Comprehensive coagulation and fibrinolytic potential in the acute phase of pediatric patients with idiopathic nephrotic syndrome evaluated by whole blood-based rotational thromboelastometry

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Abstract

Background: Venous thromboembolism is a rare, serious complication of idiopathic nephrotic syndrome (INS) in childhood. The mechanisms responsible for the hypercoagulable state in the acute phase of INS are poorly understood, however.

Aim: To assess overall coagulation and fibrinolytic function in pediatric patients with INS.

Methods: Global coagulation and fibrinolysis were examined in whole blood samples from 22 children with initial onset INS (initial-group), 22 children with relapsed INS (relapse-group) and 15 control pediatric patients using rotational thromboelastometry (ROTEM®). In the initial-group, blood samples were obtained before (week 0) and 1-4 weeks after the initiation of corticosteroid therapy. EXTEM and FIBTEM were used to assess coagulation and fibrinolysis, respectively. Clot time (CT), clot formation time (CFT), maximum clot firmness (MCF), and α-angle were determined as coagulation parameters, and lysis index at 30 and 60 min (LI30 and LI60, respectively) were assessed as fibrinolytic parameters.

Results: CT was significantly shortened, and MCF and α-angle were significantly greater than in the controls at week 0 and week 1 both in the initial-group and in the relapse-group. MCF correlated with serum albumin (r=0.70, p<0.001) and fibrinogen level (r=0.68, p<0.001). The fibrinolytic parameters (LI30 and LI60) in the initial-group were stable and higher than those in controls at all weeks (p<0.01).

Conclusion: We have shown that the hypofibrinolytic defect did not improve with effective NS treatment at the early 4-week time-point. Additionally, a likely pre-thrombotic state was evident in the period before initial onset and 1 week after corticosteroid therapy in pediatric INS.

Key Words: Idiopathic nephrotic syndrome, rotational thromboelastometry, fibrinolysis, hypercoagulability, children

Introduction

Nephrotic syndrome (NS) is the most common glomerular disease in children, characterized by massive proteinuria, serum hypoalbuminemia, and edema. Venous thromboembolism (VTE) and serious complications including cerebral venous sinus thrombosis and pulmonary thromboembolism have been reported in approximately 3% of pediatric NS [1-12]. The mechanism(s) that cause VTE in NS remains to be elucidated, however, and the establishment of prophylactic antithrombotic therapy could be highly beneficial.

Previous studies have suggested that the hypercoagulable state in NS patients is not associated with any single pathology but appears to be governed by multiple factors such as coagulation-related and/or fibrinolysis-related disturbances, hemoconcentration, acceleration of platelet function, and drug-related events including corticosteroid (CS) therapy and diuretics [1,3,5,13,14]. Nevertheless, among these etiologies, imbalances in coagulation and fibrinolysis are considered to be the primary triggers of VTE [1,3,4]. Changes in blood concentrations of relevant clotting proteins may reflect the sum of clearance, involving, for example, leakage into urine and synthesis in the liver. In particular, the important changes in plasma hemostatic protein, including high concentrations of fibrinogen and low levels of antithrombin, are known to be associated with hypercoagulability of NS [1], although few studies have assessed

coagulation-fibrinolysis potential in whole blood samples from patients with NS.

Rotational thromboelastometry (ROTEM®) is a computerized, viscoelastic test, which examines the global coagulation process in whole blood from the beginning of clot formation to the conclusion of fibrinolysis in real-time [15-17]. ROTEM has been widely used as a point-of-care tool to evaluate comprehensive coagulation and fibrinolysis in various clinical circumstances including pregnancy, post-surgery, trauma/injury, and hemorrhagic and thrombotic disorders [17-23]. In addition, recent reports demonstrated that ROTEM was useful for assessing hypercoagulability in canine models of protein-losing nephropathies, rodent models with NS, and adult patients with different pathologic characteristics of NS [24-26]. There is little information, however, assessing clot formation and subsequent lysis by ROTEM in pediatric idiopathic NS (INS). The present study was designed, therefore, to investigate comprehensive coagulation and fibrinolytic potentials in whole blood samples from pediatric patients with INS using the automated ROTEM technique.

Materials and Methods

The present study was approved by the Medical Research Ethics Committee of Nara Medical University (No.2365). Blood samples were obtained after informed consent according to the

ethical guidelines of Nara Medical University.

Patients - Forty-four children with INS participated in the present study. Twenty-two children were initial onset INS (termed as the initial group), and 22 children were relapsed cases (termed as the relapse group). All patients were admitted to Nara Medical University Hospital between August 2016 and December 2020. In the initial group, the diagnosis of NS was defined as proteinuria >40 mg/m^2/hr or urinary protein to creatinine ratio (UP/Cr) ≥200 mg/mmol (2 mg/mg) on a first morning urine sample, and hypoalbuminemia (<2.5 g/dL) according to the International Study of Kidney Disease in Children [27]. Patients in the initial group received high-dose prednisolone at a dose of 60 mg/m^2/day for 4 weeks (max. 60 mg/day). Relapse was defined in patients with a urine dipstick ≥3+ on 3 consecutive days or UP/Cr ≥200 mg/mmol (2 mg/mg) on a first morning urine sample [28]. Patients in relapse group received prednisolone at a dose of 60 mg/m^2/day (max. 60 mg/day) until 3 days after complete remission was achieved. Complete remission was defined as UP/Cr (based on first morning void or 24 h urine sample) ≤20 mg/mmol (0.2 mg/mg) or negative or trace dipstick on three or more consecutive occasions. Steroid sensitive was defined as complete remission within 4 weeks of prednisone at standard dose (60 mg/m^2/day, max. 60 mg/day) according to IPNA clinical practice recommendation [28].

Exclusion criteria included the use of anticoagulant or antiplatelet drugs, and other therapy such as cyclosporine that might influence coagulation, from two weeks before the onset of INS to the end of blood collection, and the use of CS within two weeks prior to the onset of INS. In addition, patients, having any signs of secondary NS and were aged <1 year at onset were considered as congenital NS and were excluded. At the onset of nephrotic syndrome, none of them had hypertension, hypocomplementemia, renal insufficiency or hematuria (>20 erythrocytes/high-power field) for which a renal biopsy is recommended.

