Original Research Article

A mini volume loading test (mVLT) using 2.5-mL kg^{-1} boluses of crystalloid for indication of perioperative changes in hydration status

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ABSTRACT

Background and objective: A mini volume loading test (mVLT) evaluating hemodilution during step-wise crystalloid infusion has established that the arterio-capillary plasma dilution difference is inversely correlated to the body hydration level of subjects. This observational study aimed to test whether this can be replicated in a perioperative setting using a 2.5-mL kg^{-1} boluses.

Materials and methods: The mVLT was performed before induction of regional anesthesia and 24 h later. Step-wise infusion implied six mini fluid challenges. These consisted of 2.5-mL kg^{-1} boluses of Ringer's acetate infused during 2–3 min and followed by 5-min periods with no fluids. Invasive (arterial) and noninvasive (capillary) measurements of hemoglobin were performed before and after each mini fluid challenge, as well as after a 20-min period without fluid following the last bolus. Hemoglobins were used to calculate the arterio-capillary plasma dilution difference which is used as an indication of changes in body hydration level. The 24-h fluid balance was calculated.

Results: Subjects were 69.5 (6.0) years old, their height was 1.62 m (1.56–1.65), weight was 87.0 kg (75.5–97.5) and body mass index (BMI) was 33.5 kg/m^2 (31.0–35.1). Preoperative arterio-capillary plasma dilution difference was significantly higher than postoperative (0.085 [0.012–0.141] vs. 0.006 [−0.059 to 0.101], $P = 0.000$). The perioperative 24-h fluid balance was 1976 mL (870–2545).

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Conclusions: The mVLT using 2.5-mL kg^{-1} boluses of crystalloid was able to detect the higher postoperative body hydration level in total knee arthroplasty patients.

1.   Introduction

Fluid management is an important part of perioperative care which will, if correctly administered, enhance recovery after surgery [1]. However, it has been estimated that up to 20% of surgical patients are subject to inappropriate fluid therapy [2]. The administration of fluids is equivalent to the administration of drugs and should be performed with care and appropriate monitoring to avoid harm [3]. Currently, individual fluid requirements are estimated [4] and basic questions regarding fluid therapy such as 'what, when and how (much)' are still unanswered [5,6].

The goal directed therapy (GDT) has evolved with an aim to determine individual fluid requirements and infuse appropriate volume by administering step-wise infusion based on the fluid responsiveness of various target parameters [5]. However, an increase in cardiac output or stroke volume as a response to fluid loading may not necessarily reflect an improved microcirculation. [7]. To study the hydration level of peripheral tissues other measurements are necessary [8].

The fluid infusion induced changes in microcirculation are especially important because delivery of fluid, oxygen and nutrients to the tissues and removing waste products of metabolism depends on the perfusion of true capillaries. However, in addition to limited ability of current clinical techniques to discriminate between perfusion of true capillaries (transcapillary exchange vessels) and metarteriolae (arteriolar-venular shunts), the monitored changes in microcirculation lack specificity and may be explained by many causes other than fluid infusion. Interstitial edema poses direct threat to microcirculation [9] because both crystalloids [10] and colloids [11] may cause excessive fluid accumulation in such tissues. Interstitial edema itself is associated with increased morbidity and mortality [12,13]. Therefore, a clinically useful method for evaluation of interstitial fluid expansion and establishing its correlation with the whole body hydration level is warranted.

A mini volume loading test (mVLT) has previously been found to detect changes in body hydration levels by evaluating hemodilution response to mini fluid challenges [14,15]. In this method, the assessment of hemodilution response is based on non-invasive (capillary) and invasive (large vessel) measurements of hemoglobin. Hemoglobins are used to calculate the difference in dilution between large vessels and capillaries which is used as an indication of changes in body hydration level. There is some evidence that noninvasively evaluated changes in hemoglobin can provide enough information for the mVLT [16].

Previous studies have established that the veno-capillary [14] and arterio-capillary [15] plasma dilution differences are inversely correlated to the body hydration level of subjects.

This observational study aimed to test whether this can be replicated in a perioperative setting using a 2.5-mL kg^{-1} boluses. In our institution pre-study records show that perioperative 24-h fluid balance in elective primary total knee arthroplasty (TKA) patients usually is positive. Thus, under the assumption that preoperatively subjects are less hydrated than postoperatively 24 h later we tested the hypothesis that the arterio-capillary plasma dilution difference (acPDD) is higher preoperatively.

2.   Materials and methods

2.1.   Study design and settings

This study is part of a primary randomized controlled double-blinded clinical trial (primary RCT) conducted at Vilnius University Emergency Hospital, Vilnius, Lithuania (Fig. 1). The primary RCT was approved by Vilnius Regional Bioethics Committee, Lithuania (Ethical Committee N° 158200-9-071-22) and registered at Clinicaltrials.Gov (trial identifier: NCT01355900).

2.2.   Eligibility and enrolment

All patients scheduled for TKA at our hospital were screened. Inclusion criteria were ASA II physical state adult patients scheduled for TKA surgery at 8 AM, operation performed by the same lead surgeon and anesthesia provided by the same anesthetist. Exclusion criteria included patients with body mass index <20 or >40 kg/m^2, rheumatoid arthritis, diabetes, history of bleeding disorders or thromboembolic events and severe deformity of the knee. A detailed list is described elsewhere [17]. A written informed consent for participation in the study was obtained from all subjects.

2.3.   Perioperative care

2.3.1.   Enhanced recovery pathway
Perioperative care was conducted in compliance with our institution's enhanced recovery (ER) program 2009. An ER pathway included carbohydrate rich diet before surgery, tromboprophylaxis, antibiotic prophylaxis, prevention of nausea and vomiting, ''fit-for-purpose'' fluid therapy, spinal anesthesia, epidural analgesia, early postoperative nutrition and physical rehabilitation, early removal of catheters and restrictive transfusion strategy.

2.3.2.   Interventions
During the preoperative night rest, all subjects were deprived of food but were allowed to drink clear fluids (Fig. 1). The patients arrived in the operating room at 7:00 AM. On arrival,
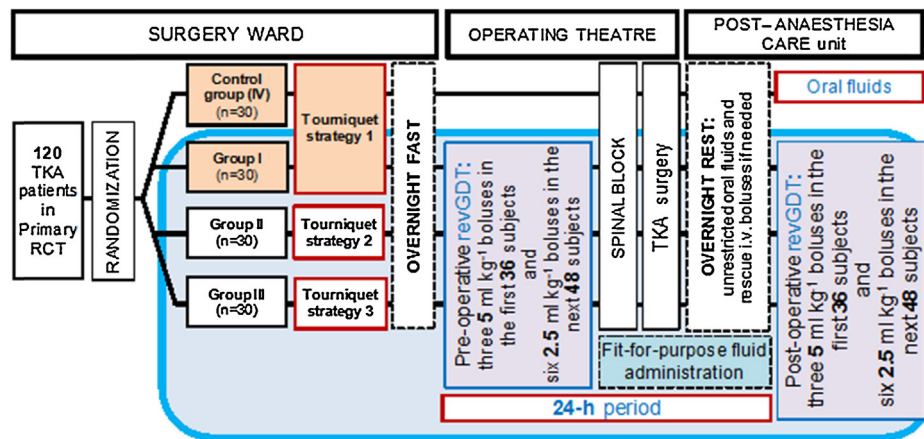
Fig. 1 – The study protocol. A snapshot of the study protocol of a primary RCT. The study protocol of the present observational study is part of the primary RCT (see area highlighted in blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

standard monitors (electrocardiography, pulse-oximetry and non-invasive arterial blood pressure) were applied. Oxygen was provided by a facemask and the FiO_2 was continuously adjusted toward target oxygen saturation of 98%–100%.

