Supplementary Text 1. 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. 1A).

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 we designate 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, we obtain 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 (Hct1 and Hct2) and the volume of infused crystalloid fluid (N) (Fig. 1B).

We 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, we 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]. We 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 cal culate 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] = $Hct_1 \times$ RBV …④"

Similarly,

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

∴ [Volume of red blood cells, final] = $Hct_2 \times$ [Volume of blood, final] …⑤'

Meanwhile, the blood volume has increased by $k \times$ N. In other words,

[Volume of blood, final] = RBV + $k \times$ N …⑥

Incorporating ⑥ into ⑤', we find that

[Volume of red blood cells, final] = $Hct_2 \times (RBV + k \times N)$ …⑤"

As we have assumed that blood loss is not ongoing in this study,

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

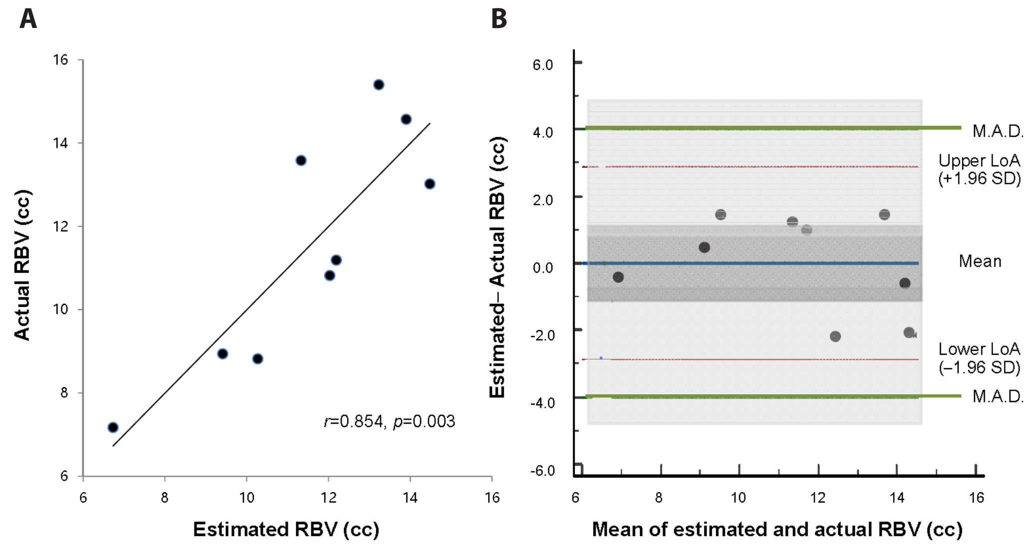
Incorporating formulas ④" and ⑤" into ⑦, we obtain the following:

$Hct_1 \times RBV = Hct_2 \times (RBV + k \times N)$

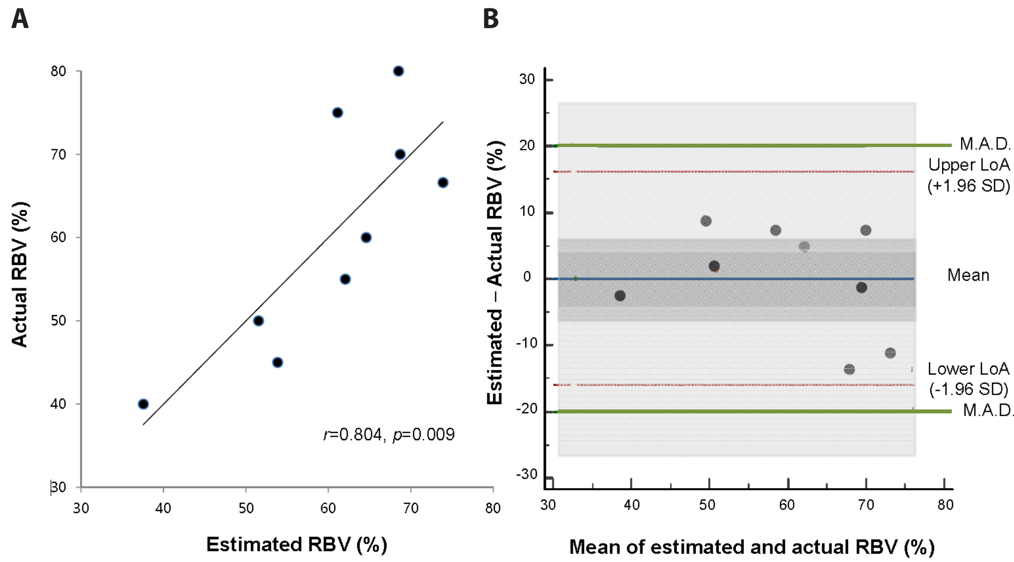
$\therefore (Hct_1 / Hct_2) \times RBV = RBV + k \times N$

