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간이식 환자에서 항트롬빈 제제의 투여 방법에 따른 농도 변화

Continuous versus Intermittent Infusion of Human Antithrombin III Concentrate in the Immediate Postoperative Period after Liver Transplantation

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김 보 림

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이 논문을 의학박사 학위논문으로 제출함

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Abstract

Continuous versus Intermittent Infusion of Human Antithrombin III Concentrate in the Immediate Postoperative Period after Liver Transplantation

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Background: Antithrombin-III (AT-III) concentrates have been used in the immediate postoperative period after liver transplantation to prevent critical thrombosis. In the preceding study that retrospectively analyzed the pharmacokinetics of AT-III in liver recipients, plasma AT-III activity level was expected to be more stably maintained in the target range (80-120%) with a continuous infusion method than with a conventional intermittent infusion method. Thus, this study was performed to investigate whether the continuous infusion is more appropriate method for AT-III concentrate administration to maintain plasma AT-III activity level within targeted range compared to intermittent infusion method.

Methods: In this randomized controlled trial, 130 adult patients undergoing living-donor liver transplantation were randomized to either intermittent group or continuous group. In intermittent group, 500 international units (IU) of AT-III concentrate were administered after liver transplantation and repeated every 6 hours for 72 hours. In continuous group, 3000 IU of AT-III was continuously infused for 71 hours after a loading dose of 2000 IU over 1 hour. Plasma AT-III activity level was measured at 12, 24, 48, 72, and 84 hours from the first AT-III administration. The primary outcome was the target attainment rate at 72 hours. Target attainment rates at other time points and associated complications were collected as secondary outcomes.

Results: A total of 107 patients were included in the analysis. The target attainment rates at 72 hours post-dose were 30% and 62% in intermittent group and continuous group, respectively. (p = 0.003) Compared to intermittent group, patients in

continuous group reached the target level more rapidly (12 vs. 24 hours, median time, $p < 0.001$) and was more likely to remain in the target range until 84 hours.

Conclusions: For maintaining the target plasma AT-III activity level after living-donor liver transplantation, continuous infusion of AT-III seemed to be more appropriate compared to the conventional intermittent infusion regimen.

Keywords : antithrombin, coagulation, dose, liver, thrombosis, transplantation

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PRECEDING STUDY

Pharmacokinetics of Human Antithrombin III Concentrate in the Immediate Postoperative Period after Liver Transplantation

Background

Antithrombin III (AT-III) is a liver-synthesized glycoprotein that acts as a natural anticoagulant and functions as a major inhibitor of thrombin and other components of the coagulation cascade such as factors IXa, Xa, XIa, and XIIa, as well as plasmin [1, 2]. Inactivation of coagulation factors by AT-III is accelerated in the presence of heparin, making AT-III a key component in heparin-mediated anticoagulation [3]. AT-III deficiency or decreased activity may result from congenital deficiency or acquired conditions such as liver dysfunction, sepsis, cardiopulmonary bypass or major surgical procedures. Since decreased AT-III activity level is associated with increased risk of vascular thrombosis or embolism [4, 5], AT-III replacement is recommended for high-risk patients, especially in the presence of thrombophilic conditions [6]. Moreover, previous studies have reported positive effects of AT-III supplementation on anticoagulation in such patient population [7-9].

Hepatic artery thrombosis (HAT) is the most critical complication in the immediate postoperative period after liver transplantation, with a reported incidence of 4-15% [10]. Portal vein thrombosis (PVT), which has been reported to occur in 2-7% of liver recipients, is another technical complication that may compromise graft survival [11]. Both HAT and PVT may lead to acute graft failure, sepsis, and biliary complications, requiring endovascular or surgical interventions, and ultimately re-transplantation. Risk factors of thrombotic complications include hypercoagulability, poor surgical technique, and anatomical variation [10, 12, 13]. A hypercoagulable state due to the imbalance between stimulated coagulation activity and impaired fibrinolysis activity is often observed in the early postoperative period after liver transplantation [14]. Preoperative prolonged hepatic dysfunction and delayed recovery of the

transplanted graft may lead to depletion of anticoagulation factors. Plasma AT-III activity levels have been reported to decrease to as low as 80% of the preoperative level in the early post-transplant period and gradually normalize in the first 2 weeks after transplantation [3, 14, 15]. Since lower plasma activity levels of AT-III might be associated with hepatic artery thrombosis, AT-III supplementation has been widely considered. However, the optimal range of plasma AT-III activity level in the immediate postoperative period after liver transplantation is not known.

AT-III concentrate is a human plasma-derived agent obtained from healthy donors that is processed with modified Cohn ethanol separation and heated for viral inactivation [16, 17]. Intensive anticoagulation therapy including AT-III was reported to markedly decrease the incidence of HAT after pediatric liver transplantation [18]. Kaneko et al. advocated AT-III administration early after liver transplantation based on their results of a pilot study that it might reduce fibrin degradation product D-dimer (FDP-DD) levels [14]. Another study showed that lower AT-III activity was associated with portal vein thrombosis after splenectomy in patients with liver cirrhosis [19]. AT-III concentrate administration might help prevent post-operative PVT in these patients. Furthermore, AT-III concentrate may potentially be an effective treatment for PVT in patients with liver disease [20].

AT-III concentrates have been used to prevent PVT or HAT in the early post-operative period after liver transplantation. With little evidence to guide AT-III therapy, the dosage has been determined based on the surgeon's preference. Consequences of inadequate titration may result in potentially serious complications such as postoperative bleeding. Therefore, the relationship between the AT-III dosage and the plasma activity levels of AT-III during the immediate postoperative period after liver transplantation was evaluated.

Methods

Study design

This study was a retrospective cohort study and the study protocol was approved by the institutional review board of Seoul National University Hospital (H-1804-114-939). Informed consent was waived by the institutional review board due to the retrospective nature of the study design.

Patient selection and data collection

Adult Patients (>18 years) who underwent living or deceased donor liver transplantation at Seoul National University Hospital from January 2017 to September 2018 were identified. Patients who received AT-III concentrates postoperatively and had records of pre- and post-dose AT-III activity levels were included in the analysis. Pediatric liver transplant patients were excluded due to separate postoperative anticoagulation protocol regarding smaller vessel size and complex vascular reconstruction [21, 22]. Patients with incomplete data or who underwent liver re-transplantation were excluded as well to avoid potential bias. Patients who underwent liver transplantation from January 2017 to May 2018 were included in the model development dataset, whereas patients who underwent liver transplantation between June 2018 and September 2018 were included in the external validation dataset.

Data were extracted from electronic medical records from April to June of 2018 through retrospective chart review and clinical data warehouse. Baseline patient characteristics including age, sex, weight, diagnosis, donor status (living or deceased), graft-recipient weight ratio, and concomitant administration status of heparin were recorded. The dose and administration time of AT-III, as well as the plasma AT-III activity level and the sampling time were retrieved from the electronic medical records.

Antithrombin III administration protocol

During the first 7 days after liver transplantation, 500 international units (IU) of AT-III were infused every 6 hour per protocol unless there were evidence of postoperative bleeding or hypotension requiring vasopressors. Since there is no sufficient evidence regarding AT-III concentrate dosing for liver transplantation recipients, this protocol is based on clinician's experience and preference. The initial dose of AT-III concentrate was administered as soon as the patient was transferred to intensive care unit after the operation.

Antithrombin III measurement protocol

Baseline (pre-dose) plasma AT-III activity level was the plasma AT-III activity level routinely measured at the end of liver transplantation in the operating room. Subsequent sampling was performed from postoperative day (POD) 1, just before the first AT-III concentrate administration of the day. Additional sample was collected from POD 2 to POD 7, as clinically required. Exact time for each drug administration and blood draw were recorded.

Plasma AT-III activity level was measured with chromogenic assay using ACL-TOP 750 CTS (Instrumentation Laboratory, Lexington, MA, USA) based on factor Xa as the enzymatic source. Factor Xa forms a complex with functionally active AT-III when added to the plasma. When the chromogenic substrate (HemosIL Liquid Antithrombin, Instrumentation Laboratory) is added, the residual factor Xa generates color which is inversely proportional to the active AT-III level.

Population pharmacokinetic analysis

Nonlinear mixed-effects method was used to develop a population pharmacokinetic (PK) model using the NONMEM® software version 7.4 (ICON plc, Dublin, Ireland). The model parameters were estimated using the first-order conditional estimation with interaction method. The endogenously produced AT-III levels were incorporated in the model as follows;

Observed AT–III activity level (%) = Increased AT–III activity level from exogenous AT-III concentrate (%) + Endogenous AT–III activity level (%)

Single and multiple compartment models were evaluated to determine the basic structural model. The inter-individual variability (IIV) was modeled exponentially to the model parameters, and the residual variability was modeled with various error models (additive, proportional and combined error models).

A previous study of AT-III metabolism showed that AT-III was produced at a relatively constant rate in humans [23]. The elimination or distribution of endogenous AT-III was not affected by the addition of exogenous AT-III [24]. Therefore, the recovery of endogenous AT-III activity was modeled independent of the administration of exogenous dose of AT-III in this study. The recovery of endogenous AT-III activity level after liver transplantation was modeled using an E_{max} model as follows;

$$\text{Endogenous AT–III activity level (\%)} = \text{Pre–dose AT–III activity level} + \frac{\text{AT3}_{\max} \bullet \text{POD}}{\text{T}_{50} + \text{POD}}$$

, where the $AT3_{max}$ is the maximum recovered AT-III activity level during PODs and the T_{50} is the time to recover half of $AT3_{max}$ level [14]. Every subject had a pre–dose AT–III activity level, so the observed value was used for the model. However, we could not accurately estimate the parameters ($AT3_{max}$ and T_{50}) from our dataset because there were no patients who can represent the natural recovery course of endogenous AT-III activity level (i.e., those who did not received AT-III concentrate after the liver transplantation). Instead of estimating the $AT3_{max}$ and T_{50}, both parameters were fixed based on several assumptions. First, we assumed that the fully recovered AT-III level will be about 100%. The $AT3_{max}$ was set as 70, because the mean baseline (pre-dose) AT-III level was about 30%. Secondly, the T_{50} was initially fixed at 96 hours (4 days) based on the natural endogenous AT-III recovery profile observed in a published clinical study and sensitivity analysis was performed on T_{50} to identify a reasonable parameter value [14].

After the development of structural model and the error model, the effect of various covariates (age, sex, weight, diagnosis, donor status (living or deceased), graft-recipient weight ratio, estimated glomerular filtration rate (eGFR) calculated by modification of diet in renal disease study equation, albumin and platelet count) on the PK parameters were evaluated through the covariate screening. The potential covariates were screened by plotting against the empirical Bayesian estimates of PK parameters. A generalized additive model was also used for covariate screening. The continuous covariates were centered at the median values and modeled to the PK parameters using a power model. The categorical covariates were modeled to the PK parameters using indicator variables. The covariate selection was performed considering its statistical significance, reduction in the magnitude of IIV and precision of parameter estimates.