A spectrophotometric adhesive sensor (ReSposable™ R2-25a; Masimo Inc., Irvine, CA) was attached to the nail of the middle finger of one hand to measure total hemoglobin (SpHb) and perfusion index (PI). The sensor was covered with an opaque probe cover to eliminate interference from ambient light. The sensor was further connected to a Radical-7 Pulse CO-Oximeter (Masimo Inc.; software version 7.6.2.1). The monitor was set to arterial mode and a 'short' averaging time. A radial artery cannula was placed in the same arm on which the SpHb sensor was placed. This line was used for sampling blood for laboratory analysis of arterial hemoglobin concentration (aHb), and also for direct monitoring of arterial blood pressure (DASH 3000®, GE Medical Systems Information Technologies Inc., Milwaukee, WI) and cardiac stroke volume response to mini fluid challenges (ΔSV) by the arterial pulse contour analysis technique (LiDCO Plus, London, UK). The laboratory analysis of aHb was performed by CO-Oximetry (COULTER® LH750, Beckman Coulter Inc., Chicago, IL). An antecubital cannula was placed in the same arm for fluid infusion.

In this study a revised goal directed therapy (revGDT) protocol where GDT is used supplemented by mVLT (Fig. 2). Each patient received a series of mini fluid challenges. These were 2.5 mL kg^{-1} boluses infused for 2–3 min and followed by 5-min periods with no fluids. Measurements of aHb, SpHb and PI were performed before and after each mini fluid challenge, as well as after a 20-min period without fluid following the last bolus. Data were manually recorded. In addition, hemoglobins were entered into the computer. Hemoglobins were used to calculate the arterial and capillary plasma dilutions (aPD and cPD, respectively) and their difference, the acPDD. Calculations were performed and acPDD trend was visualized on the screen (Fig. 3) using the Windows® platform-based software of our prototype automated clinical decision support system [18,19]. Calculations are explained in Appendix.

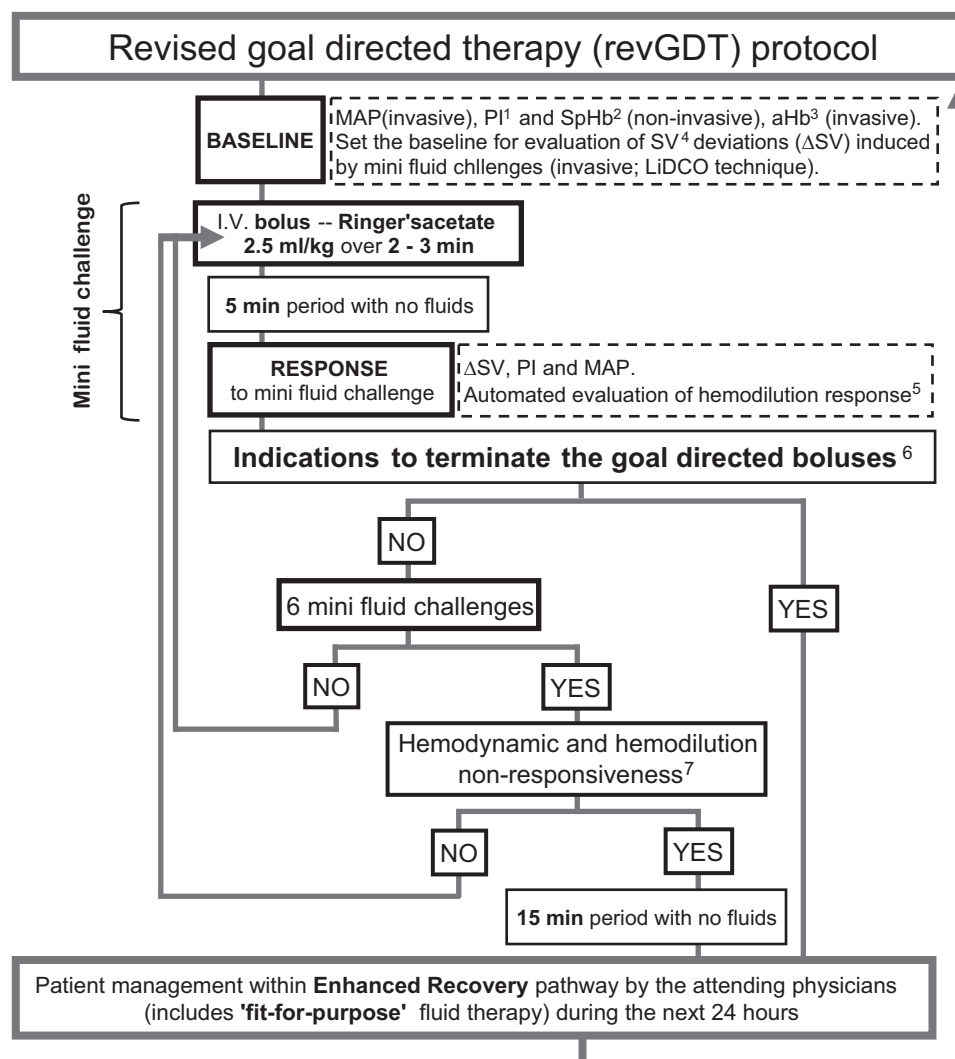Volume responsiveness was evaluated after each mini fluid challenge by measuring ΔSV (%).

The number of mini fluid challenges was decided as follows. The net 15 mL kg^{-1} volume of Ringer's acetate infused in six boluses aimed to replace the estimated basal physiological fluid deficit after preoperative overnight fast [20]. The same fluid deficit was assumed to be present before the postoperative session because of minor postoperative blood loss and negligible oral fluid intake with no i.v. infusions during the postoperative night rest. Thus, at least six 2.5-mL kg^{-1} boluses were supposed to be given in each fluid session.

