

ORIGINAL ARTICLE

A look into hemostatic characteristics during pediatric liver transplantation using the thromboelastometry (ROTEM®) test

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Abstract

There is a paucity of evidence about the coagulation profile regarding the complexity of children undergoing liver transplantation (LT). This study aimed to investigate intraoperative hemostatic changes during pediatric LT according to the etiology for LT and examine the ability of rotational thromboelastometry (ROTEM®, TEM International GmbH, Munich, Germany) as a point-of-care monitoring method. We evaluated 106 patients aged 3 months to 17 years undergoing LT for acute liver failure (ALF) and chronic liver disease, which consists of patients with cholestatic disease, metabolic/genetic disease, and cancer. A total of 731 ROTEM® measurements, including 301 ellagic acid to initiate clotting via the intrinsic pathway, 172 tissue factor to initiate the extrinsic clotting cascade (EXTEM), and 258 cytochalasin D to inhibit platelet activity reflecting fibrinogen (FIBTEM), were analyzed at predetermined time points (the preanhepatic, anhepatic, and postreperfusion phases). We simultaneously conducted conventional coagulation tests. In children with ALF, preanhepatic measurements of conventional coagulation tests and ROTEM® showed a more hypocoagulable state than other diseases. During LT, the coagulation profile was deranged, with a prolonged clotting time and reduced clot firmness, changes that were more profound in the cholestatic disease group. Maximum clot firmness (MCF) on EXTEM and FIBTEM were well correlated with the platelet count and fibrinogen concentration ($r = 0.830$, $p < 0.001$ and $r = 0.739$, $p < 0.001$, respectively). On the EXTEM, MCF with 30 mm predicted a platelet count $< 30,000/\text{mm}^3$ (area under the curve, 0.985), and 6 mm predicted a fibrinogen concentration $< 100 \text{ mg/dl}$ on the FIBTEM (area under the curve, 0.876). However, the activated partial thromboplastin time and prothrombin time were significant but only weakly correlated with the clotting time on the ROTEM®. In children undergoing LT, coagulation

Abbreviations: ALF, acute liver failure; aPTT, activated partial thromboplastin time; AUC, area under the curve; BSA, body surface area; CFT, clot formation time; CLD, chronic liver disease; CT, clotting time; ESLD, end-stage liver disease; FFP, fresh frozen plasma; INR, international normalized ratio; LT, liver transplantation; MCF, maximum clot firmness; PC, platelet concentrate; PLT, platelet count; PT, prothrombin time; RBC, red blood cell; ROC, receiver operating characteristic; ROTEM®, rotational thromboelastometry.

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profiles depend on the etiology for LT. During LT, ROTEM[®] parameters could help detect thrombocytopenia and hypofibrinogenemia and guide transfusion therapy as a point-of-care monitoring method.

INTRODUCTION

Coagulopathy in end-stage liver disease (ESLD) is characterized as rebalanced hemostasis with impaired synthesis and function of anticoagulants and procoagulants.^[1-3] Although the coagulation profile in adults with ESLD has been addressed, there is a paucity of studies conducted on children. Moreover, children with ESLD show diverse coagulation profiles according to the underlying liver disease and stage of development. On one hand, during pediatric liver transplantation (LT), thrombotic complications occur more frequently than in adults.^[4,5] On the other hand, bleeding can rapidly lead to fatal results because children with ESLD have small blood volumes. Therefore, timely and reliable monitoring of coagulation status should be performed to optimize blood transfusion and to avoid overcorrection of anticoagulation factors.^[6,7]

A point-of-care monitoring method to evaluate coagulation function, such as rotational thromboelastometry (ROTEM[®]; ROTEM[®] Delta, TEM International GmbH, Munich, Germany), measures the viscoelasticity of clots using whole blood throughout the coagulation process and provides information on clot initiation, kinetics, strength, and stability; however, conventional coagulation tests, such as plasma-based assays, only measure the effect of procoagulants and do not reflect anticoagulants or cellular components involved in hemostasis, and they provide a coagulation profile only for the clot initiation phase.^[8] Their results also require more time compared with ROTEM[®].^[9] Thus, because of its ability to provide a global coagulation profile and short turnaround times, ROTEM[®] has been widely used during adult LT and major pediatric surgeries to provide guidance for transfusion therapy.^[10-12]

However, several factors affecting the coagulation system should be considered before applying ROTEM[®] during pediatric LT. First, the hemostatic profile of children varies according to their quantitative and qualitative pro- and anticoagulant levels.^[13,14] Second, the indications for pediatric LT are different from those of adult LT; the indications for LT in children include chronic liver disease (CLD) such as cholestatic disease, metabolic/genetic disease, cancer, and acute liver failure (ALF).^[15] In addition, the clinical features of LT recipients vary widely according to the underlying liver disease,^[16,17] as seen in patients with CLD, which is mainly caused by biliary atresia, and they undergo LT at a younger age than patients with

ALF. However, it is unknown whether the underlying liver disease affects the coagulation system in children and if so to what extent. Literature on ROTEM[®] assessing the intraoperative coagulation profile during pediatric LT is scarce. Therefore, in this study, we aimed to evaluate the intraoperative coagulation profiles including conventional coagulation tests and ROTEM[®] at various stages of pediatric LT according to the etiology of liver disease. In addition, we examined whether ROTEM[®] could be a reliable tool for the point-of-care monitoring of hemostatic status during pediatric LT by comparing ROTEM[®] with conventional coagulation tests.

PATIENTS AND METHODS

Study population

This retrospective study was approved by the institutional review board of Asan Medical Center, Seoul, Republic of Korea. A total of 121 patients aged younger than 18 years underwent living or deceased donor LT at the Asan Medical Center from November 2011 to January 2018. Of these, five patients were excluded because of the absence of ROTEM[®] data or preoperative anticoagulant therapy. The remaining 106 patients were included in the study. This study was approved by the local research ethics committee (protocol number 2020-0207), with data analyzed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its amendments.

