Ph.D. Dissertation of Medicine

A Simple and Novel Equation to Estimate the Degree of Bleeding in Haemorrhagic Shock

- Mathematical Derivation and Preliminary In Vivo Validation -

출혈성 쇼크에서 출혈 정도를 예측하는 간단하고 새로운 식: 수학적 유도 및 생체 내 예비 검증

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A Simple and Novel Equation to Estimate the Degree of Bleeding in Haemorrhagic Shock

- Mathematical Derivation and Preliminary In Vivo Validation –

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이 논문을 의학박사 학위논문으로 제출함

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Abstract

Background

Determining blood loss is challenging in the management of haemorrhagic shock. The author aimed to derive an equation estimating residual blood volume (RBV, %) via serial haematocrits (Hct_1, Hct_2) and infused crystalloid fluid volume (N) and validated it in vivo. Then, blood loss (%) would be calculable as '100%–RBV (%)'.

Methods

By fixing N as [0.015×body weight(g)]cc in line with the current guidelines, the author derived an equation estimating RBV (%) using simple mathematics: $24k/[(Hct_1/Hct_2)–1]$. For validation, non-ongoing haemorrhagic shock was induced in Sprague-Dawley rats by withdrawing 20.0–60.0% of their total blood volume in 5.0% intervals (n=9). Hct_1 was checked after 10 min and normal saline (0.015×body weight(g) cc) was infused over the course of 10 min. Hct_2 was checked five minutes later. The author applied a linear equation to explain RBV (%) with $1/[(Hct_1/Hct_2)–1]$.

Results

Seven rats losing 30.0–60.0% of their TBV suffered persistent shock despite fluid resuscitation. For them, RBV (%) was updated as $5.67/[(Hct_1/Hct_2)–1]+32.8$ (95% confidence interval [CI] of the slope: 3.14–8.21, p=0.002, R^2=0.87). On a Bland-Altman plot, the difference between the estimated and actual RBV (%) was 0.00±4.03%; the 95% CIs of the limits of agreements were included within the pre-determined criterion of validation (<20%).

Conclusion

For rats suffering from persistent, non-ongoing haemorrhagic shock, the author derived and validated a simple equation estimating RBV (%). This enables the calculation of blood loss [100%–RBV (%)] via information on serial haematocrits under a fixed N. Human clinical validation is required before utilisation for emergency care of haemorrhagic shock.

Keyword: Blood volume determination; Haematocrit; Haemorrhagic shock; Isotonic solutions

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Chapter 1. Introduction

1. 1. Study Background

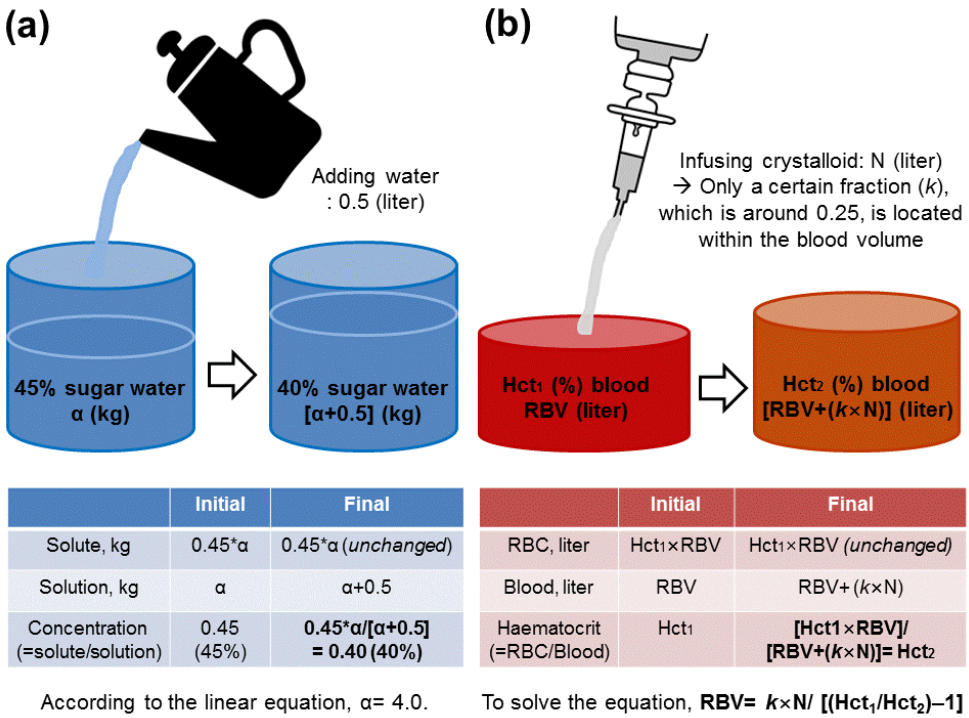
Haemorrhagic shock has various etiologies, including trauma, maternal haemorrhage, peptic ulcers, perioperative haemorrhage, and ruptured aortic aneurysms [1]. This medical condition causes 1.9 million deaths annually (with trauma as the leading cause; there are 1.5 million trauma-induced haemorrhagic shock deaths annually worldwide) and affects the young disproportionately raising a socioeconomic issue [2]. When trauma-related haemorrhage deteriorates, death occurs at a median of approximately 2.6 h after initial presentation addressing the importance of initial management [3, 4]. Initial management is also critical for reducing delayed mortality and repaying oxygen debt before shock becomes irreversible [5]. For clinicians, prompt and correct determination of the degree of blood loss (%) is critical.

The blood loss (%) is calculated as '100%–residual blood volume (RBV) (%)'. For example, when RBV (%) is 65%, blood loss (%) is 35%. RBV (%) is defined as RBV/total blood volume (TBV). TBV, the denominator, is easily estimable via body weight [6]. Therefore, once RBV, the numerator, is also known, RBV (%) and thus blood loss (%) can be estimated.

The gold standard to determine RBV is a dilution method using radioactive chromium (^{51}Cr); briefly after transfusing a small, fixed quantity of ^{51}Cr-labelled red blood cells, the radioactivity of the blood is measured to calculate RBV [7]. The carbon monoxide rebreathing technique, which shows high reproducibility without using radioactive materials, is based on a fixed amount of

an inspired oxygen-carbon monoxide gas mixture and traces the carboxyhaemoglobin (HbCO) difference to estimate RBV [8, 9]. However, neither method is applicable to real-world haemorrhagic shock patients. Clinicians estimate RBV (%) or blood loss (%) considering multiple factors such as vital signs, haemoglobin/haematocrit, central venous or pulmonary capillary wedge pressure (CVP/PCWP), ultrasonography, and visual estimation [10-16]. However, these methods provide only rough estimations.

Previously, the author mathematically derived an equation to estimate RBV for acute, non-ongoing haemorrhagic shock patients [17]. In mathematics class, middle school students are asked the following question: “There is a cup of sugar water with a concentration of 45%; 0.5 kg of water is poured into this mixture. The concentration of the sugar water changed to 40%. Can you calculate the initial mass of the sugar water?”. Once the initial and final concentration of sugar water and the mass of water poured into the mixture is known, it is possible to calculate the initial mass of the sugar water through a linear equation (Fig 1.a; see text S1.a for a detailed mathematical explanation). The author paid attention to the fact that this sugar water scenario is similar to that of initial management of haemorrhagic shock patients.



	Initial	Final
Solute, kg	0.45*α	0.45*α (*unchanged*)
Solution, kg	α	α+0.5
Concentration (=solute/solution)	0.45 (45%)	**0.45*α/[α+0.5] = 0.40 (40%)**

According to the linear equation, α= 4.0.

	Initial	Final
RBC, liter	Hct_1×RBV	Hct_1×RBV *(unchanged)*
Blood, liter	RBV	RBV+(*k*×N)
Haematocrit (=RBC/Blood)	Hct_1	**[Hct1×RBV]/ [RBV+(*k*×N)]= Hct_2**

To solve the equation, **RBV= *k*×N/ [(Hct_1/Hct_2)–1]**

Fig 1. Analogy between the change of concentration of sugar water after adding some water and that of haematocrit of blood after crystalloid fluid infusion

(a) Change of concentration of sugar water after adding some water. Once you know the initial and final concentration of sugar water and the mass of water poured into it, you can tell the initial mass of the sugar water by building a linear equation. (See text S1.a for detailed explanation).

(b) Change of haematocrit of blood after crystalloid fluid infusion. Likewise, if there is no blood or fluid loss via the circulation system, residual blood volume (RBV) would be calculable with serial haematocrits (Hct_1 and Hct_2) and the volume of crystalloid fluid infused in-between (N). The only difference from (A) is that only a certain fraction (*k*, which is approximately 0.25 for men) would be distributed into the intravascular volume (See text S1.b for detailed explanation).

For patients presenting at the emergency department (ED) with haemorrhagic shock, clinicians control bleeding, request laboratory tests, infuse crystalloid fluid restrictively, and start transfusion as soon as materials are available [1, 13, 18]. Along with blood type, arterial blood gas analysis (ABGA), lactate, electrolytes, coagulation profiles, thromboelastography/thromboelastometry, and complete blood counts should be checked initially as point-of-care tests (POCT) [1]. With this standard management of haemorrhagic shock, the initial and final concentration of the blood, that is, the serial haematocrits (Hct_1 and Hct_2), become available immediately. In addition, clinicians themselves determine the volume of crystalloid fluid (N), which is infused as an initial resuscitative effort. As with the sugar water story solved by a linear equation, the author derived the following equation to determine the initial blood volume (RBV) at the time of ED arrival using the information on Hct_1, Hct_2, and N, which are the key elements of standard management [1, 13] (Fig 1.b; see text S1.b for a detailed mathematical explanation):

$$RBV = k \times N / [(Hct_1/Hct_2) - 1]$$

(*k*: the fraction of N distributed in the intravascular volume)

The only difference between this approach and the sugar water example is *k*, which is approximately 0.25 for men; only a fraction of crystalloid fluid is distributed in the intravascular volume, leaving the remnant within the interstitial compartment [19].

