EDITORIAL

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Venous-to-arterial carbon dioxide difference: an experimental model or a bedside clinical tool?

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In the present issue of *Intensive Care Medicine*, Ospina-Tascón et al. present an intriguing observational study in which they assessed the association between venous-to-arterial carbon dioxide difference (Pv-aCO$_2$) and microvascular perfusion in patients with early septic shock [1]. A total of 75 adult patients with septic shock from a 60-bed mixed ICU in Columbia were included in the study. Potentially eligible patients with septic shock in the ICU were screened, and those eligible had a pulmonary artery catheter (PAC) inserted and were included in the study. Arterial and mixed venous blood samples were collected at the insertion of the PAC (T0) and at 6 h (T6), and Pv-aCO$_2$ was defined as the difference between mixed-venous and arterial CO$_2$ partial pressures. A side-stream dark-field imaging device was used to evaluate the microcirculation of the tongue at T0 and T6, and the association between Pv-aCO$_2$ and the microcirculation of the tongue was assessed. Furthermore, the association between Pv-aCO$_2$ and global haemodynamic variables was evaluated. The authors conclude that Pv-aCO$_2$ was closely related to microcirculatory blood flow parameters during the early phase of septic shock, whereas Pv-aCO$_2$ was poorly related to systemic haemodynamic variables [1].

Septic shock is a serious and frequent condition in the ICU, and early recognition and adequate treatment of tissue hypoperfusion is crucial [2, 3]. Oxygen-derived parameters such as central venous oxygen saturation (ScVO$_2$) have failed to demonstrate clinical benefit as resuscitation targets in recent large randomised controlled trials and systematic reviews [4–6]. Mean baseline ScVO$_2$ was above 70 % in all the trials, which may be explained by early recognition and aggressive treatment of shock. Nevertheless, while a low ScVO$_2$ can prompt further resuscitation [3], in the context of normal or high ScVO$_2$ its role is more questionable. Indeed, one of the limitations of using ScVO$_2$ as a marker of hypoperfusion is that normal to high values cannot discriminate whether oxygen delivery is adequate or in excess of demand. High ScVO$_2$ in the context of high lactate has for instance been shown to be associated with poor survival rates [7]. In this situation other tissue perfusion variables such as Pv-aCO$_2$ have been proposed [8, 9]. In normal circumstances the difference between the arterial and the venous carbon dioxide is less than 6 mmHg. However, in states of low perfusion this difference can increase. In areas of the microcirculation that are poorly perfused there is an increased local production of CO$_2$. Despite poor perfusion, since CO$_2$ is about 20 times more soluble than O$_2$, the likelihood of CO$_2$ diffusing out of ischemic tissues and into the venous effluent is high, making it a very sensitive marker of hypoperfusion. Vallée et al. demonstrated that in septic patients with normalized ScVO$_2$, high central venous-to-arterial CO$_2$ difference (Pcv-aCO$_2$) was associated with worse outcomes in terms of lower lactate clearance, lower cardiac index, and higher sepsis-related organ failure assessment (SOFA) score [8]. Ospina-Tascón et al.'s study adds to the evidence that