Controls - A control group matched for age and gender was selected from patients attending in our hospital. The inclusion criteria for controls were as follows; (i) male or females aged 1-18 years old, (ii) no kidney and liver insufficiency, (iii) no proteinuria, (iv) no history of thrombosis and coagulation disorders, (v) no medication that may have affected coagulation at the time of sample collection.

Blood samples - Blood samples were taken from the 44 pediatric patients with INS and 15 pediatric control patients. Blood samples were obtained from the initial and relapse groups by

venipuncture at the onset of INS just before CS therapy (terms as 0W in initial group). In addition, whole blood samples were obtained at four time-points (one-, two-, three-, and four-weeks) after CS therapy (termed as 1W, 2W, 3W, and 4W, respectively) in the initial group. Blood sample in controls was obtained by venipuncture when the patient was visiting our hospital under healthy conditions. Whole blood samples were collected into plastic tubes containing 3.2% sodium citrate at a ratio of 9:1 (Fuso Pharmaceutical Industries, Osaka, Japan).

Conventional laboratory tests - The following general laboratory data were recorded; hemoglobin (Hb), hematocrit (Ht), platelet counts (Plt), serum total protein (TP) and albumin (Alb), urinary total protein/creatinine (UP/Cr). Coagulation parameters, including prothrombin time-international normalized ratio (PT-INR), activated partial thromboplastin time (APTT), fibrinogen, antithrombin (AT), fibrinogen/fibrin degradation products (FDP), D-dimer, plasminogen, α2-plasmin inhibitor (α2PI), thrombin-antithrombin complex (TAT), plasmin-α2PI-complex (PIC), total plasminogen activator inhibitor-1 (tPAI-1) were estimated using standard commercially available methods.

ROTEM - ROTEM was performed using ROTEM delta (Tem Innovations GmbH, Munich, Germany). Citrated whole blood samples were incubated for 30 min at 22°C, followed by

ROTEM, using two alternative-triggered tests (EXTEM and FIBTEM) [17,18].

(i) **EXTEM** (triggered by tissue factor and Ca^{2+}) - Coagulation interactions were initiated by the addition of 20 µL $CaCl_2$ (final concentration (f.c.) 12.5 mM) together with 2.5 µL tissue factor (TF; f.c. 0.5 pM, Innovin®; Dade Behring, Marburg, Germany) to the citrated whole blood samples (280 µL). **Figure 1A** illustrates a representative thromboelastogram pattern of clot formation with the four measured parameters. The clotting time (CT) was estimated as the time from the start of the test until reaching 2-mm amplitude. The clot formation time (CFT) was determined as the time between 2-mm and 20-mm amplitude. The α-angle was defined as the angle between the baseline and a tangent to the clotting curve through the 2 mm time-point. The maximum clot firmness (MCF) was defined as the maximum amplitude observed.

(ii) **FIBTEM** (triggered by TF, tPA and Ca^{2+}) - Fibrinolytic responses were assessed after the addition of 20 µL $CaCl_2$ (f.c. 12.5 mM), 2.5 µL TF (f.c. 0.5 pM), and 2.5 µL tPA (f.c. 2 nM) to the citrated whole blood samples (280 µL). **Figure 1B** illustrates a representative thromboelastogram pattern of clot lysis with the two specific parameters. The Lysis Index was determined as the residual clot firmness amplitude at 30 min (LI30) and 60 min (LI60) after the CT.

Statistical analysis - Data analyses were performed using JMP®10 (SAS Institute Inc., Cary, NC,

USA). All data are illustrated as the median and interquartile ranges (IQR). The numeric variables of laboratory data were analyzed by the Steel-Dwass test. For the ROTEM parameters, the Dunnett's multiple comparison tests were used to identify statistically significant differences vs. the control group. Correlations between the laboratory data and ROTEM parameters were investigated by Pearson's correlation coefficient test and linear regression analysis. *P* values of <0.05 were considered to be statistically significant.

Results

Patients' clinical characteristics - Forty-four children with INS (30 boys: 14 girls) and 15 controls (6 boys: 9 girls) were enrolled. Of the 44 patients, 22 (13 boys: 9 girls) were identified in the initial group, and 22 (17 boys: 5 girls) in the relapse group. All patients were steroid-sensitive and no overt, clinically observed thrombotic events. The clinical characteristics of the enrolled patients are summarized in **Table 1**. The median age of initial group at admission was 5.1 years (IQR; 2.9-10.0 years). The median age of relapse group was 7.5 years (4.6-10.6 years). The median age of the control group was 5.0 years (1.0-8.0 years). The time to remission after CS therapy was 9.0 days in the initial group and 10.0 days in the relapse group.

Table 2 summarizes conventional laboratory data in the initial group (at 0W) just before CS

therapy, the relapse group, and the control group. Measurements of Hb, Ht, and UP/Cr in both the initial and relapse groups were higher than in the control group ($p<0.05$). Hb and Ht values were within reference range, however. Fibrinogen levels in the initial and relapse groups were higher than in controls ($p<0.05$) and were higher in the initial group than in relapse ($p<0.05$). TP, Alb, and AT assays in initial group were lower than those in both the relapse and control groups ($p<0.05$), and were lower in relapse group compared to control ($p<0.05$). FDP and D-dimer values in the initial group were higher than in the relapse and control groups ($p<0.05$). No significant differences between the groups were evident with the other parameters.

Table 3 summarizes the changes of laboratory characteristics in the initial group during follow-up. TP and Alb measurements were lowest at 0W, but recovered time-dependently close to the control range. Compared to the control group, the median of Hb and Plt remained high throughout the observation period. Fibrinogen levels were highest at 0W, decreased at 2W, and then gradually fell to below or near the lower limit of the normal range. AT levels were lower than controls at 0W but were increased at 1W and remained higher than controls thereafter. The UP/Cr ratios were high at 0W and returned to the normal range at 2W in most cases.