A likelihood ratio test, graphical evaluation, visual predictive checks (VPCs) were performed to assess the goodness of fit of model and the prediction performance of model. The precision of parameter estimates was evaluated by bootstrapping. The model parameters were estimated using 1000 bootstrap-resampled data and its median and 95% confidence interval (CI) were compared to the original

parameter estimates. The prediction performance of model for external validation dataset was evaluated by VPCs and by calculating percentage prediction errors (PE) as follows:

$$\mathrm{PE_{ij}}(\%) = \frac{(\mathrm{Cobs_{ij}} - \mathrm{Cipred_{ij}})}{\mathrm{Cipred_{ij}}} \bullet 100$$

, where the $\mathrm{Cobs_{ij}}$ is the observed plasma AT-III activity level in sample j from patient i, and $\mathrm{Cipred_{ij}}$ is the individual predicted AT-III activity level for that sample by the model. The series of PEs and absolute PEs (APE) were graphically presented. To evaluate bias and precision of the model prediction, the median PE (MDPE) and median APE (MDAPE) were calculated, respectively. If the MDPE value was between -20% to 20% and MDAPE value was lower than 30% were the prediction performance of PK model was regarded as clinically acceptable, based on the performance evaluation criteria of a typical target-controlled infusion model [25].

Simulations to optimize AT-III dose regimen

Using the population PK model, Monte Carlo simulations were performed to explore the plasma AT-III activity levels upon various dosing scenarios. The change of endogenous AT-III activity level during the PODs, and the increment of AT-III activity level by administration of AT-III concentrate were simulated. The simulated dose regimens included a published clinical study dose regimen (1500 IU per day intermittently, POD 0 through 2) and several alternative dose regimens required to fall within normal AT-III activity level range (80-120%) were explored [14].

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

Results

Data from 198 liver transplant patients were included in the analysis. Data from 161 patients were

included in the model development dataset and used to develop the population PK model and perform internal validation. Data from 37 patients were included in validation dataset and used to perform external validation for the model. Patient characteristics are summarized in table 1 and were similar between the model development dataset and the validation dataset except for body weight. There were no patients who were administered heparin during the liver transplantation or postoperative periods.

Population pharmacokinetic analysis results

To develop a population PK model and to externally validate the model, 516 and 185 plasma AT-III activity level-time data were used, respectively. The median (interquartile range, IQR) number of samples per patient were 3 (2 - 4) including one baseline sample, and the median (IQR) sampling time was 15 hours (0 - 68 hour) after the first dose. Most of the samples were taken during the first 5 days after liver transplantation (Figure 1). The sampling time points were regular and evenly distributed as the dosing intervals were regular (Figure 2).

The plasma AT-III activity levels were best described by a single compartment model with first order elimination kinetics. The model was parameterized in terms of the $AT3_{max}$, T_{50}, the volume of distribution (V) and the clearance (CL). The inter-individual variability was modeled exponentially to the, V, and CL (Figure 3). A proportional error model best described the residual variability.

The typical value of CL and V were 0.129 L h^{-1} and 3.86 L, respectively. The inter-individual variability (coefficient of variation, %) of CL and V were 66.6%, and 39.9%, respectively. Several T_{50} values were tested in sensitivity analysis, and the objective function value of model was lowest when the T_{50} values were fixed at 72 hours after the first dose (Table 2). Thereby the T_{50} value in the final model was fixed at 72 hours.

From the covariate screening, several covariates showed a significant association with CL and V (Table 3). The patient's body weight, donor status, sex, serum albumin and diagnosed disease had a significant association with CL. Serum albumin and body weight were included in the final model considering their statistical significance, reduction in the magnitude of IIV and precision of parameter estimates. The reduction of IIV were highest when the effect of albumin was included in the model. The

patient's body weight was highly correlated with sex, so only the body weight was included in the model to avoid multicollinearity. The effect of body weight on CL was modeled by a power model using 0.75 for exponents because body weight has a well-described scientific background and because the allometric relationship had been successfully applied in previous studies [26, 27]. The effect of donor status was not significant when the effect of albumin was included in the model. The precision of parameters was unacceptable when diagnosed disease were included in the model. The screened covariates did not result in decrease of IIV of V so the effect of covariates was considered only for CL (Table 4). The formulas for final model are as follows:

$$Y = f(\theta; t) + f(\theta; t) \bullet \varepsilon$$

$$f(\theta; t) = \frac{Dose}{V} \cdot e^{-\frac{CL}{V} \cdot t} + \text{Endogenous AT–III activity level}$$

$$\text{CL (L/h)} = 0.129 \bullet (\text{Albumin}/3)^{-2.26} \bullet (\text{Weight}/70)^{0.75} \bullet \exp(\eta)$$

$$\text{V (L)} = 3.86 \bullet \exp(\eta)$$

$$\text{Endogenous AT–III activity level (\%)} = \text{Pre–dose AT–III activity level} + \frac{70 \bullet \text{POD}}{72 + \text{POD}}$$

Based on goodness-of-fit plots, VPCs, and bootstrapping results (Figure 4, Figure 5, and Table 4), the established final model was robust and showed adequate prediction performance. The high observed AT-III levels (levels more than 150%) were under predicted on a population level, but those were well predicted on an individual level (Figure 4a and Figure 4b). The proposed final model also showed good prediction performance for external validation dataset based on VPCs, PEs, and APEs (Figure 5 and Figure 6). The MDPE and MDAPE were 8.1% and 26.2% respectively, and the prediction performance of PK model for external validation dataset was regarded as clinically acceptable.

Simulated plasma AT-III activity levels and proposed dosing regimens

Various dosing scenarios were simulated using the final population PK model to explain natural course of endogenous AT-III recovery after liver transplantation and to suggest optimal AT-III concentrate dosing regimens. The simulated endogenous AT-III activity level gradually normalized during the postoperative period, but it required more than 7 days after liver transplantation (Figure 7a).

The simulated serum AT-III activity level using regimen from a published clinical study (1500 IU per day intermittently, from POD 0 to POD 2) was successful in maintaining normal AT-III activity level range after the second dose (Figure 7b) [14]. A modified intermittent dose regimen (2000 IU at POD 0, followed by 1000 IU per day for POD 1 and 2) was also successful in maintaining normal AT-III activity level range. Moreover, the 95% prediction interval for simulated concentrations of the modified regimen was narrower than that of the previous regimen (Figure 7c). A continuous infusion regimen following a loading dose (loading dose, 2000 IU for 1 hours; maintenance dose, 3000 IU for 72 hours) was also successful in maintaining normal AT-III activity level in the early postoperative period after liver transplantation (Figure 7d).

Table 1 Patient characteristics of the preceding study

	Model development (n=161)	**Validation (n=37)**	***P*-value**
Age (years)	56 (19, 76)	58 (22, 76)	0.314
Height (meters)	1.65 (1.36, 1.81)	1.67 (1.42, 1.78)	0.991
Weight (kg)	64 (41, 106)	58 (42, 78)	0.002
Graft-recipient weight ratio	1.09 (0.39, 4.11)	1.23 (0.76, 1.78)	0.105
Baseline AT-III activity level (%)	30 (7, 83)	29 (12, 56)	0.525
eGFR (ml/min)	94.9 (6.8, 250.4)	106.3 (28.6, 174.4)	0.933
Serum Albumin (mg/dL)	3.1 (1.1, 4.8)	3.4 (1.9, 4.2)	0.213
Platelet count ($\times 10^3$/mL)	67 (12, 551)	58 (22, 170)	0.265
Sex			0.684
Male	112 (69.6)	27 (73)	
Female	49 (30.4)	10 (27)	
Diagnosis			
Hepatitis B liver cirrhosis	78 (48.4)	23 (62.2)	0.132
Hepatitis C liver cirrhosis	10 (6.2)	5 (13.5)	0.130
Alcoholic liver cirrhosis	52 (32.3)	9 (24.3)	0.343
Hepatocellular carcinoma	72 (44.7)	18 (48.6)	0.665
Hepatitis	4 (2.5)	0 (0.0)	0.333
Others	25 (15.5)	4 (10.8)	0.464
Donor status			0.546
Living donor	137 (85.1)	30 (81.1)	
Deceased donor	24 (14.9)	7 (18.9)	

Data are expressed as median (min, max) or number (%)

Abbreviations: AT-III, antithrombin III; eGFR, estimated glomerular filtration rate; GRWR, graft-recipient weight ratio

Table 2. Sensitivity analysis result for T_{50} [1]

	Model 201	Model 202	Model 203	Model 204	Model 205	Model 206	Model 207	Model 208	Model 209
Objective function value	2996.63	3028.689	3007.061	2999.38	2996.876	2997.467	2998.844	3000.474	3002.202
ΔOFV with Model 201	-	32.059	10.431	2.75	0.246	0.837	2.214	3.844	5.572
Parameters estimate (%RSE)									
CL (L/h)	0.126 (7.2)	0.199 (13.6)	0.172 (9.8)	0.151 (8.5)	0.137 (7.7)	0.119 (6.8)	0.112 (6.7)	0.107 (6.4)	0.103 (6.5)
V (L)	3.76 (6.3)	7.29 (11.1)	5.14 (8.6)	4.35 (7.3)	3.97 (6.7)	3.62 (6)	3.53 (5.8)	3.47 (5.6)	3.42 (5.6)
$AT3_{max}$ (%)	70*	70*	70*	70*	70*	70*	70*	70*	70*
T_{50} (h)	72*	24*	36*	48*	60*	84*	96*	108*	120*
Inter-individual variability (IIV)									
IIV of CL (%)	76.4 (9.4)	160 (12.6)	108.4 (10.5)	91.1 (9.9)	82.1 (9.6)	72.6 (9.3)	69.8 (9.2)	67.6 (9.2)	65.9 (9.2)
IIV of V (%)	39.4 (24.9)	58.7 (18.9)	47.8 (23.5)	42.8 (24.6)	40.5 (24.9)	38.8 (24.7)	38.5 (24.4)	38.3 (24.1)	38.2 (23.8)
Residual variability (ε)									
Proportional residual error (%)**	12.7 (13.5)	13.7 (11)	13.1 (12.2)	12.9 (12.7)	12.8 (13.1)	12.6 (13.7)	12.6 (13.9)	12.5 (14.1)	12.5 (14.2)

* Parameters were fixed at this value

** $Y = f(\theta; x) + f(\theta; x) \bullet \varepsilon$

Abbreviations: OFV, objective function value; RSE, relative standard error (%); CL, clearance; V, volume of distribution; $AT3_{max}$, maximum increased Antithrombin III level by endogenous production; T_{50}, Time to reach half of the maximum Antithrombin III level by endogenous production, IIV, inter-individual variability (coefficient of variation, %)

[1] The table was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

Table 3. Covariate screening result [2]

Parameter	Covariate	Functional form	ΔOFV	Degree of freedom	*P*-value	ΔIIV (%)
-	Base model	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-	-	-	-
CL	Weight	$CL = \theta_{CL} \bullet (Weight/70)^{0.75} \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-4.589	1	0.0322	-1.6
	Age	$CL = \theta_{CL} \bullet (Age/57)^{\theta Age} \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-0.002	1	0.9643	0
	Donor status	$CL = \theta_{CL} \bullet (1 + \theta_{Donor}) \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-4.593	1	0.0321	-2.2
	eGFR	$CL = \theta_{CL} \bullet (eGFR/88.6)^{\theta eGFR} \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-2.498	1	0.114	2.5
	Sex	$CL = \theta_{CL} \bullet (1 + \theta_{Sex}) \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-7.907	1	0.0049	-2.3
	Albumin	$CL = \theta_{CL} \bullet (Albumin/3)^{\theta Albumin} \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-6.836	1	0.0089	-7.1
	GRWR	$CL = \theta_{CL} \bullet (GRWR)^{\theta GRWR} \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-3.753	1	0.0527	-2
	Diagnosis	$CL = \theta_{CL} \bullet (1 + \theta_{Dx1} \bullet Dx1 + \theta_{Dx2} \bullet Dx2 + \theta_{Dx3} \bullet Dx3 + \theta_{Dx4} \bullet Dx4 + \theta_{Dx5} \bullet Dx5 + \theta_{Dx6} \bullet Dx6) \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-13.288	5	0.0208	-6.7