Indication to stop boluses after the sixth mini fluid challenge was the presence of two criteria: (1) *hemodynamic non-response* defined as ΔSV < 10% (macrocirculation non-response), (2) *transcapillary hemodilution* defined as negative value of acPDD (when tissues become saturated with fluid during bolus infusion, after entering capillaries arterial blood is diluted by increased transcapillary shift of interstitial fluid during the 5-min period without infusion; the process labeled as *transcapillary fluid reflux*). The acPDD was visualized on the screen (Fig. 3) of our prototype automated clinical decision support system [18,19]. For safety reasons, termination of the study protocol was indicated at any stage if there were signs of patient distress including an increase or decrease in mean arterial pressure (MAP) by more than 30%, SV decrease for more than 10% from baseline (suggests fluid overload), an increase of heart rate >110 bpm, complaints of dizziness or a change of mental state.

2.3.3. Patient care during 24-h period between the fluid sessions

After completing the preoperative fluid session, spinal anesthesia was administered and the surgery commenced. Other measures have been described elsewhere [15,17].

Intravenous fluid administration during the 24 h between pre- and postoperative fluid sessions was at the discretion of the attending physicians (anesthesiologists and surgeons). The ''fit-for-purpose'' principles of fluid administration [3] within our ER pathway were applied to all subjects during the

Revised goal directed therapy (revGDT) protocol

BASELINE — MAP(invasive), PI[1] and SpHb[2] (non-invasive), aHb[3] (invasive). Set the baseline for evaluation of SV[4] deviations (ΔSV) induced by mini fluid chllenges (invasive; LiDCO technique).

Mini fluid challenge

I.V. **bolus** -- **Ringer'sacetate 2.5 ml/kg** over **2 - 3 min**

5 min period with no fluids

RESPONSE to mini fluid challenge — ΔSV, PI and MAP. Automated evaluation of hemodilution response[5]

Indications to terminate the goal directed boluses [6]

NO

6 mini fluid challenges

NO   YES   YES

Hemodynamic and hemodilution non-responsiveness[7]

NO   YES

15 min period with no fluids

Patient management within **Enhanced Recovery** pathway by the attending physicians (includes **'fit-for-purpose'** fluid therapy) during the next 24 hours

[1] **PI** - perfusion index (Radical 7 monitor from Masimo Inc., Irvine, CA, USA).

[2] **SpHb** - non-invasive total (capillary) hemoglobin (Radical 7 monitor from Masimo Inc.).

[3] **aHb** - invasive arterial hemoglobin concentration.

[4] **ΔSV** - stroke volume deviation measured by arterial pulse contour analysis (LiDCO Plus, London, UK).

[5] **Hemodinamic and hemodilution response** -- ΔSV, aHb and SpHb, arterial (aPD) and capillary (cPD) plasma dilutions, arterio-capillary plasma dilution difference (acPDD), arterial (aPDE) and capillary (cPDE) plasma dilution efficies, arterio-capillary plasmadilution efficacy difference (acPED).

[6] **Signs of patient distress** includes an increase or decrease in mean arterial blood pressure (MAP) by more than 30%, a decrease in SV by >10% from initial baseline, an increase of heart rate >110 bpm, complaints of dizziness or changes in mental status.

[7] **Hemodinamic and hemodilution non-responsiveness** includes ΔSV < 10 % and negative values of arterio-capillary plasma dilution difference (acPDD) and/or arterio-capillary plasmadilution efficacy difference (acPED) in response to a mini fluid challenge.

Fig. 2 – A revised goal directed therapy (revGDT) protocol.

24 h period between the fluid sessions (Fig. 1). This therapy commenced during the induction of spinal anesthesia (about 15 min after the fluid session). It started with a 1–2 mL kg^{-1} h^{-1} infusion of 0.9% NaCl or Ringer's acetate ('maintenance'). Choice of crystalloid was on the sole discretion of attending physicians. Boluses of colloids or crystalloids were not recommended for the treatment of arterial hypotension during surgery. Epinephrine infusion had to be infused for that purpose. In order to prevent postoperative hypotension episodes, a prophylactic 500 mL bolus of hydroxy-ethyl-starch (HES 130/0.4; Voluven; Fresenius Kabi Polska Sp.zo.o., Kutno, Poland) was infused after the deflation of tourniquet and 6 h
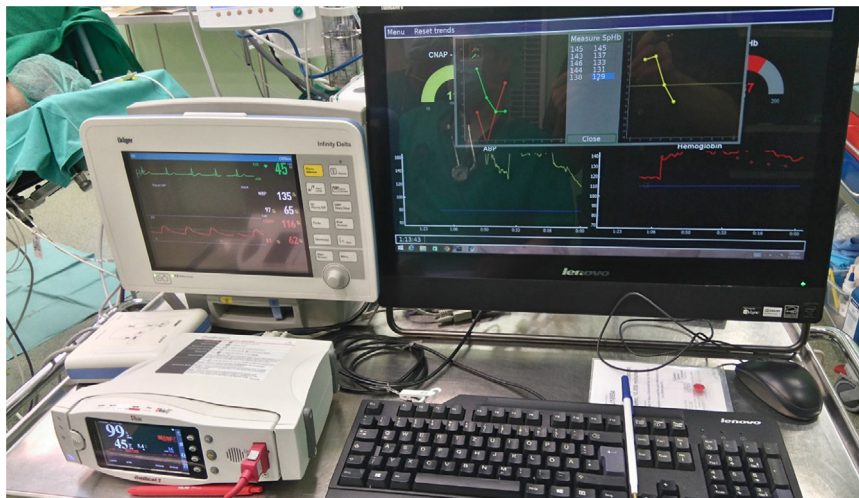
Fig. 3 – Prototype automated clinical decision support system. The acPDD was visualized on the screen of our prototype automated clinical decision support system (white arrow points to the acPDD trend).

later ("optimization"). In a postoperative period, a bolus of up to 500 mL of HES over about 15 min ("rescue") with or without ephedrine was allowed for the treatment of arterial hypotension. The maximum allowed 24-h volume of HES was 2000 mL including the standardized administration of 1000 mL in compliance with the study protocol. Intravenous fluids were stopped at 7 PM, but 'rescue' boluses of up to 500 mL of HES or crystalloids (when the 24-h volume limit for colloids is reached) were allowed thereafter if an event of arterial hypotension should occur. Red blood cell transfusion was indicated in case of anemia intolerance in the presence of normal arterial blood pressure and aHb values <90 g/L.

The postoperative revGDT session commenced at 7 AM and was finished about 8 AM. The study protocol was terminated thereafter. The net perioperative fluid balance during the study period was calculated with and without considering the estimated blood loss (EBL) as part of net fluid loss (Table). The EBL was calculated using the modified Gross formula as described elsewhere [17].

