Microcirculatory dysfunction and the venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) in the septic shock: A translational medicine model



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Chapter 1

OUTLINE OF THIS THESIS

Shock is a life-threatening condition in which the circulatory system is unable to deliver sufficient oxygen to maintain the metabolic demands of tissues, resulting in cellular dysfunction. Unfortunately, the monitoring of systemic oxygen-derived parameters and macro hemodynamic variables are not sufficient to ensure an adequate tissue perfusion and cellular respiration. Many studies have emphasized the importance of convective and diffusive microcirculatory blood flow derangements in the genesis of multiorgan dysfunction, whereby direct exploration of microcirculation could identify such "in vivo" alterations and guide therapeutic interventions. Nevertheless, despite improvements in techniques evaluating microcirculation at the bedside, an immediate and reliable quantification of microcirculatory alterations is still limited. Thus, surrogates reflecting the adequacy of microvascular blood flow might be highly valuable.

According to previous studies demonstrating the link between systemic venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) and cardiac output during experimental conditions of hemorrhagic, hypovolemic and obstructive shock, we first explored the clinical value of $Pv\text{-}aCO_2$ changes during the initial resuscitation of septic shock, appealing for its capability to track macro blood flow variations. In this initial study, persistently increased $Pv\text{-}aCO_2$ values were associated with more severe multiorgan dysfunction and unfavorable clinical outcomes even in patients attaining normal oxygen-derived parameters. Importantly, variations in CO_2 were faster than lactate kinetics, which makes $Pv\text{-}aCO_2$ an attractive variable to monitor, especially during the early stages of resuscitation. Our observations demonstrated, however, a lack of agreement between $Pv\text{-}aCO_2$ and cardiac output in septic shock, contrary to that observed during non-inflammatory conditions. We subsequently explored the link between $Pv\text{-}aCO_2$ variations and blood flow alterations at the microcirculatory level, keeping the hypothesis that venous CO_2 accumulation is a phenomenon explained by blood flow stagnation rather than by tissue disoxia. Accordingly, we evaluated the relationships between $Pv\text{-}aCO_2$ changes and the microcirculatory blood flow during early phases of resuscitation in humans with septic shock. For this, we used current

arterial and mixed-venous gases analysis to calculate Pv-CO_2 and a hand-held video-microscopy system to visualize in-vivo microcirculation at the bedside. We found a good correlation between Pv-aCO_2 values and microcirculatory alterations. Indeed, changes in Pv-aCO_2 were found to reflect the variations in microvascular blood flow during the early stages of resuscitation, independently of macro hemodynamic changes.

In order to understand the pathophysiological mechanisms of Pv-aCO_2 increasing, we experimentally recreated the alterations of microcirculatory blood flow distribution, regional oxygen extraction ratio, venous-to-arterial and tissue-to-arterial CO_2 differences, macrohemodynamics and lactate dynamics during septic shock. Thus, we designed an experimental swine model of severe hypodynamic septic shock induced by peritonitis, aiming to evaluate the importance of the heterogeneity of microvascular blood flow distribution on the regional splanchnic oxygen extraction ratio during early resuscitation with fluids and the random use of low fixed-doses of dobutamine. Simultaneously, we evaluated the ability of systemic (mixed-venous) carbon dioxide (CO_2) accumulation to reflect local (intestinal mucosal) and regional (splanchnic) CO_2 accumulation and these in turn, to reflect microcirculatory blood flow alterations at the intestinal mucosa and serosa. Increasing heterogeneity of intestinal microvascular blood flow was directly related to altered oxygen extraction ratio capabilities. Notably, this phenomenon was potentially reversed by using dobutamine and was reflected by variations in tissue and mesenteric-to-arterial CO_2 differences and these, in turn, by the systemic venous-to-arterial CO_2 differences.

We also proposed a pathophysiological challenge in which the venous-arterial carbon dioxide to arterial-venous oxygen contents differences ratio (Cv-aCO_2/Ca-vO_2) could be used as a surrogate of respiratory quotient (VCO_2/VO_2) in order to detect non-aerobic metabolism during the early stages of resuscitation from septic shock. According to the Fick principle, both the venous-to-arterial CO_2 and the arterial-to-venous O_2 content differences are influenced by macro and micro blood flow, which

suggest that increases in Cv-aCO_2/Ca-vO_2 ratio are, to some extent, independent of flow alterations. Accordingly, we evaluate, in a subsequent study, the additive value of the Cv-aCO_2/Ca-vO_2 ratio to lactate levels during the early stages of resuscitation, hypothesizing that increased Cv-aCO_2/Ca-vO_2 ratios should reflect ongoing anaerobic metabolism.

Finally, we propose a theoretical model advocating that an increased Cv-aCO_2/Ca-vO_2 ratio reflects the relative increase of VCO_2 over VO_2 secondary to the buffering of hydrogen ions due to anaerobic metabolism, whilst the isolated Pv-aCO_2 (or Cv-aCO_2) reflects the blood flow conditions at both the macro and microvascular levels.

Outline of this thesis

This thesis addresses the issues concerning the heterogeneity of microvascular blood flow and functional capillary density as mechanisms that lead to deranged diffusive and convective oxygen transport during septic shock and the ability of regional and systemic Pv-aCO_2 differences to detect such alterations. We also propose potential mechanisms for, and the clinical use of the Cv-aCO_2/Ca-vO_2 ratio during early phases of resuscitation from septic shock.

Chapter 2: General Background: Fundamentals of microcirculatory blood flow monitoring to guide resuscitation in septic shock

Hemodynamic monitoring during shock states mainly focuses on detection of pressure-derived hemodynamic variables related to systemic circulation and oxygen-derived parameters. This chapter briefly describes the limitations of such type of monitoring and emphasizes the importance of microcirculatory blood flow during resuscitation from septic shock. Despite demonstrating the theoretical benefits of

monitoring microcirculation and the development of new devices to explore it, bedside evaluation of microvascular blood flow is, however, still limited. Thus, we propose that practical surrogates of microcirculatory blood flow alterations are highly valuable and deserve more intense research efforts.

Chapter 3: The clinical problem and the potential use of the venous-to-arterial carbon dioxide difference (Pv-aCO_2)

Since hemodynamic and oxygen systemic parameters are not sufficient to ensure an adequate tissue perfusion during septic shock, we explored the clinical value of the venous-to-arterial carbon dioxide difference (Pv-aCO_2) variations during the very early phases of resuscitation from septic shock. Multiorgan dysfunction development and clinical outcomes at day-28 were studied in a group of patients with septic shock who were subjected to a quantitative protocol-guided resuscitation. The clinical significance of Pv-aCO_2 variations in patients attaining normal oxygen-derived goals and the relationships between Pv-aCO_2 and cardiac output variations were also studied. Calculations of venous-to-arterial CO_2 differences were also performed using central-venous and mixed-venous blood samples in order to determine their interchangeability. Finally, Pv-aCO_2 changes were compared with arterial lactate variations in order to test the kinetics of these two variables during the early stages of resuscitation.

Chapter 4. The pathophysiological challenge: searching for markers of anaerobic metabolism

Monitoring tissue perfusion and cellular respiration are authentic challenges in critical care. During steady state conditions, carbon dioxide generation should not exceed oxygen consumption. Thus, a disproportionate increase in CO_2 would suggest the presence of a non-aerobic CO_2 source. We explore the combination of arterial

lactate levels (the traditional marker of anaerobic metabolism) with the venous-arterial CO_2 to arterial-venous O_2 content differences ratio ($Cv\text{-}aCO_2/Ca\text{-}vO_2$) as a surrogate of the respiratory quotient (i.e., VCO_2/VO_2 ratio), in a population of patients with septic shock who had been subjected to a protocolled quantitative early goal-directed resuscitation. Multiorgan failure and clinical outcomes at day-28 were explored in four groups defined by the combination of normal and abnormal arterial lactate values with normal or theoretically elevated $Cv\text{-}aCO_2/Ca\text{-}vO_2$ ratio. The additional prognostic value of the $Cv\text{-}aCO_2/Ca\text{-}vO_2$ ratio was also calculated and a discussion of using content CO_2 differences but not CO_2 pressures was also presented.

Chapter 5. Increased $Pv\text{-}aCO_2$ and microcirculatory dysfunction: the human septic shock model

In a population of patients with septic shock subjected to early-directed goal resuscitation therapy, we evaluated the development of macro hemodynamics, oxygen-derived parameters, $Pv\text{-}aCO_2$, and $Cv\text{-}aCO_2/Ca\text{-}vO_2$ ratio during the first hours of resuscitation. Simultaneously, we explored the microcirculatory blood flow of septic shock patients at the bedside using hand-held video microscopy. Thus, the relationships among microcirculatory alterations, $Pv\text{-}aCO_2$, oxygen-derived parameters, macro hemodynamics, and $Cv\text{-}aCO_2/Ca\text{-}vO_2$ ratio at static points of resuscitation were evaluated, and the variations between initial measurements and six-hours after resuscitation interventions were considered. The relationships between $Pv\text{-}aCO_2$ and the proportion of perfused small vessels and the functional capillary density and heterogeneity of microvascular blood flow were evaluated. Furthermore, the apparent independence between microcirculatory variables and $Cv\text{-}aCO_2/Ca\text{-}vO_2$ ratio was described.

Chapter 6. The key role of heterogeneity of microvascular blood flow on the tissue oxygen extraction and the link with PCO_2 gaps: the animal experimental model

In order to study the mechanistic relationship between the heterogeneity of microvascular blood flow and the tissue oxygen extraction ratio at the intestinal level, an experimental swine model of septic shock induced by fecal peritonitis was developed. In this experiment, we evaluated the variations of heterogeneity of microvascular blood flow at the intestinal mucosa and serosa and their relationship with the oxygen extraction ratio during random use of dobutamine at low-fixed dose or placebo. Alternatively, the ability of systemic Pv-aCO_2 to track gut-mucosal and regional-splanchnic CO_2 accumulation was evaluated and these were used, in turn, to track the proportion of perfused small vessels, the functional capillary density and the heterogeneity of microcirculatory blood flow at both the sublingual and intestinal mucosa. Accordingly, all models were subjected to goal-directed resuscitation with fluids and were randomly assigned to low fixed-doses of dobutamine or placebo. Throughout the experiment, all microcirculatory variables were evaluated along with regional and systemic oxygen consumption ratios, in order to establish their dynamic relationships during resuscitation maneuvers. Additionally, the variations of the Cv-aCO_2/Ca-vO_2 ratio and microcirculatory variables were also evaluated during the resuscitation interventions.

Chapter 7. The theoretical model: understanding the pathophysiological issues

According to data obtained from our human and animal experimental models, a theoretical model about the mechanisms and clinical significance of Cv-aCO_2/Ca-vO_2 ratio was constructed.

Chapter 8. General discussion

This chapter presents an integrative discussion about the step-by-step construction of this thesis. In total, four original papers, an editorial expert comment and a pathophysiological theoretical model are discussed and harmonized.

Chapter 9. General Conclusions

Global conclusions from the data accumulated from human and animal experimental septic shock models are presented.

Chapter 10. Clinical implications and future considerations

This chapter presents some points about possible future research on the mechanisms involved in anaerobic CO_2 generation, in vivo cellular redox potential determination and the practical use, in clinical settings, of these markers of tissue perfusion.

Chapter 2

GENERAL INTRODUCTION:

FUNDAMENTALS OF MICROCIRCULATORY BLOOD FLOW MONITORING TO GUIDE RESUSCITATION IN SEPTIC SHOCK

PAPER No. 1 (COMMENTARY)

SHOULD MICROCIRCULATION MONITORING BE USED TO GUIDE FLUID RESUSCITATION IN SEVERE SEPSIS AND SEPTIC SHOCK?

Rev Bras Ter Intensiva. 2015;27(2):92-95

2.1 Should microcirculation monitoring be used to guide fluid resuscitation in severe sepsis and septic shock?

Hypoperfusion due to macro and microcirculatory blood flow alterations represents a key factor determining development of multi-organ failure (MOF) and clinical outcomes in septic shock [1,2]. However, how to identify organ perfusion abnormalities at the bedside and select the appropriate interventions to improve tissue hypoxia remain highly controversial. Traditionally, clinical signs, such as reduced blood pressure and urinary output, altered consciousness, and mottled skin, have been used to identify tissue perfusion abnormalities. Consequently, current hemodynamic monitoring during shock states mainly focuses on detection of pressure-derived hemodynamic variables related to systemic circulation. However, it has been largely recognized that monitoring these macro-hemodynamic variables is not sufficient to rule out persistent abnormalities of tissue oxygenation. Indeed, the usefulness of resuscitation targets, such as global oxygen-derived parameters, has been strongly questioned [3] and recent data have failed to demonstrate beneficial effects of using central venous oxygen saturation as a goal of resuscitation [4-6].

Fluid resuscitation therapy primarily aims to optimize cardiac output on the assumption that increasing macro blood flow can improve the convective transport of oxygen to the tissues maintaining cellular respiration and supporting organ function[7,8]. Thus, fluid therapy targeting central venous pressure has been widely recommended to achieve adequate cardiac performance [9]. However, high positive fluid balances have also been associated with unfavorable clinical outcomes [10]. In this sense, dynamic approaches to assessing volume responsiveness to fluid administration seem to be superior to static variables [11,12]. Unfortunately, macro-hemodynamic optimization guided by either dynamic or static variables does not guarantee adequate tissue perfusion or adequate cellular respiration making

necessary more sensible markers of tissue perfusion and cellular respiration.

2.1.1 Importance of microcirculation during resuscitation of septic shock

Oxygen transport to tissues is governed by convective and diffusive components. The convective component is determined by the microcirculatory blood flow itself, i.e., the number of red blood cells (RBCs) entering the microcirculation, and by the oxygen content. In normal conditions, inflow and outflow pressures control the driving pressure at the microvascular level. Thus, the convective transport is regulated upstream at the arteriolar level through microcirculatory inflow changes with subsequent micro-hematocrit modifications and is limited downstream by venous pressure. Meanwhile, the diffusive component of oxygen transport is determined by the gradient between the capillary and mitochondrial oxygen partial pressures, the diffusional distance and the area available for gas exchange, according to Fick´s law.

Unfortunately, current resuscitation procedures are based on the assumption that defects in oxygen transport arise from a lack of perfusion. Thus, resuscitation efforts are mostly focused on the promotion of convective flow based on the assumption that hypovolemia is the main limiting factor of blood flow. However, a substantial contribution of oxygen tissue transport is determined by the capacity of diffusion of oxygen from RBCs to the cells, and this can be quantified by functional capillary density (FCD), i.e., the density of capillaries with owing RBCs carrying oxygen. In normal conditions, the microvascular blood flow is carefully matched to the metabolic demands of tissues. However, septic shock is characterized by decreased FCD in addition to increased heterogeneity of blood flow, with zones of well-perfused vessels in close proximity to non-perfused capillaries [13]. The persistence of such alterations has been related to multiorgan dysfunction, even when global hemodynamics appear to be optimal [14]. Indeed, increases in cardiac output may be insufficient to correct tissue hypoxemia because microcirculation alterations might persist. Thus, global hemodynamic targets should be integrated with functional microcirculatory goals, as this would optimize both convective and diffusive components in order to maximize

oxygen delivery to cells. Microcirculatory responsiveness should be defined as an increase in convective microvascular flow in response to fluid load in addition to a reduction of the heterogeneity of flow, resulting in a more balanced distribution of oxygen to the tissues (Figure 1). Nevertheless, efforts to increase blood flow by fluid administration could be counterbalanced by a decrease in FCD resulting in a limited oxygen diffusive capacity (Figure 2). Thus, excessive fluid administration could increase the distance between capillaries, reducing oxygen diffusion to the tissues and finally to the mitochondria.

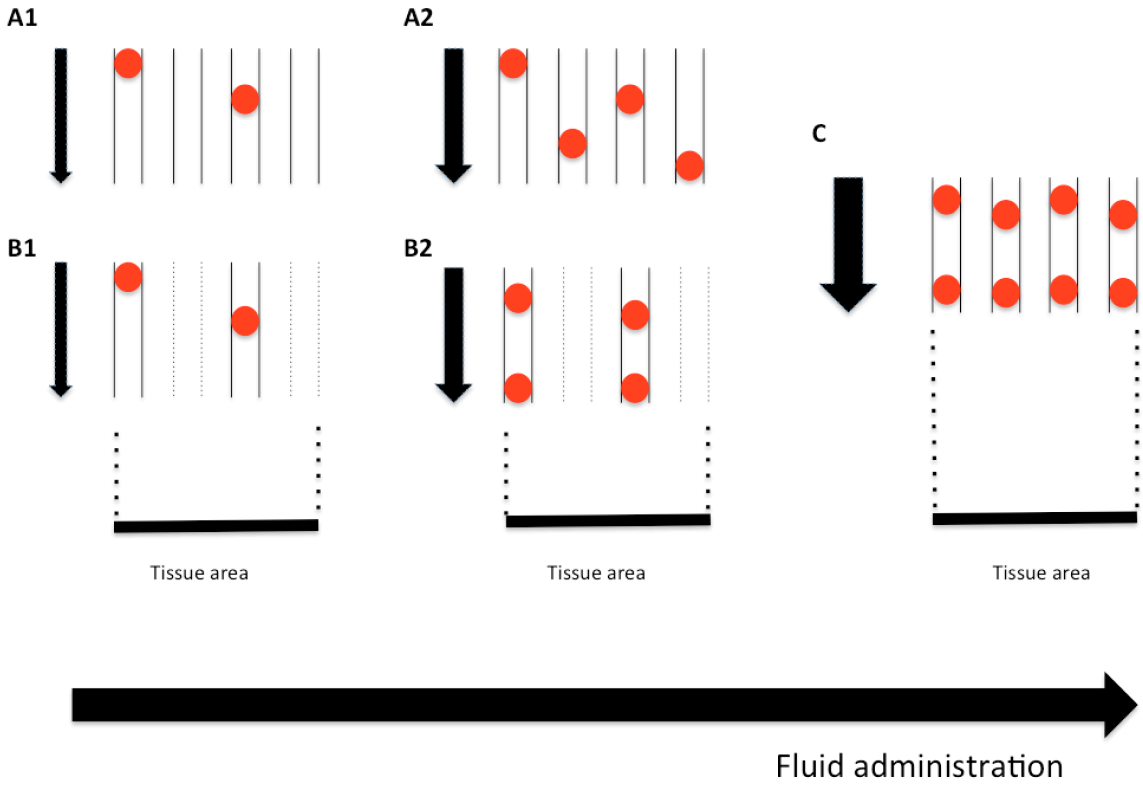
2.1.2 Bedside techniques to evaluate "In vivo" microcirculatory blood flow

Orthogonal polarization spectral and the side-stream dark Field imaging techniques have helped us gain a better understanding of microcirculatory derangements during severe sepsis and septic shock at the bedside. In fact, deeper microvascular alterations, such as a reduced percentage of small perfused vessels (PPV), decreased FCD and increased heterogeneity of flow, have been shown to be related to more severe organ dysfunctions and unfavorable outcomes [13,15,16]. Interestingly, reductions in FCD in sepsis are completely explained by decreased PPV, while RBC velocities are similar in both survivors and non-survivors [16]. These findings suggest that variables that describe the diffusional component of oxygen transport, i.e., FCD and heterogeneity of microvascular blood flow, are more closely related to clinical outcomes than pure convective components, such as RBC velocity [16]. Remarkably, these derangements are amenable to correction over time and are dissociated from global hemodynamics. Consequently, micro-hemodynamics cannot be predicted by the typical systemic hemodynamic parameters, although more severe microcirculatory alterations coexist with high lactate levels and the requirement of higher doses of vasopressors [17].

Theoretically, direct evaluation of microcirculation at the bedside would be a more physiological-based approach to fluid administration and therapeutic interventions.

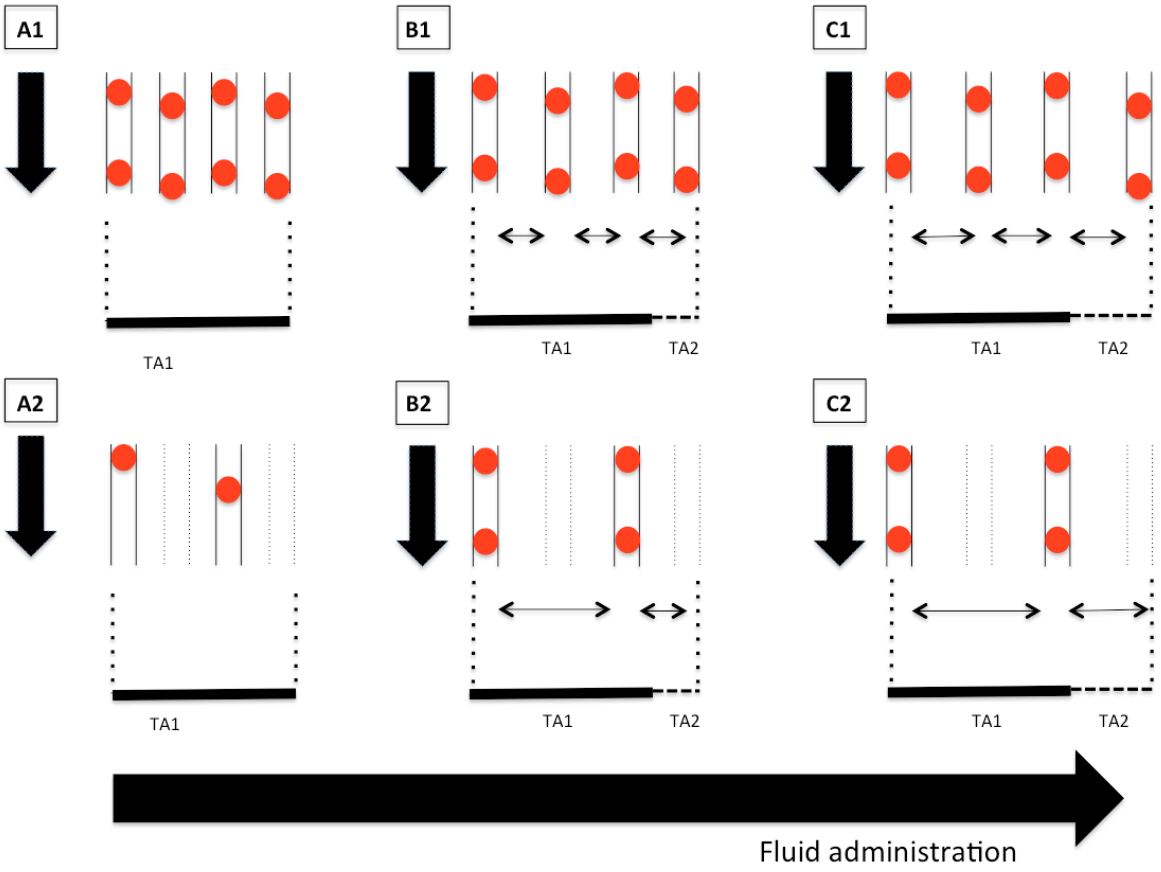
However, portable devices to evaluate microcirculation are not widely used in the clinical setting and remain restricted to research purposes.

Figure 1. Effects of adequate fluid administration on microvascular blood flow



Progressive increasing of convective flow after fluid loading during microcirculatory conditions of pure convective flow derangements (A1, A2) and combined with increased heterogeneity of blood flow (B1, B2). An optimal fluid administration corrects convective and heterogeneity blood flow disturbances (C). Note that the number of vessels with adequate flow per tissue area represents the functional capillary density (FCD), i.e., the major determinant of the diffusive component of oxygen transport to tissues. Black arrows pointing down represent the magnitude of the blood flow.

Figure 2. Effects of fluid overload on microvascular blood flow.



Inadequate fluid overload conducts to progressive decrease in functional capillary density in cases of homogeneous (A1, B1) or heterogeneous (A2, B2) microcirculatory blood flow. Progressive increase in distance between capillaries impairs oxygen diffusive capacities to tissues despite apparently normal convective flow (A1, B1) or in cases of apparent corrected convective component with persistence of increased heterogeneity (B2, C2). TA1 depicts the original tissue area. TA2 depicts the increased tissue area due to edema. Black arrows pointing down represent the magnitude of blood flow.

2.2 Searching for surrogates of microcirculatory blood flow distribution: the role of the venous-to-arterial carbon dioxide difference

The coexistence of venous acidemia and increased venous carbon dioxide during cardiac arrest was described in both animals [18] and critically ill humans [19]. Subsequently, increases in the venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) were described during hypovolemic, cardiogenic, obstructive, and septic shock [10-12]. In fact, it was described an inverse curvilinear relationship between $Pv\text{-}aCO_2$ and cardiac output in these situations, highlighting the decisive link between blood flow on venous CO_2 accumulation [20,21]. Thus, the $Pv\text{-}aCO_2$ aroused clinical interest as a marker of global perfusion during shock states. However, some models evaluating the mechanisms implied on venous CO_2 accumulation during non-inflammatory conditions [22-24] questioned the ability of $Pv\text{-}aCO_2$ to identify tissue dysoxia because venous CO_2 only rises during ischemic hypoxia, but not during hypoxic or anemic hypoxia for comparable declines in oxygen delivery (DO_2) and consumption (VO_2). Nonetheless, recent data suggest that an increased $Pv\text{-}aCO_2$ could identify patients inadequately resuscitated despite attaining oxygen metabolism targets, reinforcing the idea that $Pv\text{-}aCO_2$ can reflect global perfusion [25] and even anaerobic CO_2 generation [26].

Mechanisms implicated in the elevation of $Pv\text{-}aCO_2$ during inflammatory states are not completely understood and the interpretation of $Pv\text{-}aCO_2$ variations during resuscitation of septic shock is frequently difficult. However, $Pv\text{-}aCO_2$ resembles linked to blood flow variations and it could be used to track the adequacy of blood flow during resuscitation of shock. Alternatively, the combination of carbon dioxide and oxygen parameters could be used to detect ongoing anaerobic metabolism during states of shock in which isolated oxygen parameters seem apparently corrected.

Thus, we proposed to study the prognostic value and clinical relevance of the $Pv\text{-}aCO_2$ during the early phases of resuscitation of septic shock. Subsequently, we aimed to

explain the mechanisms implied in increasing Pv-aCO_2, emphasizing on the influence of microvascular blood flow alterations on venous and tissue CO_2 accumulation. Using translational medicine principles, we explored the microcirculation in humans employing technology previously limited to experimental laboratory purposes. Thus, bedside evaluation of microcirculation was performed in humans with septic shock during resuscitation maneuvers in conjunction with macro hemodynamic and oxygen-derived parameters. We simultaneously built a new experimental laboratory for critical care research at the ICESI University designed to facilitate bidirectional translation of physiological concepts applying the "bench-to-bedside" approach. Thus, we recreated a hypodynamic septic shock model aimed to explore the relationships between microcirculation, macro hemodynamics, carbon dioxide and oxygen-derived parameters during resuscitation with fluids alone or accompanied by low fixed doses of dobutamine. In a parallel study, we evaluated the prognostic clinical value of the Cv-aCO_2/Da-vO_2 ratio as surrogate of respiratory quotient (i.e., VCO_2/VO_2 ratio) hypothesizing that value > 1.0 might reflect anaerobic CO_2 generation. The relationships of Cv-aCO_2/Da-vO_2 ratio with microcirculatory blood flow were also evaluated during experimental conditions at the animal laboratory. Finally, a theoretical model was constructed with data coming from our successive studies, clarifying the significance of Pv-aCO_2 and Cv-aCO_2/Da-vO_2 ratio, possible mechanisms and potential clinical use.

2.3 REFERENCES

1. Vincent JL, De Backer D. Microvascular dysfunction as a cause of organ dysfunction in severe sepsis. *Crit Care.* 2005;9 Suppl 4:S9-12.
2. Cecconi M, De Backer D, Antonelli M, et al. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. *Intensive Care Med.* 2014;40(12):1795-1815.
3. Bellomo R, Reade MC, Warrillow SJ. The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. *Crit Care.* 2008;12(2):130.
4. Yealy DM, Kellum JA, Huang DT, et al. A randomized trial of protocol-based care for early septic shock. *N Engl J Med.* 2014;370(18):1683-1693.
5. Peake SL, Bailey M, Bellomo R, et al. Australasian resuscitation of sepsis evaluation (ARISE): A multi-centre, prospective, inception cohort study. *Resuscitation.* 2009;80(7):811-818.
6. Mouncey PR, Osborn TM, Power GS, et al. Trial of early, goal-directed resuscitation for septic shock. *N Engl J Med.* 2015;372(14):1301-1311.
7. Santry HP, Alam HB. Fluid resuscitation: past, present, and the future. *Shock.* 2010;33(3):229-241.
8. Vincent JL, Weil MH. Fluid challenge revisited. *Crit Care Med.* 2006;34(5):1333-1337.
9. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med.* 2013;39(2):165-228.
10. Boyd JH, Forbes J, Nakada TA, Walley KR, Russell JA. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. *Crit Care Med.* 2011;39(2):259-265.
11. Pinsky MR, Payen D. Functional hemodynamic monitoring. *Crit Care.* 2005;9(6):566-572.
12. Teboul JL, Monnet X. Detecting volume responsiveness and unresponsiveness in intensive care unit patients: two different problems, only one solution. *Crit Care.* 2009;13(4):175.
13. De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent JL. Monitoring the microcirculation in the critically ill patient: current methods and future approaches. *Intensive Care Med.* 2010;36(11):1813-1825.
14. Doerschug KC, Delsing AS, Schmidt GA, Haynes WG. Impairments in microvascular reactivity are related to organ failure in human sepsis. *Am J Physiol Heart Circ Physiol.* 2007;293(2):H1065-1071.
15. Trzeciak S, Dellinger RP, Parrillo JE, et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Ann Emerg Med.* 2007;49(1):88-98, 98.e81-82.

16. Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. *Crit Care Med.* 2012;40(5):1443-1448.
17. Hernandez G, Boerma EC, Dubin A, et al. Severe abnormalities in microvascular perfused vessel density are associated to organ dysfunctions and mortality and can be predicted by hyperlactatemia and norepinephrine requirements in septic shock patients. *J Crit Care.* 2013;28(4):538.e539-514.
18. Grundler W, Weil MH, Rackow EC. Arteriovenous carbon dioxide and pH gradients during cardiac arrest. *Circulation.* 1986;74(5):1071-1074.
19. Weil MH, Rackow EC, Trevino R, Grundler W, Falk JL, Griffel MI. Difference in acid-base state between venous and arterial blood during cardiopulmonary resuscitation. *The New England journal of medicine.* 1986;315(3):153-156.
20. Teboul JL, Mercat A, Lenique F, Berton C, Richard C. Value of the venous-arterial PCO2 gradient to reflect the oxygen supply to demand in humans: effects of dobutamine. *Critical care medicine.* 1998;26(6):1007-1010.
21. Bakker J, Vincent JL, Gris P, Leon M, Coffernils M, Kahn RJ. Veno-arterial carbon dioxide gradient in human septic shock. *Chest.* 1992;101(2):509-515.
22. Vallet B, Teboul JL, Cain S, Curtis S. Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol.* 2000;89(4):1317-1321.
23. Neviere R, Chagnon JL, Teboul JL, Vallet B, Wattel F. Small intestine intramucosal PCO(2) and microvascular blood flow during hypoxic and ischemic hypoxia. *Critical care medicine.* 2002;30(2):379-384.
24. Dubin A, Estenssoro E, Murias G, et al. Intramucosal-arterial Pco2 gradient does not reflect intestinal dysoxia in anemic hypoxia. *The Journal of trauma.* 2004;57(6):1211-1217.
25. Vallee F, Vallet B, Mathe O, et al. Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? *Intensive care medicine.* 2008;34(12):2218-2225.
26. Mekontso-Dessap A, Castelain V, Anguel N, et al. Combination of venoarterial PCO2 difference with arteriovenous O2 content difference to detect anaerobic metabolism in patients. *Intensive care medicine.* 2002;28(3):272-277.

Chapter 3

THE CLINICAL PROBLEM AND THE POTENTIAL USE OF THE VENOUS-TO-ARTERIAL CARBON DIOXIDE DIFFERENCE ($Pv\text{-}aCO_2$)

PAPER No. 2 (Original paper)

PERSISTENTLY HIGH VENOUS-TO-ARTERIAL CARBON DIOXIDE DIFFERENCES DURING EARLY RESUSCITATION ARE ASSOCIATED WITH POOR OUTCOMES IN SEPTIC SHOCK

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ABSTRACT

Background: Venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) may reflect the adequacy of blood flow during shock states. We sought to test whether the development of $Pv\text{-}aCO_2$ during the very early phases of resuscitation is related to multi-organ dysfunction and outcomes in a population of septic shock patients resuscitated targeting the usual oxygen-derived and hemodynamic parameters.

Methods: Prospective observational study in a 60-bed mixed ICU in a University affiliated Hospital. Eighty-five patients with a new septic shock episode were included. A $Pv\text{-}aCO_2$ value ≥ 6 mmHg was considered to be high. Patients were classified in four predefined groups according to the $Pv\text{-}aCO_2$ evolution during the first 6 hours of resuscitation: (1) persistently high $Pv\text{-}aCO2$ (high at T0 and T6); (2) increasing $Pv\text{-}aCO_2$ (normal at T0, high at T6); (3) decreasing $Pv\text{-}aCO_2$ (high at T0, normal at T6); and (4) persistently normal $Pv\text{-}aCO_2$ (normal at T0 and T6). Multiorgan dysfunction at day-3 was compared for predefined groups and a Kaplan Meier curve was constructed to show the survival probabilities at day-28 using a log-rank test to evaluate differences between groups. A Spearman-Rho was used to test the agreement between cardiac output and $Pv\text{-}aCO_2$. Finally, we calculated the mortality risk ratios at day-28 among patients attaining normal oxygen parameters but with a concomitantly increased $Pv\text{-}aCO_2$.

Results: Patients with persistently high and increasing $Pv\text{-}aCO_2$ at T6 had significant higher SOFA scores at day-3 ($p<0.001$) and higher mortality rates at day-28 (log rank test: 19.21, $p<0.001$) compared with patients who evolved with normal $Pv\text{-}aCO_2$ at T6. A poor agreement between cardiac output and $Pv\text{-}aCO_2$ was observed ($r^2=0.025$, $p<0.01$) at different points of resuscitation. Patients who reached a $ScvO_2$ ≥ 70% or SvO_2 ≥ 65% but with concomitantly high $Pv\text{-}aCO_2$ at different developmental points

(i.e., T0, T6 and T12) had a significant mortality risk ratio at day-28.

Conclusion: The persistence of high Pv-aCO_2 during the early resuscitation of septic shock was associated with more severe multi-organ dysfunction and worse outcomes at day-28. Although mechanisms conducting to increase Pv-aCO2 during septic shock are insufficiently understood, Pv-aCO_2 could identify a high risk of death in apparently resuscitated patients.

Keywords: venous-to-arterial carbon dioxide difference, multiorgan dysfunction, cardiac output, septic shock.

RESUMEN

Antecedentes: La diferencia veno-arterial de dióxido de Carbono ($Pv\text{-}aCO_2$) puede reflejar qué tan adecuado es el flujo sanguíneo durante los estados de shock. Nosotros evaluamos si la evolución de la $Pv\text{-}aCO_2$ durante las fases iniciales de la reanimación está relacionada con la aparición de disfunción multiorgánica y desenlaces clínicos en un población de pacientes con shock séptico sometidos a una reanimación guiada por la metas usuales de oxígeno y parámetros hemodinámicos.

Métodos: Estudio prospectivo observacional en una UCI mixta de 60 camas en un hospital con afiliación universitaria. Ochenta y cinco pacientes con un nuevo episodio de shock séptico fueron incluidos. Un valor de $Pv\text{-}aCO_2 \geq 6$ mmHg fue considerado como elevado. <los pacientes fueron clasificados en cuatro grupos predefinidos de acuerdo a la evolución de la $Pv\text{-}aCO_2$ durante las primeras 6 horas de reanimación, así: (1) $Pv\text{-}aCO_2$ persistentemente elevada (elevada al T0 y T6); (2) $Pv\text{-}aCO_2$ en incremento (normal al T0 y elevada al T6); (3) $Pv\text{-}aCO_2$ en descenso (elevada al T0, normal al T6); y (4) $Pv\text{-}aCO_2$ persistentemente normal (normal al T0 yT6). El grado de disfunción multiorgánica al día 3 fue comprada entre los grupos predefinidos y además, se construyeron gráficas de Kaplan Meier para mostrar las probabilidades de sobrevida al día 28, usando un log-rank test para evaluar las diferencias entre grupos. Un test de Spearman-Rho fue utilizado para evaluar la concordancia entre el gasto cardiaco y la $Pv\text{-}aCO_2$. Finalmente, nosotros calculamos las razones de riesgo de mortalidad al día 28 en los pacientes que alcanzaron las metas de oxígeno y concomitantemente permanecieron con elevación de la $Pv\text{-}aCO_2$.

Resultados: Los pacientes con una elevación persistente o con incremento de la $Pv\text{-}aCO_2$ al T6 tuvieron más altos puntajes de SOFA al día 3 ($p<0.001$) y mayor mortalidad al día 28 (log rank test: 19.21, $p<0.001$) que aquellos que evolucionaron con una $Pv\text{-}aCO_2$ normal en el T6. Una pobre concordancia entre gasto cardiaco y Pv-

aCO_2 (r^2=0.025, p<0.01) fue observada a diferentes puntos de la reanimación. Los pacientes que alcanzaron una $ScvO_2$ ≥ 70% o SvO_2 ≥ 65% pero con una concomitante Pv-aCO_2 elevada en diferentes tiempos de evolución (es decir, T0, T6 y T12) tuvieron una significativa razón de riesgo para mortalidad al día 28.

Conclusión: Una Pv-aCO_2 persistentemente elevada durante las etapas tempranas de reanimación estuvo asociada con una más severa disfunción multiorgánica y más desfavorables desenlaces clínicos al día 28. Aunque los mecanismos que conducen a la elevación de la Pv-aCO_2 son insuficientemente entendidos, la Pv-aCO_2 podría identificar un incremento del riesgo de muerte en pacientes con shock séptico aparentemente reanimados.

Palabras clave: diferencia veno-arterial de dióxido de carbono, disfunción multiorgánica, gasto cardiac, shock séptico.

3.2 INTRODUCTION

Inadequate tissue perfusion is a pivotal factor in the pathogenesis and clinical course of multi-organ failure in critically ill [1]. Current techniques for monitoring tissue perfusion have largely focused on systemic blood flow and the balance between O_2 demands and supply [2,3]. An early hemodynamic optimization that targets central venous saturation ($ScvO_2$) and systemic hemodynamic parameters improves outcomes in severe sepsis and septic shock [4] reinforcing the idea that tissue perfusion abnormalities are flow dependent at least during very early stages. However, normalizing systemic hemodynamic parameters does not guarantee adequate tissue perfusion [5-7] and in fact, a substantial number of patients still progress to multi-organ dysfunction and death despite meeting $ScvO_2$ targets [4].

In the past, authors described the coexistence of venous acidemia and increased venous carbon dioxide during cardiac arrest in both animals [8] and humans critically ill [9]. Thereafter, increases in the venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) were reported during hypovolemic, cardiogenic, obstructive, and septic shock [10-12]. Interestingly, an inverse curvilinear relationship between $Pv\text{-}aCO_2$ and cardiac output was described, highlighting the importance of blood flow on venous CO_2 accumulation [13,14]. Thus, the $Pv\text{-}aCO_2$ aroused clinical interest as a marker of global perfusion during shock states although some studies questioned its prognostic value [14]. In fact, some in vivo models evaluating the mechanisms conducting to venous CO_2 accumulation during non-inflammatory conditions [15-17] challenged the ability of $Pv\text{-}aCO_2$ to identify tissue dysoxia because it only rises during ischemic hypoxia, but not during hypoxic or anemic hypoxia for comparable declines in oxygen delivery (DO_2) and consumption (VO_2). However, more recent data suggest that high $Pv\text{-}aCO_2$ could identify septic patients who remain inadequately resuscitated despite achieving oxygen metabolism

targets reinforcing the notion of Pv-aCO_2 as a marker of global perfusion due to its ability to track blood flow alterations [18] or even detect anaerobic CO_2 generation [19]. Furthermore, patterns of recovery or derangement of Pv-aCO_2 during very early stages of resuscitation of septic shock have not been widely described and recent studies trying to demonstrate the reliability of Pv-aCO_2 as a tool in resuscitation of septic patients could have been influenced by selection bias as not all potential patients were elected to catheter insertion and goal-directed therapy [20,21].

Recent publications in critical care demonstrate that oxygen-derived parameters as $ScvO_2$ or SvO_2 are commonly normalized at ICU admission [22] and maneuvers as emergent intubation can quickly improve $ScvO_2$ despite regional and tissue perfusion derangements are ongoing [23]. Because global and regional hypoperfusion have been incriminated in the development of multiple organ failure, investigation on surrogate markers of such phenomenon remains important in critical care.

3.3 OBJECTIVES

3.2.1 General objective

To evaluate the prognostic value of the Pv-aCO_2 during the early stages of resuscitation and its relationships with macro hemodynamic and oxygen-derived parameters in a population of septic shock patients resuscitated targeting the usual oxygen-derived and hemodynamic parameters.

3.2.2 Specific objectives

- To evaluate whether the early development of Pv-aCO_2 during goal-directed resuscitation in septic shock is related to the progress of multiorgan dysfunction and to determinate if Pv-aCO_2 can add prognostic information in patients with septic shock attaining macro hemodynamic and oxygen-derived goals

- To evaluate the relationships between Pv-aCO_2 and the cardiac output as representative of macrovascular blood flow.

3.4 MATERIALS AND METHODS

This prospective observational study was performed in a 60-bed mixed ICU in a university-affiliated hospital. We examined all patients with a new septic shock episode admitted to the emergency room or proceeding from clinical wards during a 24-month period. Septic shock was defined using the criteria of the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference [24]. Patients were excluded if they were younger than 18 years old, pregnant, had severe chronic obstructive pulmonary disease (GOLD 3 and 4 categories according to the current classification at the time of our study) or advanced liver cirrhosis (Child-Pugh C).

3.4.1 General management

All patients admitted to the emergency room or proceeding from clinical wards fulfilling the diagnosis criteria for septic shock were evaluated by the "ICU rapid response team" according to our local procedures. Each patient was equipped with an arterial cannula and a pulmonary artery catheter (CCO Swan-Ganz catheter; Irvine, CA). Our early goal-directed therapy included a bundle of interventions that sought to obtain (a) mean arterial pressure (MAP) ≥ 65 mmHg; (b) urine output ≥ 0.5 ml/kg/min; (c) normalization of serum lactate; and (d) $ScvO_2$ ≥ 70% or SvO_2 ≥ 65%. The use of vasopressors (dopamine or norepinephrine) was standardized to maintain a MAP ≥ 65 mmHg, and repeated fluid challenges with crystalloids or colloids were used to optimize stroke volume as well as allow the lowest dose of vasopressors and pulse-pressure variability lower than 12%. Dobutamine was added for persistent

$ScvO_2$ ≤ 70% or SvO_2 ≤ 65% after fluid resuscitation. A low dose of hydrocortisone was given within 6 hours of resuscitation when use of vasopressors persisted after an adequate fluid restitution. Mechanical ventilation was provided when needed under light sedation (midazolam) and analgesia (fentanyl); tidal volume was limited to 6 – 8 ml/kg. Glycemic control was adjusted to maintain glucose levels < 150 mg/dl. Finally, stress ulcer and venous thrombosis prophylaxis were provided according to international recommendations [25].

3.4.2 Study protocol

The Fundación Valle del Lili´s Ethical and Biomedical research committee approved the current study. A written informed consent was waived as no new therapeutic interventions were performed and all measurements and procedures routinely followed the local protocols for the management of severe sepsis and septic shock.

Time 0 (T0) was declared when the pulmonary artery catheter (PAC) was inserted using common monitoring tracings to place the distal port in the pulmonary artery and the proximal port in the right atrium, approximately 3 cm above the tricuspid valve. In order to standardize the T0, we recorded the total volume of fluids administered and the time elapsed between the start of resuscitation (first hypotension episode) and the PAC insertion (T0).

We collected arterial and (central and mixed) venous blood samples for arterial-venous gases (ABL 300, Radiometer Copenhagen, Denmark) and arterial lactate measurements at Time 0 (T0), 6 hours (T6), 12 hours (T12) and 24 hours (T24) later. We simultaneously registered hemodynamic and respiratory variables at each measurement. We defined Pv-aCO_2 as the difference between the mixed venous CO_2 partial pressure and the arterial CO_2 partial pressure. Previous studies considered Pv-aCO_2 ≥ 6 mmHg as abnormal [14]. Hence, we classified the patients according to the Pva-CO_2 development during the first 6 hours of resuscitation: (1) persistently high Pv-

aCO_2 (high at T0 and T6); (2) increasing Pv-aCO_2 (normal at T0, high at T6); (3) decreasing Pv-aCO_2 (high at T0, normal at T6); and (4) persistently normal Pv-aCO2 (normal at T0 and T6). The SOFA score [26] was used to describe multi-organ dysfunction at day-3 and we also described mortality at day-28 for the pre-defined groups.

3.4.3 Data analysis

After exclusion of a normal distribution of the data by the Kolmogorov–Smirnov test, we used a Kruskal-Wallis test to compare continuous variables (followed by Bonferroni correction for multiple comparisons) and Chi^2 (or Fisher´s exact test, when appropriated) for discrete variables. Survival probabilities at day-28 were described using a Kaplan-Meier curve and differences between groups were calculated using a log-rank test before and after adjusting for SvO_2 at T6. The development of SvO_2, $ScvO_2$, lactate, cardiac output, mean arterial pressure and Pv-aCO_2 during the first 24 hours were analyzed using a repeated-measures analysis of variance. A Spearman-Rho was used to test the agreement between cardiac output and Pv-aCO_2. We also calculated the mortality risk ratios at day-28 in patients who attained a $ScvO_2 \geq 70\%$ or $SvO_2 \geq 65\%$ but maintained persistently high Pv-aCO_2 at different points during resuscitation (T0, T6, and T12). Data were expressed as medians and 25-75% interquartile ranges. A p value ≤ 0,05 (2-tailed) was considered significant.

3.5 RESULTS

During the 24-month period, 108 patients older than 18 years with a new episode of septic shock were screened. Patients with advanced cirrhosis (n=4), severe COPD (n=8) and pregnant women (n=4) were not included for analysis; additionally, a central catheter could not be placed in four patients, and three refused the procedure.

The final sample was 85 patients. The median length of ICU stay for all patients was 6 days (IQ25-75, 3 – 11 days), and the 28-day mortality rate was 37.6%. The median time elapsed from sepsis-induced hypotension to catheter insertion was 3.0 hours (IQ25-75, 1.0 – 4.0 hours) and the median volume of fluids received before catheter insertion was 2,079 ml (IQ25-75, 1,184 – 3,135 ml) for all patients.

Thirty-six patients had a Pv-aCO_2 < 6.0 mmHg at T0 and T6 and seventeen had a high $PvaCO_2$ at T0 but it fell below 6 mmHg at T6 (a total of fifty-three patients had a Pv-aCO_2 < 6 mmHg at T6); on the other hand, twenty-four had a persistently high Pv-aCO_2 during the first 6 hours and the remaining eight evolved from normal at T0 to high $PvaCO_2$ at T6 (thirty-two had a Pv-aCO_2 ≥ 6 mmHg at T6).

We did not find any significant difference with regard to APACHE II, comorbidities, demographics, or respiratory and hemodynamic variables between groups (Tables 1 and 2) neither for the volume of fluids received before inclusion (T0). Dose of vasopressors or inotropic received were similar for the groups both at T0 and T6 (Table 2).

Multi-organ dysfunction at day-3 was significantly higher among patients with persistently high Pv-aCO_2 compared with those with persistently normal or decreasing Pv-aCO_2 during the first 6 hours of resuscitation (Kruskal-Wallis test, $p<0.001$) (Figure 1). Likewise, patients with persistently high Pv-aCO_2 during the first 6 hours of resuscitation had a significant lower survival at day-28 compared with those who normalized Pv-aCO_2 during this period (log rank, Mantel-Cox: 19.21, $p<0.001$; Figure 2). These results were maintained after adjusting for the SvO_2 achieved at T6 (log rank test, $p<0.001$).

Table 1. Patient characteristics.

Variable	Group 1 H - H (n=24)	Group 2 L –H (n=8)	Group 3 H - L (n=17)	Group 4 L - L (n=36)	P
Age (years)	63.0 (54.7-75.0)	63.5 (50.5-76.5)	55.0 (53.0-76.0)	62.0 (49.3-71.8)	0.83
Gender: Male (%)	17 (70.8)	4 (50.0)	10 (58.8)	22 (61.1)	0.72
APACHE II	24.4 (21.2-26.0)	25.2 (21.0-27.0)	23.4 (21.2-25.6)	24.8 (22.2-25.8)	0.11
Time between diagnosis to catheter insertion (T0)	2.0 (2.0 – 4.0)	1.0 (1.0 – 3.3)	3.0 (1.0 – 4.0)	3.0 (2.0 – 4.0)	0.44
Fluids received before catheter insertion	2039 (1343-2834)	1934 (849-4575)	2500 (1430-3628)	2000 (1025-3153)	0.76
Temperature (°C)	37.5 (37.2-37.9)	37.4 (37.1-37.9)	37.6 (37.0-38.0)	37.4 (37.4-37.8)	0.74
Hemoglobin (gr/dl)	9.4 (8.7-11.6)	8.8 (7.4-11.4)	9.8 (9.1-11.4)	9.9 (9.1-11.4)	0.47
Source of infection, n (%)					
Pneumonia					
Abdominal	9	3	4	10	
Urinary	8	4	6	14	
Soft tissue	2	1	3	4	
No specific site	2	0	1	2	
Other	3	0	2	4	
	0	0	1	2	
Culture positive, n (%)	20 (83.3)	5 (62.5)	12 (70.6)	19 (52.8)	0.11
Antibiotics given at T0, n (%)	24 (100)	7 (87.5)	16 (94.1)	32(88.9)	0.38
Antibiotics adequate, n (%)	23 (95.8)	7 (87.5)	16 (94.1)	27 (75.0)	0.10

Hydrocortisone, n (%)	22 (91.7)	8 (100)	15 (88.2)	31 (86.1)	0.68
Transfusion RBC, n (%)	4 (16.7)	3 (37.5)	3 (17.6)	4 (11.1)	0.35
Fluids and vasoactive agents					
Fluids, ml (IQ 25-75)					
T0	2039 (1343 - 2834)	1934 (849-4575)	2500 (1430-3628)	2000 (1025-3153)	0.76
T6	4733 (3196 – 6360)	4082 (1576 – 7584)	4845 (2550 – 6560)	4673 (3380 – 7348)	0.83
Norepinephrine, ugr/kg/min (IQ 25-75), n					
T0	0.25 (0.19-0.36), 24	0.29 (0.14-1.20), 6	0.19 (0.10-0,37), 16	0.23 (0.10-0.38), 30	0.81
T6	0.20 (0.11-0.40), 24	0.36 (0.09-0.49), 6	0.12 (0.11-0.18), 15	0.19 (0.08-0.39), 31	0.45
Dopamin, ugr/kg/min (IQ 25-75), n					
T0	--	7.1 (7.1-7.3), 2	10.7 (10.7-10.7), 1	5.1 (2.8-6.6), 5	0.14
T6	--	--	8.0 (8.0-8.0), 1	5.3 (2.5-6.4), 4	0.16
Dobutamine, ugr/kg/min (IQ 25-75), n					
T0	3.1 (2.7-4.5), 5	---	5.9 (5.1-6.7), 2	3.3 (3.0-4.2), 3	0.15
T6	3.1 (2.7-5.6), 3	---	5.1 (4.7-6.3), 4	3.8 (3.3-4.8), 3	0.21

H-H: Pv-aCO_2 high at T0 and T6; L-H: Pv-aCO_2 normal at T0 and high at T6; H-L: Pv-aCO_2 high at T0 and normal at T6; and L-L Pv-aCO_2 normal at T0 and T6

Table 2. Hemodynamic, oxygen, and ventilatory parameters.

Variable	Group 1 H - H (n=24)	Group 2 L –H (n=8)	Group 3 H - L (n=17)	Group 4 L - L (n=36)	p
Hemodynamic variables					
HR, beats/min (IQ 25-75)					
T0	112 (103-129)	111 (77-138)	104 (86-126)	109 (93-118)	0.31
T6	114 (101-125)	103 (73-127)	98 (85-114)	103 (88-118)	0.14
CI, L/min/m2 (IQ 25-75)					
T0	3.0 (2.3-4.2)	3.6 (2.7-4.6)	3.0 (2.5-5.0)	3.5 (2.7-4.5)	0.63
T6	3.2 (2.2-4.3)	3.3 (2.4-3.8)	3.7 (2.2-5.5)	3.6 (2.8-4.3)	0.75
MAP, mmHg (IQ 25-75)					
T0	65.0 (61.0-72.8) **	67.0 (65.3-69.5)	73.0 (68.0-82.5) **	72.0 (64.5-77.0)	0.02
T6	69.0 (65.3-75.8)	77.5 (70.5-96.0)	68.0 (63.5-72.0)	71.5 (65.0-78.8)	0.06
CVP, mmHg (IQ 25-75)					
T0	14.5 (10.3-17.0)	10.0 (9.0-12.8)	12.0 (8.5-15.0)	10.0 (7.0-14.0)	0.10
T6	13.5 (10.0-15.8) **	15.0 (10.0-17.0)	10.0 (5.0-12.5) **	10.0 (7.3-13.0)	0.03
PAOP, mmHg (IQ 25-75)					
T0	17.0 (14.0-20.0)	12.5 (10.0-15.0)	17.0 (12.0-22.0)	15.0 (11.0-20.0)	0.52
T6	19.0 (15.0-24.0) **	14.5 (10.0-19.0)	13.0 (10.0-15.0) **	16.0 (12.0-20.0)	0.03
Blood gases and O_2 variables					
pH, (IQ 25-75)					
T0	7.30 (7.18–7.37)	7.31 (7.22-7.40)	7.34 (7.22-7.41)	7.32 (7.24-7.40)	0.85
T6	7.32 (7.20-7.36)	7.35 (7.30-7.42)	7.35 (7.26-7.39)	7.34 (7.30-7.39)	0.33

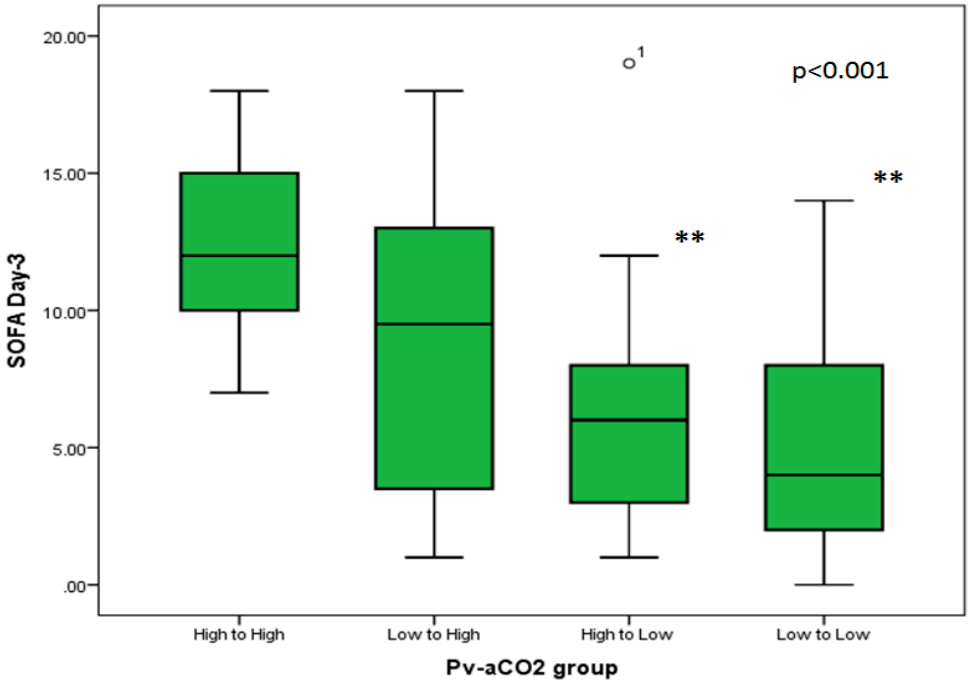
$PaCO_2$, mmHg (IQ 25-75)					
T0	30.5 (24.1-36.6)	34.2 (24.7-36.9)	31.0 (23.6-33.6)	27.5 (23.7-36.5)	0.81
T6	26.1 (22.4-32.7)	31.0 (21.6-34.0)	31.3 (26.8-33.6)	27.5 (21.7-32.1)	0.25
$PvmCO_2$, mmHg (IQ 25-75)					
T0	40.6 (33.9-46.5) ¶	29.5 (25.0-35.3)	39.7 (35.5-47.1)	32.8 (27.3-40.9) ¶	0.03
T6	38.0 (29.9-39.7) ¶	38.3 (28.0-46.2)	34.3 (30.4-36.8)	30.0 (25.5-35.5) ¶	0.02
$PvcCO_2$, mmHg (IQ 25-75)					
T0	38.2 (29.1-50.5)	40.1 (34.2-43.4)	36.7 (35.5-47.1)	32.5 (28.1-38.4)	0.12
T6	33.7 (28.3-36.9)	33.8 (31.4-41.4)	33.9 (30.4-36.8)	29.0 (25.1-35.8)	0.20
PaO_2, mmHg (IQ 25-75)					
T0	112.2 (79.3-167.3)	117.1 (77.5-139.5)	115.7 (81.4-140.8)	100 (86.0-123.9)	0.70
T6	113.6 (83.0-147.3)	132.5 (109.8-197.8)	105.0 (83.5-127.4)	111.5 (86.2-135.7)	0.34
PaO_2/FiO_2, mmHg (IQ 25-75)					
T0	188.9 (117.1-265.0)	166.9 (143.8-244.1)	233.7 (184.6-314.0)	214.3 (141.6-368.0)	0.28
T6	177.9 (136.4-298.4)	265.0 (185.5-383.5)	239.3 (185.1-306.3)	253.5 (181.6-368.0)	0.17
Lactate, mmol/L, (IQ 25-75)					
T0	4.3 (2.0-7.9)	2.9 (2.0-9.7)	2.7 (1.5-3.9)	2.9 (1.7-4.7)	0.16
T6	3.3 (2.1-6.8) **¶	2.9 (1.1-7.1)	1.3 (0.9-2.3) **	2.0 (1.0-3.5) ¶	0.002
$ScvO_2$, %, (IQ 25-75)					
T0	64.0 (54.7-75.1)	66.8 (59.7-74.5)	73.0 (70.8-76.7)	67.1 (62.1-75.9)	0.36
T6	70.8 (66.4-73.5)	73.4 (69.3-78.0)	72.3 (67.2-75.9)	76.0 (60.0-77.9)	0.53
SvO_2, %, (IQ 25-75)					
T0	66.0 (56.8-71.4)	68.0 (54.9-81.0)	68.1 (57.0-71.0)	69.7 (62.1-75.9)	0.39
T6	69.0 (63.3-72.9)	70.6 (63.4-73.8)	71.2 (64.4-75.1)	68.0 (58.7-74.3)	0.77

ERO_2, (IQ 25-75)					
T0	34.7 (28.3-44.3)	30.8 (22.7-38.4)	29.4 (25.0-36.8)	30.7 (24.9-37.5)	0.36
T6	29.8 (26.4-36.6)	30.2 (24.9-31.3)	29.3 (24.1-33.3)	30.7 (23.4-39.3)	0.68
Pv-aCO_2, mmHg (IQ 25-75)					
T0	8.2 (7.0-10.6) ***¶**	1.4 (1.1-5.3) ***φ**	6.3 (6.1-7.2) **φ ξ**	2.7 (2.0-4.0) **¶ ξ**	<0.001
T6	7.6 (6.4-9.4) ****¶**	8.6 (6.5-11.7) **φ Ψ**	3.5 (0.9-4.3) ****φ**	3.3 (2.2-4.1) **¶ Ψ**	<0.001
Pvc-aCO_2, mmHg (IQ 25-75)					
T0	9.7 (7.0-12.1) ¶	4.6 (3.3-6.1)	7.3 (4.6-9.1)	4.4 (2.7-5.4) ¶	<0.001
T6	7.0 (5.8-9.7)	8.5 (5.6-9.6) **Ψ**	4.4 (2.1-5.5)	4.0 (1.7-5.4) **Ψ**	0.003
Ventilatory parameters					
Mechanical ventilation					
T0	24	5	11	23	0.01
T6	24	5	11	23	0.01
PEEP, mmHg, n					
T0	6.0 (5.0-8.0), 24	5.0 (5.0-6.0), 5	5.0 (5.0-8.0), 11	6.0 (5.0-8.0), 23	0.48
T6	6.5 (5.0-8.0), 24	5.0 (5.0-7.5), 5	5.0 (5.0-8.0), 11	8.0 (5.0-10.0), 23	0.64
Vt (ml/kg), (IQ 25-75)					
T0	7.1 (6.3-7.7)	7.3 (6.3-7.6), 5	7.0 (6.5-8.0), 11	7.5 (6.6-8.0), 23	0.65
T6	7.2 (6.3-7.9)	7.2 (6.6-7.6), 5	7.5 (6.8-8.0), 11	7.5 (6.9-8.0), 23	0.65

HR: Heart rate; CI= cardiac Index; MAP: mean arterial pressure; CVP: central venous pressure; PAOP: pulmonary artery occlusion pressure; $ScvO_2$: central venous oxygen saturation; SvO_2: mixed venous oxygen saturation; ERO_2: oxygen extraction ratio; $PvmCO_2$: mixed venous carbon dioxide pressure; $PvcCO_2$: central venous carbon dioxide pressure; Pv-aCO_2: mixed venous-to-arterial carbon dioxide difference; Pvc-aCO_2: central venous-to-arterial carbon dioxide difference Vt: tidal volume.

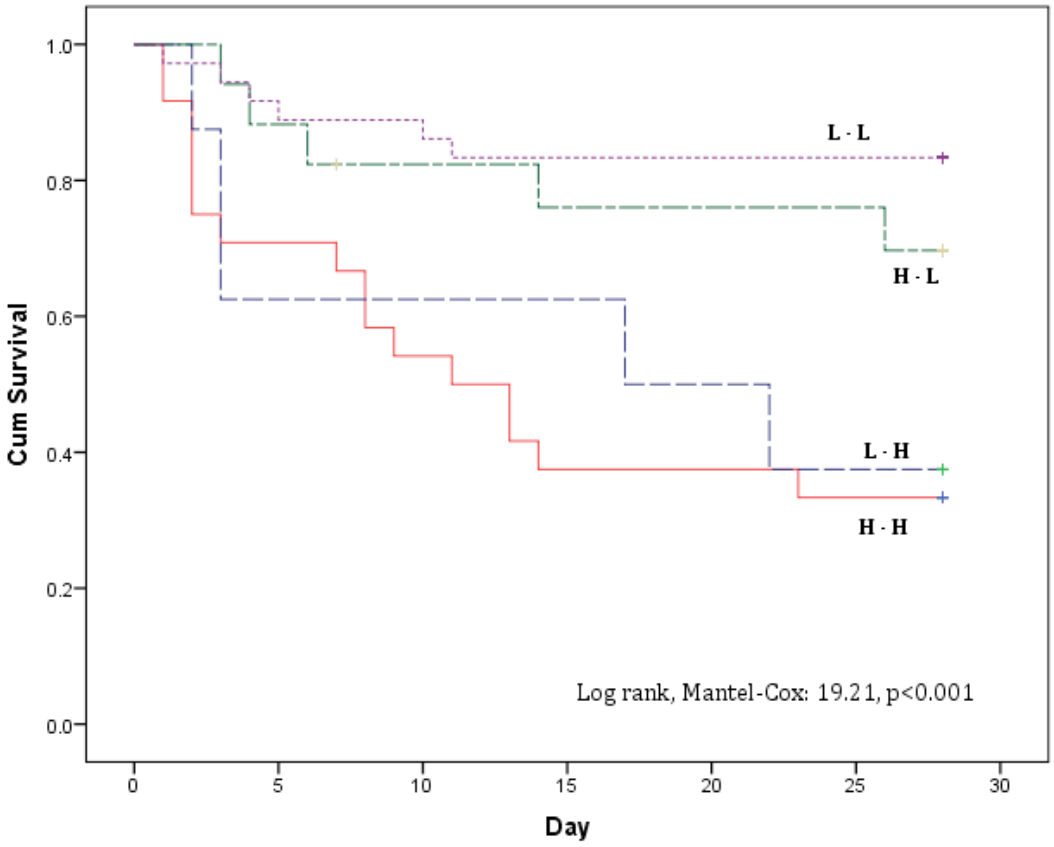
***** p<0.05 for Groups 1 vs. 2; ****** p<0.05 for Groups 1 vs. 3; **¶** p<0.05 for Groups 1 vs. 4; **φ** p<0.05 for Groups 2 vs. 3; **Ψ** p<0.05 for Groups 2 vs. 4; and **ξ** p<0.05 for Groups 3 vs. 4

Figure 1. SOFA scores at Day-3 according to the early development (first 6 hours) of Pv-aCO_2.



Kruskal-Wallis test, p<0.001. **Significant differences between persistently high Pv-aCO_2 and low Pv-aCO_2 group; and between persistently high Pv-aCO_2 and decreasing Pv-aCO_2 group after Bonferroni correction. H-H: Pv-aCO_2 high at T0 and T6; L-H: Pv-aCO_2 normal at T0 and high at T6; H-L: Pv-aCO_2 high at T0 and normal at T6; and L-L Pv-aCO_2 normal at T0 and T6.

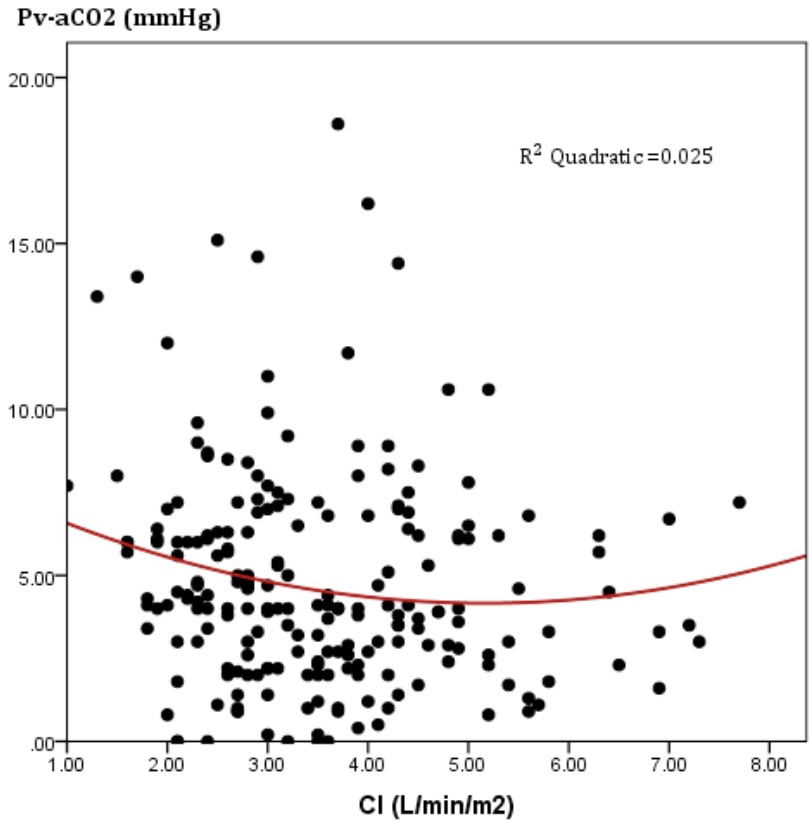
Figure 2. Survival probabilities at Day 28 by the development of Pv-aCO_2 during the first 6 hours of resuscitation.



Log rank, Mantel-Cox: 19.21, p<0.001. H-H: Pv-aCO_2 high at T0 and T6; L-H: Pv-aCO_2 normal at T0 and high at T6; H-L: Pv-aCO_2 high at T0 and normal at T6; and L-L Pv-aCO_2 normal at T0 and T6.

The time course of SvO_2, $ScvO_2$ and cardiac output did not significantly differ among Pv-aCO_2 groups (Table 2) or between survivors and non-survivors at Day-28 (Annex A. Suppl. Figures 1a, 1b, and 2). Interestingly, a poor agreement between cardiac output and Pv-aCO_2 was observed at T6 (Annex A. Suppl. Figure 3) and when pooled all data (r^2=0.025, p<0.01) (Figure 3).

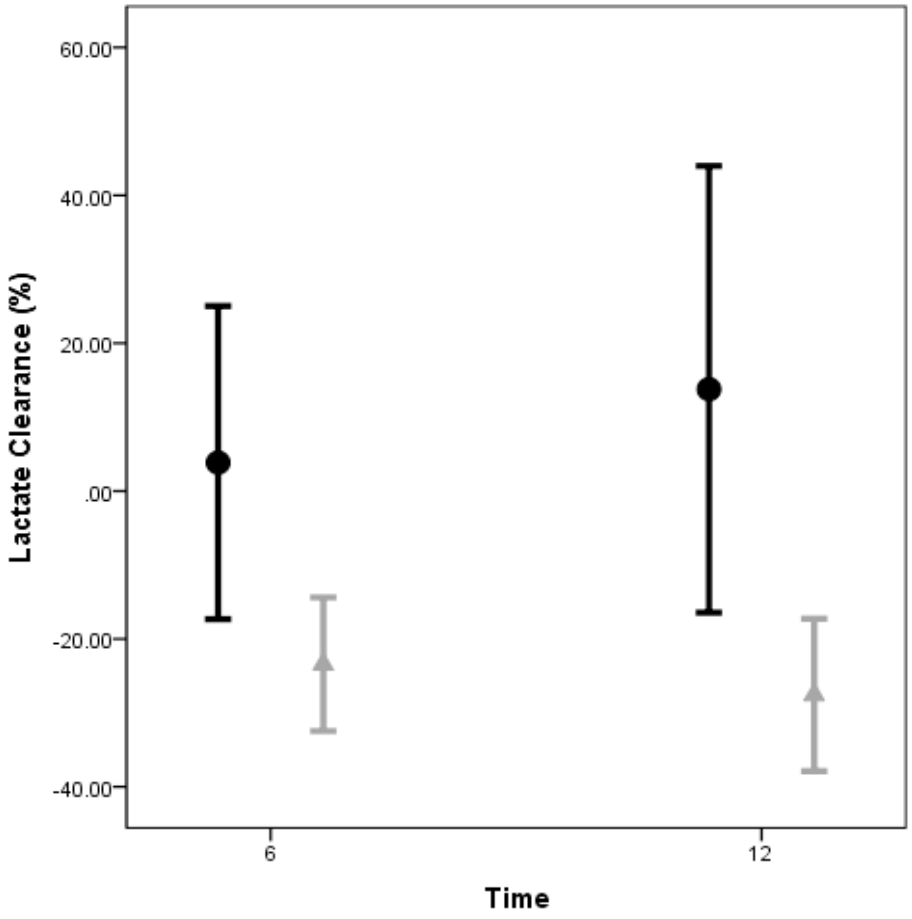
Figure 3. Scatter plot between cardiac index and Pv-aCO_2 (all patients at T0, T6, T12 and T24)



Pearson correlation: 0.16; r^2=0.025; p<0.01

Patients with elevated Pv-aCO_2 at T6 had slower lactate clearances at T6 and T12 than patients with attaining a normal Pv-aCO_2 during the first 6 hours of resuscitation (Fig. 4).

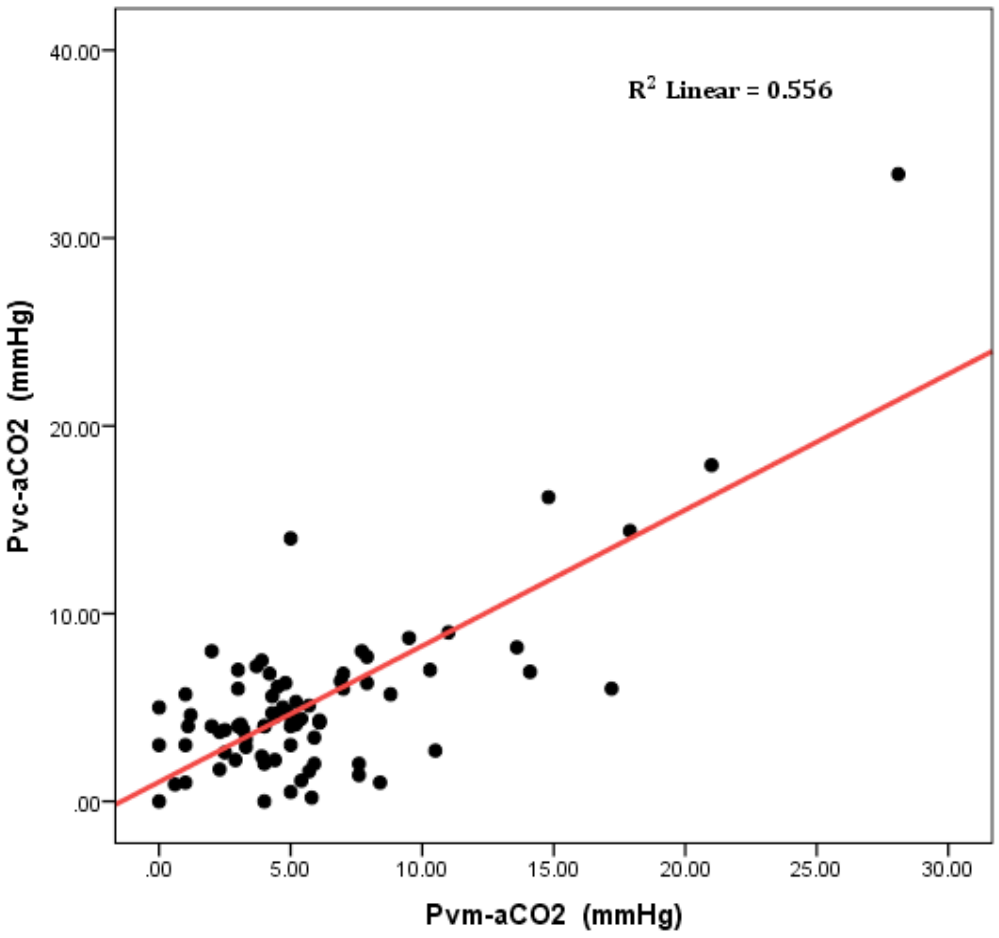
Figure 4. Lactate clearance (%) at T6 and T12 for patients with normal or high Pv-aCO_2 at T6.



Significant differences for lactate clearance (T0 to T6 and T0 to T12) between patients with persistently high (i.e., high-to-high and normal-to-high groups) and normalized Pv-aCO2 at T6 (high-to-normal and persistently low groups). A negative % clearance indicates a reduction in lactate levels.

We also observed a significant linear correlation between mixed-venous to arterial pCO_2 and central-venous to arterial pCO_2 (Pearson correlation: 0.71, 95% CI: 0.47 – 0.86; $p<0.001$) but with moderate agreement between them ($R^2=0.556$, $p<0.001$) (Figure 5).

Figure 5. Correlation between Pvm-aCO_2 and Pvc-aCO_2



Scatter plot representing the mixed-venous to arterial CO_2 difference vs. the central-venous to arterial CO_2 difference. Pearson Correlation: 0.71 (95% CI: 0.47 – 0.86); R^2=0.55, p<0.001

Additionally, significant differences were observed for the time-course of Pv-aCO_2 and Pvc-aCO_2 during the first 24 hours for survivor and non-survivors at day-28 (Repeated measures analysis of variance, p=0.003 and p=0.03, respectively; Annex A. Suppl. Figures 4a and 4b).

Finally, patients who achieved $ScvO_2$ ≥ 70% or SvO_2 ≥ 65% but maintained high Pv-aCO_2 at T0, T6 and T12 had a higher mortality risk at Day 28 (Table 3).

Table 3. Mortality risk ratio for patients with $SvO_2 \geq 65\%$ but with Pv-$aCO_2 \geq 6$ mmHg at Day 28.

Time	RR	CI	P
0 h	1.77	0.97-3.22	0.06
6 h	2.23	1.20-4.13	0.01
12 h	2.41	1.42-4.10	0.001

RR: relative mortality risk at Day 28. CI: confidence interval

3.6 DISCUSSION

We studied a cohort of patients during the very early phases of septic shock who were subjected to a comprehensive resuscitation aimed to target the usual hemodynamic and oxygen metabolism parameters. A recent study demonstrated how Pv-aCO_2 could be a tool to detect persistent inadequate resuscitation during septic shock [18] although it was not conducted during very early stages of resuscitation. Even though faster enrollment, our study showed that a number of patients had approximately normal SvO_2 and $ScvO_2$ at catheter insertion (T0) as it has recently been reported at ICU admissions [22] and most of them reached normal oxygen-derived parameters at 6 hours. However, despite attaining SvO_2 and $ScvO_2$ targets (and after adjusting for SvO_2) and an apparent global hemodynamic normalization in most patients, those with persistently high Pv-aCO_2 developed more severe multi-organ dysfunction at day-3 than patients evolving with normal Pv-aCO_2 during the first 6 hours of resuscitation or those who evolved from high to normal $PvaCO_2$. Additionally, we

observed that persistently high Pv-aCO_2 was associated with a lower survival at day-28.

Venous hypercarbia is a marker of limited blood flow during cardiac arrest and shock states [8-19]. Recent observations have suggested that Pv-aCO_2 might identify septic patients who remain inadequately resuscitated despite achieving $ScvO_2$ goals [15]. Consistent with these findings, we found that patients in septic shock achieving $ScvO_2$ ≥ 70% or SvO_2 ≥ 65% had worse outcomes when a concomitant high Pv-aCO_2 was observed. These data reinforce the idea that Pv-aCO_2 provides additional information to hemodynamic and oxygen parameters habitually used during resuscitation of septic shock. Nevertheless, the underlying mechanisms that explain increases in Pv-aCO_2 during septic shock are incompletely understood but, to the current knowledge, an increased Pv-aCO_2 result from the interactions between blood flow to the tissues, aerobic and anaerobic CO_2 generation, and CO_2 dissociation curve.

According to Fick equation, during steady state the CO_2 excretion equals the product of cardiac output by the difference between mixed venous blood CO_2 content ($CvCO_2$) and arterial blood CO_2 content ($CaCO_2$). Some studies have emphasized on the key role of cardiac output on venous to arterial CO_2 content differences and indeed a curvilinear relationship between these two variables has been described [14]. However, in our study we found a poorer concordance between cardiac output and Pv-aCO_2 at each time-point of resuscitation (Fig. 3) and in fact, the cardiac output remained normal or even high during the first 24 hours of resuscitation (Annex A, Suppl. Fig. 2) suggesting some independence between Pv-aCO_2 and macro-vascular blood flow changes. While in non-inflammatory low flow states, tissue and regional hypercarbia can be easily explained by "CO_2 stagnation phenomenon" [15,16], the interpretation of an increased tissue and/or regional PCO_2 during inflammatory conditions is more complex. Thereby, sepsis may be associated with the coexistence of normal or even high cardiac output, inter- and intra-organ blood flow redistribution, and altered

microvascular and oxygen extraction capabilities. All of these alterations can influence the tissue CO_2 production and elimination. Thus, a study by Neviere et al [27] demonstrated the key role of microvascular blood flow on gastric PCO_2 accumulation. Similarly, using simultaneous gastric tonometry and laser Doppler flowmetry, Elizalde et al [28] demonstrated the association between gastric mucosal pH and mucosal blood flow, regardless of macro-hemodynamic variations. Likewise, Tugtekin et al [29] demonstrated in a porcine sepsis model that the increase of mucosal to arterial CO_2 gap was related to the heterogeneity of gut mucosal blood flow, even though cardiac output and mesenteric blood flow were maintained. Meanwhile, Creteur et al [30] found a significant correlation among sublingual CO_2 ($PslCO_2$), gastric mucosal CO_2 (PgCO2) and microcirculatory heterogeneity in human septic shock during dobutamine infusion and they suggested that the primary determinant of tissue CO_2 accumulation was the microcirculatory blood flow. Hence, there is an evident link between blood flow and tissue or local CO_2 accumulations conducting to increase tissue or venous to arterial CO_2 differences but sometimes, normal macro-hemodynamics does not prevent an elevated Pv-aCO_2. The near normalization of the oxygen and hemodynamic parameters between the subgroups in our study suggests that venous CO_2 accumulation encloses more complex mechanisms than macrovascular stagnation and we could hypothesize that microvascular blood flow distribution is one of several factors potentially influencing the behavior of Pv-aCO_2 during inflammatory conditions in which the heterogeneity of microvascular blood flow is increased. However, this hypothesis should be confirmed in future studies.

Interpretation of hyperlactatemia in sepsis is very complex especially in septic shock [31] since anaerobic metabolism, non-anaerobic generation and slow clearance can conduct to lactate accumulation. We observed higher lactate levels and slower lactate clearance at T6 and T12 in patients with persistently high Pv-aCO_2 during the first 6 hours of resuscitation. Interestingly, Pv-aCO_2 (and Pvc-aCO_2) kinetics seems anticipate to a slower lactate clearance (Annex A. Suppl. Figure 5). A high Pv-aCO_2 could indicate

a decrease in global or microvascular blood flow conducting to slow lactate clearance. However, a high Pv-aCO_2 could also reflect the persistence of anaerobic metabolism as result of bicarbonate buffering of protons derived from fixed acids [32]. Thus, an increased Pv-aCO_2 to VO_2 ratio could reflect global anaerobic metabolism as proposed by Mekontso-Dessap et al [19]. However, even in the presence of anaerobic metabolism, a high efferent venous blood flow could be sufficient to wash out the global CO_2 generation from the hypoperfused peripheral tissues and this way, Pv-aCO_2 could not increase. In fact, hypoperfusion could persist in some of our patients even oxygen parameters, global hemodynamics or Pv-aCO_2 remain normal.

Finally, we found a significant linear correlation but moderate agreement between venous-arterial CO_2 differences obtained from mixed venous and central venous samples which agrees with recent observations published simultaneously to the review of our paper [21]. Even though Pvc-aCO_2 can be easily obtained and speedily usable in the emergency room, the point about if Pvc-aCO_2 and Pvm-aCO_2 (Pv-aCO_2 in our study) are really interchangeable should be addressed in future studies.

Our study has some limitations. First, our observations are restricted to macro-hemodynamic variables, and Pv-aCO_2 is another global variable that does not necessarily represent tissue or regional vascular perfusion at different beds. We did not describe regional perfusion variables as gastric tonometry or local tissue CO_2 accumulation; hence, normal Pv-aCO_2 might also occur when regional hypoperfusion is ongoing. Second, we suggest that persistently high Pv-aCO_2 reflects tissue or regional hypoperfusion. We hypothesized that Pv-aCO_2 could reflect the venous CO_2 accumulation due to the heterogeneous microcirculatory blood flow when cardiac output and oxygen parameters remain normal or even high or eventually, Pv-aCO_2 could reflect anaerobic CO_2 generation. However, mechanism conducting to venous CO_2 accumulation during inflammatory conditions should be explored in future studies. Third, during conditions of tissue hypoxia but with preserved blood flow

(even though VCO_2 decreasing is lower than VO_2 during anaerobic metabolism), venous blood flow might be high enough to ensure adequate washout of the CO_2 produced by hypoxic cells, thereby preventing a Pv-aCO_2 increase. Fourth, we assumed that a linear relationship exist between partial CO_2 pressure and CO_2 content at the venous and arterial levels [33,34]. Thus, Pv-aCO_2 could be used as a surrogate for the Cv-aCO_2. However, previous research has shown that the Haldane effect causes paradoxical increases in Pv-aCO_2 during blood flow increases [33,34]. Unfortunately, the calculation of CO_2 content is complex and subject to errors due to the number of variables included in the formulas. Simplified formulas are easy to use, but wide differences in venous and arterial acid-base status (e.g., ischemic hypoxia) can preclude their use. Nevertheless, some authors consider that the Haldane effect exerts a minor influence, and in most cases, Pv-aCO_2 and CO_2 content differences develop similarly [35].

Finally, our observations were restricted to a small sample of patients in septic shock. Although our findings seem logical and biologically plausible, they should be confirmed in future studies.

3.7 CONCLUSION

The persistence of high Pv-aCO_2 during the early resuscitation of patients in septic shock is associated with significant higher multi-organ dysfunction and poor outcomes. Although underlying mechanisms that increase Pv-aCO_2 among patients in septic shock must be clarified, Pv-aCO_2 might identify a high risk of death in apparently resuscitated patients. Future studies should test Pv-aCO_2 as a perfusion goal during early phases of the resuscitation of patients in septic shock.

3.8 REFERENCES

1. Beal AL, Cerra FB. Multiple organ failure syndrome in the 1990s. Systemic inflammatory response and organ dysfunction. *JAMA : the journal of the American Medical Association.* 1994;271(3):226-233.
2. Shoemaker WC, Appel PL, Kram HB. Tissue oxygen debt as a determinant of lethal and nonlethal postoperative organ failure. *Critical care medicine.* 1988;16(11):1117-1120.
3. Vallet B. Vascular reactivity and tissue oxygenation. *Intensive care medicine.* 1998;24(1):3-11.
4. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *The New England journal of medicine.* 2001;345(19):1368-1377.
5. Third European Consensus Conference in Intensive Care Medicine. Tissue hypoxia: How to detect, how to correct, how to prevent. Societe de Reanimation de Langue Francaise. The American Thoracic Society. European Society of Intensive Care Medicine. *American journal of respiratory and critical care medicine.* 1996;154(5):1573-1578.
6. Russell JA, Phang PT. The oxygen delivery/consumption controversy. Approaches to management of the critically ill. *American journal of respiratory and critical care medicine.* 1994;149(2 Pt 1):533-537.
7. Marik PE, Bankov A. Sublingual capnometry versus traditional markers of tissue oxygenation in critically ill patients. *Critical care medicine.* 2003;31(3):818-822.
8. Grundler W, Weil MH, Rackow EC. Arteriovenous carbon dioxide and pH gradients during cardiac arrest. *Circulation.* 1986;74(5):1071-1074.
9. Weil MH, Rackow EC, Trevino R, Grundler W, Falk JL, Griffel MI. Difference in acid-base state between venous and arterial blood during cardiopulmonary resuscitation. *The New England journal of medicine.* 1986;315(3):153-156.
10. Mecher CE, Rackow EC, Astiz ME, Weil MH. Venous hypercarbia associated with severe sepsis and systemic hypoperfusion. *Critical care medicine.* 1990;18(6):585-589.
11. Adrogue HJ, Rashad MN, Gorin AB, Yacoub J, Madias NE. Assessing acid-base status in circulatory failure. Differences between arterial and central venous blood. *The New England journal of medicine.* 1989;320(20):1312-1316.
12. Kazarian KK, Del Guercio LR. The use of mixed venous blood gas determinations in traumatic shock. *Annals of emergency medicine.* 1980;9(4):179-182.
13. Teboul JL, Mercat A, Lenique F, Berton C, Richard C. Value of the venous-arterial PCO2 gradient to reflect the oxygen supply to demand in humans: effects of dobutamine. *Critical care medicine.* 1998;26(6):1007-1010.
14. Bakker J, Vincent JL, Gris P, Leon M, Coffernils M, Kahn RJ. Veno-arterial carbon dioxide gradient in human septic shock. *Chest.* 1992;101(2):509-515.
15. Vallet B, Teboul JL, Cain S, Curtis S. Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol.* 2000;89(4):1317-1321.
16. Neviere R, Chagnon JL, Teboul JL, Vallet B, Wattel F. Small intestine intramucosal PCO(2) and microvascular blood flow during hypoxic and ischemic hypoxia. *Critical care medicine.* 2002;30(2):379-384.