Anesthetic management

General anesthesia was carried out according to our institutional protocol.^[18] Anesthesia was induced with intravenous bolus of thiopental, vecuronium, or rocuronium and fentanyl and maintained using 1%–2% sevoflurane, 50% oxygen in medical air, and continuous infusion of vecuronium or rocuronium and fentanyl. Radial arterial and central venous catheters were placed to monitor the hemodynamic parameters and to obtain blood samples. During LT, balanced crystalloid and 5% or 20% albumin were administered. Transfusion of packed red blood cells (RBCs), fresh frozen plasma (FFP), platelet concentrate (PC), and cryoprecipitate was based on the clinical situation or guided by conventional coagulation tests. In brief, according to our

institutional transfusion protocol for pediatric LT, blood products were provided to maintain a hematocrit of 25%–28%, prothrombin time (PT) <2.5 international normalized ratio (INR), platelet count (PLT) >30,000/mm³, and fibrinogen concentration >100 mg/dl and when clinical bleeding existed.

Blood sampling and conventional coagulation tests

Blood sampling for conventional coagulation tests and ROTEM[®] was performed simultaneously from the radial artery catheter at the following predetermined time points: preanhepatic phase (1 h after surgical incision), anhepatic phase (30 min after hepatectomy), and neohepatic phase (30 min after graft reperfusion). Conventional coagulation tests included activated partial thromboplastin time (aPTT), PT, PLT, and fibrinogen. PLT was determined using an automated hematology analyzer (Sysmex XE-2100, Siemens Healthcare Diagnostics, GmbH, Marburg, Germany) with flow cytometry. aPTT, PT, and fibrinogen concentration were assayed with an automatic coagulation analyzer (Sysmex CA-7000, Siemens Healthcare Diagnostics) using Thromborel S kits (Siemens Healthcare Diagnostics) and the Dade Thrombin Reagent (Siemens Healthcare Diagnostics), respectively.

ROTEM[®] measurements

ROTEM[®] was performed as instructed by the manufacturer using reagents and equipment provided by TEM International GmbH. Briefly, ROTEM[®] was performed using 300 µl of citrated whole blood after the addition of

recalcification reagent (0.2 mol/L CaCl₂) and 20 µl of the respective reagents (tissue factor to initiate the extrinsic clotting cascade [INTEM], ellagic acid; tissue factor to initiate the extrinsic clotting cascade [EXTEM], tissue factor; and cytochalasin D to inhibit platelet activity reflecting fibrinogen [FIBTEM], tissue factor with cytochalasin D). The ROTEM[®] parameters were obtained as follows (Figure 1): clotting time (CT), the time (s) until the initiation of clotting (clot amplitude of 2 mm); clot formation time (CFT), the time (s) from the initiation of clotting to a clot amplitude of 20 mm; and maximum clot firmness (MCF). The ROTEM[®] device was in the operating theater, and all tests were conducted by anesthesia nurses skilled in conducting ROTEM[®] tests. Among the ROTEM[®] assays obtained, any with an inadequate run time or technical irregularities in the traces and patients who received heparin or recombinant coagulation factor VII_a during LT were excluded.

To assess the correlations between the conventional coagulation tests and the ROTEM[®] parameters, the following comparisons were performed:

1. aPTT was compared with both INTEM CT and CFT.
2. PT was compared with both EXTEM CT and CFT.
3. PLT was compared with MCF on EXTEM.
4. The fibrinogen concentration was compared with MCF on FIBTEM.

Coagulation profiles according to the etiology of the liver disease

To understand the characteristics of the baseline coagulation profiles and the changes in coagulation profiles

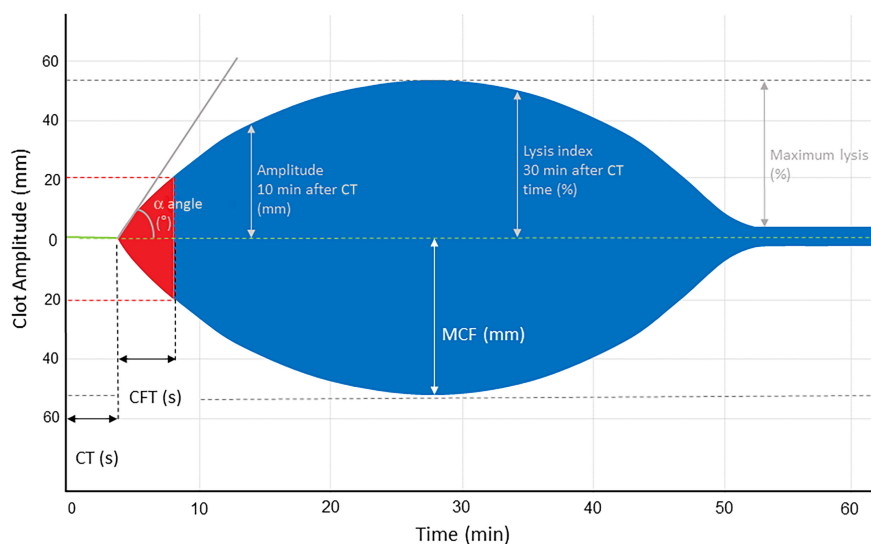


FIGURE 1 Schematic ROTEM[®] trace showing its parameters. CT represents the time (s) until the initiation of clotting (clot amplitude of 2 mm). CFT indicates the time (s) from the initiation of clotting to a clot amplitude of 20 mm, and MCF indicates the maximal amplitude (mm) of clot firmness in the graphical trace

during LT according to the etiology of the liver disease, the patients were divided into four groups based on relevant clinical features: ALF and CLD which was subdivided into cholestatic disease, metabolic/genetic disease, and cancer. Pediatric ALF was defined according to the Pediatric Acute Liver Failure study group criteria, regardless of what caused liver failure.^[19] We compared conventional coagulation tests and ROTEM[®] parameters between the groups at each stage during LT and within each group across the LT procedure.

