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Volume kinetics in a translational porcine model of stabilized sepsis with fluid accumulation

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Abstract

Background Fluid dynamics during and after a septic event is complex, but better knowledge could guide both fluid resuscitation and fluid removal. We aimed to compare fluid dynamics before and after sepsis in a clinically relevant mono-bacterial porcine model.

Methods Twelve sows with a mean body weight of 56 kg were anesthetized, mechanically ventilated, and invasively monitored. Sepsis was induced with an intravenous infusion of *P. aeruginosa*. Animals were resuscitated during the acute septic phase according to a protocolized algorithm. Volume kinetics was studied before the bacterial infusion (baseline) and 24 h later (late sepsis), and both consisted of an infusion of 1,500 mL of 0.9% saline over 20 min with repeated hemoglobin and albumin measurements and urine quantification.

Results The kinetic analysis at baseline showed transient volume expansion of the central fluid compartment (the plasma) and a fast-exchange interstitial space, while gradually more fluid accumulated in the remote "third fluid space" with very slow turnover. In the late sepsis phase, hypoalbuminemia and slight hypovolemia was observed. As compared with baseline, fluid kinetics showed improved plasma expansion, and more expansion of the fast-exchange interstitial space rather than the slow-exchange space. The rate constant k_{21} describing return flow to the circulation was increased during the late sepsis phase, and hemoglobin-albumin dilution difference suggested that interstitial albumin recruitment occurred with the fluid infusion. The model predicted that high cardiac index and sepsis-induced weight gain were associated with greater fast-exchange compartment expansion.

Conclusion After sepsis, fluid was accumulated in the slow-exchange compartment, and further fluid administration distributed preferentially to the fast-exchange compartment with acceleration of lymph flow, improved plasma expansion, and recruitment of interstitial albumin.

Keywords Sepsis, Pharmacokinetics, Fluid accumulation, Isotonic saline, Volume kinetic, Preclinical study

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Introduction

The ROSE framework (Resuscitation, Optimization, Stabilization, and Evacuation) describes successive stages of fluid administration [1] in critically ill patients. This conceptualization acknowledges the fluid accumulation syndrome or fluid overload, notoriously associated with poor outcomes [2]. The transition from fluid accumulation to evacuation can be spontaneous in critically ill patients, with the achievement of a negative fluid balance. However, therapeutic intervention is often needed for fluid removal [3] as conceptualized within the ROSE framework [4, 5]. Nevertheless, the benefit of de-resuscitation strategies has yet to be proven [6].

The mechanisms underlying fluid accumulation remain elusive. In the acute phase of shock, the need for resuscitation is mostly due to increased vascular permeability. In the stabilization/evacuation phase, less is known about the determinants of fluid dynamics. Potential contributors include resolution of vascular permeability, equilibration with interstitial pressure, and enhancedlymphatic flow [7]. Better knowledge of these changes could improve the clinicians' decisions on de-resuscitation timing and suitable strategy for fluid removal [5].

Fluid dynamics in critically ill individuals can be modeled using population pharmacokinetic principles to assess the effects of a fluid bolus. The distribution of the fluid bolus is analyzed in a multi-compartment model based on the fluid-induced dilution of blood hemoglobin and quantification of urine output [8]. A population-based analysis allows a more precise prediction of the input parameters than analysis of individual experiments in isolation [9]. This approach known as "volume kinetics" has been used to study the distribution of fluid in various context including healthy volunteers [10], patients undergoing surgery [11, 12], and volunteers with dehydration and hypovolemia [13].

Few previous works have focused on the distribution and clearance of crystalloid fluids during sepsis, especially during the late phase when fluid accumulation is present. Therefore, we aimed to describe fluid kinetics in the late phase of sepsis by using population volume kinetic analysis in a translational porcine sepsis model with fluid accumulation. The hypothesis was that the distribution and elimination of infused fluid after the septic episode would differ from a pre-sepsis infusion in the same animals.

Methods

Ethical statement

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals and all procedures performed on animals were approved by the Animal Care Committee of VetAgro Sup, Marcy l'Etoile, France (authorization n° 2403). All procedures adhered to the guidelines set forth by Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

Animals

This study included healthy sows [*Sus scrofa domestica*, Yuna breed] aged between three and five months, weighing between 55 and 60 kg. The sows were housed during one week in a conventional facility before the experiment. Pigs were fed with standard food and had ad libitum access to water. Food was withdrawn 12 h before anesthesia.

Overview of the protocol

The protocol is described in Fig. 1. The animals were under general anesthesia for 24 h. After induction, they first underwent a 30-min fluid challenge that was monitored during 2 h. Thereafter, sepsis was induced. Finally, the monitored fluid challenge was repeated during the last 2 h of the 24-h period. We also included 4 additional sows that served as controls for the resuscitation protocol. They underwent the same procedures as the septic pigs, but sepsis was not induced.

