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# Optimization of tissue oxygenation by using the metabolic approach in acute circulatory failure states

Jihad Mallat

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## THÈSE

Pour obtenir le diplôme de doctorat

Spécialité RECHERCHE CLINIQUE, INNOVATION TECHNOLOGIQUE, SANTE PUBLIQUE

Préparée au sein de l'Université de Caen Normandie

**Optimisation de l'oxygénation tissulaire par l'approche métabolique dans les états d'insuffisances circulatoires aiguës**

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**Thèse soutenue le 27/10/2022  
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UR 4650



Physiopathologie et  
Stratégie d'  
Imagerie du  
Remodelage cardiovasculaire



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in acute circulatory failure states**

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## Abstract (French)

L'état de choc est une forme d'insuffisance circulatoire aiguë qui est associée à une inadéquation entre le transport artériel en oxygène et la consommation tissulaire en oxygène aboutissant à une hypoxie tissulaire. Une reconnaissance précoce et une réanimation adéquate de l'hypoperfusion tissulaire revêtent une importance particulière dans la gestion du choc, afin d'éviter le développement d'hypoxie tissulaire et la progression vers la défaillance multi-viscérale.

Le transport artériel en oxygène ( $DO_2$ ) aux tissus est déterminé par les mécanismes de convection et diffusion. La capacité de l'organisme à ajuster l'extraction d'oxygène ( $ERO_2$ ) en réponse aux changements de  $DO_2$  est crucial pour maintenir constante la consommation tissulaire en oxygène ( $VO_2$ ). La capacité d'augmenter l' $ERO_2$  est le résultat de la régulation de la circulation sanguine régionale et l'activation simultanée des facteurs centraux et locaux. L'endothélium joue un rôle crucial dans l'adaptation de l'apport en oxygène aux besoins tissulaires dans les situations de baisse aiguë de l'oxygénation tissulaire. Lorsque le  $DO_2$  est gravement compromis, une valeur critique de  $DO_2$  est atteinte en dessous de laquelle le  $VO_2$  diminue et devient dépendant du  $DO_2$ , entraînant une hypoxie tissulaire. Les différents mécanismes d'hypoxie tissulaire sont circulatoires, anémiques et hypoxiques, caractérisés par une  $DO_2$  diminuée mais une capacité préservée d'augmentation de  $ERO_2$ . L'hypoxie cytopathique est un autre mécanisme d'hypoxie tissulaire dû à des altérations dans la respiration mitochondriale que l'on peut observer dans des conditions septiques avec une  $DO_2$  globale normale.

Chez les patients en insuffisance circulatoire aiguë, l'un des objectifs du traitement est d'augmenter le débit cardiaque. L'objectif en est d'améliorer l'apport en oxygène aux tissus et de corriger le déséquilibre entre la demande et le transport en oxygène. Cependant, aucune valeur absolue du débit cardiaque ou de  $DO_2$  ne peut être définie, car leur valeurs adéquates dépendent essentiellement des besoins tissulaires en oxygène à un instant donné. La valeur adaptée du débit cardiaque est celle qui assure un débit d'oxygène qui répond à la demande métabolique. Ainsi, tout traitement visant à modifier le débit cardiaque, tel qu'un remplissage vasculaire ou l'utilisation d'agents inotropes, doit être déterminé par l'évaluation de l'adéquation entre la demande et le transport en oxygène.

Pour évaluer cette adéquation entre  $DO_2$  et  $VO_2$ , l'examen clinique a encore une valeur limitée. La diurèse peut refléter la perfusion rénale, mais de nombreux autres facteurs peuvent l'altérer au cours des états de choc. De plus, la présence d'une insuffisance rénale antérieure modifie l'interprétation et le diagnostic de la nécrose tubulaire aiguë. Les signes d'hypoperfusion cutanée comme les marbrures ont été corrélées à une diminution de l'oxygénation tissulaire et à une mortalité augmentée chez les patients en état de choc septique. Cependant, la présence des marbrures n'est pas constante chez les patients en insuffisance circulatoire aiguë, puisque seulement la moitié des patients en choc septique présentent des marbrures. Un temps de recoloration élevé, suggérant une diminution de perfusion tissulaire, a été associé, chez les patients en insuffisance circulatoire aiguë, à une mortalité plus élevée que chez les patients avec un temps de recoloration normal. Néanmoins, seulement 30% des patients en état de choc septique avaient un temps de recoloration élevé. Un taux de lactate sanguin élevé est souvent reconnu comme un marqueur d'activation de métabolisme anaérobique (dépendance  $VO_2/DO_2$ )

et donc d'hypoxie tissulaire. Cependant, le lactate sanguin peut augmenter en raison de nombreux mécanismes non liés à l'oxygénation tissulaire, ce qui conduit à de faux positifs. De plus, la concentration de lactate dans le sang dépend de l'équilibre entre la production et la clairance du lactate, de sorte que le retard requis par son métabolisme empêche l'utilisation du lactate en tant que marqueur en temps réel du métabolisme tissulaire. Ainsi, étant donné le caractère non spécifique de l'élévation du taux de lactate, l'hyperlactatémie n'est pas un facteur discriminant dans l'établissement de la cause de l'insuffisance circulatoire. Récemment, il a été démontré qu'une stratégie de réanimation visant à normaliser le temps de recoloration cutané était associée à une réduction de dysfonction d'organe qu'une approche visant à normaliser ou à diminuer le taux de lactate chez les patients en choc septique.

Plusieurs techniques ont été proposées pour détecter et monitorer les situations d'hypoperfusion ou d'hypoxie tissulaire comme le « Laser Doppler Flowmetry », le « Orthogonal polarization spectral (OPS ) » et le « Sidestream Dark Field (SDF) ». Chaque technique a ses avantages et ses inconvénients, mais le facteur limitant majeur est le besoin des appareils spécifiques qui ne sont pas disponibles dans de nombreux services de réanimations et leurs utilisations sont limitées à la recherche clinique.

La saturation veineuse mêlée en oxygène ( $SvO_2$ ) a été proposée comme un marqueur indirect d'oxygénation tissulaire. La  $SvO_2$  reflète la balance entre  $DO_2$  et  $VO_2$ . Il a été démontré qu'une optimisation précoce de l'hémodynamique en utilisant un protocole de réanimation visant à augmenter la saturation veineuse central en oxygène ( $ScvO_2$ ) > 70% était lié à une réduction importante de la mortalité chez les patients en choc septique. Cependant, trois grandes études multicentriques n'ont démontré aucun bénéfice de cette approche thérapeutique ciblée précoce.

Néanmoins, la conception de ces essais ne visait pas à répondre à la question de savoir si une cible  $ScvO_2 > 70\%$  était efficace pour réduire la morbidité et la mortalité. Dans ces mêmes études, les valeurs moyennes initiales de  $ScvO_2$  étaient déjà au-dessus de 70%. Ainsi, ces résultats n'indiquent pas que les cliniciens doivent cesser de surveiller la  $ScvO_2$  et d'ajuster la  $DO_2$  en optimisant les niveaux de  $ScvO_2$ , en particulier chez les patients en état de choc septique avec une  $ScvO_2$  basse, qui sont les plus à risque de mortalité. D'autre part, la normalisation de  $ScvO_2$  peut ne pas exclure une hypoperfusion tissulaire persistante et l'évolution vers un dysfonctionnement multi-organe et la mortalité. Dans des conditions septiques, des valeurs de  $ScvO_2$  normales ou élevées pourraient être dues à l'hétérogénéité de la microcirculation générant un shunt capillaire et/ou une atteinte mitochondriale responsables de perturbations de l'extraction d'oxygène tissulaire.

Dans ce contexte, les indices dérivés de la pression partielle du sang artériel et du sang veineux central ou mixte en dioxyde de carbone ( $CO_2$ ) ont été proposés pour surmonter les limites des variables précédentes afin d'indiquer l'adéquation de l'apport et des besoins en oxygène. Le gradient veino-artériel de  $PCO_2$  est la différence de pression partielle de  $CO_2$  entre le compartiment veineux mêlé ou veineux central ( $\Delta PCO_2$ ) et le compartiment artériel. Plusieurs études ont montré l'utilité de ce gradient à détecter les situations d'hypoperfusion tissulaire chez les patients en insuffisance circulatoire aiguë même avec une  $ScvO_2$  normale ( $> 70\%$ ). Le rapport de  $\Delta PCO_2$  sur la différence artérioveineuse de contenu en oxygène ( $\Delta PCO_2 / \Delta O_2$ ), considéré comme le reflet du quotient respiratoire, a été démontré comme étant un indice qui permet de prédire de façon fiable l'activation du métabolisme anaérobie et donc la présence d'hypoxie

tissulaire. Cependant les résultats ne sont pas très clairs chez les patients après chirurgie cardiaque notamment ceux qui ont le recours à une circulation extracorporelle.

D'un autre côté, il a été mis en évidence que l'augmentation de  $\Delta\text{PCO}_2$  et la diminution de la  $\text{ScvO}_2$  lors d'une épreuve de ventilation spontanée prédit de façon fiable l'échec de l'extubation chez les patients en ventilation mécanique invasive. En effet, les changements de ces paramètres durant une épreuve de ventilation spontanée reflètent l'incapacité du débit cardiaque à s'adapter à l'augmentation de la consommation d'oxygène induite par la ventilation spontanée. Cette analyse multiparamétrique ( $\Delta\text{PCO}_2$  et  $\text{ScvO}_2$ ) était plus prédictive que chaque paramètre pris séparément et que les paramètres non métaboliques comme les critères échographiques et le pro-BNP. Aussi, il a été démontré que les changements de  $\Delta\text{PCO}_2$  et  $\text{ScvO}_2$  après un remplissage vasculaire pourraient prédire l'augmentation du débit cardiaque induite par le remplissage vasculaire.

Il existe plusieurs limitations à l'utilisation des variables dérivées du  $\text{CO}_2$  qui sont essentiellement liées à la relation entre le contenu de  $\text{CO}_2$  et la  $\text{PCO}_2$ . En effet, plusieurs facteurs peuvent affecter cette relation notamment l'acidose métabolique, la concentration d'hémoglobine, et la saturation veineuse en oxygène. Ces facteurs peuvent changer le  $\Delta\text{PCO}_2$  indépendamment du changement de la perfusion tissulaire. Aussi, les variations aiguës de la  $\text{PCO}_2$  induites par des changements aigus de la ventilation alvéolaire peuvent aussi modifier le  $\Delta\text{PCO}_2$  indépendamment du débit cardiaque. Le clinicien doit connaître ces limitations afin de pouvoir interpréter ces paramètres de façon appropriée.

Il semble donc que l'intégration des paramètres métaboliques  $ScvO_2$ ,  $\Delta PCO_2$ , et  $\Delta PCO_2/\Delta O_2$  dans la réanimation précoce des patients en état de choc septique offre des possibilités intéressantes d'optimisation hémodynamique, mais nécessite des travaux de validation clinique à travers des études randomisées contrôlées multicentriques pour préciser les modalités d'utilisation de ces paramètres.

**Mots-Clés :** Hypoxie tissulaire ; oxygénation tissulaire ; transport artérielle en oxygène ; consommation tissulaire en oxygène ; extraction d'oxygène ; insuffisance circulatoire aigue ; choc septique ; saturation veineuse en oxygène ; gradient veino-artériel de  $PCO_2$  ; rapport de gradient veino-artériel de  $PCO_2$  sur la différence artérioveineuse de contenu en oxygène ; quotient respiratoire ; effet Haldane ; monitoring de la microcirculation ; complications postopératoires.

## Abstract (English)

Acute circulatory failure is associated with an inadequacy between arterial oxygen delivery ( $DO_2$ ) and oxygen consumption ( $VO_2$ ) that, if left untreated, leads to tissue hypoxia and, ultimately, multi-organ failure. Tissue oxygenation is adequate when tissue oxygen demand is met. When  $DO_2$  is severely compromised, a critical  $DO_2$  level is reached, below which  $VO_2$  falls and becomes dependent on  $DO_2$ , leading to tissue hypoxia. The different mechanisms of tissue hypoxia are circulatory, anemic, and hypoxic, characterized by reduced  $DO_2$  but with a preserved capacity to increase oxygen extraction. Cytopathic hypoxia is another tissue hypoxia mechanism caused by impairment of mitochondrial respiration and can be observed in septic conditions with normal overall  $DO_2$ . Rapid detection of tissue hypoperfusion and tissue hypoxia is crucial for early management to increase the likelihood of survival. Also, monitoring the effectiveness of treatments is essential to ensure the appropriateness of the management. Different parameters and tools were proposed for this purpose. This review describes the advantages and limitations of these tools with a focus on the carbon dioxide ( $CO_2$ ) and oxygen-derived variables approach (metabolic approach) to detect and monitor the treatments of tissue hypoperfusion/tissue hypoxia in patients with acute circulatory failure.

**Key-words:** Tissue hypoxia; oxygen delivery; oxygen consumption; oxygen extraction; tissue oxygenation; acute circulatory failure; septic shock; venous oxygen saturation; venous-to-arterial  $PCO_2$  difference; venous-to-arterial  $PCO_2$  difference over difference in oxygen content ratio; respiratory quotient; postoperative complications; microcirculation monitoring.

## Introduction

A shock is a form of acute circulatory failure which is associated with an imbalance between arterial oxygen transport ( $DO_2$ ) and oxygen consumption ( $VO_2$ ), resulting in tissue hypoxia [1]. Early recognition and adequate resuscitation of tissue hypoperfusion are of particular importance in the management of shock to prevent the development of tissue hypoxia and progression to multi-organ failure.

In patients with acute circulatory failure, one of the goals of treatment is to increase cardiac output. This aims to improve the oxygen supply to the tissues and correct the imbalance between oxygen demand and supply [2]. However, no absolute values of cardiac output or  $DO_2$  can be defined since their adequate values depend essentially on the oxygen requirements of the tissue. The correct value of cardiac output is that which ensures an oxygen flow that meets the metabolic demand. Thus, any treatment aimed at modifying cardiac output, such as fluid replacement or inotropes, must be determined by assessing the adequacy between oxygen demand and supply.

The clinical examination still has a limited value in assessing the adequacy between  $DO_2$  and  $VO_2$ . Urine output may reflect renal perfusion, but many other factors may alter its value in shock states. In addition, it depends on the presence or absence of previous renal failure, and it can no longer be used as an indicator of renal perfusion in acute tubular necrosis [3]. Different tools exist to detect and monitor tissue hypoperfusion or tissue hypoxia. However, most of them require specific devices and are used for research purposes. The metabolic approach is based on carbon dioxide ( $CO_2$ ), and oxygen ( $O_2$ ) derived variables such as venous to arterial partial pressure in  $CO_2$  ( $PCO_2$ ) difference ( $\Delta PCO_2$ ) [4] and  $\Delta PCO_2$  over the difference between arterial and venous  $O_2$

content ( $\Delta\text{PCO}_2/\Delta\text{O}_2$ ) ratio [5] have been proposed to assess the adequacy between oxygen intake and requirements and to guide the hemodynamic management in acute circulatory failure patients [6].

In this work, the first section reviewed the concept of  $\text{DO}_2$ , the relationship between  $\text{VO}_2$  and  $\text{DO}_2$ , the mechanisms of tissue oxygen alterations, and different therapeutic approaches. The second section described the various tools of tissue perfusion/oxygenation monitoring and their limitations. The third section presented the metabolic approach for tissue oxygenation monitoring in patients with acute circulatory failure. Finally, the last section reviewed the different perspectives of the metabolic approach and its limitations.

# **First Part**

## **Pathophysiology of altered tissue oxygenation**

## I. Background

Vital cellular activities, including deoxyribonucleic acid (DNA) and protein synthesis, need energy, which is mainly derived from the breakdown of adenosine triphosphate (ATP) [7]. Aerobic ATP production happens in the mitochondria through oxidative phosphorylation, which is generated by the electron transport chain, in which the final electron acceptor is oxygen [8]. Therefore, a constant oxygen supply is required to support vital cell functions.  $DO_2$  is the total amount of oxygen delivered to the tissues per minute. The end goal of  $DO_2$  is to meet the oxygen needs of the cells. During  $DO_2$  reduction,  $VO_2$  remains constant due to the progressive increase the oxygen extraction ( $ERO_2$ ). The ability of the body to increase  $ERO_2$  is the result of the regulation of the circulation and the effects of the simultaneous activation of both central and local factors. The endothelium plays a crucial role in matching tissue oxygen supply to demand in conditions of an acute decline in tissue oxygenation. When  $DO_2$  falls below a critically low value ( $DO_{2crit}$ ),  $VO_2$  begins to fall and becomes dependent on  $DO_2$ , leading to tissue hypoxia and anaerobic metabolism. Also, if oxygen utilization by the cell is altered, cellular  $VO_2$  may drop, leading to tissue hypoxia.

There are three mechanisms of tissue hypoxia based on the  $DO_2$  determinants: (1) hypoxic hypoxia where the arterial oxygen pressure is inadequately low; (2) anemic hypoxia where hemoglobin level is inadequately low; or (3) ischemic hypoxia where cardiac output is inadequately low. These mechanisms are characterized by a diminished  $DO_2$  but preserved capacity of increasing  $ERO_2$ . Cytopathic hypoxia is another tissue hypoxia mechanism that is caused by impairment in mitochondrial respiration with normal overall  $DO_2$ . Alterations in  $ERO_2$  due to microcirculatory impairments with decreased functional capillary density and increased

heterogeneity between the areas with large intercapillary distance leading to the development of tissue hypoxia can be observed in sepsis situations.

In the below manuscript [9], we reviewed the concept of oxygen delivery, the relationship between oxygen consumption and delivery, tissue oxygen alterations' mechanisms, and different therapeutic approaches.



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## Review article

### Pathophysiology, mechanisms, and managements of tissue hypoxia

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#### ABSTRACT

Oxygen is needed to generate aerobic adenosine triphosphate and energy that is required to support vital cellular functions. Oxygen delivery ( $DO_2$ ) to the tissues is determined by convective and diffusive processes. The ability of the body to adjust oxygen extraction ( $ERO_2$ ) in response to changes in  $DO_2$  is crucial to maintain constant tissue oxygen consumption ( $VO_2$ ). The capability to increase  $ERO_2$  is the result of the regulation of the circulation and the effects of the simultaneous activation of both central and local factors. The endothelium plays a crucial role in matching tissue oxygen supply to demand in situations of acute drop in tissue oxygenation. Tissue oxygenation is adequate when tissue oxygen demand is met. When  $DO_2$  is severely compromised, a critical  $DO_2$  value is reached below which  $VO_2$  falls and becomes dependent on  $DO_2$ , resulting in tissue hypoxia. The different mechanisms of tissue hypoxia are circulatory, anaemic, and hypoxic, characterised by a diminished  $DO_2$  but preserved capacity of increasing  $ERO_2$ . Cytopathic hypoxia is another mechanism of tissue hypoxia that is due to impairment in mitochondrial respiration that can be observed in septic conditions with normal overall  $DO_2$ . Sepsis induces microcirculatory alterations with decreased functional capillary density, increased number of stopped-flow capillaries, and marked heterogeneity between the areas with large intercapillary distance, resulting in impairment of the tissue to extract oxygen and to satisfy the increased tissue oxygen demand, leading to the development of tissue hypoxia. Different therapeutic approaches exist to increase  $DO_2$  and improve microcirculation, such as fluid therapy, transfusion, vasopressors, inotropes, and vasodilators. However, the effects of these agents on microcirculation are quite variable.

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List of abbreviations:  $DO_2$ , oxygen delivery;  $DO_{2crit}$ , critical value of  $DO_2$ ;  $VO_2$ , oxygen consumption;  $ERO_2$ , oxygen extraction; CO, cardiac output; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Pi, inorganic phosphate; DNA, deoxyribonucleic acid; Hb, haemoglobin;  $SaO_2$ , arterial oxygen saturation;  $CaO_2$ , arterial oxygen content;  $CvO_2$ , venous oxygen content;  $PaO_2$ , arterial oxygen partial pressure;  $PCO_2$ , carbon dioxide partial pressure; 2,3-DPG, 2,3 diphosphoglycerate; iNOS, inducible nitric oxide synthase; NO, nitric oxide; NADH, nicotinic adenine dinucleotide; FADH<sub>2</sub>, flavine adenine dinucleotide; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid cycle;  $O_2^-$ , oxygen anion; ONOO<sup>-</sup>, peroxynitrite; PARP-1, poly-ADP-riboseyl polymerase; LPS, lipopolysaccharide; CLP, cecal ligation and puncture; RBC, red blood cells; MAP, mean arterial pressure; RCT, randomised controlled trial; SSC, Surviving Sepsis Campaign; SVR, systemic vascular resistance; CI, confidence interval; PGI<sub>2</sub>, Prostaglandin I<sub>2</sub>.

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**1. Introduction**

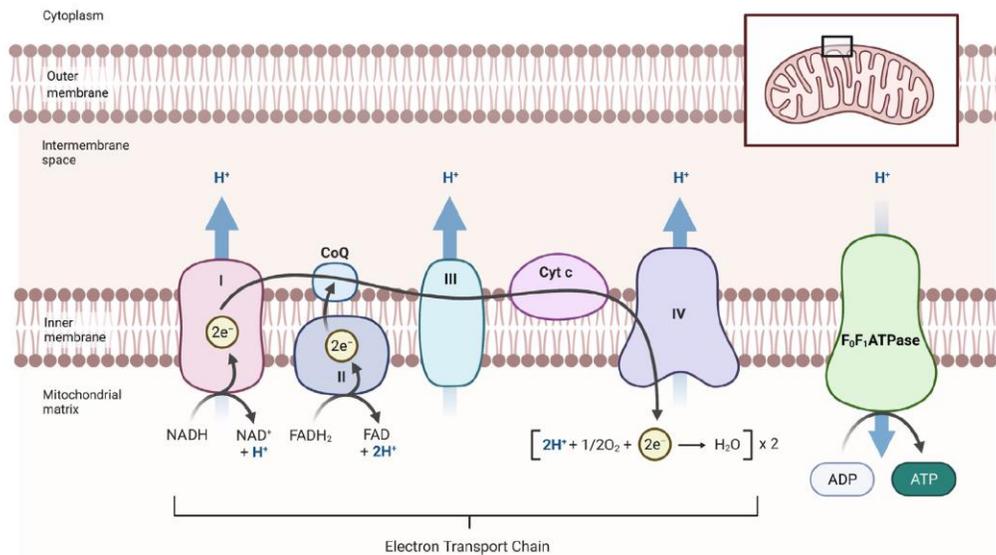
Important cellular processes, ranging from deoxyribonucleic acid (DNA) and protein synthesis, require energy primarily obtained from the degradation of adenosine triphosphate (ATP) to form adenosine diphosphate (ADP) and inorganic phosphate (Pi) [1]. Aerobic generation of ATP occurs in the mitochondria through oxidative phosphorylation. This is a complex reaction powered by the proton gradient across the mitochondrial inner membrane, which is generated by the electron transport chain (mitochondrial respiration), in which the final electron acceptor is oxygen (Fig. 1) [2]. Therefore, a steady supply of oxygen is needed to generate the energy required to support vital cellular functions. If oxygen availability is limited or oxygen utilisation by the cell is altered, cellular oxygen consumption may fall, leading to organ dysfunction. Vital cellular functions can no longer be sustained, and irreversible impairments may develop if the situation persists. Moreover, tissue hypoxia occurs when tissue cells have abnormal oxygen utilisation, resulting in anaerobic metabolism [3].

Tissues have no storage system for oxygen. Thus, it is essential to maintain adequate oxygen supply to the cells and conserve their oxygen utilisation capacity to preserve organ function and avoid tissue hypoxia and death. We herein review the concept of oxygen delivery, the relationship between oxygen consumption and oxygen delivery, the mechanisms of tissue oxygen alterations, and different therapeutic approaches.

**2. The concept of oxygen delivery**

Oxygen delivery (DO<sub>2</sub>) is determined by cardiac output (CO), haemoglobin concentration (Hb), and arterial oxygen saturation (SaO<sub>2</sub>) [4]. DO<sub>2</sub> can be calculated using the following formula:

$$DO_2 = CaO_2 \times CO \times 10 = (1.34 \times Hb \times SaO_2 + (0.003 \times PaO_2)) \times CO \times 10$$



**Fig. 1.** Schematic representation of the mitochondrial oxidative phosphorylation. Electrons (e<sup>-</sup>) donated from the reducing agent, nicotinic adenine dinucleotide (NADH), move progressively from Complex I through Coenzyme Q to Complex III and then through cytochrome c with oxygen (O<sub>2</sub>) being the terminal acceptor at Complex IV. Electrons donated from another reducing agent, flavin adenine dinucleotide (FADH<sub>2</sub>), move from Complex II to Complex III by way of Coenzyme Q. This movement of electrons results in a shift of protons (H<sup>+</sup>) across the inner mitochondrial membrane into the intermembrane space by Complexes I, III, and IV generating the electrochemical gradient (energy) necessary for F<sub>0</sub>F<sub>1</sub>ATPase to produce adenosine triphosphate (ATP) from adenosine diphosphate (ADP).

Where  $CaO_2$  is the arterial oxygen content,  $PaO_2$  is the arterial oxygen partial pressure, and 1.34 are the millilitres of oxygen bound to 1 g of Hb.  $CaO_2$  mainly depends on  $SaO_2$  and Hb concentration, as the amount of dissolved oxygen in the blood is minimal (0.003 mL of oxygen dissolved in 100 mL of blood for 1 mmHg of  $PO_2$ ). The level of  $SaO_2$  is determined by the oxygen-haemoglobin dissociation curve (Fig. 2). Many factors such as temperature, pH, carbon dioxide partial pressure ( $PCO_2$ ), and 2,3 diphosphoglycerate (2,3-DPG) affect the Hb affinity to oxygen. These factors shift the oxygen-haemoglobin dissociation curve. A right shift of the curve as seen in situations of fever, acidosis, hypercapnia, or elevated 2,3-DPG indicates a decreased Hb affinity for oxygen, which facilitates the release of oxygen to the cells. The opposite changes in these factors induce a left shift of the oxygen-haemoglobin dissociation curve indicating an increased affinity of Hb for oxygen, but with reducing tissue oxygen availability. From the  $DO_2$  equation, it may appear that manipulating  $SaO_2$  and Hb levels are as effective as manipulating cardiac output (CO). However,  $SaO_2$  usually is close to 100%, and Hb concentration cannot change acutely. Also, a decrease in  $SaO_2$  or Hb concentration can be compensated for by an increase in CO, while the reverse is not valid [5]. Therefore,  $DO_2$  is fundamentally dependent on CO, and  $SaO_2$  or Hb concentration levels have a meagre contribution to  $DO_2$ , except in situations with extreme hypoxaemia.

Diffusion is the mechanism by which oxygen is transferred from blood to tissue cells. It is dependent on the gradient in  $PO_2$  between the capillary blood and mitochondria, and the diffusion distance between vessels to cells. Diffusion distance is determined by the capillary density and the degree of tissue oedema (Fig. 3). Mathematical models of tissue hypoxia show that the drop in cellular oxygen resulting from an increase in intercapillary distance is more severe if the decrease in tissue  $DO_2$  is caused by a fall in  $PaO_2$  rather than a fall in flow or Hb [6].

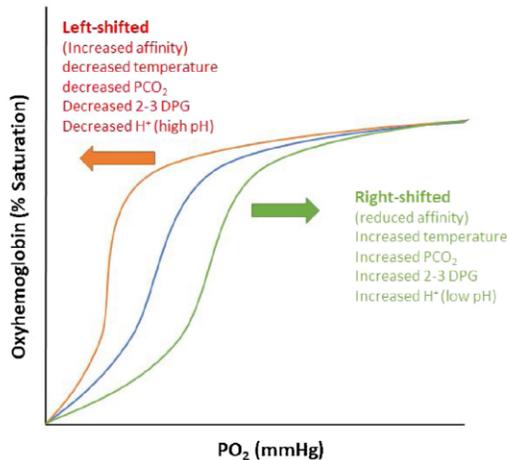


Fig. 2. Oxyhaemoglobin dissociation curve. The blue line shows the normal standard curve. Conditions that shift the curve to the right (green curve) and decrease the oxygen affinity are fever, hypercapnia, acidosis, and increased 2,3 diphosphoglycerate (2,3-DPG). Conditions that shift the curve to the left (red curve) and increase the oxygen affinity are hypothermia, hypocapnia, alkalosis, and decreased 2,3-DPG.

### 3. Relationship between oxygen consumption and oxygen delivery

Global oxygen consumption ( $VO_2$ ) is the rate at which oxygen is taken up from the blood and consumed by the tissues.  $VO_2$  can be derived from the CO and arterial and venous oxygen contents ( $CvO_2$ ) according to the Fick equation:

$$VO_2 = CO \times (CaO_2 - CvO_2) \times 10$$

Tissue oxygenation is adequate when tissue oxygen demand is satisfied. The fundamental characterisation of the relationship between  $VO_2$  and  $DO_2$  came from the findings of experimental animal studies by Cain [7–9], and others [10–12]. Tissue  $VO_2$  is determined by cellular metabolic needs, and it is independent of  $DO_2$  over a wide range of values because compensatory adjustments occur to satisfy the oxygen requirements of peripheral tissues. When  $DO_2$  is acutely decreased by a drop in blood flow (CO), Hb concentration, or  $SaO_2$  levels, the oxygen extraction increases, and  $VO_2$  can therefore be maintained stable for a long time (supply-independency). It is only when  $DO_2$  drops below a critically low value ( $DO_{2crit}$ ) that  $VO_2$  starts to decrease (supply-dependency) (Fig. 4). At  $DO_{2crit}$ , cells shift toward anaerobic metabolism as reflected by the development of tissue metabolic acidosis.

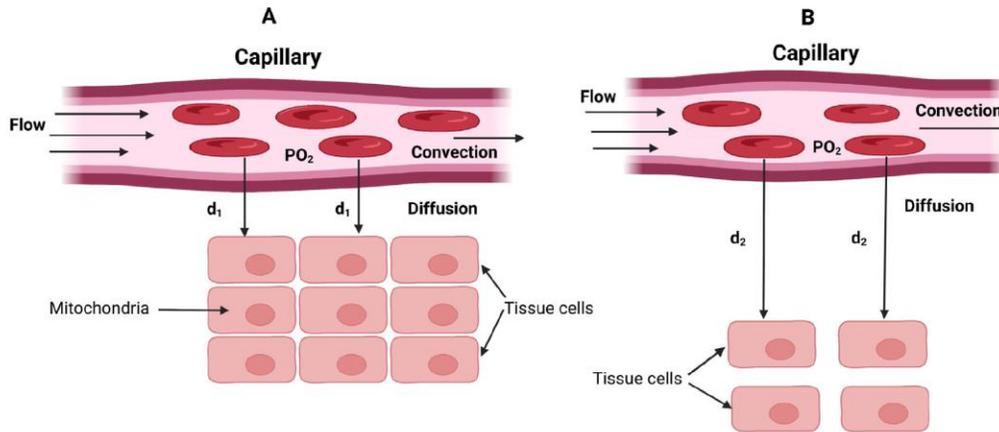
### 4. Physiology of tissue oxygen extraction

The oxygen extraction ratio ( $ERO_2$ ) is the proportion of arterial oxygen that is removed from the blood as it passes the microcirculation:

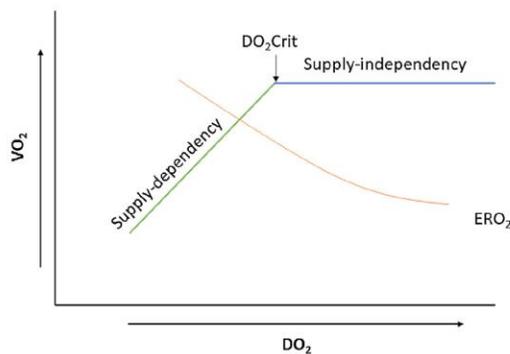
$$ERO_2 = VO_2 / DO_2$$

Normal  $ERO_2$  ranges from 0.25 to 0.3 [4].

The body adjusts  $ERO_2$  in response to changes in  $DO_2$  through two complementary mechanisms that act to prevent the occurrence of tissue hypoxia. First, blood flow into organs is regulated by the vascular tone of the vessels, including the arteriolar system (flow-controlling vessels). These vessels are encircled by smooth muscles that can constrict or dilate in response to different stimulations [13]. Under normal conditions, blood flow to each organ is paired to current demands such that each of them gets a given fraction of whole-body  $DO_2$ . However, when  $DO_2$  is impaired, blood flow is redistributed to metabolically active organs due to an increase in sympathetically induced vasoconstrictor, preventing “vascular steal” of blood flow overperfusing organs with relatively low oxygen requirements [8,14,15]. Cain demonstrated that the use of alpha-blockade with phenoxybenzamine attenuated the increase in  $ERO_2$ , indicating the importance of a strong vasoconstrictor sympathetic tone to promote oxygen tissue extraction during global tissue hypoxia in anaesthetised dogs [16,17]. Second, even if arteriolar blood flow goes to the right organs proportionally to their demands, there is no assurance that the capillary bed gets this blood according to tissue requirements. Therefore, in reference to the metabolic concept of microvascular regulation, tissues with rather elevated metabolic rates induce metabolic vasodilation that opposes the sympathetic vasoconstrictor tone, thus preventing the imbalance between cellular oxygen supply and demand. Local distribution of flow is determined downstream by precapillary (distribution vessels) sphincters in tissues. Any condition with decreased tissue  $PO_2$  would induce a vascular dilation, increase the perfused capillary density, and restore normal tissue oxygenation [18–20]. In response to local tissue hypoxia, capillary recruitment can reduce intercapillary spaces, thus permitting tissues to extract



**Fig. 3.** Diagram showing the importance of tissue oedema in determining the capillary oxygen delivery and intracellular partial oxygen pressure (PO<sub>2</sub>). Oxygen diffusion from capillary red blood cells (RBC) to tissue cell mitochondria is determined by the oxygen gradient from RBC to mitochondria and the diffusion distance (d<sub>1</sub> and d<sub>2</sub>) from RBC to mitochondria. A, Blood flow through microcirculation under normal conditions with no tissue oedema. B, Blood flow through microcirculation under conditions with tissue oedema and increased diffusion distance [d<sub>2</sub>] between capillary RBC and tissue cells mitochondria.



**Fig. 4.** Relationship between oxygen delivery (DO<sub>2</sub>) and oxygen consumption (VO<sub>2</sub>). When DO<sub>2</sub> decreases, VO<sub>2</sub> remains constant over a wide range of DO<sub>2</sub> (supply-independency, blue line) because of the increases in oxygen extraction (ERO<sub>2</sub>, red line). When DO<sub>2</sub> decreases further beyond a critical value DO<sub>2</sub> (DO<sub>2</sub> Crit), VO<sub>2</sub> decreases along with the decreases in DO<sub>2</sub> (supply-dependency, green line), which defines the occurrence of tissue hypoxia and the activation of anaerobic metabolism.

oxygen effectively by reducing the capillary-to-cell diffusion distance [21].

In situations of tissue hypoxia, there is an indication that parenchymal cells generate high quantities of metabolites such as lactate, hydrogen ions, adenosine, and potassium ions that accumulate in the tissue. These cause vascular smooth muscle to relax, probably through activation of ATP-sensitive K<sup>+</sup> channels, leading to increased blood flow to the hypoxic tissue [22]. However, this suggests that the cells need to experience some degree of hypoxia before the organ blood flow is changed. Consequently, other sources of control should exist. The endothelial cell is an essential element of the microvascular system. It plays a crucial function as a signal transducer of shear stress [23,24] and

vasoactive agents [25], including catecholamines, acetylcholine, prostaglandins, and others. In response to decrease in PO<sub>2</sub>, endothelial cells release autacoid substances, including prostacyclin, nitric oxide (NO), and the endothelium-dependent hyperpolarising factor that have strong vasodilator effects on the vascular tone by stimulating soluble guanylate cyclase in vascular smooth muscle directly [26–28]. Furthermore, it has been shown that endothelial cells are implicated in tissue ERO<sub>2</sub> capability [29]. An endothelial oxygen detector in the vessel would allow the vasculature system to react quickly to a decline in arteriolar PO<sub>2</sub> without the requirement for metabolites to accumulate, with the inherent risk of hypoxia-induced cell injury. Therefore, endothelium plays a crucial role in matching tissue oxygen supply to demand in situations of acute drop in tissue oxygenation.

In summary, increased ERO<sub>2</sub> is the result of the regulation of the circulation and the effects of the concomitant activation of both central and local factors [8]. Central factors provoke a regional redistribution of blood flow between organs via sympathetic vasoconstrictor tone. In contrast, local factors cause an increase in the density of perfused capillaries within tissues via vasodilator tone after releasing vasodilating substances that are part of the general response termed autoregulation [30]. Altered vascular reactivity with an abnormal balance between vasoconstrictor and vasodilator tone should, therefore, fail to control blood flow distribution between and within tissues, favouring hyperperfusion of some tissue beds at the cost of hypoperfused areas, with reduced oxygen extraction capability.

### 5. Mechanisms of tissue hypoxia

The method by which DO<sub>2</sub> is altered determines the mechanism of tissue hypoxia. According to the DO<sub>2</sub> formula, hypoxic hypoxia (drop in SaO<sub>2</sub>), anaemic hypoxia (drop in Hb), and circulatory hypoxia (drop in CO) are the different pathophysiological mechanisms of tissue hypoxia. It is not rare for such conditions to be concomitantly seen in critically ill patients. Another proposed mechanism to explain tissue hypoxia in situations of preserved DO<sub>2</sub> is cytopathic hypoxia [31].

### 5.1. Hypoxic, anaemic, and low flow tissue hypoxia

In normal conditions, tissue  $VO_2$  is determined by cellular metabolic requirements and is not influenced by the quantity of oxygen delivered because of progressive increases in  $ERO_2$  [32,33]. When  $DO_2$  is severely compromised, a critical  $DO_2$  is reached below which  $VO_2$  falls and becomes dependent on  $DO_2$  (oxygen-supply dependency), resulting in anaerobic metabolism activation [7], leading to severe acidosis and eventual cell death, if not corrected. This is due to the fact that  $ERO_2$  does not increase proportionally to the decrease in  $DO_2$ . This biphasic nature of  $DO_2$  and  $VO_2$  has been observed in normal intestinal tissue and during ischaemia. There is no one  $DO_{2crit}$  value for the whole organism. Each organ has its own  $DO_{2crit}$  according to its capability of oxygen extraction. Hence, tissue hypoxia is an earlier phenomenon in the skin, whereas it is delayed in the brain and heart during reductions in  $DO_2$ .

Circulatory (hypovolaemic, cardiogenic, and obstructive types of shock), anaemic and hypoxic tissue hypoxia are characterised by a diminished  $DO_2$  but preserved capacity of increasing  $ERO_2$  (physiological oxygen supply dependency). In an experimental study of splenectomised dogs that were made progressively anaemic (reaching haematocrit as low as 10%) by replacement of blood with colloid (anaemic hypoxia model), and another group with progressive hypoxia (hypoxic hypoxia model) by breathing air mixed with nitrogen (reaching oxygen inspired fraction as low as 6%), Cain observed that excess lactate production appeared at about the same level of  $DO_2$  ( $DO_{2crit} = 12$  mL/kg/min) in both models [34]. In a more detailed study with isovolumic haemodilution anaemic dogs and hypoxic hypoxia dogs, Cain observed that  $VO_2$  was limited at a higher mixed venous  $PO_2$  level in the anaemic model than in the hypoxic model (45 mmHg vs. 17 mmHg, respectively). However, oxygen supply dependency occurred at the same  $DO_{2crit}$  level (9.8 mL/kg/min) in both models [7]. Schwartz et al. investigated the effects of haemorrhagic (hypovolaemic), normovolaemic anaemia, and hypoxia models in dogs [35]. They found that with progressively decreasing  $DO_2$ ,  $ERO_2$  increased to the same extent in each model and continued to increase even after reaching  $DO_{2crit}$  and decreased only at very low  $DO_2$  values in the three experiments. There were differences in the haemodynamic responses to the three types of decreases in  $DO_2$ . Indeed, CO increased in the anaemic model and greater rise in systemic and pulmonary vascular resistances in the haemorrhagic model. In that study,  $DO_{2crit}$  level was also the same in the three models of tissue hypoxia around 10 mL/kg/min, and the  $VO_2/DO_2$  dependency phenomenon (precipitous drop in  $VO_2$ ) occurred only at near-death [35].

Intestinal tissue oxygenation is relatively more resilient to hypoxic hypoxia than to reduced blood flow (hypoxic ischaemia). The ability to endure pure hypoxia is dependent on preserved blood flow to intestinal tissues. Indeed, during progressive ischaemia, in intact dog intestine,  $VO_2$  decreased when  $DO_2$  reduced to 60% of its baseline value [36]. However, when  $DO_2$  was compromised by pure hypoxia,  $VO_2$  did not fall and did not become dependent on  $DO_2$  despite a decrease to 36% of its baseline.  $ERO_2$  increased with decreasing  $DO_2$  but did not reach a plateau, suggesting no diffusion limitation.  $VO_2$  stayed independent to a very low level of  $DO_2$  because the blood flow part of  $DO_2$  was maintained. Also, in another experimental study,  $VO_2$  was found to be independent of  $DO_2$  in a pure hypoxic model, in fetal lamb intestines, despite decreases in  $DO_2$  to 43% of baseline [37]. The capacity of the intestines to keep appropriate oxygenation in the face of hypoxia might be explained in part by the strong vasodilatory effects of hypoxia in the small bowel. It has been shown that arterial hypoxia induces an increase in blood flow up to

146% of control group in intestinal small bowel [38]. In an effort to maintain adequate tissue oxygenation, ischaemic, anaemic, or hypoxic intestinal tissue will react by modulating the blood flow by acting on the local arteriolar tone [39,40] and by controlling the pre-capillary sphincters, which allows to increase the number of perfused capillaries in the surface area and decrease the capillary-to-cell diffusion distance leading to increase in  $ERO_2$ .

### 5.2. Altered tissue oxygen extraction

The reduced capability of oxygen extraction has been observed in animal models of sepsis and septic patients. In a model of haemorrhagic shock with stepwise decrease in  $DO_2$ ,  $DO_{2crit}$  value was high, and  $ERO_2$  was low in the whole body of anaesthetised dogs that were challenged with either live bacteria or LPS [9,41]. This suggests that sepsis caused by bacterial infection may induce regional alterations in vascular tones. Several studies reported impairments of contractile responses to  $\alpha$ -adrenergic agonists such as angiotensin II and serotonin in vasculature tissue from endotoxaemic animals [42–45]. It was reported that iNOS inhibitors reinstate the contractile responses of the animals' vessels to agonists [43]. These observations align with the pathophysiologic hallmarks of sepsis in clinical and experimental conditions, which is intense vasodilation with dysregulation of blood flow distribution between organs. However, in vivo endotoxaemic animal models, blocking iNOS failed to reinstate a normal  $ERO_2$ , and to improve survival [46,47]. Furthermore, increasing  $\alpha$ -adrenergic tones with treatments was not enough to improve  $ERO_2$  during septic shock [48]. These results suggest that impaired vasoconstriction cannot entirely explain the altered  $ERO_2$  observed in septic shock, and derangements in vasodilator tones should occur since a normal  $ERO_2$  response results from an adequate balance between vasoconstrictor and vasodilator tones.

Attenuated acetyl-choline-induced dilation in vascular rings isolated from large arteries from endotoxaemic animals was reported by many studies [44]. These observations suggest impaired NO secretion by endothelial cells secondary to a defect in the expression of the constitutive NOS isoform. This was also demonstrated in the isolated small intestine arterioles after CLP of rats [49]. Moreover, the density of tissue perfused capillaries decreased, and the spatial distribution was more heterogeneous with an increase in the number of capillaries with stop-flow and large intercapillary distance in the skeletal muscles in rats that were made septic by CLP [50]. In endotoxaemic pigs, gut capillary transit-time heterogeneity significantly increased and was associated with lower critical  $ERO_2$  than in control animals [51]. The remaining functional capillaries compensate for the reduced capillary density by delivering more oxygen to the adjacent tissue. However, augmented oxygen flow heterogeneity apparently impairs oxygen extraction by raising critical  $DO_2$  and reducing the critical  $ERO_2$  [52]. Reactive hyperaemia is a test used to assess an organ's maximal capacity to raise its blood flow when oxygen demand has been maximally augmented. The rise in blood flow is due to an increase in flow in the same patent capillaries and by recruiting other capillaries [53,54]. This response is due to vasoactive agents affecting the arteriole smooth muscle and those acting through the arteriole endothelium [54,55]. Using laser Doppler flowmetry, it has been shown that after stopping the leg blood flow by inflating a pneumatic cuff, the reactive hyperaemia was decreased in critically ill septic patients despite increase in  $DO_2$  of the whole body [56]. Possible mechanisms that might explain these findings are impaired vascular reactivity to vasodilate and microvascular obstruction limiting capillary recruitment ability. Using orthogonal polarisation spectral imaging technique to assess the sublingual microcirculation, De Backer

et al. found that the density of all vessels was significantly decreased, the proportion of perfused small vessels was significantly reduced, and a more significant number of non-perfused and intermittently perfused small vessels with a marked heterogeneity between the areas in severe sepsis critically ill patients compared to volunteers patients [57]. In a mathematical model of the determinants of  $\text{DO}_2$  and  $\text{VO}_2$ , a rise in blood flow heterogeneity was associated with a rise in  $\text{DO}_{2\text{crit}}$  with decreased  $\text{ERO}_2$  [58].

Capillary recruitment resulting from local hypoxia can efficiently decrease intercapillary space, thus permitting more tissues oxygen extraction. Deranged  $\text{ERO}_2$  abilities in sepsis might result from a failure to adjust capillary density according to the local oxygen supply to demand requirements.

### 5.3. Cytopathic tissue hypoxia

The classical mechanisms of tissue hypoxia (hypoxic hypoxia, anaemic hypoxia, and stagnant hypoxia) were insufficient to explain the reduced ATP biosynthesis and organ dysfunction in sepsis. Thus, acquired intrinsic impairment in cellular respiration was proposed as a potential mechanism that contributes to ATP production derangement despite normal (or supranormal)  $\text{PO}_2$  values around mitochondria inside cells. This mechanism was named "cytopathic hypoxia" by Mitchell Fink [59,60].

The evidence supporting the concept of cytopathic hypoxia came from a considerable body of experimental studies in the context of sepsis with tissue  $\text{PO}_2$  measurements [1,61–66]. For example, in a model of septic peritonitis with cecal ligation and puncture (CLP) in rats, the mean skeletal  $\text{PO}_2$  was not different between the resuscitated septic animals and in the normal controls [1]. Also, in anaesthetised pigs that were challenged with lipopolysaccharide (LPS), the mucosal  $\text{PO}_2$  significantly increased after restoring CO with fluid resuscitation [62]. Furthermore, the  $\text{PO}_2$  distribution in skeletal muscle was shifted to supranormal values in patients with septic shock, whereas it was shifted to low values in cardiogenic shock patients as expected [64]. These data suggest that tissue  $\text{PO}_2$  levels are normal/high in resuscitated septic patients. In addition, several studies demonstrated that mitochondrial function is deranged in animals with sepsis [67,68]. Indeed, the hepatocytes'  $\text{VO}_2$  was significantly less when the hepatocytes were isolated from septic rats than from non-septic control rats [67]. Also, the amount of  $\text{VO}_2$  was significantly lower in ileal mucosa tissue from endotoxaemic rats that was given LPS compared with control rats [68]. These findings support the concept of impaired cellular oxygen use in sepsis condition with normal/high  $\text{PO}_2$  levels. Interestingly, the ileal mucosa  $\text{VO}_2$  rate was preserved when LPS challenged rats were administered with aminoguanidine to block the inducible nitric oxide synthase (iNOS), suggesting that the occurrence of cytopathic hypoxia in endotoxaemic rats needs iNOS-dependent NO production.

Aerobic production of ATP happens in mitochondria through oxidative phosphorylation (Fig. 1). The reduced forms of nicotinic adenine dinucleotide (NADH) and flavine adenine dinucleotide ( $\text{FADH}_2$ ) are generated during glycolysis and the tricarboxylic acid cycle (TAC) in cells are oxidised by molecular oxygen. The energy liberated by the process is used to actively pump protons from the mitochondrial matrix into the intermediate space creating an electrochemical gradient across the inner mitochondrial membrane. This gradient pushes hydrogen ions through the  $\text{F}_0\text{F}_1$ ATPase mitochondrial enzyme that catalyses the phosphorylation of ADP to form ATP (Fig. 1).

Several different but jointly potential mechanisms might participate in the occurrence of cytopathic hypoxia in sepsis. These mechanisms include the inhibition of pyruvate dehydrogenase (PDH) by activation of PDH kinase [69–72], limiting the

delivery of pyruvate into the mitochondrial TCA leading to excess pyruvate accumulation and increased lactate production that is not related to impaired  $\text{DO}_2$  [70]. NO-induced inactivation of cytochrome *a*,*a*3 is another possible mechanism of sepsis-mediated cytopathic hypoxia. Sepsis is associated with increased iNOS synthesis that results in increased generation of NO. Experimental data showed that NO generated endogenously is able to cause reversible inactivation of the enzymatic activity of cytochrome *a*,*a*3, which is the terminal complex of the mitochondrial electron transport chain, leading to mitochondrial dysfunction [72–74]. Acute inflammatory states, including sepsis and ischemia/reperfusion, are appropriate situations where NO reacts with oxygen anion ( $\text{O}_2^-$ ) to form a potent oxidising and nitrosating agent, which is peroxynitrite ( $\text{ONOO}^-$ ) [75–77]. It has been shown that  $\text{ONOO}^-$  inhibits different components of the mitochondrial function, including the mitochondrial  $\text{F}_0\text{F}_1$ ATPase that drives phosphorylation of ADP to produce ATP and the mitochondrial enzyme complexes I and II that are involved in electron transport [78] (Fig. 1). In a rat animal model, endogenous generation of  $\text{ONOO}^-$  following in vivo LPS injection resulted in mitochondrial respiration dysfunction in the rat diaphragm [79]. The most important mechanism contributing to cytopathic tissue hypoxia in sepsis is the poly-ADP-ribosyl polymerase (PARP-1), a nuclear enzyme involved in various cellular functions, including DNA repair, replication, and cellular apoptosis [80–82]. PARP-1 is activated by single-strand breaks in nuclear DNA, leading to the degradation of  $\text{NAD}^+$  [83], and resulting in  $\text{NAD}^+/\text{NADH}$  depletion in cells. Consequently, PARP-1 activation can result in a severe derangement of oxygen utilisation by cells, leading to cytopathic hypoxia. In acute inflammation conditions, reactive oxygen species such as  $\text{ONOO}^-$  can activate PARP-1 by inducing single-strand breaks in nuclear DNA [84]. Incubation of human enterocytic cells with cytokines (tumour necrosis factor- $\alpha$ , interleukin-1  $\beta$ , and interferon- $\gamma$ ) reduced  $\text{VO}_2$  that was measured directly using an oxygen-sensitive optode by 50% [85]. Interestingly, this phenomenon was entirely reversible when cytokines were removed. Also, PARP-1 knockout mice were resistant to the lethal LPS effects [86,87]. Moreover, blocking PARP-1 using a pharmacological agent increased survival in a porcine model of lethal bacterial peritonitis [88].

## 6. Therapeutic approach

### 6.1. Fluid therapy

Fluid infusion continues to be an indispensable part of the management of all types of shock. Originally held for hypovolaemic conditions, and to some degree septic shock, cardiogenic shock also requires fluid infusion as the vasoconstrictive state leads to extravasation of fluid into the interstitium. Thus, treatment of cardiogenic shock using careful fluid administration has become a standard, even in the presence of cardiogenic pulmonary oedema [89,90].

The goal of volume expansion is to increase CO and improve tissue perfusion. This implies correcting the oxygen accessibility in the microcirculation after microcirculatory hypoperfusion in shock states [91]. Thus, this means that adequate tissue perfusion should guarantee an adequate supply of oxygen to the tissue cells.

Fluid therapy can enhance the microcirculation if administered in a timely manner and in suitable quantities. Fluids improved microvascular perfusion by increasing the proportion of perfused small vessels and small vessels density, thus reducing perfusion heterogeneity in septic patients [92,93]. Importantly, improvement in microcirculation was mainly observed when fluid was given early (within 24 h) whereas late administration (after 48 h)

did not ameliorate the microvascular perfusion [92]. Also, the microcirculatory effects of volume expansion were relatively independent of systemic effects [92,93]. Interestingly, in preload-dependent patients, organ dysfunction improved only in those who improved their microcirculation in response to fluid administration [94]. However, excessive fluid resuscitation might be harmful by inducing tissue oedema and increasing the distance between capillaries and cells, resulting in a decrease in oxygen diffusivity (Fig. 3). Several experimental and clinical studies have demonstrated the detrimental effects of aggressive fluid administration on the outcome of sepsis [95–106]. It is unknown how much fluid should be given. The latest version of the Surviving Sepsis Campaign (SSC) guidelines still recommending the administration of 30 mL/kg of intravenous crystalloid fluid to be given within the first 3 h of resuscitation in septic patients with signs of tissue hypoperfusion [107]. However, many experts have questioned this approach as a fixed dose of 30 mL/kg was based upon a low quality of evidence, and as “one size does not fit all,” initial fluid resuscitation should be individualised to each patient’s needs [107–109]. Thus, fluid administration should be guided by dynamic tests (based on heart-lung interaction) [110–112], passive leg raising test [113,114], end-expiratory occlusion test [115], mini-fluid challenge techniques [116–118], or hypoperfusion parameters [119,120]. In situations of tissue hypoperfusion, fluid resuscitation should only be given to fluid responsive patients (preload-dependent) in order to avoid fluid accumulation [121].

In this regard, fluid withdrawal by administration of diuretics resulted in improved microvascular perfusion by decreasing interstitial oedema, and thus, increasing the diffusive capacity of the microcirculation by increasing its functional capillary density [122]. Multiple trials showed the feasibility of a restrictive fluid approach in the early phase of resuscitation of septic shock patients with suggestions toward benefit with fluid restriction in some studies [123–127]. However, these studies were not powered to identify differences in patient-centred outcomes. The ongoing Crystalloid Liberal or Vasopressors Early Resuscitation in Sepsis (CLOVERS) trial and the Conservative vs. liberal fluid therapy in septic shock (CLASSIC) trial will shed some light to this issue [128,129].

### 6.2. Blood transfusion

A sufficient number of RBC should reach the microcirculation in order to increase the oxygen carrying capacity of blood and improve tissue oxygenation. To obtain this, resuscitation should achieve an adequate amount of Hb-loaded red blood cells (RBC) entering the microcirculation to meet the tissue cells’ metabolic requirements since Hb concentration is the main factor of oxygen availability [130]. Therefore, the idea of sufficient tissue perfusion should enclose the RBC’s convection entering the microcirculation and attain an adequate density of RBC-filled capillaries to guarantee the equally important diffusion of oxygen from the RBCs to the tissue cells [13]. These two features of tissue perfusion would be more appropriately combined into one variable, which is called tissue RBC perfusion. This theory reflects the necessity of having a sufficient capillary haematocrit and functional capillary density (diffusion capacity) integrated with adequate convection or RBC flow in the microcirculation [131].

RBC transfusion improved the sublingual microcirculation by significantly increasing the proportion of perfused vessels and the functional capillary density, and by significantly decreasing the blood flow heterogeneity in haemorrhagic shock patients [132]. Interestingly, these observed effects of transfusion on microcirculation were independent of the Hb level or the systemic effects [132]. However, in septic patients, RBC transfusion improved microvascular perfusion only in those with altered capillary

perfusion at baseline [133]. In another study performed in critically ill patients, RBC transfusion improved the microvascular perfusion in patients with impaired microcirculation but had opposite effects on patients with normal microcirculation before transfusion [134]. Interestingly, RBC transfusion at both 75 g/L and 90 g/L of Hb can ameliorate or damage the microvascular perfusion, challenging the concept of arbitrary transfusion thresholds [134].

It remains a matter of debate when we should transfuse a patient. The recent transfusion guidelines in non-bleeding critically ill patients recommend adopting a restrictive transfusion approach (Hb threshold of 70 g/L) over a liberal transfusion approach (Hb threshold 90 g/L) in the majority of critically ill patients with non-bleeding anaemia, except in patients with an acute coronary syndrome where a liberal transfusion strategy (Hb threshold of 90–100 g/L) is preferred to a restrictive strategy (Hb threshold of 70 g/L) [107,135]. No recommendations were made for elderly and oncologic or haemato-oncologic critically ill patients or for those undergoing extracorporeal membrane oxygenation [135].

In critically ill bleeding patients, the recent guidelines of the European Society of Intensive Care Medicine (ESICM) suggest adopting a restrictive RBC transfusion threshold: 70 g/L vs. 90 g/L for non-massive gastrointestinal bleeding, and 75–80 g/L vs. 100 g/L for non-massive bleeding after vascular surgery [136].

In a recent meta-analysis, RBC transfusions resulted in significant increases in  $DO_2$ ,  $VO_2$ , and venous oxygen saturation and a significant decrease in  $ERO_2$  without effects on CO [137]. Also, recently, Fischer et al. demonstrated that an individualised RBC transfusion strategy based on a central venous oxygen saturation threshold of 70% resulted in a significantly lower transfusion rate with no differences in postoperative morbidity or 6-month mortality compared to liberal strategy in patients admitted to intensive care unit after cardiac surgery with postoperative Hb less than 90 g/L [138]. These findings suggest that the decision to transfuse in critically ill patients should not be based on a Hb level alone but also guided by oxygenation derived variables when it is possible. Today, an acceptable strategy is that RBC transfusion can be safely withheld when the Hb is  $> 90$  g/L and should be administered when Hb is  $< 70$  g/L. Transfusion decisions should be individualised if the Hb is between these two values [90].

### 6.3. Vasopressors

Vasopressors are drugs used to increase arterial blood pressure by peripheral vasoconstriction. Different vasopressor agents have been proposed. Norepinephrine is a potent  $\alpha$ -1 receptor agonist and mild-moderate  $\beta$ -1 receptor agonist with minimal  $\beta$ -2 receptor activity [139]. Its haemodynamic effects are dominated by  $\alpha$ -1 receptor-induced vasoconstriction and increased systemic vascular resistance (SVR), which results in increased mean arterial pressure (MAP) with minimal effects on heart rate and CO. At high doses, norepinephrine may increase CO due to  $\beta$ -1 receptor activation and increases in venous return mediated by rising in the mean systemic filling pressure [140]. However, like a pure vasoconstrictor, norepinephrine may decrease CO in untreated hypovolaemia cases or in patients with cardiac dysfunction owing to the potent rise in afterload. Nevertheless, many patients with cardiogenic shock can keep CO during norepinephrine treatment [141,142]. Dopamine exerts its effects in a dose-dependent manner on dopamine-1,  $\alpha$ -1, and  $\beta$ -1 receptors. At lower doses, dopamine induces vasodilation through dopamine-1 receptor activity in the kidney, splanchnic, brain, and coronary circulation. At higher doses, the activity of dopamine’s  $\alpha$  receptor prevails, which leads to vasoconstriction and increased SVR. Its  $\beta$ -1 receptor activity can cause dose-dependent arrhythmias [139]. Epinephrine is a potent  $\alpha$ -1 and  $\beta$ -1 receptors agonists with stronger  $\beta$ -2

receptor activity than norepinephrine (Table 1). At low doses, epinephrine activity is mainly related to its effects on  $\beta$ -1 receptors resulting in increased CO and heart rate with variable responses on MAP. At higher doses, epinephrine infusion leads to an increase in SVR, MAP, and CO [139]. Potential side effects of epinephrine comprise arrhythmias and impaired splanchnic circulation [143]. Phenylephrine is a pure  $\alpha$ -1 receptor agonist with no  $\beta$ -1/ $\beta$ -2 receptors activity that increases arterial and venous resistance. Vasopressin is a natural hormone with strong vasoconstrictive effects. Its vasoconstrictor effects are due to activation of V1a receptors on vascular smooth muscles, resulting in increased SVR and MAP [139,144]. At high doses, vasopressin has been associated with cardiac, digital, and splanchnic ischaemia [145].