Coagulation potential assessed by EXTEM in pediatric INS - Comprehensive coagulation

potential was assessed in pediatric INS patients using the EXTEM viscoelastic method. Representative thromboelastograms of EXTEM **(Figure 2A)** in controls (*panel* **a**) and INS at 0W (*panel* **b**) demonstrate that the CT and CFT were shorter, and MCF and α-angle were greater in the INS patients than in the controls.

These coagulation parameters (CT, CFT, MCF, and α angle) obtained by EXTEM in the initial INS group (0-4W) and at relapse are illustrated in detail in **Figure 3 (***panels* **a-d)**. In the initial group, CT and CFT at 0W and 1W were shorter than that in controls ($p<0.01$). Thereafter, both CT and CFT returned to within the normal range. In contrast, CFT at 3W and 4W appeared to be longer than in controls ($p<0.05$). MCF and α-angle at 0W and 1W were significantly greater than those in controls ($p<0.01$), and both decreased to near normal limits after 2W. MCF, estimated at 3W and 4W, were lower than in controls ($p<0.01$), however. In the relapsed patients, CT was significantly shorter than that in controls ($p<0.01$), and although the CFT appeared to be low in the INS group, the differences were not statistically significant. Both MCF and α-angle were greater in the relapsed group than in controls ($p<0.01$).

Overall, these EXTEM parameters at the initial onset of INS (within 1W) and at relapse were consistent with a likely hypercoagulable state.

Fibrinolytic potential determined by FIBTEM in pediatric INS patients - The principles of ROTEM were also extended to examine global fibrinolytic potential (FIBTEM) in pediatric INS. As above, representative thromboelastograms obtained by FIBTEM **(Figure 2B)** in the controls (*panel* **a**) and the INS patients at 0W (*panel* **b**) illustrate that the fibrinolytic parameters were greater in the INS patients than in controls.

These fibrinolytic parameters (LI30 and LI60) obtained by FIBTEM in the initial INS patients (0-4W) and at relapse are highlighted in **Figure 3** (*panels* **e-f**). In the initial group, LI30 and LI60 at 0-4W were markedly greater than in controls ($p<0.01$). In the relapse group, LI30 was significantly greater than in controls ($p<0.05$), but, although the LI60 in the relapsed patients tended to be greater than in controls, the differences were not statistically significant ($p=0.06$). These results were in keeping with the concept that fibrinolysis was more defective at the initial onset of pediatric INS (0-4W) than at relapse.

Relationship between EXTEM/FIBTEM parameters and conventional laboratory assays - The results of these analyses of global hemostasis were further assessed to examine the relationship between the ROTEM parameters and conventional laboratory tests. The effects of

CS therapy on coagulation mechanisms could have influenced the data, however, and comparisons were limited, therefore, using samples at 0-1W in the initial group. The EXTEM parameters (**Figure 4A**), demonstrated that both the MCF and α-angle correlated with Alb and fibrinogen (r=0.70, p<0.001; r=0.68, p<0.001; and r=0.50, p<0.007; r=0.50, p=0.001, respectively). The FIBTEM parameters (**Figure 4B**), suggested that LI60 correlated weakly with IgG and fibrinogen (r=0.43, p=0.021; r=0.45, p=0.004, respectively), but no significant correlations were evident between the L130 or L160 parameters and the specific fibrinolysis markers, D-dimer, PIC, and α2-PI (L130, p=0.67, 0.74, and 0.05, respectively and LI60, p=0.36, 0.07, and 0.37, respectively; data not shown).

Discussion

VTE is a rare but life-threatening complication of INS in childhood. For example, some findings indicated that the incidence of VTE in adults ranged from 20-50% whereas the rate was approximately 3% in pediatric patients [1]. A later report demonstrated, however, that VTE could be detected in pediatric patients by radiographic technology even in the absence of clinical findings [9]. The pathogenesis of thrombosis appears likely to involve a combination of multiple mechanisms including those involved in coagulation and fibrinolysis, platelet function, vascular endothelial disturbances, and blood viscosity. Furthermore, a wide range of underlying

diseases are known to contribute to thrombotic complications, and critical disturbances in hemostasis may vary depending on the underlying disease. In this context, the clarification of thrombotic mechanism(s) is especially needed to establish prophylactic antithrombotic therapy in pediatric INS patients. Plasma-based measurements, such as PT, APTT, and fibrinogen, are generally used to evaluate blood coagulation, but these *in vitro* tests provide only limited information about *in vivo*, physiological processes. Global coagulation assay such as TEG, ROTEM, and thrombin generation [29], therefore, have focused on whole blood techniques to assess the complex role of interacting mechanisms governing hemostasis, including platelets and other blood cell components.

Consequently, thromboelastography (TEG) was devised as a rheological assay to estimate overall coagulation and fibrinolytic potential in whole blood, and assessments in various clinical situations, including trauma and postoperative management, have confirmed the effectiveness of the technique for evaluating disordered coagulation pathology [17,18]. TEG has been recently reported to be informative for assessing comprehensive coagulability in adult patients with different pathological types of NS [26]. Moreover, Huang *et al.* [30,31] reported that patients with membranous nephropathy tend to be more hypercoagulable than normal individuals and patients with MCD. ROTEM is a more recent modification of TEG that provides a visual

assessment of clot formation and subsequent lysis under low shear, similar to those present under venous flow conditions [15-18,22]. In particular, ROTEM parameters appeared to correlate better with fibrinogen concentration and hyperfibrinolysis relative to TEG [32]. Moreover, Kerlin *et al.* [25] used ROTEM in a puromycin aminonucleoside-induced rat nephrosis model to demonstrate that proteinuria and hypoalbuminemia could be clinically meaningful surrogate biomarkers of hypercoagulopathy. In the present study, therefore, ROTEM was adapted to assess the overall coagulation and fibrinolytic potential in pediatric INS patients, and the results indicated that a hypercoagulable state and decreased fibrinolytic activity co-existed in the acute phase of this syndrome. In addition, dynamic changes in blood coagulation were observed within 4 weeks post-initiation of the CS therapy.