1.2. Purpose of Research

Clinicians prefer to know blood loss (%) or RBV (%) rather than RBV itself. In this study, the author mathematically derived an equation to determine the RBV (%) (and thus the blood loss (%)) by modifying the above equation and then validated it *in vivo*. Additionally, the author also validated the original equation estimating RBV in vivo.

Chapter 2. Method

2.1. Ethics Approval

This study was approved by the Institutional Animal Care and Use Committee (approval number: IACUC210053) and the author observed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guideline in conducting this study [20].

2.2. Mathematical Derivation of the Equation Estimating RBV (%)

By definition, RBV (%) is calculated as 'RBV/TBV'. Incorporating this relationship into the original equation estimating RBV, the author derives that:

$$RBV = TBV \times RBV\ (\%) = k \times N / [(Hct_1/Hct_2) - 1].$$

$$\therefore\ RBV\ (\%) = k \times N/TBV/[(Hct_1/Hct_2) - 1] \times 100(\%)$$

Among the components, N/TBV can be substituted with a constant as follows.

In this study, the author fixed N as 0.015×body weight(g)(cc) [13, 21]. The author calculated TBV as 0.06×body weight(g)+0.77(cc) as reported by Lee and Blaufox (r=0.99, n=70, p<0.001) [22].

$$\therefore\ N/TBV = 0.015 \times \text{body weight(g)(cc)} / 0.06 \times \text{body weight(g)} + 0.77\text{(cc)}$$

$$= 0.015/(0.06 + 0.77/\text{body weight})$$

≒0.24 (when body weight ranges between 280–350 g, as described below).

Incorporating this information, the equation to estimate the RBV (%) becomes far simpler:

$$\text{RBV}\ (\%) = k \times (\text{N}/\text{TBV}) / [(\text{Hct}_1/\text{Hct}_2) - 1] \times 100(\%)$$

$$= 24k / [(\text{Hct}_1/\text{Hct}_2) - 1](\%)$$

This indicates that the RBV (%) can be determined solely by information on serial haematocrits when N is fixed.

2.3. In Vivo Validation of the Equation Estimating RBV (%)

The above equation the author aimed to evaluate is a type of linear equation explaining RBV (%), the dependent variable, with $1/[(\text{Hct}_1/\text{Hct}_2) - 1]$ as the independent variable and $24k$ as the slope. To validate it, the author induced varying degrees of haemorrhagic shock in a rat model. Then, the author performed a linear regression analysis to obtain a regression equation in the following form: $\text{RBV}\ (\%) = 24k / [(\text{Hct}_1/\text{Hct}_2) - 1] + \alpha$. As is common during updates and validations, the author expected that a y-intercept, α, would be added to the original equation [23].

Using this updated equation, the author estimated the RBV (%) for each rat and compared it with the ‘actual’ RBV (%). The author could determine the ‘actual’ RBV (%) by pre-determining the blood loss (%), which is 100%–RBV (%), in each experiment.

The author performed a correlation analysis between the actual and estimated RBVs (%) by drawing a calibration plot. Finally, drawing a Bland-Altman plot, the author compared the estimated RBV (%) with the actual RBV (%) [24, 25]. The author expected the mean and standard deviation of their difference to be 0.0% of the TBV (0.0 cc) and 4.0% of the TBV (around 1.6 cc), respectively. In this preliminary, concept-validation study with a small sample size, the author set the absolute maximum allowed difference as 20.0% of the TBV (4.0 cc). When the 95% CI of the upper and lower limits of agreement were included in these maximum-allowed differences, the equation was considered validated.

2.4. In Vivo Validation of the Original Equation Estimating RBV

The author validated the original equation, $RBV=k\times N/[(Hct_1/Hct_2)-1]$, in the same way as for the RBV (%) equation. Trying to explain the RBV in terms of $N/[(Hct_1/Hct_2)-1]$, the author generated a regression equation with a slope of k and with the addition of a y-intercept. Using this updated equation, the author estimated the RBV for each experiment and compared it with the actual value. After checking the degree of correlation on a calibration plot, a Bland-Altman plot was drawn to validate the equation.

2.5. Animal Preparation

Male Sprague-Dawley rats weighing 280–350 g were used in this study. They were housed in a controlled environment with free access to food and water for one week prior to the experiments.

2.6. Design, Procedure, and Variables

A very strong correlation was defined as $r \geq 0.80$ [26]. To accommodate an α error <0.05, a β error <0.20 and $r \geq 0.80$, ≥ 9 rats were required. This is similar to the minimum sample size, 8, required for a Bland-Altman plot with a difference of $0.0 \pm 4.0\%$ and an absolute maximum allowed difference $<20.0\%$.

Considering the sample size, the author simulated a 30.0% loss of TBV as well as increased blood loss in 5.0% increments (35.0%, 40.0%, and so on) within each experiment. When a rat died at a certain degree of blood loss (for example, 65.0% of TBV), the author performed the same experiment again with another rat. If the next rat died, the author designated the previous degree of blood loss (60.0% of TBV) as the upper limit of blood loss. The author then decreased blood loss by 5.0% (25.0%, 20.0%, and so on). Similarly, when two consecutive rats failed to show signs of shock (mean arterial pressure≤65 mmHg or lactate≥2 mmol/L) given a certain degree of haemorrhage (e.g., 15.0% of the TBV), the author designated the previous degree (e.g., 20.0% of the TBV) as the lower limit of blood loss. The author expected that rats bleeding at the level of 20.0–60.0% of TBV would be included in the current investigation, fulfilling the minimum sample size of n=9 [11, 27].

The author divided the experiments into five sections, modifying a previously published model [28]. These study components were (1) preparation (baseline), (2) induction of haemorrhagic shock, (3) observation without further treatment, (4) restricted crystalloid fluid resuscitation, and (5) follow-up testing (Fig 2). The author used the subscripts $_0$, $_1$, and $_2$ to denote baseline before bleeding, initial ED presentation after bleeding and before fluid resuscitation, and post-fluid resuscitation status, respectively, throughout the study description.

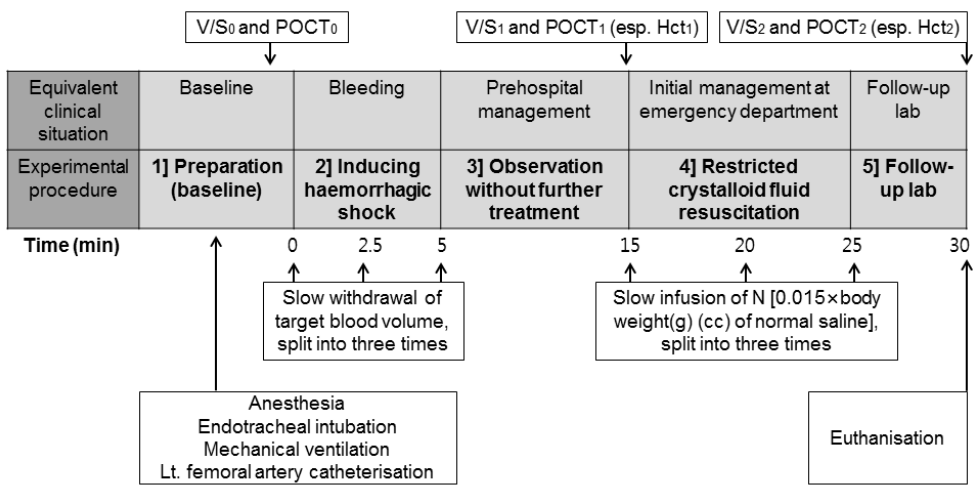


Fig 2. Study protocol

POCT, point-of-care test including arterial blood gas analysis, haematocrit, and lactate; V/S, vital signs including systolic, diastolic, and mean arterial blood pressure and heart rate

Subscripts $_0$, $_1$, and $_2$ denote baseline before bleeding, status just before fluid resuscitation, and status after fluid resuscitation, respectively.

During the study preparation phase (baseline), the author injected intramuscular anesthesia into the Sprague-Dawley rats: zoletil (50 mg/kg, Virbac, Carros, France) and xylazine (10 mg/kg, Bayer, Seoul, Korea). Endotracheal intubation was performed with a 14-gauge catheter (BD Insyte, Autoguard, NJ, US) [29]. To avoid hypoxemia and maintain normo-ventilation [13], a mechanical ventilator (Harvard rodent ventilator model 645, Harvard Apparatus, Holliston, MA) was applied with a tidal volume of 2.5 mL, a respiratory rate of 50 breaths/min, and 0.21 as the fraction of inspired oxygen. A 24-gauge catheter (BD Insyte, Autoguard, NJ, US) was introduced into the left femoral artery after sterile cut-down procedure to withdraw blood, replace/infuse fluid, and monitor heart rate (HR) and systolic, diastolic, and mean arterial blood pressure (SBP, DBP, and MAP). After administering anesthesia, the procedure itself took ≤5 min. After the disposal of 0.2 cc within the arterial line, the author performed a baseline $POCT_0$ (ABL90 FLEX PLUS, Radiometer Medical, Copenhagen, Denmark) with the next 0.2 cc of blood to check $ABGA_0$, $lactate_0$, $haemoglobin_0$, and Hct_0 levels. Following this, 0.2 cc of normal saline was replaced to avoid intra-catheter clotting. Guided by $ABGA_0$, tidal volume was adjusted to a target pH level of 7.35–7.45 and a $PaCO_2$ level of 35–45 mmHg. Vital $signs_0$ (SBP_0, DBP_0, MAP_0, and HR_0) were recorded throughout the procedure.

For the second phase of the study, the author induced haemorrhagic shock after pre-determining the target blood loss volume as TBV×target blood loss (%). The author split this target volume to lose into three. Each third was shed slowly every 2.5 min; 0.6 cc of blood had already been shed during the preparation phase (specifically, 0.2 cc of blood was used for filling the catheter hub during initial

catheterisation and subsequently discarded, and 0.4 cc was used to check $POCT_0$ levels). The author compensated for this loss by subtracting 0.6 cc from the first third of blood loss volume. After each blood withdrawal, 0.1 cc of normal saline was replaced to prevent intra-catheter clotting.