Fig. 1 Algorithm for the assessment of patients with shock
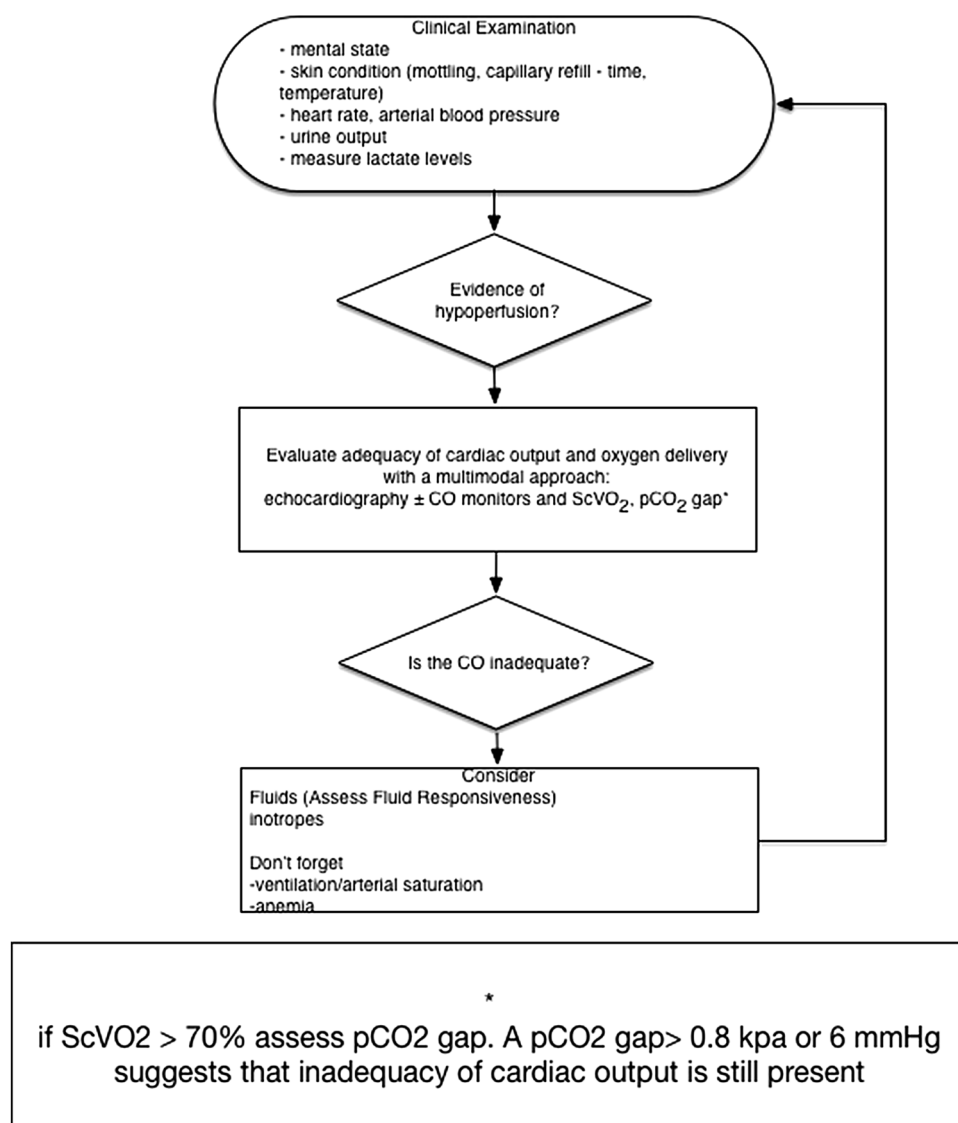
P(c)v-aCO$_2$ may be a useful tool to identify patients who remain inadequately resuscitated when an ScVO$_2$ of 70 % has been reached [10]. Consequently, Pv-aCO$_2$ may prove useful in the assessment of patients with shock (Fig. 1).

While there seems to be convincing evidence that P(c)v-aCO$_2$ is a marker of (microcirculatory) hypoperfusion, a number of unanswered questions and limitations regarding routine clinical bedside use of Pv-aCO$_2$ exist. First, use of Pv-aCO$_2$ has not been tested in (randomised) clinical trials. Consequently, there is a risk of falsely inflated estimates [11], and the balance between benefits and harms are unknown. Second, there is a lack of Pv-aCO$_2$ studies assessing patient-important outcome measures with adequate follow-up [12]. Trials using non-patient-important outcome measures (surrogate outcomes) overestimate the intervention effect by 40–50 % [13]. Third, existing studies have mainly been conducted in small single-centre institutions, which increases the risk of reporting falsely inflated estimates [14, 15]. Finally, the unblinded assessment of the association between Pv-aCO$_2$ and the microcirculation increases the risk of selection bias [15].

In conclusion, Pv-aCO$_2$ is an interesting and potentially important new tool for evaluation of the microcirculation in critically ill patients with shock; however, additional clinical evaluation, including adequate assessment of benefits and harms in high-quality randomised clinical trials, is needed prior to routine clinical use at the bedside.

Compliance with ethical standards

Conflicts of interest None.

