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Hemodynamic and perfusion variables during experimental septic shock treated with goal-directed fluid resuscitation

Evolução de variáveis hemodinâmicas e perfusionais durante o choque séptico experimental tratado com ressuscitação volêmica guiada por metas

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ABSTRACT

Objectives: Although fluid resuscitation guided by central venous oxygen saturation (SvO₂) is currently considered the gold standard in sepsis therapy, few studies have described hemodynamic and perfusion parameters during this procedure. This study aims to describe these parameters during septic shock without resuscitation and after 12 hours of goal-directed resuscitation.

Methods: Thirteen anesthetized pigs (35-45 kg) had peritonitis caused by fecal inoculation (0.75 g/kg). After developing persistent hypotension, both groups were given antibiotics and randomized either to the control group (n=7) or the experimental group (n=6). In the control group, hemodynamic control was optimized to maintain a central venous pressure of 8-12 mmHg, a urinary output above 0.5 mL/kg/hour and a mean arterial blood pressure above 65 mmHg. The experimental group received the above target therapy in addition to maintaining a SvO₂ above 65%. The interventions included lactated

Ringer's solution and norepinephrine for both groups and dobutamine in the SvO₂ group. The animals were treated for 12 hours or until death.

Results: Untreated sepsis was associated with significant reductions in SvO₂, PvO₂, cardiac output and central venous pressure in addition to increased arteriovenous oxygen saturation and veno-arterial CO₂ differences. Following resuscitation, these parameters were corrected in both groups. Goal-directed resuscitation was associated with a better hemodynamic profile, characterized by higher SvO₂, cardiac output and central venous pressure.

Conclusions: Non-resuscitated sepsis showed a hemodynamic profile suggesting hypovolemia, with worsened perfusion and hemodynamics, which is reversed upon fluid resuscitation. Goal-directed resuscitation is associated with significantly improved hemodynamic and perfusion parameters.

Keywords: Shock, septic/blood; Oxygen/blood; Oximetry/methods; Lactates/blood; Resuscitation/methods; Hemodynamics/physiology; Pigs

Study conducted at the Intensive Care Medicine and Anesthesiology Research Laboratory, Instituto Sírio-Libanês de Ensino e Pesquisa, São Paulo (SP), Brazil.

Conflicts of interest: None.

Submitted on August 2, 2011
Accepted on September 6, 2011

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INTRODUCTION

Hemodynamic optimization in a critical patient after systemic insults, such as surgery, severe trauma or sepsis, has frequently been discussed in the medical literature. It has been suggested that appropriate hemodynamic resuscitation, using clear parameters such as lactate, central/mixed venous oxygen saturation or base deficit, is the cornerstone of improved tissue perfusion and oxygenation, with consequential benefits with respect to clinical outcome.⁽¹⁾

For instance, hemodynamic resuscitation in sepsis has been repeatedly

evaluated during this last decade. Essentially, this type of resuscitation consists of a multi-step protocol, including fluid replacement, use of vasoactive agents, blood transfusion and administration of inotropes, that target pre-determined hemodynamic and metabolic parameters.^(2,3) The most used parameter to guide reanimation is central venous oxygen saturation (SvcO₂). It has been shown that maintaining a value of SvcO₂ above 70% during the first 6 hours of therapy (the so-called “golden hours”) is associated with survival benefits.⁽²⁾ More recently, therapy guidelines have suggested that mixed venous oxygen saturation (SvO₂) values above 65% may be a surrogate for SvcO₂ during the “golden hours”.^(2,3) After these studies and therapy guidelines were published, goal-directed fluid resuscitation, including the use of SvcO₂ values, started to be used in sepsis therapy in worldwide clinical practice. Although widely used for sepsis resuscitation, few studies have assessed the course of hemodynamic tissue perfusion markers used in this strategy. Therefore, this study’s aim was to describe the time course of the main perfusion and hemodynamic parameters during a clinically relevant experimental model of non-resuscitated sepsis.