Statistical analysis

All statistical analyses were performed using R (Version 4.1.0; R Foundation for Statistical Computing, Vienna, Austria) and MedCalc (MedCalc Software, Ostend, Belgium). As the reference interval for coagulation parameters in children was dependent on age, we used a reference range based on the patient's individual age group as given in a previous study.^[20] Continuous variables were expressed as median (25th–75th percentiles) or mean \pm standard deviation. For comparisons between groups, chi-square tests or Fisher's exact tests and *t* tests or Mann–Whitney U tests were used as appropriate. For analysis of the change in the coagulation parameters over the LT phases, linear mixed models of repeated measures were performed. Bonferroni's test was used as a post hoc test to ascertain the difference

between each operation phase of LT. After confirming the nonparametric distribution of data using the Shapiro–Wilk test, Spearman's correlation coefficients were calculated to assess the correlation between the conventional coagulation tests and the ROTEM[®] parameters. Receiver operating characteristic (ROC) curve analysis with area under the curve (AUC) was used to calculate the cutoff values of MCF on EXTEM and FIBTEM predicting PLTs $<30,000/\text{mm}^3$ and $<50,000/\text{mm}^3$ and fibrinogen concentrations <100 and <150 mg/dl. The cutoff values of PLT and fibrinogen were based on our institutional protocol and the thresholds widely used in major pediatric surgery.^[15,21,22] Optimal cutoff values were determined based on the Youden index. To validate optimal cutoff values in ROC curve analysis, a bootstrap method was used based on 10,000 resamples with replacement in the “cutpoint” R package.^[23–25] A *p* < 0.05 was considered statistically significant.

RESULTS

Patient demographics and intraoperative data

A total of 731 ROTEM[®] measurements that included 301 INTEM, 172 EXTEM, and 258 FIBTEM measurements from 106 patients were used for the final analysis. The patient characteristics and the intraoperative

TABLE 1 Patient characteristics and intraoperative transfusion

	Total, N = 106	ALF, n = 20	Cholestatic disease, n = 63	Metabolic/genetic disease, n = 12	Cancer, n = 11	<i>p</i> value
Baseline characteristics						
Age, years	2.1 (0.9–6.0)	5.5 (2.0–11.0)	1.2 (0.8–2.6)*	5.8 (2.9–11.0)**	2.5 (1.7–8.0)	<0.001
Male/female	50/56 (47/53)	13/7 (65/35)	22/41 (35/65)	9/3 (75/25)	6/5 (55/45)	0.02
Weight, kg	11.6 (8.5–18.5)	20.4 (13.4–40.1)	8.9 (7.8–13.4)*	17.6 (13.4–30.3)**	13.5 (10.5–20.2)	<0.001
Height, cm	83.1 (70.6–111.2)	118.0 (89.5–149.7)	73.5 (68.5–89.9)*	105.8 (91.0–123.2)**	87.7 (80.2–114.4)	<0.001
BSA, m ²	0.53 (0.41–0.76)	0.82 (0.58–1.29)	0.43 (0.39–0.58)*	0.73 (0.59–1.02)**	0.56 (0.48–0.80)	<0.001
Child-Turcotte-Pugh Class A/B/C	25/27/54	0/1/19	6/25/32	10/0/2	9/1/1	<0.001
Donor type, living	62 (58.5)	15 (75.0)	30 (47.6)	7 (58.3)	10 (90.9)	0.02
Intraoperative transfusion						
RBCs	95 (89.6)	20 (100.0)	56 (88.9)	11 (91.7)	8 (72.7)	0.12
Unit/kg	0.10 (0.05–0.16)	0.12 (0.07–0.19)	0.11 (0.06–0.22)	0.05 (0.03–0.08)**	0.04 (0.03–0.05)***	<0.001
FFP	65 (61.3)	20 (100.0)	35 (55.6)*	8 (66.7)	2 (18.2)*	<0.001
Unit/kg	0.13 (0.07–0.23)	0.15 (0.10–0.22)	0.14 (0.08–0.25)	0.07 (0.05–0.13)	0.08 (0.05–0.12)	0.12
PC	21 (19.8)	8 (40.0)	10 (15.9)	2 (16.7)	1 (9.1)	0.08
Unit/kg	0.20 (0.14–0.37)	0.21 (0.13–0.45)	0.25 (0.18–0.37)	0.14 (0.12–0.15)	0.14 (0.14–0.14)	0.24
Cryoprecipitate	41 (38.7)	16 (80.0)	16 (25.4)*	6 (50.0)	3 (27.3)*	<0.001
Unit/kg	0.18 (0.13–0.28)	0.18 (0.13–0.23)	0.25 (0.14–0.41)	0.15 (0.12–0.21)	0.10 (0.10–0.12)	0.08

Note: Data are presented as median (25th–75th percentiles) or number (percentage) as appropriate.

Abbreviations: ALF, acute liver failure; BSA, body surface area; FFP, fresh frozen plasma; PC, platelet concentrate; RBCs, red blood cells.

p* < 0.05 versus ALF; *p* < 0.05 versus cholestatic disease by analysis using Bonferroni's method.

transfusion data are shown in Table 1. The median age of all patients was 2.1 years (0.9–6.0 years), and their etiologies of liver disease were composed of ALF ($n = 20$), cholestatic disease ($n = 63$), metabolic/genetic disease ($n = 12$), and cancer ($n = 11$). More details on the etiologies of liver disease are described in Table S1. The patients in the cholestatic disease group were younger than the patients in the ALF and metabolic/genetic disease groups. Compared with the cholestatic disease group, the ALF group had higher Child-Turcotte-Pugh scores, whereas the metabolic/genetic disease and cancer groups had lower Child-Turcotte-Pugh scores.

Coagulation profiles according to the etiology of liver disease

Intraoperative trends in the parameters of the conventional coagulation tests and ROTEM[®] throughout the LT procedure are presented in Table 2. In patients with ALF, preanhepatic measurements of conventional coagulation tests and ROTEM[®] showed a hypocoagulable state, presenting a prolonged time for clotting and a reduced clot strength. Throughout the LT phases, PLT was significantly lower in the ALF group compared with the cholestatic disease group (Figure 2). Fibrinogen concentration was also lower in ALF group than in the cholestatic disease and cancer groups. Similarly, in ROTEM[®] measurements at the preanhepatic phase (Figure 3), the ALF group showed significantly lower values of EXTEM MCF and FIBTEM MCF than both the cholestatic disease and cancer groups. In patients with ALF, the ROTEM[®] parameters were below the reference range (55% for INTEM CT, 85% for INTEM CFT, and 63% for EXTEM MCF). From the preanhepatic phase to the neohepatic phase, there were no statistically significant differences in coagulation profiles on the ROTEM[®] measurements among the four groups. Particularly in patients with cholestatic disease, the coagulation profiles were deranged as there was a prolonged CT and reduced clot firmness across the LT phases.