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ORIGINAL

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/mm²			
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/mm²			
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
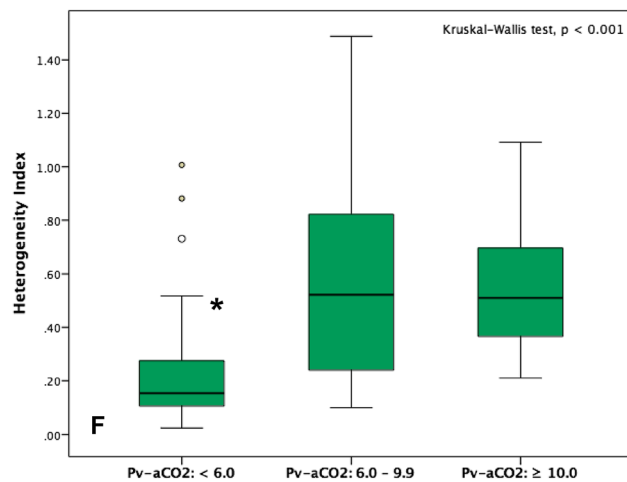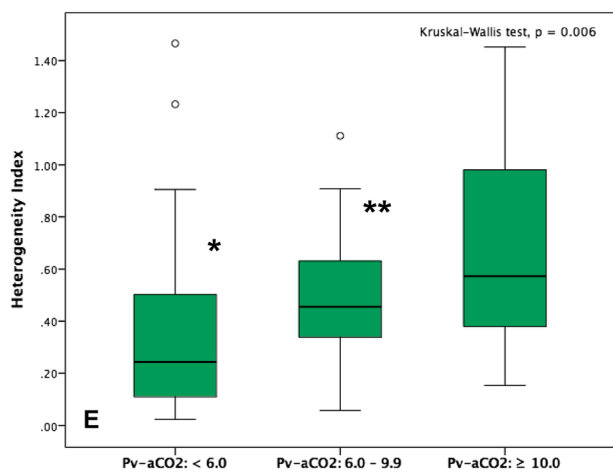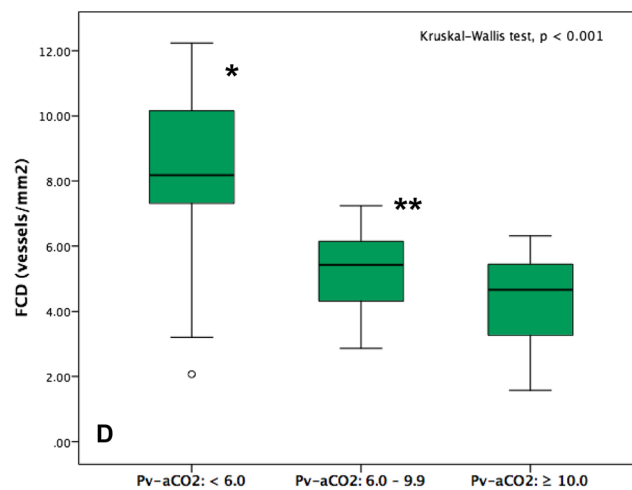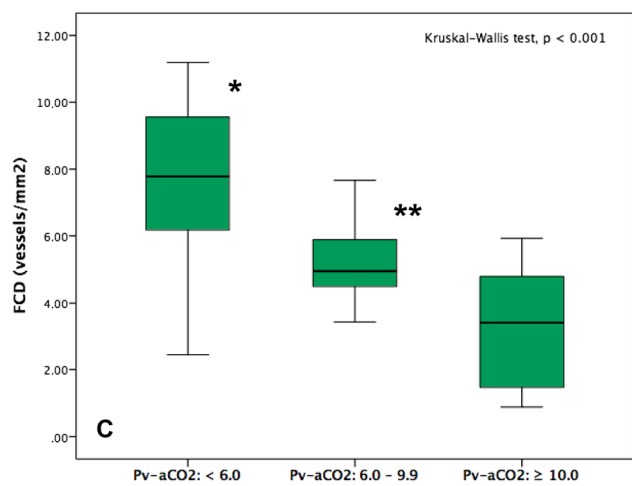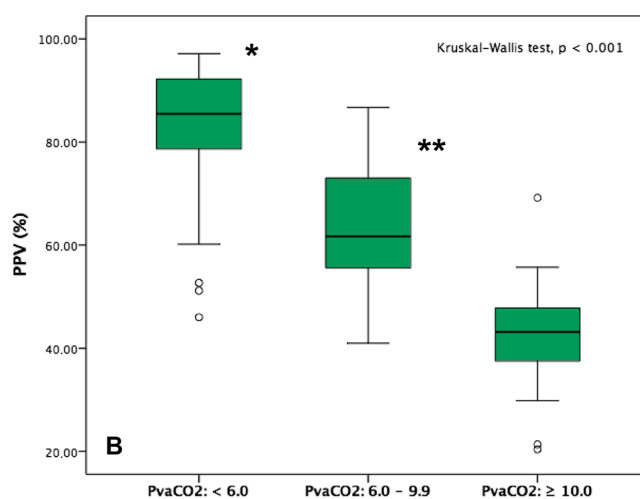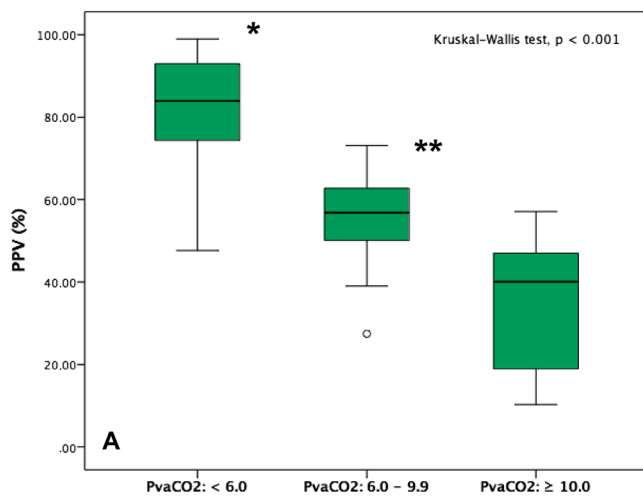