METHODS

This study was conducted at the Intensive Care Medicine and Anesthesiology Research Laboratory of the Instituto Sírio-Libanês de Ensino e Pesquisa and appropriately approved by the institution’s ethics committee. All experimental procedures were conducted in compliance with the Brazilian Instituto Nacional de Saúde’s guidelines for the use and care of experimental animals. This study was based on a previously described experimental fecal peritonitis model.⁽⁴⁾ Part of the results, which used a different sample size and different parameters than those reported in this article, was accepted for publication in *Shock*.⁽⁵⁾

Preparation of the animals

Thirteen male *Agroceres* pigs with an average body weight of 40 kg were fasted for 18 hours and had free access to water. The animals were pre-anesthetized with intramuscular midazolam and acepromazine. Following premedication, the pigs were anesthetized with thiopental and an orotracheal tube was inserted. After successfully intubating the animals, an infusion of thiopental (10 mg/kg/hour) and Fentanyl (10 mcg/kg/hour) was maintained, and pancuronium (0.15 mg/kg starting dose followed by an infusion of 0.25 mg/kg/

hour) for neuromuscular blockade during the surgical procedure was used. After stabilization, the animals were maintained with thiopental and pancuronium in the dosages described above. The animals were then connected to a mechanical ventilator (Evita XL, Dräger Medical, Lübeck, Germany) with a fixed positive end-expiratory pressure (PEEP) of 5 cm H₂O and a FiO₂ of 30%. A tidal volume of approximately 8 mL/kg and a respiratory rate adjusted for PaCO₂ between 35 and 45 mmHg was maintained. In case of significantly reduced arterial oxygen saturation and/or PaO₂ during the study, FiO₂ was increased up to the minimum necessary to maintain arterial saturation above 92%. Multi-parameter monitoring was provided for the following variables: heart rate, arterial saturation and central venous and pulmonary artery pressure curves after a pulmonary artery catheter was inserted (DX2020, Dixtal Biomédica, Manaus, Brazil).

Next, vascular accesses were dissected. A pulmonary artery catheter was inserted via the right external jugular for continuous measurement of pressure curves, cardiac output and venous oxygen saturation, as well as right-ventricular end-diastolic pressure and ejection fraction (Vigilance VD®, Edwards Lifesciences LLC, Irvine, California, United States of America). A catheter was inserted via the right femoral artery for invasive blood pressure monitoring and arterial blood sample collection. The right femoral vein was catheterized for intravenous fluid administration, drug infusion and venous blood collection. A catheter was inserted via cystostomy for urinary output assessment.

Immediately following installation of the monitoring devices, the animals underwent a median laparotomy. The incision was approximately 4 cm. The descending colon was identified and a 2 cm incision was made. At this time, 0.75 g/kg of feces was removed and the intestine was sutured. Two large gauge catheters were installed into the abdominal cavity bilaterally to the parietocolic gutters, and the laparotomy was sutured. After 60 minutes of stabilization, an amount equivalent to 0.75 g/kg of feces was diluted into 200 mL of 37°C saline solution and introduced into the abdominal cavity via the catheters. After fluid resuscitation was started, all study animals were given intravenous amikacin (250 mg every 12 hours) and metronidazole (500 mg every 8 hours) for the duration of the experiment.

Immediately before the peritonitis induction, and then every 60 minutes thereafter, the following parameters were assessed: heart rate, thermodilution cardiac output, central venous pressure (CVP),

pulmonary capillary pressure, pulmonary artery pressure (PAP), mean blood pressure (MBP), SvO₂, central temperature and urinary output. Arterial and venous blood gas, arterial lactate and hemoglobin/hematocrit concentrations were measured with a blood gas analyzer (ABL 700 Radiometer, Copenhagen, Denmark) before sepsis induction and then every 3 hours until the end of the experiment or death.