The updated version of the SSC guidelines continues to recommend norepinephrine as the first-line vasopressor for septic shock, and there is enough proof to back this recommendation [107]. Norepinephrine is also the first-line vasopressor in most types of shock, including cardiogenic shock [146]. Norepinephrine improved microvascular perfusion when used to correct severe hypotension by restoring a minimal perfusion pressure to organs [147,148]. However, increasing MAP higher than 65 mmHg (75 or 85 mmHg) had variables effects on microvascular perfusion [149,150]. Interestingly, increasing MAP with norepinephrine improved microvascular perfusion only in patients with severe impairment in sublingual microcirculation, whereas it was detrimental in patients with close to normal baseline microcirculation [150]. The SSC recommends targeting a MAP of 65 mmHg during the initial resuscitation of septic shock [107]. Although the optimal MAP target in shock states has been a subject of intense research [151,152], no universally accepted value has been recognised. Thus, target values of MAP should be personalised for a given patient considering several factors, including history of chronic hypertension, and persistent signs of tissue hypoperfusion [153].

When to start norepinephrine is still a matter of debate. In the past, vasopressor was administered only when it was deemed that the patient was fluid unresponsive. However, even untreated transient hypotension can be associated with organ dysfunction [154] or increased mortality [155]. Early administration of norepinephrine during resuscitation of severely hypotensive septic shock patients increased CO through an increase in venous return by enhancing the mean systemic filling pressure, and consequently cardiac preload, and also by improving cardiac contractility [156–158]. Early initiation of norepinephrine was associated with better outcomes in septic shock patients. In a retrospective study that included 213 septic shock patients, the 28-day mortality rate was significantly higher when norepinephrine infusion was initiated  $\geq 2$  h after septic shock onset. Also, every 1-h delay in norepinephrine starting during the first 6 h after septic shock onset was associated with a 5.3% increase in mortality. Further-

more, the duration of hypotension and norepinephrine requirements was significantly shorter in the early norepinephrine initiation group [159]. A phase II randomised controlled trial (RCT) with 310 septic shock patients showed that early administration of norepinephrine (median 1.5 h) was significantly associated with higher shock control by 6 h [160]. In another prospective study with propensity score-based analysis, early administration of norepinephrine (within 6 h of septic shock onset) was associated with significantly lower 24-h net fluid balance and lower risk of death at day 28 with no significant increase in acute renal failure or renal replacement therapy requirements in 186 septic shock patients [161]. These results were supported by the findings of a recent meta-analysis that included 929 patients [162]. These results suggest that norepinephrine might be used as an adjunct to fluids to increase CO and MAP in hypotensive patients. However, large-scale RCTs are required to confirm these findings. Also, the optimal timing of norepinephrine initiation is yet to be determined.

Patients with more severe septic shock may require high doses of norepinephrine due to down regulation of  $\alpha$ 1-adrenergic receptors. High dose norepinephrine may harm myocardial cells and induce oxidative stress. Thus, a second vasopressor should be added before patients develop refractory septic shock. In a meta-analysis, the use of vasopressin was related to a reduction in the need for renal replacement therapy, a tendency toward lower 90-day mortality, but more complications such as digital ischaemia [163]. The SSC guidelines suggest adding vasopressin when the dose of norepinephrine is in the range of 0.25–0.5  $\mu$ g/kg/min [107]. In cases of myocardial dysfunction, epinephrine is a reasonable alternative [107].

Other novel vasopressor drugs (selepressin and angiotensin II) have been developed recently [164–166]. Their potential indications in septic shock patients are still under investigation.

#### 6.4. Inotropes

In septic shock patients with persistent signs of tissue hypoperfusion after adequate fluid resuscitation and MAP and myocardial dysfunction based on suspected or measured low CO and elevated cardiac filling pressure, the SSC guidelines suggest adding dobutamine to norepinephrine or using epinephrine alone [107]. However, there are no RCTs that compared dobutamine to placebo in these patients. A network meta-analysis found that the combination of dobutamine and norepinephrine had no effect on mortality when compared to no dobutamine [167]. Nevertheless, none of the included trials directly compared the association of dobutamine and norepinephrine to norepinephrine alone. Also, there was no evidence to support the superiority of the combination of dobutamine and norepinephrine over epinephrine alone in terms of mortality [167]. The ongoing ADAPT multicentre RCT (NCT04166331) will shed light on the efficacy of dobutamine

**Table 1**  
Vasopressors and inotropes receptors potency.

Agents	$\alpha$ -1 receptors	$\beta$ -1 receptors	$\beta$ -2 receptors	Dopamine-1 receptors	Vasopressin-1a receptors
Dobutamine	+	++++	+++	0	0
Dopamine	+++	++++	++	++++	0
Epinephrine	++++	++++	+++	0	0
Milrinone	0	0	0	0	0
Levosimendan	0	0	0	0	0
Norepinephrine	++++	+++	++	0	0
Phenylephrine	++++	0	0	0	0
Vasopressin	0	0	0	0	++++

0: no significant receptor affinity; + through ++++: minimal to maximal receptor affinity.

as an adjunctive treatment in septic shock patients with septic cardiomyopathy and tissue hypoperfusion.

Levosimendan is a calcium-sensitising drug with inotropic and vasodilatory properties. In a meta-analysis of seven RCTs, no significant difference in the mortality rate between levosimendan and dobutamine was found in sepsis and septic shock patients [168]. The SSC guidelines suggest against using levosimendan in septic shock patients and cardiac dysfunction with signs of tissue hypoperfusion despite adequate fluid resuscitation and MAP [107].

In patients with cardiogenic shock, the first line inotropic agent is dobutamine [146,169,170]. Levosimendan may be used in cardiogenic shock patients already on chronic beta-blocker therapy and in patients with cardiogenic shock with acute right ventricular failure or pulmonary hypertension due to its positive effects on pulmonary vascular resistance mediated by potent phosphodiesterase-3 inhibition [146]. Milrinone, a selective phosphodiesterase-3 inhibitor, and dobutamine had similar effectiveness and safety profiles [171], but may be harmful in ischaemic decompensated heart failure [172].

### 6.5. Additional therapies

#### a) Corticosteroids

Corticosteroids enhance cardiovascular function by restoring adequate blood volume through augmented mineralocorticoid activity and by improving SVR, vascular contractile, and blood pressure responses to  $\alpha$ 1-agonists, an effect that is partially linked to endothelial glucocorticoid receptors [173]. A meta-analysis that included 22 RCTs and 7297 septic shock patients, low dose of corticosteroids accelerated the resolution of shock (mean difference: 1.52 days; 95% CI: 1.71–1.32), reduced duration of invasive mechanical ventilation (mean difference: 1.38 days; 95% CI: 0.8–1.96), and ICU stay (mean difference: 1.34 days; 95% CI: 0.75; 95% CI: 0.17–1.34). However, low dose corticosteroids had no effect on short- or long-term mortality. Also, the risk of experiencing any adverse event was higher with corticosteroids [174]. The overall quality of evidence was moderate. However, the experts judged that the beneficial effects outweigh the undesirable effects. Thus, the SSC guidelines suggest using low dose systemic corticosteroids in septic shock patients with ongoing requirements of vasopressor treatment [107]. It is suggested to start intravenous hydrocortisone at dose of 200 mg/day administered as 50 mg every 6 h or as a continuous infusion in septic shock patients with norepinephrine dose  $\geq$  0.25  $\mu$ g/kg/min for at least 4 h after initiation to maintain the target MAP [107]. However, the optimal dose, timing of administration, and duration of corticosteroids remain uncertain.

#### b) Vitamin C/vitamin B1

Vitamin C (ascorbic acid) has pleiotropic properties, including antioxidant and anti-inflammatory effects. It acts as a cofactor in the biosynthesis of norepinephrine and vasopressin, enhances catecholamine sensitivity, and protects the microcirculation [175,176]. Potential side effects of high doses of intravenous vitamin C are oxalate nephropathy, and haemolysis in patients with glucose-6-phosphate deficiency [175]. Vitamin B1 (thiamine) is an essential cofactor in energy metabolism, and vitamin B1 deficit induces mitochondrial dysfunction and oxidative stress [175]. Vitamin C and vitamin B1 are depleted in sepsis [177,178]. Therefore, the supplementation of vitamin C and vitamin B1 has been suggested as a therapy for improving organ function in septic shock patients [179–181].

Whether vitamin C, corticosteroids, and vitamin B1 alone or in combination improve septic patients' clinical outcomes has been a topic of extensive investigation. In 2017, a single-centre retrospective before-after study found an impressive decrease in-hospital mortality (odds ratio: 0.13; 95% CI: 0.04–0.48), improved organ function, and shorter duration of vasopressor treatment in sepsis and septic shock patients who received the combination of vitamin C, vitamin B1, and hydrocortisone compared to control patients [182]. Following this study, 8 RCTs and many meta-analyses of RCTs [183–188] were published with conflicting results. All the meta-analyses showed that the combination of vitamin C, vitamin B1, and hydrocortisone had no effect on sepsis and septic shock mortality [183–188]. Also, most of the meta-analyses reported either a significant reduction in organ dysfunction (SOFA score) or shorter duration of vasopressors or both in septic patients who received these treatments than the control group [183–186,188]. However, in most of the included RCTs [189–195], the patients in the control group received placebo and a few of them received hydrocortisone as co-intervention. Therefore, these meta-analyses [183–186,188] cannot answer whether the adjunction of vitamin C or vitamin B1 to hydrocortisone is superior to hydrocortisone alone in terms of shock resolution or improvement in organs function. The recent network meta-analysis [187] did not find any evidence that the combination of vitamin C, vitamin B1, and corticosteroids results in shorter vasopressors treatment duration or reduction in organ dysfunction than corticosteroids alone [187]. However, these results should be interpreted with caution, as data for this comparison are scarce. Until further evidence from ongoing RCTs, the SSC guidelines suggest against using vitamin C in septic shock patients [107].

#### c) Vasodilators

Vasodilator agents can play a role in manipulating the microcirculation as the reduced vascular density and stopped-flow capillaries can be due to exaggerated vasoconstriction. The topical administration of acetylcholine, an endothelium-dependent vasodilating agent, directly on the sublingual area, restored the microvascular blood flow derangements by recruiting capillaries of the sublingual microcirculation in septic patients [57]. Also, the infusion of nitroglycerin intravenously improved the sublingual microvascular flow in a small series of fluid resuscitated septic shock patients [196]. However, these findings were not confirmed by a randomised controlled trial that included 70 severe sepsis and septic shock patients where the authors found no effects of intravenous nitroglycerin on the sublingual microcirculatory blood flow [197]. This observation may be due to the fact that the sublingual microcirculation was minimally damaged at baseline with a normal proportion of perfused capillaries, leaving no space for further improvement [197]. In patients with circulatory shock and persistent tissue hypoperfusion after 6 h of resuscitation, and macrohaemodynamic stabilisation, the nitroglycerin infusion improved the peripheral perfusion [198]. However, in a randomised trial that included 46 septic shock patients, after achieving normal macrohaemodynamic targets, inhaled NO (40 ppm) did not improve the sublingual microcirculation compared to placebo [199]. Prostaglandin  $I_2$  ( $PGI_2$ ) analogs act as a potent arteriolar vasodilator and have been used in critical limb ischaemia and frostbite to improve tissue perfusion by restoring the microcirculation. In an experimental sepsis model, Iloprost, a  $PGI_2$ -analog, improved kidney perfusion and function [200]. In a case series study of four septic shock patients with persistent signs of tissue hypoperfusion (mottling skin and increased capillary refill time) after optimising the macrocirculation, Iloprost infusion resulted in improved microvascular perfusion and almost complete resolution

of hypoperfusion signs [201], suggesting promising findings. However, clinical evidence is lacking, and Iloprost is currently being investigated in a multicentre RCT (NCT03788837). Therefore, until further evidence, vasodilating agents use cannot be recommended. One of the reasons for this relative failure is the absence of selectivity of these agents, which dilate both the non-perfused and perfused vessels, resulting in unwarranted over perfusion of some areas.

## 7. Conclusions

Under stable conditions of oxygen requirement,  $VO_2$  can be maintained despite a wide range of variation in  $DO_2$  by adjusting  $ERO_2$  with different mechanisms, including the regulation of the circulation and the endothelium effects. When  $DO_2$  is severely compromised, a critical  $DO_2$  value is reached below which  $VO_2$  falls and becomes dependent on  $DO_2$ , resulting in tissue hypoxia. The different mechanisms of tissue hypoxia are circulatory, anaemic, and hypoxic, characterised by a diminished  $DO_2$  but preserved capacity of increasing  $ERO_2$ . Cytopathic hypoxia is another mechanism of tissue hypoxia that is due to impairment in mitochondrial respiration that can be observed in septic conditions with normal overall  $DO_2$ . Altered tissue oxygen extraction, caused by impaired systemic compensatory vasoconstriction and microvascular reactivity to vasodilate with limited capillary recruitment, is also reported in inflammatory situations due to the release of vasoactive substances, and can result in tissue hypoxia by an inability to satisfy increased tissue oxygen demand. Different therapeutic approaches exist to increase  $DO_2$ , such as fluid therapy, transfusion, and inotropes. Also, there is little therapeutic ability to manipulate the other determinants of tissue hypoxia. Vasopressors, corticosteroids, vitamin C, and vasodilators were proposed to improve microcirculation. However, the effect of these agents on microcirculation and clinical outcomes are quite variable.

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Not applicable.

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## Authors' contributions

JM and MOF conceived the manuscript.

JM drafted the manuscript.

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## References

- Astiz M, Rackow EC, Weil MH, Schumer W. Early impairment of oxidative metabolism and energy production in severe sepsis. *Circ Shock* 1988;26(3):311–20.
- Bertram R, Gram Pedersen M, Luciani DS, Sherman A. A simplified model for mitochondrial ATP production. *J Theor Biol* 2006;243(4):575–86.
- Richard C. Tissue hypoxia. How to detect, how to correct, how to prevent? *Intensive Care Med* 1996;22(11):1250–7.
- Castro RH, Bakker J. Oxygen transport and tissue utilization. In: *Book: Monitoring Tissue Perfusion in Shock*. Springer; 2018.
- Vincent JL, De Backer D. My paper 20 years later: effects of dobutamine on the  $VO_2/DO_2$  relationship. *Intensive Care Med* 2014;40(11):1643–8.
- Schumacker PT, Samsel RW. Analysis of oxygen delivery and uptake relationships in the Krogh tissue model. *J Appl Physiol* (1985) 1989;67(3):1234–44.
- Cain SM. Oxygen delivery and uptake in dogs during anemic and hypoxic hypoxia. *J Appl Physiol Respir Environ Exerc Physiol* 1977;42(2):228–34.
- Schumacker PT, Cain SM. The concept of a critical oxygen delivery. *Intensive Care Med* 1987;13(4):223–9.
- Nelson DP, Samsel RW, Wood LD, Schumacker PT. Pathological supply dependence of systemic and intestinal  $O_2$  uptake during endotoxemia. *J Appl Physiol* (1985) 1988;64(6):2410–9.
- Van der Linden P, Gilbert E, Paques P, Simon C, Vincent JL. Influence of hematocrit on tissue  $O_2$  extraction capabilities during acute hemorrhage. *Am J Physiol* 1993;264(6 Pt 2):H1942–7.
- De Backer D, Roman A, Van der Linden P, Armistead C, Schiltz G, Vincent JL. The effects of balloon filling into the inferior vena cava on the  $VO_2/DO_2$  relationship. *J Crit Care* 1992;7(3):167–73.
- Zhang H, Spapen H, Benlabeled M, Vincent JL. Systemic oxygen extraction can be improved during repeated episodes of cardiac tamponade. *J Crit Care* 1993;8(2):93–9.
- Bateman RM, Sharpe MD, Ellis CG. Bench-to-bedside review: microvascular dysfunction in sepsis—hemodynamics, oxygen transport, and nitric oxide. *Crit Care* 2003;7(5):359–73.
- Vallet B. Vascular reactivity and tissue oxygenation. *Intensive Care Med* 1998;24(1):3–11.
- Schumacker PT, Samsel RW. Oxygen delivery and uptake by peripheral tissues: physiology and pathophysiology. *Crit Care Clin* 1989;5(2):255–69.
- Cain SM. Effects of time and vasoconstrictor tone on  $O_2$  extraction during hypoxic hypoxia. *J Appl Physiol Respir Environ Exerc Physiol* 1978;45(2):219–24.
- Cain SM, Chapler CK.  $O_2$  extraction by canine hindlimb during alpha-adrenergic blockade and hypoxic hypoxia. *J Appl Physiol Respir Environ Exerc Physiol* 1980;48(4):630–5.
- Shepherd AP. Role of capillary recruitment in the regulation of intestinal oxygenation. *Am J Physiol* 1982;242(5):G435–41.
- Lindbom L, Tuma RF, Arfors KE. Influence of oxygen on perfused capillary density and capillary red cell velocity in rabbit skeletal muscle. *Microvasc Res* 1980;19(2):197–208.
- Duling BR, Klitzman B. Local control of microvascular function: role in tissue oxygen supply. *Annu Rev Physiol* 1980;42:373–82.
- Grum CM. Tissue oxygenation in low flow states and during hypoxemia. *Crit Care Med* 1993;21(2 Suppl):S44–9.
- Nakhostine N, Lamontagne D. Adenosine contributes to hypoxia-induced vasodilation through ATP-sensitive  $K^+$  channel activation. *Am J Physiol* 1993;265(4 Pt 2):H1289–93.
- Griffith TM. Endothelial control of vascular tone by nitric oxide and gap junctions: a haemodynamic perspective. *Biorheology* 2002;39(3–4):307–18.
- Fisher AB, Chien S, Barakat AI, Nerem RM. Endothelial cellular response to altered shear stress. *Am J Physiol Lung Cell Mol Physiol* 2001;281(3):L529–33.
- Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288(5789):373–6.
- Pohl U, Busse R. Hypoxia stimulates release of endothelium-derived relaxant factor. *Am J Physiol* 1989;256(6 Pt 2):H1595–600.
- Michiels C, Arnould T, Knott I, Dieu M, Remacle J. Stimulation of prostaglandin synthesis by human endothelial cells exposed to hypoxia. *Am J Physiol* 1993;264(4 Pt 1):C866–74.
- Mantelli L, Amerini S, Ledda F. Roles of nitric oxide and endothelium-derived hyperpolarizing factor in vasorelaxant effect of acetylcholine as influenced by aging and hypertension. *J Cardiovasc Pharmacol* 1995;25(4):595–602.
- Curtis SE, Vallet B, Winn MJ, Caufield JB, King CE, Chapler CK, et al. Role of the vascular endothelium in  $O_2$  extraction during progressive ischemia in canine skeletal muscle. *J Appl Physiol* (1985) 1995;79(4):1351–60.
- Jones CJ, Kuo L, Davis MJ, Chilian WM. Regulation of coronary blood flow: coordination of heterogeneous control mechanisms in vascular microdomains. *Cardiovasc Res* 1995;29(5):585–96.
- Fink MP. Cytopathic hypoxia: Is oxygen use impaired in sepsis as a result of an acquired intrinsic derangement in cellular respiration? *Crit Care Clin* 2002;18(1):165–75.
- Cain SM. Peripheral oxygen uptake and delivery in health and disease. *Clin Chest Med* 1983;4(2):139–48.
- Gutierrez G. The relationship of tissue oxygenation to cellular bioenergetics. *Adv Exp Med Biol* 1988;227:183–205.

- [34] Cain SM. Appearance of excess lactate in anesthetized dogs during anemic and hypoxic hypoxia. *Am J Physiol* 1965;209(3):604–10.
- [35] Schwartz S, Frantz RA, Shoemaker WC. Sequential hemodynamic and oxygen transport responses in hypovolemia, anemia, and hypoxia. *Am J Physiol* 1981;241(6):H864–71.
- [36] Grum CM, Fiddian-Green RG, Pittenger GL, Grant BJ, Rothman ED, Dantzer DR. Adequacy of tissue oxygenation in intact dog intestine. *J Appl Physiol Respir Environ Exerc Physiol* 1984;56(4):1065–9.
- [37] Edelstone DL, Holzman IR. Fetal intestinal oxygen consumption at various levels of oxygenation. *Am J Physiol* 1982;242(1):H50–4.
- [38] Shepherd AP. Intestinal O<sub>2</sub> consumption and 86Rb extraction during arterial hypoxia. *Am J Physiol* 1978;234(3):E248–51.
- [39] Granger HJ, Nyhof RA. Dynamics of intestinal oxygenation: interactions between oxygen supply and uptake. *Am J Physiol* 1982;243(2):G91–6.
- [40] Granger HJ, Shepherd Jr AP. Intrinsic microvascular control of tissue oxygen delivery. *Microvasc Res* 1973;5(1):49–72.
- [41] Nelson DP, Beyer C, Samsel RW, Wood LD, Schumacker PT. Pathological supply dependence of O<sub>2</sub> uptake during bacteremia in dogs. *J Appl Physiol* (1985) 1987;63(4):1487–92.
- [42] Wakabayashi I, Hatake K, Kakishita E, Nagai K. Diminution of contractile response of the aorta from endotoxin-injected rats. *Eur J Pharmacol* 1987;141(1):117–22.
- [43] Julou-Schaeffer G, Gray GA, Fleming I, Schott C, Parratt JR, Stoclet JC. Loss of vascular responsiveness induced by endotoxin involves L-arginine pathway. *Am J Physiol* 1990;259(4 Pt 2):H1038–43.
- [44] Parker JL, Keller RS, DeFily DV, Laughlin MH, Novotny MJ, Adams HR. Coronary vascular smooth muscle function in E. coli endotoxemia in dogs. *Am J Physiol* 1991;260(3 Pt 2):H832–41.
- [45] Umans JG, Wylam ME, Samsel RW, Edwards J, Schumacker PT. Effects of endotoxin in vivo on endothelial and smooth-muscle function in rabbit and rat aorta. *Am Rev Respir Dis* 1993;148(6 Pt 1):1638–45.
- [46] Schumacker PT, Kazaglis J, Connolly HV, Samsel RW, O'Connor MF, Umans JG. Systemic and gut O<sub>2</sub> extraction during endotoxemia. Role of nitric oxide synthesis. *Am J Respir Crit Care Med* 1995;151(1):107–15.
- [47] Cobb JP, Natanson C, Quezado ZM, Hoffman WD, Koev CA, Banks S, et al. Differential hemodynamic effects of L-NMMA in endotoxemic and normal dogs. *Am J Physiol* 1995;268(4 Pt 2):H1634–42.
- [48] Zhang H, De Jongh R, De Backer D, Cherkaoui S, Vray B, Vincent JL. Effects of alpha- and beta-adrenergic stimulation on hepatosplanchnic perfusion and oxygen extraction in endotoxic shock. *Crit Care Med* 2001;29(3):581–8.
- [49] Wang P, Ba ZF, Chaudry IH. Endothelium-dependent relaxation is depressed at the macro- and microcirculatory levels during sepsis. *Am J Physiol* 1995;269(5 Pt 2):R988–94.
- [50] Lam C, Tymk K, Martin C, Sibbald W. Microvascular perfusion is impaired in a rat model of normotensive sepsis. *J Clin Invest* 1994;94(5):2077–83.
- [51] 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.
- [52] Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R. Effect of a maldistribution of microvascular blood flow on capillary O<sub>2</sub> extraction in sepsis. *Am J Physiol Heart Circ Physiol* 2002;282(1):H156–64.
- [53] Burton KS, Johnson PC. Reactive hyperemia in individual capillaries of skeletal muscle. *Am J Physiol* 1972;223(3):517–24.
- [54] Ward ME, Magder SA, Hussain SN. Role of endothelium-derived relaxing factor in reactive hyperemia in canine diaphragm. *J Appl Physiol* (1985) 1993;74(4):1606–12.
- [55] Koller A, Kaley G. Role of endothelium in reactive dilation of skeletal muscle arterioles. *Am J Physiol* 1990;259(5 Pt 2):H1313–6.
- [56] Neviere R, Mathieu D, Chagnon JL, Lebleu N, Millien JP, Wattel F. Skeletal muscle microvascular blood flow and oxygen transport in patients with severe sepsis. *Am J Respir Crit Care Med* 1996;153(1):191–5.
- [57] 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.
- [58] Walley KR. Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. *J Appl Physiol* (1985) 1996;81(2):885–94.
- [59] Fink M. Cytopathic hypoxia in sepsis. *Acta Anaesthesiol Scand Suppl* 1997;110:87–95.
- [60] Fink MP. Cytopathic hypoxia. Mitochondrial dysfunction as mechanism contributing to organ dysfunction in sepsis. *Crit Care Clin* 2001;17(1):219–37.
- [61] Hotchkiss RS, Rust RS, Dence CS, Wasserman TH, Song SK, Hwang DR, et al. Evaluation of the role of cellular hypoxia in sepsis by the hypoxic marker [18F]fluoromisonidazole. *Am J Physiol* 1991;261(4 Pt 2):R965–72.
- [62] VanderMeer TJ, Wang H, Fink MP. Endotoxemia causes ileal mucosal acidosis in the absence of mucosal hypoxia in a normodynamic porcine model of septic shock. *Crit Care Med* 1995;23(7):1217–26.
- [63] Rosser DM, Stidwill RP, Jacobson D, Singer M. Oxygen tension in the bladder epithelium rises in both high and low cardiac output endotoxemic sepsis. *J Appl Physiol* (1985) 1995;79(6):1878–82.
- [64] Boekstegers P, Weidenhöfer S, Pilz G, Werdan K. Peripheral oxygen availability within skeletal muscle in sepsis and septic shock: comparison to limited infection and cardiogenic shock. *Infection* 1991;19(5):317–23.
- [65] Sair M, Etherington PJ, Peter Winlove C, Evans TW. Tissue oxygenation and perfusion in patients with systemic sepsis. *Crit Care Med* 2001;29(7):1343–9.
- [66] Vallet B, Lund N, Curtis SE, Kelly D, Cain SM. Gut and muscle tissue PO<sub>2</sub> in endotoxemic dogs during shock and resuscitation. *J Appl Physiol* (1985) 1994;76(2):793–800.
- [67] Kantrow SP, Taylor DE, Carraway MS, Piantadosi CA. Oxidative metabolism in rat hepatocytes and mitochondria during sepsis. *Arch Biochem Biophys* 1997;345(2):278–88.
- [68] King CJ, Tytgat S, Delude RL, Fink MP. Ileal mucosal oxygen consumption is decreased in endotoxemic rats but is restored toward normal by treatment with aminoguanidine. *Crit Care Med* 1999;27(11):2518–24.
- [69] Vary TC, Siegel JH, Nakatani T, Sato T, Aoyama H. Effect of sepsis on activity of pyruvate dehydrogenase complex in skeletal muscle and liver. *Am J Physiol* 1986;250(6 Pt 1):E634–40.
- [70] Vary TC. Sepsis-induced alterations in pyruvate dehydrogenase complex activity in rat skeletal muscle: effects on plasma lactate. *Shock* 1996;6(2):89–94.
- [71] Vary TC, Hazen S. Sepsis alters pyruvate dehydrogenase kinase activity in skeletal muscle. *Mol Cell Biochem* 1999;198(1–2):113–8.
- [72] Giuffrè A, Sarti P, D'Itri E, Buse G, Soulimane T, Brunori M. On the mechanism of inhibition of cytochrome c oxidase by nitric oxide. *J Biol Chem* 1996;271(52):33404–8.
- [73] Torres J, Darley-Usmar V, Wilson MT. Inhibition of cytochrome c oxidase in turnover by nitric oxide: mechanism and implications for control of respiration. *Biochem J* 1995;312(Pt 1):169–73.
- [74] Brown GC, Bolaños JP, Heales SJ, Clark JB. Nitric oxide produced by activated astrocytes rapidly and reversibly inhibits cellular respiration. *Neurosci Lett* 1995;193(3):201–4.
- [75] Wink DA, Mitchell JB. Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radic Biol Med* 1998;25(4–5):434–56.
- [76] Lu M, Wang WH. Reaction of nitric oxide with superoxide inhibits basolateral K<sup>+</sup> channels in the rat CCD. *Am J Physiol* 1998;275(1):C309–16.
- [77] Radi R, Peluffo G, Alvarez MN, Naviliat M, Cayota A. Unraveling peroxynitrite formation in biological systems. *Free Radic Biol Med* 2001;30(5):463–88.
- [78] Rubbo H, Denicola A, Radi R. Peroxynitrite inactivates thiol-containing enzymes of *Trypanosoma cruzi* energetic metabolism and inhibits cell respiration. *Arch Biochem Biophys* 1994;308(1):96–102.
- [79] Boczkowski J, Lisdero CL, Lanone S, Samb A, Carreras MC, Boveris A, et al. Endogenous peroxynitrite mediates mitochondrial dysfunction in rat diaphragm during endotoxemia. *FASEB J* 1999;13(12):1637–46.
- [80] Durkacz BW, Omidiji O, Gray DA, Shall S. (ADP-ribose)<sup>n</sup> participates in DNA excision repair. *Nature* 1980;283(5747):593–6.
- [81] Satoh MS, Poirier GG, Lindahl T. Dual function for poly(ADP-ribose) synthesis in response to DNA strand breakage. *Biochemistry* 1994;33(23):7099–106.
- [82] Simbulan-Rosenthal CM, Rosenthal DS, Iyer S, Boulares H, Smulson ME. Involvement of PARP and poly(ADP-ribose)ylation in the early stages of apoptosis and DNA replication. *Mol Cell Biochem* 1999;193(1–2):137–48.
- [83] D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribose)ylation reactions in the regulation of nuclear functions. *Biochem J* 1999;342(Pt 2):249–68.
- [84] Fink MP. Bench-to bedside review: cytopathic hypoxia. *Crit Care* 2002;6(6):491–9.
- [85] Khan AU, Delude RL, Han YY, Sappington PL, Han X, Carcillo JA, et al. Liposomal NAD(+) prevents diminished O<sub>2</sub> consumption by immunostimulated Caco-2 cells. *Am J Physiol Lung Cell Mol Physiol* 2002;282(5):L1082–91.
- [86] Kühnle S, Nicotera P, Wendel A, Leist M. Prevention of endotoxin-induced lethality, but not of liver apoptosis in poly(ADP-ribose) polymerase-deficient mice. *Biochem Biophys Res Commun* 1999;263(2):433–8.
- [87] Oliver FJ, Ménessier-de Murcia J, Nacci C, Decker P, Andriantsitohaina R, Muller S, et al. Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice. *EMBO J* 1999;18(16):4446–54.
- [88] Goldfarb RD, Mairton A, Szabó E, Virág L, Salzman AL, Glock D, et al. Protective effect of a novel, potent inhibitor of poly(adenosine 5'-diphosphate-ribose) synthetase in a porcine model of severe bacterial sepsis. *Crit Care Med* 2002;30(5):974–80.
- [89] Figueras J, Weil MH. Hypovolemia and hypotension complicating management of acute cardiogenic pulmonary edema. *Am J Cardiol* 1979;44(7):1349–55.
- [90] Vincent JL, Joosten A, Saugel B. Hemodynamic monitoring and support. *Crit Care Med* 2021;49(10):1638–50.
- [91] Ceconi M, Hernandez G, Dunser M, Antonelli M, Baker T, Bakker J, et al. Fluid administration for acute circulatory dysfunction using basic monitoring: narrative review and expert panel recommendations from an ESICM task force. *Intensive Care Med* 2019;45(1):21–32.
- [92] 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.
- [93] Pottecher J, Derudder S, Teboul JL, Georger JF, Laplace C, Benhamou D, et al. Both passive leg raising and intravascular volume expansion improve sublingual microcirculatory perfusion in severe sepsis and septic shock patients. *Intensive Care Med* 2010;36(11):1867–74.
- [94] 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.

- [95] Brandt S, Regueira T, Bracht H, Porta F, Djafarzadeh S, Takala J, et al. Effect of fluid resuscitation on mortality and organ function in experimental sepsis models. *Crit Care* 2009;13(6):R186.
- [96] Rehberg S, Yamamoto Y, Sousse L, Bartha E, Jonkam C, Hasselbach AK, et al. Selective V(1a) agonism attenuates vascular dysfunction and fluid accumulation in ovine severe sepsis. *Am J Physiol Heart Circ Physiol* 2012;303(10):H1245–54.
- [97] Rosenberg AL, Dechert RE, Park PK, Bartlett RH. Review of a large clinical series: association of cumulative fluid balance on outcome in acute lung injury: a retrospective review of the ARDSnet tidal volume study cohort. *J Intensive Care Med* 2009;24(1):35–46.
- [98] Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, et al. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med* 2006;34(2):344–53.
- [99] Alsous F, Khamees M, DeGirolo A, Amoateng-Adjepong Y, Manthous CA. Negative fluid balance predicts survival in patients with septic shock: a retrospective pilot study. *Chest* 2000;117(6):1749–54.
- [100] Murphy CV, Schramm GE, Doherty JA, Reichly RM, Gajic O, Afessa B, et al. The importance of fluid management in acute lung injury secondary to septic shock. *Chest* 2009;136(1):102–9.
- [101] Payen D, de Pont AC, Sakr Y, Spies C, Reinhart K, Vincent JL. A positive fluid balance is associated with a worse outcome in patients with acute renal failure. *Crit Care* 2008;12(3):R74.
- [102] Bouchard J, Soroko SB, Chertow GM, Himmelfarb J, Iklizler TA, Paganini EP, et al. Fluid accumulation, survival and recovery of kidney function in critically ill patients with acute kidney injury. *Kidney Int* 2009;76(4):422–7.
- [103] Micek ST, McEvoy C, McKenzie M, Hampton N, Doherty JA, Kollef MH. Fluid balance and cardiac function in septic shock as predictors of hospital mortality. *Crit Care* 2013;17(5):R246.
- [104] 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.
- [105] Acheampong A, Vincent JL. A positive fluid balance is an independent prognostic factor in patients with sepsis. *Crit Care* 2015;19(1):251.
- [106] van Mourik N, Geerts BF, Binnekade JM, Veelo DP, Bos LDJ, Wiersinga WJ, et al. A higher fluid balance in the days after septic shock reversal is associated with increased mortality: an observational cohort study. *Crit Care Explor* 2020;2(10):e0219.
- [107] Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med* 2021;47(11):1181–247.
- [108] Marik PE, Byrne L, van Haren F. Fluid resuscitation in sepsis: the great 30 mL per kg hoax. *J Thorac Dis* 2020;12(Suppl 1):S37–47.
- [109] Marik PE, Malbrain M. The SEP-1 quality mandate may be harmful: How to drown a patient with 30 mL per kg fluid! *Anaesthesiology* 2017;26(5):323–8.
- [110] Michard F, Boussat S, Chemla D, Anguel N, Mercat A, Lecarpentier Y, et al. Relation between respiratory changes in arterial pulse pressure and fluid responsiveness in septic patients with acute circulatory failure. *Am J Respir Crit Care Med* 2000;162(1):134–8.
- [111] Myatra SN, Prabu NR, Divatia JV, Monnet X, Kulkarni AP, Teboul JL. The changes in pulse pressure variation or stroke volume variation after a "tidal volume challenge" reliably predict fluid responsiveness during low tidal volume ventilation. *Crit Care Med* 2017;45(3):415–21.
- [112] Yang X, Du B. Does pulse pressure variation predict fluid responsiveness in critically ill patients? A systematic review and meta-analysis. *Crit Care* 2014;18(6):650.
- [113] Monnet X, Marik P, Teboul JL. Passive leg raising for predicting fluid responsiveness: a systematic review and meta-analysis. *Intensive Care Med* 2016;42(12):1935–47.
- [114] Monnet X, Teboul JL. Passive leg raising: five rules, not a drop of fluid! *Crit Care* 2015;19(1):18.
- [115] Gavelli F, Shi R, Teboul JL, Azzolina D, Monnet X. The end-expiratory occlusion test for detecting preload responsiveness: a systematic review and meta-analysis. *Ann Intensive Care* 2020;10(1):65.
- [116] Biasi M, de Courson H, Lanchon R, Pereira B, Bardonneau G, Griton M, et al. Mini-fluid challenge of 100 mL of crystalloid predicts fluid responsiveness in the operating room. *Anesthesiology* 2017;127(3):450–6.
- [117] Muller L, Toumi M, Bousquet PJ, Riu-Poulenc B, Louart G, Candela D, et al. An increase in aortic blood flow after an infusion of 100 mL colloid over 1 minute can predict fluid responsiveness: the mini-fluid challenge study. *Anesthesiology* 2011;115(3):541–7.
- [118] Mallat J, Meddour M, Durville E, Lemyze M, Pepy F, Temime J, et al. Decrease in pulse pressure and stroke volume variations after mini-fluid challenge accurately predicts fluid responsiveness. *Br J Anaesth* 2015;115(3):449–56.
- [119] Hernández G, Ospina-Tascón GA, Damiani LP, Estenssoro E, Dubin A, Hurtado J, et al. Effect of a resuscitation strategy targeting peripheral perfusion status vs serum lactate levels on 28-day mortality among patients with septic shock: the ANDROMEDA-SHOCK Randomized Clinical Trial. *Jama* 2019;321(7):654–64.
- [120] Zampieri FG, Damiani LP, Bakker J, Ospina-Tascón GA, Castro R, Cavalcanti AB, et al. Effects of a resuscitation strategy targeting peripheral perfusion status versus serum lactate levels among patients with septic shock: A Bayesian reanalysis of the ANDROMEDA-Shock Trial. *Am J Respir Crit Care Med* 2020;201(4):423–9.
- [121] 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.
- [122] Uz Z, Ince C, Guerci P, Ince Y, Araujo RP, Ergin B, et al. Recruitment of sublingual microcirculation using handheld incident dark field imaging as a routine measurement tool during the postoperative de-escalation phase—a pilot study in post ICU cardiac surgery patients. *Periop Med (Lond)* 2018;7:18.
- [123] Chen C, Kollef MH. Targeted fluid minimization following initial resuscitation in septic shock: a pilot study. *Chest* 2015;148(6):1462–9.
- [124] Corl KA, Prodromou M, Merchant RC, Gareen I, Marks S, Banerjee D, et al. The restrictive IV fluid trial in severe sepsis and septic shock (RIFTS): a randomized pilot study. *Crit Care Med* 2019;47(7):951–9.
- [125] Hjortrup PB, Haase N, Bundgaard H, Thomsen SL, Winding R, Pettilä V, et al. Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial. *Intensive Care Med* 2016;42(11):1695–705.
- [126] Macdonald SP, Keijzers G, Taylor DM, Kinneer F, Arendts G, Fatovich DM, et al. Restricted fluid resuscitation in suspected sepsis associated hypotension (REFRESH): a pilot randomised controlled trial. *Intensive Care Med* 2018;44(12):2070–8.
- [127] Semler MW, Janz DR, Casey JD, Self WH, Rice TW. Conservative fluid management after sepsis resuscitation: a pilot randomized trial. *J Intensive Care Med* 2020;35(12):1374–82.
- [128] Meyhoff TS, Hjortrup PB, Møller MH, Wetterslev J, Lange T, Kjaer MN, et al. Conservative vs liberal fluid therapy in septic shock (CLASSIC) trial-protocol and statistical analysis plan. *Acta Anaesthesiol Scand* 2019;63(9):1262–71.
- [129] Self WH, Semler MW, Bellomo R, Brown SM, de Boisblanc BP, Exline MC, et al. Liberal versus restrictive intravenous fluid therapy for early septic shock: rationale for a randomized trial. *Ann Emerg Med* 2018;72(4):457–66.
- [130] Siam J, Kadan M, Flaishon R, Barnea O. Blood flow during hemotocrit in optimization of oxygen transfer to tissue during fluid resuscitation. *Cardiovasc Eng Technol* 2015;6(4):474–84.
- [131] Ince C, De Backer D, Mayeux PR. Microvascular dysfunction in the critically ill. *Crit Care Clin* 2020;36(2):323–31.
- [132] Tanaka S, Escudier E, Hamada S, Harrois A, Leblanc PE, Vicaut E, et al. Effect of RBC transfusion on sublingual microcirculation in hemorrhagic shock patients: a pilot study. *Crit Care Med* 2017;45(2):e154–60.
- [133] Sakr Y, Chierego M, Piagnerelli M, Verdant C, Dubois MJ, Koch M, et al. Microvascular response to red blood cell transfusion in patients with severe sepsis. *Crit Care Med* 2007;35(7):1639–44.
- [134] Scheuzger J, Zehnder A, Meier V, Yeginsoy D, Flükiger J, Siegemund M. Sublingual microcirculation does not reflect red blood cell transfusion thresholds in the intensive care unit—a prospective observational study in the intensive care unit. *Crit Care* 2020;24(1):18.
- [135] Vlaar AP, Oczkowski S, de Bruin S, Wijnberge M, Antonelli M, Aubron C, et al. Transfusion strategies in non-bleeding critically ill adults: a clinical practice guideline from the European Society of Intensive Care Medicine. *Intensive Care Med* 2020;46(4):673–96.
- [136] Vlaar APJ, Dionne JC, de Bruin S, Wijnberge M, Raasveldt SJ, van Baarle F, et al. Transfusion strategies in bleeding critically ill adults: a clinical practice guideline from the European Society of Intensive Care Medicine. *Intensive Care Med* 2021;47(12):1368–92.
- [137] Cavalcanti Dos Santos E, Orbegozo D, Mongkolpun W, Galfó V, Nan W, Gouvêa Bogossian E, et al. Systematic review and meta-analysis of effects of transfusion on hemodynamic and oxygenation variables. *Crit Care Med* 2020;48(2):241–8.
- [138] Fischer MO, Guinot PG, Debreczi S, Huette P, Beyls C, Babatasi G, et al. Individualised or liberal red blood cell transfusion after cardiac surgery: a randomised controlled trial. *Br J Anaesth* 2022;128(1):37–44.
- [139] Jentzer JC, Coons JC, Link CB, Schmidhofer M. Pharmacotherapy update on the use of vasopressors and inotropes in the intensive care unit. *J Cardiovasc Pharmacol Ther* 2015;20(3):249–60.
- [140] Persichini R, Silva S, Teboul JL, Jozwiak M, Chemla D, Richard C, et al. Effects of norepinephrine on mean systemic pressure and venous return in human septic shock. *Crit Care Med* 2012;40(12):3146–53.
- [141] Rokytka Jr R, Tesařová J, Pechman V, Gajdos P, Kroužek A. The effects of short-term norepinephrine up-titration on hemodynamics in cardiogenic shock. *Physiol Res* 2010;59(3):373–8.
- [142] Maas JJ, Pinsky MR, de Wilde RB, de Jonge E, Jansen JR. Cardiac output response to norepinephrine in postoperative cardiac surgery patients: interpretation with venous return and cardiac function curves. *Crit Care Med* 2013;41(1):143–50.
- [143] De Backer D, Creteur J, Silva E, Vincent JL. Effects of dopamine, norepinephrine, and epinephrine on the splanchnic circulation in septic shock: which is best? *Crit Care Med* 2003;31(6):1659–67.
- [144] Demiselle J, Fage N, Radermacher P, Asfar P. Vasopressin and its analogues in shock states: a review. *Ann Intensive Care* 2020;10(1):9.
- [145] Dünser MW, Mayr AJ, Tür A, Pajk W, Barbara F, Knotzer H, et al. Ischemic skin lesions as a complication of continuous vasopressin infusion in catecholamine-resistant vasodilatory shock: incidence and risk factors. *Crit Care Med* 2003;31(5):1394–8.
- [146] Chioncel O, Parissis J, Mebazaa A, Thiele H, Desch S, Bauersachs J, et al. Epidemiology, pathophysiology and contemporary management of cardio-

- genic shock - a position statement from the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail* 2020;22(8):1315–41.
- [147] Nakajima Y, Baudry N, Duranteau J, Vicaute E. Effects of vasopressin, norepinephrine, and L-arginine on intestinal microcirculation in endotoxemia. *Crit Care Med* 2006;34(6):1752–7.
- [148] Georger JF, Hamzaoui O, Chaari A, Maizel J, Richard C, Teboul JL. Restoring arterial pressure with norepinephrine improves muscle tissue oxygenation assessed by near-infrared spectroscopy in severely hypotensive septic patients. *Intensive Care Med* 2010;36(11):1882–9.
- [149] Thooft A, Favory R, Salgado DR, Taccone FS, Donadello K, De Backer D, et al. Effects of changes in arterial pressure on organ perfusion during septic shock. *Crit Care* 2011;15(5):R222.
- [150] Dubin A, Pozo MO, Casabella CA, Pálizas Jr F, Murias G, Moseinco MC, et al. Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study. *Crit Care* 2009;13(3):R92.
- [151] Asfar P, Meziani F, Hamel JF, Grelon F, Megarbane B, Anguel N, et al. High versus low blood-pressure target in patients with septic shock. *N Engl J Med* 2014;370(17):1583–93.
- [152] Lamontagne F, Richards-Belle A, Thomas K, Harrison DA, Sadique MZ, Grieve RD, et al. Effect of reduced exposure to vasopressors on 90-day mortality in older critically ill patients with vasodilatory hypotension: a randomized clinical trial. *Jama* 2020;323(10):938–49.
- [153] Saugel B, Vincent JL, Wagner JY. Personalized hemodynamic management. *Curr Opin Crit Care* 2017;23(4):334–41.
- [154] Panwar R, Tarvade S, Lanyon N, Saxena M, Bush D, Hardie M, et al. Relative hypotension and adverse kidney-related outcomes among critically ill patients with shock. A multicenter, prospective cohort study. *Am J Respir Crit Care Med* 2020;202(10):1407–18.
- [155] Vincent JL, Nielsen ND, Shapiro NI, Gerbasi ME, Grossman A, Doroff R, et al. Mean arterial pressure and mortality in patients with distributive shock: a retrospective analysis of the MIMIC-III database. *Ann Intensive Care* 2018;8(1):107.
- [156] Hamzaoui O, Georger JF, Monnet X, Ksouri H, Maizel J, Richard C, et al. Early administration of norepinephrine increases cardiac preload and cardiac output in septic patients with life-threatening hypotension. *Crit Care* 2010;14(4):R142.
- [157] Adda I, Lai C, Teboul JL, Guerin L, Gavelli F, Monnet X. Norepinephrine potentiates the efficacy of volume expansion on mean systemic pressure in septic shock. *Crit Care* 2021;25(1):302.
- [158] Hamzaoui O, Jozwiak M, Geffriaud T, Sztzyrmf B, Prat D, Jacobs F, et al. Norepinephrine exerts an inotropic effect during the early phase of human septic shock. *Br J Anaesth* 2018;120(3):517–24.
- [159] Bai X, Yu W, Ji W, Lin Z, Tan S, Duan K, et al. Early versus delayed administration of norepinephrine in patients with septic shock. *Crit Care* 2014;18(5):532.
- [160] Permpikul C, Tongyoo S, Viarasilpa T, Trainarongsakul T, Chakorn T, Udompanturak S. Early use of norepinephrine in septic shock resuscitation (CEN-SER). A randomized trial. *Am J Respir Crit Care Med* 2019;199(9):1097–105.
- [161] Ospina-Tascón GA, Hernandez G, Alvarez I, Calderón-Tapia LE, Manzano-Nunez R, Sánchez-Ortiz AI, et al. Effects of very early start of norepinephrine in patients with septic shock: a propensity score-based analysis. *Crit Care* 2020;24(1):52.
- [162] Li Y, Li H, Zhang D. Timing of norepinephrine initiation in patients with septic shock: a systematic review and meta-analysis. *Crit Care* 2020;24(1):488.
- [163] Nagendran M, Russell JA, Walley KR, Brett SJ, Perkins GD, Hajjar L, et al. Vasopressin in septic shock: an individual patient data meta-analysis of randomised controlled trials. *Intensive Care Med* 2019;45(6):844–55.
- [164] Russell JA, Vincent JL, Kjølbjerg AL, Olsson H, Blemings A, Spapen H, et al. Selapressin, a novel selective vasopressin V(1A) agonist, is an effective substitute for norepinephrine in a phase IIa randomized, placebo-controlled trial in septic shock patients. *Crit Care* 2017;21(1):213.
- [165] Laterre PF, Berry SM, Blemings A, Carlsen JE, François B, Graves T, et al. Effect of selapressin vs placebo on ventilator- and vasopressor-free days in patients with septic shock: the SEPSIS-ACT randomized clinical trial. *Jama* 2019;322(15):1476–85.
- [166] Khanna A, English SW, Wang XS, Ham K, Tumlin J, Szerlip H, et al. Angiotensin II for the treatment of vasodilatory shock. *N Engl J Med* 2017;377(5):419–30.
- [167] Belletti A, Benedetto U, Biondi-Zoccai G, Leggieri C, Silvani P, Angelini GD, et al. The effect of vasoactive drugs on mortality in patients with severe sepsis and septic shock. A network meta-analysis of randomized trials. *J Crit Care* 2017;37:91–8.
- [168] Bhattacharjee S, Soni KD, Maitra S, Baidya DK. Levosimendan does not provide mortality benefit over dobutamine in adult patients with septic shock: a meta-analysis of randomized controlled trials. *J Clin Anesth* 2017;39:67–72.
- [169] Mebazaa A, Tolppanen H, Mueller C, Lassus J, DiSomma S, Bakstye G, et al. Acute heart failure and cardiogenic shock: a multidisciplinary practical guidance. *Intensive Care Med* 2016;42(2):147–63.
- [170] Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for The diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016;18(8):891–975.
- [171] Lewis TC, Aberle C, Altschuler D, Piper GL, Papadopoulos J. Comparative effectiveness and safety between milrinone or dobutamine as initial inotropic therapy in cardiogenic shock. *J Cardiovasc Pharmacol Ther* 2019;24(2):130–8.
- [172] Felker GM, Benza RL, Chandler AB, Leimberger JD, Cuffe MS, Califf RM, et al. Heart failure etiology and response to milrinone in decompensated heart failure: results from the OPTIME-CHF study. *J Am Coll Cardiol* 2003;41(6):997–1003.
- [173] Annane D. The role of ACTH and corticosteroids for sepsis and septic shock: an update. *Front Endocrinol (Lausanne)* 2016;7:70.
- [174] Rygård SL, Butler E, Granholm A, Møller MH, Cohen J, Finfer S, et al. Low-dose corticosteroids for adult patients with septic shock: a systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med* 2018;44(7):1003–16.
- [175] Spoelstra-de Man AME, Elbers PWG, Oudemans-Van Straaten HM. Vitamin C: should we supplement? *Curr Opin Crit Care* 2018;24(4):248–55.
- [176] Rozemeijer S, van der Horst FAL, de Man AME. Measuring vitamin C in critically ill patients: clinical importance and practical difficulties—is it time for a surrogate marker? *Crit Care* 2021;25(1):310.
- [177] Carr AC, Rosengrave PC, Bayer S, Chambers S, Mehrrens J, Shaw GM. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. *Crit Care* 2017;21(1):300.
- [178] Donnino MW, Carney E, Cocchi MN, Barbash I, Chase M, Joyce N, et al. Thiamine deficiency in critically ill patients with sepsis. *J Crit Care* 2010;25(4):576–81.
- [179] Donnino MW, Andersen LW, Chase M, Berg KM, Tidswell M, Giberson T, et al. Randomized, double-blind, placebo-controlled trial of thiamine as a metabolic resuscitator in septic shock: a pilot study. *Crit Care Med* 2016;44(2):360–7.
- [180] Mallat J, Lemyze M, Thevenin D. Do not forget to give thiamine to your septic shock patient! *J Thorac Dis* 2016;8(6):1062–6.
- [181] Fowler 3rd AA, Syed AA, Knowlson S, Sculthorpe R, Farthing D, DeWilde C, et al. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med* 2014;12:32.
- [182] Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, vitamin C, and thiamine for the treatment of severe sepsis and septic shock: a retrospective before-after study. *Chest* 2017;151(6):1229–38.
- [183] Zayed Y, Alzghoul BN, Banifadel M, Venigandla H, Hyde R, Sutchu S, et al. Vitamin C, thiamine, and hydrocortisone in the treatment of sepsis: a meta-analysis and trial sequential analysis of randomized controlled trials. *J Intensive Care Med* 2021. 885066620987809.
- [184] Assouline B, Faivre A, Verissimo T, Sangla F, Berchtold L, Giraud R, et al. Thiamine, ascorbic acid, and hydrocortisone as a metabolic resuscitation cocktail in sepsis: a meta-analysis of randomized controlled trials with trial sequential analysis. *Crit Care Med* 2021;49(12):2112–20.
- [185] Wu T, Hu C, Huang W, Xu Q, Hu B, Li J. Effect of combined hydrocortisone, ascorbic acid and thiamine for patients with sepsis and septic shock: a systematic review and meta-analysis. *Shock* 2021;56(6):880–9.
- [186] Ge Z, Huang J, Liu Y, Xiang J, Gao Y, Walline JH, et al. Thiamine combined with vitamin C in sepsis or septic shock: a systematic review and meta-analysis. *Eur J Emerg Med* 2021;28(3):189–95.
- [187] Fujii T, Salanti G, Belletti A, Bellomo R, Carr A, Furukawa TA, et al. Effect of adjunctive vitamin C, glucocorticoids, and vitamin B1 on longer-term mortality in adults with sepsis or septic shock: a systematic review and a component network meta-analysis. *Intensive Care Med* 2022;48(1):16–24.
- [188] Renqi Yao YZ, Yu Yue, Li Zhixuan, Wang Lixue, Zheng Liyu, Li Jianguan, et al. Combination therapy of thiamine, vitamin C and hydrocortisone in treating patients with sepsis and septic shock: a meta-analysis and trial sequential analysis. *Burns Trauma* 2021;9.
- [189] Iglesias J, Vassallo AV, Patel VV, Sullivan JB, Cavanaugh J, Elbaga Y. Outcomes of metabolic resuscitation using ascorbic acid, thiamine, and glucocorticoids in the early treatment of sepsis: the ORANGES trial. *Chest* 2020;158(1):164–73.
- [190] Chang P, Liao Y, Guan J, Guo Y, Zhao M, Hu J, et al. Combined treatment with hydrocortisone, vitamin C, and thiamine for sepsis and septic shock: a randomized controlled trial. *Chest* 2020;158(1):174–82.
- [191] Hwang SY, Ryoo SM, Park JE, Jo YH, Jang DH, Suh GJ, et al. Combination therapy of vitamin C and thiamine for septic shock: a multi-centre, double-blinded randomized, controlled study. *Intensive Care Med* 2020;46(11):2015–25.
- [192] Moskowitz A, Huang DT, Hou PC, Gong J, Doshi PB, Grossestreuer AV, et al. Effect of ascorbic acid, corticosteroids, and thiamine on organ injury in septic shock: the ACTS randomized clinical trial. *Jama* 2020;324(7):642–50.
- [193] Wani SJ, Mufti SA, Jan RA, Shah SU, Qadri SM, Khan UH, et al. Combination of vitamin C, thiamine and hydrocortisone added to standard treatment in the management of sepsis: results from an open label randomised controlled clinical trial and a review of the literature. *Infect Dis (Lond)* 2020;52(4):271–8.
- [194] Mohamed ZU, Prasannan P, Moni M, Edathadathil F, Prasanna P, Menon A, et al. Vitamin C therapy for routine care in septic shock (VICTOR) trial: effect of intravenous vitamin C, thiamine, and hydrocortisone administration on inpatient mortality among patients with septic shock. *Indian J Crit Care Med* 2020;24(8):653–61.
- [195] Sevransky JE, Rothman RE, Hager DN, Bernard GR, Brown SM, Buchman TG, et al. Effect of vitamin C, thiamine, and hydrocortisone on ventilator- and vasopressor-free days in patients with sepsis: the VICTAS randomized clinical trial. *Jama* 2021;325(8):742–50.

- [196] Spronk PE, Ince C, Gardien MJ, Mathura KR, Oudemans-van Straaten HM, Zandstra DF. Nitroglycerin in septic shock after intravascular volume resuscitation. *Lancet* 2002;360(9343):1395–6.
- [197] Boerma EC, Koopmans M, Konijn A, Kaiferova K, Bakker AJ, van Roon EN, et al. Effects of nitroglycerin on sublingual microcirculatory blood flow in patients with severe sepsis/septic shock after a strict resuscitation protocol: a double-blind randomized placebo controlled trial. *Crit Care Med* 2010;38(1):93–100.
- [198] Lima A, van Genderen ME, van Bommel J, Klijn E, Janssen T, Bakker J. Nitroglycerin reverts clinical manifestations of poor peripheral perfusion in patients with circulatory shock. *Crit Care* 2014;18(3):R126.
- [199] Trzeciak S, Glaspey LJ, Dellinger RP, Durlinger P, Anderson K, DeZfulian C, et al. Randomized controlled trial of inhaled nitric oxide for the treatment of microcirculatory dysfunction in patients with sepsis\*. *Crit Care Med* 2014;42(12):2482–92.
- [200] Johannes T, Ince C, Klingel K, Unertl KE, Mik EG. Iloprost preserves renal oxygenation and restores kidney function in endotoxemia-related acute renal failure in the rat. *Crit Care Med* 2009;37(4):1423–32.
- [201] Dépret F, Sitbon A, Soussi S, De Tymowski C, Blet A, Fratani A, et al. Intravenous iloprost to recruit the microcirculation in septic shock patients? *Intensive Care Med* 2018;44(1):121–2.

# **Second Part**

## **Limitations of tissue oxygenation monitoring**

## I. Background

Resuscitation goals in shocked patients traditionally aim to normalize the systemic hemodynamic variables and oxygenation (macrocirculation). While this approach is necessary, it might not result in a parallel improvement of the microcirculation perfusion and restoration of tissue oxygenation. Indeed, in conditions with damaged microcirculation, tissue hypoperfusion can persist despite correcting the systemic hemodynamic variables by fluid resuscitation and vasopressor drugs leading to tissue hypoxia and death. This situation is defined as a loss of hemodynamic coherence. Many studies reported situations of a loss of hemodynamic coherence where the perfusion of the microcirculation and tissue oxygenation did not improve after the normalization of systemic circulation variables [10-14].

Resuscitation targeting microcirculatory and tissue oxygenation variables is needed to restore the hemodynamic coherence and improve the organs' function. However, this requires the use of different specific technologies to be able to monitor tissue perfusion and oxygenation. This section will discuss the different methods used for this purpose with their advantages and limitations.