[2] The table was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

	Platelet	$CL = \theta_{CL} \bullet (Platelet/64K)^{\theta Platelet} \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-2.524	1	0.1121	-3.1
V	Weight	$CL = \theta_{CL} \bullet (Weight/70)^{1} \bullet \exp(\eta)$ $V = \theta_V \bullet \exp(\eta)$	-4.75	1	0.0293	1.8
	Age	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet (Age/57)^{\theta Age} \bullet \exp(\eta)$	-0.732	1	0.3922	-0.5
	Donor status	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet (1 + \theta_{Donor}) \bullet \exp(\eta)$	-1.698	1	0.1925	0.1
	eGFR	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet (eGFR/88.6)^{\theta eGFR} \bullet \exp(\eta)$	-13.005	1	0.0003	1.1
	Sex	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet (1 + \theta_{Sex}) \bullet \exp(\eta)$	-4.248	1	0.0393	0.2
	Albumin	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet (Albumin/3)^{\theta Albumin} \bullet \exp(\eta)$	-0.217	1	0.6413	-0.6
	GRWR	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet (GRWR)^{\theta GRWR} \bullet \exp(\eta)$	-0.043	1	0.8357	0
	Diagnosis	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet (1 + \theta_{Dx1} \bullet Dx1 + \theta_{Dx2} \bullet Dx2 + \theta_{Dx3} \bullet Dx3 + \theta_{Dx4} \bullet Dx4 + \theta_{Dx5} \bullet Dx5 + \theta_{Dx6} \bullet Dx6) \bullet \exp(\eta)$	-12.351	5	0.0303	-3.7
	Platelet	$CL = \theta_{CL} \bullet \exp(\eta)$ $V = \theta_V \bullet (Platelet/64K)^{\theta Platelet} \bullet \exp(\eta)$	-13.117	1	0.0003	3.8

Abbreviations: OFV, Objective function value; IIV, inter-individual variability (coefficient of variation, %); CL, clearance; V, volume of distribution; eGFR, estimated glomerular filtration rate; GRWR, graft-recipient weight ratio

Table 4 Final population pharmacokinetic model parameters [3]

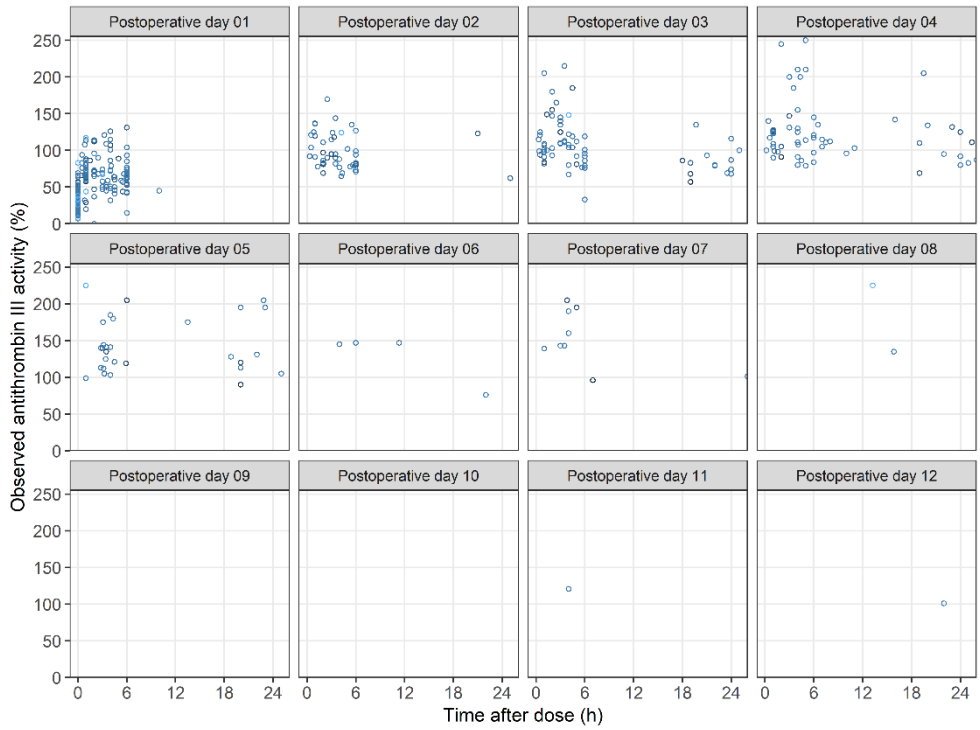
	Model Estimate		Bootstrap Result	
Parameters	Estimate (%RSE)	95% CI	Median	95% CI
CL (L/h)	0.129 (7)	(0.111 to 0.147)	0.129	(0.111 to 0.149)
Serum albumin on CL	-2.26 (30)	(-3.58 to -0.94)	-2.18	(-3.84 to -0.82)
V (L)	3.86 (6)	(3.40 to 4.32)	3.84	(3.42 to 4.41)
$AT3_{max}$ (%)	Fixed at 70		Fixed at 70	
T_{50} (h)	Fixed at 72		Fixed at 72	
Inter-individual variability (IIV)				
IIV of CL (%)	66.6 (11)	(47.8 to 83.4)	66.2	(47.2 to 85)
IIV of V (%)	39.9 (23)	(12.6 to 56.9)	39.6	(15.5 to 60.9)
Residual variability (ε)				
Proportional residual error (%)*	12.8 (13)	(9.5 to 16.1)	12.5	(9.1 to 15.8)

*$Y = f(\theta; x) + f(\theta; x) \cdot \varepsilon$

Abbreviations: RSE, relative standard error (%); CI, confidence interval; CL, clearance; V, volume of distribution; $AT3_{max}$, maximum increased antithrombin III level by endogenous production; T_{50}, Time to reach half of the maximum antithrombin III level by endogenous production; IIV, inter-individual variability (coefficient of variation, %)

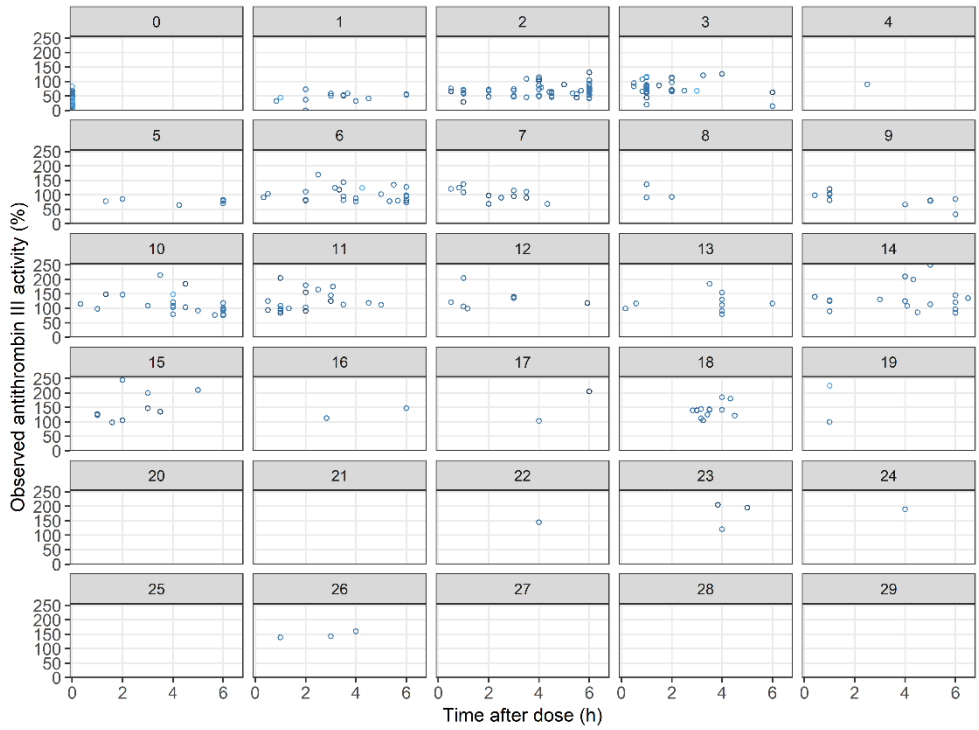
[3] The table was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

Figure 1. Distribution of plasma antithrombin III levels in the liver transplantation patients during postoperative days [4]



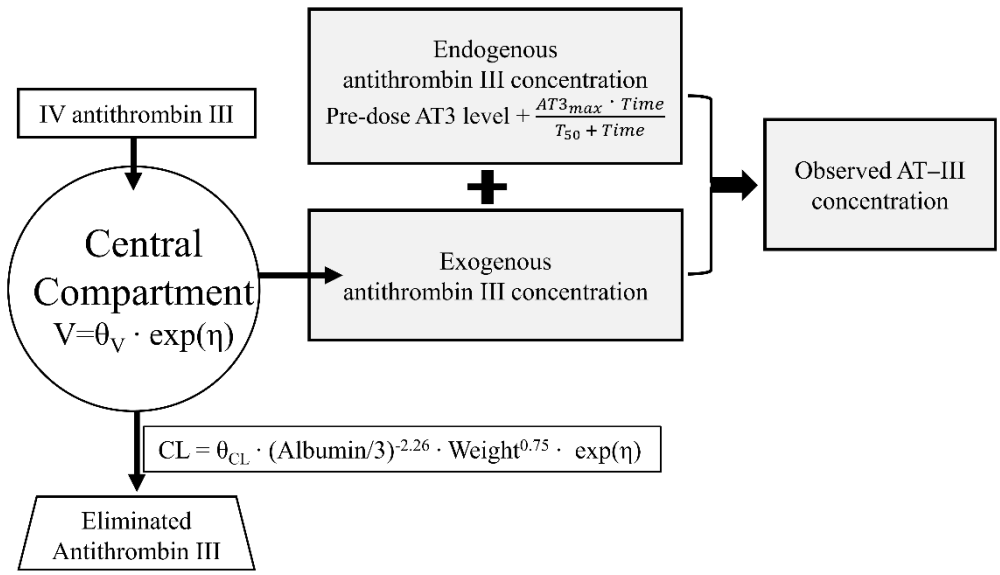
[4] The figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

Figure 2. Distribution of plasma antithrombin III levels in the liver transplantation patients during dosing intervals [5]