◀ **Fig. 1** Percentage of small vessels perfused (PPV), functional capillary density (FCD), and heterogeneity index (HI) for the predefined Pv-aCO$_2$ groups. *Box plots* depicting differences in PPV, FCD, and HI for predefined Pv-aCO$_2$ groups (group 1, <6.0 mmHg; group 2, 6.0–9.9 mmHg; group 3, ≥10 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).
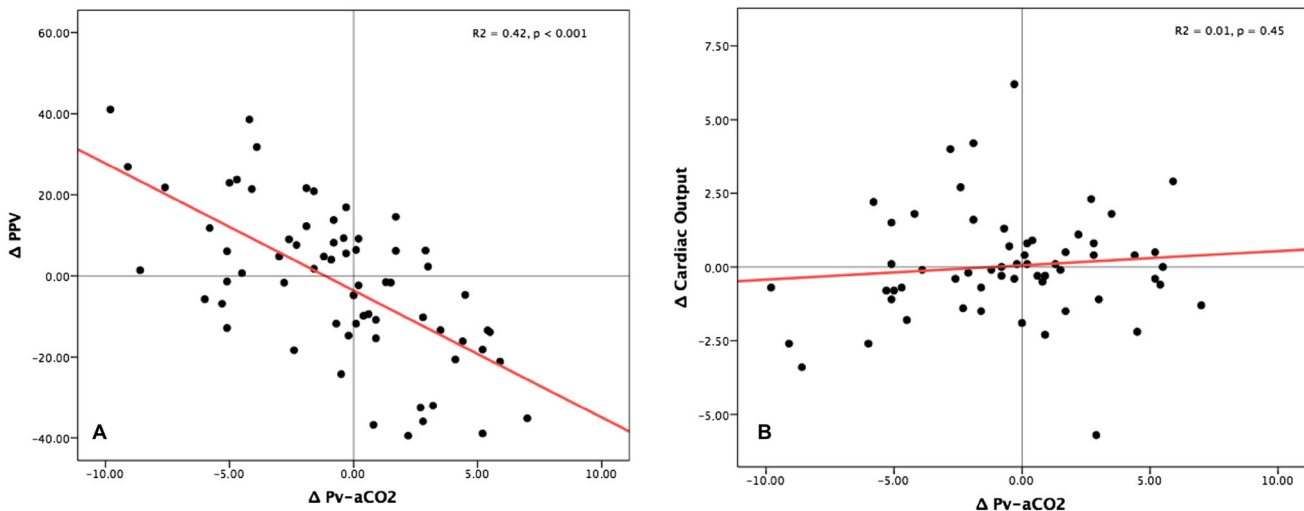