Anesthesia and instrumentation

Animals were sedated with an intramuscular tiletamine/zolazepam (5mg/kg). They were intubated and mechanically ventilated after anesthesia induction with propofol, which was followed by intravenous infusions of propofol, midazolam and morphine (3, 0.5 and 0.1 mg/kg/h, respectively). The tidal volume was set at 8 ml/kg and the respiratory rate was adjusted to achieve an expired CO_2 fraction between 35 and 45 mmHg.

Instrumentation was performed under sterile conditions and consisted of a pulmonary artery catheter for monitoring of central hemodynamics and a two-lumen central venous line in the left jugular for fluid and drug administration. An arterial catheter was placed in the right femoral artery to facilitate invasive blood pressure monitoring and blood sampling. A Foley catheter was inserted into the bladder via the urethra to allow collection of urine.

Sepsis induction and therapeutic protocol

Sepsis was induced by a loading dose infusion of live *Pseudomonas aeruginosa* (ATCC® 27,853™) through the central venous catheter (5×10^8 CFU/ml at 0.3 mL/20 kg/min) over ninety minutes. A continuous bacterial infusion was initiated if hypotension did not occur at the end of the loading dose but was stopped if the animal showed signs of poor tolerance such as cardiac arrhythmias or pulmonary hypertension (pulmonary

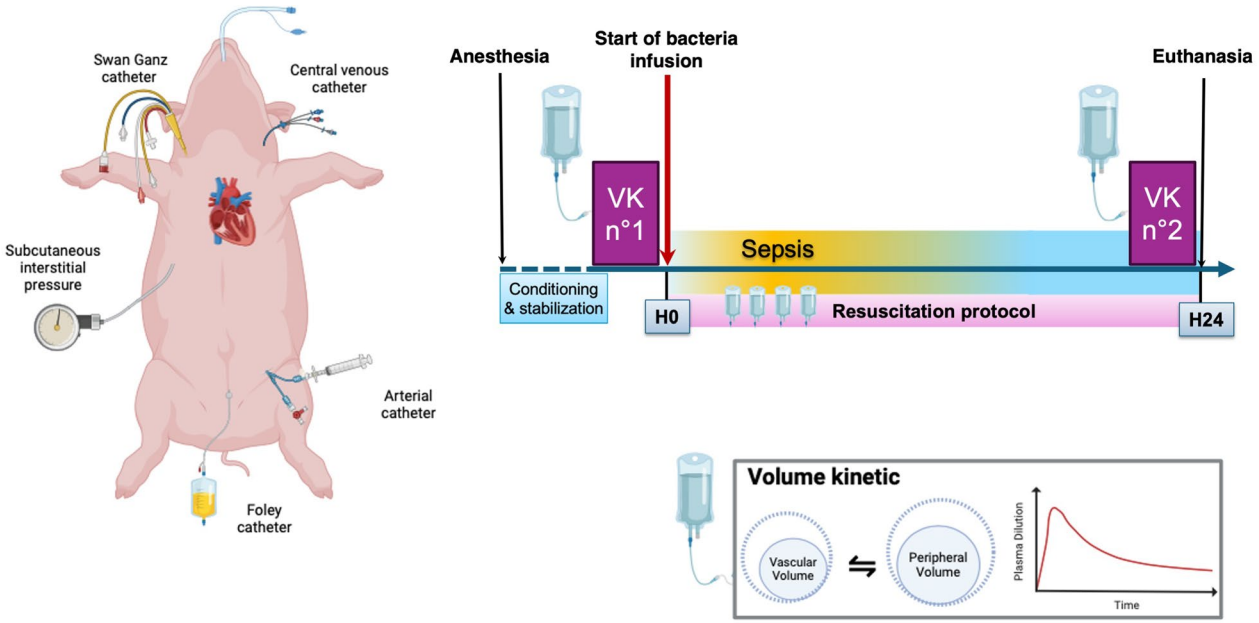

Fig. 1 Study Design. VK: volume kinetic

systolic pressure > 80 mmHg). Animals were monitored and resuscitated for 24 h following a rigorous protocol (Fig. S1 in the Supplementary File). Briefly, the first hypotension episode triggered an initial fluid bolus of 10 ml/kg of isotonic saline, after which subsequent boluses were administered using preload responsiveness indices (pulse-pressure variation and cardiac output response to a mini-fluid challenge) to avoid unnecessary fluid administration. No fluid was given before the first experiment, but thereafter 2 ml/h of isotonic saline was given as maintenance. Norepinephrine infusion was started after a total fluid load of 30 ml/kg of isotonic saline or earlier if the diastolic arterial pressure decreased to below 40 mmHg.

Measurements

Hemodynamic parameters were recorded throughout the experiment, either continuously (heart rate, systemic and pulmonary arterial pressure, temperature) or intermittently (central venous pressure, pulmonary capillary wedge pressure and diuresis). Cardiac output was calibrated every 2 h and after each hypotensive episode, using intermittent pulmonary thermodilution on the Swan Ganz catheter. Arterial and venous blood gases were collected hourly for 4 h and then every 2 h. Complete blood count, creatinine, liver enzymes, and coagulation parameters were assessed at baseline and after 12 and 24 h. The animals were euthanized when the last blood sample had been taken.