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

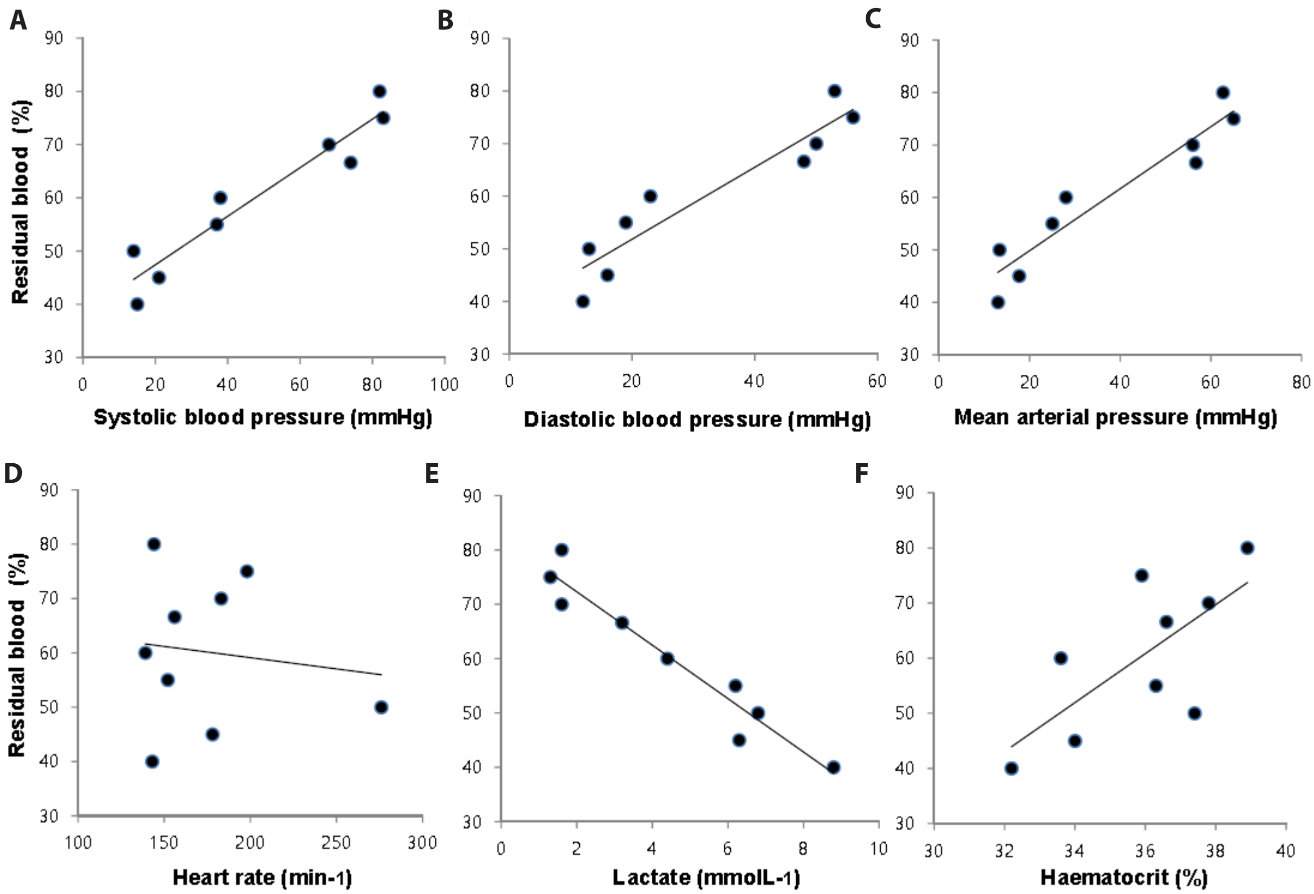
$\therefore RBV = k \times N / [(Hct_1 / Hct_2) - 1]$