Correlations between conventional coagulation tests and ROTEM[®] parameters

PT and EXTEM CT and CFT had significant but weak linear correlations ($r = 0.434$; $p < 0.001$ and $r = 0.466$; $p < 0.001$, respectively). There was a slightly higher correlation between aPTT and INTEM CT and CFT ($r = 0.726$ [$p < 0.001$] and $r = 0.565$ [$p < 0.001$], respectively). However, aPTT showed a discrepancy with INTEM CT and CFT, especially in the range of high aPTT levels. PLT was strongly correlated with EXTEM

MCF ($r = 0.830$, $p < 0.001$). The fibrinogen concentration was also highly correlated with MCF on FIBTEM ($r = 0.739$, $p < 0.001$). When the data were analyzed according to the etiology of liver disease (ALF, cholestatic disease, metabolic/genetic disease, and cancer groups), MCF of EXTEM and FIBTEM were also significantly correlated with PLT and fibrinogen concentration, respectively (Figure 4).

Cutoff values of ROTEM[®] for predicting thrombocytopenia and hypofibrinogenemia

This study showed that marked prolongation of preanhepatic PT (≥ 2.0 INR) was more frequently observed in the ALF group (80%) compared with the other groups (21%, 25%, and 9% for cholestatic disease, metabolic/genetic disease, and cancer groups, respectively). More severe thrombocytopenia ($< 50,000/\text{mm}^3$) and hypofibrinogenemia (< 100 mg/dl) were also found in 30% and 53% in the ALF group relative to other groups.

Throughout the LT procedure, 4% and 12% of the measurements showed PLT $< 30,000$ and $< 50,000/\text{mm}^3$, respectively. ROC curve analysis showed that EXTEM MCF with a cutoff value of 30 mm (sensitivity, 100%; specificity, 94.4%; AUC, 0.985; $p < 0.001$) predicted PLT $< 30,000/\text{mm}^3$. EXTEM MCF with a cutoff value of 44 mm (sensitivity, 100%; specificity, 66.9%; AUC, 0.905; $p < 0.001$) predicted PLT $< 50,000/\text{mm}^3$. Fibrinogen concentrations < 100 and < 150 mg/dl were observed in 50% and 78%. The cutoff value of FIBTEM MCF predicting a fibrinogen concentration < 100 mg/dl was 6 mm (sensitivity, 79.7%; specificity, 82.4%; AUC, 0.876; $p < 0.001$). In addition, the cutoff value of FIBTEM MCF to predict the fibrinogen concentration < 150 mg/dl was 8 mm (sensitivity, 78.8%; specificity, 94.5%; AUC, 0.918; $p < 0.001$; Figure 5). We conducted internal validation for ROC curve analysis using the bootstrap method with 10,000 resamples and replacements (Table S2). It showed the optimal cutoff value as well as its Youden index determined from bootstrap samples, which were consistent with those from the full samples.

DISCUSSION

In pediatric LT, hemostatic profiles are distinguished on the basis of etiology for ESLD. In ALF, all coagulation tests either by conventional methods or by ROTEM[®] revealed remarkable hypocoagulability during the preanhepatic phase. Children with CLD, including cholestatic disease, metabolic/genetic disease, and cancer, had relative near-normal preanhepatic coagulation profiles. Throughout LT phases, coagulopathy existed, and there was no difference in hemostasis between liver

TABLE 2 Intraoperative changes in parameters of conventional coagulation tests and ROTEM® according to the stages of LT

	Preanhepatic			Anhepatic			Neohepatic					
	ALF	Cholestatic disease	Metabolic/genetic disease	Cancer	ALF	Cholestatic disease	Metabolic/genetic disease	Cancer	ALF	Cholestatic disease	Metabolic/genetic disease	Cancer
Conventional coagulation tests												
aPTT, s	54 (49–62)	40 (32–54)	36 (31–67)	35 (28–42)	109**** (75–180)	70**** (48–180)	55* (39–70)	59**** (39–180)	86**** (74–122)	98**** (70–180)	137**** (42–180)	180**** (50–180)
PT, INR	2.53 (2.07–3.60)	1.57 (1.35–1.91)	1.24 (1.19–2.01)	1.16 (1.12–1.19)	2.40 (1.97–3.30)	2.00 (1.75–2.73)	1.61 (1.48–1.88)	1.54 (1.29–1.82)	2.19 (1.94–2.57)	2.42 (2.06–2.97)	1.77**** (1.57–2.47)	2.28 (1.57–2.57)
PLT, ×10 ⁹ /mm ³	66 (40–101)	144* (90–234)	132 (76–216)	168 (122–214)	65 (40–86)	134**** (79–205)	99 (68–218)	138 (95–193)	55 (42–89)	118**** (72–184)	110 (77–152)	108**** (91–191)
Fibrinogen, mg/dl	98 (90–104)	159* (107–231)	116** (85–123)	189**** (158–230)	73 (62–98)	95**** (69–136)	78 (67–114)	129**** (86–145)	90 (78–102)	81**** (60–108)	71 (46–124)	84**** (60–112)
ROTEM® measurements												
INTEM CT, s	246 (224–279)	209 (182–245)	212 (184–276)	209 (171–226)	263 (226–310)	240**** (200–307)	218 (202–242)	216 (175–293)	267 (222–290)	286**** (225–329)	255 (230–300)	262 (234–275)
INTEM CFT, s	230 (140–366)	118 (65–194)	196 (144–306)	104 (82–122)	236 (193–380)	147 (89–227)	300 (188–371)	170 (108–244)	278 (233–338)	189**** (124–326)	278 (170–383)	248 (121–403)
EXTEM CT, s	91 (73–104)	67 (53–92)	76 (62–100)	57 (54–63)	70 (50–90)	67 (52–123)	86 (57–115)	86 (80–89)	66 (62–82)	88 (59–129)	76 (76–76)	113 (88–116)
EXTEM CFT, s	227 (148–328)	125 (84–216)	178 (136–318)	108 (86–126)	283 (221–360)	165 (111–341)	393 (330–456)	194 (164–254)	235 (161–270)	216**** (148–404)	181 (181–181)	341 (296–384)
EXTEM MCF, mm	42 ± 9	53 ± 14*	47 ± 13	56 ± 8*	39 ± 7	44 ± 17****	32 ± 2	44 ± 14	40 ± 10	42 ± 13****	45 ± 0	37 ± 5****
FIBTEM MCF, mm	6.0 (5.5–7.5)	12.0* (8.0–15.0)	4.0 (4.0–13.0)	11.5* (10.0–16.0)	5.0 (4.0–7.0)	7.0**** (5.0–11.0)	4.0 (3.0–5.0)	7.0**** (5.5–13.0)	5.0 (5.0–7.0)	6.0**** (4.0–8.0)	7.0 (3.0–8.0)	5.5**** (4.0–7.0)

Note: Data are presented as median (25th–75th percentiles) or mean ± standard deviation.

