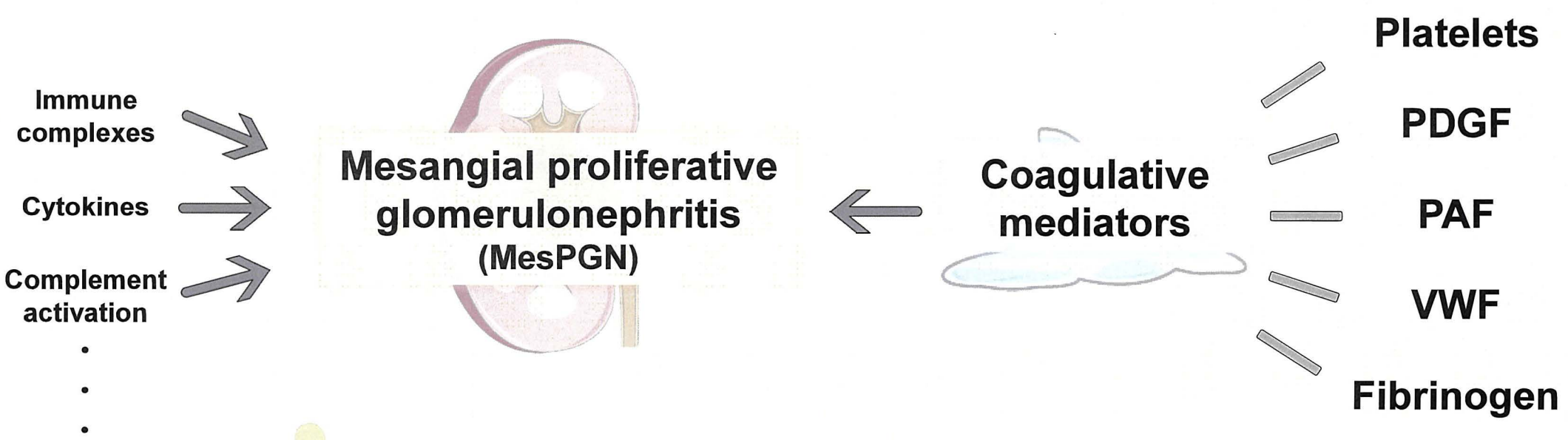
Plain Language Summary

Mesangial proliferative glomerulonephritis (MesPGN) is one of the most freaquent kidney diseases, and the pathogenesis of this disease is complex and involves several pathways. Blood coagulation is associated with MesPGN pathophysiology. Many coagulative mediators associated with these mechanisms have been evaluated and reported, individually. However, few studies have evaluated whole blood samples, especially in children, and the precise underlying mechanism(s) remains unclear. The total thrombus formation system (T-TAS®), new system, is a flow chamber system using a microchip for analyzing platelet thrombus formation (PTF), a coagulable state, under variable flow conditions, and can evaluate more closely living body. In this study, we assessed PTF in pediatric patients with MesPGN under flow condition using T-TAS. Twenty-nine pediatric patients definitively diagnosed with MesPGN by renal biopsy at Nara Medical University Hospital between 2015 and 2022 were evaluated. We assesed the PTF usig T-TAS before, durig, and after treatment, and compared with controls. The results showed that PTF of MesPGN patients was enhanced before treatment and was improved after the end of treatment.

Conclusions: Pediatric MesPGN increased the potential for PTF under flow conditions.

Pediatric mesangial proliferative glomerulonephritis has increased the platelet thrombus formation potentials under high-shear flow condition

Karger



Aim; Evaluation of the coagulable state in **pediatric patients with MesPGN**

Methods; Using flow chamber system (**T-TAS®**)

Results; Increased functional potential of **platelet thrombus formation**

Omae.T, et al.