Volume kinetics

A volume kinetic experiment was performed at two different points in time: first, at the end of the stabilization and conditioning period (i.e., before the bacterial infusion); and second, during the last 2 h of the 24-h monitoring (Fig. 1).

The kinetic experiment was conducted as described elsewhere [12, 13]. Briefly, a 1,500 mL (≈ 25 ml/kg) bolus infusion of normal saline was administered over 20 min using a peristaltic pump. Blood hemoglobin (Hb) and plasma albumin were measured every 5 min for 1 h and at 70, 90, and 120 min. Urine output was measured and sampled every 30 min during these 120 min.

The three-volume "base model" we used is summarized in Fig. 2A**.** Briefly, five rate constants (k_{12}, k_{21}, k_{23}, k_{32}, and k_{10}) and one scaling factor between dilution and volume (V_c, central volume) were fitted to the two dependent variables, which were the measured urinary output, and the plasma dilution derived from the serial Hb values [8]. This kinetic model was constructed to mimic human physiology using symbolism adopted from Gabrielsson & Weiner [14]. Fluid is infused into the plasma (V_c), from which distribution occurs to a fast-exchange interstitial space (V_{t1}) and can then either return to V_c or be further distributed to a more remote interstitial, slow-exchange fluid space (V_{t2}, the "third fluid space"). Elimination from V_c occurs by the measured urinary excretion in proportion the expansion of V_c. The flow between compartments were obtained as the product of a rate constant signifying the flow and the volume expansion of the

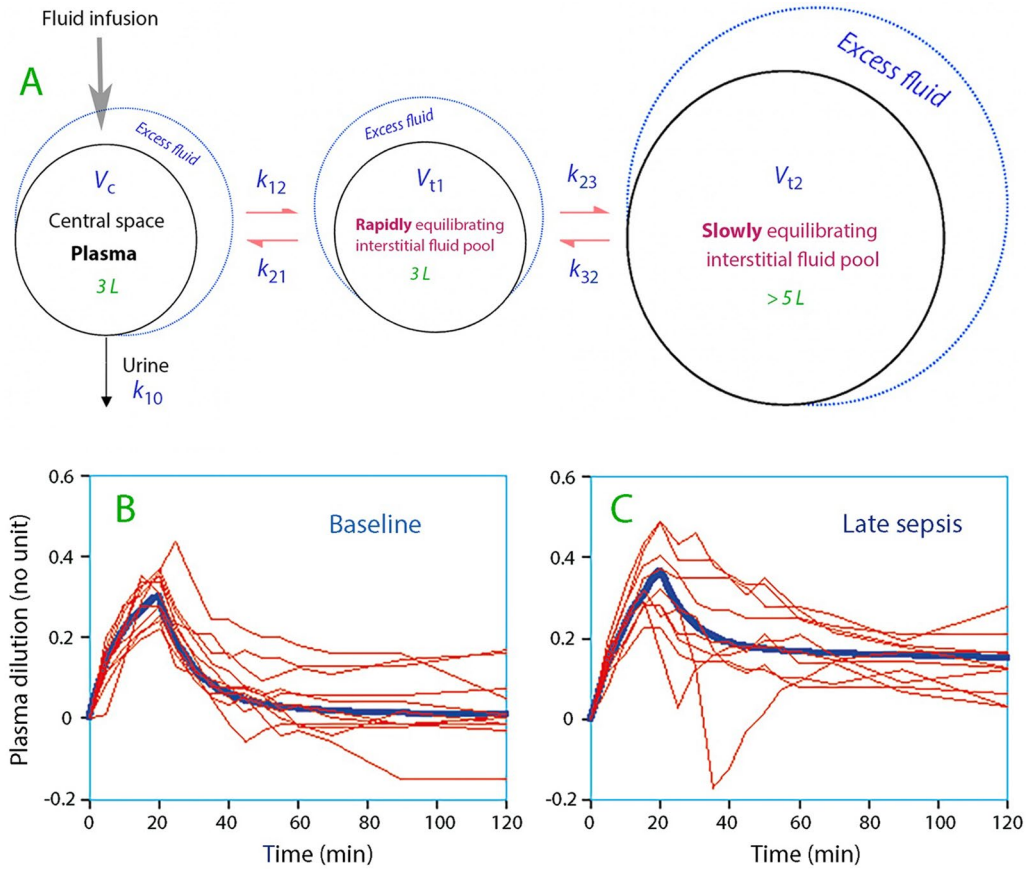

Fig. 2 **A** The kinetic model used for the analysis of fluid distribution. **B** The plasma dilution during the baseline experiments. The thin lines are the measured values and the thick line the modeled average. **C** Same plot as in B but for the "late sepsis" experiments

body fluid compartment from where the flow originated. Hence, the flow varied over time in proportion to the filling of that fluid space.

The parameter estimates in the kinetic "base model", which was valid for all experiments, could be modified by individual-specific *covariates*, such as the body weight and hemodynamic variables. How these covariates were identified and validated is, together with the differential equations describing the kinetic model, described in the Supplementary File.