[5] The figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

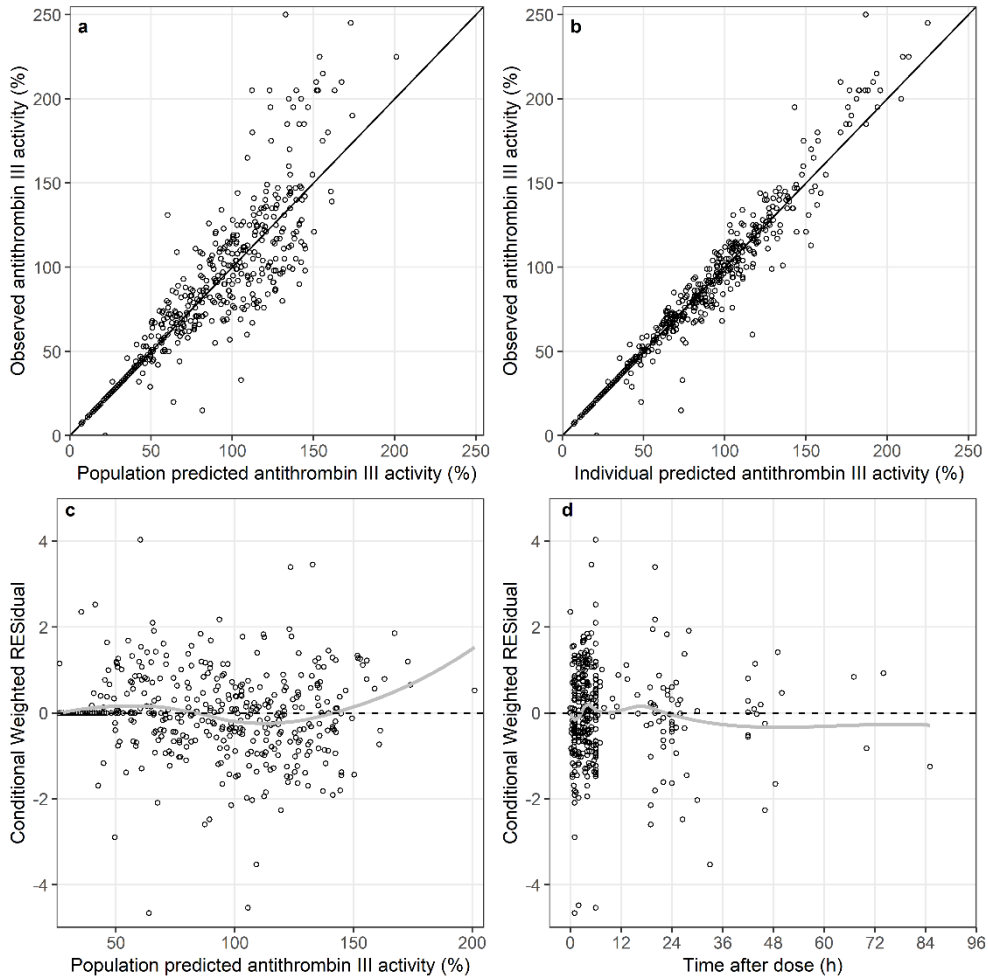
Figure 3. Final model scheme for the population pharmacokinetic analysis of AT-III[6]



Abbreviations: AT-III, antithrombin III; $AT3_{max}$, maximum increased antithrombin III level by endogenous production; CL, clearance; T_{50}, Time to reach half of the maximum antithrombin III level by endogenous production; V, volume of distribution, η, inter-individual variability; θ, typical population parameter values

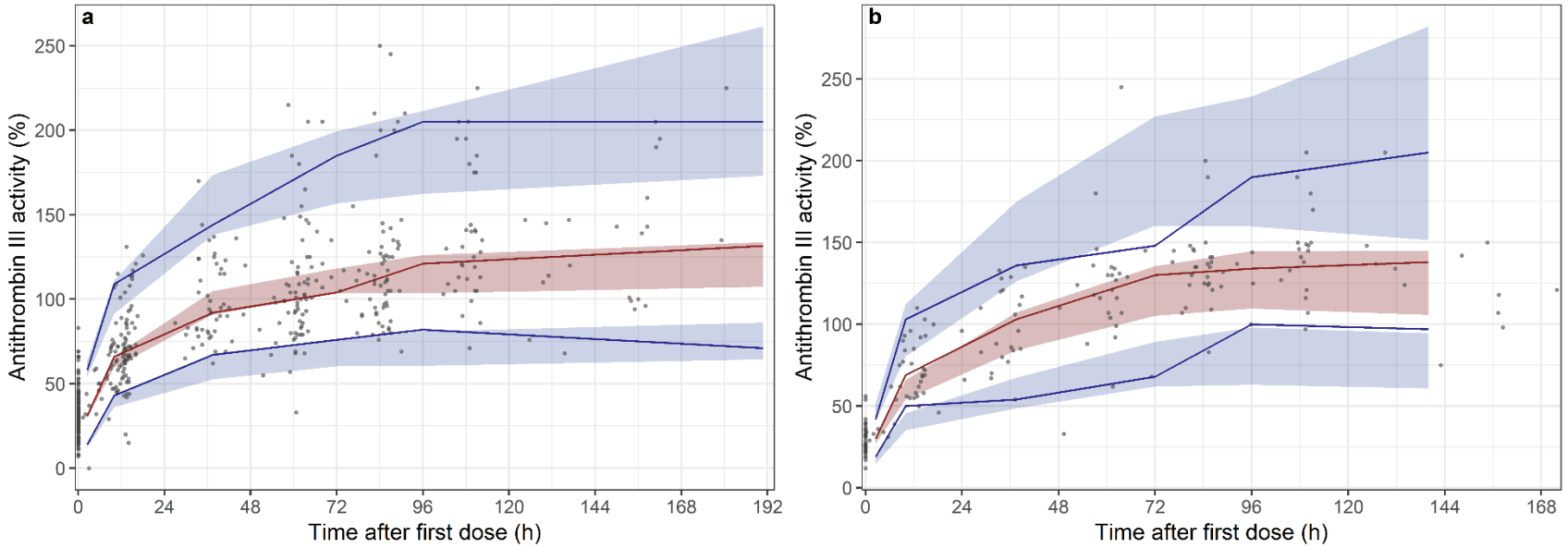
[6] The figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

Figure 4. Goodness of fit plots for the final model [7]



[7] This figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

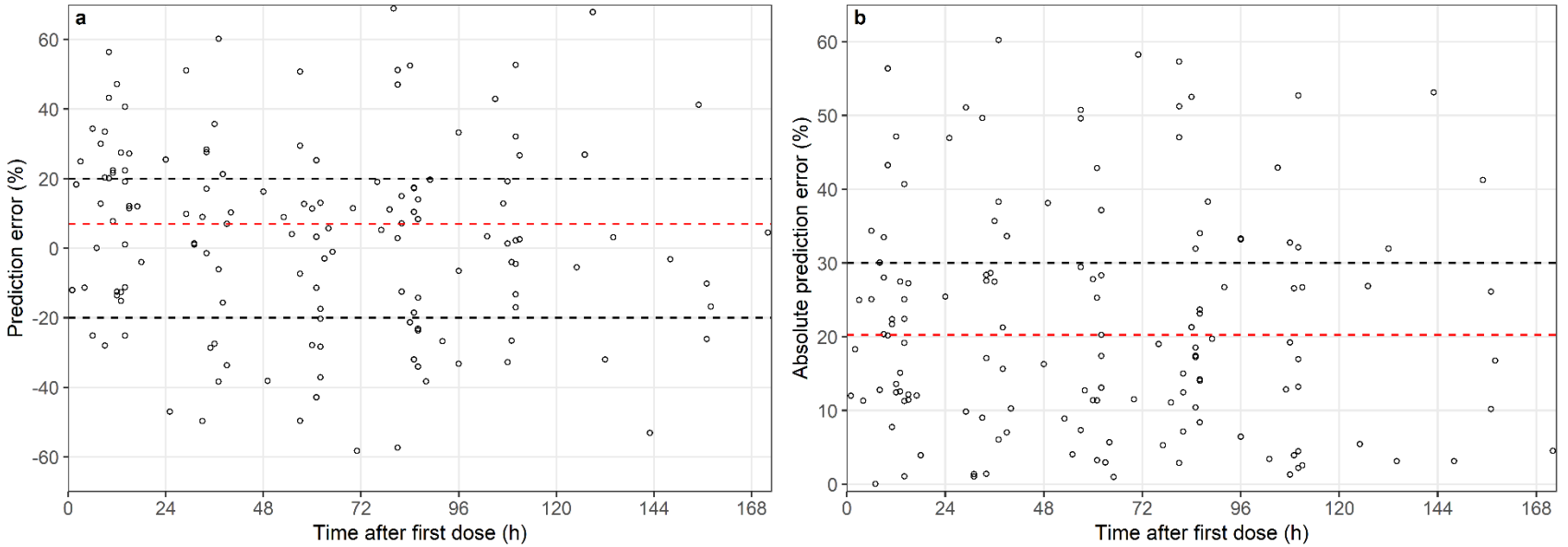
Figure 5. Visual predictive check plot for model development dataset (a) and external validation dataset (b) [8]



Circles represent observed antithrombin III plasma activity levels. The lines represent the median (red) and the 5th and 95th percentiles (blue) of the observed plasma activity levels. The shaded areas represent the 95% confidence intervals for the median (red) and the 5th and 95th percentiles (blue) of the simulated plasma activity levels

[8] This figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

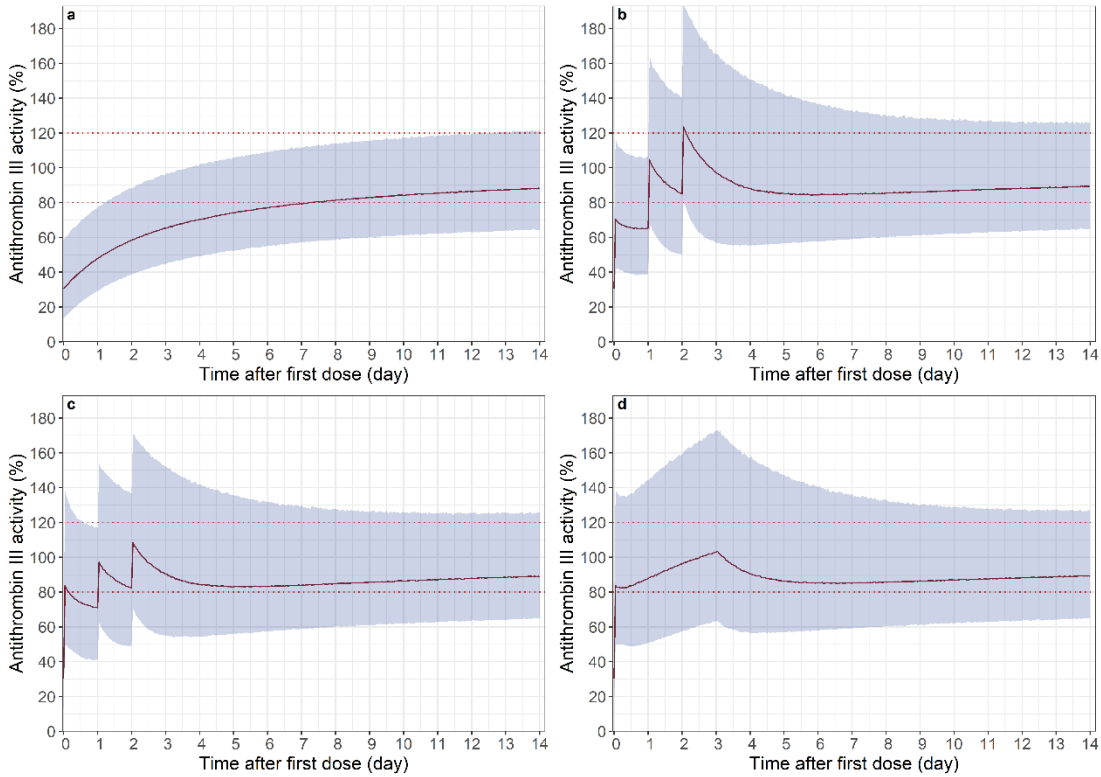
Figure 6. Prediction errors (a) and absolute prediction errors (b) of the final population pharmacokinetic model when applied to external validation dataset[9]



