Prediction of colloid osmotic pressure from albumin and/or hemodynamics in pigs undergoing hemorrhagic shock

Steven Brantlov, Asger Granfeldt, Else Tønnesen, Aage Kristian Olsen Alstrup, Michael Winterdahl

Abstract

***Background and purpose*: Colloid osmotic pressure (COP) is strongly affected during hemorrhagic shock (HS) and imbalances may result in pulmonary and systematic oedema. Measurement of COP is therefore crucial in order to monitor changes and to react to critical levels. The aims of this study were to examine the time-course effects on COP and albumin in pigs undergoing controlled HS, and to investigate whether albumin and/or hemodynamic parameters are relevant predictors of COP during HS.**

***Methods*: 18 pigs randomly assigned in a blinded manner to one of three groups. Group 1: sham (n=4); group 2: hemorrhage control (n=7) and group 3: hemorrhage + adenosine, lidocaine and Mg2+/adenosine, lidocaine (ALM/AL) (n=7). COP was measured using a commercially available oncometer.**

***Results*: Group 2 experienced the greatest change in absolute and relative COP-values during the experiment, while group 1, as expected, experienced the smallest change. Strong correlations were seen between COP and albumin for group 2 (0.84, p<0.0001) and for group 3 (0.82, p<0.0001), whereas moderate to negligible correlations were seen between COP and the hemodynamic parameters.**

***Conclusion*: Pigs subjected to HS compensated unexpectedly well compared to pigs not exposed to HS (sham). It is possible to predict COP from albumin, which may be clinically relevant in situations where an oncometer is not accessible. Further studies are needed if these findings are to be transferred to humans.**

Key words: Hemorrhagic shock, resuscitation, colloid osmotic pressure, hemodynamics, swine.

Introduction

Hemorrhagic shock (HS) is the leading cause of death in civilian and military traumas. (1) Severe HS is characterized by hemodynamic instability, decreased tissue perfusion, cellular hypoxia, organ damage, and can result in death. (2) Therefore, rapid volume replacement is critical for patient survival, and infusing blood and intravenous fluids is essential in restoring fluid volume deficits. (3) Such deficits affect the balance between hydrostatic pressure and the colloid osmotic pressure (COP) across the capillary wall, (4,5) and where COP plays a major role in the transcapillary fluid shift. Under normal physiological conditions, plasma contains high amounts of proteins, where albumin is the principal oncotically active component, accounting for approximately 80% of COP. (4) The extravascular space only contains small amounts of albumin. (6) In addition, HS initiates a systemic inflammatory response that may cause capillary leakage of proteins from the intravascular to the extravascular space, (7) hereby decreasing the effect of COP and consequently the risk of local or generalized oedema. (8,9) This relationship has been seen in critically ill patients, where COP is a reliable predictor of pulmonary (10,11) and generalized oedema. (12) Determination of COP can easily be made with instruments called oncometers. The aims of this study were to examine the time-course effects on COP and albumin in pigs undergoing controlled HS, and to clarify the clinical potential of using albumin and/or hemodynamic parameters as predictors of COP, in cases where an oncometer is not available.

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Materials and methods

Values of albumin and hemodynamics are taken from a previous study, (13) investigating the effects of adenocaine and Mg2+ on the maintenance of mean arterial pressure. COP measurements have not been used previously.

Animals

The current study was approved by The Danish Experimental Animal Inspectorate and conducted in accordance with the "Principles of Laboratory Animal Care". (14) Eighteen female crossbred Landrace/Yorkshire/Duroc pigs (35-42 kg) were fasted overnight with ad libitum water. Anaesthesia was induced with midazolam (20 mg) and s-ketamine (250 mg), and was maintained with intravenous infusion of fentanyl (60 μg/kg/h) and midazolam (6 mg/kg/h). The animals were intubated and volume-controlled ventilated (S/5 Avance, Datex Ohmeda, WI, USA) with a positive end-expiratory pressure of 5 cmH2O, FiO2 of 0.35, and a tidal volume of 10 ml/kg. PaCO2 was kept between 41-45 mmHg and body temperature approximately 38.5 °C. Animals received a bolus of Ringers acetate (20 ml/kg) before surgical procedures and a maintenance rate of 10 ml/kg/h starting 1 hour after re-infusion of shed blood.