### 1. Near-Infrared Spectroscopy

Near-Infrared Spectroscopy (NIRS) is a system consisting of a light source, optodes for light emission and reception, a processor, and a display system. NIRS technology used near-infrared light (700-900 nanometers wavelengths) to measure chromophores (hemoglobin, myoglobin, and cytochrome aa3) present in the sampled tissue. By choosing specific lengths, the impact of myoglobin and cytochrome is minimized. Thus, NIRS measures the concentrations of oxyhemoglobin and deoxyhemoglobin based on light attenuation using a predefined algorithm.

Tissue oxygen saturation ( $StO_2$ ) is calculated from the fractions of oxyhemoglobin and deoxyhemoglobin and displayed on the NIRS screen device [15]. By the Beer-Lambert law, the NIRS signal is limited to vessels with a diameter  $< 1$  mm (arterioles, capillaries, and venules).

Skeletal muscle  $StO_2$  is assumed to be a potential early sensor for occult hypoperfusion. The thenar eminence is the primary area where  $StO_2$  is measured, as this site is less affected by subcutaneous fat thickness and edema [15]. In normal conditions, around 75% of blood in skeletal muscle is venous; therefore, the NIRS  $StO_2$  value reflects mainly the venous oxygen saturation rather than that of the tissue.  $StO_2$  represents the balance between local oxygen supply and consumption, and therefore, changes in  $StO_2$  may be related to a change in microcirculatory perfusion and/or variation in regional oxygen consumption [16].

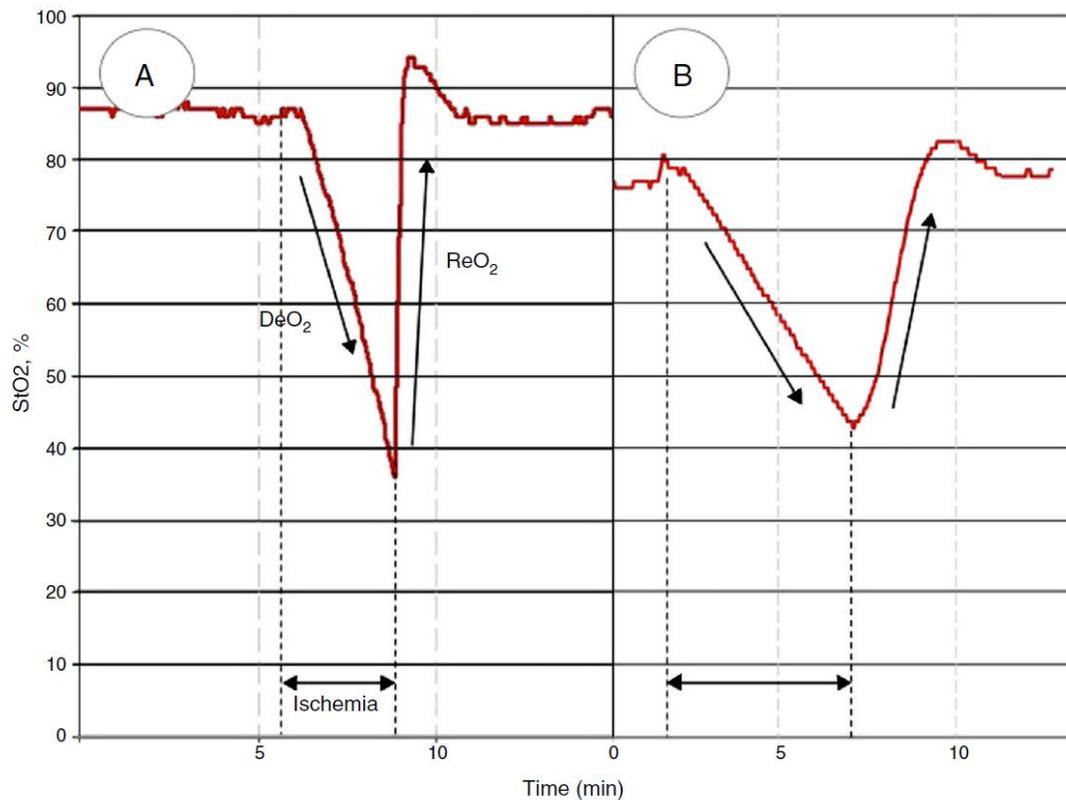
$StO_2$  values were lower in septic patients compared to healthy subjects. However, considerable overlap was observed between the two populations [17, 18]. These findings may be explained by the heterogeneity of microcirculatory perfusion resulting in the co-occurrence of ischemic and well perfused/highly oxygenated areas (microcirculatory shunting). However, some authors reported that persistent low  $StO_2$  values after initial fluid resuscitation were associated with increased mortality [19, 20]. More utility can be derived from the analysis of changes in  $StO_2$  during a dynamic vascular occlusion test (VOT) that consists of applying an arterial occlusion proximal to the NIRS probe until a pre-defined ischemic point is achieved, and then after the occlusion is removed. An initial de-oxygenation slope ( $DeO_2$ ) is observed following ischemia and has been suggested as an indicator of regional oxygen extraction. A re-oxygenation slope ( $ReO_2$ ) is observed after the occlusion is unleashed, which has been suggested to reflect the endothelial function as it depends on regional blood flow and capillary recruitment after the hypoxic stimulus

[21]. Interestingly, a correlation between  $ReO_2$  and perfusion pressure was reported, suggesting that  $ReO_2$  is derived from the interaction between the endothelium and regional perfusion pressure [22].

VOT-derived variables have shown much better performance when considering prognosis than the baseline  $StO_2$ . Some studies observed that a slighter decrease in  $DeO_2$ , which reflects impairment in regional oxygen extraction, was associated with organ dysfunction and increased mortality [23, 24]. Furthermore, decreased  $ReO_2$  slope, which reflects vascular reactivity derangements, was linked to increased mortality in septic shock patients (Figure 1) [17, 24-26].

Several methodological and technological limitations exist to the routine use of NIRS at the bedside. NIRS requires a specific device that might not be routinely available in ICUs. Also, NIRS-derived measurements are affected by adipose tissue thickness and the existence of edema. Furthermore, baseline  $StO_2$  values showed high variability among healthy subjects, which makes NIRS a valuable device for trend monitoring rather than based on absolute  $StO_2$  values. Moreover, NIRS technology does not directly measure microcirculatory blood flow. In addition,

different NIRS devices differ in some technological points and are not interchangeable.



**Figure 1.** Distinct  $StO_2$  patterns in response to a vascular occlusion test (VOT) in healthy subjects and septic shock. Figure A shows a normal  $StO_2$  response to a VOT, whereas figure B shows an impaired response in a septic shock patient. Note that, independently of the baseline absolute  $StO_2$  value, the response to ischemia is different. The  $DeO_2$  slope is slower, and a longer period is needed to reach a predetermined ischemic threshold. The vascular reactivity is also altered, resulting in a slower  $StO_2$  recovery rate ( $ReO_2$ ) and a blunted hyperemic response before returning to baseline  $StO_2$  [24].

## 2. Laser Doppler Flowmetry

Laser Doppler Flowmetry (LDF) is a non-invasive method that continuously measures the microvascular blood flow in different types of tissue, including muscle, skin, and intestine. The principle of this technique is to measure the doppler shift, which is the change in frequency that laser light experiences when it reflects off moving objects, such as red blood cells. LDF operates

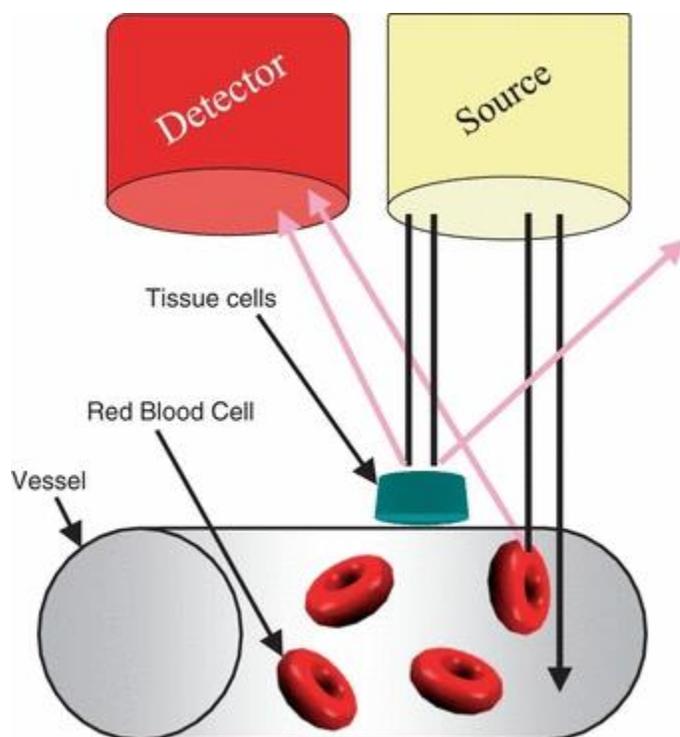
by lighting the tissue under examination with a monochromatic laser probe. Laser light is delivered fiber-optically and diffusely scatters through tissue, some reflecting back with no change in wavelength and some encountering moving red blood cells undergo a Doppler shift that is also detected by the monitor. As a result, LDF provides an output signal proportional to the microcirculatory blood flow [27] (Figure 2). Depending on the device, LDF can be used to evaluate blood flow in muscle and gastric, rectal, and vagina mucosa [28, 29]. However, as a non-invasive measurement of peripheral blood flow, its use is restricted to the skin [27].

LDF has been helpful in assessing endothelium-dependent vascular responses in the skin microcirculation during reactive hyperemia after transient ischemia obtained arterial occlusion with a cuff positioned around the arm [30]. The rate of flow restoration is primarily determined by the capacity of the microvasculature to recruit arterioles and capillaries. The ascending slope after transient occlusion is a marker of endothelial reactivity and consequently can be utilized as a substitute for the functional integrity of the microvasculature [31, 32]. A decreased hyperemic response was observed in septic patients, and a link between changes in vasculature tone and severity of sepsis was also reported [33-35]. Furthermore, restoration of vasoreactivity in septic patients assessed by LDF appears to be linked with a better outcome [34]. The capacity of LDF to assess altered skin perfusion in sepsis could be of clinical utility for the early detection of microcirculatory disorders in high-risk patients.

Scanning laser Doppler and reflection-mode confocal laser scanning microscopy are attractive developments, as they both can visualize the field of interest and allow semi-quantitative assessment of perfusion heterogeneity. With a confocal technique, vessel density, diameter, and

blood flow measurements can be obtained. Due to the device's size, it can currently only be used on humans to study skin blood flow.

LDF technique has numerous limitations. It provides blood flow measurements in relative units; thus, only relative changes from baseline can be evaluated. Also, this technique cannot consider the microvascular heterogeneity as the device measures the average blood flow in all vessels (at least 50 vessels, including arterioles, capillaries, and venules of variable size, direction, and perfusion) of the sampling volume.



**Figure 2.** Principle of Laser Doppler Flowmetry. red light is emitted from a light source; if the light beam is scattered-off of stationary tissue or cells, there is no shift in the light spectrum. If, however, the light hits a moving cell in a blood vessel there is a shift in the light spectrum of the scattered light according to the Doppler flowmetry.

### 3. Videomicroscopic imaging techniques

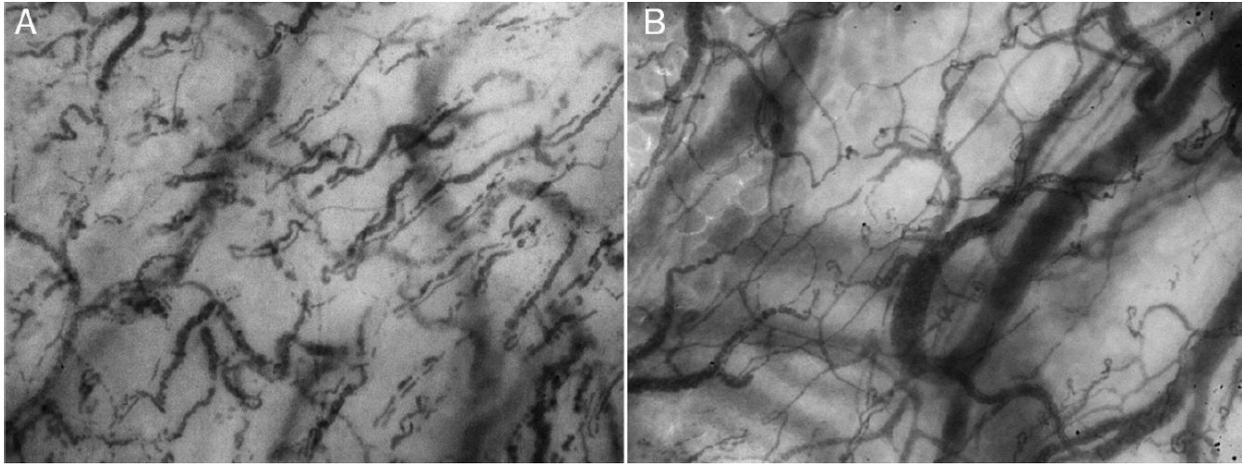
Orthogonal polarization spectral (OPS) and sidestream darkfield (SDF) are two video-microscopic imaging methods incorporated into handheld microscopes that can be employed to directly assess the microcirculation at the bedside. After applying a light source on a surface, the light reflects off the deeper layers of tissue, delivering trans-illumination of the superficial layers of tissue (thus restricting its use to organs or tissue surfaces covered by slim layers of epithelium). As the selected wavelength (530 nm) is absorbed by the hemoglobin, red blood cells look like black/gray bodies streaming inside capillaries (absorbed light) over a white tissue environment (reflected light). Hence, only functional capillaries (with red blood cells flow) will be seen as opposed to nonfunctional capillaries (with no red blood cells flow). In OPS, an externally filtered light source illuminates the organ surface with linearly polarized light and reflected light is stopped by an orthogonally polarized analyzer [36, 37]. OPS devices are no longer commercially available. The SDF technique uses pulsed green light, and illumination is achieved by surrounding the tip of the light guide with light-emitting diodes creating dark-field illumination [38]. Both devices provide good-quality images of microvascular vessels filled with red blood cells. However, SDF delivers a more precise visualization of capillaries, with more sharp and less granular images than OPS [38]. These techniques can only be used on organs that are covered by a thin layer of epithelium. In the sublingual area, which has been the most studied area, capillaries and venules of different sizes (2–3  $\mu\text{m}$ ) can be visualized; arterioles are usually not seen because they are situated in deeper layers. Red blood cells are recognized as black bodies, and tissue perfusion can be characterized in individual vessels. Microcirculatory image analysis has been challenging, primarily due to hardware limitations but also because different scoring systems have been

suggested. According to the second expert consensus conference [39], the microcirculation analysis report should evaluate microvascular blood flow, vascular density, and perfusion heterogeneity. Microcirculatory perfusion is evaluated by assessing the microvascular flow index (MFI) and the proportion of perfused vessels (PPV). Vascular density, which is related to the diffusion distance of oxygen between the red blood cells and the tissue cells, is evaluated by assessing the functional capillary density (FCD), including total vessel density (TVD) and perfused vessel density (PVD). Notably, tissue perfusion depends on FCD and blood flow (reflected by MFI). Lastly, heterogeneity of perfusion is reflected by PPV in the examined area and the heterogeneity index in the studied organ. Evaluating perfusion heterogeneity is a crucial key aspect of assessing the shunted fraction in septic shock [40]. Most of these variables are quantitative, except for the flow-related parameters, which are semi-quantitative but have nonetheless been shown in the literature to be sensitive enough to assess microcirculatory performance.

A significant reduction in vessel density and a rise of non-perfused/under-perfused vessels were observed in the sublingual microcirculation in the early period of sepsis and septic shock patients compared to the control group [11, 41]. Also, increased heterogeneity of blood flow and vascular density between concurrent areas was reported in those studies. Furthermore, these derangements were more pronounced in non-survivors, and the speedy recovery of these microcirculatory alterations following interventional treatment was also linked to improved outcomes, including mortality [10, 41, 42]. On the contrary, the persistence of these microcirculatory derangements beyond the first 24 hours was strongly and independently associated with mortality related to circulatory failure in the initial stage and to multi-organ failure in the late stage [43].

Handheld videomicroscopes present several limitations. Optimal sublingual videomicroscopy is only possible in cooperative or sedated patients. It is also not feasible to evaluate the sublingual microcirculation in patients treated with non-invasive mechanical ventilation. Furthermore, motion artifacts can also change the quality of the recorded video and make image analysis more complex, making stable video recording imperative. In addition, avoiding excessive pressure while applying the videomicroscope to the tissue surface is essential, as this can impair flow in the microvessels and result in underlining microcirculatory perfusion [39].

Moreover, the microcirculation cannot be monitored continuously with a videomicroscope, and recorded video clips should be analyzed offline. However, with the introduction of a third-generation portable video microscope called Cytocam (Incident Dark Field) Imaging [44], a real-time bedside examination of microcirculation is available with a reasonable agreement with traditional image analysis (Figure 3) [45]. A point of care microcirculation (POEM) 5-point-scoring system for real-time assessment of sublingual microcirculation was introduced by Naumann et al. [46]. These authors demonstrated that the POEM scoring system could be used at the bedside to assess sublingual microcirculatory flow and heterogeneity with minimal inter-user variability amongst healthcare professionals [46]. However, despite the new significant technological advancements that have been accomplished in this domain, additional developments are required before it can be integrated into routine clinical practice.



**Figure 3.** Cytocam-Incident Dark Field (IDF) images of sublingual microcirculation. A: early phase septic shock patient. A decrease of vessel density and of perfused vessels, and an increase of non-perfused/under-perfused vessels can be observed. B: healthy volunteer. A normal vessel density and proportion of perfused vessels can be observed [24].

#### 4. PO<sub>2</sub> electrodes

Tissue oxygen tension (tPO<sub>2</sub>) measures the partial pressure of oxygen in the interstitial space of a sampling volume of tissue. It represents the balance between local oxygen supply (dependent on microcirculation) and consumption (dependent on mitochondrial respiration). tPO<sub>2</sub> can be measured with Clark electrodes, which usually comprise a platinum cathode and a silver anode connected by a salt bridge. Such electrodes consume oxygen, which can be disadvantageous when tPO<sub>2</sub> is low. Newer techniques use dynamic luminescence-based oxygen-sensing optodes, and this process consumes no oxygen. These optodes consequently reliably determine low tPO<sub>2</sub> values.

tPO<sub>2</sub> monitoring has been extensively investigated in animal models in multiple organs, including the brain, gut, kidney, liver, muscle, and bladder [47-49], and peripheral tissues such as skin or skeletal muscle in human studies. Even though it seems to be a sensitive marker of organ perfusion, static tPO<sub>2</sub> values show an inconsistent response between organs and different shock

states, as well as discrepancies depending on the timing of sepsis progression and the use of simultaneous supportive treatments (fluid resuscitation) [48]. This high inconsistency has led to using a dynamic challenge to reveal tissue hypoxia, the so-called oxygen challenge test (OCT). The test consists of analyzing the aptitude of the vascular system to transport high  $PO_2$  to the tissues, which is reflected in a downstream increase in  $tPO_2$  after a rise in  $PaO_2$  [50]. A poor increase in  $tPO_2$  would reflect an inadequate blood flow. Thus, the adequacy of tissue perfusion can be evaluated by examining the appropriateness of response observed in all organs to the OCT. Accordingly, the existence of an altered  $tPO_2$  response to an OCT may signify local microcirculatory dysfunction in well-resuscitated septic patients. Indeed, it has been observed that the rise in  $tPO_2$  induced by an OCT was associated with less organ failure and decreased mortality in septic shock patients [51]. Also, the inclusion of OCT as an endpoint in a resuscitation protocol was associated with better survival compared to resuscitation according to the hemodynamic parameters of oxygen delivery and mixed venous oxygen saturation in patients with septic shock [52].

Several limitations of  $tPO_2$  should be acknowledged. Matched rises or declines in local oxygen delivery and consumption will not influence  $tPO_2$ . Tissue  $PO_2$  electrodes measure  $PO_2$  on a tissue surface of  $8 \text{ mm}^2$  over a depth of a few microns [53]. This corresponds to a sampling volume of at least  $0.5 \text{ mm}^3$ . Such a volume encompasses at least 100 microvessels, including arterioles, capillaries, venules, interstitium, and other cells, all of which contribute to  $PO_2$ . Even if  $tPO_2$  monitoring may be of potential interest, it still needs to be fully validated before it evolves into a bedside tool for the clinical management of septic patients.

## 5. Temperature gradients

Skin temperature is a standard marker of peripheral vasoconstriction. It has been reported that critically ill patients with cool extremities had a significantly lower cardiac index and higher lactate levels than patients with warm skin temperature [54]. However, temperature gradients are a better subjective assessment of peripheral perfusion [14, 55].

Central-to-toe temperature is the difference between central temperature measured at the tympanic membrane and temperature at the ventral surface of the big toe measured by a skin probe. This gradient has been used as a measure of peripheral vasoconstriction. However, it has the disadvantage of being affected by hypothermia and room temperature [14]. The difference between different peripheral skin temperature differences has also been used as a marker of skin perfusion in various studies. Using two skin probes, the gradient between the forearm and fingertip (Tskin-diff) is often used to evaluate peripheral blood flow. Tskin-diff greater than 2 and 4 °C demonstrates the existence of moderate and severe vasoconstriction, respectively [56]. The advantage of Tskin-diff is that both fingertip and forearm are equally affected by ambient temperature [14, 56]. Tskin-diff has been reported to be associated with skin laser Doppler flow. Also, it has been observed that increased Tskin-diff is associated with worsening organ failure [14]. In addition, Tskin-diff was linked to worse outcomes in patients following major abdominal surgery [56]. While using of peripheral perfusion parameters, including Tskin-diff, as guidelines in clinical resuscitation were shown to be very promising [57], more studies are needed before adopting this technique in critically ill patients. Furthermore, this method requires more complex technology.

## 6. Capillary refill time

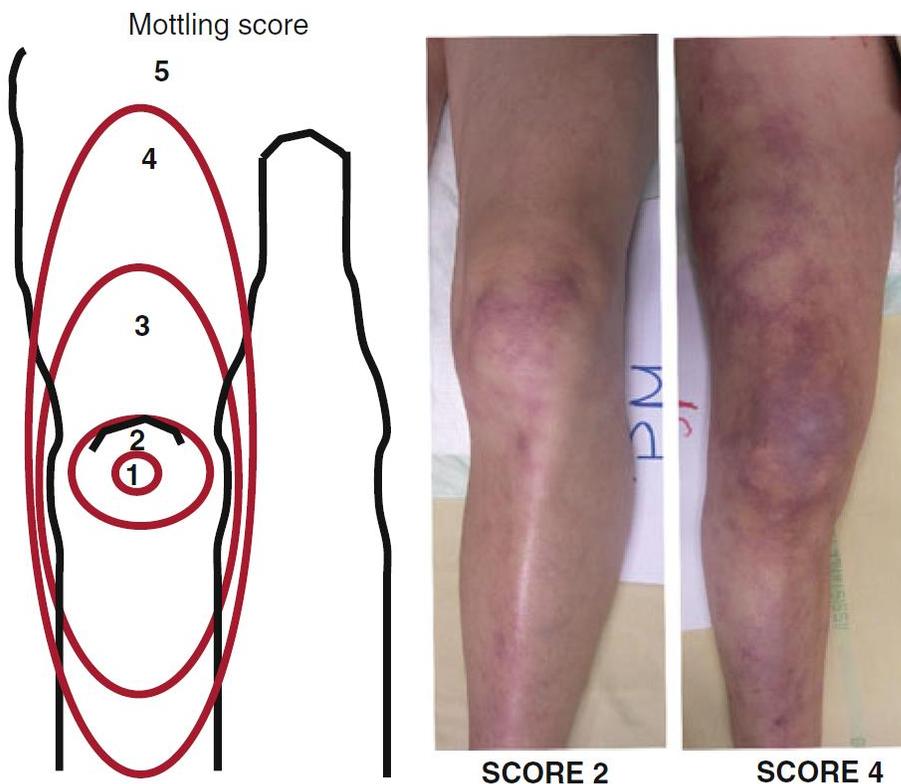
Capillary refill time (CRT) is the time it takes for skin color to return to baseline after applying blanching pressure [55]. CRT can be clinically measured over the fingertip [56, 58] or over the knee area [58]. CRT reflects peripheral capillary blood flow.

After initial resuscitation of septic shock patients, CRT was a strong predictor of 14-day mortality with a cutoff value of 2.4 s when applied on the fingertip and 4.9 s when used on the knee area [58]. Interestingly, in this study, CRT showed a good correlation with other tissue perfusion parameters such as urinary output and serum lactate levels. However, in patients following abdominal surgery, the fingertip CRT cutoff value that was associated with postoperative complications and death was 5 s [56]. In a multicenter randomized controlled trial conducted at 28 ICUs (ANDROMEDA-SHOCK trial) and included 424 patients in their early phase of septic shock, CRT-targeted resuscitation during the first 8 hours was associated with lower 28-day mortality compared to lactate-targeted resuscitation (34.9% vs. 43.4%,  $p= 0.06$ ) [59]. Also, CRT-targeted resuscitation was associated with less organ dysfunction at 72 hours. The superiority of this strategy was also supported by a subsequent Bayesian analysis [60]. However, 25 to 30% of septic shock patients had a normal CRT at baseline before fluid resuscitation [59, 61] and might not benefit from the CRT strategy.

## 7. Skin mottling

Skin mottling is described as patchy skin discoloration that generally displays on the area around the knees but can develop into other peripheral circulations like fingers and ears [55]. In the absence of diffuse intravascular coagulation yielding to complete microcirculatory block, skin mottling is a marker of skin hypoperfusion. It has been shown that perfusion and tissue

oxygenation were compromised in mottling zones by using Laser Doppler Flowmetry and NIRS technologies [62, 63]. A higher mottling score (range from 0 to 5) was associated with increased mortality in critically ill patients [64] and septic shock patients [63, 65] independently of vasopressor doses and other tissue perfusion parameters (Figure 4) [66]. Also, a decrease in mottling score during resuscitation was significantly associated with better outcomes [66]. However, the main limitation of using this method is that the incidence of skin mottling is only 29% in the general ICU population and 49% in the subset of patients admitted for septic shock [64]. Also, it is not helpful in patients with burns, amputations, and dark skin.



**Figure 4.** Left: the mottling score is based on a mottling area extension on the legs. Score 0 indicates no mottling; score 1, a modest mottling area (coin size) localized to the center of the knee; score 2, a moderate mottling area that does not exceed the superior edge of the kneecap;

score 3, a mild mottling area that does not exceed the middle thigh; score 4, a severe mottling area that does not go beyond the fold of the groin; score 5, an extremely severe mottling area that goes beyond the fold of the groin. Right: Examples of the mottling score [62].

## 8. Lactate

During glycolysis, a series of cytosolic enzymatic reactions converts glucose to pyruvate. Pyruvate is either moved into the mitochondria and converted to acetyl-CoA by pyruvate dehydrogenase (PDH) to enter the tricarboxylic acid cycle, which along with O<sub>2</sub>, drives the synthesis of adenosine triphosphate (ATP), the primary energy source for cellular metabolism, or transformed to lactate by the enzyme lactate dehydrogenase. Produced lactate can be either used locally or secreted into the blood. With these mechanisms (Figure 5) in mind, it is easy to comprehend why lactate levels can rise during shock or other critical illness-related physiological stress.

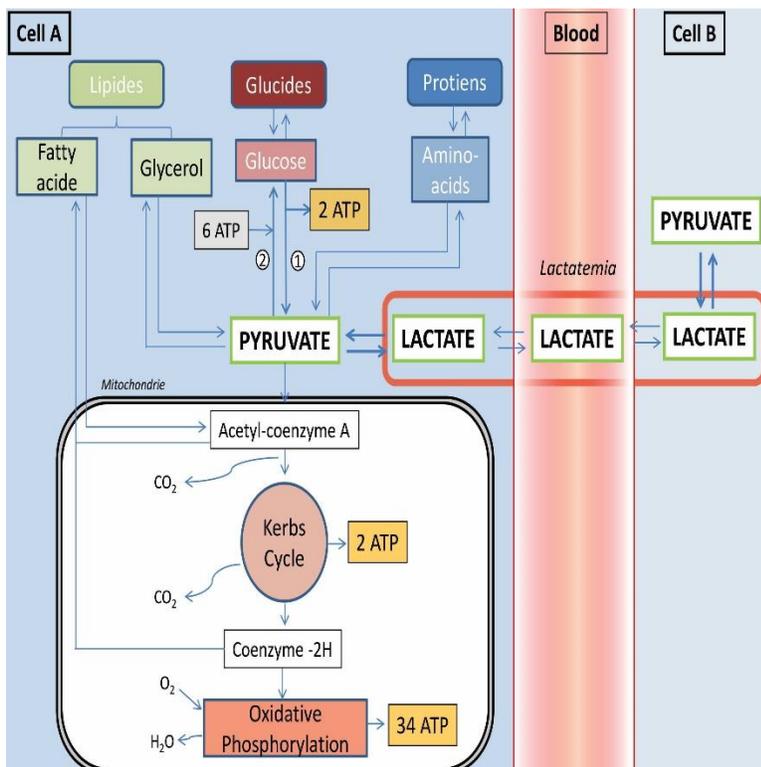
In situations where oxygen delivery cannot meet the oxygen demand or when cells cannot utilize oxygen, oxygen consumption falls, and tissue hypoxia occurs, resulting in a sharp increase in lactate levels [67]. Microcirculatory alterations, particularly in sepsis, may result in insufficient oxygen that is delivered to the cell, thereby increasing lactate levels [68]. This is indirectly demonstrated by the observation that elevated lactate levels have been associated with deranged microcirculatory perfusion [69] and that normalization of capillary perfusion was linked to a decrease in lactate levels in septic shock patients, unrelated to changes in systemic hemodynamic variables [70]. In addition, restoration of tissue perfusion parameters was linked to a rapid reduction in lactate levels [71]. For this reason, hyperlactatemia has traditionally been considered a hallmark of circulatory dysfunction and tissue hypoxia. Elevated lactate level was associated with increased mortality risk in sepsis and in general ICU population [72-74].

The effects of lactate-guided therapy on outcomes are controversial. Two randomized clinical trials (RCT) have used hemodynamic approaches based on lactate reduction. Jansen et al. randomized 348 patients with blood lactate  $\geq 3$  mmol/L to two hemodynamic strategies, one of which targeted a reduction in lactate level of  $\geq 20\%$  every 2 hours for the first 8 hours of admission [75]. The lactate-guided therapy group received a larger fluid volume over the 8 hours. However, the lactate levels over the 72 hours did not differ between the two groups. Also, there was no significant difference in the unadjusted mortality rate even though hospital mortality was decreased when adjusted for risk factors. In the second RCT that included 300 patients with severe sepsis, the lactate-guided hemodynamic strategy did not improve patients' outcomes compared to a strategy based on the central venous oxygen saturation [76]. However, different meta-analyses that included these RCTs observed that early lactate clearance-guided therapy was associated with an improved survival rate [77, 78].

However, elevated blood lactate level or persistent hyperlactatemia is particularly difficult to interpret. At least four possible pathophysiological mechanisms might be involved: anaerobic glycolysis in tissue hypoperfusion areas, particularly in the presence of severe microcirculatory derangements [78]; stress-related adrenergic-induced aerobic glycolysis; impaired hepatic lactate clearance; and mitochondrial dysfunction limiting pyruvate metabolism [79]. Identifying a clinical pattern of hypoperfusion-related hyperlactatemia is important as optimizing systemic blood flow in this situation could reverse persistent hypoperfusion and ameliorate the prognosis. On the opposite, in cases unrelated to hypoperfusion, additional resuscitation could result in the harmful of over-resuscitation. In line with this idea, a study [80] suggested that the time course of lactate normalization during a successful resuscitation follows a biphasic curve: an early rapid

response (a flow-responsive phase) followed by a later slower recovery trend potentially explained by non-flow-dependent mechanisms. In addition, it has been observed that limiting fluid resuscitation in patients with a persistent increased lactate level but with normal peripheral perfusion was safe and associated with an improvement in organ function [57].

Therefore, a general recommendation to target the circulation in patients with increased lactate levels is too simplistic and not adequately supported by clinical studies. The complexity of lactate as a molecule, substrate, biomarker, and energy source, makes it inconceivable to define what goal it should be a marker or target of. Attempting to reduce lactate levels (by whatever means, given the numerous events regulating its blood levels) has no credibility or logic in terms of hemodynamics, bioenergetics, or tissue protection[81].



**Figure 5.** Lactate metabolism.

## 9. Venous oxygen saturation

Assessment of mixed venous oxygen saturation ( $SvO_2$ ) from a pulmonary artery catheter has been proposed as an indirect marker of global tissue oxygenation [82].  $SvO_2$  reflects the balance between oxygen demand and supply. A low  $SvO_2$  represents a high  $ERO_2$  in order to maintain aerobic metabolism and  $VO_2$  constant in response to an acute decrease in  $DO_2$ . However, when  $DO_2$  drops under a critical value,  $ERO_2$  is no longer capable of upholding  $VO_2$ , and global tissue hypoxia appears, as indicated by the occurrence of lactic acidosis [68, 83, 84].

Since the assessment of central venous oxygen saturation ( $ScvO_2$ ) can be achieved more easily and is less risky than from a pulmonary artery catheter, it would be helpful if  $ScvO_2$  could function as an accurate reflection of  $SvO_2$ . In fact,  $SvO_2$  is not similar to  $ScvO_2$  because the latter primarily reflects the oxygenation of the upper side of the body. In normal patients,  $ScvO_2$  is lower than  $SvO_2$  by about 2% to 3%, largely because of the less rate of oxygen extraction by the kidneys [85]. In shock state, the absolute value of  $ScvO_2$  was more often reported to be higher than  $ScvO_2$ , probably due to increased oxygen extraction in splanchnic and renal tissues [86-89]. This suggests that the existence of a decreased  $ScvO_2$  implies an even smaller  $SvO_2$ . Because of the lack of agreement regarding absolute values, some authors questioned the clinical utility of  $ScvO_2$  [90, 91]. However, despite absolute values differ, trends in  $ScvO_2$  closely mirror trends in  $SvO_2$  [87, 92], suggesting that monitoring  $ScvO_2$  makes sense in critically ill patients.

It has been shown that an early hemodynamic optimization using a resuscitation bundle aimed at increasing  $ScvO_2 > 70\%$  was related to an important reduction in septic shock mortality [93]. However, three large multicenter studies [94-96] failed to demonstrate any benefits of the early goal-directed therapy approach. Nevertheless, the design of these trials was not to answer the

question of whether targeting an ScvO<sub>2</sub> > 70% was effective. Also, in these studies, the mean baseline ScvO<sub>2</sub> values were already above 70%. Thus, these findings do not indicate that clinicians should stop monitoring ScvO<sub>2</sub> and adjust DO<sub>2</sub> by optimizing ScvO<sub>2</sub> levels, particularly in septic shock patients with low ScvO<sub>2</sub>, who are at the highest risk of death [97].

On the other hand, normalization of ScvO<sub>2</sub> does not rule out persistent tissue hypoperfusion and does not preclude evolution to multi-organ dysfunction and death [98]. The obvious limitation of ScvO<sub>2</sub> is that normal/high values cannot distinguish if DO<sub>2</sub> is sufficient or in excess to demand. In septic conditions, normal/high ScvO<sub>2</sub> values might be due to the heterogeneity of the microcirculation that generates capillary shunting and/or mitochondrial damage responsible of disturbances in tissue oxygen extraction. Because ScvO<sub>2</sub> is measured downstream from tissues, when a given tissue receives inadequate DO<sub>2</sub>, the resulting low local oxygen venous saturations may be “masked” by admixture with highly saturated venous blood from tissues with better perfusion and DO<sub>2</sub>, resulting overall in normal or even high ScvO<sub>2</sub>. Although ScvO<sub>2</sub> may thus not miss any global DO<sub>2</sub> dysfunction, it may stay “blind” to local perfusion disturbances, which exist in abundance in sepsis due to damaged microcirculation. Indeed, high ScvO<sub>2</sub> values have been associated with increased mortality in septic shock patients [99, 100]. Thus, in some circumstances, using ScvO<sub>2</sub> might erroneously drive a clinician to conclude that the patient's physiologic state has improved when, in fact, it may not have improved.

### Tissue PCO<sub>2</sub> (Capnometry)

Tissue CO<sub>2</sub> monitoring has been, for decades, the cornerstone of regional and microcirculatory evaluation in critically ill patients. This technology is based on the principle that tissue CO<sub>2</sub> depends on three factors: tissue CO<sub>2</sub> production, blood flow to the tissue, and arterial CO<sub>2</sub>

content. Presuming that arterial CO<sub>2</sub> is constant (stable respiratory conditions), tissue CO<sub>2</sub> reflects the balance between local CO<sub>2</sub> production (dependent on basal metabolism) and blood flow to the tissue (CO<sub>2</sub> washout phenomenon). Given that arterial CO<sub>2</sub> influences tissue CO<sub>2</sub>, the gradient between the two is usually calculated and expressed as the PCO<sub>2</sub> gap. This parameter reflects the adequacy of blood flow to the tissue and has been proposed as a hypoperfusion marker and an additional endpoint for resuscitation [101].

Gastric intramucosal PCO<sub>2</sub>, as measured by gastric tonometry, has been recognized to be of clinical value as a prognostic factor in assessing the effects of particular therapeutic interventions and as an end-point of resuscitation. However, this technique has several limitations that have hampered its implementation in clinical practice. The sublingual tissue bed has been shown to be damaged in models of shock, and microcirculatory changes in this area may indicate imminent changes in other important organs. Sublingual mucosal PCO<sub>2</sub> (PslCO<sub>2</sub>) measurement by sublingual capnography is technically simple, noninvasive, and gives near-instantaneous results. Clinical studies have established that high PslCO<sub>2</sub> values and, more especially, high PslCO<sub>2</sub> gap (PslCO<sub>2</sub> - arterial PCO<sub>2</sub>) values are correlated with impaired microcirculatory blood flow and a poor outcome in critically ill patients. Sublingual capnography seems to be the ideal noninvasive monitoring tool to evaluate the severity of shock states and the adequacy of tissue perfusion. However, clinical studies are needed to determine the clinical utility of PslCO<sub>2</sub> gap monitoring as an endpoint target to guide resuscitation in critically ill patients. In the below manuscript, we reviewed the tissue capnometry for assessing tissue hypoperfusion in critically ill patients [102].

## REVIEW

Mucosal and cutaneous capnometry  
for the assessment of tissue hypoperfusionJihad MALLAT<sup>1</sup>\*, Benoit VALLET<sup>2</sup><sup>1</sup>Service of Réanimation, Department of Anesthesiology and Critical Care Medicine, Dr. Schaffner Hospital, Lens, France; <sup>2</sup>Department of Anesthesiology and Critical Care Medicine, Lille University Hospital, Lille, France

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## ABSTRACT

In critically ill patients, tissue hypoperfusion is an important cause leading to multi-organ dysfunction and death, and it cannot always be detected by measuring standard global hemodynamic and oxygen-derived parameters. Gastric intramucosal partial pressure of carbon dioxide (PCO<sub>2</sub>) as measured by gastric tonometry has been recognized to be of clinical value as a prognostic factor, in assessing the effects of particular therapeutic interventions, and as an end-point of resuscitation. However, this technique has several limitations that have hampered its implementation in clinical practice. The sublingual tissue bed has been shown to be damaged in models of shock, and microcirculatory changes in this area may indicate imminent changes in other important organs. The measurement of sublingual mucosal PCO<sub>2</sub> (PslCO<sub>2</sub>) by sublingual capnography is technically simple, noninvasive and gives near instantaneous results. Clinical studies have established that high PslCO<sub>2</sub> values and, more especially, high PslCO<sub>2</sub> gap (PslCO<sub>2</sub> - arterial PCO<sub>2</sub>) values are correlated with impaired microcirculatory blood flow and a poor outcome in critically ill patients. Sublingual capnography seems to be the ideal noninvasive monitoring tool to evaluate the severity of shock states and the adequacy of tissue perfusion. However, clinical studies are needed to determine the clinical utility of PslCO<sub>2</sub> gap monitoring as end-point target to guide resuscitation in critically ill patients.

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**Key words:** Perfusion - Carbon dioxide - Manometry - Capnography - Critical care - Shock - Microcirculation.

Early recognition and adequate correction of tissue hypoperfusion are of great importance in the management of critically ill patients with shock to prevent the development of multiple organ dysfunctions and to improve outcome.<sup>1,2</sup> Early aggressive resuscitation targeting macrocirculatory parameters has been recommended for the management of shock states.<sup>3,4</sup> However, even if optimizations of macrocirculatory parameters are achieved, inadequate oxygen supply and compromised tissue perfusion can persist, leading to organ failure.<sup>5,6</sup> Indeed, global hemodynamic and

oxygen-derived variables may be normal despite evidence of tissue hypoxia and, therefore, fail to reflect both the imbalance between oxygen demand/oxygen supply and the microcirculatory deficit, especially in sepsis.<sup>7</sup> This causes difficulties in interpreting oxygen-derived variables in revealing tissue hypoxia: a low oxygen consumption may be due to a diminished oxygen demand without hypoxia,<sup>8</sup> or to tissue hypoxia by a whatsoever process (e.g., sepsis, hypovolemia). This may be a consequence of the microcirculatory shunting and/or the inability of the tissues to use oxy-

gen (cytopathic hypoxia).<sup>9-11</sup> Thus, systemic oxygen-derived variables such as venous oxygen saturation are nonspecific and not capable of detecting regional hypoperfusion, and they are not specifically intended to detect regional hypoperfusion.

While commonly measured in critically ill patients, elevated blood lactate is an insensitive marker of tissue hypoxia and hypoperfusion.<sup>12-18</sup> Other non-hypoxic mechanisms such as accelerated aerobic glycolysis induced by sepsis-induced inflammation,<sup>19</sup> inhibition of pyruvate dehydrogenase,<sup>20</sup> and impaired lactate clearance<sup>21</sup> may contribute to increased blood lactate level found in septic patients. In endotoxic states, lactate levels failed to distinguish between hypoxia and aerobiosis.<sup>22</sup> Moreover, given the nonspecific nature of lactate elevation, hyperlactatemia alone is not a discriminatory factor in defining the nature of the circulatory failure.

Increase in tissue partial pressure of carbon dioxide (PCO<sub>2</sub>) has been observed in low-flow states of cardiac prolonged resuscitation for a long time.<sup>23, 24</sup> It has been demonstrated that the PCO<sub>2</sub> of the heart, the liver parenchyma, the kidney, and the cerebral cortex were increased during the low-flow states of circulatory shock and cardiac arrest.<sup>25-27</sup> The rise in tissue PCO<sub>2</sub> has been proposed to be an early and better marker of tissue hypoxia than global metabolic markers,<sup>28-30</sup> although potential mechanisms involved remain debated. These findings underlined the potential importance of monitoring tissue PCO<sub>2</sub>, which may allow the detection of early signs of microcirculatory hypoperfusion. The measurement of tissue PCO<sub>2</sub> can be performed by using different technologies that can be applied to a variety of sites that are accessible for use in clinical practice (gastric, sublingual, and skin).

In the present review, we will first discuss the determinants of tissue PCO<sub>2</sub> in shock states and then review the current knowledge about the several available regional capnography methods for measuring tissue PCO<sub>2</sub>, their applicability, and limitations, to evaluate organ perfusion in critically ill patients.

### Physiological background of increase in tissue PCO<sub>2</sub>

Augmented tissue PCO<sub>2</sub> has been mainly used to detect tissue hypoxia, the situation in which oxygen supply (DO<sub>2</sub>) can no longer maintain oxygen consumption (VO<sub>2</sub>).<sup>31</sup> However, the interpretation of tissue PCO<sub>2</sub> is relatively complex and depends on three variables: regional blood flow, arterial CO<sub>2</sub> content, and tissue CO<sub>2</sub> production. In stable respiratory situations when arterial CO<sub>2</sub> content is constant, tissue CO<sub>2</sub> content mainly reflects the balance between tissue blood flow and local CO<sub>2</sub> production. Theoretically, tissue PCO<sub>2</sub> can increase by two mechanisms.<sup>32</sup> First, rises in aerobic metabolism are associated with greater CO<sub>2</sub> generation by the cells, which is usually linked to similar rises in blood flow, due to the regulatory role of oxygen demand in tissues, so that tissue PCO<sub>2</sub> does not change (washout phenomenon). Conversely, in low flow situations, tissue CO<sub>2</sub> content rises because of an imbalance between CO<sub>2</sub> generation and a decreased CO<sub>2</sub> clearance,<sup>33</sup> even in the absence of tissue hypoxia. Indeed, due to the decreasing of transit time, a higher than usual addition of CO<sub>2</sub> per unit of blood passing the peripheral efferent microvessels results in venous and tissue hypercarbia (CO<sub>2</sub> stagnation phenomenon). Second, under conditions of tissue hypoxia, there is an increased generation of H<sup>+</sup> ions from an excessive generation of lactic acid due to an acceleration of anaerobic glycolysis, and the hydrolysis of high-energy phosphates.<sup>34</sup> These H<sup>+</sup> ions will then be buffered by bicarbonate existing in the cells so that CO<sub>2</sub> will be produced, which may result in an increased PCO<sub>2</sub> that would reflect tissue hypoxia in such case. Experimental studies showed evidence supporting intramucosal PCO<sub>2</sub> as an indicator of tissue hypoxia in low-flow models when VO<sub>2</sub> decreases.<sup>32, 35</sup> Schlichtig and Bowles,<sup>32</sup> in a dog model of cardiac tamponade, demonstrated that below critical oxygen supply threshold, mucosal PCO<sub>2</sub> increases because of anaerobic CO<sub>2</sub> production. Also, Dubin *et al.*<sup>35</sup> identified an anaerobic source of gut intramucosal CO<sub>2</sub> production

with increased PCO<sub>2</sub> in a model of hemorrhagic shock.

However, all studies that had addressed the issue of detecting tissue hypoxia by analysis of tissue PCO<sub>2</sub> had used experimental protocols of decreasing blood flow to produce reduced VO<sub>2</sub>, which may act as a potential confounder because of the impossibility of separating tissue hypoxia from hypoperfusion.<sup>36</sup> Moreover, anaerobic CO<sub>2</sub> generation in hypoxic tissues is not simple to identify. Indeed, under situations of tissue hypoxia, with a reduced VO<sub>2</sub>, there is a decreased aerobic CO<sub>2</sub> generation counterbalanced by an anaerobic CO<sub>2</sub> production with the net result is a reduction in total CO<sub>2</sub> generation. As a consequence, the resultant effect on tissue PCO<sub>2</sub> depends mainly on the flow state. Therefore, when tissue hypoxia is associated with a preserved blood flow, tissue PCO<sub>2</sub> should remain relatively unchanged because the smaller quantity of CO<sub>2</sub> generated should be easily removed by a normal tissue blood flow. Conversely, in stagnant hypoxia where blood flow is decreased, increased tissue PCO<sub>2</sub> should be observed due to altered clearance (Figure 1).

Vallet *et al.*<sup>36</sup> have addressed this issue in a canine model of isolated dog hind limb. These authors nicely demonstrated that when oxygen delivery was decreased below the

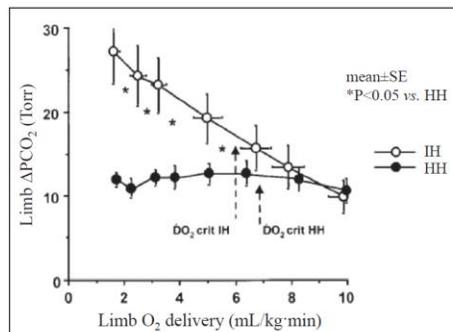


Figure 2.—Hindlimb PCO<sub>2</sub> gap ( $\Delta$ PCO<sub>2</sub>) as function of limb oxygen delivery (DO<sub>2</sub>) for ischemic hypoxia (IH) and hypoxic hypoxia (HH). Critical DO<sub>2</sub> (DO<sub>2 crit</sub>) was not different in IH and HH (from Vallet *et al.*<sup>36</sup>).

critical threshold by reducing blood flow (ischemic hypoxia), this was associated with an increased venous-to-arterial PCO<sub>2</sub> gap in the limb because of the tissue CO<sub>2</sub>-stagnation phenomenon even if the total CO<sub>2</sub> production decreased. Conversely, when blood flow was maintained but arterial PO<sub>2</sub> was reduced by decreasing the input oxygen concentration (hypoxic hypoxia), the PCO<sub>2</sub> gap did not increase in spite of similar declines in DO<sub>2</sub> and VO<sub>2</sub>, implying similar degrees of tissue hypoxia. This because the maintained blood flow was sufficient to clear the CO<sub>2</sub> generated in excess (anaerobic CO<sub>2</sub> production) (Figure 2). Nevrière *et al.*<sup>37</sup> and Dubin *et al.*<sup>38, 39</sup> both in their experimental studies confirmed that increased intramucosal-arterial PCO<sub>2</sub> gap was mainly related to the decrease in blood flow as the PCO<sub>2</sub> gap was increased during ischemic hypoxia, but not during hypoxia. Interestingly, Creteur *et al.*<sup>40</sup> found that sublingual tissue PCO<sub>2</sub> correlated with the proportion of sublingual perfused capillaries (evaluated using orthogonal polarized spectroscopy) and the reperfusion of impaired microcirculation was associated with normalized sublingual tissue PCO<sub>2</sub> in septic shock patients (Figure 3).

In summary, taken together, these findings support the concept that the widening of tissue-arterial PCO<sub>2</sub> gap reflects only microcirculatory stagnation, not tissue hypoxia. Tissue

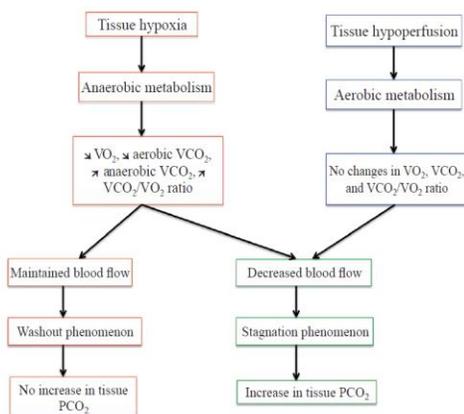


Figure 1.—Diagram illustrating the physiological background of increased tissue PCO<sub>2</sub>.

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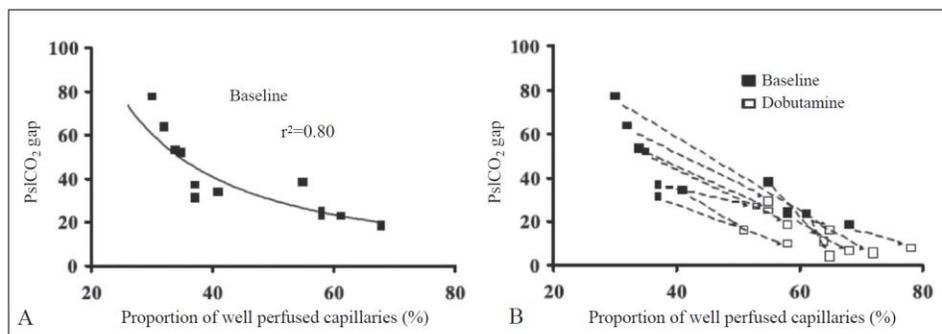


Figure 3.—A) Relation between sublingual to arterial PCO<sub>2</sub> gradient (PslCO<sub>2</sub> gap) and the proportion of well-perfused capillaries; B) individual effects of a dobutamine infusion on PslCO<sub>2</sub> gap and the proportion of well-perfused capillaries (from Creteur *et al.*<sup>40</sup>).

PCO<sub>2</sub> is an insensitive indicator of hypoxia and purely a marker of tissue hypoperfusion (Figure 1). These results were confirmed by a mathematical model.<sup>41</sup>

### Gastric tonometry

Gastric tonometry (Table I) is a technique aimed to measure PCO<sub>2</sub> in the lumen of the stomach by inserting a nasogastric tube with a silicone balloon at its distal end. The CO<sub>2</sub> generated in the mucosal cells can freely diffuse into the lumen of the stomach and equilibrates with the content inside the balloon (saline or gas) across its semipermeable membrane

(Supplementary Figure 1, online content only). The PCO<sub>2</sub> within the balloon can be measured by one of two ways: 1) saline tonometry, where the balloon is filled with saline solution, and then withdrawn after an equilibration period and the PCO<sub>2</sub> of the fluid determined using a blood gas analyzer; or 2) air tonometry, where air is aspirated through the balloon and the PCO<sub>2</sub> is semi-continuously measured by an infrared analyzer. The advantages of air tonometry are a quicker full equilibration and automated sampling and measurement, which may eliminate potential sources of error associated with saline tonometry.

The gut mucosa is highly sensitive to re-

TABLE I.—Techniques used for regional CO<sub>2</sub> measurements.

Approach	Advantages	Main limitations
Gastric tonometry	<ul style="list-style-type: none"> <li>- Stomach is easy to access</li> <li>- Measurement of gastric mucosal PCO<sub>2</sub></li> <li>- Reflects inadequate perfusion of the gut, which is highly sensitive to tissue hypoperfusion</li> <li>- May help early detection of splanchnic ischemia</li> </ul>	<ul style="list-style-type: none"> <li>- Discontinuation of enteral feeding</li> <li>- Need for premedication (H<sub>2</sub>-blockers)</li> <li>- Off-market</li> </ul>
Sublingual capnography	<ul style="list-style-type: none"> <li>- Noninvasive method</li> <li>- Does not need premedication and interruption of enteral feeding</li> <li>- Under tongue PCO<sub>2</sub> measurement</li> <li>- Reflects inadequate perfusion of the sublingual circulation, which is part of the splanchnic region</li> <li>- May help early detection of splanchnic ischemia</li> </ul>	<ul style="list-style-type: none"> <li>- Availability limited</li> <li>- Limited clinical data/off-market</li> </ul>
Cutaneous	<ul style="list-style-type: none"> <li>- Simple and noninvasive technique</li> <li>- Cutaneous PCO<sub>2</sub> measurement</li> <li>- Reflects inadequate perfusion</li> <li>- May help early detection of tissue hypoperfusion</li> <li>- Two available devices in the marketplace</li> </ul>	<ul style="list-style-type: none"> <li>- Arterialized PCO<sub>2</sub></li> <li>- Limited clinical data</li> </ul>

PCO<sub>2</sub>: partial pressure of carbon dioxide.

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duced tissue perfusion because of the counter-current flow of its microcirculation and the higher critical O<sub>2</sub> requirements of its cells than other vital organs.<sup>42</sup> Splanchnic hypoperfusion may cause the release of inflammatory cytokines and structural changes in the gut mucosa with augmented permeability and bacterial translocation, which has been strongly associated with the development of multi-organ failure, and death in critically ill patients.<sup>43-45</sup> It has been suggested that the gastrointestinal tract may be the “canary of the body,” with gastrointestinal ischemia as “an early cautionary of imminent danger.”<sup>46</sup> The stomach is a relatively easy organ to access and may deliver crucial information to the rest of the splanchnic bed. Monitoring the splanchnic perfusion by using gastric tonometry may help lessen or avoid incidents of mesenteric ischemia and ameliorate the outcome in critically ill patients. Indeed, temporary normotensive hypovolemia may result in splanchnic vasoconstriction,<sup>47</sup> and this early alteration could be detected by gastric tonometry.<sup>48</sup>

#### *Prognostic capability of gastric tonometry*

Monitoring of gastric intramucosal PCO<sub>2</sub> (PgCO<sub>2</sub>) and gastric intramucosal pH (pHi) was initially proposed to assess splanchnic hypoxia. Gastric intramucosal hypercarbia and acidosis have been demonstrated to be an index of gastric mucosal hypoxia and a predictor of morbidity and mortality in critically ill patients,<sup>15, 17</sup> with a strong prognostic value.<sup>49, 50</sup> However, the determination of pHi assumes that serum arterial bicarbonate equals gastric mucosal bicarbonate, which may be incorrect. Indeed, simulations of splanchnic ischemia show that use of the arterial bicarbonate will result in errors in the calculation of gastric pHi.<sup>51</sup> Furthermore, acid-base disorders could lead to low pHi without an excess accumulation of gastric CO<sub>2</sub>.<sup>52</sup> Consequently, the calculation of pHi was abandoned, and interest was turned to the increase in PgCO<sub>2</sub>.<sup>53</sup> Nevertheless, since gastric PCO<sub>2</sub> is straightforwardly linked to arterial PCO<sub>2</sub>, it is, indeed, the difference between PgCO<sub>2</sub> and PaCO<sub>2</sub> (PaCO<sub>2</sub> gap)

that should be considered to determine perfusion to the stomach as it more precisely reflects the adequacy of gastric mucosal blood flow.<sup>54</sup>

Levy *et al.*<sup>30</sup> measured the PgCO<sub>2</sub> gap using air-automated tonometer in 95 consecutive critically ill patients on admission to the ICU and at 24 hours. Interestingly, the authors found that the PgCO<sub>2</sub> gap and the organ failure score measured at 24 hours after admission were independent prognostic factors for 28-day mortality. The best threshold value for PgCO<sub>2</sub> gap to predict 28-day mortality was 20 mmHg.<sup>30</sup> This study suggests that the persistence of splanchnic hypoperfusion within 24 hours after ICU admission is associated with worse outcome.

Monitoring gastric tonometry-derived variables intra-operatively also has a prognostic value in the prediction of postoperative complications.<sup>55, 56</sup> It has been demonstrated an association between intraoperative splanchnic hypoperfusion and increased intestinal permeability, exaggerated acute phase response, and postoperative septic complications in patients undergoing esophagectomy.<sup>57</sup> Lebuffe *et al.*<sup>58</sup> in a European multicenter observational study, found that the intraoperative gradient between PgCO<sub>2</sub> and end-tidal PCO<sub>2</sub>, which was considered as a surrogate of arterial PCO<sub>2</sub>, could be used as a prognostic index to predict postoperative morbidity, in high-risk surgical patients during major surgery. In multiple trauma patients, it has been demonstrated the superiority of gastric capnography over other clinical variables in predicting the development of multiple organ dysfunction syndrome and death.<sup>59</sup>

#### *Gastric tonometry as a guide to therapy*

Some reports have tested the usefulness of gastric tonometry as a guide to therapy using pHi as a goal of resuscitation.<sup>28, 60-64</sup> Unluckily, these studies yielded conflictive results, mainly as some of the resuscitation strategies that were used failed to alter effectively pHi.<sup>62</sup>

In a large multicenter trial, Gutierrez *et al.*<sup>28</sup> randomized 260 critically ill patients to a standard resuscitation protocol group or a protocol group in which resuscitation was guided to

maintain  $\text{pHi} \geq 7.35$ . Those individuals with an initial  $\text{pHi} \geq 7.35$  and whose resuscitation was titrated by  $\text{pHi}$  had a greater 28-day survival compared to those patients who were treated according to a standard protocol. This study gives additional support to the argument that early detection and treatment of splanchnic tissue hypoperfusion may influence the outcome of critically ill patients. However, five other randomized controlled trials<sup>60-64</sup> failed to establish patients benefit from this treatment strategy. Indeed, Gomersall *et al.*<sup>62</sup> did not notice a difference in survival when resuscitation to a gastric  $\text{pHi} > 7.35$  was compared to a standard resuscitation protocol in critically ill patients with diverse illness. Also, the Miami Trauma Clinical Trials Group was a prospective randomized study that compared a therapeutic approach to treat hypoperfusion guided by gastric tonometry with a standard approach to shock management based on conventional indicators of hypoperfusion, in 151 trauma patients admitted to ICU.<sup>63</sup> There were no statistically significant differences in mortality rates, ventilator days, or length of stay between the two approaches. Furthermore, in a randomized controlled trial of 130 septic shock patients, Palizas *et al.*<sup>64</sup> compared a gastric intramucosal  $\text{pHi}$ -guided resuscitation protocol aimed to obtain  $\text{pHi} \geq 7.32$  with a standard approach targeted at normalizing cardiac index. These authors failed to demonstrate any survival benefit of using  $\text{pHi}$  compared with the cardiac index as resuscitation goal in septic shock patients. Nevertheless, a normalization of  $\text{pHi}$  within 24 hours of resuscitation was a strong sign of therapeutic success, and in contrast, a persistent low  $\text{pHi}$  despite treatment was associated with a very poor prognosis.