17. Dubin A, Estenssoro E, Murias G, et al. Intramucosal-arterial Pco2 gradient does not reflect intestinal dysoxia in anemic hypoxia. *The Journal of trauma.* 2004;57(6):1211-1217.
18. Vallee F, Vallet B, Mathe O, et al. Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? *Intensive care medicine.* 2008;34(12):2218-2225.
19. Mekontso-Dessap A, Castelain V, Anguel N, et al. Combination of venoarterial PCO2 difference with arteriovenous O2 content difference to detect anaerobic metabolism in patients. *Intensive care medicine.* 2002;28(3):272-277.
20. van Beest PA, van Ingen J, Boerma EC, et al. No agreement of mixed venous and central venous saturation in sepsis, independent of sepsis origin. *Crit Care.* 2010;14(6):R219.
21. van Beest PA, Lont MC, Holman ND, Loef B, Kuiper MA, Boerma EC. Central venous-arterial pCO(2) difference as a tool in resuscitation of septic patients. *Intensive care medicine.* 2013;39(6):1034-1039.
22. van Beest PA, Hofstra JJ, Schultz MJ, Boerma EC, Spronk PE, Kuiper MA. The incidence of low venous oxygen saturation on admission to the intensive care unit: a multi-center observational study in The Netherlands. *Crit Care.* 2008;12(2):R33.
23. Hernandez G, Pena H, Cornejo R, et al. Impact of emergency intubation on central venous oxygen saturation in critically ill patients: a multicenter observational study. *Crit Care.* 2009;13(3):R63.
24. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Critical care medicine.* 2003;31(4):1250-1256.
25. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Critical care medicine.* 2008;36(1):296-327.
26. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Critical care medicine.* 1998;26(11):1793-1800.
27. Neviere R, Mathieu D, Chagnon JL, Lebleu N, Wattel F. The contrasting effects of dobutamine and dopamine on gastric mucosal perfusion in septic patients. *American journal of respiratory and critical care medicine.* 1996;154(6 Pt 1):1684-1688.
28. Elizalde JI, Hernandez C, Llach J, et al. Gastric intramucosal acidosis in mechanically ventilated patients: role of mucosal blood flow. *Critical care medicine.* 1998;26(5):827-832.
29. Tugtekin IF, Radermacher P, Theisen M, et al. Increased ileal-mucosal-arterial PCO2 gap is associated with impaired villus microcirculation in endotoxic pigs. *Intensive care medicine.* 2001;27(4):757-766.
30. Creteur J, De Backer D, Sakr Y, Koch M, Vincent JL. Sublingual capnometry tracks microcirculatory changes in septic patients. *Intensive care medicine.* 2006;32(4):516-523.
31. De Backer D. Lactic acidosis. *Intensive care medicine.* 2003;29(5):699-702.
32. Raza O, Schlichtig R. Metabolic component of intestinal PCO(2) during dysoxia. *J Appl Physiol.* 2000;89(6):2422-2429.
33. Jakob SM, Kosonen P, Ruokonen E, Parviainen I, Takala J. The Haldane effect--an alternative explanation for increasing gastric mucosal PCO2 gradients? *British journal of anaesthesia.* 1999;83(5):740-746.

34. Hurley R, Mythen MG. The Haldane effect--an explanation for increasing gastric mucosal PCO2 gradients? *British journal of anaesthesia.* 2000;85(1):167-169.
35. Schlichtig R, Bowles SA. Distinguishing between aerobic and anaerobic appearance of dissolved CO2 in intestine during low flow. *J Appl Physiol.* 1994;76(6):2443-2451.

Chapter 4

THE PATHOPHYSIOLOGICAL CHALLENGE: SEARCHING FOR MARKERS OF ANAEROBIC METABOLISM

PAPER No. 3 (Original paper)

COMBINATION OF ARTERIAL LACTATE LEVELS AND VENOUS-ARTERIAL CO_2 TO ARTERIAL-VENOUS O_2 CONTENT DIFFERENCE RATIO AS MARKERS OF RESUSCITATION IN PATIENTS WITH SEPTIC SHOCK

Intensive Care Med. 2015 May;41(5):796-805

ABSTRACT

Purpose: To evaluate the prognostic value of the Cv-aCO_2/Da-vO_2 ratio combined with lactate levels during the early phases of resuscitation in septic shock.

Methods: Prospective observational study in a 60-bed mixed ICU. One hundred thirty-five patients with septic shock were included. The resuscitation protocol targeted mean arterial pressure, pulse pressure variations or central venous pressure, mixed venous oxygen saturation and lactate levels. Patients were classified in four groups according to lactate levels and Cv-aCO_2/Da-vO_2 ratio at 6 hours of resuscitation (T6): Group 1: Lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 >1.0; Group 2: Lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ≤1.0; Group 3: Lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 > 1.0; and Group 4: Lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ≤1.0.

Results: Combination of hyperlactatemia and high Cv-aCO_2/Da-vO_2 ratio was associated with the worst SOFA scores and lower survival rates at day-28 (Log rank [Mantel-Cox]=31.39, p<0.0001). Normalization of both variables was associated with the best outcomes. Patients with a high Cv-aCO_2/Da-vO_2 ratio and lactate <2.0 mmol/L had similar outcomes to hyperlactatemic patients with low Cv-aCO_2/Da-vO_2 ratio. The multivariate analysis revealed that Cv-aCO_2/Da-vO_2 ratio at both T0 (RR 3.85; 95% CI, 1.60-9.23) and T6 (RR 3.97; 95% CI, 1.54-10.23) was an independent predictor for mortality at day-28, as well as lactate levels at T6 (RR 1.58; 95% CI, 1.13-2.22).

Conclusion: Complementing lactate assessment with Cv-aCO_2/Da-vO_2 ratio during early stages of resuscitation of septic shock can better identify patients at high risk of adverse outcomes. The Cv-aCO_2/Da-vO_2 ratio may become a potential resuscitation

goal in patients with septic shock.

Key words: venous-arterial carbon dioxide to arterial-venous oxygen content differences ratio, venous-to-arterial carbon dioxide difference, septic shock, lactate, hyperlactatemia, tissue perfusion

RESUMEN

Propósito: Evaluar el valor pronóstico de la relación Cv-aCO_2/Da-vO_2 al combinarse con los niveles de lactato, durante las fases tempranas de reanimación del shock séptico.

Métodos: Estudio prospectivo observacional en una UCI mixta de 60 camas. Ciento treinta y cinco pacientes con shock séptico fueron incluidos. El protocolo de reanimación tuvo como metas la presión arterial media, variaciones de la presión de pulso y/o la presión venosa central, saturación de oxígeno en sangre venosa mixta y niveles de lactato. Para su análisis, los pacientes fueron clasificados en 4 grupos de acuerdo a los niveles de lactato y relación Cv-aCO_2/Da-vO_2 alcanzados a las 6 horas de reanimación (T6): Grupo 1: Lactato ≥2.0 mmol/L y Cv-aCO_2/Da-vO_2 >1.0; Grupo 2: Lactato ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ≤1.0; Grupo 3: Lactato <2.0 mmol/L and Cv-aCO_2/Da-vO_2 > 1.0; and Grupo 4: Lactato <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ≤1.0.

Resultados: La combinación de hiperlactatemia y elevación de la relación Cv-aCO_2/Da-vO_2 estuvo asociada con más severa disfunción orgánica medida con el puntaje de SOFA y con menor probabilidad d e sobrevida al día 28 (Log rank [Mantel-Cox]=31.39, p<0.0001). La normalización concomitante de ambas variables se relacionó con los mejores desenlaces. Pacientes con una relación Cv-aCO_2/Da-vO_2 elevada y un lactato <2.0 mmol/L tuvieron desenlaces clínicos similares a aquellos con hiperlactatemia y relación Cv-aCO_2/Da-vO_2 baja. El análisis multivariable reveló que la relación Cv-aCO_2/Da-vO_2 fue un predictor independiente de mortalidad a 28 días tanto a su medición al T0 (RR 3.85; 95% CI, 1.60-9.23) y T6 (RR 3.97; 95% CI, 1.54-10.23), también como lo fue el lactato al T6 (RR 1.58; 95% CI, 1.13-2.22).

Conclusión: Complementar con la relación Cv-aCO_2/Da-vO_2 la información dada por el lactato durante las etapas temprana de la reanimación del shock séptico, puede identificar mejor aquellos pacientes en alto riesgo de muerte y desenlaces clínicos desfavorables. La relación Cv-aCO_2/Da-vO_2 podría convertirse en una potencial meta de reanimación en pacientes con shock séptico.

Palabras clave: relación veno-arterial de dióxido de carbono / diferencia arterio-venosa de oxígeno, diferencia veno-arterial de dióxido de carbono, shock séptico, lactato, hiperlactatemia, perfusión tisular

4.2 INTRODUCTION

Early identification of tissue hypoperfusion and adequate resuscitation are key factors in the management of patients with shock [1,2]. Although early resuscitation seem to improve outcomes in severe sepsis and septic shock, the relative value of resuscitation goals continue to be highly debated [3-6]. Monitoring of $ScvO_2$ is widely recommended [3-7] although strongly challenged by others [8,9]. In an early trial, Rivers et al. [3] observed a significant decrease in mortality when used a resuscitation bundle targeting $ScvO_2$>70%. Conversely, recent data failed to confirm any benefit with this approach [10]. However, it should be noted that $ScvO_2$ was normal or near normal at inclusion in a number of patients in these trials [10] as it has frequently been reported on admissions to the intensive care unit in studies subsequent to the River's trial [11]. Moreover, normalization of systemic hemodynamic and oxygen metabolism variables does not ensure an adequate tissue perfusion and does not prevent progression to multiorgan dysfunction and death [12]. Lactate has also been proposed as a target for resuscitation therapy. In fact, not only baseline lactate levels [13] but also its evolution under the influence of therapy [14] have been associated to clinical outcomes. Despite promising results observed in one trial [15], no consistent advantages have been found for lactate-based resuscitation bundles over resuscitation guided by oxygen parameters [15-17]. Accordingly, additional markers of inadequate perfusion should be explored, especially when $ScvO_2$ values are close to normal.

Recently, the venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) has been proposed as an alternative marker of tissue hypoperfusion [18,19]. In fact, persistently high $Pv\text{-}aCO_2$ predicts adverse clinical outcomes independently of oxygen-derived parameters and it could anticipate lactate variations [20]. However, the $Pv\text{-}aCO_2$ may be

normal despite the presence of significant hypoperfusion in high cardiac output states such as septic shock, where high flows might prevent venous CO_2 accumulation or inversely, Pv-aCO_2 can increase in absence of hypoperfusion, in part due to the Haldane effect [21]. Consequently, CO_2 variations must be evaluated according to O_2 changes. Indeed, CO_2 production should not exceed O_2 availability during aerobic metabolism. Thus, the ratio between the Pv-aCO_2 and the arterial-to-venous oxygen content difference (Da-vO_2), as surrogate of VCO_2/VO_2 ratio (i.e., the respiratory quotient), may identify patients at risk of anaerobic metabolism. Using this rationale, Mekontso-Dessap et al [22] demonstrated that a Pv-aCO_2 to Da-vO_2 ratio > 1.4 was superior to Pv-aCO_2, SvO_2 and Da-vO_2 to predict hyperlactatemia in a cohort of critically ill patients. Importantly, Pv-aCO_2/Da-vO_2 ratio variations are faster than lactate kinetics, which make it an attractive variable to monitor. However, CO_2 partial pressure (PCO_2) is not equivalent to CO_2 content (CCO_2), particularly when O_2 saturation varies (Haldane effect). Thus, Cv-aCO_2/Da-vO_2 variations should better reflect variations in VO_2 than Pv-aCO_2/Da-vO_2, especially when $ScvO_2$ or SvO_2 are low. As Cv-aCO_2 to Da-vO_2 ratio could reflect ongoing anaerobic metabolism, we hypothesized that an increased Cv-aCO_2/Da-vO_2 could be used to identify patients at risk of adverse outcomes during early stages of septic shock and that this variable could provide additional information when combined with lactate levels.

4.3 OBJECTIVE

To evaluate the ability of the Cv-aCO_2/Da-vO_2 variations as surrogate of respiratory quotient in combination with lactate levels to identify high risk of adverse clinical outcomes when calculated during the early stages of resuscitation of septic shock

4.4 MATERIALS AND METHODS

We conducted a prospective observational study in a 60-bed mixed ICU in a University-affiliated Hospital. The Fundación Valle del Lili's Ethical and Biomedical research committee approved the current study (Protocol number: 710; approval number: 093-2014). A written informed consent was waived because all measurements and procedures routinely followed the local protocols for the management of severe sepsis and septic shock and no new therapeutic interventions were performed. Our "rapid-response team" evaluated all patients with suspected septic shock at the emergency room and clinical wards. Resuscitation was immediately started and these patients were rapidly admitted to the ICU. Presence of infection was established using the Centers for Diseases Control and Prevention criteria [23] and septic shock was defined according to the criteria of American College of Chest Physicians / Society of Critical Care Medicine Consensus Conference [24]. Antibiotics were started within one hour of diagnosis of sepsis. An arterial catheter was placed in the radial or femoral artery while a pulmonary artery catheter (PAC) was inserted via the jugular or subclavian vein when deemed appropriate according to clinical judgment and local indications for the use of hemodynamic monitoring. All patients with a new episode of septic shock and equipped by a PAC were included for the study. Patients with a previous episode of severe sepsis or septic shock within the last 3 months, younger than 18 years old, pregnant women, with limitations of care, with liver cirrhosis Child-Pugh C or severe chronic obstructive pulmonary disease, were excluded.

4.4.1 General management

All patients followed an early quantitative resuscitation protocol adapted from the Surviving Sepsis Campaign [7] in order to target: (a) mean arterial pressure (MAP) ≥ 65 mmHg; (b) urine output ≥ 0.5 ml/kg/min; (c) SvO_2 ≥ 65%; (d) normalization of lactate levels. In the case of attaining SvO_2 goal but with persistently high lactate levels, additional efforts were performed to attain normalization of the latter. Pulse pressure variations were used to indicate fluid responsiveness whenever applicable. In other cases, filling pressures and clinical judgment were used. Fluid resuscitation was conducted by repeated fluid challenges with crystalloids and/or albumin 4%. Hydroxyethyl starches (HES) were not used.

Norepinephrine was the first-choice vasopressor to maintain MAP goals. Vasopressin titrated to a maximum of 0.03 UI/minute was allowed in order to raise MAP or to decrease norepinephrine dose but never as a single vasopressor. Titrated dobutamine up to 20 ugr/kg/min was used when myocardial dysfunction was demonstrated or when SvO_2 goals were not achieved despite adequate intravascular volume and MAP. Mechanical ventilation was provided (when needed) under light sedation (midazolam) and analgesia (fentanyl, morphine); tidal volume was limited to 6 – 8 ml/kg. Low dose hydrocortisone was indicated if vasopressor requirement did not decrease during the first 6 hours of resuscitation despite ensuring adequate intravascular volume. Glycemic control was adjusted to maintain glucose levels < 150 mg/dl. Finally, stress ulcer and venous thrombosis prophylaxis were provided according to international recommendations [7] .

4.4.2 Study protocol

Time 0 (T0) was stated at the PAC insertion. We recorded the total volume of fluids received and the time elapsed between the first episode of hypotension and T0. We performed complete hemodynamic measurements and drew blood samples for arterial and mixed-venous gases analysis (ABL 300, Radiometer Copenhagen, Denmark) and arterial lactate at T0, and 6 hours (T6), and around 12 hours (T12) and 24 hours (T24) after. Vasopressors and inotropic doses, respiratory parameters and total fluids were also registered at each measurement time. Organ dysfunction at day-3 was evaluated using the Sequential Organ Failure Assessment (SOFA) score [25]. We also calculated the ventilator-free days and survival at day-28.

4.4.3 Carbon dioxide and oxygen variables

We calculated CO_2 and O_2 variables at T0, T6, T12 and T24, as follows:

- $DO_2 = CaO_2 \times CI$

- $VO_2 = (CaO_2 – CvO_2) \times CI$
- $ERO_2 = (CaO_2 – CvO_2) / CaO_2$
- $CaO_2 = (Hg \times SaO_2 \times 1.34) + (PaO_2 \times 0.003)$
- $CvO_2 = (Hg \times SvO_2 \times 1.34) + (PvO_2 \times 0.003)$
- $Pv\text{-}aCO_2 = PvCO_2 – PaCO_2$
- $Da\text{-}vO_2 = CaO_2 - CvO_2$

Where CaO_2 and CvO_2 are the arterial and venous O_2 content while PaO_2 and PvO_2 represent their arterial and venous partial pressures respectively, while CI represents the cardiac index and ERO_2 represents the oxygen extraction ratio.

We also calculated CO_2 contents according to the Douglas´s formula [26]:

Blood CO_2 content (Blood CCO_2) = Plasma CCO_2

$$\times \left| 1 - \left[0.0289 \times [Hb] \right] \div \left[\left[3.352 - 0.456 \times SpO2 \right] \times \left[8.142 - pH \right] \right] \right|$$

Where Plasma $CCO_2 = 2.226 \times S \times$ plasma $PCO_2 \times (1 + 10^{pH - pK'})$; In turn, *s* (plasma CO_2 solubility) and pK´(apparent pK) are temperature (T, expressed as °C) dependent and calculated according to previous calculations [27] :

$$S = 0.0307 + [0.00057 \times (37 - T)] + [0.00002 \times (37 - T)^2]$$

$$pK' \quad 6.086 + |0.042 \times (7.4 \quad pH)| + \left| |(38 \quad T)| \times \{0.00472 + 0.00139 \; x \; |7.4 \quad pH|\} \right|$$

4.4.4 Definitions of the four groups

Considering that in aerobic conditions VCO_2 should not exceed VO_2, we considered a $Cv\text{-}aCO_2$ / $Da\text{-}vO_2$ ratio > 1.0 as abnormal. Hence, we analyzed hemodynamic and oxygen metabolism parameters for four predetermined groups according to lactate levels and $Cv\text{-}aCO_2$ / $Da\text{-}vO_2$ attained after the first 6 hours of resuscitation: Group 1: Lactate ≥2.0 mmol/L and $Cv\text{-}aCO_2/Da\text{-}vO_2$ ratio >1.0; Group 2: Lactate ≥2.0 mmol/L

and Cv-aCO_2/Da-vO_2 ratio ≤1.0; Group 3: Lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio > 1.0; and Group 4: Lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0.

4.4.5 Statistics

After demonstrating a non-normal distribution of data with a Kolmogorov-Smirnoff test, we used a Kruskal-Wallis to compare continuous variables with a Tukey-Kramer test for multiple comparisons among the predefined groups. Chi^2 test was used to compare discrete variables (or Fisher´s exact test, when appropriate). Multiorgan dysfunction at day-3 was evaluated using the Sequential Organ Failure Assessment (SOFA) score [25] for the predefined groups. Survival curves up to day-28 were estimated using the Kaplan-Meier method and log-rank (Mantel-Cox) test was used to estimate differences among the predefined groups.

In a further analysis, variables were introduced in a multivariate model if significantly associated to mortality at day-28 at the univariate analysis when a p value was < 0.2. General demographics, hemodynamics, vasopressor use, fluids and blood gases parameters at T0 and T6 were used in the model, previously testing for collinearity. These analyses were also conducted in those patients attaining SvO_2 ≥ 65%. A Hosmer and Lemeshow test was used to assess the goodness of fit of the model. Receiver operating characteristic (ROC) curves for the original model (i.e., the *"large model"*) and a second one excluding the Cv-aCO_2/Da-vO_2 ratio (i.e., the *"short model"*) were constructed in order to test the adding value of Cv-aCO_2/Da-vO_2 ratio to predict mortality at day-28. The ROC curves were compared using the method described by DeLong and colleagues [28]. Additional logistic regression models including Pv-aCO_2/Da-vO_2 instead of Cv-aCO_2/Da-vO_2 and other one including simultaneously Pv-aCO_2/Da-vO_2 and Cv-aCO_2/Da-vO_2 were also conducted to explore its relationship with mortality at day-28.

Finally, we described the time-course for oxygen metabolism variables, Pv-aCO_2/Da-vO_2 and Cv-aCO_2/Da-vO_2 during the first 24 hours for both survivors and non-

survivors at day-28. Data are presented as median [percentiles 25-75]. Risk assessments are presented as risk ratios with 95% confidence intervals. A p value ≤ 0.05 (2-tailed) was considered significant.

4.5 RESULTS

Selection of patients is shown in the Annex B, Suppl. Figure 1. A total of 135 patients were included in the study during a period of eighteen months. Mortality at day-28 in this cohort was 42 % and ICU length of stay was 6 [2-10] days. The time from first hypotension episode to catheter insertion and blood sampling (i.e., T0) was 3.0 [2.5 – 4.0] hours and the median amount of fluids received before T0 was 1,977 [1,200-2,800] ml.

After the first 6 hours of resuscitation, 110 (81%) patients achieved a MAP> 65mmHg and 98 (73%) a SvO_2 >65%. However, 84 (62%) patients still had an arterial lactate ≥2.0 mmol/L and 65 (48%) had a Cv-aCO_2 / Da-vO_2 ratio > 1.0. Accordingly, 42 patients were classified into group 1, 42 into group 2, 23 into group 3 and 28 into group 4. Patients in groups 1 and 2 had higher APACHE II scores and required higher vasopressor doses at T0 (Table 1).

No significant differences were found in demographic data or other hemodynamic variables at T0 (Table 1 and Annex B. Suppl. Table 1). All hemodynamic, blood gases, oxygen parameters and ventilator settings at both T0 and T6 are presented in the Annex B. Suppl. Table 1. Patients from groups 1 and 2 had more acidosis at T0 and T6. Regarding to clinical outcomes, patients from group 1 evolved with higher SOFA scores (Kruskal-Wallis, $p<0.001$; post-hoc test demonstrated significant differences among groups 1 vs. 3 and 1 vs. 4) (Figure 1) and they also had the lowest survival

rates at day-28 (Log rank [Mantel-Cox]=31.39, p<0.0001) (Figure 2). Intriguingly, patients in groups 2 and 3 had similar SOFA scores and outcomes at day-28 (Table 2). Furthermore, patients from group 1 had the lowest VO_2 at T6 and T12 compared to all other groups, even though cardiac output, SvO_2, and DO_2, were not different (Annex B. Suppl. Fig.2). Multivariate logistic regression analysis at T0 demonstrated that Cv-aCO_2/Da-vO_2 was an independent predictor of mortality at day-28 (RR 3.85; 95% CI, 1.60-9.27). When analysis was performed using the same variables at T6, Cv-aCO_2/Da-vO_2 was again related to higher mortality at day-28 (RR 3.97; 95% CI, 1.54-10.24), in addition to lactate levels (RR 1.58; 95% CI, 1.123-2.22) (Table 3). An additional multivariate analysis performed in patients attaining SvO_2 > 65% showed that lactate levels (RR 2.41; 95% CI, 1.22-4.76) and Cv-aCO_2/Da-vO_2 (RR 5.71; 95% CI, 1.20-27.19) remained predictors of mortality at day-28 (Table 3). The area under ROC curves for models including or excluding the Cv-aCO_2/Da-vO_2 ratio (i.e., the *"large"* and "short" model respectively) were significantly different (AUC_{large}: 0.8542; 95%CI, 0.77973-0.92860 vs. AUC_{short}: 0.7943; 95%CI, 0.70504-0.88361. LR test: X^2: 17.81, p<0.001; C-statistic, X^2: 4.52, p=0.03) (Fig. 3).

We also found significant differences in the time-course of Pv-aCO_2, Pv-aCO_2/Da-vO_2, lactate levels and Cv-aCO_2/Da-vO_2 during the first 24 hours of resuscitation between survivors and non-survivors at day 28 (Repeated measurements ANOVA, p<0.05) (Annex B. Suppl. Fig. 3 to 5).

Table 1. Patient characteristics.

	Group 1 (n= 42) Lactate ≥ 2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio >1.0	Group 2 (n= 42) Lactate ≥ 2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio ≤1.0	Group 3 (n= 23) Lactate < 2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio >1.0	Group 4 (n= 28) Lactate < 2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio ≤1.0	P
Age (years)	68 [59-75]	64 [54-74]	64 [52-79]	67 [51-76]	0.62
APACHE II	25.0 [21.0 - 34.3] **	24.5 [19.0 - 29.0]	21.0 [19.0 - 23.0] **	20.0 [18.0 - 26.0]	< 0.001
SOFA, Day 1	14.5 [10.0 – 16.3] **¶	13.0 [9.0 – 15.0] ‡	9.0 [8.0 -11.0] **	7.5 [5.8 – 10.0] ‡,¶	< 0.001
Time between diagnosis to catheter insertion (T0)	3.0 [2.4 – 4.0]	3.0 [2.5 – 3.3]	3.0 [2.0 – 4.0]	3.0 [2.5 – 4.0]	0.57
Fluids received before catheter insertion	2,100 [1,542 – 2,818]	1,988 [1,178 – 3,000]	1,600 [1,200 – 2,200]	1,550 [900 – 2,700]	0.32
Temperature (°C)	37.0 [36.1 - 37.7]	37.0 [37.1-37.9]	37.0 [36.4 - 37.7]	37.4 [36.8 - 37.9]	0.23
Source of infection, n					
Pneumonia					
Abdominal	12	13	8	8	
Urinary	11	12	5	9	0.48
Soft tissue	8	8	5	4	
No specific site	6	6	2	1	
Other	5	3	2	4	
	0	0	1	2	
Culture positive, n (%)	31 (73.8)	32 (76.2)	18 (78.3)	23 (82.1)	0.45
Antibiotics given at T0, n (%)	42 (100)	40 (95.2)	22 (95.7)	28 (100)	0.35
Antibiotics adequate, n (%)	42 (100)	40 (95.2)	22 (95.7)	28 (100)	0.35

Steroids, n (%)	36 (85.7) **¶	37 (88.1) †	15 (65.2) †	18 (64.3) ¶‡	0.02
Vasopressin, n (%)	20 (47.6) **¶	23 (54.8) †	3 (13) †	2 (7.1) ¶‡	< 0.001
Red blood cell transfusion, n (%)	12 (28.6)	7 (16.7)	4 (17.4)	6 (21.4)	0.23

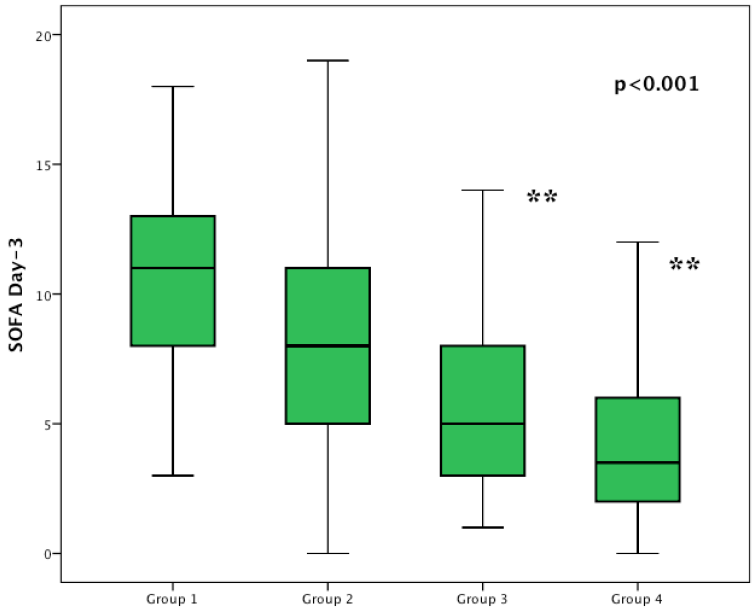
Fluids and vasoactive agents

Fluids, ml [IQ 25-75]					
T0	2,100 [1,542 – 2,818]	1,988 [1,178 – 3,000]	1,600 [1,200 – 2,200]	1,550 (900 – 2,700]	0.32
T6	3,896 [2,950 – 5,000]	4,100 [2,826 – 6,119] †	2,700 [2,150 – 4,400] †	3,176 (2,100 – 4,450]	0.02
Norepinephrine, ugr/kg/min [IQ 25-75], n					
T0	0.32 [0.19 – 0.54], 38**¶	0.34 [0.18 – 0.41], 39	0.15 [0.10 – 0.29], 21**	0.16 (0.07 – 0.23], 26¶	< 0.001
T6	0.36 [0.19 – 0.62], 38**¶	0.35 [0.17 – 0.53], 39 †,‡	0.13 [0.08 – 0.20], 22** †	0.12 (0.05 – 0.17], 24¶ ‡	< 0.001
Dobutamine, ugr/kg/min [IQ 25-75], n					
T0	2.74 [1.38 – 9.0], 4	3.10 [2.22 – 8.70], 6	8.56 [5.13 – 12.00], 5	6.34 (5.23 – 10.45], 5	0.26
T6	5.30 [4.84 – 8.12], 12	4.90 [3.33 – 9.35], 9	6.10 [5.13 – 12.0], 5	5.95 (2.50 – 9.20], 6	0.72

SOFA: sequential organ failure assessment; APACHE II: Acute Physiology and Chronic Health Evaluation II

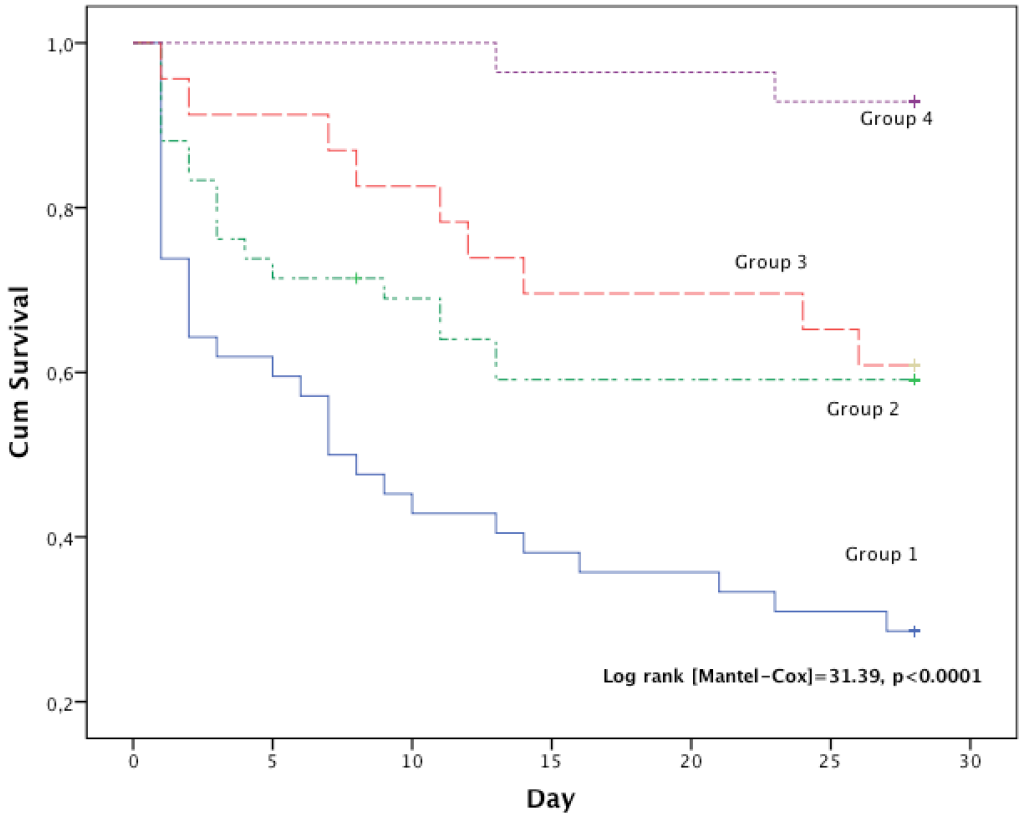
***** p<0.05 for Groups 1 vs. 2; ****** p<0.05 for Groups 1 vs. 3; ¶ p<0.05 for Groups 1 vs. 4; † p<0.05 for Groups 2 vs. 3; ‡ p<0.05 for Groups 2 vs. 4; and **ξ** p<0.05 for Groups 3 vs. 4

Figure 1. Sequential Organ Failure Assessment (SOFA) scores at 3-day for predefined groups.



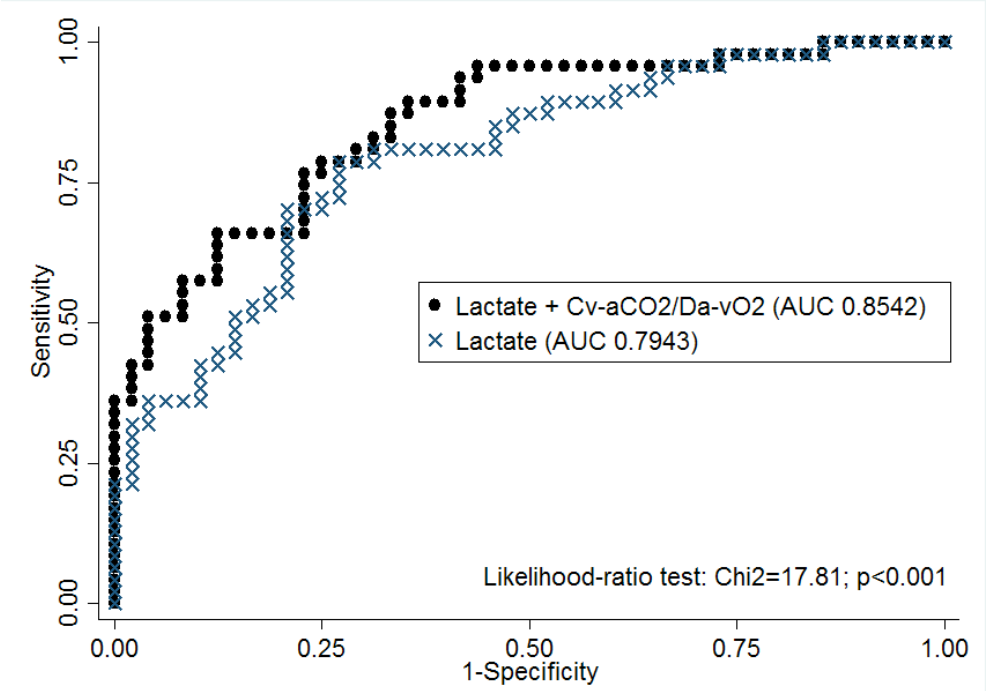
Data presented as median (percentiles). Patients were separated in four groups according to measured lactate and Cv-aCO_2/Da-vO_2 ratio after the first 6 hours of resuscitation: Group 1: Lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; Group 2: Lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0; Group 3: Lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio > 1.0; and Group 4: Lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0. Kruskal-Wallis One-way ANOVA, p<0.001. * p<0.01 by Tukey Kramer showing differences between groups 1 vs. 3 and 1 vs. 4.

Figure 2. Survival probabilities up to 28-day according to lactate and Cv-aCO_2/Da-vO_2 after 6 hours of resuscitation.



Log rank [Mantel-Cox]=31.39, p<0.0001. Group 1: Lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; Group 2: Lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0; Group 3: Lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio > 1.0; and Group 4: Lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0.

Figure 3. Receiver operating characteristics (ROC) curves for prediction of mortality at 28-day for models including or not Cv-aCO_2/Da-vO_2 ratio.



The "*large model*" included Cv-aCO_2/Da-vO_2 + lactate levels. The "*short model*" included lactate levels but not Cv-aCO_2/Da-vO_2. Both models also included: oxygen consumption (VO_2), oxygen delivery (DO_2), mixed-venous oxygen saturation (SvO_2), cardiac index, APACHE II, age, time before T0, gender, fluids administered, norepinephrine dose and mean arterial pressure.
Likelihood ratio test, X^2=17.81, p<0.001.
Differences between AUCs, C-statistic, X^2: 4.52, p=0.03

4.6 DISCUSSION

We observed that persistent hyperlactatemia combined with a high Cv-aCO_2/Da-vO_2 were associated with the most severe organ dysfunction and worst clinical outcomes while simultaneous normalization of lactate and Cv-aCO_2/Da-vO_2 ratio were associated with the best outcomes. Interestingly, patients attaining lactate levels < 2.0 mmol/L combined with Cv-aCO_2/Da-vO_2 >1.0 had similar outcomes than patients with persistent hyperlactatemia and low Cv-aCO_2/Da-vO_2 ratio.

We hypothesized that a Cv-aCO_2/Da-vO_2 >1.0 reflects anaerobic metabolism as VCO_2 should not be higher than VO_2 during aerobic conditions. Indeed, occurrence of a high VCO_2/VO_2 has been previously reported in experimental conditions, where lower reductions in VCO_2 than in VO_2 have been associated with other markers of tissue hypoxia, suggesting the involvement of a non-aerobic source of CO_2 [29,30]. Consequently, a Cv-aCO_2/Da-vO_2 ratio >1.0 (as surrogate of VCO_2/VO_2 ratio) could identify an excess of CO_2 generation probably due to anaerobic metabolism and this condition could be associated with more unfavorable clinical outcomes as we report in our study. Using a similar rationale, Mekontso-Dessap et al [22] tested the hypothesis that Pv-aCO_2/Da-vO_2 better detects anaerobic metabolism than other parameters derived from PAC measurements in critically ill patients. They found a significant agreement between Pv-aCO_2/Da-vO_2 and lactate levels. However, the agreement between Pv-aCO_2/Da-vO_2 and lactate levels should not necessarily be considered as representative of anaerobic metabolism since hyperlactatemia is not always of hypoxic origin [31,32]. Interestingly, our data demonstrated that a high Cv-aCO_2/Da-vO_2 might be present with normal or high lactate levels suggesting that these variables evolve independently probably because lactate kinetics can be slower than Cv-aCO_2/Da-vO_2 variations. Thus, in the presence of hyperlactatemia a high Cv-aCO_2/Da-vO_2 may favor anaerobic metabolism as the possible source of lactate, while a normal Cv-aCO_2/Da-vO_2 may suggest that lactate accumulation is due to other causes [33-36].

Searching for other markers of ongoing tissue hypoxia could increase the information given by lactate levels during resuscitation of septic shock. Recently, Rimachi et al [37] reported the presence of hyperlactatemia in 65% of patients with septic shock, but only 75% of these patients exhibited increased lactate/pyruvate ratio, confirming that hyperlactatemia may be not due to hypoxia, especially during the early stages of shock. Consistent with that study, 71% of the patients in our study had hyperlactatemia at T0 and half of them had an elevated Cv-aCO_2/Da-vO_2. Interestingly, hyperlactatemic patients evolving with a high Cv-aCO_2/Da-vO_2 at T6 (i.e., after initial resuscitation) had a lower VO_2 compared with those evolving with normal Cv-

$aCO_2/Da\text{-}vO_2$, despite similar DO_2 values. This suggests that a high $Cv\text{-}aCO_2/Da\text{-}vO_2$ coupled with hyperlactatemia could identify ongoing VO_2/DO_2 dependence. In agreement with this concept, Monnet et al [38] recently reported that VO_2 increased after fluid administration only in patients with a pre-fluid high $Pv\text{-}aCO2_2/DavO2_2$ ratio. In other words, a VCO_2/VO_2 ratio estimated by the $Pv\text{-}aCO2_2/DavO2_2$ or the $Cv\text{-}aCO2_2/DavO2_2$ could be used to predict fluid responsiveness at the tissue level.

Intriguingly, both $Cv\text{-}aCO_2/Da\text{-}vO_2$ and lactate levels were independent factors determining clinical outcomes, at T0 and T6. As expected, patients with combined increase in $Cv\text{-}aCO_2/Da\text{-}vO_2$ and lactate had the worse outcome, while patients with both variables normal had the best outcomes. Interestingly, patients attaining normal lactate levels at T6 but with a high $Cv\text{-}aCO_2/Da\text{-}vO_2$ had a similar incidence of multiorgan dysfunction and unfavorable clinical outcomes than hyperlactatemic patients with a $Cv\text{-}aCO_2/Da\text{-}vO_2 \leq 1.0$. This further emphasizes the additive value of both indices.

Recent human studies suggest that $Pv\text{-}aCO_2$ may identify persistent perfusion derangements in apparently resuscitated septic shock patients [18,20,39]. Simplicity of $Pv\text{-}aCO_2$ measurement makes it an attractive tool to guide resuscitation in the clinical setting. However, the $Pv\text{-}aCO_2$ is a measurement physiologically complex, as the relationship between the CO_2 partial pressure (PCO_2) and the CO_2 content (CCO_2) is affected by O_2 saturation, i.e., the Haldane effect (19). Accordingly, clinical interpretation of the $Pv\text{-}aCO_2$ can be difficult since its increase can be observed in both aerobic and anaerobic conditions.

Other important question is whether $Pv\text{-}aCO_2/Da\text{-}vO_2$ can be used as a surrogate of $Cv\text{-}aCO_2/Da\text{-}vO_2$. This approach, used by several investigators [22,38] assumes that CO_2 partial pressure (PCO_2) keeps a quasi-linear relationship with CO_2 content (CCO_2) over physiological range of PCO_2, i.e., along the steep portion of the CO_2 dissociation curve. However, the relationship between PCO_2 and CCO_2 becomes non linear if oxygen saturation, arterial-venous pH difference, and/or hemoglobin concentrations change. In this respect, several studies [21,40] reported dissociation between CCO_2 and

PCO_2 in the splanchnic region when CCO_2 decreased in the venous splanchnic effluent while PCO_2 paradoxically increased during increases of splanchnic blood flow. In fact, they showed that venous to arterial PCO_2 differences could increase or decrease for identical blood flow increases. Thus, depending on the basal venous oxygen saturation, the Haldane effect may cause a decrease or increase in the respective venous to arterial PCO_2 difference in response to the same changes in blood flow and metabolism [40]. Thus, theoretically Cv-aCO_2/Da-vO_2 is not equivalent to Pv-aCO_2/Da-vO_2 especially during low PCO_2 and SvO_2 conditions. Importantly, despite the similarities in the time course of the Pv-aCO_2/Da-vO_2 and the Cv-aCO_2/Da-vO_2 (Annex B. Suppl. Figures 3 and 4), we did not find significant association with 28-day mortality for the Pv-aCO_2/Da-vO_2 when it was included instead of Cv-aCO_2/Da-vO_2 in the logistic regression (Annex B. Suppl. Table 2). An additional analysis including simultaneously the Pv-aCO_2/Da-vO_2 and the Cv-aCO_2/Da-vO_2 demonstrated that despite the former was significantly associated to mortality in the univariate analysis, it was not maintained in the multivariate analysis (Annex B. Suppl. Table 3). Nevertheless, despite having ruled out mathematical collinearity between Pv-aCO_2/Da-vO_2 and Cv-aCO_2/Da-vO_2 in the model, it would be debatable to refuse that any collinearity might exist between two variables tightly related. However, we admit that Pv-aCO_2/Da-vO_2 could be equivalent to Cv-aCO_2/Da-vO_2 when PCO_2, pH and SvO_2 approximates to normality, which occurs frequently. Cv-a$CO2_2$/Dav$O2_2$ is an approximation of respiratory quotient, and thus it has a strong physiological meaning. Even though more complicated to compute, it is easier to interpret, with values above 1 suggesting anaerobic metabolism. Admittedly, computation of CO_2 content and DavO_2 are cumbersome and subject to an important risk of errors due to the number of variables included in the formulas. Nevertheless, our data suggest that the influence of measurements errors is limited as it correctly identified patients at increased risk of death.

Nowadays critically ill patients admitted to the ICU often exhibit normal or near normal venous oxygen saturations [11]. Interestingly, when we studied only the patients attaining SvO_2 >65%, Cv-aCO_2/Da-vO_2 and lactate levels were still independent

predictors of outcomes. Thus, Cv-aCO_2/Da-vO_2 could be a useful resuscitation variable in both low and normal SvO_2 conditions.

We acknowledge some limitations of our study. First, both Cv-aCO_2 and Da-vO_2 are global variables and they may not represent regional or local perfusion derangements. Thus, tissue hypoperfusion inducing local CO_2 accumulation may occur even when systemic venous CO_2 remains normal. Second, the Cv-aCO_2 may not increase during conditions of tissue hypoxia associated with high blood flow, even if CO_2 production is increased due to anaerobic metabolism, as venous blood flow may be sufficient to washout the CO_2 generated by hypoxic cells [41]. In these patients, the combination with lactate levels is useful to overcome this shortcoming. Finally, our observations were restricted to a relatively small sample of septic shock patients and although our results sound biologically plausible, they should be confirmed in future physiological studies to better understand the Cv-aCO_2/Da-vO_2 ratio significance during early stages of septic shock.

4.7 CONCLUSION

Combination of Cv-aCO_2/Da-vO_2 and lactate measurements at early stages of resuscitation can identify risk of adverse outcomes in septic shock. The Cv-aCO_2/Da-vO_2 ratio may become a potential resuscitation goal in patients with septic shock.

4.8 REFERENCES

1. Vincent JL, De Backer D. Circulatory shock. *N Engl J Med.* 2013;369(18):1726-1734.
2. Cecconi M, De Backer D, Antonelli M, et al. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. *Intensive Care Med.* 2014;40(12):1795-1815.
3. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med.* 2001;345(19):1368-1377.
4. Trzeciak S, Dellinger RP, Abate NL, et al. Translating research to clinical practice: a 1-year experience with implementing early goal-directed therapy for septic shock in the emergency department. *Chest.* 2006;129(2):225-232.
5. Jones AE, Focht A, Horton JM, Kline JA. Prospective external validation of the clinical effectiveness of an emergency department-based early goal-directed therapy protocol for severe sepsis and septic shock. *Chest.* 2007;132(2):425-432.
6. Shapiro NI, Howell MD, Talmor D, et al. Implementation and outcomes of the Multiple Urgent Sepsis Therapies (MUST) protocol. *Crit Care Med.* 2006;34(4):1025-1032.
7. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med.* 2013;39(2):165-228.
8. Bellomo R, Reade MC, Warrillow SJ. The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. *Crit Care.* 2008;12(2):130.
9. Perel A. Bench-to-bedside review: the initial hemodynamic resuscitation of the septic patient according to Surviving Sepsis Campaign guidelines--does one size fit all? *Crit Care.* 2008;12(5):223.
10. Yealy DM, Kellum JA, Huang DT, et al. A randomized trial of protocol-based care for early septic shock. *N Engl J Med.* 2014;370(18):1683-1693.
11. van Beest PA, Hofstra JJ, Schultz MJ, Boerma EC, Spronk PE, Kuiper MA. The incidence of low venous oxygen saturation on admission to the intensive care unit: a multi-center observational study in The Netherlands. *Crit Care.* 2008;12(2):R33.
12. Puskarich MA, Trzeciak S, Shapiro NI, et al. Outcomes of patients undergoing early sepsis resuscitation for cryptic shock compared with overt shock. *Resuscitation.* 2011;82(10):1289-1293.
13. Shapiro NI, Howell MD, Talmor D, et al. Serum lactate as a predictor of mortality in emergency department patients with infection. *Ann Emerg Med.* 2005;45(5):524-528.
14. Puskarich MA, Trzeciak S, Shapiro NI, et al. Whole blood lactate kinetics in patients undergoing quantitative resuscitation for severe sepsis and septic shock. *Chest.* 2013;143(6):1548-1553.
15. Jansen TC, van Bommel J, Schoonderbeek FJ, et al. Early lactate-guided therapy in intensive care unit patients: a multicenter, open-label, randomized controlled trial. *Am J Respir Crit Care Med.* 2010;182(6):752-761.
16. Nguyen HB, Kuan WS, Batech M, et al. Outcome effectiveness of the severe sepsis resuscitation bundle with addition of lactate clearance as a bundle item: a multi-national evaluation. *Crit Care.* 2011;15(5):R229.
17. Jones AE, Shapiro NI, Trzeciak S, et al. Lactate clearance vs central venous oxygen saturation as goals of early sepsis therapy: a randomized clinical trial. *JAMA.* 2010;303(8):739-746.

18. van Beest PA, Lont MC, Holman ND, Loef B, Kuiper MA, Boerma EC. Central venous-arterial pCO_2 difference as a tool in resuscitation of septic patients. *Intensive Care Med.* 2013;39(6):1034-1039.
19. Vallet B, Pinsky MR, Cecconi M. Resuscitation of patients with septic shock: please "mind the gap"! *Intensive Care Med.* 2013;39(9):1653-1655.
20. Ospina-Tascón GA, Bautista-Rincón DF, Umaña M, et al. Persistently high venous-to-arterial carbon dioxide differences during early resuscitation are associated with poor outcomes in septic shock. *Crit Care.* 2013;17(6):R294.
21. Jakob SM, Kosonen P, Ruokonen E, Parviainen I, Takala J. The Haldane effect--an alternative explanation for increasing gastric mucosal PCO2 gradients? *Br J Anaesth.* 1999;83(5):740-746.
22. Mekontso-Dessap A, Castelain V, Anguel N, et al. Combination of venoarterial PCO2 difference with arteriovenous O2 content difference to detect anaerobic metabolism in patients. *Intensive Care Med.* 2002;28(3):272-277.
23. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control.* 2008;36(5):309-332.
24. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med.* 2003;31(4):1250-1256.
25. Vincent JL, de Mendonça A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med.* 1998;26(11):1793-1800.
26. Douglas AR, Jones NL, Reed JW. Calculation of whole blood CO2 content. *J Appl Physiol (1985).* 1988;65(1):473-477.
27. Austin WH, Lacombe E, Rand PW, Chatterjee M. Solubility of carbon dioxide in serum from 15 to 38 C. *J Appl Physiol.* 1963;18:301-304.
28. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics.* 1988;44(3):837-845.
29. Groeneveld AB, Vermeij CG, Thijs LG. Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end-expiratory pressure in the pig. *Anesth Analg.* 1991;73(5):576-582.
30. Dubin A, Murias G, Estenssoro E, et al. End-tidal CO2 pressure determinants during hemorrhagic shock. *Intensive Care Med.* 2000;26(11):1619-1623.
31. James JH, Luchette FA, McCarter FD, Fischer JE. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. *Lancet.* 1999;354(9177):505-508.
32. Levraut J, Ciebiera JP, Chave S, et al. Mild hyperlactatemia in stable septic patients is due to impaired lactate clearance rather than overproduction. *Am J Respir Crit Care Med.* 1998;157(4 Pt 1):1021-1026.
33. Severin PN, Uhing MR, Beno DW, Kimura RE. Endotoxin-induced hyperlactatemia results from decreased lactate clearance in hemodynamically stable rats. *Crit Care Med.* 2002;30(11):2509-2514.
34. De Backer D, Creteur J, Zhang H, Norrenberg M, Vincent JL. Lactate production by the lungs in acute lung injury. *Am J Respir Crit Care Med.* 1997;156(4 Pt 1):1099-1104.
35. Vallet B, Teboul JL, Cain S, Curtis S. Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol (1985).* 2000;89(4):1317-1321.
36. Nevière R, Chagnon JL, Teboul JL, Vallet B, Wattel F. Small intestine intramucosal PCO(2) and microvascular blood flow during hypoxic and ischemic hypoxia. *Crit Care Med.* 2002;30(2):379-384.

37. Rimachi R, Bruzzi de Carvahlo F, Orellano-Jimenez C, Cotton F, Vincent JL, De Backer D. Lactate/pyruvate ratio as a marker of tissue hypoxia in circulatory and septic shock. *Anaesth Intensive Care.* 2012;40(3):427-432.
38. Monnet X, Julien F, Ait-Hamou N, et al. Lactate and venoarterial carbon dioxide difference/arterial-venous oxygen difference ratio, but not central venous oxygen saturation, predict increase in oxygen consumption in fluid responders. *Crit Care Med.* 2013;41(6):1412-1420.
39. Vallée F, Vallet B, Mathe O, et al. Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? *Intensive Care Med.* 2008;34(12):2218-2225.
40. Hurley R, Mythen MG. The Haldane effect--an explanation for increasing gastric mucosal PCO2 gradients? *Br J Anaesth.* 2000;85(1):167-169.
41. Dubin A, Estenssoro E, Murias G, et al. Intramucosal-arterial Pco2 gradient does not reflect intestinal dysoxia in anemic hypoxia. *J Trauma.* 2004;57(6):1211-1217.

Chapter 5

INCREASED Pv-aCO2 AND THE MICROCIRCULATORY DYSFUNCTION:

THE HUMAN SEPTIC SHOCK MODEL

PAPER No. 4 (Original paper)

CAN VENOUS-TO-ARTERIAL CARBON DIOXIDE DIFFERENCES REFLECT MICROCIRCULATORY ALTERATIONS IN PATIENTS WITH SEPTIC SHOCK?

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ABSTRACT

Purpose: Septic shock has been associated with microvascular alterations, and these in turn, with the development of organ dysfunction. Despite advances in video-microscopic techniques, evaluation of microcirculation at bedside is still limited. Venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) may be increased even when venous O_2 saturation (SvO_2) and cardiac output looks normal, which could suggest microvascular derangements. We sought to evaluate whether $Pv\text{-}aCO_2$ can reflect the adequacy of microvascular perfusion during the early stages of resuscitation of septic shock.

Methods: Prospective observational study including 75 patients with septic shock in a 60-bed mixed-ICU. Arterial and mixed-venous blood gases and hemodynamic variables were obtained at catheter insertion (T0) and six-hours after (T6). Using a Side-stream dark field device, we simultaneously acquired sublingual microcirculatory images for blinded semi-quantitative analysis. $Pv\text{-}aCO_2$ was defined as the difference between mixed-venous and arterial CO_2 partial pressures.

Results: Progressively lower percentages of small-perfused vessels (PPV), lower functional capillary density and higher heterogeneity of microvascular blood flow were observed at higher $Pv\text{-}aCO_2$ values at both T0 and T6. $Pv\text{-}aCO_2$ was significantly correlated to PPV in the linear model (T0: coefficient:-5.35 IC95%:-6.41 to -4.29, $p<0.001$; T6: coefficient:-3.49 IC95%:-4.43 to -2.55, $p<0.001$) and changes in $Pv\text{-}aCO_2$ between T0 and T6 were significantly related to changes in PPV ($R^2=0.42$, $p<0.001$). Absolute values and changes in $Pv\text{-}aCO_2$ were not related to global-hemodynamic variables. Good agreement between venous-to-arterial CO_2 and PPV was maintained even after corrections for the Haldane effect.

Conclusions: During early phases of resuscitation of septic shock, Pv-aCO_2 could reflect the adequacy of microvascular blood flow.

Key words: Venous-to-arterial carbon dioxide difference, microcirculation, microcirculatory blood flow, septic shock.

RESUMEN

Propósito: El shock séptico ha sido asociado a alteraciones microvasculares y estas a su vez, con el desarrollo de disfunción multiorgánica. A pesar de los avances en las técnicas de video-microscopía, la evaluación de la microcirculación en tiempo real y a la cabecera del paciente es aún limitada. La diferencia veno-arterial de dióxido de carbono ($Pv\text{-}aCO_2$) puede verse incrementada aún cuando la saturación venosa de oxígeno (SvO_2) y el gasto cardiaco parezcan normales, lo cual sugeriría la presencia de alteraciones microvasculares. Así, nos propusimos evaluar si la $Pv\text{-}aCO_2$ puede reflejar la suficiencia de la perfusión microvascular durante las etapas tempranas del shock séptico.

Métodos: Estudio prospectivo observacional en el que se incluyeron 7 5pacientes con shock séptico en una UCI mixta de 60 camas. Se realizó análisis de gases arteriales y venosos mixtos además de variables macro hemodinámicas al momento de la inserción del catéter de arteria pulmonar (T0) y 6 horas después (T6). Usando el dispositivo *Side-stream dark field* (SDF), se obtuvieron de forma simultánea imágenes de la microcirculación sublingual para la realización de un análisis semicuantitativo ulterior. La $Pv\text{-}aCO_2$ fue definida como la diferencia entre las presiones parciales venosa-mixta y arterial de CO_2.

Resultados: Se observó un porcentaje progresivamente menor de vasos pequeños perfundidos (PPV), disminución de la densidad capilar funcional e incremento de la heterogeneidad del flujo sanguíneo microvascular en la medida que se incrementó la $Pv\text{-}aCO_2$ tanto al T0 como al T6. Se encontró además, una correlación significativa entre la $Pv\text{-}aCO_2$ y la PPV en el modelo de regresión linear (T0: coeficiente:-5.35 IC95%:-6.41 a -4.29, $p<0.001$; T6: coeficiente:-3.49 IC95%:-4.43 a -2.55, $p<0.001$), mientras que los cambios en la $Pv\text{-}aCO_2$ entre el T0 y T6 se relacionaron inversamente

y de forma significativa con los cambios en la PPV (R^2=0.42, p<0.001). Los valores absolutos y los cambios en la Pv-aCO_2 no se correlacionaron con las variables hemodinámicas globales. La buena concordancia entre Pv-aCO_2 y la PPV se mantuvo aún después de las correcciones para el efecto Haldane.

Conclusiones: La Pv-aCO_2 podría reflejar la suficiencia del flujo sanguíneo microvascular durante las etapas tempranas de la reanimación del shock séptico.

Palabras clave: Diferencia veno-arterial de dióxido de carbono, microcirculación, flujo sanguíneo microcirculatorio, shock séptico.

5.2 INTRODUCTION

Septic shock remains associated with a high mortality and early recognition of signs of tissue hypoperfusion is crucial in its management [1,2]. Usefulness of oxygen-derived parameters as resuscitation targets has been strongly questioned [3-5] and recent data have failed to demonstrate clinical benefits [4-6]. Indeed, venous oxygen saturation (SvO_2) is often normal or near normal at admission to ICU [7]. Hence, other resuscitation goals such as venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) has been proposed due to its simplicity and ability to predict adverse clinical outcomes in patients achieving normal oxygen-derived parameters during the early phases of resuscitation of septic shock [8-10]. Nevertheless, the mechanisms implicated in the elevation of $Pv\text{-}aCO_2$ during inflammatory states are not completely understood and interpretation of $Pv\text{-}aCO_2$ values during resuscitation of septic shock could be sometimes difficult.

In cardiac arrest $Pv\text{-}aCO_2$ increases as a result of vascular stagnation [11,12]. Similarly, $Pv\text{-}aCO_2$ and cardiac output are inversely related in experimental models of hemorrhagic, hypovolemic and obstructive shock, highlighting the importance of blood flow on increasing venous CO_2 [13-16]. Conversely, agreement between cardiac output variations and $Pv\text{-}aCO_2$ during septic shock is weak [17,18], suggesting that macro hemodynamic alterations cannot explain such $Pv\text{-}aCO_2$ variations [8,9]. Alternatively, $Pv\text{-}aCO_2$ could be related to tissue oxygenation derangements or oxygen consumption capabilities [13,14], but this concept was questioned when others demonstrated that ischemic but not hypoxic or anemic hypoxia leads to $Pv\text{-}aCO_2$ increments [19,20]. Hence, physiological variables different to macro hemodynamics and tissue dysoxia should be associated to variations of $Pv\text{-}aCO_2$ gradients during septic shock.

Microcirculatory derangements such as decreases in functional capillary density and increased heterogeneity of blood flow are common findings in patients with severe

sepsis and septic shock [21,22], even when global oxygen parameters seem adequate. Importantly, these abnormalities might be associated with the development of multiple organ dysfunction and death [23]. Nevertheless, despite improvements in techniques evaluating microcirculation at the bedside, immediate and reliable quantification of microcirculatory derangements is still limited. Thus, surrogates reflecting adequacy of microvascular blood flow might be highly valuable.

Tissue CO_2 accumulation has been related to microcirculatory alterations [24,25]. However, the relationship between Pv-aCO_2 and microcirculatory derangements during human septic shock has not been widely studied.

5.3 OBJECTIVE

To evaluate the ability of the venous-to-arterial carbon dioxide differences (Pv-aCO_2) to reflect the adequacy of the microcirculatory blood flow distribution during the early stages of resuscitation in patients with septic shock.

5.4 MATERIALS AND METHODS

This prospective observational study was conducted in a 60-bed mixed ICU at a University hospital (Fundación Valle del Lili, Cali, Colombia) during fifteen consecutive months (April 2012 to July 2013). We received the approval by the Fundación Valle del Lili´s Ethical and Biomedical research committee (protocol number: 563; approval number: 091-2012). Written informed consent was waived as no new or invasive procedures were conducted for the study purposes. However, in all cases the patients and/or their relatives were informed about the study and were given the opportunity to refuse the use of the data. Infection was defined according to the Centers for Diseases Control and Prevention criteria [26] while septic shock followed the criteria of the American College of Chest Physicians / Society of Critical Care

Medicine Consensus Conference [27]. All consecutive patients fulfilling diagnostic criteria of septic shock and elected by the attending physician to use a pulmonary artery catheter (PAC) within the six hours from the first hypotensive episode were included for the study. The decision of using a PAC was exclusively taken by the attending physician according to the local protocols for hemodynamic monitoring. We did not include patients with a preceding episode of septic shock within the last 3 months, < 18 years old, pregnant women, patients with limitation of therapeutic effort orders, or severe chronic obstructive pulmonary disease. Patients using cardiac output monitoring systems other than PAC were not included. For assessment of microcirculation, we did not include patients who were under non-invasive mechanical ventilation or breathing with a facemask with high FiO_2, as well as patients who were agitated or not collaborative when we first attempted to visualize sublingual microcirculation.

5.4.1 General management

Patients were managed according to an early-resuscitation protocol modified from the Surviving Sepsis Campaign [28], with the aim of achieving: (a) mean arterial pressure ≥ 65 mmHg; (b) urine output ≥ 0,5 ml/kg/min; (c) SvO_2 ≥ 65%; (d) normalization of lactate levels. Repeated fluid loads with crystalloids and/or albumin 4% guided by dynamic predictors of fluid responsiveness were performed in order to optimize cardiac preload. Dynamic predictors of fluid response were preferred over static parameters (i.e., PAOP or CVP). However, when the use of dynamic predictors was not appropriate, cardiac filling pressures and clinical judgment were used to guide fluid administration. We did not use Hydroxyethyl starches (HES). Norepinephrine was titrated to maintain mean arterial pressure (MAP) >65 mmHg. Low doses of Vasopressin titrated up to maximum of 0.03 UI/minute were allowed to increase MAP or to decrease norepinephrine requirements but never as single vasopressor. Dobutamine was titrated up to 20 μgr/kg/min when SvO_2 goals were not achieved after fluid load optimization and normalization of MAP. Mechanical ventilation support was provided when needed under a targeted sedation and analgesia protocol

with midazolam and fentanyl according to the local procedures. Use of low doses hydrocortisone (up to 200 mg/day) was indicated in the case of persistence of vasopressors requirement despite ensuring an adequate intravascular volume. Glycemic control, venous thrombosis prophylaxis and gastric ulcer stress prevention were provided according to Surviving Sepsis Guidelines [28].

5.4.2 Study protocol

All potentially eligible patients from the emergency room or clinical/surgical wards were screened by our "rapid response team" (which was available 24/7) and promptly transferred to the ICU where attending physicians decided on the type of hemodynamic monitoring needed. Those equipped with PAC monitoring were consecutively selected for inclusion. Time 0 (T0) was set when PAC was inserted. Arterial and mixed venous blood samples were drawn for gases analysis (ABL300, Radiometer; Copenhagen, Denmark) at T0 and 6 hours after (T6). We defined Pv-aCO_2 as the difference between mixed-venous and arterial CO_2 partial pressures. Complete hemodynamic and respiratory parameters, inotropic / vasopressor doses and volume of resuscitation fluids were also registered at these points. Simultaneously, sublingual microcirculation images were also recorded and stored under a random number for ulterior analysis [29].

5.4.3 Microcirculatory measurements

We used a Sidestream dark-field (SDF) imaging device (Micro Scan; MicroVision Medical, Amsterdam, the Netherlands) to explore microcirculation at T0 and T6. The SDF device was softly applied to the lateral side of the tongue covering an area approximately of 2.5 to 4 cm from the tip of tongue after gentle removal of secretions with gauze. At each time of measurements, we recorded five sequences of video of 20 seconds each from different adjacent mucosa areas using a videocard (MicroVideo; Pinnacle system, Mountain Views, CA, U.S.A.). These sequences of video were stored under a random number and later analyzed by two investigators blinded to the origin of sequences (J.D.V. and G.O.T.). Vessels were classified as large or small using a cutoff

value of 20 μm. Microvessels with continuous flow were considered as normal while sluggish, intermittent and stopped flows were considered as abnormal. According to the consensus for the evaluation of microcirculation [29], we calculated the proportion of small-perfused vessels (< 20μm diameter), the microvascular flow index (MFI), the heterogeneity index (HI), the total vascular density (all vessels) and the functional capillary density (vessels < 20μm diameter). The intra and inter-observer variability were determined by using five sequences analyzed five times at eight-week intervals by two observers (J.D.V. and G.O.T.). We calculated the intra-observer and inter-observer coefficient of variability for both total number of vessels and the proportion of perfused vessels. An expanded description of images acquisition and analysis are provided in the Annex 3.

5.4.4 Outcome measures

The primary outcome was the agreement between Pv-aCO_2 and PPV at T0 and T6. Secondary outcomes included the relationships between Pv-aCO_2 and hemodynamic systemic variables, and the relationships between changes in Pv-aCO_2 and changes in cardiac output and PPV observed between T0 and T6.

5.4.5 Sample calculation

This was an exploratory trial. Since not data exist on the potential relationship between PvaCO2 and PPV (primary hypothesis), sample size computation could be not feasible. However, we tried to make an estimation based on the cutoffs of normality for Pv-aCO_2 and the incidence of abnormal Pv-aCO_2 in our department. Previous studies demonstrated that Pv-aCO_2 > 6.0 mmHg represents a cutoff for abnormality. Thus, a sample of 71 patients was calculated to demonstrate a Pv-aCO_2 variation of 1 mmHg (from 6 to 5 mmHg), knowing a SD of 3 mmHg and estimating an error of 0.05 and power of 80%. This sample was readjusted until 76 estimating a 10% of losses.

Formula used to calculate sample was:

$$N = \left[\frac{(Z\alpha - Z\beta)\sigma}{\mu 1 - \mu 2}\right]^2$$

Where, Z_α and Z_β represents the Z values for 0.05 and 0.80; σ represents the standard deviation; and μ_1 and μ_2 the means of Pv-aCO_2.

5.4.6 Statistics

As the primary hypothesis has not been tested before, it was difficult to compute a sample size. Based on previous data from our department [8], we estimated that 75 patients would allow having a good range of dispersion of Pv-aCO_2 and PPV for looking at relationship between variables.

Distribution of data was tested using the Kolmogorov-Smirnoff test. We explored the relationship between microcirculatory and systemic hemodynamic variables using the Spearman-Rho test and calculating the coefficient of determination (R^2) to establish the strength of such associations. The distribution of microcirculatory variables, systemic hemodynamics, blood gases, and oxygen / tissue perfusion parameters at T0 and T6 was also evaluated for three predefined groups classified according to the Pv-aCO_2 range values in agreement with previous observations [8]: (a) < 6.0 mmHg (b) 6.0 to 9.9 mmHg, and (c) ≥ 10 mmHg. Differences among groups were assessed using the Kruskal-Wallis test with *pos-hoc* Mann-Whitney analysis with adjustment for multiple comparisons.

We calculated the delta of variation of Pv-aCO_2, PPV and cardiac outputs between T0 and T6. Spearman Rho test and R^2 were used to evaluate the agreement and strength between Pv-aCO_2 vs. PPV, Pv-aCO_2 vs. cardiac output and PPV vs. cardiac output variations.

A multiple linear regression model was used to determinate the association between the Pv-aCO_2, cardiac index, SvO_2, and mean arterial pressure with the percentage of small-perfused vessels (PPV). Subsequent multiple linear regression models were

constructed to test the relation between Pv-aCO_2 and the other systemic resuscitation goals with PPV in patients with SvO_2 < or ≥ 65%. Goodness of fit of each model was evaluated using the coefficient of determination. Through the Spearman-Rho test and calculating R^2, we also evaluated the agreement between PPV and Pv-aCO_2, venous-to-arterial carbon dioxide content (Cv-aCO_2) and venous-to-arterial carbon dioxide content to arterial-to-venous oxygen content ratio (Cv-aCO_2/Da-vO_2).

Receiver operating characteristics (ROC) curves were constructed to compare the accuracy of Pv-aCO_2, lactate, SvO_2 and cardiac output in predicting altered PPVs at different abnormal cutoffs points (PPV50%, PPV60% and PPV70%). Areas under the ROC curves (AUCs) and their respective 95% confidence intervals (CIs) were calculated and compared using non-parametric statistics. Data are presented as median [percentiles 25-75]. A p value ≤ 0.05 (2-tailed) was considered significant.

5.5 RESULTS

A total of 75 patients fulfilling the inclusion criteria were included in the study (Annex D, Suppl. Figure E1). General characteristics according to pre-defined Pv-aCO_2 groups are presented in Table 1. Mortality at day-28 in this cohort was 34.7 % and the ICU length of stay was 6.0 [2.0 – 10.0] days. Time elapsed from first hypotension episode until PAC insertion and blood sampling (i.e., T0) was 3.0 [3.0 – 4.0] hours and amount of fluids received at that point was 1,650 [1,200 – 2,400] ml.

A total of 39, 25 and 11 patients were analyzed into the three predefined Pv-aCO_2 groups at T0, and 36, 23 and 13 at T6, respectively. Of these, 49/75patients (65.3%) had SvO_2 >65% at the T0 and 58/72 (82.9%) attained an SvO_2 >65% at the T6. Hemodynamic and respiratory variables, arterial and mixed-venous blood gases and oxygen-derived parameters for the predefined Pv-aCO_2 groups at both T0 and T6 are shown in the Annex D (Suppl. Table E1).

Microcirculatory blood flow parameters are shown in the Table 2. We observed a progressive lower percentage of small-vessels perfused (PPV) with a subsequent decrease in functional capillary density (FCD) and increased heterogeneity index (HI) at progressive higher Pv-aCO_2 values at both T0 and T6 (Figure 1). Microcirculatory blood flow index (MFI) was also lower at higher Pv-aCO_2 values while total capillary density was normal only in patients with normal Pv-aCO_2 (Annex D, Suppl. Figure E2). We observed a significant agreement between the Pv-aCO_2 and PPV (T0:R^2=0.61, p<0.001; T6:R^2=0.55, p<0.001; Annex D, Suppl. Figure E3). Conversely, we found lack of agreement between the cardiac index (representing the macro blood flow) and the PPV (Annex D, Suppl. Figure E4). Other resuscitation and hemodynamic goals such as SvO_2 and MAP were also poorly correlated to the microcirculatory blood flow (Annex D, Suppl. Figures E5, E6).

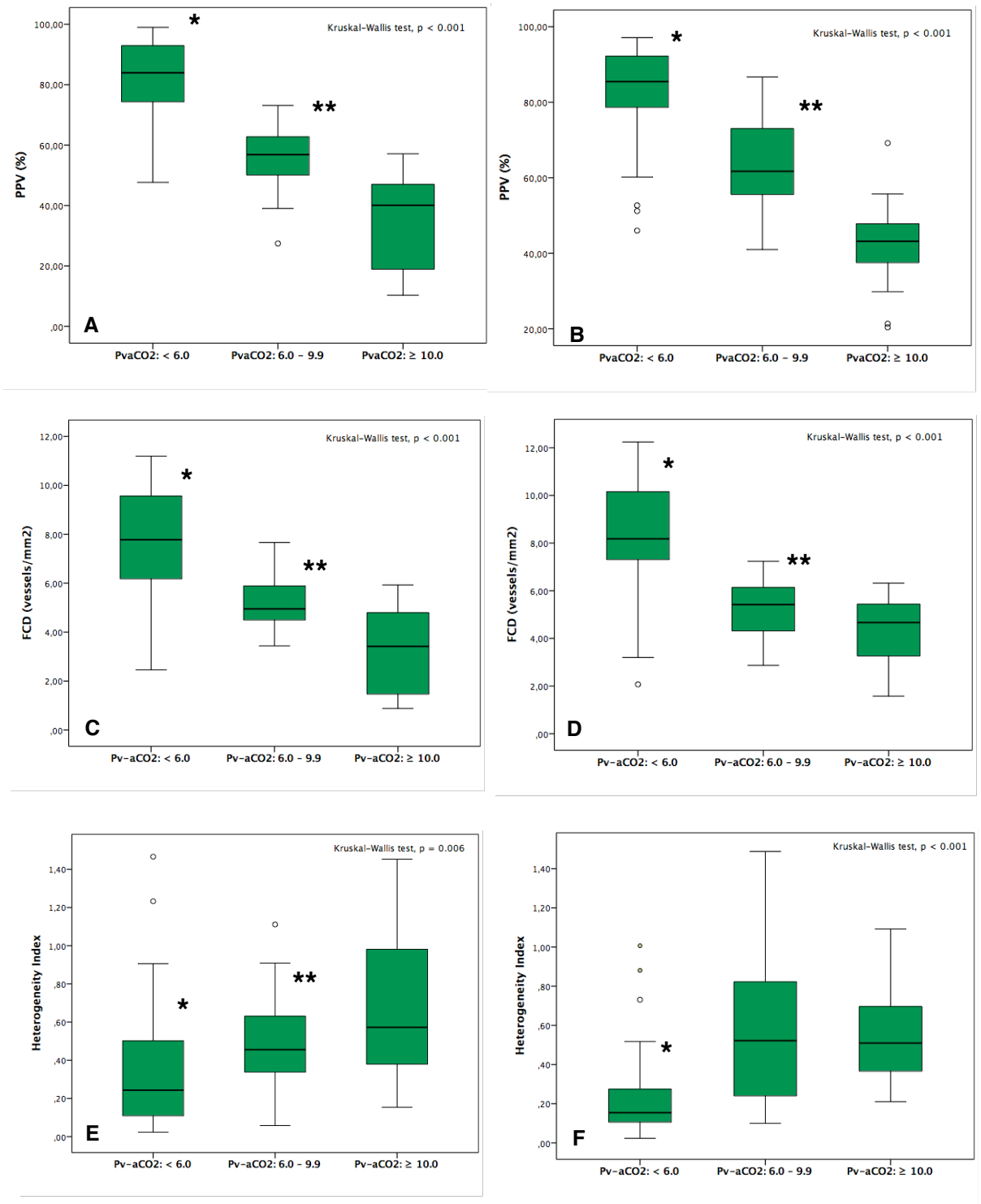
Changes in Pv-aCO_2 values between T0 and T6 were significant related with changes in PPV, while changes in cardiac output were not related to changes in Pv-aCO_2 or PPV (Figure 2; Annex D, Suppl. Figure E7). A multiple linear regression model including all patients revealed that Pv-aCO_2 was independently related to PPV at T0 and T6, while other systemic hemodynamic parameters such as cardiac output, SvO_2 or MAP were not significantly related to microcirculatory blood flow (Table 3). Likewise, when linear regression models were performed separately in patients with SvO_2 < 65% or SvO_2 ≥ 65%, Pv-aCO_2 was again the only variable related to PPV at both T0 and T6 (Table 3).

Even though Pv-aCO_2 was apparently not correlated with cardiac index in this septic shock group (Annex D, Suppl. Figure E8), we observed lower cardiac indexes in predefined groups with abnormal Pv-aCO_2 values at T6 (Annex D, Suppl. Figure E9). Similarly, lower MAP was observed in groups with higher Pv-aCO_2 values at T6 (Annex D, Suppl. Figure E10). We also observed a trend towards lower SvO_2 at higher Pv-aCO_2 values, without achieving significant differences (Annex D, Suppl. Figure E11). Pv-aCO_2 exhibited the highest area under the ROC curve (AUC) for predicting abnormal PPV at different cutoffs at both T0 and T6. Other macro-hemodynamic or tissue

perfusion parameters were not related to PPV (Annex D, Suppl. Figure E12; Annex D, Suppl. Table E2).

Venous-to-arterial carbon dioxide differences calculated in terms of partial pressures ($Pv\text{-}aCO_2$) were significantly related to those calculated by blood contents ($Cv\text{-}aCO_2$) (T0: R^2=0.52, p<0.001; T6: R^2=0.42, p<0.001) (Annex D, Suppl. Figure E13). In addition to the significant relation between PPV and $Pv\text{-}aCO_2$, we also observed a linear relationship between PPV and $Cv\text{-}aCO_2$ (T0:R^2=0.42, p<0.001, T6: R^2=0.32, p<0.001) and between PPV and the $Cv\text{-}aCO_2$ / $Da\text{-}vO_2$ ratio (T0: R^2=0.18, p<0.001) (Figure 3; Annex D, Suppl. Figure E14). When data was analyzed considering the $Pv\text{-}aCO_2$ as dependent variable, PPV was independently related to $Pv\text{-}aCO_2$ (SM-Table E3). Remarkably, cardiac index was also related to $Pv\text{-}aCO_2$ but only in patients with SvO_2 < 65% at T6 (Annex D, Suppl. Table E3).

Figure 1. Percentage of small vessels perfused (PPV), functional capillary density (FCD) and heterogeneity index (HI) for the predefined Pv-aCO_2 groups



Boxplots depicting differences in PPV, FCD and HI for predefined Pv-aCO_2 groups (Group 1: < 6.0 mmHg, Group 2: 6.0 to 9.9 mmHg and Group 3: ≥ 10 mmHg) at both T0 (A) and T6 (B). Kruskal-Wallis test, $p < 0.001$.

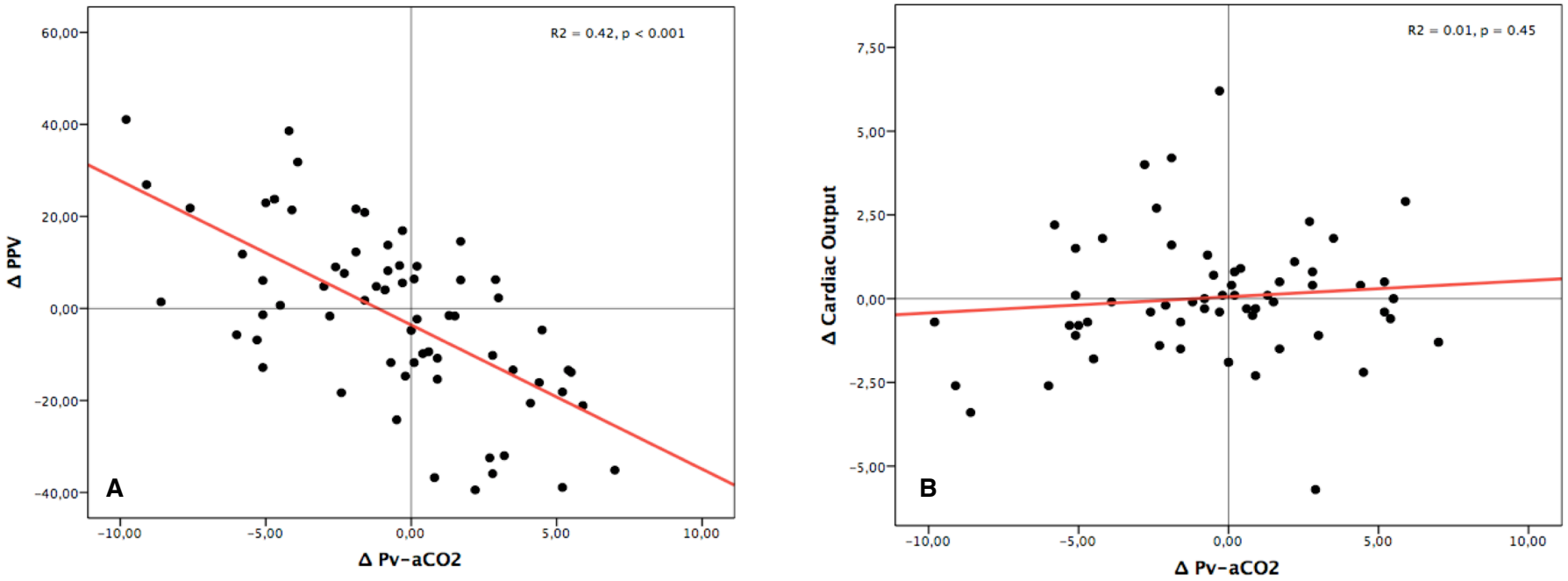
* *post hoc* Mann-Whitney analysis adjusted for multiple comparisons; $p < 0.05$ for Group 1 vs. 2 and 1 vs. 3

** *post hoc* Mann-Whitney analysis adjusted for multiple comparisons; $p < 0.05$ for Group 2 vs. 3.

Boxes denote interquartile range, horizontal line in the boxes represents the median values, and whiskers extend 1.5 times the interquartile range above and below the 25 – 75 percentiles.

PPV: percentage of small vessels perfused; Pv-aCO_2: venous-to-arterial carbon dioxide difference; FCD: Functional capillary density; HI: heterogeneity index

Figure 2. Correlation of changes in Pv-aCO$_2$ vs. changes in PPV and changes in cardiac output between T0 and T6



Panel A: Scatterplot showing the correlation of variations observed between changes in venous-to-arterial CO_2 partial pressure differences (Δ Pv-aCO_2) and changes in percentage of small vessels perfused (Δ PPV) between measurements performed at T0 and T6. R^2=0.42, p<0.001

Panel B: Scatterplot showing the correlation of variations observed between changes in venous-to-arterial CO_2 partial pressure differences (Δ Pv-aCO_2) and changes in cardiac output (Δ cardiac output) between measurements performed at T0 and T6. R^2=0.01, p=0.45

Table 1. General characteristics

	All patients (n = 75)	**Pv-aCO_2 < 6.0 (n = 39)**	**Pv-aCO_2 = 6.0 – 9.9 (n = 25)**	**Pv-aCO_2 ≥ 10.0 (n = 11)**	**p**
Age (years)	67 (58 – 77)	66 (60 – 74)	73 (65 – 81)	54 (43 – 71) *	0.02
Male (%)	45 (60)	23 (59)	15 (60)	7 (64)	0.96
APACHE II	25 (20 – 32)	24 (20 – 28)	28 (20 – 34)	25 (20 – 34)	0.55
SOFA, Day 1	11 (8 – 15)	10 (6 – 15)	11 (9 – 15)	11 (10 – 14)	0.22
Time from first hypotension episode to catheter insertion (hours)	3 (3 – 4)	3(3 – 4)	4 (2 – 4)	3 (2 – 4)	0.69
Fluids before catheter insertion (ml)	1,650 (1,200 – 2,400)	1,600 (1,200 – 2,400)	1,600 (1,025 – 2,300)	2,000 (1,500 – 2,700)	0.66
Temperature (ºC)	37.5 (37.4 – 38.4)	37.4 (37.2 – 38.2)	37.8 (37.6 – 38.4)	37.6 (37.5 – 38.6)	0.54

Source of infection, n (%)					0.25
Pneumonia	30 (40.0)	18 (46.2)	10 (40.0)	2 (18.2)	
Abdominal	17 (22.7)	8 (20.5)	7 (28.0)	2 (18.2)	
Urinary	10 (13.3)	4 (10.3)	3 (12.0)	3 (27.3)	
Soft tissue	2 (2.7)	2 (5.1)	0 (0.0)	0 (0.0)	
No specific site	7 (9.3)	1 (2.6)	3 (12.0)	3 (27.3)	
Other	10 (13.3)	6 (15.4)	2 (8.0)	1 (9.1)	
Medical, n (%) / surgical, n (%)	48 (64.0) / 27 (36.0)	25 (64.1) / 14 (35.9)	15 (60.0) / 10 (40.0)	8 (72.7) / 3 (27.3)	0.76
Mechanical ventilation, n (%)	57 (76.0)	34 (87.2)	24 (96.0)	9 (81.8)	0.37
Renal replacement therapy, n (%)	12 (16.0)	7 (17.9)	3 (12.0)	2 (18.2)	0.80

* $p < 0.05$ for $Pv\text{-}aCO_2 \geq 10.0$ vs. $Pv\text{-}aCO_2 = 6.0 – 9.9$

Values represent median (25^{th} -75^{th} range) or absolute number (%) when indicated

Table 2. Microcirculatory blood flow variables for the predefined Pv-aCO_2 groups

	Pv-aCO_2 < 6.0 (n_{T0} = 39) (n_{T6} = 36)	**Pv-aCO_2 = 6.0 – 9.9** (n_{T0} = 25) (n_{T6} = 23)	**Pv-aCO_2 ≥ 10.0** (n_{T0} = 11) (n_{T6} = 13)
PPV, %			
T0	83.9 (74.2 – 93.2)* †	56.8 (47.9 – 62.9) ‡	40.1 (27.5 – 48.7)
T6	85.5 (77.8 – 92.3)* †	61.7 (54.5 – 74.5) ‡	43.2 (33.7 – 49.6)
LPV, %			
T0	100 (97.0 – 100) * †	96.0 (91.2 – 100)	90.0 (80.0 – 100)
T6	100 (96.0 – 100) †	96.4 (91.7 – 100)	92.5 (72.3 – 98.8)
MFI			
T0	2.4 (2.1 – 2.9)* †	1.6 (1.2 – 1.9)	1.4 (1.1 – 1.9)
T6	2.4 (1.8 – 2.8) * †	1.9 (1.4 – 2.3) ‡	1.3 (1.0 – 1.7)
TCD, n/mm^2			
T0	11.5 (10.3 – 12.5) †	10.8 (10.4 -11.4)	10.2 (9.3 – 11.3)
T6	11.5 (10.3 -13.3) *	10.1 (8.9 – 10.8)	10.8 (9.8- 11.6)
FCD, n/mm^2			
T0	7.8 (6.2 – 9.6) * †	4.9 (4.4 – 5.9) ‡	3.4 (1.4 – 4.9)
T6	8.2 (7.2 – 10.2) * †	5.4 (4.2 – 6.2) ‡	4.7 (3.2 – 5.6)
Heterogeneity			
index	0.24 (0.10 – 0.51) * †	0.46 (0.33 – 0.63)	0.57 (0.29 – 1.04)
T0	0.15 (0.11 – 0.28) * †	0.52 (0.21 – 0.84)	0.54 (0.37 – 0.80)
T6			

* $p<0.05$ Pv-aCO_2 < 6.0 vs. Pv-aCO_2 = 6.0 – 9.9
† $p<0.05$ Pv-aCO_2 < 6.0 vs. Pv-aCO_2 ≥ 10.0
** $p<0.05$ Pv-aCO_2 = 6.0 – 9.9 vs. Pv-aCO_2 ≥ 10.0

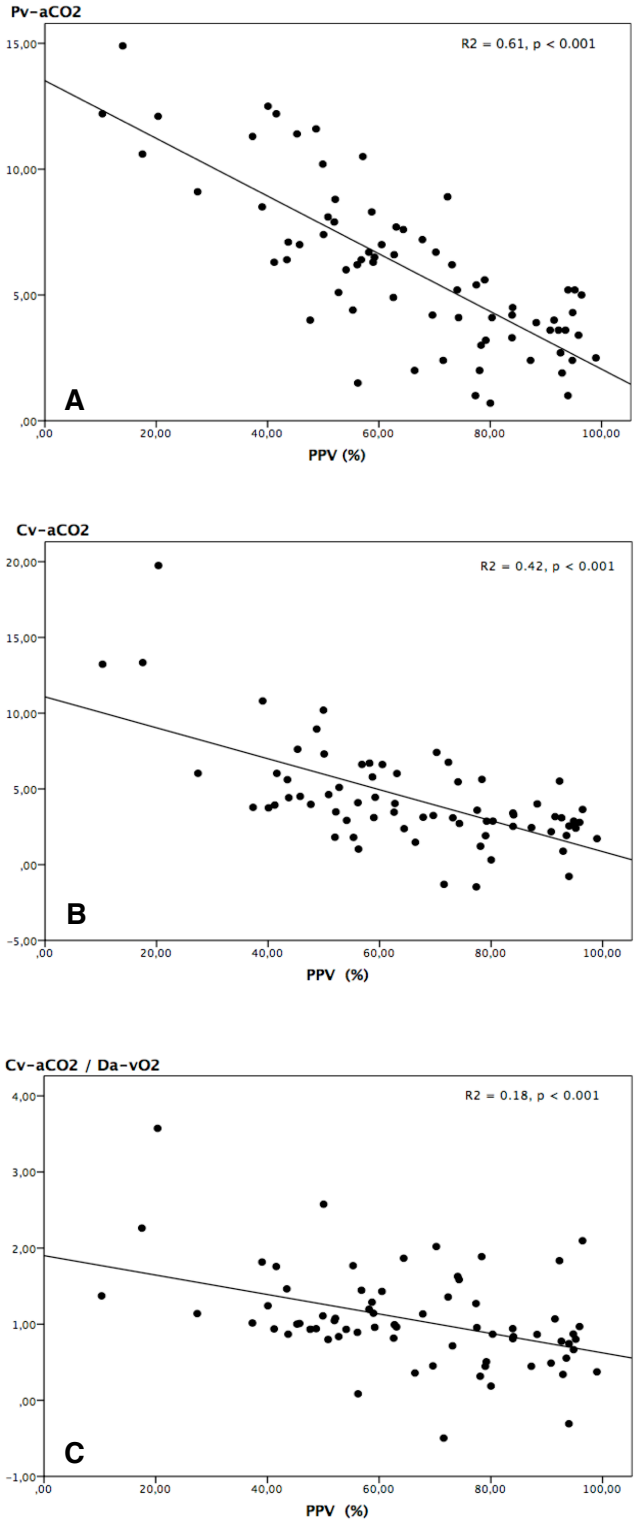
PPV: percentage of small-perfused vessels; LPV: percentage of large-perfused vessels; MFI: microvascular flow index; TCD: total capillary density; FCD: functional capillary density

Table 3. Multiple linear regression models for variables related to the percentage of small-perfused vessels (PPV)

	T0				T6			
	Coefficient	95% CI		p value	Coefficient	95% CI		p value
All patients								
Pv-aCO_2	-5.35	-6.41	-4.29	<0.001	-3.49	-4.43	-2.55	<0.001
SvO_2, %	0.12	-0.16	0.41	0.39	0.05	-0.25	0.35	0.75
CI, L/min	-0.83	-3.47	1.81	0.53	-0.11	-4.29	4.06	0.96
MAP	0.23	-0.04	0.51	0.93	0.30	-0.04	0.64	0.08
R^2 for the model				0.66				0.58
$SvO_2 \geq 65\%$								
Pv-aCO_2	-5.11	-6.46	-3.76	<0.001	-3.92	-5.02	-2.82	<0.001
CI, L/min	-0.31	-3.53	2.91	0.85	2.27	-2.48	7.02	0.34
MAP	0.32	-0.03	0.63	0.06	0.19	-0.21	0.59	0.34
R^2 for the model				0.63				0.61
$SvO_2 < 65\%$								
Pv-aCO_2	-5.91	-7.63	-4.19	<0.001	-3.22	-5.39	-1.05	0.007
CI, L/min	-2.18	-7.37	3.01	0.39	-3.68	-13.43	6.07	0.43
MAP	-0.07	-0.65	0.52	0.82	0.39	-0.35	1.13	0.28
R^2 for the model				0.71				0.57

Pv-aCO_2: venous to arterial carbon dioxide difference; SvO_2: mixed venous oxygen saturation; CI: cardiac index; MAP: mean arterial pressure; R^2: coefficient of determination

Figure 3. Relationships between Pv-aCO_2, Cv-aCO_2 and Cv-aCO_2/Da-$vO_2$with the Percentage of small vessels perfused (PPV) at T0



Scatterplots depicting the relationships between the percentage of small vessels perfused (PPV) and (A) the venous-to-arterial CO_2 partial pressure difference (Pv-aCO_2), (B) the venous-to-arterial CO_2 content difference (Pv-aCO_2), and (C) the venous-to-arterial CO_2 to arterial-venous O_2 content difference ratio (Cv-aCO_2/Da-vO_2).