Data are shared at https://figshare.com/s/9be3a7425ce63ae 354fc.
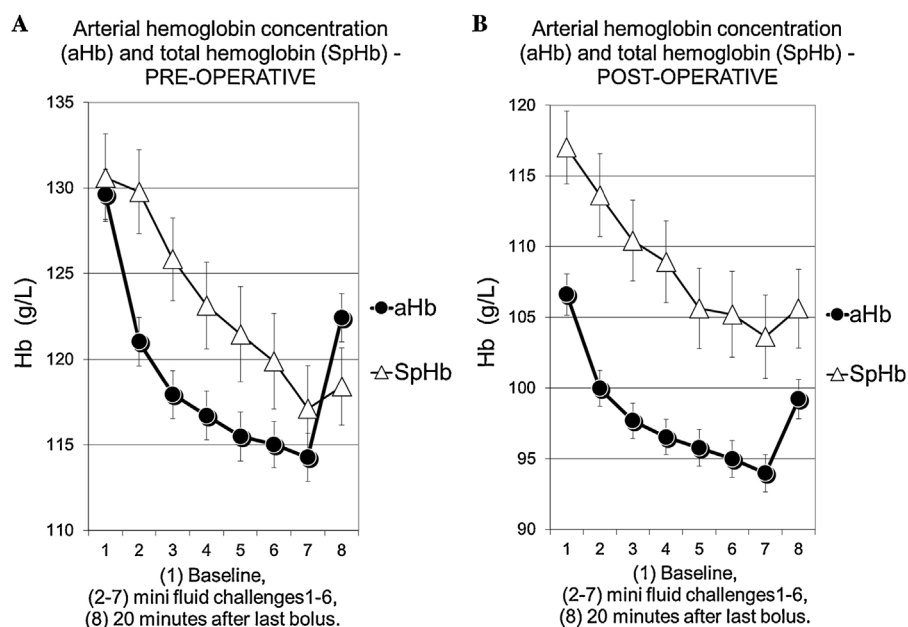
2.4.   Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate the pooled data for normality. The data are presented as mean (SD) where appropriate and median [25th and 75th percentiles] for non-normally distributed data. The Wilcoxon signed rank test was applied for non-normally distributed data. A statistical analysis was performed using PASW (PASW Statistics 17, SPSS, IBM Corporation, NY). The significance level was set to alpha = 0.05 (two-sided). The primary outcome measure was acPDD.

3.   Results

A total of 54 ASA II physical state adult patients scheduled for TKA surgeries were enrolled in the intervention group of the primary RCT from October 2011 to January 2014, and of them, 48 (41 females and 7 males) completed the study. Six patients who dropped out of the study had a malfunctioning radial artery catheter ($n = 4$) or there were deviations from the surgical protocol – synovectomy ($n = 2$). Subjects were 69.5 (6.0) years old, their height was 1.62 m [1.56–1.65], weight was 87.0 kg [75.5–97.5] and body mass index (BMI) was 33.5 kg/m² [31.0–35.1].

The study comprised 96 fluid sessions altogether (48 preoperative and 48 postoperative). The preoperative session commenced at significantly higher aHb level than postoperative (130.2 [121.0–137.0] vs. 106.0 [102.0–113.8], $P = 0.000$) (baseline in Fig. 4). There was no significant difference between PI in pre- and postoperative sessions.

Termination of the fluid loading was never indicated before the completion of sixth mini fluid challenge since there were no signs of patient distress (see distress criteria in Section 2). All the subjects were both SV and hemodilution

Variable	Median (25th and 75th percentiles)
Table – The 24-h fluid balance.	
Ringer's acetate (RA)	1500 (1500–2500)
NaCl 0.9% (NS)	1500 (1000–2000)
Urine volume (UV)	1585 (903–2875)
HES[a]	Fixed volume (1000 mL for each subject)
Fluid balance[b]	2990 (1538–3658)
EBL[c]	933 (709–1154)
Modified fluid balance[d]	1976 (870–2545)

[a] Hydroxyethyl starch; each subject received 1000 mL in 2 boluses (first 500 mL bolus after deflation of tourniquet and another 6 h later).
[b] RA + NS + HES − UV.
[c] Estimated blood loss; calculated using the modified Gross formula.
[d] SV + RA + HES − UV − EBL.

A Arterial hemoglobin concentration (aHb) and total hemoglobin (SpHb) - PRE-OPERATIVE

B Arterial hemoglobin concentration (aHb) and total hemoglobin (SpHb) - POST-OPERATIVE

(1) Baseline,
(2-7) mini fluid challenges1-6,
(8) 20 minutes after last bolus.

Fig. 4 – Invasively (arterial) and non-invasively (capillary) determined hemoglobins. (A) Preoperative and (B) postoperative arterial hemoglobin concentration (aHb) and total hemoglobin (SpHb). Data point referred to as baseline is the measure before the first bolus. Data points referred to as mini fluid challenges N. 1–6 are hemoglobins determined after the 5 min period with no fluids which followed each bolus. Data are presented as means ± SEM.

non-responders to the sixth mini fluid challenge (see responsiveness criteria in Section 2). Thus, according to the revGDT protocol (Fig. 2), there was never any indication to administer a seventh bolus.

3.1. Primary endpoint

The preoperative acPDD was significantly higher than post-operative (0.085 [0.012–0.141] vs. 0.006 [−0.059 to 0.101], $P = 0.000$) (pooled data from time points 1–6 in Fig. 5B). The observations of acPDD in 6 mini fluid challenges revealed that preoperative acPDD was significantly higher than postoperative in each of the first 5 mini fluid challenges ($P = 0.002$, 0.004, 0.015, 0.001 and 0.043, respectively) (Figs. 5 and 6B).

In the preoperative session the acPDD decreased from 0.114 (0.058–0.149) after the 1st mini fluid challenge to 0.044 (−0.056 to 0.119) after the 6th ($P = 0.000$). After the 20-min period without fluid it decreased further to −0.064 (−0.151 to −0.010, $P = 0.000$). The median acPDD was negative only after the 20 min period with no fluid after the last bolus.

In the postoperative session the acPDD decreased significantly from 0.042 (−0.009 to 0.110) after the 1st mini fluid challenge to −0.006 (−0.100 to 0.101) after the 6th ($P = 0.002$). After the 20-min period without fluid it decreased further to −0.064 (−0.134 to 0.020, $P = 0.000$). The median acPDD was negative in 4th to 6th mini fluid challenges and also after the 20-min period with no fluid after the last bolus.

3.2. Secondary endpoints

The preoperative aPD was higher than postoperative ($P = 0.000$; pooled data from time points 2–7 in Fig. 6A). The preoperative

cPD was lower than postoperative ($P = 0.009$; pooled data from time points 2–7 in Fig. 6A).

The preoperative cPD was lower than aPD ($P = 0.000$; pooled data from time points 2–7 in Fig. 6A). The comparisons after each of the 6 mini fluid challenges revealed that aPD was higher than cPD in 1st to 5th mini fluid challenges but did not differ after the 6th, and aPD became lower than cPD after the 20 min period with no fluids after the last (6th) bolus (Fig. 6 A). See Appendix for more median and P values.