Unfortunately, most of these studies did not have the statistical power to detect significant differences in resuscitation approaches. Nevertheless, a constant finding in all of these reports has been that a decreased gastric  $\text{pHi}$  correlates with outcome (Table II).<sup>28, 30, 55, 58-64</sup> Recently, Zhang *et al.*<sup>65</sup> undertook a systematic review and meta-analysis of these six studies to investigate whether gastric tonometry-guided therapy could be of benefit for critical care patients. The authors found that resuscitations

guided by gastric  $\text{pHi}$  reduced total mortality of critically ill patients when compared with control groups (OR=0.732, 95% CI: 0.536-0.999; P=0.049). However, there was no difference regarding ICU and hospital mortalities neither ICU and hospital length of stay.

Silva *et al.*<sup>66</sup> reported the effects of fluid challenge on  $\text{PgCO}_2$  gap and systemic hemodynamic and global tissue oxygenation variables in septic shock patients. While the fluid challenge was associated with an increase in cardiac index and a decrease in  $\text{PgCO}_2$ , global measures of tissue oxygenation remained unchanged. Furthermore, neither changes in cardiac index and  $\text{PgCO}_2$  gap were related nor baseline indices of preload and changes in the  $\text{PgCO}_2$  gap. However, the changes in  $\text{PgCO}_2$  gap were highly associated with the baseline  $\text{PgCO}_2$ . These findings provide further evidence that regional markers of tissue hypoperfusion (such as  $\text{PgCO}_2$  gap) rather than global parameters should be used when starting and titrating resuscitative measures in critically ill patients (Table II).

Gastric tonometry has many limitations, even after the arrival of air-automated tonometry,<sup>67</sup> including discontinuation of enteral feeding and the need for the concomitant use of  $\text{H}_2$ -blockers. All these drawbacks have prevented its widespread adoption as a practical tool for routine tissue  $\text{PCO}_2$  monitoring.

### Sublingual capnography

It has been hypothesized that the sublingual space, the very proximal and easily accessible part of the gastrointestinal tract, may be used as a suitable location for measurement of tissue  $\text{PCO}_2$ .<sup>68</sup> The vascularization of the sublingual mucosa emanates from branches of the external carotid arteries, and thus, the sublingual area is not part of the splanchnic region. However, the sublingual mucosa, which shares a similar embryologic origin with the digestive mucosa, may present identical alterations. Interestingly, experimental studies have demonstrated a good correlation between  $\text{PgCO}_2$  and sublingual mucosa  $\text{PCO}_2$  ( $\text{PslCO}_2$ ).<sup>68-70</sup> These studies observed that changes in  $\text{PslCO}_2$  dur-

TABLE II.—Summary of clinical studies using gastric tonometry.<sup>28, 30, 55, 58-64</sup>

Study	Population	Study design
Gutierrez <i>et al.</i> <sup>28</sup>	Medical and surgical ICU patients	Multicenter RCT pH <sub>t</sub> -guided: 135 patients, resuscitation was guided to maintain pH <sub>t</sub> ≥7.35 Control: 125 patients, no specific resuscitation protocol
Ivatury <i>et al.</i> <sup>60</sup>	Patients with trauma injury and hypotension	Single-center RCT pH <sub>t</sub> -guided: 30 patients, resuscitation was guided to achieve and maintain pH <sub>t</sub> ≥7.30 Control: 27 patients, the goal of therapy was to achieve and maintain DO <sub>2</sub> ≥600 mL/min/m <sup>2</sup> or VO <sub>2</sub> ≥150 mL/min/m <sup>2</sup>
Pargger <i>et al.</i> <sup>61</sup>	Patients scheduled for elective repair of abdominal aortic aneurysms	Single-center RCT pH <sub>t</sub> -guided: 29 patients, resuscitation was guided to achieve and maintain pH <sub>t</sub> ≥7.32 Control: 26 patients, resuscitation was performed according to the usual clinical guidelines
Gomersall <i>et al.</i> <sup>62</sup>	Emergency ICU admission	Single-center RCT pH <sub>t</sub> -guided: 104 patients, after resuscitation according to standard guidelines, patients were treated to achieve pH <sub>t</sub> ≥7.35 Control: 106 patients were treated to achieve basic targets
Hameed <i>et al.</i> <sup>63</sup>	Trauma patients admitted to ICU	Single-center RCT pH <sub>t</sub> -guided: 50 patients, resuscitation was guided to achieve and maintain pH <sub>t</sub> ≥7.25 Control: 54 patients were resuscitated based on conventional parameters and systemic markers of hypoperfusion (lactate, base deficit, SvO <sub>2</sub> )
Palizas <i>et al.</i> <sup>64</sup>	Adult septic shock patients	Single center RCT pH <sub>t</sub> -guided: 64 patients, after achieving basic targets, patients were treated to achieve pH <sub>t</sub> ≥7.32 Control: 66 patients were resuscitated to achieve the common physiological targets (cardiac index >3.4 L/min/m <sup>2</sup> )
Levy <i>et al.</i> <sup>30</sup>	95 critically ill patients	Prospective observational study
Bennett-Guerrero <i>et al.</i> <sup>55</sup>	100 cardiac surgery patients	Prospective observational study
Lebuffe <i>et al.</i> <sup>58</sup>	290 ASA III-IV patients scheduled for elective, non-cardiac and non-neurological surgery	Multicenter prospective observational study
Kirton <i>et al.</i> <sup>59</sup>	19 consecutive critically ill trauma patients with signs of systemic hypoperfusion	Single-center prospective randomized study

ICU: intensive care unit; pH<sub>t</sub>: gastric intra-mucosal pH; RCT: randomized controlled trial; PgCO<sub>2</sub> gap: difference between gastric and arterial PCO<sub>2</sub>; LOS: length of stay; PgCO<sub>2</sub>-etCO<sub>2</sub>: difference between gastric and end tidal expiratory PCO<sub>2</sub>; CI: confidence interval; MOSF: multiple organ systemic failure; DO<sub>2</sub>: oxygen delivery.

ing hemorrhagic and septic shock were parallel to variations in PgCO<sub>2</sub> and systemic indicators of hypoperfusion such as arterial blood lactate concentration in different animal models. Sublingual capnography has many advantages over gastric tonometry. The method is easy to implement, noninvasive, does not need premedication and interruption of enteral feeding, yields an instant result, and therefore, it is a simple technique of monitoring tissue perfusion at the bedside in ICU (Table I).

Two different devices have been used for PslCO<sub>2</sub> measurement: MI-720 CO<sub>2</sub> electrode

(Microelectrodes; Londonderry, UK) and CapnoProbe SL Monitoring System (Nellcor, Pleasanton, CA, USA). MI-720 is a CO<sub>2</sub> electrode that requires being calibrated in standard gasses with known percent values of CO<sub>2</sub> before use, and it was essentially utilized in animal studies for PslCO<sub>2</sub> measurements.<sup>69, 70</sup> The CapnoProbe was mainly designed for analysis of PslCO<sub>2</sub> and has been used in most of the clinical studies on this topic.<sup>29, 40, 71, 72</sup> It consists of a disposable PCO<sub>2</sub> sensor (placed under the tongue), which is actually a CO<sub>2</sub>-sensing optode. The optode contains a fluorescent indicator,

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Results	Conclusions
Hospital survivor was greater in the protocol than in the control group (58% vs. 42%; P<0.01)	Therapy guided by pHi measurement improved survival in patients whose ICU admission pHi was normal
3/44 (6.8%) patients with pHi ≥7.30 at 24 hours died compared with 7/13 (53.9%) patients in whom pHi was not optimized (P=0.006)	Monitoring of gastric mucosal pH may be an important early marker to assess the adequacy of resuscitation
Patients with pHi<7.32 had more major complications during SICU stay than patients with pHi≥7.32 (26.1% vs. 3.1%)	Low and persistence pHi values are predictors of major SICU complications
No significant differences in hospital mortality between the two groups (45.3% in the control group vs. 42.3% in the intervention group)	The routine use of treatment titrated against pHi in the management of critically ill patients cannot be supported
No significant differences in ICU LOS and number of ventilator days between the two groups (13±21 days in control groups vs. 13±20 days in the intervention group) and (12±22 days in control groups vs. 11±20 days in the intervention group), respectively	Techniques of optimization of splanchnic perfusion were not advantageous relative to standard resuscitation measures guided by conventional measures of hypoperfusion in the therapy of occult and clinical shock in trauma patients
No significant differences in 28-day mortality and ICU LOS between the two groups (30.3% in control groups vs. 28.1% in the intervention group) and (12.6±8.2 days in control groups vs. 16±12.4 days in the intervention group), respectively	No survival benefit of using pHi compared with CI as resuscitation goal in septic-shock patients
PgCO <sub>2</sub> gap at 24 hours was found to be an independent predictor of 28-day mortality (OR=1.57; 95% CI: 1.10-2.24)	PgCO <sub>2</sub> gap is a marker of mortality in ventilated ICU mortality
In the postoperative period, pHi and PgCO <sub>2</sub> gap were strongly associated with postoperative complications	Several gastric tonometry-derived variables were predictive of postoperative cardiac surgery complications
Pg-etCO <sub>2</sub> gradient was found to be the best predictor of postoperative functional recovery delay at a cut-off of 21 mmHg	Pg-etCO <sub>2</sub> gradient may be a useful prognostic index of postoperative morbidity in a high-risk surgical population
A gastric pHi<7.32 at 24 hours carried a relative risk (RR) of 5.4 for occurrence of MOSF and 4.5 for death (P<0.01), compared with achievement of supra-normal DO <sub>2</sub> (RR=1.4, P>0.05)	A low gastric pHi appears to be a marker of post resuscitative morbidity

which is excited by light conducted through an optical fiber, which then transmits the fluorescent back to the instrument where they are converted to a numerical value of PCO<sub>2</sub>.<sup>73</sup>

Five clinical studies using sublingual capnography in critically ill patients have been reported (Table III).<sup>29, 40, 71, 72, 74, 75</sup> Weil *et al.*<sup>74</sup> found higher PslCO<sub>2</sub> values in patients with acute circulatory shock and observed that PslCO<sub>2</sub> value at admission was predictive of the probability of hospital survival. Initial PslCO<sub>2</sub> values were very well associated with blood lactate levels but diminished more rapidly dur-

ing resuscitation, implying that reductions in PslCO<sub>2</sub> arise faster than blood lactate concentration. The authors concluded that sublingual capnography was a trustworthy technique for diagnosis and assessment of severity of acute circulatory failure in critically ill patients.

Similarly, Marik *et al.*<sup>29, 71</sup> tested the clinical usefulness of PslCO<sub>2</sub> gap (PslCO<sub>2</sub> - arterial PCO<sub>2</sub>) as an indicator of tissue hypoperfusion in a prospective study of hemodynamically unstable critically ill patients. They observed that PslCO<sub>2</sub> gap was a better prognostic factor of outcome than traditional markers of tissue hypoxia

TABLE III.—Summary of clinical study using sublingual capnography and transcutaneous CO<sub>2</sub> measurement.<sup>29, 40, 71, 72, 74, 75</sup>

Study	Population	Study design	Results	Conclusions
Weil <i>et al.</i> <sup>74</sup>	46 patients with acute life-threatening illness or injuries	Prospective observational study	PslCO <sub>2</sub> at admission was higher (P<0.001) in non-survivors (96.2±26.6 mmHg) compared with survivors (58.4±11.3 mmHg). A PslCO <sub>2</sub> value cut-off of 70 mmHg was predictive of severity of the circulatory shock state and the likelihood of hospital survival	PslCO <sub>2</sub> may serve as a technically simple and noninvasive clinical measurement for the diagnosis and estimation of the severity of circulatory shock states.
Marik <sup>71</sup>	22 septic and cardiogenic shock patients	Prospective observational study	PslCO <sub>2</sub> gap at admission was higher (P=0.04) in non-survivors (17.8±11.5 mmHg) compared with survivors (9.2±5.0 mmHg)	PslCO <sub>2</sub> may serve as a technically simple and noninvasive clinical measurement of tissue dysoxia in critically ill and injured patients
Rackow <i>et al.</i> <sup>72</sup>	19 sepsis and 6 cardiac failure patients	Prospective observational study	PslCO <sub>2</sub> gap at 24 hours was higher (P<0.05) in non-survivors (29±4 mmHg) compared with survivors (14±3 mmHg)	PslCO <sub>2</sub> gap may be useful as indexes of the severity of perfusion failure.
Marik <i>et al.</i> <sup>29</sup>	54 hemodynamically unstable critically ill patients	Prospective observational study	PslCO <sub>2</sub> gap at the time of Swan-Ganz insertion was higher (P=0.0004) in non-survivors (35.3±18.3 mmHg) compared with survivors (19.0±12.8 mmHg)	PslCO <sub>2</sub> gap may prove to be a useful marker for goal-directed therapy and for assessing the response to clinical interventions aimed at improving tissue oxygenation
Creteur <i>et al.</i> <sup>40</sup>	18 consecutive septic shock patients, sublingual microcirculation assessed with OPS imaging technique	Prospective observational study	At baseline, PslCO <sub>2</sub> gap correlated well with the proportion of well-perfused capillaries (r <sup>2</sup> =0.80; P<0.01). The decrease in PslCO <sub>2</sub> gap was associated with the increase in the proportion of well-perfused capillaries in each patient	Sublingual capnometry could represent a simple, non-invasive method to monitor these microcirculatory alterations in septic patients.
Vallee <i>et al.</i> <sup>75</sup>	46 ventilated septic shock patients	Prospective observational study	At hour 24, Pc-aCO <sub>2</sub> gradient was higher in non-survivors than in survivors (29.7±22.5 vs. 10.9±7.3 mmHg; P<0.001, respectively). A cut-off of 16 mmHg for Pc-aCO <sub>2</sub> gradient discriminated survivors from non-survivors with a sensitivity of 83% and a specificity of 90%. Pc-aCO <sub>2</sub> variations during fluid challenge were inversely correlated with microcirculatory skin blood flow (r <sup>2</sup> =0.74, P<0.001)	Ear lobe cutaneous PCO <sub>2</sub> at 37 °C represents a noninvasive technique to assess tissue PCO <sub>2</sub> measurement. Pc-aCO <sub>2</sub> and was related to outcome and provide continuous information on microperfusion in patients with septic shock

PslCO<sub>2</sub>: sublingual mucosa PCO<sub>2</sub>; PslCO<sub>2</sub> gap: difference between sublingual and arterial PCO<sub>2</sub>; Pc-aCO<sub>2</sub>: difference between cutaneous and arterial PCO<sub>2</sub>; OPS: orthogonal polarization spectral.

(mixed venous oxygen saturation, cardiac index, oxygen delivery, and arterial lactate level) and more responsive to therapeutic interventions.

Using the orthogonal polarization spectral (OPS) imaging technique, De Backer *et al.*<sup>76</sup> observed that microcirculatory impairments in the sublingual area were common in septic shock patients with decreases in capillary density and in the percentage of perfused capillaries compared with control patients. In septic shock patients, it has also been demonstrated that microvascular blood flow quickly improved in survivors but persisted altered in non-survivors.<sup>77</sup> These results suggest that

persistent impairments in microcirculation are incriminated in the development of multiple organ failure and death in septic patients. Interestingly, in an elegant study of patients in septic shock, Creteur *et al.*<sup>40</sup> examined the association between impairment in sublingual microcirculatory perfusion (assessing using OPS) and PslCO<sub>2</sub> gap. They showed that the reperfusion of damaged sublingual microcirculation was associated with normalized PslCO<sub>2</sub> gap levels (Figure 3). The authors concluded that sublingual capnography could serve as a simple, noninvasive tool to monitor the sepsis-induced microcirculation impairments during

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the resuscitation of septic shock. However, no study has evaluated the clinical utility of PslCO<sub>2</sub> gap as an end-point resuscitation target in critically ill patients. Also, unfortunately, CapnoProbe is no longer commercially accessible. A newly developed sublingual tonometric method using a specially coiled silicone rubber tube is under investigation,<sup>78</sup> but it is not yet available in the marketplace (Supplementary Figure 2, online content only).

### Transcutaneous CO<sub>2</sub> measurement

Severinghaus is the first who described the measurement of PCO<sub>2</sub> on human skin surfaces.<sup>79</sup> Transcutaneous measurement of PCO<sub>2</sub> is based on the fact that CO<sub>2</sub> gas diffuses throughout body tissue and skin and can be detected by a sensor at the skin surface.<sup>79</sup> By heating the skin, a local hyperemia is produced, which rises the delivery of arterial blood to the dermal capillary area beneath the sensor (Supplementary Figure 3, online content only). Overall, the transcutaneous PCO<sub>2</sub> values correlate well with the corresponding arterial PCO<sub>2</sub> values after a correcting algorithm is applied to take into consideration the elevated temperature of the sensor (usually 42-44 °C).<sup>80</sup> According to Severinghaus and Bradley method,<sup>81</sup> CO<sub>2</sub> is measured potentiometrically by determining the pH of an electrolyte layer separated from the skin by a highly permeable membrane. A change in the pH is proportional to the logarithm of PCO<sub>2</sub> change.

Technical advances have led to the production of transcutaneous PCO<sub>2</sub> monitors designed for adults. Among them, the TOSCA® 500 (Linde Medical Systems, Basel, Switzerland) device, which is based on a heated (42 °C) Stow-Severinghaus-type CO<sub>2</sub> sensor including a pulse oximetry sensor, and attached by a low-pressure clip to the earlobe.<sup>82</sup> This device has been evaluated in ICU patients and has been shown to be safe with good accuracy between earlobe PCO<sub>2</sub> and arterial PCO<sub>2</sub> in the majority of clinical situations.<sup>83-85</sup>

Recently, Vallée *et al.*<sup>75</sup> used this device to examine whether cutaneous earlobe PCO<sub>2</sub> could be used to assess tissue perfusion in sep-

tic shock patients. In that study, the sensor was heated at 37 °C to limit the impact of arterial PCO<sub>2</sub> on cutaneous PCO<sub>2</sub> because the arterialization of the blood is minimal compared with the effect when warming the sensor to 42 °C. The authors found that a threshold value of 16 mmHg for the gradient between the earlobe PCO<sub>2</sub> and arterial PCO<sub>2</sub> discriminated patients reliably with septic shock from the patients in control group with a sensitivity of 83% and a specificity of 90%. Furthermore, it has been found that fluid challenge induced a decrease in the earlobe-to-arterial PCO<sub>2</sub> gradient in parallel with the improvement in the earlobe microcirculatory blood flow. Interestingly, where a significant reduction in earlobe-to-arterial PCO<sub>2</sub> gradient was observed in survivors compared to non-survivors, no significant changes were found with traditional macrocirculatory parameters (cardiac output and central venous oxygen saturation) (Table III).

### Conclusions

In critically ill patients, tissue hypoperfusion is an important cause leading to multi-organ dysfunction and death, and it cannot always be detected by measuring global hemodynamic and oxygen-derived parameters. Experimental and clinical studies have demonstrated that

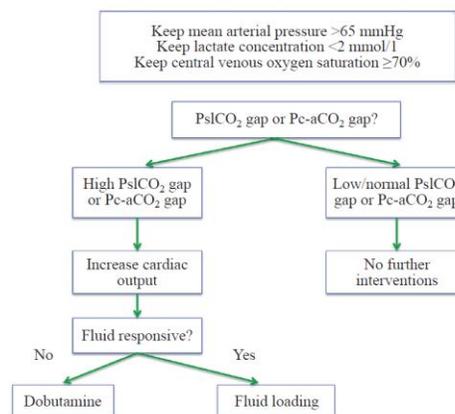


Figure 4.—Tissue PCO<sub>2</sub> gap-guided protocol. PslCO<sub>2</sub> gap: sublingual to arterial PCO<sub>2</sub> difference; Pc-aCO<sub>2</sub>: cutaneous to arterial PCO<sub>2</sub> difference.

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low-flow states are consistently associated with important PCO<sub>2</sub> increase affecting virtually all tissues. Monitoring regional PCO<sub>2</sub> by gastric tonometry has been recognized to be useful as a prognostic factor and as an end-point of therapeutic interventions. However, this technique has several limitations that have hampered its implementation in clinical practice. Sublingual capnography and transcutaneous PCO<sub>2</sub> seem to be the ideal noninvasive monitoring tools to evaluate the severity of shock states and the adequacy of tissue perfusion. The clinical experience with these devices is, however, limited, and further studies are needed to determine the clinical utility of PslCO<sub>2</sub> and transcutaneous PCO<sub>2</sub> monitoring, particularly as an end-point to guide resuscitation (Figure 4).

#### Key messages

— Tissue hypoperfusion is a major cause of multiple-organ failure in critically ill patients, and cannot always be predicted by measuring classic global hemodynamic parameters.

— Tissue PCO<sub>2</sub> gap is a good marker of adequacy of venous blood flow to remove the CO<sub>2</sub> generated by the peripheral tissues and can be used to detect tissue hypoperfusion.

— Monitoring regional PCO<sub>2</sub> by gastric tonometry has been recognized to be useful as a prognostic factor and as an end-point of therapeutic interventions, but is no longer used in clinical practice.

— Sublingual capnography and transcutaneous PCO<sub>2</sub> seem to be the ideal non-invasive monitoring tools to evaluate the severity of shock states and the adequacy of tissue perfusion.

#### References

- Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, *et al*. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001;345:1368-77.
- Vincent JL, De Backer D. Circulatory shock. *N Engl J Med* 2013;369:1726-34.
- Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, *et al*. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013;39:165-228.
- 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;40:1795-815.
- 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;32:1825-31.
- Vincent JL, De Backer D. Microvascular dysfunction as a cause of organ dysfunction in severe sepsis. *Crit Care Med* 2005;4:S9-12.
- Trzeciak S, Cline I, Phillip Dellinger R, Shapiro NI, Arnold RC, Parrillo JE, *et al*. Resuscitating the microcirculation in sepsis: the central role of nitric oxide, emerging concepts for novel therapies, and challenges for clinical trials. *Acad Emerg Med* 2008;15:399-413.
- Mekontso-Dessap A, Castelain V, Anguel N, Bahloul M, Schaulvliege F, Richard C, *et al*. Combination of veno-arterial PCO<sub>2</sub> difference with arteriovenous O<sub>2</sub> content difference to detect anaerobic metabolism in patients. *Intensive Care Med* 2002;28:272-7.
- Ince C, Sinaasappel M. Microcirculatory oxygenation and shunting in sepsis and shock. *Crit Care Med* 1999;27:1369-77.
- 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;166:98-104.
- Fink MP. Bench-to bedside review: Cytopathic hypoxia. *Crit Care* 2002;6:491-9.
- Hotchkiss RS, Karl IE. Reevaluation of the role of cellular hypoxia and bioenergetic failure in sepsis. *JAMA* 1992;267:1503-10.
- James JH, Luchette FA, McCarter FD, Fischer JE. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. *Lancet* 1999;354:505-8.
- Garcia-Alvarez M, Marik P, Bellomo R. Sepsis-associated hyperlactatemia. *Crit Care* 2014;18:503.
- Marik PE. Gastric intramucosal pH. A better predictor of multiorgan dysfunction syndrome and death than oxygen-derived variables in patients with sepsis. *Chest* 1993;104:225-9.
- De Backer D. Lactic acidosis. *Intensive Care Med* 2003;29:699-702.
- Marik PE. Regional carbon dioxide monitoring to assess the adequacy of tissue perfusion. *Curr Opin Crit Care* 2005;11:245-51.
- 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;40:427-32.
- Gore DC, Jahoor F, Hibbert JM, DeMaria EJ. Lactic acidosis during sepsis is related to increased pyruvate production, not deficits in tissue oxygen availability. *Ann Surg* 1996;224:97-102.
- Vary TC, Siegel JH, Nakatani T, Sato T, Aoyama H. Effect of sepsis on activity of pyruvate dehydrogenase complex in skeletal muscle and liver. *Am J Physiol* 1986;250:E634-40.
- Levrant J, Ciebiera JP, Chave S, Rabary O, Jambou P, Carles M, *et al*. Mild hyperlactatemia in stable septic patients is due to impaired lactate clearance rather than overproduction. *Am J Respir Crit Care Med* 1998;157:1021-6.
- Curtis SE, Cain SM. Regional and systemic oxygen delivery/uptake relations and lactate flux in hyperdynamic, endotoxin-treated dogs. *Am Rev Respir Dis* 1992;145:348-54.

23. Grundler W, Weil MH, Rackow EC. Arteriovenous carbon dioxide and pH gradients during cardiac arrest. *Circulation* 1986;74:1071-4.
24. 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;315:153-6.
25. Kette F, Weil MH, Gazmuri RJ, Bisera J, Rackow EC. Intramyocardial hypercarbic acidosis during cardiac arrest and resuscitation. *Crit Care Med* 1993;21:901-6.
26. Gudipati CV, Weil MH, Gazmuri RJ, Deshmukh HG, Bisera J, Rackow EC. Increases in coronary vein CO<sub>2</sub> during cardiac resuscitation. *J Appl Physiol* (1985) 1990;68:1405-8.
27. Desai VS, Weil MH, Tang W, Gazmuri R, Bisera J. Hepatic, renal, and cerebral tissue hypercarbia during sepsis and shock in rats. *J Lab Clin Med* 1995;125:456-61.
28. Gutierrez G, Palizas F, Doglio G, Wainsztein N, Gallesio A, Pacin J, *et al*. Gastric intramucosal pH as a therapeutic index of tissue oxygenation in critically ill patients. *Lancet* 1992;339:195-9.
29. Marik PE, Bankov A. Sublingual capnometry *versus* traditional markers of tissue oxygenation in critically ill patients. *Crit Care Med* 2003;31:818-22.
30. Levy B, Gawalkiewicz P, Vallet B, Briancon S, Nace L, Bollaert PE. Gastric capnometry with air-automated tonometry predicts outcome in critically ill patients. *Crit Care Med* 2003;31:474-80.
31. Connert RJ, Honig CR, Gayeski TE, Brooks GA. Defining hypoxia: a systems view of VO<sub>2</sub>, glycolysis, energetics, and intracellular PO<sub>2</sub>. *J Appl Physiol* (1985) 1990;68:833-42.
32. Schlichtig R, Bowles SA. Distinguishing between aerobic and anaerobic appearance of dissolved CO<sub>2</sub> in intestine during low flow. *J Appl Physiol* (1985) 1994;76:2443-51.
33. Teboul JL, Michard F, Richard C. Critical analysis of venoarterial CO<sub>2</sub> gradient as a marker of tissue hypoxia. In: Vincent JL, editor. *Yearbook of intensive care and emergency medicine*. Heidelberg: Springer; 1996. p. 296-307.
34. Randall HM Jr, Cohen JJ. Anaerobic CO<sub>2</sub> production by dog kidney in vitro. *Am J Physiol* 1966;211:493-505.
35. Dubin A, Estenssoro E, Murias G, Canales H, Sottile P, Badie J, *et al*. Effects of hemorrhage on gastrointestinal oxygenation. *Intensive Care Med* 2001;27:1931-6.
36. Vallet B, Teboul JL, Cain S, Curtis S. Venoarterial CO<sub>2</sub> difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol* (1985) 2000;89:1317-21.
37. Nevière R, Chagnon JL, Teboul JL, Vallet B, Wattel F. Small intestine intramucosal PCO<sub>2</sub> and microvascular blood flow during hypoxic and ischemic hypoxia. *Crit Care Med* 2002;30:379-84.
38. Dubin A, Estenssoro E, Murias G, Pozo MO, Sottile JP, Barán M, *et al*. Intramucosal-arterial Pco<sub>2</sub> gradient does not reflect intestinal dysoxia in anemic hypoxia. *J Trauma* 2004;57:1211-7.
39. Dubin A, Murias G, Estenssoro E, Canales H, Badie J, Pozo M, *et al*. Intramucosal-arterial PCO<sub>2</sub> gap fails to reflect intestinal dysoxia in hypoxic hypoxia. *Crit Care* 2002;6:514-20.
40. Creteur J, De Backer D, Sakr Y, Koch M, Vincent JL. Sublingual capnometry tracks microcirculatory changes in septic patients. *Intensive Care Med* 2006;32:516-23.
41. Gutierrez G. A mathematical model of tissue-blood carbon dioxide exchange during hypoxia. *Am J Respir Crit Care Med* 2004;169:525-33.
42. Nelson DP, Beyer C, Samsel RW, Wood LD, Schumacker PT. Pathological supply dependence of O<sub>2</sub> uptake during bacteremia in dogs. *J Appl Physiol* (1985) 1987;63:1487-92.
43. Pastores SM, Katz DP, Kvetan V. Splanchnic ischemia and gut mucosal injury in sepsis and the multiple organ dysfunction syndrome. *Am J Gastroenterol* 1996;91:1697-710.
44. Doig CJ, Sutherland LR, Sandham JD, Fick GH, Verhoef M, Meddings JB. Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. *Am J Respir Crit Care Med* 1998;158:444-51.
45. Meakins JL, Marshall JC. The gastrointestinal tract: the motor of MOF. *Archives of Surgery* 1986;121:197-201.
46. Dantzker DR. The gastrointestinal tract. The canary of the body? *JAMA* 1993;270:1247-8.
47. Edouard AR, Degrémont AC, Duranteau J, Pussard E, Berdeaux A, Samii K. Heterogeneous regional vascular responses to simulated transient hypovolemia in man. *Intensive Care Med* 1994;20:414-20.
48. Hamilton-Davies C, Mythen MG, Salmon JB, Jacobson D, Shukla A, Webb AR. Comparison of commonly used clinical indicators of hypovolaemia with gastrointestinal tonometry. *Intensive Care Med* 1997;23:276-81.
49. Friedman G, Berlot G, Kahn RJ, Vincent JL. Combined measurements of blood lactate concentrations and gastric intramucosal pH in patients with severe sepsis. *Crit Care Med* 1995;23:1184-93.
50. Oud L, Haupt MT. Persistent gastric intramucosal ischemia in patients with sepsis following resuscitation from shock. *Chest* 1999;115:1390-6.
51. Morgan TJ, Venkatesh B, Endre ZH. Accuracy of intramucosal pH calculated from arterial bicarbonate and the Henderson-Hasselbalch equation: assessment using simulated ischemia. *Crit Care Med* 1999;27:2495-9.
52. Pernat A, Weil MH, Tang W, Yamaguchi H, Pernat AM, Sun S, *et al*. Effects of hyper- and hypoventilation on gastric and sublingual PCO<sub>2</sub>. *J Appl Physiol* (1985) 1999;87:933-7.
53. Vincent JL, Creteur J. Gastric mucosal pH is definitely obsolete--please tell us more about gastric mucosal PCO<sub>2</sub>. *Crit Care Med* 1998;26:1479-81.
54. Schlichtig R, Mehta N, Gayowski TJ. Tissue-arterial PCO<sub>2</sub> difference is a better marker of ischemia than intramural pH (pHi) or arterial pH-pHi difference. *J Crit Care* 1996;11:51-6.
55. Bennett-Guerrero E, Panah MH, Bodian CA, Methikalani BJ, Alfaroni JR, DePerio M, *et al*. Automated detection of gastric luminal partial pressure of carbon dioxide during cardiovascular surgery using the Tonocap. *Anesthesiology* 2000;92:38-45.
56. Lebuffé G, Decoene C, Pol A, Prat A, Vallet B. Regional capnometry with air-automated tonometry detects circulatory failure earlier than conventional hemodynamics after cardiac surgery. *Anesth Analg* 1999;89:1084-90.
57. Holland J, Carey M, Hughes N, Sweeney K, Byrne PJ, Healy M, *et al*. Intraoperative splanchnic hypoperfusion, increased intestinal permeability, down-regulation of monocyte class II major histocompatibility complex expression, exaggerated acute phase response, and sepsis. *Am J Surg* 2005;190:393-400.
58. Lebuffé G, Vallet B, Takala J, Hartstein G, Lamy M, Mythen M, *et al*. A European, multicenter, observational study to assess the value of gastric-to-end tidal PCO<sub>2</sub> difference in predicting postoperative complications. *Anesth Analg* 2004;99:166-72.
59. Kirton OC, Windsor J, Wedderburn R, Hudson-Civetta J, Shatz DV, Mataragas NR, *et al*. Failure of splanchnic resuscitation in the acutely injured trauma patient correlates with multiple organ system failure and length of stay in the ICU. *Chest* 1998;113:1064-9.
60. Ivatury RR, Simon RJ, Islam S, Fuego A, Rohman M, Stahl WM. A prospective randomized study of end points of resuscitation after major trauma: global oxygen transport indices versus organ-specific gastric mucosal pH. *J Am Coll Surg* 1996;183:145-54.
61. Pargger H, Hampl KF, Christen P, Staender S, Scheidegger D. Gastric intramucosal pH-guided therapy in patients af-

- ter elective repair of infrarenal abdominal aneurysms: is it beneficial? *Intensive Care Med* 1998;24:769-76.
62. Gomersall CD, Joynt GM, Freebairn RC, Hung V, Buckley TA, Oh TE. Resuscitation of critically ill patients based on the results of gastric tonometry: a prospective, randomized, controlled trial. *Crit Care Med* 2000;28:607-14.
  63. Miami Trauma Clinical Trials G. Splanchnic hypoperfusion-directed therapies in trauma: a prospective, randomized trial. *Am Surg* 2005;71:252-60.
  64. Palizas F, Dubin A, Regueira T, Bruhn A, Knobel E, Lazzari S, *et al*. Gastric tonometry versus cardiac index as resuscitation goals in septic shock: a multicenter, randomized, controlled trial. *Crit Care* 2009;13:R44.
  65. Zhang X, Xuan W, Yin P, Wang L, Wu X, Wu Q. Gastric tonometry guided therapy in critical care patients: a systematic review and meta-analysis. *Crit Care* 2015;19:22.
  66. Silva E, De Backer D, Creteur J, Vincent JL. Effects of fluid challenge on gastric mucosal PCO<sub>2</sub> in septic patients. *Intensive Care Med* 2004;30:423-9.
  67. Creteur J. Gastric and sublingual capnometry. *Curr Opin Crit Care* 2006;12:272-7.
  68. Jin X, Weil MH, Sun S, Tang W, Bisera J, Mason EJ. Decreases in organ blood flows associated with increases in sublingual PCO<sub>2</sub> during hemorrhagic shock. *J Appl Physiol* (1985) 1998;85:2360-4.
  69. Nakagawa Y, Weil MH, Tang W, Sun S, Yamaguchi H, Jin X, *et al*. Sublingual capnometry for diagnosis and quantitation of circulatory shock. *Am J Respir Crit Care Med* 1998;157:1838-43.
  70. Povoas HP, Weil MH, Tang W, Moran B, Kamohara T, Bisera J. Comparisons between sublingual and gastric tonometry during hemorrhagic shock. *Chest* 2000;118:1127-32.
  71. Marik PE. Sublingual capnography: a clinical validation study. *Chest* 2001;120:923-7.
  72. Rackow EC, O'Neil P, Astiz ME, Carpati CM. Sublingual capnometry and indexes of tissue perfusion in patients with circulatory failure. *Chest* 2001;120:1633-8.
  73. Maciel AT, Creteur J, Vincent JL. Tissue capnometry: does the answer lie under the tongue? *Intensive Care Med* 2004;30:2157-65.
  74. Weil MH, Nakagawa Y, Tang W, Sato Y, Ercoli F, Finigan R, *et al*. Sublingual capnometry: a new noninvasive measurement for diagnosis and quantitation of severity of circulatory shock. *Crit Care Med* 1999;27:1225-9.
  75. Vallee F, Mateo J, Dubreuil G, Poussant T, Tachon G, Ouanounou I, *et al*. Cutaneous ear lobe Pco<sub>2</sub> at 37°C to evaluate microperfusion in patients with septic shock. *Chest* 2010;138:1062-70.
  76. 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;166:98-104.
  77. 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;32:1825-31.
  78. Palágyi P, Kaszaki J, Rostás A, Erces D, Németh M, Boros M, *et al*. Monitoring Microcirculatory Blood Flow with a New Sublingual Tonometer in a Porcine Model of Hemorrhagic Shock. *Biomed Res Int* 2015;2015:847152.
  79. Severinghaus JW. Methods of measurement of blood and gas carbon dioxide during anaesthesia. *Anesthesiology* 1960;21:717-26.
  80. Eberhard P. The design, use, and results of transcutaneous carbon dioxide analysis: current and future directions. *Anesth Analg* 2007;105:S48-52.
  81. Severinghaus JW, Bradley AF. Electrodes for blood pO<sub>2</sub> and pCO<sub>2</sub> determination. *J Appl Physiol* 1958;13:515-20.
  82. Eberhard P, Gisiger PA, Gardaz JP, Spahn DR. Combining transcutaneous blood gas measurement and pulse oximetry. *Anesth Analg* 2002;94:S76-S80.
  83. Bendjelid K, Schutz N, Stotz M, Gerard I, Suter PM, Romand JA. Transcutaneous PCO<sub>2</sub> monitoring in critically ill adults: clinical evaluation of a new sensor. *Crit Care Med* 2005;33:2203-6.
  84. Maniscalco M, Zedda A, Faraone S, Carratù P, Sofia M. Evaluation of a transcutaneous carbon dioxide monitor in severe obesity. *Intensive Care Med* 2008 Jul; 34:1340-4.
  85. Gancel PE, Roupie E, Guittet L, Laplume S, Terzi N. Accuracy of a transcutaneous carbon dioxide pressure monitoring device in emergency room patients with acute respiratory failure. *Intensive Care Med* 2011 Feb; 37:348-51.

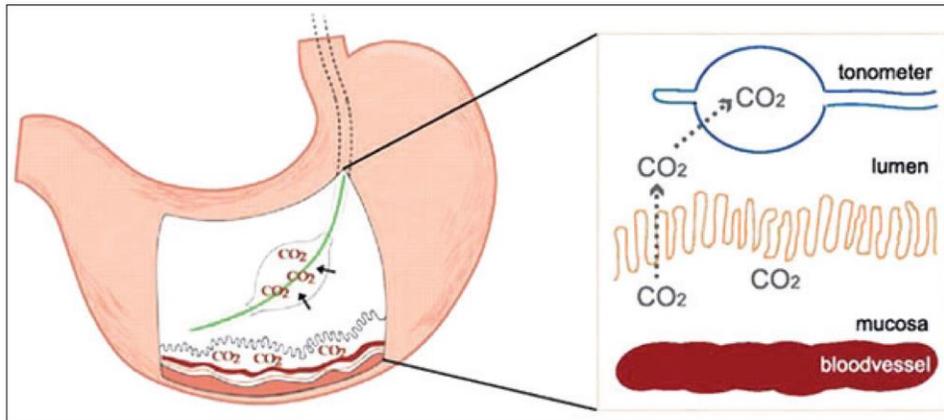
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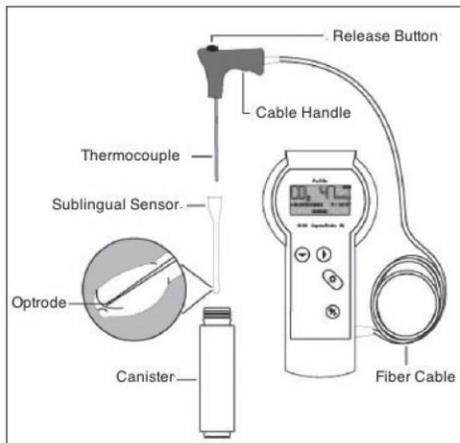
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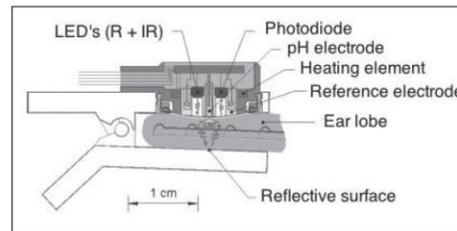
SUPPLEMENTARY MATERIALS



Supplementary Figure 1.—Principle of gastric tonometry (from van Wijck K, Lenaerts K, Grootjans J, Wijnands KA, Poeze M, van Loon LJ, *et al.* Physiology and pathophysiology of splanchnic hypoperfusion and intestinal injury during exercise: strategies for evaluation and prevention. *Am J Physiol Gastrointest Liver Physiol* 2012;303:G155-68).



Supplementary Figure 2.—Principle of sublingual capnography (from Marik PE. Sublingual capnometry: a non-invasive measure of microcirculatory dysfunction and tissue hypoxia. *Physiol Meas* 2006;27:R37-47).



Supplementary Figure 3.—Transcutaneous PCO<sub>2</sub> sensor at the ear lobe (from Eberhard P. The Design, Use, and Results of Transcutaneous Carbon Dioxide Analysis: Current and Future Directions. *Anesth Analg* 2007;105(6 Suppl):S48-52).

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**Third Part**  
**Metabolic approach for**  
**assessment of tissue**  
**oxygenation**

## I. Background

CO<sub>2</sub> is transported in the blood in three figures [103]: Dissolved, in combination with proteins as carbamino compounds, and as bicarbonate. Physically dissolved CO<sub>2</sub> is a function of CO<sub>2</sub> solubility in blood, which is about 20 times that of O<sub>2</sub>; therefore, considerably more CO<sub>2</sub> than O<sub>2</sub> is present in simple solution at equal partial pressures. However, dissolved CO<sub>2</sub> shares only around 5% of the whole CO<sub>2</sub> concentration in arterial blood.

The bicarbonate ion (HCO<sub>3</sub><sup>-</sup>) is the most significant form of CO<sub>2</sub> carriage in the blood. CO<sub>2</sub> combines with water (H<sub>2</sub>O) to form carbonic acid (H<sub>2</sub>CO<sub>3</sub>), and this dissociates to HCO<sub>3</sub><sup>-</sup> and hydrogen ion (H<sup>+</sup>):  $\text{CO}_2 + \text{H}_2\text{O} = \text{H}_2\text{CO}_3 = \text{HCO}_3^- + \text{H}^+$ . Carbonic anhydrase is the enzyme that catalyzes the first reaction, making it almost instantaneous. Carbonic anhydrase occurs mainly in red blood cells, but it also occurs on pulmonary capillary endothelial cells, which accelerates the reaction in plasma and in the lungs. The uncatalyzed reaction will occur in plasma but at a much slower rate. The second reaction happens immediately inside the red blood cells and does not require any enzyme. The H<sub>2</sub>CO<sub>3</sub> dissociates to H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup>, and the H<sup>+</sup> is buffered primarily by hemoglobin while the excess HCO<sub>3</sub><sup>-</sup> is transported out of the red blood cells into plasma by an electrically neutral bicarbonate-chloride exchanger. The fast conversion of CO<sub>2</sub> to HCO<sub>3</sub><sup>-</sup> results in nearly 90% of the CO<sub>2</sub> in arterial blood being transported in that manner.

Hemoglobin-O<sub>2</sub> saturation is the main factor affecting the capacity of hemoglobin to fix CO<sub>2</sub> (Haldane effect). Consequently, CO<sub>2</sub> concentration increases when blood is deoxygenated, or CO<sub>2</sub> concentration diminishes when blood is oxygenated at any assumed PCO<sub>2</sub> [103]. H<sup>+</sup> ions from CO<sub>2</sub> can be deemed as competing with O<sub>2</sub> for hemoglobin binding. Accordingly, rising oxygen reduces the affinity of hemoglobin for H<sup>+</sup> and blood CO<sub>2</sub> concentration (Haldane effect). The

physiological assets of the Haldane effect are that it promotes removing CO<sub>2</sub> in the lungs when blood is oxygenated and CO<sub>2</sub> filling in the blood when oxygen is delivered to tissues. Additionally, the Haldane effect leads to a sharper physiologic CO<sub>2</sub> blood equilibrium curve that has the physiologic interest of rising CO<sub>2</sub> concentration differences for a given PCO<sub>2</sub> difference.

### 1. Determinant of venous-to-arterial CO<sub>2</sub> tension difference

The mixed  $\Delta\text{PCO}_2$  ( $\Delta\text{PCO}_2_{\text{Mix}}$ ) is the gradient between PCO<sub>2</sub> in mixed venous blood (PvCO<sub>2</sub>) and PCO<sub>2</sub> in arterial blood (PaCO<sub>2</sub>):  $\Delta\text{PCO}_2_{\text{Mix}} = \text{PvCO}_2 - \text{PaCO}_2$ ; PvCO<sub>2</sub> and PaCO<sub>2</sub> are partial pressures of the dissolved CO<sub>2</sub> in the mixed venous and arterial blood, respectively. The application of the Fick equation to CO<sub>2</sub> shows that the CO<sub>2</sub> elimination (identical to CO<sub>2</sub> generation in a stable condition) equals the product of the difference between mixed venous blood CO<sub>2</sub> content (CvCO<sub>2</sub>) and arterial blood CO<sub>2</sub> content (CaCO<sub>2</sub>) and cardiac output: Total CO<sub>2</sub> production (VCO<sub>2</sub>) = cardiac output  $\times$  (CvCO<sub>2</sub> - CaCO<sub>2</sub>). Despite of a global curvilinear shape of the relation between PCO<sub>2</sub> and the total CCO<sub>2</sub>, there is a rather linear association between CCO<sub>2</sub> and PCO<sub>2</sub> over the general physiological range of CO<sub>2</sub> content so that CCO<sub>2</sub> can be substituted by PCO<sub>2</sub> ( $\text{PCO}_2 = k \times \text{CCO}_2$ ) [104-106]. Therefore, VCO<sub>2</sub> can be calculated from a modified Fick equation as:  $\text{VCO}_2 = \text{cardiac output} \times k \times \Delta\text{PCO}_2_{\text{Mix}}$  so that  $\Delta\text{PCO}_2_{\text{Mix}} = k \times \text{VCO}_2 / \text{cardiac output}$ , where k is the pseudo-linear coefficient supposed to be constant in physiological states [104]. Therefore,  $\Delta\text{PCO}_2_{\text{Mix}}$  would be linearly linked to CO<sub>2</sub> generation and inversely associated with cardiac output. Under normal conditions,  $\Delta\text{PCO}_2_{\text{Mix}}$  values range between 2 and 6 mmHg [107].

Under steady states of both VO<sub>2</sub> and VCO<sub>2</sub>,  $\Delta\text{PCO}_2_{\text{Mix}}$  was observed to increase in parallel with the reduction in cardiac output [4, 108, 109]. In other words, when cardiac output is adapted to VO<sub>2</sub>,  $\Delta\text{PCO}_2_{\text{Mix}}$  should not increase due to increased clearance of CO<sub>2</sub>, whereas  $\Delta\text{PCO}_2_{\text{Mix}}$

should rise following cardiac output reduction because of a low flow-induced tissue CO<sub>2</sub> stagnation phenomenon. Due to the decreasing of transit time, a higher than usual addition of CO<sub>2</sub> per unit of blood passing the efferent microvessels results in hypercapnia in the venous blood. As long as alveolar respiration is sufficient, a gradient will occur between PvCO<sub>2</sub> and PaCO<sub>2</sub>. However, during spontaneous breathing, hyperventilation, stimulated by the decreased blood flow, may reduce PaCO<sub>2</sub> and thus may prevent the CO<sub>2</sub> stagnation-induced rise in PvCO<sub>2</sub> [110]. This finding underscores the utility of calculating  $\Delta\text{PCO}_2\text{_{Mix}}$  rather than simply assessing PvCO<sub>2</sub>, particularly in the case of spontaneous breathing [111].

The relationship between PCO<sub>2</sub> and the total blood CCO<sub>2</sub> is curvilinear even though more linear than the oxygen dissociation curve [103]. Oxygen saturation, hematocrit, temperature, and the degree of metabolic acidosis influence the PCO<sub>2</sub>/CCO<sub>2</sub> relationship [103]. Hence, for a given value of CCO<sub>2</sub>, PCO<sub>2</sub> is higher in the case of metabolic acidosis than in the case of normal pH.

## 2. Can $\Delta\text{PCO}_2\text{_{Mix}}$ be used as a marker of tissue hypoxia?

It has been suggested that  $\Delta\text{PCO}_2\text{_{Mix}}$  can be used to detect the presence of tissue hypoxia in patients with acute circulatory failure [4, 108]. In fact, under conditions of tissue hypoxia with a decreased VO<sub>2</sub>, the relationship between changes in cardiac output and  $\Delta\text{PCO}_2\text{_{Mix}}$  is much more complex. Indeed, in these circumstances, the increase in CO<sub>2</sub> production related to the anaerobic pathway is counterbalanced by a reduced aerobic CO<sub>2</sub> production, so that VCO<sub>2</sub> and hence  $\Delta\text{PCO}_2\text{_{Mix}}$  could be at best unchanged or decreased [109]. Nevertheless, since the k factor should rise during tissue hypoxia [4] while VCO<sub>2</sub> must fall, the resultant effect on  $\Delta\text{PCO}_2\text{_{Mix}}$  depends mainly on the flow state (cardiac output) [104].

Under conditions of tissue hypoxia with a maintained flow state, venous blood flow should be sufficiently elevated to assure adequate clearance of the CO<sub>2</sub> generated by the hypoxic cells so that  $\Delta\text{PCO}_2_{\text{Mix}}$  should not increase even if the CO<sub>2</sub> production is not decreased. Conversely, low flow states can result in a widening of  $\Delta\text{PCO}_2_{\text{Mix}}$  due to the tissue CO<sub>2</sub> stagnation phenomenon [112], even if no additional CO<sub>2</sub> production occurs. This point was nicely demonstrated by Vallet et al. [113] in a canine model of an isolated limb in which a diminished DO<sub>2</sub> by reducing blood flow (ischemic hypoxia) was related to a rise in regional  $\Delta\text{PCO}_2$ . On the other hand, when blood flow was preserved, but arterial PO<sub>2</sub> was decreased by lowering the input oxygen concentration (hypoxic hypoxia),  $\Delta\text{PCO}_2$  did not rise despite a significant decline in VO<sub>2</sub>. This is because the preserved blood flow was sufficient to clear the generated CO<sub>2</sub> [114] (Figure). Accordingly, Nevière et al. [115] demonstrated that for the same level of induced oxygen supply dependency, regional  $\Delta\text{PCO}_2$  was risen only in ischemic hypoxia but not in hypoxic hypoxia, indicating that elevated  $\Delta\text{PCO}_2$  was mainly linked to the reduction in cardiac output. These studies clearly show that the absence of elevated  $\Delta\text{PCO}_2$  does not preclude the presence of tissue hypoxia and hence underline the good value of  $\Delta\text{PCO}_2$  to detect inadequate tissue perfusion related to its metabolic production but also its poor sensitivity to detect tissue hypoxia. A mathematical model analysis also established that cardiac output plays a crucial role in the widening of  $\Delta\text{PCO}_2$  [116].

### 3. Ratio of $\Delta\text{PCO}_2_{\text{Mix}}$ to $\Delta\text{O}_2_{\text{Mix}}$ ( $\Delta\text{PCO}_2_{\text{Mix}}/\Delta\text{O}_2_{\text{Mix}}$ ) as a marker of tissue hypoxia

In experimental conditions of tissue hypoxia, the drop in VO<sub>2</sub> leads to decreased total VCO<sub>2</sub> generation, mainly related to the decrease in aerobic CO<sub>2</sub> production. However, in situations of hypoxia, tissue CO<sub>2</sub> increases as hydrogen ions generated by anaerobic sources of energy (hydrolysis of high-energy phosphates) are buffered by bicarbonate existing in the cells

(anaerobic CO<sub>2</sub> production) [108]. Therefore, VCO<sub>2</sub> being reduced less than VO<sub>2</sub>, the respiratory quotient (VCO<sub>2</sub>/VO<sub>2</sub>) should increase. Accordingly, the increase in respiratory quotient has been shown to be a valuable marker of global tissue hypoxia [109, 117]. Indeed, Groeneveld et al. [109] observed, in an experimental model of a graded increase in positive end-expiratory pressure-induced a decrease in cardiac output and oxygen delivery in pigs, that the decline in VCO<sub>2</sub> (by 21 ± 2%) was less than in VO<sub>2</sub> (by 27 ± 2%). Cohen et al. [117] reported in an experimental graded hemorrhage in swine that the airway respiratory quotient (VCO<sub>2</sub>/VO<sub>2</sub>) raised from 0.87 ± 0.07 to 1.16 ± 0.07 at the maximum bleeding due to a lesser decrease in VCO<sub>2</sub> than of VO<sub>2</sub> [117]. After retransfusion of the blood, the respiratory quotient came back to its baseline value [117]. Furthermore, Ferrera et al. [118] observed an increase in airway respiratory quotient (median 0.96 [IQR: 0.91-1.06]) during the last step of graded hemorrhage in sheep, and it was normalized after retransfusion.

Because VO<sub>2</sub> is equal to the product of cardiac output by the difference between arterial and mixed venous O<sub>2</sub> content  $\Delta O_2\_Mix$ , and VCO<sub>2</sub> is proportional to the product of cardiac output and  $\Delta PCO_2\_Mix$  so the  $\Delta PCO_2\_Mix/\Delta O_2\_Mix$  ratio could be utilized as a surrogate of respiratory quotient and an indicator of the presence of global tissue hypoxia in critically ill patient. In this line, Dubin et al. [119] found that in sheep that were subject to graded hemorrhage,  $\Delta PCO_2\_Mix/\Delta O_2\_Mix$  significantly increased at the time of the fall in VO<sub>2</sub> and the sharp increase in respiratory quotient (measured by indirect calorimetry), and thus, it was a good indicator of anaerobic metabolism.

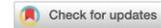
Nevertheless, the use of  $\Delta PCO_2\_Mix/\Delta O_2\_Mix$  ratio as a surrogate of respiratory quotient supposes that the PCO<sub>2</sub>/CCO<sub>2</sub> relationship is quasi-linear, which may be true over the

physiological range of  $PCO_2$  [120]. However, this relationship can be influenced by the degree of metabolic acidosis [121], the hematocrit [122], and oxygen saturation (Haldane effect) [123, 124], and it becomes nonlinear if these factors change [106]. Indeed, severe metabolic acidosis, low hematocrit, and high oxygen saturation can increase  $PCO_2$  for a given  $CO_2$  content since less  $CO_2$  is bound to hemoglobin [123]. Furthermore, these effects are more pronounced at a higher range of  $CCO_2$  since the  $PCO_2/CCO_2$  relationship becomes flatter. Thus,  $\Delta PCO_2\_Mix/\Delta O_2\_Mix$  ratio might be increased due to several factors unrelated to anaerobic metabolism. Therefore, some authors questioned its reliability in reflecting the development of anaerobic metabolism in some specific conditions [118, 119]. In this regard, Dubin et al. found, in a normovolemic hemodilution model of tissue hypoxia in pigs, that  $\Delta PCO_2\_Mix/\Delta O_2\_Mix$  ratio was a misleading indicator of anaerobic metabolism [119]. Indeed, the authors found that this ratio was increased before the occurrence of oxygen supply dependency phenomenon. The authors attributed the rise in the ratio, even in the absence of  $VO_2/DO_2$  dependency, to the effects of anemia on  $\Delta O_2\_Mix$  and on  $CO_2$  hemoglobin dissociation curve that led to enlarged  $\Delta PCO_2\_Mix$  [122]. However, according to the results of that study [119], the slight increase in  $\Delta PCO_2\_Mix$  compared to the baseline in the hemodilution group was noted to be statistically significant only at the end of the protocol ( $DO_2 \approx 5$  mL/min/kg) far beyond the occurrence of oxygen supply dependency ( $DO_2$  critique  $\approx 12$  mL/min/kg). Thus, it seems that the effects of anemia on  $\Delta PCO_2\_Mix$  were minimal, and the increase in  $\Delta PCO_2\_Mix/\Delta O_2\_Mix$  ratio observed before the  $VO_2/DO_2$  dependency could mainly be explained by the decrease in  $\Delta O_2\_Mix$  induced by the severe hemodilution anemia [125]. Of note, in that study, the  $\Delta PCO_2\_Mix/\Delta O_2\_Mix$  ratio increased only at the appearance of  $VO_2/DO_2$  dependency, at the same time as the increase in airway respiratory quotient, and, therefore, it

was a very good indicator of the development of anaerobic metabolism in the hemorrhage group [119]. The same team, in a recent experimental graded bleeding and blood retransfusion study, in mechanically ventilated sheep [118], found that the time course of  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$  ratio was different from the airway respiratory quotient. Nevertheless, the results of that study show that, in the hemorrhagic group, the  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$  ratio suddenly increased along with the airway respiratory quotient at the occurrence of oxygen supply dependency (90 minutes after bleeding). However, the ratio took a bit longer time to return to its baseline value after retransfusion (30 minutes) than the airway respiratory quotient (2 minutes) [118]. Thus, we believe that those findings are instead in favor of the  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$  ratio as a good indicator to depict the development of global anaerobic metabolism.

Moreover, other authors suggested that  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$  ratio might not rise during tissue hypoxia conditions when associated with normal/high blood flow because venous blood flow seemed to guarantee a sufficient clearance of  $\text{CO}_2$  generated by the anaerobic metabolism [126]. In an experimental model of progressive tissue hypoxia induced by reducing either flow [ischemic hypoxia (IH)] or arterial oxygen tension [hypoxic hypoxia (HH)], we observed [127] that regional  $\Delta\text{PCO}_2/\Delta\text{O}_2$  significantly increased at critical  $\text{DO}_2$  value where  $\text{VO}_2$  start to decrease ( $\text{DO}_{2\text{crit}}$ ) and not before, suggesting that these variables were able to depict the occurrence of oxygen supply dependency ( $\text{DO}_{2\text{crit}}$ ) in both IH and HH groups. The increases in these variables were mainly due to the decline in  $\Delta\text{O}_2$  in the HH group and the rise in  $\Delta\text{PCO}_2$  in the IH group induced by the decrease in blood flow. We also found that metabolic acidosis significantly influenced the  $\text{PCO}_2/\text{CO}_2$  content relationship, but not the Haldane effect. Furthermore, at very low  $\text{DO}_2$  values,  $\Delta\text{PCO}_2/\Delta\text{O}_2$  did not only reflect the ongoing anaerobic metabolism; it was confounded by the

effects of metabolic acidosis on the CO<sub>2</sub>-hemoglobin dissociation curve, and then it should be interpreted with caution.