All measurements of plasma dilution and urinary excretion were simultaneously fitted to the kinetic model (including the base model and the covariates) using the Phoenix software version 8.3.4 for nonlinear mixed effects (Phoenix NLME, Pharsight, St. Louis, MO) with the First-Order Conditional Estimation Extended Least Squares (FOCE ELS) as search routine. This routine operates slowly but provides very precise parameter estimates.

The behavior of the model (goodness-of-fit) was studied by residual plots, weighted conditional weighted residuals [15] and predictive checks.

Statistics

Hemodynamic variables were reported as the mean (standard deviation) (SD). Repeated-measures ANOVA was used to compare measurements performed at different points in time in the same animal. Kinetic parameters are reported as the best estimate and 95% confidence interval (CI) according to the output from the Phoenix program. All other statistics were performed using R studio software.

Results

Outcomes

Twelve sows with a mean body weight of 56 (6) kg were included. All animals met the sepsis criteria using the porcine-derived five-domain SOFA score (PaO_2 to FiO_2 ratio, platelets and bilirubin were measured at baseline, 12 and 24 h) [16]. Two of them died before the end of the experiment and therefore did not undergo the second volume kinetic experiment. Five animals were more tolerant to bacteremia and received a continuous bacteria infusion as per our protocol, allowing to obtain

sepsis criteria. The infusion was stopped when the second experiment was initiated.

Table 1 summarizes the hemodynamic and laboratory data. Complete hemodynamic data from the infusion experiments is shown in Tables S1 and S2. Sepsis occurred during the first 6 h. During the septic phase, the arterial pressure decreased while plasma lactate increased by 2.6 (1.1) mmol/l. Urine output was impaired soon after bacteria infusion (Table 1, total averaged diuresis from beginning of bacteria infusion: 0.46 (0.38) ml/kg/h).

Most surviving animals had recovered from the acute sepsis phase 24 h after the onset of the bacteria infusion, with an increase in blood pressure and a decrease in serum lactate. However, the urine output did not recover fully in every animal. There was evidence for fluid accumulation as the average body weight had increased by 5.1 (2.8) kg, which corresponded to 8.3 (4.4) % of the weight at baseline. Cultures of target organs (kidney, lung, liver) were performed after the autopsy, and revealed Pseudomonas growth in every animal (except for one kidney in an individual).

Four control animals underwent the 24-h anesthesia without receiving bacteria, as a control of the resuscitation and anesthesia protocol. They received 1.7 (0.491) L fluid for a weight gain of 0.8 (0.21) kg during the procedure (4.3 kg in the septic pigs). The MAP was higher at the end of the procedure (from 85 to 91 mmHg, at H0 and H22, compared to 85 to 79 mmHg in the septic pigs), and cardiac index was also decreased but relatively preserved compared to septic pigs (decrease of 34% and 24% in septic and control pigs, respectively).

Volume kinetics

The kinetic model is illustrated in Fig. 2A. Analyses of the infused 1,500 mL was first made separately for the two experiments (Fig. 2B, C).

Table 1 Hemodynamic and laboratory data recorded just before the first and second infusion study (Baseline and Late sepsis), and 4 h after start of bacterial infusion (Early Sepsis)

Variable	Baseline (H-2)	Early Sepsis (H4)	Late sepsis (H22)	*P* value [a]
	N = 12	N = 12	N = 10	
Mean arterial pressure (mmHg)	92 (12)	84 (20)	79 (12)	0.07
Norepinephrine support (n (%))	0 (0)	4 (33)	2 (20)	
SVRI (dyn.s.cm^{-5}.m^{-2})	1324 (348)	2126 (810)	1438 (468)	0.73
Mean pulmonary arterial pressure (mmHg)	17 (3)	33 (7)	26 (4)	0.2
Pulmonary artery occlusion pressure (mmHg)	4 (1)	3 (2)	7 (3)	< 0.001
Central venous pressure (mmHg)	3 (2)	2 (2)	6 (2)	0.01
Heart rate (bpm)	102 (15)	157 (30)	101 (21)	0.29
Cardiac index (L/min/m^2)	5.2 (1)	3.3 (0.7)	4.4 (1.5)	0.6
Temperature (°C)	37.4 (0.7)	38.8 (1.3)	39.4 (0.7)	< 0.001
Fluid balance (mL)	0	2336 (1170)	6705 (2887)	< 0.001
Urine output (cumulated, mL)	0	286 (241)	631 (428)	
Lactate (mmol/L)	1.8 (0.6)	4.5 (1.1)	1.0 (0.7)	0.03
Albumin (g/L)	31.6 (1.7)	28.6 (2.3) [b]	27.3 (2.5)	< 0.001
Hematocrit (%)	30.0 (2.2)	43.9 (12.3) [b]	30.9 (2.6)	0.2
Hemoglobin (g/dL)	9.1 (0.6)	12.8 (3.4) [b]	9.5 (0.8)	0.28
Sodium (mmol/L)	137 (1)	132 (2)	132 (5)	< 0.001
Chloride (mmol/L)	102 (1)	102 (4)	103 (4)	0.33
Creatinine (µmol/L)	84.2 (27.6)	296.7 (82.8) [b]	439.9 (179.5)	< 0.001
Platelet count (G/L)	299 (73)	61 (37.2) [b]	30.6 (16.5)	< 0.001
Bilirubin (µmol/L)	1.0 (0.55)	7.6 (3.2)	4.2 (1.5)	< 0.001