Abbreviations: ALF, acute liver failure; aPTT, activated partial thromboplastin; CFT, clot formation time; INR, international normalized ratio; MCF, maximum clot firmness; PLT, platelet count; PT, prothrombin time; ROTEM®, rotational thromboelastometry.

*p < 0.05 versus ALF; **p < 0.05 versus cholestatic disease; ***p < 0.05 versus metabolic/genetic disease; ****p < 0.05 versus anhepatic by analysis using Bonferroni's method.

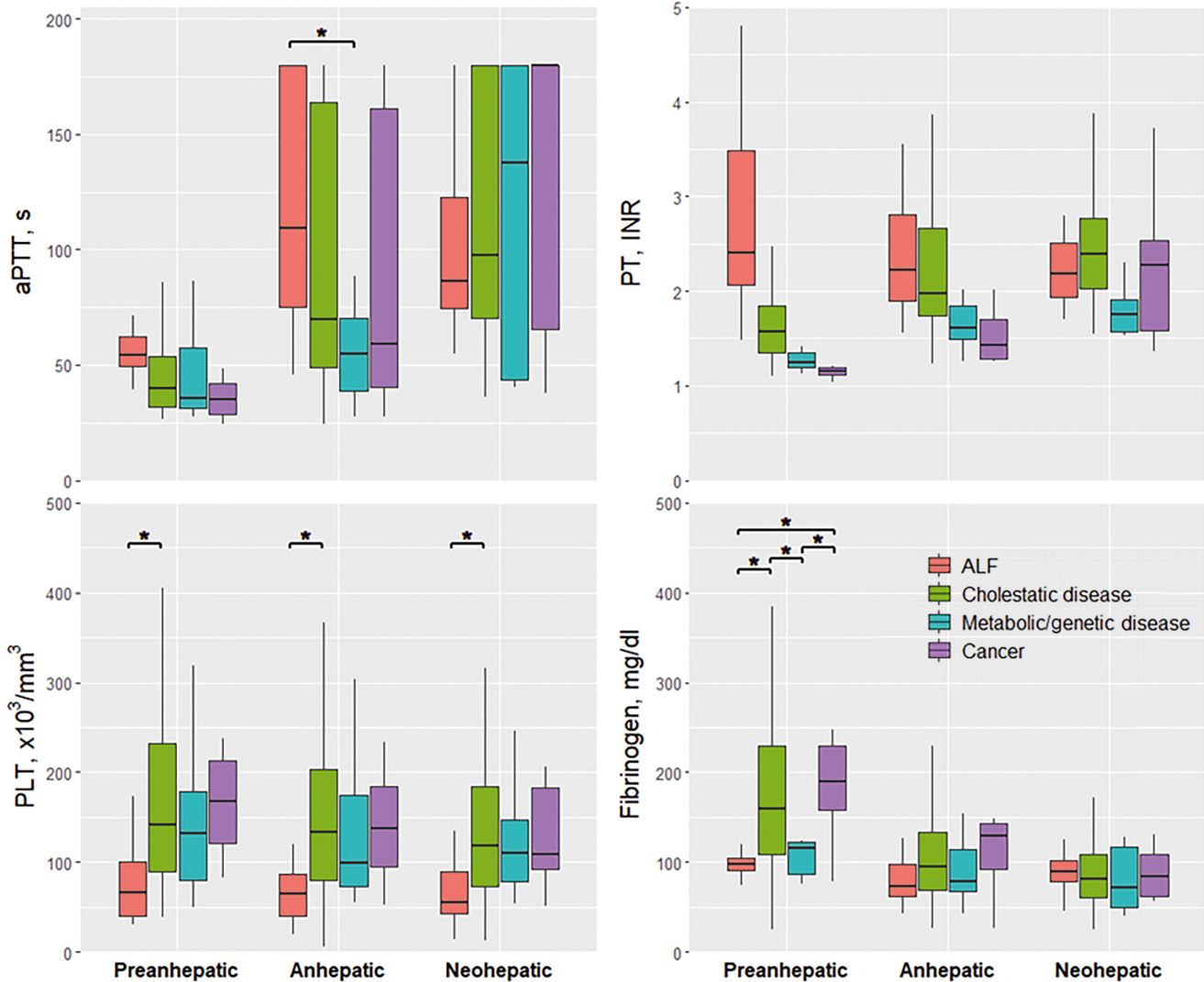


FIGURE 2 Trends of conventional coagulation tests during pediatric LT. In children with ALF, preanhepatic measurements of conventional coagulation tests revealed more hypocoagulable states than those with other liver diseases (cholestatic disease, metabolic/genetic disease, and cancer). Throughout the LT phases, PLT was significantly lower in ALF compared with cholestatic disease. The fibrinogen level was also lower in ALF than in the cholestatic disease and cancer groups. During the neohepatic phase, there was no significant difference in conventional coagulation tests between the groups except for the PLT. The median value is shown as a solid line, whereas the box indicates the 25th–75th percentile range. Whiskers extend to the 5th and 95th percentiles. * $p < 0.05$

disease groups across the anhepatic and neohepatic phases except aPTT and PLT. We also found that MCF on EXTEM and FIBTEM could reliably predict thrombocytopenia and hypofibrinogenemia, respectively. Meanwhile, CT on INTEM and EXTEM showed poor associations with aPTT and PT, especially within the high limits of aPTT and PT. Marked prolongation of aPTT or PT may not necessarily be accompanied by an increase in CT or CFT and vice versa.