Phase (3) of the study comprised observation without further treatment over the course of 10 min, simulating the prehospital situation in which a 'scoop-and-run' treatment approach is preferred to a 'stay-and-play' approach in order to prevent unnecessary delays of definitive care [1, 13, 30]. Haemorrhage control, which is strongly recommended within medical guidelines, was accomplished per this protocol (i.e., the author did not allow further bleeding).

The author recorded vital $signs_1$ immediately before phase (4) of the study, which comprised restricting crystalloid fluid resuscitation. After discarding 0.2 cc of blood within the line, 0.2 cc of blood was sampled to check $POCT_1$ levels (especially Hct_1). The author determined the volume of normal saline necessary to infuse N as 0.015×body weight(g)(cc), which corresponds to approximately 1 L for a 70 kg adult [21, 30-32]. The author split N into three groups and infused fluid slowly every 5 min; The first bolus was subtracted by 0.5 cc: 0.2 cc had already been replaced after sampling for $POCT_0$ during the preparation phase and 0.3 cc was replaced during blood loss induction.

Five minutes after completing fluid resuscitation, the author initiated component (5) of the study (i.e., study follow-up). The author checked $POCT_2$ levels (including Hct_2) along with vital $signs_2$. The rats were then euthanised via cervical dislocation.

At this point, except for *k*, all variables for estimating the RBV became available for inclusion in the linear equations (specifically, Hct_1, Hct_2, and N).

Due to a calculation mistake, the author withdrew 33.4% of the TBV from a rat assigned to lose 35.0% of its TBV. The author analyzed this erroneous observation as though it was purposeful (i.e., the author did not perform any statistical corrections and did not remove the rat from the study).

2.7. Statistics

Results for body weight, V/S, and POCT were calculated as means±standard deviations.

Linear regression analysis was performed to generate a regression equation explaining RBV in terms of $k \times N/[(Hct_1/Hct_2)-1]$ as well as with the addition of a y-intercept. Using this updated equation, the author estimated the RBV for each experiment, drew a calibration plot to compare the estimated values with the actual observed values, and calculated *r*. Following this, a Bland-Altman plot was drawn as the final step of validation. The same procedure was used to update and validate the equations for RBV (%).

Among the nine rats that experienced haemorrhagic shock, two recovered from shock after fluid resuscitation. The author performed the main analysis with seven rats showing persistent shock despite fluid resuscitation.

As a supplementary analysis, the author re-conducted the analysis including all the rats regardless of persistent shock. Additionally, the author performed linear regression analyses to explain RBV (%) with the following

potential predictive covariates: initial and final values and interval changes for vital signs, haematocrit, and lactate.

All statistical analyses were performed using IBM SPSS statistical software, version 26 (SPSS Inc., Chicago, IL, US) and MedCalc Statistical Software, version 19.2.6 (MedCalc Software Ltd., Ostend, Belgium). Statistical significance was set at a threshold of $p<0.05$.

Chapter 3. Results

The rats suffered shock when losing ≥20.0% of their TBV. However, those shedding 20.0–25.0% of their TBV recovered from shock via fluid resuscitation (Fig 3 and 4.c). Rats bleeding out 60.0% of their TBV barely survived the study protocol. A total of seven rats shedding 30.0–60.0% of their TBV were ultimately included in the main analysis.

The rats included in the analysis weighed between 285 and 334 g and their TBV ranged from 17.87 to 20.81 cc; N spanned 4.27–5.01 cc. The mean SBP_0, DBP_0, and MAP_0 levels were 110±11, 71±7, and 84±8 mmHg, respectively and the mean HR_0 was 215±18 beats/min. Mean $haemoglobin_0$, $haematocrit_0$, and $lactate_0$ levels were 13.2±0.8 g/dL, 40.6±2.5 %, and 0.9±0.3 mmol/L, respectively. Changes in vital signs and POCT findings according to the study timeline are shown in Fig 3 and 4, respectively.

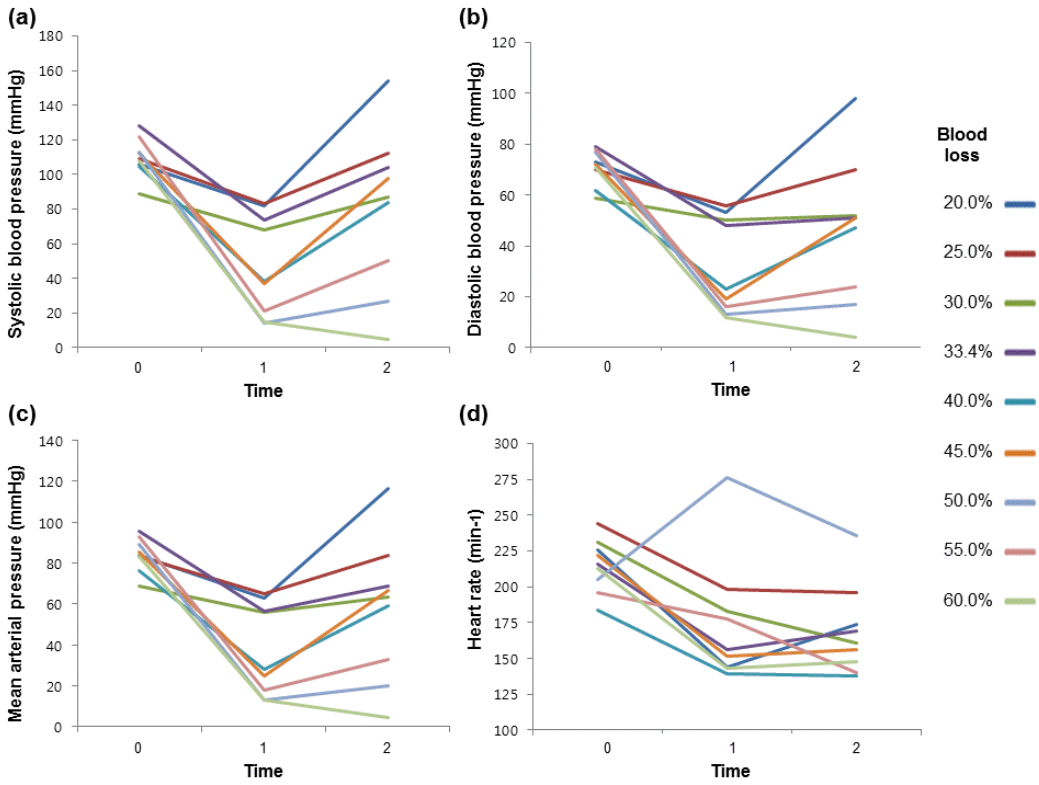


Fig 3. Vital signs at the time of baseline (time0), before (time1), and after (time2) crystalloid fluid resuscitation

(a) Systolic blood pressure

(b) Diastolic blood pressure

(c) Mean arterial pressure

(d) Heart rate

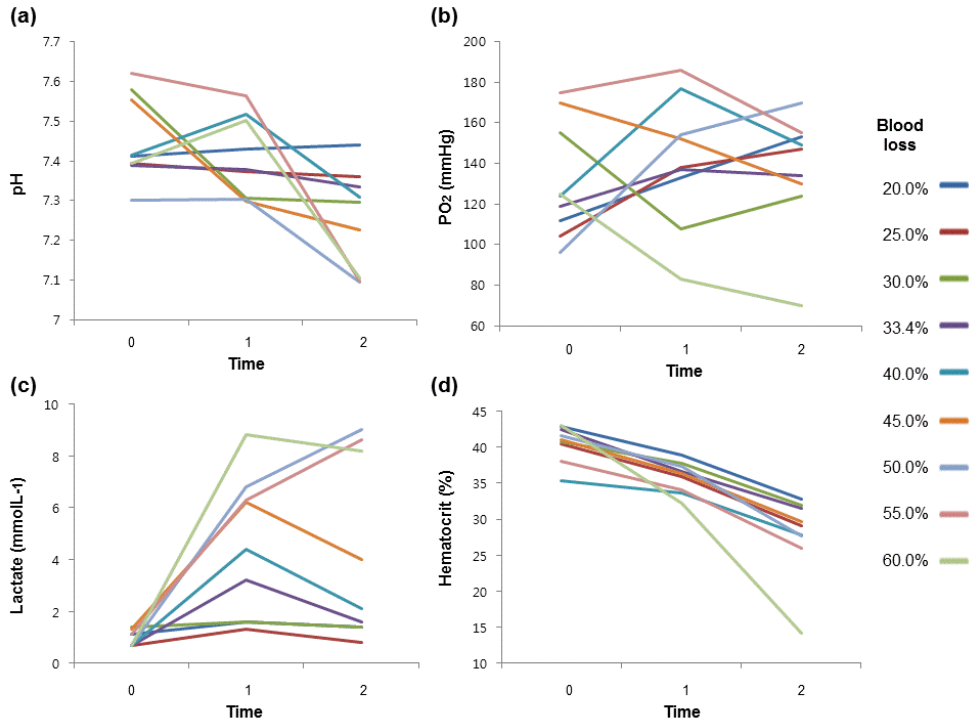


Fig 4. Laboratory findings at the time of baseline (time0), before (time1), and after (time2) crystalloid fluid resuscitation

(a) pH

(b) Partial oxygen pressure (PO2)

(c) Lactate

(d) Haematocrit

Within a linear regression analysis among the rats shedding 30.0–60.0% of their TBV, the equation to estimate RBV was updated as $0.272N/[(Hct_1/Hct_2)–1]+5.64$ (95% CI of k: 0.164–0.380, p=0.001, R^2=0.89). In the correlation analysis between the actual and estimated RBV, r was 0.945 (p=0.001) (Fig 5.a). On a Bland-Altman plot, the difference was 0.00±0.84 cc (95% CI: –0.77, 0.78) with lower and upper limits of agreement of –1.64 (95% CI: –3.04, –0.24) cc and 1.65 (95% CI: 0.25, 3.05) cc, respectively (Fig 5.b). The pre-determined value of ±4.0 cc included the 95% CI of these limits, thereby validating the equation.