Coefficient of determination (R^2) was calculated to assess the strength of correlations.

5.6 DISCUSSION

Our observations reveal that Pv-aCO_2 is closely related to microcirculatory blood flow parameters during the early phases of resuscitation of septic shock. Indeed, Pv-aCO_2 was the best predictor of the microvascular blood flow misdistribution as indicated by the alterations in the percentage of small vessels perfused, the heterogeneity blood flow index and consequently, functional capillary density. In addition, changes in Pv-aCO_2 were significantly related to changes in PPV. Meanwhile, Pv-aCO_2 was poorly related with systemic hemodynamic variables.

Microcirculatory dysfunction in septic shock is a generalized phenomenon characterized by decreasing functional capillary density associated to increasing heterogeneity of blood flow consisting in zones with well-perfused vessels adjacent to non-perfused capillaries [21,22]. Those derangements seem to trigger the development of organ dysfunctions since capillary blood flow alterations precede cellular stress [30,31] and hypoxia-inducible gene expression [32]. In fact, microcirculatory alterations are stronger determinants of outcomes than global hemodynamic parameters, with progressive increase in the risk of death in quartiles representing more severe microcirculatory disturbances [33]. Nevertheless, some authors argue against microcirculatory dysfunction as the primary event leading to organ failure since microcirculatory derangements might be coupled with cellular and mitochondrial

dysfunction and merely reflect an adaptive response [34]. Despite controversies about the causal relationship between microcirculatory alterations and organ dysfunction, the quest for appropriate monitoring microcirculation could potentially improve resuscitation results in septic shock. However, despite of advances in techniques to evaluate microcirculation at the bedside, an immediate and reliable method to quantify microcirculatory alterations is not yet available [35]. In our study, Pv-aCO_2 was independently related to PPV (and consequently, to FCD and MFI) at both T0 and T6 and this relationship was also maintained when we evaluated separately patients with SvO_2 < or ≥ 65%. Furthermore, Pv-aCO_2 was better at predicting abnormal PPV values at different cutoffs, and was superior to macro hemodynamic or oxygen metabolism goals. Similarly, Pv-aCO_2 >6.0 was related with an increased heterogeneity index (HI) and therefore a decreased functional capillary density (FCD). In normal conditions, the heterogeneity of microvascular blood flow is negligible [36], and matching of perfusion to metabolism usually improves during hypoxic or low flow states [37]. However, increases in the heterogeneity of the microcirculatory blood flow with the subsequent reduction in the functional capillary density could be responsible for altered oxygen extraction capabilities in sepsis [38]. In fact, the heterogeneous flow cessation of individual capillaries could be an important factor determining the oxygen supply dependence phenomenon during the most severe cases of septic shock [38,39] and this heterogeneity of microvascular blood flow could be tracked by Pv-aCO_2 variations during the early stages of resuscitation. In fact, changes observed in Pv-aCO_2 between T0 and T6 were significantly related to those exhibited by PPV during the same time interval.

It has been suggested that microcirculatory derangements can occur in absence of macro-hemodynamic derangements even when global oxygen delivery seems adequate [21,40]. However, other small-size studies have insinuated that microcirculatory impairment is related to decreasing mean arterial pressure or increasing dose of vasopressors [41]. Our data also suggest some independence between

microcirculatory variables and macro-hemodynamics, which is in line with previous observations [32, 39]. Indeed, changes exhibited by Pv-aCO_2 between T0 and T6 were not related to variations in cardiac output. Nevertheless, patients with a Pv-$aCO_2 \geq 6.0$ exhibited a trend in lower CI and MAP values than those with Pv-$aCO_2 < 6.0$. Furthermore, CI was significant but weakly related to Pv-aCO_2 in patients with $SvO_2 <$ 65% but not in those with normal SvO_2.

Pv-aCO_2 can also be influenced by the CO_2 dissociation curve. In this regard, we observed a good agreement between Pv-aCO_2 and the venous-to-arterial CO_2 content difference (Cv-aCO_2), especially at normal ranges of Pv-aCO_2 (i.e., Pv-$aCO_2 < 6.0$ mmHg). Interestingly, Cv-aCO_2 was mostly dispersed at higher Pv-aCO_2 values, suggesting a deeper impact of the Haldane effect at abnormal Pv-aCO_2 values. Similarly, difference between slopes described by the linear regression of PPV vs. Pv-aCO_2 and PPV vs. Cv-aCO_2 relationships should also be influenced by Haldane effect. Even though, the also significant relationship between PPV vs. Cv-aCO_2 suggests that CO_2 accumulation is, at least in part, related to the abnormal microcirculatory blood flow occurring during inflammatory conditions.

It has been recently suggested that combination of venous-to-arterial CO_2 content to arterial-venous O_2 difference ratio (Cv-aCO_2/Da-vO_2) can add prognostic value to lactate measurements during early stages of septic shock [42] since it could be used as a surrogate for the VCO_2/VO_2 ratio (i.e., the respiratory quotient), to potentially detect non-aerobic CO_2 generation [43]. We explored the association between the Cv-aCO_2/Da-vO_2 ratio and microcirculatory alterations, and although we found a significant correlation, the strength of such relationship was notoriously weaker than that observed between Pv-aCO_2 (or even Cv-aCO_2) and PPV. We acknowledge that Cv-aCO_2/Da-vO_2 ratio could be more subjected to error calculations than Pv-aCO_2, but differences in the slope of the Cv-aCO_2/Da-vO_2 ratio vs. PPV compared with the Pv-aCO_2 vs. PPV relationship, suggests the involvement of other physiological variables different to microcirculatory blood flow. Indeed, Cv-aCO_2/Da-vO_2 ratio was

abnormally increased in a number of patients through a wide range of PPV values. Therefore, it is not surprising that Cv-aCO_2/Da-vO_2 ratio be superior to Pv-aCO_2/Da-vO_2 ratio at the clinical setting [41] since under hypoxic conditions, disparity between Pv-aCO_2 and Cv-aCO_2 increases, and when coupling Pv-aCO_2 to different DO_2/VO_2 values, pH, temperature and hemoglobin levels, the Pv-aCO_2/Da-vO_2 may acquire different cutoff normality values (a situation obviated when the numerator is corrected with the determinants of Haldane effect).

We recognize limitations to our study. First, we used mixed-venous blood to assess Pv-aCO_2 gradients, thus our results may not apply to central-venous blood Pv-aCO_2 gradients. Indeed, even though a reasonable agreement is often reported in trials comparing both sampling sites [44], significant discrepancy can sometimes be observed and should not be neglected. Of note, this concordance between sampling sites has mostly be evaluated for SvO_2 more than for PCO_2 gradients. Second, variations of Pv-aCO_2, macro hemodynamics and microcirculatory variables between T0 and T6 were not the result of targeted interventions but simply changing during resuscitation. Nevertheless, relationships exhibited between Pv-aCO_2 and PPV allows hypothesize that some specific therapeutic interventions modifying microcirculation might reliable be tracked by Pv-aCO_2. Third, the relationship observed between microcirculatory variables and Pv-aCO_2 does not establish a causal association even though the physiological reasoning leading to our conclusions seems logical. Finally, there was no pre-experimental statistical plan published in a pre-specified registry or a journal. However, observational research is, by nature, exploratory and requires considerable flexibility to explore novel findings and unexpected signals in the data [45,46]

5.7 CONCLUSION

Increased Pv-aCO_2 values are associated with microcirculatory dysfunction in septic shock, even when SvO_2 is within normal values. Whether changes in Pv-aCO_2 can be used to track changes in microvascular perfusion remains to be determined.

5.8 REFERENCES

1. Vincent JL, De Backer D. Circulatory shock. *N Engl J Med.* 2013;369(18):1726-1734.
2. Cecconi M, De Backer D, Antonelli M, et al. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. *Intensive Care Med.* 2014;40(12):1795-1815.
3. Bellomo R, Reade MC, Warrillow SJ. The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. *Crit Care.* 2008;12(2):130.
4. Yealy DM, Kellum JA, Huang DT, et al. A randomized trial of protocol-based care for early septic shock. *N Engl J Med.* 2014;370(18):1683-1693.
5. Peake SL, Bailey M, Bellomo R, et al. Australasian resuscitation of sepsis evaluation (ARISE): A multi-centre, prospective, inception cohort study. *Resuscitation.* 2009;80(7):811-818.
6. Mouncey PR, Osborn TM, Power GS, et al. Trial of early, goal-directed resuscitation for septic shock. *N Engl J Med.* 2015;372(14):1301-1311.
7. van Beest PA, Hofstra JJ, Schultz MJ, Boerma EC, Spronk PE, Kuiper MA. The incidence of low venous oxygen saturation on admission to the intensive care unit: a multi-center observational study in The Netherlands. *Crit Care.* 2008;12(2):R33.
8. Ospina-Tascón GA, Bautista-Rincón DF, Umaña M, et al. Persistently high venous-to-arterial carbon dioxide differences during early resuscitation are associated with poor outcomes in septic shock. *Crit Care.* 2013;17(6):R294.
9. van Beest PA, Lont MC, Holman ND, Loef B, Kuiper MA, Boerma EC. Central venous-arterial pCO_2 difference as a tool in resuscitation of septic patients. *Intensive Care Med.* 2013;39(6):1034-1039.
10. Vallée F, Vallet B, Mathe O, et al. Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? *Intensive Care Med.* 2008;34(12):2218-2225.
11. Grundler W, Weil MH, Rackow EC. Arteriovenous carbon dioxide and pH gradients during cardiac arrest. *Circulation.* 1986;74(5):1071-1074.
12. Weil MH, Rackow EC, Trevino R, Grundler W, Falk JL, Griffel MI. Difference in acid-base state between venous and arterial blood during cardiopulmonary resuscitation. *N Engl J Med.* 1986;315(3):153-156.
13. Zhang H, Vincent JL. Arteriovenous differences in PCO2 and pH are good indicators of critical hypoperfusion. *Am Rev Respir Dis.* 1993;148(4 Pt 1):867-871.
14. Van der Linden P, Rausin I, Deltell A, et al. Detection of tissue hypoxia by arteriovenous gradient for PCO2 and pH in anesthetized dogs during progressive hemorrhage. *Anesth Analg.* 1995;80(2):269-275.
15. Groeneveld AB, Vermeij CG, Thijs LG. Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end-expiratory pressure in the pig. *Anesth Analg.* 1991;73(5):576-582.
16. Teboul JL, Mercat A, Lenique F, Berton C, Richard C. Value of the venous-arterial PCO2 gradient to reflect the oxygen supply to demand in humans: effects of dobutamine. *Crit Care Med.* 1998;26(6):1007-1010.
17. Bakker J, Vincent JL, Gris P, Leon M, Coffernils M, Kahn RJ. Veno-arterial carbon dioxide gradient in human septic shock. *Chest.* 1992;101(2):509-515.

18. Mecher CE, Rackow EC, Astiz ME, Weil MH. Venous hypercarbia associated with severe sepsis and systemic hypoperfusion. *Crit Care Med.* 1990;18(6):585-589.
19. Vallet B, Teboul JL, Cain S, Curtis S. Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol.* 2000;89(4):1317-1321.
20. Dubin A, Estenssoro E, Murias G, et al. Intramucosal-arterial Pco2 gradient does not reflect intestinal dysoxia in anemic hypoxia. *J Trauma.* 2004;57(6):1211-1217.
21. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med.* 2002;166(1):98-104.
22. De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent JL. Monitoring the microcirculation in the critically ill patient: current methods and future approaches. *Intensive Care Med.* 2010;36(11):1813-1825.
23. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med.* 2004;32(9):1825-1831.
24. Creteur J, De Backer D, Sakr Y, Koch M, Vincent JL. Sublingual capnometry tracks microcirculatory changes in septic patients. *Intensive Care Med.* 2006;32(4):516-523.
25. Dubin A, Edul VS, Pozo MO, et al. Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. *Crit Care Med.* 2008;36(2):535-542.
26. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control.* 2008;36(5):309-332.
27. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med.* 2003;31(4):1250-1256.
28. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med.* 2013;41(2):580-637.
29. De Backer D, Hollenberg S, Boerma C, et al. How to evaluate the microcirculation: report of a round table conference. *Crit Care.* 2007;11(5):R101.
30. Wu L, Tiwari MM, Messer KJ, et al. Peritubular capillary dysfunction and renal tubular epithelial cell stress following lipopolysaccharide administration in mice. *Am J Physiol Renal Physiol.* 2007;292(1):F261-268.
31. Fink T, Heymann P, Taha-Melitz S, et al. Dobutamine pretreatment improves survival, liver function, and hepatic microcirculation after polymicrobial sepsis in rat. *Shock.* 2013;40(2):129-135.
32. Bateman RM, Tokunaga C, Kareco T, Dorscheid DR, Walley KR. Myocardial hypoxia-inducible HIF-1alpha, VEGF, and GLUT1 gene expression is associated with microvascular and ICAM-1 heterogeneity during endotoxemia. *Am J Physiol Heart Circ Physiol.* 2007;293(1):H448-456.
33. De Backer D, Donadello K, Sakr Y, et al. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. *Crit Care Med.* 2013;41(3):791-799.
34. Moore JP, Dyson A, Singer M, Fraser J. Microcirculatory dysfunction and resuscitation: why, when, and how. *Br J Anaesth.* 2015;115(3):366-375.
35. Ince C. The rationale for microcirculatory guided fluid therapy. *Curr Opin Crit Care.* 2014;20(3):301-308.
36. Zuurbier CJ, van Iterson M, Ince C. Functional heterogeneity of oxygen supply-consumption ratio in the heart. *Cardiovasc Res.* 1999;44(3):488-497.

37. Stein JC, Ellis CG, Ellsworth ML. Relationship between capillary and systemic venous PO2 during nonhypoxic and hypoxic ventilation. *Am J Physiol.* 1993;265(2 Pt 2):H537-542.
38. Goldman D, Bateman RM, Ellis CG. Effect of decreased O2 supply on skeletal muscle oxygenation and O2 consumption during sepsis: role of heterogeneous capillary spacing and blood flow. *Am J Physiol Heart Circ Physiol.* 2006;290(6):H2277-2285.
39. Walley KR. Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. *J Appl Physiol.* 1996;81(2):885-894.
40. De Backer D, Creteur J, Dubois MJ, et al. The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med.* 2006;34(2):403-408.
41. Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. *Crit Care Med.* 2012;40(5):1443-1448.
42. Ospina-Tascon GA, Umana M, Bermudez W, et al. Combination of arterial lactate levels and venous-arterial CO2 to arterial-venous O 2 content difference ratio as markers of resuscitation in patients with septic shock. *Intensive Care Med.* 2015;41(5):796-805.
43. Jakob SM, Groeneveld AB, Teboul JL. Venous-arterial CO2 to arterial-venous O2 difference ratio as a resuscitation target in shock states? *Intensive Care Med.* 2015;41(5):936-938.
44. Cuschieri J, Rivers EP, Donnino MW, et al. Central venous-arterial carbon dioxide difference as an indicator of cardiac index. *Intensive Care Med.* 2005;31(6):818-822.
45. Vandenbroucke JP. Observational research, randomised trials, and two views of medical science. *PLoS Med.* 2008;5(3):e67.
46. Pearce N. Registration of protocols for observational research is unnecessary and would do more harm than good. *Occup Environ Med.* 2011;68(2):86-88.

Chapter 6

THE KEY ROLE OF THE HETEROGENEITY OF MICROVASCULAR BLOOD FLOW ON THE TISSUE OXYGEN EXTRACTION AND THE LINK WITH PCO_2 GAPS:

THE ANIMAL EXPERIMENTAL MODEL

PAPER No. 5 (Original paper)

EFFECTS OF DOBUTAMINE ON INTESTINAL MICROVASCULAR BLOOD FLOW HETEROGENEITY AND OXYGEN EXTRACTION IN A HYPODYNAMIC SEPTIC SHOCK MODEL

(Submitted)

ABSTRACT

Introduction: Derangements on microvascular blood flow distribution have been implied on impaired of oxygen extraction by peripheral tissues. We evaluated the effects of dobutamine on the microvascular blood flow heterogeneity at intestinal mucosa and its relationship with mesenteric oxygen extraction, in a severe hypodynamic septic shock model.

Methods: Fifteen landrace pigs were anesthetized and mechanically ventilated. Catheters were inserted into carotid artery, pulmonary artery and portal vein for blood sampling, and for invasive pressures and cardiac output monitoring. A gas-tonometer was placed into jejunum for mucosal-CO_2 determination while a jejunostomy was prepared to visualize gut-mucosal and serosa microcirculation. At each measurement time-point, arterial, mesenteric-venous and mixed-venous blood samples were obtained for blood gases analysis. Simultaneously, images of sublingual and intestinal microcirculation were acquired using the Side-stream dark Field technique. Video-sequences thus obtained were stored under a random number for ulterior blinded analysis. After baseline measurements (BL), fecal peritonitis was induced. When hypotension was not responsive to fluid resuscitation, norepinephrine was started and shock was declared (TS). Then, the pigs were randomly assigned to a fixed-dose of dobutamine at 5 μg/kg/min (n=6) or placebo (n=6). New measurements were performed 2 (T2H) and 6 hours (T6H) afterwards. Three sham animals subjected to identical monitoring served as time-matched controls.

Results: We observed a significant decrease in the proportion of intestinal-villi perfused (%villi-PPV) with increasing mesenteric oxygen extraction ratio (mes-ERO_2) at TS, in both dobutamine and placebo groups. Cardiac output and oxygen delivery

behaved similarly in both experimental groups. After starting resuscitation, %villi-PPV increased significantly in dobutamine group while mes-ERO_2 decreased, also significantly (Friedman repeated-measures analysis of variance, p=0.01 and p=0.04 for time*group interactions for %villi-PPV and mes-ERO_2, respectively). Variations of %villi-PPV between BL and each measurement time-point (BL-to-TS; BL-to-T2H; BL-to-T6H) exhibited good agreement with variations in mesenteric oxygen extraction ratio (R^2=0.67, p<0.001). Mesenteric lactate levels were also well correlated with variations in %villi-PPV (Spearman-Rho test, R^2=0.69, p<0.001).

Conclusion: Dobutamine improved gut microvascular blood flow heterogeneity during severe hypodynamic septic shock. Decreasing flow heterogeneity was associated with improved in regional oxygen extraction, suggesting the crucial role of microvascular blood flow distribution on oxygen consumption in sepsis.

Key words: Septic shock; microcirculation; microcirculatory blood flow; villi perfusion; oxygen extraction ratio; oxygen consumption; gut mucosal perfusion

RESUMEN

Introducción: Las alteraciones de la distribución del flujo sanguíneo microvascular han sido implicadas con un empeoramiento de la extracción de oxígeno por parte de los tejidos periféricos. Nosotros evaluamos los efectos de la dobutamina sobre la heterogeneidad del flujo sanguíneo microvascular en la mucosa intestinal y su relación con la extracción mesentérica de oxígeno en un modelo de shock séptico severamente hipodinámico.

Métodos: Quince cerdos de raza Landrace fueron anestesiados y sometidos a ventilación mecánica. Se insertaron catéteres en la arteria carótida, arterial pulmonar y vena porta para la toma de muestras sanguíneas y para medición de presiones invasivas y monitoria del gasto cardiaco. Un tonómetro gaseoso fue localizado en el yeyuno para la determinación del CO_2 mucoso, mientras que se preparó una yeyunostomía para visualizar directamente la microcirculación mucosa y serosa durante el resto del experimento. En cada momento de medición, nosotros obtuvimos muestra de sangre arterial, venosa mesentérica y venosa mixta para el análisis de gases sanguíneos. Simultáneamente, se obtuvieron imágenes de la microcirculación sublingual e intestinal, utilizando la técnica *Side-stream dark field* (SDF). Las secuencias de video obtenidas fueron almacenadas bajo un número aleatorio para un análisis cegado y ulterior de las mismas. Después de las mediciones basales (BL), se indujo peritonitis. Cuando la hipotensión no respondió más a la infusión de líquidos, se inició soporte con norepinefrina y se declaró la aparición del shock (TS). Entonces, los cerdos fueron asignados aleatoriamente al uso de una dosis fija de dobutamina a 5 ás a la infusión de líquidos, se inició soporte con norepinefrina y se declaró la aparición del shock (TS). Entonces, los cerdos fueron asignados aleatoriamente al uso de una dosis fija de dobutamina a 5 μg/kg/min (n=6) or placebo (n=6). Tres animales

"sham" sometidos a monitoría idéntica, sirvieron como controles ajustados por tiempo.

Resultados: Observamos una disminución significativa de la proporción de vellosidades intestinales perfundidas (%villi-PPV) junto a un incremento de la tasa de extracción de oxígeno mesentérico (mes-ERO_2) en el TS, en los grupos dobutamina y placebo. El comportamiento del gasto cardiaco y el transporte de oxígeno fue similar en ambos grupos experimentales. Después del inicio de la reanimación, el %villi-PPV se incrementó de manera significativa mientras que la mes-ERO_2 disminuyó también significativamente (Análisis de varianza - mediciones repetidas de Friedman, p=0.01 and p=0.04 para interacciones tiempo*grupo de %villi-PPV y mes-ERO_2, respectivamente). Las variaciones del %villi-PPV entre el BL y cada punto de medición (BL a TS; BL a T2H; BL a T6H) mostraron una buena concordancia con las variaciones en la tasa de extracción de oxígeno mesentérico (R^2=0.67, p<0.001). Los niveles de lactato también se correlacionaron muy bien con las variaciones en el %villi-PPV (Spearman-Rho test, R^2=0.69, p<0.001).

Conclusión: La dobutamina mejora la heterogeneidad del flujo sanguíneo microvascular durante condiciones de shock séptico hipodinámico severo. La disminución de la heterogeneidad de flujo estuvieron asociadas con una mejoría en la extracción regional de oxígeno, sugiriendo un papel crucial de la heterogeneidad de la distribución del flujo microvascular en el consumo de oxígeno durante la sepsis.

Palabras clave: Shock séptico; microcirculación; flujo sanguíneo microcirculatorio; perfusión vellosa intestinal; tasa de extracción de oxígeno; consumo de oxígeno; perfusión mucosa intestinal

6.2 INTRODUCTION

Hypoperfusion due to macro and microcirculatory blood flow alterations is a key factor in the development of multi-organ failure (MOF) in septic shock [1]. The gastrointestinal tract is particularly prone to be affected during low flow states [2,3], whereby, it has been hypothesized that improvement of splanchnic perfusion prevents the progression of shock. Dobutamine is a synthetic catecholamine capable of increasing cardiac output and oxygen delivery, but its impact on regional splanchnic and intestinal microcirculation remain controversial, with favorable [4-8], unchanged [9] or even negative effects have been described [10,11] on splanchnic macrovascular blood flow and there have been descriptions of apparently beneficial [12] or unaffected [13] total microcirculatory blood flow at intestinal mucosa [12,13] and other intraabdominal organs [13]. Despite these different results on macro or microvascular blood flow, dobutamine has been shown to have favorable effects on intestinal oxygenation [14], although mucosal oxygenation might in turn be dissociated from splanchnic oxygen delivery when dobutamine is infused [7,15], which suggest microcirculatory blood flow distribution alterations more than total microcirculatory flow reduction.

Microcirculatory blood flow is profoundly altered in sepsis, with decreased functional capillary density and increased blood flow heterogeneity as their main features [16,17]. Theoretically, oxygen delivery heterogeneity impairs tissue oxygen extraction by peripheral tissues [18], a condition demonstrated in ex-vivo intestinal tissue from endotoxemic pigs [19]. Consequently, the misdistribution of microvascular blood flow could leads to oxygen capillary extraction derangements during sepsis [20] , so that heterogeneous flow cessation of individual capillaries could determine oxygen supply dependence during the most severe cases of septic shock [21]. Interestingly, persistent villi hypoperfusion might be detected even after restoring mean arterial pressure and

mesenteric arterial blood flow by colloid resuscitation during endotoxemia [22]. On the other hand, low fixed doses of dobutamine demonstrated favorable effects on sublingual microcirculatory blood flow distribution in patients with septic shock [23] and maintained villus blood flow in normotensive endotoxemic rats [24]. Others have not confirmed such beneficial effects [25,26]. Thus, there is not widely available information on the link between microcirculatory blood flow distribution and tissue oxygen consumption at intestinal level during resuscitation in septic shock.

6.3 OBJECTIVE

To evaluate the effects of dobutamine on intestinal microcirculatory alterations, focusing on the blood flow distribution heterogeneity at intestinal mucosa and its impact on regional oxygen extraction ratio in a septic shock model of fecal peritonitis subjected to hemodynamic goal-directed fluid resuscitation, hypothesizing that variations in microcirculatory heterogeneity are closely related to changes in local oxygen consumption independently of macro hemodynamic changes.

6.4 METHODS

6.4.1 Animal preparation

Our institution´s Animal Research Committee (CIECUAE) approved the present study (Res. 001-12). Fifteen female Landrace pigs (35 – 42 kg) were kept fasting for a 12 hours period but with free access to water. After this pre-conditioning period, they were initially sedated with intramuscular injections of ketamine (5 - 10 mg/kg) and xylazine (0.1 mg/kg) and translated from the bioterio to the laboratory (ANNEX E). Then, a venous-access in the ear (Insyte Autoguard, Infusion therapy system; Sandy, Utah, U.S.A.) was inserted for medicament and fluids administration. After intravenous dose of propofol (2 - 4 mg/kg) and fentanyl (5 μgr/kg), an endotracheal

tube and mainstream volumetric capnography were placed (Infinity $EtCO_2$ + respiratory mechanics module, Dräger Medical Systems, Telford, USA). Animals were connected to mechanical ventilation (Siemens Servo 900C, Engström – Solna, Sweeden) in assist control mode, tidal volume of 12 ml/kg and adjusting volume minute to maintain arterial PCO_2 at 38 ± 5 torr. Anesthesia was maintained throughout the experiment with midazolam (3 – 5 μgr/kg/min), fentanyl (0.03 – 0.05 μgr/kg/min) and propofol (50 μgr/kg/min). Muscular paralysis was also provided with pancuronium bromide (5 μgr/kg/min) during the entire experiment. Neck vessels were accessed by direct dissection and catheters were inserted into the carotid artery (Single lumen central venous 7-Fr catheter; Arrow International Inc. Bernville Road, Pennsylvania, USA) for monitoring arterial pressure and blood sampling and through the left internal jugular vein for fluids infusion. We also advanced a continuous-cardiac-output (CCO) pulmonary artery catheter (7.5-Fr, Edwards Swan-Ganz CCO, Baxter Edwards Critical Care, Irvine, CA, USA) via the right internal jugular vein to measure pulmonary arterial pressures and continuous cardiac output as well as to withdraw mixed-venous blood samples. Core temperature was continuously monitored by a thermistor in the pulmonary artery catheter and external heating was used to maintain a central temperature of 36.5 ± 1ºC. Continuous electrocardiographic, pulsioximetry and invasive pressures were also provided throughout the experiment (Drägger Infinity Vista XL, Drägger Medical system. Lübeck, Germany).

A midline laparotomy was performed and a gas-tonometer catheter (TRIP Tonometry Catheter, 8-Fr. Tonometrics Division, Instrumentarium; Helsinki, Finland) was placed into jejunal lumen at 50 cm beyond the Treitz angle for local tissue CO_2 and pHi measurements. A double-lumen catheter (Two lumen central venous 7-Fr gauge catheter; Arrow International Inc. Bernville Road, Pennsylvania. USA) was inserted in the superior mesenteric vein through the splenic vein after splenectomy and local constriction with epinephrine, ensuring its position under echography guide. An infusion with SS0.9% through this catheter was provided during the experiment to

ensure its permeability. A surgical cystostomy was also done to monitor urinary output. Cecal ligation and puncture with 16-gauge needle followed by peritoneal contamination (1.5 gr/kg of feces) was performed in all study models (except for sham animals). Finally, a loop of jejunum was cautiously exteriorized through the midline incision, and a small segment was opened along its antimesenteric border using an electrocautery. After careful hemostasis, the abdominal contents were returned to the cavity and the abdomen was closed keeping out the jejunostomy loop and covering it with a moistened compresses and anti-adherent bag to avoid heat and fluid loss.

6.4.2 General monitoring

Mean arterial pressure was monitored throughout the experiment and pulse pressure variations (dPP) were calculated during the respiratory cycle as: PPinsp – PPexp / (PPinsp + PPexp / 2) (Drägger Infinity Vista XL, Drägger Medical system. Lübeck, Germany). Mean pulmonary artery, central venous and pulmonary arterial occlusion pressures were measured at the end of expiration and referenced to the mid-chest level. Cardiac output was continuously measured by thermodilution principle using a thermal filament on the pulmonary artery catheter (Vigilance, Baxter - Edwards Critical Care, Irvine, CA, USA). A mainstream capnography and respiratory module (Dräger Medical Systems, Telford, USA) was used to measure airway and alveolar dead-space fraction, end-tidal CO_2 and complete respiratory mechanics.

6.4.3 Calculation of carbon dioxide and oxygen variables

Simultaneous arterial (a), mixed-venous ($\bar{v}$) and mesenteric-venous (mes) blood samples were withdrawn at each measurement time-point for determination of blood gases (The Alere Epoc® blood analysis system. Alere Inc., Waltham, MA. USA), hemoglobin concentration and lactate concentration (The Alere Epoc® blood analysis

system. Alere Inc., Waltham, MA. USA). Concurrent mucosal jejunal CO_2 was measured by gas-tonometry (The Tonocap®, Datex-Ohmeda; Tonometrics Division, Instrumentarium Corp. Helsinki, Finland) as described elsewhere [27]. Oxygen and CO_2 parameters were calculated as follows:

- $CaO_2 = (Hg \times SaO_2 \times 1.34) + (PaO_2 \times 0.003)$
- $CvO_2 = (Hg \times SvO_2 \times 1.34) + (PvO_2 \times 0.003)$
- $Da\text{-}vO_2 = CaO_2 - CvO_2$
- $DO_2 = CaO_2 \times CO$
- $VO_2 = (CaO_2 - CvO_2) \times CO$
- $ERO_2 = (CaO_2 - CvO_2) / CaO_2$
- $mes\text{-}ERO_2 = (CaO_2 - CvmesO_2) / CaO_2$
- Mixed-venous-to-arterial CO_2 difference ($P\bar{v}\text{-}aCO_2$)= $P\bar{v}CO_2 - PaCO_2$
- Mesenteric-venous-to-arterial CO_2 difference ($Pmes\text{-}aCO_2$)= $PmesCO_2 - PaCO_2$
- Jejunum mucosal-to-arterial CO_2 difference ($Ptis\text{-}aCO_2$)= $PtisCO_2 - PaCO_2$

Where, CaO_2 and CvO_2 are the arterial and venous O_2 content while PaO_2 and PvO_2 represent their arterial and venous partial pressures respectively, while CO represents the cardiac output. ERO_2 and $mes\text{-}ERO_2$ represent the total and mesenteric oxygen extraction ratio, respectively.

6.4.4 Microcirculatory measurements

We used a Sidestream dark-field (SDF) imaging device (Micro Scan; MicroVision Medical, Amsterdam, the Netherlands) to explore microcirculation at each time-point of measurements. The SDF device was softly applied to the jejunal mucosa and adjacent serosa after gentle washing with warm water. Each block of measurements consisted in the recording of five sequences of video of 30 seconds each from different adjacent mucosa areas using a videocard (MicroVideo; Pinnacle system, Mountain Views, CA, U.S.A.). These video-sequences were stored under a random number for

ulterior analysis by two investigators blinded to the origin of sequences (E.Q and G.O.T.). For mucosal microcirculation analysis, the number of villi in each image were counted and individual villi microcirculation was semi-quantitatively classified according to its predominant blood flow as: normal-perfused (continuous blood flow), hypoperfused (intermittent or sluggish blood flow) or non-perfused (stopped blood flow). Thus, we quantified the percentage of normal-perfused villi in each video-sequence at each time-point of measurement. Meanwhile, for serosa and sublingual microcirculation analysis, we used a cutoff value of 20 μm to classify the vessels as large or small. Microvessels with continuous flow were considered as normal while sluggish, intermittent and stopped flows were considered as abnormal. Serosa and sublingual microcirculatory blood flow were evaluated according to the consensus for the evaluation of microcirculation [28] and we calculated the proportion of small-perfused vessels (< 20μm diameter), the heterogeneity index (HI), the total vascular density (all vessels) and the functional capillary density (vessels < 20μm diameter). The intra and inter-observer variability were determined by using five sequences analyzed five times at eight-week intervals by two observers (E.Q. and G.O.T.). We calculated the intra-observer and inter-observer coefficient of variability for both total number of vessels and the proportion of perfused vessels.

6.4.5 Experimental protocol

After the initial preparation, a stabilization period of 30 minutes was ensured and baseline measurements (BL) were performed. Then, animals were randomly allocated to dobutamine (n=6), placebo (n=6) or sham group (n=3). Cecal ligation and puncture followed by peritoneal contamination was performed in the dobutamine and placebo models. All animals received intravenous Lactate Ringer infusion at 3 cc/kg during the entire experiment. When hypotension developed and it was not corrected by crystalloid boluses of at least 30 cc/kg, norepinephrine (NE) infusion was started and titrated to maintain mean arterial pressure (MAP) > 65 mmHg. Once NE dose

remained stable for at least 30 minutes, time-shock (TS) was declared and a new set of measurements was done. At this moment, resuscitation was started using successive fluid loads of crystalloid (at 10 cc/kg) guided by dynamic predictors of fluid responsiveness in order to optimize cardiac output. Concomitantly, fixed doses of dobutamine (5 μgr/kg/min) or placebo (SS0.9% isovolumetric dose) were started until the end of the experiment. A new set of measurements was performed two-hours (T2) and six-hours (T6). Sham animals received identical monitoring than the other groups and measurements were performed after initial preparation (BL), 12, 14 and 18 hours after. Euthanasia was performed at the end of the experiment using Euthanex® according to the local regulations for animal research. More detailed information about the experimental protocol is presented in ANNEX E.

6.4.6 Statistical analysis

Data are reported as medians and interquartile range. Intragroup differences were evaluated using the repeated-measures Friedman analysis of variance on ranks with subsequent Student-Newman-Keuls test for multiple comparisons. Meanwhile, Mann-Whitney rank sum for unpaired samples test was used to test intergroup differences. Relationship between microcirculatory and oxygen extraction were tested using the Spearman-Rho test while coefficient of determination (R^2) was calculated to establish the strength of such associations. A p value ≤ 0.05 (2-tailed) was considered significant.

6.5 RESULTS

6.5.1 Systemic hemodynamics and oxygen transport

General hemodynamics are presented in table 1. A profound hypodynamic shock was developed 12 [10 – 14] hours after induction of peritonitis in the dobutamine (D-

group) and placebo (P-group), with similar falls in cardiac output (91.2 [84.8 – 94.1] vs. 75.0 [68.2 – 95.1] $ml.kg^{-1}.min^{-1}$, for P and D group, respectively; p=0.21) and systemic oxygen delivery (13.4 [12.5 – 15.1] vs. 13.9 [10.9 – 15.0] $ml.kg^{-1}.min^{-1}$, for P and D groups respectively; p=0.88) (Table 1). Later, cardiac output and systemic oxygen delivery exhibited a similar improvement in D and P groups at T2H and T6H (Repeated measurements – Friedman analysis, p=0.63 and p=0.84 for time*group interactions for CO and DO_2, respectively) (Figure 1).

6.5.2 Microcirculatory blood flow

All microcirculatory parameters are presented in the table 2. Time course of the proportion of jejunal-villi with well-perfused vessels (%Villi-PPV) was significantly different between groups (Repeated measures – Friedman analysis, p=0.006 for time*group interactions; S.N.K. test, $p<0.05$ for sham vs. P and sham vs. D groups at TS, T2H and T6H; $p<0.05$ for P vs. D at T2H and T6H) (Figure 2; Annex F, Suppl. Figures 1 and 2). Excess in stopped vessels at intestinal mucosa explained the decreasing %Villi-PPV at TS in P and D groups. Thereafter, excess of stopped vessels was predominant in P group at T2H and T6H, while D group exhibited a significant improvement in microvascular blood flow showing increasing in normal perfused villi and moving from stopped to intermittent flow is other areas (Figure 3; Annex F, Suppl. Figure 2), but without attaining complete normalization of flow.

Meanwhile, jejunal serosa was severely hypoperfused at TS in P and D groups, demonstrating partial and delayed recovery at T6H just in D group (Annex F, Suppl. Figure 3). These alterations at intestinal serosa were parallel by functional capillary density (Ser-FCD) and inversely mirrored by the serosa heterogeneity index (Ser-HI) (Annex F, Suppl. Figure 3). The percentage of small vessels perfused at sublingual mucosa (SL-PPV) exhibited similar variations to %villi-PPV, with significant improvement in D group but without attaining complete normalization at the end of the experiment (Annex F, Suppl. Figure 3).

6.5.3 Oxygen extraction ratio (ERO_2) and its relationships with microcirculatory blood flow

Mesenteric ERO_2 (mes-ERO_2) showed a severe and parallel increase in both P and D groups at TS (P:0.65 [0.64 – 0.65] vs. D:0.68 [0.64 – 0.68], Mann-Whitney Rank sum test, $p>0.05$). Although we observed a significant recovery after starting resuscitation in both experimental groups, the slope of mes-ERO_2 recovery was significantly steeper in D group (Repeated measures – Friedman test; $p=0.04$ for time*group interaction. S.N.K. test, $p<0.05$ for sham vs. P and D at BL; $p<0.05$ for P vs. D at T2H and T6H) (Figure 4, Panel A). Variations of %villi-PPV between baseline measurement (BL) and each measurement time-point (BL-to-TS; BL-to-T2H; BL-to-T6H) revealed a very good agreement with changes in mes-ERO_2 (Spearman Rho test, $R^2= 0.67$, $p<0.001$) (Figure 4, Panel B). Meanwhile, systemic ERO_2 also exhibited significant differences between dobutamine and placebo groups at both T2H and T6H (Annex F, Suppl. Figure 4).

Table 1. Hemodynamics, blood gases, and oxygen / carbon dioxide parameters

	Baseline	TS	T2H	T6H
Hemodynamic variables				
HR, beats/min				
Placebo	90 [81– 130]	154 [131 -180]	155 [153 – 168]	164 (132 – 174]
Dobutamine	97 [87 – 128]	187 [113 – 205]	156 [142 – 189]	172 (146 –197]
Sham	146 [140 – 151]	108 [96 – 120]		70 [54 – 85]
CO, ml/kg/min				
Placebo	122.9 [111.8 – 138.2]	91.2 [84.8 – 94.1]	125.7 [120.6 – 138.2]	138.2 [128.6 – 145.5]
Dobutamine	100.0 [90.9 – 120.0]	75.0 [68.2 – 95.1]	106.8 [90.9 – 144.1]	113.2 [124.5 – 152.7]
Sham	104.3 [100.0 – 108.6]	108.7 [108.6 – 108.8]		113.0 [111.8 – 114.3]
MAP, mmHg				
Placebo	102 [92 – 114]	70 [69 - 77]	71 [69 – 82]	65 [63 – 97]
Dobutamine	100 [88 – 105]	81 [77 – 87]	67 [65 – 71]	77 [60 – 82]
Sham	97 [87 – 106]	100 [81 – 118]		129 [123 – 135]
CVP, mmHg				
Placebo	12 [7– 21]	13 [10 – 15]	13 [11 – 18]	14 [12 – 22]
Dobutamine	10 [5 – 14]	9 [8 – 10]	11 [10 – 12]	12 [10 – 15]
Sham	14 [13 – 15]	15 [13 – 16]		15 [11 – 18]
PAOP, mmHg				
Placebo	12 [9 – 16]	16 [13 – 18]	18 [15 – 20]	17 [16 – 22]
Dobutamine	10 [7 – 12]	11 [10 – 12]	12 [11 – 13]	12 [11 – 13]
Sham	16 [13 – 19]	19 [13 – 24]		15 [13 – 16]
Temperature, ºC				
Placebo	35.8 [34.3 – 36.4]	38.1 [35.1 – 38.3]	37.8 [34.3 – 38.1]	36.8 [33.8 – 38.4]
Dobutamine	35.9 [34.9 – 37.0]	38.2 [37.1 – 39.8]	37.0 [36.4 – 37.6]	36.6 [36.3 – 37.3]
Sham	34.7 [33.8 – 35.6]	34.6 [33.5 – 35.7]		34.5 [33.1 – 35.6]

Blood gases and O_2 variables

pH (arterial)				
Placebo	7.38 [7.37 – 7.40]	7.34 [7.28 – 7.36]	7.31 [7.24 – 7.41]	7.20 [7.10 – 7.42]
Dobutamine	7.38 [7.38 – 7.39]	7.27 [7.21 – 7.28]	7.20 [7.14 – 7.26]	7.29 [7.27 – 7.32]
Sham	7.39 [7.38 – 7.40]	7.42 [7.40 – 7.43]		7.42 [7.40 – 7.45]
PaO_2, mmHg				
Placebo	156.0 [149.8 – 187.8]	111.3 [109.3 – 113.7]	106.8 [102.3 – 125.9]	101.5 [96.6 – 127.3]
Dobutamine	113.7 [113.3 – 170.1]	102.0 [91.4 – 116.5]	84.6 [81.8 – 86.9]	86.0 [80.2 – 125.8]
Sham	131.1 [124.0 – 138.2]	114.0 [110.0 – 118.0]		168.7 [150.0 – 187.3]
$PaCO_2$, mmHg				
Placebo	34.6 [32.9 – 37.5]	40.1 [35.0 – 40.2]	37.6 [36.2 – 42.4]	38.6 [37.2 – 40.3]
Dobutamine	29.9 [29.5 – 33.2]	41.7 [36.3 – 50.5]	42.2 [41.6 – 46.3]	39.1 [36.0 – 39.8]
Sham	34.1 [33.6 – 34.5]	34.7 [34.2 – 35.1]		35.0 [34.6 – 35.3]
pH vmx, mmHg				
Placebo	7.37 [7.33 – 7.41]	7.22 [7.20 – 7.26]	7.17 [7.14 – 7.36]	7.07 [7.05 – 7.35]
Dobutamine	7.43 [7.37 – 7.43]	7.32 [7.22 – 7.32]	7.32 [7.25 – 7.33]	7.27 [7.27 – 7.30]
Sham	7.37 [7.25 – 7.49]	7.44 [7.41 – 7.47]		7.49 [7.40 – 7.59]
$PvmxO_2$, mmHg				
Placebo	41.2 [39.9 – 42.9]	28.4 [28.2 – 32.1]	35.8 [33.9 – 42.1]	44.7 [41.6 – 56.5]
Dobutamine	42.5 [40.1 – 42.6]	27.0 [26.4 – 27.8]	34.7 [33.4 – 38.7]	38.8 [36.8 – 46.0]
Sham	46.1 [45.8 – 46.4]	40.2 [37.9 – 42.4]		45.1 [41.1 – 49.1]
$PvmxCO_2$, mmHg				
Placebo	38.8 [35.6 – 40.5]	50.5 [49.8 – 54.5]	53.8 [46.2 – 57.4]	51.4 [46.9 – 52.7]
Dobutamine	34.6 [33.9 – 38.1]	55.3 [52.5 – 63.4]	51.4 [49.7 – 52.5]	43.7 [43.5 – 45.6]
Sham	37.6 [36.9 – 38.2]	38.7 [38.6 – 38.8]		
pH mes, mmHg				
Placebo	7.40 [7.34 – 7.46]	7.26 [7.20 – 7.30]	7.26 [7.12 – 7.38]	7.21 [7.00 – 7.37]
Dobutamine	7.46 [7.44 – 7.48]	7.21 [7.10 – 7.33]	7.24 [7.10 – 7.34]	7.26 [7.13 – 7.30]
Sham	7.44 [7.42 – 7.47]	7.44 [7.43 – 7.45]		7.42 [7.40 – 7.45]
$PvmesO_2$, mmHg				
Placebo	47.3 [42.3 – 48.6]	64.5 [61.7 – 64.6]	63.8 [62.0 – 64.8]	68.5 [65.0 – 70.5]
Dobutamine	47.3 [46.6 – 47.5]	58.4 [54.9 – 58.7]	58.6 [55.8 – 60.6]	57.7 [57.2 – 58.8]
Sham	37.4 [31.2 – 43.6]	37.8 [33.6 – 42.0]		45.3 [42.7 – 47.8]

$PvmesCO_2$, mmHg	41.2 [40.4 – 42.8]	57.2 [56.7 – 58.6]	58.6 [47.3 – 63.3]	56.4 [49.6 – 57.8]
Placebo	35.3 [34.9 – 36.3]	65.1 [55.8 – 68.8]	52.0 [49.2 – 59.3]	42.8 [41.6 – 45.7]
Dobutamine	40.3 [39.3 – 41.3]	38.3 [37.7 – 38.8]		39.6 [37.3 – 41.8]
Sham				
$PtisCO_2$, mmHg	45 [44 – 52]	68 [67 – 68]	69 [58 – 73]	72 [65 – 83]
Placebo	41 [35 – 42]	70 [67 – 86]	62 [59 – 67]	52 [52 – 56]
Dobutamine	43 [43 – 44]	41 [39 – 43]		48 [47 – 49]
Sham				
Pv-aCO_2 , mmHg	3.0 [2.7 – 4.2]	12.0 [11.0 – 15.5]	11.7 [11.6 – 16.0]	9.9 [9.3 – 14.1]
Placebo	4.9 [4.5 – 5.1]	13.6 [12.1 – 16.6]	9.2 [6.6 – 10.2]	6.5 [4.7 – 7.5]
Dobutamine	3.5 [3.3 – 3.7]	4.1 [3.7 – 4.4]		4.4 [4.0 – 4.7]
Sham				
Pvmes-aCO_2 , mmHg	5.8 [3.7 – 6.8]	18.4 [18.2 – 18.9]	17.6 [11.0 – 22.4]	19.2 [9.3 – 19.2]
Placebo	5.8 [3.8 – 6.4]	20.3 [17.5 – 23.4]	7.0 [5.7 – 11.1]	5.1 [3.7 – 5.9]
Dobutamine	6.3 [4.8 – 7.7]	3.6 [3.5 – 3.7]		4.6 [2.7 – 6.5]
Sham				
Ptis-aCO_2 , mmHg	10.4 [6.4 – 11.1]	32.0 [25.9 – 34.0]	23.4 [23.3 – 30.6]	33.4 [24.7 – 34.8]
Placebo	11.1 [4.5 – 14.0]	30.7 [28.5 – 34.7]	20.4 [19.4 – 24.3]	16.0 [14.2 – 16.9]
Dobutamine	9.5 [8.5 – 10.4]	6.4 [3.9 – 8.8]		13.1 [12.4 – 13.7]
Sham				
Pv-aCO_2/Da-vO_2 , mmHg	0.74 [0.61 – 0.89]	2.08 [1.61 – 2.95]	2.13 [1.79 – 2.45]	1.69 [1.54 – 1.92]
Placebo	0.96 [0.83 – 1.06]	1.96 [1.89 – 2.12]	1.76 [1.01 – 1.97]	1.46 [1.15 – 1.55]
Dobutamine	0.87 [0.85 – 0.90]			0.99 [0.90 -1.09]
Sham				
Pvmes-aCO_2/Da-vO_2, mmHg	1.69 [1.12 – 1.83]	1.89 [1.77 – 2.06]	1.92 [1.50 – 3.01]	2.68 [1.93 – 2.91]
Placebo	1.42 [1.19 – 1.75]	1.87 [1.60 – 2.15]	1.47 [1.04 – 2.18]	1.12 [0.94 – 1.17]
Dobutamine	1.47 [1.05 – 1.90]			1.22 [0.78 – 1.66]
Sham				

Perfusion /oxygen variables

Lactate Art, mmol/L,				
Placebo	1.8 [1.7 – 2.3]	7.4 [7.1 – 7.8]	8.3 [6.7 – 9.9]	8.9 [8.4 – 9.3]
Dobutamine	1.9 [1.9 – 2.1]	7.5 [7.5 – 8.1]	4.4 [3.8 – 7.4]	2.3 [2.1 – 3.9]
Sham	1.4 [1.1 – 1.7]	0.9 [0.8 – 1.0]		1.2 [0.7 – 1.8]
Lactate Mes, mmol/L,				
Placebo	2.6 [2.0 – 2.6]	8.2 [7.9 – 8.5]	8.9 [5.6 – 12.2]	7.3 [4.6 – 10.3]
Dobutamine	2.1 [1.8 – 2.2]	7.6 [7.3 – 8.2]	6.4 [3.8 – 7.1]	4.2 [3.4 – 4.8]
Sham	1.6 [1.2 – 2.0]	1.0 [1.0 – 1.1]		1.1 [0.8 – 1.4]
SvO_2, %,				
Placebo	67.6 [61.4 – 70.3]	68.8 [55.6 – 72.5]	73.7 [73.4 – 74.8]	77.5 [71.4 – 77.8]
Dobutamine	62.5 [53.0 – 71.1]	62.0 [61.2 – 71.7]	66.1 [62.6 – 66.4]	65.8 [64.5 – 66.0]
Sham	76.4 [70.8 – 81.9]	72.7 [68.3 – 77.1]		77.1 [74.5 – 79.6]
$SvmesO_2$, %,				
Placebo	74.4 [72.3 – 75.0]	34.4 [32.5 – 36.0]	38.4 [35.1 – 52.7]	52.2 [48.9 – 57.2]
Dobutamine	75.5 [74.9 – 78.0]	32.0 [30.5 – 32.5]	58.7 [45.0 – 60.5]	67.5 [64.4 – 68.4]
Sham	65.0 [64.3 – 65.6]	67.5 [66.1 – 68.8]		70.8 [69.9 – 71.6]
DO_2, ml/kg/min				
Placebo	17.0 [14.1 – 18.9]	13.4 [12.5 – 15.1]	17.1 [15.8 – 21.9]	18.4 [17.7 – 18.7]
Dobutamine	14.5 [13.5 – 17.3]	13.9 [10.9 – 15.0]	18.6 [12.2 – 19.3]	18.0 [15.4 – 20.0]
Sham	12.5 [12.4 – 12.5]	12.9 [12.3 – 13.5]		13.0 [12.1 – 13.8]
VO_2, ml/kg/min				
Placebo	5.9 [4.4 – 7.0]	5.8 [4.9 – 6.2]	8.2 [7.8 – 8.3]	8.9 [7.1 -8.9]
Dobutamine	5.6 [4.7 – 5.7]	6.0 [4.7 – 6.5]	4.9 [7.3 – 7.9]	6.2 [6.1 – 7.1]
Sham	4.2 [4.0 – 4.4]	4.7 [4.7 – 4.8]		4.7 [4.4 – 5.0]
Systemic ERO_2,				
Placebo	0.34 [0.30 – 0.40]	0.42 [0.37 – 0.46]	0.47 [0.41 – 0.48]	0.48 [0.40 – 0.55]
Dobutamine	0.36 [0.35 – 0.39]	0.40 [0.39 – 0.47]	0.41 [0.40 – 0.41]	0.40 [0.36 – 0.40]
Sham	0.33 [0.32 – 0.35]	0.37 [0.35 – 0.38]		0.36 [0.36 – 0.37]

Mesenteric ERO_2,				
Placebo	0.27 [0.27 – 0.29]	0.65 [0.64 – 0.65]	0.62 [0.47 – 0.63]	0.44 [0.44 – 0.46]
Dobutamine	0.26 [0.23 – 0.26]	0.68 [0.64 – 0.68]	0.37 [0.37 – 0.49]	0.32 [0.25 – 0.35]
Sham	0.36 [0.35 – 0.36]	0.33 [0.32 – 0.35]		0.31 [0.29 – 0.32]

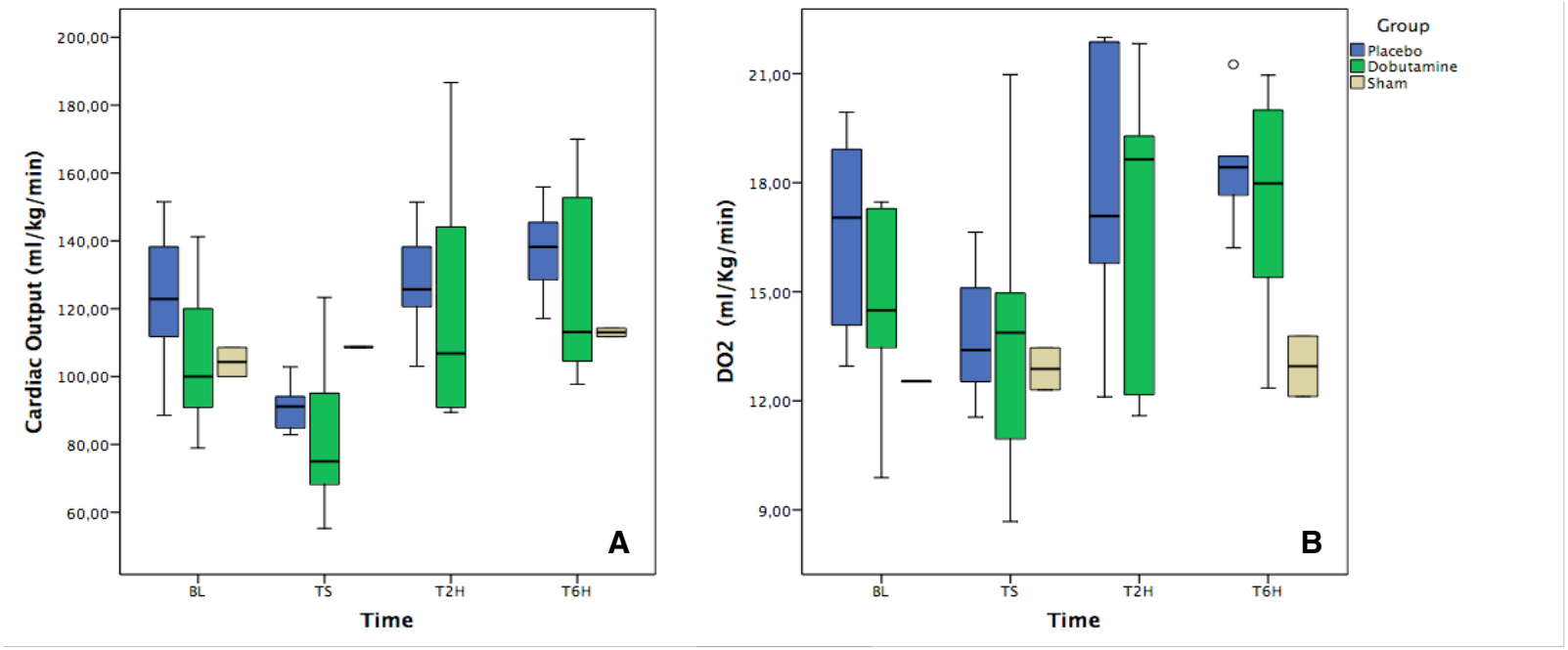
All data are presented as median [percentiles 25_75]. **HR**: Heart rate; **CO**: cardiac output; **MAP**: mean arterial pressure; **CVP**: central venous pressure; **PAOP**: pulmonary artery occlusion pressure; **pH**: arterial pH; **PaO_2**: arterial oxygen partial pressure; **$PaCO_2$**: arterial carbon dioxide partial pressure; **pH vmx**: mixed-venous pH; **$PvmxO_2$**: mixed-venous oxygen partial pressure; **$PvmxCO_2$**: mixed-venous carbon dioxide partial pressure; **pH mes**: mesenteric blood venous pH; **$PvmesO_2$**: mesenteric venous oxygen partial pressure; **$PvmesCO_2$**: mesenteric venous carbon dioxide partial pressure; **$PtisCO_2$**: tissue (jejunal mucosa) carbon dioxide pressure; **Pv-aCO_2**: mixed venous-to-arterial carbon dioxide difference; **Pvmes-aCO_2**: mesenteric venous-to-arterial carbon dioxide difference; **Ptis-aCO_2**: tissue(jejunal mucosa)-to-arterial carbon dioxide difference; **Pv-aCO_2/Da-vO_2**: mixed venous-to-arterial carbon dioxide to arterial-venous oxygen pressure differences ratio; **Pvmes-aCO_2/Da-vO_2**: mesenteric blood venous-to-arterial carbon dioxide to arterial-venous oxygen pressure difference ratio; **Lactate Art**: arterial lactate; **Lactate mes**: mesenteric lactate; **SvO_2**: mixed-venous oxygen saturation; **DO_2**: systemic oxygen delivery; **VO_2**: systemic oxygen consumption; **Systemic ERO_2**: systemic oxygen extraction ratio; **Mesenteric ERO_2**: mesenteric oxygen extraction ratio.

Table 2. Microcirculatory blood flow parameters

	Baseline	TS	T2H	T6H
% Villi-PPV, %				
Placebo	100.0 [97.5 – 100.0]	0.0 [0.0 – 6.8]	6.9 [0.0 – 28.9]	18.8 [17.0 – 31.8]
Dobutamine	98.5 [98.0 – 100.0]	0.0 [0.0 – 13.9]	48.9 [16.7 – 76.5]	79.0 [67.4 – 79.6]
Sham	97.5 [96.5 – 98.5]	97.5 [96.5 – 98.5]		94.8 [94.6 – 95.0]
Ser-PPV, %				
Placebo	97.4 [90.1 – 99.7]	31.2 [16.7 – 37.8]	34.3 [22.6 – 49.2]	32.7 [6.5 – 58.7]
Dobutamine	100.0 [87.9 – 100.0]	16.7 [0.0 – 20.8]	45.4 [7.4 – 45.7]	85.7 [75.7 – 91.5]
Sham	68.7 [67.8 – 69.6]	89.0 [88.5 – 89.4]		97.8 [95.6 – 100.0]
Ser-FCD, vessels/mm^2				
Placebo	6.8 [5.6 – 7.9]	1.3 [0.4 – 2.2]	2.0 [1.1 – 2.3]	2.5 [2.1 – 2.7]
Dobutamine	6.3 [5.9 – 8.8]	0.9 [0.0 – 1.2]	2.1 [0.4 – 2.4]	5.2 [3.5 – 5.6]
Sham	5.5 [5.3 – 5.6]	6.8 [6.7 – 6.8]		6.3 [5.2 – 7.4]
Ser-HI				
Placebo	0.2 [0.0 – 0.2]	1.5 [0.8 – 2.0]	1.0 [1.0 – 1.5]	2.0 [1.5 – 2.6]
Dobutamine	0.0 [0.0 – 0.3]	2.0 [0.0 – 2.8]	0.5 [0.2 – 0.6]	0.1 [0.0 – 0.3]
Sham	0.7 [0.3 – 1.1]	0.3 [0.2 – 0.4]		0.3 [0.0 – 0.6]
SL-PPV, %				
Placebo	87.0 [85.9 – 95.7]	31.8 [21.2 – 32.1]	34.5 [8.2 – 67.2]	22.8 [22.7 – 67.0]
Dobutamine	86.2 [81.0 – 95.1]	20.7 [13.2 – 29.9]	68.3 [47.5 – 71.1]	69.1 [58.2 – 70.3]
Sham	83.3 [79.5 – 87.2]	88.3 [86.1 – 90.4]		97.6 [96.3 – 98.9]
SL- FCD, vessels/mm^2				
Placebo	7.0 [6.9 – 7.1]	2.5 [1.6 – 3.0]	2.8 [0.5 – 3.8]	2.1 [1.9 – 4.5]
Dobutamine	6.5 [6.0 – 6.9]	1.7 [0.8 – 2.2]	4.6 [4.0 – 5.7]	4.6 [4.5 – 5.7]
Sham	7.6 [7.4 – 7.8]	9.1 [8.9 – 9.3]		10.9 [10.9 – 11.0]
SL-HI				
Placebo	0.3 [0.1 – 0.4]	2.1 [2.0 – 3.1]	1.5 [0.8 – 2.3]	1.7 [1.1 – 2.5]
Dobutamine	0.8 [0.8 – 0.9]	1.5 [1.5 – 2.6]	0.9 [0.8 – 1.2]	0.9 [0.4 – 1.0]
Sham	0.4 [0.3 – 0.4]	0.3 [0.2 – 0.3]		0.1 [0.1 – 0.1]

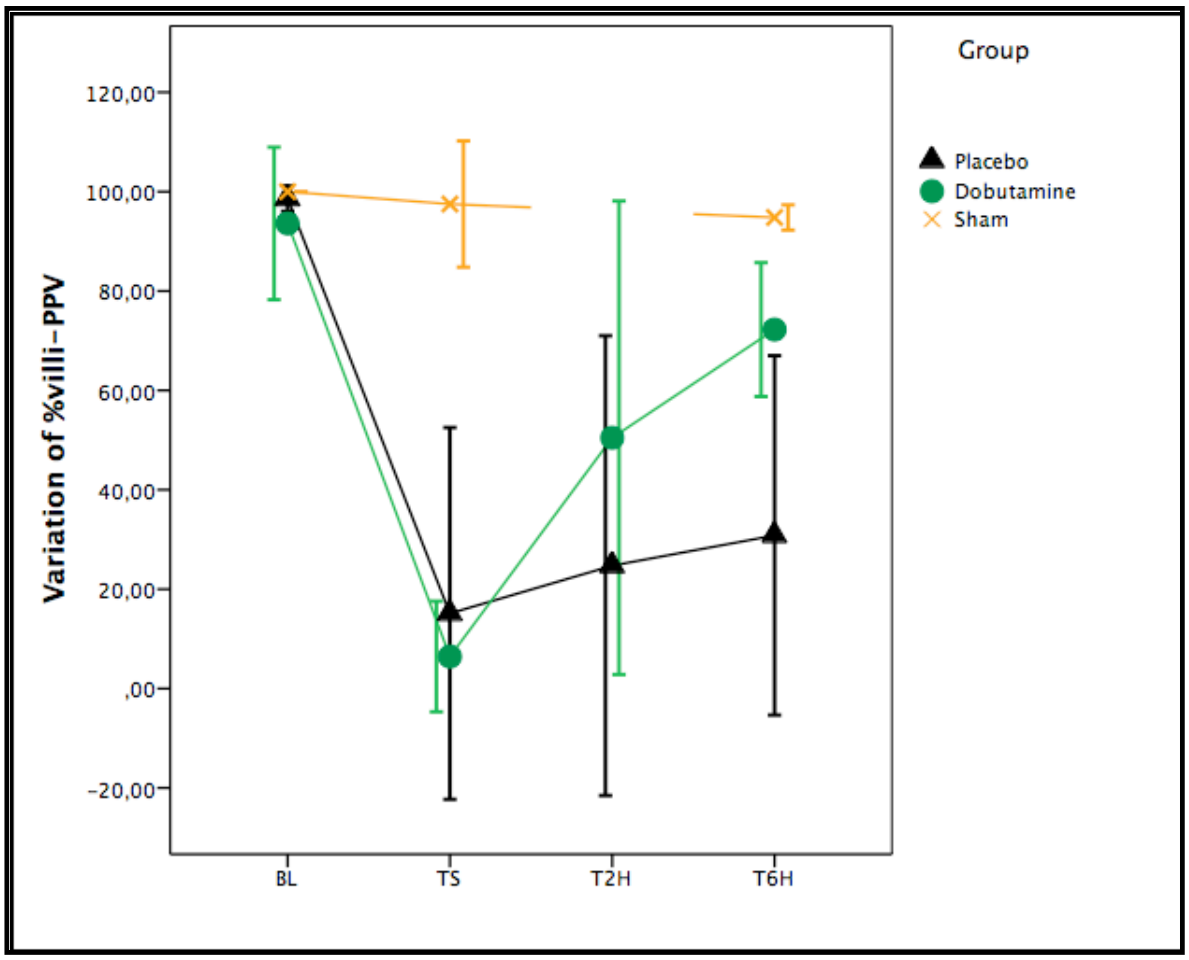
% Villi-PPV: proportion of jejunal-villi with well-perfused vessels; **Ser-PPV**: percentage of small vessels perfused at jejunal serosa; **Ser-FCD**: functional capillary density at jejunal serosa; **Ser-HI**: heterogeneity index at jejunal serosa; **SL-PPV**: percentage of small vessels perfused at sublingual mucosa; **SL-FCD**: functional capillary density at sublingual mucosa; **SL-HI**: heterogeneity index at sublingual mucosa.

Figure 1. Time-course of cardiac output and systemic oxygen delivery



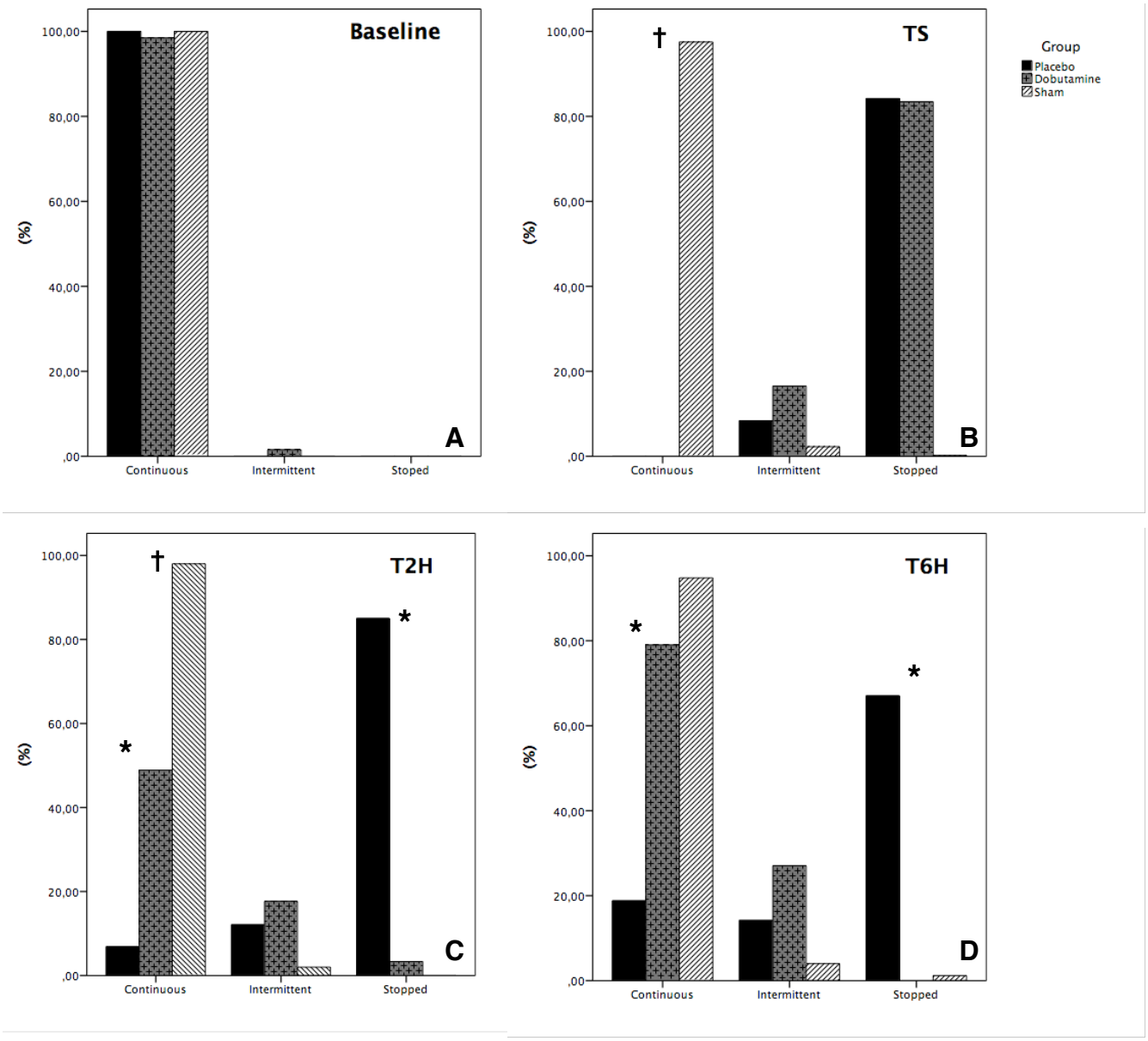
Panel A. Cardiac Output, Repeated measurements – Friedman analysis, p=0.21 for time*group interactions
Panel B. Systemic oxygen delivery, Repeated measurements – Friedman analysis, p=0.88 for time*group interactions

Figure 2. Time-course of %Villi-PPV variations



%villi-PPV: percentage of villi with predominantly well-perfused small vessels. Repeated measurements – Friedman analysis, p=0.006, for time*group interactions
p<0.05, between dobutamine vs. placebo groups at T2H and T6H

Figure 3. Type of predominant microvascular flow in jejunal villi at each measurement time

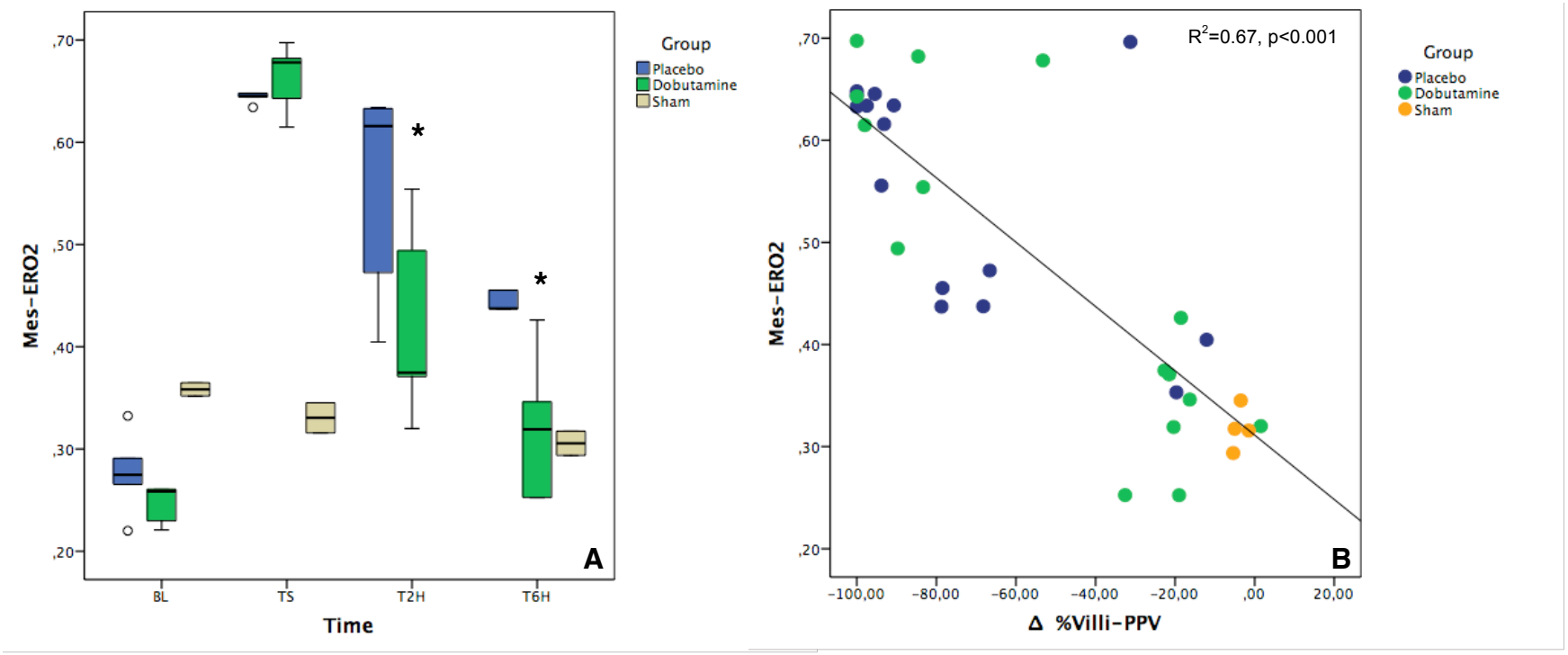


Percentage distribution of type of microvascular blood flow in jejunal villi at baseline **(Panel A),** time of shock –TS (**Panel B),** two-hours after starting resuscitation – T2H **(Panel C)** and six-hours after starting resuscitation – T6H **(Panel D).**

† p<0.05, between sham vs. dobutamine and placebo groups

* p<0.05, between dobutamine vs. placebo groups

Figure 4. Time-course of Mes-ERO$_2$ and its relationship with %villi-PPV



Panel A. Time-course of Mes-ERO$_2$ (mesenteric oxygen extraction ratio). Repeated measurements – Friedman analysis, p=0.04, for time*group interactions. **Panel B.** Scatterplot for delta %villi-PPV (delta of percentage of jejunal villi with normal flow) vs. Mes-ERO$_2$ (mesenteric oxygen extraction ratio). Delta of %villi-PPV was calculated as %villi-PPV between baseline measurement (BL) and each measurement time-point (BL-to-TS; BL-to-T2H; BL-to-T6H) (Spearman Rho test, R^2= 0.67, p<0.001)

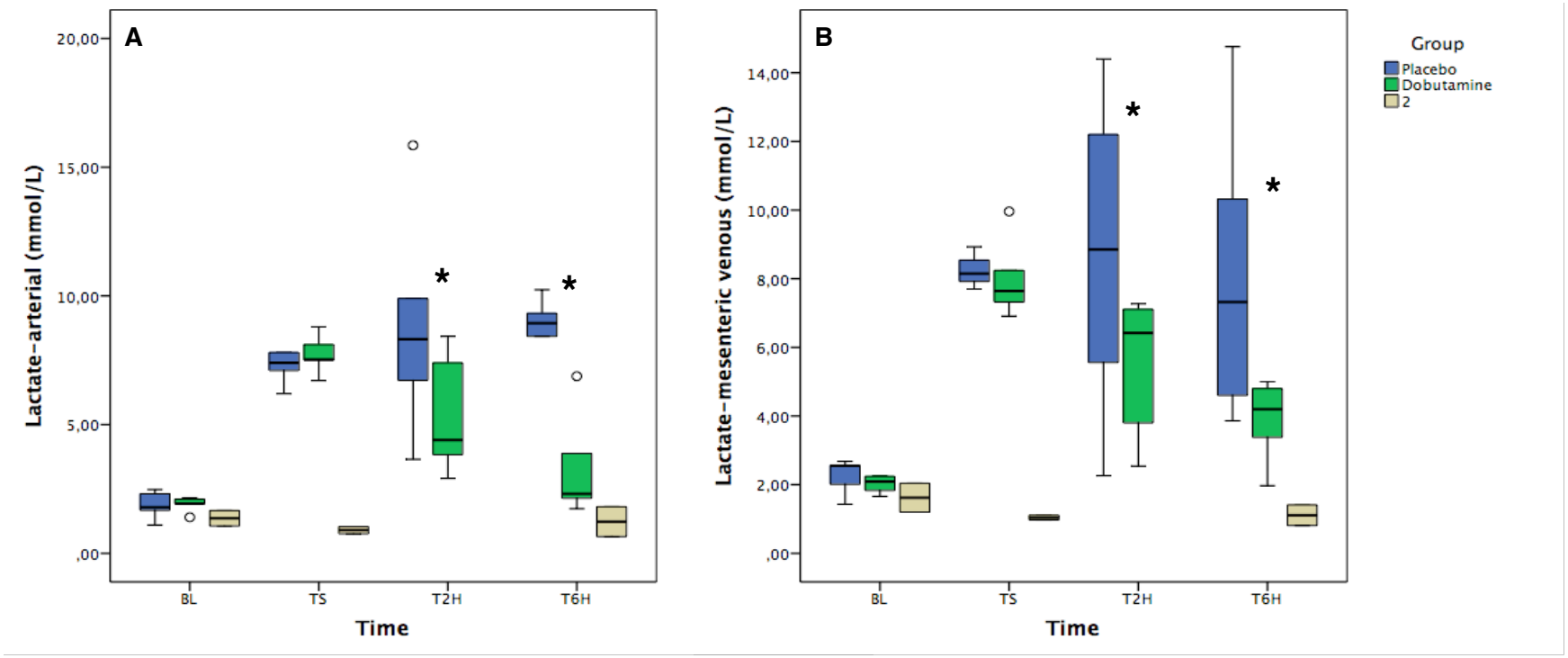
6.5.4 Systemic, regional and local carbon dioxide to arterial differences

Variations in the tissue-to-arterial carbon dioxide difference (Pti-aCO_2) were well reflected by regional changes (Pmes-aCO_2) and these in turn, by the systemic venous-to-arterial carbon dioxide differences obtained by mixed-venous blood sampling (P$\bar{v}$-aCO_2) (Table 1; Annex F, Suppl. Figure 5). Pti-aCO_2 and Pmes-aCO_2 exhibited a good agreement with %Villi-PPV (Spearman-Rho test, R^2=0.69 and 0.63 respectively, p<0.001), and discrete agreement with Ser-PPV (Spearman-Rho test, R^2=0.48 and 0.43 respectively, p<0.001) (Annex F, Suppl. Figure 6).

6.5.5 Lactate and venous-arterial CO_2 to arterial-venous O_2 pressures differences ratio

Significant increases in arterial and venous mesenteric lactate levels were observed at TS. Models subjected to dobutamine infusion showed significant decrease at T2H and T6H in both arterial (Repeated measures – Friedman test, p=0.01 for time effect; p=0.02 for time*group interaction; S.N.K. test, p<0.05 for P vs. D at T2H and T6H) and venous mesenteric lactate levels (Repeated measures – Friedman test, p=0.04 for time effect; p=0.12 for time*group interaction; S.N.K. test, p<0.05 for P vs. D at T2H and T6H), while lactate levels remained high in placebo group (Figure 5). A good agreement between %Villi-PPV with venous mesenteric and arterial lactate levels was also observed (Annex F, Suppl. Figure 7). Mesenteric venous-arterial carbon dioxide to arterial-venous oxygen pressure differences exhibited parallel developments to mesenteric lactate levels (Annex F, Suppl. Figure 8).

Figure 5. Time-course of arterial and venous-mesenteric lactate levels



Panel A. Repeated measures – Friedman test, p=0.01 for time effect; p=0.02 for time*group interaction; S.N.K. test, p<0.05 for P vs. D at T2H and T6
Panel B. Repeated measures – Friedman test, p=0.04 for time effect; p=0.12 for time*group interaction; S.N.K. test, p<0.05 for P vs. D at T2H and T6H

* p<0.05 for placebo vs. dobutamine groups

6.6 DISCUSSION

Our results suggest that variations in the distribution of intestinal microcirculatory blood flow are closely related to splanchnic oxygen extraction ratio during septic shock. Although cardiac output and systemic oxygen delivery evolved similarly in both dobutamine and placebo groups, dobutamine infusion partially reversed microcirculatory blood flow heterogeneity at jejunal mucosa, and these changes were followed by an improvement of oxygen extraction and lactate levels.

The assumption stating that tissue oxygenation can be preserved by maintaining its blood supply is derived from models with uniformly perfused capillaries. However, sepsis and septic shock are characterized by inhomogeneity of microvascular blood flow with zones perfused and underperfused in close vicinity. In our study, we observed a severe decline in the proportion of small vessels perfused at both jejunal and sublingual mucosa with a concurrent decreased functional capillary density, when shock appeared. Importantly, this heterogeneity of flow progressively decreased after starting dobutamine infusion while remained almost unchanged in the placebo group. Theoretically, heterogeneity of oxygen delivery responds in part for tissue oxygen derangements in peripheral tissues [18]. Indeed, an animal model demonstrated that heterogeneity of gut capillary transit times in ex-vivo intestinal pieces is responsible for impaired tissue oxygenation [19]. Subsequent studies demonstrated that heterogeneity of oxygen delivery is associated to microvascular dysfunction [20] such as it was suggested by our observations. Actually, our experiment suggests that variations in %villi-PPV are closely related with mesenteric ERO_2 and microvascular blood flow distribution could be potentially intervened. Interestingly, we observed a significant increase in stopped vessels at shock appearance and it was related to the worse mesenteric ERO_2, almost reaching the maximal oxygen extraction capacity. Resuscitation with combined fluids and dobutamine progressively decreased the proportion of stopped vessels to the extent that stopped were gradually replaced by

intermittent and well-perfused small vessels, increasing in turn the functional capillary density. Some authors propose that heterogeneous flow cessation of individual capillaries could determine oxygen supply dependence during septic shock [20]. Importantly, mathematical modeling of this phenomenon [21] has demonstrated that oxygenation derangements are more severe when capillary blood flow is totally stopped that when it is intermittent, which agrees with our results.

Inhomogeneity of microcirculatory blood flow leads to increase intercapillary distances with subsequent decreased oxygen diffusion. In the past, some authors highlighted the importance of this phenomenon on the oxygen uptake [29] and in fact, a decreased in functional capillary density (FCD) with reduction in blood flow is necessary to observe increase in supply dependence during stagnant hypoxia [21]. Our results reveal a decrease in FCD during septic shock, which could favor the alteration of oxygen utilization and agrees with previous mathematical simulations [21,29]. Dobutamine has been related to contradictory results on macrovascular splanchnic [4-11], total intestinal microvascular blood flow [12,13], and sublingual microcirculation [23,25,26]. Conversely, information about the effect of dobutamine on microcirculatory blood flow distribution at intestinal villi during sepsis or endotoxemia is restricted but usually favorable [24]. However, far beyond the splanchnic macrohemodynamic effects, our model demonstrates that low fixed doses of dobutamine can dynamically modify the blood flow distribution at intestinal villi under conditions of severe hypoperfusion.

Microcirculatory alterations observed in our experimental models were accompanied by increases in jejunal-mucosa and regional-mesenteric to arterial CO_2 differences and simultaneously, these were reflected by systemic venous CO_2 accumulation. Interestingly, the grade of recovery of microcirculation was mirrored by decreases in Pti-aCO_2 and Pmes-aCO_2, thus supposing a link between microcirculatory blood flow and CO_2 accumulation, as it as been recently suggested by observations in human

septic shock [30]. Additionally, our results reveal that microvascular blood flow alterations are linked to mesenteric and arterial lactate accumulation, which reinforce the idea that distribution of blood flow is a key factor on tissue oxygen distribution and this in turn, on appearance of anaerobic metabolism. Interestingly, the mesenteric-venous CO_2 to arterial-venous O_2 pressures differences parallel evolved with regional lactate accumulation, suggesting the presence of anaerobic carbon dioxide generation.

It could be argued that the thermogenic effects induced by dobutamine could explain the increases in oxygen extraction ratio in our experiment [31,32]. However, others have argued against it [33], while VO_2 responses to prostacyclin [34] and nitroprusside [35] are quite similar to dobutamine in septic patients and healthy volunteers. Additionally, thermogenic effects of catecholamines are related to progressively increased doses, a situation prevented when low fixed doses are used.

Our model also had limitations. First, we focused on microcirculatory blood flow alterations while resuscitation was guided by macro hemodynamic variations, such as occur during resuscitation of human septic shock. Nonetheless, macrohemodynamics evolved similarly in dobutamine and placebo groups suggesting that effects of dobutamine were most apparent at microcirculatory level. Second, we did not measure variations of arterial mesenteric blood flow, which would suppose a limitation for comparing our results with previous ones. However, we directly evaluated the microcirculatory blood flow distribution more than total microcirculatory flow at intestinal mucosa and serosa. Thus, variations in oxygen extraction could effectively be more related to microvascular blood flow distribution than to regional splanchnic blood flow variations. Third, measurements of oxygen extraction were performed at regional but not at local level. Nevertheless, although microcirculatory measurements sound as really local, we acquired mucosal and serosa images in at least five different points proximal and distal to jejunostomy and the final report at each time-point of measurements was the mean of these different

anatomical sites. Fourth, our experimental model reproduces a very hypodynamic septic shock, which could distance from usual clinical observations. However, our model closely recreates the initial phases of non-resuscitated septic shock with a complete spectrum of enteric pathogens present and immunological and macro hemodynamic disturbances occurring gradually.

6.7 CONCLUSION

Increased heterogeneity of microcirculatory blood flow at jejunal mucosa is associated to worse regional splanchnic oxygen extraction ratio, thus suggesting the key role of microvascular blood flow distribution on oxygen utilization in septic shock. Low fixed doses of dobutamine can decrease such flow heterogeneity, improving tissue oxygen consumption.