Regarding conventional laboratory data, AT and fibrinogen levels showed biphasic trends, normalizing once and then shifting in the opposite side of the reference value after CS treatment. TP and Alb levels, which affect blood viscosity, gradually normalized over time, while Hb and Plt counts remained higher than those in the control group throughout the observation period, but the phenomenon appeared unlikely to be clinically meaningful. FDP and D-dimer levels were elevated, while TAT and PIC levels were not significantly elevated compared to the control group. Whereas, Kerlin *et al.* reported that in the PAN-induced rat nephrosis model, TAT and D-

dimer levels were not significantly elevated and the hemostatic system remained quiescent in vivo in the absence of insults that would activate the hemostatic mechanism [25]. The insults that cause thrombosis include trauma, obesity, cardiovascular disease, venous catheter-related endothelial injury, or venous stasis related to edema, poor perfusion, bed rest, and so on. The precise mechanism(s) for the FDP and D-dimer were elevated while the TAT and PIC were not, is unclear. One possibility, however, may be the influence of the presence of ascites or pleural effusion. Agarwal *et al.* [33] suggested the possibility of ascites as a cause of high D-dimer levels in blood and ascites in cirrhotic patients. The elevated blood levels of FDP and D-dimer formed in ascites and pleural effusion may be due to their migration into the circulating blood, but may not be the state of systemic coagulation activation as reported by Kerlin *et al* [25]. With such complicated changes of many factors in the laboratory data, it was difficult to accurately assess the changes in blood coagulation.

The EXTEM test of ROTEM reflects extrinsic coagulation, and our investigations using this method revealed a hypercoagulable state both in the initial INS group at 0-1W and in the relapse group. In the initial group, the coagulation potential returned to normal at 2W, but the CFT was prolonged and the MCF was decreased at 3-4W compared to controls. These findings suggested delayed thrombus formation and weak clot firmness during these times. Waller *et al.* [34]

analyzed blood samples from patients with childhood NS using thrombin generation and have reported that CS treatment reduced NS-related thrombotic risk in children. Importantly, the experiments demonstrated for the first time that the pathophysiology of hypercoagulability in the initial group was significantly changed during CS therapy using ROTEM. Comparisons of the ROTEM parameters with conventional laboratory assays identified a strong negative correlation between MCF with Alb levels and a strong positive correlation with fibrinogen concentrations. In addition, α-angle showed negative correlations with Alb and AT levels. In particular, our results illustrated that fibrinogen and Alb levels correlated well with ROTEM parameters. These data were in keeping with earlier reports demonstrating that hypoalbuminemia, hyperfibrinogenemia, and low AT levels were risk factors for VTE in NS [3,4,14,35], and that fibrinogen and Alb could be useful markers of the overall coagulability in pediatric INS patients.

The FIBTEM technique was originally devised to study fibrinolysis potential. In the present study, we utilized additional reagent of tPA to evaluate clot stability for hyperfibrinolytic conditions. Several groups have modified ROTEM to induce fibrinolysis such that they would become more sensitive to changes in the fibrinolytic system [36,37]. This method is artificial for assessment of fibrinolysis, however. The current data suggested a predominant decrease in

fibrinolytic activity both in the initial group at 0-4W and in relapse group. Lisman *et al.* [38,39] reported the involvement of hypercoagulability and hypofibrinolysis in the thrombotic pathogenesis, for example, in COVID19 and acute-on-chronic liver failure. In NS patients, fibrinolytic activity is known to decrease in association with hypercoagulability [3,40], but in our initial group the parameters of fibrinolytic activity at 2-4W were decreased despite the absence of hypercoagulability. Hence, unlike earlier reports, the coagulability and fibrinolytic activity appeared to vary independently. In addition, the LI60 parameter correlated weakly with IgG and fibrinogen levels, but there were no significant correlations between the FIBTEM parameters and standard markers of fibrinolysis including D-dimer, PIC, and α2PI. The findings indicated, therefore, that conventional fibrinolytic measurements appeared unlikely to adequately reflect fibrinolytic activity in pediatric INS patients.

The new observation from the present study was that CS therapy did not correct the hypofibrinolytic state at least by 4 weeks after treatment. One reason may be the effect of CS therapy-mediated PAI-1 induction on fibrinolytic activity. Sartori *et al.* [35] reported that fibrinolytic activity was significantly decreased in renal-transplant recipients treated with CS, suggesting the likely significant influence of CS on fibrinolytic function. Similarly, other reports have demonstrated that CS increased levels of PAI-1, leading to decreased fibrinolytic activity

[41-43]. In the present study, since PAI-1 was not measured during CS therapy, its involvement is unclear. Another reason may be related to the synthetic rates of various components on fibrinolytic system, and the etiology remains an open question.

There are some limitations with the present study. Firstly, the number of patients involved was relatively low, and no VTE events were observed. Secondly, the principles of the ROTEM technique do not take account of the role of physiological blood flow or the localized effects of disturbed vascular endothelial cells. In recent years, automated systems for analyzing whole blood coagulation under variable shear-flow have been developed [44-46], and these have been applied clinically to assess total thrombus formation. Studies utilizing this technology are under development in our laboratory. Finally, positive controls were not available for the present study, although ROTEM has been adopted to evaluate hypercoagulability in Cushing's syndrome and in severe COVID-19 pneumonia [49,50]. Experiments of this nature have not been fully standardized, however, and the influence of other pathophysiological on ROTEM data remains to be thoroughly compared. Consequently, prophylactic antithrombotic protocols to prevent the onset of VTE in pediatric INS patients are controversial. Nevertheless, our current results were consistent with an important role for hypercoagulable and hypofibrinolytic mechanisms possibly associated with an increased risk of VTE in the acute phase of INS. Our findings could

help to provide appropriate predictive treatment for VTE in INS.

Declarations

Conflict of interest; None of the authors have a conflict of interest.