The actual RBV (%) was expressed as $5.67/[(Hct_1/Hct_2)–1]+32.8\%$ (95% CI of the slope: 3.14–8.21, p=0.002, R^2=0.87). A calibration plot revealed that the r between the two RBV (%) was 0.932 (p=0.002) (Fig 6.a). On a Bland-Altman plot, the difference was 0.00±4.03% (95% CI: –3.71, 3.71), with lower and upper limits of agreement of –7.85% (95% CI: –14.5%, –1.18%) and 7.85% (95% CI: 1.18%, 14.5%), respectively (Fig 6.b). The 95% CIs of these limits were included within ±20.0%, thereby validating this equation as well.

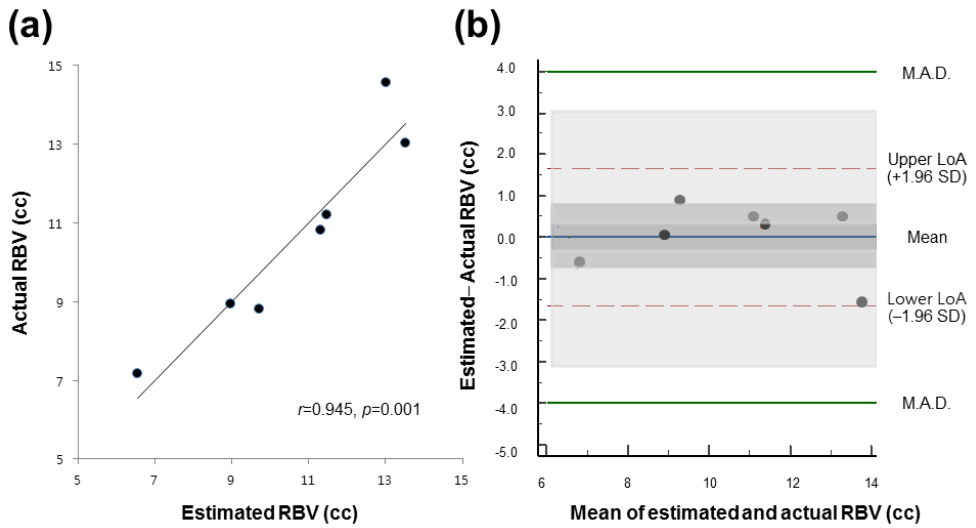


Fig 5. Relation between actual and estimated RBV among the seven rats that showed persistent shock despite fluid resuscitation

(a) Relation between actual RBV and estimated RBV calculated as $0.272N/ [(Hct_1/ Hct_2)–1]+5.64$

(b) Bland-Altman plot with shades showing 95% CI of mean, upper and lower limits of agreement

Hct_1, initial haematocrit; Hct_2, subsequent haematocrit; LoA, limit of agreement; M.A.D., maximum allowed difference (pre-determined); N, volume of crystalloid fluid infused in-between; SD, standard deviation

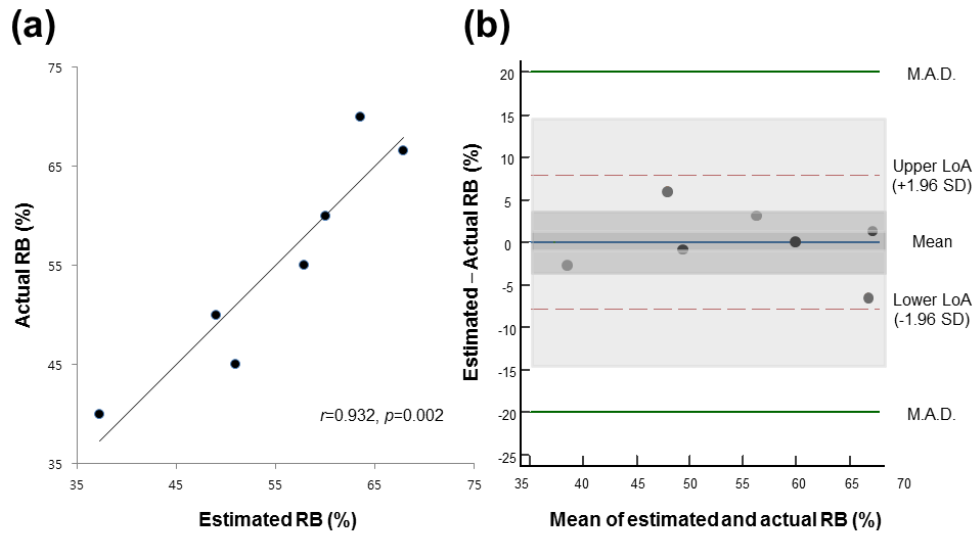


Fig 6. Relation between actual and estimated RBV (%) among the seven rats that showed persistent shock despite fluid resuscitation

(a) Relation between actual RBV (%) and estimated RBV (%) calculated as 6.74/ [(Hct_1/Hct_2)–1]+ 32.3

(b) Bland-Altman plot with shades showing 95% CI of mean, upper and lower limits of agreement

Hct_1, initial haematocrit; Hct_2, subsequent haematocrit; LoA, limit of agreement; M.A.D., maximum allowed difference (pre-determined); SD, standard deviation

As supplementary analyses, the author performed the same analyses including all nine rats that initially suffered haemorrhagic shock after bleeding. RBV was estimated as $0.302N/[(Hct_1/ Hct_2)–1]+5.72$ (95% CI of the slope: 0.138–0.466, p=0.003, R^2=0.73). On calibration, the r between the actual and estimated RBV was 0.854 (p=0.003) (Fig. S1.a). A Bland-Altman plot revealed a difference of 0.00 (95% CI: –1.12, 1.14)±1.48 cc with lower and upper limits of agreement of –2.88 (95% CI: –4.89, –0.87) cc and 2.89 (95% CI: 0.88, 4.90) cc, respectively (Fig S1.b). Actual RBV (%) was expressed as $6.74/[(Hct_1/Hct_2)–1]+32.3\%$ (95% CI of the slope: 2.29–11.2, p=0.009, R^2=0.65). A calibration plot revealed that the r between the actual and estimated RBV (%) was 0.804 (p=0.009) (Fig S2.a). On a Bland-Altman plot, the difference was 0.02±8.21% (95% CI: –6.28, 6.32), with lower and upper limits of agreement of –16.0% (95% CI: –27.2%, –4.83%) and 16.1% (95% CI: 4.88%, 27.30%), respectively (Fig S2.b). As the 95% CIs of the limits of agreement exceeded ±4.0cc and ±20.0% (the pre-determined values of validation), neither equation was validated.

The results of the regression analyses examining factors associated with RBV (%) are shown in Figs S3, S4, and S5, respectively. These figures present initial and final values and interval changes for vital signs, haematocrit, and lactate. The relevant statistics are summarised in Table S1.

Chapter 4. Discussion

This preliminary study aimed to mathematically derive a simple equation estimating RBV (%) mathematically via serial haematocrit measurements and volumes of infused crystalloids and to validate it *in vivo*. For the rats that shed 30.0–60.0% of their TBV and suffered persistent shock despite fluid resuscitation, the equation was updated and subsequently validated: RBV (%)=6.74/[(Hct_1/Hct_2)–1]+32.3%. In addition, the original equation was also updated and validated: RBV=0.272N/[(Hct_1/Hct_2)–1]+5.64

To our knowledge, this is the first study to suggest an equation to estimate RBV (%) mathematically in order to promptly and correctly calculate blood loss (%) [=100%–RBV (%)] and to update and validate this equation *in vivo*. In addition, this is the first *in vivo* study to validate a mathematically derived equation estimating RBV. As all the involved variables are established components of standard haemorrhagic shock management, these equations do not require an additional apparatus or specialised testing and thus have maximal clinical applicability. If validated in human studies, these equations may help clinicians design an optimal treatment plan for patients suffering from acute, non-ongoing haemorrhagic shock at the earliest possible phase.

The author conducted a regression analysis to explain RBV as a function of N/[(Hct_1/Hct_2)–1], generating a y-intercepts of 5.64. Modification of a prediction rule with the addition of a y-intercept is commonly implemented to fit a new target population during external validation [23, 33]. The original equation to estimate RBV included *k* (the fraction of crystalloid fluid distributed in the intravascular volume) as a slope. A *k* of 0.272 (95% CI: 0.164–0.380) for rats shedding 30.0–

60.0% of their TBV was observed for this experimental group. This seems to match the *k* values reported for humans, which is reported to be approximately 0.25 [19].

Supplementary analyses revealed that the regression equations implemented for rats suffering from persistent shock despite fluid resuscitation were superior to those implemented among all the rats regardless of persistent shock shedding (i.e., 20.0–60.0% TBV). In estimating RBV (%), the former showed greater a R^2 (0.87 vs. 0.65) and a narrower 95% CI of the slope (5.67 [3.14–8.21] vs. 6.74 [2.29–11.2]). By excluding the two rats shedding 20.0–25.0% of their TBV, the equation provided a superior explanation of RBV (%) via the equation $24k/[(Hct_1/Hct_2)–1]$ and specified the slope more precisely. Though the author are unsure why the rats that lost 20.0–25.0% of their TBV distorted the equations, the following observations as well as knowledge of the relevant literature provide important context for interpreting these findings. Just before crystalloid fluid resuscitation, their MAP_1 levels were 63 and 65 mmHg, respectively (Fig 3.c). After fluid resuscitation, their MAP_2 levels increased to 117 and 84 mmHg, respectively, exceeding 65 mmHg (the criterion of shock). Their lactate levels were persistently <2.0 mmol/L, failing to fulfill another criterion of shock (Fig 4.c). The more MAP out-ranges above shock level, the more urine is excreted [34, 35]. This leakage of the circulatory system via the urinary system violates the basic assumptions of the current equations and may have isolated these two rats as outliers [17].