6.8 REFERENCES

1. Vincent JL, De Backer D. Microvascular dysfunction as a cause of organ dysfunction in severe sepsis. *Crit Care.* 2005;9 Suppl 4:S9-12.
2. Desai VS, Weil MH, Tang W, Yang G, Bisera J. Gastric intramural PCO2 during peritonitis and shock. *Chest.* 1993;104(4):1254-1258.
3. Tang W, Weil MH, Sun S, Noc M, Gazmuri RJ, Bisera J. Gastric intramural PCO2 as monitor of perfusion failure during hemorrhagic and anaphylactic shock. *J Appl Physiol (1985).* 1994;76(2):572-577.
4. Uusaro A, Ruokonen E, Takala J. Splanchnic oxygen transport after cardiac surgery: evidence for inadequate tissue perfusion after stabilization of hemodynamics. *Intensive Care Med.* 1996;22(1):26-33.
5. Biro GP, Douglas JR, Keon WJ, Taichman GC. Changes in regional blood flow distribution induced by infusions of dopexamine hydrochloride or dobutamine in anesthetized dogs. *Am J Cardiol.* 1988;62(5):30c-36c.
6. Schneider AJ, Groeneveld AB, Teule GJ, Wesdorp RI, Thijs LG. Total body blood volume redistribution in porcine E. coli septic shock: effect of volume loading, dobutamine, and norepinephrine. *Circ Shock.* 1991;35(4):215-222.
7. Parviainen I, Ruokonen E, Takala J. Dobutamine-induced dissociation between changes in splanchnic blood flow and gastric intramucosal pH after cardiac surgery. *Br J Anaesth.* 1995;74(3):277-282.
8. Ruokonen E, Takala J, Kari A. Regional blood flow and oxygen transport in patients with the low cardiac output syndrome after cardiac surgery. *Crit Care Med.* 1993;21(9):1304-1311.
9. Priebe HJ, Noldge GF, Armbruster K, Geiger K. Differential effects of dobutamine, dopamine, and noradrenaline on splanchnic haemodynamics and oxygenation in the pig. *Acta Anaesthesiol Scand.* 1995;39(8):1088-1096.
10. Bersten AD, Hersch M, Cheung H, Rutledge FS, Sibbald WJ. The effect of various sympathomimetics on the regional circulations in hyperdynamic sepsis. *Surgery.* 1992;112(3):549-561.
11. Ferrara JJ, Dyess DL, Peeples GL, et al. Effects of dopamine and dobutamine on regional blood flow distribution in the neonatal piglet. *Ann Surg.* 1995;221(5):531-540; discussion 540-532.
12. Neviere R, Chagnon JL, Vallet B, et al. Dobutamine improves gastrointestinal mucosal blood flow in a porcine model of endotoxic shock. *Crit Care Med.* 1997;25(8):1371-1377.
13. Hiltebrand LB, Krejci V, Sigurdsson GH. Effects of dopamine, dobutamine, and dopexamine on microcirculatory blood flow in the gastrointestinal tract during sepsis and anesthesia. *Anesthesiology.* 2004;100(5):1188-1197.
14. Germann R, Haisjackl M, Schwarz B, et al. Inotropic treatment and intestinal mucosal tissue oxygenation in a model of porcine endotoxemia. *Crit Care Med.* 1997;25(7):1191-1197.
15. Shepherd AP, Kiel JW. A model of countercurrent shunting of oxygen in the intestinal villus. *Am J Physiol.* 1992;262(4 Pt 2):H1136-1142.
16. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med.* 2002;166(1):98-104.

17. Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. *Crit Care Med.* 2012;40(5):1443-1448.
18. Walley KR. Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. *J Appl Physiol.* 1996;81(2):885-894.
19. Humer MF, Phang PT, Friesen BP, Allard MF, Goddard CM, Walley KR. Heterogeneity of gut capillary transit times and impaired gut oxygen extraction in endotoxemic pigs. *J Appl Physiol (1985).* 1996;81(2):895-904.
20. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R. Effect of a maldistribution of microvascular blood flow on capillary O(2) extraction in sepsis. *Am J Physiol Heart Circ Physiol.* 2002;282(1):H156-164.
21. Goldman D, Bateman RM, Ellis CG. Effect of decreased O2 supply on skeletal muscle oxygenation and O2 consumption during sepsis: role of heterogeneous capillary spacing and blood flow. *Am J Physiol Heart Circ Physiol.* 2006;290(6):H2277-2285.
22. Dubin A, Edul VS, Pozo MO, et al. Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. *Crit Care Med.* 2008;36(2):535-542.
23. De Backer D, Creteur J, Dubois MJ, et al. The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med.* 2006;34(2):403-408.
24. Secchi A, Wellmann R, Martin E, Schmidt H. Dobutamine maintains intestinal villus blood flow during normotensive endotoxemia: an intravital microscopic study in the rat. *J Crit Care.* 1997;12(3):137-141.
25. Enrico C, Kanoore Edul VS, Vazquez AR, et al. Systemic and microcirculatory effects of dobutamine in patients with septic shock. *J Crit Care.* 2012;27(6):630-638.
26. Hernandez G, Bruhn A, Luengo C, et al. Effects of dobutamine on systemic, regional and microcirculatory perfusion parameters in septic shock: a randomized, placebo-controlled, double-blind, crossover study. *Intensive Care Med.* 2013;39(8):1435-1443.
27. Creteur J, De Backer D, Vincent JL. Monitoring gastric mucosal carbon dioxide pressure using gas tonometry: in vitro and in vivo validation studies. *Anesthesiology.* 1997;87(3):504-510.
28. De Backer D, Hollenberg S, Boerma C, et al. How to evaluate the microcirculation: report of a round table conference. *Crit Care.* 2007;11(5):R101.
29. Schumacker PT, Samsel RW. Analysis of oxygen delivery and uptake relationships in the Krogh tissue model. *J Appl Physiol (1985).* 1989;67(3):1234-1244.
30. Ospina-Tascon GA, Umana M, Bermudez WF, et al. Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock? *Intensive Care Med.* 2016;42(2):211-221.
31. Gilbert EM, Haupt MT, Mandanas RY, Huaringa AJ, Carlson RW. The effect of fluid loading, blood transfusion, and catecholamine infusion on oxygen delivery and consumption in patients with sepsis. *Am Rev Respir Dis.* 1986;134(5):873-878.
32. Green CJ, Frazer RS, Underhill S, Maycock P, Fairhurst JA, Campbell IT. Metabolic effects of dobutamine in normal man. *Clin Sci (Lond).* 1992;82(1):77-83.
33. Ronco JJ, Fenwick JC, Wiggs BR, Phang PT, Russell JA, Tweeddale MG. Oxygen consumption is independent of increases in oxygen delivery by dobutamine in septic patients who have normal or increased plasma lactate. *Am Rev Respir Dis.* 1993;147(1):25-31.

34. De Backer D, Berre J, Zhang H, Kahn RJ, Vincent JL. Relationship between oxygen uptake and oxygen delivery in septic patients: effects of prostacyclin versus dobutamine. *Crit Care Med.* 1993;21(11):1658-1664.
35. De Backer D, Berre J, Moraine JJ, Melot C, Vanfraechem J, Vincent JL. Effects of dobutamine on the relationship between oxygen consumption and delivery in healthy volunteers: comparison with sodium nitroprusside. *Clin Sci (Lond).* 1996;90(2):105-111.

Chapter 7

THE THEORETICAL MODEL:

UNDERSTANDING THE PATHOPHYSIOLOGICAL ISSUES

PAPER No. 6 (Expert opinion paper)

UNDERSTANDING THE VENOUS-ARTERIAL CO_2 TO ARTERIAL-VENOUS O_2 CONTENT DIFFERENCE RATIO

Intensive Care Med. 2016 Feb 12. [Epub ahead of print]
PMID: 26873834

Early identification of tissue hypoperfusion is a cornerstone in the management of septic shock [1]. However, normal macrohemodynamic and oxygen-derived parameters do not rule out the presence of tissue hypoxia [2]. In this setting, carbon dioxide (CO_2) derived variables could provide important information about macro and micro vascular blood flow [3] and probably, about the presence of anaerobic metabolism [4,5] .

7.1 The rational of Cv-aCO_2 to Da-vO_2 ratio

According to the Fick equation, oxygen consumption (VO_2) and carbon dioxide production (VCO_2) depend on cardiac output and their respective arterial-to-venous and venous-to-arterial content differences. Thus, under aerobic steady-state conditions, VCO_2 approximates to VO_2, and venous-to-arterial CO_2 content difference (Cv-aCO_2) approximates to arterial-to-venous O_2 content difference (Da-vO_2). In other words, CO_2 production should not be higher beyond O_2 availability, consequently VCO_2/VO_2 ratio (i.e., the respiratory quotient) should not be higher than 1.0. Nonetheless, during some situations such as progressive exercise, in which VO_2 is increased in response to metabolic demands, VCO_2 might overcome VO_2 when anaerobic threshold is reached [6], because of the disproportionate increase in CO_2 production released through the buffering of excess hydrogen ions, most of them coming from ATP hydrolysis [7] and the excessive liberation of protons related to accelerated anaerobic glycolysis [8]. Thus, during progressive exercise, both VCO_2 and respiratory quotient increase. Interestingly, experimental blockage of mitochondrial O_2 utilization [9] also leads to simultaneous but not symmetric decrease in VCO_2 with subsequent increasing of the respiratory quotient suggesting non-aerobic CO_2 generation.

Under tissue hypoxia, a global decrease in VO_2 should be accompanied by a reduction in aerobic CO_2 production. However, experimental models demonstrated that VCO_2 exhibits a slighter drop than VO_2 [10,11], thus pathologically increasing the VCO_2/VO_2

ratio and representing predominantly anaerobic metabolism (Fig1). Interestingly, after shock reversion, VCO_2/VO_2 returns to normal values, suggesting the potential reversibility of this phenomenon, at least during early stages of shock.

Cv-aCO_2/Da-vO_2 ratio could be used as a surrogate of VCO_2/VO_2. Remarkably, both the venous-to-arterial carbon dioxide difference and the arterial-to-venous O_2 content difference i.e., numerator and denominator of such quotient, could be influenced by macro and micro blood flow alterations. Low cardiac output might increase venous CO_2 partial pressure ($PvCO_2$) even in absence of extra CO_2 production due to venous stagnation phenomenon leading to venous hypercarbia [12]. Likewise, microcirculatory blood flow alterations such as decreased percentage of perfused small-vessels, decreased functional capillary density and increased heterogeneity are associated with progressively increased Pv-aCO_2 and Cv-aCO_2 during septic shock [3]. Analogously, increased heterogeneity of microvascular blood flow impairs oxygen extraction [13] as well as insufficient cardiac output might limit VO_2 during oxygen-supply dependency. Consequently, Cv-aCO_2/Da-vO_2 ratio is an integrative flow-independent variable, since cardiac output is included in calculations for both VO_2 and VCO_2 according to the Fick´s equation whereas microvascular blood flow distribution is linked to Cv-aCO_2 and Da-vO_2. Accordingly, when cardiac output and oxygen metabolism variables attain apparent normal or even high values as occurs during resuscitated septic shock, venous CO_2 could increase the respiratory quotient due to relative rising of VCO_2 over VO_2, probably secondary to the buffering of hydrogen ions [14].

7.2 Cv-aCO_2 to Da-vO_2 ratio and its relationship with anaerobic metabolism during tissue hipoxia

Clinical detection of CO_2 coming from anaerobic metabolism is difficult to determinate during hypoxic conditions since the bulk of anaerobic CO_2 is relatively small compared with the already reduced total VCO_2. However, Mekontso-Dessap et al. [4]

demonstrated a good correlation between Pv-aCO_2/Da-vO_2 ratio (a surrogate of Cv-aCO_2 to Da-vO_2 ratio) and lactate levels [4] assuming a lactate ≥ 2.0 mmol/L as a marker of anaerobic metabolism. Nevertheless, hyperlactatemia may not always represent anaerobic metabolism [15] and interpretation of lactate levels during resuscitation and post-resuscitation period is sometimes very difficult. In a recent study, Ospina-Tascón et al. [5] demonstrated the strong prognostic significance of persistent hyperlactatemia combined with an increased Cv-aCO_2/Da-vO_2 ratio when compared to hyperlactemia with normal ratio [15] . Therefore, Cv-aCO_2 to Da-vO_2 ratio could be a tool to differenciate an hypoxic-generated lactate accumulation versus other non-flow-dependent causes of hyperlactatemia.

7.3 Does Cv-aCO_2/Da-O_2 ratio differs from Pv-aCO_2/Da-O_2 ratio?

Over physiological range of CO_2 contents, i.e., along the steep portion of the CO_2 dissociation curve, partial CO_2 pressure (PCO_2) maintains a quasi-linear relationship with CO_2 content (CCO_2), so Pv-aCO_2 could be theoretically used as a surrogate for the Cv-aCO_2. However, the relationship between PCO_2 and CCO_2 becomes non linear at abnormal Pv-aCO_2 values [16]. Thus, Cv-aCO_2/Da-vO_2 ratio could be superior to Pv-aCO_2/Da-vO_2 ratio in predicting outcomes during very early phases of resuscitation of septic shock [5] since at deeper tissue hypoxia, disparity between Pv-aCO_2 and Cv-aCO_2 increases according to the Haldane effect.

7.4 Limitations in the clinical practice

First, calculating VCO_2 according to Fick´s approach is valid under steady-state conditions. Thus, increasing cardiac output could overestimate VCO_2 leading to increases in VCO_2/VO_2. However, Cv-aCO_2/Da-vO_2 ratio couples global CO_2

accumulation and O_2 consumption with some independence of macro or micro blood flow and it should also be less influenced by pulmonary ventilation/perfusion abnormalities. Second, calculation of Cv-aCO_2/Da-vO_2 ratio is cumbersome and subjected to an important risk of errors. Nevertheless, the influence of such potential errors is limited as it correctly identifies patients at increased risk of death [5]. Third, calculating Pv-aCO_2/Da-vO_2 is easier and it should be equivalent to Cv-aCO_2/Da-vO_2 when PCO_2 and SvO_2 approximates to normality, which occurs frequently. However, Pv-aCO_2/Da-vO_2 ratio is mostly subjected to Haldane effect determinants. Thus, its interchangeability with Cv-aCO_2/Da-vO_2 ratio could be debatable. Fourth, information regarding to global CO_2/O_2 relationships comes from pulmonary artery catheter monitoring [4,5], so its equivalence with central venous sample calculations is not yet proved.

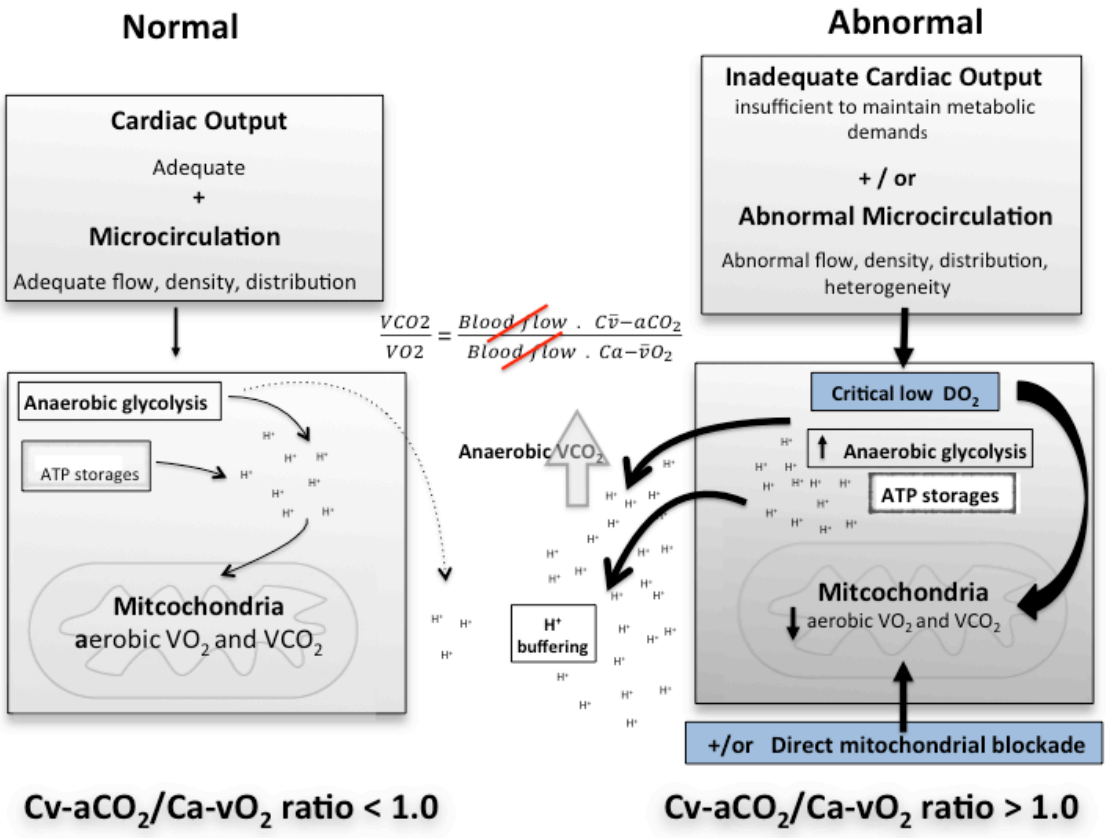


Fig. 1 A comparison of Cv-aCO_2/Da-vO_2 ratio during normal tissue perfusion (left side) and progressive hypoperfusion (right side). Normal aerobic mitochondrial function leads to normal ratio. In contrast, during progressive hypoperfusion, both O_2 consumption and aerobic CO_2 generation are depressed but the Cv-aCO_2/Da-vO_2 ratio increases (>1.0) due to an increase in anaerobic CO_2 generation as consequence of the buffering of hydrogen ions. For more de

7.5 CONCLUSION

Cv-aCO_2/DavO_2 ratio can be used as an approximation of respiratory quotient and could detect ongoing anaerobic CO_2 generation in patients with septic shock. Admittedly, computation of CO_2 and O_2 contents are cumbersome. However, Cv-aCO_2/DavO_2 ratio significance and biological plausibility during resuscitation of septic shock deserves future research efforts.

7.6 REFERENCES

1. Cecconi M, De Backer D, Antonelli M, et al. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. *Intensive Care Med.* 2014;40(12):1795-1815.
2. Bellomo R, Reade MC, Warrillow SJ. The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. *Crit Care.* 2008;12(2):130.
3. Ospina-Tascon GA, Umana M, Bermudez WF, et al. Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock? *Intensive Care Med.* 2016;42(2):211-221.
4. Mekontso-Dessap A, Castelain V, Anguel N, et al. Combination of venoarterial PCO2 difference with arteriovenous O2 content difference to detect anaerobic metabolism in patients. *Intensive Care Med.* 2002;28(3):272-277.
5. Ospina-Tascon GA, Umana M, Bermudez W, et al. Combination of arterial lactate levels and venous-arterial CO2 to arterial-venous O 2 content difference ratio as markers of resuscitation in patients with septic shock. *Intensive Care Med.* 2015;41(5):796-805.
6. Wasserman K, Beaver WL, Whipp BJ. Gas exchange theory and the lactic acidosis (anaerobic) threshold. *Circulation.* 1990;81(1 Suppl):Ii14-30.
7. Randall HM, Jr., Cohen JJ. Anaerobic CO2 production by dog kidney in vitro. *Am J Physiol.* 1966;211(2):493-505.
8. Stringer W, Wasserman K, Casaburi R. The VCO2/VO2 relationship during heavy, constant work rate exercise reflects the rate of lactic acid accumulation. *Eur J Appl Physiol Occup Physiol.* 1995;72(1-2):25-31.
9. Breen PH, Isserles SA, Westley J, Roizen MF, Taitelman UZ. Combined carbon monoxide and cyanide poisoning: a place for treatment. *Anesth Analg.* 1995;80(4):671-677.
10. Groeneveld AB, Vermeij CG, Thijs LG. Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end-expiratory pressure in the pig. *Anesth Analg.* 1991;73(5):576-582.
11. Cohen IL, Sheikh FM, Perkins RJ, Feustel PJ, Foster ED. Effect of hemorrhagic shock and reperfusion on the respiratory quotient in swine. *Crit Care Med.* 1995;23(3):545-552.
12. Austin WH, Lacombe E, Rand PW, Chatterjee M. Solubility of carbon dioxide in serum from 15 to 38 C. *J Appl Physiol.* 1963;18:301-304.
13. Goldman D, Bateman RM, Ellis CG. Effect of decreased O2 supply on skeletal muscle oxygenation and O2 consumption during sepsis: role of heterogeneous capillary spacing and blood flow. *Am J Physiol Heart Circ Physiol.* 2006;290(6):H2277-2285.
14. Raza O, Schlichtig R. Metabolic component of intestinal PCO(2) during dysoxia. *J Appl Physiol (1985).* 2000;89(6):2422-2429.
15. James JH, Luchette FA, McCarter FD, Fischer JE. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. *Lancet.* 1999;354(9177):505-508.
16. Ospina-Tascon GA, Umana M, Bermudez WF, et al. Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock? *Intensive Care Med.* 2015.

Chapter 8

GENERAL DISCUSSION

Enhancing convective and diffusive oxygen transport should theoretically reduce tissue hypoxia during shock states, as the early identification of inadequate tissue perfusion has been considered key factor in the management of septic shock[1]. Normalizing systemic hemodynamic parameters does not, however, guarantee an adequate tissue perfusion and, indeed, a substantial number of patients who have received such treatment still progress to multi-organ dysfunction and death.

Traditional resuscitation approaches have been based on targeting macro hemodynamic and oxygen-derived parameters, since it is assumed that blood flow is uniformly distributed at the microcirculatory level leading to normal oxygen diffusion to cells, thus preserving cellular respiration. Accordingly, the assumption stating that tissue oxygenation is maintained by blood supply is derived from models that assume uniformity in capillary perfusion. However, in animal models and human patients with sepsis and septic shock, microcirculation is characterized by inhomogeneity of microvascular blood flow with perfused and underperfused zones in close proximity[2]. Mathematical simulations suggest that heterogeneity of oxygen delivery is a major determinant of oxygen consumption disturbances in peripheral tissues[3], whilst experimental endotoxemic models using ex vivo intestinal tissue confirm that heterogeneity of capillary transit times is responsible for impaired tissue oxygenation[4]. Consequently, heterogeneous flow cessation of individual capillaries could determine oxygen supply dependence during septic shock[5]. Other mathematical models suggest that more severe oxygenation derangements occur when capillary blood flow is totally stopped[6]. Consequently, we conducted an experimental model of severe hypodynamic septic shock induced by peritonitis in which hemodynamic goal-directed resuscitation was randomly combined with low fixed doses of dobutamine. Our results suggest that, effectively, microvascular blood flow distribution at the jejunal mucosa is closely related to alterations in mesenteric oxygen extraction. Higher percentages of stopped small-vessels at the jejunal mucosa are significantly

related to the most severe oxygen extraction alterations. Fluid resuscitation with only crystalloids did not correct villi hypoperfusion and tissue-to-arterial carbon dioxide differences remained high. Conversely, when low fixed-doses of dobutamine were randomly added, microvascular blood flow exhibited a progressive recovery in the number of perfused small vessels and functional capillary density, which led to decreases in the heterogeneity index. Improvement in microvascular blood flow was reflected by progressive decreases in tissue (jejunal) and regional (mesenteric) -to-arterial carbon dioxide differences and these, in turn, were mirrored by systemic venous-to-arterial carbon dioxide differences (Pv-aCO_2). Thus, we experimentally confirmed the assumptions posed by mathematical modeling and *ex vivo* experimental results, which suggest that heterogeneity of microvascular blood flow is a key determinant of oxygen consumption during septic shock. Most importantly, our data indicate that microcirculatory alterations at the gut mucosa might be potentially reversed, leading to improvements in regional oxygen consumption.

Previously, authors have described the coexistence of venous acidemia and increased venous carbon dioxide during cardiac arrest in both animals[7] and critically ill humans[8]. Thereafter, increases in the venous-to-arterial carbon dioxide difference (Pv-aCO_2) were reported during hypovolemic, cardiogenic, obstructive, and septic shock[9-11]. Interestingly, an inverse curvilinear relationship between Pv-aCO_2 and cardiac output was described, highlighting the importance of blood flow for venous CO_2 accumulation[12,13]. Thus, the Pv-aCO_2 aroused clinical interest as a marker of global perfusion during shock states, although some studies questioned its prognostic value[13]. In fact, some in vivo models evaluating the mechanisms leading to venous CO_2 accumulation during non-inflammatory conditions[14-16] challenged the ability of Pv-aCO_2 to identify tissue dysoxia because this only appears during ischemic hypoxia, not during hypoxic or anemic hypoxia, for comparable declines in oxygen delivery (DO_2) and consumption (VO_2). More recent data suggests, however, that high Pv-aCO_2 could identify septic patients who remain inadequately resuscitated despite achieving

oxygen metabolism targets, reinforcing the notion of Pv-aCO_2 as a marker of global perfusion, due to its ability to track blood flow alterations[17] or even detect anaerobic CO_2 generation[18].

Our initial clinical observations suggested that persistence of increased Pv-aCO_2 during the early stages of resuscitation from septic shock is related to more severe multiorgan dysfunction and to poorer clinical outcomes, even in patients attaining normal SvO_2 or $ScvO_2$. We also observed a lack of concordance between Pv-aCO_2 and macro vascular blood flow at each time-point of measurements, with normal or even high cardiac output values (Annex A, Suppl. Fig. 2), challenging previous observations performed in non inflammatory conditions.

Subsequently, we explored the mechanisms behind Pv-aCO_2 increasing during septic shock in humans, using a hand-held video microscopy to assess the microcirculation at the bedside. For that, we evaluated the development of macro hemodynamics, oxygen-derived parameters, Pv-aCO_2, and Cv-aCO_2/Ca-vO_2 ratio during the first hours of resuscitation, with simultaneous microcirculatory blood flow evaluation by means of the Side-stream dark field technique. Our observations revealed that Pv-aCO_2 is closely related to microcirculatory blood flow. Indeed, Pv-aCO_2 was the best predictor of the microvascular blood flow misdistribution, as indicated by alterations in the percentage of perfused small vessels, increasing heterogeneity of the blood flow index and, consequently, decreasing functional capillary density. In addition, changes in Pv-aCO_2 over time were found to be significantly related to changes in the percentage of perfused small-vessels (PPV), while Pv-aCO_2 was found to be poorly related to systemic hemodynamic variables.

Interpretation of hyperlactatemia during septic shock is very complex since anaerobic metabolism, non-anaerobic generation and slow reuptake can all lead to lactate accumulation[19]. Due to this, it would be interesting to explore signals indicating when

hyperlactatemia is of anaerobic origin, which would guide maneuvers aiming to increase blood flow, supposing that this would improve tissue perfusion and cellular respiration. Isolated Pv-aCO_2 does not, however, represent tissue dysoxia[14-16], but combining this with oxygen consumption could give some idea about cellular respiration. During steady state conditions, carbon dioxide generation should not exceed oxygen consumption. Thus, a disproportionate increase in CO_2 would indicate the presence of a non-aerobic CO_2 source. Clinical detection of CO_2 coming from anaerobic metabolism is, however, difficult to determinate during hypoxic conditions, since the bulk of anaerobic CO_2 is relatively small compared with the already reduced total VCO_2. We therefore explore the combination of arterial lactate levels (the traditional marker of anaerobic metabolism) with the venous-arterial CO_2 to arterial-venous O_2 content differences ratio (Cv-aCO_2/Ca-vO_2) as a surrogate for the respiratory quotient (i.e., VCO_2/VO_2 ratio), in a population of patients with septic shock subjected to a protocolled quantitative early goal-directed resuscitation. Our data suggest that persistent hyperlactatemia combined with a high Cv-aCO_2/Da-vO_2 is associated with the most severe organ dysfunction and the worst clinical outcomes, while simultaneous normalization of lactate and Cv-aCO_2/Da-vO_2 ratio is associated with the best outcomes. Interestingly, patients attaining lactate levels < 2.0 mmol/L combined with Cv-aCO_2/Da-vO_2 >1.0 exhibit similar outcomes than patients with persistent hyperlactatemia and low Cv-aCO_2/Da-vO_2 ratio. Cv-aCO_2/Da-vO_2 ratio is thus an integrative flow-independent variable, since cardiac output is included in calculations for both VO_2 and VCO_2, according to the Fick´s equation, whereas microvascular blood flow distribution is linked to Cv-aCO_2 and Da-vO_2. Accordingly, when cardiac output and oxygen metabolism variables attain apparently normal or even high values, as occurs during resuscitated septic shock, venous CO_2 could increase the respiratory quotient due to the relative increase of VCO_2 over VO_2, probably secondary to the buffering of hydrogen ions. More research is needed in order to clarify the mechanisms involved in the increasing Cv-aCO_2/Da-vO_2 ratio during septic shock, although its clinical value seem to be biologically plausible.

8.1 REFERENCES

1. Cecconi M, De Backer D, Antonelli M, et al. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. *Intensive Care Med.* 2014;40(12):1795-1815.
2. De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent JL. Monitoring the microcirculation in the critically ill patient: current methods and future approaches. *Intensive Care Med.* 2010;36(11):1813-1825.
3. Walley KR. Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. *J Appl Physiol.* 1996;81(2):885-894.
4. Humer MF, Phang PT, Friesen BP, Allard MF, Goddard CM, Walley KR. Heterogeneity of gut capillary transit times and impaired gut oxygen extraction in endotoxemic pigs. *J Appl Physiol (1985).* 1996;81(2):895-904.
5. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R. Effect of a maldistribution of microvascular blood flow on capillary O(2) extraction in sepsis. *Am J Physiol Heart Circ Physiol.* 2002;282(1):H156-164.
6. Goldman D, Bateman RM, Ellis CG. Effect of decreased O2 supply on skeletal muscle oxygenation and O2 consumption during sepsis: role of heterogeneous capillary spacing and blood flow. *Am J Physiol Heart Circ Physiol.* 2006;290(6):H2277-2285.
7. Grundler W, Weil MH, Rackow EC. Arteriovenous carbon dioxide and pH gradients during cardiac arrest. *Circulation.* 1986;74(5):1071-1074.
8. Weil MH, Rackow EC, Trevino R, Grundler W, Falk JL, Griffel MI. Difference in acid-base state between venous and arterial blood during cardiopulmonary resuscitation. *The New England journal of medicine.* 1986;315(3):153-156.
9. Mecher CE, Rackow EC, Astiz ME, Weil MH. Venous hypercarbia associated with severe sepsis and systemic hypoperfusion. *Critical care medicine.* 1990;18(6):585-589.
10. Adrogue HJ, Rashad MN, Gorin AB, Yacoub J, Madias NE. Assessing acid-base status in circulatory failure. Differences between arterial and central venous blood. *The New England journal of medicine.* 1989;320(20):1312-1316.
11. Kazarian KK, Del Guercio LR. The use of mixed venous blood gas determinations in traumatic shock. *Annals of emergency medicine.* 1980;9(4):179-182.
12. Teboul JL, Mercat A, Lenique F, Berton C, Richard C. Value of the venous-arterial PCO2 gradient to reflect the oxygen supply to demand in humans: effects of dobutamine. *Critical care medicine.* 1998;26(6):1007-1010.
13. Bakker J, Vincent JL, Gris P, Leon M, Coffernils M, Kahn RJ. Veno-arterial carbon dioxide gradient in human septic shock. *Chest.* 1992;101(2):509-515.
14. Vallet B, Teboul JL, Cain S, Curtis S. Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol.* 2000;89(4):1317-1321.

15. Neviere R, Chagnon JL, Teboul JL, Vallet B, Wattel F. Small intestine intramucosal PCO(2) and microvascular blood flow during hypoxic and ischemic hypoxia. *Critical care medicine.* 2002;30(2):379-384.
16. Dubin A, Estenssoro E, Murias G, et al. Intramucosal-arterial Pco2 gradient does not reflect intestinal dysoxia in anemic hypoxia. *The Journal of trauma.* 2004;57(6):1211-1217.
17. Vallee F, Vallet B, Mathe O, et al. Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? *Intensive care medicine.* 2008;34(12):2218-2225.
18. Mekontso-Dessap A, Castelain V, Anguel N, et al. Combination of venoarterial PCO2 difference with arteriovenous O2 content difference to detect anaerobic metabolism in patients. *Intensive care medicine.* 2002;28(3):272-277.
19. De Backer D. Lactic acidosis. *Intensive care medicine.* 2003;29(5):699-702.

Chapter 9

GENERAL CONCLUSIONS

Traditional assessment of tissue perfusion and cellular respiration has been widely based on hemodynamic pressure-derived variables, cardiac output variations and systemic oxygen-derived parameters. This type of monitoring approach seems, however, to be insufficient to ensure an adequate tissue perfusion and to promote the normalization of cellular function during shock states.

According to the sequential construction of this thesis, the following conclusions are proposed:

1. Despite controversies about the causal relationship between microcirculatory alterations and organ dysfunction, the quest for appropriate monitoring of microcirculation could potentially improve resuscitation results in septic shock. Thus, the direct evaluation of microcirculation at the bedside would be a more physiologically based approach to guide fluid administration and therapeutic interventions during resuscitation. However, despite advances in the techniques to evaluate microcirculation at the bedside, an immediate and reliable method to quantify microcirculatory alterations is not yet available. Surrogates reflecting the adequacy of microvascular blood flow might be highly valuable.

2. Persistence of a high Pv-aCO_2 during the early phases of resuscitation in septic shock is associated with more severe multi-organ dysfunction and a higher chance of unfavorable outcomes even in patients attaining normal central or mixed-venous oxygen saturation, which makes Pv-aCO_2 a potentially useful variable to monitor in the clinical setting. Furthermore, abnormal values of Pv-aCO_2 can anticipate delays in lactate normalization and, contrary to observations performed in non-inflammatory conditions, there is a lack of agreement between Pv-aCO_2 and cardiac output changes in septic shock, which suggests that mechanisms other than macro blood flow are important in venous carbon dioxide accumulation.

3. Complementing lactate assessment with Cv-aCO_2/Da-vO_2 ratio during the early stages of resuscitation from septic shock can help to better identify patients at high risk of adverse outcomes. Unfortunately, the Cv-aCO_2/Da-vO_2 ratio is not clinically equivalent to the Pv-aCO_2/Da-vO_2 ratio (i.e., when venous-to-arterial CO_2 difference is calculated in terms of CO_2 pressures but not in terms of contents), which suggests an important influence of the Haldane effect. Admittedly, computation of CO_2 content and Da-vO_2 are cumbersome and subject to an important risk of error due to the number of variables included in the formulas, but our data suggest that the influence of such measurements errors is limited, as the formulas correctly identify patients at increased risk of death. Even though it is more complicated to compute, the Cv-aCO_2/Da-vO_2 ratio is easier to interpret, with values above 1 suggesting anaerobic metabolism. Thus, the Cv-aCO_2/Da-vO_2 ratio may become a potential resuscitation goal in patients with septic shock, since it could reflect non-aerobic carbon dioxide generation (a mechanistic hypothesis to be tested in the future).

4. Increased Pv-aCO_2 values might reflect microcirculatory dysfunction in septic shock, even when SvO_2 and cardiac output appear to have been corrected. Particularly, progressively higher Pv-aCO_2 values are related to a more altered microcirculatory blood flow in terms of the percentage of perfused small vessels, functional capillary density and higher heterogeneity of flow. Once again, Pv-aCO_2 did not agree with cardiac output, mean arterial pressure or mixed-venous oxygen saturation. Extremely high Pv-aCO_2 values occurred, however, in patients with more severely altered macro-hemodynamic variables.

5. Dynamic variations of Pv-aCO_2 could track microcirculatory alterations during early stages of resuscitation in septic shock. Pv-aCO_2 could reflect the sufficiency of microvascular blood flow even when macro hemodynamics appear to have been

corrected. Conversely, microcirculatory alterations did not agree with the Cv-aCO_2/Da-vO_2 ratio, suggesting that the latter is a non-dependent flow phenomenon.

6. Heterogeneity of microvascular blood flow distribution is implied on impaired oxygen extraction capabilities by the peripheral tissues. Particularly, increased heterogeneity of microvascular blood flow at the intestinal mucosa is inversely related to regional oxygen extraction ratios. This microvascular heterogeneity is significantly reduced with low doses of dobutamine, leading to improvement in oxygen consumption. Systemic Pv-aCO_2 tracks tissue (intestinal mucosa) and regional splanchnic CO_2 accumulation and these in turn, reflect microcirculatory alterations at the gut mucosa during hypodynamic septic shock. Low fixed doses of dobutamine are related with improving villi microcirculatory blood flow and this, in turn, with progressive decreases of local, regional and systemic-to-arterial CO_2 differences.

7. The Cv-aCO_2/Da-vO_2 ratio might be used as a surrogate of the respiratory quotient whereby increasing the Cv-aCO_2/Da-vO_2 ratio could detect ongoing non-aerobic generation of carbon dioxide in patients with septic shock. Computations of CO_2 and O_2 contents are admittedly cumbersome, but the significance of the Cv-aCO_2/Da-vO_2 ratio and its biological plausibility warrants future research efforts.

CONCLUSIONES GENERALES

La evaluación tradicional del estado de perfusión tisular y respiración celular durante los estados de shock ha sido ampliamente basado en variables macro-hemodinámicas derivadas de presiones, variaciones del gasto cardiaco y parámetros sistémicos derivados del oxígeno. Sin embargo, este tipo de aproximación de monitoría parecen ser insuficientes para asegurar una adecuada perfusión tisular y de esta forma, para promover la normalización de la función celular durante los estados de shock.

De acuerdo a la construcción secuencial de esta tesis, propongo las siguientes conclusiones:

1. A pesar de las controversias acerca de la relación causal entre las alteraciones microcirculatorias y la disfunción orgánica, la búsqueda de métodos apropiados para monitorear la microcirculación podría mejorar los resultados de la reanimación en el shock séptico. En este sentido, la evaluación directa de la microcirculación a la cabecera del paciente críticamente enfermo sería una aproximación más fisiológica para guiar la administración de líquidos e intervenciones terapéuticas durante la reanimación. Sin embargo, a pesar de los avances en las técnicas portátiles de monitoria de la microcirculación, una método confiable y reproducible para cuantificar las alteraciones microcirculatorias no está todavía disponible, por lo cual, equivalente biológicos capaces de reflejar la suficiencia de la microcirculación gozan de gran importancia.

2. La persistencia de una $Pv\text{-}aCO_2$ elevada durante las etapas tempranas de

reanimación del shock séptico está asociada con una más severa disfunción de múltiples órganos y desenlaces clínicos desfavorables, aún en pacientes que logran la normalización de la saturación venosa central y/o mixta, lo cual hace de la Pv-aCO_2 una variable potencialmente utilizable en monitoría. Además, valores anormales de Pv-aCO_2 pueden anticipar el retraso de la normalización de los niveles de lactato y contrario a las observaciones realizadas en condiciones no inflamatorias, existe una falta de concordancia entre la Pv-aCO_2 y los cambios en el gasto cardiaco, lo cual sugiere que otros mecanismos diferentes a las variaciones en el flujo macro vascular están implicadas en la acumulación venosa de dióxido de carbono.

3. La adición de la relación Cv-aCO_2/Da-vO_2 a los niveles de lactato durante las etapas tempranas de reanimación del shock séptico, puede mejorar la capacidad de identificación de pacientes en alto riesgo de desenlaces desfavorables. Desafortunadamente, la relación Cv-aCO_2/Da-vO_2 no es clínicamente equivalente a la Pv-aCO_2/Da-vO_2 (es decir, cuando la diferencia veno-arterial es calculada en términos de presiones parciales de CO_2 y no en términos de contenidos), lo cual sugiere una importante influencia del efecto Haldane. Debe admitirse que el cálculo de los contenidos de CO_2 y la diferencia arterio-venosa de O_2 son complicados y sujetos a una alta posibilidad de error dado el número de variables incluidas en la fórmula; sin embargo, nuestros datos sugieren que la influencia de tales errores de medición son limitados en la medida en que al relación Cv-aCO_2/Da-vO_2 identifica correctamente a aquellos en alto riesgo de muerte. Aunque más complicado de calcular, la relación Cv-aCO_2/Da-vO_2 es más fácil de interpretar, con valores por encima de 1 sugiriendo presencia de metabolismo anaeróbico. Así, una relación Cv-aCO_2/Da-vO_2 podría convertirse en una meta potencial de reanimación en pacientes con shock séptico, al reflejar probablemente, generación anaeróbica de CO_2 (hipótesis a ser evaluada en el futuro).

4. Valores elevados de Pv-aCO_2 puede reflejar la presencia de disfunción

microcirculatoria durante el shock séptico, aún cuando la SvO_2 y el gasto cardiaco luzcan aparentemente corregidos. Particularmente, valores progresivamente mayores de Pv-aCO_2 están relacionados con u flujo microcirculatorio progresivamente más alterado en términos de porcentaje de vasos pequeños perfundidos, densidad capilar funcional e incremento de la heterogeneidad de flujo. Un vez más, la Pv-aCO_2 no tuvo una buena concordancia con el gasto cardiaco, presión arterial media o la saturación venosa mixta de oxígeno. Sin embargo, valores extremadamente altos de Pv-aCO_2 ocurrieron en pacientes con alteraciones macro hemodinámicas más severas.

5. Las variaciones dinámicas de la Pv-aCO_2 pueden reflejar las alteraciones microcirculatorias durante las etapas tempranas de reanimación del shock séptico. Así, la Pv-aCO_2 puede reflejar la suficiencia del flujo sanguíneo microvascular cuando la macro hemodinamia luce aparentemente corregida.
Contrariamente, las alteraciones microcirculatorias no mostraron una buena concordancia con la relación Cv-aCO_2/Da-vO_2, lo que sugiere que ésta última variable se trata de un fenómeno no dependiente de flujo.

6. La heterogeneidad de la distribución del flujo sanguíneo microcirculatorio está implicada en el empeoramiento de la capacidad de extracción de oxígeno por parte de los tejidos periféricos. Particularmente, el incremento de la heterogeneidad del flujo microcirculatorio a nivel de la mucosa intestinal está relacionada inversamente con la tasa de extracción de oxígeno regional. Esta heterogeneidad microcirculatoria es significativamente reducida con el uso de dosis bajas fijas de dobutamina, lo que conduce a una mejoría en el consumo de oxígeno.
La Pv-aCO_2 sistémica refleja la acumulación tisular (mucosa intestinal) y regional esplácnica de CO_2 y estas a su vez, reflejan las alteraciones de la microcirculación a nivel de la mucosa intestinal durante el shock séptico hipodinámico. Dosis bajas fijas de dobutamina provocan mejoría del flujo sanguíneo microvascular en las vellosidades intestinales y esto se relaciona a su vez con una disminución progresiva

de las diferencias local, regional y sistémica-arterial de CO_2.

7. La relación Cv-aCO_2/Da-vO_2 puede ser utilizada como un equivalente del cociente respiratorio, por lo que un incremento de la Cv-aCO_2/Da-vO_2 podría detectar la producción anaeróbica de CO_2 en pacientes con shock séptico. El cálculo de los contenidos de CO_2 y O_2 son complicados, pero la significancia de la relación Cv-aCO_2/Da-vO_2 y su plausibilidad biológica ameritan futuros esfuerzos de investigación.

Chapter 10

CLINICAL IMPLICATIONS AND FUTURE CONSIDERATIONS

The end-point of fluid resuscitation during septic shock has been focused on optimize tissue perfusion supposing that enhancing convective and diffusive oxygen transport will reduce tissue hypoxia. However, usual monitoring emphasizes on macro hemodynamic and systemic oxygen-derived parameters, assuming that blood flow will be uniformly distributed at microcirculatory level and oxygen will diffuse to cells, thus maintaining cellular respiration.

Venous-to-arterial carbon dioxide difference might reflect micro blood flow variations in apparently resuscitated patients with septic shock. However, despite its biological plausibility, the capacity of Pv-aCO_2 to guide resuscitation in patients with septic shock has not been tested in clinical trials. Similarly, although Cv-aCO_2/Da-vO_2 ratio might differentiate anaerobic from non-anaerobic lactate increases because of its ability to detect ongoing anaerobic CO_2 generation, Cv-aCO_2/Da-vO_2 ratio-guided resuscitation has not been evaluated in clinical trials. Therein, both Pv-aCO_2 and the Cv-aCO_2/Da-vO_2 ratio will be part of the multimodal monitoring protocol to be tested in the ANDROMEDA trial, a multicenter randomized-controlled study aiming to evaluate if peripheral perfusion-guided resuscitation can improve clinical outcomes in septic shock. For this, centers in Brazil, Colombia, Chile, Uruguay, Argentina, France and The Netherlands will enroll septic shock patients with persistent clinical signs of hypoperfusion in order to test whether some therapeutic interventions can improve clinical peripheral perfusion. Data from ANDROMEDA will clarify the role of Pv-aCO_2 and the Cv-aCO_2/Da-vO_2 ratio as resuscitation goals during septic shock.

Our data reinforce the idea asserting that microvascular blood flow distribution is a key aspect on peripheral oxygen consumption during septic shock. Furthermore, these alterations in oxygen consumption are potentially reversible when some therapeutic interventions are applied, at least during early stages of resuscitation. Thus, the most physiological approach to guide resuscitation in septic shock seems to use multimodal microcirculatory blood flow monitoring. However, a number of

patients survive without attaining normal microvascular blood flow, which supposes the existence of some degree of adaptation to deranged convective and diffusive oxygen transport. In this sense, it should be clarified when and why this adaptation appears and how it can be clinically detected. For that, efforts should be aimed to better understand the kinetics of tissue oxygen distribution and cellular redox potential in order to understand not only the dynamics of convective and diffusive oxygen transport, but also how oxygen is used in cellular respiration during shock conditions. Understanding the variations in cellular redox potential during "in vivo" conditions could clarify the mechanisms involved in altered oxygen consumption and whether microcirculatory blood flow derangements are causal or simply an adaptive phenomenon to variations in cellular respiration. However, measuring cellular redox potential in "in vivo" conditions supposes many technical challenges, since this implies measurements of some intracellular metabolic intermediates. In this regard, intravital fluorescence techniques can offer the possibility to evaluate the cellular redox potential while coupling with microcirculatory blood flow monitoring and tissue oxygenation in order to study the interactions between blood flow distribution, tissue oxygenation and cellular respiration during the development and resuscitation of shock.

Mechanisms of increasing Cv-aCO_2/Da-vO_2 ratio must also be clarified. Our theoretical construction has been made on the basis of previous research, which we hypothesized that increases in Cv-aCO_2/Da-vO_2 ratio reflect anaerobic CO_2 generation. However, demonstrating that such CO_2 increases come from anaerobic production when oxygen mitochondrial availability is limited or even mitochondrial function is inappropriate, implies using energetic metabolic radiolabeled substrates to determinate whether "additional" CO_2 is effectively generated by bicarbonate tamponade of excess of hydrogen ions not participating in the normal respiratory chain.

The experience constructing this thesis confirms that understanding of some

phenomena in clinical practice requires the junction with basic research, and knowledge extracted of the latter should in turn be disposable for resolution of clinical problems.

GENERAL BIBLIOGRAPHY

- ADROGUE HJ, RASHAD MN, GORIN AB, YACOUB J, MADIAS NE. Assessing acid-base status in circulatory failure. Differences between arterial and central venous blood. *The New England journal of medicine.* 1989;320(20):1312-1316.

- AUSTIN WH, LACOMBE E, RAND PW, CHATTERJEE M. Solubility of carbon dioxide in serum from 15 to 38 C. *J Appl Physiol.* 1963;18:301-304.

- BAKKER J, VINCENT JL, GRIS P, LEON M, COFFERNILS M, KAHN RJ. Veno-arterial carbon dioxide gradient in human septic shock. *Chest.* 1992;101(2):509-515.

- BATEMAN RM, TOKUNAGA C, KARECO T, DORSCHEID DR, WALLEY KR. Myocardial hypoxia-inducible HIF-1alpha, VEGF, and GLUT1 gene expression is associated with microvascular and ICAM-1 heterogeneity during endotoxemia. *Am J Physiol Heart Circ Physiol.* 2007;293(1):H448-456.

- BEAL AL, CERRA FB. Multiple organ failure syndrome in the 1990s. Systemic inflammatory response and organ dysfunction. *JAMA : the journal of the American Medical Association.* 1994;271(3):226-233.

- BELLOMO R, READE MC, WARRILLOW SJ. The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. *Crit Care.* 2008;12(2):130.

- BERSTEN AD, HERSCH M, CHEUNG H, RUTLEDGE FS, SIBBALD WJ. The effect of various sympathomimetics on the regional circulations in hyperdynamic sepsis. *Surgery.* 1992;112(3):549-561.

- BIRO GP, DOUGLAS JR, KEON WJ, TAICHMAN GC. Changes in regional blood flow distribution induced by infusions of dopexamine hydrochloride or dobutamine in anesthetized dogs. *Am J Cardiol.* 1988;62(5):30c-36c.

- BOYD JH, FORBES J, NAKADA TA, WALLEY KR, RUSSELL JA. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. *Crit Care Med.* 2011;39(2):259-265.

- BRACHT H, KREJCI V, HILTEBRAND L, et al. Orthogonal polarization spectroscopy to detect mesenteric hypoperfusion. *Intensive Care Med.* 2008;34(10):1883-1890.

- BREEN PH, ISSERLES SA, WESTLEY J, ROIZEN MF, TAITELMAN UZ. Combined carbon monoxide and cyanide poisoning: a place for treatment. *Anesth Analg.* 1995;80(4):671-677.

- BÜCHELE GL, SILVA E, OSPINA-TASCÓN GA, VINCENT JL, DE BACKER D. Effects of hydrocortisone on microcirculatory alterations in patients with septic shock. *Crit Care Med.* 2009;37(4):1341-1347.

- CECCONI M, DE BACKER D, ANTONELLI M, et al. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. *Intensive Care Med.* 2014;40(12):1795-1815.
- COHEN IL, SHEIKH FM, PERKINS RJ, FEUSTEL PJ, FOSTER ED. Effect of hemorrhagic shock and reperfusion on the respiratory quotient in swine. *Crit Care Med.* 1995;23(3):545-552.

- CRETEUR J, DE BACKER D, SAKR Y, KOCH M, VINCENT JL. Sublingual capnometry tracks microcirculatory changes in septic patients. *Intensive care medicine.* 2006;32(4):516-523.

- CRETEUR J, DE BACKER D, VINCENT JL. Monitoring gastric mucosal carbon dioxide pressure using gas tonometry: in vitro and in vivo validation studies. *Anesthesiology.* 1997;87(3):504-510.

- CUSCHIERI J, RIVERS EP, DONNINO MW, et al. Central venous-arterial carbon dioxide difference as an indicator of cardiac index. *Intensive Care Med.* 2005;31(6):818-822.

- DE BACKER D, BERRE J, MORAINE JJ, MELOT C, VANFRAECHEM J, VINCENT JL. Effects of dobutamine on the relationship between oxygen consumption and delivery in healthy volunteers: comparison with sodium nitroprusside. *Clin Sci (Lond).* 1996;90(2):105-111.

- DE BACKER D, BERRE J, ZHANG H, KAHN RJ, VINCENT JL. Relationship between oxygen uptake and oxygen delivery in septic patients: effects of prostacyclin versus dobutamine. *Crit Care Med.* 1993;21(11):1658-1664.

- DE BACKER D, CRETEUR J, DUBOIS MJ, et al. The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med.* 2006;34(2):403-408.

- DE BACKER D, CRETEUR J, PREISER JC, DUBOIS MJ, VINCENT JL. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med.* 2002;166(1):98-104.

- DE BACKER D, CRETEUR J, ZHANG H, NORRENBERG M, VINCENT JL. Lactate production by the lungs in acute lung injury. *Am J Respir Crit Care Med.* 1997;156(4 Pt 1):1099-1104.

- DE BACKER D, DONADELLO K, SAKR Y, et al. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. *Crit Care Med.* 2013;41(3):791-799.

- DE BACKER D, HOLLENBERG S, BOERMA C, et al. How to evaluate the microcirculation: report of a round table conference. *Crit Care.* 2007;11(5):R101.

- DE BACKER D, OSPINA-TASCON G, SALGADO D, FAVORY R, CRETEUR J, VINCENT JL. Monitoring the microcirculation in the critically ill patient: current methods and future approaches. *Intensive Care Med.* 2010;36(11):1813-1825.

- DE BACKER D. Lactic acidosis. *Intensive care medicine.* 2003;29(5):699-702.

- DELLINGER RP, LEVY MM, CARLET JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Critical care medicine.* 2008;36(1):296-327.
- DELLINGER RP, LEVY MM, RHODES A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med.* 2013;39(2):165-228.

- DELLINGER RP, LEVY MM, RHODES A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med.* 2013;41(2):580-637.

- DELONG ER, DELONG DM, CLARKE-PEARSON DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics.* 1988;44(3):837-845.

- DESAI VS, WEIL MH, TANG W, YANG G, BISERA J. Gastric intramural PCO2 during peritonitis and shock. *Chest.* 1993;104(4):1254-1258.

- DOERSCHUG KC, DELSING AS, SCHMIDT GA, HAYNES WG. Impairments in microvascular reactivity are related to organ failure in human sepsis. *Am J Physiol Heart Circ Physiol.* 2007;293(2):H1065-1071.

- DOUGLAS AR, JONES NL, REED JW. Calculation of whole blood CO2 content. *J Appl Physiol (1985).* 1988;65(1):473-477.

- DUBIN A, EDUL VS, POZO MO, et al. Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. *Crit Care Med.* 2008;36(2):535-542.

- DUBIN A, ESTENSSORO E, MURIAS G, et al. Intramucosal-arterial Pco2 gradient does not reflect intestinal dysoxia in anemic hypoxia. *J Trauma.* 2004;57(6):1211-1217.

- DUBIN A, MURIAS G, ESTENSSORO E, et al. End-tidal CO2 pressure determinants during hemorrhagic shock. *Intensive Care Med.* 2000;26(11):1619-1623.

- EDUL VS, ENRICO C, LAVIOLLE B, VAZQUEZ AR, INCE C, DUBIN A. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. *Crit Care Med.* 2012;40(5):1443-1448.

- ELIZALDE JI, HERNANDEZ C, LLACH J, et al. Gastric intramucosal acidosis in mechanically ventilated patients: role of mucosal blood flow. *Critical care medicine.* 1998;26(5):827-832.

- ELLIS CG, BATEMAN RM, SHARPE MD, SIBBALD WJ, GILL R. Effect of a maldistribution of microvascular blood flow on capillary O(2) extraction in sepsis. *Am J Physiol Heart Circ Physiol.* 2002;282(1):H156-164.

- ENRICO C, KANOORE EDUL VS, VAZQUEZ AR, et al. Systemic and microcirculatory effects of dobutamine in patients with septic shock. *J Crit Care.* 2012;27(6):630-638.

- FERRARA JJ, DYESS DL, PEEPLES GL, et al. Effects of dopamine and dobutamine on regional blood flow distribution in the neonatal piglet. *Ann Surg.* 1995;221(5):531-540; discussion 540-532.
- FINK T, HEYMANN P, TAHA-MELITZ S, et al. Dobutamine pretreatment improves survival, liver function, and hepatic microcirculation after polymicrobial sepsis in rat. *Shock.* 2013;40(2):129-135.

- GERMANN R, HAISJACKL M, SCHWARZ B, et al. Inotropic treatment and intestinal mucosal tissue oxygenation in a model of porcine endotoxemia. *Crit Care Med.* 1997;25(7):1191-1197.

- GILBERT EM, HAUPT MT, MANDANAS RY, HUARINGA AJ, CARLSON RW. The effect of fluid loading, blood transfusion, and catecholamine infusion on oxygen delivery and consumption in patients with sepsis. *Am Rev Respir Dis.* 1986;134(5):873-878.

- GOLDMAN D, BATEMAN RM, ELLIS CG. Effect of decreased O2 supply on skeletal muscle oxygenation and O2 consumption during sepsis: role of heterogeneous capillary spacing and blood flow. *Am J Physiol Heart Circ Physiol.* 2006;290(6):H2277-2285.

- GREEN CJ, FRAZER RS, UNDERHILL S, MAYCOCK P, FAIRHURST JA, CAMPBELL IT. Metabolic effects of dobutamine in normal man. *Clin Sci (Lond).* 1992;82(1):77-83.

- GROENEVELD AB, VERMEIJ CG, THIJS LG. Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end-expiratory pressure in the pig. *Anesth Analg.* 1991;73(5):576-582.

- GRUNDLER W, WEIL MH, RACKOW EC. Arteriovenous carbon dioxide and pH gradients during cardiac arrest. *Circulation.* 1986;74(5):1071-1074.

- HERNANDEZ G, BOERMA EC, DUBIN A, et al. Severe abnormalities in microvascular perfused vessel density are associated to organ dysfunctions and mortality and can be

predicted by hyperlactatemia and norepinephrine requirements in septic shock patients. *J Crit Care.* 2013;28(4):538.e539-514.

- HERNANDEZ G, BRUHN A, LUENGO C, et al. Effects of dobutamine on systemic, regional and microcirculatory perfusion parameters in septic shock: a randomized, placebo-controlled, double-blind, crossover study. *Intensive Care Med.* 2013;39(8):1435-1443.

- HERNANDEZ G, PENA H, CORNEJO R, et al. Impact of emergency intubation on central venous oxygen saturation in critically ill patients: a multicenter observational study. *Crit Care.* 2009;13(3):R63.

- HILTEBRAND LB, KREJCI V, SIGURDSSON GH. Effects of dopamine, dobutamine, and dopexamine on microcirculatory blood flow in the gastrointestinal tract during sepsis and anesthesia. *Anesthesiology.* 2004;100(5):1188-1197.

- HORAN TC, ANDRUS M, DUDECK MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control.* 2008;36(5):309-332.

- HUMER MF, PHANG PT, FRIESEN BP, ALLARD MF, GODDARD CM, WALLEY KR. Heterogeneity of gut capillary transit times and impaired gut oxygen extraction in endotoxemic pigs. *J Appl Physiol (1985).* 1996;81(2):895-904.
- HURLEY R, MYTHEN MG. The Haldane effect--an explanation for increasing gastric mucosal PCO2 gradients? *Br J Anaesth.* 2000;85(1):167-169.

- INCE C. The rationale for microcirculatory guided fluid therapy. *Curr Opin Crit Care.* 2014;20(3):301-308.

- JAKOB SM, GROENEVELD AB, TEBOUL JL. Venous-arterial CO2 to arterial-venous O2 difference ratio as a resuscitation target in shock states? *Intensive Care Med.* 2015;41(5):936-938.

- JAKOB SM, KOSONEN P, RUOKONEN E, PARVIAINEN I, TAKALA J. The Haldane effect--an alternative explanation for increasing gastric mucosal PCO2 gradients? *Br J Anaesth.* 1999;83(5):740-746.

- JAMES JH, LUCHETTE FA, MCCARTER FD, FISCHER JE. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. *Lancet.* 1999;354(9177):505-508.

- JANSEN TC, VAN BOMMEL J, SCHOONDERBEEK FJ, et al. Early lactate-guided therapy in intensive care unit patients: a multicenter, open-label, randomized controlled trial. *Am J Respir Crit Care Med.* 2010;182(6):752-761.

- JONES AE, FOCHT A, HORTON JM, KLINE JA. Prospective external validation of the clinical effectiveness of an emergency department-based early goal-directed therapy protocol for severe sepsis and septic shock. *Chest.* 2007;132(2):425-432.

- JONES AE, SHAPIRO NI, TRZECIAK S, et al. Lactate clearance vs central venous oxygen saturation as goals of early sepsis therapy: a randomized clinical trial. *JAMA.* 2010;303(8):739-746.

- KAZARIAN KK, DEL GUERCIO LR. The use of mixed venous blood gas determinations in traumatic shock. *Annals of emergency medicine.* 1980;9(4):179-182.

- LEVRAUT J, CIEBIERA JP, CHAVE S, et al. Mild hyperlactatemia in stable septic patients is due to impaired lactate clearance rather than overproduction. *Am J Respir Crit Care Med.* 1998;157(4 Pt 1):1021-1026.

- LEVY MM, FINK MP, MARSHALL JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med.* 2003;31(4):1250-1256.

- MARIK PE, BANKOV A. Sublingual capnometry versus traditional markers of tissue oxygenation in critically ill patients. *Critical care medicine.* 2003;31(3):818-822.

- MECHER CE, RACKOW EC, ASTIZ ME, WEIL MH. Venous hypercarbia associated with severe sepsis and systemic hypoperfusion. *Crit Care Med.* 1990;18(6):585-589.

- MEKONTSO-DESSAP A, CASTELAIN V, ANGUEL N, et al. Combination of venoarterial PCO2 difference with arteriovenous O2 content difference to detect anaerobic metabolism in patients. *Intensive Care Med.* 2002;28(3):272-277.

- MONNET X, JULIEN F, AIT-HAMOU N, et al. Lactate and venoarterial carbon dioxide difference/arterial-venous oxygen difference ratio, but not central venous oxygen saturation, predict increase in oxygen consumption in fluid responders. *Crit Care Med.* 2013;41(6):1412-1420.

- MOORE JP, DYSON A, SINGER M, FRASER J. Microcirculatory dysfunction and resuscitation: why, when, and how. *Br J Anaesth.* 2015;115(3):366-375.

- MOUNCEY PR, OSBORN TM, POWER GS, et al. Trial of early, goal-directed resuscitation for septic shock. *N Engl J Med.* 2015;372(14):1301-1311.

- NEVIÈRE R, CHAGNON JL, TEBOUL JL, VALLET B, WATTEL F. Small intestine intramucosal PCO(2) and microvascular blood flow during hypoxic and ischemic hypoxia. *Crit Care Med.* 2002;30(2):379-384.

- NEVIERE R, CHAGNON JL, VALLET B, et al. Dobutamine improves gastrointestinal mucosal blood flow in a porcine model of endotoxic shock. *Crit Care Med.* 1997;25(8):1371-1377.

- NEVIERE R, MATHIEU D, CHAGNON JL, LEBLEU N, WATTEL F. The contrasting effects of dobutamine and dopamine on gastric mucosal perfusion in septic patients.

American journal of respiratory and critical care medicine. 1996;154(6 Pt 1):1684-1688.

- NGUYEN HB, KUAN WS, BATECH M, et al. Outcome effectiveness of the severe sepsis resuscitation bundle with addition of lactate clearance as a bundle item: a multi-national evaluation. *Crit Care.* 2011;15(5):R229.

- OSPINA-TASCÓN GA, BAUTISTA-RINCÓN DF, UMAÑA M, et al. Persistently high venous-to-arterial carbon dioxide differences during early resuscitation are associated with poor outcomes in septic shock. *Crit Care.* 2013;17(6):R294.

- OSPINA-TASCON GA, UMANA M, BERMUDEZ W, et al. Combination of arterial lactate levels and venous-arterial CO2 to arterial-venous O 2 content difference ratio as markers of resuscitation in patients with septic shock. *Intensive Care Med.* 2015;41(5):796-805.

- OSPINA-TASCON GA, UMANA M, BERMUDEZ WF, et al. Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock? *Intensive Care Med.* 2016;42(2):211-221.

- PARVIAINEN I, RUOKONEN E, TAKALA J. Dobutamine-induced dissociation between changes in splanchnic blood flow and gastric intramucosal pH after cardiac surgery. *Br J Anaesth.* 1995;74(3):277-282.

- PEAKE SL, BAILEY M, BELLOMO R, et al. Australasian resuscitation of sepsis evaluation (ARISE): A multi-centre, prospective, inception cohort study. *Resuscitation.* 2009;80(7):811-818.

- PEARCE N. Registration of protocols for observational research is unnecessary and would do more harm than good. *Occup Environ Med.* 2011;68(2):86-88.

- PEREL A. Bench-to-bedside review: the initial hemodynamic resuscitation of the septic patient according to Surviving Sepsis Campaign guidelines--does one size fit all? *Crit Care.* 2008;12(5):223.
- PINSKY MR, PAYEN D. Functional hemodynamic monitoring. *Crit Care.* 2005;9(6):566-572.

- PRIEBE HJ, NOLDGE GF, ARMBRUSTER K, GEIGER K. Differential effects of dobutamine, dopamine, and noradrenaline on splanchnic haemodynamics and oxygenation in the pig. *Acta Anaesthesiol Scand.* 1995;39(8):1088-1096.

- PUSKARICH MA, TRZECIAK S, SHAPIRO NI, et al. Outcomes of patients undergoing early sepsis resuscitation for cryptic shock compared with overt shock. *Resuscitation.* 2011;82(10):1289-1293.

- RANDALL HM, JR., COHEN JJ. Anaerobic CO2 production by dog kidney in vitro. *Am J Physiol.* 1966;211(2):493-505.

- RAZA O, SCHLICHTIG R. Metabolic component of intestinal PCO(2) during dysoxia. *J Appl Physiol (1985).* 2000;89(6):2422-2429.

- RIMACHI R, BRUZZI DE CARVAHLO F, ORELLANO-JIMENEZ C, COTTON F, VINCENT JL, DE BACKER D. Lactate/pyruvate ratio as a marker of tissue hypoxia in circulatory and septic shock. *Anaesth Intensive Care.* 2012;40(3):427-432.

- RIVERS E, NGUYEN B, HAVSTAD S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med.* 2001;345(19):1368-1377.

- RONCO JJ, FENWICK JC, WIGGS BR, PHANG PT, RUSSELL JA, TWEEDDALE MG. Oxygen consumption is independent of increases in oxygen delivery by dobutamine in septic patients who have normal or increased plasma lactate. *Am Rev Respir Dis.* 1993;147(1):25-31.

- RUOKONEN E, TAKALA J, KARI A. Regional blood flow and oxygen transport in patients with the low cardiac output syndrome after cardiac surgery. *Crit Care Med.* 1993;21(9):1304-1311.

- RUSSELL JA, PHANG PT. The oxygen delivery/consumption controversy. Approaches to management of the critically ill. *American journal of respiratory and critical care medicine.* 1994;149(2 Pt 1):533-537.

- SAKR Y, DUBOIS MJ, DE BACKER D, CRETEUR J, VINCENT JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med.* 2004;32(9):1825-1831.

- SANTRY HP, ALAM HB. Fluid resuscitation: past, present, and the future. *Shock.* 2010;33(3):229-241.

- SCHLICHTIG R, BOWLES SA. Distinguishing between aerobic and anaerobic appearance of dissolved CO2 in intestine during low flow. *J Appl Physiol.* 1994;76(6):2443-2451.

- SCHNEIDER AJ, GROENEVELD AB, TEULE GJ, WESDORP RI, THIJS LG. Total body blood volume redistribution in porcine E. coli septic shock: effect of volume loading, dobutamine, and norepinephrine. *Circ Shock.* 1991;35(4):215-222.
- SCHUMACKER PT, SAMSEL RW. Analysis of oxygen delivery and uptake relationships in the Krogh tissue model. *J Appl Physiol (1985).* 1989;67(3):1234-1244.

- SECCHI A, WELLMANN R, MARTIN E, SCHMIDT H. Dobutamine maintains intestinal villus blood flow during normotensive endotoxemia: an intravital microscopic study in the rat. *J Crit Care.* 1997;12(3):137-141.

- SEVERIN PN, UHING MR, BENO DW, KIMURA RE. Endotoxin-induced hyperlactatemia results from decreased lactate clearance in hemodynamically stable rats. *Crit Care Med.* 2002;30(11):2509-2514.

- SHAPIRO NI, HOWELL MD, TALMOR D, et al. Implementation and outcomes of the Multiple Urgent Sepsis Therapies (MUST) protocol. *Crit Care Med.* 2006;34(4):1025-1032.

- SHAPIRO NI, HOWELL MD, TALMOR D, et al. Serum lactate as a predictor of mortality in emergency department patients with infection. *Ann Emerg Med.* 2005;45(5):524-528.

- SHEPHERD AP, KIEL JW. A model of countercurrent shunting of oxygen in the intestinal villus. *Am J Physiol.* 1992;262(4 Pt 2):H1136-1142.

- SHOEMAKER WC, APPEL PL, KRAM HB. Tissue oxygen debt as a determinant of lethal and nonlethal postoperative organ failure. *Critical care medicine.* 1988;16(11):1117-1120.

- STEIN JC, ELLIS CG, ELLSWORTH ML. Relationship between capillary and systemic venous PO2 during nonhypoxic and hypoxic ventilation. *Am J Physiol.* 1993;265(2 Pt 2):H537-542.

- STRINGER W, WASSERMAN K, CASABURI R. The VCO2/VO2 relationship during heavy, constant work rate exercise reflects the rate of lactic acid accumulation. *Eur J Appl Physiol Occup Physiol.* 1995;72(1-2):25-31.

- TANG W, WEIL MH, SUN S, NOC M, GAZMURI RJ, BISERA J. Gastric intramural PCO2 as monitor of perfusion failure during hemorrhagic and anaphylactic shock. *J Appl Physiol (1985).* 1994;76(2):572-577.

- TEBOUL JL, MERCAT A, LENIQUE F, BERTON C, RICHARD C. Value of the venous-arterial PCO2 gradient to reflect the oxygen supply to demand in humans: effects of dobutamine. *Crit Care Med.* 1998;26(6):1007-1010.

- TEBOUL JL, MONNET X. Detecting volume responsiveness and unresponsiveness in intensive care unit patients: two different problems, only one solution. *Crit Care.* 2009;13(4):175.

- THIRD EUROPEAN CONSENSUS CONFERENCE IN INTENSIVE CARE MEDICINE. Tissue hypoxia: How to detect, how to correct, how to prevent. Societe de Reanimation de Langue Francaise. The American Thoracic Society. European Society of Intensive Care Medicine. *American journal of respiratory and critical care medicine.* 1996;154(5):1573-1578.

- TRZECIAK S, DELLINGER RP, ABATE NL, et al. Translating research to clinical practice: a 1-year experience with implementing early goal-directed therapy for septic shock in the emergency department. *Chest.* 2006;129(2):225-232.

- TRZECIAK S, DELLINGER RP, PARRILLO JE, et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Ann Emerg Med.* 2007;49(1):88-98, 98.e81-82.

- TUGTEKIN IF, RADERMACHER P, THEISEN M, et al. Increased ileal-mucosal-arterial PCO2 gap is associated with impaired villus microcirculation in endotoxic pigs. *Intensive care medicine.* 2001;27(4):757-766.

- UUSARO A, RUOKONEN E, TAKALA J. Splanchnic oxygen transport after cardiac surgery: evidence for inadequate tissue perfusion after stabilization of hemodynamics. *Intensive Care Med.* 1996;22(1):26-33.

- VALLÉE F, VALLET B, MATHE O, ET AL. Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? *Intensive Care Med.* 2008;34(12):2218-2225.

- VALLET B, PINSKY MR, CECCONI M. Resuscitation of patients with septic shock: please "mind the gap"! *Intensive Care Med.* 2013;39(9):1653-1655.

- VALLET B, TEBOUL JL, CAIN S, CURTIS S. Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol (1985).* 2000;89(4):1317-1321.

- VALLET B. Vascular reactivity and tissue oxygenation. *Intensive care medicine.* 1998;24(1):3-11.

- VAN BEEST PA, HOFSTRA JJ, SCHULTZ MJ, BOERMA EC, SPRONK PE, KUIPER MA. The incidence of low venous oxygen saturation on admission to the intensive care unit: a multi-center observational study in The Netherlands. *Crit Care.* 2008;12(2):R33.

- VAN BEEST PA, LONT MC, HOLMAN ND, LOEF B, KUIPER MA, BOERMA EC. Central venous-arterial pCO_2 difference as a tool in resuscitation of septic patients. *Intensive Care Med.* 2013;39(6):1034-1039.

- VAN BEEST PA, VAN INGEN J, BOERMA EC, et al. No agreement of mixed venous and central venous saturation in sepsis, independent of sepsis origin. *Crit Care.* 2010;14(6):R219.

- VAN DER LINDEN P, RAUSIN I, DELTELL A, et al. Detection of tissue hypoxia by arteriovenous gradient for PCO2 and pH in anesthetized dogs during progressive hemorrhage. *Anesth Analg.* 1995;80(2):269-275.

- VANDENBROUCKE JP. Observational research, randomised trials, and two views of medical science. *PLoS Med.* 2008;5(3):e67.

- VINCENT JL, DE BACKER D. Circulatory shock. *N Engl J Med.* 2013;369(18):1726-1734.
- VINCENT JL, DE BACKER D. Microvascular dysfunction as a cause of organ dysfunction in severe sepsis. *Crit Care.* 2005;9 Suppl 4:S9-12.

- VINCENT JL, DE MENDONÇA A, CANTRAINE F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med.* 1998;26(11):1793-1800.

- VINCENT JL, WEIL MH. Fluid challenge revisited. *Crit Care Med.* 2006;34(5):1333-1337.

- WALLEY KR. Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. *J Appl Physiol.* 1996;81(2):885-894.

- WASSERMAN K, BEAVER WL, WHIPP BJ. Gas exchange theory and the lactic acidosis (anaerobic) threshold. *Circulation.* 1990;81(1 Suppl):Ii14-30.

- WEIL MH, RACKOW EC, TREVINO R, GRUNDLER W, FALK JL, GRIFFEL MI. Difference in acid-base state between venous and arterial blood during cardiopulmonary resuscitation. *N Engl J Med.* 1986;315(3):153-156.

- WU L, TIWARI MM, MESSER KJ, et al. Peritubular capillary dysfunction and renal tubular epithelial cell stress following lipopolysaccharide administration in mice. *Am J Physiol Renal Physiol.* 2007;292(1):F261-268.

- YEALY DM, KELLUM JA, HUANG DT, et al. A randomized trial of protocol-based care for early septic shock. *N Engl J Med.* 2014;370(18):1683-1693.

- ZHANG H, VINCENT JL. Arteriovenous differences in PCO2 and pH are good indicators of critical hypoperfusion. *Am Rev Respir Dis.* 1993;148(4 Pt 1):867-871.

- ZUURBIER CJ, VAN ITERSON M, INCE C. Functional heterogeneity of oxygen supply-consumption ratio in the heart. *Cardiovasc Res.* 1999;44(3):488-497.

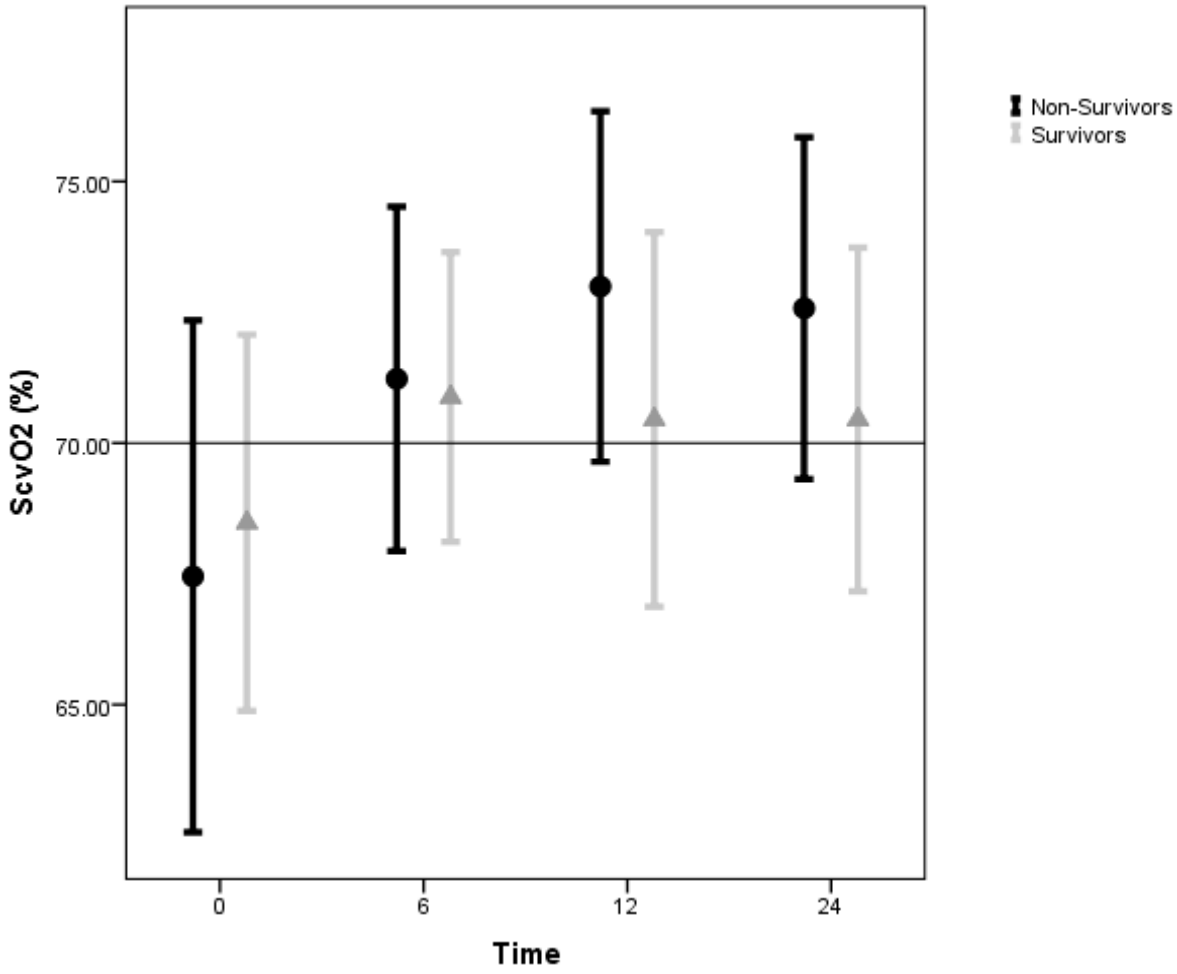
ANNEX A

PERSISTENTLY HIGH VENOUS-TO-ARTERIAL CARBON DIOXIDE DIFFERENCES DURING EARLY RESUSCITATION ARE ASSOCIATED WITH POOR OUTCOMES IN SEPTIC SHOCK

Crit Care. 2013 Dec 13;17(6):R294.

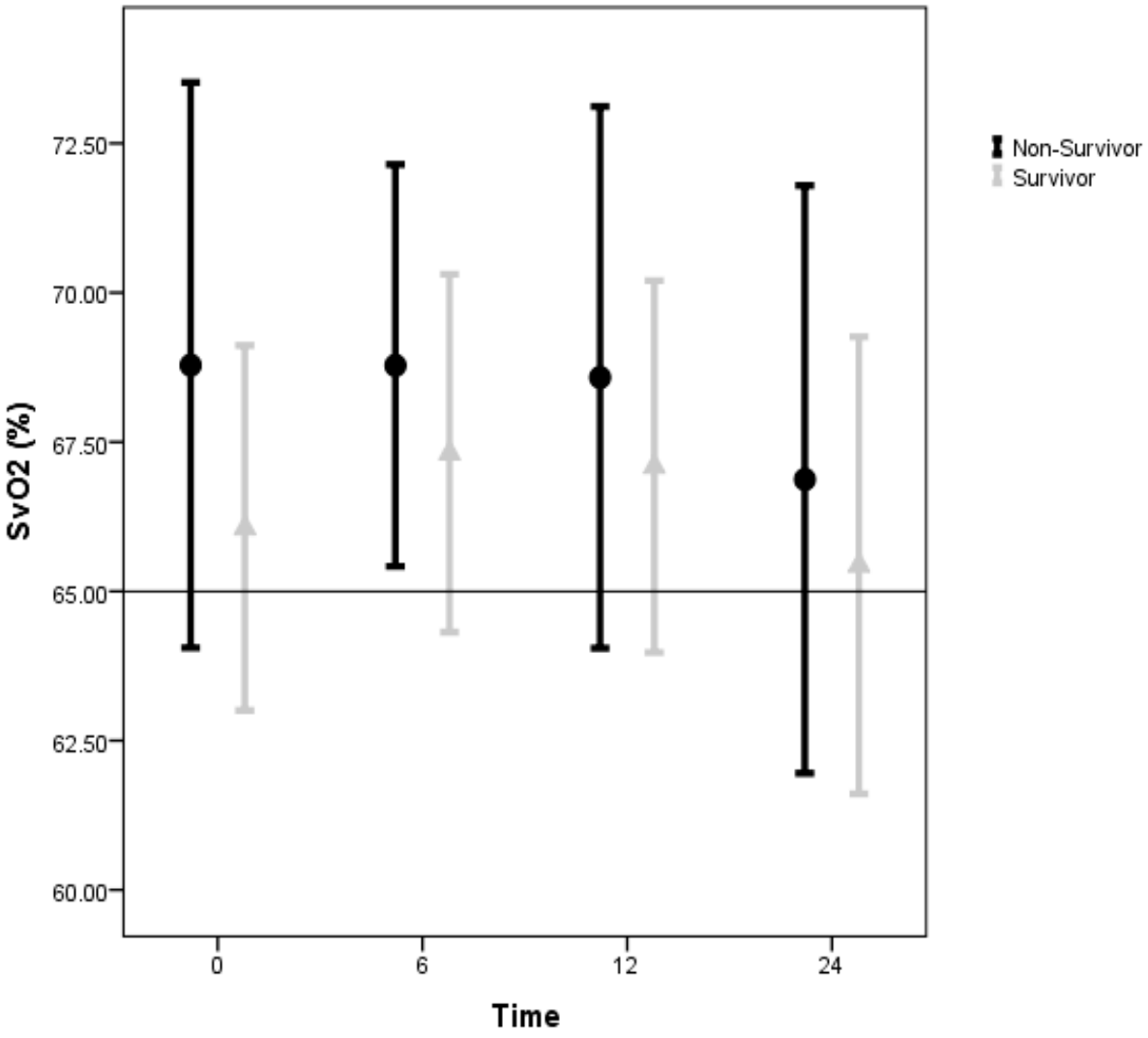
Supplementary Material

Suppl. Figure 1a. Time-course of ScvO2 (%) during the first 24 hours for survivors and non-survivors at day-28.



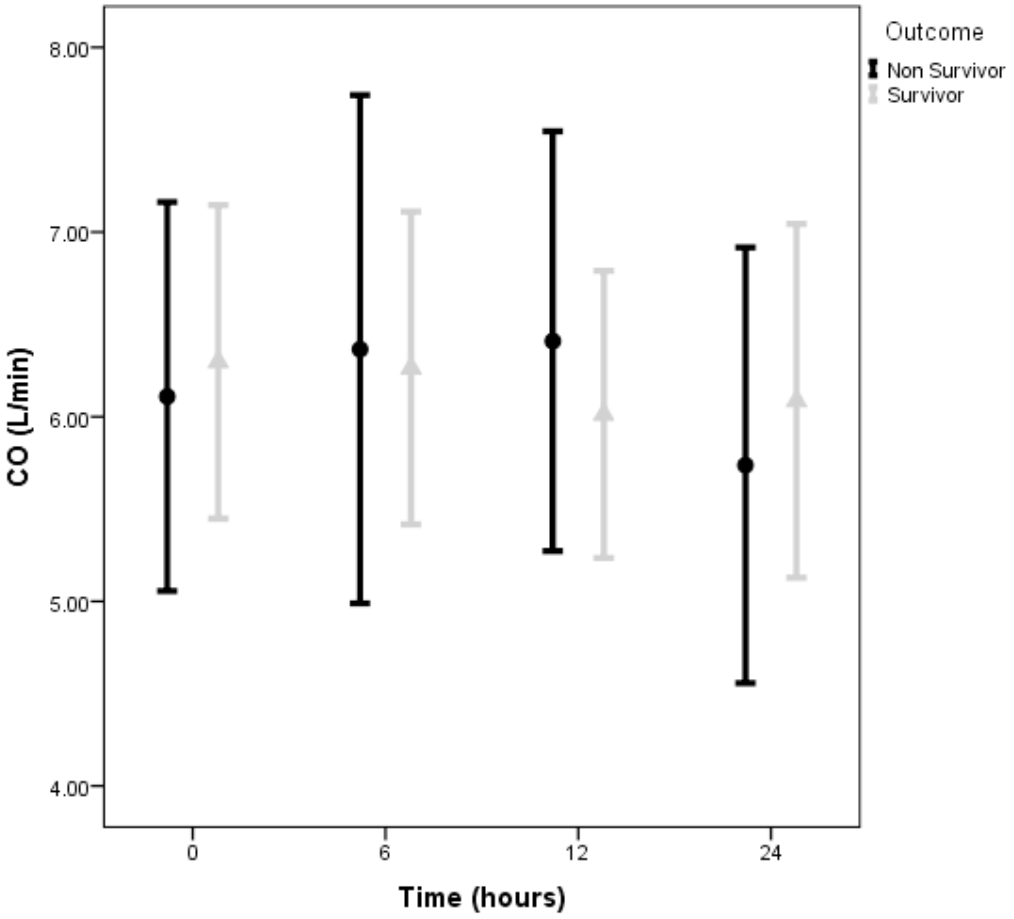
Repeated measures analysis of variance, p=0.22.

Suppl. Figure 1b. Time-course of SvO2 (%) during the first 24 hours for survivors and non-survivors at day-28.



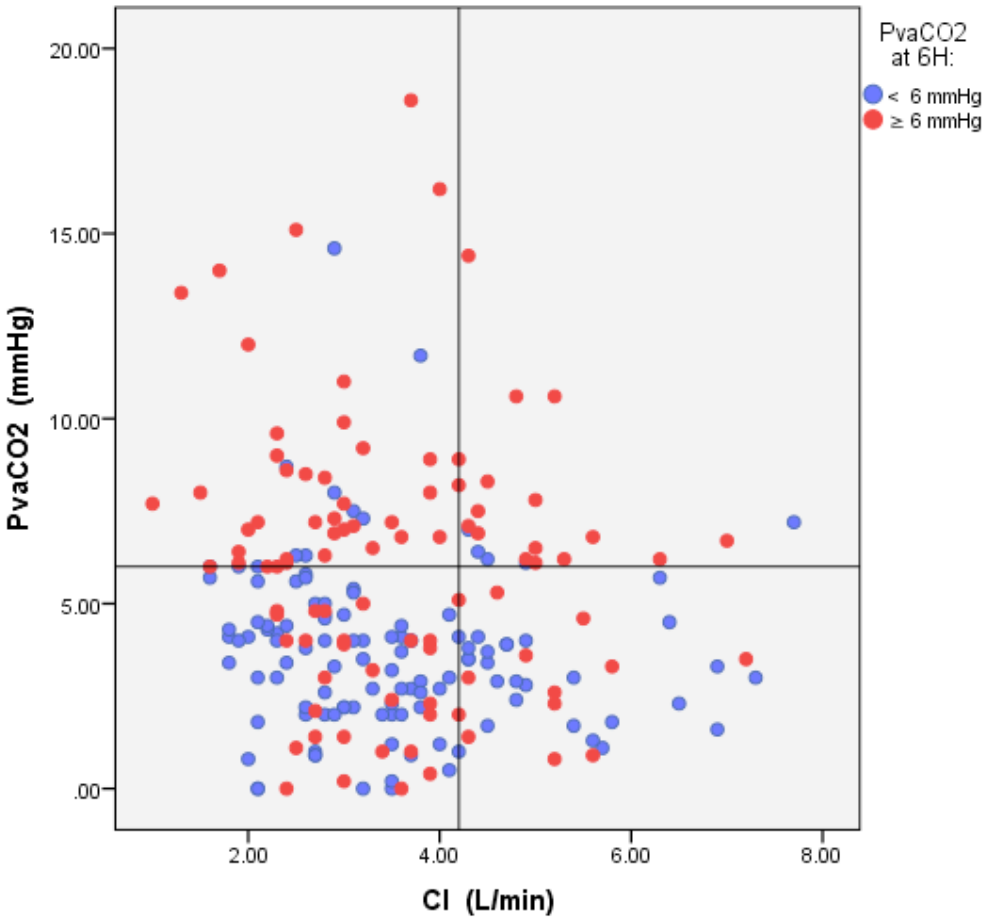
Repeated measures analysis of variance, p=0.65.

Suppl. Figure 2. Time-course of cardiac output (L/min) during the first 24 hours for survivors and non-survivors at day-28.