Baseline correspond to the time values recorded just before the first volume kinetic experiment. Early sepsis values were recorded 4 h after the start of bacteria infusion. Late sepsis values were recorded just before the second volume kinetic experiment. Values are the mean (standard deviation). [a]: Repeated—measures ANOVA between three time points in 10 pigs; [b]: measured at H12 instead of H4

Figure 3 shows the result of this kinetic analysis at each time point. The central space (V_c, the plasma) was better maintained during the "late sepsis" experiment, suggesting improved plasma volume expansion by the fluid bolus with longer half-life, as compared with baseline (Table S2). In parallel, infused fluid predominantly accumulated in V_{t1} in the late sepsis phase, as opposed to the baseline experiment when transcapillary filtration was mostly directed toward the V_{t2} compartment (Fig. 3).

A population kinetic analysis was then made based on the pooled data from both series of infusions, allowing to build a model and to estimate the effect of the timing of the experiment (baseline or late sepsis) and other clinical covariates on the rate constants. The final kinetic output is given in Table S4, and the outcome of the goodness-of-fit tests are shown in subplots A-D of Fig. S2 of the Supplementary File. In this model, "late sepsis" was associated with a significant increase in the rate constant k_{21} describing the return flow from V_{t1} to V_c, often assimilated to lymph flow ($P<0.01$). Late sepsis is also associated with decreased urine output, compared to baseline experiment: 199 [164; 309] vs. 13 [1; 181] mL in the 2 h period, $p=0.025$. These two differences explain why the plasma volume was better maintained during the late sepsis experiments than at baseline.

Cardiac index was negatively correlated with k_{23}, i.e. a high cardiac index decreased the flow from V_{t1} to V_{t2} ($P<0.001$). The central venous pressure slightly accelerated the capillary filtration (via k_{12}, $P<0.04$). The body weight increase between the experiments greatly reduced flow to V_{t2} (via k_{23}, $P<0.006$). These covariate effects are shown numerically in Table S3 and are also illustrated graphically in subplots E–H of Fig. S2.

Figure 4, top row, shows the influence of cardiac index on the fluid distribution during the baseline experiment. Figure 4, lower row, illustrates the influence of the increase in body weight between the first and the second experiment after standardizing for cardiac index. Importantly, only a small or no increase in body weight created a fluid distribution that was similar to the baseline experiment with the sole exception of the low urine output.

The relationship between the volume expansion of V_{t1} and V_{t2} over time showed that rapid filling of V_{t2} was initiated when V_{t1} had been expanded by approximately 1,200 mL in the baseline and by 850 mL during the "late sepsis" experiments (Fig. S3).

Calculated half-lives did not differ greatly between the experiments, except for a longer half-life of the plasma volume expansion in the late sepsis phase compared with baseline (24 versus 5.5 h, respectively, Table S3).

P-creatinine increased over time (Table 1) and was as strong covariate as sepsis to the rate constant for urine output (k_{10}). Hence, they were alternatives. An approximation of k_{10} can be obtained from the ratio 0.5/P-creatinine which, in turn, yields the urine flow rate when multiplied by the plasma volume expansion.

Hypovolemia and albumin balance

The volume kinetics model allowed estimation of the size of the central volume (V_c) which was 7.5% lower when the second infusion was initiated as compared to the baseline infusion [2,041 (127) mL vs. 2,194 (136) mL, $P=0.31$] (N.B: the total volume of blood drawn for Hb and Albumin measurements was of 34 mL for one kinetic experiment, plus 94 mL during the 24 h experiment for the other analyses).

The Hb concentration was higher when the second infusion was initiated (9.53 vs. 9.08 g/dL; $P=0.17$) which, after correction for the baseline hematocrit of 29.8%,