Clinicians have estimated RBV (%) or blood loss (%) using vital signs, haemoglobin/haematocrit measurements, CVP/PCWP, ultrasonography, and visual estimation. Although useful, these methods provide only rough estimations.

Tachycardia and hypotension, occurring within class I, II (mild), III (moderate), and IV (severe) haemorrhagic shock, are less reliable indicators for patients receiving antihypertensive medications (especially beta or calcium channel blocking medications) and their sensitivities are unsatisfactory [11, 16]. Haematocrit does not reflect acute haemorrhage adequately as the plasma volume fails to increase sufficiently for achieving a euvolemic state [36]. Neither CVP nor PCWP predicts ventricular preload (which correlates with RBV) [37]. Although ultrasonography provides some hints regarding preload with respect to the diameter and collapsibility of the inferior vena cava as well as fluid responsiveness, these indicate RBV (%) indirectly; fluid challenge is less applicable for haemorrhagic shock patients whose fluid resuscitation should be restricted [18, 38]. Meanwhile, visual estimation of blood loss is inaccurate and unreliable even in the operating room [14]. Due to these limitations, researchers combined these variables to enhance diagnostic accuracy [10, 12]. For example, Callcut and colleagues suggested that massive transfusion is indicated when two of following factors are present: an international normalised ratio (INR)>1.5, SBP<90 mmHg, haemoglobin<11 g/dL, a base deficit of ≥6 mmol/L, and fluid revealed on focused assessment with sonography for trauma (sensitivity 85%, specificity 41%) [10]. However, these rules are relatively non-specific and cannot differentiate RBV (%) quantitatively.

Some researchers previously investigated the volume of infused crystalloid fluid or serial haematocrits (the key variables of the current study) as tools for RBV (%) estimation. The response to initial fluid resuscitation is suggested to help estimate blood loss (%), with rapid, transient, and minimal/no

response correspond to minimal (<15%), moderate and ongoing (15–40%), and severe (>40%) loss of TBV, respectively [11]. However, this approach cannot estimate RBV (%) quantitatively in order to guide fluid/blood resuscitation delicately, as required for successful haemorrhagic shock management.

Thorson and colleagues reported that $Hct_1–Hct_2$ >6% reliably indicate ongoing bleeding [39]. However, only 3.9% (9/232) of their study participants suffered shock and the interval to check the serial haematocrits was 120±63 min even for patients with ongoing bleeding. These rendered their results less applicable for haemorrhagic shock, which required much faster fluid resuscitation followed by a repeat haematocrit measurement; 60% of patients die within 3 h after ED presentation for haemorrhagic shock [4]. Meanwhile, the current study (that dealt with the earliest phase of haemorrhagic shock) showed some correlation between $Hct_1–Hct_2$ and RBV (%) (Fig S5.f, Table S1). This association may be explained mathematically using our equation:

$$\text{RBV (\%)} = 24k/[(Hct_1/Hct_2)–1]$$
$$= 24k/[(Hct_1–Hct_2)/Hct_2]$$
$$= 24k \times Hct_2/(Hct_1–Hct_2)$$

As mentioned above, the equation to estimate RBV (%) contains $Hct_1–Hct_2$ as a denominator. However, considering the effect of the numerator (Hct_2) on the whole equation, the equation including both the numerator and denominator is more robust than $Hct_1–Hct_2$ alone. The R^2 of our regression equation (0.87) is greater than that including $Hct_1–Hct_2$ alone (0.59), supporting its superiority in terms of explaining RBV (%).

By replacing N/TBV with 0.24 in rats, the author simplified the equation to estimate RBV (%) from k×N/TBV/[(Hct_1/Hct_2)–1]×100(%) to 24k/[(Hct_1/Hct_2)–1] (%). The only condition was pre-determination of N in terms of body weight (0.015 cc/g in this study). This suggests that RBV (%), and thus blood loss (%) (100%–RBV [%]) can be determined by Hct_1 and Hct_2 when a fixed N is infused. For a human, TBV (L/kg) is approximately 0.075×(body weight) for men and 0.065×(body weight) for women [6]. When N is fixed as 0.015 L/kg, which corresponds to 1 L for 70 kg adults (in line with standard management of haemorrhagic shock), N/TBV is 0.20 for men and 0.23 for women. If k is 0.25, as reported previously [19], the following equations may be applicable for non-ongoing haemorrhagic shock patients:

RBV (%)=5.0/[(Hct_1/Hct_2)–1] for men, and

5.8/ [(Hct_1/Hct_2)–1] for women

Of course, further clinical studies are required to modify these equations, including adjustment of the slope and the addition of a y-intercept [23].

In supplementary analyses, RBV (%) was closely associated with both initial and final values of SBP, DBP, MAP, lactate, and haematocrit (R^2: 0.50–0.91, with all p<0.05; Table S1 and Figs. S1 and S2). However, the author regarded these results as inapplicable in practice. For instance, the author strictly controlled the time of bleeding, observation, and fluid resuscitation in this animal study. However, haemorrhagic shock patients arrive at the ED at various times following the time of initial bleeding. Because blood pressure, lactate, and haematocrit change over time even in the same patient [28], these variables measured at strict timelines in a laboratory setting are not applicable to real haemorrhagic shock patients.

Chapter 5. Limitation

5.1. Limited Indication of 'Non-ongoing' Haemorrhagic Shock

This study had several limitations. First, this study dealt with 'non-ongoing' haemorrhagic shock. This confines the indication of this work to patients for whom instant haemostasis is achievable (for example, patients with penetrating extremity wounds, peptic ulcers, or perioperative bleeding). For most blunt trauma, maternal haemorrhage, and ruptured aortic aneurysm cases (i.e., the other major causes of mortality due to haemorrhagic shock), instant haemostasis may be difficult to achieve, thus rendering our study results less applicable [1, 3]. However, the current equations may have some value even for ongoing haemorrhagic shock patients; for example, Hct_2 would be lower among ongoing haemorrhagic shock patients than among non-ongoing haemorrhagic shock patients (e.g., 30.0% vs. 32.0%). Incorporating this lowered Hct_2 into the equation as $6.74/[(Hct_1/Hct_2)–1]+32.3(\%)$, while assuming Hct_1=40.0% in this example, would cause RBV (%) to be underestimated (52.5% vs. 59.3%). Therefore, for patients with ongoing haemorrhagic shock, the actual initial RBV (%) must be larger than the value estimated by the equation (52.5% in this example). Clinicians may not know whether bleeding is ongoing. Even in this situation, they may guess that the initial RBV (%) would be at least equal to the estimated value (52.5% in case of a non-ongoing haemorrhage) or larger (in case of an ongoing haemorrhage).

5.2. Limited Indication of 'Persistent Haemorrhagic Shock despite Fluid Resuscitation without Mortality'

Second, according to the study, the degree of haemorrhage may be categorised as follows: <20% of TBV without shock; 20-30% of TBV with shock which can be reversed by crystalloid fluid resuscitation of 0.015cc/g; 30-60% of TBV with persistent shock despite fluid resuscitation; and >60% of TBV with mortality despite fluid resuscitation. Among these four categories, this study focused on the third one losing 30-60% of TBV with persistent shock despite the fluid resuscitation. Clinicians would be more interested in the patients that suffer from haemorrhagic shock probably by shedding >20% of TBV. Although the response after initial fluid resuscitation –recovery from shock, persistent shock, and mortality- may give a hint to the degree of haemorrhage, clinicians should keep in mind that the equation the author proposed in this study is applicable only to those with persistent shock despite initial fluid resuscitation.

5.3. Use of Penetrating Trauma with Low Energy to Induce Haemorrhagic Shock

Third, bleeding was induced simply by puncturing the left femoral artery. With this low energy injury, the author could assume that *k* would not vary significantly. However, in severe trauma, broken endothelial glycocalyx layers and coagulopathy caused by oxygen debt lead to increased vascular permeability and extravasation of

intravascular fluid into the interstitial space (especially under lower oncotic pressure), thus lowering *k* [40-42]. In this situation, *k* might fluctuate according to the type and severity of the injury, thus making the equations less applicable in their current forms.

5.4. Small Sample Size

Fourth, in this preliminary, concept-validating study, the author set the absolute maximum allowed difference between the estimated and actual residual blood as <20.0%, assuming a mean difference of 0.0±4.0%. For rats shedding 30.0–60.0% of their TBV, the actual maximum difference in this study was –6.5%, far smaller than the pre-determined value of ±20%. However, considering the small sample size in this study, the author had to compensate for the wide 95% CIs of the upper and lower limits of agreement. The issue of sample size needs to be considered carefully within further clinical studies conducted to validate the concept of this study.

5.5. Use of a Small Animal

Fifth, the author used rats in this study. To monitor haemodynamic variables, larger animals such as dogs or pigs would have yielded more information in a far stable fashion. Using rats, the author had to compensate even 0.2 cc of blood sampling, which estimated almost 1% of TBV. As the sugar water story was based on the presumption that the container is intact without leakage, the author assumed that

there would be negligible leakage via urination while an animal suffers from a persistent haemorrhagic shock. However, without Foley catheterisation, the author could not measure urine output from rats, which might have been measurable from larger animals.