Author contribution; TI designed the research, assessed the patients clinically, performed the experiments, analyzed the data, prepared the figures, and wrote the paper; **YN** interpreted the data, and wrote the paper; **TO** performed the experiments, and assessed the patients clinically; **KO** interpreted the data, and supervised the study; **KN** designed the research, interpreted the data, prepared the figures, wrote the paper, edited the manuscript and approved the final version to be published.

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Data Availability Statement; The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1. Demographic and clinical characteristics in the pediatric INS patients enrolled

	Initial group n=22	Relapse group n=22	Control n=15
Age (yrs.)	5.1 [2.9-10.0]	7.5 [4.7-10.6]	5.0 [1.0-8.0]
Sex; Male (%)	13 (59)	17 (77)	6 (36)
Time to remission after CST (days)	9.0 [8.5-11.5]	10.0 [8.0-12.0]	–

Data are presented as the median [IQR].

CST; corticosteroid therapy

Table 2. Comparison of laboratory findings between the pediatric INS group and control group

	Initial group		Relapse group		Control group		*P* value
Hb (g/dL)	13.4	(12.9-14.1)	13.4	(12.7-14.6)	12.5	(12.0-13.6)	*/‡
Ht (%)	40.3	(39.2-42.2)	41.0	(38.6-44.0)	37.4	(34.9-39.7)	*/‡
Plt ($\times 10^4$/dL)	27.0	(30.6-40.3)	30.8	(28.1-33.1)	29.4	(27.2-34.6)	NS
T-TP (g/dL)	3.9	(3.6-4.3)	5.6	(5.3-5.9)	6.8	(6.6-7.1)	*/†/‡
S-Alb (g/dL)	1.8	(1.6-2.1)	3.4	(3.1-3.6)	4.5	(4.4-4.6)	*/†/‡
UP/Cr (g/gCr)	13.4	(8.2-18.0)	12.1	(3.3-14.9)	0.02	(0.02-0.02)	*/‡
PT-INR	0.99	(0.95-1.00)	1.01	(0.99-1.04)	1.04	(0.97-1.06)	NS
APTT (sec)	31.8	(27.9-34.7)	29.9	(28.1-32.3)	28.0	(26.1-30.4)	NS
Fibrinogen (mg/dL)	710	(561-801)	395	(323-425)	244	(227-273)	*/†/‡
Antithrombin (%)	68.1	(57.5-82.5)	93.6	(81.5-106)	111	(110-113)	*/†/‡
FDP (μg/mL)	5.6	(3.4-6.1)	2.7	(2.5-2.8)	2.5	(2.5-2.5)	*/†
D-dimer (μg/mL)	2.1	(1.1-2.4)	0.7	(0.6-0.8)	0.59	(0.5-0.7)	*/†

Plasminogen (%)	98.3	(81-106)	97.1	(93-104)	–	NS
α2PI (%)	101	(93-113)	110	(100-122)	–	
TAT (ng/mL)	2.8	(1.5-3.4)	4.7	(1.4-3.6)	–	NS
PIC (μg/mL)	0.6	(0.3-0.9)	0.3	(0.2-0.4)	–	†
Total PAI-1 (ng/mL)	17.6	(10.0-22.3)	19.0	(10.0-25.0)	–	NS

Data are presented as the median (IQR).

* ; Initial group vs Control group † ; Initial group vs Relapse group ‡ ; Relapse group vs Control group

Statistical significance of differences among the three groups was calculated using Steel-Dwass test.

P values of <0.05 were considered to be statistically significant.

Table 3. Change of laboratory data during the follow-up period in the initial group

	Initial group					Control
	0W	1W	2W	3W	4W	
TP (g/dL)	3.9 (3.6-4.5)	4.5 (4.1-5.0)	5.6 (5.3-6.1)	6.0 (5.6-6.2)	6.1 (5.6-6.5)	6.8 (6.6-7.1)
Alb (g/dL)	1.8 (1.6-2.1)	2.4 (2.2-2.7)	3.3 (3.1-3.5)	3.7 (3.6-3.9)	4.1 (3.7-4.2)	4.5 (4.4-4.6)
Hb (g/dL)	13.4 (12.9-14.1)	13.6 (13.0-14.2)	13.6 (13.2-14.5)	13.5 (13.2-14.3)	13.5 (12.7-14.2)	12.5 (12.0-13.6)
Plt ($\times 10^4/\mu L$)	35.7 (29.6-40.3)	47.4 (38.2-54.9)	45.2 (41.7-60.9)	35.7 (29.4-47.6)	32.1 (28.9-38.6)	29.4 (27.2-34.6)
Fibrinogen (mg/dL)	624 (574-736)	355 (327-426)	208 (167-280)	160 (142-191)	168 (147-227)	244 (227-273)
Antithrombin (%)	68 (58-84)	135 (90-140)	150 (140-150)	150 (147-150)	150 (140-150)	111 (110-113)
UP/Cr (g/gCre)	12.6 (8.5-17.9)	0.91 (0.20-11.5)	0.12 (0.09-0.21)	0.11 (0.06-0.14)	0.10 (0.06-0.13)	0.02 (0.02-0.02)

Data are presented as the median (IQR).

0W, 1W, 2W, 3W, 4W; Whole blood samples at one-, two-, three- and four-week after the CS therapy, respectively.