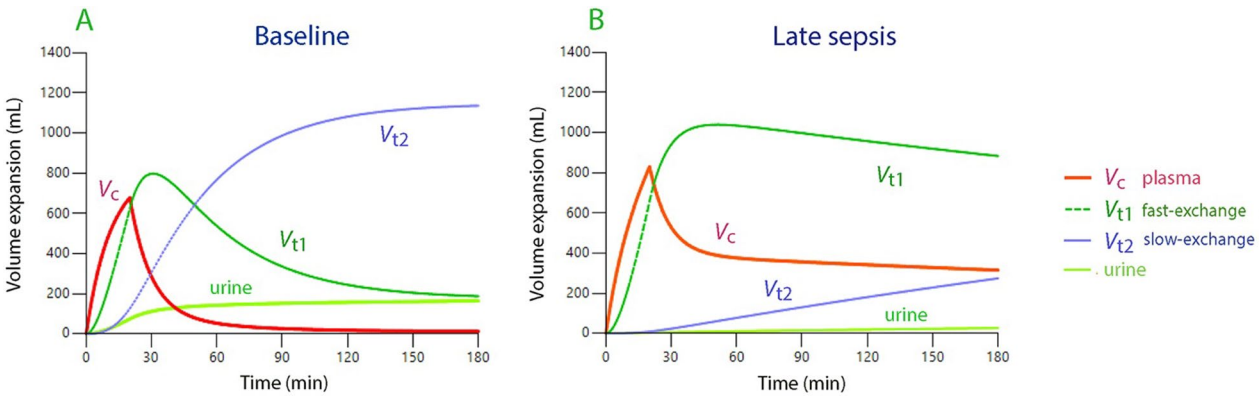

Fig. 3 **A** Distribution of the infused 1,500 mL of crystalloid fluid over 20 min between the extracellular body fluid compartments [plasma, fast-exchange interstitial, and slow-exchange interstitial] in the baseline experiments. **B** Same plot as in A but for the "late sepsis" experiments. The base model without covariates was applied for the two series of experiments separately. Simulations were prolonged to 180 min

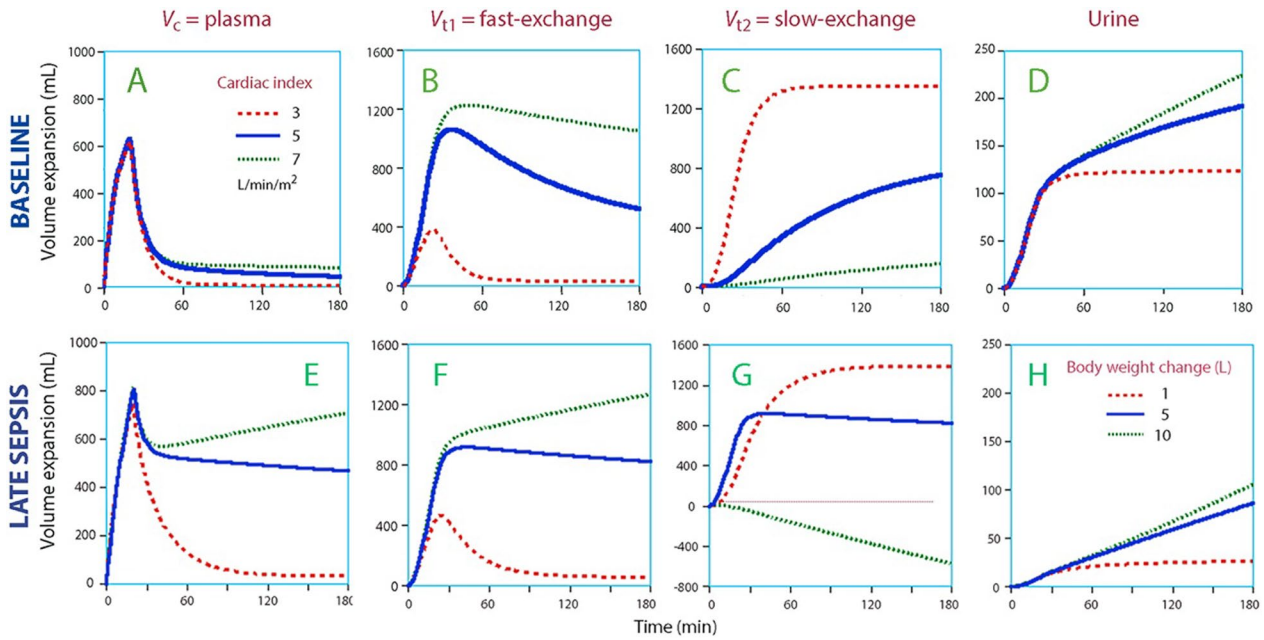

Fig. 4 The predicted distribution of 1500 mL of crystalloid fluid infused over 20 min in the baseline experiments (**A–D**) depending on three levels of cardiac index. The lower row (**E–H**) shows the same infusion in the "late sepsis" setting depending on the increase in body weight since the first infusion. Cardiac index was pre-set to 5 L/min/m^2 as this variable affects k_{23}, too. The plots were created by simulations based on the kinetic data for all experiments shown in Table S3. Group-wise correction was made for the mean central venous pressure and the lower row was also standardized for the mean cardiac index. Plots are extended one hour ahead of the performed experiment time. Note different scales on the y-axis, although the two rows use the same scaling

indicated that the plasma volume had decreased by 7.0% between the experiments.

Plasma albumin changed in the direction opposite to Hb and decreased by 16% (from 31.7 (1.7) to 27.3 (2.5) g/L; $P<0.001$) which, when considering the reduced plasma volume, implies that up to 19.3% of the circulating albumin may have been translocated to the interstitium (from 69.4 to 55.7 g = 13.7 g, as given by the product of V_c and plasma albumin). Moreover, the difference in fluid-induced dilution between Hb and plasma albumin could be computed over time as both were analyzed on the same repeated samples.