Chapter 6. Suggestion

Considering these limitations, the author believes that this preliminary concept-validation study is a starting point for further investigations. First, the equation to estimate the RBV (%) needs to be established clinically in non-ongoing, haemorrhagic shock patients and in studies with a larger sample size. Although the author proposed $5.0/[(Hct_1/Hct_2)–1](\%)$ for men and $5.8/[(Hct_1/Hct_2)–1]$ (%) for women (assuming a *k* of 0.25), these equations need clinical validation including adjustment of the *k* value and assignment of a y-intercept [23]. Second, preclinical or clinical studies aiming to broaden the indications of the current equations are required, including those for ongoing haemorrhage and high-energy blunt injury. And if animal studies are to be performed, the author suggests that larger animals be used to get better and more haemodynamic information. In contrast to animal studies wherein researchers can freely pre-determine the degree of bleeding, it may be difficult to determine the 'actual' RBV (or blood loss), which is the reference value to compare the 'estimated' RBV with, among the actual haemorrhagic shock patients. As previously mentioned, the reference standard method to measure RBV is the dilution method using radioactive chromium (^{51}Cr), and the carbon monoxide rebreathing technique is also applicable [7-9]. If permitted ethically, these may be used to determine the 'actual' RBV. However, in case these methods are ethically debatable, the author proposes that, among the patients undergoing major surgery that tends to cause profuse bleeding, the concept of this study can be validated while the anaesthesiologists monitor input/output of fluid, vital signs, and POCT on a real time basis. More practically, the ability to predict the need for massive

transfusion among haemorrhagic shock patients may be compared using the equation the author suggests in this study and the current indexes composed of several variables at ED [10, 12]. Meanwhile, whether the equation the author proposes is associated with the clinical indexes such as SOFA (sequential organ failure assessment) and SAPS (simplified acute physiologic score) II may be also investigated. These indexes are expected to be worse in patients with more blood loss because of the hypoperfusion secondary to multiple organ injuries [43-45]. Furthermore, if the equation shows a direct relationship with the occurrence of multiple organ failure and mortality, it can potentially be easily adopted by clinicians considering its immediate availability and simplicity.

Chapter 7. Conclusion

This concept-derivation and preliminary in-vivo validation study demonstrates that RBV (%) and thus blood loss (%) may be calculable via information on serial haematocrits and the volume of crystalloid fluid infused for rats suffering from acute, non-going haemorrhagic shock. The equations the author suggests in the study seem to apply best for rats suffering from persistent haemorrhagic shock despite crystalloid fluid resuscitation. Further studies are required to validate the clinical applicability of these equations and to widen their indications, regardless of ongoing haemorrhage, injury mechanism, and severity.

Chapter 8. Abbreviations

ABGA: arterial blood gas analysis

ARRIVE: Animal Research: Reporting of In Vivo Experiments

^{51}Cr: radioactive chromium

CVP: central venous pressure

DBP: diastolic blood pressure

ED: emergency department

HbCO: carboxyhaemoglobin

Hct: haematocrits

HR: heart rate

IACUC: Institutional Animal Care and Use Committee

k: the fraction of N distributed in the intravascular volume

MAP: mean arterial blood pressure

N: infused crystalloid fluid volume

PCWP: pulmonary capillary wedge pressure

POCT: point-of-care tests

RBV: residual blood volume

SAPS: simplified acute physiologic score

SBP: systolic blood pressure

SOFA: sequential organ failure assessment

TBV: total blood volume

* Subscripts $_0$, $_1$, and $_2$ to denote baseline before bleeding, initial ED presentation after bleeding and before fluid resuscitation, and post-fluid resuscitation status.

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Chapter 10. Supplementary text

Text S1. A mathematical explanation for how to calculate the initial residual blood volume via information on serial haematocrits and the volume of infused crystalloid fluid.

(a) A solution for estimating the initial mass of sugar water based on initial and final concentrations and the mass of water poured into the mixture (Fig 1.a).

In mathematics class, middle school students are asked the following question: "There is a cup of sugar water with a concentration of 45%; 0.5 kg of water is poured into this mixture. The concentration of the sugar water changed to 40%. Can you calculate the initial mass of the sugar water?"

This is a typical question that deals with a linear equation. The concentration of sugar water is defined as [Mass of the sugar] / [Mass of the sugar water]. When the author designates the initial volume of the sugar water as α (kg), the following equation can be applied to calculate the final concentration of the sugar water mixture:

[Concentration of sugar water, final] = [Mass of the sugar, final] / [Mass of the sugar water, final] …①

Meanwhile,

[Mass of the sugar, final] remains the same as before, that is 0.45α (kg). …②

[Mass of the sugar water, final] = (α+ 0.5) kg …③

Incorporating formulae ② and ③ into ①, the author obtains the following result:

$0.40 = 0.45\alpha / (\alpha + 0.5)$

$0.40 \times (\alpha + 0.5) = 0.45\alpha$

$8 \times (\alpha + 0.5) = 9\alpha$

$\therefore\ \alpha = 4.0$

Therefore, the initial mass of the sugar water was 4.0 (kg).

The key point of this quiz is that once the initial and final concentration of a solution and the mass of water poured into it is known, the initial mass of the solution can be determined. Thus, the degree of dilution of a solution after adding a known amount of solvent can reveal the initial mass of the solution.

(b) Our solution to estimate the residual blood volume (RBV) via information on serial haematocrits (Hct_1 and Hct_2) and the volume of infused crystalloid fluid (N) (Fig 1.b).

The author paid attention to the fact that the sugar water scenario described above is similar to the scenario of initial management of haemorrhagic shock patients. Mathematically, the author derived an equation to estimate the RBV via information on Hct_1, Hct_2, and N.

The haematocrit of blood is defined as [Volume of red blood cells] / [Volume of whole blood]. The author defined the initial blood volume of a haemorrhagic shock patient at the time of hospital arrival as the RBV. Using these definitions, the following equations are applicable to calculate blood volumes before (equation ④) and after (equation ⑤) crystalloid fluid resuscitation:

[Haematocrit of blood, initial] = [Volume of red blood cells, initial] / [Volume of blood, initial] …④

In other words,

Hct_1 = [Volume of red blood cells, initial] / RBV …④'

∴ [Volume of red blood cells, initial] = Hct_1 × RBV …④''

Similarly,

[Haematocrit of blood, final] = [Volume of red blood cells, final] / [Volume of blood, final] …⑤

∴ [Volume of red blood cells, final] = Hct_2 × [Volume of blood, final] …⑤'

Meanwhile, the blood volume has increased by k×N. In other words,

[Volume of blood, final] = RBV + k×N …⑥

Incorporating ⑥ into ⑤', the author finds that

[Volume of red blood cells, final] = Hct_2 × (RBV + k×N) …⑤''

As the author has assumed that blood loss is not ongoing in this study,

[Volume of red blood cells, final] = [Volume of red blood cells, initial] …⑦

Incorporating formulas ④'' and ⑤'' into ⑦, the following is obtained:

Hct_1 × RBV = Hct_2 × (RBV + k×N)

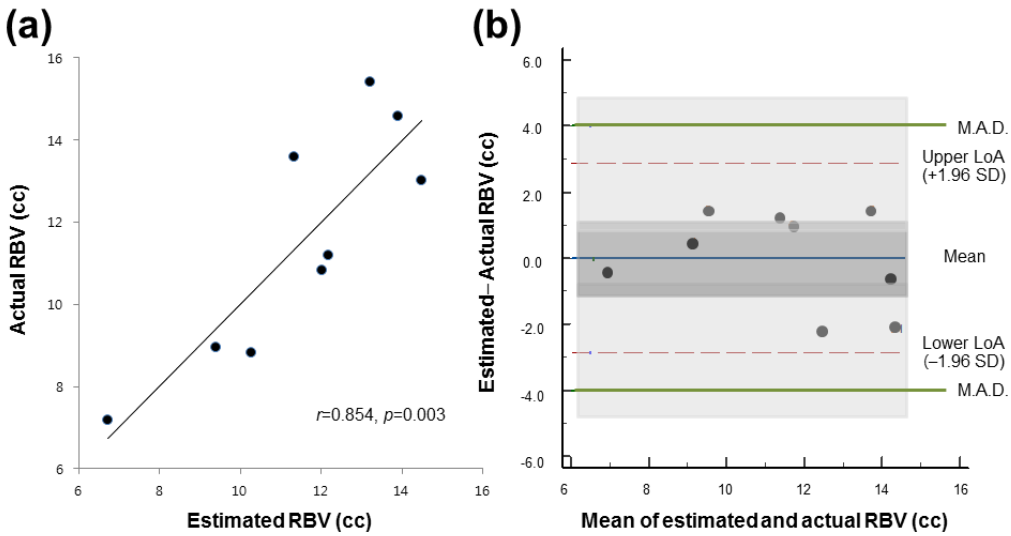
∴ (Hct_1 / Hct_2) × RBV = RBV+ k×N

∴ [(Hct_1 / Hct_2)–1] × RBV = k×N

∴ RBV = k×N / [(Hct_1 / Hct_2)–1]

Chapter 11. Supplementary figures

Fig S1. Relation between actual and estimated RBV among the whole nine rats

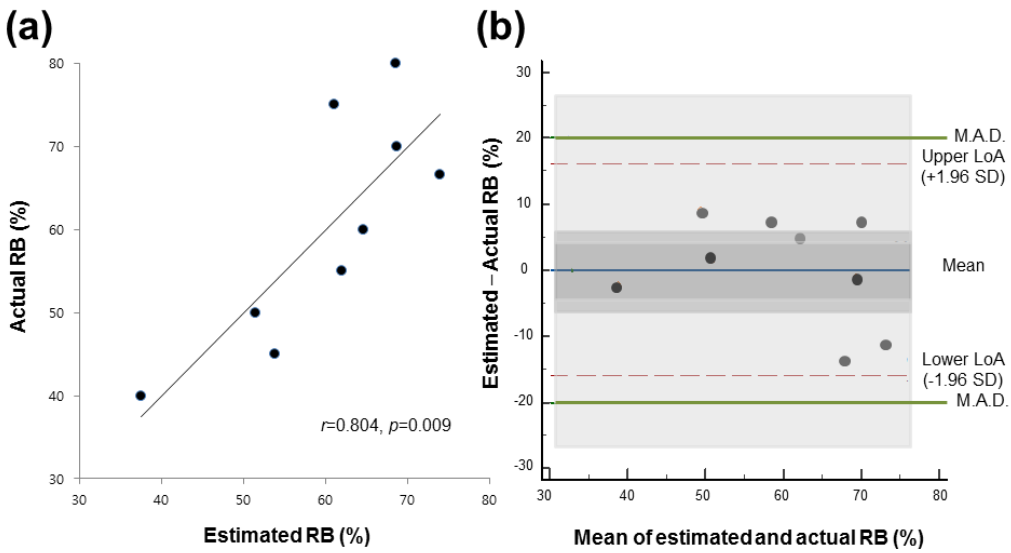


(a) Actual RBV vs. estimated RBV calculated as $0.302N/ [(Hct_1/ Hct_2)–1]+ 5.72$