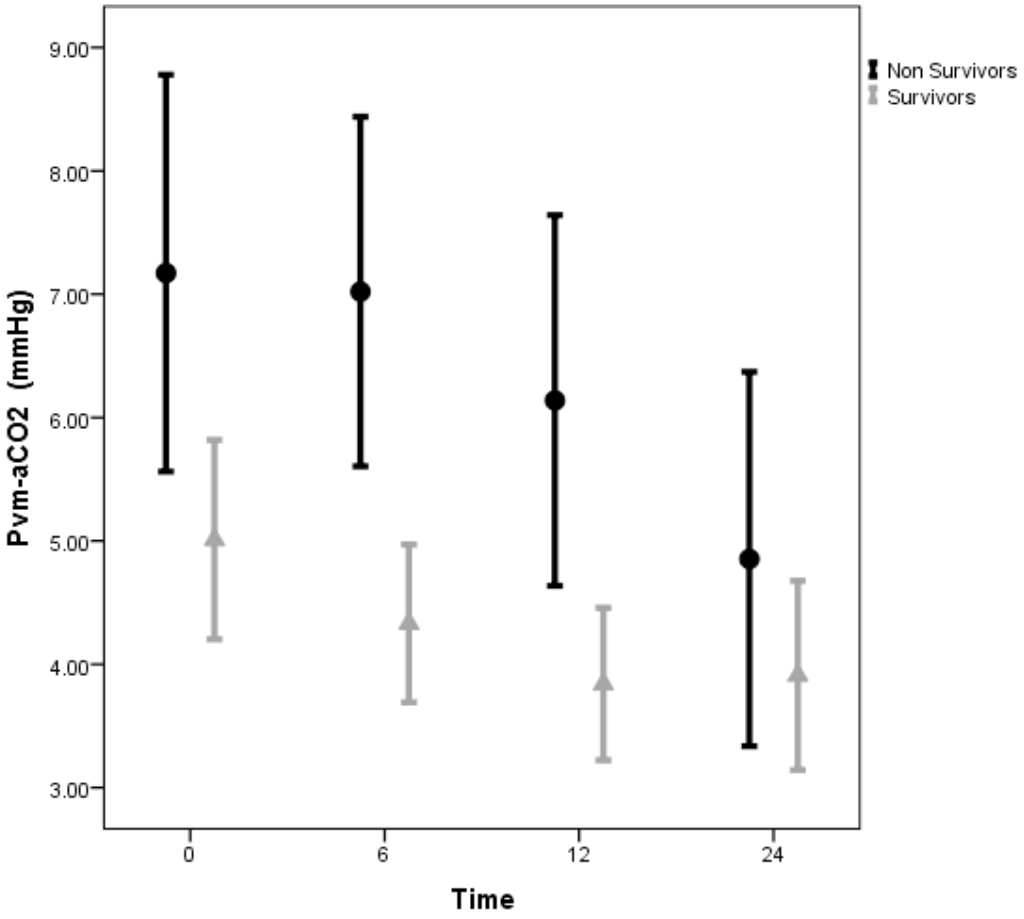
Repeated measures analysis of variance, p=0.37.

Suppl. Figure 3. Scatter plot between cardiac index and Pv-aCO_2 (according to Pv-aCO_2 at T6)



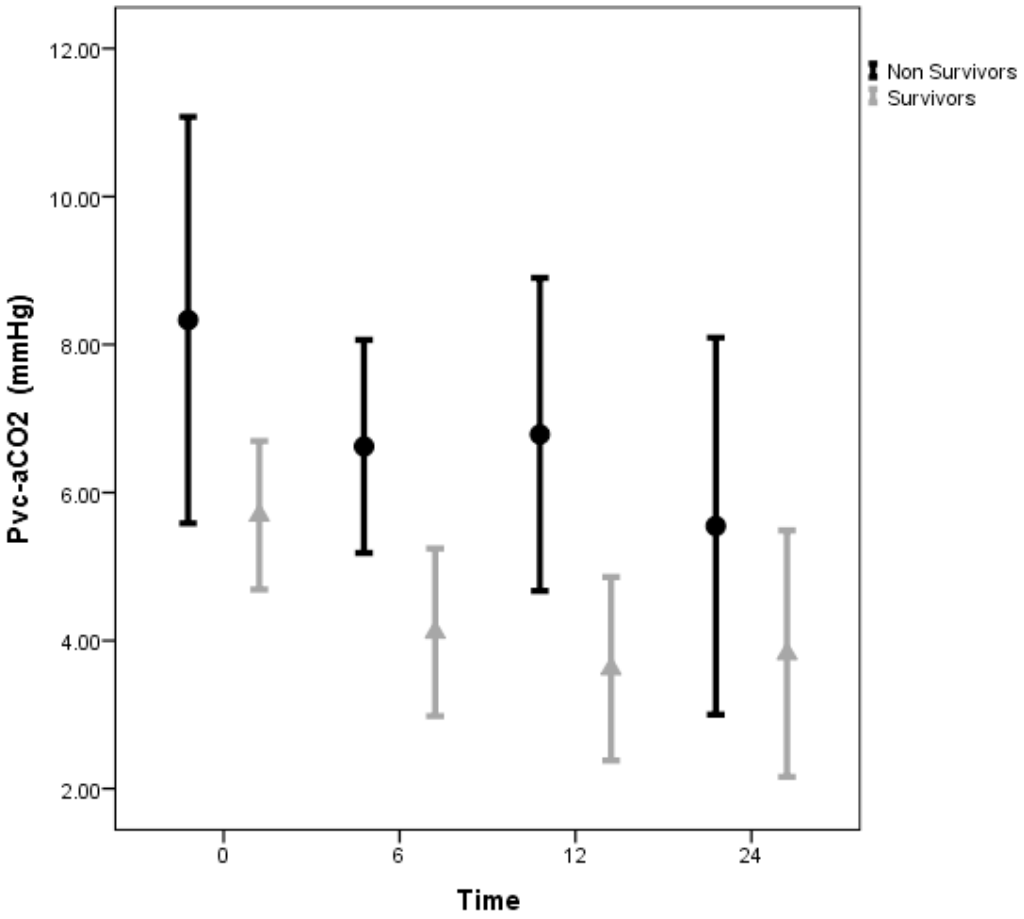
Blue points: Pv-aCO_2 < 6 mmHg; Red points: Pv-aCO_2 ≥ 6 mmHg

Suppl. Figure 4a. Time-course of Pv-aCO2 (mmHg) during the first 24 hours for survivors and non-survivors at day-28.



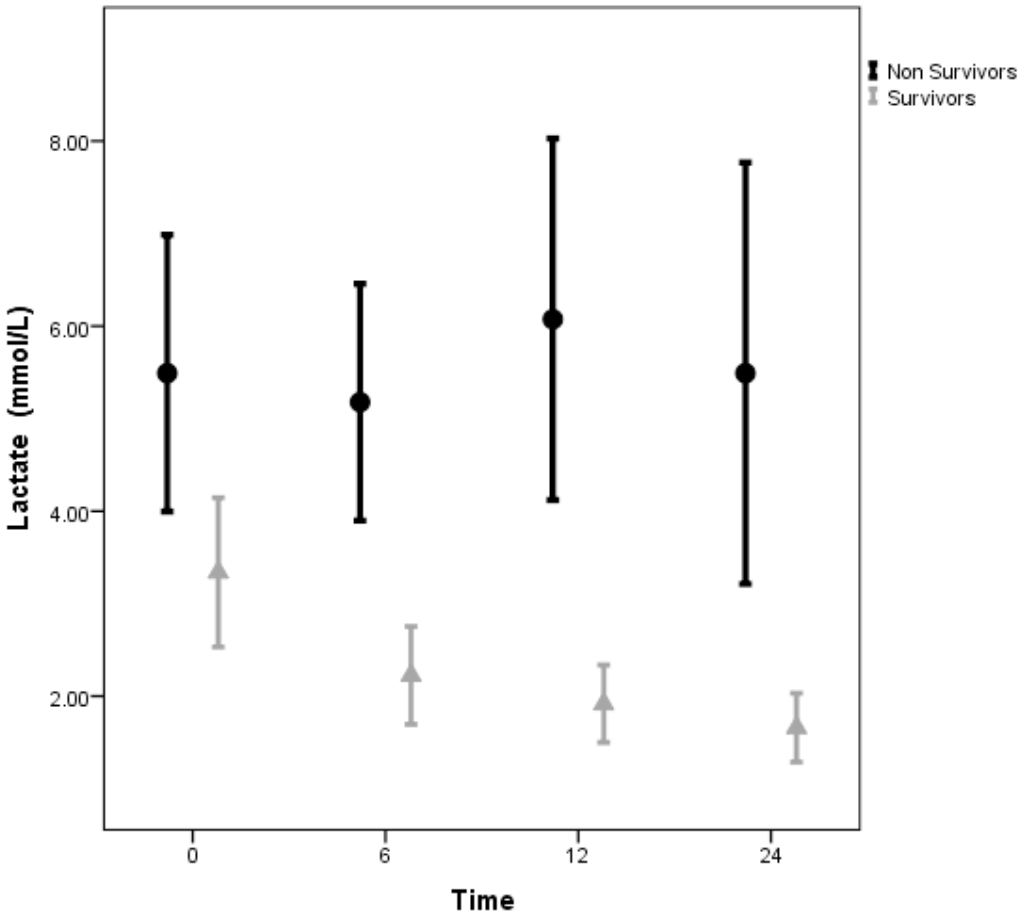
Repeated measures analysis of variance, p=0.003. Pv-aCO_2 denotes mixed-venous to arterial pCO_2 difference.

Suppl. Figure 4b. Time-course of Pvc-aCO2 (mmHg) during the first 24 hours for survivors and non-survivors at day-28.



Repeated measures analysis of variance, p=0.03. Pvc-aCO_2 denotes central-venous to arterial pCO_2 difference.

Suppl. Figure 5. Time-course of lactate levels (mmol/L) during the first 24 hours for survivors and non-survivors at day-28.



Repeated measures analysis of variance, $p<0.0001$.

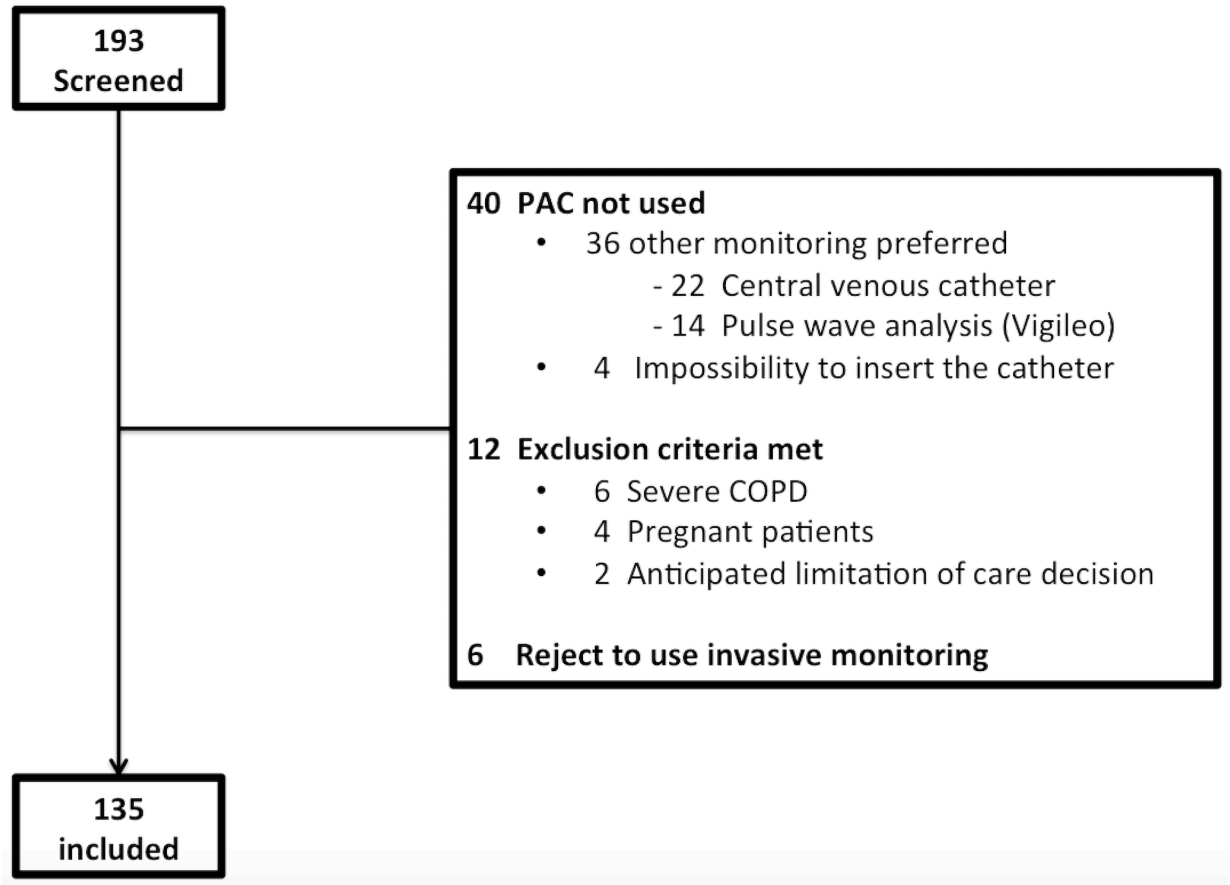
ANNEX B

COMBINATION OF ARTERIAL LACTATE LEVELS AND VENOUS-ARTERIAL CO_2 TO ARTERIAL-VENOUS O_2 CONTENT DIFFERENCE RATIO AS MARKERS OF RESUSCITATION IN PATIENTS WITH SEPTIC SHOCK

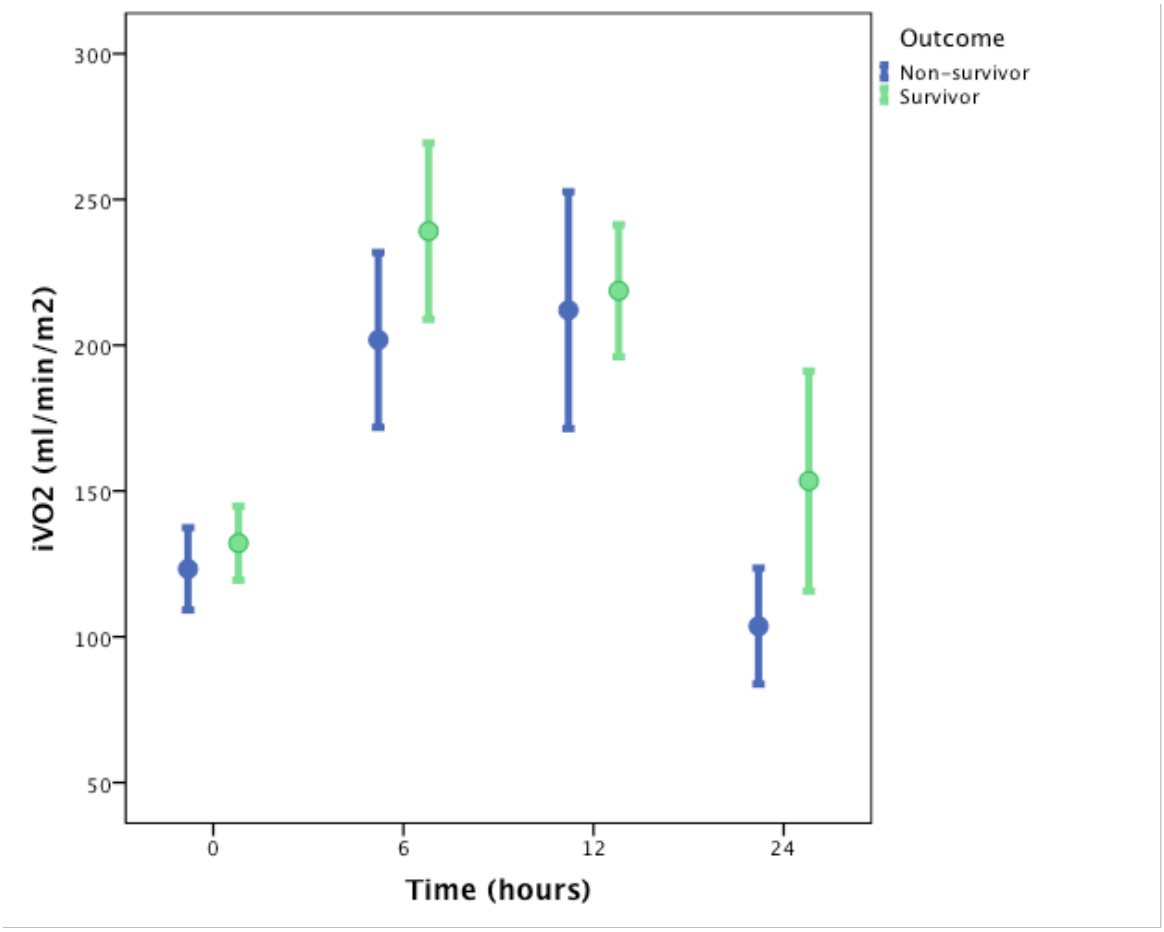
Intensive Care Med. 2015 May;41(5):796-805

Supplementary Material

Suppl. Figure 1. Selection of patients.

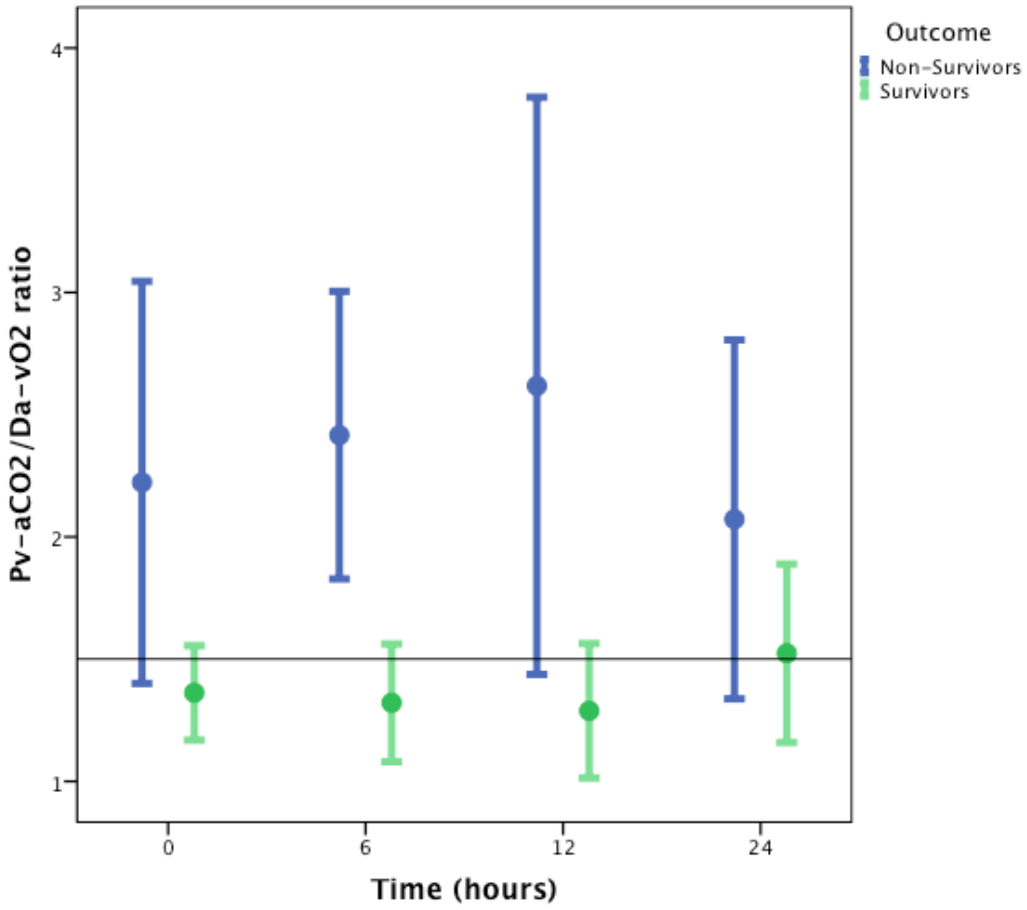


Suppl. Figure 2. Time-course of VO_2 (ml/min/m²) during the first 24 hours in survivors and non-survivors at day-28.



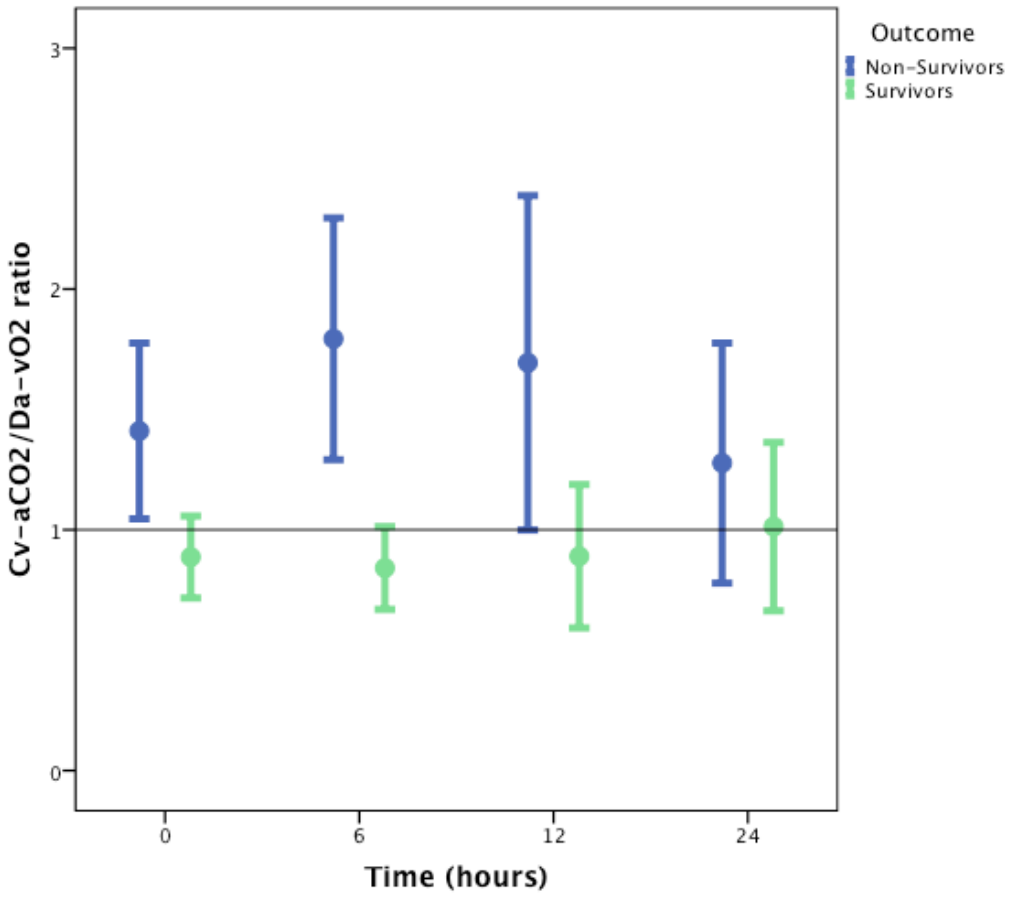
Bars represent the mean and 95% confidence interval. One-way ANOVA, p=0.011 for T6, T12 and T24 vs. baseline. Repeated measures of ANOVA, p=0.56 for survivors vs. non-survivors (Group x Time interaction).

Suppl. Figure 3. Time-course of Pv-aCO_2/Da-vO_2 ratio during the first 24 hours in survivors and non-survivors at day-28.



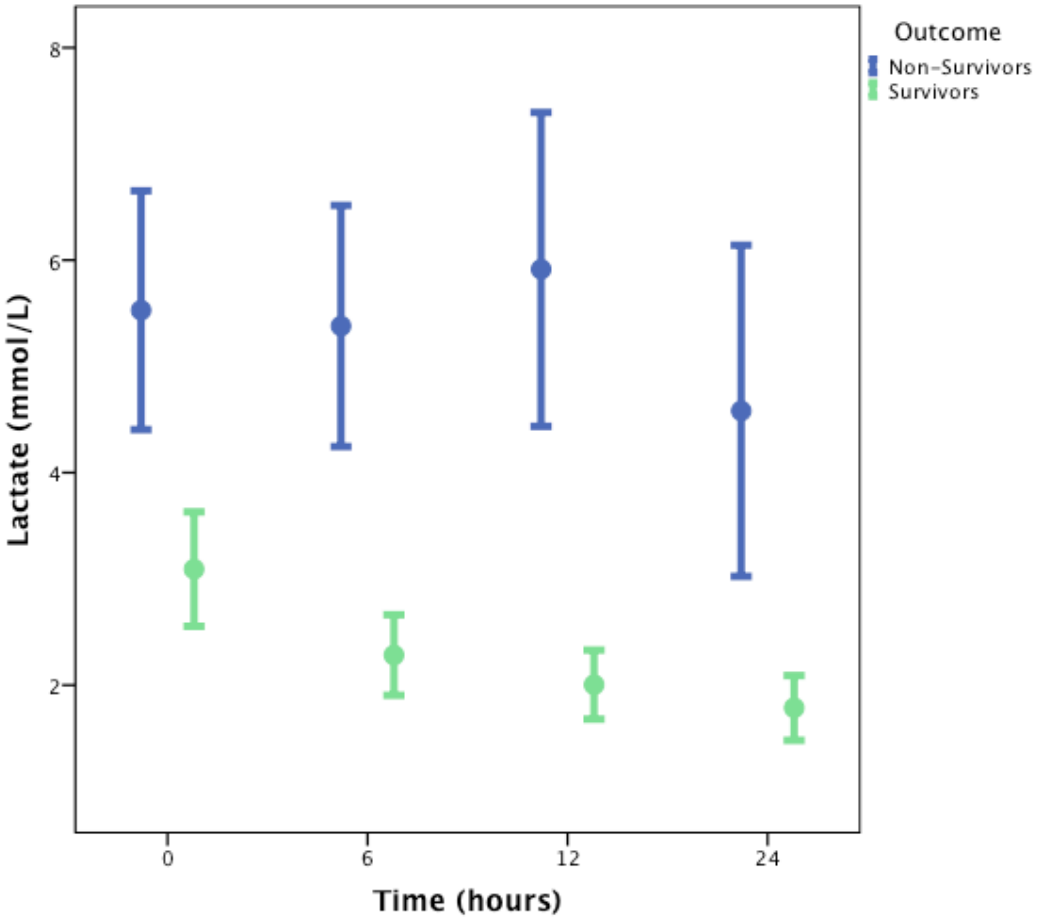
Bars represent the mean and 95% confidence interval. One-way ANOVA, p >0.05 for T6, T12 and T24 vs. baseline. The horizontal line at 1.5 represents the upper limit of normal values. Repeated measures of ANOVA, p=0.0001 for survivors vs. non-survivors (Group x Time interaction).

Suppl. Figure 4. Time-course of Cv-aCO_2/Da-vO_2 ratio during the first 24 hours in survivors and non-survivors at day-28.



Bars represent the mean and 95% confidence interval. One-way ANOVA, p >0.05 for T6, T12, and T24 vs. baseline. The horizontal line at 1.0 represents the upper limit of normal values. Repeated measures of ANOVA, p=0.0001 for survivors vs. non-survivors (Group x Time interaction).

Suppl. Figure 5. Time-course of lactate (mmol/L) during the first 24 hours in survivors and non-survivors at day-28.



Bars represent the mean and 95% confidence interval. One-way ANOVA, p<0.001 for T6, T12 and T24 vs. baseline. Repeated measures of ANOVA, p=0.0001 for survivors vs. non-survivors (Group x Time interaction).

Suppl. Table 1. Hemodynamics, blood gases, oxygen parameters and ventilator settings

	Group 1 (n= 42) Lactate ≥ 2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio >1.0	Group 2 (n= 42) Lactate ≥ 2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio ≤1.0	Group 3 (n= 23) Lactate < 2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio >1.0	Group 4 (n= 28) Lactate < 2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio ≤1.0	p
Hemodynamic variables					
HR, beats/min	110 [96.5 – 125,5]	114.5 [96.5 -128.5] †	96 [77 – 110] †	102 (76.8 – 114.5]	0.02
T0	105 [97 – 126]	107 [93.5 – 123.5] †	89 [77 – 115] †	97 (81 – 112.3]	0.017
T6					
CI, L/min/m2					
T0	3.1 [2.3 – 4.5]	3.3 [2.5 – 4.4]	3.3 [2.5 – 3.8]	3.4 [2.5 – 4.1]	0.94
T6	3.0 [2.7 – 3.9]	3.6 [2.8 – 4.3]	3.2 [2.4 – 3.6]	3.4 [2.7 – 4.2]	0.70
MAP, mmHg					
T0	69.0 [62.5 – 76.8]	71.0 [64.0 - 77.0]	70.0 [64.0 – 79.0]	70.5 [66.0 -78.5]	0.74
T6	68.0 [62.5 – 72.5]	69.0 [64.0 – 75.0]	72.0 [66.0 – 79.0]	73.0 [67.5 – 81.3]	0.02
CVP, mmHg					
T0	13.0 [8.0 – 20.5]	14 [8.8 – 17.0]	12.0 [7.0 – 16.0]	10 [7.3 – 13.8]	0.16
T6	12.0 [8.0 – 15.0] ¶	11 [8.0 – 16.0] ‡	10.0 [8.0 – 14.0]	8.0 [5.3 – 11.0] ¶ ‡	0.018
PAOP, mmHg					
T0	16.5 [12.0 – 20.0]	17.0 [11.0 – 21.0]	16.0 [10.0 – 21.0]	14.0 [11.0 – 16.3]	0.30
T6	17.0 [11.0 – 20.0]	16.0 [10.0 – 20.0]	13.0 [11.0 – 16.0]	13.0 [10.0 – 16.0]	0.22

Blood gases and O_2 variables

pH,					
T0	7.28 [7.18 – 7.34] ** ¶	7.28 [7.14 – 7.36] †,,‡	7.32 [7.28 – 7.40] ** †	7.39 [7.13 – 7.42] ¶‡	< 0.001
T6	7.31 [7.21 – 7.39] ¶	7.28 [7.17 – 7.36]†,‡	7.34 [7.32 – 7.41] †	7.38 [7.35 – 7.42] ¶ ‡	< 0.001
$PaCO_2$, mmHg					
T0	30.4 [25.9 – 36.1]	30.1 [24.7 – 35.4]	30.2 [27.3 – 41.6]	30.9 [26.8 – 32.5]	0.75
T6	28.0 [25.5 – 31.1]	28.0 [23.7 – 32.4]	31.1 [27.5 – 36.4]	31.0 [26.1 – 34.1]	0.14
$PvCO_2$, mmHg					
T0	37.7 [32.7 – 42.5]	34.0 [30.5 – 42.3]	37.3 [31.0 – 46.5]	35.7 [29.6 – 39.0]	0.54
T6	35.0 [31.8 – 39.20]	32.5 [27.5 – 36.7]	34.3 [30.3 – 43.3]	34.4 [30.4 – 37.4]	0.28
PaO_2, mmHg					
T0	106.0 [91.7 – 142.3]	103.9 [79.7 – 149.0]	100.0 [84.1 – 123.0]	93.9 [82.9 – 131.9]	0.64
T6	101.5 [81.0 – 119.6]	118.0 [78.1 – 155.0]	103.1 [82.3 – 143.9]	110.8 [87.9 – 129.5]	0.47
PaO_2/FiO_2, mmHg					
T0	204.0 [142.3 – 250.8]	205.0 [108.6 – 322.7]	233.7 [121.9 – 329.7]	274.1 [186.6 – 362.3]	0.05
T6	190.2 [130.3 – 271.2]	226.0 [141.7 – 330.1]	247.8 [185.5 – 384.3]	307.6 [211.0 – 396.7]	0.001
Lactate, mmol/L,					
T0	4.48 [3.34 – 6.88] ** ¶	4.49 [3.25 – 5.78] †, ‡	1.55 [1.40 – 2.40] ** †	1.65 [0,98 – 2.13] ¶,‡	< 0.001
T6	4.06 [2.92 – 6.73] ** ¶	3.59 [2.30 – 6.57] †,‡	1.18 [0.93 – 1.45] ** ,†	1.19 [0.93 – 1.68] ¶,‡	< 0.001
SvO_2, %,					
T0	68.9 [61.1 – 75.2]	69.8 [59.6 – 76.5]	68.9 [63.4 – 74.6]	67.3 [59.4 – 70.1]	0.43
T6	70.6 [66.1 – 77.5]	68.9 [60.0 – 74.2]	71.2 [64.4 – 79.6]	66.3 [60.8 – 72.3]	0.11
ERO_2,					
T0	29.3 [23.5 – 35.4]	30.4 [21.7 – 36.4]	28.7 [24.3 – 34.3]	32.6 [27.5 – 41.0]	0.14
T6	26.5 [21.0 – 33.6]	32.2 [26.8 – 38.6]	27.9 [16.2 – 30.4]	31.8 [24.1 – 43.4]	0.01
DO_2, ml/min/m^2					
T0	384.4 (294.8 – 524.7]	429.7 (330.8 – 597.7]	449.3 (339.9 – 542.9]	394.3 (305.1 – 492.8]	0.60
T6	386.1 (320.8 – 489.9]	459.5 (378.8 – 582.4]	415.9 (312.5 – 506.5]	407.0 (320.1 – 489.9]	0.05

VO_2, ml/min/m^2					
T0	115.5 [84.8 – 149.5]	122.8 [92.5 – 167.9]	111.4 [85.7 – 157.3]	141.0 [105.0 – 155.1]	0.45
T6	188.2 [130.8 – 226.4] *	272.24 [206.73 – 347.9] * †	150.4 [116.7 – 268.6] † ξ	225.4 [149.3 – 279.8] ξ	<0.001
Pv-aCO_2, mmHg					
T0	6.6 [3.9 – 8.8]	5.2 [3.0 – 7.2]	6.0 [2.7 – 7.6]	4.4 [2.7 – 6.2]	0.08
T6	6.6 [5.0 – 10.5] *¶	4.6 [2.6 – 6.5] *	4.9 [3.1 – 6.9]	3.0 [2.0 – 5.1] ¶	< 0.001
Pv-aCO_2/Da-vO_2,					
T0	1.49 [1.18 – 2.39]	1.27 [0.68 – 1.78]	1.48 [0.84 – 1.85]	1.18 [0.54 – 1.43]	0.04
T6	2.15 [1.62 – 3.15] *¶	0.98 [0.73 – 1.46] *	1.53 [1.03 – 2.65]	0.78 [0.71 – 1.21] ¶	< 0.001
Cv-aCO_2/Da-vO_2,					
T0	1.11 [0.87 – 1.72]	0.82 [0.39 – 1.25]	0.93 [0.54 – 1.42]	0.80 [0.47 – 1.11]	0.02
T6	1.57 [1.20 – 2.73] *¶	0.55 [0.28 – 0.79] * †	1.43 [1.19 – 1.80] † ξ	0.37 [0.09 – 0.75] ¶ ξ	< 0.001

Ventilatory parameters

PEEP, mmHg,					
T0	5 [5 – 8]	7 [5 – 8]	6 [5 – 8]	5 [5 – 8]	0.78
T6	5 [5 – 8]	8 [5 – 10]	7 [5 – 8]	5 [5 – 8]	0.59
Vt (ml/kg),					
T0	7.7 [6.9 – 8.4]	7.1 [6.1 – 8.0]	7.8 [6.8 – 8.3]	7.8 [6.8 – 8.0]	0.42
T6	7.7 [7.2 – 8.4]	7.3 [6.2 – 8.0]	7.5 [6.9 – 8.8]	7.8 [7.1 – 8.0]	0.22

All data are presented as median [percentiles 25_75]. HR: Heart rate; CI= cardiac Index; MAP: mean arterial pressure; CVP: central venous pressure; PAOP: pulmonary artery occlusion pressure; pH: arterial pH; $PaCO_2$: arterial carbon dioxide pressure; $PvCO_2$: venous carbon dioxide pressure; PaO_2: arterial oxygen pressure; SvO2: mixed-venous oxygen saturation; ERO_2: oxygen extraction ratio; iDO_2: oxygen delivery indexed; iVO_2: oxygen consumption indexed; Pv-aCO_2: mixed venous-to-arterial carbon dioxide difference; Pv-CO2/Da-vO_2: mixed venous-to-arterial carbon dioxide pressure difference to arterial-venous oxygen content difference ratio; Cv-CO2/Da-vO_2: mixed venous-to-arterial carbon dioxide to arterial-venous oxygen content differences ratio; Vt: tidal volume.

* p<0.05 for Groups 1 vs. 2; ** p<0.05 for Groups 1 vs. 3; ¶ p<0.05 for Groups 1 vs. 4; † p<0.05 for Groups 2 vs. 3; ‡ p<0.05 for Groups 2 vs. 4; and ξ p<0.05 for Groups 3 vs. 4

Suppl. Table 2. Multivariate logistic regression for predictors of mortality at day-28 (including Pv-aCO_2/Da-vO_2 instead of Cv-aCO_2/Da-vO_2)

	T0			T6			T6 (for SvO_2≥65%) *		
	RR	**95%CI**	**p**	**RR**	**95%CI**	**p**	**RR**	**95%CI**	**p**
Pv-aCO_2/Da-vO_2	1.61	0.92-2.82	0.10	1.43	0.86-2.36	0.17	1.66	0.87-3.16	0.13
Lactate, mmol/L	1.15	0.96-1.38	0.13	1.52	1.13-2.05	0.01	2.23	1.21-4.11	0.01
VO_2, ml/min/m^2	1.00	0.98-1.03	0.69	0.99	0.99-1.00	0.20	1.00	0.99-1.01	0.89
DO_2, ml/min/m^2	1.00	0.99-1.01	0.92	1.00	0.99-1.01	0.38	1.00	0.99-1.01	0.94
SvO_2, %	0.97	0.90-1.05	0.41	0.96	0.89-1.03	0.23			
CI, L/min/m^2	0.95	0.51-1.75	0.86	0.85	0.40-1.82	0.68	0.99	0.35-2.81	0.99
APACHE II	1.09	0.99-1.19	0.07	1.03	0.94-1.12	0.51	0.99	0.88-1.12	0.91
Age, years	1.03	0.99-1.06	0.12	1.01	0.98-1.05	0.49	1.05	0.99-1.12	0.09
Time before T0, Hours	0.67	0.41-1.09	0.10	0.71	0.42-1.19	0.20	0.67	0.32-1.38	0.27
Gender	0.51	0.19-1.34	0.17	0.59	0.20-1.69	0.33	0.13	0.27-0.65	0.01
Fluids, ml	1.00	0.99-1.01	0.66	1.00	1.00-1.01	0.86	1.00	0.99-1.00	0.41
Norepinephrine, µgr/kg/min	2.95	0.28-30.87	0.37	0.58	0.09-3.60	0.56	0.26	0.01-1.08	0.36
MAP, mmHg	0.98	0.94-1.02	0.34	0.99	0.93-1.05	0.69	0.99	0.92-1.08	0.88

Pv-aCO_2: mixed venous-to-arterial carbon dioxide difference; Pv-CO2/Da-vO_2: mixed venous-to-arterial carbon dioxide pressure difference to arterial-venous oxygen content difference ratio; Cv-CO2/Da-vO_2: mixed venous-to-arterial carbon dioxide to arterial-venous oxygen content differences ratio; iDO_2: oxygen delivery indexed; iVO_2: oxygen consumption indexed; SvO2: mixed-venous oxygen saturation; CI: cardiac index; APACHE II: Acute Physiology and Chronic Health Evaluation II; MAP: mean arterial pressure

* Analysis just for patients attaining SvO_2 >65% at T6.

Suppl. Table 3. Multivariate logistic regression for predictors of mortality at day-28 (simultaneously including $Cv\text{-}aCO_2/Da\text{-}vO_2$ and $Pv\text{-}aCO_2/Da\text{-}vO_2$)

	T0			T6			T6 (for $SvO_2 \geq 65\%$) *		
	RR	**95%CI**	**p**	**RR**	**95%CI**	**p**	**RR**	**95%CI**	**p**
$Cv\text{-}aCO_2/Da\text{-}vO_2$	3.32	1.26-8.75	0.02	3.92	1.47-10.44	0.006	5.13	1.04-25.22	0.04
$Pv\text{-}aCO_2/Da\text{-}vO_2$	1.10	0.54-2.23	0.80	0.98	0.54-1.79	0.96	1.21	0.50-2.90	0.68
Lactate, mmol/L	1.18	0.96-1.43	0.11	1.56	1.12-2.22	0.01	2.40	1.21-4.75	0.01
VO_2, ml/min/m^2	1.01	0.99-1.03	0.55	0.99	0.99-1.01	0.35	1.01	0.99-1.02	0.28
DO_2, ml/min/m^2	1.00	0.99-1.01	0.74	1.00	0.99-1.01	0.48	1.00	0.99-1.01	0.74
SvO_2, %	0.97	0.90-1.04	0.39	0.93	0.86-1.01	0.09			
CI, L/min/m^2	0.81	0.44-1.52	0.52	0.95	0.39-2.30	0.90	1.19	0.30-4.78	0.80
APACHE II	1.09	0.99-1.20	0.08	1.03	0.93-1.14	0.54	0.94	0.80-1.10	0.43
Age, years	1.03	0.99-1.06	0.15	1.02	0.98-1.06	0.42	1.10	0.99-1.21	0.05
Time before T0, Hours	0.63	0.37-1.06	0.08	0.72	0.41-1.27	0.26	0.65	0.29-1.44	0.29
Gender	0.46	0.16-1.30	0.14	0.75	0.23-2.42	0.63	0.15	0.24-0.94	0.04
Fluids, ml	1.00	0.99-1.01	0.80	1.00	1.00-1.01	0.97	1.00	0.99-1.00	0.81
Norepinephrine, µgr/kg/min	1.89	0.19-18.58	0.59	0.42	0.06-3.10	0.39	0.28	0.01-8.94	0.47
MAP, mmHg	0.96	0.92-1.01	0.12	0.98	0.92-1.05	0.60	0.99	0.89-1.09	0.80

$Pv\text{-}aCO_2$: mixed venous-to-arterial carbon dioxide difference; $Pv\text{-}CO2/Da\text{-}vO_2$: mixed venous-to-arterial carbon dioxide pressure difference to arterial-venous oxygen content difference ratio; $Cv\text{-}CO2/Da\text{-}vO_2$: mixed venous-to-arterial carbon dioxide to arterial-venous oxygen content differences ratio; iDO_2: oxygen delivery indexed; iVO_2: oxygen consumption indexed; SvO2: mixed-venous oxygen saturation; CI: cardiac index; APACHE II: Acute Physiology and Chronic Health Evaluation II; MAP: mean arterial pressure

* Analysis just for patients attaining SvO_2 >65% at T6

ANNEX C

SUBLINGUAL MICROCIRCULATORY IMAGES ACQUISITION AND ANALYSIS OF MICROVASCULAR BLOOD FLOW IN HUMANS

We used a Sidestream dark-field (SDF) imaging device (Micro Scan; MicroVision Medical, Amsterdam, the Netherlands) to explore microcirculation (Figure 1). This portable video-microscope device uses a stroboscopic green light (around 530 nm wavelength), which is delivered to the tissues by multiple light-emitting diodes (LEDs). This wavelength of light is absorbed by hemoglobin of red blood cells, allowing their observation as dark cells flowing in the microcirculatory net while the light reflected by superficial layers does not reach the optics. As result of peripheral location of LEDs and the synchronization between light emission and camera frame rate, SDF provides a detailed visualization of open capillaries using a 5x objective and providing an on-screen magnification of x380 (Figure 2).

When sublingual mucosa was assessed, the SDF device was softly applied to the lateral side of the tongue covering an area approximately of 2 – 4 cm from the tip of tongue after gentle removal of secretions with gauze (Figure 2. Panel D). Light intensity and focus were manually adjusted until obtain the best quality in each case. Operator of SDF device was a well-trained physician (W.F.B., J.D.V., H.M.N.) exclusively dedicated to collect data information in clinical and experimental trials concerning to microcirculatory alterations in our intensive care department. At each time of measurements, we collected five sequences of video of 20 seconds each from different adjacent mucosa areas using a videocard (MicroVideo; Pinnacle system, Mountain Views, CA, U.S.A.). These sequences of video were stored under a random number and later analyzed by two investigators blinded to the origin of sequences (J.D.V., G.O.T.). For analysis, a grill of three horizontal and three vertical lines was placed in front of the screen while each sequence of video was reproduced (Figure 4, Panel A). The vascular density was calculated as the number of vessels crossing these lines divided by the total length of the lines. Total vascular density (TCD) corresponds to the total

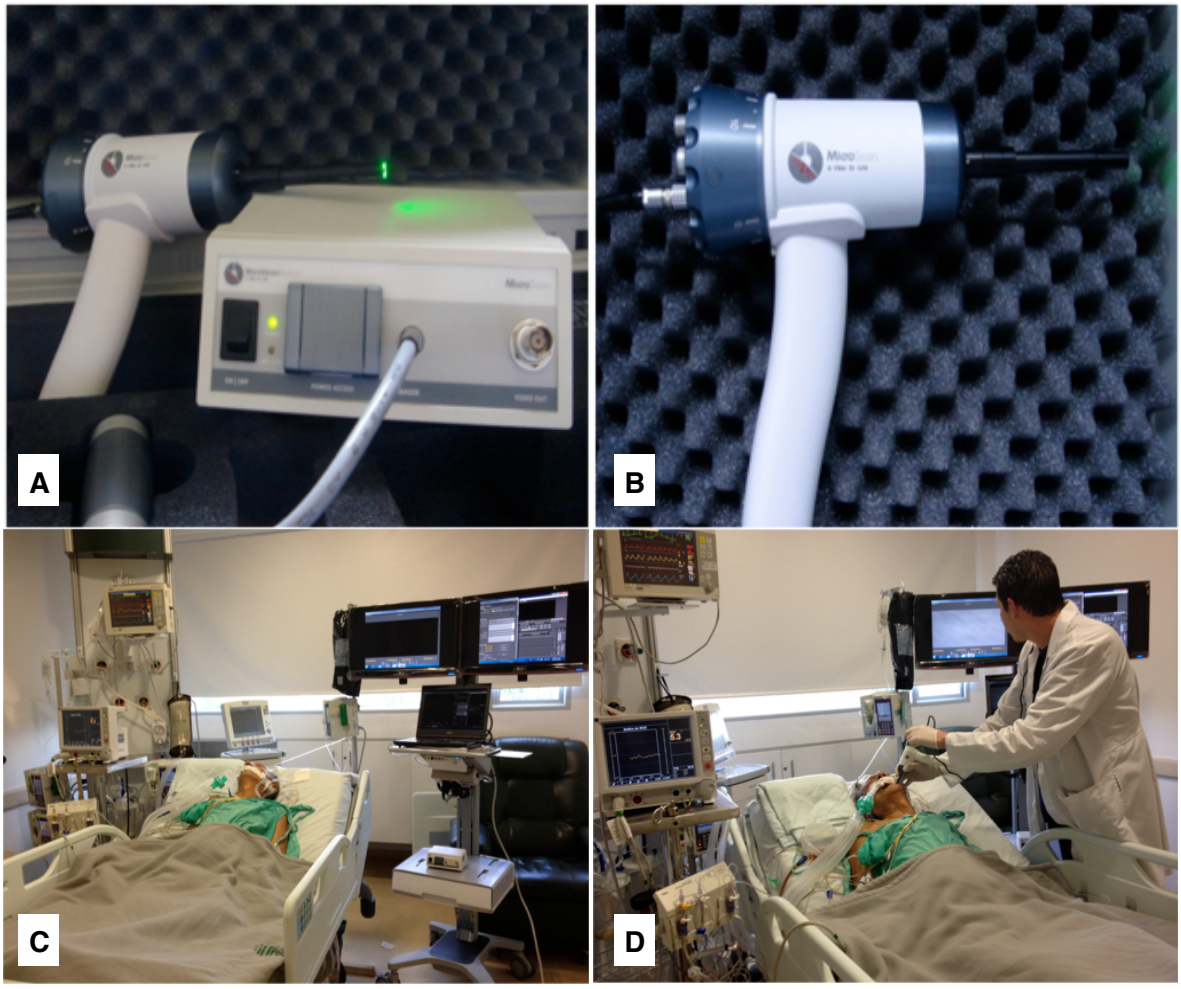
number of vessels (small and large, with and without normal flow) while functional capillary density (FCD) corresponds to the number of well-perfused small vessels (< 20 μm) per area unit. The type of flow was defined as continuous, intermittent, or absent. The vessels were separated into large (mostly venules) and small (mostly capillaries) vessels using a cut-off value of 20 μm in diameter. Vessel perfusion (total, large and small) was defined as the proportion of perfused vessels, calculated as the number of vessels continuously perfused during the 20-second observation period divided by the total number of vessels of the same type. Thus, we report the proportion of small vessels perfused (PPV) corresponding to the proportion of well-perfused vessels with a diameter < 20 μm (mainly capillaries). We also reported the blood flow heterogeneity index (HI) defined as the difference between maximal and minimal proportions of PPV evaluated at each point in the five areas divided by its own mean value. Each microvascular blood flow parameter was obtained by the average of five video sequences. Additionally, a second grill consisting in one vertical and one horizontal line was placed in front of the screen to separate the image in four quadrants. Microvascular flow was characterized as absent (0), intermittent (1), sluggish (2), or normal (3). Average of values of those four quadrants was reported as microvascular flow index (MFI) (Figure 4, Panel B).

The intra- and inter-observer variability for these methods have been demonstrated in the past [1]. Previous observations revealed a 4 (1-8)% difference between 2 baselines in 13 hemodynamically stabilized patients with septic shock, representing a coefficient of variability of 5% [2] . Other have reported a 3% coefficient of variability in the assessment of sublingual microcirculation between 2 observers [3] . Thus, given the intrinsic variability of measurements and previous data showing delineation between survivors and non-survivors [4], an absolute change of 7-10% in the proportion of perfused small vessels can be considered as clinically significant.

For the current study, the intra and inter-observer variability were determined by using five sequences analyzed five times at eight-week intervals by two observers (J.D.V. and G.O.T.). Our coefficient of variability of the determination of one video-

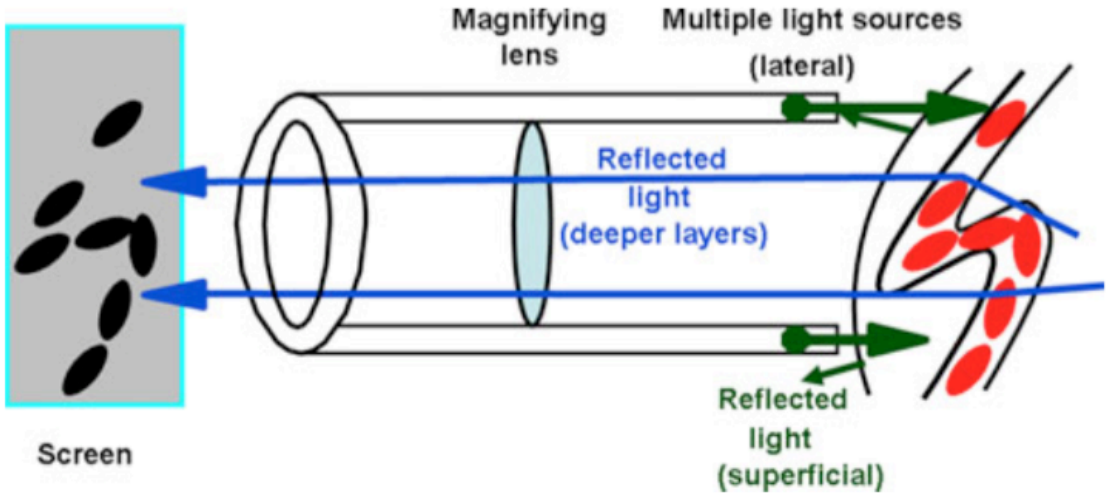
sequence ranged from 2.8 to 6.2% (intra-observer) and from 4.0 to 6.5 % (inter-observer) for the total number of vessels, and from 1.8 to 4.6% (intra-observer) and from 3.6 to 8.2% (inter-observer) for the proportion of perfused vessels (all sizes).

Figure 1. Side-stream dark field device (SDF).



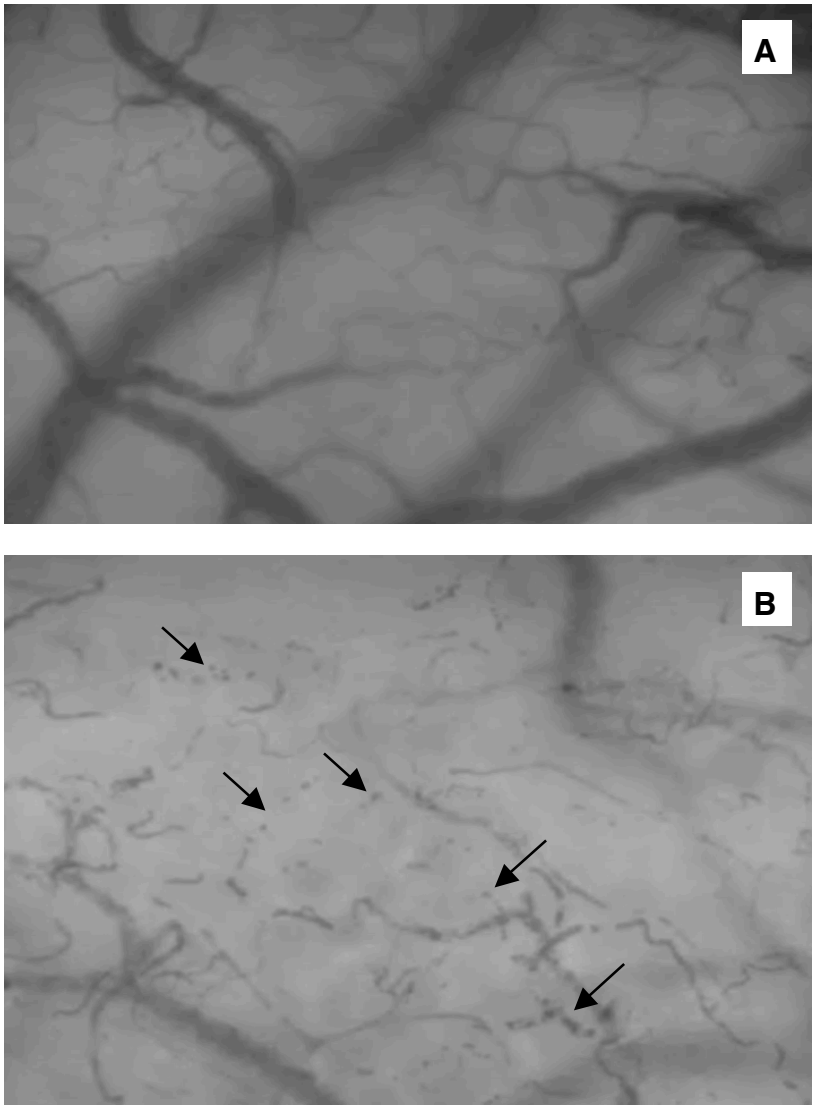
Panel A. Side-stream dark field device (SDF) **Panel B.** SDF hand-held part. **Panel C and D.** Portable mounting for bedside microcirculation assessment at the ICU (ICU, Fundación Valle del Lili. Cali, Colombia)

Figure 2. SDF imaging technique



The lateral sides of the device provide a stroboscopic green light, is absorbed by hemoglobin contained in red blood cells. The rest of the light is reflected from the depth of the tissues reaching the center of the device, being magnify by lens and subsequently captured by a camera. Video images will be seen as gray/black bodies flowing on the screen. (Reproduced with permission. De Backer D, Ospina-Tascón G, Salgado D et al. Intensive Care Med (2010) 36:1813–1825)

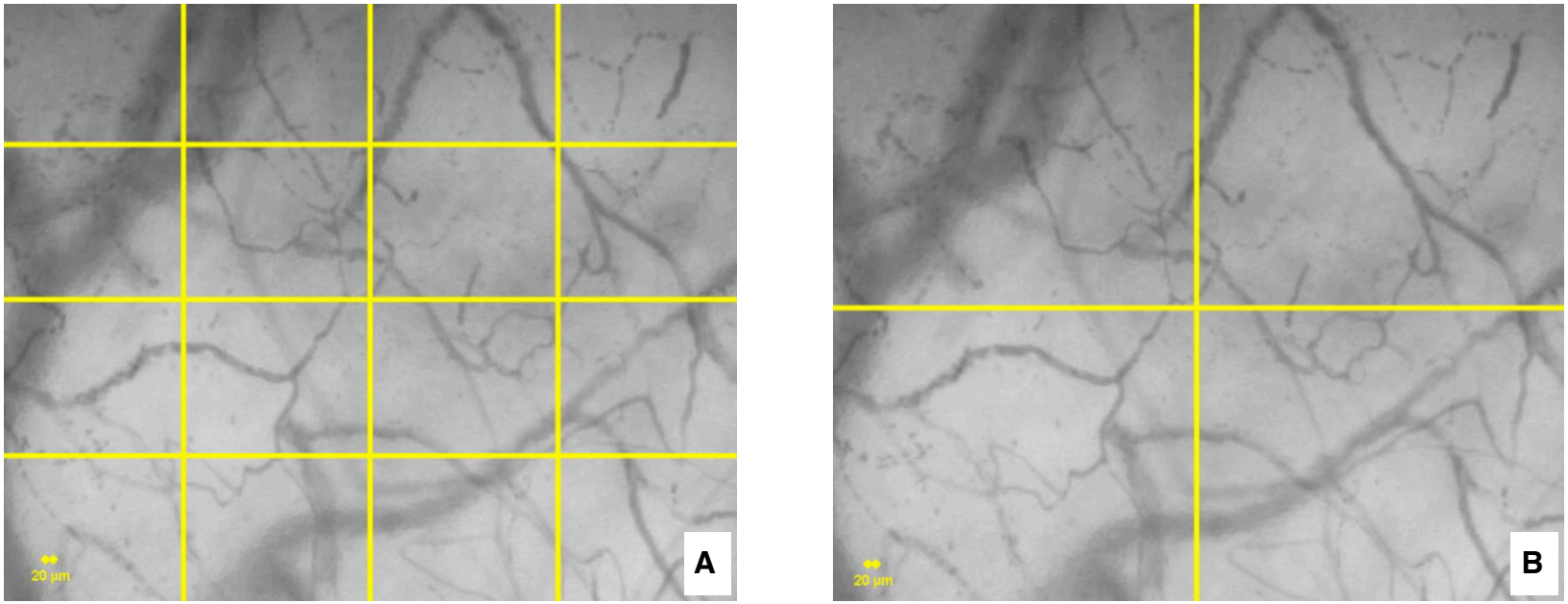
Figure 3. Images of sublingual microcirculation



Panel A. Sublingual microcirculation image obtained by Side-stream dark field (SDF) device in a volunteer. Typically, vessels have continuous flow and spacing between capillaries provides normal oxygen diffusion

Panel B. Sublingual microcirculation image obtained by Side-stream dark field (SDF) in a patient with septic shock. Arrows designate small vessels (< 20μm diameter) with abnormal (stopped and intermittent) red blood cells flow. Note that lowering of small vessels perfused leads to decreasing functional capillary density

Figure 4. Semiquantitative analysis of microcirculation



Panel A. Vessels are classified into large (mostly venules) and small (mostly capillaries) vessels using a cut-off value of 20 µm in diameter. Vessel perfusion (total, large and small) is defined as the proportion of perfused vessels, calculated as the number of vessels continuously perfused during the 20-second observation period divided by the total number of vessels of the same type. The proportion of small vessels perfused (PPV) corresponds to the proportion of well-perfused vessels with a diameter < 20 µm (mainly capillaries). The microvascular blood flow heterogeneity index (HI) is defined as the difference between maximal and minimal proportions of PPV evaluated at each point in the five areas divided by its own mean value. **Panel B.** Microvascular flow was characterized as absent (0), intermittent (1), sluggish (2), or normal (3). Average of values of those four quadrants is reported as microvascular flow index (MFI). Vessels are classified into large (mostly venules) and small (mostly capillaries) vessels using a cut-off value of 20 µm in diameter. Reproduced with permission, De Backer D et al. *Critical Care* 2007, 11:R101

REFERENCES

1. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med.* 2002;166(1):98-104.

2. Büchele GL, Silva E, Ospina-Tascón GA, Vincent JL, De Backer D. Effects of hydrocortisone on microcirculatory alterations in patients with septic shock. *Crit Care Med.* 2009;37(4):1341-1347.

3. Bracht H, Krejci V, Hiltebrand L, et al. Orthogonal polarization spectroscopy to detect mesenteric hypoperfusion. *Intensive Care Med.* 2008;34(10):1883-1890.

4. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med.* 2004;32(9):1825-1831.

ANNEX D

CAN VENOUS-TO-ARTERIAL CARBON DIOXIDE DIFFERENCES REFLECT MICROCIRCULATORY ALTERATIONS IN PATIENTS WITH SEPTIC SHOCK?

Intensive Care Med. 2016 Feb;42(2):211-21

Supplementary Material

Suppl. Table E1. Hemodynamic, ventilatory, blood gases and oxygen parameters

	All	Pv-aCO_2 < 6.0 (n = 39)	Pv-aCO_2 = 6.0 – 9.9 (n = 25)	Pv-aCO_2 ≥ 10.0 (n = 11)
Hemodynamics / Fluids / Vasoactives / Inotropes				
HR, beats/min				
T0	108 (90 – 122)	109 (90 – 118)	105 (92 – 129)	109 (75 – 136)
T6	100 (82 – 122)	99 (80 – 116)	96 (81 – 123)	100 (83 – 126)
CI, L/min/m2				
T0	3.2 (2.4 – 4.0)	3.4 (2.5 – 4.3)	3.2 (2.4 – 3.7)	2.4 (2.2 – 4.1)
T6	3.2 (2.7 – 3.8)	3.6 (3.1 – 4.1) * †	2.9 (2.6 – 3.3)	3.0 (2.2 – 3.7)
MAP, mmHg				
T0	70 (64 – 77)	72 (66 – 80) *	65 (61 – 69)	71 (66 – 78)
T6	70 (65 – 77)	75 (66– 84) * †	68 (63 – 74)	65 (61 – 71)
CVP, mmHg				
T0	11 (7 – 15)	9 (6 – 14)*	14 (11 – 18)	11 (5 – 16)
T6	9 (7 – 12)	8 (4 – 11)	10 (8 – 15)	12 (8 – 16)
PAOP, mmHg				
T0	15 (10 – 20)	13 (9 – 17)	17 (14 – 22)	15 (8 – 19)
T6	13 (10 – 18)	12 (8 – 17)	15 (12 – 18)	16 (9 – 20)
Fluids, ml [IQ 25-75]				
T0	1,650 (1,200 – 2,400)	1,600 (1,200 – 2,400)	1,600 (1,025 – 2,300)	2,100 (1,500 – 2,700)
T6	3,000 (2,200 – 3,850)	3,000 (2,125 – 3,812)	3,100 (2,100 – 4,200)	3,350 (2,700 – 4,250)
Norepinephrine, ugr/kg/min [IQ 25-75], n				
T0	0.24 (0.13 – 0.52)	0.17 (0.10 – 0.32)* †	0.38 (0.20 – 0.71)	0.38 (0.14 – 0.72)
T6	0.14 (0.08 – 0.48)	0.10 (0.05 – 0.33) †	0.16 (0.08 – 0.52)	0.48 (0.20 – 0.74)

Dobutamine, ugr/kg/min [IQ 25-75], n				
T0	9.2 (3.4 – 11.5)	10.6 (2.5 – 11.8)	3.7 (2.4 – 3.8)	10.6 (2.5 – 11.8)
T6	10.9 (5.6 – 12.0)	8.9 (4.3 – 12.0)	5.8 (5.0 – 7.9)	10.2 (5.3 – 15.0)
Mechanical ventilation				
VT, ml/kg, [IQ 25-75], n				
T0	6.2 (6.0 – 6.6), 56	6.6 (6.4 – 7.0), 24	6.0 (5.2 – 6.5), 21	6.2 (5.8 – 6.6), 11
T6	6.2 (6.0 – 6.5), 64	6.0 (5.7 – 6.4), 33	6.2 (5.4 – 6.4), 18	6.2 (5.8 – 6.5), 13
PEEP, mmHg				
T0	6 (5 – 8)	5 (5 – 8)	7 (5 – 8)	8 (6 – 10)
T6	7 (5 – 8)	7 (5 – 8)	8 (5 – 8)	7 (5 – 10)
Blood Gases				
pH (arterial)				
T0	7.30 (7.20 – 7.39)	7.35 (7.25 – 7.40)	7.29 (7.20 – 7.40)	7.29 (7.09 – 7.33)
T6	7.34 (7.24 – 7.40)	7.39 (7.32 – 7.43) †	7.30 (7.18 – 7.34)	7.24 (7.06 – 7.35)
pH (venous)				
T0	7.27 (7.19 – 7.35)	7.33 (7.24 – 7.37) †	7.25 (7.16 – 7.34)	7.17 (6.98 – 7.27)
T6	7.31 (7.22 – 7.38)	7.35 (7.30 – 7.40) †	7.27 (7.17 – 7.34)	7.23 (7.02 – 7.30)
$PaCO_2$, mmHg				
T0	30.3 (26.8 – 36.0)	29.7 (26.9 – 35.4)	32.4 (27.2 – 37.6)	28.2 (24.0 – 45.3)
T6	30.1 (26.9 – 33.8)	29.4 (25.5 – 31.7)	31.8 (28.2 – 37.1)	28.3 (24.2 – 34.9)
$PvCO_2$, mmHg				
T0	36.1 (32.2 – 41.6)	33.9 (30.5 – 38.0)	38.8 (34.9 – 46.3)	39.6 (34.7 – 60.2)
T6	34.5 (31.4 – 39.2)	33.4 (29.4 – 35.3)	39.6 (34.3 – 43.7)	36.1 (27.1 – 44.2)
PaO_2, mmHg				
T0	102 (82 – 128)	102 (82 – 123)	93 (78 – 129)	104 (91 – 194)
T6	102 (80 – 129)	100 (81 – 134)	98 (75 – 115)	103 (84 – 129)

PaO_2/FiO_2, mmHg				
T0	248 (140 – 332)	259 (175 – 339)	176.0 (92 – 259)	252 (115 – 458)
T6	258 (163 – 353)	304 (168 – 412)	206 (127 – 314)	226 (120 – 281)
Oxygen/ Perfusion parameters				
Lactate, mmol/L,				
T0	3.0 (1.6 – 4.4)	2.7 (1.5 – 3.7)	3.6 (1.8 – 5.9)	3.4 (2.4 – 4.5)
T6	2.3 (1.2 – 4.1)	1.8 (1.2 – 3.3) †	3.4 (1.6 – 6.6)	2.1 (0.9 – 4.2)
SvO_2, %,				
T0	68.8 (63.0 – 74.4)	69.7 (64.6 – 74.6)	66.8 (62.3 – 73.5)	61.1 (35.6 – 75.7)
T6	68.6 (61.5 – 75.5)	71.8 (65.5 – 72.8)	68.6 (65.5 – 72.8)	66.8 (55.7 – 76.0)
ERO_2,				
T0	29.7 (24.2 – 35.3)	29.6 (24.2 – 34.4)	29.0 (24.0 – 36.0)	33.7 (24.2 – 61.2)
T6	26.9 (14.4 – 34.6)	24.9 (14.6 – 31.8)	27.4 (15.4 – 35.5)	33.9 (18.3 – 47.8)
DO_2, ml/min/m^2				
T0	383 (293 – 496)	415 (284 – 549)	383 (340 – 548)	275 (179 – 528)
T6	396 (322 – 468)	412 (368 – 430)	340 (304 – 430)	447 (356 – 447)
VO_2, ml/min/m^2				
T0	125 (87 – 150)	125 (86 – 152)	125 (90 – 147)	107 (84 – 153)
T6	188 (140 – 249)	183 (116 – 240)	192 (147 – 239)	247 (140 – 290)
Pv-aCO_2, mmHg				
T0	5.4 (3.6 – 7.7)	3.7 (2.4 – 4.4) * †	7.0 (6.4 – 8.0) **	11.6 (10.6 – 12.2)
T6	5.9 (3.0 – 8.6)	3.5 (2.3 – 4.9) * †	6.8 (6.1 – 7.8) **	11.9 (10.5 – 14.6)
Pv-aCO_2/Da-vO_2, mmHg				
T0	1.40 (0.98 – 1.94)	1.06 (0.65 – 1.27) * †	1.67 (1.40 – 2.50) **	3.00 (2.00 – 4.30)
T6	1.40 (0.77 – 2.19)	0.93 (0.73 – 1.54) * †	1.85 (1.20 – 2.60) **	2.59 (1.69 – 3.89)

* $p<0.05$ Pv-$aCO_2 < 6.0$ vs. Pv-$aCO_2 = 6.0 – 9.9$; † $p<0.05$ Pv-$aCO_2 < 6.0$ vs. Pv-$aCO_2 \geq 10.0$; ** $p<0.05$ Pv-$aCO_2 = 6.0 – 9.9$ vs. Pv-$aCO_2 \geq 10.0$

HR: heart rate; CI: cardiac index; MAP: mean arterial pressure; CVP: central venous pressure; PAOP: pulmonary artery occlusion pressure; $PaCO_2$: arterial carbon dioxide partial pressure; $PvCO_2$: venous carbon dioxide partial pressure; PaO_2: arterial oxygen partial pressure; SvO_2: mixed-venous oxygen saturation; ERO_2: oxygen extraction ration; DO_2: oxygen delivery; VO_2: oxygen consumption; Pv-aCO_2: venous to arterial carbon dioxide difference; Pv-aCO_2/Da-vO_2: venous-arterial carbon dioxide difference to arterial-venous oxygen content difference ratio.

Suppl. Table E2. Area under curve for the prediction of PPV

Timing	PPV Cutoff	Variable	Area	95% CI		p
T0	50%	**Pv-aCO$_2$**	0.896	0.806	0.987	< 0.001
		MAP	0.460	0.303	0.617	0.626
		SvO$_2$	0.420	0.241	0.600	0.326
		Lactate	0.704	0.560	0.848	0.012
		CI	0.374	0.206	0.542	0.120
	60%	**Pv-aCO$_2$**	0.898	0.827	0.968	< 0.001
		MAP	0.387	0.255	0.520	0.109
		SvO$_2$	0.436	0.297	0.575	0.366
		Lactate	0.647	0.511	0.783	0.036
		CI	0.396	0.262	0.529	0.138
	70%	**Pv-aCO$_2$**	0.914	0.844	0.985	< 0.001
		MAP	0.392	0.255	0.529	0.126
		SvO$_2$	0.357	0.227	0.488	0.043
		Lactate	0.718	0.595	0.841	0.002
		CI	0.385	0.249	0.520	0.101

	50%	**$Pv\text{-}aCO_2$**	0.936	0.839	0.999	< 0.001
		MAP	0.267	0.114	0.419	0.015
		SvO_2	0.421	0.207	0.636	0.414
		Lactate	0.657	0.456	0.858	0.102
		CI	0.417	0.210	0.625	0.390
T6	**60%**	**$Pv\text{-}aCO_2$**	0.899	0.819	0.978	< 0.001
		MAP	0.290	0.160	0.420	0.006
		SvO_2	0.313	0.173	0.452	0.071
		Lactate	0.725	0.589	0.861	0.069
		CI	0.356	0.213	0.498	0.073
	70%	**$Pv\text{-}aCO_2$**	0.907	0.837	0.977	< 0.001
		MAP	0.347	0.214	0.480	0.033
		SvO_2	0.339	0.204	0.475	0.025
		Lactate	0.700	0.573	0.828	0.005
		CI	0.316	0.186	0.445	0.010

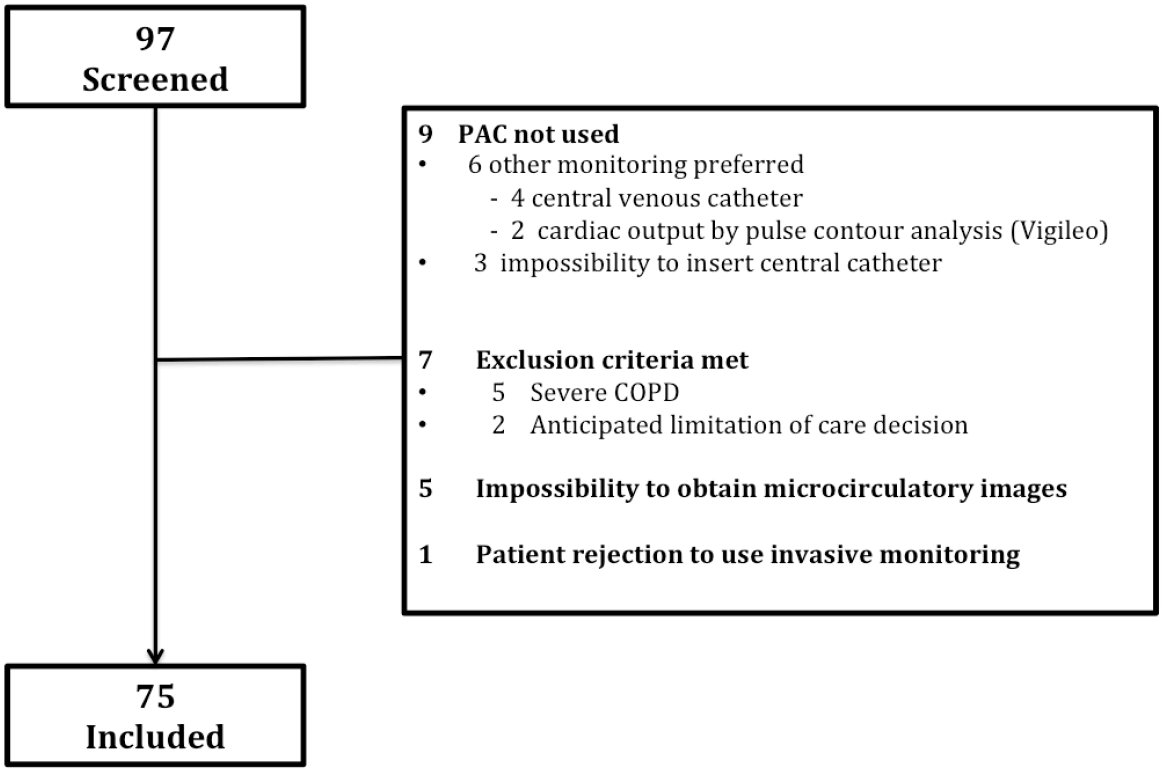
Suppl. Table E3. Multiple linear regression models for prediction of Pv-aCO_2

	T0				T6			
	Coefficient	95% CI		p value	Coefficient	95% CI		p value
All Patients								
PPV, %	-0.111	-0.137	-0.085	<0.001	-0.164	-0.211	-0.118	<0.001
LVP, %	-0.014	-0.066	0.038	0.595	0.070	-0.002	0.143	0.057
SvO_2, %	-0.009	-0.051	0.033	0.664	-0.019	-0.077	0.039	0.523
CI, L/min	-0.274	-0.657	0.110	0.159	-0.625	-1.415	0.165	0.119
MAP, mmHg	0.023	-0.018	0.064	0.262	-0.003	-0.071	0.065	0.929
R^2 for the model				0.67				0.61
SvO_2 ≥ 65%								
PPV, %	-0.114	-0.150	-0.077	<0.001	-0.162	-0.214	-0.111	<0.001
LVP, %	0.010	-0.089	0.068	0.793	0.061	-0.021	0.143	0.141
CI, L/min	-0.280	-0.764	0.204	0.249	-0.038	-0.844	0.920	0.931
MAP, mmHg	0.034	-0.016	0.084	0.178	-0.022	-0.095	0.052	0.553
R^2 for the model				0.62				0.61

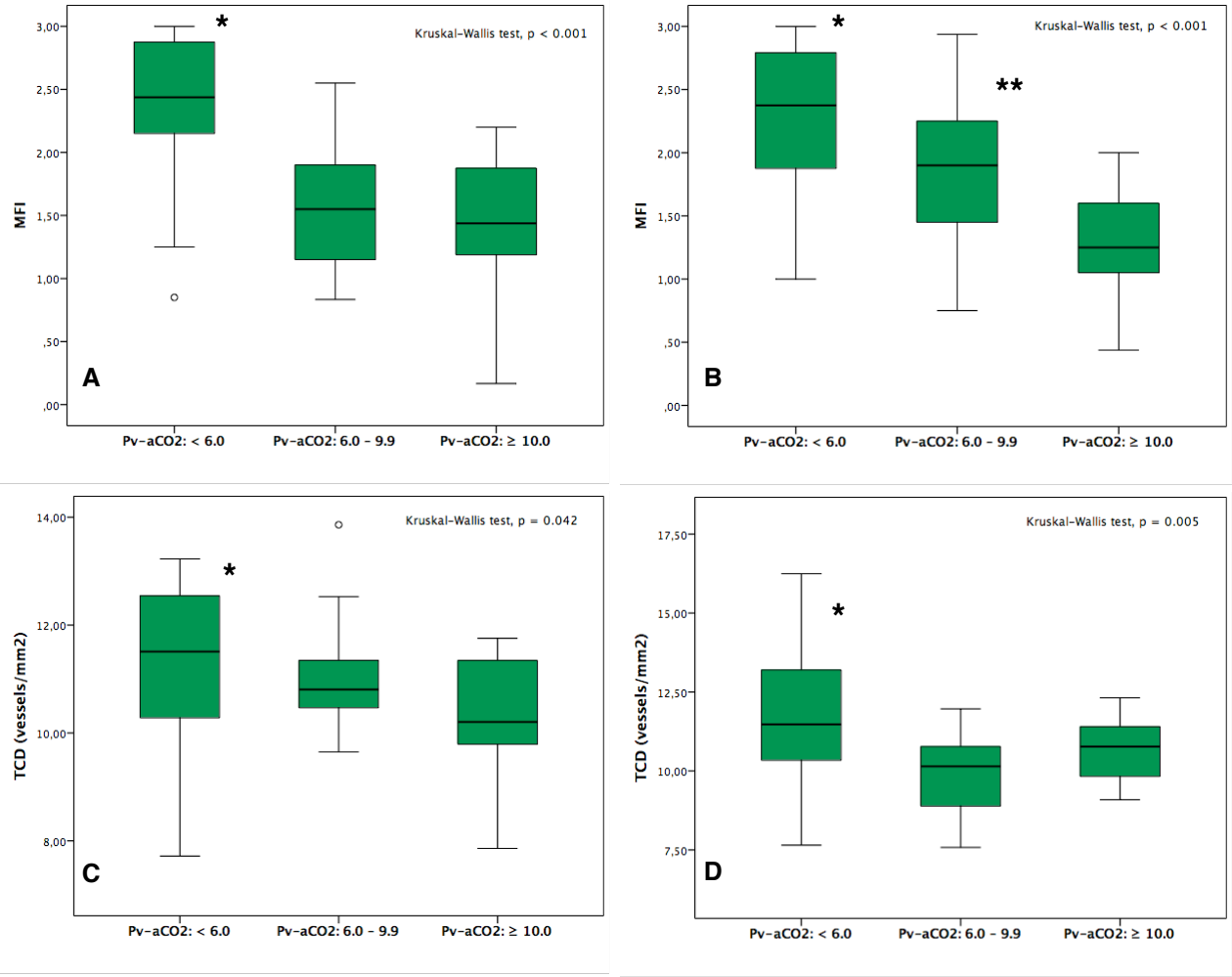
SvO_2 < 65%								
PPV, %	-0.115	-0.154	-0.075	<0.001	-0.165	-0.272	-0.057	0.006
LVP, %	-0.023	-0.098	0.052	0.533	0.085	-0.086	0.256	0.302
CI, L/min	-0.446	-1.183	0.292	0.222	-2.000	-3.670	-0.330	0.023
MAP, mmHg	-0.014	-0.099	0.072	0.742	0.033	-0.134	0.199	0.676
R^2 for the model				0.73				0.71

PPV: % small-perfused vessels; LVP: % large-perfused vessels; SvO_2: mixed-venous oxygen saturation; CI: cardiac index; MAP: mean arterial pressure

Suppl. Figure E1. Selection of patients



Suppl. Figure E2. Microvascular blood flow index (MFI) and total capillary density (TCD) for the predefined Pv-aCO_2 groups



Boxplots depicting differences in microcirculatory blood flow parameters for predefined Pv-aCO_2 groups at T0 and T6 (A and B for MFI; C and D for TCD, respectively). Group 1: Pv-aCO_2 < 6.0; Group 2: Pv-aCO_2 6.0 - 9.9; Groups 3: Pv-aCO_2 > 10.0.

Kruskal-Wallis test, indicated at each figure.

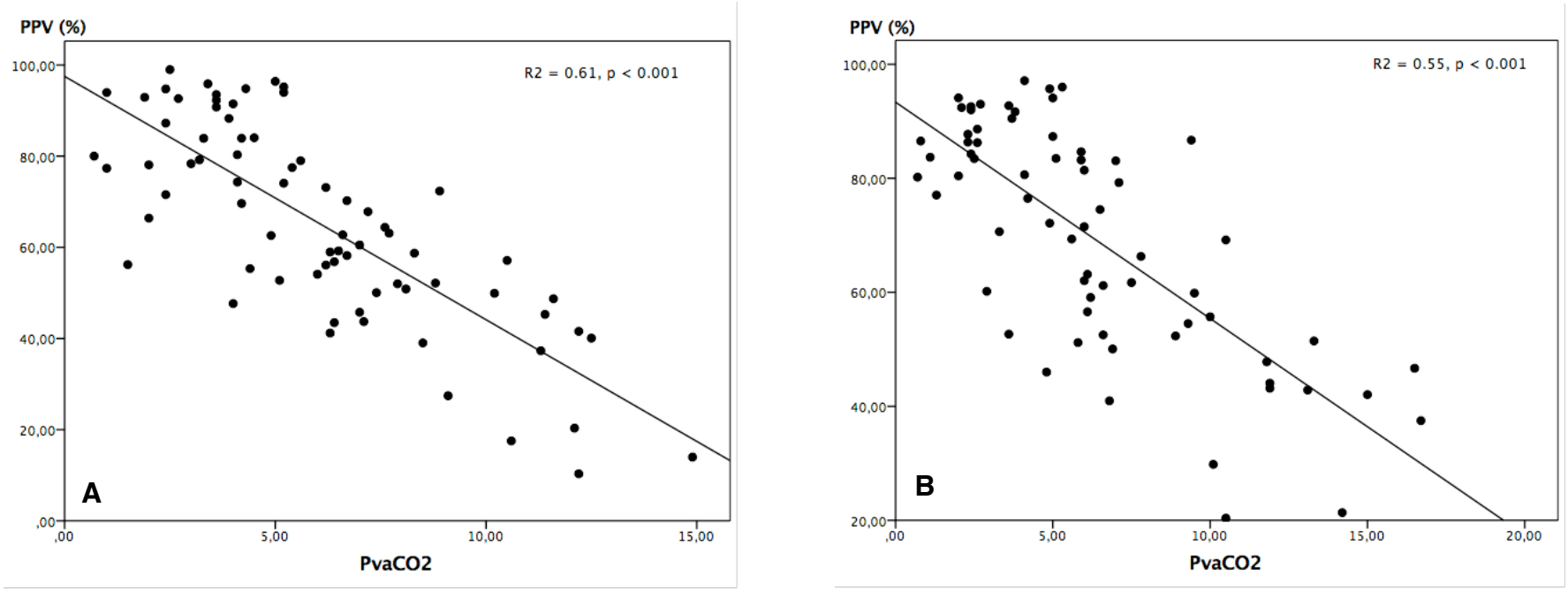
* *pos hoc* analysis for multiple comparisons; p < 0.05 for Group 1 vs. 2 and 1 vs. 3

** *pos hoc* analysis for multiple comparisons; p < 0.05 for Group 2 vs. 3

Boxes denote interquartile range, horizontal line in the boxes represents the median values, and whiskers extend 1.5 times the interquartile range above and below the 25 – 75 percentiles.

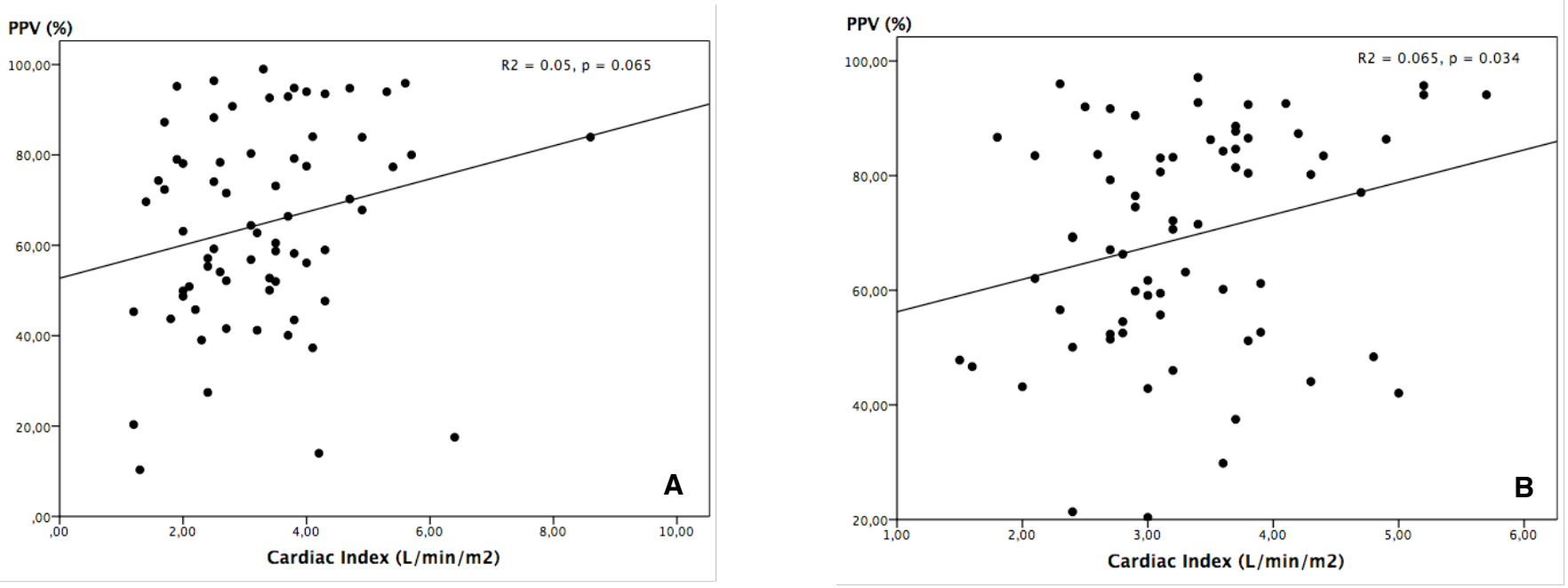
MFI: microvascular blood flow index; TCD: total capillary density; Pv-aCO_2: venous-to-arterial carbon dioxide difference.

