Intensive Care Med (2015) 41:796–805
DOI 10.1007/s00134-015-3720-6

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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

Received: 8 December 2014
Accepted: 25 February 2015
Published online: 20 March 2015

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
The online version of this article (doi: 10.1007/s00134-015-3720-6) contains supplementary material, which is available to authorized users.

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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

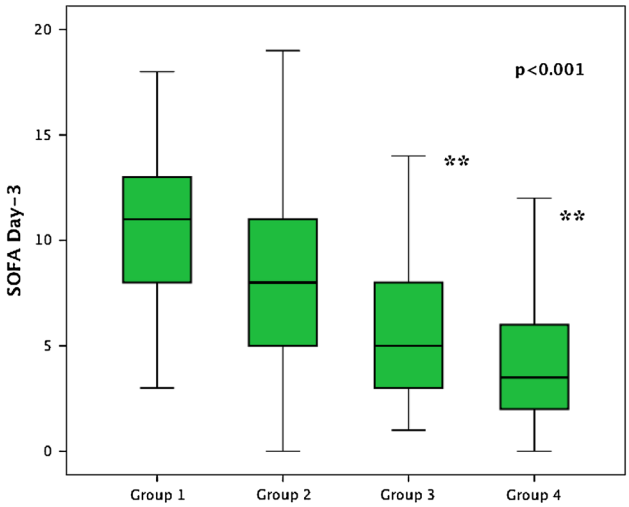

Fig. 1 Sequential Organ Failure Assessment (SOFA) scores at day 3 for predefined groups. Data presented as median (percentiles). Patients were separated into four groups according to lactate and Cv-aCO_2/Da-vO_2 ratio measured after the first 6 h of resuscitation: group 1, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 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

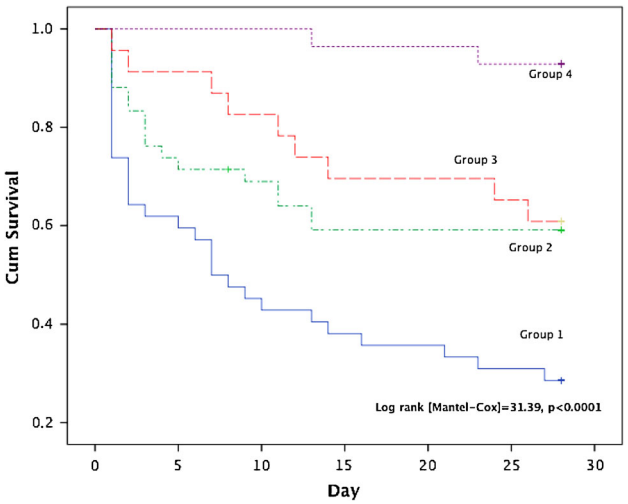

Fig. 2 Survival probabilities up to day 28 according to lactate and Cv-aCO_2/Da-vO_2 after 6 h of resuscitation. Log rank (Mantel–Cox) = 31.39, $p < 0.0001$. Group 1, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio >1.0; group 2, lactate ≥2.0 mmol/L and Cv-aCO_2/Da-vO_2 ratio ≤1.0; group 3, lactate <2.0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

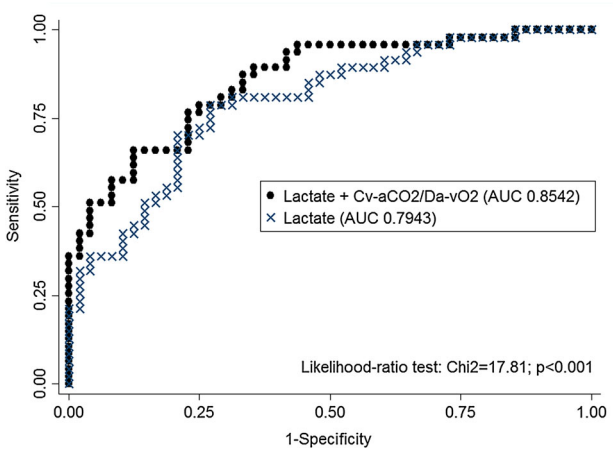

Fig. 3 Receiver operating characteristics (ROC) curves for prediction of mortality at day 28 for models including or not Cv-aCO_2/Da-vO_2 ratio. The "large model" included Cv-aCO_2/Da-vO_2 + lactate levels. The "short model" included lactate levels but not Cv-aCO_2/Da-vO_2. Both models also included 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.

Acknowledgments The authors kindly thank Dr. Jairo Osorno and Dr. Fernando Rosso (Centro de Investigaciones Clínicas, Fundación Valle del Lili-Universidad ICESI, Cali, Colombia) for their contribution to enhancing the manuscript quality and Dr. Yuri Takeuchi (Fundación Valle del Lili-Universidad ICESI) for her unconditional support to the research.

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

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