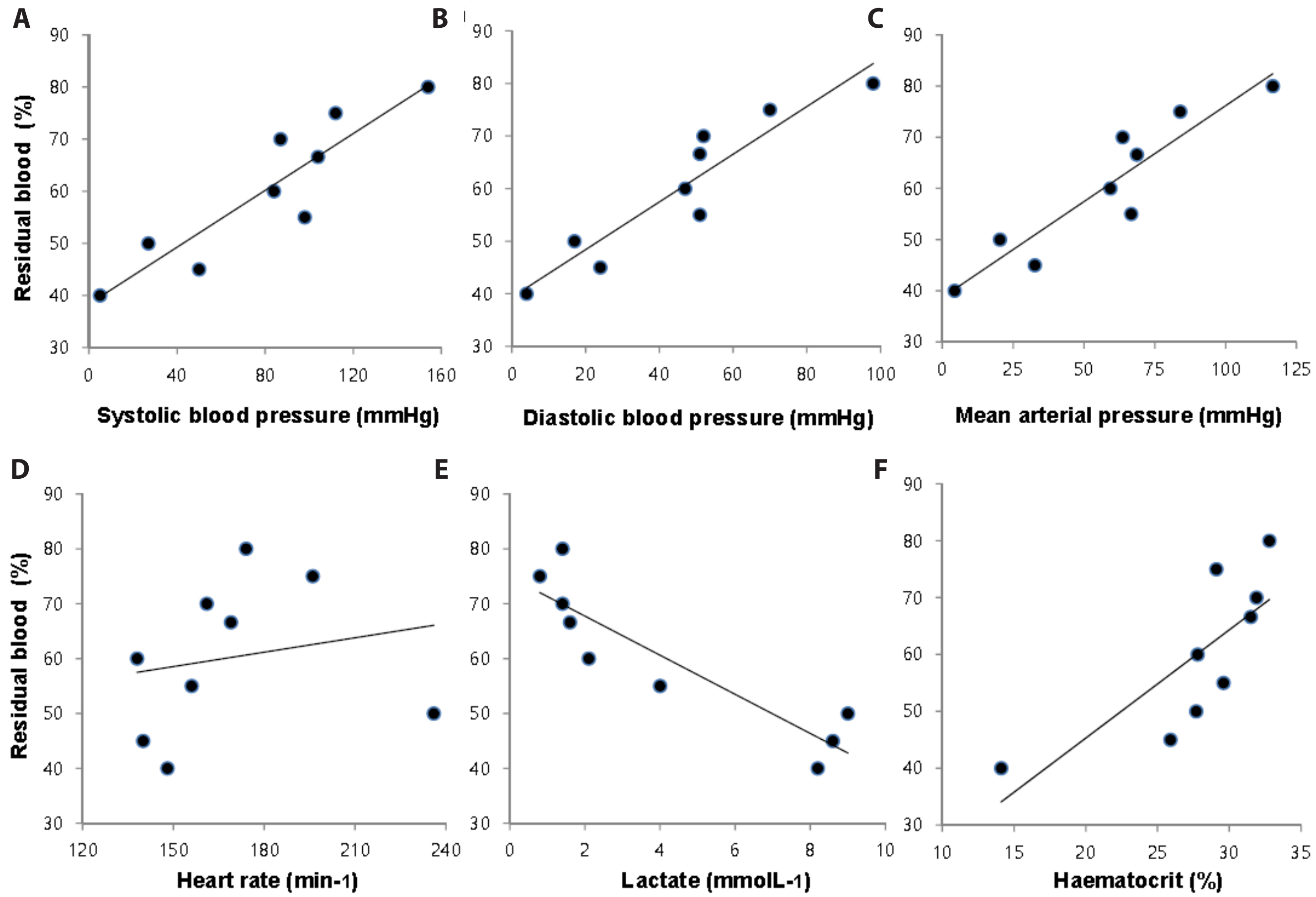
Supplementary Fig. 1. Relation between actual and estimated residual blood volume (RBV) among the whole nine rats. (A) Actual RBV *vs.* estimated RBV calculated as 0.302 N / [(Hct_1 / Hct_2) – 1] + 5.72. (B) Bland-Altman plot with shades showing 95% CI of mean, upper and lower LoA. Hct_1, initial haematocrit; Hct_2, subsequent haematocrit; LoA, limit of agreement; M.A.D., maximum allowed difference (pre-determined); SD, standard deviation; CI, confidence interval.