There was no difference between cPD and aPD in the postoperative session (pooled data from time points 2–7 in Fig. 6A). The aPD was higher than cPD only after the 1st and after the 2nd boluses but there were no differences after the next (last) 4 boluses. The aPD became lower than cPD after the 20-min period with no fluids after the last (6th) bolus (Fig. 6A). See Appendix for more median and P values.

Secondary endpoints were used for the theoretical interpretation of the results (Fig. 7).

3.3. SV response

Preoperatively one subject responded with SV increase >10% in the first mini fluid challenge and 4, 1, 0, 2, 1 subjects responded so in the other five mini fluid challenges, respectively. Postoperatively 3, 5, 1, 4, 4 and 2 subjects responded that way in six mini fluid challenges, respectively.

3.4. 24-h fluid balance

The modified fluid balance (when EBL was added to losses) was 1715 mL (753–3162) (Table 1) considering i.v. infusions of
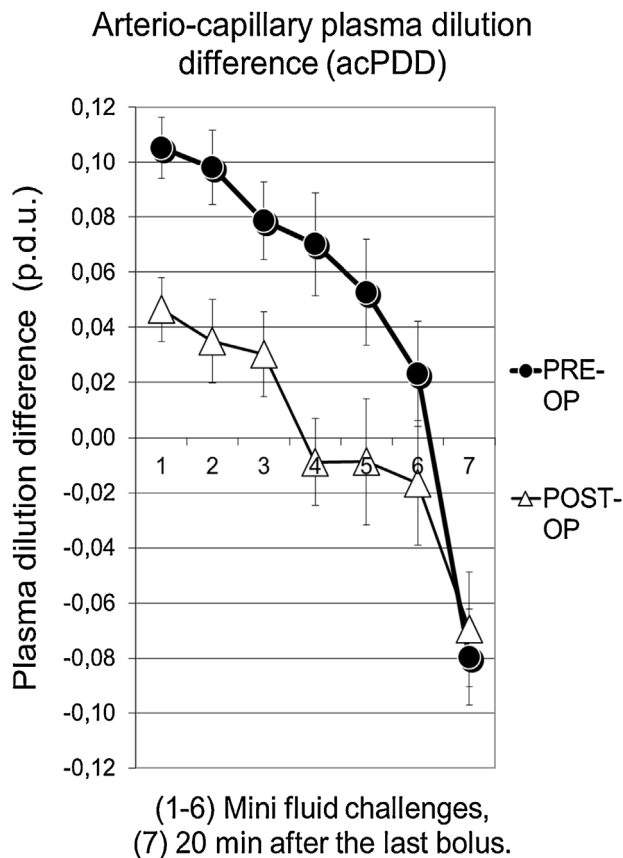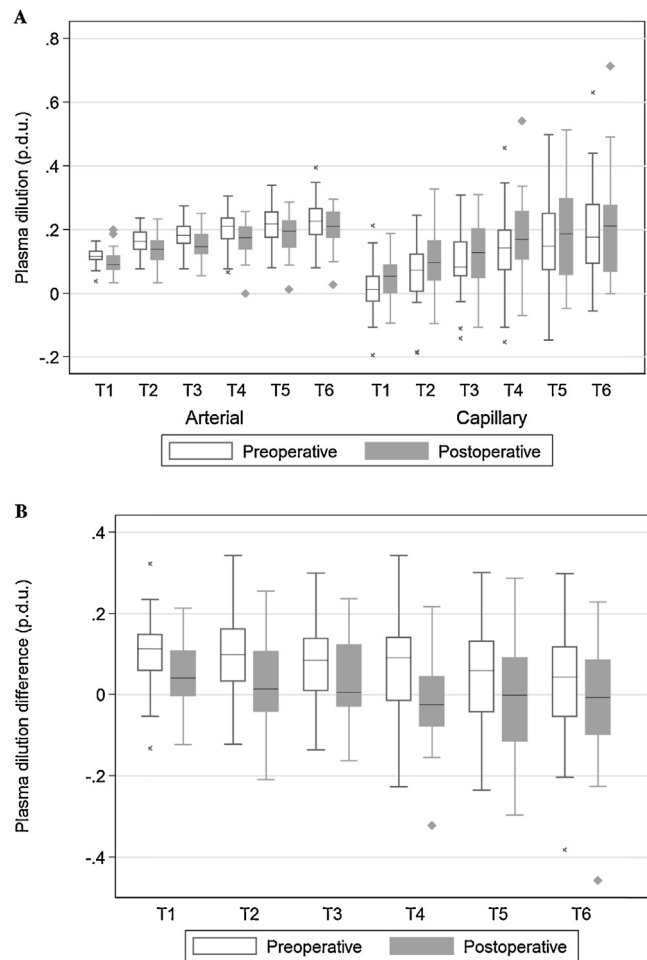
Fig. 5 – Arterio-capillary plasma dilution difference (acPDD). Differences between invasively (arterial) and non-invasively (capillary) determined plasma dilution during the pre and postoperative revGDT sessions. Data points referred to as mini fluid challenges N. 1–6 are the measures determined after the 5-min period with no fluids which followed each bolus. Data are presented as means ± SEM.

Fig. 6 – Box plots of nonnormally distributed variables. (A) Pre- and postoperative arterial (aPD) and capillary (cPD) plasma dilutions; (B) pre- and postoperative arterio-capillary plasma dilution difference (acPDD). Data are presented as box plots.

normal saline, acetated Ringer's and HES as fluid input and the urine volume as output was 2755 mL (1822–3553).

4. Discussion

This observational study assessed if the mVLT method could detect differences between pre- and postoperative hydration levels in TKA patients. The main finding was that arterio-capillary plasma dilution difference during mVLT using 2.5-mL kg^{-1} boluses of crystalloid was lower postoperatively than preoperatively suggesting that the postoperative hydration level was higher. That finding was supported by a positive 24-h fluid balance (Table 1).