Figure E3. Correlation between Pv-aCO_2 and percentage of small-perfused vessels (PPV) at T0 and T6



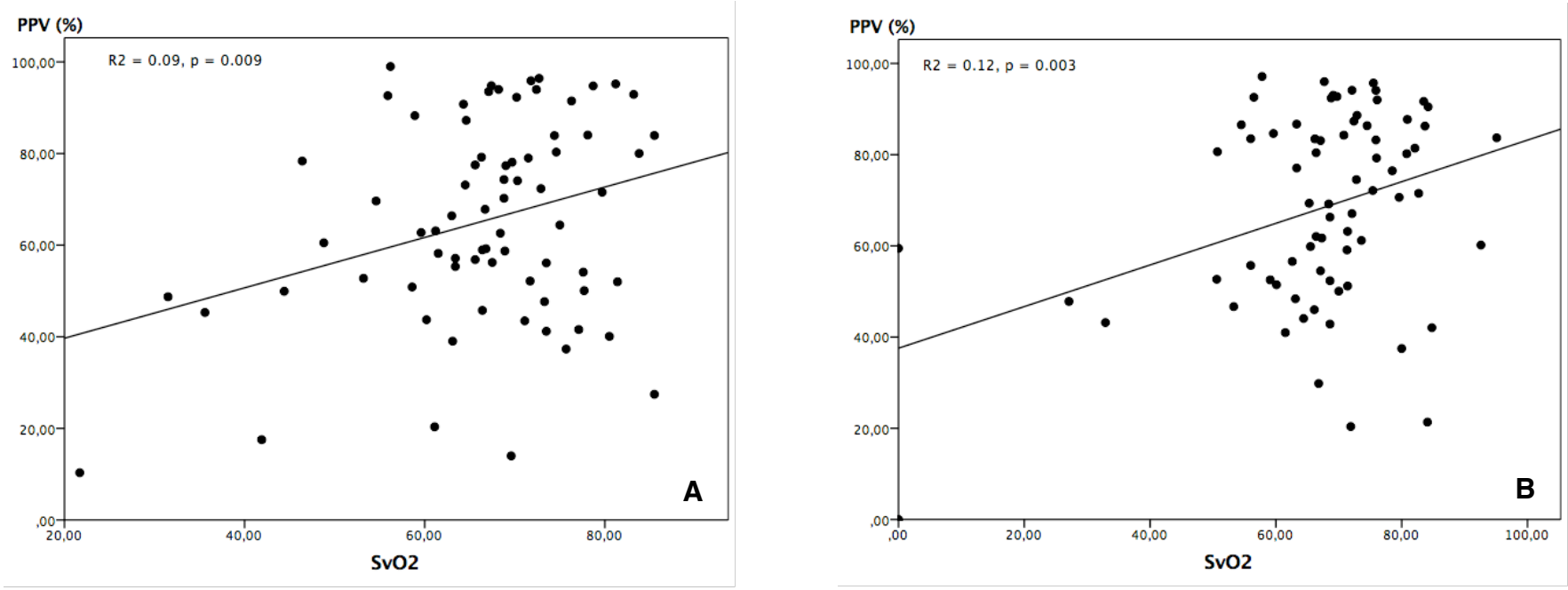
Scatter plot depicting the correlation between Pv-aCO_2 vs. PPV at T0 (A) and T6 (B).
Coefficient of determination (R^2) was calculated to assess the strength of correlations.
Pv-aCO_2: venous-to-arterial carbon dioxide difference

Suppl. Figure E4. Correlation between cardiac index and percentage of small vessels perfused (PPV) at T0 and T6



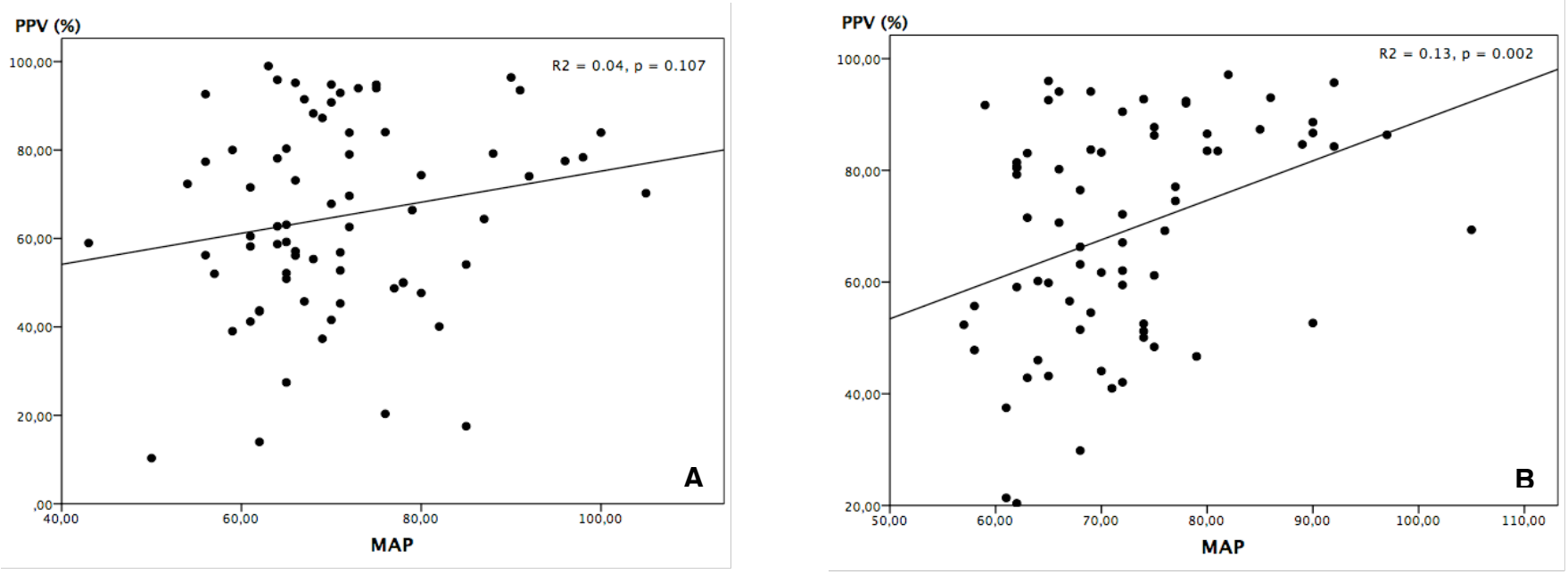
Scatter plot depicting the correlation between cardiac index vs. PPV at T0 (Panel A) and T6 (Panel B).
Coefficient of determination (R^2) was calculated to assess the strength of correlations.

Suppl. Figure E5. Correlation between SvO_2 and percentage of small-perfused vessels (PPV) at T0 and T6



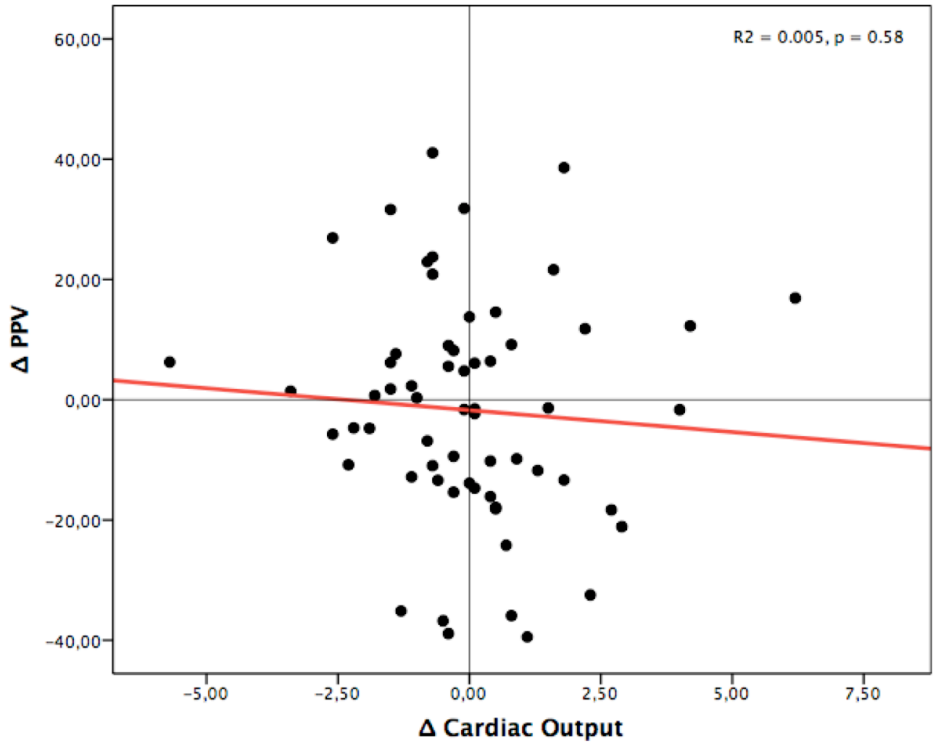
Scatter plot depicting the correlation between SvO_2 vs. PPV at T0 (Panel A) and T6 (Panel B).
Coefficient of determination (R^2) was calculated to assess the strength of correlations.
SvO_2: mixed-venous oxygen saturation

Suppl. Figure E6. Correlation between mean arterial pressure (MAP) and percentage of small-perfused vessels (PPV) at T0 and T6



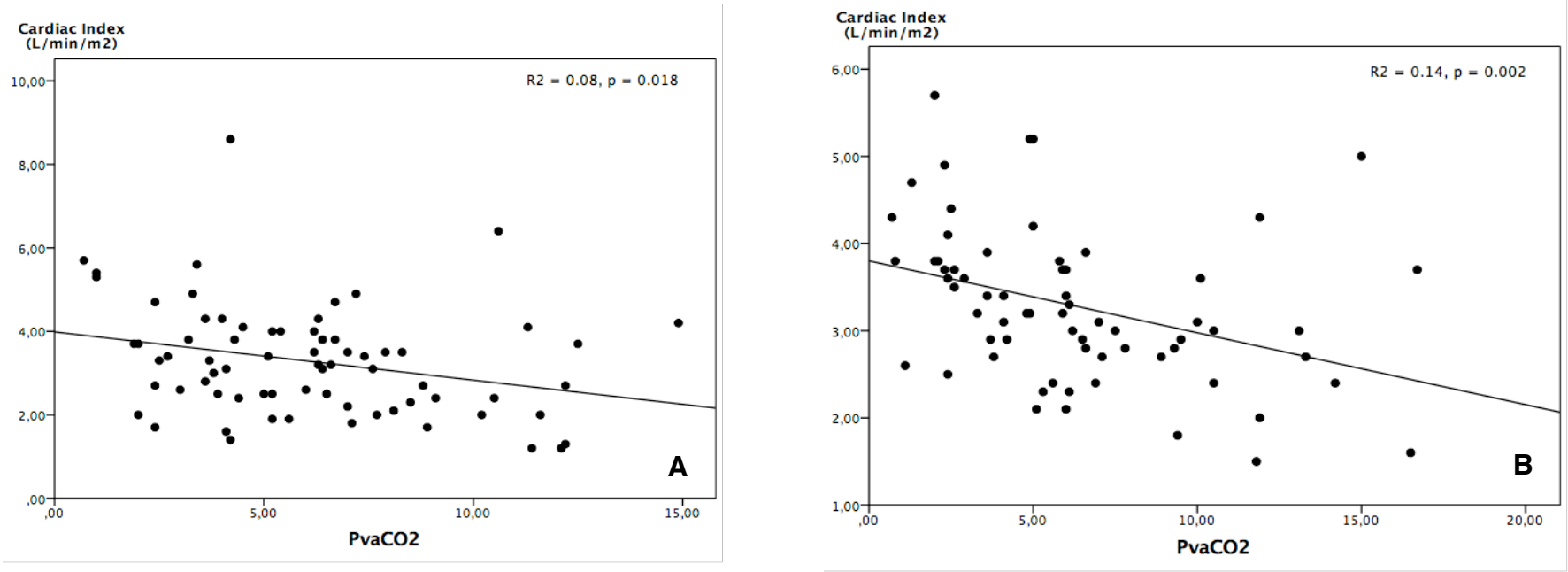
Scatter plot depicting the correlation between MAP vs. PPV at T0 (A) and T6 (B).
Coefficient of determination (R^2) was calculated to assess the strength of correlations.
MAP: mean arterial pressure

Suppl. Figure E7. Correlation of Δ cardiac output vs. Δ PPV variations between T0 and T6



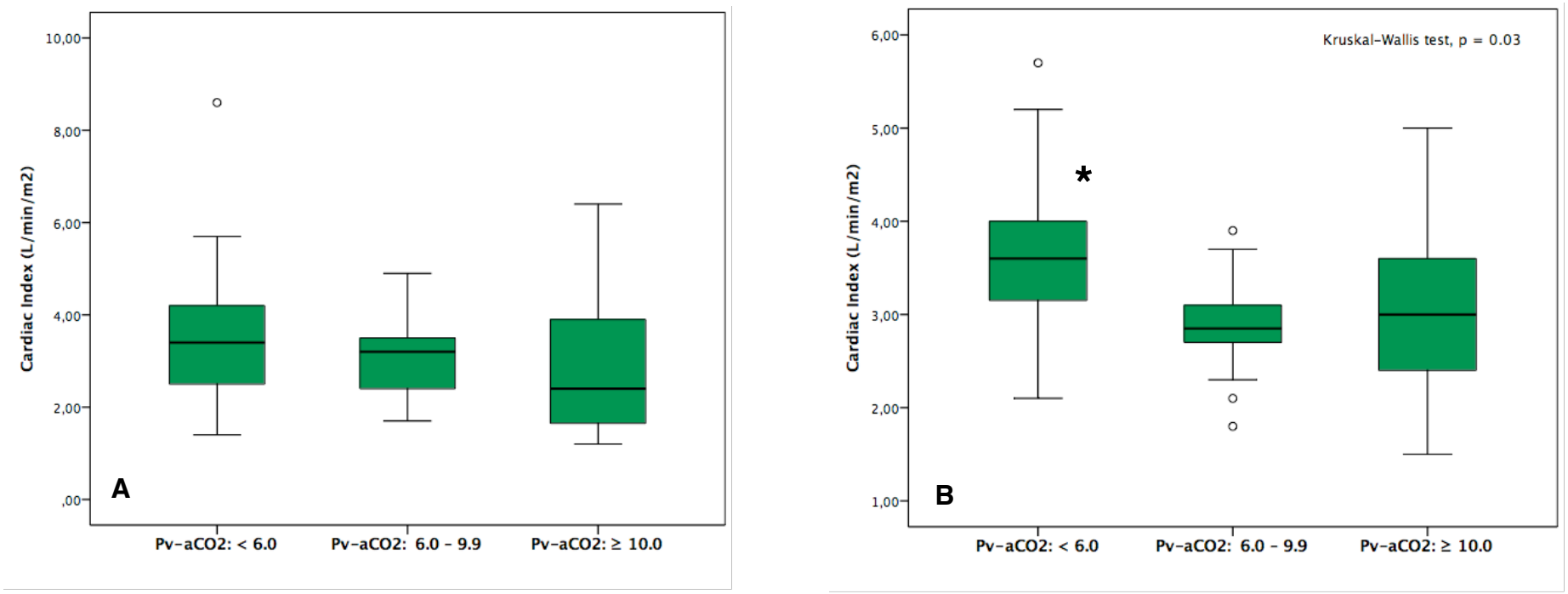
Scatterplot depicting the correlation of variations observed between measurements at T0 and T6 for Δ cardiac output vs. Δ PPV (R^2=0.005, p=0.58)
Coefficient of determination (R^2) was calculated to assess the strength of correlations.
Δ PPV: change of % of small-perfused vessels between T0 and T6

Suppl. Figure E8. Correlation between Pv-aCO_2 and cardiac index at T0 and T6



Scatter plot depicting the correlation between Pv-aCO_2 vs. cardiac index at T0 (A) and T6 (B).
Coefficient of determination (R^2) was calculated to assess the strength of correlations.
Pv-aCO_2: venous-to-arterial carbon dioxide difference

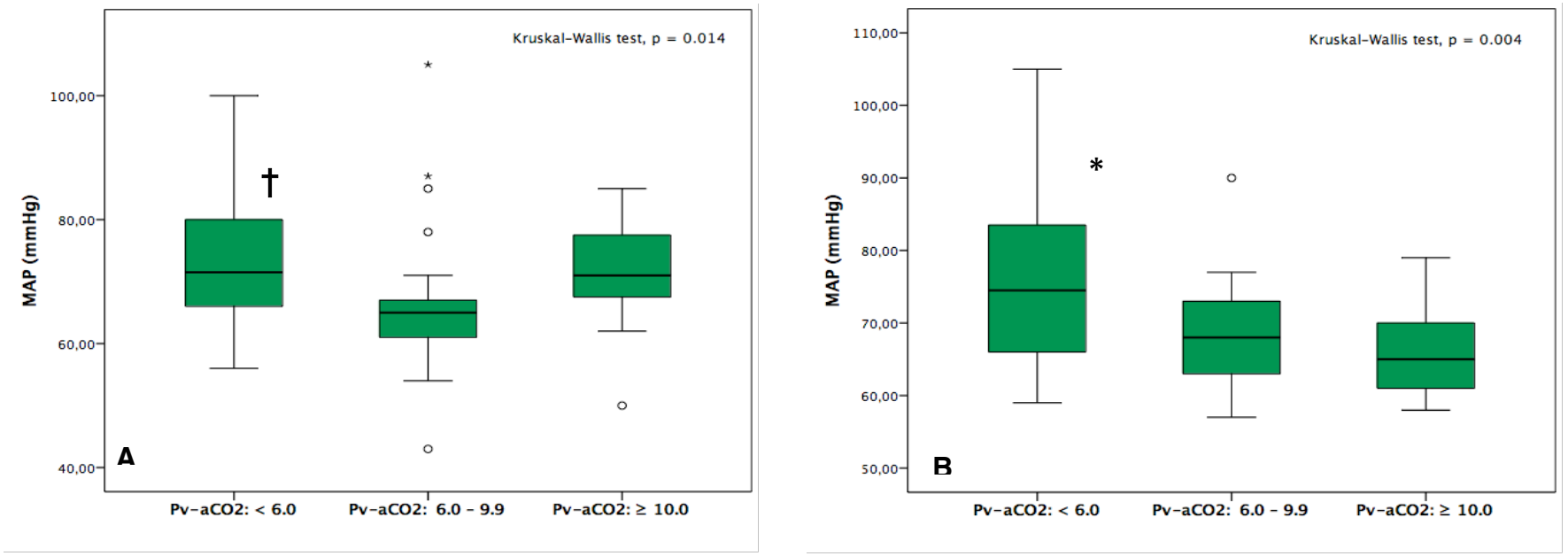
Suppl. Figure E9. Cardiac index for the predefined Pv-aCO_2 groups



Boxplots depicting differences in cardiac index for predefined Pv-aCO_2 groups at T0 (A) and T6 (B). Group 1: Pv-aCO_2 < 6.0; Group 2: Pv-aCO_2 6.0 - 9.9; Groups 3: Pv-aCO_2 > 10.0.
Kruskal-Wallis test, $p < 0.03$ (at T6).
* *pos hoc* analysis for multiple comparisons; $p < 0.05$ for Group 1 vs. 2 and 1 vs. 3

Boxes denote interquartile range, horizontal line in the boxes represents the median values, and whiskers extend 1.5 times the interquartile range above and below the 25 – 75 percentiles.
Pv-aCO_2: venous-to-arterial carbon dioxide difference

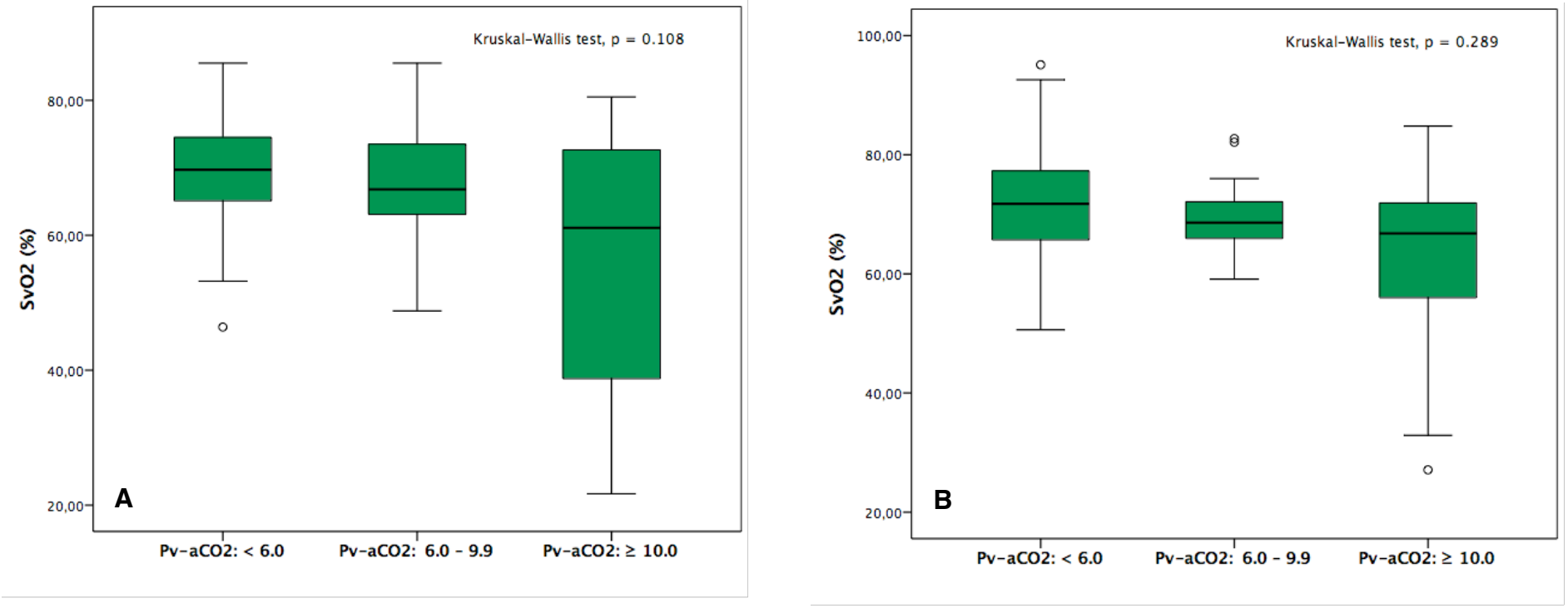
Suppl. Figure E10. Mean arterial pressure for the predefined Pv-aCO_2 groups



Boxplots depicting differences in mean arterial pressure for predefined Pv-aCO_2 groups at T0 (A) and T6 (B). Group 1: Pv-aCO_2 < 6.0; Group 2: Pv-aCO_2 6.0 - 9.9; Groups 3: Pv-aCO_2 > 10.0.
Kruskal-Wallis test, p = 0.014 at T0 and p = 0.004 at T6.
* *pos hoc* analysis for multiple comparisons; p < 0.05 for Group 1 vs. 2 and 1 vs. 3 (at T6)
† *pos hoc* analysis for multiple comparisons; p < 0.05 for Group 1 vs. 2 (at T0)

Boxes denote interquartile range, horizontal line in the boxes represents the median values, and whiskers extend 1.5 times the interquartile range above and below the 25 – 75 percentiles.
MAP: mean arterial pressure; Pv-aCO_2: venous-to-arterial carbon dioxide difference

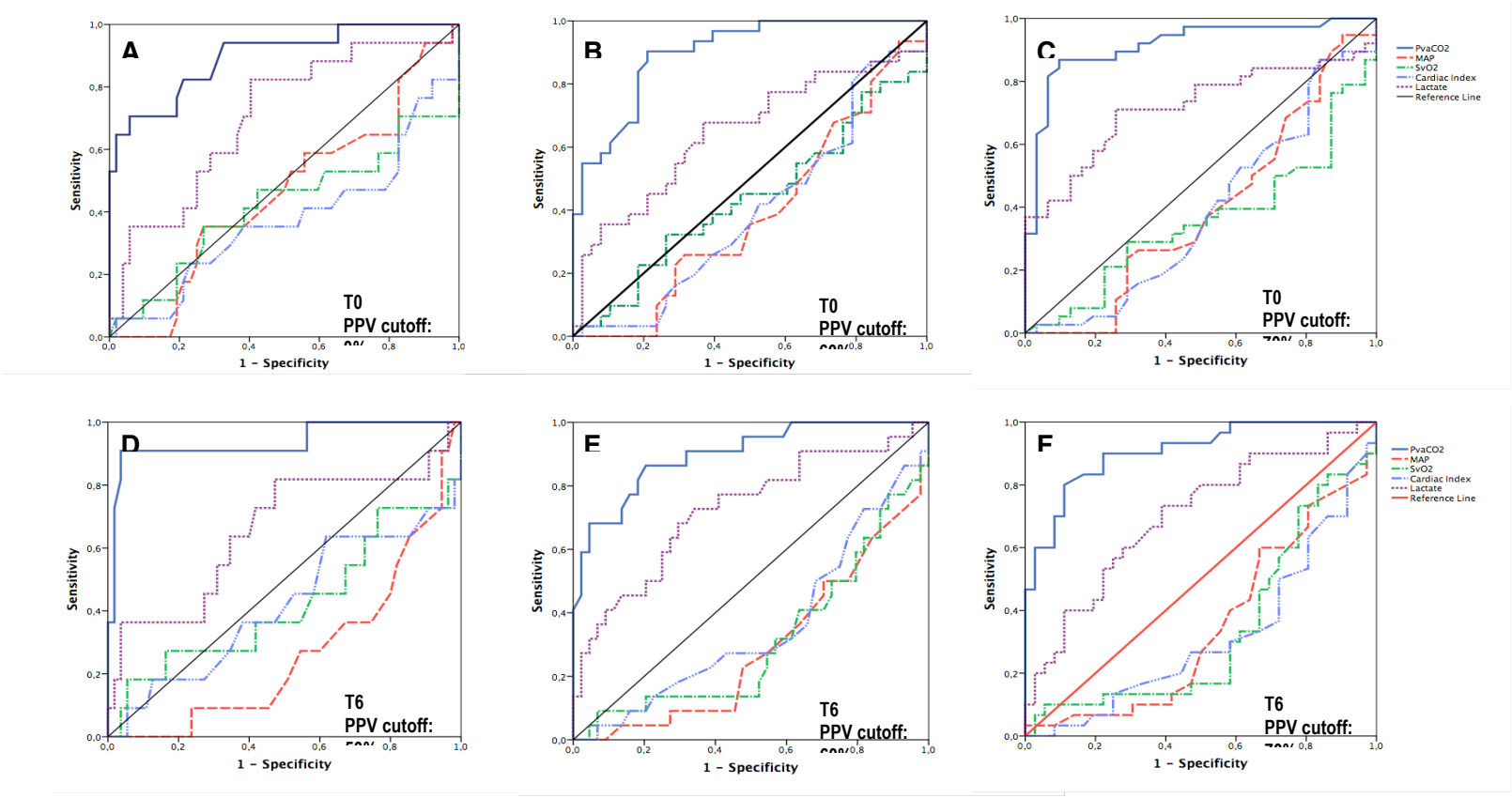
Suppl. Figure E11. SvO_2 for the predefined Pv-aCO_2 groups



Boxplots depicting SvO_2 differences for predefined Pv-aCO_2 groups at T0 (A) and T6 (B). Group 1: Pv-aCO_2 < 6.0; Group 2: Pv-aCO_2 6.0 - 9.9; Groups 3: Pv-aCO_2 > 10.0. Kruskal-Wallis test, p = 0.108 for T0 and p = 0.289 for T6.

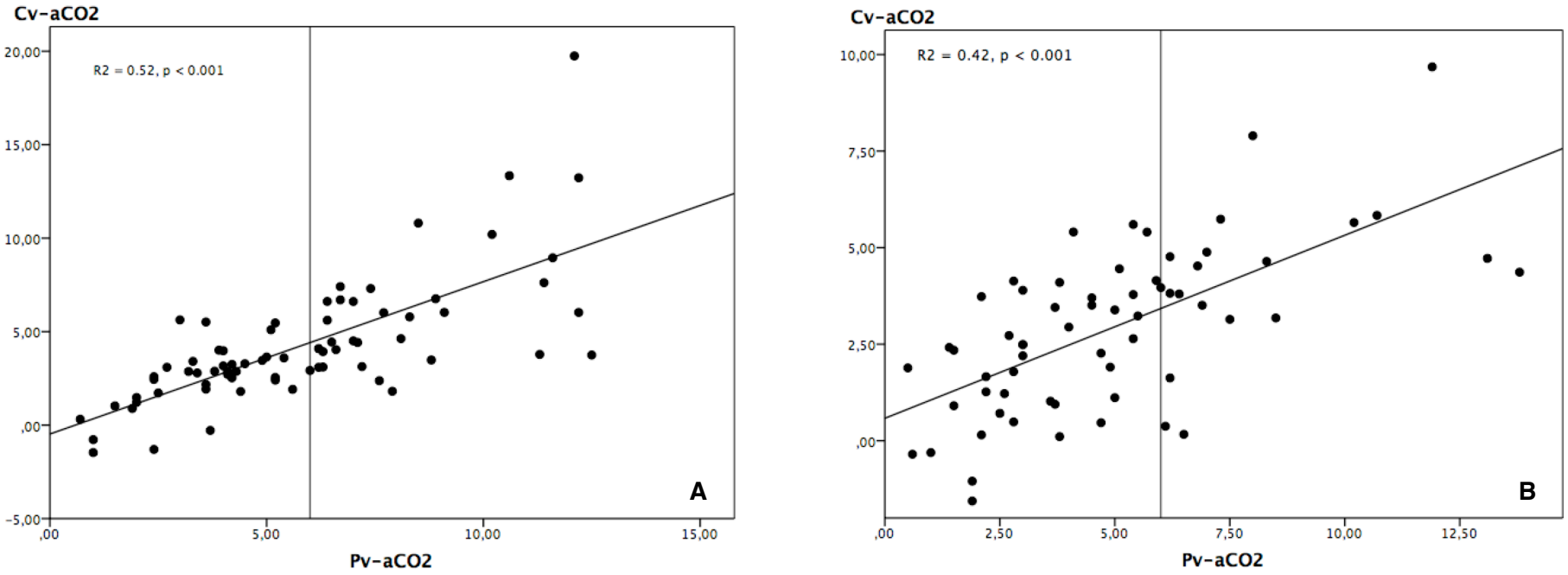
Boxes denote interquartile range, horizontal line in the boxes represents the median values, and whiskers extend 1.5 times the interquartile range above and below the 25 – 75 percentiles.
SvO_2: mixed-venous oxygen saturation; Pv-aCO_2: venous-to-arterial carbon dioxide difference

Suppl. Figure E12. Receiver operating characteristics (ROC) curve for predictors of percentage of small vessels perfused (PPV) at T0 and T6



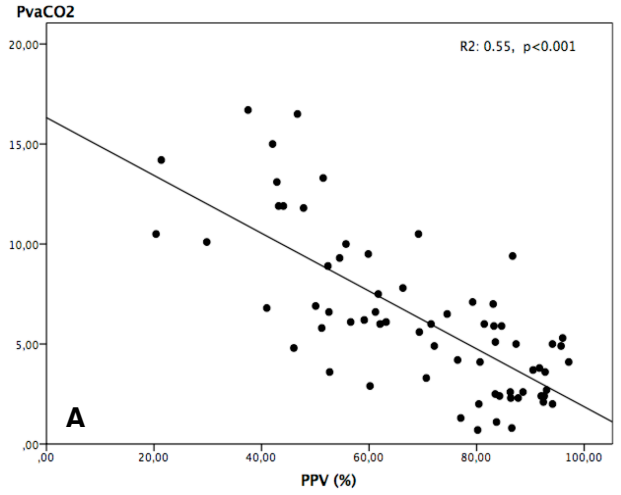
ROC curves at different cutoffs of PPV at T0: (A) PPV 50%; (B) PPV 60%; (C) PPV 70%; and at T6: (D) PPV 50%; (E) PPV 60%; (F) PPV 70%
Pv-aCO_2: venous-to-arterial carbon dioxide difference; MAP: mean arterial pressure; SvO_2: mixed-venous oxygen saturation; CI: cardiac index

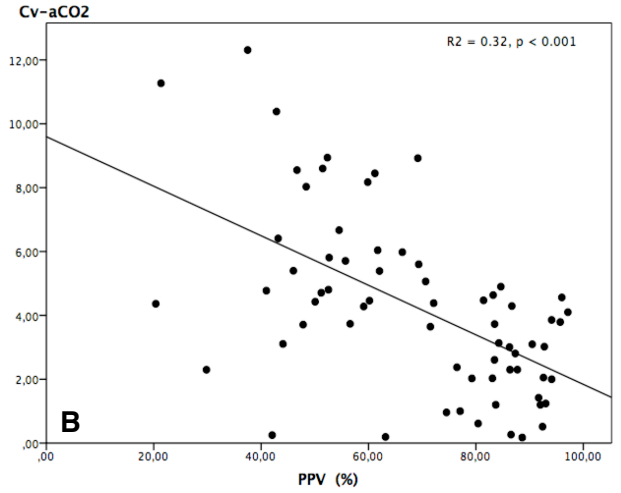
Suppl. Figure E13. Correlation between Pv-aCO_2 and Cv-aCO_2 at T0 and T6

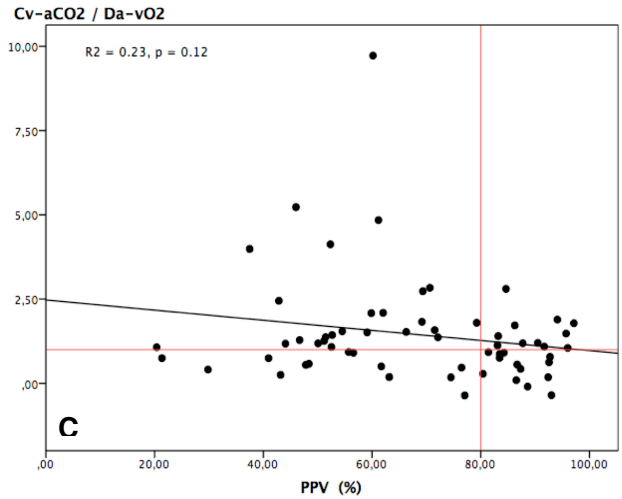


Scatter plot depicting the correlation between Pv-aCO_2 vs. Cv-aCO_2 at T0 (A) and T6 (B).
Coefficient of determination (R^2) was calculated to assess the strength of correlations.
Pv-aCO_2: venous-to-arterial carbon dioxide partial pressure difference; Cv-aCO_2: venous-to-arterial carbon dioxide content difference

Suppl. Figure E14. Relationships between Pv-aCO_2, Cv-aCO_2 and Cv-aCO_2/Da-vO_2 with the Percentage of small vessels perfused (PPV) at T6







Scatterplots depicting the relationships between the percentage of small vessels perfused (PPV) and (A) the venous-to-arterial CO_2 partial pressure difference (Pv-aCO_2), (B) the venous-to-arterial CO_2 content difference (Pv-aCO_2), and (C) the venous-to-arterial CO_2 to arterial-venous O_2 content difference ratio (Cv-aCO_2/Da-vO_2) at T6. In the panel C, vertical red line represents PPV 80%, while horizontal represents Cv-aCO_2/Da-vO_2 ratio = 1.
Coefficient of determination (R^2) was calculated to assess the strength of correlation

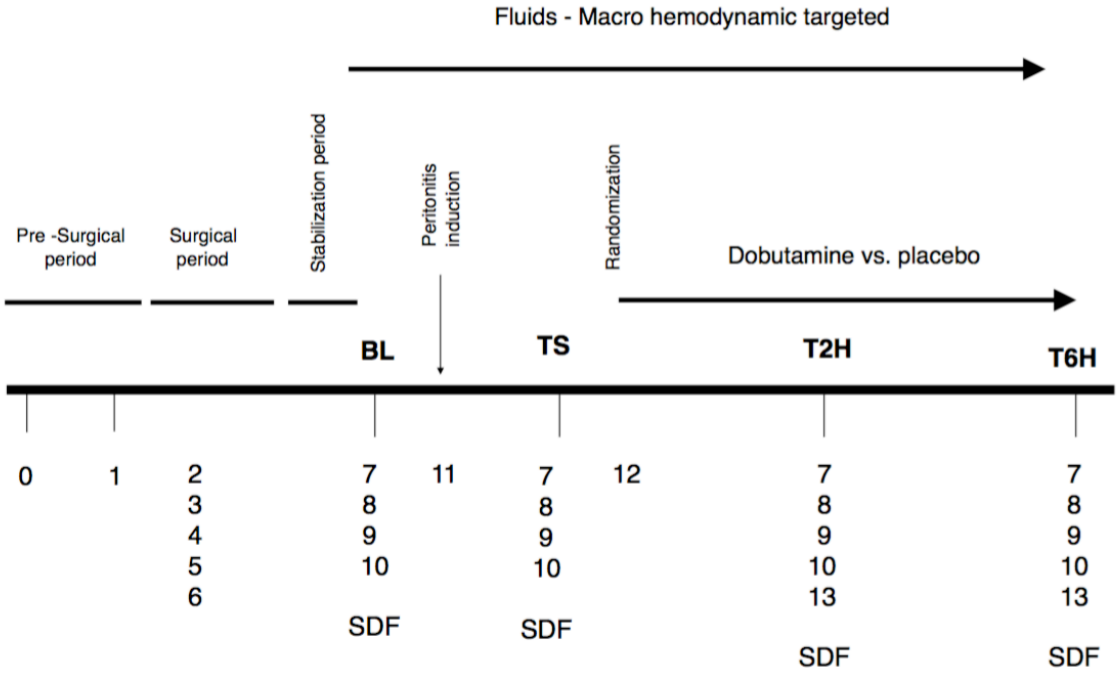
ANNEX E

INTESTINAL MICROCIRCULATORY IMAGES ACQUISITION AND ANALYSIS OF MICROVASCULAR BLOOD FLOW IN THE ANIMAL MODEL

We used a Sidestream dark-field (SDF) imaging device (Micro Scan; MicroVision Medical, Amsterdam, the Netherlands) to explore microcirculation at jejunal mucosa, jejunal serosa and sublingual mucosa at each point of measurements in both experimental and sham groups (Figures 1 and 2). The SDF device was softly applied to the jejunal mucosa and adjacent serosa after gentle washing with warm water (Figure 6). At each time-point of measurements, we five sequences of video of 30 seconds each from different adjacent mucosa and serosa areas using a videocard (MicroVideo; Pinnacle system, Mountain Views, CA, U.S.A.). These video-sequences were stored under a random number for ulterior analysis by two investigators blinded to the origin of sequences (E.Q and G.O.T.). For mucosal microcirculation analysis, the number of villi in each image were counted and individual villi microcirculation was semi-quantitatively classified according to its predominant blood flow as: (a) normal-perfused (continuous blood flow); (b) hypoperfused (intermittent or sluggish blood flow); or (c) non-perfused (stopped blood flow). Thus, we quantified the percentage of normal-perfused villi in each video-sequence at each time-point of measurement (Figure 7). Meanwhile, for serosa and sublingual microcirculation analysis, we used a cutoff value of 20 μm to classify the vessels as large or small (mostly, venules and capillaries respectively). Microvessels with continuous flow were considered as normal while sluggish, intermittent and stopped flows were considered as abnormal.

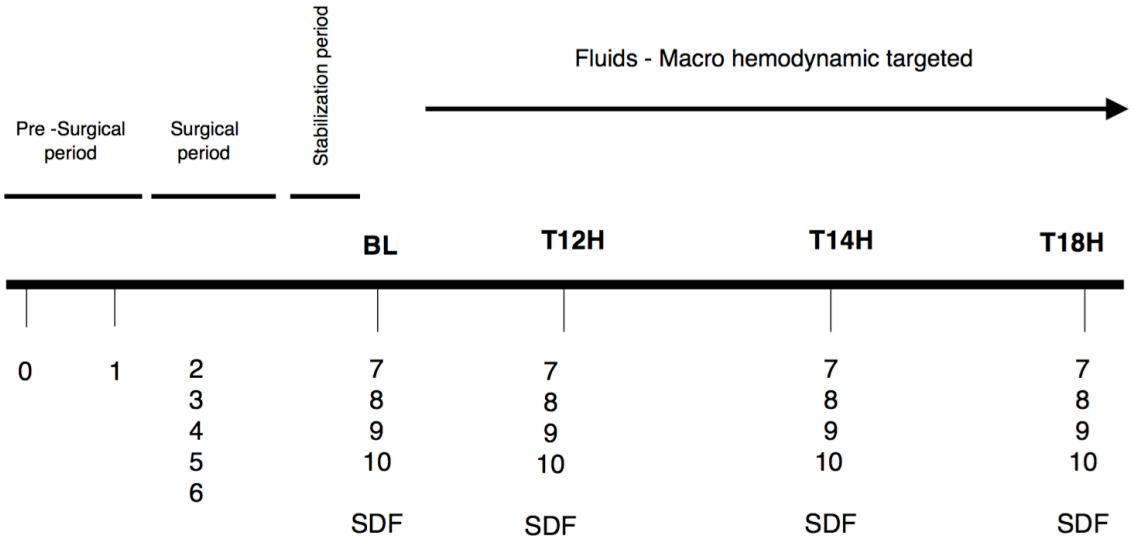
Serosa and sublingual microcirculatory blood flow were evaluated according to the consensus for the evaluation of microcirculation and we calculated the proportion of small-perfused vessels (< 20µm diameter), the heterogeneity index (HI), the total vascular density (all vessels) and the functional capillary density (vessels < 20µm diameter) (Figure 8). The intra and inter-observer variability were determined by using five sequences analyzed five times at eight-week intervals by two observers (E.Q. and G.O.T.). We calculated the intra-observer and inter-observer coefficient of variability for both total number of vessels and the proportion of perfused vessels.

Figure 1. Experimental protocol (dobutamine and placebo groups)



0. Acclimatization
1. Pharmacologic paralysis
2. Pharmacologic paralysis
3. Orotracheal Intubation
4. Muscle Relaxation
5. Carotid arterial / Pulmonary Artery catheter and Internal yugular vein cannulation
6. Laparotomy: superior mesenteric vein cannulation; jejunal ostomy preparation, jejunal tonometer insertion
7. Arterial blood sample: gases analysis and lactate
8. Mixed-venous blood sample: gases analysis and lactate
9. Mesenteric-venous blood sample: gases analysis and lactate
10. Jejunal tonometry measurements
11. Cecal ligation and perforation; fecal contamination
12. Randomization: dobutamine vs. placebo
13. Infusion: dobutamine vs. Placebo
14. Euthanasia
15. SDF video microscopy images: sublingual mucosa, jejunal mucosa and jejunal serosa.

Figure 2. Experimental protocol (sham group)



0. Acclimatization
1. Pharmacologic paralysis
2. Pharmacologic paralysis
3. Orotracheal Intubation
4. Muscle Relaxation
5. Carotid arterial / Pulmonary Artery catheter and Internal yugular vein cannulation
6. Laparotomy: superior mesenteric vein cannulation; jejunal ostomy preparation, jejunal tonometer insertion
7. Arterial blood sample: gases analysis and lactate
8. Mixed-venous blood sample: gases analysis and lactate
9. Mesenteric-venous blood sample: gases analysis and lactate
10. Jejunal tonometry measurements
11. Euthanasia
12. SDF video microscopy images: sublingual mucosa, jejunal mucosa and jejunal serosa.

Figure 3. Experimental surgery and critical care laboratory. Universidad ICESI, Cali. Colombia



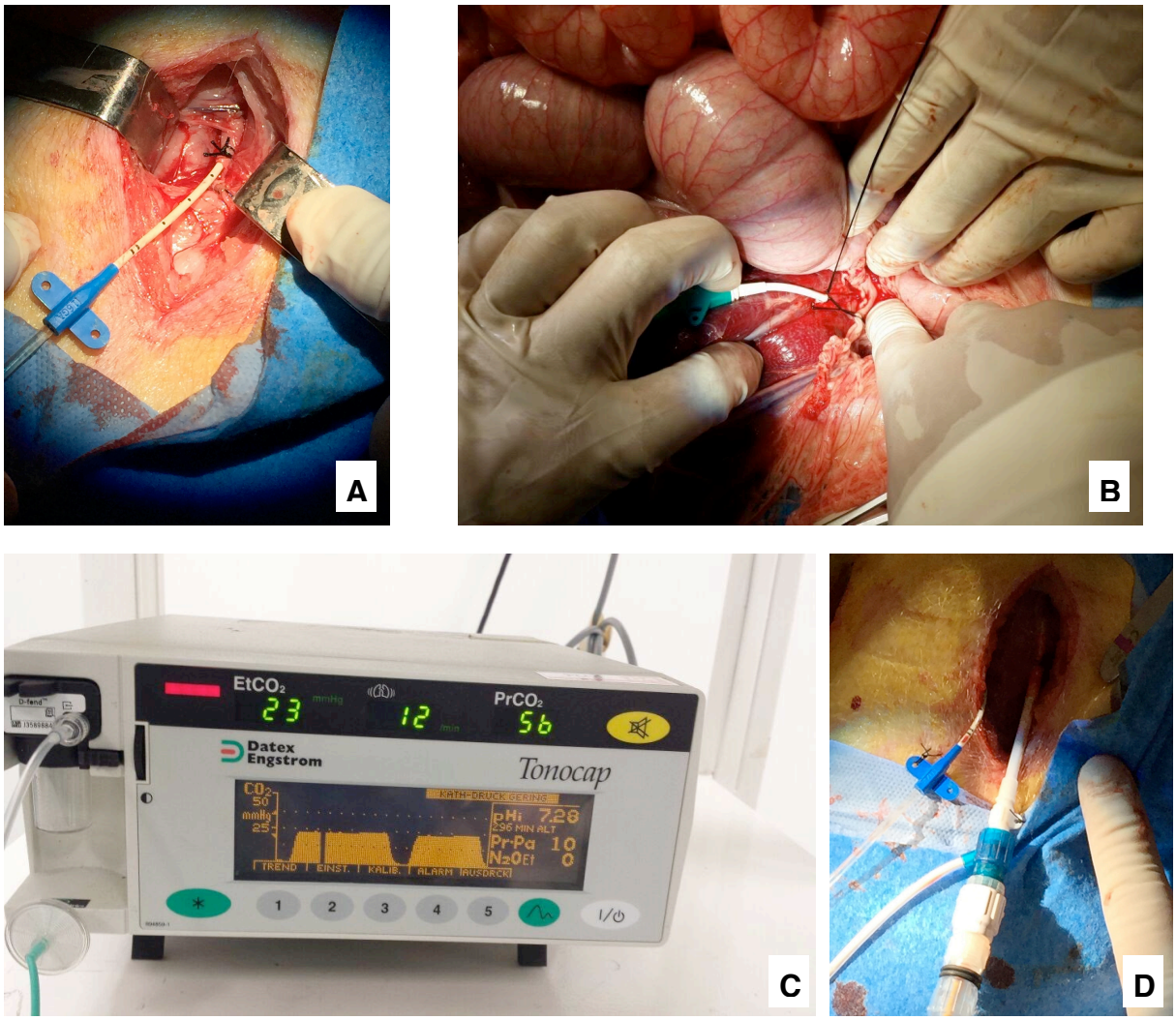


Figure 4. Preparation and surgical period



Panel A. Animal preparation – pre surgical period. Superficial-venous catheter insertion. **Panel B and C.** Surgical preparation. **Panel D.** Ending surgical preparation. Complete hemodynamic monitoring and mechanical ventilation. (Experimental surgery and critical care laboratory. Universidad ICESI, Cali. Colombia)

Figure 5. General macro hemodynamic monitoring



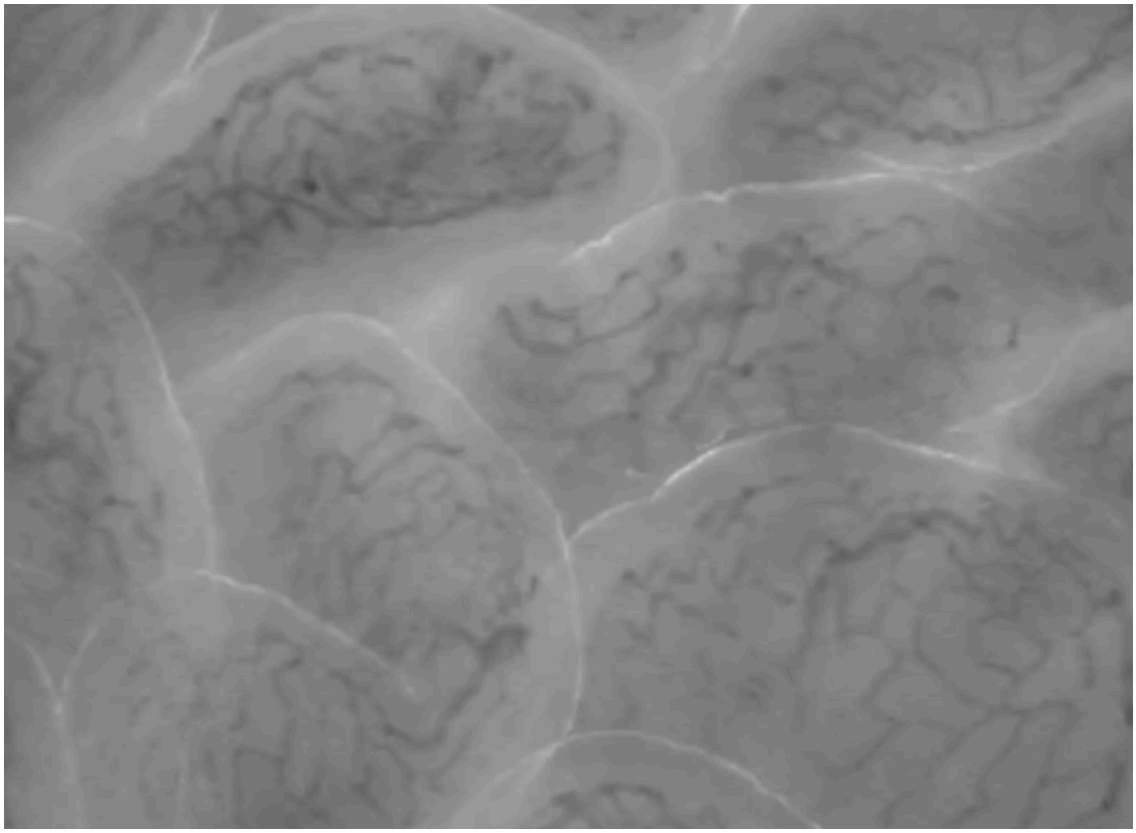
Panel A. Carotid arterial catheter insertion; **Panel B.** Mesenteric venous catheter insertion; **Panel C.** Gas-tonometer and end-tidal CO_2 monitoring; **Panel D.** Pulmonary artery catheter insertion (Experimental surgery and critical care laboratory. Universidad ICESI, Cali. Colombia)

Figure 6. Intestinal microcirculation images acquisition



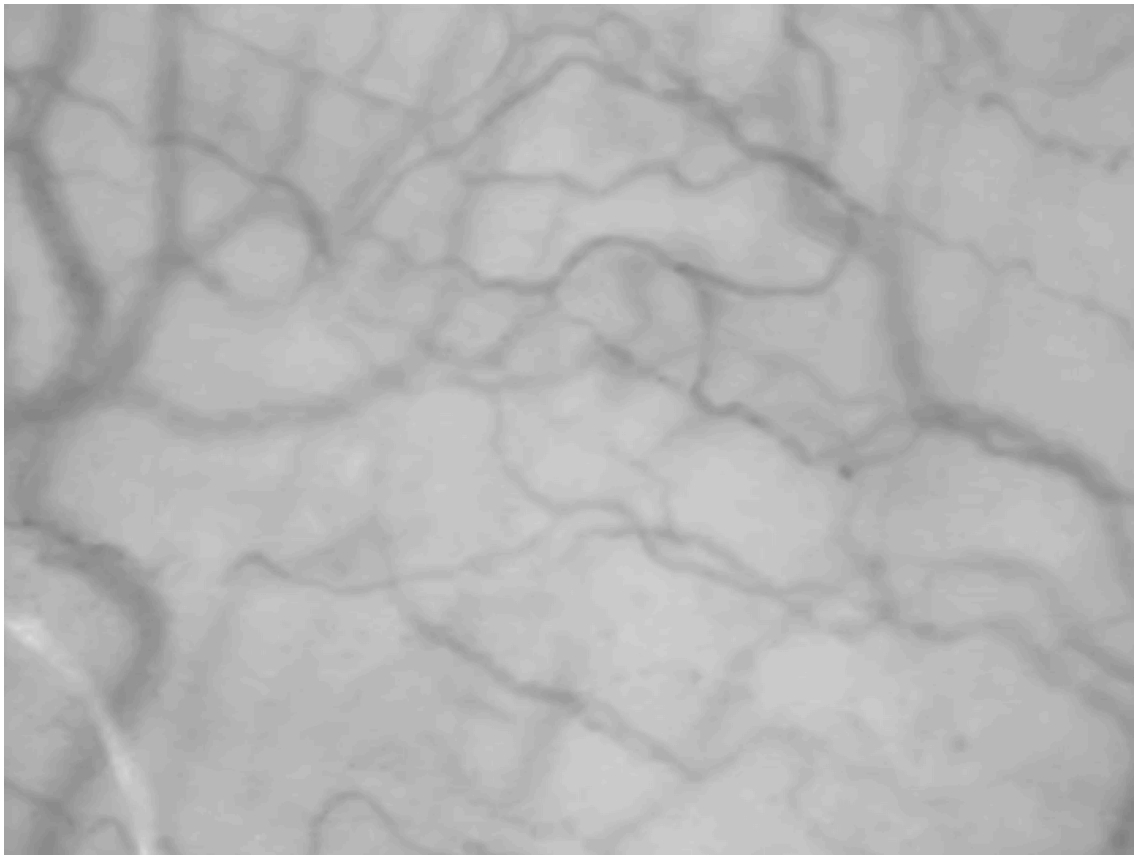
Jejunal mucosa and serosa images were obtained through a Side-stream dark field (SDF) device at baseline (BL), time of shock (TS), and 2 (T2H) and 6 hours (T6H) after randomization to dobutamine or placebo. (Experimental surgery and critical care laboratory. Universidad ICESI, Cali. Colombia)

Figure 7. Jejunal mucosa microcirculation image captured through Side-stream dark field technique



The number of villi in each image were counted and individual villi microcirculation was semi-quantitatively classified according to its predominant blood flow as: (a) normal-perfused (continuous blood flow); (b) hypoperfused (intermittent or sluggish blood flow); or (c) non-perfused (stopped blood flow). Thus, the percentage of normal-perfused villi in each video-sequence was calculated (%villi-PPV). (Experimental surgery and critical care laboratory. Universidad ICESI, Cali. Colombia)

Figure 8. Jejunal serosa microcirculation image captured through Side-stream dark field technique



Vessels are classified into large (mostly venules) and small (mostly capillaries) vessels using a cut-off value of 20 μm in diameter. Vessel perfusion (total, large and small) is defined as the proportion of perfused vessels, calculated as the number of vessels continuously perfused during the 20-second observation period divided by the total number of vessels of the same type. The proportion of small vessels perfused (PPV) corresponds to the proportion of well-perfused vessels with a diameter < 20 μm (mainly capillaries). The microvascular blood flow heterogeneity index (HI) is defined as the difference between maximal and minimal proportions of PPV evaluated at each point in the five areas divided by its own mean value. (Experimental surgery and critical care laboratory. Universidad ICESI, Cali. Colombia)

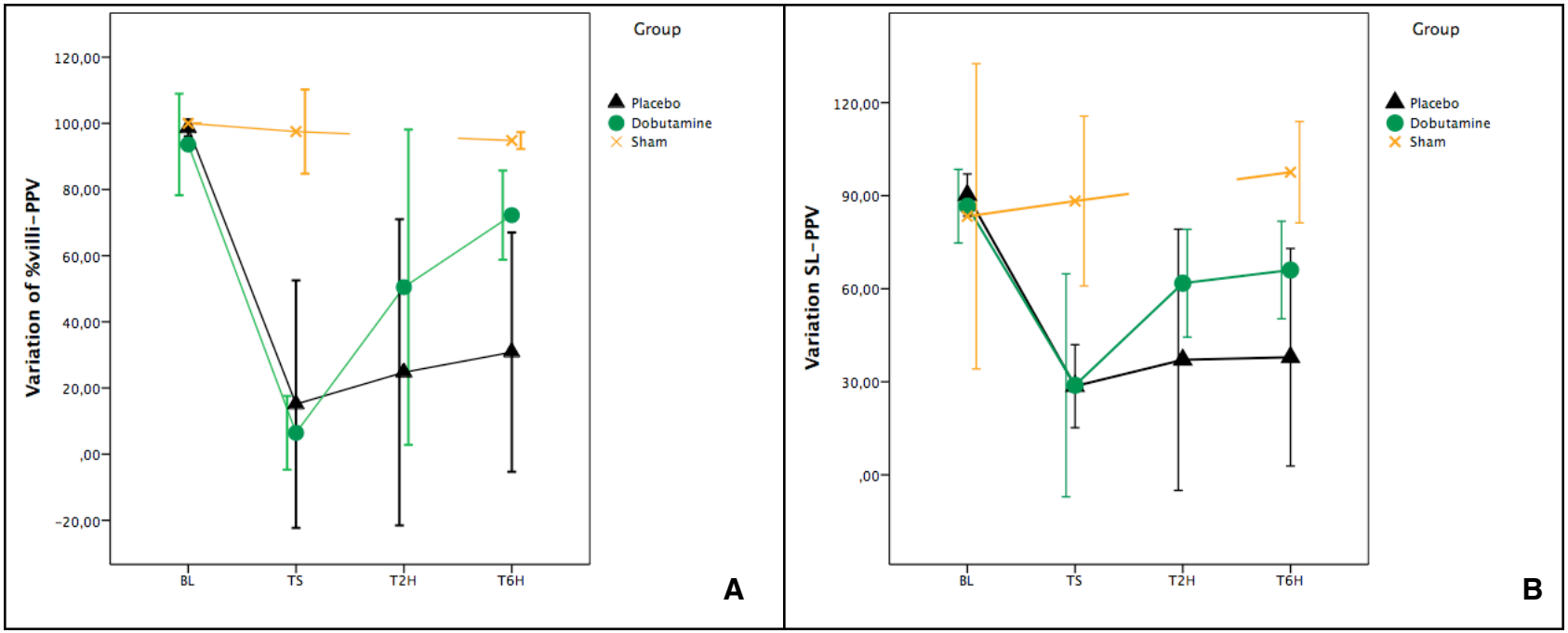
ANNEX F

EFFECTS OF DOBUTAMINE ON INTESTINAL MICROVASCULAR BLOOD FLOW HETEROGENEITY AND OXYGEN EXTRACTION IN A HYPODYNAMIC SEPTIC SHOCK MODEL

(Submitted)

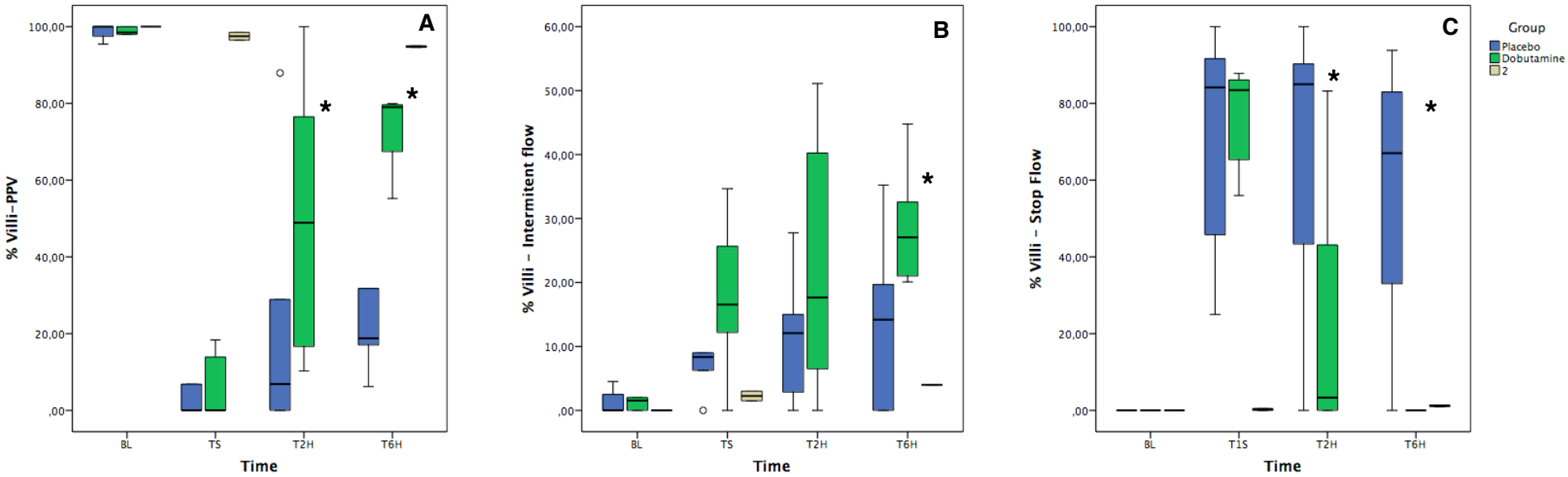
Supplementary Material

Suppl. Figure 1. Time-course of variations in %villi-PPV and SL-PPV



Panel A. %Villi-PPV (percentage of villi with predominantly well-perfused small vessels): Repeated measurements – Friedman analysis, p=0.006, for time*group interactions
Panel B. SL-PPV (percentage of small vessels perfused at sublingual mucosa): Repeated measures – Friedman test, p<0.001 for time effect; p=0.12 for time*group interaction.

Suppl. Figure 2. Time-course of %Villi-PPV, %Villi with intermittent and stopped flow

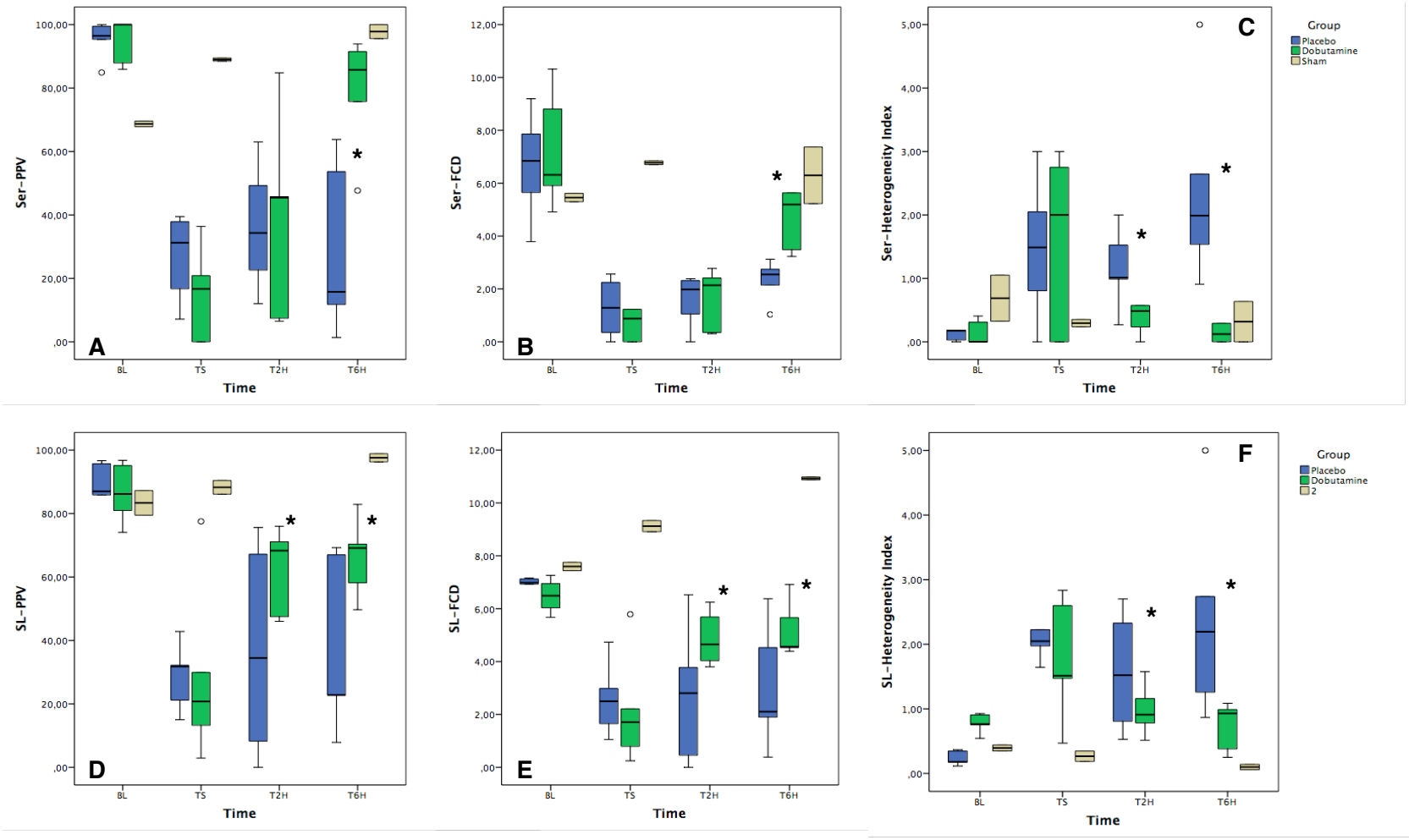


Panel A. %Villi-PPV: percentage of villi with predominantly well-perfused small vessels; **Panel B.** % villi – Intermittent Flow: percentage of villi with predominantly intermittent flow; **Panel C.** % villi – Intermittent Flow: percentage of villi with predominantly stopped flow.

Panel A, B, and C: Repeated measurements – Friedman analysis, p=0.006, P=0.15 and p=0.007 for time*group interactions respectively

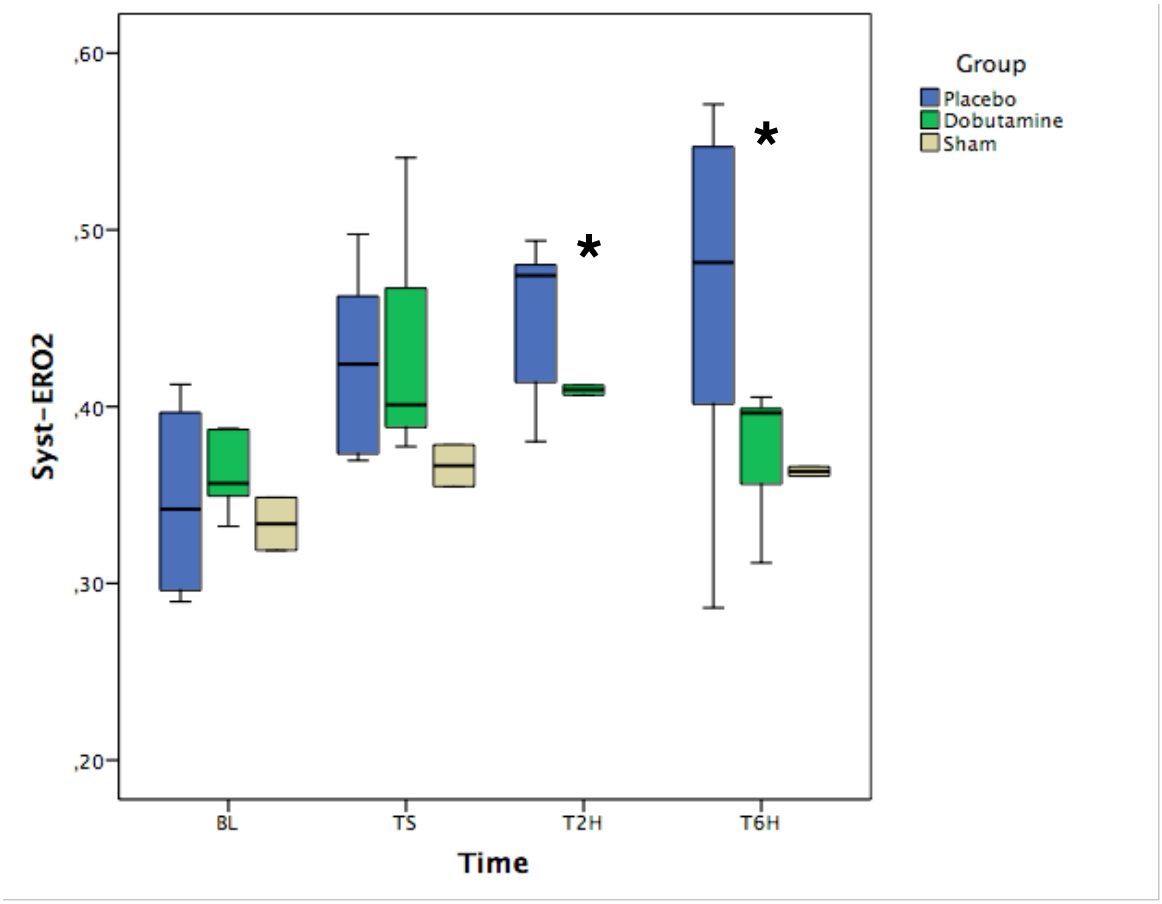
* p<0.05, between dobutamine vs. placebo groups

Suppl. Figure 3. Time course of microcirculatory blood flow at jejunal villi



Panel A. Ser-PPV: percentage of small vessels perfused at jejunal serosa. Repeated measures – Friedman test, p=0.016 for time effect; p=0.07 for time*group interaction.
Panel B. Ser-FCD: functional capillary density at jejunal serosa. Repeated measures – Friedman test, p<0.001 for time effect; p=0.12 for time*group interaction.
Panel C. Ser-Heterogeneity Index (Ser-HI): heterogeneity index at jejunal serosa. Repeated measures – Friedman test, p=0.05 for time effect; p=0.04 for time*group interaction.
Panel D. SL-PPV: percentage of small vessels perfused at sublingual mucosa. Repeated measures – Friedman test, p<0.001 for time effect; p=0.12 for time*group interaction.
Panel E. SL-FCD: functional capillary density at sublingual mucosa. Repeated measures – Friedman test, p=0.04 for time effect; p=0.07 for time*group interaction.
Panel F. SL- Heterogeneity Index (SL-HI): heterogeneity index at sublingual mucosa. Repeated measures – Friedman test, p=0.08 for time effect; p=0.01 for time*group interaction.
***** $p < 0.05$ for placebo vs. dobutamine group (S.N.K test)

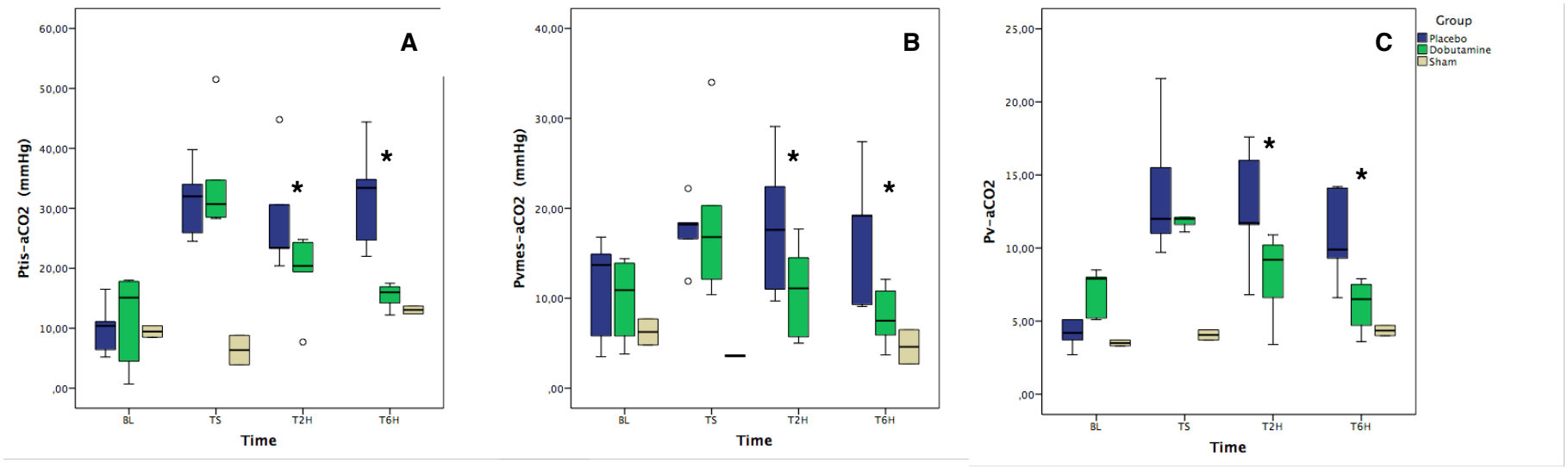
Suppl. Figure 4. Time-course of systemic ERO_2



Syst-ERO_2: systemic oxygen extraction ratio. Repeated measures – Friedman test, p=0.06 for time effect; p=0.07 for time*group interaction.

***** p<0.05 for placebo vs. dobutamine group

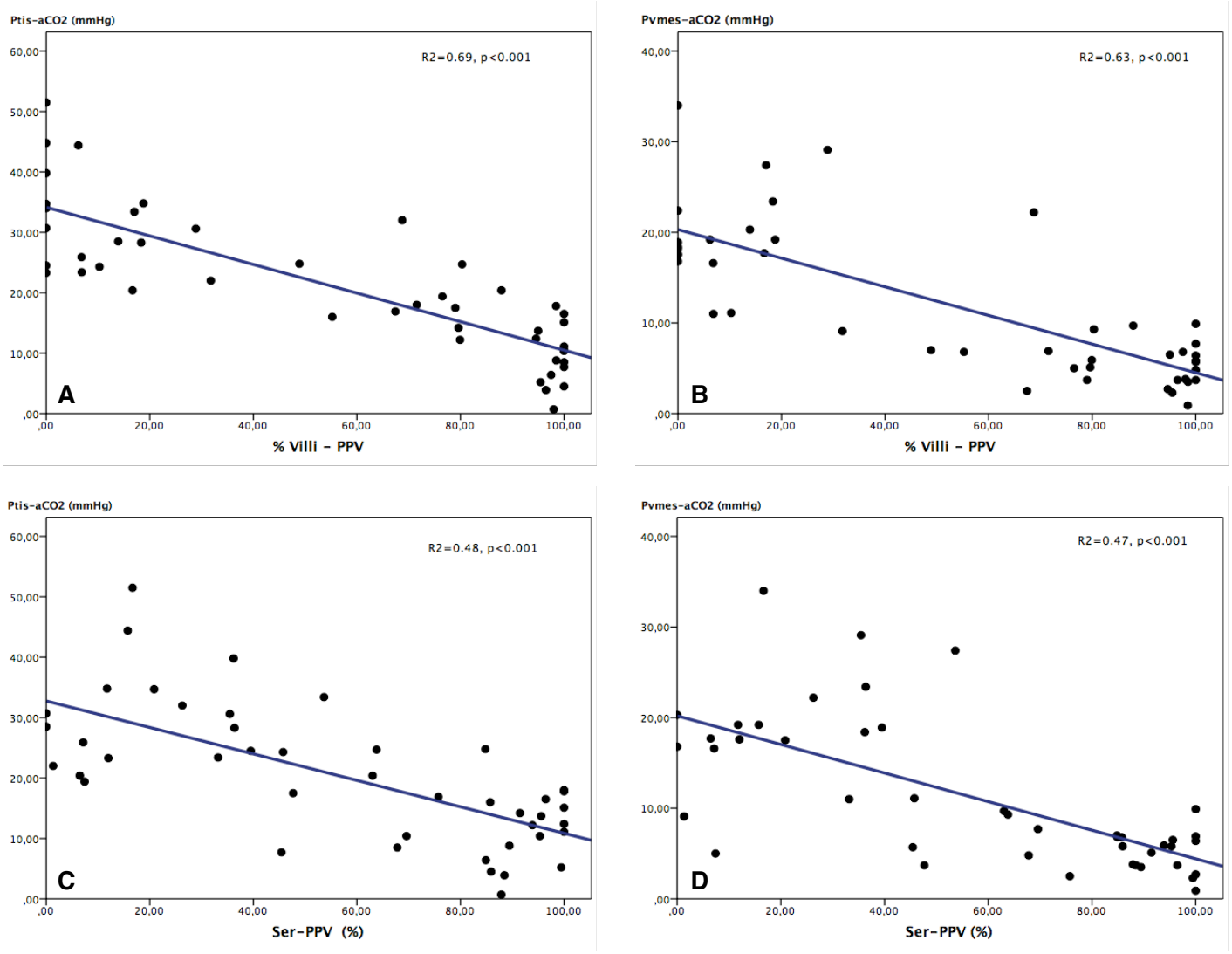
Suppl. Figure 5. Time-course of Ptis-aCO_2, Pmes-aCO_2, and Pv-aCO_2 for placebo, dobutamine and sham groups.



Panel A. Ptis-aCO_2: tissue (jejunal mucosa)-to-arterial carbon dioxide difference. Repeated measures – Friedman test, p=0.01 for time effect; p=0.01 for time*group interaction.
Panel B. Pvmes-aCO_2: mesenteric venous-to-arterial carbon dioxide difference. Repeated measures – Friedman test, p<0.001 for time effect; p=0.001 for time*group interaction.
Panel C. Pv-aCO_2: mixed venous-to-arterial carbon dioxide difference. Repeated measures – Friedman test, p=0.04 for time effect; p=0.02 for time*group interaction.

* p<0.05 for placebo vs. dobutamine group

Suppl. Figure 6. Relationships between %villi-PPV and Ser-PPV vs. Ptis-aCO_2 and Pmes-aCO_2



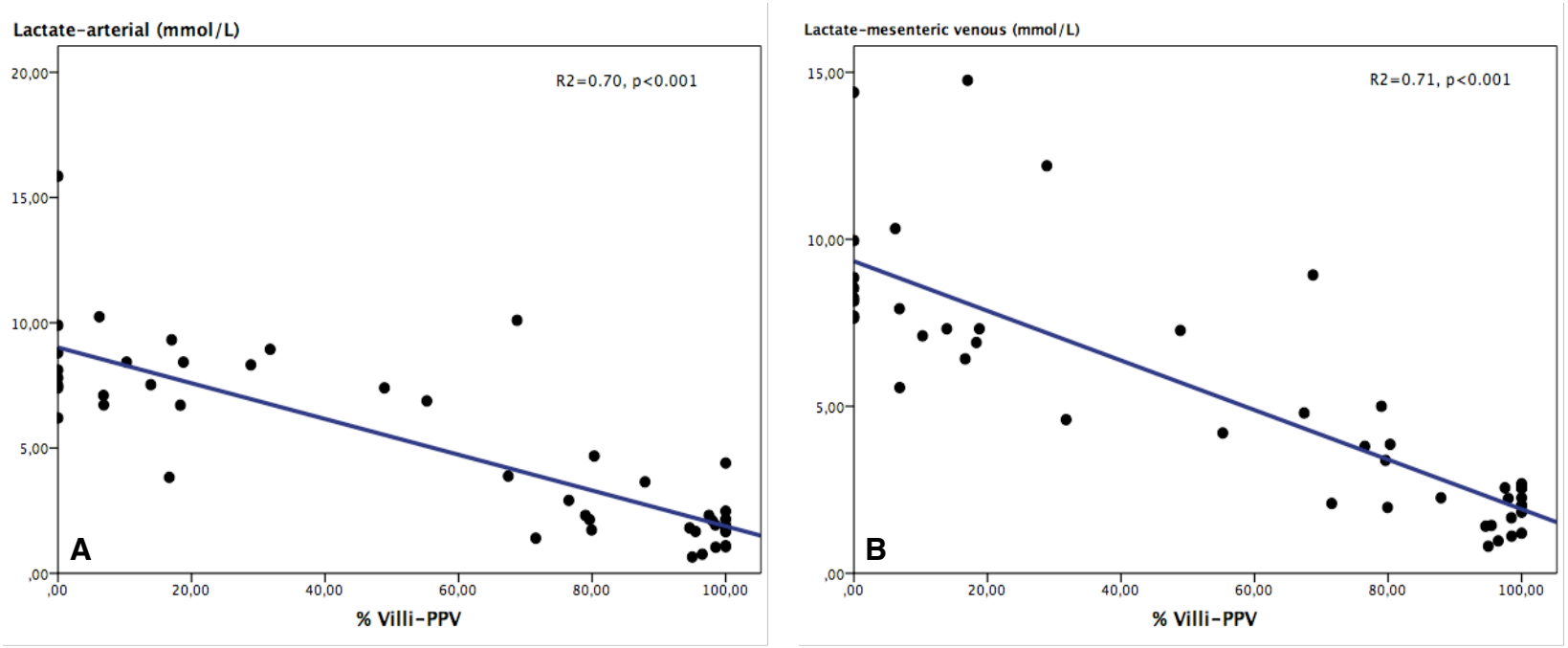
Panel A. Scatterplot for %Villi-PPV (percentage of villi with predominantly well-perfused small vessels) vs. Ptis-aCO_2 (tissue [jejunal mucosa]-to-arterial carbon dioxide difference). Spearman-Rho test, R^2=0.69, p<0.001

Panel B. Scatterplot for %Villi-PPV (percentage of villi with predominantly well-perfused small vessels) vs. Pvmes-aCO_2 (mesenteric venous-to-arterial carbon dioxide difference). Spearman-Rho test, R^2=0.63, p<0.001

Panel C. Scatterplot for Ser-PPV (percentage of small vessels perfused at jejunal serosa) vs. Ptis-aCO_2 (tissue [jejunal mucosa]-to-arterial carbon dioxide difference). Spearman-Rho test, R^2=0.69, p<0.001

Panel D. Scatterplot for Ser-PPV (percentage of small vessels perfused at jejunal serosa) vs. Pvmes-aCO_2 (mesenteric venous-to-arterial carbon dioxide difference). Spearman-Rho test, R^2=0.63, p<0.001

Suppl. Figure 7. Relationships between % Villi-PPV and lactate (arterial and mesenteric venous)



Panel A. Scatterplot for %Villi-PPV (percentage of villi with predominantly well-perfused small vessels) vs. arterial lactate. Spearman-Rho test, R^2=0.70, p<0.001

Panel B. Scatterplot for %Villi-PPV (percentage of villi with predominantly well-perfused small vessels) vs. mesenteric-venous lactate. Spearman-Rho test, R^2=0.71, p<0.001

ANNEX G

ACCEPTED PAPERS

PAPER No. 1 (COMMENTARY)

SHOULD MICROCIRCULATION MONITORING BE USED TO GUIDE FLUID RESUSCITATION IN SEVERE SEPSIS AND SEPTIC SHOCK?

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Should microcirculation monitoring be used to guide fluid resuscitation in severe sepsis and septic shock?

A ressuscitação volêmica na sepse grave e choque séptico deve ser guiada pela microcirculação?

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2. Universidad ICESI - Cali, Colombia.

Conflicts of interest: None.

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Tissue hypoperfusion and subsequent limited oxygen transport are critical features conducting to organ failure during shock states. Therefore, early identification of tissue hypoperfusion and adequate resuscitation are key for improving the probability of survival after septic shock.[1,2] However, how to identify organ perfusion abnormalities at the bedside and select the type and amount of fluids required to improve tissue hypoxia remain highly controversial. Traditionally, clinical signs, such as reduced blood pressure and urinary output, altered consciousness, and mottled skin, have been used to identify tissue perfusion abnormalities. Consequently, current hemodynamic monitoring during shock states mainly focuses on detection of pressure-derived hemodynamic variables related to systemic circulation. However, it has been largely recognized that monitoring these macro-hemodynamic variables is not sufficient to rule out persistent abnormalities of tissue oxygenation. Indeed, the usefulness of resuscitation targets, such as global oxygen-derived parameters, has been strongly questioned,[3] and recent data have failed to demonstrate beneficial effects of using central venous oxygen saturation as a goal of resuscitation.[4-6]

Fluid resuscitation therapy primarily aims to optimize cardiac output on the assumption that increasing macro blood flow can improve the convective transport of oxygen to the tissues and, therefore, maintain cellular respiration and support organ function.[7,8] Thus, fluid therapy targeting central venous pressure has been widely recommended to achieve adequate cardiac performance.[9] However, high positive fluid balances have also been associated with unfavorable clinical outcomes.[10] In this sense, dynamic approaches to assessing volume responsiveness to fluid administration seem to be superior to static variables.[11,12] Unfortunately, macro-hemodynamic optimization guided by either dynamic or static variables does not guarantee adequate tissue perfusion or adequate cellular respiration.

Oxygen transport to tissues is governed by convective and diffusive components. The convective component is determined by the microcirculatory blood flow itself, i.e., the number of red blood cells (RBCs) entering the microcirculation, and by oxygen content. In normal conditions, inflow and outflow pressures control the driving pressure at the microvascular level. Thus, the convective transport is regulated upstream at the arteriolar level through microcirculatory inflow changes with subsequent micro-hematocrit

modifications and is limited downstream by venous pressure. Meanwhile, the diffusive component of oxygen transport is determined by the gradient between the capillary and mitochondrial oxygen partial pressures, the diffusional distance and the area available for gas exchange, according to Fick´s law.

Unfortunately, current resuscitation procedures are based on the assumption that defects in oxygen transport arise from a lack of perfusion. Thus, resuscitation efforts are mostly focused on the promotion of convective flow based on the assumption that hypovolemia is the main limiting factor of blood flow. However, a substantial contribution of oxygen tissue transport is determined by the capacity of diffusion of oxygen from RBCs to the cells, and this can be quantified by functional capillary density (FCD), i.e., the density of capillaries with flowing RBCs carrying oxygen. In normal conditions, the microvascular blood flow is carefully matched to the metabolic demands of tissues. However, septic shock is characterized by decreased FCD in addition to increased heterogeneity of blood flow, with zones of well-perfused vessels in close proximity to non-perfused capillaries.[13] The persistence of such alterations has been shown to be related to multiorgan dysfunction, even when global hemodynamics appear to be optimal.[14] Indeed, increases in cardiac output may be insufficient to correct tissue hypoxemia because microcirculation alterations might persist. Thus, global hemodynamic targets should be integrated with functional microcirculatory goals, as this would optimize both convective and diffusive components in order to maximize oxygen delivery to cells. Microcirculatory responsiveness should be defined as an increase in convective microvascular flow in response to fluid load in addition to a reduction of the heterogeneity of flow, resulting in a more balanced distribution of oxygen to the tissues (Figure 1). Nevertheless, efforts to increase blood flow by fluid administration could be counterbalanced by a decrease in FCD resulting in a limited oxygen diffusive capacity (Figure 2). Thus, excessive fluid administration could increase the distance between capillaries, reducing oxygen diffusion to the tissues and finally to the mitochondria.

Orthogonal polarization spectral and sidestream dark field imaging techniques have helped us gain a better understanding of microcirculatory derangements during severe sepsis and septic shock at the bedside. In fact, deeper microvascular alterations, such as a reduced percentage of small perfused vessels (PPV), decreased FCD and increased heterogeneity of flow, have been shown to be related to more severe organ dysfunctions and unfavorable outcomes.[13,15,16] Interestingly, reductions in FCD in sepsis are completely explained by decreased PPV, while RBC velocities are similar in both survivors and non-survivors.[16] These findings suggest that variables that describe the diffusional component of oxygen transport, i.e., FCD and heterogeneity of microvascular blood flow, are more closely related to clinical outcomes than pure convective components, such as RBC velocity.[16] Remarkably, these derangements are amenable to correction over time and are dissociated from global hemodynamics. Consequently, micro-hemodynamics cannot be predicted by the typical systemic hemodynamic parameters, although more severe microcirculatory alterations coexist with high lactate levels and the requirement of higher doses of vasopressors.[17]

In a recent study, Ospina-Tascón et al.[18] explored the effect of fluids on microcirculatory blood flow using the sidestream dark field imaging technique in severe sepsis and septic shock. They found that early, but not late fluid challenge can increase the PPV, with subsequent improvement in FCD and decreased heterogeneity of blood flow. Analogous to macro-hemodynamic fluid responsiveness predictors, the prior status of the microcirculation is strongly related to the response to volume expansion.[18,19] Indeed, a recent study[20] demonstrated that the effects of fluid load on the microcirculation are dependent on both basal microvascular perfusion and the magnitude of the increase in cardiac output.

Theoretically, direct evaluation of microcirculation at the bedside would be a more physiological-based approach to fluid administration that may predict fluid responsiveness at the microvascular level and avoid unnecessary and unfavorable fluid overload. However, current data regarding the use of devices to evaluate microcirculation at the bedside only support pathophysiological concepts and require confirmation through clinical trials.

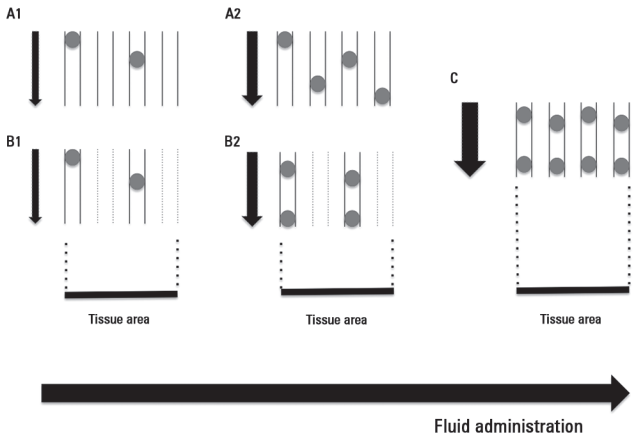


Figure 1 - Effects of adequate fluid administration on microvascular blood flow. Progressive increasing of convective flow after fluid loading during microcirculatory conditions of pure convective flow derangements (A1, A2) and combined with increased heterogeneity of blood flow (B1, B2). An optimal fluid administration corrects convective and heterogeneity blood flow disturbances (C). Note that the number of vessels with adequate flow per tissue area represents the functional capillary density (FCD), i.e., the major determinant of the diffusive component of oxygen transport to tissues. Black arrows pointing down represent the magnitude of the blood flow.