Figure 5 shows Hb-albumin dilution difference during the fluid infusion, which reveals the "interstitial washdown" phenomenon, described elsewhere [17]. After a peak corresponding to the ingress of interstitial albumin in the circulation via lymphatic return, the two experimental conditions show clear divergence. Indeed, the relative increase of albumin concentration fades rapidly to its original value in the baseline experiment (as described in volunteers and during anesthesia), whereas recruited albumin appears to remain in the circulation after the infusion in the late sepsis phase.

According to these data, approximately 5.5 g (i.e., one third of the leaked albumin) was returned to the plasma where it remained at least 2 h after the second infusion.

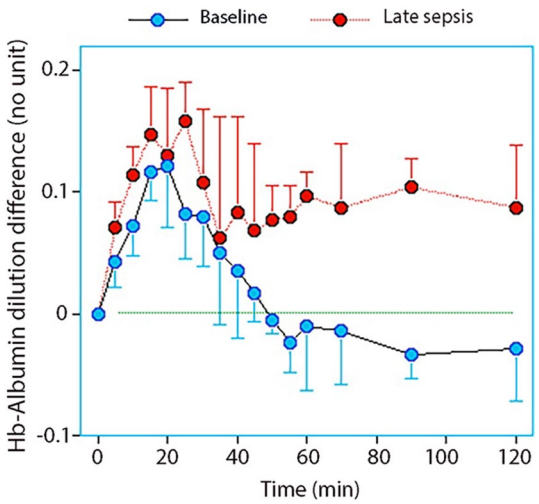

Fig. 5 The Hb-Albumin dilution difference at baseline and in the late sepsis phase. A positive value implies that more albumin enters the plasma via the lymph than is filtered out to the interstitium. Median and IQR are shown (for clarity, 25th percentile only for baseline and 75.th only for late sepsis)

This amount is given by comparing the Hb-derived plasma dilution, which was 20% and corresponded to 400 mL during the steady state period post-infusion (Fig. 2C and 3B), with the albumin-derived plasma dilution, which

was only half as great (Fig. 5**)**. This albumin recruitment then equals the albumin content of 200 mL of plasma.

Discussion

This study demonstrates differences in hemodynamics and in the distribution of crystalloid fluid between a baseline state with isolated general anesthesia and an infusion administered one day after a septic episode serious enough to kill 2 out of 12 pigs.

The baseline experiment showed a fluid distribution pattern known from infusion experiments in humans [10], which changed dramatically during the late sepsis phase. This could be explained by hemodynamic factors, sepsis-unique changes, and previous fluid accumulation. The hemodynamic influences from cardiac index and central venous pressure had the same quality in both series of experiments but occurred at different levels depending on the hemodynamic scenario. Two covariate effects were unique for the post-septic setting: depressed urine output (lower k_{10}), and faster return flow of distributed fluid to the plasma (higher k_{21}). Finally, retention of fluid between the experiments inhibited the entrance of fluid to V_{t2}.

Population volume kinetics has previously been used in an ovine model of early sepsis to demonstrate sepsis-induced capillary leak and influence of various therapeutic agents [18]. In that model of early sepsis, capillary filtration increased but the distributed fluid hardly returned from the extravascular space at all (low k_{21}), indicating decreased lymphatic flow at the acute phase. In addition to increased capillary filtration, this finding allowed to explain fluid accumulation in the interstitium.

To our knowledge, the present study is the first to address fluid kinetics in a stabilized, late sepsis model after significant fluid accumulation has taken place. We observed contrasting results compared to the acute phase models: first, capillary leakage of fluid (via k_{12}) was not increased, second, lymphatic flow (k_{21}) was increased above baseline values during fluid infusion and was accelerated by the pronounced expansion of V_{t1} which is assumed to correspond to the volume of the interstitial free fluid pool plus the lymphatics. Thus, the characteristic fluid kinetic pattern of acute sepsis (i.e. increased transcapillary filtration and decreased lymphatic return) had subsided, with an even increased lymphatic return. We also observed a persistent increase of albumin concentration with the crystalloid bolus, due to the phenomenon of "interstitial washdown", which is usually short-lived [17, 19]. The persistent inflow of albumin to the plasma during the "late sepsis" experiments probably reflects a higher albumin concentration in V_{t1} and perhaps also that the pathological distribution of albumin began to normalize. The capillary leakage of albumin increases during sepsis [20] with a four-fold increase measured in septic shock [21]. The decreased plasma albumin concentration, without central volume expansion (V_c was slightly lower) at the late sepsis phase suggested that albumin leaked during the acute sepsis, to an estimated amount of 14 g.

Our analysis shows that the fluid overload of 5.1 kg that developed between the sessions was a key factor explaining the fluid kinetics during the "late sepsis" experiments. The present data offers clues to where this fluid was located. First, the plasma volume can be ruled out as it was not expanded. Second, V_{t2} tends to open for rapid filling at a specific rise in interstitial pressure (probably at zero pressure) [10] and the V_{t1}-V_{t2} plot in Fig. S3 shows that V_{t2} opened 350–400 mL earlier during "late sepsis" compared to the baseline experiments. This volume might represent fluid that had been added between the experiments. Therefore, nearly all the accumulated resuscitation fluid would be located to V_{t2}. This space serves as an overflow reservoir that greatly prolongs the persistence of infused fluid in the body [10] and probably corresponds to the interstitial gel phase in which fluid moves slowly and is not freely distributed [22, 23]. By contrast, the fast-exchange interstitial fluid pool named V_{t1} might be the interstitial free fluid pool plus the lymphatics.