In children with ALF, hemostatic status by conventional coagulation tests indicates hypocoagulability represented by the prolongation of aPTT and PT. All coagulation factors are markedly decreased during the synthetic functional deficiency of the liver parenchyma during fulminant liver failure.^[26] Our results also

showed that all parameters of the conventional coagulation tests in the ALF group were abnormally reduced or prolonged. Most measurements of ROTEM[®] in the ALF group were out of the reference range^[20] (55% for increased INTEM CT, 85% for increased INTEM CFT, and 63% for decreased EXTEM MCF). However, the further deterioration of coagulation profile across LT phases was not found in ALF because of baseline severe coagulopathy in ALF and the subsequent high transfusion rates of FFP, PC, and cryoprecipitate during LT.

On the other hand, clinical features of cholestatic cirrhosis in children include ascites, splenomegaly, and thrombocytopenia attributed to portal hypertension rather than severe coagulopathy. In our study,

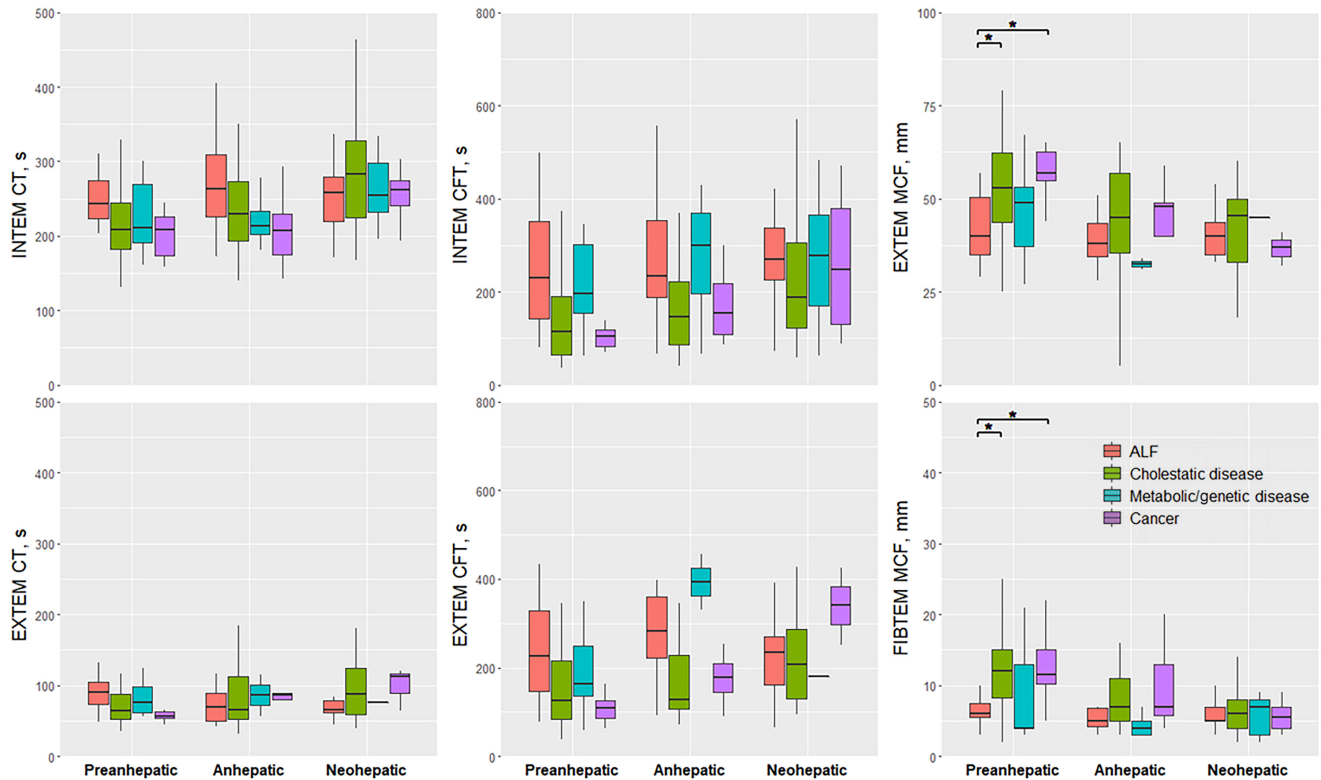


FIGURE 3 Changes in ROTEM® parameters during pediatric LT. Preanhepatic ROTEM® measurements show more hypocoagulable states in children with ALF than in those with other liver diseases (cholestatic disease, metabolic/genetic disease, and cancer). Notably, ALF has lower EXTEM and FIBTEM MCF values than the cholestatic disease and cancer groups. However, the differences disappear in the anhepatic and neohepatic phases with escalating coagulopathy in patients with other liver diseases. The median value is shown as a solid line, whereas the box indicates the 25th–75th percentile range. Whiskers extend to the 5th and 95th percentiles. * $p < 0.05$.