(b) Bland-Altman plot with shades showing 95% CI of mean, upper and lower LoA

LoA, limit of agreement; M.A.D., maximum allowed difference; RB, residual blood

Fig S2. Relation between actual and estimated RB (%) among the whole nine rats



(a) Actual RB vs. estimated RB calculated as $0.302N/ [(Hct_1/ Hct_2)–1]+ 5.72$

(b) Bland-Altman plot with shades showing 95% CI of mean, upper and lower LoA

LoA, limit of agreement; M.A.D., maximum allowed difference; RB, residual blood

Fig S3. Relation between residual blood (%) and initial (a) systolic, (b) diastolic, (c) mean arterial pressure, (d) heart rate, (e) lactate, and (f) haematocrit before fluid resuscitation

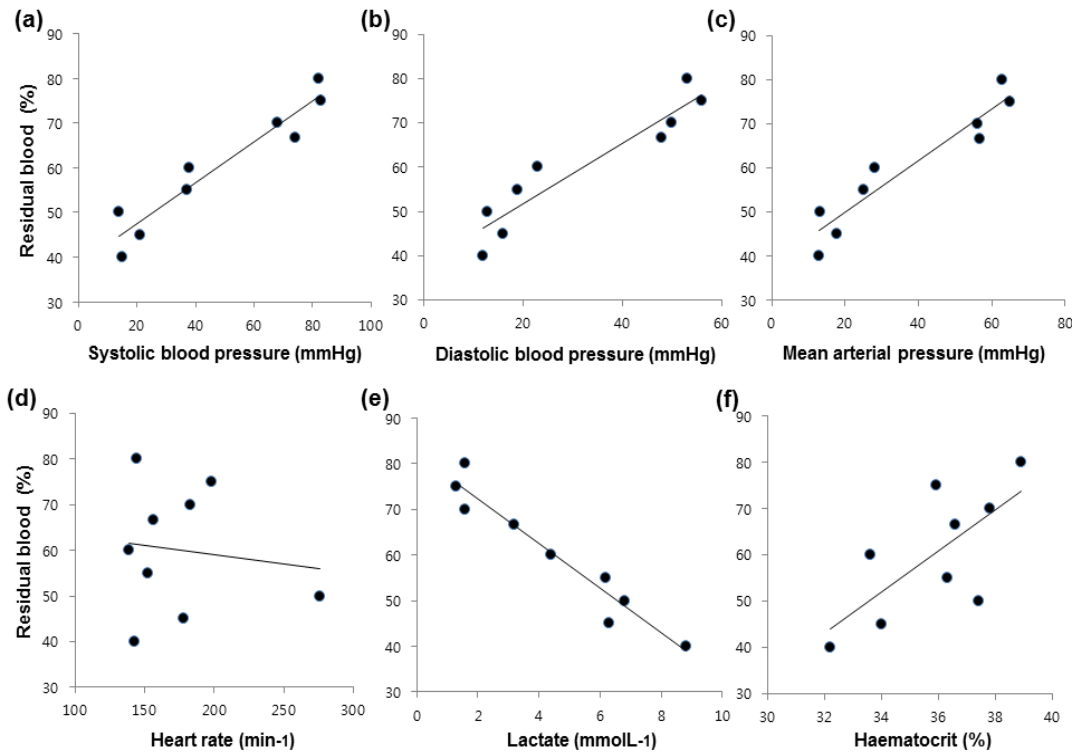


Fig S4. Relation between residual blood (%) and follow-up (a) systolic, (b) diastolic, (c) mean arterial pressure, (d) heart rate, (e) lactate and (f) haematocrit after fluid resuscitation

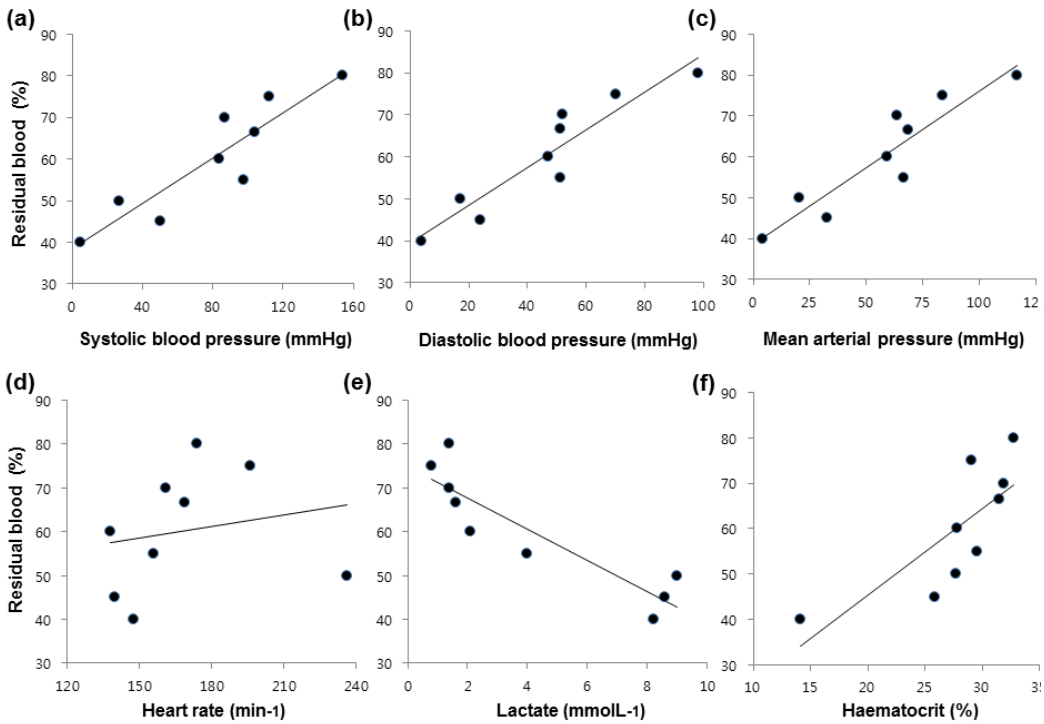
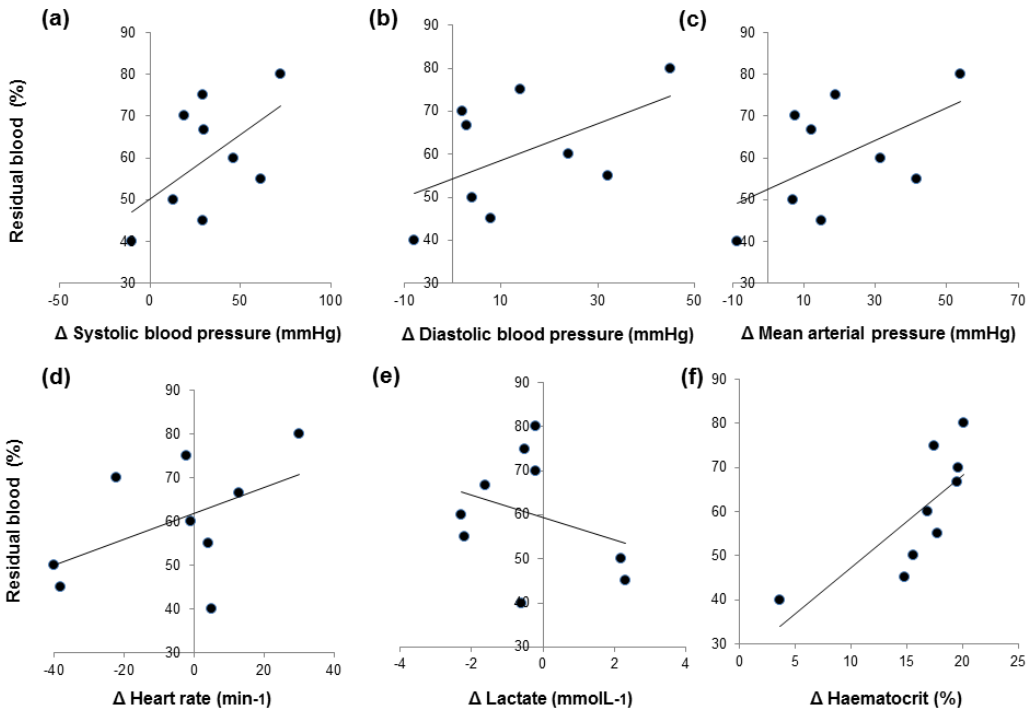


Fig S5. Relation between residual blood (%) and interval change (Δ) of (a) systolic, (b) diastolic, (c) mean arterial pressure, (d) heart rate, (e) lactate and (f) haematocrit



Chapter 12. Supplementary table

Table S1. Linear regression analysis to express residual blood (%) in terms of vital signs, lactate, and haematocrit