Measurements

Hemodynamics

Vascular sheaths were inserted into the carotid artery, the external jugular vein and the femoral vessels. A conductance pressure-volume (P-V) catheter (Millar Instruments, Houston, Tex, USA) was inserted into the left ventricle through the carotid artery. A pulmonary artery catheter (CCOmbo, Edwards Lifesciences, CA, USA) was inserted through the jugular vein to monitor cardiac index (CI) and core temperature. Through the femoral artery a pigtail catheter (Medtronic, Minneapolis, MN, USA) was placed in the left ventricle for injection of microspheres. Finally a PTS sizing balloon (NMT Medical Boston MA, USA) was inserted through the femoral vein for occlusion of the inferior caval vein. The placement of catheters was fluoroscopically guided. The following hemodynamic parameters were measured throughout the experiment: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), central venous pressure (CVP), pulmonary capillary wedge pressure (PCWP) and cardiac output (CO).

Urine and blood analysis

A bladder catheter was placed for urine collection. Analysis of blood and urine sample: arterial and mixed venous blood gases were analyzed continually through the study (ABL 725, Radiometer, Denmark). Animals were treated with 200 U/kg of heparin and supplemented hourly (100 U/kg) for anticoagulation.

Colloid osmotic pressure (COP)

COP was measured using a commercial oncometer (Gonotec, Osmomat 050, Berlin, Germany) with a semi-permeable membrane and a cut-off of 20 kDa (Gonotec, Berlin, Germany). COP was measured using arterial plasma. (12) Plasma was following centrifuged at 4 °C for 10 minutes at 3000 rpm. In accordance with manufacturer recommendations, the following was carried out: the sample volume was examined visually for bubbles prior to use, and the samples were injected into the measuring cell using three equal cycles of approximately 50 μl (150 μl in total), and calibration was performed prior to each measurement series by creating a hydrostatic pressure difference of 10 mmHg. Five percent human albumin (Human Albumin CSL Behring 5%) was used to check membrane precision.

Experimental protocol

All experiments were conducted in an internationally accepted pig bleeding model. (15)

Pigs were randomly assigned in a blinded manner to one of three groups: group 1: sham (exposed to surgery and anesthesia only) (n=4), group 2: hemorrhage control (n=7) and group 3: hemorrhage + adenosine, lidocaine and Mg2+/adenosine, lidocaine (ALM/AL) (n=7). The experimental protocol was based on eight measurements and structured in the following way:

- T=0: Baseline measurement.
- T=1: End of 90 min of HS, where the HS pigs were bled through the femoral artery to a MAP of 35 mmHg at a rate of 2.15 mL/kg/min over 7 min, and then 1.15 mL/kg/min over the remaining period. (11) The pigs were kept at a MAP of 30-35 mmHg by withdrawing or infusing shed blood that was stored at 37 °C in a citrated glucose solution (concentration: citric acid monohydrate 8 mg, sodium citrate 22 mg, glucose monohydrate 25 mg).
- T=2: Animals were resuscitated with Ringers acetate at a rate of 120 ml/min to reach a target MAP of 50 mmHg.

Group 2 (hemorrhage control) was simultaneously infused with 20 ml of 7.5% NaCl, and group 3

(hemorrhage + ALM/AL) was infused with 20 ml of 7.5% NaCl + ALM (adenosine [0.23 mg/kg], lidocaine [0.64 mg/kg] and MgCl2 [0.41 mg/kg]). In the event that MAP fell below 50 mmHg, 30 ml of Ringers acetate was infused as a bolus. After 30 min of hypotensive resuscitation, 75% of the shed blood volume was re-infused at a rate of 60 ml/min (ml/min/kg), and the pigs were observed for 6 hours (T=3 to T=7). After re-infusion of shed blood, 10 ml 0.9% NaCl containing a higher concentration of adenosine (0.82 mg/kg), lidocaine (1.66 mg/kg) (AL) was infused in group 3 (hemorrhage + ALM/AL), whereas group 2 (hemorrhage controls) received only 10 ml 0.9% NaCl.

Data analysis

Data was tested for normality using Q-Q plots and the Shapiro-Wilk test. Absolute and relative changes in COP, albumin and hemodynamic parameters during the HS study were analyzed as follows:

1) Absolute change (mmHg) = End value (T=7) - Baseline value (T=0)
2) Relative change in percent (%) = ([T=7 - T=0] / T=0) x 100%

The relation between COP and albumin and the hemodynamic parameters (SBP, DBP, MAP, MPAP, CVP, PCWP and CO) were analyzed using Pearsson's correlation coefficients (r) and linear regression with 95% prediction intervals. Statistical significance was set at $p<0.05$. Statistical tests were performed with MedCalc® (Version 8.0.0.1, Medcalc Software, Ostend, Belgium) and regression plots were prepared using SigmaPlot® (Version 10.0, Systat Software, Inc., San Jose, CA, USA).