We postulate that a lower postoperative acPDD indicates a more activated renal fluid elimination and saturated interstitium postoperatively. The increased urine production may reduce aPD, while a more profound release of interstitial fluid from saturated tissues into capillaries may increase cPD. This may possibly explain why preoperative cPD was lower than postoperative, which is contrary to the finding that

preoperative aPD was higher than postoperative (Fig. 7). A possible explanation for the increased gap between the cPD and aPD after the 20-min period without fluids is that activated renal fluid elimination may significantly reduce aPD since no more boluses are given. Previously infused boluses have already undergone partial distribution and been eliminated from plasma. Meanwhile cPD decreased much slower because hemoconcentration in arteries has negligible impact on cPD. This is because, in the capillary bed, the arterial dilution correlates to dilution in metarterioles (arteriolar-venular shunts) but may be significantly different in true capillaries where dilution is affected by metabolism and neuro-humoral stimuli guided transcapillary filtration absorption ratio (FAR). The cPD is calculated from changes in SpHb, which is a spectrophotometric measure of total hemoglobin under the sensor. Thus, total hemoglobin is an indication of net hemoglobin content in metarterioles and true capillaries. Since true capillaries occupy the largest area of the capillary bed, the true capillary hemoglobin content is the main determinant of the SpHb value and consequently the cPD. Therefore, we postulate that the change in acPDD is an
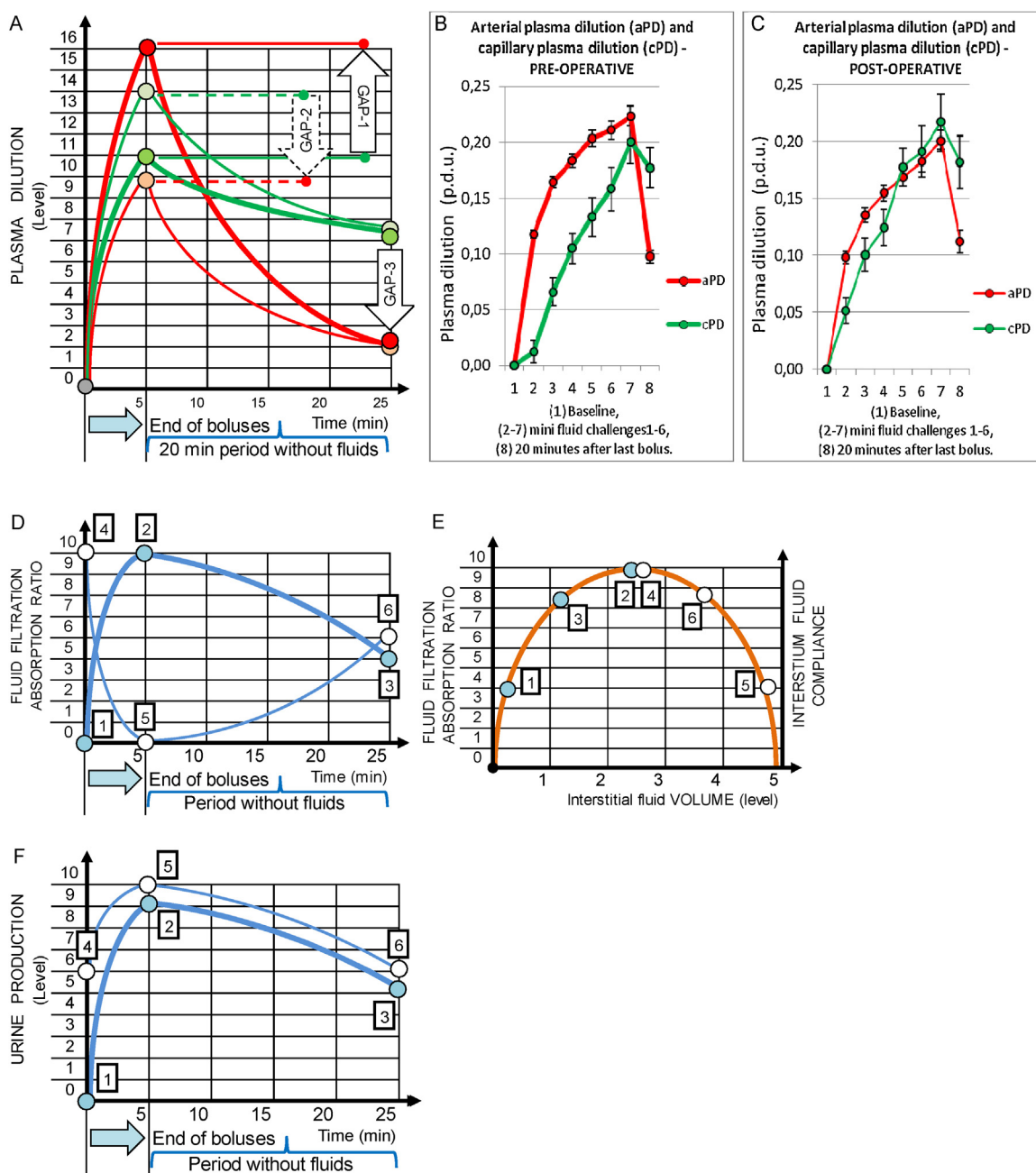
Fig. 7 – The model for the explanation of the dynamic gap between the arterial and capillary plasma dilutions reported in the present study. (A) Theoretical graphs to illustrate changes in arterio-capillary plasma dilution difference (acPDD labeled as GAP arrows) during the pre- and postoperative fluid sessions (B and C); time point 5 min or end of boluses refers to plasma dilutions after the 6th mini fluid challenge, and time point 25 min refers to plasma dilutions after 20 min following the 6th mini fluid challenge; the GAP-1 is positive but GAP-2 and GAP-3 are negative. (B) Preoperative arterial (aPD) and capillary (cPD) plasma dilutions; (C) postoperative aPD and cPD. Data point referred to as baseline is the measure before the first bolus. Data points referred to as mini fluid challenges N. 1–6 are the measures determined after the 5 min period with no fluids which followed each bolus. Data are presented as means ± SEM. (D) The time-course of transcapillary fluid filtration absorption ratio (FAR). (E) The relationship between FAR, interstitial fluid volume and interstitial fluid compliance. (F) The time-course of urine production (activation of renal elimination). See "Supplement to Fig. 7" for the detailed explanation of the model and the graphs (available in Appendix).

indication of changes in FAR where acPDD could detect changes in transcapillary fluid equilibration with an implication for evaluation of changes in interstitial hydration level under the SpHb sensor. As in previous studies [14,15], we found

that acPDD inversely correlates with the whole body hydration level of subjects. A possible explanation is that fluid accumulation in a small segment of derma under the SpHb sensor may correlate with fluid accumulation in all the derma

of the skin, which is the largest organ. Furthermore, fluid compliance in derma is similar to compliance, and thus fluid accumulation, in skeletal muscles [21]. However, it may be different in other physiological and clinical settings and therefore warrants further research.

As in previous studies in healthy volunteers [14] and patients [15], our findings indicate that the intensity of the flux of fluid into capillaries depends on the pre-existing hydration level. An earlier occurring state where aPD is higher than cPD (negative value of acPDD) during mVLT may indicate an increased flux of interstitial fluid into capillaries. It occurred earlier postoperatively. The aPD was higher than cPD after each of the first five mini fluid challenges preoperatively but only after the 1st postoperatively (Fig. 6). That is not obvious in Figs. 5 and 7B, C, because acPDD, aPD and cPD data are presented as means while statistical comparison required the use of medians as presented in box-plots in Fig. 6.