Fluid resuscitation

For the study duration, the animals received a maintenance infusion every 24 hours of 100 mL of 10% glucose solution containing 25 mEq of potassium chloride and 75 mEq of sodium chloride, to maintain blood glucose, potassium and sodium levels as close to normal as possible.

During the surgery and the stabilization period, the animals were given a lactated Ringer solution. The protocol was started with a CVP above 8 mmHg and venous oxygen saturation above 70%. After peritonitis induction, the animals were given no volume replacement solution. Upon mean arterial blood pressure (MBP) reaching less than 65 mmHg for 30 minutes, the animals were divided into two groups:

a) Control group: In this group, fluid replacement was started with a 500 mL lactated Ringer bolus every 30 minutes, targeted to reach and maintain the following hemodynamic parameters: MBP above 65 mmHg, CVP between 8 and 12 mmHg and urinary output above 0.5 mL/kg/hour. Norepinephrine was added after 30 minutes of hypotension if volume replacement was deemed unsatisfactory. Initially, the infusion was started at 0.05 mcg/kg/minute, and then it was increased by 0.025 mcg/kg/minute increments every 15 minutes, as necessary, to maintain MBP above 65 mmHg.

b) SvO₂-guided resuscitation group: In this group, fluid resuscitation was based on the mixed venous oxygen saturation values obtained from the pulmonary artery catheter with continued venous saturation, in addition to the hemodynamic goals mentioned above. Therefore, appropriate resuscitation criteria were: MBP above 65 mmHg, urinary output above 0.5 mL/kg/hour, CVP 8-12 mmHg and SvO₂ above 65%. These hemodynamic targets were maintained for 12 hours or until death. Initially the animals were given 500 mL lactated Ringer solution every 30 minutes until reaching a CVP above 8 mmHg. In those animals still maintaining an SvO₂ below 65%, dobutamine was started by an infusion of 2.5 mcg/kg/minute with 2.5 mcg/kg/minute increments every

30 minutes for a maximal dose of 20 mcg/kg/minute. If CVP dropped during the dobutamine infusion, additional crystalloid solution boluses were given to reach the previously described CVP levels. Dobutamine infusion increments were limited by tachycardia above 180 bpm. Norepinephrine was used as necessary, as described above.

The animals were maintained with these parameters until death or for 12 hours (counted from the start of resuscitation), and then were euthanized with an overdose of potassium chloride after deepening anesthesia.

Statistical analysis

The data were tested for normality using the Shapiro-Wilk test and are presented as the mean ± standard deviation. For assessment of time course until shock, both groups were considered together and the statistical significance was assessed with repeated-measures analysis of variance (RM-ANOVA). After the start of shock and the randomization process, comparisons were made using a two-way analysis of variance (two-way ANOVA). *Post hoc* ANOVA analyses were performed using the Tukey Honest Significant Differences (HSD) test. Correlation analyses were performed using the Pearson's test. The R open source statistical package (Vienna, Austria, 2009) was used for statistical analysis and graphics plotting.

RESULTS

Thirteen animals were studied, 6 in the SvO₂ group and 7 in the control group. The median time to hypotension after sepsis induction was 11 hours (range: 7-21 hours).

Figure 1 (A to D) shows hemodynamic and perfusion variable progression during non-resuscitated and treated sepsis. Peritonitis was associated with a significant worsening of perfusion and hemodynamic parameters during the observation time, including reduced SvO₂ and PvO₂ and increased arteriovenous oxygen saturation and venoarterial CO₂ gradients. Table 1 identifies some of the study-related hemodynamic and perfusion variables and the effects of fluid resuscitation. Non-resuscitated sepsis is characterized by significantly reduced cardiac output and systolic index, CVP and MBP. Following resuscitation, these parameters were significantly improved; however, the SvO₂ guided resuscitation group showed better perfusion and hemodynamic parameters, including cardiac output,

CVP, MBP, SvO₂, PvO₂ and arteriovenous oxygen saturation gradient.