# OPEN Ratio of venous-to-arterial PCO<sub>2</sub> to arteriovenous oxygen content difference during regional ischemic or hypoxic hypoxia

Jihad Mallat<sup>1,2,3✉</sup> & Benoit Vallet<sup>4</sup>

The purpose of the study was to evaluate the behavior of the venous-to-arterial CO<sub>2</sub> tension difference ( $\Delta\text{PCO}_2$ ) over the arterial-to-venous oxygen content difference ( $\Delta\text{O}_2$ ) ratio ( $\Delta\text{PCO}_2/\Delta\text{O}_2$ ) and the difference between venous-to-arterial CO<sub>2</sub> content calculated with the Douglas' equation ( $\Delta\text{CCO}_{2D}$ ) over  $\Delta\text{O}_2$  ratio ( $\Delta\text{CCO}_{2D}/\Delta\text{O}_2$ ) and their abilities to reflect the occurrence of anaerobic metabolism in two experimental models of tissue hypoxia: ischemic hypoxia (IH) and hypoxic hypoxia (HH). We also aimed to assess the influence of metabolic acidosis and Haldane effects on the PCO<sub>2</sub>/CO<sub>2</sub> content relationship. In a vascularly isolated, innervated dog hindlimb perfused with a pump-membrane oxygenator system, the oxygen delivery (DO<sub>2</sub>) was lowered in a stepwise manner to decrease it beyond critical DO<sub>2</sub> (DO<sub>2crit</sub>) by lowering either arterial PO<sub>2</sub> (HH-model) or flow (IH-model). Twelve anesthetized and mechanically ventilated dogs were studied, 6 in each model. Limb DO<sub>2</sub>, oxygen consumption ( $\dot{V}\text{O}_2$ ),  $\Delta\text{PCO}_2/\Delta\text{O}_2$ , and  $\Delta\text{CCO}_{2D}/\Delta\text{O}_2$  were obtained every 15 min. Beyond DO<sub>2crit</sub>,  $\dot{V}\text{O}_2$  decreased, indicating dysoxia.  $\Delta\text{PCO}_2/\Delta\text{O}_2$  and  $\Delta\text{CCO}_{2D}/\Delta\text{O}_2$  increased significantly only after reaching DO<sub>2crit</sub> in both models. At DO<sub>2crit</sub>,  $\Delta\text{PCO}_2/\Delta\text{O}_2$  was significantly higher in the HH-model than in the IH-model ( $1.82 \pm 0.09$  vs.  $1.39 \pm 0.06$ ,  $p = 0.002$ ). At DO<sub>2crit</sub>,  $\Delta\text{CCO}_{2D}/\Delta\text{O}_2$  was not significantly different between the two groups ( $0.87 \pm 0.05$  for IH vs.  $1.01 \pm 0.06$  for HH,  $p = 0.09$ ). Below DO<sub>2crit</sub>, we observed a discrepancy between the behavior of the two indices. In both models,  $\Delta\text{PCO}_2/\Delta\text{O}_2$  continued to increase significantly (higher in the HH-model), whereas  $\Delta\text{CCO}_{2D}/\Delta\text{O}_2$  tended to decrease to become not significantly different from its baseline in the IH-model. Metabolic acidosis significantly influenced the PCO<sub>2</sub>/CO<sub>2</sub> content relationship, but not the Haldane effect.  $\Delta\text{PCO}_2/\Delta\text{O}_2$  was able to depict the occurrence of anaerobic metabolism in both tissue hypoxia models. However, at very low DO<sub>2</sub> values,  $\Delta\text{PCO}_2/\Delta\text{O}_2$  did not only reflect the ongoing anaerobic metabolism; it was confounded by the effects of metabolic acidosis on the CO<sub>2</sub>-hemoglobin dissociation curve, and then it should be interpreted with caution.

## Abbreviations

CO <sub>2</sub>	Carbon dioxide
VO <sub>2</sub>	Oxygen consumption
VCO <sub>2</sub>	Carbon dioxide production
DO <sub>2</sub>	Oxygen delivery
RQ	Respiratory quotient
$\Delta\text{PCO}_2$	Venous-to-arterial carbon dioxide tension difference
CCO <sub>2</sub>	CO <sub>2</sub> content
$\Delta\text{CCO}_2$	Venous-to-arterial carbon dioxide content difference
CCvCO <sub>2</sub>	Venous CO <sub>2</sub> content
CCaCO <sub>2</sub>	Arterial CO <sub>2</sub> content
$\Delta\text{O}_2$	Arterial-to-venous oxygen content difference
PaCO <sub>2</sub>	Partial arterial carbon dioxide tension

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PvCO <sub>2</sub>	Partial venous carbon dioxide tension
SvO <sub>2</sub>	Venous oxygen saturation
SaO <sub>2</sub>	Arterial oxygen saturation
PaO <sub>2</sub>	Partial arterial oxygen tension
PvO <sub>2</sub>	Partial venous oxygen tension
Hb	Hemoglobin

In a landmark study, Vallet et al. demonstrated the determinant role of blood flow in the tissue hypoxia-induced increased venous-to-arterial CO<sub>2</sub> tension difference ( $\Delta$ PCO<sub>2</sub>)<sup>1</sup>. Their data supported the hypothesis that increases in the venous PCO<sub>2</sub> are primarily a function of changes in regional blood flow, independently of the degree of hypoxia. Gutierrez G has confirmed this conclusion in a mathematical model of tissue-to-blood CO<sub>2</sub> exchange during hypoxia<sup>2</sup>. In these previous publications, the behavior of  $\Delta$ PCO<sub>2</sub> over the arterial-to-venous oxygen content difference ( $\Delta$ O<sub>2</sub>) ratio ( $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub>), and the difference between venous-to-arterial CO<sub>2</sub> content ( $\Delta$ CCO<sub>2</sub>) over  $\Delta$ O<sub>2</sub> ratio ( $\Delta$ CCO<sub>2</sub>/ $\Delta$ O<sub>2</sub>) in a model of progressive tissue hypoxia generated by reducing either flow [ischemic hypoxia (IH)] or arterial oxygen tension [hypoxic hypoxia (HH)], were not investigated<sup>1,2</sup>.

Several clinical studies<sup>3–7</sup> have shown that  $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratio, taken as a surrogate of respiratory quotient (RQ), was associated with elevated lactate levels and oxygen supply dependency considered, in those studies, as indices of global anaerobic metabolism in critically ill patients with tissue hypoperfusion. However, in an experimental study, Dubin et al. found that  $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratio was a poor indicator of anaerobic metabolism in the hemodilution model of tissue hypoxia, where anemia was associated with preserved blood flow<sup>8</sup>. Similarly, other authors suggested that  $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratio might not rise during tissue hypoxia conditions when associated with normal/high blood flow because venous blood flow seemed to guarantee a sufficient clearance of CO<sub>2</sub> generated by the anaerobic metabolism<sup>9</sup>. Thus, it is unclear if the  $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratio would be able to depict the presence of anaerobic metabolism in patients with maintained blood flow (cardiac output).

Furthermore, one estimates that the  $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratio might be affected by other factors than anaerobic metabolism by influencing the relationship between CO<sub>2</sub> content (CCO<sub>2</sub>) and PCO<sub>2</sub>. Indeed, metabolic acidosis can change the PCO<sub>2</sub>/CCO<sub>2</sub> relationship so that PCO<sub>2</sub> is higher for a given CCO<sub>2</sub>. Low oxygen saturation, by promoting more CO<sub>2</sub> binding to hemoglobin (Haldane effect), increases the CCO<sub>2</sub> for a given PCO<sub>2</sub><sup>10</sup>. It is not completely clear to what extent these factors would impact the PCO<sub>2</sub>/CCO<sub>2</sub> relationship and influence the  $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratio. Answering this question would help to define the applicability of this ratio in different clinical situations.

Therefore, we used, in secondary analysis, the original study published by Vallet et al.<sup>1</sup> with the aim to assess the behavior of  $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratio,  $\Delta$ CCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratio, and their components in the regional model of progressive tissue hypoxia generated by IH or HH<sup>1</sup>. We also investigated the metabolic acidosis (pH) and Haldane effects on the PCO<sub>2</sub>/CCO<sub>2</sub> relationship. Since the flow was maintained unchanged in the HH model, we hypothesized that  $\Delta$ PCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> and  $\Delta$ CCO<sub>2</sub>/ $\Delta$ O<sub>2</sub> ratios might not be able to detect the occurrence of anaerobic metabolism as the sustained blood flow would be sufficient to wash out the CO<sub>2</sub> generated by hypoxic cells in that model.

## Methods

**Animal preparation.** The original study was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. The study is reported in accordance with the ARRIVE guidelines. All experiments were performed in accordance with relevant guidelines and regulations. Twelve dogs of either sex and mixed breed were used<sup>1</sup>. All animals were anesthetized with intravenous 30 mg/kg of sodium phenobarbital and mechanically ventilated with a Harvard animal respirator at 10 breaths/min. Lamps suspended above the operating table were used to maintain core temperature near 37 °C. Tidal volume was varied to maintain systemic arterial PCO<sub>2</sub> between 30 and 35 mmHg. The ventilator setting was kept unchanged during the rest of the experiment. A 20 mg of succinylcholine chloride was given intramuscularly and a continuous infusion (0.1 mg/mL/min) was begun. Anesthesia depth was checked regularly by vigorous toe pinching, and additional anesthetic was given if systemic blood pressure and heart rate responded.

Catheters were inserted into the pulmonary artery (through the internal jugular vein) and common carotid artery for continuous measurements of vascular pressures and blood sampling. Arterial inflow (Q) and venous outflow from the left hindlimb were isolated, as previously described<sup>1,11</sup> (Supplemental Digital Content 1, Appendix). A roller occlusive pump directed blood flow from the right hindlimb femoral artery to the femoral artery of the vascularly isolated left hindlimb. A sampling port and pressure transducer were placed in this circuit proximal to the limb. A membrane oxygenator (model 0800-2A, Sci Med) was interposed in the perfusion circuit. A gas flow mixer (model GF-3, Cameron Instruments) supplied O<sub>2</sub>, N<sub>2</sub>, and CO<sub>2</sub> to the oxygenator, as needed, to produce normoxia or hypoxia with normocapnia in the blood supply to the hindlimb. A water bath warmed the oxygenator so that perfusion to the isolated hindlimb was at 37 °C after heat loss through the tubing.

**Measurements.** Blood samples from the carotid, femoral, and pulmonary arteries and femoral vein were obtained simultaneously. Blood gas tensions and pH were measured in an acid–base analyzer (ABL-30, Radiometer, Westlake, OH) at 37 °C and later corrected to esophageal temperature at the time of sampling. Oxygen saturation was measured with a co-oximeter calibrated for dog blood (IL-282, Instrumentation Lab, Lexington, MA). Arterial oxygen content was calculated as CaO<sub>2</sub> (mL) = 1.34 × Hb (g/dL) × SaO<sub>2</sub> + 0.0031 × PaO<sub>2</sub> (mmHg), where SaO<sub>2</sub> is the oxygen saturation of arterial blood, Hb the hemoglobin concentration, and PaO<sub>2</sub> the arterial oxygen tension. Hindlimb venous oxygen content was calculated as CvO<sub>2</sub> (mL) = 1.34 × Hb (g/dL) × SvO<sub>2</sub> + 0.0031 × PvO<sub>2</sub> (mmHg), where PvO<sub>2</sub> is the hindlimb venous oxygen tension, and SvO<sub>2</sub> is the hindlimb venous oxygen saturation.  $\Delta$ O<sub>2</sub> was calculated as CaO<sub>2</sub> – CvO<sub>2</sub>. Hindlimb VO<sub>2</sub> ( $\dot{V}$ O<sub>2</sub>) was calculated

as the product of  $Q$  (leg blood flow) and  $\Delta O_2$ . Hindlimb oxygen delivery ( $DO_2$ ) was calculated by using the formula:  $DO_2$  (mL/min) =  $CaO_2 \times Q \times 10$ . Hindlimb oxygen extraction (OE) was defined as:  $OE = \dot{V}O_2/DO_2$ .

$\Delta PCO_2$  was calculated as the difference between the hindlimb venous carbon dioxide tension ( $PvCO_2$ ) and hindlimb arterial  $PCO_2$  ( $PaCO_2$ ). In the original study, the hindlimb difference between venous-to-arterial  $CO_2$  content ( $CvCO_2 - CaCO_2$ ) was calculated with the McHardy equation (as proposed by Neviere et al.<sup>12</sup>):  $\Delta CCO_2 = 11.02 \times [(PvCO_2)^{0.396} - (PaCO_2)^{0.396}] - (15 - Hb) \times 0.015 \times (PvCO_2 - PaCO_2) - (95 - SaO_2) \times 0.064$ . However, the most used equation to calculate the blood  $CO_2$  content is the Douglas equation<sup>13</sup>, which includes pH:

$$\begin{aligned} \text{Blood } CO_{2D} \text{ content [blood Douglas } CCO_2 \text{ (mL)]} \\ = \text{Plasma } CCO_2 \times [1 - 0.0289 \times (Hb) / (3.352 - 0.456 \times SO_2) \times (8.142 - pH)] \end{aligned}$$

where plasma  $CCO_2 = 2.226 \times S \times \text{plasma } PCO_2 \times (1 + 10^{pH - pK'})$ ,  $CCO_2$  is  $CO_2$  content,  $SO_2$  is oxygen saturation,  $S$  is the plasma  $CO_2$  solubility coefficient, and  $pK'$  is the apparent  $pK$ .

$S$  and  $pK'$  were calculated as follow:

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

and

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

where  $T$  is the temperature expressed as  $^{\circ}C$ .

The difference between venous-to-arterial  $CCO_2$  calculated with the Douglas equation was:  $\Delta CCO_{2D} = CvCO_{2D} - CaCO_{2D}$ .

To investigate the metabolic acidosis and Haldane effects on the  $PCO_2/CCO_2$  relationship, default (Def) values of blood  $CCO_2$  were calculated with the Douglas's equation by using only the resting values of pH and  $SvO_2$  for each dog as following:  $DefpH - \Delta CCO_{2D} = DefpH - CvCO_{2D} - DefpH - CaCO_{2D}$ , and  $DefSvO_2 - \Delta CCO_{2D} = DefSvO_2 - CvCO_{2D} - DefSvO_2 - CaCO_{2D}$ .

Leg blood flow,  $DO_2$ , and  $\dot{V}O_2$  were reported per kilogram of muscle mass.

We also calculated the hindlimb  $\Delta PCO_2/\Delta O_2$ ,  $\Delta CCO_2/\Delta O_2$ , and  $\Delta CCO_{2D}/\Delta O_2$  ratios.

**Experimental protocol.** The experimental model was already described previously<sup>4</sup>. After all pressures and flows were stable for at least 30 min, the experiment began with a 30-min control period, during which measurements were obtained every 15 min. In the progressive ischemic hypoxia (IH) group,  $Q$  was then decreased every 15 min to produce  $Q$  values of ~60, 45, 40, 30, 20, 15, and 10 mL/kg/min. In the hypoxic hypoxia (HH) group,  $Q$  was set at 60 mL/kg/min and limb  $DO_2$  was reduced by decreasing arterial  $PO_2$  from 100 to ~15 mmHg (i.e.,  $CaO_2$  of 17 to 2 mL  $O_2/100$  mL) in eight steps at 15-min intervals. A flow rate of 60 mL/kg/min was chosen for progressive hypoxia because it is within the range of resting blood flow to normal skeletal muscle and for the practical reason that a moderate flow was necessary to achieve the desired low  $PO_2$  values using the membrane oxygenator. Oxygen and  $CO_2$ -derived variables were determined every 15 min, 13 min after the change in hindlimb arterial flow or  $PO_2$ .

For each experiment, regression lines were fitted to the delivery independent and dependent portions of the delivery-uptake curve using a dual-line, least squares method<sup>14</sup>. The intercept of these two lines defined the critical  $DO_2$  ( $DO_{2crit}$ ), that is, the delivery at which  $\dot{V}O_2$  began to fall with any further decline in  $DO_2$ .

**Statistical analysis.** All data are expressed as mean  $\pm$  SEM after assessed for normality using the Kolmogorov-Smirnov test.

Comparisons of data within and between groups were performed using a mixed ANOVA. Post-hoc paired and unpaired  $t$  tests were used, as appropriate, for one-time comparisons. The Bonferroni method was used to adjust for multiple comparisons.

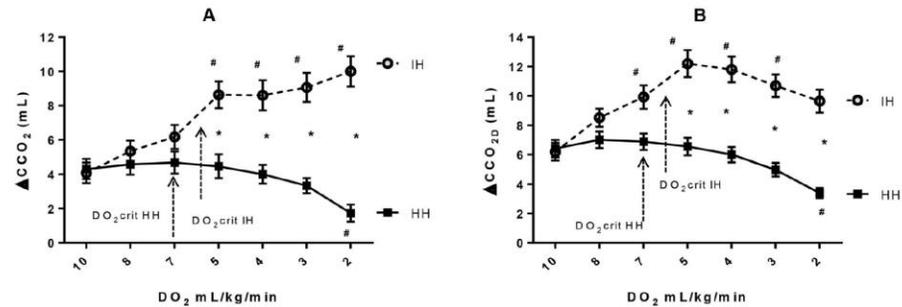
Statistical analysis was performed using GraphPad Prism 6.0 software for windows (San Diego, California, USA).  $p < 0.006$  and  $p < 0.007$  were considered statistically significant for the between-group and within-group (with the baseline) comparisons, respectively. All reported  $p$  values are two-sided.

## Results

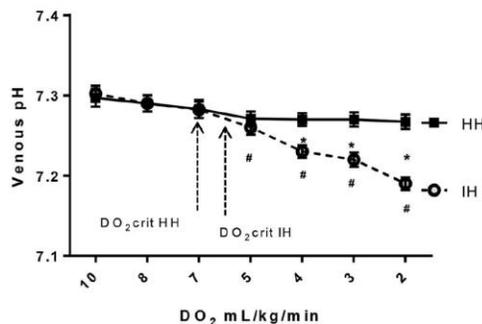
Systemic hemodynamics and oxygen-derived variables remain unchanged throughout the protocol with no differences between the IH and HH models (Supplemental Digital Content 2, Table S1).

In both groups, the  $\dot{V}O_2/DO_2$  graph depicts the typical biphasic relationship (Supplemental Digital Content 3, Figure S1). There was no statistically significant difference between the mean  $DO_{2crit}$  in the HH and IH models ( $6.9 \pm 0.6$  vs.  $6.0 \pm 0.5$  mL/kg/min,  $p = 0.28$ , respectively).  $SvO_2$  at  $DO_{2crit}$  was not statistically different between the two groups ( $25 \pm 1.7\%$  in HH vs.  $26 \pm 1.5\%$  in IH,  $p = 0.66$ ). However, for the lower  $DO_2$  values,  $SvO_2$  was significantly higher in the IH model than in the HH group (Supplemental Digital Content 4, Figure S2).  $EO_2$  at  $DO_{2crit}$  was significantly higher in the IH group than in the HH model ( $74 \pm 2\%$  vs.  $60 \pm 4\%$ ,  $p = 0.01$ ) and increased continuously and similarly in both groups (Supplemental Digital Content 5, Figure S3).  $\Delta PCO_2$  risen significantly in the IH model and did not change in the HH model (Supplemental Digital Content 6, Figure S4).

**Time course of venous-to-arterial  $CCO_2$  difference.**  $\Delta CCO_2$  calculated with the McHardy equation increased progressively along with the decrease in  $DO_2$  in the IH group but remained unchanged and even significantly decreased at the lowest  $DO_2$  value on the HH group (Fig. 1A). At  $DO_{2crit}$ ,  $\Delta CCO_2$  was significantly



**Figure 1.** Hindlimb venous-to-arterial CO<sub>2</sub> content difference ( $\Delta\text{CCO}_2$ ) calculated with McHardy equation (A) and with Douglas equation ( $\Delta\text{CCO}_{2D}$ ) (B) as a function of hindlimb oxygen delivery ( $\text{DO}_2$ ) for ischemic hypoxia model (IH) and hypoxic hypoxia model (HH). \* $p < 0.006$  vs. HH, # $p < 0.007$  vs. baseline, mixed ANOVA.



**Figure 2.** Hindlimb venous pH as a function of hindlimb oxygen delivery ( $\text{DO}_2$ ) for ischemic hypoxia model (IH) and hypoxic hypoxia model (HH). \* $p < 0.006$  vs. HH, # $p < 0.007$  vs. baseline, mixed ANOVA.

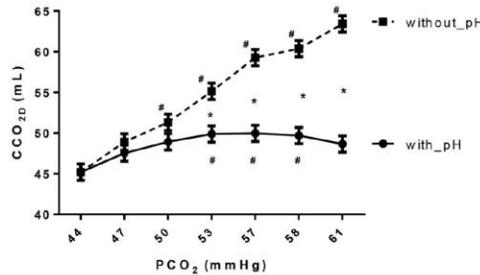
higher in the IH group than in the HH group ( $7.5 \pm 0.66$  vs.  $4.6 \pm 0.5$  mL,  $p = 0.006$ , respectively), and it was significantly different from the baseline only in the IH group ( $p = 0.0023$ ).

$\Delta\text{CCO}_{2D}$  calculated with the Douglas equation, in the IH group, increased with the decrease in  $\text{DO}_2$  down to  $\text{DO}_{2\text{crit}}$ . However, beyond  $\text{DO}_{2\text{crit}}$ ,  $\Delta\text{CCO}_{2D}$  started to decrease with the further decline in  $\text{DO}_2$  to become not significantly different from its baseline value at the lowest value of  $\text{DO}_2$  (Fig. 1B). In the HH group,  $\Delta\text{CCO}_{2D}$  had the same pattern as  $\Delta\text{CCO}_2$  calculated with the McHardy equation (Fig. 1A,B), which remained unchanged in parallel with the decreases in  $\text{DO}_2$  to become significantly lower than its baseline ( $p < 0.001$ ) only at the end of the experiment. At  $\text{DO}_{2\text{crit}}$ ,  $\Delta\text{CCO}_{2D}$  was greater in the IH group compared to the HH group ( $11.0 \pm 0.88$  vs.  $7.0 \pm 0.56$  mL,  $p = 0.003$ , respectively), and it was significantly higher than its baseline value ( $p < 0.001$ ) only in the IH group (Fig. 1B).

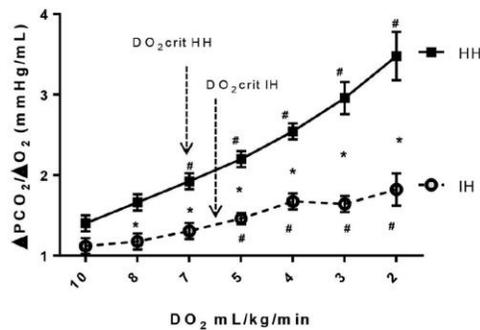
**pH and Haldane effects on the  $\text{PCO}_2/\text{CCO}_2$  relationship.** Hindlimb venous pH ( $\text{pH}_v$ ) remained unchanged with the decline in  $\text{DO}_2$  down to  $\text{DO}_{2\text{crit}}$  in both groups (Fig. 2). However, beyond  $\text{DO}_{2\text{crit}}$ ,  $\text{pH}_v$  decreased significantly only in the IH group and remained stable in the HH group (Fig. 2).

The venous  $\text{CCO}_2$  calculated, with the Douglas equation, by acknowledging the changes in  $\text{pH}_v$  ( $\text{CvCO}_{2D}$ ) increased first with the rise in  $\text{PvCO}_2$ , but then after, it stabilized despite further increases in  $\text{PvCO}_2$ , due to the fall in  $\text{pH}_v$ . Eventually, despite the continuously increasing  $\text{PvCO}_2$ ,  $\text{CvCO}_{2D}$  decreased due to the marked decline in  $\text{pH}_v$  (Fig. 3). On the contrary, there was almost a linear increase in  $\text{DefpH-CvCO}_{2D}$  (without accounting for the changes in  $\text{pH}_v$ ) with the increase in  $\text{PvCO}_2$  (Fig. 4). Also,  $\text{DefpH-}\Delta\text{CCO}_{2D}$  increased linearly with the decreases in  $\text{DO}_2$  in the IH group, while it remained unchanged in the HH group (Supplemental Digital Content 7, Figure S5).

The relationship between  $\text{PvCO}_2$  and  $\text{CCO}_2$  calculated without accounting for the changes in  $\text{SvO}_2$  was the same as that if we acknowledged the variations in  $\text{SvO}_2$  (Supplemental Digital Content 8, Figure S6).



**Figure 3.** Hindlimb venous CO<sub>2</sub> content (CCO<sub>2v</sub>) as a function of hindlimb venous PCO<sub>2</sub> for CCO<sub>2v</sub> calculated with accounting for pH changes (with\_pH) and without accounting for pH changes (without\_pH) using Douglas equation (CCO<sub>2D</sub>). \**p* < 0.006 vs. HH, #*p* < 0.007 vs. baseline, mixed ANOVA.



**Figure 4.** Hindlimb venous-to-arterial PCO<sub>2</sub> difference (ΔPCO<sub>2</sub>) over the arterial-to-venous O<sub>2</sub> difference (ΔO<sub>2</sub>) ratio (ΔPCO<sub>2</sub>/ΔO<sub>2</sub>) as a function of hindlimb oxygen delivery (DO<sub>2</sub>) for ischemic hypoxia model (IH) and hypoxic hypoxia model (HH). At DO<sub>2crit</sub>, ΔPCO<sub>2</sub>/ΔO<sub>2</sub> was significantly higher in HH model (1.82 ± 0.09) than IH model (1.39 ± 0.06). \**p* < 0.006 vs. HH, #*p* < 0.007 vs. baseline, mixed ANOVA.

**Time course of ΔPCO<sub>2</sub>/ΔO<sub>2</sub>, ΔCCO<sub>2D</sub>/ΔO<sub>2</sub>, and ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> ratios.** ΔO<sub>2</sub> increased significantly in the IH and decreased in the HH in parallel with the decreases in DO<sub>2</sub> (Supplemental Digital Content 9, Figure S7).

At DO<sub>2crit</sub>, ΔPCO<sub>2</sub>/ΔO<sub>2</sub> ratio was significantly higher in the HH group than in the IH group (1.82 ± 0.09 mmHg/mL vs. 1.39 ± 0.06 mmHg/mL, *p* = 0.002, respectively). In both groups, ΔPCO<sub>2</sub>/ΔO<sub>2</sub> ratio increased significantly only after reaching DO<sub>2crit</sub> (Fig. 4). Also, the increase in ΔPCO<sub>2</sub>/ΔO<sub>2</sub> ratio was significantly higher in the HH than in the IH group.

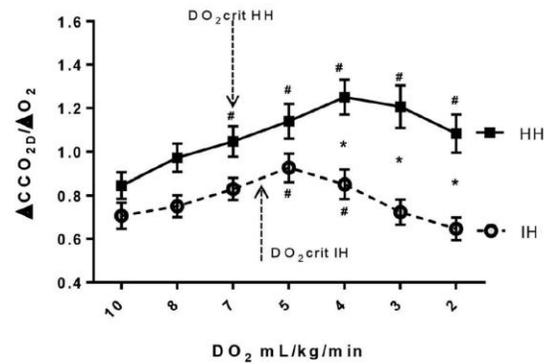
ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> ratio increased after DO<sub>2crit</sub> was reached in both groups, with a trend to decrease by the end of the experiment in the HH group (Supplemental Digital Content 10, Figure S8). At DO<sub>2crit</sub>, there was no significant difference between the two groups (IH: 0.59 ± 0.02 vs. HH: 0.67 ± 0.03, *p* = 0.05).

In both groups, ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> ratio increased significantly after reaching DO<sub>2crit</sub>. However, in the HH group, at lower values of DO<sub>2</sub>, ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> ratio started to decline but remained significantly higher than its baseline value. In the IH group, beyond DO<sub>2crit</sub>, ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> ratio began to decrease at a higher value of DO<sub>2</sub> than in the HH group, to become not significantly different from its baseline value at the end of the experiment (Fig. 5). At DO<sub>2crit</sub>, ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> was not significantly different between the two groups (0.87 ± 0.05 for IH vs. 1.01 ± 0.06 for HH, *p* = 0.09).

In both groups, DefpH-ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> (without accounting for pH changes) increased similarly and linearly in parallel with the decrease in DO<sub>2</sub> (Supplemental Digital Content 11, Figure S9). The increase in DefpH-ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> in IH occurred before reaching DO<sub>2crit</sub>.

### Discussion

The main findings of our study were that: (1) in both groups, ΔPCO<sub>2</sub>/ΔO<sub>2</sub> as well as ΔCCO<sub>2D</sub>/ΔO<sub>2</sub>, and ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> increases significantly in parallel with the decreases in DO<sub>2</sub> only after reaching DO<sub>2crit</sub>; (2) beyond DO<sub>2crit</sub>, the time course of ΔPCO<sub>2</sub>/ΔO<sub>2</sub> ratio was different from that of ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> or ΔCCO<sub>2D</sub>/ΔO<sub>2</sub> ratio, in both



**Figure 5.** Hindlimb venous-to-arterial CO<sub>2</sub> content difference calculated with Douglas equation ( $\Delta\text{CCO}_{2D}$ ) over the arterial-to-venous O<sub>2</sub> difference ( $\Delta\text{O}_2$ ) ratio ( $\Delta\text{CCO}_{2D}/\Delta\text{O}_2$ ) as a function of hindlimb oxygen delivery (DO<sub>2</sub>) for ischemic hypoxia model (IH) and hypoxic hypoxia model (HH). At DO<sub>2</sub>crit, there was no significant difference between HH model ( $1.01 \pm 0.06$ ) and IH model ( $0.87 \pm 0.05$ ). \* $p < 0.006$  vs. HH, # $p < 0.007$  vs. baseline, mixed ANOVA.

groups; (3) metabolic acidosis, but not Haldane effect influenced significantly the PCO<sub>2</sub>/CCO<sub>2</sub> relationship explaining the discrepancy between  $\Delta\text{PCO}_2$  and  $\Delta\text{CCO}_{2D}$ ; (4) the method of CCO<sub>2</sub> calculation had a considerable impact on the results and yielded different conclusions.

Anaerobic metabolism occurrence is usually due to cellular hypoxia<sup>15</sup>. Whenever oxygen delivery decreases relative to demand, and the compensatory mechanism is exhausted, extra-mitochondrial anaerobic glycolysis occurs, and lactic acidosis develops<sup>16</sup>. We aimed to investigate if  $\Delta\text{PCO}_2/\Delta\text{O}_2$  and  $\Delta\text{CCO}_{2D}/\Delta\text{O}_2$  could reflect the development of anaerobic metabolism in two regional models of tissue hypoxia: IH, where the oxygen delivery progressively decreased by decreasing the blood flow, and HH, where the blood flow was maintained unchanged, and the oxygen delivery was reduced by decreasing the arterial oxygen content.

In experimental conditions of tissue hypoxia, the drop in VO<sub>2</sub> leads to decreased total VCO<sub>2</sub> generation, mainly related to the decrease in aerobic CO<sub>2</sub> production. However, under situations of hypoxia, tissue CO<sub>2</sub> increases as hydrogen ions generated by anaerobic sources of energy (hydrolysis of high-energy phosphates) are buffering by bicarbonate existing in the cells (anaerobic CO<sub>2</sub> production)<sup>17</sup>. Therefore, VCO<sub>2</sub> being reduced less than VO<sub>2</sub>, the RQ (VCO<sub>2</sub>/VO<sub>2</sub>) should increase. Accordingly, the increase in RQ has been shown to be a useful marker of global tissue hypoxia<sup>18,19</sup>. Indeed, Groeneveld et al.<sup>18</sup> observed, in an experimental model of a graded increase in positive end-expiratory pressure-induced a decrease in cardiac output and oxygen delivery in pigs, that the decline in VCO<sub>2</sub> (by  $21 \pm 2\%$ ) was less than in VO<sub>2</sub> (by  $27 \pm 2\%$ ).

However, airway RQ measurement necessitates a specific monitoring device (indirect calorimetry) that many hospitals might not have. Recently, there has been a growing interest in the  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio as a surrogate of the RQ to detect the development of global anaerobic metabolism in critically ill patients<sup>3-7</sup>. Indeed, several studies found an association between increased  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio and hyperlactatemia<sup>5</sup> and decreased lactate clearance<sup>6,7</sup>, which were taken as markers of anaerobic metabolism activation. We<sup>4</sup> and other authors<sup>3</sup> have also shown that  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio had an excellent ability to detect the presence of VO<sub>2</sub>/DO<sub>2</sub> dependency phenomenon, better than central venous oxygen saturation and blood lactate levels, in septic shock patients. Recently, Mesquida et al.<sup>20</sup> reported an association between  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio and ICU mortality in septic shock patients. In contrast, in other studies,  $\Delta\text{PCO}_2/\Delta\text{O}_2$  was unable to predict hyperlactatemia, poor lactate clearance, or VO<sub>2</sub>/DO<sub>2</sub> dependency and was not associated with outcome in septic shock or cardiac surgery patients<sup>9,21-23</sup>. Thus, the relationship between  $\Delta\text{PCO}_2/\Delta\text{O}_2$  and the presence of tissue hypoxia is controversial.

Indeed, the use of  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio as a surrogate of RQ supposes that the PCO<sub>2</sub>/CCO<sub>2</sub> relationship is quasi-linear, which may be true over the physiological range of PCO<sub>2</sub><sup>24</sup>. However, this relationship can be influenced by the degree of metabolic acidosis<sup>25</sup>, hematocrit<sup>26</sup>, and oxygen saturation (Haldane effect)<sup>8,27</sup>, and it becomes nonlinear if these factors change<sup>28</sup>. Indeed, severe metabolic acidosis, low hematocrit, and high oxygen saturation can increase PCO<sub>2</sub> for a given CCO<sub>2</sub> since less CO<sub>2</sub> is bound to hemoglobin<sup>8</sup>. Thus,  $\Delta\text{PCO}_2$  and  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio might be increased due to several factors unrelated to the blood flow and anaerobic metabolism. We found that metabolic acidosis influenced the PCO<sub>2</sub>/CCO<sub>2</sub> relationship significantly. Indeed, when the changes in pH were ignored, the PCO<sub>2</sub>/CCO<sub>2</sub> relationship was almost linear (Fig. 3). However, CCO<sub>2</sub> was not linearly related to PCO<sub>2</sub> when the changes in pH were acknowledged. In fact, PCO<sub>2</sub> and CCO<sub>2</sub> changed in opposite directions as metabolic acid was added to the blood by the hypoxic cells (Fig. 3). That is because metabolic acidosis causes plasma and red blood cell CCO<sub>2</sub> and bicarbonates to decrease<sup>29</sup>. In our study, the Haldane effect did not influence the PCO<sub>2</sub>/CCO<sub>2</sub> relationship as the latter was the same, taking into account or not for the changes in SvO<sub>2</sub> (Supplemental Digital Content 8, Figure S6).

Our findings suggest that, in situations with moderate/severe metabolic acidosis, an elevated  $\Delta\text{PCO}_2$  might not reflect only low or inadequate blood flow but could also be ascribed to modifications of the  $\text{CO}_2$ -hemoglobin dissociation curve. Our results are in line with previous studies. Indeed, Sun et al.<sup>29</sup> found that, in healthy subjects, during heavy exercise, changes in pH had a significant influence on the  $\text{PCO}_2/\text{CCO}_2$  relationship with  $\text{CCO}_2$  not linearly related to  $\text{PCO}_2$  and even varied in opposite directions after the lactic acidosis threshold was reached. However, in that study, changes in  $\text{SO}_2$  (Haldane effect) had a minor influence on the  $\text{PCO}_2/\text{CCO}_2$  relationship. Also, in septic shock patients, Mesquida et al.<sup>30</sup> observed that pH was the only best predictor of the discrepancy found between  $\Delta\text{PCO}_2/\Delta\text{O}_2$  and  $\Delta\text{CCO}_{2\text{D}}/\Delta\text{O}_2$ ; venous oxygen saturation (Haldane effect) had a minimal effect.

We observed that  $\Delta\text{PCO}_2/\Delta\text{O}_2$ , and  $\Delta\text{CCO}_{2\text{D}}/\Delta\text{O}_2$  significantly increased at  $\text{DO}_{2\text{crit}}$  and not before (Figs. 4 and 5), suggesting that these variables were able to depict the occurrence of oxygen supply dependency ( $\text{DO}_{2\text{crit}}$ ) in both IH and HH groups. The increases in these variables were mainly due to the decline in  $\Delta\text{O}_2$  in the HH group and the rise in  $\Delta\text{PCO}_2$  and  $\Delta\text{CCO}_2$  in the IH group induced by the decrease in blood flow. In contrast, in an experimental study of hemodilution model of tissue hypoxia, Dubin et al.<sup>8</sup> found that  $\Delta\text{PCO}_2/\Delta\text{O}_2$  significantly increased before the fall in  $\text{VO}_2$  and the sharp increase in RQ (measured by indirect calorimetry), and thus, it was a misleading indicator of anaerobic metabolism. The authors explained this finding by the effects of low hemoglobin on the  $\text{CO}_2$ -hemoglobin dissociation curve<sup>8</sup>. However, it is hard to compare these results together as the two tissue hypoxia models (HH and hemodilution) are different. Indeed, the effects of anemia on the  $\text{CO}_2$ -hemoglobin dissociation curve could be different from that of the low oxygen saturation (Haldane effect). Also, the magnitude of the decrease in venous oxygen saturation would be much more pronounced in the HH model, where the flow was maintained constant, than in the hemodilution model, where cardiac output increased by 126%<sup>8</sup>. Beyond  $\text{DO}_{2\text{crit}}$ , we observed a discrepancy between the evolutions of  $\Delta\text{PCO}_2/\Delta\text{O}_2$  and  $\Delta\text{CCO}_{2\text{D}}/\Delta\text{O}_2$  in both groups (Figs. 4 and 5). That might be explained by the different behavior of  $\Delta\text{PCO}_2$  and  $\Delta\text{CCO}_{2\text{D}}$  at lower  $\text{DO}_2$  values. Indeed, in the IH group, these two variables changed in opposite directions:  $\Delta\text{PCO}_2$  continued to increase, whereas  $\Delta\text{CCO}_{2\text{D}}$  fell caused by metabolic acidosis (decreases in bicarbonate levels). In the HH model,  $\Delta\text{PCO}_2$  remained unchanged, whereas  $\Delta\text{CCO}_{2\text{D}}$  decreased at lower  $\text{DO}_2$  values (Fig. 1B and Supplemental Digital Content 6, Figure S4). Therefore, below  $\text{DO}_{2\text{crit}}$  and at very low  $\text{DO}_2$  values,  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio is confounded by the changes in the  $\text{CO}_2$ -hemoglobin curve induced by metabolic acidosis, and it does not reliably reflect the oxygen supply dependency phenomenon and the activation of anaerobic metabolism, especially in the IH tissue hypoxia model. However, in clinical practice, in such cases with very low  $\text{DO}_2$ , the clinical diagnosis of tissue hypoxia would be obvious without the need for such markers.

It is worth to note that the method of calculation of the difference in  $\text{CCO}_2$  matters as the McHardy equation<sup>12</sup>, and Douglas equation<sup>13</sup> yielded different findings (Figs. 5 and Supplemental Digital Content 10, Figure S8). However, we think that the Douglas equation is much more used in research papers, and more accurate as it accounts for much more factors such as pH.

There is no reported data, in the literature, on the behavior of  $\Delta\text{CCO}_{2\text{D}}/\Delta\text{O}_2$  ratio beyond  $\text{DO}_{2\text{crit}}$  at very low  $\text{DO}_2$  values. This ratio tended to decrease in both tissue hypoxia models, even in the presence of anaerobic  $\text{CO}_2$  production. It is possible that in case of advanced tissue hypoxia with massive decreases in  $\text{VO}_2$ , the anaerobic sources of  $\text{CO}_2$  becoming much less important than the dramatically decreased aerobic ones leading to a reduction in  $\text{VCO}_2/\text{VO}_2$  ratio.

We acknowledge several limitations to our study. First, our study was a secondary analysis that is subject to inherent limitations. Second, computation of  $\text{CCO}_2$  is subject to an important potential risk of measurement errors due to the number of variables included in the equation<sup>30</sup> that might amplify during the calculation of  $\Delta\text{CCO}_{2\text{D}}$ . Nevertheless,  $\Delta\text{CCO}_{2\text{D}}/\Delta\text{O}_2$  ratio was already shown to be associated with mortality in septic shock patients<sup>9</sup>, suggesting that the influence of measurement errors might be limited.

## Conclusions

In both IH and HH regional models of tissue hypoxia,  $\Delta\text{PCO}_2/\Delta\text{O}_2$  and  $\Delta\text{CCO}_{2\text{D}}/\Delta\text{O}_2$  ratios both widened significantly only at the beginning of oxygen supply dependency. The hypoxic tissue hypoxia model yielded higher increases in  $\Delta\text{PCO}_2/\Delta\text{O}_2$  than the IH model. At advanced stages of tissue hypoxia (very low  $\text{DO}_2$ ),  $\Delta\text{PCO}_2/\Delta\text{O}_2$  did not only reflect the ongoing anaerobic metabolism, but it was confounded by the effects of metabolic acidosis on the  $\text{CO}_2$ -hemoglobin dissociation curve, and then it should be interpreted with caution. For clinical practice, in severe metabolic acidosis situations, elevated  $\Delta\text{PCO}_2$  may not reflect the degree of tissue hypoperfusion. In these cases, calculating the difference in  $\text{CCO}_2$  with the Douglas equation is advisable.

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## References

1. Vallet, B., Teboul, J. L., Cain, S. & Curtis, S. Venoarterial  $\text{CO}_2$  difference during regional ischemic or hypoxic hypoxia. *J. Appl. Physiol.* **89**, 1317–1321 (2000).
2. Gutierrez, G. A mathematical model of tissue-blood carbon dioxide exchange during hypoxia. *Am. J. Respir. Crit. Care Med.* **169**, 525–533 (2004).
3. Monnet, X. 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.* **41**, 1412–1420 (2013).
4. Mallat, J. et al. Ratios of central venous-to-arterial carbon dioxide content or tension to arteriovenous oxygen content are better markers of global anaerobic metabolism than lactate in septic shock patients. *Ann. Intensive Care* **6**, 10 (2016).
5. Mekontso-Dessap, A. et al. Combination of venoarterial  $\text{PCO}_2$  difference with arteriovenous  $\text{O}_2$  content difference to detect anaerobic metabolism in patients. *Intensive Care Med.* **28**, 272–277 (2002).

6. Mesquida, J. *et al.* Central venous-to-arterial carbon dioxide difference combined with arterial-to-venous oxygen content difference is associated with lactate evolution in the hemodynamic resuscitation process in early septic shock. *Crit. Care* **19**, 126 (2015).
7. He, H. W., Liu, D. W., Long, Y. & Wang, X. T. High central venous-to-arterial CO<sub>2</sub> difference/arterial-central venous O<sub>2</sub> difference ratio is associated with poor lactate clearance in septic patients after resuscitation. *J. Crit. Care* **31**, 76–81 (2016).
8. Dubin, A. *et al.* Venoarterial PCO<sub>2</sub>-to-arteriovenous oxygen content difference ratio is a poor surrogate for anaerobic metabolism in hemodilution: An experimental study. *Ann. Intensive Care* **7**, 65 (2017).
9. Ospina-Tascón, G. A. *et al.* Combination of arterial lactate levels and venous-arterial CO<sub>2</sub> to arterial-venous O<sub>2</sub> content difference ratio as markers of resuscitation in patients with septic shock. *Intensive Care Med.* **41**, 796–805 (2015).
10. Teboul, J. L. & Scheeren, T. Understanding the Haldane effect. *Intensive Care Med.* **43**, 91–93 (2016).
11. Cain, S. M. & Chapler, C. K. Oxygen extraction by canine hindlimb during hypoxic hypoxia. *J. Appl. Physiol.* **46**, 1023–1028 (1979).
12. Neviere, R. *et al.* Carbon dioxide rebreathing method of cardiac output measurement during acute respiratory failure in patients with chronic obstructive pulmonary disease. *Crit. Care Med.* **22**, 81–85 (1994).
13. Douglas, A. R., Jones, N. L. & Reed, J. W. Calculation of whole blood CO<sub>2</sub> content. *J. Appl. Physiol.* **65**, 473–477 (1988).
14. Samsel, R. & Schumacker, P. T. Determination of the critical O<sub>2</sub> delivery from experimental data: Sensitivity to error. *J. Appl. Physiol.* **64**, 2074–2082 (1988).
15. Cohen, P. J. The metabolic function of oxygen and biochemical lesions of hypoxia. *Anesthesiology* **37**, 148–177 (1972).
16. Ronco, J. J. *et al.* Identification of the critical oxygen delivery for anaerobic metabolism in critically ill septic and nonseptic humans. *JAMA* **270**, 1724–1730 (1993).
17. Mallat, J., Lemyze, M., Tronchon, L., Vallet, B. & Thevenin, D. Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock. *World J. Crit. Care Med.* **5**, 47–56 (2016).
18. Groeneveld, A. B., Vermeij, C. G. & Thijs, L. G. Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end-expiratory pressure in the pig. *Anesth. Analg.* **73**, 576–582 (1991).
19. Cohen, I. L., Sheikh, F. M., Perkins, R. J., Feustel, P. J. & Foster, E. D. Effect of hemorrhagic shock and reperfusion on the respiratory quotient in swine. *Crit. Care Med.* **23**, 545–552 (1995).
20. Mesquida, J. *et al.* Respiratory quotient estimations as additional prognostic tools in early septic shock. *J. Clin. Monit. Comput.* **32**, 1065–1072 (2018).
21. Muller, G. *et al.* Prognostic significance of central venous-to-arterial carbon dioxide difference during the first 24 hours of septic shock in patients with and without impaired cardiac function. *Br. J. Anaesth.* **119**, 239–248 (2017).
22. Abou-Arab, O. *et al.* The ratios of central venous to arterial carbon dioxide content and tension to arteriovenous oxygen content are not associated with overall anaerobic metabolism in postoperative cardiac surgery patients. *PLoS ONE* **13**, e0205950 (2018).
23. Fischer, M. O. *et al.* Assessment of macro- and micro-oxygenation parameters during fractional fluid infusion: A pilot study. *J. Crit. Care* **40**, 91–98 (2017).
24. Cavaliere, F. *et al.* Comparison of two methods to assess blood CO<sub>2</sub> equilibration curve in mechanically ventilated patients. *Respir. Physiol. Neurobiol.* **146**, 77–83 (2005).
25. Jakob, S. M., Groeneveld, A. B. & Teboul, J. L. Venous-arterial CO<sub>2</sub> to arterial-venous O<sub>2</sub> difference ratio as a resuscitation target in shock states?. *Intensive Care Med.* **41**, 91–93 (2015).
26. Chiarla, C. *et al.* Significance of hemoglobin concentration in determining blood CO<sub>2</sub> binding capacity in critical illness. *Respir. Physiol. Neurobiol.* **172**, 32–36 (2010).
27. Jakob, S. M., Kosonen, P., Ruokonen, E., Parviainen, I. & Takala, J. The Haldane effect—An alternative explanation for increasing gastric mucosal PCO<sub>2</sub> gradients?. *Br. J. Anaesth.* **83**, 740–746 (1999).
28. Mchardy, G. J. The relationship between the differences in pressure and content of carbon dioxide in arterial and venous blood. *Clin. Sci.* **32**, 299–309 (1967).
29. Sun, X. G., Hansen, J. E., Stringer, W. W., Ting, H. & Wasserman, K. Carbon dioxide pressure-concentration relationship in arterial and mixed venous blood during exercise. *J. Appl. Physiol.* **90**, 1798–1810 (2001).
30. Mallat, J. *et al.* Repeatability of blood gas parameters, PCO<sub>2</sub> gap, and PCO<sub>2</sub> gap to arterial-to-venous oxygen content difference in critically ill adult patients. *Medicine (Baltimore)* **94**, e415 (2015).

#### Author contributions

J.M., and B.V. designed the study. J.M. conducted statistical analyses. J.M. and B.V. participated in manuscript writing and reviewing. All authors read and approved the final manuscript.

#### Competing interests

The authors declare no competing interests.

#### Additional information

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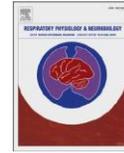
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Metformin inhibits mitochondrial respiration in the liver and other tissues in a dose-dependent manner [128, 129]. Thus, metformin intoxication induces the occurrence of anaerobic metabolism and lactate overproduction (metformin-associated lactic acidosis) by decreasing global oxygen extraction and consumption without affecting the oxygen delivery [128, 129]. In an experimental model of metformin intoxications in pigs [130], we investigated if the  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$  ratio could reflect the development of anaerobic metabolism when the oxygen delivery and cardiac output remained unchanged. We found an excellent individual correlation coefficient ( $R=0.8$ ) between the metformin-induced increases in lactate levels and the increases in  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$ . We, also, observed that  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$  detected overt lactic acidosis with an area under the ROC curve of 0.81 (95%-CI: 0.70-0.92) ( $p < 0.001$ ) with a best cut-off value of 2.0 mmHg/ml/dl, sensitivity was 0.81 (95%-CI: 0.65-0.94) and specificity 0.74 (95%-CI: 0.46-0.94).



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## Increased ratio of P[v-a]CO<sub>2</sub> to C[a-v]O<sub>2</sub> without global hypoxia: the case of metformin-induced lactic acidosis

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## ABSTRACT

The ratio of venoarterial CO<sub>2</sub> tension to arteriovenous O<sub>2</sub> content difference (P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub>) increases when lactic acidosis is due to inadequate oxygen supply (hypoxia); we aimed to verify whether it also increases when lactic acidosis develops because of mitochondrial dysfunction (dysoxia) with constant oxygen delivery. Twelve anaesthetised, mechanically ventilated pigs were intoxicated with IV metformin (4.0 to 6.4 g over 2.5 to 4.0 h). Saline and norepinephrine were used to preserve oxygen delivery. Lactate and P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> were measured every one or two hours (arterial and mixed venous blood). During metformin intoxication, lactate increased from 0.8 (0.6–0.9) to 8.5 (5.0–10.9) mmol/l (p < 0.001), even if oxygen delivery remained constant (from 352 ± 78 to 343 ± 97 ml/min, p = 0.098). P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> increased from 1.6 (1.2–1.8) to 2.3 (1.9–3.2) mmHg/ml/dl (p = 0.004). The intraclass correlation coefficient between lactate and P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> was 0.72 (p < 0.001). We conclude that P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> increases when lactic acidosis is due to dysoxia. Therefore, a high P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> may not discriminate hypoxia from dysoxia as the cause of lactic acidosis.

## 1. Introduction

Lactic acidosis (*i.e.* hyperlactataemia with metabolic acidosis) in critically ill patients is commonly attributed to hypoxia (Mizock, 1989). Based on a widely accepted model of acid-base changes during exercise (Wasserman *et al.*, 1990), when oxygen supply becomes inadequate, mitochondrial carbon dioxide production and oxygen consumption decline in parallel as they are both linked to cellular respiration (Gutierrez, 2004). The ratio between the two – the respiratory quotient, ideally measured in the cells – remains constant, provided substrates oxidised do not change. At the same time, cytoplasmic glycolysis accelerates and hyperlactataemia develops together with metabolic acidosis (Cilley *et al.*, 1991; Ronco *et al.*, 1993). As bicarbonate buffers hydrogen ions in excess (Beaver *et al.*, 1986), some extra-mitochondrial

or anaerobic carbon dioxide is released (Gutierrez, 2004), reaches the lungs and is exhaled together with carbon dioxide of mitochondrial or aerobic origin. Several details of this model remain controversial (Péronnet and Aguilaniu, 2006). Nonetheless, there is general agreement that the onset of lactic acidosis due to hypoxia is accompanied by an acute increase in the respiratory exchange ratio, *i.e.* the ratio between whole-body carbon dioxide output and oxygen uptake measured at the airway with indirect calorimetry (Cohen *et al.*, 1995; Groeneweld *et al.*, 1991).

According to the Fick principle, the respiratory exchange ratio can also be measured without indirect calorimetry, as the ratio between mixed-venous-to-arterial carbon dioxide content difference (C[v-a]CO<sub>2</sub>) (times cardiac output) and arterial-to-mixed-venous oxygen content difference (C[a-v]O<sub>2</sub>) (times cardiac output). As long as blood carbon

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dioxide tension reflects content, it can also be estimated from the ratio of mixed-venous-to-arterial carbon dioxide tension difference ( $P[v-a]CO_2$ ) to  $C[a-v]O_2$ . In line with this model, a high  $P[v-a]CO_2/C[a-v]O_2$  may signal hypoxia (Mekontso-Dessap et al., 2002; Monnet et al., 2013; Mesquida et al., 2015; Mallat et al., 2016a; Ospina-Tascón and Madriñán, 2019), and a normal  $P[v-a]CO_2/C[a-v]O_2$  might be a reasonable target of resuscitation of critically ill patients (Du et al., 2015; Su et al., 2018).

Lactic acidosis can also occur without hypoxia (James et al., 1999; Garcia-Alvarez et al., 2014). For example, when catecholamines stimulate glycolysis and lactate production (Levy et al., 2005); when fulminant liver failure limits lactate clearance (Murphy et al., 2001); or when mitochondria become unable to use oxygen. This latter condition, known as *dysoxia*, is common to several critical diseases including sepsis (Fink, 2001; Brealey et al., 2002), thiamine deficiency (Depeint et al., 2006), metformin intoxication (Protti et al., 2010, 2012; Protti et al., 2012b), and linezolid intoxication (Protti et al., 2016).

Distinguishing hypoxia from dysoxia is important because the treatment of lactic acidosis should be based on its cause. If this is hypoxia, then arterial oxygen content and/or blood flow should be augmented (Rivers et al., 2001; Protti et al., 2018). Otherwise, there is no physiological rationale to do so. Unmotivated and excessive fluid resuscitation, transfusion of red blood cells and/or use of inotropes may cause fluid overload, pulmonary oedema, intra-abdominal hypertension, cardiac arrhythmias, myocardial ischaemia and even increase mortality (Hayes et al., 1994; Andreis and Singer, 2016; Marik et al., 2017; Hernández et al., 2019).

Whether  $P[v-a]CO_2/C[a-v]O_2$  discriminates hypoxia from dysoxia is unknown. In animals intoxicated with cyanide or rotenone, two well-known mitochondrial blockers, the respiratory exchange ratio measured with indirect calorimetry increases (Breen et al., 1995; Karlsson et al., 2016). We therefore hypothesised that  $P[v-a]CO_2/C[a-v]O_2$  increases not only with hypoxia but also with dysoxia (Ospina-Tascón et al., 2016a). This study aimed to verify this hypothesis by monitoring  $P[v-a]CO_2/C[a-v]O_2$  in a preclinical model of metformin-induced lactic acidosis, a syndrome that is mainly due to dysoxia (Protti et al., 2010, 2012; Protti et al., 2012b).

In a first set of experiments (metformin intoxication), we analysed  $P[v-a]CO_2/C[a-v]O_2$  in a group of healthy pigs infused with a large dose of metformin. In a second set of experiments (chloride intoxication), we aimed to exclude the possible confounding role of hyperchloraemic acidosis which developed during metformin intoxication, possibly as a result of resuscitation with chloride-rich normal saline.

## 2. Materials and methods

This is a secondary sub-analysis of a series of experiments showing that mitochondrial dysfunction plays a key role in lactic acidosis during metformin intoxication. It was stimulated by recent works suggesting that a high  $P[v-a]CO_2/C[a-v]O_2$  in critically ill patients signals hypoxia or the need to increase oxygen delivery (Mekontso-Dessap et al., 2002; Monnet et al., 2013; Mesquida et al., 2015; Mallat et al., 2016a; Ospina-Tascón and Madriñán, 2019). As detailed in *Supplemental Digital File 1*, some of those experiments were included in this present analysis and some others were not. The original studies complied with international recommendations (Institute of Laboratory Animal Resources, 1996) and were approved by the Italian Ministry of Health (protocol number 212).

### 2.1. Metformin intoxication

Twelve healthy pigs ( $21 \pm 2$  kg) were maintained under general anaesthesia and paralysis with IV propofol, medetomidine, and pancuronium bromide. Surgery included tracheostomy, urinary bladder catheterisation, and cannulation of the carotid artery, internal jugular vein, and pulmonary artery. Ventilation was delivered with a tidal

volume of 10–15 ml/kg, respiratory rate of 12–18 breaths/min and fraction of inspired oxygen of 0.5 (Engstrom Carestation; GE Healthcare; Madison, WI, USA).

After a few hours of recovery from surgery and baseline recordings (time 0), animals received 4.0 to 6.4 g of metformin hydrochloride (Sigma-Aldrich; St. Louis, MO, USA) as an IV infusion over 2.5 to 4.0 h (1.6 g of metformin in 20 ml of saline per hour). Anaesthesia and ventilation were kept constant. Normal saline, with 154 mmol/l of sodium and chloride, was infused to match urinary output. Hypotension (mean arterial pressure <60 mmHg) was treated with additional saline and IV norepinephrine. Hypothermia (body core temperature <37 °C) was treated with active warming.

Variables of interest were recorded at the start of the infusion of metformin (time 0) and every one or two hours thereafter, for four hours in total. Arterial and mixed venous blood gas analyses, including sodium, potassium, and lactate, were measured in all animals (GEM 3000, Instrumentation Laboratory; Milan, Italy or ABL 800 FLEX, Radiometer; Copenhagen, Denmark). Once we purchased an indirect calorimeter (COVX Metabolic Module, GE Healthcare) and the appropriate blood gas analyser (ABL 800 FLEX, Radiometer), we started measuring whole-body carbon dioxide output and oxygen uptake (in the last eight animals) and arterial chloride level (in the last six animals).

In line with other authors (Mekontso-Dessap, 2002; Monnet, 2013; Mesquida, 2015; Mallat, 2016; Mesquida, 2019),  $P[v-a]CO_2$  was calculated using the following standard formulas.  $P[v-a]CO_2$  was the difference between mixed venous and arterial carbon dioxide tensions. Arterial oxygen content was calculated as  $CaO_2$  (ml/dl) =  $1.34$  (ml/g)  $\times$  Hb (g/dl)  $\times$  SaO<sub>2</sub> +  $0.003$  [ml/(dl  $\times$  mmHg)]  $\times$  PaO<sub>2</sub> (mmHg), where SaO<sub>2</sub> is the oxygen saturation and PaO<sub>2</sub> the oxygen tension of arterial blood. Mixed venous oxygen content was calculated as  $CvO_2$  (ml/dl) =  $1.34$  (ml/g)  $\times$  Hb (g/dl)  $\times$  SvO<sub>2</sub> +  $0.003$  [ml/(dl  $\times$  mmHg)]  $\times$  PvO<sub>2</sub> (mmHg), where SvO<sub>2</sub> is the oxygen saturation and PvO<sub>2</sub> the oxygen tension of mixed venous blood (Hall and Hall, 2020). In both formulas, Hb is the mean haemoglobin concentration of arterial and mixed venous blood taken simultaneously.  $C[a-v]O_2$  (ml/dl) was calculated as  $CaO_2 - CvO_2$ . Cardiac output was measured in triplicate with thermodilution technique. Oxygen delivery was calculated as  $DO_2$  (ml/min) =  $CaO_2$  (ml/dl)  $\times$  CO (l/min)  $\times$  10. The respiratory exchange ratio was the ratio of carbon dioxide output to oxygen uptake measured with indirect calorimetry. The arteriovenous CO<sub>2</sub> content difference ( $C[v-a]CO_2$ ) was computed as the ratio between carbon dioxide output (indirect calorimetry) and cardiac output (thermodilution technique). By comparing it with the corresponding  $P[v-a]CO_2$  we estimated the carbon dioxide-binding capacity of blood.