Fig. 2 Scatter plots showing the correlation of variations observed between changes in venous-to-arterial CO_2 partial pressure differences ($\Delta Pv-aCO_2$) and **a** changes in percentage of small vessels perfused (ΔPPV) between measurements performed at T0 and T6 ($R^2 = 0.42$, $p < 0.001$) and **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
$SvO_2 \geq 65$ %								
$Pv-aCO_2$	−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

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 $SvO_2 \geq 65$ %, $Pv-aCO_2$ 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
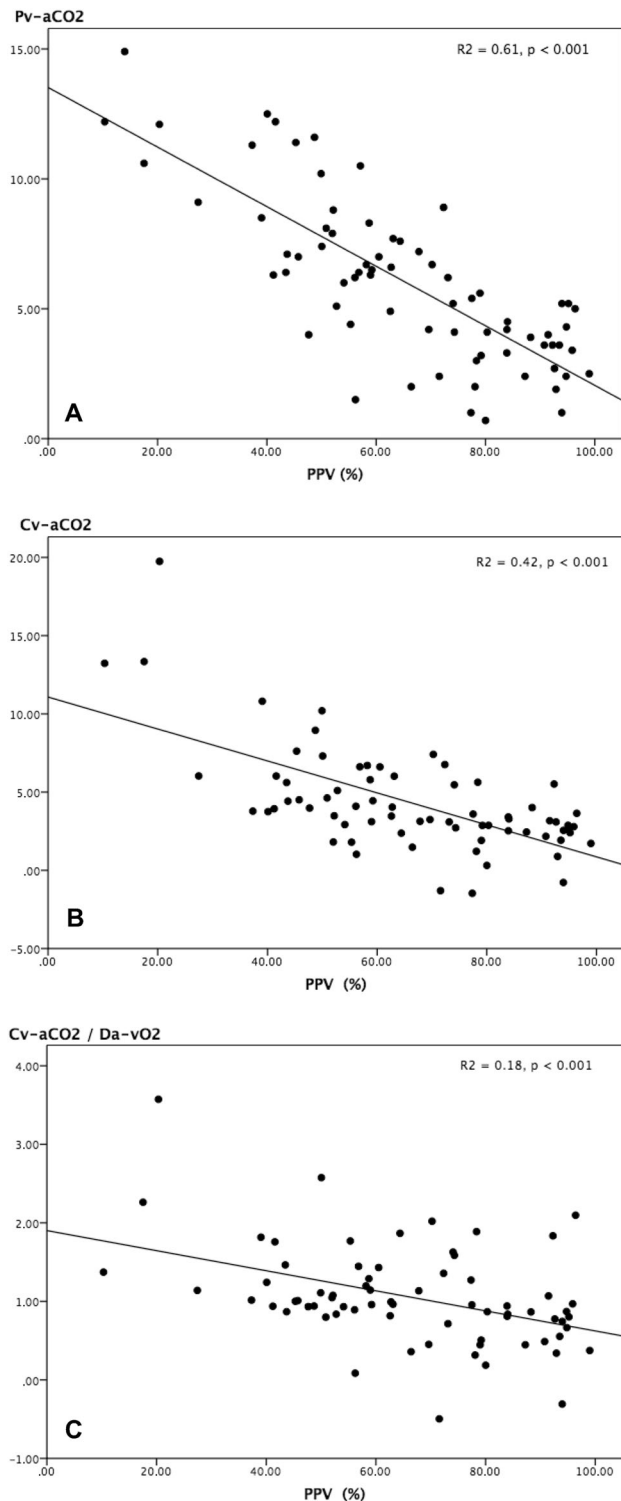
Fig. 3 Scatter plots depicting the relationships between the percentage of small vessels perfused (PPV) at T0 and **a** the venous-to-arterial CO_2 partial pressure difference (Pv-aCO_2), **b** the venous-to-arterial CO_2 content difference (Pv-aCO_2), and **c** the venous-to-arterial CO_2 to arterial-venous 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 $\geq$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 $\geq$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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