Figure 2 shows the correlation between several perfusion variables and cardiac output. Of the analyzed parameters, venoarterial CO₂ gradient showed the

best coefficient of correlation, as shown in figure 2A. Lactate failed to show a significant correlation (Figure 2B) while the oxygen-dependent variables showed a significant correlation, albeit with a poor coefficient of correlation (Figure 2 C and D).

Table 1 – Hemodynamic characteristics during the study

	Non-resuscitated sepsis				Sepsis resuscitation			
	Baseline	3 hours	Shock	p value	Group	1 hour	Final	p value
CO (L/min)	6.9 ± 1.4	4.5 ± 0.8 #	2.9 ± 0.9 #	< 0.001	SvO ₂	6.8 ± 1.1 §	7.4 ± 1.7 §	0.489 †
					Control	4.5 ± 1.6	5.2 ± 1.5	< 0.001 &
HR (bat/min)	126 ± 21	151 ± 32	185 ± 32 #	< 0.001	SvO ₂	194 ± 23	177 ± 28	0.965 †
					Control	175 ± 40	172 ± 46	0.428 &
SV (mL/beat)	55 ± 12	31 ± 7 #	16 ± 4 #	< 0.001	SvO ₂	36 8	42 9	0.425 †
					Control	26 9	32 13	< 0.001
mPAP (mmHg)	28 ± 6	27 ± 7	27 ± 6	0.886	SvO ₂	33 ± 7	32 ± 8	0.138 †
					Control	31 ± 4	27 ± 4	0.231 &
MBP (mmHg)	113 ± 20	98 ± 12 #	58 ± 7 #	< 0.001	SvO ₂	78 ± 13	84 ± 10 §	0.355 †
					Control	70 ± 6	76 ± 5	< 0.001 &
CVP (mmHg)	9 ± 1	7 ± 3	5 ± 1 #	0.001	SvO ₂	9 ± 2	10 ± 2 §	0.131 †
					Control	7 ± 1	8 ± 2	< 0.001 &
SVR (Dynas/s/cm ⁵ /kg)	1280 ± 390	1675 ± 412 #	1547 ± 424	< 0.001	SvO ₂	859 ± 207	795 ± 106 §	0.622 †
					Control	1260 ± 494	1087 ± 294	0.002 &
Diuresis (mL / h)	142 ± 105	51 ± 47 #	15 ± 22 #	< 0.001	SvO ₂	238 ± 424 §	327 ± 206 §	0.086 †
					Control	54 ± 70	106 ± 86	0.002 &
Lactate (mEq/L)	2.0 ± 1.2	1.8 ± 0.9	2.1 ± 0.9	0.818	SvO ₂	3.9 ± 3.1	2.9 ± 1.7	0.873 †
					Control	5.8 ± 2.1	3.8 ± 2.3	0.389 &
Norepinephrine (mcg/kg/min)	0	0	0	-----	SvO ₂	0.42 ± 0.51	0.33 ± 0.29	0.716 †
					Control	0.54 ± 0.18	0.87 ± 1.14	0.242 &
Dobutamine (mcg/kg/min)	0	0	0	-----	SvO ₂	2.0 ± 1.0	5.0 ± 2.0	-----
					Control	0	0	-----
Extra volume	0	0	0	-----	SvO ₂	1458 ± 332	1000 ± 316 §	< 0.001 †
					Control	1357 ± 377	428 ± 120 *	< 0.001 &

CO – cardiac output; HR – heart rate; SV – stroke volume; MBP – mean systemic blood pressure; CVP – central venous pressure; mPAP – mean pulmonary artery pressure; SVR – systemic vascular resistance. Intra and inter-group two-way ANOVA. No factor-time interaction. # *Post-hoc* Tukey HSD analysis: p < 0.05 vs. baseline. § *Post-hoc* Tukey HSD analysis: p < 0.05 vs. control group. * *Post-hoc* Tukey HSD analysis: p < 0.05 vs. 1 hour.