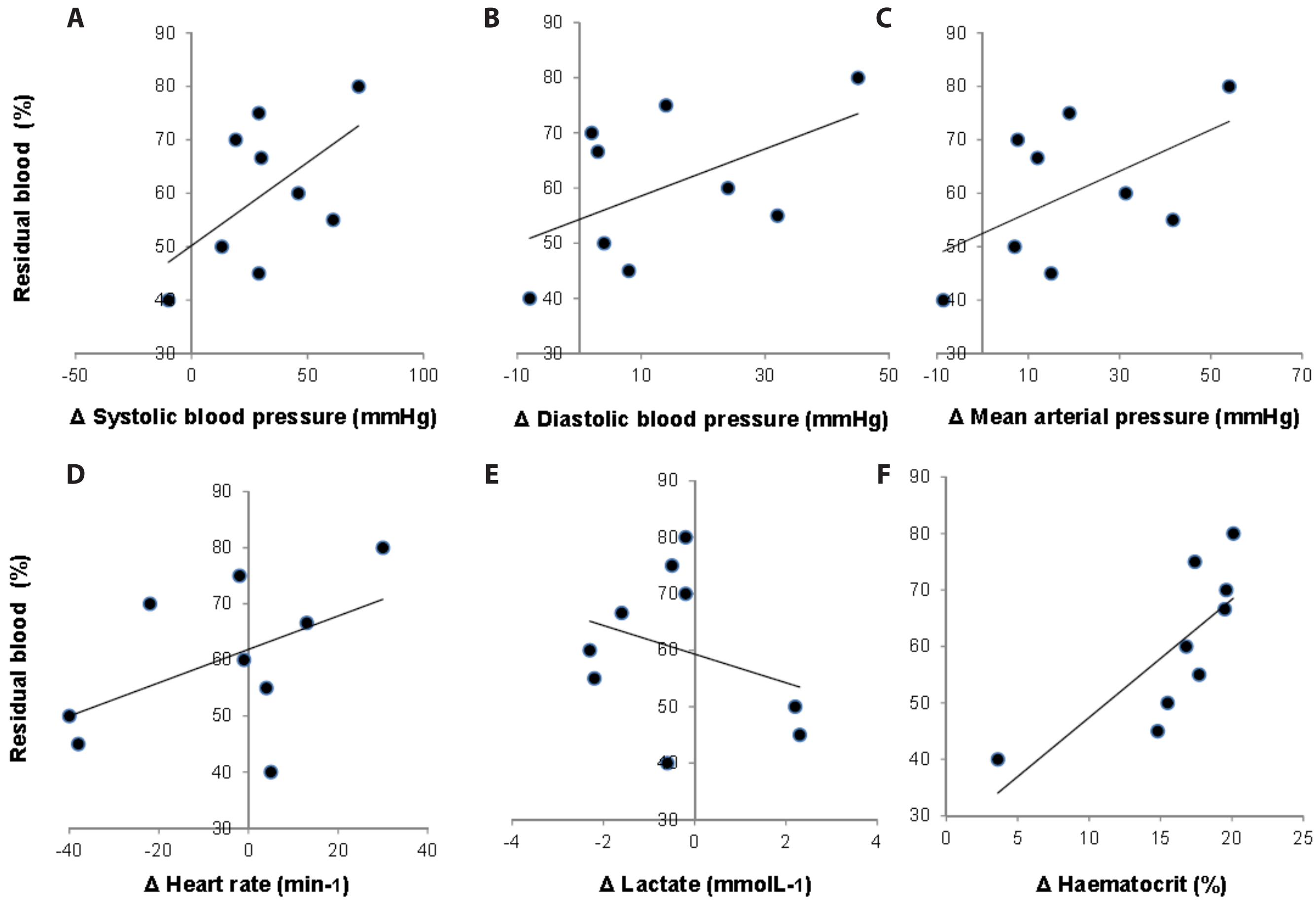
Supplementary Fig. 2. Relation between actual and estimated residual blood volume (RBV) (%) among the whole nine rats. (A) Actual RBV *vs.* estimated RBV calculated as 0.302 N / [(Hct_1 / Hct_2) – 1] + 5.72. (B) Bland-Altman plot with shades showing 95% CI of mean, upper and lower LoA. Hct_1, initial haematocrit; Hct_2, subsequent haematocrit; LoA, limit of agreement; M.A.D., maximum allowed difference (pre-determined); SD, standard deviation; CI, confidence interval.