Red line denotes median prediction error (a) and median absolute prediction error (b). Hairline denotes proposed acceptable ranges for median prediction error and median absolute prediction error

[9] This figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

Figure 7. Simulated plasma AT-III activity levels according to various dosing scenarios[10]



Line, median prediction; area, 95% prediction interval; hairline, normal AT-III activity level range (80-120%)

(a) change of endogenous AT-III activity level during the postoperative periods, (b) 1500 IU per day intermittently, for postoperative day 0 through 2, (c) 2000 IU per day for postoperative day 0, followed by 1000 IU per day for day 1 and 2, (d) a continuous IV infusion regimen after a loading dose (loading dose, 2000 IU for 1 hours; maintenance dose, 3000 IU for 71 hours)

Abbreviations: AT-III, antithrombin III; IU, international units

[10] The figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

INTRODUCTION

Liver transplantation is a life-saving treatment option for patients with end-stage liver disease. The most recent 5-year survival rate after liver transplantation in the US reached 81.2% with advances in perioperative care including immunosuppressive agents, antibiotics, tissue preservation, and anticoagulation [28]. During the immediate postoperative period, AT-III has been advocated as an adjunct therapy to prevent critical thrombosis and improve surgical outcomes [3, 14, 29].

AT-III is a glycoprotein produced in the liver that inhibits thrombin and other factors of the coagulation cascade such as IXa, Xa, XIa, and XIIa, and plasmin [1, 2]. It is primarily eliminated from the circulation by hepatic clearance and degraded inside the hepatocytes [30]. The impact of surgery on the impaired coagulation function of a liver transplant recipient may cause further dysfunction of the coagulation system. After liver transplantation, plasma AT-III activity level may decrease to 80% of the pre-operative baseline level after liver transplantation and gradually recover with the resumed function of the liver graft over a few days [15, 31, 32]. In the immediate postoperative phase, there may be dysfunction of the anti-coagulation mechanism with reduced plasma AT-III activity level, increasing the possibility of thrombosis of anastomosed major vessels such as the hepatic artery and portal vein. The incidences of post-transplantation hepatic artery and portal vein thrombosis have been reported to be around 4-15% and 2-7%, respectively, which might be fatal to the prognosis [10, 11].

When using AT-III, titration of plasma AT-III activity level is essential as an

excessive dose of AT-III concentrate may promote postoperative bleeding and an insufficient dose may not be effective. On that account, we performed a preceding study that retrospectively analyzed the pharmacokinetics of AT-III in liver transplantation recipients and showed simulations of various dosing scenarios according to the developed model. According to the simulations, the plasma AT-III activity level was expected to be maintained more stably within the targeted range (80-120%) with a smaller total dose by the continuous infusion method with a loading dose than by the conventional intermittent infusion method (Figure 7d, Figure 8) [33].

Therefore, a randomized controlled trial was performed to compare the two AT-III administration methods in liver transplantation recipients. We hypothesized that the continuous infusion method of AT-III concentrate would be more appropriate in maintaining the plasma AT-III activity level within the target range compared to the intermittent infusion method.

METHODS

Patients

The study was approved by the Institutional Review Board of Seoul National University Hospital (IRB number: H-1911-100-1081) and registered to ClinicalTrials.gov (NCT 04219579) before recruitment. The study was designed and executed adhering to the guidelines for Good Clinical Practice and the Consolidated Standards of Reporting Trials.

Adult patients (>18 years old) scheduled to undergo elective living-donor liver transplantation between December 2019 and June 2022 were assessed for eligibility and written informed consent was obtained. Patients who refused to participate in the study or decided to participate in other clinical trials were excluded. Additionally, patients with high risk of significant postoperative bleeding, canceled operation or protocol violation were dropped out. In case of bleeding or thrombosis requiring intervention/re-operation, patients were excluded from the trial and the complications were collected as secondary outcomes.

Patients were randomly allocated into an intermittent group or a continuous group according to the computer-generated randomization table in a 1:1 ratio. An assistant who was not involved in the study performed the randomization, concealed the allocation in the opaque envelopes, and passed it on to the assigned nurse in the intensive care unit. The assigned nurse administered AT-III concentrate to the patients following the pre-determined protocol according to the group allocation. The investigators were not concerned in the AT-III concentrate administration

during the study period for concealment of the group assignment. The patients were also unaware of the group allocation as the drug was administered behind the screen shield over the patients' heads.

AT-III concentrate administration

AT-III concentrate was administered to all participants immediately after admission to the intensive care unit after liver transplantation. In intermittent group, 500 IU of AT-III concentrate were injected over an hour every 6 hours for 72 hours after admission to the ICU. In continuous group, a loading dose of 2000 IU was administered over an hour, followed by continuous infusion of 3000 IU of AT-III over 71 hours. Following the manufacturer's protocol, the syringe for continuous infusion was replaced every 12 hours to maintain the stability of reconstituted AT-III. In total, 6000 IU and 5000 IU of AT-III concentrate for intermittent group and continuous group were administered, respectively (Figure 9).

The plasma AT-III activity level at the end of the liver transplantation was recorded as the baseline value ($AT\text{-}III_{base}$). Plasma AT-III activity level was analyzed at 12, 24, 48, 72, and 84 hours (before the dose in the intermittent group) and the measured values were labeled as $AT\text{-}III_{12hr}$, $AT\text{-}III_{24hr}$, $AT\text{-}III_{48hr}$, $AT\text{-}III_{72hr}$, and $AT\text{-}III_{84hr}$, respectively (Figure 9). If the plasma AT-III activity level at a specific time point was higher than 120%, AT-III concentrate administration was withheld until the next measure plasma AT-III activity level was 120% or lower, at which AT-III concentrate administration was resumed.

Determination of plasma AT-III activity level

The plasma AT-III activity levels in plasma were determined by chromogenic assay using ACL-TOP 750 CTS (Instrumentation Laboratory, Lexington, MA, USA) and the factor Xa was used as enzymatic source. When factor Xa is added to plasma sample, it forms a complex with functionally active AT-III. Chromogenic substrate (HemosIL Liquid Antithrombin, Instrumentation Laboratory) was added to the mixture, and the active AT-III level was determined by the color change generated by residual factor Xa.

Outcome measurement

The primary endpoint was the proportion of patients whose plasma AT-III activity level at 72 hours was in the target range of 80-120%. The proportions of patients who showed the plasma AT-III activity level within the target range at 12, 24, 48, and 84 hours were collected as the secondary endpoints. In addition, the proportion of plasma AT-III activity level values within the target range among all collected samples, the time required for plasma AT-III activity level to reach the target range, and the incidence of postoperative bleeding requiring intervention or thrombosis events were also collected.

Safety evaluation

The safety was evaluated based on the emergence rate of bleeding or thrombosis event requiring intervention and clinical laboratory tests. Prothrombin time, platelet count, hemoglobin level and serum albumin were evaluated at baseline and 12, 24, 48, 72, 84 hours after admission to the ICU.

Statistical Analysis

Based on the pharmacokinetic model of the preceding study, the target attainment rates at 72 hours after liver transplantation were 40 % and 60 % for intermittent group and continuous group, respectively [33]. The sample size to show superiority of the primary endpoint in continuous group compared to the intermittent group was 57 patients in each treatment group with 70% power at a significance level of 0.05. Assuming a 15% dropout rate, a total of 130 subjects were purposed to be recruited.

SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses. Patients' demographics, baseline characteristics, results of primary and secondary endpoints, and safety data were summarized and presented by descriptive statistics. Two sample T-test was used to compare the demographics and baseline characteristics between the treatment groups. A mixed-effect model was developed to compare plasma AT-III activity levels between the treatment groups. Chi-square test was used to compare the proportion data in the primary and the secondary endpoints between the treatment groups. The time to reach the target plasma AT-III activity level range was compared between the treatment groups using Kruskal-Wallis Rank Sum Test. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Patients characteristics

A total of 157 liver transplantation recipients were screened for eligibility. After excluding 27 patients, 130 patients were enrolled and randomly assigned to either the intermittent group (n=64) or the continuous group (n=66) (Figure 10). AT-III concentrate was administered in accordance with the allocated group in 58 patients in each group and 9 patients additionally excluded from the analysis due to complications or protocol violations. The remaining 107 patients completed the study as planned and were included in the final analysis. Baseline patient characteristics were similar between the intermittent group and continuous group, except for sex and body weight (Table 5). Perioperative laboratory data on liver function, including model for end-stage liver disease score and prothrombin time showed no significant intergroup difference (Table 5). Total AT-III dose during 72 hours were significantly lower in the continuous group compared to the intermittent group (Table 5).

Plasma AT-III activity levels after admission to the ICU

In the continuous group, the plasma AT-III activity level reached the targeted r ange (80-120%) more rapidly and more stably remained within the target ra nge 84 hours postoperatively compared to the intermittent group (Figure 11a). The median time to reach target concentration was 12 and 24 hours in the continuous

group and intermittent group, respectively ($p < 0.001$, Figure 12). The target attainment rate at 72-hours postoperatively was significantly higher in the continuous group compared to the intermittent group (62% vs 30%, $p = 0.003$). The target attainment rates at 12 (72% vs. 24%, $p < 0.001$) and 48 hours (77% vs. 52%, $p = 0.019$) postoperatively were also significantly higher in the continuous group (Figure 11b and Table 6). The proportion of patients who did not reach the target range at 12 hours postoperatively were significantly higher in the intermittent group compared to the continuous group (76% vs. 11%, $p < 0.001$, Table 6). Also, the proportion of patients who surpassed the target range at 48 (33% vs. 2%, $p < 0.001$) and 72 hours (63% vs. 26%, $p < 0.001$) postoperatively were significantly higher in the intermittent group compared to the continuous group. The proportion of the plasma AT-III activity levels within target range was significantly higher in the continuous group. (53% vs. 31%, $p < 0.001$, Figure 13 and Table 6)

Safety

The number of patients who developed postoperative bleeding requiring intervention during the study period was similar with 2 patients in intermittent group and 4 patients in continuous group (3.1% *vs*. 6.1%, $p = 0.425$). Critical thrombosis requiring intervention was observed in two patients (3.1%), both in the intermittent group. ($p = 0.496$) One patient developed hepatic artery, portal vein, and hepatic vein thromboses on postoperative day 1 and underwent surgical thrombectomy. The other patient underwent balloon angioplasty on postoperative day 3 due to portal vein stenosis and thrombosis. The overall recovery was similar between the 2 groups as

shown by the similar improving trends in prothrombin time, hemoglobin levels, platelet counts, and serum albumin (Figure 14).