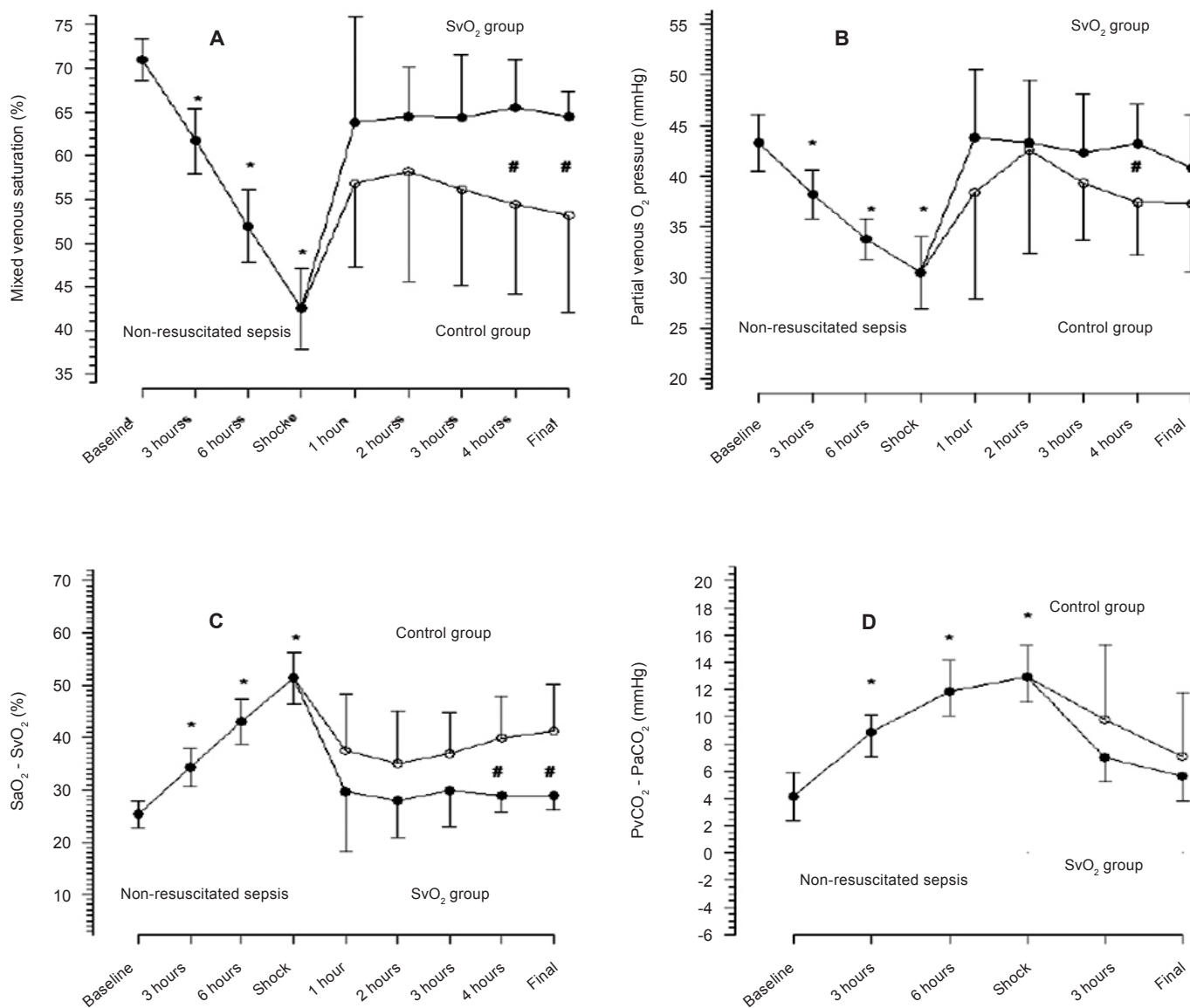


Figure 1 - Progression of the assessed variables during non-resuscitated sepsis and after resuscitation for SvO₂ and control groups. A) SvO₂ progression (one-way RM-ANOVA for non-resuscitated sepsis data $p < 0.001$; two-way ANOVA for SvO₂ and control comparison, intra-group comparison $p = 0.973$, inter-group comparison $p < 0.001$ and factor-time interaction $p = 0.951$). B) Partial oxygen venous pressure (one-way RM-ANOVA for non-resuscitated sepsis data $p < 0.001$; two-way ANOVA for SvO₂ and control comparison, intra-group $p = 0.726$, inter-group $p = 0.048$ and factor-time interaction $p = 0.914$). C) The difference between arterial and mixed venous oxygen saturation (one-way RM-ANOVA for non-resuscitated sepsis data $p < 0.001$ and factor-time interaction non-resuscitated $p = 0.907$) and D) The venoarterial CO₂ gradient (one-way RM-ANOVA for sepsis $p = 0.452$; inter-group $p = 0.465$ and factor-time interaction $p = 0.716$). *Post-hoc analysis, Tukey HSD $p < 0.05$ vs. baseline. # Post-hoc analysis, Tukey HSD $p < 0.05$ between groups.