Results

Absolute and relative changes in COP, albumin and hemodynamic parameters are shown in **Table 1**. **Figure 1** illustrated the experimental set-up and the time-course for COP for all groups. Group 2 experienced the greatest change in absolute and relative COP-values during the experiment, while group 1 experienced the smallest change. Correlation coefficients for all parameters were found to be predominantly positive (**Table 2**). Strong correlations were seen between COP and albumin for group 2 (0.84, $p<0.001$) and for group 3 (0.82, $p<0.001$) (**Table 3**). Other correlation coefficients ranged from moderate to negligible. The linear relation between COP and albumin was depicted in **Figure 2**. No statistically significant differences were found between COP and albumin data from group 2 and group 3 ($p>0.05$ - unpublished data). Data from these two groups were therefore pooled in **Figure 2**.

Discussion

This study presents the time-course effects on COP and albumin in pigs undergoing controlled HS, and the clinical potential of using albumin and/or hemodynamic parameters as predictors of COP. The time-course effects on COP corresponds well with earlier findings done in pigs. (16,17) Eighteen female crossbred Landrace/Yorkshire/Duroc pigs were enrolled in this study. Compared to normal COP ranges (12.7 to 19.7 mmHg), (18) the pigs enrolled in this study were slightly below. An explanation for this might be that the pigs were fasted overnight with ad libitum water or the choice of a semi-permeable membrane with a cut-off of 20 kDa. Such membranes are provided in 10, 20 and 30 kDa, which potentially could influence COP results. Therefore, future methodological studies should investigate the effect of membrane size on COP measurements. It has been shown that HS may initiate vascular trauma resulting in increased capillary permeability of proteins (e.g. albumin) from the intra- to the extravascular space, and thus influence the COP balance across the vascular wall. (7) However, in the current pig study, the large compensation of the pigs in the HS-groups led to COP and albumin levels comparable to that of pigs in group 1 (sham). The greatest change in absolute and relative COP-values during the experiment was seen in group 2, where group 1 experienced the smallest change, which was as expected. We expected noticeable decreases in COP in the HS-groups, since capillary leakage induced by inflammation has been confirmed in earlier papers. (7,19) In order to comment on changes in interstitial COP, it is necessary to apply the so-called Wick method, where a small tube is inserted in the subcutis to collect samples of the interstitial COP. (20) However, since the use of this technique was beyond the scope of this study, it will be highly relevant in future studies to investigate capillary leakage by use of the Wick method. We found a strong linear relationship between the COP and albumin for group 2 and group 3. This suggests that albumin may predicts COP, which has a clinical potential in situations where an oncometer is not accessible. Determination of COP, alternatively albumin, may be a tool for clinicians in determining critical COP levels and when to start and

stop IV infusion of plasma protein solutions. (10,21) However, in order to obtain absolute COP values, it is recommended that measurements are performed directly using an oncometer. We did not find any significant correlations between COP and hemodynamic parameters. These parameters are therefore not suitable as predictors of COP. To extrapolate our findings to humans requires further studies, although pigs share similar anatomic and physiologic characteristics, e.g. the cardiovascular system, with humans. (22) In conclusion, pigs subjected to HS compensated unexpectedly well by altering COP when compared to pigs not exposed to HS (sham). Albumin was found to be able to predict COP in pigs undergoing HS. This may be clinically relevant in cases an oncometer is not accessible. Furthers studies are needed in order to transfer these findings to humans.

Conflicts of interest

Steven Brantlov, Asger Granfeldt, Else Tønnesen, Aage Kristian Olsen Alstrup, and Michael Winterdahl declare that they have no conflicts of interest.

Compliance with ethical requirements

The current study was approved by The Danish Experimental Animal Inspectorate and conducted in accordance with the "Principles of Laboratory Animal Care" and the Helsinki Declaration of 1975 (revised in 2000) concerning Human and Animal Rights.