To clarify the origin of metabolic acidosis in animals infused with metformin, we used the physicochemical or Stewart approach (Stewart, 1983). According to it, metabolic acidosis is either due to the accumulation of weak non-volatile acids (in practice albumin and phosphate) or the reduction of the strong ion difference. The strong ion difference can be estimated as  $[SID] = [sodium] + [potassium] - [chloride] - [lactate]$  where square brackets refer to concentrations. Assuming constant weak non-volatile acids (we could not measure them), metabolic acidosis results from a reduction of the strong ion difference. In pure lactic acidosis, this is equivalent to the increase in [lactate]; [sodium], [potassium] and [chloride] do not change.

Other details on animal preparation and study design can be found in ref. 24.

### 2.2. Chloride intoxication

Animals infused with metformin hydrochloride and resuscitated with (chloride-rich) saline developed not only lactic but also hyperchloraemic acidosis (Langer et al., 2012). To isolate the impact of this disorder on  $P[v-a]CO_2/C[a-v]O_2$ , we then intoxicated six other animals with hydrochloric acid, as reported in *Supplemental Digital File 1*.

### 2.3. Statistical analysis

The sample size of this retrospective analysis was not planned; it was based on the number of past experiments fulfilling the inclusion and exclusion criteria reported in *Supplemental Digital File 1*.

Data are reported as mean  $\pm$  SD or median and interquartile range depending on their distribution. Changes over time in variables of interest were analysed with paired *t*-test, one-way repeated measures analysis of variance (RM ANOVA) or one-way RM ANOVA on ranks. Post-hoc multiple comparisons against baseline recording (time 0) were adjusted with Bonferroni or Dunn's methods. The increase of arterial chloride from the start to the end of experiments was compared between animals intoxicated with metformin or hydrochloric acid with unpaired *t*-test.

P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> and arterial lactate were measured several times on the same animals. We then studied the relationship between these two variables with univariable linear regressions considering animals one by one, and with two-variable linear regression analysis considering all data together, and adding subjects to the model as dummy variables (Bland and Altman, 1994). Results are expressed as individual (R) and intraclass correlation coefficients, respectively.

The ability of P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> to detect the onset of overt lactic acidosis (arterial lactate  $\geq$ 4 mmol/l) was studied with the Receiver Operating Characteristic (ROC) curve analysis considering all data from all animals at all time points. The value with the highest sensitivity and specificity (Youden's index) was considered the best cut-off point. The area under the ROC curve, sensitivity, specificity, negative, and positive predictive values and diagnostic accuracy are reported with 95%-confidence intervals calculated with bootstrapping as proposed by Liu et al. (2005). The overall statistical significance of the ROC curve was calculated with two-variable logistic regression analysis: P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> was the predictor, animals were included as dummy variables to account for the repeated measures design of the study, and overt lactic acidosis was the outcome.

Statistical analysis was performed with SigmaPlot 11.0 (Jandel Scientific, San Jose, CA) and JMP Pro 14 (SAS Inc., Cary, NC). All tests were two-tailed. A *p*-value  $<$ 0.05 was considered statistically significant.

## 3. Results

### 3.1. Metformin intoxication

One animal was infused with 4.0 g of metformin over 2.5 h; the other

eleven were infused with 6.0 to 6.4 g of metformin over 3.75 to 4.0 h. Variables of interest, including the P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> and arterial lactate, were recorded every hour from eleven animals (although two mixed venous blood gas analyses turned out to be missing) and every two hours from one animal.

Overt lactic acidosis developed in all animals even if oxygen delivery remained quite constant (Fig. 1). Mixed venous oxygen saturation increased and body core temperature declined over time (Fig. 1 and Table 1).

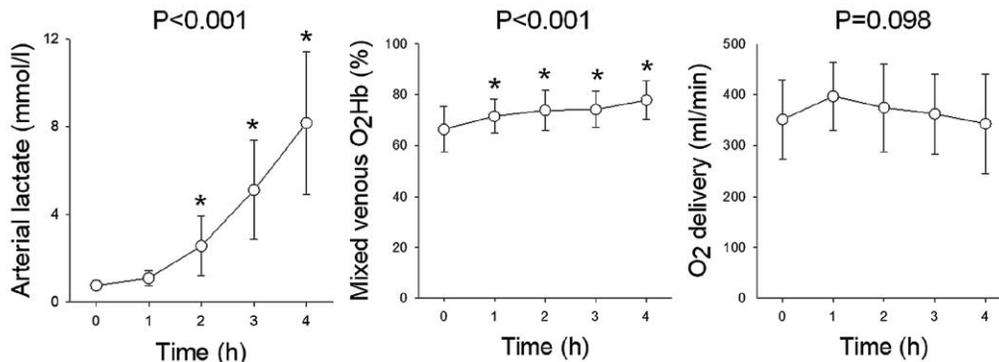
In eight animals studied with indirect calorimetry, carbon dioxide output decreased less than oxygen uptake so that the respiratory exchange ratio increased (Fig. 2).

In the whole study population, P[v-a]CO<sub>2</sub> slightly increased, C[a-v]O<sub>2</sub> decreased, and P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> accordingly increased (Table 1 and Fig. 3). As shown in *Supplemental Table 1 (Supplemental Digital File 1)*, carbon dioxide-binding capacity of blood tended to decline over time: for each ml of carbon dioxide added to 100 ml of blood, P[v-a]CO<sub>2</sub> increased by 2.1 (1.8–2.6) mmHg at the start and by 3.0 (2.5–3.3) mmHg at the end of the experiments (*p* = 0.120).

By the end of the study, mean arterial pressure was lower than 60 (but still higher than 50) mmHg in four animals, including three treated with norepinephrine. Results were similar when analyses were restricted to the eight animals without hypotension at the end of the study (final mean arterial pressure  $\geq$ 60 mmHg). Even there, P[v-a]CO<sub>2</sub> tended to increase (from 7.2  $\pm$  1.6 to 8.8  $\pm$  3.3 mmHg; *p* = 0.218); C[a-v]O<sub>2</sub> decreased (from 4.7  $\pm$  1.1 to 3.7  $\pm$  0.9 ml/dl; *p* = 0.002); and P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> increased [from 1.7 (1.1–1.8) to 2.1 (1.8–3.2) mmHg/ml/dl; *p* = 0.012]. Details on these analyses are reported in *Supplemental Table 2 (Supplemental Digital File 1)*.

The relationship between P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> and arterial lactate level in each animal is described in Fig. 4 and in *Supplemental Fig. 1 (Supplemental Digital File 1)*. P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> usually rose with lactate: median individual correlation coefficient (R) was 0.80 (0.37–0.90). The rate of increase in P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> per each mmol/l increase in arterial lactate level ( $\beta$ ) ranged from -0.01 up to 0.24 mmHg/ml/dl; on average, it was 0.11 (0.05–0.15) mmHg/ml/dl. The intraclass correlation coefficient between P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> and arterial lactate level within all animals was 0.72 (*p*  $<$  0.001).

As shown in *Supplemental Fig. 2 (Supplemental Digital File 1)*, P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> detected overt lactic acidosis with an area under the ROC curve of 0.81 (95%-CI: 0.70–0.92) (*p*  $<$  0.001). With a best cut-off value of 2.0 mmHg/ml/dl, sensitivity was 0.81 (95%-CI: 0.65–0.94), specificity 0.74 (95%-CI: 0.46–0.94), negative predictive value 0.87 (95%-

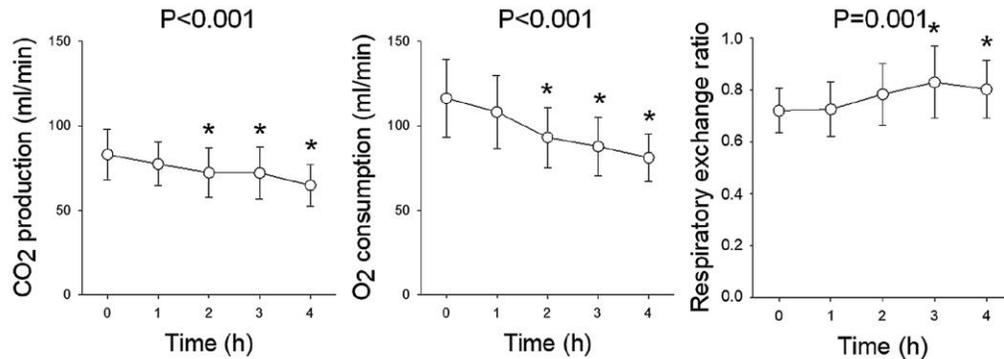


**Fig. 1.** Impact of metformin intoxication on arterial lactate, mixed venous oxygen saturation and systemic oxygen delivery. Twelve animals were studied during continuous IV infusion of metformin. Arterial lactate level, mixed venous oxygen saturation (O<sub>2</sub>Hb) and systemic oxygen (O<sub>2</sub>) delivery were recorded before the start of infusion (time 0) and every one or two hours thereafter, for four hours in total. Systemic O<sub>2</sub> delivery was not available for one animal. *P* values refer to RM ANOVA or RM ANOVA on ranks. \* Adjusted *p*  $<$  0.05 versus time 0 at post-hoc comparisons. Arterial lactate level was not normally distributed: median (IQR) values are reported in Table 1.

**Table 1**  
Impact of metformin infusion on arterial blood gas analysis, mixed venous blood gas analysis and haemodynamics.

Time (h)	0	1	2	3	4	p
<i>Arterial blood gas analysis</i>						
pHa	7.56 ± 0.07	7.56 ± 0.06	7.50 ± 0.04	7.40 ± 0.08*	7.30 ± 0.12*	<0.001
PaCO <sub>2</sub> (mmHg)	33 ± 4	31 ± 5*	30 ± 4*	30 ± 5*	29 ± 5*	<0.001
PaO <sub>2</sub> (mmHg)	212 ± 30	223 ± 33	214 ± 32	211 ± 39	233 ± 32	0.046
HCO <sub>3</sub> <sup>-</sup> -a (mmol/l)	30 ± 2	27 ± 2	23 ± 3*	19 ± 3*	15 ± 4*	<0.001
BEa (mmol/l)	7 ± 3	5 ± 2	1 ± 3*	-5 ± 4*	-11 ± 6*	<0.001
Haemoglobin (g/dl)	9.6 ± 1.2	9.9 ± 1.2	10.3 ± 1.4*	10.5 ± 1.4*	10.7 ± 0.3*	<0.001
SaO <sub>2</sub> (%)	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1	0.463
CaO <sub>2</sub> (ml/dl)	13.5 ± 1.6	14.2 ± 1.8	14.3 ± 1.6*	15.1 ± 2.0*	15.1 ± 1.9*	<0.001
<i>Mixed venous blood gas analysis</i>						
pHv	7.51 ± 0.06	7.50 ± 0.05	7.45 ± 0.04	7.35 ± 0.07*	7.24 ± 0.12*	<0.001
PvCO <sub>2</sub> (mmHg)	40 ± 4	39 ± 5	37 ± 4*	39 ± 4*	38 ± 6*	0.006
PvO <sub>2</sub> (mmHg)	38 ± 5	42 ± 5	45 ± 6*	49 ± 7*	57 ± 10*	<0.001
HCO <sub>3</sub> <sup>-</sup> -v (mmol/l)	32 ± 2	30 ± 2	26 ± 2*	22 ± 3*	16 ± 4*	<0.001
BEv (mmol/l)	8 ± 3	6 ± 2	2 ± 3*	-3 ± 4*	-11 ± 7*	<0.001
Haemoglobin (g/dl)	9.5 ± 1.2	10.1 ± 1.4*	10.2 ± 1.2*	10.9 ± 1.5*	10.7 ± 1.5*	<0.001
SvO <sub>2</sub> (%)	66 ± 8	71 ± 7*	74 ± 8*	74 ± 7*	78 ± 8*	<0.001
CvO <sub>2</sub> (ml/dl)	8.8 ± 2.0	10.0 ± 1.9*	10.4 ± 1.9*	11.1 ± 2.4*	11.6 ± 2.4*	<0.001
<i>Haemodynamics</i>						
Heart rate (bpm)	101 ± 20	106 ± 17	113 ± 21	117 ± 24	112 ± 26	0.213
Mean arterial pressure (mmHg)	98 ± 14	83 ± 14*	77 ± 10*	67 ± 8*	62 ± 11*	<0.001
Cardiac output (l/min)	2.6 ± 0.6	2.8 ± 0.5	2.7 ± 0.7	2.4 ± 0.4	2.3 ± 0.4	0.002
Oxygen delivery (ml/min)	352 ± 78	397 ± 68	374 ± 86	362 ± 78	343 ± 97	0.098
Urinary output (ml/h)	104 ± 80	139 ± 90	293 ± 144*	431 ± 148*	445 ± 197*	<0.001
Saline infused (ml/h)	50 (50–95)	80 (50–100)	175 (100–250)	335 (194–465)*	480 (373–600)*	<0.001
With additional saline (n)	0/12	1/12	1/12	1/12	2/12	–
Norepinephrine infusion (µg/kg/min)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.2)	0.017
With norepinephrine (n)	0/12	0/12	0/12	0/12	3/12	–
Body core temperature (°C)	38.6 (37.0–39.2)	38.3 (37.2–39.5)	38.0 (36.9–38.6)	37.8 (36.6–38.1)*	37.2 (36.4–37.8)*	<0.001
<i>Main outcomes</i>						
Lactate (mmol/l)	0.8 (0.6–0.9)	1.2 (0.8–1.3)	2.4 (1.4–3.3)*	5.4 (3.4–6.2)*	8.5 (5.0–10.9)*	<0.001
P[v-a]CO <sub>2</sub> (mmHg)	7.0 ± 1.5	7.4 ± 1.6	7.1 ± 1.5	7.8 ± 1.4	8.7 ± 2.8	0.097
C[a-v]O <sub>2</sub> (ml/dl)	4.7 ± 0.9	4.2 ± 0.8	3.9 ± 1.0*	4.0 ± 0.7*	3.5 ± 0.8*	<0.001
P[v-a]CO <sub>2</sub> /C[a-v]O <sub>2</sub> (mmHg/ml/dl)	1.6 (1.1–1.8)	1.7 (1.3–2.4)	2.0 (1.4–2.3)	2.1 (1.6–2.1)	2.3 (1.9–3.2)*	0.004

Twelve animals were studied during continuous IV infusion of metformin. Physiological variables were recorded at the start of infusion (time 0) and every one or two hours thereafter, for four hours in total. Cardiac output and oxygen delivery were measured in all animals but one (early malfunction of the pulmonary artery catheter). Mixed venous blood gas analysis, and all related variables including P[v-a]CO<sub>2</sub>, C[a-v]O<sub>2</sub> and P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub>, were not available in one animal at time 1, one animal at time 2 and two animals at time 3. BE: base excess; CaO<sub>2</sub>: arterial blood oxygen content; CvO<sub>2</sub>: mixed venous blood oxygen content. With additional saline (n): animals given saline to treat hypotension, on top of that given to match urinary output. P values refer to RM ANOVA or RM ANOVA on ranks. \* Adjusted P < 0.05 versus time 0 at post-hoc comparisons.



**Fig. 2.** Impact of metformin intoxication on whole-body carbon dioxide output and oxygen uptake.

Eight animals were studied with indirect calorimetry during continuous IV infusion of metformin. Whole-body carbon dioxide (CO<sub>2</sub>) output and oxygen (O<sub>2</sub>) uptake were recorded before the start of infusion (time 0) and every one or two hours thereafter, for four hours in total. Respiratory exchange ratio (RER) was the ratio of CO<sub>2</sub> production to O<sub>2</sub> consumption. Dots are means; bars are standard deviations. P values refer to RM ANOVA or RM ANOVA on ranks. \* Adjusted P < 0.05 versus time 0 at post-hoc comparisons.

CI: 0.81–1.00), positive predictive value 0.65 (95%-CI: 0.46–0.91), and diagnostic accuracy 0.77 (95%-CI: 0.63–0.89).

### 3.2. Chloride intoxication

In the last six animals intoxicated with metformin (those with known arterial chloride levels), we estimated the strong ion difference to better

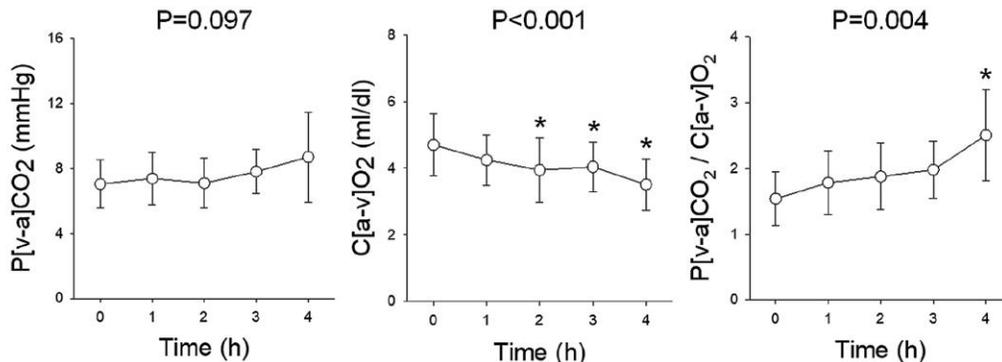


Fig. 3. Impact of metformin intoxication on carbon dioxide and oxygen-derived variables.

Twelve animals were studied during continuous IV infusion of metformin. Mixed-venous-to-arterial carbon dioxide tension difference ( $P[v-a]CO_2$ ) and arterial-to-mixed-venous oxygen content difference ( $C[a-v]O_2$ ) were recorded before the start of infusion (time 0) and every one or two hours thereafter, for four hours in total.  $P[v-a]CO_2/C[a-v]O_2$  was the ratio between the two; it was measured in mmHg/ml/dl. Dots are means; bars are standard deviations. P values refer to RM ANOVA or RM ANOVA on ranks. \* Adjusted  $p < 0.05$  versus time 0 at post-hoc comparisons.  $P[v-a]CO_2/C[a-v]O_2$  was not normally distributed; median (IQR) values are reported in Table 1.

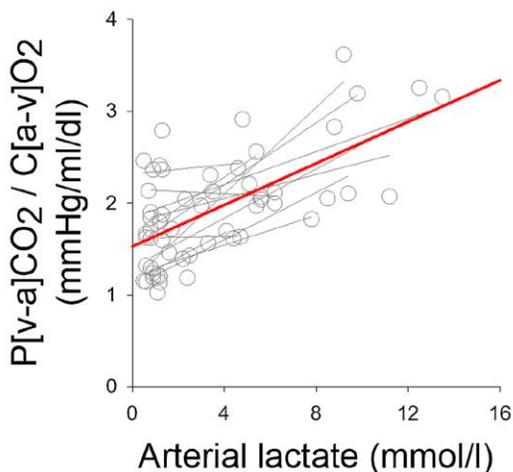


Fig. 4. Relationship between  $P[v-a]CO_2/C[a-v]O_2$  and arterial lactate level in each animal.

Twelve animals were studied during continuous IV infusion of metformin. Mixed-venous-to-arterial carbon dioxide tension difference ( $P[v-a]CO_2$ ) and arterial-to-mixed-venous oxygen content difference ( $C[a-v]O_2$ ) were recorded before the start of infusion (time 0) and every one or two hours thereafter, for four hours in total.  $P[v-a]CO_2/C[a-v]O_2$  was the ratio between the two. Dots refer to simultaneous recordings of arterial lactate and  $P[v-a]CO_2/C[a-v]O_2$ . Lines describe the (linear) relationship between data recorded from single animals.

clarify the origin of their metabolic acidosis. As shown in Table 2, the strong ion difference decreased, so that metabolic acidosis developed, not only because lactate but also chloride level increased (by  $10 \pm 3$  mEq/l). Sodium and potassium levels did not change. This hyperchloraemic acidosis, possibly due to the infusion of large volumes of saline (Table 1), confounded the association between  $P[v-a]CO_2/C[a-v]O_2$  and arterial lactate.

We then infused six other animals with hydrochloric acid over four hours. Their chloride level increased by  $10 \pm 3$  mmol/l similarly to

animals intoxicated with metformin ( $p > 0.999$ ). Sodium and potassium levels did not change. Arterial lactate level remained constantly low ( $< 1$  mmol/l).  $P[v-a]CO_2$  increased (from  $7.1 \pm 1.1$  to  $10.4 \pm 1.1$  mmHg;  $p = 0.005$ ), but in line with  $C[a-v]O_2$  (from  $5.3 \pm 0.7$  to  $6.6 \pm 1.0$  ml/dl;  $p = 0.039$ ); accordingly,  $P[v-a]CO_2/C[a-v]O_2$  did not significantly change over time [from 1.3 (1.2–1.5) to 1.5 (1.4–1.9) mmHg/ml/dl;  $p = 0.209$ ] (Supplemental Table 3 in Supplemental Digital File 1).

Based on these findings, we conclude that hyperchloraemic acidosis *per se* did not explain the larger increase in  $P[v-a]CO_2/C[a-v]O_2$  noted during metformin intoxication [from 1.6 (1.2–1.8) to 2.3 (1.9–3.2) mmHg/ml/dl;  $p = 0.004$ ].

#### 4. Discussion

Our retrospective analysis suggests that  $P[v-a]CO_2/C[a-v]O_2$  increases also when lactic acidosis is primarily due to dysoxia.

Before discussing the results of this study, it is important to comment on its design. First, we aimed to verify whether  $P[v-a]CO_2/C[a-v]O_2$  increases during dysoxia and animals with metformin-induced lactic acidosis seemed a valid model for this purpose. At a very high dose, metformin is a mitochondrial poison similar to cyanide. *In vitro*, it dose-dependently inhibits mitochondrial respiration of cells incubated in room air (Protti et al., 2012a; El-Mir et al., 2000; Owen et al., 2000). Herein, in intact living pigs, it increased mixed venous oxygen saturation and decreased body core temperature despite active warming, as if it diminished whole-body oxygen extraction and metabolism. By the end of our original experiments (which lasted nine hours in total, as reported in Supplemental Digital File 1), intoxicated pigs had generalised mitochondrial dysfunction and elevated lactate-to-pyruvate ratio in blood (Protti et al., 2012a). Humans with lactic acidosis due to accidental metformin accumulation equally have abnormally low whole-body oxygen consumption, high mixed or central venous oxygen saturation, low body core temperature and dysfunctional mitochondria in their circulating platelets (Protti et al., 2010; Protti et al., 2012b). Second, to minimise the risk of concomitant tissue hypoxia, we focused only on the initial part of our original experiments. Four hours after their start, average systemic oxygen delivery was normal and mean arterial pressure generally higher than 60 mmHg. When we further restricted our analysis to those animals that never developed hypotension, the results did not differ from the overall population (Supplemental Table 2 in Supplemental Digital File 1). Third, metformin-induced lactic acidosis is very rare but dysoxia is probably not, especially in critical illness, when

**Table 2**  
Impact of metformin infusion on arterial electrolytes and the strong ion difference.

Time (h)	0	1	2	3	4	p
Sodium (mmol/l)	139 ± 3	139 ± 3	139 ± 3	139 ± 4	140 ± 4	0.351
Potassium (mmol/l)	3.8 ± 0.3	3.8 ± 0.7	3.7 ± 0.9	3.8 ± 1.2	3.8 ± 1.0	0.967
Chloride (mmol/l)	105 ± 3	106 ± 4	110 ± 4*	112 ± 5*	115 ± 5*	<0.001
Lactate (mmol/l)	0.9 (0.6–1.1)	1.1 (0.7–1.2)	1.5 (1.2–2.3)	3.4 (2.4–4.6)*	5.1 (4.6–8.2)*	<0.001
Strong ion difference (mmol/l)	37 ± 6	35 ± 5	31 ± 5*	28 ± 6*	23 ± 6*	<0.001

Arterial chloride was measured and the strong ion difference estimated in six animals during continuous IV infusion of metformin. Arterial electrolytes were recorded at the start of infusion (time 0) and every hour thereafter, for four hours in total. The strong ion difference was estimated as the difference between strong cations (sodium and potassium) and anions (chloride and lactate). P values refer to RM ANOVA or RM ANOVA on ranks. \* Adjusted P < 0.05 versus time 0 at post-hoc comparisons.

discriminating hypoxia from dysoxia may prevent harm from overzealous resuscitation.

During metformin intoxication, the respiratory exchange ratio increased. When metabolic acidosis develops, bicarbonate combines with hydrogen ions and forms dissolved carbon dioxide (Beaver et al., 1986), a volatile acid that can be readily eliminated from the body. This additional source of carbon dioxide produces all the changes that define an “unsteady” state (Fahri and Rahn, 1960), such as occurs with heavy exercise: carbon dioxide output exceeds mitochondrial production; whole-body carbon dioxide stores decline; the respiratory exchange ratio exceeds the respiratory quotient by an amount equal to the ratio of extra-mitochondrial carbon dioxide production to oxygen consumption.

P[v-a]CO<sub>2</sub> depends on the venoarterial carbon dioxide content difference and carbon dioxide-binding capacity of the blood. According to the Fick principle, the former reflects the balance between total carbon dioxide production and blood flow. The latter is mainly influenced by pH, carbon dioxide tension, haemoglobin concentration and oxygenation, and temperature (Christiansen et al., 1914; Austin et al., 1963; Mallat et al., 2016b). An elevated P[v-a]CO<sub>2</sub> can indicate hypoperfusion, with supranormal addition of carbon dioxide per unit of blood (Weil et al., 1986; Adrogue et al., 1989; Cuschieri et al., 2005). But this is not always the case. For example, during hydrochloric acid infusion, P[v-a]CO<sub>2</sub> became larger even if the cardiac output was constant, probably because bicarbonate buffering increased with acidosis, metabolic rate increased with the discharge of endogenous catecholamines (as suggested by spontaneous arterial hypertension [Malm et al., 1966]), and the capacity of blood to combine with carbon dioxide diminished. Therefore, an elevated P[v-a]CO<sub>2</sub> is not specific for hypoxia due to hypoperfusion.

Compared to P[v-a]CO<sub>2</sub>, P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> depends less on cardiac output and metabolic rate. Under the assumption of constant respiratory quotient and carbon dioxide-binding capacity of the blood, changes in blood flow and cellular respiration affect the venoarterial carbon dioxide content difference, the P[v-a]CO<sub>2</sub> and the C[a-v]O<sub>2</sub> to a similar extent. Therefore, an increased P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> may be more specific for hypoxia than P[v-a]CO<sub>2</sub> (Mekontso-Dessap et al., 2002; Monnet et al., 2013; Mesquida et al., 2015; Mallat et al., 2016a). For example, in isolated dog hindlimbs where hypoxia was induced by lowering arterial oxygenation with constant blood flow, P[v-a]CO<sub>2</sub> did not increase although oxygen consumption and C[a-v]O<sub>2</sub> progressively diminished (Vallet et al., 2000). Even if not explicitly reported in the manuscript, P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> most likely increased as a result of the isolated decrease in the denominator. Herein we show that similar changes occur during dysoxia. Overt lactic acidosis induced by metformin was generally associated with a P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> higher than 2.0 mmHg/ml/dl. In critically ill patients, values above 1.6–1.8 mmHg/ml/dl have been associated with global oxygen supply dependency (a sign of hypoxia) (Monnet et al., 2013; Mallat et al., 2016a; Du et al., 2015), or with impaired muscle oxygen use (a possible sign of dysoxia) (Mesquida et al., 2019). Therefore, P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> can increase independently of hypoperfusion, during either hypoxia or dysoxia, when bicarbonate contributes to buffering (lactic) acidosis. This finding might explain why a high P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> does not

always predict oxygen-supply dependency (Abou-Arab et al., 2018; Mahendran et al., 2020).

The decrease in C[a-v]O<sub>2</sub> made a fundamental contribution to elevating the P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> during metformin intoxication. For any given increase in the rate of extra-mitochondrial carbon dioxide production and the corresponding fraction of P[v-a]CO<sub>2</sub>, P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> will increase more or less depending on C[a-v]O<sub>2</sub>. If this becomes smaller, as during dysoxia (when oxygen extraction is low), P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> will markedly increase. If it becomes larger, as with hydrochloric acid infusion or hypoxia (when oxygen extraction is high), it will not. Based on this model, P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> could be even more sensitive to dysoxia than to hypoxia.

Some of the limitations of this study deserve a comment. First, this was a secondary analysis of a series of experiments with another aim. Because of that, not all potential confounders were strictly controlled. For example, haemoglobin concentration increased over time by 10% possibly as a result of polyuria. As haemoglobin can bind carbon dioxide, this change may have mitigated the increase in P[v-a]CO<sub>2</sub> and P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> (Chiara et al., 2010). More importantly, cardiac output declined by 10–15% and arterial blood pressure even more, as for vasodilation. Therefore, we cannot exclude some regional hypoperfusion, which may have contributed to increasing P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> (Ospina-Tascón et al., 2016a, b). Our results thus need to be confirmed in a more controlled setting. Second, experiments with hydrochloric acid (where the strong difference decreased by 9 mmol/l) were not designed to assess the consequences of acidosis as severe as that finally obtained with metformin (where the strong ion difference decreased by 14 mmol/l). The larger increase in P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> in animals intoxicated with metformin may have merely reflected more severe metabolic acidosis. However, C[a-v]O<sub>2</sub> became smaller with metformin and larger with hydrochloric acid. Therefore, we hypothesise that even with an equal degree of acidosis, P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> would have increased more with metformin than with hydrochloric acid. This hypothesis needs to be verified.

In conclusion, P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> increases during metformin-induced lactic acidosis, a syndrome that is primarily due to dysoxia. A high P[v-a]CO<sub>2</sub>/C[a-v]O<sub>2</sub> in a critically ill patient may not signal hypoxia and should not automatically trigger efforts to increase systemic oxygen delivery.

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#### Author contributions

Davide T Andreis, Jihad Mallat and Alessandro Protti conceived the study and designed the experiments. Davide T Andreis, Jihad Mallat, Mauro Tettamanti, Carlo Chiarla, Ivo Giovannini, Stefano Gatti and Alessandro Protti acquired, analysed, and interpreted experimental data. Davide T Andreis and Alessandro Protti drafted the paper. Jihad Mallat, Mauro Tettamanti, Carlo Chiarla, Ivo Giovannini, and Stefano

Gatti revised the paper for important intellectual content.

#### Declaration of Competing Interest

None.

#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.resp.2020.103586>.

#### References

- Abou-Arab, O., Braik, R., Huette, P., Bouhemad, B., Lorne, E., Guinot, P.G., 2018. The ratios of central venous to arterial carbon dioxide content and tension to arteriovenous oxygen content are not associated with overall anaerobic metabolism in postoperative cardiac surgery patients. *PLoS One* 13, e0205950.
- Adrogué, J.J., Rashad, M.N., Gorin, A.B., Yacoub, J., Madras, N.E., 1989. Assessing acid-base status in circulatory failure. Differences between arterial and central venous blood. *N. Engl. J. Med.* 320, 1312–1326.
- Andreis, D.T., Singer, M., 2016. Catecholamines for inflammatory shock: a Jekyll-and-Hyde conundrum. *Intensive Care Med.* 42, 1387–1397.
- Austin, W.H., Lacombe, E., Rand, P.W., Chatterjee, M., 1963. Solubility of carbon dioxide in serum from 15 to 38 °C. *J. Appl. Physiol.* 18, 301–304.
- Beaver, W.L., Wasserman, K., Whipp, B.J., 1986. Bicarbonate buffering of lactic acid generated during exercise. *J. Appl. Physiol.* 60, 472–478.
- Bland, J.M., Altman, D.G., 1994. Statistics notes: correlation, regression, and repeated data. *BMJ* 308, 896.
- Brealey, D., Brand, M., Hargreaves, I., Heales, S., Land, J., Smolenski, R., Davies, N.A., Cooper, C.E., Singer, M., 2002. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet.* 360, 219–223.
- Breen, P.H., Isseries, S.A., Westley, J., Roixen, M.F., Taitelman, U.Z., 1995. Combined carbon monoxide and cyanide poisoning: a place for treatment. *Anesth. Analg.* 80, 671–677.
- Chiarla, C., Giovannini, L., Giuliano, F., Vellone, M., Ardito, F., Tenhunen, J., Nuzzo, G., 2010. Significance of hemoglobin concentration in determining blood CO<sub>2</sub> binding capacity in critical illness. *Respir. Physiol. Neurobiol.* 172, 32–36.
- Christiansen, J., Douglas, G.G., Haldane, J.S., 1914. The absorption and dissociation of carbon dioxide by human blood. *J. Physiol.* 48, 244–271.
- Cilley, R.E., Scharenberg, A.M., Bongiorno, P.F., Guire, K.E., Bartlett, R.H., 1991. Low oxygen delivery produced by anemia, hypoxia, and low cardiac output. *J. Surg. Res.* 51, 425–433.
- Cohen, L.L., Sheikh, F.M., Perkins, R.J., Feustel, P.J., Foster, E.D., 1995. Effect of hemorrhagic shock and reperfusion on the respiratory quotient in swine. *Crit. Care Med.* 23, 545–552.
- Cuschieri, J., Rivers, E.P., Donnino, M.W., Katillius, M., Jacobsen, G., Nguyen, H.B., Pamukov, N., Horst, H.M., 2005. Central venous-arterial carbon dioxide difference as an indicator of cardiac index. *Intensive Care Med.* 31, 818–822.
- Depeint, F., Bruce, W.R., Shangari, N., Mehta, R., O'Brien, P.J., 2006. Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism. *Chem. Biol. Interact.* 163, 94–112.
- Du, W., Long, Y., Wang, X.T., Liu, D.W., 2015. The use of the ratio between the venoarterial carbon dioxide difference and the arterial-venous oxygen difference to guide resuscitation in cardiac surgery patients with hyperlactatemia and normal central venous oxygen saturation. *Chin. Med. J. (Engl.)* 128, 1306–1313.
- El-Mir, M.Y., Nogueira, V., Fontaine, E., Avéret, N., Rigoutet, M., Leverve, X., 2000. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. *J. Biol. Chem.* 275, 223–228.
- Fahri, L.E., Rahn, H., 1960. Dynamics of changes in carbon dioxide stores. *Anesthesiology* 21, 604–614.
- Fink, M.P., 2001. Cytopathic hypoxia. Mitochondrial dysfunction as mechanism contributing to organ dysfunction in sepsis. *Crit. Care Clin.* 17, 219–237.
- García-Alvarez, M., Marić, P., Bellomo, R., 2014. Stress hyperlactataemia: present understanding and controversy. *Lancet Diabetes Endocrinol.* 2, 339–347.
- Groeneveld, A.B., Vermeij, C.G., Thijs, L.G., 1991. Arterial and mixed venous blood acid-base balance during hyperperfusion with incremental positive end expiratory pressure in the pig. *Anesth. Analg.* 73, 576–582.
- Gutierrez, G., 2004. A mathematical model of tissue-blood carbon dioxide exchange during hypoxia. *Am. J. Respir. Crit. Care Med.* 169, 525–533.
- Hall, J.E., Hall, M.E., 2020. Transport of oxygen and carbon dioxide in blood and tissue fluids. Guyton and Hall Textbook of Medical Physiology, 14th edition. Elsevier, Philadelphia, PA.
- Hayes, M.A., Timmins, A.C., Yau, E.H., Palazzo, M., Hinds, C.J., Watson, D., 1994. Elevation of systemic oxygen delivery in the treatment of critically ill patients. *N. Engl. J. Med.* 330, 1717–1722.
- Hernández, G., Ospina-Tascón, G.A., Damiani, L.P., Estenssoro, E., Dubin, A., Hurtado, J., Friedman, G., Castro, R., Alegría, L., Teboul, J.L., Cecconi, M., Ferri, G., Jibaja, M., Pairumani, R., Fernández, P., Barahona, D., Granda-Luna, V., Cavalcanti, A.B., Bakker, J., 2019. Effect of a resuscitation strategy targeting peripheral perfusion status vs serum lactate levels on 28-day mortality among patients with septic shock: the ANDROMEDA SHOCK randomized clinical trial. *JAMA* 321, 654–664.
- Institute of Laboratory Animal Resources, 1996. Commission on Life Sciences, National Research Council. Guide for the care and use of laboratory animals, Washington.
- James, J.H., Luchette, F.A., McCarter, F.D., Fischer, J.E., 1999. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. *Lancet* 354, 505–508.
- Karlsson, M., Ehinger, J.K., Piel, S., Sjövall, F., Henriksnäs, J., Höglund, U., Hansson, M. J., Elmér, E., 2016. Changes in energy metabolism due to acute rotenone-induced mitochondrial complex I dysfunction – An in vivo large animal model. *Mitochondrion* 31, 56–62.
- Langer, T., Carlesso, E., Protti, A., Monti, M., Comibi, B., Zani I. Andreis, D.T., Iapichino, G.E., Dondossola, D., Caironi, P., Gatti, S., Gattinoni, L., 2012. In vivo conditioning of acid-base equilibrium by crystalloid solutions: an experimental study on pigs. *Intensive Care Med.* 38, 686–693.
- Levy, B., Gibot, S., Franck, P., Cravoisy, A., Bollaert, P.E., 2005. Relation between muscle Na<sup>+</sup>K<sup>+</sup> ATPase activity and raised lactate concentrations in septic shock: a prospective study. *Lancet* 365, 871–875.
- Liu, H., Li, G., Cumberland, W.G., Tongtong, W., 2005. Testing statistical significance of the area under a receiving operating characteristics curve for repeated measures design with bootstrapping. *J. Data Sci.* 3, 257–278.
- Mahendran, S., Nguyen, J., Butler, E., Aneman, A., 2020. Prospective, observational study of carbon dioxide gaps and free energy change and their association with fluid therapy following cardiac surgery. *Acta Anaesthesiol. Scand.* 64, 202–210.
- Mallat, J., Lemzye, M., Meddour, M., Pepy, F., Gasan, G., Barrailler, S., Durville, E., Temime, J., Vangrunderbeeck, N., Tronchon, L., Vallet, B., Thevenin, D., 2016a. Ratios of central venous to arterial carbon dioxide content or tension to arteriovenous oxygen content are better markers of global anaerobic metabolism than lactate in septic shock patients. *Ann. Intensive Care* 6, 10.
- Mallat, J., Lemzye, M., Tronchon, L., Vallet, B., Thevenin, D., 2016b. Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock. *World J. Crit. Care Med.* 5, 47–56.
- Maln, J.R., Manger, W.M., Sullivan, S.F., Papper, E.M., Nahas, G.G., 1966. The effect of acidosis on sympatho-adrenal stimulation. Particular reference to cardiopulmonary bypass. *JAMA* 197, 121–125.
- Marik, P.E., Linde-Zwirble, W.T., Bittner, E.A., Sahafijian, J., Hansell, D., 2017. Fluid administration in severe sepsis and septic shock, patterns and outcomes: an analysis of a large national database. *Intensive Care Med.* 43, 625–632.
- Mekontso-Dessap, A., Castelain, V., Anguel, N., Bahloul, M., Schavuliege, F., Richard, C., Teboul, J.L., 2002. Combination of venoarterial PCO<sub>2</sub> difference with arteriovenous O<sub>2</sub> content difference to detect anaerobic metabolism in patients. *Intensive Care Med.* 28, 272–277.
- Mesquida, J., Saludes, P., Gruartmoner, G., Espinal, C., Torrents, E., Baigorri, F., Artigas, A., 2015. Central venous-to-arterial carbon dioxide difference combined with arterial-to-venous oxygen content difference is associated with lactate evolution in the hemodynamic resuscitation process in early septic shock. *Crit. Care* 19, 126.
- Mesquida, J., Espinal, C., Saludes, P., Cortés, E., Pérez-Madrugal, A., Gruartmoner, G., 2019. Central venous-to-arterial carbon dioxide difference combined with arterial-to-venous oxygen content difference (P(cva)CO<sub>2</sub>/C(av)O<sub>2</sub>) reflects microcirculatory oxygenation alterations in early septic shock. *J. Crit. Care* 53, 162–168.
- Mizock, B.A., 1989. Lactic acidosis. *Dis.* 35, 233–300.
- Monnet, X., Julien, F., Ait-Hamou, N., Leguoy, M., Gosset, C., Jozwiak, M., Persichini, R., Anguel, N., Richard, C., Teboul, J.L., 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.
- Murphy, N.D., Kodakati, S.K., Wendon, J.A., Jooste, C.A., Mutesau, P., Rele, M., Heaton, N.D., 2001. Liver and intestinal lactate metabolism in patients with acute hepatic failure undergoing liver transplantation. *Crit. Care Med.* 29, 2111–2118.
- Ospina-Tascón, G.A., Madriñán, H.J., 2019. Combination of O<sub>2</sub> and CO<sub>2</sub>-derived variables to detect tissue hypoxia in the critically ill patient. *J. Thorac. Dis.* 11, S1544–S1550.
- Ospina-Tascón, G.A., Hernández, G., Cecconi, M., 2016a. Understanding the venous-arterial CO<sub>2</sub> to arterial-venous O<sub>2</sub> content difference ratio. *Intensive Care Med.* 42, 1801–1804.
- Ospina-Tascón, G.A., Umaña, M., Bermúdez, W.F., Bautista-Rincón, D.F., Valencia, J.D., Madriñán, H.J., Hernández, G., Bruhn, A., Arango-Dávila, C., De Backer, D., 2016b. Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock? *Intensive Care Med.* 42, 211–221.
- Owen, M.R., Doran, E., Halestrap, A.P., 2000. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex I of the mitochondrial respiratory chain. *Biochem. J.* 348, 607–614.
- Péronnet, F., Aguilaniu, B., 2006. Lactic acid buffering, nonmetabolic CO<sub>2</sub> and exercise hyperventilation: a critical reappraisal. *Respir. Physiol. Neurobiol.* 150, 4–18.
- Protti, A., Russo, R., Tagliabue, P., Vecchio, S., Singer, M., Rudiger, A., Foti, G., Rossi, A., Mistrarelli, G., Gattinoni, L., 2010. Oxygen consumption is depressed in patients with lactic acidosis due to biguanide intoxication. *Crit. Care* 14, R22.
- Protti, A., Fortunato, F., Monti, M., Vecchio, S., Gatti, S., Comi, G.P., De Giuseppe, R., Gattinoni, L., 2012a. Metformin overdose, but not lactic acidosis per se, inhibits oxygen consumption in pigs. *Crit. Care* 16, R75.
- Protti, A., Lecchi, A., Fortunato, F., Artoni, A., Greppi, N., Vecchio, S., Fagioli, G., Moggio, M., Comi, G.P., Mistrarelli, G., Lantichina, B., Faraldi, L., Gattinoni, L., 2012b. Metformin overdose causes platelet mitochondrial dysfunction in humans. *Crit. Care* 16, R180.
- Protti, A., Ronchi, D., Bassi, G., Fortunato, F., Bordoni, A., Rizzuti, T., Fumagalli, R., 2016. Changes in whole-body oxygen consumption and skeletal muscle mitochondria during linezolid-induced lactic acidosis. *Crit. Care Med.* 44, e579–582.

## II. Clinical studies

### 1. Venous-to-arterial PCO<sub>2</sub> difference

Results from clinical studies in septic shock patients have also supported that the decreased cardiac output is the primary determinant in the elevation of  $\Delta\text{PCO}_2_{\text{Mix}}$ . Indeed, septic patients with  $\Delta\text{PCO}_2_{\text{Mix}} > 6$  mmHg had a significantly lower mean cardiac output when compared to patients with  $\Delta\text{PCO}_2_{\text{Mix}} \leq 6$  mmHg [131]. Moreover, the changes in cardiac output induced by volume expansion were correlated with changes in  $\Delta\text{PCO}_2_{\text{Mix}}$  ( $r = 0.46$ ,  $P < 0.01$ ). The authors rightly concluded that in patients with septic shock, an elevated  $\Delta\text{PCO}_2_{\text{Mix}}$  is related to a decreased systemic blood flow. Similarly, Bakker et al. found a significant negative correlation between cardiac output and  $\Delta\text{PCO}_2_{\text{Mix}}$ . Therefore, a strong association between cardiac output and  $\Delta\text{PCO}_2_{\text{Mix}}$  is also well documented in septic shock [132]. Interestingly, many patients in those studies [131, 132] had normal  $\Delta\text{PCO}_2_{\text{Mix}}$  despite the presence of tissue hypoxia, presumably since their elevated cardiac output had simply washed out the CO<sub>2</sub> generated in the peripheral circulation.

Creteur et al. [133] examined the association between impairment in microcirculatory perfusion and tissue PCO<sub>2</sub>. They showed that the reperfusion of damaged microcirculation (assessed using orthogonal polarized spectroscopy) was associated with normalized sublingual tissue PCO<sub>2</sub> levels. Thus, there is a clear relation between tissue CO<sub>2</sub> accumulation and blood flow, leading to increasing venous-arterial CO<sub>2</sub> gradients. Furthermore, Ospina-Tascon et al. [134], using a sidestream dark-field device, observed that sublingual  $\Delta\text{PCO}_2$  was closely associated with microvascular blood flow parameters during the early phases of resuscitation of septic shock. Indeed, sublingual  $\Delta\text{PCO}_2$  was the best predictor of the microcirculatory blood flow

maldistribution as suggested by the alterations in the percentage of perfused small vessels (PPV), the heterogeneity blood flow index, and consequently functional capillary density. In addition, changes in sublingual  $\Delta\text{PCO}_2$  were significantly associated with changes in PPV. However, sublingual  $\Delta\text{PCO}_2$  was poorly related to systemic hemodynamic variables such as cardiac output, which was also not associated with microcirculatory variables.

Ospina-Tascon et al. [135] have shown that the persistence of high  $\Delta\text{PCO}_2_{\text{Mix}} (\geq 6 \text{ mmHg})$  during the first six hours of resuscitation of septic shock patients was linked to more severe multiple organ failure and higher mortality rate (Relative Risk = 2.23, P = 0.01). However, The measurement of  $\Delta\text{PCO}_2_{\text{Mix}}$  requires the presence of a pulmonary artery catheter, which is rarely practiced nowadays [136]. Since the central venous catheter is implanted in most septic shock patients, using central venous-arterial carbon dioxide partial pressure difference ( $\Delta\text{PCO}_2_{\text{Cent}}$ ) is significantly easier and similarly helpful. Interestingly, a strong agreement between  $\Delta\text{PCO}_2_{\text{Mix}}$  and  $\Delta\text{PCO}_2_{\text{Cent}}$ , calculated as the difference between central venous  $\text{PCO}_2$  sampled from a central vein catheter and arterial  $\text{PCO}_2$ , was reported in critically ill patients [137] and severe sepsis and septic shock patients [138].

As emphasized previously, high values of  $\text{ScvO}_2$  do not preclude the presence of tissue hypoperfusion and hypoxia in cases of impaired  $\text{ERO}_2$  capabilities that can occur in septic shock [99, 100]. Since the solubility of  $\text{CO}_2$  is very high (around 20 times that of  $\text{O}_2$ ), its capability of spreading out of ischemic tissues into the efferent veins is phenomenal, making it an extremely sensitive indicator of hypoperfusion. Consequently, in conditions with  $\text{O}_2$  diffusion difficulties (resulting from shunted and obstructed capillaries), “covering” reduced  $\text{ERO}_2$  and increased tissue  $\text{O}_2$  debt,  $\text{CO}_2$  still diffuses to the efferent veins, “uncovering” the hypoperfusion situation

for the clinician when  $\Delta\text{PCO}_2$  is evaluated [139]. Accordingly, Vallée et al. [140] tested the hypothesis that the  $\Delta\text{PCO}_2$ \_Cent can be used as a global indicator of tissue hypoperfusion in resuscitated septic shock patients in whom  $\text{ScvO}_2$  was already greater than 70%. They showed that despite a normalized  $\text{DO}_2/\text{VO}_2$  ratio, patients who had impaired tissue perfusion with blood lactate concentration  $> 2$  mmol/L remained with an elevated  $\Delta\text{PCO}_2$ \_Cent ( $> 6$  mmHg). Also, patients with low  $\Delta\text{PCO}_2$ \_Cent values had greater lactate decrease and cardiac index values and exhibited a significantly higher reduction in SOFA score than patients with high  $\Delta\text{PCO}_2$ \_Cent. In a prospective study that included 80 patients, we recently examined the usefulness of measuring  $\Delta\text{PCO}_2$ \_Cent during the initial resuscitation period of septic shock [141]. We found that during the very early period of septic shock, patients who reached a normal  $\Delta\text{PCO}_2$ \_Cent ( $\leq 6$  mmHg) after six hours of resuscitation had greater decreases in blood lactate and in SOFA score than those who failed to normalize  $\Delta\text{PCO}_2$ \_Cent ( $> 6$  mmHg). Interestingly, patients who achieved the goals of both  $\Delta\text{PCO}_2$ \_Cent  $\leq 6$  mmHg and  $\text{ScvO}_2 > 70\%$  after the first six hours of resuscitation had the greatest blood lactate decrease, which was found to be an independent prognostic factor of ICU mortality [141]. In addition, Du et al. [142], in a retrospective study, showed that the normalization of both  $\text{ScvO}_2$  and  $\Delta\text{PCO}_2$ \_Cent seems to be a better prognostic factor of outcome after reanimation from septic shock than  $\text{ScvO}_2$  only. Patients who achieved both targets seemed to clear blood lactate more efficiently [142]. Recently, Wang et al. [143] reported that in septic patients secondary to a bloodstream infection,  $\Delta\text{PCO}_2$ \_Cent was independently associated with 28-day mortality in multivariable regression analysis.

Several observational studies found an association between  $\Delta\text{PCO}_2$ \_Cent and outcomes in high-risk non-cardiac surgical patients. In a retrospective study that included 70 patients who

underwent major abdominal surgery with an individualized goal-directed fluid replacement therapy, Futier et al. [144] observed that  $\Delta\text{PCO}_2_{\text{Cent}}$  was the only parameter associated with postoperative septic complications that occurred in 34% of cases. Also, a  $\Delta\text{PCO}_2_{\text{Cent}} > 5$  mmHg was able to predict postoperative complications with an AUROC of 0.785. In a prospective study that included 66 high-risk non-cardiac surgical patients [145], the authors found that  $\Delta\text{PCO}_2_{\text{Cent}}$  values  $> 5$  mmHg preoperatively were associated with worse postoperatively outcomes. Later, Robin et al. [146] performed a prospective study that included 115 high-risk non-cardiac surgery patients (mainly abdominal surgery). The authors reported that  $\Delta\text{PCO}_2_{\text{Cent}}$  on ICU admission immediately after surgery was significantly higher in patients who developed postoperative complications compared to those who did not. In addition,  $\Delta\text{PCO}_2_{\text{Cent}}$  on ICU admission predicted the development of postoperative complications with an AUROC of 0.86 and a best cuff value of 5.8 mmHg. Furthermore, the ability of  $\Delta\text{PCO}_2_{\text{Cent}}$  to predict postoperative complications was significantly better than the arterial lactate levels.

However, clinical studies have shown contradicting findings in cardiac surgery patients. In a retrospective study that included 60 cardiac surgery patients, high  $\Delta\text{PCO}_2_{\text{Cent}}$  values ( $\geq 8$  mmHg) were associated with worse postoperative outcomes, including worse splanchnic functions, longer mechanical ventilation, and longer ICU stays [147]. In another retrospective study that included 220 cardiac surgery patients, the authors reported a low ability of  $\Delta\text{PCO}_2_{\text{Cent}}$  to predict outcomes [148]. Also,  $\Delta\text{PCO}_2_{\text{Cent}}$  did not predict the occurrence of major postoperative complications and was poorly correlated with tissue perfusion parameters and arterial lactate clearance in a prospective study that included 339 cardiac surgery patients [149]. In addition, in a prospective study that included 308 cardiac surgery patients, Moussa et

al. observed that  $\Delta\text{PCO}_2_{\text{Cent}}$  was independently associated with lower major postoperative complications [150]. However, the ability of  $\Delta\text{PCO}_2_{\text{Cent}}$  to predict major postoperative complications was not good (AUROC of 0.64). In contrast, a retrospective study that included 1,019 cardiac surgery patients showed that  $\Delta\text{PCO}_2_{\text{Cent}}$  on ICU admission was independently associated with ICU mortality [151]. Also, recent studies reported the good ability of  $\Delta\text{PCO}_2_{\text{Cent}}$  to predict adverse outcomes after cardiac surgery. Mukai et al., in a prospective study that included 110 cardiac surgery patients, found that postoperatively  $\Delta\text{PCO}_2_{\text{Mix}}$  was independently associated with major organ morbidity and mortality (MOMM). Also,  $\Delta\text{PCO}_2_{\text{Mix}}$  was the best predictor of MOMM with an AUROC of 0.80 and a cut-off of 5.2 mmHg [152]. In a propensity-matched study, Chen et al. found that  $\Delta\text{PCO}_2_{\text{Mix}}$  predicted the occurrence of poor outcomes with an AUROC of 0.84 and a cut-off of 7.1 mmHg [153]. Nevertheless, in a recent retrospective study that included 1,933 patients, we found that  $\Delta\text{PCO}_2_{\text{Cent}}$  had poor diagnostic performance (AUROC of 0.55) to predict postoperative complications even though it was independently associated with postoperative adverse outcomes [154].



## Central venous-to-arterial CO<sub>2</sub> difference is a poor tool to predict adverse outcomes after cardiac surgery: a retrospective study

### La différence entre le CO<sub>2</sub> veineux central et artériel est un outil médiocre pour prédire les devenir défavorables après une chirurgie cardiaque : une étude rétrospective

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#### Abstract

**Purpose** The venous-to-arterial carbon dioxide partial pressure difference (CO<sub>2</sub> gap) has been reported to be a sensitive indicator of cardiac output adequacy. We aimed to assess whether the CO<sub>2</sub> gap can predict postoperative adverse outcomes after cardiac surgery.

**Methods** A retrospective study was conducted of 5,151 patients from our departmental database who underwent cardiac surgery from 1 January 2008 to 31 December 2018. Lactate level (mmol·L<sup>-1</sup>), central venous oxygen saturation (ScVO<sub>2</sub>) (%), and the venous-to-arterial carbon dioxide

difference (CO<sub>2</sub> gap) were measured at intensive care unit (ICU) admission and on days 1 and 2 after cardiac surgery. The following postoperative adverse outcomes were collected: ICU mortality, hemopericardium or tamponade, resuscitated cardiac arrest, acute kidney injury, major bleeding, acute hepatic failure, mesenteric ischemia, and pneumonia. The primary outcome was the presence of at least one postoperative adverse outcome. Logistic regression was used to assess the association between ScVO<sub>2</sub>, lactate, and the CO<sub>2</sub> gap with adverse outcomes. Their diagnostic performance was compared using a receiver operating characteristic (ROC) curve.

**Results** There were 1,933 patients (38%) with an adverse outcome. Cardiopulmonary bypass (CPB) parameters were similar between groups. The CO<sub>2</sub> gap was slightly higher for the “adverse outcomes” group than for the “no adverse outcomes” group. Arterial lactate at admission, day 1, and day 2 was also slightly higher in patients with adverse outcomes. Central venous oxygen saturation was not significantly different between patients with and without adverse outcomes. The area under the ROC curve to predict outcomes after CPB for the CO<sub>2</sub> gap at admission, day 1, and day 2 were 0.52, 0.55, and 0.53, respectively.

**Conclusion** After cardiac surgery with CPB, the CO<sub>2</sub> gap at ICU admission, day 1, and day 2 was associated with postoperative adverse outcomes but showed poor diagnostic performance.

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#### Résumé

**Objectif** La différence de pression partielle de dioxyde de carbone veineux versus artériel (gradient de CO<sub>2</sub>) a été

rapportée comme étant un indicateur sensible d'un débit cardiaque adéquat. Nous avons tenté d'évaluer si le gradient de  $CO_2$  pouvait prédire les devenir postopératoires défavorables après une chirurgie cardiaque.

**Méthode** Une étude rétrospective a été réalisée en se basant sur les dossiers de 5151 patients issus de notre base de données départementale ayant subi une chirurgie cardiaque entre le 1<sup>er</sup> janvier 2008 et le 31 décembre 2018. Les taux de lactate ( $mmol \cdot L^{-1}$ ), la saturation en oxygène veineux central (ScVO<sub>2</sub>) (%), et la différence de dioxyde de carbone veineux versus artériel (gradient de  $CO_2$ ) ont été mesurés lors de l'admission en réanimation (ICU) et aux jours 1 et 2 après la chirurgie cardiaque. Les complications postopératoires suivantes ont été colligées : mortalité en réanimation, hémopéricarde ou tamponnade, arrêt cardiaque récupéré, insuffisance rénale aiguë, saignements majeurs, insuffisance hépatique aiguë, ischémie mésentérique et pneumonie. Le critère d'évaluation principal était la présence d'au moins une complication postopératoire. La régression logistique a été utilisée pour évaluer l'association entre ScVO<sub>2</sub>, taux de lactate et gradient de  $CO_2$  et les complications. Leur performance diagnostique a été comparée à l'aide d'une courbe ROC (receiver operating characteristic).

**Résultats** Des complications sont survenues chez 1933 patients (38 %). Les paramètres de circulation extracorporelle (CEC) étaient semblables entre les groupes. Le gradient de  $CO_2$  était légèrement plus élevé dans le groupe « complications » que dans le groupe « pas de complication ». Les taux de lactate artériels à l'admission, au jour 1 et au jour 2 étaient également légèrement plus élevés chez les patients ayant subi des complications. La différence de saturation en oxygène veineux central n'était pas significative entre les patients avec ou sans complications. L'aire sous la courbe ROC pour prédire les devenir après la CEC pour le gradient de  $CO_2$  à l'admission, au jour 1 et au jour 2 était de 0,52, 0,55 et 0,53, respectivement.

**Conclusion** Après une chirurgie cardiaque avec CEC, le gradient de  $CO_2$  à l'admission en réanimation, au jour 1 et au jour 2 était associé aux complications postopératoires, mais sa performance diagnostique était médiocre.