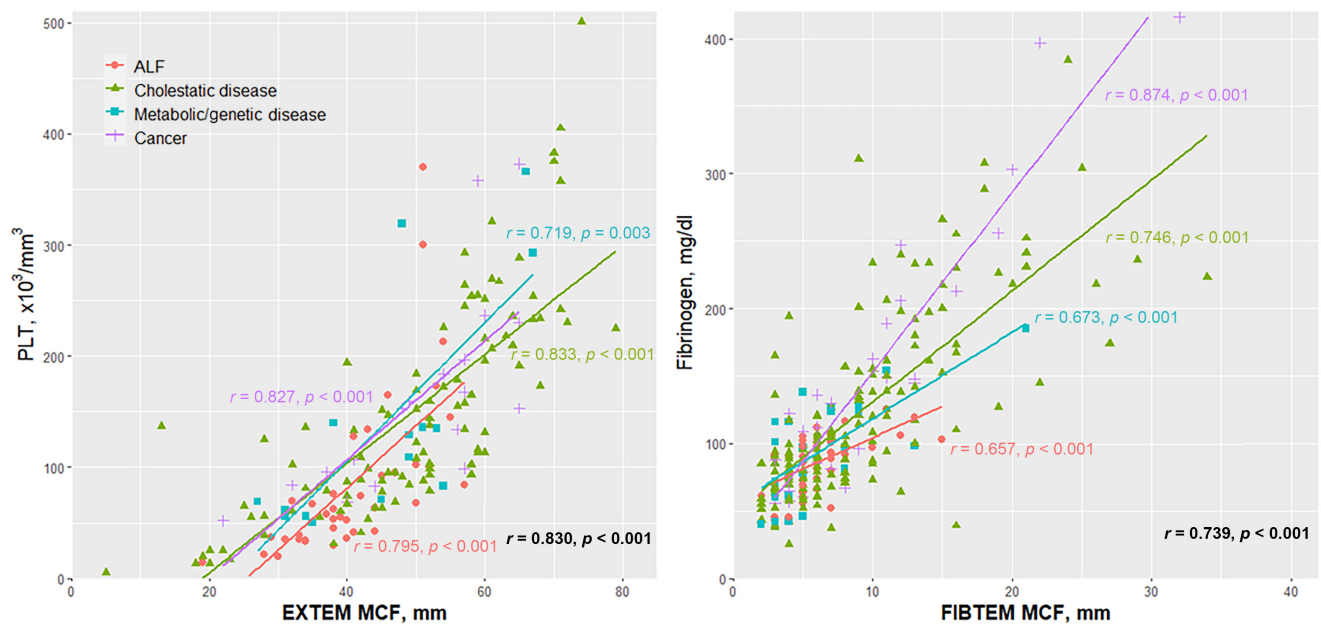


FIGURE 4 Correlations between conventional coagulation tests and ROTEM® parameters. PLT is significantly correlated with the EXTEM MCF ($r = 0.830$, $p < 0.001$). The fibrinogen concentration is also highly correlated with the MCF on FIBTEM ($r = 0.739$, $p < 0.001$). The correlations between the conventional coagulation tests and the ROTEM® parameters are also significant when the data are analyzed according to the etiology of the liver disease (ALF, red circle; cholestatic disease, green triangle; metabolic/genetic disease, blue square; and cancer, purple cross)

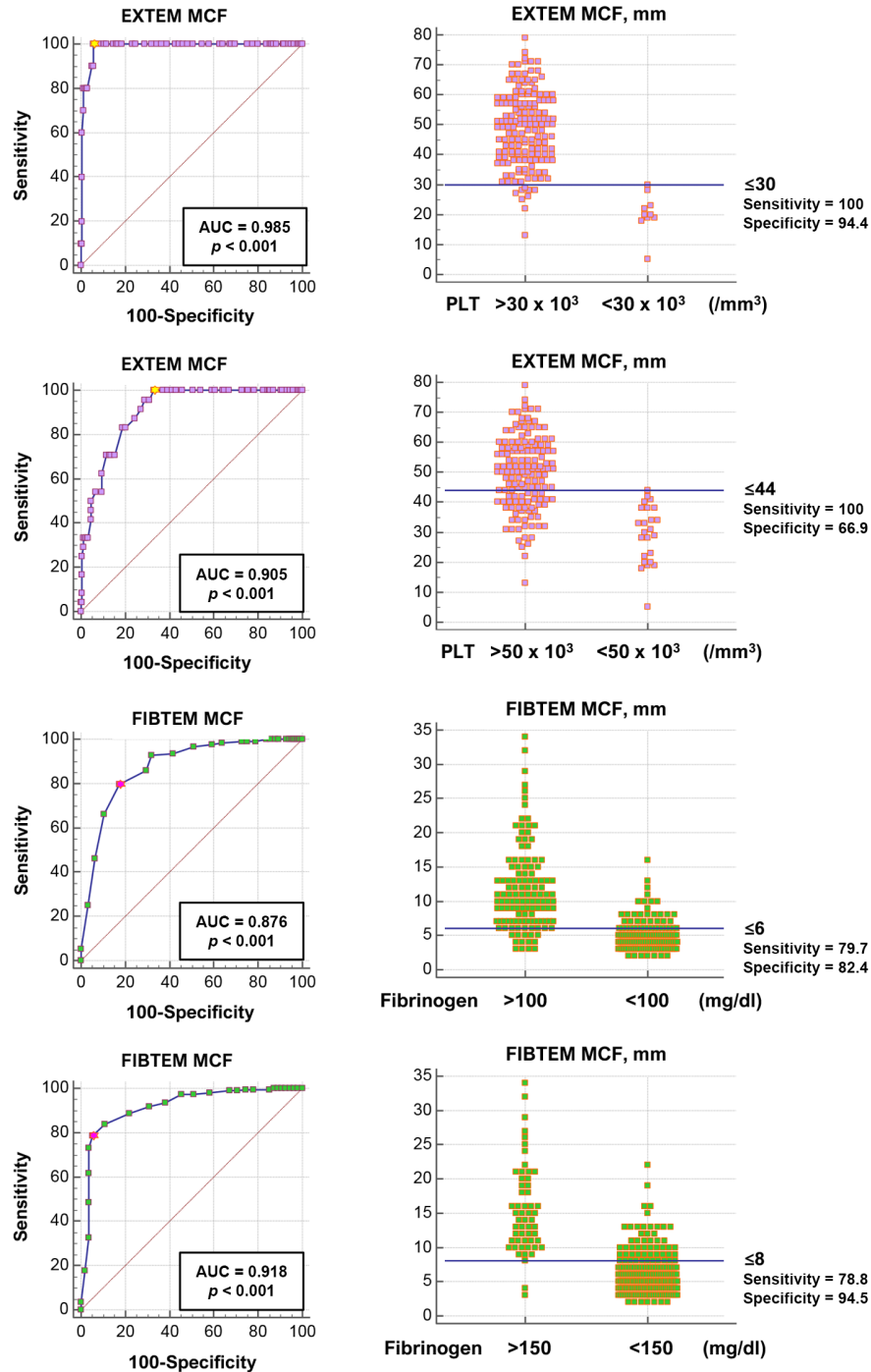


FIGURE 5 ROC curve analysis with the AUC of EXTEM and FIBTEM predicting thrombocytopenia and hypofibrinogenemia. On the EXTEM, MCF with 30 mm predicts PLT $<30,000/\text{mm}^3$ (AUC, 0.985), and 6 mm of MCF predicts fibrinogen concentration <100 mg/dl on the FIBTEM (AUC, 0.876)