Table 5. Patient characteristics of the main study

	Intermittent group (n=54)	**Continuous group (n=53)**	***P*-value**
Age (years)	57 ± 8	54 ± 11	0.115
Sex			
Male	41 (75.9)	25 (47.2)	0.004
Female	13 (24.1)	28 (52.8)	
Height (m)	1.65 ± 0.09	1.62 ± 0.09	0.152
Weight (kg)	67.4 ± 13.5	61.3 ± 13.5	0.022
Diagnosis			
Hepatitis B liver cirrhosis	29 (53.7)	19 (35.8)	0.063
Hepatitis C liver cirrhosis	3 (5.6)	6 (11.3)	0.283
Alcoholic liver cirrhosis	11 (20.4)	9 (17.0)	0.653
Other liver cirrhosis	11 (20.4)	18 (34.0)	0.114
Hepatocellular carcinoma	36 (66.7)	29 (54.7)	0.206
Others	0 (0.0)	1 (1.9)	0.311
Underlying diseases			
Hypertension	17 (31.5)	9 (17)	0.080
Diabetes mellitus	15 (27.8)	11 (20.8)	0.397
Chronic kidney disease	3 (5.6)	3 (5.7)	>0.999
Coronary artery disease	1 (1.9)	2 (3.8)	0.080
Vascular disease	4 (7.4)	5 (9.4)	0.742
Hematologic disease	1 (1.9)	6 (11.3)	0.060
Graft-recipient weight ratio	1.14 ± 0.31	1.15 ± 0.40	0.930
Preoperative laboratory data			
Serum albumin (g/dL)	3.13 ± 0.43	3.04 ± 0.55	0.362
Hemoglobin (g/dL)	9.45 ± 1.52	9.06 ± 1.50	0.181
Platelet count (x 10^3/μL)	83 ± 48	79 ± 40	0.681
Prothrombin time (INR)	1.88 ± 0.45	1.86 ± 0.41	0.769
MELD score	10.67 ± 4.37	10.25 ± 3.71	0.374
Baseline AT-III activity level (%)	33 ± 14	32 ± 11	0.566
Anesthesia time (minutes)	523 ± 164	506 ± 130	0.561
Operation Time (minutes)	455 ± 154	444 ± 133	0.697

Prothrombin time (INR) at 72 hours	1.22 ± 0.19	1.27 ± 0.32	0.361
Total AT-III dose during 72 hours (IU)	5824 ± 425	4764 ± 551	<0.001

Data are presented as arithmetic mean ± standard deviation for continuous data and number (percentage) for categorical data.

Abbreviations: MELD, model for end-stage liver disease; INR, international normalized ratio; AT-III, antithrombin-III; IU, international unit.

Table 6. AT-III plasma activity levels and target attainment rates of patients after ICU admission[11]

Variables	Time after ICU admission	Intermittent dosing group (n=54)	Continuous dosing group (n=53)	*P*-value*
AT-III plasma activity level (%)	Baseline	33 ± 14	32 ± 11	0.633
	12 hours	67 ± 20	101 ± 25	
	24 hours	81 ± 23	92 ± 19	
	48 hours	108 ± 25	94 ± 18	
	72 hours	124 ± 33	104 ± 22	
	84 hours	118 ± 49	101 ± 39	
Proportion of patients within target range (%)	Baseline	0	0	-
	12 hours	24	72	<0.001
	24 hours	48	64	0.210
	48 hours	52	77	0.019
	72 hours	30	62	0.003
	84 hours	31	42	0.471
Proportion of patients with lower than target range (%)	Baseline	100	100	-
	12 hours	76	11	<0.001
	24 hours	48	26	0.057
	48 hours	15	21	0.610
	72 hours	7	11	0.662
	84 hours	13	21	0.472
Proportion of patients with higher than target range (%)	Baseline	0	0	-
	12 hours	0	17	0.006
	24 hours	4	9	0.411
	48 hours	33	2	<0.001
	72 hours	63	26	<0.001
	84 hours	56	38	0.153

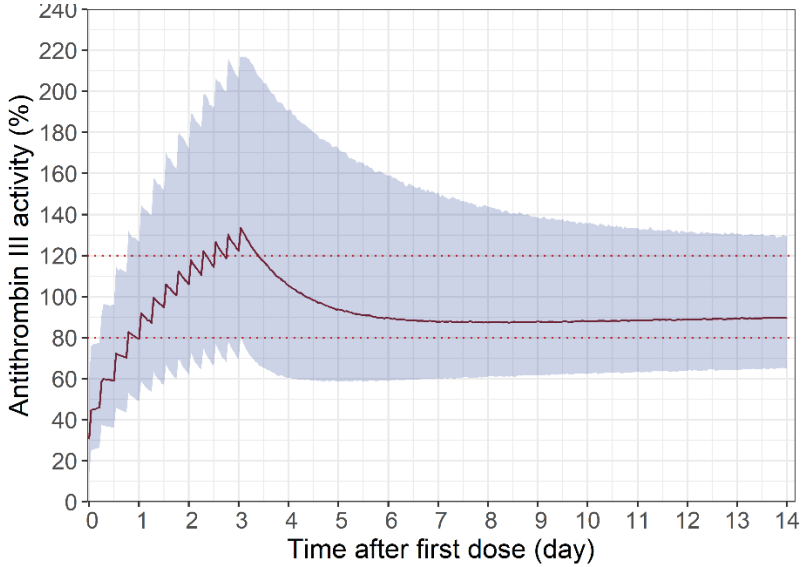
AT-III plasma activity levels are presented as arithmetic mean ± standard deviation

* P-value from mixed-effect model for AT-III plasma activity level and chi-square test for categorical data

Abbreviations: AT-III, antithrombin-III; ICU, intensive care unit

[11] This table was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

Figure 8. Simulation of the intermittent infusion regimen[12]

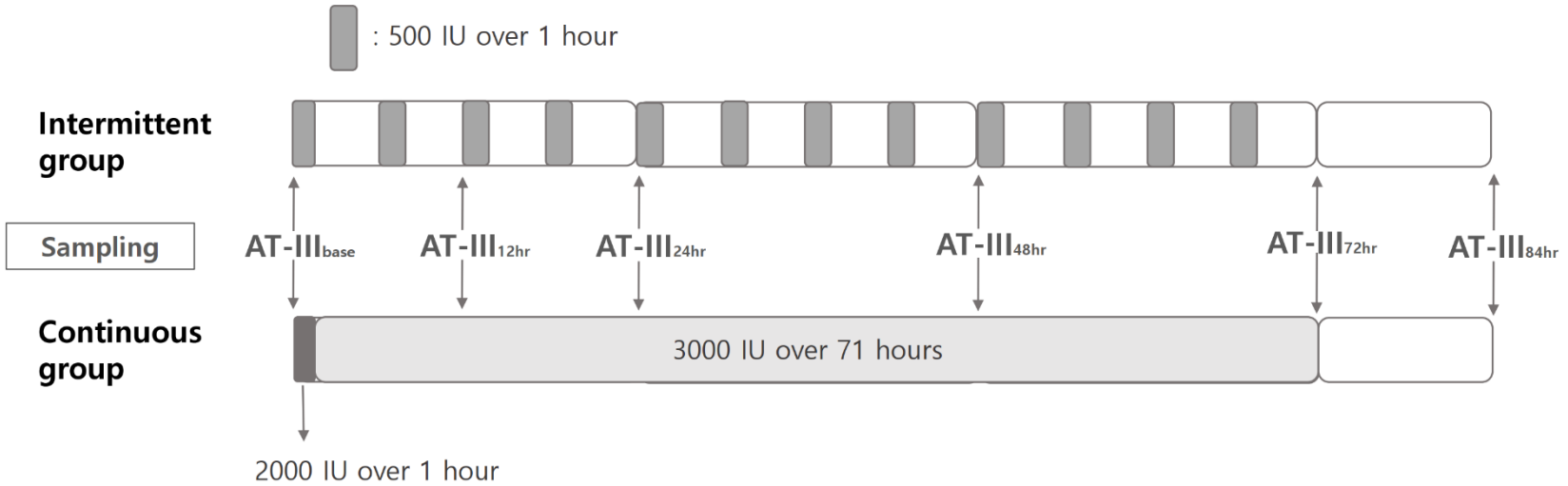


Intermittent infusion of 500 IU AT-III concentrate every 6 hours for postoperative day 0 through 2. Line, median prediction; area, 95% prediction interval; hairline, target AT-III activity level range (80-120%)

Abbreviations: IU, international unit; AT-III, antithrombin III.

[12] This figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

Figure 9. Schematic diagram of the study protocol.



In the intermittent group, 500 IU of AT-III concentrate were injected over an hour every 6 hours for 72 hours. In the continuous group, a loading dose of 2000 IU was administered over an hour, followed by continuous infusion of 3000 IU of AT-III over 71 hours. Plasma AT-III activity level was analyzed at baseline, 12, 24, 48, 72, and 84 hours, and the measured values were labeled as AT-III$_{base}$, AT-III$_{12hr}$, AT-III$_{24hr}$, AT-III$_{48hr}$, AT-III$_{72hr}$, and AT-III$_{84hr}$, respectively.

Abbreviations: IU, international unit; AT-III, antithrombin III.