Independent variable	Slope (95% CI)	Y-intercept	*p*	R^2	Figure
Initial value after bleeding before crystalloid fluid resuscitation					
SBP_1	0.456 (0.331, 0.582)	38.3	<0.001**	0.91	S3.a
DBP_1	0.685 (0.463, 0.906)	38.1	<0.001**	0.88	S3.b
MAP_1	0.591 (0.418, 0.764)	38.0	<0.001**	0.90	S3.c
HR_1	–0.041 (–0.324, 0.241)	67.4	0.74	0.02	S3.d
$Lactate_1$	–4.91 (–6.10, –3.72)	82.1	<0.001**	0.93	S3.e
Hct_1	4.44 (0.43, 8.45)	–99.1	0.034*	0.50	S3.f
Subsequent value after crystalloid fluid resuscitation					
SBP_2	0.273 (0.159, 0.386)	38.3	0.001**	0.82	S4.a
DBP_2	0.454 (0.300, 0.607)	39.3	<0.001**	0.88	S4.b
MAP_2	0.375 (0.239, 0.511)	38.7	<0.001**	0.86	S4.c
HR_2	0.088 (–0.301, 0.476)	45.4	0.611	0.04	S4.d
$Lactate_2$	–3.57 (–5.11, –2.02)	74.9	0.001**	0.81	S4.e
Hct_2	1.90 (0.518, 3.29)	7.20	0.014*	0.60	S4.f
Interval changes between before and after crystalloid fluid resuscitation					
$SBP_2 – SBP_1$	0.311 (–0.098, 0.720)	50.2	0.115	0.32	S5.a
$DBP_2 – DBP_1$	0.426 (–0.197, 1.05)	54.3	0.150	0.27	S5.b
$MAP_2 – MAP_1$	0.388 (–0.146, 0.923)	52.5	0.130	0.30	S5.c
$HR_2 – HR_1$	0.297 (–0.159, 0.753)	61.9	0.167	0.25	S5.d
$Lactate_2 – Lactate_1$	–2.54 (–9.53, 4.45)	59.3	0.419	0.10	S5.e
$Hct_2 – Hct_1$	2.09 (0.517, 3.67)	26.5	0.016*	0.59	S5.f

CI, confidence interval; DBP, diastolic blood pressure; Hct, haematocrit; HR, heart rate; MAP, mean arterial pressure; R^2, coefficient of determination; SBP, systolic blood pressure

Subscript $_1$ and $_2$ denote the status just before and after fluid resuscitation, respectively.

* $p<0.05$

** $p<0.01$

Chapter 13. Abstract in Korean

배경

출혈성 쇼크의 처치에 있어, 출혈 정도 결정은 도전적 과제다. 저자는 연속적으로 측정한 헤마토크릿 값들과 (Hct_1, Hct_2) 주입된 등장성 수액의 부피를 (N) 이용, 잔존혈액량의 정도를 (RBV, %) 추정하는 예측식을 수학적으로 유도한 뒤, 이를 생체 내에서 검증하고자 하였다. 이 때, 출혈량의 정도는, '100%-잔존혈액략(%)'로 역산이 가능할 것이다.

방법

현재 가이드라인에 의거, 주입할 등장성 수액의 부피를 (N) '0.015×체중(g)'cc 로 고정함으로써, 저자는 잔존혈액량의 정도를 다음 수식으로 나타낼 수 있음을 수학적으로 유도하였다. $24k/[(Hct_1/Hct_2)-1]$ (%) (단, k는 주입된 등장성 수액이 혈관 내에 분포되는 정도). 검증을 위해, Sprague-Dawley 백서 9마리에 대해 20.0%에서 60.0%까지 5.0% 간격으로 비진행성 출혈성 쇼크를 유발하였다. 쇼크 유발 10분 뒤 Hct_1 을 확인하고서, 생리식염수 N [=0.015×체중(g)] cc를 10분에 걸쳐 주입하였다. 주입 종료 5분 후, Hct_2 를 측정하였다. 저자는 잔존혈액량을 종속변수, '$1/[(Hct_1/Hct_2)-1]$'를 독립변수로 하는 선형회귀분석을 실시, 이들 간의 관계식을 도출하였다.

결과

전체 혈액량의 30.0-60.0%를 실혈한 일곱 마리만 수액 처치에도 지속되는 출혈성 쇼크를 보였다. 이들의 예측 잔존혈액량은

‘5.67/[(Hct_1/Hct_2)−1]+ 32.8’ (%)로 개정되었다 (기울기의 95% 신뢰구간: 3.14–8.21, p=0.002, R^2=0.87). Bland-Altman 플롯에서 실제 잔존혈액량과 예측 잔존혈액량의 차이는 0.00±4.03%였다. 둘 사이 일치도의 한계치의 95% 신뢰구간은, 미리 정해둔 기준 ±20% 범위에 포함, 유도된 잔존혈액량 예측식이 검증되었다.

결론

수액 처치에도 지속되는, 비진행성 출혈성 쇼크 하의 백서에 대해, 저자는 잔존혈액량을 예측하는 식을 수학적으로 유도한 후, 생체 내 검증하였다. 이를 통해, 주입 수액량 지정 시, 연속적 헤마토크릿 값만 있으면 잔존혈액량의 정도를 구할 수 있고, 이를 통해 실혈 정도를 [=100%−잔존혈액량 (%)] 쉽게 역산해 낼 수 있다. 출혈성 쇼크에 빠진 환자들에 대한 응급처치에 이를 활용하기 위해서는, 임상 검증 연구가 필요하겠다.

핵심어: 혈액량 결정, 헤마토크릿, 출혈성 쇼크, 등장성 수액

학번: 2013-30572

Chapter 14. Acknowledgements

2013 년에 시작한 박사 과정이 10 년째인 2022 년에 드디어 마무리되려나 봅니다.

지난 10 년간 제겐 굉장히 많은 변화들이 있었습니다. 모교에 진료교수로 부임했다가, 이역만리 UAE SKSH 에서 셋업 과장으로 혼신의 힘을 다 쏟았으나, 그마저도 여의치 않아서 귀국, 현 근무지 분당차병원에 재직한 지 벌써 6 년째가 되었습니다.
그러나, 이런 경력의 변화는, 그간의 인생관 변화에 비하면 오히려 작다 하겠습니다. 스스로 somebody 라 착각했던 제가, 스스로 자초한 인생의 험로들 겪으며 nobody 임을 깨달았습니다. 그러나, 깨닫는 것과 그를 토대로 살아가는 것은 별개 문제였습니다. 어쩜 지금도 저는 제 자신이 nobody 임을 받아들이는 문제와 씨름하고 있는 지도 모르겠습니다. 이 문제를 해결해야 비로소 참 자유를 누릴 텐데, 그게 쉽지 않아 고민이 많은 요즘입니다.

이 인생관의 변화와 맞물렸기에, 이 학위 논문은 제게 큰 의미를 갖습니다. 2016 년에 아이디어(가설)를 brief communication 형태로 Journal of Korean Medical Science 에 출판했던 것을, 이론적으로 정교하게 다듬고 생체 내에서 증명한 것이 이 논문입니다. 작년 7 월, 초고 탈고 후, 저는 이 논문이 출혈성 쇼크 환자의 잔존혈액량 추정에

도움 주는 결정적 문헌이 될 거란 과대망상에 빠졌었습니다. 그러나, 그리 시작한 투고가 desk rejection 9 회를 포함, 총 12 회 거절당하고, 마침내 금년 2 월 The Korean Journal of Physiology and Pharmacology 에서 처음으로 minor revision 판정을 받음으로써 이 논문은 겨우 기사회생하였습니다. (참고로, 해당 저널 2022 년 5 월호에 게재되었습니다.) 12 는 완전수라던가요? 저널들에서 이미 수 차례 거절당하던 작년 2021 년 늦가을 어느 날, 문득 초연해졌습니다: '아, 내 뜻대로 되는 것은 하나도 없구나.'. 여전히 somebody 임을 증명하려던 제 자신이 nobody 임을 재인증하게끔 항복하게 한 결정적 계기가 바로 이 논문입니다. 제 인생에 굉장히 중요한 변곡점 이룬 이 논문 덕에, 저는 삶 자체를 완전히 revision 하기로 했습니다.

이미 12 번의 rejection 으로 증명된 바, 여러모로 부족한 논문임에도, 기획, 실행, 작성 과정 중 참 많은 분께 도움 받았습니다.
우선, 지도교수 신상도 교수님께 감사드립니다. 굉장히 바쁜 와중에 주셨던 촌철살인의 충고들은 논문의 방향성은 물론, 제 삶을 뒤돌아보게 만들었습니다. 또한, 심사위원장으로 애써주신, 전공의 시절부터의 스승 서길준 교수님께 심심한 감사의 말씀드립니다. 후배 의사의 졸고를 타과적 관점에서 조망, 따뜻한 격려와 조언 주신 내과 이상민 교수님과 외과 박도중 교수님께도 감사드립니다. 한편, 전공의 시절부터 늘 멋진

선배였던 성균관의대 조익준 교수님께서 함께 심사해주셔서 참 뜻 깊었습니다.

실험 연구를 경험이 없던 제게 기회와 장을 열어주고, 바쁜 시간 쪼개 실험을 진두지휘해 주신 김규석 교수님 덕분에 연구가 성립될 수 있었습니다. 또한 이민지, 박예진 연구원의 도움 없이 진행된 실험은 단 1 회도 없었습니다. 2016 년 JKMS 논문의 1 저자이자 제 연구의 멘토이신 오원섭 교수님 및 연구의 기획 함께 한 권준명 선생님께도 참 감사드립니다.

그렇게도 아들이 박사 되는 것 보고 싶어하셨던 아버지 고 천윤원 장로님, 어머니 고 김은숙 권사님을 추모합니다. 또 이제 가족이란 인연으로는 가장 긴 세월 함께 한 동생 지현에게 사랑한다는 인사를 전합니다. 한편, 지난 18 여년 늘 불안정한 행보 보인 사위를 묵묵히 지켜보며 응원해주신 장인 최봉오 목사님과 장모 이옥수 권사님께도 감사의 말씀 올립니다. 또한, 부족한 아빠 때문에 힘든 시기 겪었지만 잘 헤쳐나와준 사랑하는 아들 재희와 자기 일에 늘 최선을 다하는 자랑스런 딸 예희에게 고마움을 표합니다. 예희는 그림 디자인도 도와주었습니다. 그리고, 평생의 반려자로서 제 삶의 기쁨의 너무도 큰 이유인 아내 최진선에게 사랑한다 고백합니다.

끝으로, 백지수표 드린 바, 앞으로 제 삶의 revision 을 책임지실, 그래서 그 언젠가 기꺼이 acceptance 해 주실, 제 삶의 교신저자 하나님께 영광과 감사를 돌립니다.

2022. 초여름

천성빈