Fig 1. Representative thromboelastograms together with coagulation and fibrinolysis parameters obtained by ROTEM

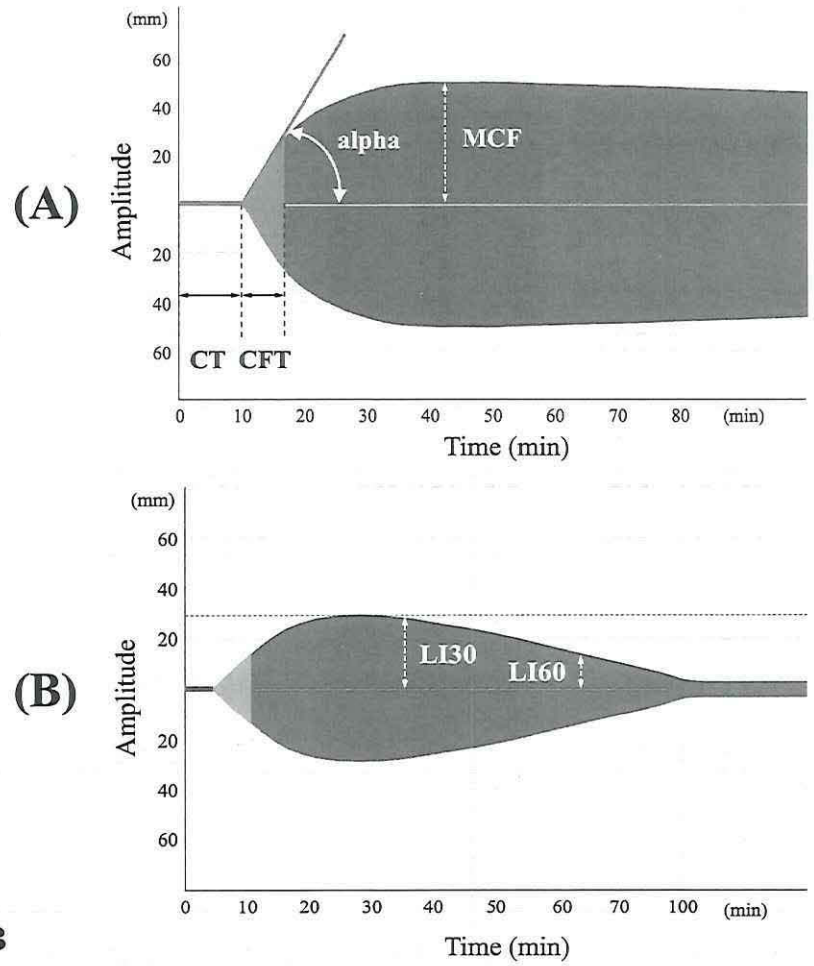

Figure 1A,B

Fig 2. Representative EXTEM and FIBTEM thromboelastograms in a control individual and a pediatric INS patient

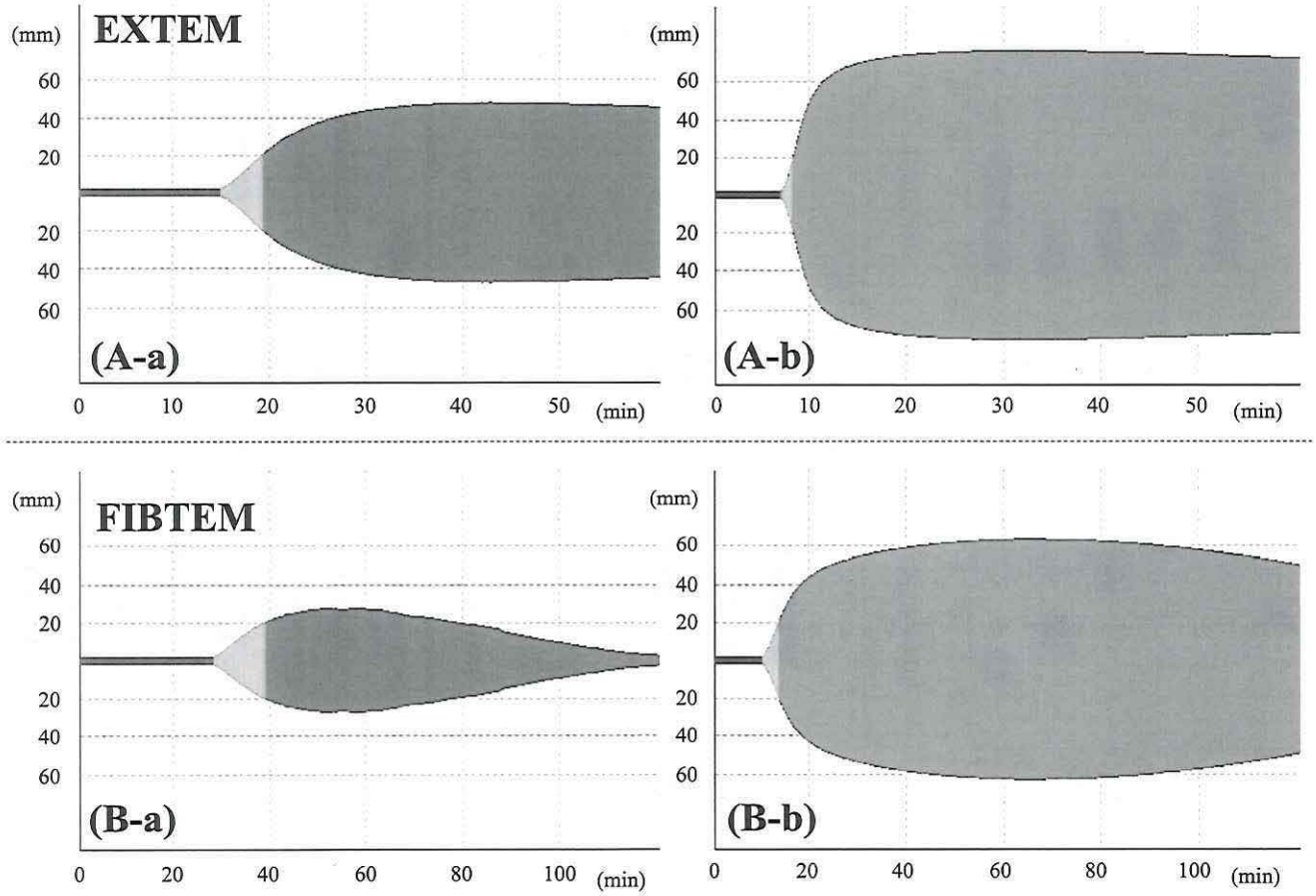

Figure 2A,B

Fig 3. Changes in EXTEM and FIBTEM parameters over time in pediatric INS patients