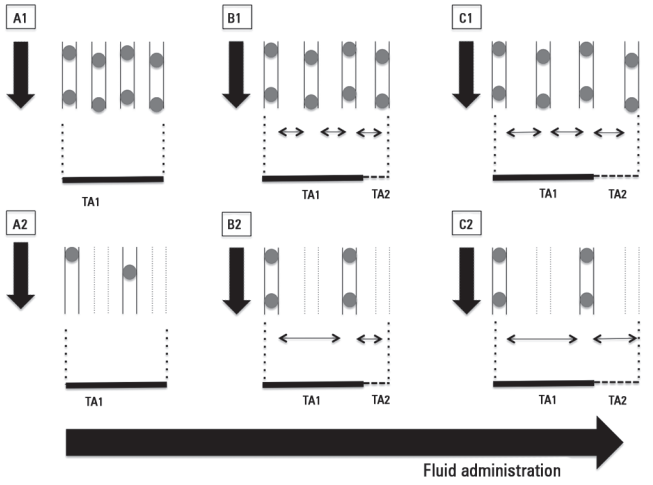


Figure 2 - Effects of fluid overload on microvascular blood flow. Inadequate fluid overload conducts to progressive decrease in functional capillary density in cases of homogeneous (A1, B1, C1) or heterogeneous (A2, B2, C2) microcirculatory blood flow. Progressive increase in distance between capillaries impairs oxygen diffusive capacities to tissues despite apparently normal convective flow (A1, B1, C1) or in cases of apparent corrected convective component with persistence of increased heterogeneity (B2, C2). TA1 depicts the original tissue area. TA2 depicts the increased tissue area due to edema. Black arrows pointing down represent the magnitude of blood flow.

REFERENCES

1. Vincent JL, De Backer D. Circulatory shock. N Engl J Med. 2013; 369(18):1726-34.
2. Cecconi M, De Backer D, Antonelli M, Beale R, Bakker J, Hofer C, et al. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. Intensive Care Med. 2014;40(12):1795-815.
3. Bellomo R, Reade MC, Warrillow SJ. The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. Crit Care. 2008;12(2):130.
4. ProCESS Investigators, Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, et al. A randomized trial of protocol-based care for early septic shock. N Engl J Med. 2014;370(18):1683-93.
5. Peake SL, Bailey M, Bellomo R, Cameron PA, Cross A, Delaney A, Finfer S, Higgins A, Jones DA, Myburgh JA, Syres GA, Webb SA, Williams P; ARISE Investigators, for the Australian and New Zealand Intensive Care Society Clinical Trials Group. Australasian resuscitation of sepsis evaluation (ARISE): A multi-centre, prospective, inception cohort study. Resuscitation. 2009;80(7):811-8.
6. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, Jahan R, Harvey SE, Bell D, Bion JF, Coats TJ, Singer M, Young JD, Rowan KM; ProMISe Trial Investigators. Trial of early, goal-directed resuscitation for septic shock. N Engl J Med. 2015;372(14):1301-11.
7. Santry HP, Alam HB. Fluid resuscitation: past, present, and the future. Shock. 2010;33(3):229-41. Review.
8. Vincent JL, Weil MH. Fluid challenge revisited. Crit Care Med. 2006;34(5):1333-7.
9. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R; Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med. 2013;39(2):165-228.
10. Boyd JH, Forbes J, Nakada TA, Walley KR, Russell JA. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. Crit Care Med. 2011;39(2):259-65.
11. Pinsky MR, Payen D. Functional hemodynamic monitoring. Crit Care. 2005;9(6):566-72.
12. Teboul JL, Monnet X. Detecting volume responsiveness and unresponsiveness in intensive care unit patients: two different problems, only one solution. Crit Care. 2009;13(4):175.
13. De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent JL. Monitoring the microcirculation in the critically ill patient: current methods and future approaches. Intensive Care Med. 2010;36(11):1813-25.
14. Doerschug KC, Delsing AS, Schmidt GA, Haynes WG. Impairments in microvascular reactivity are related to organ failure in human sepsis. Am J Physiol Heart Circ Physiol. 2007;293(2):H1065-71.
15. Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, Arnold RC, Colilla S, Zanotti S, Hollenberg SM; Microcirculatory Alterations in Resuscitation and Shock Investigators. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med. 2007;49(1):88-98, 98.e1-2.

16. Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. Crit Care Med. 2012;40(5):1443-8.
17. Hernandez G, Boerma EC, Dubin A, Bruhn A, Koopmans M, Edul VK, et al. Severe abnormalities in microvascular perfused vessel density are associated to organ dysfunctions and mortality and can be predicted by hyperlactatemia and norepinephrine requirements in septic shock patients. J Crit Care. 2013;28(4):538.e9-14.
18. Ospina-Tascon G, Neves AP, Occhipinti G, Donadello K, Büchele G, Simion D, et al. Effects of fluids on microvascular perfusion in patients with severe sepsis. Intensive Care Med. 2010;36(6):949-55.
19. Pranskunas A, Koopmans M, Koetsier PM, Pilvinis V, Boerma EC. Microcirculatory blood flow as a tool to select ICU patients eligible for fluid therapy. Intensive Care Med. 2013;39(4):612-9.
20. Edul VS, Ince C, Navarro N, Previgliano L, Risso-Vazquez A, Rubatto PN, et al. Dissociation between sublingual and gut microcirculation in the response to a fluid challenge in postoperative patients with abdominal sepsis. Ann Intensive Care. 2014;4:39.

PAPER No. 2 (Original paper)

PERSISTENTLY HIGH VENOUS-TO-ARTERIAL CARBON DIOXIDE DIFFERENCES DURING EARLY RESUSCITATION ARE ASSOCIATED WITH POOR OUTCOMES IN SEPTIC SHOCK

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CRITICAL CARE

RESEARCH Open Access

Persistently high venous-to-arterial carbon dioxide differences during early resuscitation are associated with poor outcomes in septic shock

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Abstract

Introduction: Venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) may reflect the adequacy of blood flow during shock states. We sought to test whether the development of $Pv\text{-}aCO_2$ during the very early phases of resuscitation is related to multi-organ dysfunction and outcomes in a population of septic shock patients resuscitated targeting the usual oxygen-derived and hemodynamic parameters.

Methods: We conducted a prospective observational study in a 60-bed mixed ICU in a University affiliated Hospital. 85 patients with a new septic shock episode were included. A Pv-aCO2 value ≥ 6 mmHg was considered to be high. Patients were classified in four predefined groups according to the Pv-aCO2 evolution during the first 6 hours of resuscitation: (1) persistently high Pv-aCO2 (high at T0 and T6); (2) increasing Pv-aCO2 (normal at T0, high at T6); (3) decreasing Pv-aCO2 (high at T0, normal at T6); and (4) persistently normal Pv-aCO2 (normal at T0 and T6). Multiorgan dysfunction at day-3 was compared for predefined groups and a Kaplan Meier curve was constructed to show the survival probabilities at day-28 using a log-rank test to evaluate differences between groups. A Spearman-Rho was used to test the agreement between cardiac output and Pv-aCO2. Finally, we calculated the mortality risk ratios at day-28 among patients attaining normal oxygen parameters but with a concomitantly increased Pv-aCO2.

Results: Patients with persistently high and increasing $Pv\text{-}aCO_2$ at T6 had significant higher SOFA scores at day-3 ($p < 0.001$) and higher mortality rates at day-28 (log rank test: 19.21, $p < 0.001$) compared with patients who evolved with normal $Pv\text{-}aCO_2$ at T6. Interestingly, a poor agreement between cardiac output and $Pv\text{-}aCO_2$ was observed ($r^2 = 0.025$, $p < 0.01$) at different points of resuscitation. Patients who reached a central venous saturation $(ScvO)_2 \geq 70\%$ or mixed venous oxygen saturation $(SvO_2) \geq 65\%$ but with concomitantly high $Pv\text{-}aCO_2$ at different developmental points (i.e., T0, T6 and T12) had a significant mortality risk ratio at day-28.

Conclusion: The persistence of high $Pv\text{-}aCO_2$ during the early resuscitation of septic shock was associated with more severe multi-organ dysfunction and worse outcomes at day-28. Although mechanisms conducting to increase Pv-aCO2 during septic shock are insufficiently understood, $Pv\text{-}aCO_2$ could identify a high risk of death in apparently resuscitated patients.

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Introduction

Inadequate tissue perfusion is a pivotal factor in the pathogenesis and clinical course of multiorgan failure in the critically ill [1]. Current techniques for monitoring tissue perfusion have largely focused on systemic blood flow and the balance between oxygen demand and supply [2,3]. An early hemodynamic optimization that targets central venous oxygen saturation ($ScvO_2$) and systemic hemodynamic parameters improves outcomes in severe sepsis and septic shock [4], reinforcing the idea that tissue perfusion abnormalities are flow dependent at least during the very early stages. However, normalizing systemic hemodynamic parameters does not guarantee adequate tissue perfusion [5-7], and in fact a substantial number of patients still progress to multiorgan dysfunction and death despite meeting $ScvO_2$ targets [4].

In the past, authors described the coexistence of venous acidemia and increased venous carbon dioxide (CO_2) during cardiac arrest in both animals [8] and critically ill humans [9]. Thereafter, increases in the venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) were reported during hypovolemic, cardiogenic, obstructive, and septic shock [10-12]. Interestingly, an inverse curvilinear relationship between $Pv\text{-}aCO_2$ and cardiac output was described, highlighting the importance of blood flow on venous CO_2 accumulation [13,14]. $Pv\text{-}aCO_2$ thus aroused clinical interest as a marker of global perfusion during shock states, although some studies questioned its prognostic value [14]. In fact, some *in vivo* models evaluating the mechanisms conducting to venous CO_2 accumulation during non-inflammatory conditions [15-17] challenged the ability of $Pv\text{-}aCO_2$ to identify tissue dysoxia because it only rises during ischemic hypoxia, but not during hypoxic or anemic hypoxia for comparable declines in oxygen delivery and oxygen consumption. However, more recent data suggest that high $Pv\text{-}aCO_2$ could identify septic patients who remain inadequately resuscitated despite achieving oxygen metabolism targets, reinforcing the notion of $Pv\text{-}aCO_2$ as a marker of global perfusion due to its ability to track blood flow alterations [18] or even detect anaerobic CO_2 generation [19]. Furthermore, patterns of recovery or derangement of $Pv\text{-}aCO_2$ during very early stages of resuscitation of septic shock have not been widely described and recent studies trying to demonstrate the reliability of $Pv\text{-}aCO_2$ as a tool in resuscitation of septic patients could have been influenced by selection bias because not all potential patients were elected to catheter insertion and goal-directed therapy [20,21].

Recent publications in critical care demonstrate that oxygen-derived parameters such as $ScvO_2$ or mixed venous oxygen saturation (SvO_2) are commonly normalized at ICU admission [22] and maneuvers such as emergent intubation can quickly improve $ScvO_2$ despite regional and tissue perfusion derangements [23]. Because global and regional hypoperfusion have been incriminated in the development of multiple organ failure, investigation on surrogate markers of such phenomenon remains important in critical care. Given that $Pv\text{-}aCO_2$ can track the adequacy of systemic perfusion during shock states, we sought to test whether the time course of $Pv\text{-}aCO_2$ during the early phases of resuscitation is related to the development of more severe multiorgan dysfunction and worse outcomes in a population of septic shock patients resuscitated by targeting the usual oxygen-derived and hemodynamic parameters.

Materials and methods

This prospective observational study was performed in a 60-bed mixed ICU in a university-affiliated hospital. We examined all patients with a new septic shock episode admitted to the emergency room or proceeding from clinical wards during a 24-month period. Septic shock was defined using the criteria of the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference [24]. Patients were excluded if they were younger than 18 years old, pregnant, had severe chronic obstructive pulmonary disease (GOLD 3 and 4 categories according to the current classification at the time of our study) or advanced liver cirrhosis (Child–Pugh C).

General management

All patients admitted to the emergency room or proceeding from clinical wards who fulfilled the diagnosis criteria for septic shock were evaluated by the ICU rapid response team according to our local procedures. Each patient was equipped with an arterial cannula and a pulmonary artery catheter (CCO Swan–Ganz catheter; Edwards Life sciences, Irvine, CA, USA). Our early goal-directed therapy included a bundle of interventions that sought to obtain: mean arterial pressure ≥65 mmHg; urine output ≥0.5 ml/kg/minute; normalization of serum lactate; and $ScvO_2$ ≥70% or SvO_2 ≥65%. The use of vasopressors (dopamine or norepinephrine) was standardized to maintain a mean arterial pressure ≥65 mmHg, and repeated fluid challenges with crystalloids or colloids were used to optimize the stroke volume as well as to allow the lowest dose of vasopressors and pulse-pressure variability <12%. Dobutamine was added for persistent $ScvO_2$ ≤70% or SvO_2 ≤65% after fluid resuscitation. A low dose of hydrocortisone was given within 6 hours of resuscitation when use of vasopressors persisted after an adequate fluid restitution. Mechanical ventilation was provided when needed under light sedation (midazolam) and analgesia (fentanyl); the tidal volume was limited to 6 to 8 ml/kg. Glycemic control was adjusted to maintain glucose levels <150 mg/dl. Finally, stress ulcer and venous thrombosis prophylaxis were provided according to international recommendations [25].

Study protocol

The Fundación Valle del Lili's Ethical and Biomedical research committee approved the current study. A written informed consent was waived because no new therapeutic interventions were performed and all measurements and procedures routinely followed the local protocols for the management of severe sepsis and septic shock.

Time 0 (T0) was declared when the pulmonary artery catheter was inserted using common monitoring tracings to place the distal port in the pulmonary artery and the proximal port in the right atrium, approximately 3 cm above the tricuspid valve. In order to standardize T0, we recorded the total volume of fluids administered and the time elapsed between the start of resuscitation (first hypotension episode) and the pulmonary artery catheter insertion (T0).

We collected arterial venous blood samples and central and mixed venous blood samples for arterial–venous gases (ABL 300; Radiometer Copenhagen, Denmark) and arterial lactate measurements at T0, and 6 hours (T6), 12 hours (T12) and 24 hours (T24) later. We simultaneously registered hemodynamic and respiratory variables at each measurement. We defined Pv-aCO_2 as the difference between the mixed venous CO_2 partial pressure and the arterial CO_2 partial pressure. Previous studies considered Pv-aCO_2 ≥6 mmHg abnormal [14]. Hence, we classified the patients according to the Pva-CO_2 development during the first 6 hours of resuscitation: persistently high Pv-aCO_2 (high at T0 and T6); increasing Pv-aCO_2 (normal at T0, high at T6); decreasing Pv-aCO_2 (high at T0, normal at T6); and persistently normal Pv-aCO2 (normal at T0 and T6). The Sequential Organ Failure Assessment score [26] was used to describe multiorgan dysfunction at day 3 and we also described mortality at day 28 for the pre-defined groups.

Data analysis

After exclusion of a normal distribution of the data by the Kolmogorov–Smirnov test, we used a Kruskal–Wallis test to compare continuous variables (followed by Bonferroni correction for multiple comparisons) and *a chi-squared test* (or Fisher's exact test, when appropriate) for discrete variables. Survival probabilities at day 28 were described using a Kaplan–Meier curve and differences between groups were calculated using a log-rank test before and after adjusting for SvO_2 at T6. The development of SvO_2, $ScvO_2$, lactate, cardiac output, mean arterial pressure and Pv-aCO_2 during the first 24 hours were analyzed using a repeated-measures analysis of variance. Spearman's rho was used to test the agreement between cardiac output and Pv-aCO_2. We also calculated the mortality risk ratios at day 28 in patients who attained $ScvO_2$ ≥70% or SvO_2 ≥65% but maintained persistently high Pv-aCO_2 at different points during resuscitation (T0, T6, and T12). Data were expressed as medians and 25 to 75% interquartile ranges. P ≤0.05 (two-tailed) was considered significant.

Results

During the 24-month period, 108 patients older than 18 years with a new episode of septic shock were screened. Patients with advanced cirrhosis (n = 4), patients with severe chronic obstructive pulmonary disease (n = 8) and pregnant women (n = 4) were not included for analysis; additionally, a central catheter could not be placed in four patients, and three refused the procedure. The final sample was therefore 85 patients. The median length of ICU stay for all patients was 6 days (25 to 75% interquartile range, 3 to 11 days), and the 28-day mortality rate was 37.6%. The median time elapsed from sepsis-induced hypotension to catheter insertion was 3.0 hours (25 to 75% interquartile range, 1.0 to 4.0 hours) and the median volume of fluids received before catheter insertion was 2,079 ml (25 to 75% interquartile range, 1,184 to 3,135 ml) for all patients.

Thirty-six patients had Pv-aCO_2 <6.0 mmHg at T0 and T6, and 17 patients had a high PvaCO_2 at T0 but it fell below 6 mmHg at T6 (a total of 53 patients had Pv-aCO_2 <6 mmHg at T6); on the other hand, 24 patients had a persistently high Pv-aCO_2 during the first 6 hours and the remaining eight evolved from normal at T0 to high PvaCO_2 at T6 (32 patients had Pv-aCO_2 ≥6 mmHg at T6).

We did not find any significant difference with regard to Acute Physiology and Chronic Health Evaluation II score, comorbidities, demographics, or respiratory and hemodynamic variables between groups (Tables 1 and 2), and neither for the volume of fluids received before inclusion (T0). Doses of vasopressors or inotropic received were similar for the groups both at T0 and T6 (Table 2). Multiorgan dysfunction at day 3 was significantly higher among patients with persistently high Pv-aCO_2 compared with those with persistently normal or decreasing Pv-aCO_2 during the first 6 hours of resuscitation (Kruskal–Wallis test, P <0.001) (Figure 1). Likewise, patients with persistently high Pv-aCO_2 during the first 6 hours of resuscitation had a significant lower survival at day 28 compared with those who normalized Pv-aCO_2 during this period (log-rank, Mantel–Cox: 19.21, P <0.001; Figure 2). These results were maintained after adjusting for the SvO_2 achieved at T6 (log-rank test, P <0.001). The time course of SvO_2, $ScvO_2$ and cardiac output did not significantly differ between Pv-aCO_2 groups (Table 2) nor between survivors and nonsurvivors at day 28 (Figures S1a,b and S2 in Additional file 1). Interestingly, a poor agreement between cardiac output and Pv-aCO_2 was observed both at each time of resuscitation and when all data were pooled (r^2 = 0.025, P <0.01) (Figure 3; Figure S3 in Additional file 1).

Table 1 Patient characteristics

Variable	Group 1, H-H (*n* = 24)	Group 2, L-H (*n* = 8)	Group 3, H-L (*n* = 17)	Group 4, L-L (*n* = 36)	*P* value
Age (years)	63.0 (54.7 to 75.0)	63.5 (50.5 to 76.5)	55.0 (53.0 to 76.0)	62.0 (49.3 to 71.8)	0.83
Gender, male (%)	17 (70.8)	4 (50.0)	10 (58.8)	22 (61.1)	0.72
APACHE II	24.4 (21.2 to 26.0)	25.2 (21.0 to 27.0)	23.4 (21.2 to 25.6)	24.8 (22.2 to 25.8)	0.11
Time between diagnosis and catheter insertion (T0)	2.0 (2.0 to 4.0)	1.0 (1.0 to 3.3)	3.0 (1.0 to 4.0)	3.0 (2.0 to 4.0)	0.44
Fluids received before catheter insertion	2,039 (1,343 to 2,834)	1,934 (849 to 4,575)	2,500 (1,430 to 3,628)	2,000 (1,025 to 3,153)	0.76
Temperature (°C)	37.5 (37.2 to 37.9)	37.4 (37.1 to 37.9)	37.6 (37.0 to 38.0)	37.4 (37.4 to 37.8)	0.74
Hemoglobin (g/dl)	9.4 (8.7 to 11.6)	8.8 (7.4 to 11.4)	9.8 (9.1 to 11.4)	9.9 (9.1 to 11.4)	0.47
Source of infection, *n* (%)					
Pneumonia	9	3	4	10	
Abdominal	8	4	6	14	
Urinary	2	1	3	4	
Soft tissue	2	0	1	2	
No specific site	3	0	2	4	
Other	0	0	1	2	
Culture positive, *n* (%)	20 (83.3)	5 (62.5)	12 (70.6)	19 (52.8)	0.11
Antibiotics given at T0, *n* (%)	24 (100)	7 (87.5)	16 (94.1)	32(88.9)	0.38
Antibiotics adequate, *n* (%)	23 (95.8)	7 (87.5)	16 (94.1)	27 (75.0)	0.10
Hydrocortisone, *n* (%)	22 (91.7)	8 (100)	15 (88.2)	31 (86.1)	0.68
Transfusion RBC, *n* (%)	4 (16.7)	3 (37.5)	3 (17.6)	4 (11.1)	0.35
Fluids and vasoactive agents					
Fluids (ml), IQ 25 to 75					
T0	2,039 (1,343 to 2,834)	1,934 (849 to 4,575)	2,500 (1,430 to 3,628)	2,000 (1,025 to 3,153)	0.76
T6	4,733 (3,196 to 6,360)	4,082 (1,576 to 7,584)	4,845 (2,550 to 6,560)	4,673 (3,380 to 7,348)	0.83
Norepinephrine (μg/kg/minute), IQ 25 to 75, *n*					
T0	0.25 (0.19 to 0.36), 24	0.29 (0.14 to 1.20), 6	0.19 (0.10 to 0,37), 16	0.23 (0.10 to 0.38), 30	0.81
T6	0.20 (0.11 to 0.40), 24	0.36 (0.09 to 0.49), 6	0.12 (0.11 to 0.18), 15	0.19 (0.08 to 0.39), 31	0.45
Dopamine (μg/kg/minute), IQ 25 to 75, *n*					
T0	–	7.1 (7.1 to 7.3), 2	10.7 (10.7 to 10.7), 1	5.1 (2.8 to 6.6), 5	0.14
T6	–	–	8.0 (8.0 to 8.0), 1	5.3 (2.5 to 6.4), 4	0.16
Dobutamine (μg/kg/minute), IQ 25 to 75, *n*					
T0	3.1 (2.7 to 4.5), 5	–	5.9 (5.1 to 6.7), 2	3.3 (3.0 to 4.2), 3	0.15
T6	3.1 (2.7 to 5.6), 3	–	5.1 (4.7 to 6.3), 4	3.8 (3.3 to 4.8), 3	0.21

H-H, venous-to-arterial carbon dioxide difference (Pv-aCO_2) high at T0 and T6; *L-H*, Pv-aCO_2 normal at T0 and high at T6; *H-L*, Pv-aCO_2 high at T0 and normal at T6; and L-L, Pv-aCO_2 normal at T0 and T6. APACHE, Acute Physiology and Chronic Health Evaluation; IQ 25 to 75, 25 to 75% interquartile range; RBC, red blood cells; T0, time 0; T6, 6 hours after Time 0.

Patients with elevated Pv-aCO_2 at T6 had slower lactate clearances at T6 and T12 than patients attaining a normal Pv-aCO_2 during the first 6 hours of resuscitation (Figure 4). We also observed a significant linear correlation between mixed-venous to arterial pCO_2 and central-venous to arterial pCO_2 (Pearson correlation: 0.71, 95% confidence interval: 0.47 to 0.86; P <0.001) but with moderate agreement between them (R^2 = 0.556, P <0.001) (Figure 5). Additionally, significant differences were observed for the time course of Pv-aCO_2 and central venous-to-arterial carbon dioxide difference (Pvc-aCO_2) during the first 24 hours for survivor and nonsurvivors at day 28 (repeated-measures analysis of variance, P = 0.003 and P = 0.03, respectively; Figure S4a,b in Additional file 1).

Finally, patients who achieved ScvO2 ≥ 70% or SvO2 ≥ 65% but maintained high Pv-aCO2 at T0, T6 and T12 had a higher mortality risk at Day 28 (Table 3).

Table 2 Hemodynamic, oxygen and ventilatory parameters

Variable	Group 1, H-H (*n* = 24)	Group 2, L-H (*n* = 8)	Group 3, H-L (*n* = 17)	Group 4, L-L (*n* = 36)	*P* value
Hemodynamic variables					
Heart rate (beats/minute)					
T0	112 (103 to 129)	111 (77 to 138)	104 (86 to 126)	109 (93 to 118)	0.31
T6	114 (101 to 125)	103 (73 to 127)	98 (85 to 114)	103 (88 to 118)	0.14
Cardiac index (l/minute/m^2)					
T0	3.0 (2.3 to 4.2)	3.6 (2.7 to 4.6)	3.0 (2.5 to 5.0)	3.5 (2.7 to 4.5)	0.63
T6	3.2 (2.2 to 4.3)	3.3 (2.4 to 3.8)	3.7 (2.2 to 5.5)	3.6 (2.8 to 4.3)	0.75
MAP (mmHg)					
T0	65.0 (61.0 to 72.8)**	67.0 (65.3 to 69.5)	73.0 (68.0 to 82.5)**	72.0 (64.5 to 77.0)	0.02
T6	69.0 (65.3 to 75.8)	77.5 (70.5 to 96.0)	68.0 (63.5 to 72.0)	71.5 (65.0 to 78.8)	0.06
CVP (mmHg)					
T0	14.5 (10.3 to 17.0)	10.0 (9.0 to 12.8)	12.0 (8.5 to 15.0)	10.0 (7.0 to 14.0)	0.10
T6	13.5 (10.0 to 15.8)**	15.0 (10.0 to 17.0)	10.0 (5.0 to 12.5)**	10.0 (7.3 to 13.0)	0.03
PAOP (mmHg)					
T0	17.0 (14.0 to 20.0)	12.5 (10.0 to 15.0)	17.0 (12.0 to 22.0)	15.0 (11.0 to 20.0)	0.52
T6	19.0 (15.0 to 24.0)**	14.5 (10.0 to 19.0)	13.0 (10.0 to 15.0)**	16.0 (12.0 to 20.0)	0.03
Blood gases and oxygen variables					
pH					
T0	7.30 (7.18 to 7.37)	7.31 (7.22 to 7.40)	7.34 (7.22 to 7.41)	7.32 (7.24 to 7.40)	0.85
T6	7.32 (7.20 to 7.36)	7.35 (7.30 to 7.42)	7.35 (7.26 to 7.39)	7.34 (7.30 to 7.39)	0.33
$PaCO_2$ (mmHg)					
T0	30.5 (24.1 to 36.6)	34.2 (24.7 to 36.9)	31.0 (23.6 to 33.6)	27.5 (23.7 to 36.5)	0.81
T6	26.1 (22.4 to 32.7)	31.0 (21.6 to 34.0)	31.3 (26.8 to 33.6)	27.5 (21.7 to 32.1)	0.25
$PvmCO_2$ (mmHg)					
T0	40.6 (33.9 to 46.5)¶	29.5 (25.0 to 35.3)	39.7 (35.5 to 47.1) 34.3	32.8 (27.3 to 40.9)¶	0.03
T6	38.0 (29.9 to 39.7)¶	38.3 (28.0 to 46.2)	(30.4 to 36.8)	30.0 (25.5 to 35.5)¶	0.02
$PvcCO_2$ (mmHg)					
T0	38.2 (29.1 to 50.5)	40.1 (34.2 to 43.4)	36.7 (35.5 to 47.1) 33.9	32.5 (28.1 to 38.4)	0.12
T6	33.7 (28.3 to 36.9)	33.8 (31.4 to 41.4)	(30.4 to 36.8)	29.0 (25.1 to 35.8)	0.20
PaO_2 (mmHg)					
T0	112.2 (79.3 to 167.3)	117.1 (77.5 to 139.5)	115.7 (81.4 to 140.8)	100 (86.0 to 123.9)	0.70
T6	113.6 (83.0 to 147.3)	132.5 (109.8 to 197.8)	105.0 (83.5 to 127.4)	111.5 (86.2 to 135.7)	0.34
PaO_2/FiO_2 (mmHg)					
T0	188.9 (117.1 to 265.0)	166.9 (143.8 to 244.1)	233.7 (184.6 to 314.0)	214.3 (141.6 to 368.0)	0.28
T6	177.9 (136.4 to 298.4)	265.0 (185.5 to 383.5)	239.3 (185.1 to 306.3)	253.5 (181.6 to 368.0)	0.17
Lactate (mmol/l)					
T0	4.3 (2.0 to 7.9)	2.9 (2.0 to 9.7)	2.7 (1.5 to 3.9)	2.9 (1.7 to 4.7)	0.16
T6	3.3 (2.1 to 6.8)**¶	2.9 (1.1 to 7.1)	1.3 (0.9 to 2.3)**	2.0 (1.0 to 3.5)¶	0.002
$ScvO_2$ (%)					
T0	64.0 (54.7 to 75.1)	66.8 (59.7 to 74.5)	73.0 (70.8 to 76.7)	67.1 (62.1 to 75.9)	0.36
T6	70.8 (66.4 to 73.5)	73.4 (69.3 to 78.0)	72.3 (67.2 to 75.9)	76.0 (60.0 to 77.9)	0.53
SvO_2 (%)					
T0	66.0 (56.8 to 71.4)	68.0 (54.9 to 81.0)	68.1 (57.0 to 71.0)	69.7 (62.1 to 75.9)	0.39

Table 2 Hemodynamic, oxygen and ventilatory parameters *(Continued)*

T6	69.0 (63.3 to 72.9)	70.6 (63.4 to 73.8)	71.2 (64.4 to 75.1)	68.0 (58.7 to 74.3)	0.77
Oxygen extraction ratio					
T0	34.7 (28.3 to 44.3)	30.8 (22.7 to 38.4)	29.4 (25.0 to 36.8)	30.7 (24.9 to 37.5)	0.36
T6	29.8 (26.4 to 36.6)	30.2 (24.9 to 31.3)	29.3 (24.1 to 33.3)	30.7 (23.4 to 39.3)	0.68
Pv-aCO_2 (mmHg)					
T0	8.2 (7.0 to 10.6)*¶	1.4 (1.1 to 5.3)^{*φ}	6.3 (6.1 to 7.2)^{φξ}	2.7 (2.0 to 4.0)^{¶ξ}	<0.001
T6	7.6 (6.4 to 9.4)**¶	8.6 (6.5 to 11.7)^{φψ}	3.5 (0.9 to 4.3)^{**φ}	3.3 (2.2 to 4.1)^{¶ψ}	<0.001
Pvc-aCO_2 (mmHg)					
T0	9.7 (7.0 to 12.1)¶	4.6 (3.3 to 6.1)	7.3 (4.6 to 9.1)	4.4 (2.7 to 5.4)¶	<0.001
T6	7.0 (5.8 to 9.7)	8.5 (5.6 to 9.6)^{ψ}	4.4 (2.1 to 5.5)	4.0 (1.7 to 5.4)^{ψ}	0.003
Ventilatory parameters					
Mechanical ventilation					
T0	24	5	11	23	0.01
T6	24	5	11	23	0.01
PEEP (mmHg), *n*			5.0 (5.0 to 8.0), 11	6.0 (5.0 to 8.0), 23	0.48
T0	6.0 (5.0 to 8.0), 24	5.0 (5.0 to 6.0), 5	5.0 (5.0 to 8.0), 11	8.0 (5.0 to 10.0), 23	
T6	6.5 (5.0 to 8.0), 24	5.0 (5.0 to 7.5), 5			
Tidal volume (ml/kg)					
T0	7.1 (6.3 to 7.7)	7.3 (6.3 to 7.6), 5	7.0 (6.5 to 8.0), 11	7.5 (6.6 to 8.0), 23	0.65
T6	7.2 (6.3 to 7.9)	7.2 (6.6 to 7.6), 5	7.5 (6.8 to 8.0), 11	7.5 (6.9 to 8.0), 23	0.65

Data presented as mean (25 to 75% interquartile range). *CVP*, central venous pressure; FiO_2, oxygen inspired fraction; *MAP*, mean arterial pressure; $PaCO_2$, arterial carbon dioxyde pressure; PaO2, arterial oxygen pressure; *PAOP*, pulmonary artery occlusion pressure; PEEP, positive end-expiratory pressure; *Pv-aCO_2*, mixed venous-to-arterial carbon dioxide difference; *Pvc-aCO_2*, central venous-to-arterial carbon dioxide difference; *PvCO_2*, central venous carbon dioxide pressure; *PvmCO_2*, mixed venous carbon dioxide pressure; *ScvO_2*, central venous oxygen saturation; *SvO_2*, mixed venous oxygen saturation; T0, time 0; T6, 6 hours after Time 0. $^{*}P$ <0.05 for Groups 1 vs. 2; $^{**}P$ <0.05 for Groups 1 vs. 3; $^{¶}P$ <0.05 for Groups 1 vs. 4; ^{φ}P <0.05 for Groups 2 vs. 3; ^{ψ}P <0.05 for Groups 2 vs. 4; and ^{ξ}P <0.05 for Groups 3 vs. 4.

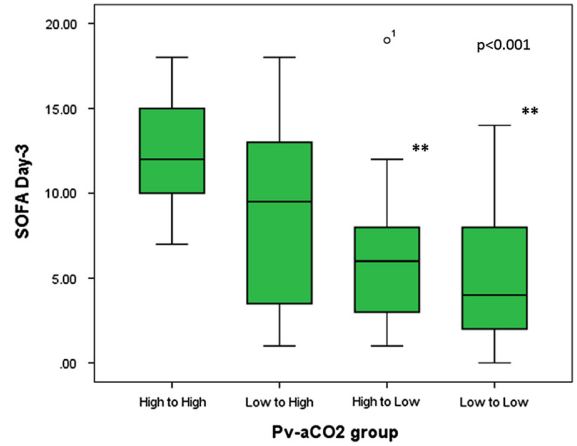


Figure 1 Sequential Organ Failure Assessment scores at day 3 by the early development (first 6 hours) of mixed venous-to-arterial carbon dioxide difference. Kruskal–Wallis test, *P* <0.001. **Significant differences between persistently high mixed venous-to-arterial carbon dioxide difference (Pv-aCO_2) and low Pv-aCO_2 group; and between persistently high Pv-aCO_2 and decreasing Pv-aCO_2 group after Bonferroni correction. H-H, Pv-aCO_2 high at Time 0 (T0) and 6 hours later (T6); L-H, Pv-aCO_2 normal at T0 and high at T6; H-L, Pv-aCO_2 high at T0 and normal at T6; and L-L, Pv-aCO_2 normal at T0 and T6. SOFA, Sequential Organ Failure Assessment.

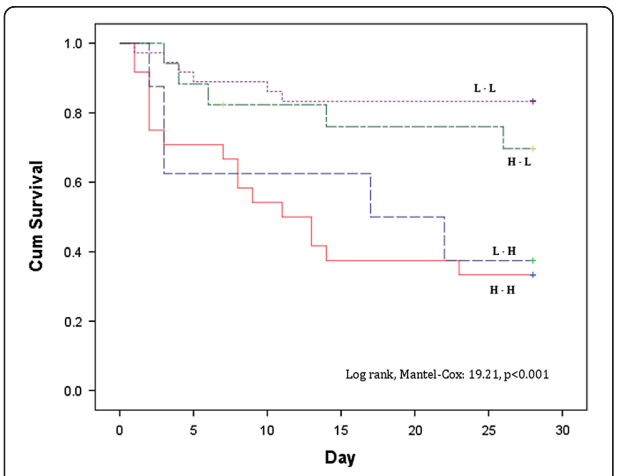


Figure 2 Survival probabilities at day 28 by the development of mixed venous-to-arterial carbon dioxide difference during the first 6 hours of resuscitation. Log-rank, Mantel–Cox: 19.21, *P* <0.001. H-H, mixed venous-to-arterial carbon dioxide difference (Pv-aCO_2) high at Time 0 (T0) and 6 hours later (T6); L-H, Pv-aCO_2 normal at T0 and high at T6; H-L, Pv-aCO_2 high at T0 and normal at T6; and L-L, Pv-aCO_2 normal at T0 and T6.

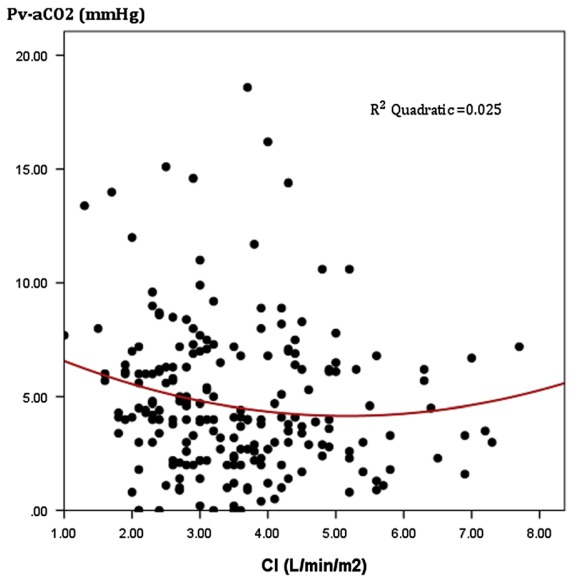


Figure 3 Scatter plot between cardiac index and mixed venous-to-arterial carbon dioxide difference. All patients at Time 0 (T0) and 6 hours (T6), 12 hours (T12) and 24 hours (T24) later. Pearson correlation: 0.16; r^2 = 0.025; P <0.01. CI, confidence interval; Pv-aCO_2, mixed venous-to-arterial carbon dioxide difference.

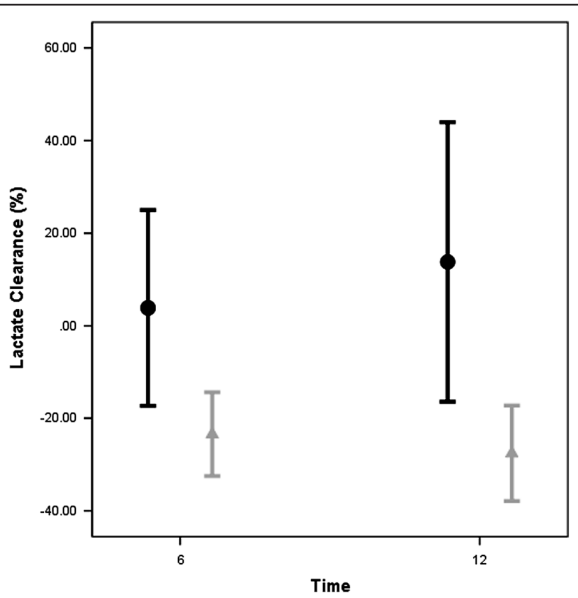


Figure 4 Lactate clearance (%) 6 and 12 hours after Time 0 for patients with normal or high mixed venous-to-arterial carbon dioxide difference at 6 hours. Significant differences for lactate clearance (Time 0 (T0) to 6 hours later (T6) and T0 to 12 hours later (T12)) between patients with persistently high (that is, high-to-high and normal-to-high groups) and normalized mixed venous-to-arterial carbon dioxide difference at T6 (high-to-normal and persistently low groups). A negative percent clearance indicates a reduction in lactate levels. Black bars: High Pv-aCO_2 at T6; Gray bars: Low Pv-aCO_2 at T6.

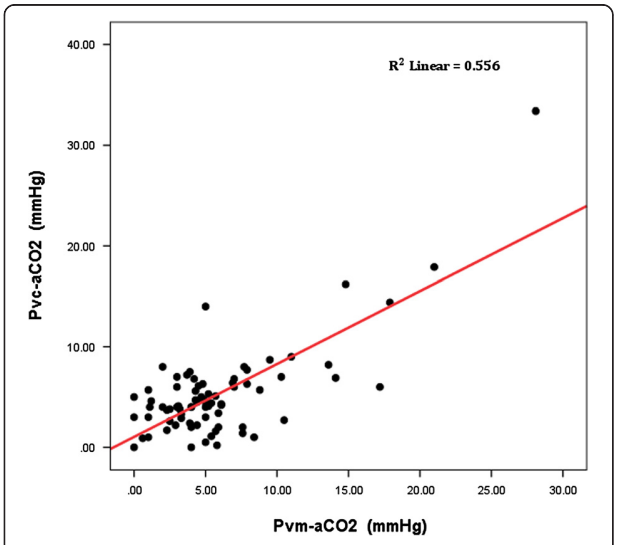


Figure 5 Correlation between mixed venous carbon dioxide pressure and central venous-to-arterial carbon dioxide difference. Scatter plot representing the mixed-venous to arterial carbon dioxide (Pvm-aCO_2) difference versus the central-venous to arterial carbon dioxide difference (Pvc-aCO_2). Pearson correlation: 0.71 (95% confidence interval: 0.47 to 0.86); R^2 = 0.55, P <0.001.

Discussion

We studied a cohort of patients during the very early phases of septic shock who were subjected to a comprehensive resuscitation aimed to target the usual hemodynamic and oxygen metabolism parameters. A recent study demonstrated how Pv-aCO_2 could be a tool to detect persistent inadequate resuscitation during septic shock [18] although it was not conducted during very early stages of resuscitation. Even a faster enrollment, our study showed that a number of patients had approximately normal SvO_2 and $ScvO_2$ at catheter insertion as it has been reported at ICU admission [22], and most of them reached normal oxygen-derived parameters at 6 hours. However, despite attaining the SvO_2 and $ScvO_2$ targets (and after adjusting for SvO_2) and an apparent global hemodynamic normalization in most patients, those with persistently high Pv-aCO_2 developed more severe multiorgan dysfunction at day 3 than patients evolving with normal Pv-aCO_2 during the first 6 hours of

Table 3 Mortality risk ratio for patients with mixed oxygen saturation ≥65% but with mixed venous-to-arterial carbon dioxide difference ≥6 mmHg at day 28

Time	Relative risk[a]	Confidence interval	*P* value
0 hours	1.77	0.97–3.22	0.06
6 hours	2.23	1.20–4.13	0.01
12 hours	2.41	1.42–4.10	0.001

[a]Relative mortality risk at day 28.

resuscitation or those who evolved from high to normal $PvaCO_2$. Additionally, we observed that persistently high $Pv\text{-}aCO_2$ was associated with a lower survival at day 28.

Venous hypercarbia is a marker of limited blood flow during cardiac arrest and shock states [8-19]. Recent observations have suggested that $Pv\text{-}aCO_2$ might identify septic patients who remain inadequately resuscitated despite achieving $ScvO_2$ goals [15]. Consistent with these findings, we found that patients in septic shock achieving $ScvO_2$ ≥70% or SvO_2 ≥65% had worse outcomes when a concomitant high $Pv\text{-}aCO_2$ was observed. These data reinforce the idea that $Pv\text{-}aCO_2$ provides additional information to hemodynamic and oxygen parameters habitually used during resuscitation of septic shock. Nevertheless, the underlying mechanisms that explain increases in $Pv\text{-}aCO_2$ during septic shock are incompletely understood; however, to current knowledge, an increased $Pv\text{-}aCO_2$ results from the interactions between blood flow to the tissues, aerobic and anaerobic CO_2 generation, and the CO_2 dissociation curve.

According to the Fick equation, during steady state the CO_2 excretion equals the product of cardiac output by the difference between mixed venous blood CO_2 content and arterial blood CO_2 content. Some studies have emphasized on the key role of cardiac output on venous to arterial CO_2 content differences and indeed a curvilinear relationship between these two variables has been described [14]. However, in our study we found a poorer concordance between cardiac output and $Pv\text{-}aCO_2$ at each time point of resuscitation (Figure 3) and, in fact, the cardiac output remained normal or even high during the first 24 hours of resuscitation (Figure S2 in Additional file 1), suggesting some independence between $Pv\text{-}aCO_2$ and macrovascular blood flow changes. While in non-inflammatory low-flow states tissue and regional hypercarbia can be easily explained by the CO_2 stagnation phenomenon [15,16], the interpretation of an increased tissue and/or regional CO_2 during inflammatory conditions is more complex. Sepsis may thereby be associated with the coexistence of normal or even high cardiac output, inter-organ and intra-organ blood flow redistribution, and altered microvascular and oxygen extraction capabilities. All of these alterations can influence the tissue CO_2 production and elimination.

A study by Neviere and colleagues thus demonstrated the key role of microvascular blood flow on gastric CO_2 accumulation [27]. Similarly, using simultaneous gastric tonometry and laser Doppler flowmetry, Elizalde and colleagues demonstrated the association between gastric mucosal pH and mucosal blood flow, regardless of macrohemodynamic variations [28]. Likewise, Tugtekin and colleagues demonstrated in a porcine sepsis model that the increase of mucosal to arterial CO_2 gap was related to the heterogeneity of gut mucosal blood flow, even though cardiac output and mesenteric blood flow were maintained [29]. Meanwhile, Creteur and colleagues found a significant correlation among sublingual CO_2, gastric mucosal CO_2 and microcirculatory heterogeneity in human septic shock during dobutamine infusion, and suggested that the primary determinant of tissue CO_2 accumulation was the microcirculatory blood flow [30]. Hence, there is an evident link between blood flow and tissue or local CO_2 accumulations conducting to increase tissue or venous-to-arterial CO_2 differences, but sometimes normal macrohemodynamics does not prevent elevation of $Pv\text{-}aCO_2$. The near normalization of the oxygen and hemodynamic parameters between the subgroups in our study suggests that venous CO_2 accumulation encloses more complex mechanisms than macrovascular stagnation, and we could hypothesize that microvascular blood flow distribution is one of several factors potentially influencing the behavior of $Pv\text{-}aCO_2$ during inflammatory conditions in which the heterogeneity of microvascular blood flow is increased. However, this hypothesis should be confirmed in future studies.

The interpretation of hyperlactatemia in sepsis is very complex, especially in septic shock [31] since anaerobic metabolism, non-anaerobic generation and slow clearance can conduct lactate accumulation. We observed higher lactate levels and slower lactate clearance at T6 and T12 in patients with persistently high $Pv\text{-}aCO_2$ during the first 6 hours of resuscitation. Interestingly, $Pv\text{-}aCO_2$ (and $Pvc\text{-}aCO_2$) kinetics seems to anticipate a slower lactate clearance (Figure S5 in Additional file 1). A high $Pv\text{-}aCO_2$ could indicate a decrease in global or microvascular blood flow conducting to slow lactate clearance. However, a high $Pv\text{-}aCO_2$ could also reflect the persistence of anaerobic metabolism as result of bicarbonate buffering of protons derived from fixed acids [32]. Thus, an increased $Pv\text{-}aCO_2$ to oxygen consumption ratio could reflect global anaerobic metabolism as was proposed by Mekontso-Dessap and colleagues [19]. However, even in the presence of anaerobic metabolism, a high efferent venous blood flow could be sufficient to wash out the global CO_2 generation from the hypoperfused peripheral tissues and, in this case, $Pv\text{-}aCO_2$ could not increase. In fact, hypoperfusion could persist in some of our patients and even oxygen parameters, global hemodynamics or $Pv\text{-}aCO_2$ remain normal.

Finally, we found a significant linear correlation but moderate agreement between venous–arterial CO_2 differences obtained from mixed venous and central venous samples that agree with recent observations published simultaneously to the review of our paper [21]. Even though $Pvc\text{-}aCO_2$ can be easily obtained and speedily usable in the emergency room, the point about whether $Pvc\text{-}aCO_2$ and mixed venous carbon dioxide pressure

($Pv\text{-}aCO_2$ in our study) are really interchangeable should be addressed in future studies.

Our study has some limitations. First, our observations are restricted to macro-hemodynamic variables, and $Pv\text{-}aCO_2$ is another global variable that does not necessarily represent tissue or regional vascular perfusion at different beds. We did not describe regional perfusion variables as gastric tonometry or local tissue CO_2 accumulation; hence, normal $Pv\text{-}aCO_2$ might also occur when regional hypoperfusion is ongoing.

Second, we suggest that persistently high $Pv\text{-}aCO_2$ reflects tissue or regional hypoperfusion. We hypothesized that $Pv\text{-}aCO_2$ could reflect the venous CO_2 accumulation due to the heterogeneous microcirculatory blood flow when cardiac output and oxygen parameters remain normal or even high or, eventually, $Pv\text{-}aCO_2$ could reflect anaerobic CO_2 generation. However, mechanism conducting to venous CO_2 accumulation during inflammatory conditions should be explored in future studies.

Third, during conditions of tissue hypoxia but with preserved blood flow (even though during anaerobic metabolism carbon dioxide production - VCO_2 - decreases less than oxygen consumption -VO_2-), venous blood flow might be high enough to ensure adequate washout of the CO_2 produced by hypoxic cells, thereby preventing a $Pv\text{-}aCO_2$ increase.

Fourth, we assumed that a linear relationship exist between partial CO_2 pressure and CO_2 content at the venous and arterial levels [33,34]. $Pv\text{-}aCO_2$ could thus be used as a surrogate for the $Cv\text{-}aCO_2$. However, previous research has shown that the Haldane effect causes paradoxical increases in $Pv\text{-}aCO_2$ during blood flow increases [33,34]. Unfortunately, the calculation of CO_2 content is complex and subject to errors due to the number of variables included in the formulas. Simplified formulas are easy to use, but wide differences in venous and arterial acid–base status (for example, ischemic hypoxia) can preclude their use. Nevertheless, some authors consider that the Haldane effect exerts a minor influence, and in most cases $Pv\text{-}aCO_2$ and CO_2 content differences develop similarly [35].

Finally, our observations were restricted to a small sample of patients in septic shock. Although our findings seem logical and biologically plausible, they should be confirmed in future studies.

Conclusions

The persistence of high $Pv\text{-}aCO_2$ during the early resuscitation of patients in septic shock is associated with significant higher multiorgan dysfunction and poor outcomes. Although underlying mechanisms that increase $Pv\text{-}aCO_2$ among patients in septic shock must be clarified, $Pv\text{-}aCO_2$ might identify a high risk of death in apparently resuscitated patients. Future studies should test $Pv\text{-}aCO_2$ as a perfusion goal during early phases of the resuscitation of patients in septic shock.

Key messages

- Persistent high $Pv\text{-}aCO_2$ is related to more severe multiorgan dysfunction and worse outcomes in apparently resuscitated septic shock patients.
- Mechanisms conducting to increase $Pv\text{-}aCO_2$ during inflammatory conditions are insufficiently understood. Variations in $Pv\text{-}aCO_2$ were independent of macro-flow variables (that is, cardiac output) or oxygen metabolism targets (that is, SvO2, oxygen extraction rate), suggesting that venous stagnation is not the single explanation for venous CO_2 accumulation.
- $Pv\text{-}aCO_2$ might identify a high risk of death in apparently resuscitated septic shock patients, and could be explored as a tissue perfusion goal during resuscitation as $Pv\text{-}aCO_2$ tracks ischemic hypoxia.

Additional file

Additional file 1: Figure S1a presenting the time course of ScvO2 (%) during the first 24 hours for survivors and nonsurvivors at day-28. Figure S1b presenting the time course of SvO2 (%) during the first 24 hours for survivors and nonsurvivors at day 28. **Figure S2** presenting the time course of cardiac output (l/minute) during the first 24 hours for survivors and nonsurvivors at day 28. **Figure S3** presenting the scatter plot between cardiac index and $Pv\text{-}aCO_2$ (according to $Pv\text{-}aCO_2$ at T6). **Figure S4a** presenting the time course of Pv-aCO2 (mmHg) during the first 24 hours for survivors and nonsurvivors at day 28. **Figure S4b** presenting the time course of Pvc-aCO2 (mmHg) during the first 24 hours for survivors and nonsurvivors at day 28. **Figure S5** presenting the time course of lactate levels (mmol/l) during the first 24 hours for survivors and nonsurvivors at day 28.

Abbreviations

CO_2: Carbon dioxide; $Pv\text{-}aCO_2$: Mixed venous-to-arterial carbon dioxide difference; $Pvc\text{-}aCO_2$: Central venous-to-arterial carbon dioxide difference; $ScvO_2$: Central venous oxygen saturation; SvO_2: Mixed venous oxygen saturation.

Competing interests

The authors declare they have no competing interests.

Authors' contributions

GAO-T contributed to the study conception, design and manuscript preparation. DFB-R, MU, JDT, AG and WB were involved in data collection and revising the manuscript. AFG, MG, CA-D and GH revised the manuscript critically. All authors read and approved the final manuscript.

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References

1. Beal AL, Cerra FB: **Multiple organ failure syndrome in the, 1990's. Systemic inflammatory response and organ dysfunction.** *JAMA* 1990, **1994:**226–233.
2. Shoemaker WC, Appel PL, Kram HB: **Tissue oxygen debt as a determinant of lethal and nonlethal postoperative organ failure.** *Crit Care Med* 1988, **16:**1117–1120.
3. Vallet B: **Vascular reactivity and tissue oxygenation.** *Intensive Care Med* 1998, **24:**3–11.
4. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M: **Early goal-directed therapy in the treatment of severe sepsis and septic shock.** *N Eng J Med* 2001, **345:**1368–1377.
5. Third European Consensus Conference in Intensive Care Medicine: **Tissue hypoxia: How to detect, how to correct, how to prevent. Societe de reanimation de langue francaise. The American thoracic society. European society of intensive care medicine.** *Am J Respir Crit Care Med* 1996, **154:**1573–1578.
6. Russell JA, Phang PT: **The oxygen delivery/consumption controversy. Approaches to management of the critically ill.** *Am J Respir Crit Care Med* 1994, **149:**533–537.
7. Marik PE, Bankov A: **Sublingual capnometry versus traditional markers of tissue oxygenation in critically ill patients.** *Crit Care Med* 2003, **31:**818–822.
8. Grundler W, Weil MH, Rackow EC: **Arteriovenous carbon dioxide and pH gradients during cardiac arrest.** *Circulation* 1986, **74:**1071–1074.
9. Weil MH, Rackow EC, Trevino R, Grundler W, Falk JL, Griffel MI: **Difference in acid–base state between venous and arterial blood during cardiopulmonary resuscitation.** *N Engl J Med* 1986, **315:**153–156.
10. Mecher CE, Rackow EC, Astiz ME, Weil MH: **Venous hypercarbia associated with severe sepsis and systemic hypoperfusion.** *Crit Care Med* 1990, **18:**585–589.
11. Adrogue HJ, Rashad MN, Gorin AB, Yacoub J, Madias NE: **Assessing acid–base status in circulatory failure. Differences between arterial and central venous blood.** *N Engl J Med* 1989, **320:**1312–1316.
12. Kazarian KK, Del Guercio LR: **The use of mixed venous blood gas determinations in traumatic shock.** *Ann Emerg Med* 1980, **9:**179–182.
13. Teboul JL, Mercat A, Lenique F, Berton C, Richard C: **Value of the venous–arterial PCO2 gradient to reflect the oxygen supply to demand in humans: effects of dobutamine.** *Crit Care Med* 1998, **26:**1007–1010.
14. Bakker J, Vincent JL, Gris P, Leon M, Coffernils M, Kahn RJ: **Veno-arterial carbon dioxide gradient in human septic shock.** *Chest* 1992, **101:**509–515.
15. Vallet B, Teboul JL, Cain S, Curtis S: **Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia.** *J Appl Physiol* 2000, **89:**1317–1321.
16. Neviere R, Chagnon JL, Teboul JL, Vallet B, Wattel F: **Small intestine intramucosal PCO(2) and microvascular blood flow during hypoxic and ischemic hypoxia.** *Crit Care Med* 2002, **30:**379–384.
17. Dubin A, Estenssoro E, Murias G, Pozo MO, Sottile JP, Baran M, Piacentini E, Canales HS, Etcheverry G: **Intramucosal-arterial Pco2 gradient does not reflect intestinal dysoxia in anemic hypoxia.** *J Trauma* 2004, **57:**1211–1217.
18. Vallee F, Vallet B, Mathe O, Parraguette J, Mari A, Silva S, Samii K, Fourcade O, Genestal M: **Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock?** *Intensive Care Med* 2008, **34:**2218–2225.
19. Mekontso-Dessap A, Castelain V, Anguel N, Bahloul M, Schauvliege F, Richard C, Teboul JL: **Combination of venoarterial PCO2 difference with arteriovenous O2 content difference to detect anaerobic metabolism in patients.** *Intensive Care Med* 2002, **28:**272–277.
20. van Beest PA, van Ingen J, Boerma EC, Holman ND, Groen H, Koopmans M, Spronk PE, Kuiper MA: **No agreement of mixed venous and central venous saturation in sepsis, independent of sepsis origin.** *Crit Care Med* 2010, **14:**R219.
21. van Beest PA, Lont MC, Holman ND, Loef B, Kuiper MA, Boerma EC: **Central venous–arterial pCO(2) difference as a tool in resuscitation of septic patients.** *Intensive Care Med* 2013, **39:**1034–1039.
22. van Beest PA, Hofstra JJ, Schultz MJ, Boerma EC, Spronk PE, Kuiper MA: **The incidence of low venous oxygen saturation on admission to the intensive care unit: a multi-center observational study in The Netherlands.** *Crit Care Med* 2008, **12:**R33.
23. Hernandez G, Pena H, Cornejo R, Rovegno M, Retamal J, Navarro JL, Aranguiz I, Castro R, Bruhn A: **Impact of emergency intubation on central venous oxygen saturation in critically ill patients: a multicenter observational study.** *Crit Care Med* 2009, **13:**R63.
24. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G: **SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference.** *Crit Care Med* 2001, **2003:**1250–1256.
25. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, *et al*: **Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2008.** *Crit Care Med* 2008, **36:**296–327.
26. Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, Sprung CL, Colardyn F, Blecher S: **Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on 'sepsis-related problems' of the European Society of Intensive Care Medicine.** *Crit Care Med* 1998, **26:**1793–1800.
27. Neviere R, Mathieu D, Chagnon JL, Lebleu N, Wattel F: **The contrasting effects of dobutamine and dopamine on gastric mucosal perfusion in septic patients.** *Am J Respir Crit Care Med* 1996, **154:**1684–1688.
28. Elizalde JI, Hernandez C, Llach J, Monton C, Bordas JM, Pique JM, Torres A: **Gastric intramucosal acidosis in mechanically ventilated patients: role of mucosal blood flow.** *Crit Care Med* 1998, **26:**827–832.
29. Tugtekin IF, Radermacher P, Theisen M, Matejovic M, Stehr A, Ploner F, Matura K, Ince C, Georgieff M, Trager K: **Increased ileal-mucosal-arterial PCO2 gap is associated with impaired villus microcirculation in endotoxic pigs.** *Intensive Care Med* 2001, **27:**757–766.
30. Creteur J, De Backer D, Sakr Y, Koch M, Vincent JL: **Sublingual capnometry tracks microcirculatory changes in septic patients.** *Intensive Care Med* 2006, **32:**516–523.
31. De Backer D: **Lactic acidosis.** *Intensive Care Med* 2003, **29:**699–702.
32. Raza O, Schlichtig R: **Metabolic component of intestinal PCO(2) during dysoxia.** *J Appl Physiol* 2000, **89:**2422–2429.
33. Jakob SM, Kosonen P, Ruokonen E, Parviainen I, Takala J: **The Haldane effect – an alternative explanation for increasing gastric mucosal PCO2 gradients?** *Br J Anaesth* 1999, **83:**740–746.
34. Hurley R, Mythen MG: **The Haldane effect – an explanation for increasing gastric mucosal PCO2 gradients?** *Br J Anaesth* 2000, **85:**167–169.
35. Schlichtig R, Bowles SA: **Distinguishing between aerobic and anaerobic appearance of dissolved CO2 in intestine during low flow.** *J Appl Physiol* 1994, **76:**2443–2451.

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PAPER No. 3 (Original paper)

COMBINATION OF ARTERIAL LACTATE LEVELS AND VENOUS-ARTERIAL CO_2 TO ARTERIAL-VENOUS O_2 CONTENT DIFFERENCE RATIO AS MARKERS OF RESUSCITATION IN PATIENTS WITH SEPTIC SHOCK

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Combination of arterial lactate levels and venous-arterial CO_2 to arterial-venous O_2 content difference ratio as markers of resuscitation in patients with septic shock

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Take-home message: The ratio between the Cv-aCO_2 and the arterial-to-venous oxygen content difference (Da-vO_2), as a surrogate of the VCO_2/VO_2 ratio (i.e., the respiratory quotient), may identify patients at risk of anaerobic metabolism.

Electronic supplementary material
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Abstract *Purpose:* To evaluate the prognostic value of the Cv-aCO_2/Da-vO_2 ratio combined with lactate levels during the early phases of resuscitation in septic shock. *Methods:* Prospective observational study in a 60-bed mixed ICU. One hundred and thirty-five patients with septic shock were included. The resuscitation protocol targeted mean arterial pressure, pulse pressure variations or central venous pressure, mixed venous oxygen saturation, and lactate levels. Patients were classified into four groups according to lactate levels and Cv-aCO_2/Da-vO_2 ratio at 6 h of resuscitation (T6): group 1, lactate $\geq$2.0 mmol/L and Cv-aCO_2/Da-vO_2 >1.0; group 2, lactate $\geq$2.0 mmol/L and Cv-aCO_2/Da-vO_2 $\leq$1.0; group 3, lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 >1.0; and group 4, lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 $\leq$1.0. *Results:* Combination of hyperlactatemia and high Cv-aCO_2/Da-vO_2 ratio was associated with the worst SOFA scores and lower survival rates at day 28 [log rank (Mantel–Cox) = 31.39, $p < 0.0001$]. Normalization of both variables was associated with the best outcomes. Patients with a high Cv-aCO_2/Da-vO_2 ratio and lactate <2.0 mmol/L had similar outcomes to hyperlactatemic patients with low Cv-aCO_2/Da-vO_2 ratio. The multivariate analysis revealed that Cv-aCO_2/Da-vO_2 ratio at both T0 (RR 3.85; 95 % CI 1.60–9.27) and T6 (RR 3.97; 95 % CI 1.54–10.24) was an independent predictor for mortality at day 28, as well as lactate levels at T6 (RR 1.58; 95 % CI 1.13–2.22). *Conclusion:* Complementing lactate assessment with Cv-aCO_2/Da-vO_2 ratio during early stages of resuscitation of septic shock can better identify patients at high risk of adverse outcomes. The Cv-aCO_2/Da-vO_2 ratio may become a potential resuscitation goal in patients with septic shock.

Keywords Lactate · Venous-to-arterial carbon dioxide difference · Oxygen consumption · Respiratory quotient · Septic shock

Introduction

Early identification of tissue hypoperfusion and adequate resuscitation are key factors in the management of patients with shock [1, 2]. Although early resuscitation seems to improve outcomes in severe sepsis and septic shock, the relative value of resuscitation goals continues to be highly debated [3–6]. Monitoring of $ScvO_2$ is widely recommended [3–7] although strongly challenged by others [8, 9]. In an early trial, Rivers et al. [3] observed a significant decrease in mortality when they used a resuscitation bundle targeting $ScvO_2$ >70 %. Conversely, recent data failed to confirm any benefit with this approach [10]. However, it should be noted that $ScvO_2$ was normal or near normal at inclusion in a number of patients in these trials [10] as it has frequently been reported on admissions to the intensive care unit in studies subsequent to the River's trial [11]. Moreover, normalization of systemic hemodynamic and oxygen metabolism variables does not ensure an adequate tissue perfusion and does not prevent progression to multiorgan dysfunction and death [12]. Lactate has also been proposed as a target for resuscitation therapy. In fact, not only baseline lactate level [13] but also its evolution under the influence of therapy [14] has been associated with clinical outcomes. Despite promising results observed in one trial [15], no consistent advantages have been found for lactate-based resuscitation bundles over resuscitation guided by oxygen parameters [15–17]. Accordingly, additional markers of inadequate perfusion should be explored, especially when $ScvO_2$ values are close to normal.

Recently, the venous-to-arterial carbon dioxide difference ($Pv\text{-}aCO_2$) has been proposed as an alternative marker of tissue hypoperfusion [18, 19]. In fact, persistently high $Pv\text{-}aCO_2$ predicts adverse clinical outcomes independently of oxygen-derived parameters and it could anticipate lactate variations [20]. However, the $Pv\text{-}aCO_2$ may be normal despite the presence of significant hypoperfusion in high cardiac output states such as septic shock, where high flows might prevent venous CO_2 accumulation; or inversely, $Pv\text{-}aCO_2$ can increase in the absence of hypoperfusion, in part due to the Haldane effect [21]. Consequently, CO_2 variations must be evaluated according to O_2 changes. Indeed, CO_2 production should not exceed O_2 availability during aerobic metabolism. Thus, the ratio between the $Pv\text{-}aCO_2$ and the arterial-to-venous oxygen content difference ($Da\text{-}vO_2$), as a surrogate of the VCO_2/VO_2 ratio (i.e., the respiratory quotient), may identify patients at risk of anaerobic metabolism. Using this rationale, Mekontso-Dessap et al. [22] demonstrated that a $Pv\text{-}aCO_2$ to $Da\text{-}vO_2$ ratio >1.4 was superior to $Pv\text{-}aCO_2$, SvO_2, and $Da\text{-}vO_2$ in predicting hyperlactatemia in a cohort of critically ill patients. Importantly, $Pv\text{-}aCO_2/Da\text{-}vO_2$ ratio variations are faster than lactate kinetics, which make it an attractive variable to monitor. However, CO_2 partial pressure (PCO_2) is not equivalent to CO_2 content (CCO_2), particularly when O_2 saturation varies (Haldane effect). Thus, $Cv\text{-}aCO_2/Da\text{-}vO_2$ variations should better reflect variations in VO_2 than $Pv\text{-}aCO_2/Da\text{-}vO_2$, especially when $ScvO_2$ or SvO_2 is low.

As the $Cv\text{-}aCO_2$ to $Da\text{-}vO_2$ ratio could reflect ongoing anaerobic metabolism, we hypothesized that an increased $Cv\text{-}aCO_2/Da\text{-}vO_2$ could be used to identify patients at risk of adverse outcomes during early stages of septic shock and that this variable could provide additional information when combined with lactate levels.

Materials and methods

We conducted a prospective observational study in a 60-bed mixed ICU in a university-affiliated hospital. The Fundación Valle del Lili's ethical and biomedical research committee approved the current study (protocol number 710; approval number 093-2014). A written informed consent was waived because all measurements and procedures routinely followed the local protocols for the management of severe sepsis and septic shock and no new therapeutic interventions were performed. Our "rapid-response team" evaluated all patients with suspected septic shock at the emergency room and clinical wards. Resuscitation was immediately started and these patients were rapidly admitted to the ICU. Presence of infection was established using the Centers for Diseases Control and Prevention criteria [23] and septic shock was defined according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [24]. Antibiotics were started within 1 h of diagnosis of sepsis. An arterial catheter was placed in the radial or femoral artery while a pulmonary artery catheter (PAC) was inserted via the jugular or subclavian vein when deemed appropriate according to clinical judgment and local indications for the use of hemodynamic monitoring. All patients with a new episode of septic shock and equipped with a PAC were included for the study. Patients with a previous episode of severe sepsis or septic shock within the last 3 months, younger than 18 years old, pregnant women, with limitations of care, with liver cirrhosis Child–Pugh C, or severe chronic obstructive pulmonary disease were excluded.

General management

All patients followed an early quantitative resuscitation protocol adapted from the Surviving Sepsis Campaign [7] in order to target (a) mean arterial pressure (MAP) ≥65 mmHg; (b) urine output ≥0.5 mL/kg/min; (c) SvO_2 ≥65 %; (d) normalization of lactate levels. In the case of attaining the SvO_2 goal but with persistently high lactate levels, additional efforts were performed to attain

normalization of the latter. Pulse pressure variations were used to indicate fluid responsiveness whenever applicable. In other cases, filling pressures and clinical judgment were used. Fluid resuscitation was conducted by repeated fluid challenges with crystalloids and/or albumin 4 %. Hydroxyethyl starches (HES) were not used.

Norepinephrine was the first-choice vasopressor to maintain MAP goals. Vasopressin titrated to a maximum of 0.03 UI/min was allowed in order to raise MAP or to decrease norepinephrine dose but never as a single vasopressor. Titrated dobutamine up to 20 μg/kg/min was used when myocardial dysfunction was demonstrated or when SvO_2 goals were not achieved despite adequate intravascular volume and MAP. Mechanical ventilation was provided (when needed) under light sedation (midazolam) and analgesia (fentanyl, morphine); tidal volume was limited to 6–8 mL/kg. Low dose hydrocortisone was indicated if vasopressor requirement did not decrease during the first 6 h of resuscitation despite ensuring adequate intravascular volume. Glycemic control was adjusted to maintain glucose levels <150 mg/dL. Finally, stress ulcer and venous thrombosis prophylaxis were provided according to international recommendations [7].

Study protocol

Time 0 (T0) was stated at the PAC insertion. We recorded the total volume of fluids received and the time elapsed between the first episode of hypotension and T0. We performed complete hemodynamic measurements and drew blood samples for arterial and mixed-venous gases analysis (ABL 300, Radiometer Copenhagen, Denmark) and arterial lactate at T0, and 6 h (T6), around 12 h (T12), and 24 h (T24) after. Vasopressors and inotropic doses, respiratory parameters, and total fluids were also registered at each measurement time. Organ dysfunction at day 3 was evaluated using the Sequential Organ Failure Assessment (SOFA) score [25]. We also calculated the ventilator-free days and survival at day 28.

Carbon dioxide and oxygen variables

We calculated CO_2 and O_2 variables at T0, T6, T12, and T24, as follows:

- $DO_2 = CaO_2 \times CI$
- $VO_2 = (CaO_2 - CvO_2) \times CI$
- $ERO_2 = (CaO_2 - CvO_2)/CaO_2$
- $CaO_2 = (Hg \times SaO_2 \times 1.34) + (PaO_2 \times 0.003)$
- $CvO_2 = (Hg \times SvO_2 \times 1.34) + (PvO_2 \times 0.003)$
- $Pv\text{-}aCO_2 = PvCO_2 - PaCO_2$
- $Da\text{-}vO_2 = CaO_2 - CvO_2$

where CaO_2 and CvO_2 are the arterial and venous O_2 content, PaO_2 and PvO_2 represent their arterial and venous partial pressures respectively, CI represents the cardiac index, and ERO_2 represents the oxygen extraction ratio.

We also calculated CO_2 contents according to the Douglas formula [26]:

$$\text{Blood } CO_2 \text{ content (Blood } CCO_2) = \text{Plasma } CCO_2 \times [1 - [0.0289 \times [Hb]] \div [[3.352 - 0.456 \times SpO_2] \times [8.142 - \text{pH}]]]$$

where plasma $CCO_2 = 2.226 \times S \times$ plasma $PCO_2 \times (1 + 10^{\text{pH} - \text{p}K'})$; In turn, S (plasma CO_2 solubility) and apparent pK (pK') are temperature (T, expressed as °C) dependent and calculated according to previous calculations [27]:

$$S = 0.0307 + [0.00057 \times (37 - T)] + [0.00002 \times (37 - T)^2]$$

$$pK' = 6.086 + [0.042 \times (7.4 - pH)] + [[(38 - T)] \times \{0.00472 + 0.00139x[7.4 - pH]\}].$$

Definitions of the four groups

Considering that in aerobic conditions VCO_2 should not exceed VO_2, we considered a Cv-aCO_2/Da-vO_2 ratio >1.0 as abnormal. Hence, we analyzed hemodynamic and oxygen metabolism parameters for four predetermined groups according to lactate levels and Cv-aCO_2/Da-vO_2 attained after the first 6 h of resuscitation: group 1, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; group 2, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0; group 3, lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; and group 4, lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0.

Statistics

After demonstrating a non-normal distribution of data with a Kolmogorov–Smirnoff test, we used a Kruskal–Wallis test to compare continuous variables with a Tukey–Kramer test for multiple comparisons among the predefined groups. χ^2 test was used to compare discrete variables (or Fisher's exact test, when appropriate). Multiorgan dysfunction at day 3 was evaluated using the Sequential Organ Failure Assessment (SOFA) score [25] for the predefined groups. Survival curves up to day 28 were estimated using the Kaplan–Meier method and log-rank (Mantel–Cox) test was used to estimate differences among the predefined groups.

In a further analysis, variables were introduced into a multivariate model if significantly associated with mortality at day 28 at the univariate analysis when a p value was <0.2. General demographics, hemodynamics, vasopressor use, fluids, and blood gases parameters at T0 and

T6 were used in the model, previously testing for collinearity. These analyses were also conducted in those patients attaining $SvO_2 \geq 65$ %. A Hosmer and Lemeshow test was used to assess the goodness of fit of the model. Receiver operating characteristic (ROC) curves for the original model (i.e., the "large model") and a second one excluding the Cv-aCO_2/Da-vO_2 ratio (i.e., the "short model") were constructed in order to test the added value of the Cv-aCO_2/Da-vO_2 ratio in predicting mortality at day 28. The ROC curves were compared using the method described by DeLong and colleagues [28].

Additional logistic regression models including Pv-aCO_2/Da-vO_2 instead of Cv-aCO_2/Da-vO_2 and another one including simultaneously Pv-aCO_2/Da-vO_2 and Cv-aCO_2/Da-vO_2 were also conducted to explore its relationship with mortality at day 28.

Finally, we described the time-course for oxygen metabolism variables, Pv-aCO_2/Da-vO_2 and Cv-aCO_2/Da-vO_2 during the first 24 h for both survivors and non-survivors at day 28.

Data are presented as median (25–75th percentiles). Risk assessments are presented as risk ratios with 95 % confidence intervals. A p value ≤ 0.05 (two-tailed) was considered significant.

Results

Selection of patients is shown in the Fig. 1 of the Electronic Supplementary Material (ESM). A total of 135 patients were included in the study during a period of 18 months. Mortality at day 28 in this cohort was 42 % and ICU length of stay was 6 (2–10) days. The time from first hypotension episode to catheter insertion and blood sampling (i.e., T0) was 3.0 (2.5–4.0) h and the median amount of fluids received before T0 was 1,977 (1,200–2,800) mL.

After the first 6 h of resuscitation, 110 (81 %) patients achieved a MAP >65 mmHg and 98 (73 %) a SvO_2 >65 %. However, 84 (62 %) patients still had an arterial lactate ≥ 2.0 mmol/L and 65 (48 %) had a Cv-aCO_2/Da-vO_2 ratio >1.0. Accordingly, 42 patients were classified into group 1, 42 into group 2, 23 into group 3, and 28 into group 4. Patients in groups 1 and 2 had higher APACHE II scores and required higher vasopressor doses at T0 (Table 1). No significant differences were found in demographic data or other hemodynamic variables at T0 (Table 1 and ESM Table 1). All hemodynamic, blood gases, oxygen parameters, and ventilator settings at both T0 and T6 are presented in the ESM Table 1. Patients from groups 1 and 2 had more acidosis at T0 and T6. Regarding the clinical outcomes, patients from group 1 evolved with higher SOFA scores (Kruskal–Wallis, $p < 0.001$; post hoc test demonstrated significant differences among groups 1 vs. 3 and 1 vs. 4) (Fig. 1) and they also had the lowest survival rates at day 28 [log rank (Mantel–Cox) = 31.39, $p < 0.0001$] (Fig. 2). Intriguingly, patients in groups 2 and 3 had similar SOFA scores and outcomes at day 28 (Table 2). Furthermore, patients from group 1 had the lowest VO_2 at T6 and T12 compared to all other groups, even though cardiac output, SvO_2, and DO_2 were not different (ESM Fig. 2).

Multivariate logistic regression analysis at T0 demonstrated that Cv-aCO_2/Da-vO_2 was an independent predictor of mortality at day 28 (RR 3.85; 95 % CI 1.60–9.27). When analysis was performed using the same variables at T6, Cv-aCO_2/Da-vO_2 was again related to higher mortality at day 28 (RR 3.97; 95 % CI 1.54–10.24), in addition to lactate levels (RR 1.58; 95 % CI 1.13–2.22) (Table 3). An additional multivariate analysis performed in patients attaining SvO_2 >65 % showed that lactate levels (RR 2.41; 95 % CI 1.22–4.76) and Cv-aCO_2/Da-vO_2 (RR 5.71; 95 % CI 1.20–27.19) remained predictors of mortality at day 28 (Table 3). The area under ROC curves for models including or excluding the Cv-aCO_2/Da-vO_2 ratio (i.e., the "large" and "short" model, respectively) were significantly different (AUC_{large} 0.8542, 95 % CI 0.7797–0.9286 vs. AUC_{short} 0.7943, 95 % CI 0.7050–0.8836. LR test, χ^2 17.81, $p < 0.001$; C statistic, χ^2 4.52, $p = 0.03$) (Fig. 3).

We also found significant differences in the time-course of Pv-aCO_2, Pv-aCO_2/Da-vO_2, lactate levels, and Cv-aCO_2/Da-vO_2 during the first 24 h of resuscitation between survivors and non-survivors at day 28 (repeated measurements ANOVA, $p < 0.05$) (ESM Figs. 3–5).

Discussion

We observed that persistent hyperlactatemia combined with a high Cv-aCO_2/Da-vO_2 was associated with the most severe organ dysfunction and worst clinical outcomes, while simultaneous normalization of lactate and Cv-aCO_2/Da-vO_2 ratio was associated with the best outcomes. Interestingly, patients attaining lactate levels <2.0 mmol/L combined with Cv-aCO_2/Da-vO_2 >1.0 had similar outcomes to patients with persistent hyperlactatemia and low Cv-aCO_2/Da-vO_2 ratio.

We hypothesized that a Cv-aCO_2/Da-vO_2 >1.0 reflects anaerobic metabolism as VCO_2 should not be higher than VO_2 during aerobic conditions. Indeed, occurrence of a high VCO_2/VO_2 has been previously reported in experimental conditions, where lower reductions in VCO_2 than in VO_2 have been associated with other markers of tissue hypoxia, suggesting the involvement of a non-aerobic source of CO_2 [29, 30]. Consequently, a Cv-aCO_2/Da-vO_2 ratio >1.0 (as a surrogate of the VCO_2/VO_2 ratio) could identify an excess of CO_2 generation probably due to anaerobic metabolism and this condition could be associated with more unfavorable clinical outcomes as we report in our study. Using a similar rationale, Mekontso-Dessap

Table 1 Patient characteristics

	Group 1 ($n = 42$) Lactate ≥2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio >1.0	Group 2 ($n = 42$) Lactate ≥2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio ≤1.0	Group 3 ($n = 23$) Lactate <2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio >1.0	Group 4 ($n = 28$) Lactate <2.0 mmol/L + Cv-aCO_2/Da-vO_2 ratio ≤1.0	p
Age (years)	68 (59–75)	64 (54–74)	64 (52–79)	67 (51–76)	0.62
APACHE II	25.0 (21.0–34.3)**	24.5 (19.0–29.0)	21.0 (19.0–23.0)**	20.0 (18.0–26.0)	<0.001
SOFA day 1	14.5 (10.0–16.3)**¶	13.0 (9.0–15.0)‡	9.0 (8.0–11.0)**	7.5 (5.8–10.0)‡¶	<0.001
Time between diagnosis to catheter insertion (T0)	3.0 (2.4–4.0)	3.0 (2.5–3.3)	3.0 (2.0–4.0)	3.0 (2.5–4.0)	0.57
Fluids received before catheter insertion	2,100 (1,542–2,818)	1,988 (1,178–3,000)	1,600 (1,200–2,200)	1,550 (900–2,700)	0.32
Temperature (°C)	37.0 (36.1–37.7)	37.0 (37.1–37.9)	37.0 (36.4–37.7)	37.4 (36.8–37.9)	0.23
Source of infection, *n* (%)					
Pneumonia	12	13	8	8	0.48
Abdominal	11	12	5	9	
Urinary	8	8	5	4	
Soft tissue	6	6	2	1	
No specific site	5	3	2	4	
Other	0	0	1	2	
Culture positive, *n* (%)	31 (73.8)	32 (76.2)	18 (78.3)	23 (82.1)	0.45
Antibiotics given at T0, *n* (%)	42 (100)	40 (95.2)	22 (95.7)	28 (100)	0.35
Antibiotics adequate, *n* (%)	42 (100)	40 (95.2)	22 (95.7)	28 (100)	0.35
Steroids, *n* (%)	36 (85.7)**¶	37 (88.1)†	15 (65.2)†	18 (64.3)¶‡	0.02
Vasopressin, *n* (%)	20 (47.6)**¶	23 (54.8)†	3 (13)†	2 (7.1)¶‡	<0.001
Red blood cell transfusion, *n* (%)	12 (28.6)	7 (16.7)	4 (17.4)	6 (21.4)	0.23
Fluids and vasoactive agents					
Fluids, mL (IQR 25–75)					
T0	2,100 (1,542–2,818)	1,988 (1,178–3,000)	1,600 (1,200–2,200)	1,550 (900–2,700)	0.32
T6	3,896 (2,950–5,000)	4,100 (2,826–6,119)†	2,700 (2,150–4,400)†	3,176 (2,100–4,450)	0.02
Norepinephrine, μg/kg/min (IQR 25–75), *n*					
T0	0.32 (0.19–0.54), 38**¶	0.34 (0.18–0.41), 39	0.15 (0.10–0.29), 21**	0.16 (0.07–0.23), 26¶	<0.001
T6	0.36 (0.19–0.62), 38**¶	0.35 (0.17–0.53), 39†‡	0.13 (0.08–0.20), 22**†	0.12 (0.05–0.17), 24¶‡	<0.001
Dobutamine, μg/kg/min (IQR 25–75), *n*					
T0	2.74 (1.38–9.0), 4	3.10 (2.22–8.70), 6	8.56 (5.13–12.00), 5	6.34 (5.23–10.45), 5	0.26
T6	5.30 (4.84–8.12), 12	4.90 (3.33–9.35), 9	6.10 (5.13–12.0), 5	5.95 (2.50–9.20), 6	0.72

Data are presented as median (IQR, i.e., 25–75th percentiles) unless specified otherwise

SOFA Sequential Organ Failure Assessment, *APACHE II* Acute Physiology and Chronic Health Evaluation II

* $p < 0.05$ for groups 1 vs. 2

** $p < 0.05$ for groups 1 vs. 3

¶ $p < 0.05$ for groups 1 vs. 4

† $p < 0.05$ for groups 2 vs. 3

‡ $p < 0.05$ for groups 2 vs. 4

ξ $p < 0.05$ for groups 3 vs. 4

Table 2 Clinical outcomes

Variable	Group 1 ($n = 42$)	Group 2 ($n = 42$)	Group 3 ($n = 23$)	Group 4 ($n = 28$)	p
SOFA day 1	14.5 (10.0–16.3)**¶	13.0 (9.0–15.0)†	9.0 (8.0 –11.0)**	7.5 (5.8–10.0)†¶	<0.001
SOFA day 3	11.0 (8.0–13.0)**¶	8.0 (5.0–11.3)†	5.0 (3.0–9.0)**	3.5 (2.0–6.0)†¶	<0.001
ICU length of stay, days	7.0 (1.0–12.0)	4.5 (2.0–10.0)	6.0 (4.0–8.0)	7.5 (3.0–12.5)	0.44
Ventilator-free days	0 (0–12)*¶**	20 (0–25)‡†	24 (14–26)‡	25 (20–28)†	<0.001
Mortality observed/expected at day 28, n (% inside group)	30 (71.4)/18 (42.9)*¶**	17 (40.5)/18 (42.9)†	9 (39.1)/10 (43.5)^{ξ}	2 (7.1)/12 (42.8)^{¶†ξ}	<0.001

Data are presented as median (25–75th percentiles) except for mortality. ICU length of stay is reported for both alive and dead patients

SOFA Sequential Organ Failure Assessment, *ICU* intensive care unit

* $p < 0.05$ for groups 1 vs. 2

** $p < 0.05$ for groups 1 vs. 3

¶ $p < 0.05$ for groups 1 vs. 4

‡ $p < 0.05$ for groups 2 vs. 3

† $p < 0.05$ for groups 2 vs. 4

ξ $p < 0.05$ for groups 3 vs. 4

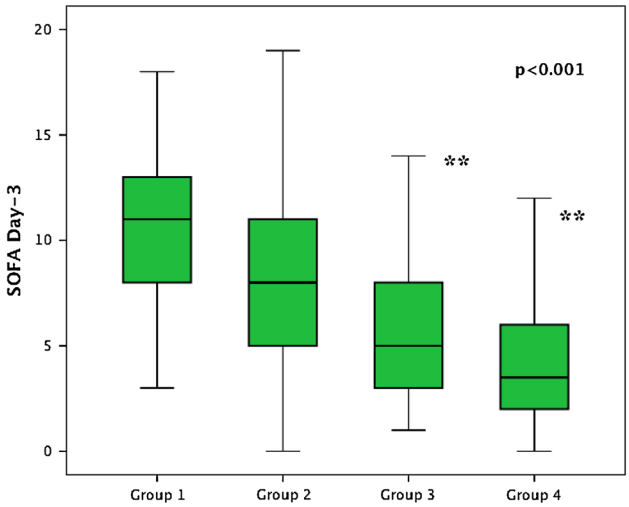


Fig. 1 Sequential Organ Failure Assessment (SOFA) scores at day 3 for predefined groups. Data presented as median (percentiles). Patients were separated into four groups according to lactate and Cv-aCO_2/Da-vO_2 ratio measured after the first 6 h of resuscitation: group 1, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; group 2, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0; group 3, lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; and group 4, lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0. Kruskal–Wallis one-way ANOVA, $p < 0.001$. **$p < 0.01$ by Tukey–Kramer showing differences between groups 1 vs. 3 and 1 vs. 4

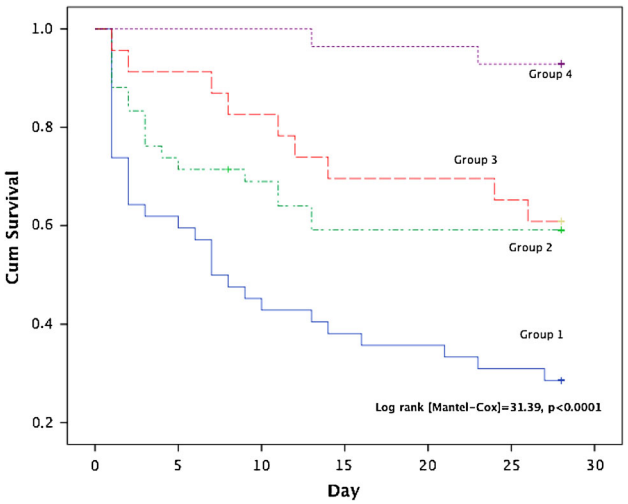


Fig. 2 Survival probabilities up to day 28 according to lactate and Cv-aCO_2/Da-vO_2 after 6 h of resuscitation. Log rank (Mantel–Cox) = 31.39, $p < 0.0001$. Group 1, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; group 2, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0; group 3, lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; and group 4, lactate <2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0

et al. [22] tested the hypothesis that Pv-aCO_2/Da-vO_2 better detects anaerobic metabolism than other parameters derived from PAC measurements in critically ill patients. They found a significant agreement between Pv-aCO_2/Da-vO_2 and lactate levels. However, the agreement between Pv-aCO_2/Da-vO_2 and lactate levels should not necessarily be considered as representative of anaerobic metabolism since hyperlactatemia is not always of hypoxic origin [31, 32]. Interestingly, our data demonstrated that a high Cv-aCO_2/Da-vO_2 might be present with normal or high lactate levels suggesting that these variables evolve independently probably because lactate kinetics can be slower than Cv-aCO_2/Da-vO_2 variations. Thus, in the presence of hyperlactatemia a high Cv-aCO_2/Da-vO_2 may favor anaerobic metabolism as the possible source of lactate, while a normal Cv-aCO_2/Da-vO_2 may suggest that lactate accumulation is due to other causes [33–36].