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Table 1. Subject characteristics

Parameter	Group 1 (n=4)	Group 2 (n=7)	Group 3 (n=7)
COP (mmHg)	10.0±0.8 (9.0-11.1)	8.9±1.3 (6.7-11.3)	9.1±1.1 (7.0-11.0)
Albumin (g/L)	24.5±1.5 (22.0-27.7)	21.1±2.5 (16.4-25.5)	21.2±2.4 (17.2-25.6)
SBP (mmHg)	94.0±18.6 (58.3-114.0)	98.6±9.8 (86.8-116.2)	87.1±13.2 (61.2-106.8)
DBP (mmHg)	50.0±16.9 (23.3-77.3)	53.3±9.2 (43.8-71.8)	44.4±13.3 (23.5-68.0)
MAP (mmHg)	66.5±19.4 (31.5-93.0)	72.2±10.6 (58.7-92.8)	60.4±15.5 (33.0-86.0)
MPAP (mmHg)	22.8±4.3 (19.0-32.3)	19.0±2.7 (16.3-24.3)	21.0±4.4 (17.6-30.3)
CVP (mmHg)	6.3±2.3 (1.5-8.5)	6.6±1.2 (4.0-8.2)	7.3±2.8 (1.6-10.2)
PCWP (mmHg)	8.0±1.8 (4.8-10.3)	8.4±0.9 (6.8-9.3)	9.5±2.6 (5.0-12.5)
CO (L/min)	3.9±1.3 (1.6-5.8)	3.4±0.5 (2.3-4.2)	3.6±1.0 (1.6-5.2)

Legend: Values are specified as mean±1 standard deviation (SD) and range (minimum-maximum); SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; MPAP=mean pulmonary arterial pressure; CVP=central venous pressure; PCWP=pulmonary capillary wedge pressure; CO=cardiac output.

Table 2. Absolute and relative changes for plasma colloid osmotic pressure (COP) and albumin

Parameter	Group 1 (n=4)	Group 2 (n=7)	Group 3 (n=7)
ΔCOP (mmHg)	-2.1 (-19.0%)	-3.2 (-27.9%)	-2.6 (-23.4%)
ΔAlbumin (g/L)	-4.6 (-17.0%)	-5.6 (-21.9%)	-5.7 (-22.4%)

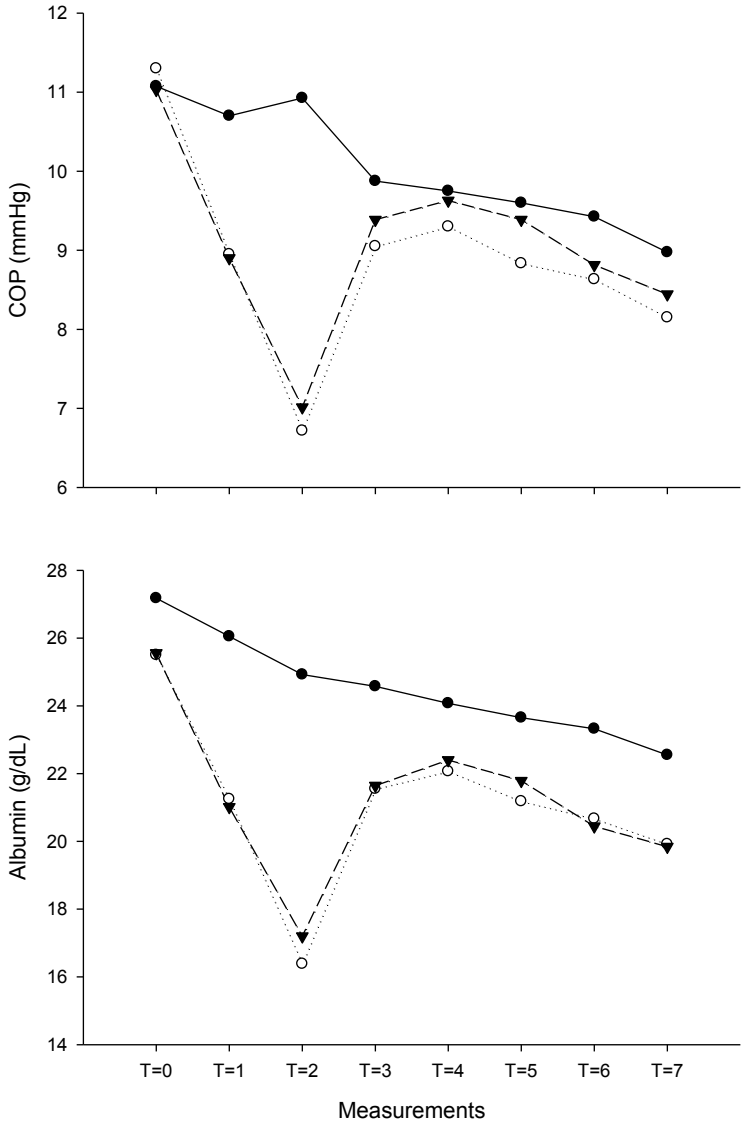
Legend: Δ=change. The numbers are specified as absolute change (relative change) of COP and albumin. Absolute change (mmHg) = end value (T=7) - baseline value (T=0); relative change in percent = ([T=7 - T=0] / T=0) x 100%.