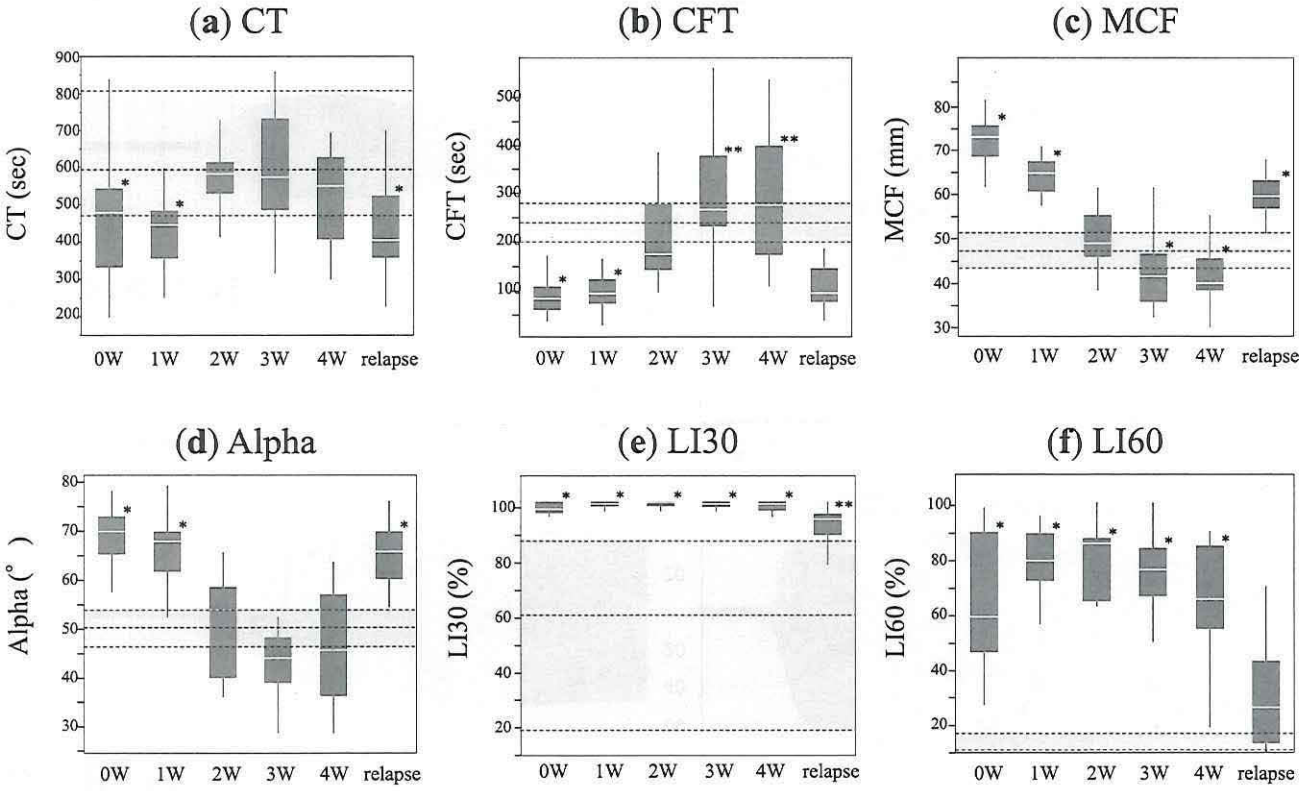

Figure 3

Fig 4. Correlation between laboratory data and coagulation and fibrinolysis parameters obtained by ROTEM in pediatric INS patients