baseline coagulation of patients with cholestatic disease was relatively preserved. This might be explained by the fact that most cases in the cholestatic disease group were biliary atresia, which involves abnormal cholestasis rather than decreased hepatic synthetic function.^[15] The metabolic/genetic disease group showed indistinct features of hemostasis because of the wide spectrum of disease entity, which was

composed of disorders with enzyme defects (ornithine transcarbamylase deficiency, primary hyperoxaluria, and methylmalonic acidemia), congenital absence of the portal vein, and Wilson's disease. Metabolic/genetic disease rarely affect coagulation system, because deficient synthesis is associated with specific metabolic pathways related to extrahepatic organ function.^[27,28] Patients with a congenital absence of

the portal vein also show normal liver function in preoperative tests and an absence of cholestasis in permanent liver biopsy.^[29] However, distinct from other metabolic diseases, the coagulation profile of Wilson's disease was associated with an acute exacerbation of liver function on chronic disease. Most cases in the cancer group were hepatoblastoma (9 of 11), which may not induce liver dysfunction and portal hypertension. However, coagulation function may be influenced by procoagulant features related to neoadjuvant cisplatin or thrombocytosis by thrombopoietin via tumor cells.^[30,31]

During LT, coagulopathy becomes further exacerbated by several causes. In the preanhepatic phase, surgical trauma, anatomical complexity, and a history of a prior operation could cause significant bleeding, resulting in the consumption of coagulation factors and dilutional coagulopathy related to massive transfusion.^[32] Hemostatic derangement in the anhepatic phase is further worsened by hyperfibrinolysis. After graft reperfusion, ischemic/reperfusion injury, heparin-like effects, and tissue plasminogen activator release also contribute to abnormalities in the coagulation system.^[33,34] Consequently, hypocoagulability in CLD group remarkably deteriorate after graft reperfusion, as well as ALF group.

Although children undergoing LT have "hypocoagulable" characteristics, their actual hemostatic function represented by clot firmness and strength on the ROTEM[®] are often preserved, which may be explained by "rebalanced hemostasis."^[35] As liver function progressively decreases, anticoagulation involving clot lysis is also reduced along with procoagulation factors. A decrease in PLT is sustained, but plasma von Willebrand factor increases with decreased a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, known as von Willebrand factor-cleaving protease to act as a facilitator of platelet adhesion.^[36,37] However, rebalanced hemostasis in liver disease is unstable and at risk of bleeding and thrombosis within a narrow functional reserve. Therefore, it is crucial to precisely assess the actual hemostatic function in real time. In this regard, conventional coagulation tests including aPTT and PT have critical shortcomings for the comprehensive assessment of coagulation function because aPTT and PT cannot reflect anticoagulation factors such as protein C, antithrombin, tissue factors, and endothelial function.^[26] In fact, children who have severely prolonged aPTT and PT show a variety of distributions of CT values on the INTEM and EXTEM, respectively. The discrepancy between aPTT/PT and INTEM/EXTEM CT is in line with a previous study on children with dilutional coagulopathy during major surgery that showed only a modest correlation between EXTEM CT and PT ($r = 0.460$) and between INTEM CT and aPTT ($r = 0.723$).^[9] Collectively, conventional coagulation time and CT from the ROTEM[®] are not interchangeable in children undergoing LT. Given the fact that there is limited reserve of hemostatic

balance in these patients, transfusions based only on the results from conventional tests may break the rebalanced state within a narrow range, resulting in bleeding or thrombosis.

Using the ROC curve analysis, we found clot firmness parameters (i.e., MCF) on EXTEM and FIBTEM could be a reliable parameter to predict thrombocytopenia and hypofibrinogenemia in children undergoing LT. In pediatric LT, the threshold of EXTEM MCF for predicting a PLT of $30,000/\text{mm}^3$ was higher (30 mm) and FIBTEM MCF predicting fibrinogen of 100 and 150 mg/dl were lower (6 and 8 mm, respectively) than those of adult LT.^[38] Considering that ROTEM[®] is a functional test, these results suggest that clot firmness might be maintained even with a range of very low PLT. On the contrary, clot firmness may be maintained at a relatively higher level of fibrinogen. Consequently, the transfusion threshold during pediatric LT should be determined according to the MCF on the ROTEM[®] and clinical bleeding rather than the PLT or fibrinogen level.

In this study, several limitations need to be considered. First, its retrospective study design could have resulted in selection and information bias. Second, when identifying the difference in baseline coagulation profiles according to the etiology of the liver disease, we analyzed samples measured during the preanhepatic phase instead of the earlier phase to include as many samples as possible in the analysis. Because our study was a retrospective observational study, there were some missed samples, especially before the preanhepatic phase. However, because the time interval from induction to the preanhepatic phase is relatively short in the pediatric population, there might be fewer possibilities of a change in the coagulation state until the preanhepatic phase. Thus, it would be allowable to adopt the time point of the preanhepatic phase for analyzing the baseline coagulation status. Third, despite the developmental changes in hemostasis in children, especially until 6 months of age,^[14] we could not consider coagulation profiles according to the age of the recipients because only a few recipients were younger than 6 months old. Fourth, although we grouped study patients into four groups based on clinical characteristics, the small number of patients in each of group might limit significant statistical differences in comparing coagulation profiles between groups. Finally, because our study was focused on pediatric LT, resulting in a small number of study samples, it was difficult to validate the ROC curve analysis by splitting samples into training and test cohorts. We performed bootstrap internal validation and identified the significant relations in the estimated cutoff values. Nevertheless, our results should be considered as preliminary, and further evaluation and external validation in a large cohort are warranted to validate our results.

In conclusion, in children undergoing LT, coagulation profiles are characterized depending on etiologies of liver disease. Throughout the phases of LT, hemostatic function is deranged in all groups, which can be timely

monitored by ROTEM[®] tests. Notably, clot firmness on thromboelastometry could reliably discriminate thrombocytopenia and hypofibrinogenemia during pediatric LT. This result suggests that ROTEM[®] can be a valuable point-of-care test for guiding transfusion of PLT and fibrinogen in children undergoing LT. Further data are needed to ascertain whether transfusion guided by thromboelastometry contributes to the outcomes related to thrombosis and bleeding in pediatric LT.

CONFLICT OF INTEREST

Nothing to report.

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SUPPORTING INFORMATION

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