**Keywords** Central venous-to-arterial  $CO_2$  difference · cardiac surgery · cardiopulmonary bypass · outcomes · tissue perfusion · arterial lactate

Over the last decades, cardiac surgery techniques have significantly improved, allowing increasingly complex procedures to be performed.<sup>1</sup> Multimodal management is

key to improving outcomes after surgery.<sup>2</sup> Hemodynamic goal-directed therapy, including various treatments, relies on protocols that increase oxygen delivery by controlling blood pressure, the cardiac index, and central venous oxygen saturation. Individualized goal-directed hemodynamic optimization during high-risk surgery has been proven to improve morbidity and mortality.<sup>3</sup> This approach aims to adapt oxygen delivery to oxygen consumption to avoid tissue hypoperfusion during surgery.<sup>4</sup> Markers of adequate tissue perfusion (lactate, ScVO<sub>2</sub>, and  $CO_2$  gap) have their limitations. Hyperlactatemia does not always reflect tissue dysoxia or anaerobic metabolism and can be aspecific.<sup>5</sup> Central venous oxygen saturation (ScVO<sub>2</sub>), used as a marker of dysoxia, can be normal despite microcirculatory impairment.<sup>6</sup>

Hence, the central venous-to-arterial  $CO_2$  partial pressure difference ( $CO_2$  gap) has been described as a parameter that reflects tissue hypoperfusion in insufficiently resuscitated critically ill patients.<sup>7</sup> Strong data support the necessity to monitor the  $CO_2$  gap during the early phase of septic shock. Indeed, in patients with septic shock, a  $CO_2$  gap > 6 mmHg and a normal ScVO<sub>2</sub> > 70% indicates a dependency on oxygen delivery with the need to pursue resuscitation.<sup>8,9</sup> In the latest guidelines on the monitoring of microcirculation, the  $CO_2$  gap was recommended to manage septic shock in the early phase.<sup>10</sup>

In the perioperative settings of cardiac surgery, data are sparse and contradictory on  $CO_2$  gap monitoring. The limits concern the small sample size, the very early postoperative time point of gap  $CO_2$  assessment and the contradictory findings. Previous studies that evaluated the association between the  $CO_2$  gap and outcomes of cardiac surgery patients showed poor diagnostic performance.<sup>11,12</sup> Thus, there is currently no clear position on the use of  $CO_2$  gap monitoring in the postoperative period of cardiac surgery.

Thus, we aimed to investigate the association between the  $CO_2$  gap and postoperative adverse outcomes in a large retrospective cohort of patients who underwent cardiac surgery with cardiopulmonary bypass (CPB). Associations were measured at different times of the intensive care unit (ICU) stay.

## Methods

### Study population

All patients who underwent cardiac surgery from 1 January 2008 to 31 December 2018 in Amiens University hospital were included. The study was approved by the appropriate institutional review board, who waived the requirement for

written informed consent. The present report was drafted in line with the STROBE statement for observational studies in epidemiology.<sup>13</sup>

#### Data collection

We identified retrospectively all patients who underwent cardiac surgery at Amiens University hospital. Since 2007, the cardiothoracic unit of Amiens has had a computerized system (Centricity Critical Care Clinisoft®, GE Healthcare, Chicago, IL, USA) that collects all patient characteristics, surgery characteristics, and postoperative outcomes.

We extracted the following data from the Clinisoft database for each patient: age, sex, height (m), weight (kg), Simplified Acute Physiology Score (SAPS II), ICU stay (days), medical history (diabetes, hypertension, coronary disease characterized by the presence of a stent, peripheral vascular disease defined by the presence of a stent of bypass graft surgery in the lower limbs, chronic kidney disease defined as a glomerular filtration rate of < 60 mL·min<sup>-1</sup>, and chronic obstructive pulmonary disease).

The following postoperative data for the first 48 hr were obtained from the same database: cumulative crystalloid infusion (mL), cumulative colloid infusion (mL), cumulative norepinephrine dose (mg), cumulative dobutamine dose (mg), and cumulative diuresis (mL).

Lactate, ScVO<sub>2</sub>, and the CO<sub>2</sub> gap were measured at ICU admission and on days 1 and 2. Venous-to-arterial carbon dioxide difference on day 1 and day 2 was measured at the same time point in all patients.

#### Definitions of the postoperative outcomes

We collected the following data from our institutional database using the French classification for medical procedures “Classification Commune des Actes Médicaux” (CCAM). Each diagnosis (outcome) is associated with a unique diagnostic code number. Medical acts and diagnoses were coded in our database by the same physician. Each outcome was defined according to standard guidelines. For each diagnosis, computed extraction was performed by a request for the diagnosis code. Cardiac arrest was defined as the cessation of cardiac mechanical activity, as confirmed by the absence of signs of circulation. Acute kidney injury was defined according to Kidney Disease Improving Global Outcomes (KDIGO) criteria as an increase in serum creatinine over 27 μmol·L<sup>-1</sup> within 48 hr or urine output lower than 0.5 mL·kg<sup>-1</sup>·hr<sup>-1</sup> (KDIGO 1).<sup>14</sup> Mesenteric ischemia was defined by surgical abdomen exploration. Hemopericardium was defined in case of requirement for surgical/mediastinal revision. Major bleeding was defined

as requirement of more than four units of red blood cell transfusion. According to French guidelines, acute hepatic failure was defined as a prothrombin ratio under 50%.<sup>15</sup> Ventilator-acquired pneumonia was defined by the prescription of antibiotic therapy for a low respiratory infection.<sup>16</sup>

#### Outcome

The primary outcome was the presence of at least one postoperative adverse outcome during the hospital stay.

#### Statistical analysis

Data were collected on 5,928 patients during the study period. The present analysis was restricted to patients with cardiac surgery under CPB (*n* = 5,151). The demographic and clinical characteristics of the study participants of both groups were compared using the t-test for continuous variables and the χ<sup>2</sup> test for discrete variables, as appropriate. A logistic regression model was used to evaluate the association between CO<sub>2</sub> gap/lactate and the outcome. We adjusted the model on differences in variables between “no outcome” and “adverse outcome” groups when the *P* value < 0.05. We built a receiver operating characteristic (ROC) curve to assess the diagnostic performance of arterial lactate, ScVO<sub>2</sub>, and the CO<sub>2</sub> gap. Statistical analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA). A *P* < 0.05 was considered significant.

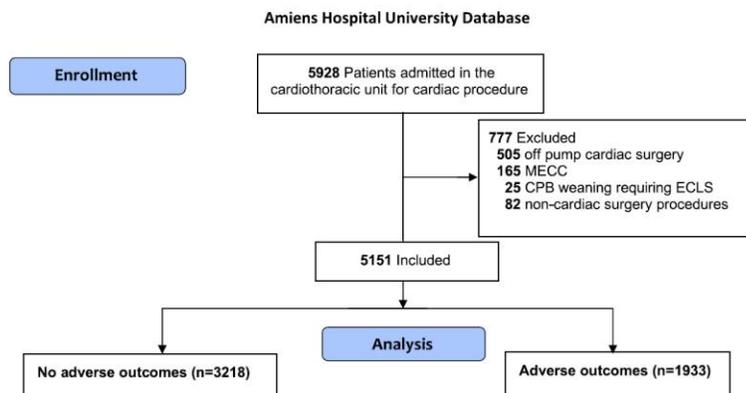
## Results

#### Study population

Between January 2008 and December 2018, 5,928 eligible patients were recorded in the database. Among them, 505 patients were excluded from analysis because of heart beating surgery, 165 because of minimal extracorporeal circulation, 25 because of CPB weaning requiring extracorporeal life support, and 82 because of a non-cardiac procedure under CPB. Five thousand one hundred and fifty-one patients were enrolled: 1,933 experienced adverse outcomes (38%) and 3,218 had no adverse outcomes (62%). The study flowchart is summarized in Fig. 1.

Baseline characteristics and surgical procedures for both groups are shown in Table 1. There were no difference in duration of CPB or aortic cross clamp between the two groups. In the “adverse outcomes” group, there were more cases of chronic kidney disease (8% vs 5%; *P* < 0.0001) and the mean (standard deviation) SAPS II were higher [41

**Fig. 1** Flow chart. CPB = cardiopulmonary bypass; ECLS = extracorporeal life support; MECC = minimal extracorporeal circulation



**TABLE 1** Demographics and intraoperative characteristics of the study population

Variables	No adverse outcomes (n = 3218)	Adverse outcomes (n = 1933)	P value
Age (yr)	66 (12)	68 (12)	0.21
BMI (kg·m <sup>-2</sup> )	28.8 (0.3)	28.2 (0.4)	0.25
Male, n (%)	2259 (70)	1289 (67)	0.01
Medical history, n (%)			
Diabetes	644 (20)	356 (18)	0.16
Hypertension	1772 (55)	1103 (57)	0.16
Coronary disease	367 (11)	— 12	0.35
Chronic kidney disease	84 (3)	151 (8)	< 0.001
Obesity	119 (4)	71 (4)	0.96
Vascular peripheral disease	149 (52)	121 (45)	0.01
Surgical type, n (%)			
CABG	1214 (38)	804 (42)	0.006
Valve surgery	1529 (47)	951 (49)	0.25
Combined surgery	475 (15)	178 (9)	0.002
SAPS II	34 (9)	41 (12)	< 0.001
Duration of CPB (min)	88 [62–119]	108 [73–145]	1
Duration of aortic clamp (min)	61 [42–86]	68 [45–99]	0.32

Data are expressed as mean (standard deviation), median [interquartile range], or numbers (percentages). BMI = body mass index, CABG = coronary artery bypass graft, CPB = cardiopulmonary bypass, SAPS = Simplified Acute Physiology Score

(12) vs 34 (9);  $P < 0.001$ ]. Also, there were fewer males (67% vs 70%;  $P = 0.01$ ), fewer cases of peripheral vascular disease (45% vs 52%;  $P = 0.011$ ), and fewer cases of combined surgery (9% vs 15%;  $P = 0.002$ ) in the “adverse outcome” group.

Association between ScVO<sub>2</sub>, arterial lactate, and the CO<sub>2</sub> gap with adverse outcomes (Table 2, Fig. 3)

The CO<sub>2</sub> gap was slightly higher for the “adverse outcomes” group than the “no adverse outcomes” group. Arterial lactate at admission, day 1, and day 2 was also slightly higher in patients with adverse outcomes. Central venous oxygen saturation was not significantly different between patients with and without adverse outcomes. The area under the curve (AUC) to predict outcomes after CPB

**TABLE 2** Association between perfusion parameters and adverse outcomes

Variables	No adverse outcomes (n = 3,218)	Adverse outcomes (n = 1,933)	P value
Arterial lactate (mmol·L <sup>-1</sup> )			
ICU	1.3 [1.1–1.7]	1.6 [1.2–2.2]	< 0.001
Day 1	1.7 [1.3–2.1]	2.1 [1.6–2.9]	< 0.001
Day 2	1.4 [1.1–1.7]	1.7 [1.3–2.2]	< 0.001
CO <sub>2</sub> gap (mmHg)			
ICU	7 [3–12]	7 [3–12]	0.01
Day 1	6 [3–10]	7 [3–10]	< 0.001
Day 2	8 [4–12]	9 [4–13]	< 0.001
ScVO <sub>2</sub> (%)			
ICU	65 [55–75]	65 [54–75]	0.26
Day 1	65 [57–72]	64 [57–72]	0.35
Day 2	65 [57–72]	64 [57–72]	0.35
Endpoints, n (%)			
Tamponade	–	316 (16)	–
Major bleeding	–	1270 (65)	–
Resuscitate cardiac arrest	–	141 (7)	–
Pneumonia	–	498 (25)	–
Mesenteric ischemia	–	100 (5)	–
Acute hepatic failure	–	285 (15)	–
AKI	–	628 (32)	–
Death	–	272 (14)	–
ICU stay (days)	2 (1)	10 (17)	< 0.001

Data are expressed as median [interquartile range IQR], mean (standard deviation), or numbers (percentages). AKI = acute kidney injury; CO<sub>2</sub> gap = venous-to-arterial carbon dioxide difference; ICU = intensive care unit. ScVO<sub>2</sub> = central venous oxygen saturation

for the CO<sub>2</sub> gap at admission, day 1, and day 2 were 0.52, 0.55, and 0.53, respectively.

Diagnostic performance of the CO<sub>2</sub> gap to predict adverse outcomes

The CO<sub>2</sub> gap was associated with postoperative adverse outcomes at ICU admission, on day 1, and on day 2. Odds ratios with 95% confidence intervals and AUCs are presented in Table 3. Receiver operating characteristic (ROC) curves for the CO<sub>2</sub> gap's diagnostic performance are presented in Fig. 2.

Diagnostic performance of arterial lactate and ScVO<sub>2</sub> to predict adverse outcomes

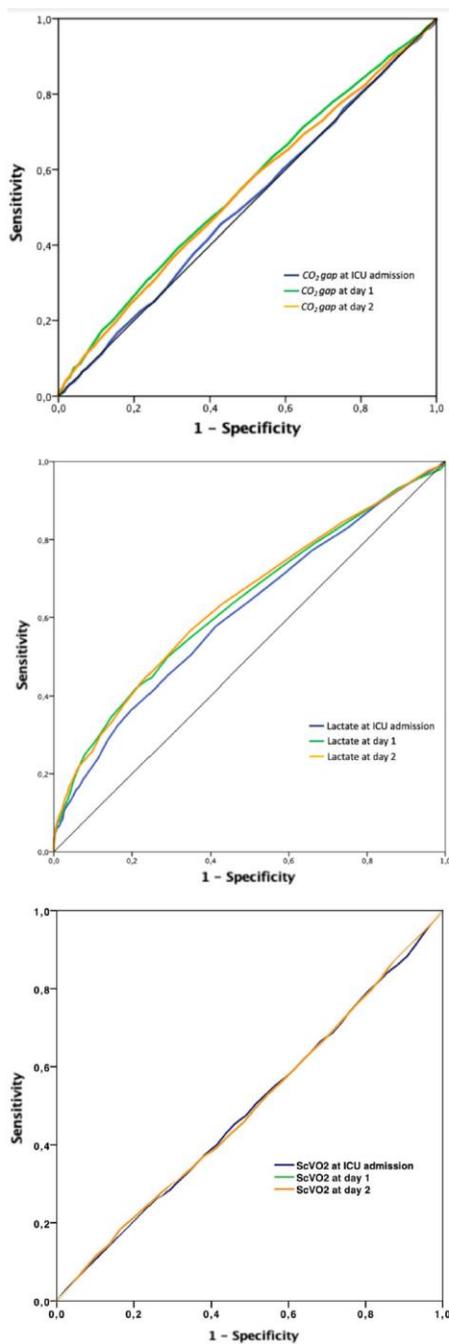
Arterial lactate was associated with postoperative adverse outcomes at ICU admission, on day 1, and on day 2. Central venous oxygen saturation was not associated with any major adverse outcomes after cardiac surgery. Odds ratios with 95% confidence intervals and AUCs are presented in Table 3. Receiver operating characteristic

curves showing the diagnostic performance of lactate and ScVO<sub>2</sub> are presented in Fig. 2.

## Discussion

Our main finding was that the CO<sub>2</sub> gap has poor predictive characteristics for postoperative adverse outcomes in cardiac surgery. Arterial lactate showed better diagnostic performance. Microcirculatory dysfunction is known to be linked to organ failure, despite adequate macro-hemodynamic stability.<sup>17</sup> Markers of adequate tissue perfusion (lactate, ScvO<sub>2</sub>, and the CO<sub>2</sub> gap) have their limitations.

Carbon dioxide production (oxygen consumption [VCO<sub>2</sub>]) is proportional to O<sub>2</sub> consumption (VO<sub>2</sub>): VCO<sub>2</sub> = R × VO<sub>2</sub>, with R as the respiratory quotient. Thus, when aerobic metabolism increases, VCO<sub>2</sub> should increase to the same extent. The CO<sub>2</sub> content (CCO<sub>2</sub>) cannot be easily calculated and in clinical practice, partial pressure of carbon dioxide (PCO<sub>2</sub>) is expressed as PCO<sub>2</sub> = k × CCO<sub>2</sub>, where k is a correction factor related to temperature,



**Fig. 2** Diagnostic performance of arterial lactate, ScVO<sub>2</sub>, and the CO<sub>2</sub> gap on postoperative adverse outcomes using a receiver operating characteristic (ROC) curve. CO<sub>2</sub> = carbon dioxide; CPB = cardiopulmonary bypass; ECLS = extracorporeal live support; MECC = minimal extracorporeal circulation; ScVO<sub>2</sub> = central venous oxygen saturation

anemia, hypoxia, and other metabolic factors. Derived from the Fick equation,  $VCO_2$  can be expressed as  $VCO_2 = CO \times CO_2 \text{ gap}$  and thus the CO<sub>2</sub> gap as  $(k \times VCO_2)/CO$ .<sup>18</sup> The CO<sub>2</sub> gap is not a good marker of global anaerobic metabolism as in hypoxia,  $VCO_2$  can decrease as a result of the decrease in  $VO_2$  and increase in the  $k$  factor.<sup>19</sup> The CO<sub>2</sub> gap is rather a marker of CO than a marker of tissue hypoxia.<sup>20</sup> The variables involved in calculating the CO<sub>2</sub> gap can vary depending on the clinical situation, resulting in divergence between non-cardiac surgery, cardiac surgery, and critically ill septic patients.<sup>21</sup>

In septic shock, the CO<sub>2</sub> gap has been shown to be a reliable marker of the cardiac index and has been proposed to guide early resuscitation.<sup>8,9</sup> Numerous studies have suggested a role for the CO<sub>2</sub> gap in identifying patients with a ScVO<sub>2</sub> > 70% who are still inadequately resuscitated.<sup>18,22,23</sup> Thus, European guidelines on septic shock management propose using the CO<sub>2</sub> gap to guide hemodynamic management during septic shock.<sup>10</sup> The CO<sub>2</sub> gap increases during ischemic hypoxia (decrease in blood flow) but not under hypoxic hypoxia conditions (normal or high blood flow),<sup>8,24</sup> and the use of the CO<sub>2</sub> gap to assess microcirculatory flow and the hypoxic state is still a matter of debate.<sup>25</sup> Non-cardiac surgery studies have reported similar findings as for septic shock, showing that the CO<sub>2</sub> gap can predict the occurrence of adverse postoperative outcomes.<sup>26,27</sup>

Clinical studies on the CO<sub>2</sub> gap in cardiac surgery have shown contradictory results. First, interpretations of results depend on the author. Moussa *et al.* concluded a positive result of the CO<sub>2</sub> gap with an AUC of 0.64 whereas Guinot *et al.* concluded a negative result with a similar AUC.<sup>11,12</sup> In contrast, recent studies reported good performance of the CO<sub>2</sub> gap in predicting adverse outcomes after cardiac surgery. Mukai *et al.* found an AUC of 0.80, with a cut-off of 5.2 mmHg<sup>29</sup> and Chen *et al.* found an AUC of 0.84 with a cut-off of 7.1.<sup>29</sup>

Several factors can explain the discrepancy between positive and negative studies. First, authors' interpretations differ from one publication to another. Biomarkers are considered to have good discriminative properties when the AUC is higher than 0.75. Therefore, an AUC under 0.75 should be considered to have low clinical relevance and not a positive result.<sup>30</sup> Secondly, selected outcomes vary from one study to the other, making the external validity more

**TABLE 3** Association of the CO<sub>2</sub> gap, arterial lactate, and ScVO<sub>2</sub> with major outcomes and area under the curve for diagnostic to predict major adverse outcomes after cardiac surgery

Variables	OR (95% CI)	P	AUC
CO <sub>2</sub> gap			
ICU admission	1.01 (1.00 to 1.02)	0.01	0.52
Day 1	1.04 (1.03 to 1.05)	< 0.001	0.55
Day 2	1.03 (1.02 to 1.04)	< 0.001	0.53
Arterial lactate			
ICU admission	1.85 (1.70 to 2.11)	< 0.001	0.63
Day 1	1.84 (1.70 to 2.00)	< 0.001	0.65
Day 2	2.26 (2.02 to 2.53)	< 0.001	0.65
ScVO <sub>2</sub>			
ICU admission	1.00 (0.998 to 1.00)	0.26	0.49
Day 1	1.00 (0.997 to 1.01)	0.36	0.49
Day 2	1.01 (0.996 to 1.02)	0.36	0.49

Multiple regression was used and adjustment was performed on male sex, chronic renal disease, SAPS II, and surgical intervention type. Data were expressed as odds ratios with 95% confidence intervals. Areas under the curve (AUCs) are expressed as proportions. Commonly-used diagnostic AUCs are: greater than 0.9 indicates high accuracy, 0.7–0.9 indicates moderate accuracy, 0.5–0.7 indicates low accuracy, and 0.5 indicates a chance result

AUC = area under the curve; CI = confidence interval; CO<sub>2</sub> gap = venous-to-arterial carbon dioxide difference; ICU = intensive care unit; OR = odds ratio; SAPS = simplified acute physiology score; ScVO<sub>2</sub> = central venous oxygen saturation

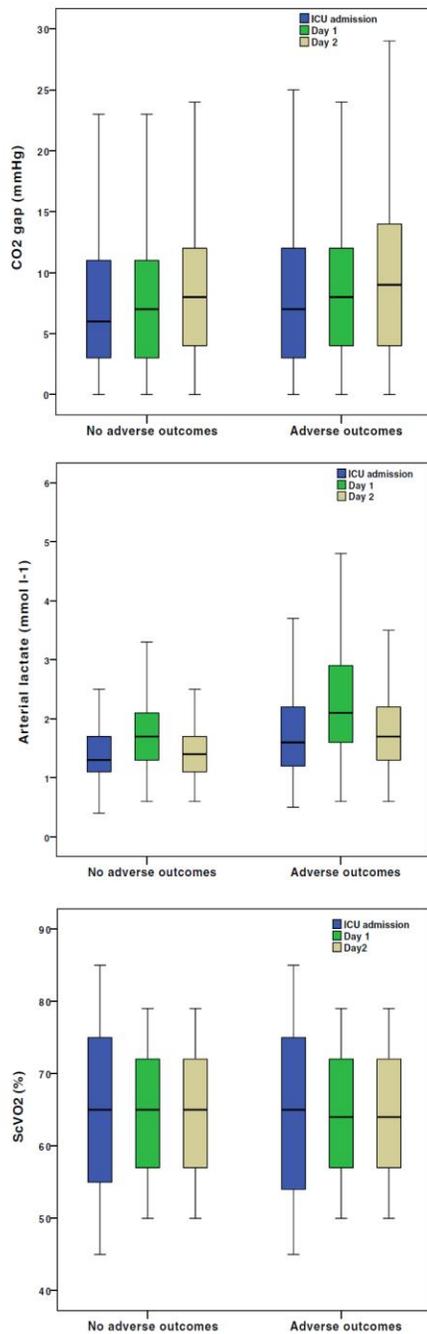
complicated. Finally, time point measurements may differ and this may account for these differences.

The heterogeneity of the findings between cardiac surgery and sepsis can be explained by possible changes in the relationship between PCO<sub>2</sub> and CO<sub>2</sub> content over time during cardiac surgery with CPB, thus altering the interpretation of the CO<sub>2</sub> gap.<sup>31,32</sup> The k factor (which defines the relationship between PCO<sub>2</sub> and CCO<sub>2</sub>) depends on the state of hypoxia, hematocrit, temperature, and anemia.<sup>33–35</sup> These factors are all in play during cardiac surgery, so the CO<sub>2</sub> gap may not reflect the CCO<sub>2</sub>. For example, the k factor increases in tissue hypoxia, increasing the CO<sub>2</sub> gap, even if the veno-arterial difference in CCO<sub>2</sub> does not change. According to Ruokonen *et al.*, an increase in the CO<sub>2</sub> gap is frequent after cardiac surgery and better reflects alterations in systemic and regional perfusion than tissue hypoxia.<sup>36</sup> Hemodilution was investigated by Dubin *et al.* and changes in the CO<sub>2</sub> gap were explained by a rightward shift of the relationship between PCO<sub>2</sub> and CCO<sub>2</sub>.<sup>37</sup> Thus, the CO<sub>2</sub> gap increases as the cardiac index decreases or as CO<sub>2</sub> production increases. This explanation requires that homogeneous perfusion reflect total CO<sub>2</sub> production, which is only partially true during cardiac surgery. It has been shown that CPB induces capillary shunting, resulting in heterogeneous organ perfusion.<sup>31,38</sup> Disturbances in organ perfusion and metabolic changes induced by CPB likely interfere with the ability of the CO<sub>2</sub> gap to detect tissue hypoxia.

Our study had several major limitations. Data on the cardiac index would have been valuable to confirm that it was not low and to provide a more complete interpretation of our results, particularly regarding the significant association with the CO<sub>2</sub> gap but not with the ScVO<sub>2</sub>. Data on the parameters that influence PCO<sub>2</sub> would also have helped us better interpret the CO<sub>2</sub> gap. Moreover, several studies have focused on the CO<sub>2</sub> gap/Ca-vO<sub>2</sub> ratio.<sup>39,40</sup> Unfortunately, we do not have this data. Another limitation was the retrospective design and that we collected data from 2008 to 2018. A lot has change during this time, including CPB management and hemodynamic management.

One of the characteristics of the CO<sub>2</sub> gap is its rapid reversibility.<sup>41,42</sup> Thus, taking measurements at a standardized time point represents measurement bias as outcome could not occur just after the measurement. Furthermore, the complication itself may lead to an increased CO<sub>2</sub> gap and, according to the time of measurement, the arrow of causation could be reversed. This bias is present in most cardiac surgery studies and makes it a limit to the use of the CO<sub>2</sub> gap to predict postoperative outcomes in cardiac surgery.

We believe that our database is reliable, as the data were recorded using the CCAM, and that these outcomes are robust, as they were easy to identify using generic keywords in the database. The strength of our study is that it is the largest sample yet focusing on this topic. External validation would be suitable, but previous studies



**Fig. 3** CO<sub>2</sub> gap, arterial lactate, and ScVO<sub>2</sub> at ICU admission and on days 1 and 2 after admission according to the occurrence of adverse outcomes. CO<sub>2</sub> gap = venous-to-arterial carbon dioxide difference; ICU = intensive care unit; ScVO<sub>2</sub> = central venous oxygen saturation

have obtained similar results.<sup>29</sup> Based on our study and on published data, the CO<sub>2</sub> gap should not be considered as a predictive marker of postoperative complications following cardiac surgery under CPB. Arterial lactate appears to show better sensitivity.

### Conclusion

After cardiac surgery with CPB, the CO<sub>2</sub> gap at ICU admission and on days 1 and 2 after ICU admission was associated with postoperative adverse outcomes but with poor diagnostic performance. The CO<sub>2</sub> gap should not be used as a prognostic marker after cardiac surgery with CPB to identify patients who are insufficiently resuscitated.

**Author contributions** Pierre Huette, Christophe Beys, Yazine Mahjoub, and Osama Abou-Arab contributed to all aspects of this manuscript, including study conception and design; acquisition, analysis, and interpretation of data; and drafting the article. Herve Dupont, Pierre-Grégoire Guinot, and Jihad Mallat contributed to the conception and design of the study. Lucie Martineau, Patricia Besserve, Guillaume Haye, and Mathieu Guilbart contributed to the acquisition of data. Momar Diouf contributed to the analysis of data.

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### References

1. Cooley DA, Frazier OH. The past 50 years of cardiovascular surgery. *Circulation* 2000; 102(20 Suppl 4): IV87-93.
2. Engelman DT, Ben Ali W, Williams JB, et al. Guidelines for Perioperative Care in Cardiac Surgery. Enhanced Recovery After Surgery Society Recommendations. *JAMA Surg* 2019; DOI: <https://doi.org/10.1001/jamasurg.2019.1153>.
3. Osawa EA, Rhodes A, Landoni G, et al. Effect of perioperative goal-directed hemodynamic resuscitation therapy on outcomes following cardiac surgery: a randomized clinical trial and systematic review. *Crit Care Med* 2016; 44: 724-33.
4. Ariza M, Gothard JW, Macnaughton P, Hooper J, Morgan CJ, Evans TW. Blood lactate and mixed venous-arterial PCO<sub>2</sub> gradient as indices of poor peripheral perfusion following cardiopulmonary bypass surgery. *Intensive Care Med* 1991; 17: 320-4.
5. Levy B, Desebbe O, Montemont C, Gibot S. Increased aerobic glycolysis through beta2 stimulation is a common mechanism

- involved in lactate formation during shock states. *Shock* 2008; 30: 417-21.
6. Habicher M, von Heymann C, Spiess CD, Wernecke KD, Sander M. Central venous-arterial pCO<sub>2</sub> difference identifies microcirculatory hypoperfusion in cardiac surgical patients with normal central venous oxygen saturation: a retrospective analysis. *J Cardiothorac Vasc Anesth* 2015; 29: 646-55.
  7. Cobianchi L, Peloso A, Filisetti C, Mojoli F, Sciutti F. Serum lactate level as a useful predictor of clinical outcome after surgery: an unfulfilled potential? *J Thorac Dis* 2016; 8: E295-7.
  8. 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: 2218-25.
  9. 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; DOI: <https://doi.org/10.1186/cc13160>.
  10. Ceconi 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: 1795-815.
  11. Moussa MD, Durand A, Leroy G, et al. Central venous-to-arterial PCO<sub>2</sub> difference, arteriovenous oxygen content and outcome after adult cardiac surgery with cardiopulmonary bypass: a prospective observational study. *Eur J Anaesthesiol* 2019; 36: 279-89.
  12. Guinot P-G, Badoux L, Bernard E, Abou-Arab O, Lorne E, Dupont H. Central venous-to-arterial carbon dioxide partial pressure difference in patients undergoing cardiac surgery is not related to postoperative outcomes. *J Cardiothorac Vasc Anesth* 2017; 31: 1190-6.
  13. von Elm E, Altman DG, Egger M, et al. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med* 2007; 147: 573-7.
  14. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract* 2012; 120: c179-84.
  15. Paugam-Burtz C, Levesque E, Louvet A, et al. Insuffisance hépatique en soins critiques. Recommandations formalisées d'expert. Société Française d'Anesthésie et de Réanimation. Available from URL: <https://sfar.org/wp-content/uploads/2018/09/RFE-IH-soins-critiques.pdf> (accessed September 2020).
  16. Torres A, Niederman MS, Chastre J, et al. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociación Latinoamericana del Tórax (ALAT). *Eur Respir J* 2017; DOI: <https://doi.org/10.1183/13993003.00582-2017>.
  17. Ince C. The microcirculation is the motor of sepsis. *Crit Care* 2005; 9(Suppl 4): S13-9.
  18. Mallat J, Lemyze M, Tronchon L, Vallet B, Thevenin D. Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock. *World J Crit Care Med* 2016; 5: 47-56.
  19. Guinot PG, Guilbart M, Hchikat AH, et al. Association between end-tidal carbon dioxide pressure and cardiac output during fluid expansion in operative patients depend on the change of oxygen extraction. *Medicine (Baltimore)* 2016; DOI: <https://doi.org/10.1097/MD.0000000000003287>.
  20. Groeneveld AB. Interpreting the venous-arterial PCO<sub>2</sub> difference. *Crit Care Med* 1998; 26: 979-80.
  21. Huette P, Ellouze O, Abou-Arab O, Guinot PG. Venous-to-arterial pCO<sub>2</sub> difference in high-risk surgical patients. *J Thorac Dis* 2019; 11(Suppl 11): S1551-7.
  22. Mesquida J, Saludes P, Gruartmoner G, et al. Central venous-to-arterial carbon dioxide difference combined with arterial-to-venous oxygen content difference is associated with lactate evolution in the hemodynamic resuscitation process in early septic shock. *Crit Care* 2015; DOI: <https://doi.org/10.1186/s13054-015-0858-0>.
  23. 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: 818-22.
  24. Bakker J, Vincent JL, Gris P, Leon M, Coffernils M, Kahn RJ. Venous-arterial carbon dioxide gradient in human septic shock. *Chest* 1992; 101: 509-15.
  25. Yuan S, He H, Long Y. Interpretation of venous-to-arterial carbon dioxide difference in the resuscitation of septic shock patients. *J Thorac Dis* 2019; 11(Suppl 11): S1538-43.
  26. Robin E, Futier E, Pires O, et al. Central venous-to-arterial carbon dioxide difference as a prognostic tool in high-risk surgical patients. *Crit Care* 2015; DOI: <https://doi.org/10.1186/s13054-015-0917-6>.
  27. Futier E, Robin E, Jabaudon M, et al. Central venous O<sub>2</sub> saturation and venous-to-arterial CO<sub>2</sub> difference as complementary tools for goal-directed therapy during high-risk surgery. *Crit Care* 2010; DOI: <https://doi.org/10.1186/cc9310>.
  28. Mukai A, Suehiro K, Kimura A, et al. Comparison of the venous-arterial CO<sub>2</sub> to arterial-venous O<sub>2</sub> content difference ratio with the venous-arterial CO<sub>2</sub> gradient for the predictability of adverse outcomes after cardiac surgery. *J Clin Monit Comput* 2020; 34: 41-53.
  29. Chen T, Pan T, Luo X, Wang D. High central venous-to-arterial CO<sub>2</sub> difference is associated with poor outcomes in patients after cardiac surgery: a propensity score analysis. *Shock* 2019; DOI: <https://doi.org/10.1097/SHK.0000000000001324>.
  30. Ray P, Le Manach Y, Riou B, Houle TT. Statistical evaluation of a biomarker. *Anesthesiology* 2010; 112: 1023-40.
  31. Koning NJ, Simon LE, Asfar P, Baufreton C, Boer C. Systemic microvascular shunting through hyperdynamic capillaries after acute physiological disturbances following cardiopulmonary bypass. *Am J Physiol Heart Circ Physiol* 2014; 307: H967-75.
  32. Takami Y, Masumoto H. Mixed venous-arterial CO<sub>2</sub> tension gradient after cardiopulmonary bypass. *Asian Cardiovasc Thorac Ann* 2005; 13: 255-60.
  33. McHardy GJ. The relationship between the differences in pressure and content of carbon dioxide in arterial and venous blood. *Clin Sci* 1967; 32: 299-309.
  34. Cavaliere F, Giovannini I, Chiarla C, et al. Comparison of two methods to assess blood CO<sub>2</sub> equilibration curve in mechanically ventilated patients. *Respir Physiol Neurobiol* 2005; 146: 77-83.
  35. Gavelli F, Teboul JL, Monnet X. How can CO<sub>2</sub>-derived indices guide resuscitation in critically ill patients? *J Thorac Dis* 2019; 11(Suppl 11): S1528-37.
  36. Ruokonen E, Soini HO, Parviainen I, Kosonen P, Takala J. Venous-arterial CO<sub>2</sub> gradient after cardiac surgery: relation to systemic and regional perfusion and oxygen transport. *Shock* 1997; 8: 335-40.
  37. Dubin A, Estenssoro E, Murias G, et al. Intramucosal-arterial Pco<sub>2</sub> gradient does not reflect intestinal dysoxia in anemic hypoxia. *J Trauma* 2004; 57: 1211-7.
  38. Koning NJ, Vonk AB, Meesters MI, et al. Microcirculatory perfusion is preserved during off-pump but not on-pump cardiac surgery. *J Cardiothorac Vasc Anesth* 2014; 28: 336-41.
  39. He H, Long Y, Liu D, Wang X, Tang B. The prognostic value of central venous-to-arterial CO<sub>2</sub> Difference-arterial-central venous

- O<sub>2</sub> difference ratio in septic shock patients with central venous O<sub>2</sub> saturation  $\geq$  80. *Shock* 2017; 48: 551-7.
40. Bar S, Grenez C, Nguyen M, et al. Predicting postoperative complications with the respiratory exchange ratio after high-risk noncardiac surgery: a prospective cohort study. *Eur J Anaesthesiol* 2019; DOI: <https://doi.org/10.1097/EJA.0000000000001111>.
41. 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: 1312-6.
42. Mathias DW, Clifford PS, Klopfenstein HS. Mixed venous blood gases are superior to arterial blood gases in assessing acid-base status and oxygenation during acute cardiac tamponade in dogs. *J Clin Invest* 1988; 82: 833-8.

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In a recent meta-analysis that included 21 studies (n= 2,155 patients) from medical (n=925), cardiovascular (n=685), surgical (n=483), and mixed (n=62) ICUs, a high  $\Delta\text{PCO}_2_{\text{Cent}}$  was associated with higher lactate levels, lower cardiac output and central venous oxygen saturation. Also, a high  $\Delta\text{PCO}_2_{\text{Cent}}$  was associated with increased mortality (OR of 2.22, 95% CI: 1.3-3.82; p=0.004) in patients with shock, but in only medical and surgical patients [155]. No association with outcomes was found for cardiac surgery patients. However, the meta-analysis included only 2 studies in cardiac surgery studies, and these negative results should be interpreted with caution. Thus, the relationship between  $\Delta\text{PCO}_2_{\text{Cent}}$  or  $\Delta\text{PCO}_2_{\text{Mix}}$  and outcomes in cardiac surgery patients remains so far unclear.

$\Delta\text{PCO}_2_{\text{Cent}}$  or  $\Delta\text{PCO}_2_{\text{Mix}}$  reflects the adequacy between cardiac index and oxygen demand and can help titrate inotropes in critically ill patients. Dobutamine is a synthetic catecholamine with strong inotropic effects on the myocardium owing to its predominately  $\beta_1$ -adrenergic properties [156]. Dobutamine administration in these patients aims to restore an appropriate cardiac index to provide adequate oxygen supply to meet the tissue oxygen requirements. However, dobutamine, in parallel to its effects on systemic hemodynamics, may increase  $\text{VO}_2$  and, therefore, tissue  $\text{VCO}_2$  through its direct cellular metabolic effects [112, 157]. Two studies investigated the impact of an incremental increase of dobutamine by 5, from 0 to 15  $\mu\text{g}/\text{kg}/\text{min}$ , one in stable septic shock patients [158] and the other in stable cardiogenic shock patients [112].  $\Delta\text{PCO}_2_{\text{Cent}}$  was a good indicator of the change of  $\text{VCO}_2$  induced by dobutamine and was able to depict the hemodynamic effects of the thermogenic effects of dobutamine in both populations [159].



## Usefulness of venous-to-arterial partial pressure of CO<sub>2</sub> difference to assess oxygen supply to demand adequacy: effects of dobutamine

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**Abstract:** The central venous O<sub>2</sub> saturation value and lactic acid levels are part of the diagnostic and therapeutic work up of patients in shock. These usual indicators of tissue hypoxia don't fully describe the adequacy of tissue perfusion. There is ample evidence that supplementing this data with the venous-to-arterial partial pressure of CO<sub>2</sub> (PCO<sub>2</sub>) difference ( $\Delta$ PCO<sub>2</sub>) complements the clinician's tools when treating patients with shock. Based on a modified Fick equation as it applies to CO<sub>2</sub>, in patients in a steady state, the  $\Delta$ PCO<sub>2</sub> reflects the cardiac output (CO). This observation has been shown to be of clinical value in resuscitating patients in shock. Moreover, the  $\Delta$ PCO<sub>2</sub> can be used to titrate inotropes, and differentiate the hemodynamic from the metabolic effect of dobutamine.

**Keywords:** Pressure of CO<sub>2</sub> (PCO<sub>2</sub>) gap; tissue hypoperfusion; venous oxygen saturation (SVO<sub>2</sub>); oxygen consumption; oxygen delivery (DO<sub>2</sub>); dobutamine

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### Introduction

Shock is defined as an imbalance between oxygen delivery (DO<sub>2</sub>) and O<sub>2</sub> demand. O<sub>2</sub> derived parameters have been historically used as reflections of the state of organ perfusion and as targets for resuscitation (1). While such parameters, including the mixed venous oxygen saturation (SVO<sub>2</sub>) and lactic acid levels, have great clinical information, studies have shown that they fail to fully describe the clinical picture and can be deficient when assisting the clinician in deciding on the next therapeutic step (2-6). In the following review, we will address the clinical utility of integrating venous-to-arterial partial pressure of CO<sub>2</sub> (PCO<sub>2</sub>) difference ( $\Delta$ PCO<sub>2</sub>) in patients in septic shock receiving dobutamine.

### *Why O<sub>2</sub> based parameters draw an incomplete picture during shock*

For patients in shock, a reduced SVO<sub>2</sub> often reflects an exaggerated O<sub>2</sub> extraction, secondary to low DO<sub>2</sub>. When DO<sub>2</sub> drops below a critical threshold, anaerobic metabolism is triggered and lactic acidosis ensues. While a reduced SVO<sub>2</sub> reflects low O<sub>2</sub> availability to the tissues, a normal SVO<sub>2</sub> does not exclude persistent tissue hypoperfusion. This observation is explained by a heterogeneous microcirculation, capillary shunting or mitochondrial dysfunction, where SVO<sub>2</sub> is normal or even elevated, despite hypoxia at the cellular level, and as is often seen in patients with sepsis (7). Similarly, lactic acid, while increased when

tissues are under perfused and anaerobic metabolism sets in, can also be increased in other conditions, such as liver dysfunction or inflammatory induced aerobic glycolysis (8).

Our current emphasis to monitor tissue perfusion by focusing on systemic blood flow, and the usual indicators of global tissue hypoxia (such as SVO<sub>2</sub> and blood lactate levels) can be misleading, especially when used alone. The ΔPCO<sub>2</sub> can assist the clinician in making adequate diagnostic and therapeutic decisions.

#### *The Fick principle as it applies to CO<sub>2</sub>*

Historically, investigators described a rise in partial pressure of venous CO<sub>2</sub> in patients in cardiac arrest or in various types of shock. This has triggered an interest in studying the ΔPCO<sub>2</sub> in these clinical scenarios.

The Fick principle allows the determination of the cardiac output (CO) based on arterial and mixed venous O<sub>2</sub> contents and O<sub>2</sub> metabolism, in patients in a steady state. According to the classical equation, CO equals O<sub>2</sub> uptake (VO<sub>2</sub>) divided by the arteriovenous O<sub>2</sub> content difference.

$$CO = VO_2 / (CaO_2 - CvO_2) \quad [1]$$

Where CaO<sub>2</sub>, arterial oxygen content; CvO<sub>2</sub>, venous oxygen content.

This same principle of conservation of mass applies to CO<sub>2</sub>. CO<sub>2</sub> is transported in the blood mostly as bicarbonate with a small percentage dissolved in plasma and another fraction bound to hemoglobin (Hb). PCO<sub>2</sub> can substitute for the CO<sub>2</sub> content over the physiologic range of PCO<sub>2</sub>, given the quasi linear relation over that range (9). This relationship can be affected by oxygenation of Hb (Haldane effect); low O<sub>2</sub> saturation favors CO<sub>2</sub> binding to Hb, increasing CO<sub>2</sub> content for the same PCO<sub>2</sub> level. Similarly, the acid base status affects carbamino hemoglobin so that metabolic acidosis increases PCO<sub>2</sub> for the same level of CO<sub>2</sub> content (10).

While the Fick equation relies on mixed venous values obtained from a pulmonary artery catheter (and reflecting total venous blood returning to the heart), central venous values obtained from a central venous catheter correlate with the mixed venous and are more readily available at the bedside (11,12). Based on these studied assumptions, we can reach the following equation:

$$CO = K \times VCO_2 / (PcvCO_2 - PaCO_2) \quad [2]$$

Where (PcvCO<sub>2</sub> - PaCO<sub>2</sub>) = ΔPCO<sub>2</sub> with PcvCO<sub>2</sub> being the central venous PCO<sub>2</sub>, PaCO<sub>2</sub> being the arterial PCO<sub>2</sub>, VCO<sub>2</sub> the CO<sub>2</sub> production by the cells, K is the pseudo-linear coefficient supposed to be constant in physiological

states.

While using the Fick equation as it applies to CO<sub>2</sub>, ΔPCO<sub>2</sub> depends on CO<sub>2</sub> production (aerobic and anaerobic metabolism) and the CO (13).

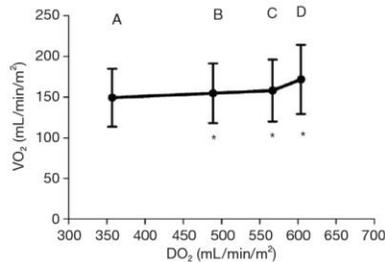
As CO<sub>2</sub> is produced in the peripheral tissues during metabolism, the venous CO<sub>2</sub> content and venous PCO<sub>2</sub> are higher than their arterial values. Under normal physiological conditions, ΔPCO<sub>2</sub> ranges between 2 and 6 mmHg (14).

#### **CO<sub>2</sub> metabolism and physiology**

The relationship between metabolism and ΔPCO<sub>2</sub> is complex. When patients develop shock and a low CO state, DO<sub>2</sub> drops. Initially aerobic metabolism is maintained, VO<sub>2</sub> remains constant as O<sub>2</sub> extraction increases in a compensatory fashion. This is reflected by a drop in SVO<sub>2</sub>. Interestingly, ΔPCO<sub>2</sub> is found to widen in this clinical scenario of hypoperfusion, and not because of metabolic changes. This is rather due to the low flow state causing CO<sub>2</sub> stagnation (i.e., reduction in CO<sub>2</sub> washout) (10). Vallet *et al.* nicely demonstrated that it is the blood flow, rather than partial pressure of oxygen (PaO<sub>2</sub>) which affects the ΔPCO<sub>2</sub> (by comparing ischemic hypoxia, where blood flow is reduced below a critical DO<sub>2</sub> threshold *vs.* hypoxic hypoxia where PaO<sub>2</sub> was reduced) (15). When DO<sub>2</sub> drops below a critical level and anaerobic metabolism is triggered, anaerobic CO<sub>2</sub> production is initiated, causing ΔPCO<sub>2</sub> to widen further. Despite the contributions from aerobic and anaerobic metabolism, the impact of CO is much larger on ΔPCO<sub>2</sub>, given the curvilinear relationship between ΔPCO<sub>2</sub> and CO.

#### **Clinical evidence**

Multiple studies observed the clinical value of ΔPCO<sub>2</sub> in patients in septic shock. As previously described through the Fick equation, ΔPCO<sub>2</sub>, in the right clinical context of a steady state, reflects the CO and its adequacy to wash out CO<sub>2</sub> relative to the patient's metabolic state (16). This correlates with observational studies showing how ΔPCO<sub>2</sub> reflects tissue perfusion parameters. Patients with high ΔPCO<sub>2</sub> on admission have high lactate levels (17). Lactate clearance is much improved in patients whose ΔPCO<sub>2</sub> normalized (it was reduced to <6 mmHg), reflecting improved organ perfusion (18). An elevated ΔPCO<sub>2</sub> (above 6 mmHg) identifies patients who are inadequately resuscitated, despite central venous oxygen saturation (SevO<sub>2</sub>) >70% (19). Reflecting the state of



**Figure 1** Time course of oxygen consumption and oxygen delivery during dobutamine infusion. Data are presented as mean  $\pm$  SD. \*,  $P < 0.001$  vs. baseline (17). A, baseline; B, dobutamine 5  $\mu\text{g}/\text{kg}/\text{min}$ ; C, dobutamine 10  $\mu\text{g}/\text{kg}/\text{min}$ ; D, dobutamine 15  $\mu\text{g}/\text{kg}/\text{min}$  (22).

organ perfusion, an exaggerated  $\Delta\text{PCO}_2$  also carries a prognostic weight and signals an increased mortality (13,17). Patients with a high  $\Delta\text{PCO}_2$  at the time of presentation have worse outcomes, perhaps because of low organ perfusion. Similarly, patients whose gap is not reduced at 6 h have a higher risk of progressing to multiorgan failure.

### The role of $\Delta\text{PCO}_2$ in titrating inotropes

The observation that the venous-to-arterial difference in  $\text{PCO}_2$  can be used clinically to reflect changes in CO has been evaluated in treating patients with septic and cardiogenic shock.

Mecher *et al.* studied 37 patients with severe sepsis, before and after fluid resuscitation (16). They found that an initial high  $\Delta\text{PCO}_2$  reflected a low CO. Upon completion of fluid resuscitation,  $\Delta\text{PCO}_2$  was reduced to  $< 6$  mmHg, as the CO was found to have increased. This opens the door for  $\Delta\text{PCO}_2$  to be used as a resuscitation target.

Other studies looked at the impact of inotropic agents, specifically dobutamine. Inotropes play an integral role in resuscitating patients with shock. While most patients with cardiogenic shock are likely to benefit from inotropic support, patients with vasodilatory shock will behave differently. In septic shock every effort is made to improve  $\text{DO}_2$ . This includes optimizing the stroke volume by increasing the intravascular volume, improving  $\text{O}_2$  content and delivery by transfusing blood, adjusting the mean arterial pressure (MAP) to enhance the perfusion pressure. Patients who continue to exhibit signs of hypoperfusion despite adequate MAP and hemoglobin concentration

could suffer from reduced cardiac function and benefit from increasing cardiac contractility and organ perfusion through the use of inotropes (20).

These investigators looked at patients with no evidence for anaerobic metabolism or global tissue hypoxia. They explicitly included patients who had signs of hypoperfusion (based on oliguria, mottled skin and  $\text{ScvO}_2 < 70\%$ ), but whose MAP was  $> 65$  mmHg, Hb was  $> 8$  g/dL, who were no longer fluid responsive and whose lactate levels were normal. These were patients who exhibited signs of low perfusion, despite optimal intravascular volume, blood  $\text{O}_2$  carrying capacity and MAP, and would potentially benefit from inotropy at this clinical stage. Two such studies have been conducted: Mallat *et al.* studied 22 patients in septic shock while Teboul *et al.* studied 10 patients with congestive heart failure (21,22). These studies investigated the impact of an incremental increase of dobutamine by 5, from 0 to 15  $\mu\text{g}/\text{kg}/\text{min}$ .

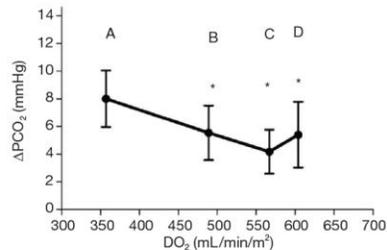
Both investigators found that increasing the rate of dobutamine infusion was accompanied by an increase in cardiac index (CI) and  $\text{DO}_2$ , as anticipated. A dichotomous response was found for dobutamine doses between 0 and 10 and 10–15  $\mu\text{g}/\text{kg}/\text{min}$ .

Between 0 and 10  $\mu\text{g}/\text{kg}/\text{min}$ , dobutamine increased the CI and  $\text{DO}_2$ , in a dose dependent fashion (CI increased from 3 to 4.6 L/min/m<sup>2</sup>).  $\text{VCO}_2$  increased, but to a much lesser extent compared to CI (Figure 1). This explains the commensurate decrease in  $\Delta\text{PCO}_2$ , from  $8 \pm 2$  to  $4.2 \pm 1.6$  mmHg (Figure 2), attributed to increased blood flow and improved  $\text{CO}_2$  wash out. As anticipated,  $\text{SVO}_2$ ,  $\text{ScvO}_2$ , and  $\text{O}_2$  extraction improved as  $\text{DO}_2$  increased to organs who were still in need for  $\text{O}_2$ . At these lower doses, dobutamine's main impact is on the patient's hemodynamics, improved organ perfusion and  $\text{CO}_2$  clearance.

On the other hand, while increasing dobutamine from 10 to 15  $\mu\text{g}/\text{kg}/\text{min}$  CI increased from 4.6 to 5.2 L/min/m<sup>2</sup>, and  $\text{VCO}_2$  was found to increase to similar levels. As such,  $\Delta\text{PCO}_2$  remained constant (Figure 2). The increase in  $\text{CO}_2$  production is explained by the  $\beta$  adrenergic stimulation effect of dobutamine. It has a direct cellular metabolic effect, increasing  $\text{O}_2$  consumption and  $\text{CO}_2$  production. This thermogenic effect is only seen at these higher doses of dobutamine (10–15  $\mu\text{g}/\text{kg}/\text{min}$ ).

### Conclusions

Tissue hypoperfusion during circulatory failure is associated with stagnation of  $\text{CO}_2$  in the tissues and



**Figure 2** Evolution of  $\Delta\text{PCO}_2$  and oxygen delivery during dobutamine infusion. \*,  $P < 0.001$  vs. baseline (17). A, baseline; B, dobutamine 5  $\mu\text{g}/\text{kg}/\text{min}$ ; C, dobutamine 10  $\mu\text{g}/\text{kg}/\text{min}$ ; D, dobutamine 15  $\mu\text{g}/\text{kg}/\text{min}$ . Data are presented as mean  $\pm$  SD (22).

increased tissues venous  $\text{PCO}_2$ . The  $\Delta\text{PCO}_2$  could be considered as a marker of adequacy of venous blood flow to remove the  $\text{CO}_2$  produced by the peripheral tissues, based on the Fick equation. This requires the patient to be in a steady state, and assumes the mixed venous values are close to the central venous values, which is the case in most patients.

In patients in cardiogenic or vasodilatory shock, without signs of global hypoxia or anaerobic metabolism,  $\Delta\text{PCO}_2$  can be used to reflect the CO. This inverse correlation is a marker of the adequacy of the venous blood efflux to remove the total  $\text{CO}_2$  produced by the peripheral tissues.  $\Delta\text{PCO}_2$  can be used to distinguish the hemodynamic from the metabolic effect of dobutamine when this inotropic agent is selected.

$\Delta\text{PCO}_2$  is easily obtained at the bedside and when included with other markers for tissue perfusion, can provide ample information to the clinician. This makes it an attractive marker in the ICU setting.

### Acknowledgments

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### Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

### References

1. 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;345:1368-77.

2. Process Investigators, Yealy DM, Kellum JA, et al. A randomized trial of protocol-based care for early septic shock. *N Engl J Med* 2014;370:1683-93.
3. ARISE Investigators, ANZICS Clinical Trials Group, Peake SL, et al. Goal-directed resuscitation for patients with early septic shock. *N Engl J Med* 2014;371:1496-506.
4. Mouncey PR, Osborn TM, Power GS, et al. Trial of early, goal-directed resuscitation for septic shock. *N Engl J Med* 2015;372:1301-11.
5. Van Beest P, Wietasch G, Scheeren T, et al. Clinical review: use of venous oxygen saturations as a goal—a yet unfinished puzzle. *Crit Care* 2011;15:232.
6. ProCESS Investigators, Yealy DM, Kellum JA, et al. A randomized trial of protocol-based care for early septic shock. *N Engl J Med* 2014;370:1683-93.
7. Charlton M, Sims M, Coats T, et al. The microcirculation and its measurement in sepsis. *J Intensive Care Soc* 2017;18:221-7.
8. Suetrong B, Walley KR. Lactic Acidosis in Sepsis: It's Not All Anaerobic: Implications for Diagnosis and Management. *Chest* 2016;149:252-61.
9. Giovannini I, Chiarla C, Boldrini G, et al. Calculation of venoarterial  $\text{CO}_2$  concentration difference. *J Appl Physiol* (1985) 1993;74:959-64.
10. Teboul JL, Scheeren T. Understanding the Haldane effect. *Intensive Care Med* 2017;43:91-3.
11. Walley KR. Use of central venous oxygen saturation to guide therapy. *Am J Resp Crit Care Med* 2011;184:514-20.
12. 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:R294.
13. Mallat J, Lemyze M, Tronchon L, et al. Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock. *World J Crit Care Med* 2016;5:47-56.
14. Diaztagle Fernández JJ, Rodríguez Murcia JC, Sprockel Díaz JJ. Venous-to-arterial carbon dioxide difference in the resuscitation of patients with severe sepsis and septic shock: A systematic review. *Med Intensiva* 2017;41:401-10.
15. Vallet B, Teboul JL, Cain S, et al. Venoarterial  $\text{CO}_2$  difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol* (1985) 2000;89:1317-21.
16. Mecher CE, Rackow EC, Astiz ME, et al. Venous hypercarbia associated with severe sepsis and systemic hypoperfusion. *Crit Care Med* 1990;18:585-9.

17. Bakker J, Coffernils M, Leon M, et al. Blood lactate levels are superior to oxygen-derived variables in predicting outcome in human septic shock. *Chest* 1991;99:956-62.
18. Mallat J, Pepy F, Lemyze M, et al. Central venous-to-arterial carbon dioxide partial pressure difference in early resuscitation from septic shock: a prospective observational study. *Eur J Anaesthesiol* 2014;31:371-80.
19. 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;34:2218-25.
20. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med* 2017;43:304-77.
21. Teboul JL, Mercat A, Lenique F, et al. Value of the venous-arterial  $PCO_2$  gradient to reflect the oxygen supply to demand in humans: effects of dobutamine. *Crit Care Med* 1998;26:1007-10.
22. Mallat J, Benzidi Y, Salleron J, et al. Time course of central venous-to-arterial carbon dioxide tension difference in septic shock patients receiving incremental doses of dobutamine. *Intensive Care Med* 2014;40:404-11.

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## 2. Combination of $\Delta\text{PCO}_2$ with $\Delta\text{O}_2$

In a retrospective study that included a mixed population of critically ill patients, Mekontso-Dessap et al. [5] found that  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$  ratio was significantly higher in patients with elevated blood arterial lactate levels ( $> 2$  mmol/L) than in patients with normal lactate levels.  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}}$  was able to detect the presence of hyperlactatemia, considered to reflect tissue hypoxia (anaerobic metabolism) with an AUROC of 0.85 and a best cutoff value of 1.4 mmHg/mL. Moreover, patients with  $\Delta\text{PCO}_2\text{_{Mix}}/\Delta\text{O}_2\text{_{Mix}} < 1.4$  mmHg/mL had better 30-day survival than patients with a ratio  $\geq 1.4$  mmHg/mL. The authors concluded that this ratio could be utilized as a reliable indicator of the presence of global anaerobic metabolism in critically ill patients. Monnet et al. [160] found that this ratio, calculated from central venous blood,  $\Delta\text{PCO}_2\text{_{Cent}}/\Delta\text{O}_2\text{_{Cent}}$ , predicted (AUROC of 0.91) an increase in  $\text{VO}_2$  after a fluid-induced increase in  $\text{DO}_2$  ( $\text{VO}_2/\text{DO}_2$  dependency), and thus, can be able to detect the presence of global tissue hypoxia as accurately as the blood lactate level and far better than  $\text{ScvO}_2$ . In a prospective study that included 98 septic shock patients, Mallat et al. also found that the baseline  $\Delta\text{PCO}_2\text{_{Cent}}/\Delta\text{O}_2\text{_{Cent}}$  ratio had an excellent ability to predict tissue hypoxia better than lactate levels (AUROC of 0.96 vs. 0.745, respectively) defined as the increase in  $\text{VO}_2 \geq 15\%$  induced by volume expansion with a best cutoff value of 1.68 mmHg/mL [161]. In a population of 35 septic shock patients with normalized mean arterial pressure and  $\text{ScvO}_2$ , Mesquida et al. [162] showed that the presence of elevated  $\Delta\text{PCO}_2\text{_{Cent}}/\Delta\text{O}_2\text{_{Cent}}$  values at baseline was associated with the absence of lactate clearance within the following hours, and this condition was also associated with mortality. However, this was a retrospective study and it was not powered to explore the prognostic value of the  $\Delta\text{PCO}_2\text{_{Cent}}/\Delta\text{O}_2\text{_{Cent}}$  ratio. In a prospective study that included 135

septic shock patients [126], OspinaTascon et al. [135] found that the mixed venous-to-arterial  $\text{CCO}_2$  difference/ $\Delta\text{O}_2$ \_Mix ratio at baseline and six hours after resuscitation was an independent prognostic factor of 28-day mortality, but not  $\Delta\text{PCO}_2$ \_Mix/ $\Delta\text{O}_2$ \_Mix ratio. The authors attributed this discrepancy to the fact that the  $\text{PCO}_2/\text{CCO}_2$  relationship is curvilinear rather than linear and is influenced by many factors such as pH and oxygen saturation (Haldane effect), and under these conditions, the mixed venous-to-arterial  $\text{CCO}_2$  difference/ $\Delta\text{O}_2$ \_Mix ratio might not be equivalent to  $\Delta\text{PCO}_2$ \_Mix/ $\Delta\text{O}_2$ \_Mix ratio.