If the net pulmonary and renal fluid elimination match the net release of excessive interstitial fluid into the capillaries, there will be no increase in aPD and this will lead to negative acPDD value. According to the mVLT method, this would be the time point during stepwise crystalloid infusion when the reflux of excessive interstitial fluid into capillaries starts. Thus, no more fluid should be given to prevent the interstitial tissue from being overfilled. If the net pulmonary and renal fluid elimination exceed the net release of excessive interstitial fluid into the capillaries, there will be a decrease in aPD and this will lead to more negative acDD value. Presumably, the interstitial fluid compliance has overcome its maximum value during six postoperative boluses, and therefore, a more intensive release of excessive interstitial fluid into capillaries explains the significantly higher postoperative cPD (Fig. 7). Our theoretical model is available in Appendix.

With an aim to explain these processes we have developed several theoretical models – the transcapillary reflux model and the dynamic GAP model. The detailed partial disclosure was published elsewhere [14,15,19]. It is noteworthy that inaccuracy of SpHb measurement is very unlikely to be responsible for the discrepancy between SpHb and aHb, and consequently between cPD and aPD values [16]. The real accuracy of Radical 7 and Pulse CO-Oximetry technique remains unknown because it cannot be evaluated by using invasive Hb measurements as a reference method. This is mainly because, in contrast to large vessels, hemodilution in capillaries under the sensor is affected by transcapillary fluid shifts that cannot be measured. The ideal reference method has to be able to simultaneously measure hemoglobin in a bunch of capillaries exactly in the same area which is scanned by the SpHb sensor [19]. It is not available to date.

We postulate that the 5-min and also 20-min periods without fluids after mini fluid challenges are appropriate for the evaluation of instant and delayed transcapillary fluid exchange as previously suggested by studies in volume kinetics [22,23]. These studies revealed that, obviously due to the transcapillary reflux, the positive arterio-venous dilution difference observed during the crystalloid infusion becomes negative after 2–3 min and continues for at least an hour following the end of infusion. Thus, we determined acPDD in a time span from 5-min up to 20-min periods without fluids after mini fluid challenges.

As previously reported, we used 3 5-mL kg^{-1} boluses for the mVLT in the first 36 intervention group subjects from the same primary RCT [15]. Such volume was appropriate for detection of preoperative dehydration. Acknowledging that bolus has to be as minimal as possible while still being sufficient for evaluation of fluid responsiveness [24], we have reduced it from 5 mL kg^{-1} to 2.5 mL kg^{-1} and increased the number of boluses from 3 to 6 in the last 48 intervention group subjects of the primary RCT (Fig. 1). It is notable that 2.5-mL kg^{-1} boluses of acetated Ringer's were previously used for the mVLT in healthy volunteers [14].

The present report addressed the optimization phase of the perioperative fluid therapy [3] for which the GDT is probably the most validated method in the intensive care and perioperative settings [8,9,25,26]. The goal in SV guided GDT is to enhance the tissue perfusion, for which systemic hemodynamic improvement is only the first step [2]. Since different mechanisms are controlling microcirculation and systemic circulation [27], changes in microcirculation can occur when hemodynamic parameters do not change and vice versa [28]. Furthermore, the fact that even healthy volunteers may be SV respondents [29] challenges the legitimacy of hemodynamic markers as sole triggers to fluid loading [7]. The currently evolving trends in GDT are therefore focusing on the concept that macro- and micro-circulatory parameters should be used in parallel to guide the goal directed resuscitation [5,7,8]. Obviously new protocols and algorithms are needed. Thus, we developed a revGDT protocol (Fig. 2). It implies two target parameters – hemodilution (microcirculation) and SV (macrocirculation). They are equivalent in the decision making regarding the need for fluid boluses. However, this protocol could not be fully implemented because the present study was an observational part of the primary RCT. Its protocol urged to infuse the minimal volume of 15 mL kg^{-1} that was our institution's standard in TKA surgery at the time of the trial. That volume was used to replace the estimated basal physiological fluid deficit after an overnight fast [20]. The only indications to infuse less were signs of fluid overload or other signs of patient deterioration. Thus, SV and hemodilution non-responses were ignored until the 6th mini fluid challenge when either SV or hemodilution response would have indicated the 7th bolus. None of the subjects received it.

This study has several weaknesses. It is a single center study. The study protocol is part of the larger primary RCT (Fig. 1). The difference in perioperative hydration level was not verified by direct measurements. The exchange of oxygen and waste products was not evaluated. We postulate that mVLT method facilitates these processes by enabling the optimization of tissue fluid accumulation and avoiding edema, thus facilitating indirectly the perfusion of tissues and transcapillary exchange therein. An overnight fasting was not in accordance with current preoperative fasting recommendations. However, it is not likely that patients would drink much or eat during the night if they are the first in the morning list.

Since direct measurement of capillary hemoglobin is not available, an off-label use of Radical-7 Pulse CO-Oximeter (Masimo Inc.) was deployed. The mVLT method may be performed using solely non-invasive hemoglobin measurements enabling the closed-loop assisted or semi-closed loop application [18,19]. However, the raw data from non-invasive

hemoglobin measurements would be more useful for the mVLT than the calibrated SpHb readings from current Pulse CO-Oximetry since these values are derived by adjusting the raw data with an aim to bring the SpHb closer to Hb in large vessels. However, invasive and non-invasive measurements are not interchangeable [19,30]. A novel device for non-invasive hemoglobin measurement [31] is even less accurate in predicting the large vessel Hb probably due to less advanced adjustment of raw data. Therefore, application of these devices for the mVLT purposes should be investigated in the future research. Ideally, both the non-adjusted and adjusted capillary hemoglobin readings could be available in such monitors. A closer and more open collaboration between researchers and industry is necessary.

Future research in fluid therapy deploying macro- and microcirculation as well as indices of metabolism such as oxygen delivery is encouraged by the present study findings.

5. Conclusions

The mVLT method indicated the higher postoperative hydration level in TKA patients. Our results suggest that series of 2.5-mL kg^{-1} boluses of crystalloid may be used for mVLT to assess the patient hydration status in the perioperative setting.

Conflict of interest

Acknowledgement

Appendix

Calculations

Fluid challenge induced plasma dilution (PD) during the mVLT is calculated from a change of hemoglobin concentration (Hb). Since we are considering the dilution of plasma, we need to adjust for the hematocrit:

$$PD_i = (Hb \times Hb_i^{-1} - 1) \times (1 - Hct)^{-1}$$

where PD_i is the plasma dilution after the fluid challenge number i, Hb is the initial hemoglobin concentration obtained before the first fluid challenge, Hb_i is the hemoglobin concentration obtained after the fluid challenge number i, and Hct is the initial hematocrit value obtained before the first fluid challenge (since noninvasive Hct is not available during the noninvasive determination of the PD the initial Hct is derived by dividing the noninvasive initial Hb by 330, which is the mean value of the normal range for the mean cell hemoglobin concentration).