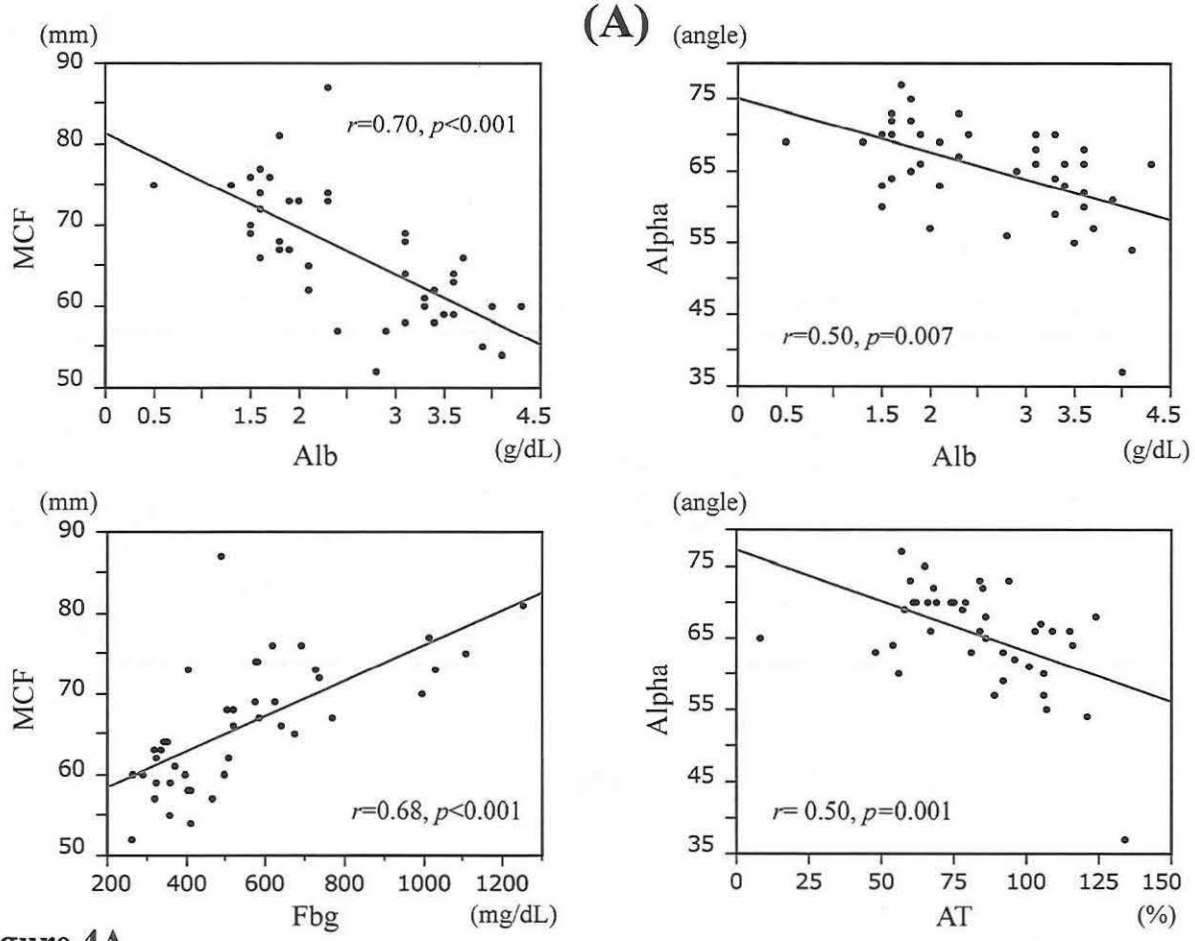

Figure 4A

(B)

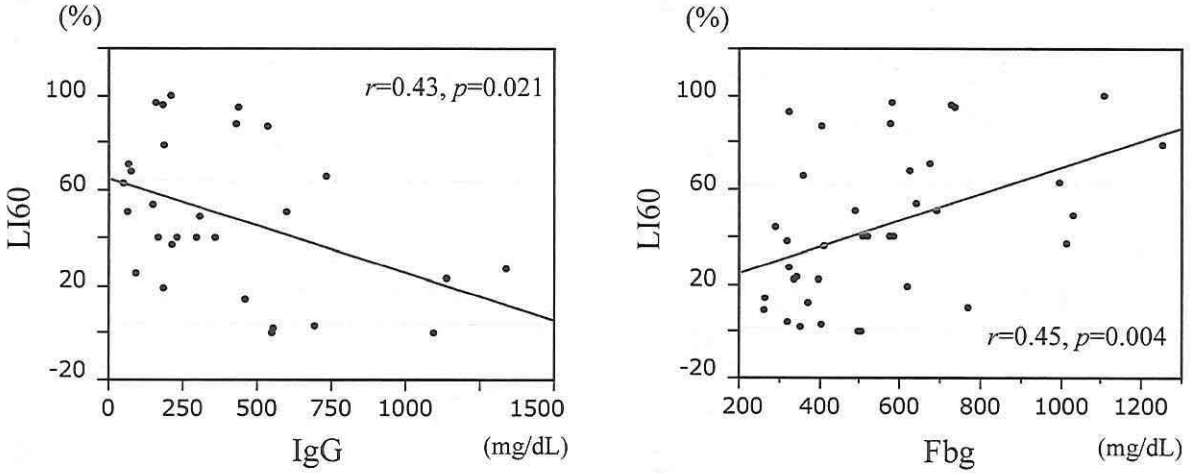
(%)
100
60
20
-20
LI60
r=0.43, p=0.021
0
250
500
750
1000
1500
IgG
(mg/dL)
(%)
100
60
20
-20
LI60
r=0.45, p=0.004
200
400
600
800
1000
1200
Fbg
(mg/dL)

Figure 4B

Figure Legends

Fig 1. Representative thromboelastograms together with coagulation and fibrinolysis parameters obtained by ROTEM

Panel (**a**) shows representative EXTEM data in a whole blood sample from a control individual. The coagulation parameters are as follows; CT; clotting time, CFT; clot formation time, MCF; maximum clot formation, alpha; α-angle. *Panel* (**b**) shows corresponding representative FIBTEM data from a control subject. The fibrinolysis parameters are as follows; LI30; lysis index at 30 min, LI60; lysis index at 60 min.

Fig 2. Representative EXTEM and FIBTEM thromboelastograms in a control individual and a pediatric INS patient

(**A**) EXTEM: TF (0.5 pM) and $CaCl_2$ were added to citrated whole blood sample for the EXTEM assay. *Panel* (**a**) shows a representative EXTEM pattern in whole blood from a control individual. *Panel* (**b**) shows a representative EXTEM pattern in whole blood from a pediatric INS patient.

(**B**) FIBTEM: TF (0.5 pM), $CaCl_2$, and tPA (2 nM) were added to citrated whole blood sample for the FIBTEM assay. *Panel* (**a**) shows a representative FIBTEM pattern in whole blood from a control individual. *Panel* (**b**) shows a representative FIBTEM pattern in whole blood from a pediatric INS patient.

Fig 3. Changes in EXTEM and FIBTEM parameters over time in pediatric INS patients

TF (0.5 pM) and $CaCl_2$ were added to citrated whole blood, followed by EXTEM analysis, and TF (0.5 pM), $CaCl_2$, and tPA (2 nM) were added to citrated whole blood, followed by the FIBTEM analysis as described in Methods. The EXTEM parameters (**a**; CT, **b**; CFT, **c**; MCF and **d**; α-angle) and the FIBTEM parameters (**e**; LI30 and **f**; LI60) in the initial group at 0-4 weeks after corticosteroid therapy and in the relapse group are shown. Each box plot represents the interquartile range with mean values (horizontal line). The gray zone shows the range of EXTEM and FIBTEM parameters in control individuals (n=15). Significant differences between control individuals and relapse group or initial group were considered as $p<0.05$. * $p<0.01$, ** <0.05.

Fig 4. Correlation between laboratory data and coagulation and fibrinolysis parameters obtained by ROTEM in pediatric INS patients

The ROTEM parameters obtained in whole blood samples from the initial group at 0-1W and shown in Figure 3 were analyzed as follows. (**A**) EXTEM parameters (α and MCF) are presented on the *y*-axis. Serum Alb, fibrinogen, and AT values are presented on the *x*-axis. **(B)** FIBTEM parameter (LI60) is presented on the *y*-axis. Fibrinogen and IgG values are presented on the *x*-axis. The correlation coefficients (*r*) are shown

(*straight line*). Significant differences between laboratory data and ROTEM parameters was shown as $p<0.05$.