Searching for other markers of ongoing tissue hypoxia could increase the information given by lactate levels during resuscitation of septic shock. Recently, Rimachi et al. [37] reported the presence of hyperlactatemia in 65 % of patients with septic shock, but only 75 % of these patients exhibited increased lactate/pyruvate ratio, confirming that hyperlactatemia may be not due to hypoxia, especially during the early stages of shock. Consistent with that study, 71 % of the patients in our study had hyperlactatemia at T0 and half of them had an elevated Cv-aCO_2/Da-vO_2. Interestingly, hyperlactatemic patients evolving with a high Cv-aCO_2/Da-vO_2 at T6 (i.e., after initial resuscitation) had a lower VO_2 compared with those evolving with normal Cv-aCO_2/Da-vO_2, despite

Table 3 Multivariate logistic regression for predictors of mortality at day 28

	T0			T6			T6 (for SvO_2 ≥65 %)[a]		
	RR	95 % CI	*p*	RR	95 % CI	*p*	RR	95 % CI	*p*
Cv-aCO_2/Da-vO_2	3.85	1.60–9.27	0.003	3.97	1.54–10.24	0.004	5.71	1.20–27.19	0.03
Lactate, mmol/L	1.19	0.98–1.44	0.09	1.58	1.13–2.22	0.008	2.41	1.22–4.76	0.01
VO_2, mL/min/m^2	1.00	0.98–1.01	0.59	0.99	0.98–1.00	0.24	1.01	0.99–1.02	0.30
DO_2, mL/min/m^2	1.00	0.99–1.00	0.69	1.00	0.99–1.01	0.43	1.00	0.99–1.01	0.67
SvO_2, %	0.97	0.90–1.04	0.35	0.93	0.86–1.01	0.06			
CI, L/min/m^2	0.82	0.44–1.53	0.54	0.94	0.39–2.26	0.89	1.28	0.33–4.96	0.72
APACHE II	1.08	0.98–1.19	0.09	1.03	0.94–1.14	0.54	0.94	0.80–1.10	0.45
Age, years	1.03	0.99–1.06	0.14	1.02	0.98–1.06	0.38	1.10	0.99–1.21	0.06
Time before T0, h	0.62	0.36–1.04	0.07	0.72	0.41–1.27	0.26	0.63	0.29–1.40	0.25
Gender	0.45	0.16–1.27	0.13	0.77	0.24–2.45	0.66	0.15	0.03–0.99	0.05
Fluids, mL	1.00	0.99–1.01	0.84	1.00	1.00–1.01	0.93	1.00	0.99–1.00	0.81
Norepinephrine, μg/kg/min	1.78	0.23–13.94	0.58	0.41	0.06–2.89	0.37	0.25	0.01–7.56	0.42
MAP, mmHg	0.96	0.92–1.01	0.09	0.98	0.92–1.05	0.58	0.98	0.89–1.09	0.71

Cv-CO_2/Da-vO_2 mixed venous-to-arterial carbon dioxide to arterial-venous oxygen content differences ratio, *DO_2* oxygen delivery, *VO_2* oxygen consumption, *SvO_2* mixed-venous oxygen saturation, *CI* cardiac index, *APACHE II* Acute Physiology and Chronic Health Evaluation II, *MAP* mean arterial pressure

[a] Analysis just for patients attaining SvO_2 >65 % at T6

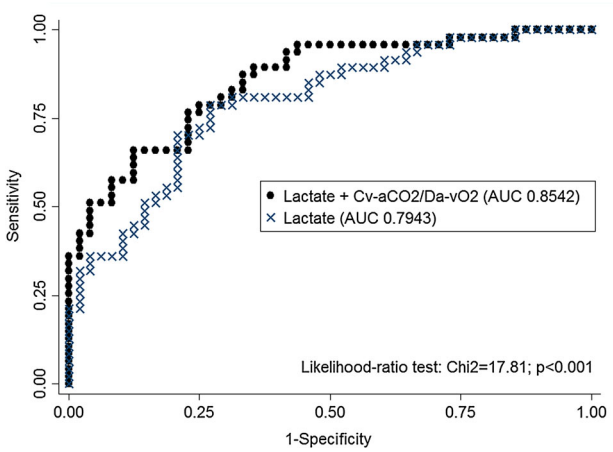


Fig. 3 Receiver operating characteristics (ROC) curves for prediction of mortality at day 28 for models including or not Cv-aCO_2/Da-vO_2 ratio. The "large model" included Cv-aCO_2/Da-vO_2 + lactate levels. The "short model" included lactate levels but not Cv-aCO_2/Da-vO_2. Both models also included oxygen consumption (VO_2), oxygen delivery (DO_2), mixed-venous oxygen saturation (SvO_2), cardiac index, APACHE II, age, time before T0, gender, fluids administered, norepinephrine dose, and mean arterial pressure. Likelihood ratio test, $\chi^2 = 17.81$, $p < 0.001$. Differences between AUCs, *C* statistic, χ^2 4.52, $p = 0.03$

similar DO_2 values. This suggests that a high Cv-aCO_2/Da-vO_2 coupled with hyperlactatemia could identify ongoing VO_2/DO_2 dependence. In agreement with this concept, Monnet et al. [38] recently reported that VO_2 increased after fluid administration only in patients with a pre-fluid high Pv-aCO_2/DavO_2 ratio. In other words, a VCO_2/VO_2 ratio estimated by the Pv-aCO_2/DavO_2 or the Cv-aCO_2/DavO_2 could be used to predict fluid responsiveness at the tissue level.

Intriguingly, both Cv-aCO_2/Da-vO_2 and lactate levels were independent factors determining clinical outcomes, at T0 and T6. As expected, patients with combined increase in Cv-aCO_2/Da-vO_2 and lactate had the worse outcome, while patients with both variables normal had the best outcomes. Interestingly, patients attaining normal lactate levels at T6 but with a high Cv-aCO_2/Da-vO_2 had a similar incidence of multiorgan dysfunction and unfavorable clinical outcomes as hyperlactatemic patients with a Cv-aCO_2/Da-vO_2 ≤1.0. This further emphasizes the additive value of both indices.

Recent human studies suggest that Pv-aCO_2 may identify persistent perfusion derangements in apparently resuscitated septic shock patients [18, 20, 39]. The simplicity of Pv-aCO_2 measurement makes it an attractive tool to guide resuscitation in the clinical setting. However, the Pv-aCO_2 is a physiologically complex measurement, as the relationship between the CO_2 partial pressure (PCO_2) and the CO_2 content (CCO_2) is affected by O_2 saturation, i.e., the Haldane effect [21]. Accordingly, clinical interpretation of the Pv-aCO_2 can be difficult since its increase can be observed in both aerobic and anaerobic conditions.

Another important question is whether Pv-aCO_2/Da-vO_2 can be used as a surrogate of Cv-aCO_2/Da-vO_2. This approach, used by several investigators [22, 38], assumes that CO_2 partial pressure (PCO_2) keeps a quasi-linear relationship with CO_2 content (CCO_2) over the physiological range of PCO_2, i.e., along the steep portion of the CO_2 dissociation curve. However, the relationship between PCO_2 and CCO_2 becomes non-linear if oxygen saturation, arterial-venous pH difference, and/or hemoglobin concentrations change. In this respect, several studies [21, 40] reported dissociation between CCO_2 and PCO_2 in the splanchnic region when CCO_2 decreased in the venous splanchnic effluent while PCO_2 paradoxically increased during increases of splanchnic blood flow. In fact, they showed that venous to arterial PCO_2 differences could increase or decrease for identical blood flow increases. Thus, depending on the basal venous oxygen saturation, the

Haldane effect may cause a decrease or increase in the respective venous to arterial PCO_2 difference in response to the same changes in blood flow and metabolism [40]. Thus, theoretically Cv-aCO_2/Da-vO_2 is not equivalent to Pv-aCO_2/Da-vO_2 especially during low PCO_2 and SvO_2 conditions. Importantly, despite the similarities in the time-course of the Pv-aCO_2/Da-vO_2 and the Cv-aCO_2/Da-vO_2 (ESM Figs. 3 and 4), we did not find significant association with day-28 mortality for the Pv-aCO_2/Da-vO_2 when it was included instead of Cv-aCO_2/Da-vO_2 in the logistic regression (ESM Table 2). An additional analysis simultaneously including the Pv-aCO_2/Da-vO_2 and the Cv-aCO_2/Da-vO_2 demonstrated that despite the former being significantly associated with mortality in the univariate analysis, it was not maintained in the multivariate analysis (ESM Table 3). Nevertheless, despite having ruled out mathematical collinearity between Pv-aCO_2/Da-vO_2 and Cv-aCO_2/Da-vO_2 in the model, it would be debatable to refuse that any collinearity might exist between two variables tightly related. However, we admit that Pv-aCO_2/Da-vO_2 could be equivalent to Cv-aCO_2/Da-vO_2 when PCO_2, pH, and SvO_2 approximate to normality, which occurs frequently. Cv-aCO_2/DavO_2 is an approximation of respiratory quotient, and thus it has a strong physiological meaning. Even though it is more complicated to compute, it is easier to interpret, with values above 1 suggesting anaerobic metabolism. Admittedly, computations of CO_2 content and DavO_2 are cumbersome and subject to an important risk of errors due to the number of variables included in the formulas. Nevertheless, our data suggest that the influence of measurement errors is limited as it correctly identified patients at increased risk of death.

Nowadays critically ill patients admitted to the ICU often exhibit normal or near-normal venous oxygen saturations [11]. Interestingly, when we studied only the patients attaining SvO_2 >65 %, Cv-aCO_2/Da-vO_2 and lactate levels were still independent predictors of outcomes. Thus, Cv-aCO_2/Da-vO_2 could be a useful resuscitation variable in both low and normal SvO_2 conditions.

We acknowledge some limitations of our study. First, both Cv-aCO_2 and Da-vO_2 are global variables and they may not represent regional or local perfusion derangements. Thus, tissue hypoperfusion inducing local CO_2 accumulation may occur even when systemic venous CO_2 remains normal. Second, the Cv-aCO_2 may not increase during conditions of tissue hypoxia associated with high blood flow, even if CO_2 production is increased due to anaerobic metabolism, as venous blood flow may be sufficient to wash out the CO_2 generated by hypoxic cells [41]. In these patients, the combination with lactate levels is useful to overcome this shortcoming. Finally, our observations were restricted to a relatively small sample of septic shock patients and although our results sound biologically plausible, they should be confirmed in future physiological studies to better understand the significance of the Cv-aCO_2/Da-vO_2 ratio during early stages of septic shock.

Conclusion

Combination of Cv-aCO_2/Da-vO_2 and lactate measurements at early stages of resuscitation can identify risk of adverse outcomes in septic shock. The Cv-aCO_2/Da-vO_2 ratio may become a potential resuscitation goal in patients with septic shock.

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Conflicts of interest The authors declare no conflict of interest for the current study.

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References

1. Vincent JL, De Backer D (2013) Circulatory shock. N Engl J Med 369:1726–1734
2. Cecconi M, De Backer D, Antonelli M, Beale R, Bakker J, Hofer C, Jaeschke R, Mebazaa A, Pinsky MR, Teboul JL, Vincent JL, Rhodes A (2014) Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. Intensive Care Med 40:1795–1815
3. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M, Group EG-DTC (2001) Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 345:1368–1377
4. Trzeciak S, Dellinger RP, Abate NL, Cowan RM, Stauss M, Kilgannon JH, Zanotti S, Parrillo JE (2006) Translating research to clinical practice: a 1-year experience with implementing early goal-directed therapy for septic shock in the emergency department. Chest 129:225–232

5. Jones AE, Focht A, Horton JM, Kline JA (2007) Prospective external validation of the clinical effectiveness of an emergency department-based early goal-directed therapy protocol for severe sepsis and septic shock. Chest 132:425–432
6. Shapiro NI, Howell MD, Talmor D, Lahey D, Ngo L, Buras J, Wolfe RE, Weiss JW, Lisbon A (2006) Implementation and outcomes of the Multiple Urgent Sepsis Therapies (MUST) protocol. Crit Care Med 34:1025–1032
7. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R, Subgroup SSCGCiTP (2013) Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 39:165–228
8. Bellomo R, Reade MC, Warrillow SJ (2008) The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. Crit Care 12:130
9. Perel A (2008) Bench-to-bedside review: the initial hemodynamic resuscitation of the septic patient according to Surviving Sepsis Campaign guidelines—does one size fit all? Crit Care 12:223
10. Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F, Filbin MR, Shapiro NI, Angus DC, Investigators P (2014) A randomized trial of protocol-based care for early septic shock. N Engl J Med 370:1683–1693
11. van Beest PA, Hofstra JJ, Schultz MJ, Boerma EC, Spronk PE, Kuiper MA (2008) The incidence of low venous oxygen saturation on admission to the intensive care unit: a multi-center observational study in the Netherlands. Crit Care 12:R33
12. Puskarich MA, Trzeciak S, Shapiro NI, Heffner AC, Kline JA, Jones AE, Emergency Medicine Shock Research Network (EMSHOCKNET) (2011) Outcomes of patients undergoing early sepsis resuscitation for cryptic shock compared with overt shock. Resuscitation 82:1289–1293
13. Shapiro NI, Howell MD, Talmor D, Nathanson LA, Lisbon A, Wolfe RE, Weiss JW (2005) Serum lactate as a predictor of mortality in emergency department patients with infection. Ann Emerg Med 45:524–528
14. Puskarich MA, Trzeciak S, Shapiro NI, Albers AB, Heffner AC, Kline JA, Jones AE (2013) Whole blood lactate kinetics in patients undergoing quantitative resuscitation for severe sepsis and septic shock. Chest 143:1548–1553
15. Jansen TC, van Bommel J, Schoonderbeek FJ, Sleeswijk Visser SJ, van der Klooster JM, Lima AP, Willemsen SP, Bakker J, group Ls (2010) Early lactate-guided therapy in intensive care unit patients: a multicenter, open-label, randomized controlled trial. Am J Respir Crit Care Med 182:752–761
16. Nguyen HB, Kuan WS, Batech M, Shrikhande P, Mahadevan M, Li CH, Ray S, Dengel A, Investigators AANtRSc (2011) Outcome effectiveness of the severe sepsis resuscitation bundle with addition of lactate clearance as a bundle item: a multi-national evaluation. Crit Care 15:R229
17. Jones AE, Shapiro NI, Trzeciak S, Arnold RC, Claremont HA, Kline JA, Investigators EMSRNE (2010) Lactate clearance vs central venous oxygen saturation as goals of early sepsis therapy: a randomized clinical trial. JAMA 303:739–746
18. van Beest PA, Lont MC, Holman ND, Loef B, Kuiper MA, Boerma EC (2013) Central venous-arterial PCO_2 difference as a tool in resuscitation of septic patients. Intensive Care Med 39:1034–1039
19. Vallet B, Pinsky MR, Cecconi M (2013) Resuscitation of patients with septic shock: please "mind the gap"! Intensive Care Med 39:1653–1655
20. Ospina-Tascón GA, Bautista-Rincón DF, Umaña M, Tafur JD, Gutiérrez A, García AF, Bermúdez W, Granados M, Arango-Dávila C, Hernández G (2013) Persistently high venous-to-arterial carbon dioxide differences during early resuscitation are associated with poor outcomes in septic shock. Crit Care 17:R294
21. Jakob SM, Kosonen P, Ruokonen E, Parviainen I, Takala J (1999) The Haldane effect—an alternative explanation for increasing gastric mucosal PCO_2 gradients? Br J Anaesth 83:740–746
22. Mekontso-Dessap A, Castelain V, Anguel N, Bahloul M, Schauvliege F, Richard C, Teboul JL (2002) Combination of venoarterial PCO_2 difference with arteriovenous O_2 content difference to detect anaerobic metabolism in patients. Intensive Care Med 28:272–277
23. Horan TC, Andrus M, Dudeck MA (2008) CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 36:309–332
24. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G, SCCM, ESICM, ACCP, ATS, SIS (2003) 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 31:1250–1256
25. Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM, Sprung CL, Colardyn F, Blecher S (1998) Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 26:1793–1800
26. Douglas AR, Jones NL, Reed JW (1985) Calculation of whole blood CO_2 content. J Appl Physiol 65:473–477
27. Austin WH, Lacombe E, Rand PW, Chatterjee M (1963) Solubility of carbon dioxide in serum from 15 to 38 C. J Appl Physiol 18:301–304
28. DeLong ER, DeLong DM, Clarke-Pearson DL (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 44:837–845
29. Groeneveld AB, Vermeij CG, Thijs LG (1991) Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end-expiratory pressure in the pig. Anesth Analg 73:576–582
30. Dubin A, Murias G, Estenssoro E, Canales H, Sottile P, Badie J, Barán M, Rossi S, Laporte M, Pálizas F, Giampieri J, Mediavilla D, Vacca E, Botta D (2000) End-tidal CO_2 pressure determinants during hemorrhagic shock. Intensive Care Med 26:1619–1623
31. James JH, Luchette FA, McCarter FD, Fischer JE (1999) Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. Lancet 354:505–508
32. Levraut J, Ciebiera JP, Chave S, Rabary O, Jambou P, Carles M, Grimaud D (1998) Mild hyperlactatemia in stable septic patients is due to impaired lactate clearance rather than overproduction. Am J Respir Crit Care Med 157:1021–1026

33. Severin PN, Uhing MR, Beno DW, Kimura RE (2002) Endotoxin-induced hyperlactatemia results from decreased lactate clearance in hemodynamically stable rats. Crit Care Med 30:2509–2514
34. De Backer D, Creteur J, Zhang H, Norrenberg M, Vincent JL (1997) Lactate production by the lungs in acute lung injury. Am J Respir Crit Care Med 156:1099–1104
35. Vallet B, Teboul JL, Cain S, Curtis S (2000) Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. J Appl Physiol 89:1317–1321
36. Nevière R, Chagnon JL, Teboul JL, Vallet B, Wattel F (2002) Small intestine intramucosal PCO(2) and microvascular blood flow during hypoxic and ischemic hypoxia. Crit Care Med 30:379–384
37. Rimachi R, Bruzzi de Carvahlo F, Orellano-Jimenez C, Cotton F, Vincent JL, De Backer D (2012) Lactate/pyruvate ratio as a marker of tissue hypoxia in circulatory and septic shock. Anaesth Intensive Care 40:427–432
38. Monnet X, Julien F, Ait-Hamou N, Lequoy M, Gosset C, Jozwiak M, Persichini R, Anguel N, Richard C, Teboul JL (2013) Lactate and venoarterial carbon dioxide difference/arterial-venous oxygen difference ratio, but not central venous oxygen saturation, predict increase in oxygen consumption in fluid responders. Crit Care Med 41:1412–1420
39. Vallée F, Vallet B, Mathe O, Parraguette J, Mari A, Silva S, Samii K, Fourcade O, Genestal M (2008) Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? Intensive Care Med 34:2218–2225
40. Hurley R, Mythen MG (2000) The Haldane effect—an explanation for increasing gastric mucosal PCO_2 gradients? Br J Anaesth 85:167–169
41. Dubin A, Estenssoro E, Murias G, Pozo MO, Sottile JP, Barán M, Piacentini E, Canales HS, Etcheverry G (2004) Intramucosal-arterial PCO_2 gradient does not reflect intestinal dysoxia in anemic hypoxia. J Trauma 57:1211–1217

PAPER No. 4 (Original paper)

CAN VENOUS-TO-ARTERIAL CARBON DIOXIDE DIFFERENCES REFLECT MICROCIRCULATORY ALTERATIONS IN PATIENTS WITH SEPTIC SHOCK?

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Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock?

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Take-home message: Pv-aCO_2 can reflect microcirculatory blood flow alterations during early stages of resuscitation in septic shock.

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Abstract *Purpose:* Septic shock has been associated with microvascular alterations and these in turn with the development of organ dysfunction. Despite advances in video microscopic techniques, evaluation of microcirculation at the bedside is still limited. Venous-to-arterial carbon dioxide difference (Pv-aCO_2) may be increased even when venous O_2 saturation (SvO_2) and cardiac output look normal, which could suggests microvascular derangements. We sought to evaluate whether Pv-aCO_2 can reflect the adequacy of microvascular perfusion during the early stages of resuscitation of septic shock. *Methods:* Prospective observational study including 75 patients with septic shock in a 60-bed mixed ICU. Arterial and mixed-venous blood gases and hemodynamic variables were obtained at catheter insertion (T0) and 6 h after (T6). Using a sidestream dark-field device, we simultaneously acquired sublingual microcirculatory images for blinded semiquantitative analysis. Pv-aCO_2 was defined as the difference between mixed-venous and arterial CO_2 partial pressures. *Results:* Progressively lower percentages of small perfused vessels (PPV), lower functional capillary density, and higher heterogeneity of microvascular blood flow were observed at higher Pv-aCO_2 values at both T0 and T6. Pv-aCO_2 was significantly correlated to PPV (T0: coefficient −5.35, 95 % CI −6.41 to −4.29, $p < 0.001$; T6: coefficient, −3.49, 95 % CI −4.43 to −2.55, $p < 0.001$) and changes in Pv-aCO_2 between T0 and T6 were significantly related to changes in PPV ($R^2 = 0.42$, $p < 0.001$). Absolute values and changes in Pv-aCO_2 were not related to global hemodynamic variables. Good agreement between venous-to-arterial CO_2 and PPV was maintained even after corrections for the Haldane effect. *Conclusions:* During early phases of resuscitation of septic shock, Pv-aCO_2 could reflect the adequacy of microvascular blood flow.

Keywords Septic shock · Venous-to-arterial carbon dioxide difference · Microcirculation · Microcirculatory blood flow

Introduction

Septic shock remains associated with high mortality and early recognition of signs of tissue hypoperfusion is crucial in its management [1, 2]. The usefulness of oxygen-derived parameters as resuscitation targets has been strongly questioned [3] and recent data have failed to demonstrate clinical benefits [4–6]. Indeed, venous oxygen saturation (SvO_2) is often normal or near normal at admission to ICU [7]. Hence, other resuscitation goals such as venous-to-arterial carbon dioxide difference (Pv-aCO_2) have been proposed owing to their simplicity and ability to predict adverse clinical outcomes in patients achieving normal oxygen-derived parameters during the early phases of resuscitation of septic shock [8–10]. Nevertheless, the mechanisms implicated in the elevation of Pv-aCO_2 during inflammatory states are not completely understood and interpretation of Pv-aCO_2 values during resuscitation of septic shock could sometimes be difficult.

In cardiac arrest Pv-aCO_2 increases as a result of vascular stagnation [11, 12]. Similarly, Pv-aCO_2 and cardiac output are inversely related in experimental models of hemorrhagic, hypovolemic, and obstructive shock, highlighting the importance of blood flow in increasing venous CO_2 [13–16]. Conversely, agreement between cardiac output variations and Pv-aCO_2 during septic shock is weak [17, 18], suggesting that macrohemodynamic alterations cannot explain such Pv-aCO_2 variations [8, 9]. Alternatively, Pv-aCO_2 could be related to tissue oxygenation derangements or oxygen consumption capabilities [13, 14], but this concept was questioned when others demonstrated that ischemic but not hypoxic or anemic hypoxia leads to Pv-aCO_2 increments [19, 20]. Hence, physiological variables different to macrohemodynamics and tissue dysoxia should be associated with variations of Pv-aCO_2 gradients during septic shock.

Microcirculatory derangements such as decreases in functional capillary density (FCD) and increased heterogeneity of blood flow are common findings in patients with severe sepsis and septic shock [21, 22], even when global oxygen parameters seem adequate. Importantly, these abnormalities might be associated with the development of multiple organ dysfunction and death [23]. Nevertheless, despite improvements in techniques evaluating microcirculation at the bedside, immediate and reliable quantification of microcirculatory derangements is still limited. Thus, surrogates reflecting adequacy of microvascular blood flow might be highly valuable.

Tissue CO_2 accumulation has been related to microcirculatory alterations [24, 25]. However, the relationship between Pv-aCO_2 and microcirculatory derangements during human septic shock has not been widely studied. Since Pv-aCO_2 has been related to blood flow variations, but cardiac output seems insufficient to explain venous CO_2 accumulation during septic shock [8, 9], we aimed to test the hypothesis that Pv-aCO_2 can reflect the adequacy of microvascular blood flow during the early stages of resuscitation in patients with septic shock.

Materials and methods

This prospective observational study was conducted in a 60-bed mixed ICU at a university hospital (Fundación Valle del Lili, Cali, Colombia) during 15 consecutive months (April 2012 to July 2013). We received approval from the Fundación Valle del Lili's ethical and biomedical research committee (protocol number 563, approval number 091-2012). The original version of the protocol is attached as Electronic Supplementary Material 2 (ESM 2). Written informed consent was waived as no new or invasive procedures were conducted for the study purposes. However, in all cases the patients and/or their relatives were informed about the study and were given the opportunity to refuse the use of their data. Infection was defined according to the Centers for Diseases Control and Prevention criteria [26], whereas septic shock followed the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [27]. All consecutive patients fulfilling diagnostic criteria of septic shock and elected by the attending physician to use a pulmonary artery catheter (PAC) within the 6 h from the first hypotensive episode were included in the study. The decision to use a PAC was exclusively taken by the attending physician according to the local protocols for hemodynamic monitoring. We did not include patients with a preceding episode of septic shock within the last 3 months, patients younger than 18 years old, pregnant women, patients with limitation of therapeutic effort orders, or severe chronic obstructive pulmonary disease. Patients using cardiac output monitoring systems other than PAC were not included. For assessment of microcirculation, we did not include patients who were under noninvasive mechanical ventilation or breathing with a face mask with high FiO_2, or patients who were agitated or not collaborative when we first attempted to visualize sublingual microcirculation.

A STROBE statement checklist for observational studies is provided in ESM Table E4.

General management

Patients were managed according to an early resuscitation protocol modified from the Surviving Sepsis Campaign [28], with the aim of achieving (1) mean arterial pressure ≥65 mmHg; (2) urine output ≥0.5 ml/kg/min; (3) SvO_2 ≥65 %; (4) normalization of lactate levels. Repeated fluid loads with crystalloids and/or albumin 4 % guided by

dynamic predictors of fluid responsiveness were performed in order to optimize cardiac preload. Dynamic predictors of fluid response were preferred over static parameters (i.e., pulmonary artery occlusion pressure or central venous pressure). However, when the use of dynamic predictors was not appropriate, cardiac filling pressures and clinical judgment were used to guide fluid administration. We did not use hydroxyethyl starches (HES). Norepinephrine was titrated to maintain mean arterial pressure (MAP) >65 mmHg. Low doses of vasopressin titrated up to maximum of 0.03 IU/min were allowed to increase MAP or to decrease norepinephrine requirements but never as a single vasopressor. Dobutamine was titrated up to 20 μg/kg/min when SvO_2 goals were not achieved after fluid load optimization and normalization of MAP. Mechanical ventilation support was provided when needed under a targeted sedation and analgesia protocol with midazolam and fentanyl

Table 1 General characteristics

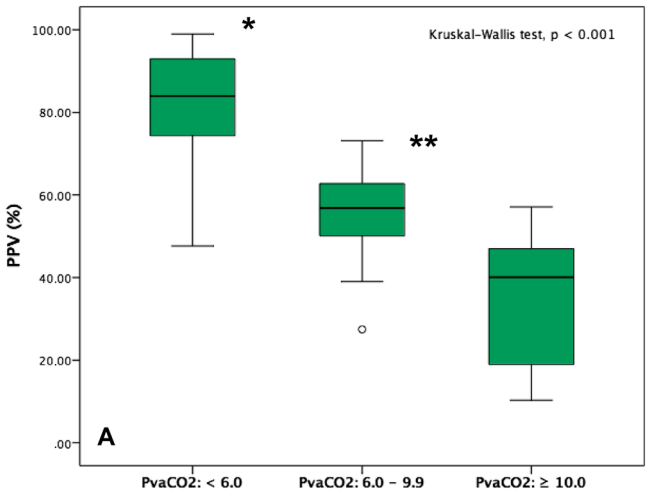
	All patients ($n = 75$)	Pv-aCO_2 <6.0 ($n = 39$)	Pv-aCO_2 = 6.0–9.9 ($n = 25$)	Pv-aCO_2 ≥10.0 ($n = 11$)	p
Age (years)	67 (58–77)	66 (60–74)	73 (65–81)	54 (43–71)*	0.02
Male (%)	45 (60)	23 (59)	15 (60)	7 (64)	0.96
APACHE II	25 (20–32)	24 (20–28)	28 (20–34)	25 (20–34)	0.55
SOFA, day 1	11 (8–15)	10 (6–15)	11 (9–15)	11 (10–14)	0.22
Time from first hypotension episode to catheter insertion (h)	3 (3–4)	3 (3–4)	4 (2–4)	3 (2–4)	0.69
Fluids before catheter insertion (ml)	1650 (1200–2400)	1600 (1200–2400)	1600 (1025–2300)	2000 (1500–2700)	0.66
Temperature (°C)	37.5 (37.4–38.4)	37.4 (37.2–38.2)	37.8 (37.6–38.4)	37.6 (37.5–38.6)	0.54
Source of infection, *n* (%)					0.25
Pneumonia	30 (40.0)	18 (46.2)	10 (40.0)	2 (18.2)	
Abdominal	17 (22.7)	8 (20.5)	7 (28.0)	2 (18.2)	
Urinary	10 (13.3)	4 (10.3)	3 (12.0)	3 (27.3)	
Soft tissue	2 (2.7)	2 (5.1)	0 (0.0)	0 (0.0)	
No specific site	7 (9.3)	1 (2.6)	3 (12.0)	3 (27.3)	
Other	9 (12.0)	6 (15.4)	2 (8.0)	1 (9.1)	
Medical, *n* (%)/surgical, *n* (%)	48 (64.0)/27 (36.0)	25 (64.1)/14 (35.9)	15 (60.0)/10 (40.0)	8 (72.7)/3 (27.3)	0.76
Mechanical ventilation, *n* (%)	57 (76.0)	34 (87.2)	24 (96.0)	9 (81.8)	0.37
Renal replacement therapy, *n* (%)	12 (16.0)	7 (17.9)	3 (12.0)	2 (18.2)	0.80

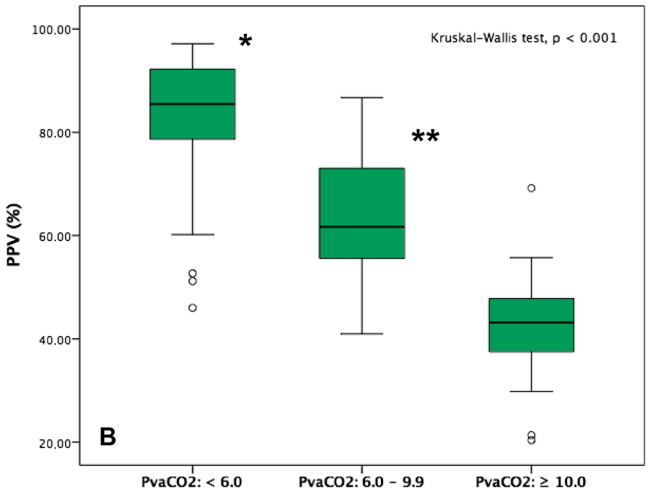
Values represent median (25–75th range) or absolute number (%) when indicated
* $p < 0.05$ for Pv-aCO_2 ≥10.0 vs. Pv-aCO_2 = 6.0–9.9

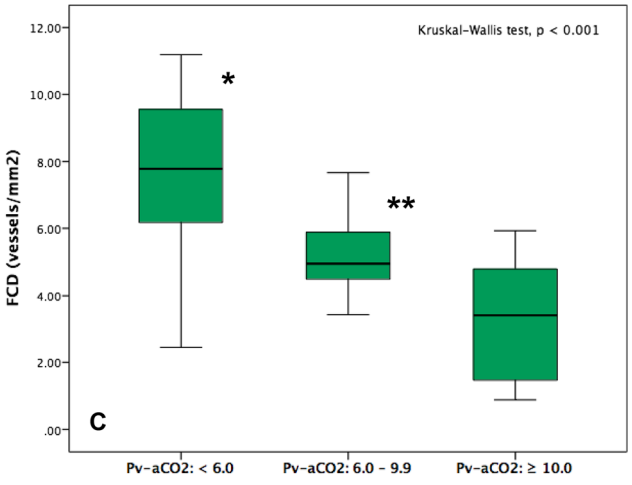
Table 2 Microcirculatory blood flow variables for the predefined Pv-aCO_2 groups

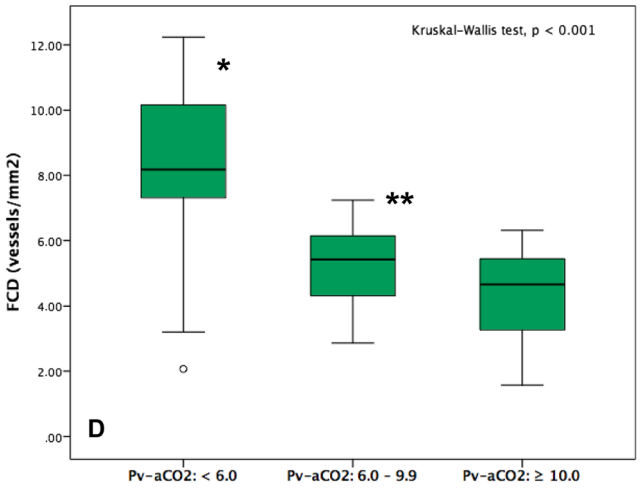
	Pv-aCO_2 <6.0 ($n_{T0} = 39$) ($n_{T6} = 36$)	Pv-aCO_2 = 6.0–9.9 ($n_{T0} = 25$) ($n_{T6} = 23$)	Pv-aCO_2 ≥10.0 ($n_{T0} = 11$) ($n_{T6} = 13$)
PPV, %			
T0	83.9 (74.2–93.2)*†	56.8 (47.9–62.9)‡	40.1 (27.5–48.7)
T6	85.5 (77.8–92.3)*†	61.7 (54.5–74.5)‡	43.2 (33.7–49.6)
LPV, %			
T0	100 (97.0–100)*†	96.0 (91.2–100)	90.0 (80.0–100)
T6	100 (96.0–100)†	96.4 (91.7–100)	92.5 (72.3–98.8)
MFI			
T0	2.4 (2.1–2.9)*†	1.6 (1.2–1.9)	1.4 (1.1–1.9)
T6	2.4 (1.8–2.8)*†	1.9 (1.4–2.3)‡	1.3 (1.0–1.7)
TCD, n/mm^2			
T0	11.5 (10.3–12.5)†	10.8 (10.4–11.4)	10.2 (9.3–11.3)
T6	11.5 (10.3–13.3)*	10.1 (8.9–10.8)	10.8 (9.8–11.6)
FCD, n/mm^2			
T0	7.8 (6.2–9.6)*†	4.9 (4.4–5.9)‡	3.4 (1.4–4.9)
T6	8.2 (7.2–10.2)*†	5.4 (4.2–6.2)‡	4.7 (3.2–5.6)
Heterogeneity index			
T0	0.24 (0.10–0.51)*†	0.46 (0.33–0.63)	0.57 (0.29–1.04)
T6	0.15 (0.11–0.28)*†	0.52 (0.21–0.84)	0.54 (0.37–0.80)

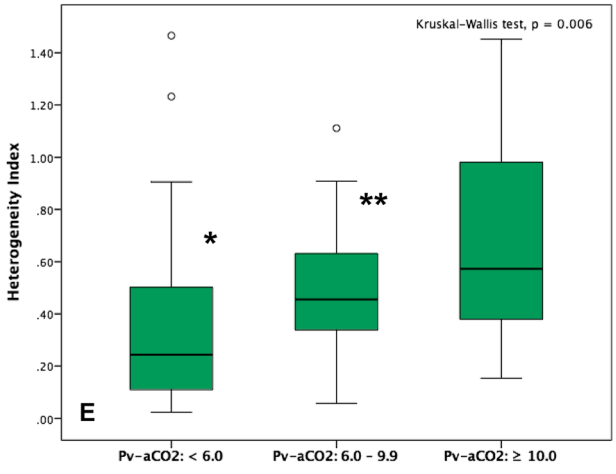
PPV percentage of small perfused vessels, *LPV* percentage of large perfused vessels, *MFI* microvascular flow index, *TCD* total capillary density, *FCD* functional capillary density
* $p < 0.05$ Pv-aCO_2 <6.0 vs. Pv-aCO_2 = 6.0–9.9
† $p < 0.05$ Pv-aCO_2 <6.0 vs. Pv-aCO_2 ≥10.0
‡ $p < 0.05$ Pv-aCO_2 = 6.0–9.9 vs. Pv-aCO_2 ≥10.0

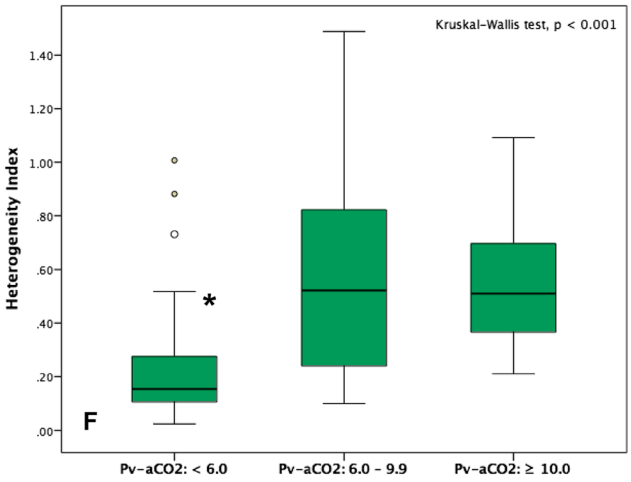

Kruskal-Wallis test, p < 0.001
PPV (%)
100.00
80.00
60.00
40.00
20.00
.00
*
**
A
PvaCO2: < 6.0
PvaCO2: 6.0 - 9.9
PvaCO2: ≥ 10.0


Kruskal-Wallis test, p < 0.001
PPV (%)
100.00
80.00
60.00
40.00
20.00
*
**
B
PvaCO2: < 6.0
PvaCO2: 6.0 - 9.9
PvaCO2: ≥ 10.0


Kruskal-Wallis test, p < 0.001
FCD (vessels/mm2)
12.00
10.00
8.00
6.00
4.00
2.00
.00
*
**
C
Pv-aCO2: < 6.0
Pv-aCO2: 6.0 - 9.9
Pv-aCO2: ≥ 10.0


Kruskal-Wallis test, p < 0.001
FCD (vessels/mm2)
12.00
10.00
8.00
6.00
4.00
2.00
.00
*
**
D
Pv-aCO2: < 6.0
Pv-aCO2: 6.0 - 9.9
Pv-aCO2: ≥ 10.0


Kruskal-Wallis test, p = 0.006
Heterogeneity Index
1.40
1.20
1.00
.80
.60
.40
.20
.00
*
**
E
Pv-aCO2: < 6.0
Pv-aCO2: 6.0 - 9.9
Pv-aCO2: ≥ 10.0


Kruskal-Wallis test, p < 0.001
Heterogeneity Index
1.40
1.20
1.00
.80
.60
.40
.20
.00
*
F
Pv-aCO2: < 6.0
Pv-aCO2: 6.0 - 9.9
Pv-aCO2: ≥ 10.0

◀ **Fig. 1** Percentage of small vessels perfused (PPV), functional capillary density (FCD), and heterogeneity index (HI) for the predefined Pv-aCO_2 groups. *Box plots* depicting differences in PPV, FCD, and HI for predefined Pv-aCO_2 groups (group 1, <6.0 mmHg; group 2, 6.0–9.9 mmHg; group 3, ≥10 mmHg) at both T0 (**a**) and T6 (**b**). Kruskal–Wallis test, $p < 0.001$. *Post hoc Mann–Whitney analysis adjusted for multiple comparisons; $p < 0.05$ for group 1 vs. 2 and 1 vs. 3, **post hoc Mann–Whitney analysis adjusted for multiple comparisons; $p < 0.05$ for group 2 vs. 3. *Boxes* denote interquartile range, *horizontal line* in the *boxes* represents the median values, and *whiskers* extend 1.5 times the interquartile range above and below the 25th and 75th percentiles. *PPV* percentage of small vessels perfused, *Pv-aCO_2* venous-to-arterial carbon dioxide difference, *FCD* functional capillary density, *HI* heterogeneity index

according to the local procedures. Use of low dose hydrocortisone (up to 200 mg/day) was indicated in the case of persistence of vasopressor requirement despite ensuring an adequate intravascular volume. Glycemic control, venous thrombosis prophylaxis, and gastric ulcer stress prevention were provided according to Surviving Sepsis Guidelines [28].

Study protocol

All potentially eligible patients from the emergency room or clinical/surgical wards were screened by our "rapid response team" (which was available 24/7) and promptly transferred to the ICU where attending physicians decided on the type of hemodynamic monitoring needed. Those equipped with PAC monitoring were consecutively selected for inclusion. Time 0 (T0) was set when the PAC was inserted. Arterial and mixed venous blood samples were drawn for gases analysis (ABL300, Radiometer; Copenhagen, Denmark) at T0 and 6 h after (T6). We defined Pv-aCO_2 as the difference between mixed-venous and arterial CO_2 partial pressures. Complete hemodynamic and respiratory parameters, inotropic/vasopressor doses, and volume of resuscitation fluids were also registered at these points. Simultaneously, sublingual microcirculation images were also recorded and stored under a random number for ulterior analysis [29].

Microcirculatory measurements

We used a sidestream dark-field (SDF) imaging device (MicroScan; MicroVision Medical, Amsterdam, the Netherlands) to explore microcirculation at T0 and T6. The SDF device was softly applied to the lateral side of the tongue covering an area of approximately 2.5–4 cm from the tip of tongue after gentle removal of secretions with gauze. At each measurement time, we recorded five sequences of video of 20 s each from different adjacent mucosa areas using a video card (Micro Video; Pinnacle system, Mountain Views, CA, USA). These sequences of video were stored under a random number and later analyzed by two investigators blinded to the origin of sequences (J.D.V. and G.O.T.). Vessels were classified as large or small using a cutoff value of 20 μm. Microvessels with continuous flow were considered as normal, whereas sluggish, intermittent, and stopped flows were considered as abnormal. In accordance with the consensus for the evaluation of microcirculation [29], we calculated the proportion of small perfused vessels (<20 μm diameter), the microvascular flow index (MFI), the heterogeneity index (HI), the total vascular density (all vessels), and the FCD (vessels <20 μm diameter). The intra- and interobserver variability were determined by using five sequences analyzed five times at 8-week intervals by two observers (J.D.V. and G.O.T.). We calculated the intra- and interobserver coefficient of variability for both the total number of vessels and the proportion of perfused vessels. An expanded description of image acquisition and analysis is provided in the ESM.

Outcome measures

The primary outcome was the agreement between Pv-aCO_2 and PPV at T0 and T6. Secondary outcomes included the relationships between Pv-aCO_2 and hemodynamic systemic variables, and the relationships between changes in Pv-aCO_2 and changes in cardiac output and PPV observed between T0 and T6.

Statistics

As the primary hypothesis has not been tested before, it was difficult to compute a sample size. On the basis of previous data from our department [8], we estimated that 75 patients would provide a good range of dispersion of Pv-aCO_2 and PPV for looking at relationship between variables.

Distribution of data was tested using the Kolmogorov–Smirnoff test. We explored the relationship between microcirculatory and systemic hemodynamic variables using the Spearman rho test and calculating the coefficient of determination (R^2) to establish the strength of such associations. The distribution of microcirculatory variables, systemic hemodynamics, blood gases, and oxygen/tissue perfusion parameters at T0 and T6 was also evaluated for three predefined groups classified according to the Pv-aCO_2 range values in agreement with previous observations [8]: (1) <6.0 mmHg (2) 6.0–9.9 mmHg, and (3) ≥10 mmHg. Differences among groups were assessed using the Kruskal–Wallis test with post hoc Mann–Whitney analysis with adjustment for multiple comparisons.

We calculated the delta of variation of Pv-aCO_2, PPV, and cardiac outputs between T0 and T6. Spearman rho test and R^2 were used to evaluate the agreement and

strength between Pv-aCO_2 vs. PPV, Pv-aCO_2 vs. cardiac output, and PPV vs. cardiac output variations.

A multiple linear regression model was used to determinate the association between the Pv-aCO_2, cardiac index, SvO_2, and mean arterial pressure with the percentage of small perfused vessels (PPV). Subsequent multiple linear regression models were constructed to test the relation between Pv-aCO_2 and the other systemic resuscitation goals with PPV in patients with SvO_2 <65 % or ≥65 %. The goodness of fit of each model was evaluated using the coefficient of determination. Through the Spearman rho test and calculating R^2, we also evaluated the agreement between PPV and Pv-aCO_2, venous-to-arterial carbon dioxide content (Cv-aCO_2), and venous-to-arterial carbon dioxide content to arterial-to-venous oxygen content ratio (Cv-aCO_2/Da-vO_2).

Receiver operating characteristics (ROC) curves were constructed to compare the accuracy of Pv-aCO_2, lactate, SvO_2, and cardiac output in predicting altered PPV at different abnormal cutoff points (PPV50, PPV60, and PPV70 %). Areas under the ROC curves (AUCs) and their respective 95 % confidence intervals (CIs) were calculated and compared using non-parametric statistics. Data are presented as median [percentiles 25–75]. A p value of 0.05 or less (2-tailed) was considered significant.

Results

A total of 75 patients fulfilling the inclusion criteria were included in the study (ESM Fig. E1). General characteristics according to predefined Pv-aCO_2 groups are presented in Table 1. Mortality at day 28 in this cohort was 34.7 % and the ICU length of stay was 6.0 (2.0–10.0) days. Time elapsed from first hypotension episode until PAC insertion and blood sampling (i.e., T0) was 3.0 (3.0–4.0) h and amount of fluids received at that point was 1650 (1200–2400) ml.

A total of 39, 25, and 11 patients were assigned to the three predefined Pv-aCO_2 groups at T0, and 36, 23, and 13 at T6, respectively. Of these, 49/75 patients (65.3 %) had SvO_2 >65 % at T0 and 58/72 (82.9 %) attained a SvO_2 >65 % at T6. Hemodynamic and respiratory variables, arterial and mixed-venous blood gases, and oxygen-derived parameters for the predefined Pv-aCO_2 groups at both T0 and T6 are shown in ESM Table E1.

Microcirculatory blood flow parameters are shown in Table 2. We observed a progressively lower PPV with a subsequent decrease in FCD and increased HI at progressively higher Pv-aCO_2 values at both T0 and T6 (Fig. 1). Microcirculatory blood flow index (MFI) was also lower at higher Pv-aCO_2 values while total capillary density was normal only in patients with normal Pv-aCO_2 (ESM Fig. E2). We observed a significant agreement between the Pv-aCO_2 and PPV (T0: $R^2 = 0.61$, $p < 0.001$; T6: $R^2 = 0.55$, $p < 0.001$; ESM Fig. E3). Conversely, we found lack of agreement between the cardiac index (representing the macro blood flow) and the PPV (ESM Fig. E4). Other resuscitation and hemodynamic goals such as SvO_2 and MAP were also poorly correlated to the microcirculatory blood flow (ESM Figs. E5, E6).

Changes in Pv-aCO_2 values between T0 and T6 were significantly related to changes in PPV, whereas changes in cardiac output were not related to changes in Pv-aCO_2 or PPV (Fig. 2, ESM Fig. E7).

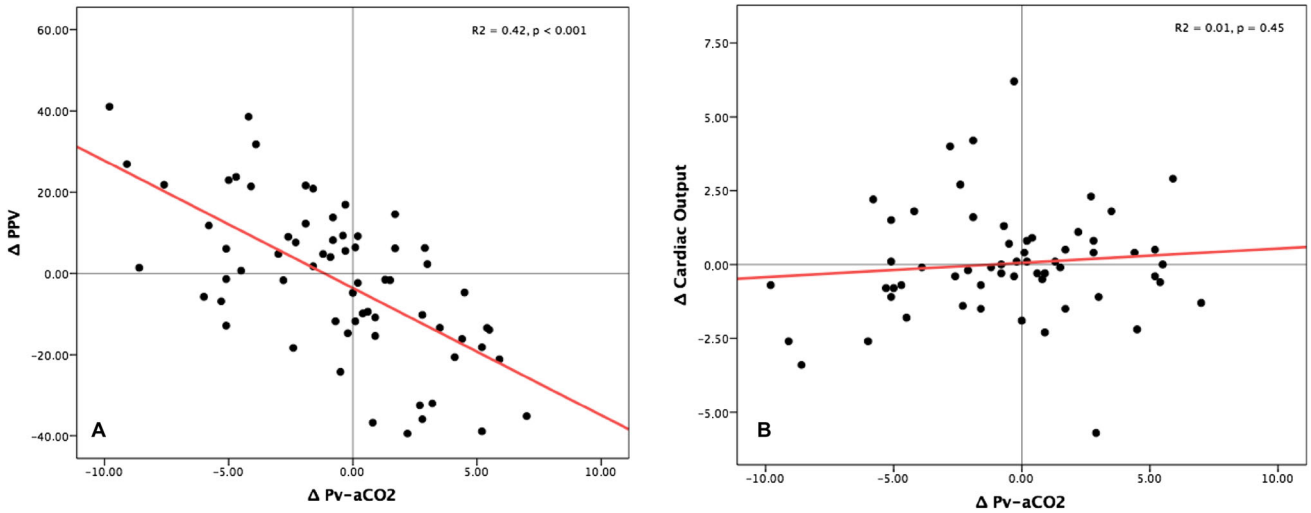


Fig. 2 Scatter plots showing the correlation of variations observed between changes in venous-to-arterial CO_2 partial pressure differences (Δ Pv-aCO_2) and **a** changes in percentage of small vessels perfused (Δ PPV) between measurements performed at T0 and T6 ($R^2 = 0.42$, $p < 0.001$) and **b** changes in cardiac output (Δ cardiac output) between measurements performed at T0 and T6 ($R^2 = 0.01$, $p = 0.45$)

Table 3 Multiple linear regression models for variables related to the percentage of small perfused vessels (PPV)

	T0				T6			
	Coefficient	95 % CI		*p* value	Coefficient	95 % CI		*p* value
All patients								
Pv-aCO$_2$	−5.35	−6.41	−4.29	<0.001	−3.49	−4.43	−2.55	<0.001
SvO$_2$, %	0.12	−0.16	0.41	0.39	0.05	−0.25	0.35	0.75
CI, L/min	−0.83	−3.47	1.81	0.53	−0.11	−4.29	4.06	0.96
MAP	0.23	−0.04	0.51	0.93	0.30	−0.04	0.64	0.08
R^2 for the model	–	–	–	0.66	–	–	–	0.58
SvO$_2$ ≥65 %								
Pv-aCO$_2$	−5.11	−6.46	−3.76	<0.001	−3.92	−5.02	−2.82	<0.001
CI, L/min	−0.31	−3.53	2.91	0.85	2.27	−2.48	7.02	0.34
MAP	0.32	−0.03	0.63	0.06	0.19	−0.21	0.59	0.34
R^2 for the model	–	–	–	0.63	–	–	–	0.61
SvO$_2$ <65 %								
Pv-aCO$_2$	−5.91	−7.63	−4.19	<0.001	−3.22	−5.39	−1.05	0.007
CI, L/min	−2.18	−7.37	3.01	0.39	−3.68	−13.43	6.07	0.43
MAP	−0.07	−0.65	0.52	0.82	0.39	−0.35	1.13	0.28
R^2 for the model	–	–	–	0.71	–	–	–	0.57

Pv-aCO$_2$ venous to arterial carbon dioxide difference, *SvO$_2$* mixed venous oxygen saturation, *CI* cardiac index, *MAP* mean arterial pressure, R^2 coefficient of determination

A multiple linear regression model including all patients revealed that Pv-aCO$_2$ was independently related to PPV at T0 and T6, whereas other systemic hemodynamic parameters such as cardiac output, SvO$_2$, or MAP were not significantly related to microcirculatory blood flow (Table 3). Likewise, when linear regression models were performed separately in patients with SvO$_2$ <65 % or SvO$_2$ ≥65 %, Pv-aCO$_2$ was again the only variable related to PPV at both T0 and T6 (Table 3).

Even though Pv-aCO$_2$ was apparently not correlated with cardiac index in this septic shock group (ESM Fig. E8), we observed lower cardiac indexes in predefined groups with abnormal Pv-aCO$_2$ values at T6 (ESM Fig. E9). Similarly, lower MAP was observed in groups with higher Pv-aCO$_2$ values at T6 (ESM Fig. E10). We also observed a trend towards lower SvO$_2$ at higher Pv-aCO$_2$ values, without achieving significant differences (ESM Fig. E11).

Pv-aCO$_2$ exhibited the highest area under the ROC curve (AUC) for predicting abnormal PPV at different cutoffs at both T0 and T6. Other macrohemodynamic or tissue perfusion parameters were not related to PPV (ESM Fig. E12, Table E2).

Venous-to-arterial carbon dioxide differences calculated in terms of partial pressures (Pv-aCO$_2$) were significantly related to those calculated by blood contents (Cv-aCO$_2$) (T0: $R^2 = 0.52$, $p < 0.001$; T6: $R^2 = 0.42$, $p < 0.001$) (ESM Fig. E13). In addition to the significant relation between PPV and Pv-aCO$_2$, we also observed a linear relationship between PPV and Cv-aCO$_2$ (T0: $R^2 = 0.42$, $p < 0.001$, T6: $R^2 = 0.32$, $p < 0.001$) and between PPV and the Cv-aCO$_2$/Da-vO$_2$ ratio (T0: $R^2 = 0.18$, $p < 0.001$) (Fig. 3, ESM Fig. E14).

When data were analyzed considering the Pv-aCO$_2$ as a dependent variable, PPV was independently related to Pv-aCO$_2$ (ESM Table E3). Remarkably, cardiac index was also related to Pv-aCO$_2$ but only in patients with SvO$_2$ <65 % at T6 (ESM Table E3).

Discussion

Our observations reveal that Pv-aCO$_2$ is closely related to microcirculatory blood flow parameters during the early phases of resuscitation of septic shock. Indeed, Pv-aCO$_2$ was the best predictor of the microvascular blood flow maldistribution as indicated by the alterations in the percentage of small vessels perfused, the heterogeneity blood flow index, and consequently FCD. In addition, changes in Pv-aCO$_2$ were significantly related to changes in PPV. Meanwhile, Pv-aCO$_2$ was poorly related to systemic hemodynamic variables.

Microcirculatory dysfunction in septic shock is a generalized phenomenon characterized by decreasing FCD associated with increasing heterogeneity of blood flow consisting in zones with well-perfused vessels adjacent to non-perfused capillaries [21, 22]. Those derangements seem to trigger the development of organ dysfunctions because capillary blood flow alterations precede cellular stress [30, 31] and hypoxia-inducible gene expression [32]. In fact, microcirculatory alterations are stronger determinants of outcomes than global hemodynamic parameters, with progressive increase in the risk of death in quartiles representing more severe microcirculatory disturbances [33]. Nevertheless, some

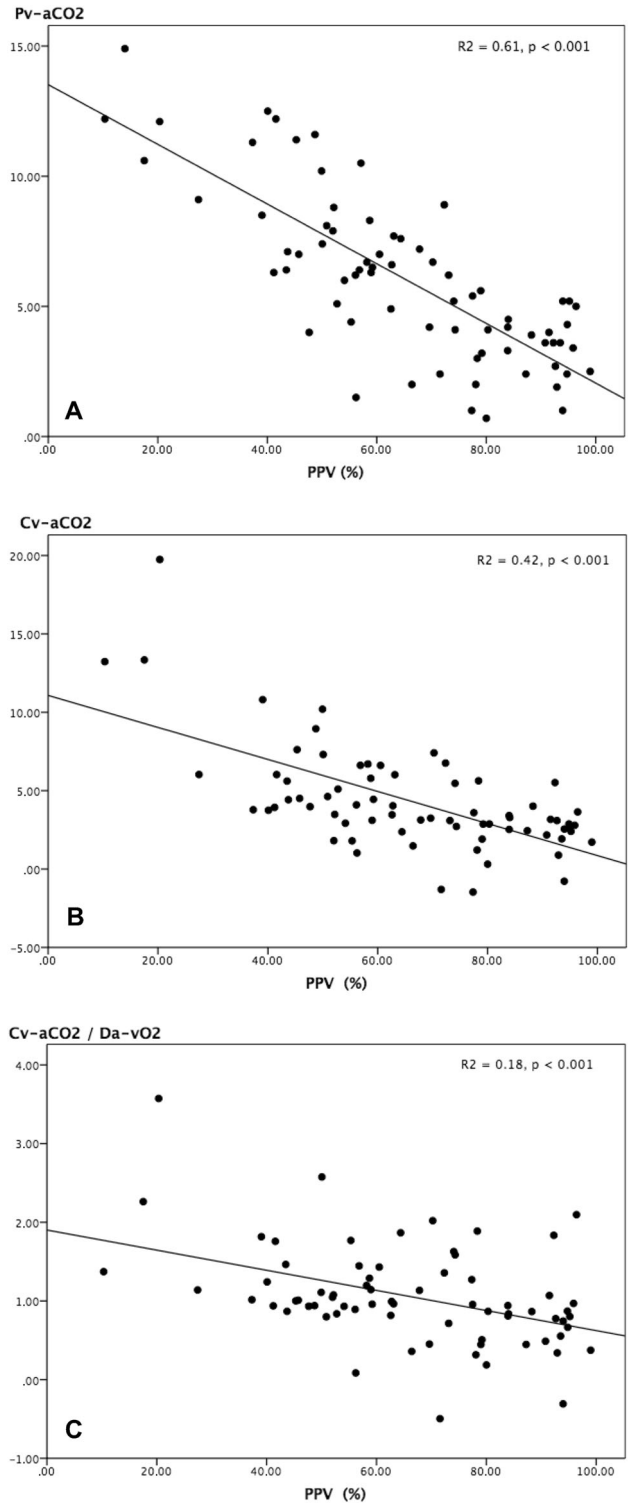


Fig. 3 Scatter plots depicting the relationships between the percentage of small vessels perfused (PPV) at T0 and **a** the venous-to-arterial CO_2 partial pressure difference (Pv-aCO_2), **b** the venous-to-arterial CO_2 content difference (Pv-aCO_2), and **c** the venous-to-arterial CO_2 to arterial-venous O_2 content difference ratio (Cv-aCO_2/Da-vO_2). Coefficient of determination (R^2) was calculated to assess the strength of correlations

authors argue against microcirculatory dysfunction as the primary event leading to organ failure because microcirculatory derangements might be coupled with cellular and mitochondrial dysfunction and merely reflect an adaptive response [34]. Despite controversies about the causal relationship between microcirculatory alterations and organ dysfunction, the quest for appropriate monitoring of microcirculation could potentially improve resuscitation results in septic shock. However, despite advances in the techniques to evaluate microcirculation at the bedside, an immediate and reliable method to quantify microcirculatory alterations is not yet available [35]. In our study, Pv-aCO_2 was independently related to PPV (and consequently to FCD and MFI) at both T0 and T6, and this relationship was also maintained when we evaluated separately patients with SvO_2 <65 % or ≥65 %. Furthermore, Pv-aCO_2 was better at predicting abnormal PPV values at different cutoffs, and it was superior to macrohemodynamic or oxygen metabolism goals. Similarly, Pv-aCO_2 >6.0 was related to an increased HI and therefore a decreased FCD. In normal conditions, the heterogeneity of microvascular blood flow is negligible [36], and matching of perfusion to metabolism usually improves during hypoxic or low flow states [37]. However, increases in the heterogeneity of the microcirculatory blood flow with the subsequent reduction in the FCD could be responsible for altered oxygen extraction capabilities in sepsis [38]. In fact, the heterogeneous flow cessation of individual capillaries could be an important factor determining the oxygen supply dependence phenomenon during the most severe cases of septic shock [38, 39] and this heterogeneity of microvascular blood flow could be tracked by Pv-aCO_2 variations during the early stages of resuscitation. In fact, changes observed in Pv-aCO_2 between T0 and T6 were significantly related to those exhibited by PPV during the same time interval.

It has been suggested that microcirculatory derangements can occur in the absence of macrohemodynamic derangements even when global oxygen delivery seems adequate [21, 40]. However, other small-sized studies have insinuated that microcirculatory impairment is related to decreasing mean arterial pressure or increasing dose of vasopressors [41]. Our data also suggest some independence between microcirculatory variables and macrohemodynamics, which is in line with previous observations [32, 39]. Indeed, changes exhibited by Pv-aCO_2 between T0 and T6 were not related to variations in cardiac output. Nevertheless, patients with a Pv-aCO_2 ≥6.0 exhibited a trend toward lower cadiac index and MAP values than those with Pv-aCO_2 <6.0. Furthermore, cadiac index was significant but weakly related to Pv-aCO_2 in patients with SvO_2 <65 % but not in those with normal SvO_2.

Pv-aCO_2 can also be influenced by the CO_2 dissociation curve. In this regard, we observed a good agreement

between Pv-aCO_2 and the venous-to-arterial CO_2 content difference (Cv-aCO_2), especially at normal ranges of Pv-aCO_2 (i.e., Pv-aCO_2 <6.0 mmHg). Interestingly, Cv-aCO_2 was mostly dispersed at higher Pv-aCO_2 values, suggesting a deeper impact of the Haldane effect at abnormal Pv-aCO_2 values. Similarly, difference between slopes described by the linear regression of PPV vs. Pv-aCO_2 and PPV vs. Cv-aCO_2 relationships should also be influenced by the Haldane effect. Nevertheless, the also significant relationship between PPV vs. Cv-aCO_2 suggests that CO_2 accumulation is, at least in part, related to the abnormal microcirculatory blood flow occurring during inflammatory conditions.

It has been recently suggested that combination of venous-to-arterial CO_2 content to arterial-venous O_2 difference ratio (Cv-aCO_2/Da-vO_2) can add prognostic value to lactate measurements during early stages of septic shock [42] because it could be used as a surrogate for the VCO_2/VO_2 ratio (i.e., the respiratory quotient), to potentially detect non-aerobic CO_2 generation [43]. We explored the association between the Cv-aCO_2/Da-vO_2 ratio and microcirculatory alterations; although, we found a significant correlation, the strength of such a relationship was notoriously weaker than that observed between Pv-aCO_2 (or even Cv-aCO_2) and PPV. We acknowledge that the Cv-aCO_2/Da-vO_2 ratio could be subjected to more error in calculations than Pv-aCO_2, but differences in the slope of the Cv-aCO_2/Da-vO_2 ratio vs. PPV compared with the Pv-aCO_2 vs. PPV relationship suggest the involvement of other physiological variables different to microcirculatory blood flow. Indeed, the Cv-aCO_2/Da-vO_2 ratio was abnormally increased in a number of patients through a wide range of PPV values. Therefore, it is not surprising that the Cv-aCO_2/Da-vO_2 ratio is superior to the Pv-aCO_2/Da-vO_2 ratio in the clinical setting [41] because under hypoxic conditions, disparity between Pv-aCO_2 and Cv-aCO_2 increases, and when coupling Pv-aCO_2 to different DO_2/VO_2 values, pH, temperature, and hemoglobin levels, the Pv-aCO_2/Da-vO_2 may acquire different cutoff normality values (a situation obviated when the numerator is corrected with the determinants of the Haldane effect).

We recognize limitations to our study. First, we used mixed venous blood to assess Pv-aCO_2 gradients, thus our results may not apply to central venous blood Pv-aCO_2 gradients. Indeed, even though a reasonable agreement is often reported in trials comparing both sampling sites [44], significant discrepancy can sometimes be observed and should not be neglected. Of note, this concordance between sampling sites has mostly be evaluated for SvO_2 more than for PCO_2 gradients. Second, variations of Pv-aCO_2, macrohemodynamics, and microcirculatory variables between T0 and T6 were not the result of targeted interventions but simply changed during resuscitation. Nevertheless, relationships exhibited between Pv-aCO_2 and PPV allow us hypothesize that some specific therapeutic interventions modifying microcirculation might reliably be tracked by Pv-aCO_2. Third, the relationship observed between microcirculatory variables and Pv-aCO_2 does not establish a causal association even though the physiological reasoning leading to our conclusions seems logical. Finally, there was no pre-experimental statistical plan published in a prespecified registry or a journal. However, observational research is, by nature, exploratory and requires considerable flexibility to explore novel findings and unexpected signals in the data [45, 46].

In conclusion, increased Pv-aCO_2 values are associated with microcirculatory dysfunction in septic shock, even when SvO_2 is within normal values. Whether changes in Pv-aCO_2 can be used to track changes in microvascular perfusion remains to be determined.

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Compliance with ethical standards

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Conflicts of interest Authors declare no conflicts of interest.

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References

1. Vincent JL, De Backer D (2013) Circulatory shock. N Engl J Med 369:1726–1734

2. Cecconi M, De Backer D, Antonelli M, Beale R, Bakker J, Hofer C, Jaeschke R, Mebazaa A, Pinsky MR, Teboul JL, Vincent JL, Rhodes A (2014) Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. Intensive Care Med 40:1795–1815

3. Bellomo R, Reade MC, Warrillow SJ (2008) The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. Crit Care 12:130
4. Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F, Filbin MR, Shapiro NI, Angus DC, Investigators P (2014) A randomized trial of protocol-based care for early septic shock. N Engl J Med 370:1683–1693
5. Peake SL, Bailey M, Bellomo R, Cameron PA, Cross A, Delaney A, Finfer S, Higgins A, Jones DA, Myburgh JA, Syres GA, Webb SA, Williams P, ARISE Investigators, for the Australian and New Zealand Intensive Care Society Clinical Trials Group (2009) Australasian resuscitation of sepsis evaluation (ARISE): a multi-centre, prospective, inception cohort study. Resuscitation 80:811–818
6. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, Jahan R, Harvey SE, Bell D, Bion JF, Coats TJ, Singer M, Young JD, Rowan KM, Investigators PT (2015) Trial of early, goal-directed resuscitation for septic shock. N Engl J Med 372:1301–1311
7. van Beest PA, Hofstra JJ, Schultz MJ, Boerma EC, Spronk PE, Kuiper MA (2008) The incidence of low venous oxygen saturation on admission to the intensive care unit: a multi-center observational study in the Netherlands. Crit Care 12:R33
8. Ospina-Tascón GA, Bautista-Rincón DF, Umaña M, Tafur JD, Gutiérrez A, García AF, Bermúdez W, Granados M, Arango-Dávila C, Hernández G (2013) Persistently high venous-to-arterial carbon dioxide differences during early resuscitation are associated with poor outcomes in septic shock. Crit Care 17:R294
9. van Beest PA, Lont MC, Holman ND, Loef B, Kuiper MA, Boerma EC (2013) Central venous-arterial pCO_2 difference as a tool in resuscitation of septic patients. Intensive Care Med 39:1034–1039
10. Vallée F, Vallet B, Mathe O, Parraguette J, Mari A, Silva S, Samii K, Fourcade O, Genestal M (2008) Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? Intensive Care Med 34:2218–2225
11. Grundler W, Weil MH, Rackow EC (1986) Arteriovenous carbon dioxide and pH gradients during cardiac arrest. Circulation 74:1071–1074
12. Weil MH, Rackow EC, Trevino R, Grundler W, Falk JL, Griffel MI (1986) Difference in acid-base state between venous and arterial blood during cardiopulmonary resuscitation. N Engl J Med 315:153–156
13. Zhang H, Vincent JL (1993) Arteriovenous differences in PCO2 and pH are good indicators of critical hypoperfusion. Am Rev Respir Dis 148:867–871
14. Van der Linden P, Rausin I, Deltell A, Bekrar Y, Gilbart E, Bakker J, Vincent JL (1995) Detection of tissue hypoxia by arteriovenous gradient for PCO2 and pH in anesthetized dogs during progressive hemorrhage. Anesth Analg 80:269–275
15. Groeneveld AB, Vermeij CG, Thijs LG (1991) Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end-expiratory pressure in the pig. Anesth Analg 73:576–582
16. Teboul JL, Mercat A, Lenique F, Berton C, Richard C (1998) Value of the venous-arterial PCO2 gradient to reflect the oxygen supply to demand in humans: effects of dobutamine. Crit Care Med 26:1007–1010
17. Bakker J, Vincent JL, Gris P, Leon M, Coffernils M, Kahn RJ (1992) Veno-arterial carbon dioxide gradient in human septic shock. Chest 101:509–515
18. Mecher CE, Rackow EC, Astiz ME, Weil MH (1990) Venous hypercarbia associated with severe sepsis and systemic hypoperfusion. Crit Care Med 18:585–589
19. Vallet B, Teboul JL, Cain S, Curtis S (2000) Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. J Appl Physiol 89:1317–1321
20. Dubin A, Estenssoro E, Murias G, Pozo MO, Sottile JP, Barán M, Piacentini E, Canales HS, Etcheverry G (2004) Intramucosal-arterial P_{CO_2} gradient does not reflect intestinal dysoxia in anemic hypoxia. J Trauma 57:1211–1217
21. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL (2002) Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med 166:98–104
22. De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent JL (2010) Monitoring the microcirculation in the critically ill patient: current methods and future approaches. Intensive Care Med 36:1813–1825
23. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL (2004) Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Crit Care Med 32:1825–1831
24. Creteur J, De Backer D, Sakr Y, Koch M, Vincent JL (2006) Sublingual capnometry tracks microcirculatory changes in septic patients. Intensive Care Med 32:516–523
25. Dubin A, Edul VS, Pozo MO, Murias G, Canullan CM, Martins EF, Ferrara G, Canales HS, Laporte M, Estenssoro E, Ince C (2008) Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. Crit Care Med 36:535–542
26. Horan TC, Andrus M, Dudeck MA (2008) CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 36:309–332
27. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G, SCCM/ESICM/ACCP/ATS/SIS (2003) 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 31:1250–1256
28. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb SA, Beale RJ, Vincent JL, Moreno R, Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup (2013) Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Crit Care Med 41:580–637
29. De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, Dobbe I, Ince C (2007) How to evaluate the microcirculation: report of a round table conference. Crit Care 11:R101
30. Wu L, Tiwari MM, Messer KJ, Holthoff JH, Gokden N, Brock RW, Mayeux PR (2007) Peritubular capillary dysfunction and renal tubular epithelial cell stress following lipopolysaccharide administration in mice. Am J Physiol Renal Physiol 292:F261–F268
31. Fink T, Heymann P, Taha-Melitz S, Taha A, Wolf B, Rensing H, Volk T, Mathes AM (2013) Dobutamine pretreatment improves survival, liver function, and hepatic microcirculation after polymicrobial sepsis in rat. Shock 40:129–135

32. Bateman RM, Tokunaga C, Kareco T, Dorscheid DR, Walley KR (2007) Myocardial hypoxia-inducible HIF-1alpha, VEGF, and GLUT1 gene expression is associated with microvascular and ICAM-1 heterogeneity during endotoxemia. Am J Physiol Heart Circ Physiol 293:H448–H456
33. De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, Vincent JL (2013) Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. Crit Care Med 41:791–799
34. Moore JP, Dyson A, Singer M, Fraser J (2015) Microcirculatory dysfunction and resuscitation: why, when, and how. Br J Anaesth 115:366–375
35. Ince C (2014) The rationale for microcirculatory guided fluid therapy. Curr Opin Crit Care 20:301–308
36. Zuurbier CJ, van Iterson M, Ince C (1999) Functional heterogeneity of oxygen supply-consumption ratio in the heart. Cardiovasc Res 44:488–497
37. Stein JC, Ellis CG, Ellsworth ML (1993) Relationship between capillary and systemic venous PO2 during nonhypoxic and hypoxic ventilation. Am J Physiol 265:H537–H542
38. Goldman D, Bateman RM, Ellis CG (2006) Effect of decreased O_2 supply on skeletal muscle oxygenation and O_2 consumption during sepsis: role of heterogeneous capillary spacing and blood flow. Am J Physiol Heart Circ Physiol 290:H2277–H2285
39. Walley KR (1996) Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. J Appl Physiol 81:885–894
40. De Backer D, Creteur J, Dubois MJ, Sakr Y, Koch M, Verdant C, Vincent JL (2006) The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. Crit Care Med 34:403–408
41. Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A (2012) Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. Crit Care Med 40:1443–1448
42. Ospina-Tascon GA, Umana M, Bermudez W, Bautista-Rincon DF, Hernandez G, Bruhn A, Granados M, Salazar B, Arango-Davila C, De Backer D (2015) Combination of arterial lactate levels and venous-arterial CO_2 to arterial-venous O_2 content difference ratio as markers of resuscitation in patients with septic shock. Intensive Care Med 41:796–805
43. Jakob SM, Groeneveld AB, Teboul JL (2015) Venous-arterial CO_2 to arterial-venous O_2 difference ratio as a resuscitation target in shock states? Intensive Care Med 41:936–938
44. Cuschieri J, Rivers EP, Donnino MW, Katilius M, Jacobsen G, Nguyen HB, Pamukov N, Horst HM (2005) Central venous-arterial carbon dioxide difference as an indicator of cardiac index. Intensive Care Med 31:818–822
45. Vandenbroucke JP (2008) Observational research, randomised trials, and two views of medical science. PLoS Med 5:e67
46. Pearce N (2011) Registration of protocols for observational research is unnecessary and would do more harm than good. Occup Environ Med 68:86–88

PAPER No. 6 (Expert opinion paper)

UNDERSTANDING THE VENOUS-ARTERIAL CO_2 TO ARTERIAL-VENOUS O_2 CONTENT DIFFERENCE RATIO

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UNDERSTANDING THE DISEASE

Understanding the venous–arterial CO_2 to arterial–venous O_2 content difference ratio

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Introduction

Early identification of tissue hypoperfusion is a cornerstone of shock management [1]. Normal macrohemodynamic and oxygen-derived parameters do not, however, rule out the presence of tissue hypoxia [2]. In this setting, carbon dioxide (CO_2)-derived variables may provide information on macro- and microvascular blood flow [3] and also on the presence of anaerobic metabolism [4, 5]. Importantly, variations in CO_2 occur more rapidly than changes in lactate kinetics, making the former an attractive biomarker for monitoring, especially during the early stages of resuscitation [6, 7].

The rationale of $C\bar{v}$–aCO_2 to Ca-$\bar{v}O_2$ ratio

According to the Fick equation, oxygen consumption (VO_2) and CO_2 production (VCO_2) are related to cardiac output and their respective arterial-to-venous and venous-to-arterial content differences. Thus, under aerobic steady-state conditions VCO_2 approximates VO_2 and, consequently, the mixed venous-to-arterial CO_2 content difference ($C\bar{v}$-aCO_2) approximates the arterial-to-mixed-venous O_2 content difference (Ca-$\bar{v}O_2$). In other words, CO_2 production should not be higher than O_2 availability and, therefore, the VCO_2/VO_2 ratio (i.e., the respiratory quotient) should not be higher than 1.0. Nonetheless, under under certain conditions, such as progressive exercise, in which VO_2 increases in response to metabolic demands, VCO_2 may surpass VO_2 when the anaerobic threshold is reached [8]. This situation arises as result of the disproportionate increase in the production of CO_2, which is released through the buffering of excess hydrogen ions, most of which derive from ATP hydrolysis [9] and the excessive liberation of protons due to accelerated anaerobic glycolysis [10]. Thus, during progressive exercise, both VO_2 and VCO_2, as well as the respiratory quotient increase. Interestingly, experimental blockage of mitochondrial O_2 utilization [11] leads to a simultaneous—but not symmetric—decrease in VO_2 and VCO_2 with subsequent increases in the respiratory quotient, also suggesting non-aerobic CO_2 generation.

During circulatory shock, a global decrease in VO_2 should be accompanied by a reduction in aerobic CO_2 production. However, experimental models demonstrate that VCO_2 exhibits a slighter decrease than VO_2 [12, 13], thus pathologically increasing the VCO_2/VO_2 ratio as consequence of predominant anaerobic metabolism (Fig. 1). Interestingly, after shock reversion, the VCO_2/VO_2 ratio returns to normal values, suggesting the potential reversibility of this phenomenon, at least during the early stages of shock.

The $C\bar{v}$-aCO_2/Ca–$\bar{v}O_2$ ratio could be used as a surrogate for VCO_2/VO_2. Remarkably, both the venous-to-arterial CO_2 difference and the arterial-to-venous O_2 content difference, i.e., the numerator and denominator of this quotient, are influenced by macro- and microblood flow alterations, which suggest that increases in the $C\bar{v}$-aCO_2/Da-$\bar{v}O_2$ ratio are to some extent independent of flow variations. It is well known that low cardiac output may increase venous CO_2 partial pressure ($PvCO_2$) even in the absence of extra CO_2 production due to the venous stagnation phenomenon [14]. Likewise, microcirculatory alterations, such as decreased percentage of perfused small vessels, decreased functional capillary density, and increased heterogeneity of flow, are associated with progressively increased $P\bar{v}$-aCO_2 and $C\bar{v}$-aCO_2 during septic shock [3]. Analogously, increasing heterogeneity of microvascular flow impairs oxygen extraction [15], and insufficient cardiac output might also limit VO_2 during periods of oxygen supply dependency. Consequently,

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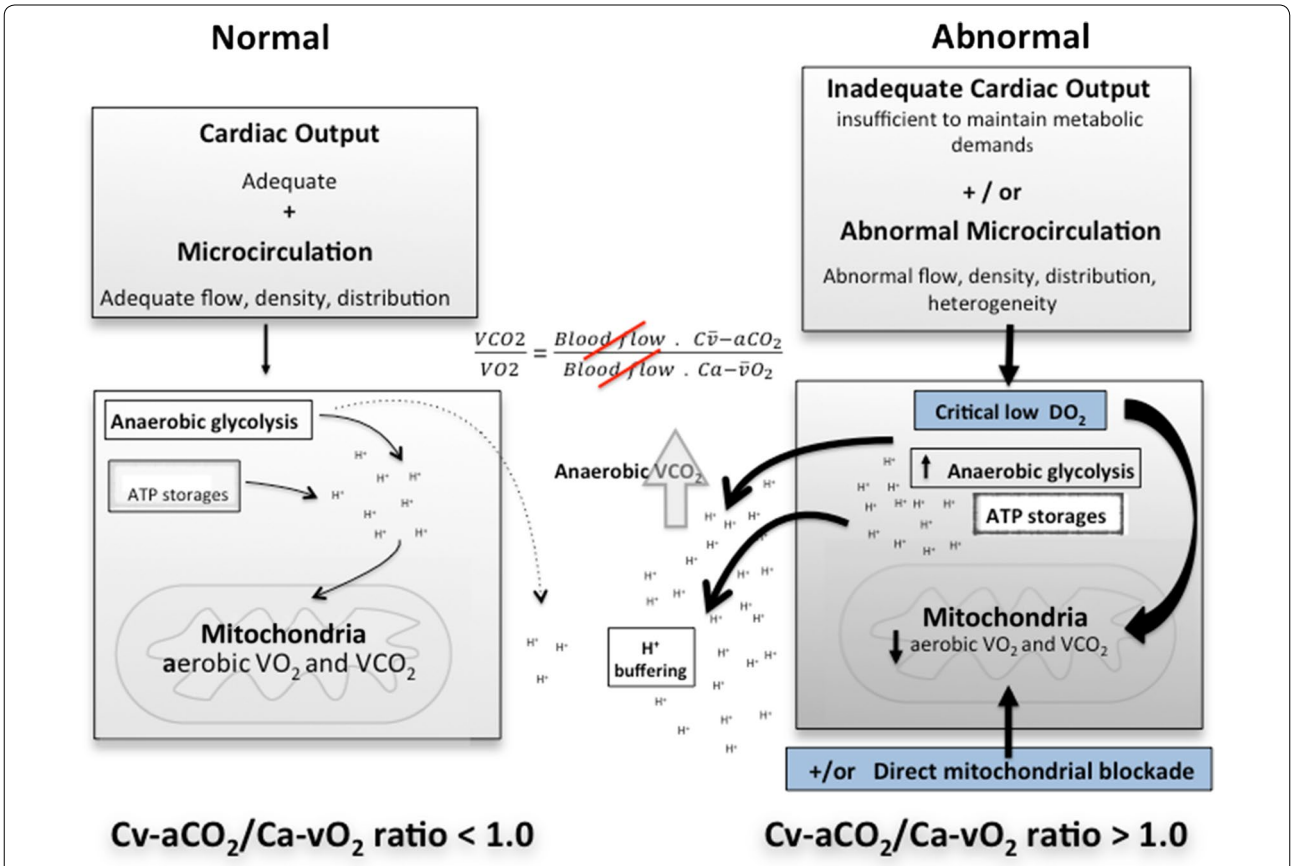


Fig. 1 A comparison of the mixed venous-to-arterial carbon dioxide (CO_2) content difference (C$\bar{v}$-aCO_2)/arterial-to-mixed-venous oxygen (O_2) content difference (Ca–$\bar{v}O_2$) ratio during normal (*left side*) and abnormal conditions (*right side*). Normal aerobic mitochondrial function leads to a normal C $\bar{v}$-aCO_2/Ca–$\bar{v}O_2$ (i.e., < 1.0). In contrast, during progressive hypoperfusion, insufficient cardiac output, abnormal microcirculation or direct mitochondrial blockade, both O_2 consumption and aerobic CO_2 generation are depressed but the C$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio increases (>1.0) due to an increase in anaerobic CO_2 generation as a consequence of the buffering of hydrogen ions (H^+) coming from anaerobic glycolysis and ATP hydrolysis . *VO_2*: oxygen consumption; *VCO_2* CO_2 production; *DO_2* oxygen delivery

an increased C$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio reflects the relative increase of VCO_2 over VO_2 secondary to the buffering of hydrogen ions due to anaerobic metabolism [16], while the isolated Pv-aCO_2 (or C$\bar{v}$-aCO_2) reflects the blood flow conditions at both the macro- and microvascular levels [3, 7].

C$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio and its relationship with anaerobic metabolism during tissue hypoxia

Interpretation of lactate levels during the resuscitation and post-resuscitation periods is sometimes very difficult since hyperlactatemia may not always represent anaerobic metabolism. Mekontso-Dessap et al. [4] demonstrated a good correlation between the P$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio (a surrogate of C$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio calculated by using CO_2 pressures instead of CO_2 contents) and lactate levels [4] using a cutoff of ≥2.0 mmol/L to indicate anaerobic metabolism. However, rather than predicting lactate elevation, the C$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio could provide important prognostic information to lactate variations during early stages of resuscitation due to its ability to detect "ongoing" anaerobic metabolism and to react faster than lactate to short-term hemodynamic changes. In a recent study, Ospina-Tascón et al. [5] demonstrated the strong prognostic significance of persistent hyperlactatemia combined with an increased C$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio when compared to hyperlactemia with a normal ratio. The C$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio could therefore be a useful tool with which to differentiate hypoxia-driven lactate accumulation versus other non-flow-dependent causes of hyperlactatemia.

Does the C$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio differ from the P$\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio?

Over the physiological range of CO_2 contents, i.e., along the steep portion of the CO_2 dissociation curve, the

partial pressure of CO_2 (PCO_2) maintains a quasi-linear relationship with CO_2 content (CCO_2); theoretically, therefore, $P\bar{v}$-aCO_2 could be used as a surrogate for the $C\bar{v}$-aCO_2 ratio. The relationship between PCO_2 and CCO_2, however, becomes non-linear at abnormal $P\bar{v}$-aCO_2 values [3]. Thus, the $C\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio may be superior to the $P\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio in predicting outcomes during very early phases of resuscitation of septic shock [5] since at deeper tissue hypoxia and acidosis, disparity between $P\bar{v}$-aCO_2 and $C\bar{v}$-aCO_2 increases according to the Haldane effect.

Limitations in clinical practice

First, calculation of VCO_2 according to Fick's approach is valid under steady-state conditions. Conversely, the regain of flow after tissue ischemia could overstimulate the VCO_2, leading to increases in VCO_2/VO_2. However, the $C\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio integrates global CO_2 accumulation, O_2 consumption, and blood flow; therefore, the $C\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio ratio should also be less influenced by pulmonary ventilation/perfusion abnormalities. Second, calculation of the $C\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio is cumbersome and subject to an important risk of error. Nevertheless, the influence of such potential error is limited as it correctly identifies patients at increased risk of death [5]. Third, calculating $P\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ is relatively easy, and the value should be equivalent to $C\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ when PCO_2 and mixed venous oxygen saturation (SvO_2) approximates normality, which occurs frequently. The $P\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio is, however, largely subject to determinants of the Haldane effect, and thus its interchangeability with the $C\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio is debatable. Fourth, information regarding global CO_2/O_2 relationships comes from studies using pulmonary artery catheter monitoring [4, 5], so its equivalence with central venous sample calculations is not yet proven, despite the relative good agreement between central venous and mixed venous CO_2 to calculate the Pv-aCO_2 [17]. Fifth, while the physiology might appear robust and the results coming from small single-center physiological studies studies sound biologically plausible, notwithstanding its complexities, the clinical applicability of the $C\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio remains to be confirmed.

Conclusion

The $C\bar{v}$-aCO_2/Ca-$\bar{v}O_2$ ratio can be used as an approximation of the respiratory quotient and may detect ongoing anaerobic CO_2 generation in patients with septic shock. Computations of CO_2 and O_2 contents are, admittedly, cumbersome, but the significance of the $C\bar{v}$-aCO_2/Ca-vO_2 ratio and its biological plausibility deserves future research efforts.

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Compliance with ethical standards

Conflicts of interest
The authors declare that they have no conflicts of interest.

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References

1. Cecconi M, De Backer D, Antonelli M, Beale R, Bakker J, Hofer C, Jaeschke R, Mebazaa A, Pinsky MR, Teboul JL, Vincent JL, Rhodes A (2014) Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. Intensive Care Med 40:1795–1815
2. Bellomo R, Reade MC, Warrillow SJ (2008) The pursuit of a high central venous oxygen saturation in sepsis: growing concerns. Crit Care 12:130
3. Ospina-Tascon GA, Umana M, Bermudez WF, Bautista-Rincon DF, Valencia JD, Madrinan HJ, Hernandez G, Bruhn A, Arango-Davila C, De Backer D (2016) Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock? Intensive Care Med 42:211–221
4. Mekontso-Dessap A, Castelain V, Anguel N, Bahloul M, Schauvliege F, Richard C, Teboul JL (2002) Combination of venoarterial PCO2 difference with arteriovenous O2 content difference to detect anaerobic metabolism in patients. Intensive Care Med 28:272–277
5. Ospina-Tascon GA, Umana M, Bermudez W, Bautista-Rincon DF, Hernandez G, Bruhn A, Granados M, Salazar B, Arango-Davila C, De Backer D (2015) Combination of arterial lactate levels and venous-arterial CO2 to arterial-venous O 2 content difference ratio as markers of resuscitation in patients with septic shock. Intensive Care Med 41:796–805
6. Ospina-Tascón GA, Bautista-Rincón DF, Umaña M, Tafur JD, Gutiérrez A, García AF, Bermúdez W, Granados M, Arango-Dávila C, Hernández G (2013) Persistently high venous-to-arterial carbon dioxide differences during early resuscitation are associated with poor outcomes in septic shock. Crit Care 17:R294
7. Vallée F, Vallet B, Mathe O, Parraguette J, Mari A, Silva S, Samii K, Fourcade O, Genestal M (2008) Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? Intensive Care Med 34:2218–2225
8. Wasserman K, Beaver WL, Whipp BJ (1990) Gas exchange theory and the lactic acidosis (anaerobic) threshold. Circulation 81[1 Supp]:II14–II30
9. Randall HM Jr, Cohen JJ (1966) Anaerobic CO_2 production by dog kidney in vitro. Am J Physiol 211:493–505
10. Stringer W, Wasserman K, Casaburi R (1995) The VCO_2/VO_2 relationship during heavy, constant work rate exercise reflects the rate of lactic acid accumulation. Eur J Appl Physiol Occup Physiol 72:25–31
11. Breen PH, Isserles SA, Westley J, Roizen MF, Taitelman UZ (1995) Combined carbon monoxide and cyanide poisoning: a place for treatment. Anesth Analg 80:671–677
12. Groeneveld AB, Vermeij CG, Thijs LG (1991) Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end-expiratory pressure in the pig. Anesth Analg 73:576–582
13. Cohen IL, Sheikh FM, Perkins RJ, Feustel PJ, Foster ED (1995) Effect of hemorrhagic shock and reperfusion on the respiratory quotient in swine. Crit Care Med 23:545–552
14. Austin WH, Lacombe E, Rand PW, Chatterjee M (1963) Solubility of carbon dioxide in serum from 15 to 38 C. J Appl Physiol 18:301–304
15. Goldman D, Bateman RM, Ellis CG (2006) Effect of decreased O2 supply on skeletal muscle oxygenation and O_2 consumption during sepsis: role

of heterogeneous capillary spacing and blood flow. Am J Physiol Heart Circ Physiol 290:H2277–H2285
16. Raza O, Schlichtig R (2000) Metabolic component of intestinal $PCO_{(2)}$ during dysoxia. J Appl Physiol 89:2422–2429
17. Cuschieri J, Rivers EP, Donnino MW, Katilius M, Jacobsen G, Nguyen HB, Pamukov N, Horst HM (2005) Central venous-arterial carbon dioxide difference as an indicator of cardiac index. Intensive Care Med 31:818–822