Table 3. Correlation matrix

Parameter	Albumin (g/L)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	MPAP (mmHg)	CVP (mmHg)	PCWP (mmHg)	CO (L/min)
COP (mmHg)								
Group 3	0.84[b]	0.07[†]	0.26[†]	0.19[†]	0.02[†]	-0.05[†]	0.08[†]	0.01[†]
Group 4	0.82[b]	0.41[a]	0.61[b]	0.64[b]	0.30[a]	0.09[†]	0.32[a]	0.15[†]

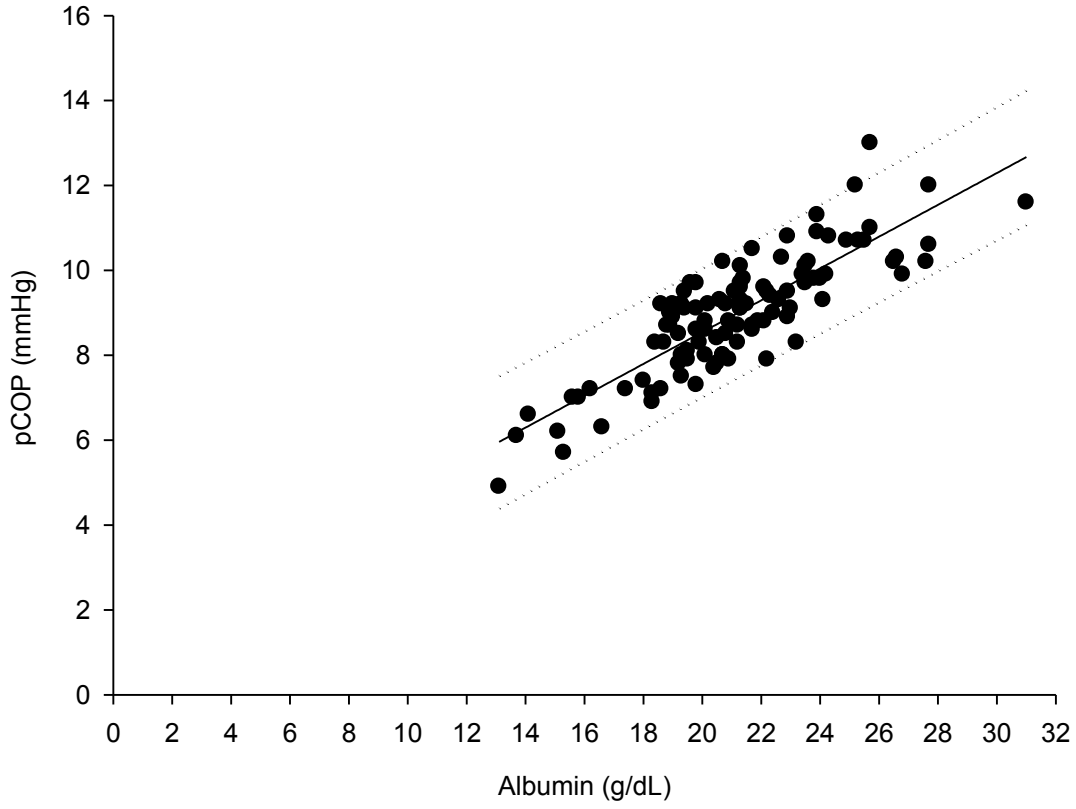
Legend: The Table shows colloid osmotic pressure (COP) correlated to albumin and hemodynamic parameters for the HS groups. Numbers are specified as Pearson's correlation coefficient (r) with p values as [a]<0.05, [b]<0.0001, [†]>0.05. Correlation data are calculated from baseline (T=0) to end value (T=7). SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; MPAP=mean pulmonary arterial pressure; CVP=central venous pressure; PCWP=pulmonary capillary wedge pressure; CO=cardiac output.

Figure 1. Study design and time-course effects on colloid osmotic pressure (COP) and albumin in pigs undergoing controlled hemorrhagic shock



Legend: ●=group 1; ○=group 2; ▼=group 3; T=0: baseline measurement; T=1: end of 90 min of hemorrhagic shock; T=2: resuscitation of the pigs with Ringers acetate. After resuscitation, 75% of the shed blood volume was re-infused, and the pigs were following observed for 6 hours (T=3 to T=7).

Figure 2: Correlation between plasma colloid osmotic pressure (pCOP) and albumin



Legend: ●=pooled data from the two hemorrhagic groups (group 2 and group 3). Linear regression with 95% prediction intervals (dotted) are shown.

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