Supplementary Fig. 3. 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.



Supplementary Fig. 4. 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.



Supplementary Fig. 5. 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.

Supplementary Table 1. Linear regression analysis to express residual blood (%) in terms of vital signs, lactate, and haematocrit

Independent variable	Slope (95% CI)	Y-intercept	p-value	R^2	Supplementary Figure No.
Initial value after bleeding before crystalloid fluid resuscitation					
SBP_1	0.456 (0.331, 0.582)	38.3	< 0.001**	0.91	3A
DBP_1	0.685 (0.463, 0.906)	38.1	< 0.001**	0.88	3B
MAP_1	0.591 (0.418, 0.764)	38.0	< 0.001**	0.90	3C
HR_1	–0.041 (–0.324, 0.241)	67.4	0.74	0.02	3D
$Lactate_1$	–4.91 (–6.10, –3.72)	82.1	< 0.001**	0.93	3E
Hct_1	4.44 (0.43, 8.45)	–99.1	0.034*	0.50	3F
Subsequent value after crystalloid fluid resuscitation					
SBP_2	0.273 (0.159, 0.386)	38.3	0.001**	0.82	4A
DBP_2	0.454 (0.300, 0.607)	39.3	< 0.001**	0.88	4B
MAP_2	0.375 (0.239, 0.511)	38.7	< 0.001**	0.86	4C
HR_2	0.088 (–0.301, 0.476)	45.4	0.611	0.04	4D
$Lactate_2$	–3.57 (–5.11, –2.02)	74.9	0.001**	0.81	4E
Hct_2	1.90 (0.518, 3.29)	7.20	0.014*	0.60	4F
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	5A
$DBP_2 – DBP_1$	0.426 (–0.197, 1.05)	54.3	0.150	0.27	5B
$MAP_2 – MAP_1$	0.388 (–0.146, 0.923)	52.5	0.130	0.30	5C
$HR_2 – HR_1$	0.297 (–0.159, 0.753)	61.9	0.167	0.25	5D
$Lactate_2 – Lactate_1$	–2.54 (–9.53, 4.45)	59.3	0.419	0.10	5E
$Hct_2 – Hct_1$	2.09 (0.517, 3.67)	26.5	0.016*	0.59	5F

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.