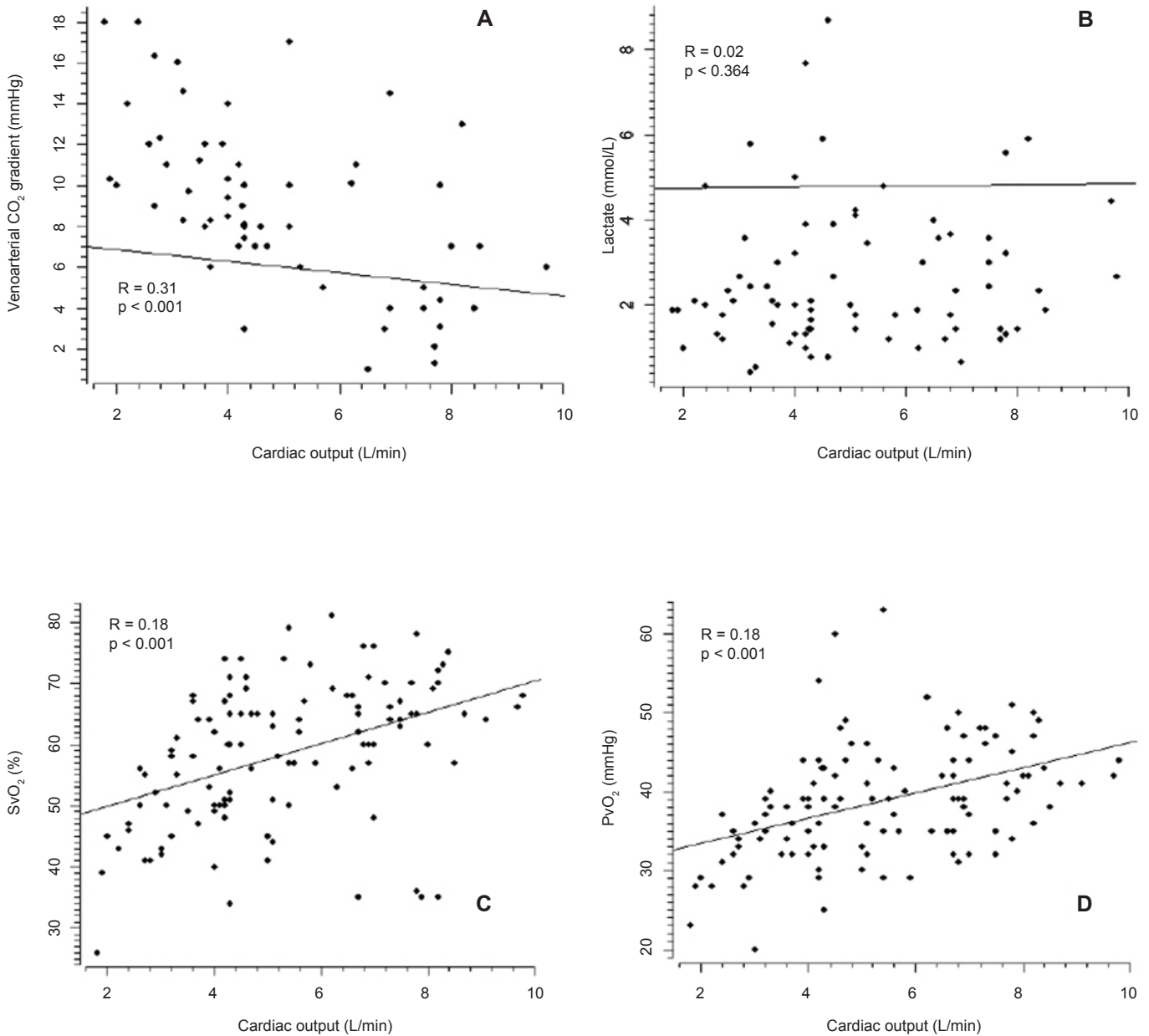


Figure 2 – Correlation between perfusion parameters and cardiac output. A) Correlation between venoarterial CO₂ gradient and cardiac output. B) Correlation between arterial lactate and cardiac output. C) Correlation between SvO₂ and cardiac output. D) Correlation between PvO₂ and cardiac output.

DISCUSSION

The time course of hemodynamic and perfusion parameters during non-resuscitated sepsis has been relatively poorly documented in the literature. In this study, we have shown that a non-resuscitated sepsis hemodynamic profile is primarily characterized by hypovolemia, possibly associated with myocardial

dysfunction. This profile has a significant impact on the perfusion parameters that are commonly measured in the ICU. Fluid, antibiotics and vasoactive drug therapy significantly improve these parameters.

In this study, the hemodynamic and inflammatory changes induced by sepsis were significant. Early non-resuscitated sepsis profiles in humans have been characterized by hypovolemia secondary to reduced

oral ingestion and increased insensitive losses due to vomiting, diarrhea or diaphoresis. In these patients, venodilation and leakage of fluid into the interstitial space due to increased endothelial capillary permeability may result in reduced cardiac preload, reduced cardiac output and inappropriate systemic oxygen supply. After fluid replacement, cardiac output is increased and systemic vascular resistance is reduced, characterizing the hyperdynamic pattern commonly associated with resuscitated septic shock.^(6,7) Clinical findings on hypovolemia were reproduced in this study, as shown by significant hypotension, tachycardia and cardiac performance indicators after sepsis (Figure 1 and Table 1). After resuscitation, the hemodynamic and perfusion data were similar to those commonly described in the hyperdynamic state profile.

Global tissue hypoxia has been described as one of the main components of early sepsis hemodynamics. It develops when systemic oxygen supply is insufficient to meet tissue requirements. Therefore, low SvO₂ (< 65%) or ScvO₂ (< 70%) and high lactate concentration suggests global tissue hypoxia, as a higher supplied-oxygen fraction is being extracted by tissues, resulting in less venous oxygen measurements. This feature, reproduced in this study, is considered to be an early phase of the disease, or hyperdynamic sepsis.⁽⁸⁻¹¹⁾ Similarly, the correlation between high SvO₂ and cardiac output has previously been established.⁽¹²⁾ As anticipated, in our study, goal-directed resuscitation was associated with better SvO₂ values but required more volume replacement and use of inotropes.