Figure 10. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study

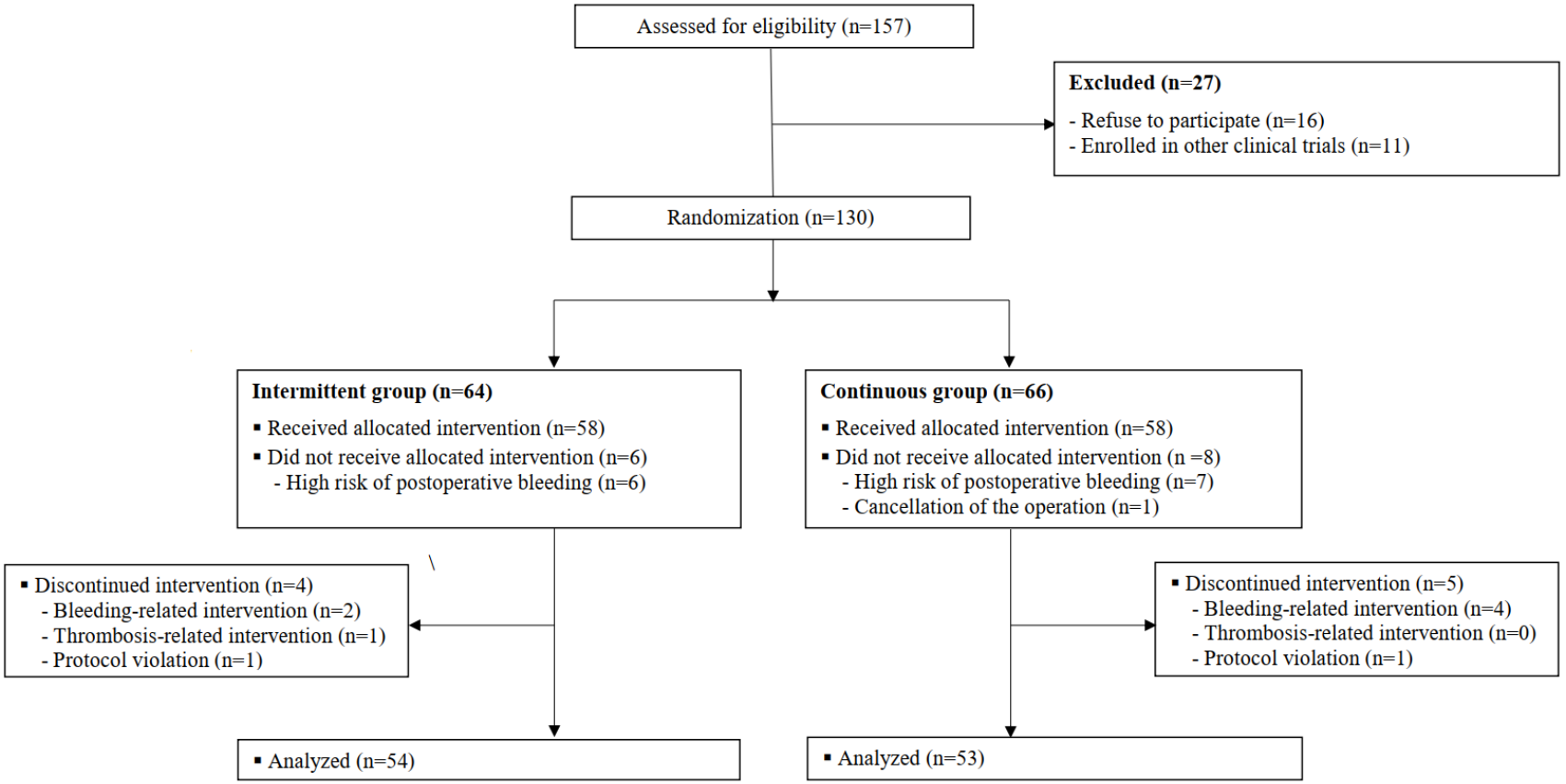
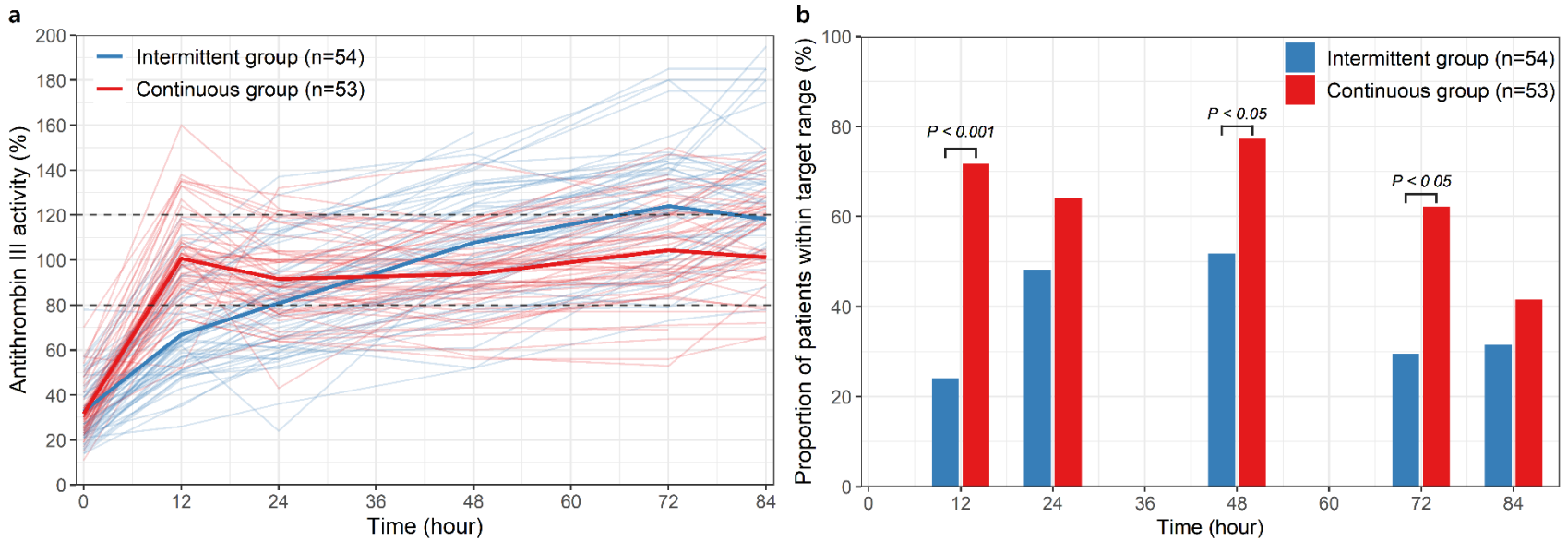


Figure 11. Observed plasma AT-III activity levels during 84 hours after liver transplantation.[13]

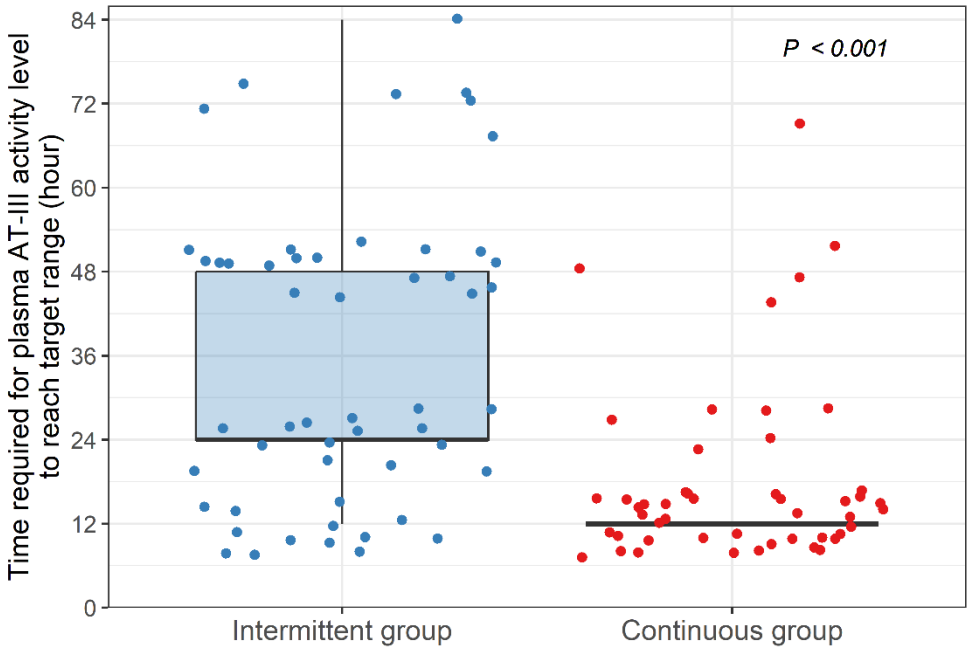


(a) Mean (bold line) and individual (hairline) plasma AT-III activity levels; dashed line, target plasma AT-III activity level range (80–120%), (b) Proportion of patients with target plasma AT-III activity level range during 84 hours after liver transplantation.

Abbreviations: AT-III, antithrombin-III.

[13] This figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

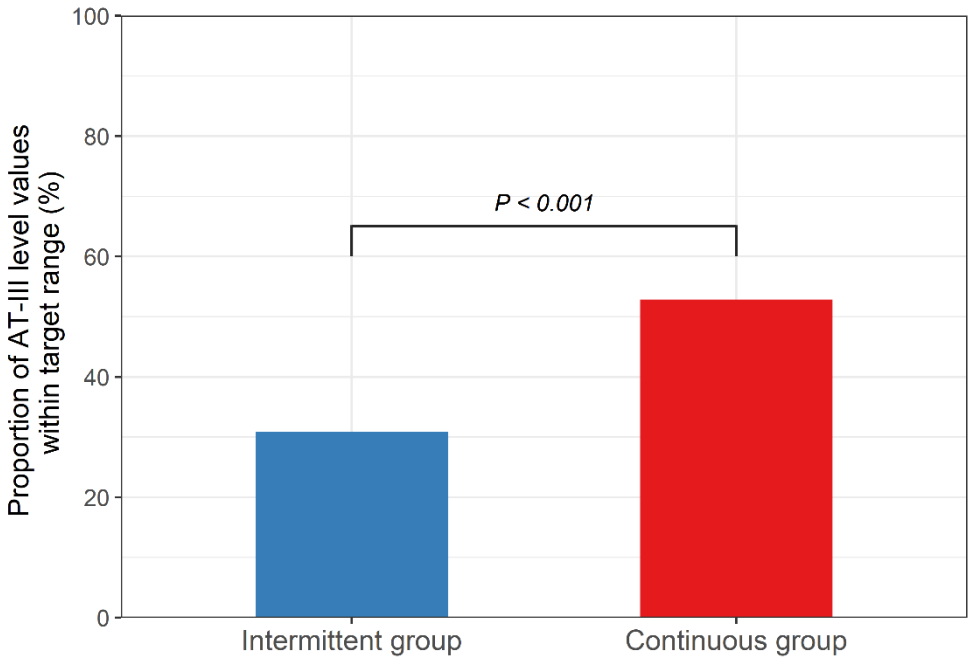
Figure 12. Time required for plasma AT-III activity level to reach target range after liver transplantation.[14]



Abbreviations: AT-III, antithrombin-III.

[14] This figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

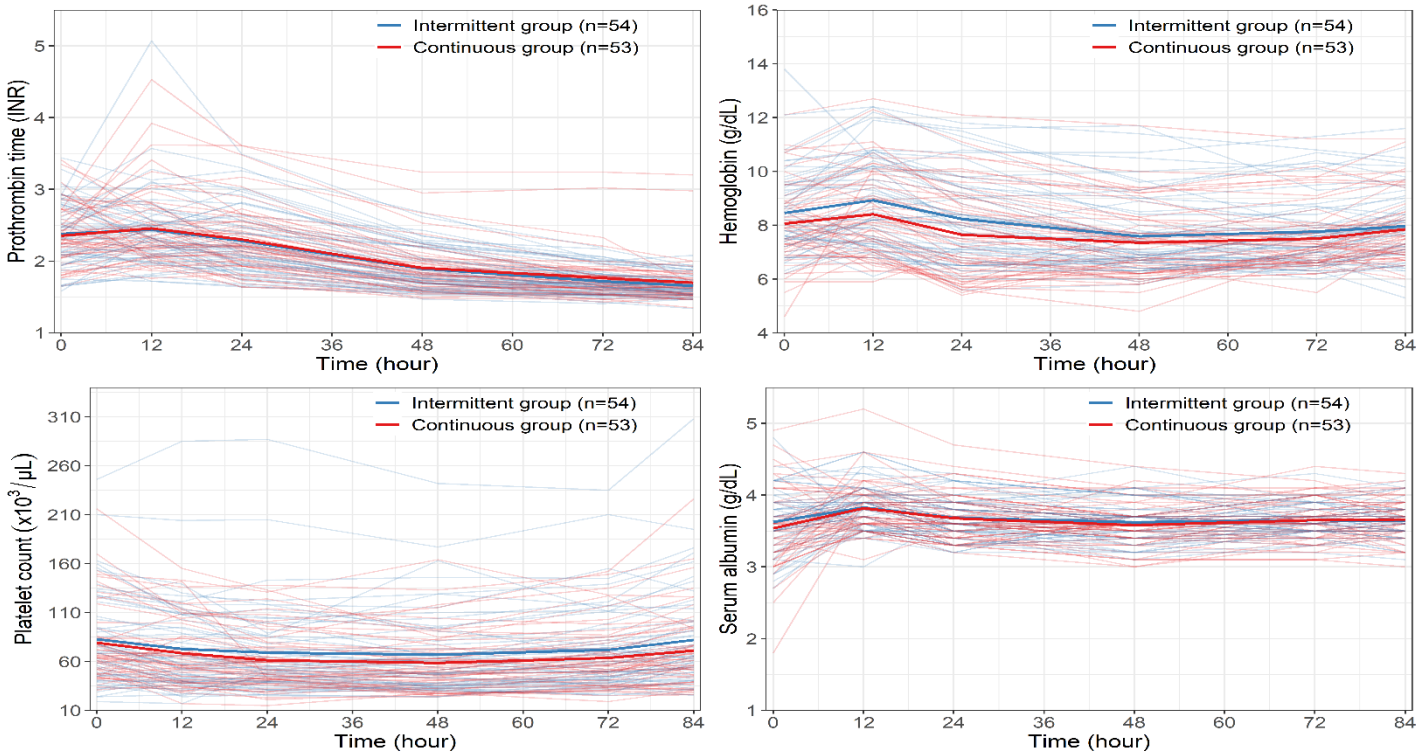
Figure 13. Proportion of total plasma AT-III activity levels within target AT-III activity range[15]



Abbreviation: AT-III, antithrombin-III.