An interesting finding in the present study is that V_{t2} cannot be filled indefinitely. Fluid accumulation in V_{t2} decreased in proportion to the degree of previous overload and even stopped at an overload of 9–10 kg (Fig. S2). Another observation is that our calculations predicted an overall long half-life of the infused fluid in the animals, which would be even longer than reported in "late sepsis" due to the carry-over of previously infused fluid.

This study brings direct insight into clinical practice. It suggests that the mechanisms leading to fluid accumulation, occurring in the early phase, may reverse rapidly. In our model, shortly after sepsis resolution, measured lymphatic return appeared to have recovered or even increased compared to baseline, although accumulated fluid indicated that lymph flow had decreased in the acute phase as it was described in other studies [24, 25]. Fluid kinetics could maybe be translated into a useful tool to screen for lymphatic function and detect the optimal timing to initiate fluid removal.

Limitations of the study include the low number of studied animals, although the model was strengthened by the fact that the analyses were based on all time points and not only on a single mean value for each experiment. Furthermore, no kinetic analysis was conducted in the acute phase, which could have allowed to better describe differences over time. This was not performed because of the required observation period of 2 h (without further fluid administration) after the fluid bolus. We feared that

animals would require more fluid and would not survive to the acute phase. However, the protocol may still be improved with the addition of an early standardized fluid. Also, the effect of prolonged anesthesia was not analyzed by volume kinetics in control animals. However, both experiments in our study were performed during general anesthesia, which implies that the comparison between the volume kinetics is still valid. Volume kinetic studies suggest that the urinary excretion would be greater, and less fluid would accumulate of in Vt1 and Vt2 in the awake state than reported here [26].

Isotonic saline was used as infusion fluid, which is not optimal. This fluid promotes metabolic acidosis and is more slowly eliminated than balanced crystalloid solutions. However, it is still widely used in anesthesia departments and ICUs. Large population studies show that that isotonic saline does not inflict more harm to ICU patients than balanced fluids [27–30], although a benefit of balanced fluids has indeed been found in septic patients [31]. Table 1 shows that persistent derangements of plasma sodium and chloride did not occur.

An important limitation is that our physiological interpretations are built on the assumption of a close agreement between the kinetic model and body physiology, which cannot be confirmed with certainty. However, estimations obtained with fluid kinetics have been confronted with in vivo measurements and showed good agreement, e.g., between V_c and plasma volume, and between k_{21}-mediated return flow to the plasma and the lymphatic flow in the thoracic duct [8]. Also, we used a bacteriemia model which may not reflect the full mechanisms involved during clinical sepsis, especially the "late sepsis" phase. In this model, the resolution of inflammation is probably much swifter, compared to clinical sepsis or peritonitis models, as bacteria infusion is stopped as soon as sepsis develops. This impairs external validity, as clinical sepsis may feature a different pattern of vascular healing (macro-, microvascular and lymphatic). However, this quick resolution was also what allowed the feasibility of investigating resolution of capillary leak in the setting of a translational model. Finally, the role of vasopressors could not be fully explored, as only two animals received norepinephrine at the time of the analysis. This may limit the clinical validity of this study as norepinephrine play a role in capillary filtration [32]. However, the higher k_{21} in the Late sepsis experiment is probably due to endogenous catecholamines stimulating the lymphatic pumping [33, 34].

Conclusion

This study explored the changes in fluid kinetics during the late sepsis phase, after significant fluid accumulation. Our findings suggest that most of the accumulated volume resided in the slow-exchange compartment (V_{t2}), and that further fluid administration was rather distributed to the fast-exchange compartment (V_{t1}) with acceleration of lymph flow, improved plasma expansion (V_c), and recruitment of interstitial albumin.

Abbreviations

CFU	Colony-forming units
IQR	Interquartile range
MAP	Mean arterial pressure
SD	Standard deviation
SOFA	Sequential organ failure assessment
SVRI	Systemic vascular resistance index
Vc	Central volume
Vt1	Fast-exchange volume compartment
Vt2	Slow-exchange compartment

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13054-025-05308-8.

Additional file1 (DOCX 4055 KB)

Author contributions

SHL and HD participated in study design, animal experiments, data acquisition and analysis, and drafting of the manuscript. RH analyzed the volume kinetics model, interpreted data, drafted the manuscript. AH participated in study design, data acquisition, supervised animal experiments and revised the manuscript. RL performed animal experiments, data acquisition, and supervised animal housing and welfare. AG and CS participated in data acquisition and analysis. BA participated in study design, supervised the team and revised the manuscript. VL participated in study design, data acquisition, supervised animal experiments and revised the manuscript. AD participated in study conception, study design, animal experiments, data acquisition and analysis, and drafting of the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal Care Committee of VetAgro Sup, Marcy l'Etoile, France according to European regulation [European Directive EU 86/609].

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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