The arterio-capillary plasma dilution difference (acPDD) is calculated as follows:

$$acPDD_i = aPD_i - cPD_i$$

where $acPDD_i$ is arterio-capillary plasma dilution difference of the fluid challenge number i, aPD_i is arterial plasma dilution of the fluid challenge number i, and cPD_i is capillary plasma dilution of the fluid challenge number i.

Secondary endpoints

Preoperative vs. postoperative variables

The preoperative aPD was higher than postoperative (0.181 [0.140–0.226] vs. 0.154 [0.108–0.200], P = 0.000) (pooled data from time points 2–7 in Fig. 3C and D). Observations after each of the 6 mini fluid challenges revealed that preoperative aPD was higher than postoperative aPD after the first 5 mini fluid challenges (P = 0.004, 0.001, 0.002, 0.001 and 0.010, respectively) (Fig. 6A).

The preoperative cPD was lower than postoperative (0.094 [0.027–0.180]) vs. 0.132 [0.046–0.230], P = 0.009) (pooled data from time points 2–7 in Fig. 3C and D). Observations after each of the 6 mini fluid challenges revealed that preoperative cPD was lower than postoperative cPD only after the 1st mini fluid challenge (P = 0.024) (Fig. 6A).

At time point T7, which is after the 20-min period with no fluids after the last (sixth) bolus, there were no differences between the pre- and postoperative aPDs, cPDs (Fig. 6A), acPDDs (Fig. 6B), aPDEs, cPDEs (Fig. 6C) or acPEDs (Fig. 6D).

There was no difference between pre- and postoperative acPED (pooled data from time points 1–6 in Figs. 5C and 6D). Observations after each of the 6 mini fluid challenges revealed that preoperative acPED was different from postoperative acPED (higher) only in the 1st mini fluid challenge (0.114 [0.058–0.149] vs. 0.042 [−0.009 to 0.110], P = 0.002) (T1 in Fig. 6D).

Preoperative cPD vs. aPD

The preoperative cPD was lower than aPD (0.094 [0.027–0.180] vs. 0.180 [0.140–0.226], P = 0.000) (pooled data from time points 2–7 in Fig. 3C). The comparisons after each of the 6 mini fluid challenges (Fig. 6A) revealed that aPD was higher than cPD after the 1st mini fluid challenge (0.116 [0.105–0.132] vs. 0.013 [−0.026 to 0.055], P = 0.000), the 2nd (0.163 [0.136–0.192] vs. 0.073 [0.003–0.126], P = 0.000), the 3rd (0.181 [0.154–0.209] vs. 0.083 [0.054–0.162], P = 0.000), the 4th (0.210 [0.169–0.237] vs. 0.142 [0.069–0.197], P = 0.001) and the 5th (0.218 [0.174–0.256] vs. 0.147 [0.072–0.252], P = 0.009). The aPD became lower than cPD

after the 20-min period with no fluids after the last (6th) bolus (0.102 [0.069–0.119] vs. 0.143 [0.113–0.237], P = 0.000).

Postoperative cPD vs. aPD

There was no difference between cPD and aPD in postoperative session (pooled data from time points 2–7 in Fig. 3D). The comparisons after each of the 6 mini fluid challenges (Fig. 6A) revealed that aPD was higher than cPD only after the 1st (0.090 [0.075–0.118] vs. 0.055 [−0.006 to 0.091], P = 0.000) and after the 2nd (0.138 [0.105–0.165] vs. 0.097 [0.040–0.165], P = 0.035) mini fluid challenges. There were no differences after the next (last) 4 mini fluid challenges. The aPD became lower than cPD after the 20-min period with no fluids after the last (6th) bolus (0.118 [0.088–0.143] vs. 0.174 [0.063–0.247], P = 0.002).

Changes in acPED during the pre- and postoperative sessions

In the preoperative session acPED decreased from 0.114 [0.058–0.149] after the 1st mini fluid challenge to −0.022 [−0.067 to 0.013] after the 6th (P = 0.000) (Fig. 6D). In the postoperative session acPED decreased from 0.042 [−0.009 to 0.110] after the 1st mini fluid challenge to −0.015 [−0.044 to 0.028] after the 6th (P = 0.001).

Supplement to Fig. 7

The model for the explanation of the dynamic gap between the arterial and capillary plasma dilutions reported in the present study. **(A)** Theoretical graphs to illustrate changes in arterio-capillary plasma dilution difference (acPDD labeled as GAP arrows) during the pre- and postoperative fluid sessions (Fig. 7B and C); time point 5 min or end of boluses refers to plasma dilutions after the 6th mini fluid challenge, and time point 25 min refers to plasma dilutions after 20 min following the 6th mini fluid challenge; the GAP-1 is positive but GAP-2 and GAP-3 are negative.

The **GAP-1** is positive due to rehydration of tissues in the preoperative session – see the shift from data point 1 to 2 in Fig. 7D–F: an increase in the transcapillary filtration absorption ratio (FAR) in Fig. 7D is an indication of increasing tissue fluid compliance in Fig. 7E (reaching maximal), and gradual activation of renal elimination in Fig. 7F explain why cPD is smaller than aPD.

The **GAP-2** is negative due to over-hydration of tissues in the postoperative session – see the shift from data point 4 to 5 in Fig. 7D–F: a decrease in FAR in Fig. 7D is an indication of decreasing tissue fluid compliance in Fig. 7E (elasticity of tissue decrease due to excessive expansion), and steep activation of already activated renal elimination in Fig. 7F explain why cPD is higher than aPD.

The **GAP-3** is negative and equivalent in both sessions because the shifts from data point 2 to 3 and from 5 to 6 in Fig. 7D show that FAR has decreased in preoperative session as an indication of increasing tissue fluid compliance (returning from maximal to sub-maximal level which is higher than before the fluid session) in Fig. 7E, while FAR (Fig. 7D) has increased in postoperative session as an indication of decreasing tissue fluid compliance (returning to less over-maximal level which is slightly higher than was before the fluid session) in Fig. 7E; gradual de-activation of renal

elimination in Fig. 7F occurs in both sessions. The persistent release of interstitial fluid into capillaries when expanded tissues release the excessive fluid is followed by activated renal elimination provides an explanation why cPD is smaller than aPD after 20 min following the last bolus.

(B) Preoperative arterial (aPD) and capillary (cPD) plasma dilutions; **(C)** postoperative aPD and cPD. Data point referred to as baseline is the measure before the first bolus. Data points referred to as mini fluid challenges N. 1–6 are the measures determined after the 5-min period with no fluids which followed each bolus. Data are presented as means ± SEM.

(D) The time-course of transcapillary fluid filtration absorption ratio (FAR).

(E) The relationship between FAR, interstitial fluid volume and interstitial fluid compliance.

(F) The time-course of urine production (activation of renal elimination).

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