[15] This figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

Figure 14. Mean (bold line) and individual (hairline) values of prothrombin time, hemoglobin, platelet counts, and serum albumin during the study period.[16]



Abbreviation: INR, international normalized ratio

[16] This figure was created in collaboration with Dr. Jaeseong Oh, who agreed to use it in this dissertation.

DISCUSSION

Compared to conventional intermittent infusion, continuous infusion of AT-III was more likely to maintain plasma AT-III activity within the targeted range after living donor liver transplantation. The AT-III level reached the target range more rapidly and remained within the range longer with continuous infusion.

From the preceding study, a PK model of AT-III for liver transplantation recipients was developed and external validation using extra dataset showed that the new model successfully predicted plasma activity levels of AT-III. The model developed in the study agrees with previous published PK models for AT-III. For the basic structural model, one- or 2-compartment model with linear elimination process were used to describe AT-III concentration according to sparse or rich PK samples, respectively [27, 34-36]. In the model development, body weight and albumin were included in the PK model as covariates. The body weight showed a positive correlation with the CL of AT-III, similar to the result observed in the population PK model developed for pediatric patients [27]. Low serum albumin was associated with low AT-III concentration in critical patients with disseminated intravascular coagulation (DIC) due to increased vascular permeability in those patients [37]. In this study, the serum albumin showed a negative correlation with CL, which results in low AT-III concentration in the patients with low serum albumin. The vascular permeability can be also increased in post-operative patients and that can be one of the reasons for the effect of serum albumin on CL of AT-III [38]. The effect of anticoagulants such as heparin was excluded from the model as patients did not receive any additional anticoagulation therapy due to the concerns of bleeding.

Various dosing scenarios of AT-III concentrate were simulated in the preceding study based on the developed population PK model to suggest optimal regimens (Figure 7) [33]. We focused on primarily to avoid underdose in the immediate postoperative period, and secondarily to avoid overdose after reaching the target range of AT-III activity. According to the simulation, approximately 1 day was required for the median value to reach the target range with intermittent infusion of 500 IU AT-III concentrate every 6 hours (Figure 8). Moreover, the lower limit of 95% prediction interval remained at extremely low values during the first day which may be related to increased risk of thrombosis (Figure 8). Another intermittent infusion regimen, 1500 IU for postoperative day 0 through 2, also showed median AT-III activity below the target level during the first postoperative day with lower limit value around 40 % (Figure 7b). In addition, upper limit of 95% interval showed spiking AT-III activity levels in accordance with the large dose of intermittent infusion (Figure 7b). A modified protocol (2000 IU at POD 0, followed by 1000 IU per day for POD 1 and 2) was predicted to be relatively successful in maintaining AT-III activity within target level (Figure 7c). However, majority of the patients were still not expected to reach the target range on the first day after the surgery (Figure 7c). In this regard, a continuous infusion protocol with a loading dose (2000 IU loading dose, 3000 IU for maintenance dose) which showed fast reach within the target level and narrow prediction interval appeared to be the most optimal dosing scenario (Figure 7d).

Several studies have investigated the administration method of AT-III concentrates in various clinical situations. A recent study compared continuous and intermittent administration of AT-III concentrate in pediatric patients using

extracorporeal membrane oxygenation. The plasma AT-III activity level remained in the target range longer when using the continuous infusion method, which was adjusted based on the daily AT-III level according to the predetermined protocol compared to the intermittent method which was based on the physician's discretion [39]. Another study that investigated AT-III concentrate regimens in severe sepsis patients showed comparable tolerability and effectiveness between the two regimens [34]. However, the plasma AT-III activity level depending on the administration strategy has not been compared in liver transplant patients in the immediate postoperative period. Our study is the first report that suggests the superiority of continuous administration over intermittent administration when using AT-III concentrate in liver transplant patients in the immediate postoperative period.

Studies regarding the use of AT-III concentrates in liver transplantation are scarce in general. A pilot study of 25 patients showed reduced fibrin degradation product D-dimer levels and platelet transfusion requirement with AT-III concentrate supplementation in the early postoperative period [14]. Furthermore, a recent retrospective study of 181 children who underwent liver transplantation suggested an association between low plasma AT-III activity level early after liver transplantation and postoperative thrombosis, advocating for postoperative AT-III supplementation [29]. In a randomized controlled trial in patients with liver disease, AT-III concentrate showed promise for preventing portal vein thrombosis in patients with low plasma AT-III activity level [20]. However, no studies have investigated the dosing strategies in liver transplantation recipients despite the significant perioperative changes in plasma AT-III activity level. In this respect, the results of this randomized controlled trial may suggest grounds for AT-III concentrate dosing

in the immediate postoperative period after liver transplantation.

The total dose of AT-III concentrate administered was on average 4,764 IU for the continuous group and 5,824 IU for intermittent group. Clinicians tend to side with overdose due to the fear of critical thrombosis and its fatal consequences since there is no clear guidance in the AT-III concentrate dosing. However, considering the high price of the drug and related personal and social economic burden, it is hard to justify the overuse of AT-III concentrate without obvious evidence on the clinical impact. Furthermore, as post-transplantation hemorrhage requiring re-operation and the number of transfused red blood cells have been known as significant risk factors for mortality, use of optimal AT-III concentrate dosage based on our results may be helpful in improving clinical outcomes with lower economic burden [40].

The plasma AT-III activity level was more likely to be within the target range in the continuous group compared to the intermittent group at 72 hours ($AT\text{-}III_{72hr}$), as well as other time points. The plasma AT-III activity level at 12 hours ($AT\text{-}III_{12hr}$) was within the target range in more than 70% of the patients in continuous group, which was in contrast to the 24.1% in the intermittent group. Based on the results, the plasma AT-III activity level seems to reach the target range quicker and stays within the target range more often in the continuous group, which may be particularly beneficial in patients with the highest risk of thrombogenesis. However, considering that it still takes time for the AT-III activity level to reach the target level, further research on intraoperative administration of AT-III concentrate might be required.

There were no significant differences in post-transplantation complications between the two groups. Therefore, it remains unclear whether the delicate control

of the plasma AT-III activity level results in the improved clinical outcomes. Two patients in the intermittent group developed thrombosis of hepatic artery and portal vein requiring intervention. The incidence was significantly lower compared to previous reports, suggesting the efficacy of AT-III concentrate in preventing thrombosis [10, 11]. However, the relatively small sample size of our study and the extremely low incidence of thrombosis makes it difficult to draw a reliable conclusion.

There are several limitations in this study that should be considered. First, the study only included living donor liver transplantations performed at a single medical center. Therefore, it may be difficult to expect similar results when introduced to different circumstances. However, considering the significant number of liver transplantations (more than 100 cases per year) performed by several surgeons in this medical center, comparable results can be anticipated in centers with surgical proficiency. Second, significant clinical advantages of AT-III concentrate after liver transplantation were not identified. Due to the low incidence of critical thrombosis, it was not easy for us to clearly demonstrate the clinical usefulness of AT-III concentrate. However, as the focus of this study was on the pharmacokinetic analysis, plasma AT-III activity levels showed a clear difference between the two groups.

In conclusion, continuous infusion of AT-III concentrate can be more efficient and safer in maintaining the plasma AT-III activity level within the target range compared to intermittent infusion in the immediate postoperative period after liver transplantation.

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국문 초록

연구 배경: 항트롬빈 제제는 간이식 수술의 중대한 합병증인 간문맥 및 간동맥 혈전을 방지하기 위해 수술 직후 사용되어 왔다. 그러나 간이식 환자에서 이 약물의 적절한 용법에 대해서 이전에 충분한 연구가 이루어지지 않았다. 이에 선행 연구에서는 항트롬빈의 혈중 농도에 대한 약동학적 분석을 시행하였으며, 본 연구에서는 항트롬빈 제제의 투약 방법에 있어 기존의 간헐적 투약 방법과 지속적 투약 방법을 비교하여 혈중 항트롬빈 농도를 목표 범위 내로 유지하기 위해 더 적절한 항트롬빈 제제의 투약 방법을 확인하고자 하였다.

연구 방법: 이 전향적, 무작위 대조군 연구는 생체 간이식을 시행받는 환자를 대상으로 하였다. 환자는 수술 전 두 군으로 무작위 배정되어 대조군의 경우 항트롬빈 제제 500 IU을 72시간 동안 6시간마다 간헐적으로 투여하였고, 시험군의 경우 항트롬빈 제제 2000 IU을 1시간 동안 부하용량으로 주입한 후 71시간에 걸쳐 3000 IU을 지속 주입하였다. 첫 항트롬빈 제제 투여 후 12, 24, 48, 72, 84시간에 혈중 항트롬빈 농도를 측정하였으며, 연구의 일차 종점은 72시간 시점에서의 혈중 항트롬빈 농도의 목표 (80-120%) 달성률이다. 또한 다른 시점에서의 혈중 항트롬빈 농도의 목표 달성률과 수술 후 합병증을 이차 종점으로 수집하였다.

연구 결과: 최종적으로 107명의 환자가 분석에 포함되었으며, 첫 항트롬빈 제제 투여 후 72시간 시점에서의 혈중 항트롬빈 농도의 목표 달성률은 대조군과 시험군에서 각각 30%, 62% 로 유의한 차이를 보였다 (P =0.003). 대조군과 비교하였을 때 시험군에서 더 빠른 시점에 목표 농도에 도달하였으며 (중앙값 12 *vs*. 24 시간, $P < 0.001$), 또한 84시간 시점에 이르기까지 목표 농도 범위에 머무르는 비율이 높았다.

결론: 생체 간이식 직후 항트롬빈의 목표 혈중 농도를 유지하기 위해서는 기존의 간헐적 주입 방식에 비해 지속적 주입 방식이 더 적절한 것으로 나타났다.

주요어: 간이식, 항트롬빈, 용법, 혈전, 응고

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