The venoarterial CO₂ gradient has been described as a potential hemodynamic parameter for sepsis resuscitation. Although commonly mentioned as a tissue hypoxia marker, recent studies have suggested that it is more closely associated with flow changes, such as cardiac output.⁽¹³⁻¹⁷⁾ This gradient increase in the context of low cardiac output can be explained using the CO₂ stagnation principle. This principle states that, due to the delayed transit time, any CO₂ increase beyond the normal in efferent vessels may lead to venous blood hypercapnia.⁽¹⁴⁾ In our study, a CO₂ gradient increase was clearly shown during non-resuscitated sepsis, as was its drop following therapy. This can be explained by the low cardiac output and the signs of hypovolemia that developed in the animals during the untreated phase. For this variable, no significant differences were seen by the type of fluid resuscitation.

This study has some strengths that should be emphasized. First, our model shows several similarities with human sepsis, including the hemodynamic profile before therapy, the administration of antibiotics, the use of perfusion parameters-guided vasoactive drugs (which is unusual in other sepsis experimental trials) and a hemodynamic therapy algorithm similar to that used in clinical practice. However, this study has limitations. Type II analysis errors may have occurred due to the relatively small sample and disease heterogeneity. These factors may have influenced the results.

CONCLUSION

This study shows that non-resuscitated sepsis has a hemodynamic profile suggesting hypovolemia, with low cardiac output, low CVP, reduced SvO₂ and PvO₂ and increased CO₂ venoarterial difference. The treatment of sepsis with antibiotics, fluids, inotropes and vasopressors significantly improves these parameters. Early goal-directed fluid resuscitation is associated with improved hemodynamic parameters when compared to a group not resuscitated using this method.

Acknowledgements: This study was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Instituto Sírio-Libanês de Ensino e Pesquisa.

RESUMO

Objetivos: Apesar da ressuscitação volêmica guiada por saturação venosa central de oxigênio (SvO₂) ser considerada atualmente padrão ouro no tratamento da sepse, poucos estudos caracterizaram o perfil evolutivo de variáveis hemodinâmicas e perfusionais durante esta abordagem terapêutica. Este estudo teve por objetivo descrever evolutivamente estes parâmetros durante o choque séptico experimental sem ressuscitação e após 12 horas de ressuscitação guiada por metas.

Métodos: Treze porcos (35-45 kg) anestesiados foram submetidos a peritonite por inoculação fecal (0,75g/kg). Após desenvolverem hipotensão persistente, ambos os grupos receberam antibióticos e foram randomizados em dois grupos: controle (n=7), com suporte hemodinâmico otimizado para pressão venosa central entre 8-12mmHg, diurese acima de 0,5ml/kg/h e pressão arterial média maior que 65mmHg; e SvO₂ (n=6), com os objetivos acima e SvO₂ acima de 65%. As intervenções incluíram ringer lactato e noradrenalina nos 2 grupos e dobutamina no grupo SvO₂. Os animais foram tratados durante doze horas ou óbito.

Resultados: A sepse não tratada associou-se a uma significativa redução da SvO₂, PvO₂, débito cardíaco e pressão venosa central e aumento da diferença arterio-venosa da saturação de oxigênio e veno-arterial de CO₂. Após ressuscitação, esses parâmetros foram corrigidos em ambos os grupos. A ressuscitação guiada por metas associou-se a um melhor perfil hemodinâmico caracterizado por maiores SvO₂, débito cardíaco e pressão venosa central.

Conclusões: A sepse não ressuscitada apresenta um perfil

hemodinâmico sugestivo de hipovolemia, com piora perfusional e hemodinâmica revertida após ressuscitação volêmica. A ressuscitação guiada por metas associa-se a uma significativa melhora dos parâmetros hemodinâmicos e perfusionais.

Descritores: Choque séptico; Oxigênio/sangue; Oximetria/métodos; Lactatos/sangue; Ressuscitação/métodos; Hemodinâmica/fisiologia; Porcos

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