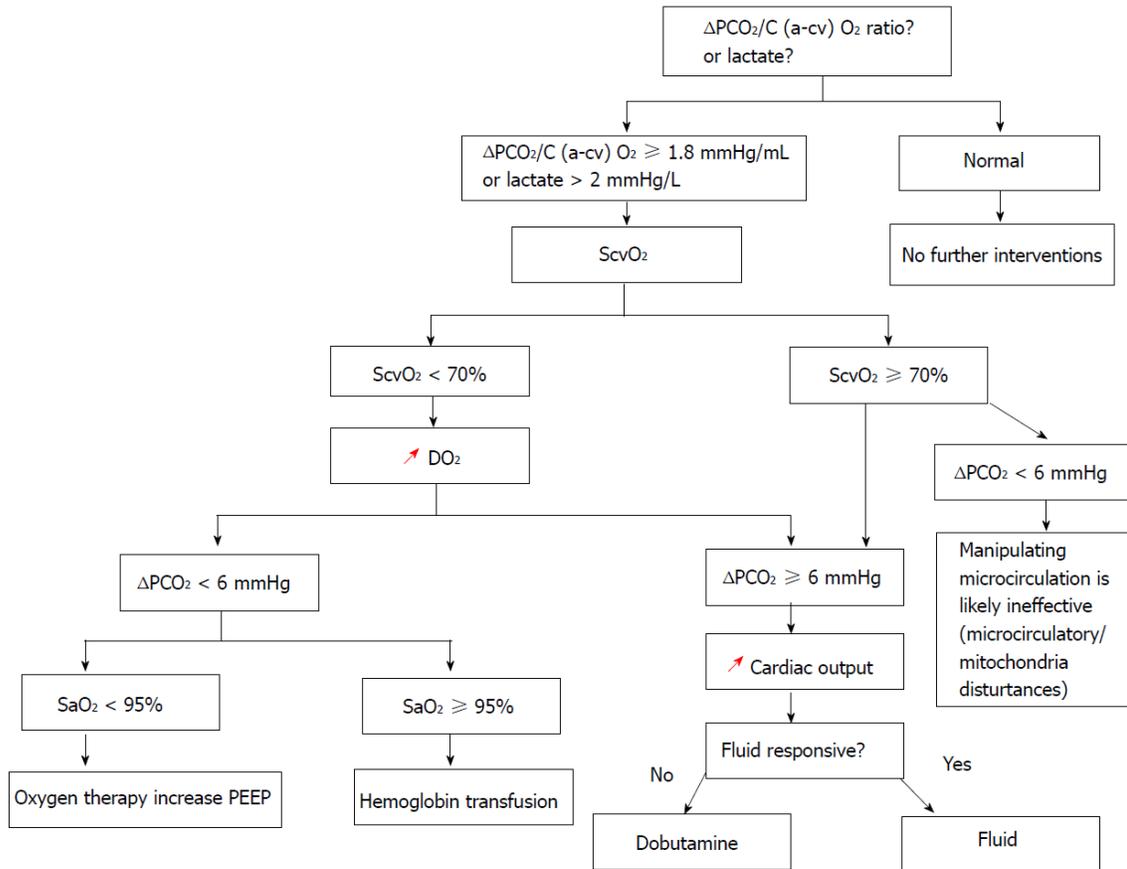
However, the relationship between  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio with tissue hypoxia is less evident in cardiac surgery patients. Indeed, in 72 cardiac surgery patients,  $\Delta\text{PCO}_2$ \_Cent/ $\Delta\text{O}_2$ \_Cent ratio was able to reliably predict the presence of tissue hypoxia defined by an increase in  $\text{VO}_2 > 10\%$  induced by fluid resuscitation with an AUROC of  $0.77 \pm 0.1$  and a best cutoff  $\geq 1.6$  mmHg/mL with a sensitivity of 68.8% and a specificity of 87.5% [163]. Nevertheless, Abou-Arab et al. found no relationship between  $\Delta\text{PCO}_2$ \_Mix/ $\Delta\text{O}_2$ \_Mix ratio and the increase in  $\text{VO}_2 \geq 15\%$  after fluid challenge considered as a marker of tissue hypoxia in 110 postoperative cardiac surgery patients [164]. Also,  $\Delta\text{PCO}_2$ \_Mix/ $\Delta\text{O}_2$ \_Mix was not associated with arterial lactate level in this study. Also, several studies did not find an association between  $\Delta\text{PCO}_2$ \_Cent/ $\Delta\text{O}_2$ \_Cent or  $\Delta\text{PCO}_2$ \_Mix/ $\Delta\text{O}_2$ \_Mix ratio and outcomes in cardiac surgery patients. In a prospective study that included 114 critically ill patients after elective cardiac surgery with cardiopulmonary bypass,  $\Delta\text{PCO}_2$ \_Cent/ $\Delta\text{O}_2$ \_Cent could not predict organ dysfunction's development 48 hours after ICU admission [165]. In another prospective study that included 308 ICU patients after cardiac surgery with cardiopulmonary bypass,  $\Delta\text{PCO}_2$ \_Cent/ $\Delta\text{O}_2$ \_Cent was not associated with major postoperative complications that occurred in 56.5% of patients [150]. The discrepancies in

findings between the septic and cardiac surgery populations might be explained by the fact that cardiac surgery with cardiopulmonary bypass is a specific physiological condition with several factors that might affect the  $PCO_2/CCO_2$  relationship.

**Fourth Part**  
**Perspective of the**  
**metabolic approach in the**  
**management of tissue**  
**oxygenation disorders**

## I. Background

Early identification and improvement of tissue hypoperfusion are critical factors in the treatment of septic shock patients. The deficit in tissue perfusion with reduced blood flow should be considered as the primary determinant of an increase in  $\Delta\text{PCO}_2$ .  $\Delta\text{PCO}_2$  should be seen as an indicator of the adequacy of venous blood flow (cardiac output) to clear the  $\text{CO}_2$  generated by the peripheral tissues rather than as a marker of tissue hypoxia. Thus, monitoring  $\Delta\text{PCO}_2$  could be a useful complementary tool to guide the resuscitation in the early phase of septic shock (Figure 6). It can also be combined with the  $\text{O}_2$ -derived parameters in order to calculate the  $\Delta\text{PCO}_2/\Delta\text{O}_2$ , which can be used to detect the presence of global anaerobic metabolism. In such a situation, the presence of low  $\text{ScvO}_2$  ( $< 70\%$ ) should push the physician to increase  $\text{DO}_2$ , and if  $\Delta\text{PCO}_2_{\text{Cent}}$  is increased ( $\geq 6$  mmHg), that indicates that increasing cardiac output is the rational choice to achieve this target (Figure 6). In the presence of a normal/high  $\text{ScvO}_2$  ( $\geq 70\%$ ), an elevated  $\Delta\text{PCO}_2_{\text{Cent}}$  still suggests that rising cardiac output can be indicated with the purpose of reducing global tissue hypoxia (Figure 6). However, if both  $\text{ScvO}_2$  and  $\Delta\text{PCO}_2_{\text{Cent}}$  are normal in a state of global anaerobic metabolism, manipulating the microcirculation will probably be ineffective in reducing oxygen deficit (Figure 6) [6]. Unfortunately, there is no RCT that compared the efficacy of hemodynamic optimization using the metabolic approach to the usual care in septic shock patients. Thus, the effectiveness of the proposed hemodynamic approach in Figure 6 needs to be validated in a multicentric RCT.



**Figure 6.** Metabolic approach guided protocol. ScvO<sub>2</sub>: Central venous oxygen saturation; ΔPCO<sub>2</sub>: Central venous-to-arterial carbon dioxide tension difference; SaO<sub>2</sub>: Arterial oxygen saturation; C(a-cv)O<sub>2</sub>: Central arteriovenous oxygen content difference; DO<sub>2</sub>: Oxygen delivery; PEEP: Positive end expiratory pressure; red arrows: Increasing [6].

## II. Metabolic approach and weaning from mechanical ventilation

The metabolic approach can be used to detect the imbalance between  $VO_2$  and  $DO_2$  in different clinical conditions, such as weaning from mechanical ventilation. Weaning from mechanical ventilation is usually associated with an increase in  $VO_2$  linked to the augmented work of breathing [166, 167]. To meet the metabolic demand of the weaning trial, increases in  $VO_2$  should be accompanied by increases in cardiac output and  $DO_2$ . Some studies found that decreases in venous oxygen saturation during spontaneous breathing trial (SBT) were associated with weaning and extubation failure [168, 169]. Mixed venous oxygen saturation dropped progressively in patients who failed SBT but not in those who succeeded SBT [168]. The drop in  $SvO_2$  was ascribed to an increase in oxygen extraction, probably by the respiratory muscles. Teixeira et al. [169] found that changes in  $ScvO_2$  between mechanical ventilation and the end of SBT were independently associated with extubation failure in patients with weaning difficulty. A decline  $> 4.5\%$  in the  $ScvO_2$  predicted extubation failure with a very good positive predictive value (93%) and negative predictive value (90%).

Since  $\Delta PCO_2$  was a reliable indicator of the adequacy between oxygen supply and oxygen demand in chronic heart failure and septic shock patients [112, 158], Mallat et al., in a prospective multicentric study [170], investigated the usefulness of the metabolic approach to predict weaning outcome from mechanical ventilation. Seventy-five patients were enrolled, and extubation failure was noted in 18 (24%) patients. The ability of the changes in  $\Delta PCO_{2\_Cent}$  ( $\Delta - \Delta PCO_{2\_Cent}$ ) and  $ScvO_2$  ( $\Delta ScvO_2$ ) during SBT to predict extubation outcomes was very good, with AUROCs of 0.865 (95% CI, 0.767–0.933) and 0.856 (95% CI, 0.756–0.926), respectively. However, the combination of  $\Delta ScvO_2$  and  $\Delta - \Delta PCO_{2\_Cent}$  led to improved extubation failure detection, with

an AUROC (0.940; 95% CI, 0.859–0.981) that was significantly better than that observed for  $\Delta\text{ScvO}_2$  ( $p = 0.04$ ) and  $\Delta-\Delta\text{PCO}_2$  ( $p = 0.03$ ). Also,  $\Delta-\Delta\text{PCO}_2\text{Cent}\Delta$  and  $\Delta\text{ScvO}_2$  were independently associated with extubation failure (odds ratio, 1.02; 95% CI, 1.01–1.05;  $p = 0.006$ , and odds ratio, 0.84; 95% CI, 0.70–0.95;  $p = 0.02$ , respectively).

# Central Venous-to-Arterial Pco<sub>2</sub> Difference and Central Venous Oxygen Saturation in the Detection of Extubation Failure in Critically Ill Patients\*

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Didier Thevenin, MD<sup>1</sup>; Marc-Olivier Fischer, MD, PhD<sup>6</sup>

**Objectives:** To evaluate the ability of central venous-to-arterial carbon dioxide pressure difference, central venous oxygen saturation, and the combination of these two parameters to detect extubation failure in critically ill patients.

**Design:** Multicentric, prospective, observational study.

**Setting:** Three ICUs.

**Patients:** All patients who received mechanical ventilation for more than 48 hours and tolerated spontaneous breathing trials with a T-piece for 60 minutes.

**Interventions:** Extubation after successful spontaneous breathing trials. Extubation failure was defined as the need for mechanical ventilation within 48 hours.

**Measurements and Main Results:** The oxygen delivery index, oxygen consumption index, central venous oxygen saturation, central venous-to-arterial carbon dioxide pressure difference, and oxygen extraction were measured immediately before spontaneous breathing trials and at 60 minutes after spontaneous breathing trials initiation. Seventy-five patients were enrolled, and extubation failure was noted in 18 (24%) patients. Oxygen consumption index increased significantly during spontaneous breathing trials in the failure group. Oxygen delivery index increased in both success and

failure groups. Oxygen extraction increased in the failure group ( $p = 0.005$ ) and decreased in the success group ( $p = 0.001$ ). Central venous oxygen saturation decreased in the failure group and increased in the success group ( $p = 0.014$ ).  $\Delta P_{CO_2}$  value increased in the extubation failure group and decreased in the success group ( $p = 0.002$ ). Changes in  $\Delta P_{CO_2}$  ( $\Delta - \Delta P_{CO_2}$ ) and central venous oxygen saturation ( $\Delta ScvO_2$ ) during spontaneous breathing trials were independently associated with extubation failure (odds ratio, 1.02; 95% CI, 1.01–1.05;  $p = 0.006$ , and odds ratio, 0.84; 95% CI, 0.70–0.95;  $p = 0.02$ , respectively).  $\Delta - \Delta P_{CO_2}$  and central venous oxygen saturation could predict extubation failure with areas under the curve of 0.865 and 0.856, respectively; however, their combined areas under the curve was better at 0.940.

**Conclusions:** We found that  $\Delta - \Delta P_{CO_2}$  and central venous oxygen saturation, during spontaneous breathing trials, were independent predictors of weaning outcomes. Combination analysis of both parameters enhanced their diagnostic performance and provided excellent predictability in extubation failure detection in critically ill patients. (*Crit Care Med* 2020; 48:1454–1461)

**Key Words:** extubation failure; oxygen consumption; oxygen delivery; spontaneous breathing trial; venous-to-arterial co<sub>2</sub> difference; venous oxygen saturation

\*See also p. 1536.

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Weaning and extubation are essential steps for the management of critically ill patients when mechanical ventilation (MV) is no longer required. Extubation failure (EF) occurs in approximately 10–30% (1, 2) of all patients meeting the readiness criteria and have tolerated a spontaneous breathing trial (SBT). EF is associated with prolonged MV, as well as increased morbidity and mortality (2). Therefore, the early identification of critically ill patients who are likely to experience EF is vital for improved outcomes.

Discontinuation of ventilatory support can be challenging for clinicians, mainly because the pathophysiology of weaning failure is complex and not fully understood. EF can result from different factors (respiratory, metabolic, neuromuscular),

particularly cardiac factor, and can be caused by the inability of the respiratory muscle pump to tolerate increases in the cardiac and respiratory load (1, 3). Weaning from MV is usually associated with an increase in oxygen consumption ( $\text{V}_{\text{O}_2}$ ) linked to the augmented work of breathing (4–7). To meet the metabolic demand of the weaning trial, increases in  $\text{V}_{\text{O}_2}$  should be accompanied by increases in cardiac output (CO) and oxygen delivery ( $\text{D}_{\text{O}_2}$ ). Venous oxygen saturation reflects the balance between  $\text{V}_{\text{O}_2}$  and  $\text{D}_{\text{O}_2}$ . In published studies, decreases in venous oxygen saturation during SBT were associated with weaning and extubation failure (8, 9); however, this was not observed in all patients with EF (10–13).

When  $\text{V}_{\text{O}_2}$  increases, the tissue carbon dioxide ( $\text{CO}_2$ ) production also increases. The mixed venous-to-arterial partial pressure difference of  $\text{CO}_2$  ( $[\text{Pmvco}_2 - \text{Paco}_2]$ ) reflects the adequacy of CO in clearing out the  $\text{CO}_2$  produced by tissues (14, 15). Interestingly,  $[\text{Pmvco}_2 - \text{Paco}_2]$  can be replaced by the central venous-to-arterial  $\text{Pco}_2$  difference ( $\Delta\text{Pco}_2$ ) in critically ill patients (16, 17), and both of them are good indicators for the assessment of the adequacy between oxygen supply and oxygen demand in chronic heart failure and septic shock patients (18, 19). Therefore,  $\Delta\text{Pco}_2$  could be useful for the evaluation of extubation outcomes. Nevertheless, it has never been tested as a diagnostic tool for EF detection.

We hypothesized that patients with EF would have increased  $\Delta\text{Pco}_2$  values during SBTs. Therefore, the aims of our study were as follows: 1) to evaluate the ability of changes in  $\Delta\text{Pco}_2$  ( $\Delta - \Delta\text{Pco}_2$ ), central venous oxygen saturation ( $\Delta\text{Scvo}_2$ ), and their combination during SBTs to predict EF and 2) to assess if  $\Delta - \Delta\text{Pco}_2$  is an independent predictor of EF.

## MATERIALS AND METHODS

This prospective multicenter study was conducted in three general adult ICUs between the end of December 2016 and February 2018. The local Ethics Committees of each center approved the study (number:160402). Informed consent was obtained from all patients or their next of kin. The study was retrospectively registered in the ISRCTN registry (registration number ISRCTN10162509).

### Patients

All patients 18 years old or older who received MV for at least 48 hours and satisfied the weaning criteria were eligible for enrollment. The readiness-to-wean criteria we employed have been described previously (1) (**Supplementary-1a**, Supplemental Digital Content 1, <http://links.lww.com/CCM/F555>). Patients also had to have an arterial catheter and a central line with the tip confirmed by radiography as being in the superior vena cava near the entrance or in the right atrium. The exclusion criteria were as follows: 1) having undergone tracheostomy, 2) do-not-reintubate orders, 3) pregnancy, 4) absence of informed consent, 5) SBT failure, and 6) inability to obtain adequate echocardiographic windows.

### Weaning Protocol

SBTs were then performed in semirecumbent patients on a T-piece (20) with supplementary humidified oxygen for the achievement of an arterial oxygen saturation value greater than 90%, as measured by pulse oximetry. The SBT duration was 60 minutes. The criteria used for poor SBT tolerance have been defined previously (20) (**Supplementary-1b**, Supplemental Digital Content 2, <http://links.lww.com/CCM/F556>). The decision to stop SBT was made by the physicians. Patients who failed SBT were reconnected to a ventilator and were not enrolled in the study.

Patients who completed the SBT were extubated and followed-up for 48 hours. The medical team (physician, nurse, and respiratory therapist) involved in the extubation decision was blinded to the  $\Delta\text{Pco}_2$  and  $\text{Scvo}_2$  results. EF was diagnosed if the patient was extubated but required MV (invasive or non-invasive) owing to respiratory failure within the following 48 hours. Criteria for poor SBT tolerance and for respiratory failure after extubation have been listed in **Supplementary-1b** (Supplemental Digital Content 2, <http://links.lww.com/CCM/F556>). The management of postextubation respiratory failure was not protocolized and was left to the physician's discretion.

### Measurements

Ventilatory and hemodynamic parameters were recorded as the average of the three measurements immediately before SBT (on MV) and at 60 minutes after SBT initiation. Echocardiography assessment was performed by board-certified physicians in echocardiography immediately before SBT and at 60 minutes after its initiation, using a General Electric Vivid3 machine (GE Healthcare, Chalfont St. Giles, Buckinghamshire, United Kingdom). The velocity–time integral (VTI) was recorded by a pulsed wave Doppler on a 5-chamber apical view, and stroke volume (SV), CO, cardiac index, and SV index were calculated as described in **Supplementary-1c** (Supplemental Digital Content 3, <http://links.lww.com/CCM/F557>). Left ventricular systolic function was assessed by the left ventricular ejection fraction, as measured with Simpson's modified rule. Left ventricular diastolic function was evaluated by the measurement of the mitral E and A waves, the mean  $e'$  wave of the lateral, medial mitral annulus, and the calculation of the  $E/e'$  ratio. The average of five consecutive VTI, SV, CO, E/A, and  $E/e'$  measurements were obtained for each step of the study (before and 60 minutes after SBT initiation).

Arterial and central venous blood gas were measured using GEM Premier 4000 (Instrumentation Laboratory Co, Paris, France) immediately before SBT and at 60 minutes after SBT initiation.  $\Delta\text{Pco}_2$  was calculated as the difference between the central venous and arterial carbon dioxide tension.  $\text{DO}_2$  index,  $\text{VO}_2$  index, and oxygen extraction ( $\text{O}_2\text{E}$ ) values were calculated as described in **Supplementary-1d** (Supplemental Digital Content 4, <http://links.lww.com/CCM/F558>).

The plasma aminoterminal-probrain-natriuretic peptide (NT-proBNP) concentration was measured immediately before SBT and at 60 minutes of SBT initiation (**Supplementary-1d**, Supplemental Digital Content 4, <http://links.lww.com/CCM/F558>).

Changes in hemodynamic and oxygenation variables were expressed as relative changes ( $([\text{parameter at the end of SBT} - \text{parameter immediately before SBT}]/\text{parameter immediately before SBT}) \times 100$ ).

### Statistical Analyses

The normality of data distribution was assessed using the Shapiro-Wilk test and by visually checking the distribution (histogram) of each variable. Data were expressed as mean  $\pm$  SD when they were normally distributed and as median and interquartile range (IQR) when they were nonnormally distributed. Proportions were used as descriptive statistics for categorical variables. Comparisons of values between the extubation success and failure groups were performed using a two-tailed Student's *t* test or Mann-Whitney *U* test, as appropriate. Analyses of discrete data were performed using the chi-square test or Fisher exact test when the numbers were small. Pairwise comparisons between the different study time periods were assessed using a paired Student's *t* test or Wilcoxon test, as appropriate. Linear correlations were tested using Pearson's or Spearman tests, as appropriate.

Receiver operating characteristics (ROC) curves were constructed to evaluate the ability of  $\Delta - \Delta\text{PCO}_2$  and  $\Delta\text{ScvO}_2$  to predict EF. The areas under the ROC curves (AUCs) were compared using the nonparametric technique described by DeLong et al (21). Previously, we showed that the upper 95% CI values of the least significant changes (LSC), which are the minimum changes that are needed to be measured by a laboratory analyzer for the recognition of a real measurement change (Supplementary-1e, Supplemental Digital Content 5, <http://links.lww.com/CCM/F559>), for  $\Delta\text{Pco}_2$  and  $\text{Scvo}_2$  were 36.5% and 5.0%, respectively (22). Thus, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR<sup>+</sup>), negative likelihood ratio (LR<sup>-</sup>), and their 95% CIs were calculated for  $\Delta - \Delta\text{PCO}_2 > 36.5\%$  and  $\Delta\text{Scvo}_2 > 5.0\%$ .

Multivariable logistic regression analysis was used to identify significant independent predictors that were associated with EF. Variables that were associated with EF ( $p < 0.1$ ) in the univariate analysis during SBT were entered into the model. Potential problems related to collinearity were evaluated using Spearman or Pearson's correlation coefficient before the analysis was performed. The model's goodness-of-fit was assessed using the Hosmer–Lemeshow test.

Usually, variables are considered to be good clinical tools (with good discriminative property) when the inferior limits of the 95% CI of their AUC are greater than 0.75 (23). For this purpose, considering an EF incidence of 17% (based on the rate in the three participating centers, unpublished data), 72 patients were required for a power of 80% and an alpha risk of 0.05.

Statistical analyses were performed using Software Foundation's R packages for Windows 3.5.1 version.  $p < 0.05$  was considered statistically significant. All reported *p* values were two sided.

## RESULTS

### Study Population

Of 677 screened patients on MV, 85 were eligible for study entry. Ten patients failed SBT. Thus, the 75 patients who successfully completed their SBT were enrolled. Of them, 18 patients developed respiratory failure within 48 hours of extubation. Thirteen of 18 were reintubated and five others were treated only with noninvasive ventilation (Supplementary-Fig. 1, Supplemental Digital Content 6, <http://links.lww.com/CCM/F560>).

The main characteristics of the cohort are summarized in Table 1. The primary reason for intubation and MV at ICU admission was acute respiratory failure caused by pneumonia (62.7%).

### Comparisons Between the Success and Failure Groups at the Baseline

Chronic obstructive pulmonary disease (COPD) diagnosis and exacerbation were associated with EF (Table 1).

Immediately before SBT, the  $\text{Vo}_2$  index and  $\Delta\text{Pco}_2$  values were significantly lower and the  $\text{Scvo}_2$  value was higher in the failure group compared to those in the success group (Table 2).

### Comparisons Between the Values at the Start and End of SBT

The cardiac index, heart rate (HR), and respiratory rate (RR) increased significantly after SBT in both groups (Table 2). However, the SV index increased significantly only in the success group (Table 2). Additionally, the magnitude of the increases in the RR and HR was greater in the failure group (Table 3).  $\text{Sao}_2$  dropped significantly in the failure group.

$\text{Scvo}_2$  decreased significantly from  $74.7 \pm 11.2\%$  to  $66.7 \pm 12.0\%$  in the failure group, whereas it increased in the success group (Table 2).  $\Delta\text{Pco}_2$  showed the opposite course; it increased significantly from 4.0 (2.0 – 5.0) mm Hg to 6.5 (5.2 – 9.7) mm Hg in the failure group and decreased in the success group (Table 2).  $\text{Do}_2$  index increased in both groups, while the  $\text{Vo}_2$  index increased significantly only in the failure group (Table 2).  $\text{O}_2\text{E}$  changed in the opposite direction; it increased in the failure group and decreased in the success group (Table 2).  $\text{E/A}$ ,  $\text{E/e'}$ , and NT-proBNP values did not change in either group.

### Correlations Between the Changes in the Hemodynamic and Oxygenation Variables

Significant correlations were observed between  $\Delta\text{Scvo}_2$  and  $\Delta\text{Vo}_2$  ( $r = -0.71$ ,  $p < 0.001$ ),  $\Delta$ -cardiac index ( $r = 0.31$ ,  $p = 0.009$ ),  $\Delta\text{O}_2\text{E}$  ( $r = -0.89$ ,  $p < 0.001$ ), and  $\Delta - \Delta\text{Pco}_2$  ( $r = -0.52$ ,  $p < 0.001$ ).

$\Delta - \Delta\text{Pco}_2$  was found to be correlated with  $\Delta\text{Vo}_2$  (0.28,  $p = 0.02$ ),  $\Delta$ -cardiac index ( $r = -0.38$ ,  $p < 0.001$ ), and  $\Delta\text{O}_2\text{E}$  ( $r = 0.50$ ,  $p < 0.001$ ).

### Multivariable Analysis for EF Prediction

Multivariable logistic regression analysis was performed to investigate if  $\Delta - \Delta\text{Pco}_2$  and  $\Delta\text{Scvo}_2$  were independently associated with extubation outcomes. In the univariate analysis (Tables 3), seven variables were associated with EF ( $p < 0.1$ ).

**TABLE 1. Baseline Characteristics of the Study Population**

Variables	All Patients (n = 75)	Success (n = 57)	Failure (n = 18)	p
Age, yr	68 ± 11	69 ± 11	65 ± 11	0.27
Weight, kg	79 (67–99)	75 (65–91)	87 (71–102)	0.14
BMI, kg/m <sup>2</sup>	26.4 (23.1–33.5)	26 (23.0–31.5)	29.4 (27.8–36.7)	0.052
BMI > 30 kg/m <sup>2</sup> , n (%)	27 (36)	20 (35.1)	7 (38.9)	0.78
Admission SAPS II	60 ± 17	62 ± 17	55 ± 17	0.25
Male, n (%)	56 (74.7)	41 (71.9)	15 (83.3)	0.51
Diabetes, n (%)	24 (32.0)	20 (35.1)	4 (22.2)	0.39
Hypertension, n (%)	46 (61.3)	32 (56.1)	14 (77.8)	0.17
COPD, n (%)	21 (28)	11 (19.3)	10 (55.6)	0.007
CHF, n (%)	30 (40)	21 (36.8)	9 (50.0)	0.47
Pneumonia, n (%)	42 (62.7)	35 (61.4)	12 (66.7)	0.90
COPD exacerbation, n (%)	6 (8.0)	2 (3.5)	4 (22.2)	0.027
Acute pulmonary edema, n (%)	10 (13.3)	9 (15.8)	1 (5.6)	0.43
Stroke, n (%)	6 (8.0)	4 (7.0)	2 (11.1)	0.62
Other, n (%)	9 (12.0)	8 (14.0)	1 (5.6)	0.68
Hemoglobin, g/dL	9.9 (9.1–10.7)	10.0 (9.2–10.7)	9.8 (8.9–11.0)	0.95
Time to SBT, d	6.0 (4.0–9.0)	6.0 (4.0–9.0)	6.5 (4.2–10.0)	0.68
Ventilatory settings at weaning trial				
PEEP, cmH <sub>2</sub> O	6 (6–7)	6 (6–7)	6 (6–8)	0.19
Inspiratory pressure, cmH <sub>2</sub> O	10 (8–12)	10 (8–12)	11 (8–12)	0.79
F <sub>IO<sub>2</sub></sub> , %	30 (25–30)	30 (25–30)	30 (25–30)	0.11
Tidal volume, mL/kg	8.0 (7.0–8.2)	8.0 (7.0–8.3)	7.7 (7.0–8.0)	0.82
Respiratory rate/tidal volume, min/L	37 (26–48)	37 (26–50)	30 (24–47)	0.34

BMI = body mass index; CHF = chronic heart failure; COPD = chronic obstructive pulmonary disease; F<sub>IO<sub>2</sub></sub> = inspired oxygen fraction; PEEP = positive end expiratory pressure; SAPS II = Simplified Acute Physiologic Score II; SBT = spontaneous breathing trial. Data are expressed as mean ± sd, median (25–75 interquartile range), or count.

However, for the reasons of the collinearity between  $\Delta\text{Scvo}_2$ ,  $\Delta\text{Vo}_2$ , and  $\Delta\text{o}_2\text{E}$ , five variables ( $\Delta\text{Scvo}_2$ ,  $\Delta - \Delta\text{Pco}_2$ ,  $\Delta\text{RR}$ ,  $\Delta\text{HR}$ , and  $\Delta\text{Sao}_2$ ) were finally entered into the multivariable logistic model (Table 4). Of these,  $\Delta\text{Scvo}_2$  and  $\Delta - \Delta\text{Pco}_2$  were independently associated with EF. The Hosmer-Lemeshow test results were not statistically significant ( $p = 0.39$ ), indicating the model's goodness of fit.  $\Delta - \Delta\text{PCO}_2$  was also independently associated with EF after adjustment for COPD presence (Supplementary-Table 1, Supplemental Digital Content 7, <http://links.lww.com/CCM/F561>).

#### Ability of $\Delta - \Delta\text{Pco}_2$ and $\Delta\text{Scvo}_2$ to predict extubation outcomes

The ability of  $\Delta - \Delta\text{Pco}_2$  and  $\Delta\text{Scvo}_2$  to predict extubation outcomes was very good, with AUCs of 0.865 (95% CI, 0.767–0.933) and 0.856 (95% CI, 0.756–0.926), respectively (Figure 1).

The best cutoff value (according to the Youden index (23)) for  $\Delta\text{Scvo}_2$  was  $-3.2\%$  (sensitivity = 83%; 95% CI, 59–96%;

specificity = 74%; 95% CI, 60–84%), which was lower than its LSC (5%) (22). Taking into account the repeatability (LSC), the best cutoff value was less than or equal to  $-5.4\%$  (sensitivity = 67%; 95% CI, 41–87%; specificity = 86%; 95% CI, 74–94%; PPV = 60%; 95% CI, 36–81%; NPV = 89%; 95% CI, 78–96%; LR<sup>+</sup> = 4.7; 95% CI, 2.3–9.8; and LR<sup>-</sup> = 0.4; 95% CI, 0.2–0.8).

The best cutoff value (according to the Youden index) for  $\Delta - \Delta\text{Pco}_2$  was greater than or equal to 40% (sensitivity = 67%; 95% CI, 41–87%; specificity = 93%; 95% CI, 83–98%; PPV = 75%; 95% CI, 48–93%; NPV = 90%; 95% CI, 79–96%; LR<sup>+</sup> = 9.5; 95% CI, 3.5–25.8; and LR<sup>-</sup> = 0.4; 95% CI, 0.2–0.7).

The AUCs of  $\Delta\text{Scvo}_2$  and  $\Delta - \Delta\text{Pco}_2$  were not significantly different ( $p = 0.88$ ). The combination of  $\Delta\text{Scvo}_2$  and  $\Delta - \Delta\text{PCO}_2$  led to improved EF detection, with an AUC (0.940; 95% CI, 0.859–0.981) that was significantly better than that observed for  $\Delta\text{Scvo}_2$  ( $p = 0.04$ ) and  $\Delta - \Delta\text{PCO}_2$  ( $p = 0.03$ ) (Fig. 1).

**TABLE 2. Hemodynamic, Respiratory, and Tissue Oxygenation Parameters During Mechanical Ventilation (MV) (Immediately Before SBT) and at 60 Minutes of SBT in the Success and Failure Groups**

Variables	Success (n = 57)		Failure (n = 18)	
	Immediately Before SBT	60 Minutes SBT	Immediately Before SBT	60 Minutes SBT
Heart rate, beats/min	86 ± 14	89 ± 17 <sup>a</sup>	86 ± 14	94 ± 12 <sup>a</sup>
Systolic arterial pressure, mm Hg	134 ± 17	134 ± 16	131 ± 17	135 ± 16
Cardiac index, L/min/m <sup>2</sup>	2.9 (2.0–4.0)	3.6 (2.6–4.8) <sup>a</sup>	2.8 (2.4–3.7)	3.2 (2.5–4.7) <sup>a</sup>
Stroke volume index, mL/m <sup>2</sup>	33.3 (24.6–48.1)	42.3 (30.6–52.0) <sup>a</sup>	33.3 (27.4–41.7)	37.6 (28.0–52.9)
Arterial pH	7.47 ± 0.05	7.47 ± 0.04	7.45 ± 0.06	7.47 ± 0.06
Bicarbonate, mmol/L	26.5 (24.4–28.8)	26.5 (24.4–28.4)	29.0 (26.6–32.6) <sup>b</sup>	28.0 (26.6–32.4)
Arterial oxygen saturation, %	97 (95–99)	97 (95–98)	97 (94–97)	94 (90–97) <sup>ab</sup>
Pao <sub>2</sub> /Fio <sub>2</sub> , mm Hg	300 (251–347)	289 (250–425)	280 (242–306)	238 (210–311) <sup>b</sup>
Paco <sub>2</sub> , mm Hg	35 (31–39)	36 (32–39)	38 (36–47) <sup>b</sup>	37 (36–43)
Respiratory rate, breath/min	22 ± 6	25 ± 5 <sup>a</sup>	22 ± 5	26 ± 6 <sup>a</sup>
Scvo <sub>2</sub> , %	67.7 ± 8.5	70.3 ± 9.1 <sup>a</sup>	74.7 ± 11.2 <sup>b</sup>	66.7 ± 12.0 <sup>a</sup>
Pcvco <sub>2</sub> , mm Hg	42 (39–46)	42 (38–46)	42 (40–51)	45 (42–55) <sup>b</sup>
DO <sub>2</sub> , mL/min/m <sup>2</sup>	373 (259–535)	449 (350–603) <sup>a</sup>	344 (280–511)	379 (314–601) <sup>a</sup>
Vo <sub>2</sub> , mL/min/m <sup>2</sup>	104 (83–135)	112 (88–136)	85 (57–95) <sup>b</sup>	115 (82–147) <sup>a</sup>
O <sub>2</sub> E, %	31 ± 9	27 ± 9 <sup>a</sup>	22 ± 12 <sup>b</sup>	29 ± 12 <sup>a</sup>
ΔPco <sub>2</sub> , mm Hg	7.0 (5.0–9.0)	6.0 (4.0–7.1) <sup>a</sup>	4.0 (2.0–5.0) <sup>b</sup>	6.5 (5.2–9.7) <sup>ab</sup>
LVEF, %	52 ± 11	50 ± 11	53 ± 9	52 ± 10
E/A	0.9 (0.8–1.2)	0.9 (0.7–1.2)	0.9 (0.7–1.4)	0.8 (0.8–1.4)
E/e'	9.9 ± 4.7	9.8 ± 4.9	9.1 ± 2.0	10.0 ± 2.7
NT-proBNP, pg/mL	2,695 (1,444–7,560)	2,766 (1,446–7,568)	2,105 (555–5,507)	1,943 (299–4,026)

SBT = spontaneous breathing trial; Pao<sub>2</sub> = arterial oxygen partial pressure; Fio<sub>2</sub> = inspired oxygen fraction; Paco<sub>2</sub> = arterial carbon dioxide partial pressure; Scvo<sub>2</sub> = central venous oxygen saturation; Pcvco<sub>2</sub> = central venous carbon dioxide partial pressure; Do<sub>2</sub> = oxygen delivery; Vo<sub>2</sub> = oxygen consumption; O<sub>2</sub>E = oxygen extraction; ΔPco<sub>2</sub> = difference between central venous and arterial carbon dioxide partial pressures; LVEF = left ventricular ejection fraction; NT-proBNP = amino terminal pro-brain natriuretic peptide.

Data are expressed as mean ± SD or as median (interquartile range, 25–75).

<sup>a</sup>p < 0.05 comparisons between during MV vs 60 min SBT.

<sup>b</sup>p < 0.05 comparisons between success vs failure.

The combination of  $\Delta - \Delta\text{PCO}_2 \geq 40\%$  and  $\Delta\text{Scvo}_2 \leq -5.4\%$  could predict EF with a sensitivity of 56% (95% CI, 31–78%), a specificity of 96 (95% CI, 88–100%), an NPV of 87% (95% CI, 80–92%), a PPV of 83% (95% CI, 55–95%), an LR<sup>+</sup> of 15.8 (95% CI, 3.8–65.7), and an LR<sup>-</sup> of 0.46 (95% CI, 0.27–0.77).

## DISCUSSION

The main findings of our study can be summarized as follows (1): the abilities of  $\Delta\text{Scvo}_2$  and  $\Delta - \Delta\text{Pco}_2$  to predict EF were similar and significantly good (2); combination analysis of these two parameters led to enhanced EF detection. Indeed,  $\Delta - \Delta\text{Pco}_2 \geq 40\%$  and  $\Delta\text{Scvo}_2 \leq -5.4\%$  during SBTs accurately predicted EF (3);  $\Delta - \Delta\text{Pco}_2$  and  $\Delta\text{Scvo}_2$  were independent predictors of EF.

The transition from MV to spontaneous ventilation leads to increases in the Vo<sub>2</sub> of respiratory muscles related to the increased work of breathing (4–7). There should be an obligatory increase in CO and Do<sub>2</sub> to match the increased Vo<sub>2</sub> demand. Venous oxygen saturation is used as a marker of adequacy between Do<sub>2</sub> and Vo<sub>2</sub>. In a previous study, the mixed venous oxygen saturation (Svo<sub>2</sub>) decreased progressively in patients with SBT failure, but not in those with SBT success (8). The Svo<sub>2</sub> decrease was attributed to an O<sub>2</sub>E increase, probably by the respiratory muscles; the O<sub>2</sub>E decreased and CO increased in patients with weaning success (8). Teixeira et al (9) demonstrated that changes in the Scvo<sub>2</sub> between MV and the end of SBT were independently associated with EF in patients with weaning difficulty. A decrease greater than 4.5% in the Scvo<sub>2</sub> predicted EF with very good PPV and NPV (9), similar

**TABLE 3. Changes in Hemodynamic, Tissue Oxygenation, and Respiratory Parameters Between the End of SBT and Immediately Before SBT**

Variables	Success (n = 57)	Failure (n = 18)	p
$\Delta - \Delta P_{CO_2}$ , %	-14.3 (-33.3 to 2.7)	130.0 (1.4 to 200.0)	< 0.001
$\Delta ScvO_2$ , %	0.9 (-3.4 to 8.7)	-8.7 (-13.7 to -4.3)	< 0.001
$\Delta CI$ , %	13.6 (7.1 to 28.2)	12.7 (0.2 to 16.6)	0.37
$\Delta Vo_2$ , %	7.2 (-9.3 to 22.9)	29.4 (14.8 to 62.8)	0.001
$\Delta Do_2$ , %	14.5 (5.3 to 25.6)	12.7 (0.2 to 15.2)	0.17
$\Delta O_2E$ , %	-8.7 (-23.2 to 5.8)	23.8 (7.8 to 36.0)	< 0.001
$\Delta RR$ , %	10.5 (3.0 to 17.4)	25.0 (7.7 to 32.4)	0.06
$\Delta HR$ , %	3.6 (0.0 to 5.7)	8.4 (2.4 to 10.5)	0.009
$\Delta SaO_2$ , %	-0.6 (-2.2 to 1.0)	-1.7 (-5.6 to 0.0)	0.08

SBT = spontaneous breathing trial;  $\Delta P_{CO_2}$  = difference between central venous and arterial carbon dioxide partial pressures;  $ScvO_2$  = central venous oxygen saturation; CI = cardiac index;  $Vo_2$  = oxygen consumption;  $O_2E$  = oxygen extraction;  $Do_2$  = oxygen delivery; RR = respiratory rate; HR = heart rate;  $SaO_2$  = arterial oxygen saturation.

All changes in parameters are expressed as relative changes. Data are expressed as mean  $\pm$  sd or as median (interquartile range, 25–75).

**TABLE 4. Multivariate Logistic Regression Analysis for Extubation Predictors**

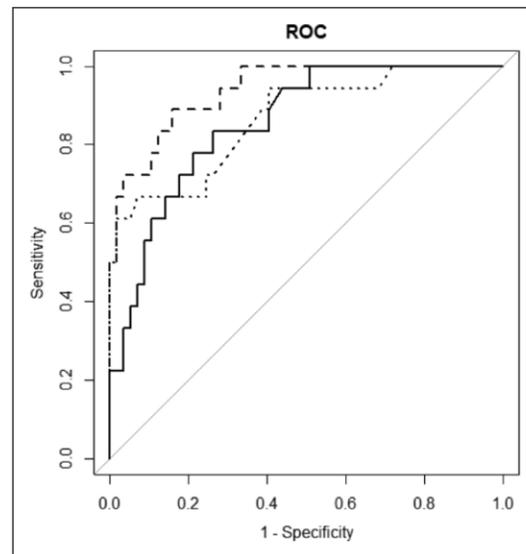
Variables	Odds Ratio (95% CI)	p
$\Delta - \Delta P_{CO_2}$ , %	1.02 (1.01–1.05)	0.006
$\Delta ScvO_2$ , %	0.84 (0.70–0.95)	0.02
$\Delta SaO_2$ , %	0.92 (0.71–1.23)	0.57
$\Delta RR$ , %	0.96 (0.89–1.02)	0.25
$\Delta HR$ , %	1.09 (0.97–1.25)	0.16

$\Delta P_{CO_2}$  = difference between central venous and arterial carbon dioxide partial pressures;  $ScvO_2$  = central venous oxygen saturation; RR = respiratory rate; HR = heart rate;  $SaO_2$  = arterial oxygen saturation.

All changes in parameters are expressed as relative changes between the end of SBT and immediately before SBT.

to our results. We found that  $\Delta ScvO_2$  was an independent factor of EF in patients with good SBT tolerance (Table 4). Additionally, a reduction of 5.4% or greater in the  $\Delta ScvO_2$  was associated with a very good NPV but only a moderate PPV in EF prediction. The reduction in the  $ScvO_2$  in the failure group can be explained by the increase in the  $Vo_2$  in response to the higher energy requirement by the respiratory muscles not being matched by an adequate increase in the CO and  $Do_2$ , causing an increase in the  $O_2E$ . Indeed, we found a strong negative correlation between  $\Delta ScvO_2$  and  $\Delta Vo_2$ . However, our findings are not in agreement with those of some previous studies (10–13). The inconsistencies between our findings and those of previous studies may stem from several factors. First, the study populations were dissimilar. Second, in our study, patients were mechanically ventilated for at least 48 hours, whereas in the other studies, they were ventilated for less than 24 hours (10, 12, 13).

According to the Fick equation applied to  $CO_2$ , the determinants of  $\Delta P_{CO_2}$  are tissue  $CO_2$  production and CO (15, 24,



**Figure 1.** Receiver operating characteristic (ROC) curves showing the ability of the changes in  $\Delta P_{CO_2}$  ( $\Delta - \Delta P_{CO_2}$ ) (dotted curve, areas under the ROC curves [AUC] = 0.865),  $ScvO_2$  ( $\Delta ScvO_2$ ) (solid curve, AUC = 0.856), and their combinations (dashed curve, AUC = 0.940) between immediately before the spontaneous breathing trial (SBT) and at 60 min of SBT to predict the weaning failure.

25).  $\Delta P_{CO_2}$  has been used as an indicator of the adequacy of venous blood flow for the removal of the  $CO_2$  produced by the peripheral tissues (26–28). Dobutamine may increase  $Vo_2$  and  $V_{CO_2}$  values through its potential thermogenic effects related to its  $\beta_1$ -adrenergic properties (29). We showed that  $\Delta P_{CO_2}$  could be used to distinguish the hemodynamic from the metabolic effects of dobutamine in septic shock patients

(19). Thus, we hypothesized that  $\Delta P_{CO_2}$ , as an index of the  $Vo_2/CO$  ratio, could be a useful marker for the assessment of the adequacy between CO and  $Vo_2$  during the weaning trial and could be able to predict extubation outcomes in critically ill patients. We found that  $\Delta P_{CO_2}$  was an independent predictor of EF, even after adjustment for  $Scvo_2$  (Table 4) and COPD (Supplementary-Table 1, Supplemental Digital Content 7, <http://links.lww.com/CCM/F561>). Furthermore,  $\Delta - \Delta P_{CO_2} \geq 40\%$  predicted EF with a very good NPV but with a moderate PPV. However, the combination of  $\Delta - \Delta P_{CO_2}$  and  $\Delta Scvo_2$  showed better EF predictability (AUC = 0.940) than did  $\Delta - \Delta P_{CO_2}$  and  $\Delta Scvo_2$  individually. Additionally,  $\Delta - \Delta P_{CO_2} \geq 40\%$  and  $\Delta Scvo_2 \leq -5.4\%$ , after a successful SBT, could predict EF with very good NPV and PPV and an excellent LR<sup>+</sup> ( $\approx 16$ ).

Our results are of clinical importance and add significant data to the existing literature. Our study is the first multicenter study to show that  $\Delta P_{CO_2}$  is a useful marker for the reliable prediction of extubation outcomes. Furthermore, for patients with central venous and arterial catheters during an SBT, the tracking of both  $\Delta P_{CO_2}$  and  $Scvo_2$  showed excellent predictability, better than that associated with each parameter separately. This combined value has the potential to assist clinicians with extubation decisions and the identification of patients at a high EF risk who may need to be extubated to noninvasive ventilation or high-flow nasal oxygen (30). Moreover, the metabolic approach ( $\Delta P_{CO_2}$  and  $Scvo_2$ ) appears to be better than the echocardiographic one ( $E/A$  and  $E/e'$ ), which failed to predict EF.

Our study presents some limitations. First,  $Vo_2$  was calculated from  $Scvo_2$  and not from  $Svo_2$ , neither was it measured by indirect calorimetry, limiting its accuracy. However, we were interested in the changes in the  $Vo_2$  observed during SBT, rather than its absolute value. Second, the increase in  $\Delta P_{CO_2}$  at the end of the SBT in the EF group could be explained by the Haldane effect (31). Although the  $Scvo_2$  decreased significantly in the failure group, it is unlikely that this change could have affected the  $P_{CO_2}/CO_2$  content relationship, because it was not strong. Indeed, changes in the  $\Delta P_{CO_2}$  may not parallel changes in  $CO_2$  content differences under conditions of very low  $Svo_2$  ( $< 30\%$ ) (32), which was not the case in our patients. Third, we included patients who had passed their first SBTs; thus, our findings should be confirmed in difficult-to-wean patients. However, 68% of our population had COPD and congestive heart failure (Table 1) and could be considered as having a high EF risk (30). Additionally, it may be helpful to compare, in a future work, the predictability of  $\Delta - \Delta P_{CO_2}$  and  $\Delta Scvo_2$  in terms of the traditional weaning parameters. Fourth, we did not fully address the causes of EF. However, the absence of changes in the  $E/e'$  and NT-proBNP values during SBT suggests that weaning-induced pulmonary edema did not cause EF. Nevertheless, this cannot be ruled out because we did not check for the development of B-lines using lung ultrasound (33). Fifth, the SBTs were performed on a 1-hour T-piece; thus, our findings may not be generalizable to other SBT modalities (34).

## CONCLUSIONS

$\Delta - \Delta P_{CO_2}$  and  $\Delta Scvo_2$  during SBTs were independent predictors of extubation outcomes. Combination analysis of both parameters led to enhanced diagnostic performance and showed excellent predictability for EF detection. The combination of  $\Delta - \Delta P_{CO_2} \geq 40\%$  and  $\Delta Scvo_2 \leq -5.4\%$  at the end of a tolerated SBT was accurate in EF detection.

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The authors declare that they have no competing interests related to the subject of the study.

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## REFERENCES

- Boles JM, Bion J, Connors A, et al: Weaning from mechanical ventilation. *Eur Respir J* 2007; 29:1033–1056
- Thille AW, Richard JC, Brochard L: The decision to extubate in the intensive care unit. *Am J Respir Crit Care Med* 2013; 187:1294–1302
- MacIntyre N: Discontinuing mechanical ventilatory support. *Chest* 2007; 132:1049–1056
- Kemper M, Weissman C, Askanazi J, et al: Metabolic and respiratory changes during weaning from mechanical ventilation. *Chest* 1987; 92:979–983
- Hubmayr RD, Loosbrock LM, Gillespie DJ, et al: Oxygen uptake during weaning from mechanical ventilation. *Chest* 1988; 94:1148–1155
- Pinsky MR: Cardiovascular effects of ventilatory support and withdrawal. *Anesth Analg* 1994; 79:567–576
- Field S, Kelly SM, Macklem PT: The oxygen cost of breathing in patients with cardiorespiratory disease. *Am Rev Respir Dis* 1982; 126:9–13
- Jubran A, Mathru M, Dries D, et al: Continuous recordings of mixed venous oxygen saturation during weaning from mechanical ventilation and the ramifications thereof. *Am J Respir Crit Care Med* 1998; 158:1763–1769
- Teixeira C, da Silva NB, Savi A, et al: Central venous saturation is a predictor of reintubation in difficult-to-wean patients. *Crit Care Med* 2010; 38:491–496
- De Backer D, El Haddad P, Preiser JC, et al: Hemodynamic responses to successful weaning from mechanical ventilation after cardiovascular surgery. *Intensive Care Med* 2000; 26:1201–1206
- Zakynthinos S, Routsis C, Vassilakopoulos T, et al: Differential cardiovascular responses during weaning failure: Effects on tissue oxygenation and lactate. *Intensive Care Med* 2005; 31:1634–1642
- Chittawattananat K, Kantha K, Tepsuwan T: Central venous oxygen saturation is not a predictor of extubation success after simple weaning from mechanical ventilation in post-cardiac surgical patients. *J Med Assoc Thai* 2016; 99(Suppl 6):S145–S152

13. Williams J, McLean A, Ahari J, et al: Decreases in mixed venous blood O<sub>2</sub> saturation in cardiac surgery patients following extubation. *J Intensive Care Med* 2017; 35:264–269
14. Vallet B, Teboul JL, Cain S, et al: Venous-arterial CO<sub>2</sub> difference during regional ischemic or hypoxic hypoxia. *J Appl Physiol (1985)* 2000; 89:1317–1321
15. Mallat J, Lemyze M, Tronchon L, et al: Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock. *World J Crit Care Med* 2016; 5:47–56
16. Cuschieri J, Rivers EP, Donnino MW, et al: Central venous-arterial carbon dioxide difference as an indicator of cardiac index. *Intensive Care Med* 2005; 31:818–822
17. van Beest PA, Lont MC, Holman ND, et al: Central venous-arterial pCO<sub>2</sub> difference as a tool in resuscitation of septic patients. *Intensive Care Med* 2013; 39:1034–1039
18. Teboul JL, Mercat A, Lenique F, et al: Value of the venous-arterial PCO<sub>2</sub> gradient to reflect the oxygen supply to demand in humans: Effects of dobutamine. *Crit Care Med* 1998; 26:1007–1010
19. Mallat J, Benzidi Y, Salleron J, et al: Time course of central venous-to-arterial carbon dioxide tension difference in septic shock patients receiving incremental doses of dobutamine. *Intensive Care Med* 2014; 40:404–411
20. Esteban A, Alia I, Tobin MJ, et al: Effect of spontaneous breathing trial duration on outcome of attempts to discontinue mechanical ventilation. Spanish Lung Failure Collaborative Group. *Am J Respir Crit Care Med* 1999; 159:512–518
21. DeLong ER, DeLong DM, Clarke-Pearson DL: Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. *Biometrics* 1988; 44:837–845
22. Mallat J, Lazkani A, Lemyze M, et al: Repeatability of blood gas parameters, PCO<sub>2</sub> gap, and PCO<sub>2</sub> gap to arterial-to-venous oxygen content difference in critically ill adult patients. *Medicine (Baltimore)* 2015; 94:e415
23. Ray P, Le Manach Y, Riou B, et al: Statistical evaluation of a biomarker. *Anesthesiology* 2010; 112:1023–1040
24. McHardy GJ: The relationship between the differences in pressure and content of carbon dioxide in arterial and venous blood. *Clin Sci* 1967; 32:299–309
25. Gavelli F, Teboul JL, Monnet X: How can CO<sub>2</sub>-derived indices guide resuscitation in critically ill patients? *J Thorac Dis* 2019; 11:S1528–S1537
26. 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; 34:2218–2225
27. Mallat J, Pepy F, Lemyze M, et al: Central venous-to-arterial carbon dioxide partial pressure difference in early resuscitation from septic shock: A prospective observational study. *Eur J Anaesthesiol* 2014; 31:371–380
28. Ospina-Tascón GA, Umaña M, Bermúdez WF, et al: Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock? *Intensive Care Med* 2016; 42:211–221
29. Ruffolo RR Jr: The pharmacology of dobutamine. *Am J Med Sci* 1987; 294:244–248
30. Thille AW, Muller G, Gacouin A, et al; HIGH-WEAN Study Group and REVA Research Network: Effect of postextubation high-flow nasal oxygen with noninvasive ventilation vs high-flow nasal oxygen alone on reintubation among patients at high risk of extubation failure: A Randomized Clinical Trial. *JAMA* 2019; 322:1465–1475
31. Teboul JL, Scheeren T: Understanding the haldane effect. *Intensive Care Med* 2017; 43:91–93
32. Jakob SM, Kosonen P, Ruokonen E, et al: The Haldane effect—An alternative explanation for increasing gastric mucosal PCO<sub>2</sub> gradients? *Br J Anaesth* 1999; 83:740–746
33. Ferré A, Guillot M, Lichtenstein D, et al: Lung ultrasound allows the diagnosis of weaning-induced pulmonary oedema. *Intensive Care Med* 2019; 45:601–608
34. Subirà C, Hernández G, Vázquez A, et al: Effect of pressure support vs t-piece ventilation strategies during spontaneous breathing trials on successful extubation among patients receiving mechanical ventilation: A Randomized Clinical Trial. *JAMA* 2019; 321:2175–2182

### III. Metabolic approach and detection of fluid responsiveness

In the same line,  $\Delta\text{PCO}_2$  might help identify patients who responded or did not to fluid challenges in cases where cardiac output monitoring is unavailable. In post-cardiac surgery sedated and mechanically ventilated patients with cardiac index  $< 2.5 \text{ L/min/m}^2$ , the changes in cardiac index ( $\Delta\text{CI}$ ) induced by fluid challenge (500-mL bolus of crystalloid given over 30 min) were found to be significantly correlated with changes in  $\Delta\text{PCO}_2\text{_{Cent}}$  ( $\Delta\text{-}\Delta\text{PCO}_2\text{_{Cent}}$ ) ( $r = -0.53, p = 0.001$ ) [171]. However, the AUROC for changes in  $\Delta\text{PCO}_2\text{_{Cent}}$  to define fluid responsiveness was not determined in that study. Also, in 30 cardiogenic shock or postoperative cardiac surgery patients, the authors observed that changes in  $\text{ScvO}_2$  ( $\Delta\text{ScvO}_2$ ) were significantly correlated with  $\Delta\text{CI}$  induced by a bolus of 500 mL of normal saline administered over 10-min ( $r = 0.67, p < 0.001$ )<sup>14</sup>. Also,  $\Delta\text{ScvO}_2$  had an excellent ability to define an increase in  $\text{CI} \geq 15\%$  after volume expansion (fluid responsiveness) with an AUROC of 0.90 [172].

Recently, in a prospective study, we investigated if  $\Delta\text{PCO}_2\text{_{Cent}}$  or  $\text{ScvO}_2$  can help classify responders and non-responders to fluid challenge in 49 septic shock patients [173]. Fluid responsiveness was defined as an increase in  $\text{CI} > 10\%$  after fluid challenge. We found that in overall population,  $\Delta\text{-}\Delta\text{PCO}_2\text{_{Cent}}$  and  $\Delta\text{ScvO}_2$  were significantly correlated with  $\Delta\text{CI}$  after fluid challenge ( $r = -0.30, p = 0.03$  and  $r = 0.42, p = 0.003$ , respectively). The AUROCs for  $\Delta\text{-}\Delta\text{PCO}_2\text{_{Cent}}$  and  $\Delta\text{ScvO}_2$  to define fluid responsiveness were 0.76 ( $p < 0.001$ ) and 0.68 ( $p = 0.02$ ), respectively. However, in the subgroup of patients in whom the oxygen consumption slightly increased ( $\leq 10\%$ ) after fluid challenge (no tissue hypoxia), the abilities of  $\Delta\text{-}\Delta\text{PCO}_2\text{_{Cent}}$  and  $\Delta\text{ScvO}_2$  to define fluid responsiveness improved (AUROC= 0.83,  $p < 0.001$ , and 0.73,  $p = 0.006$ ; respectively). The fact that  $\Delta\text{ScvO}_2$  was less reliable in classifying fluid responders compared to the results obtained in

cardiogenic shock postoperative cardiac surgery patients [172] might be due to the reason that venous oxygen saturation may not be a good indicator of tissue hypoperfusion in the setting of sepsis, due to microcirculatory shunting and mitochondrial dysfunction that can result in oxygen extraction abnormalities [139].



# OPEN Central venous-to-arterial PCO<sub>2</sub> difference as a marker to identify fluid responsiveness in septic shock

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Defining the hemodynamic response to volume therapy is integral to managing critically ill patients with acute circulatory failure, especially in the absence of cardiac index (CI) measurement. This study aimed at investigating whether changes in central venous-to-arterial CO<sub>2</sub> difference ( $\Delta\text{-}\Delta\text{PCO}_2$ ) and central venous oxygen saturation ( $\Delta\text{ScvO}_2$ ) induced by volume expansion (VE) are reliable parameters to define fluid responsiveness in sedated and mechanically ventilated septic patients. We prospectively studied 49 critically ill septic patients in whom VE was indicated because of circulatory failure and clinical indices. CI,  $\Delta\text{PCO}_2$ ,  $\text{ScvO}_2$ , and oxygen consumption ( $\text{VO}_2$ ) were measured before and after VE. Responders were defined as patients with a > 10% increase in CI (transpulmonary thermodilution) after VE. We calculated areas under the receiver operating characteristic curves (AUCs) for  $\Delta\text{-}\Delta\text{PCO}_2$ ,  $\Delta\text{ScvO}_2$ , and changes in CI ( $\Delta\text{CI}$ ) after VE in the whole population and in the subgroup of patients with an increase in  $\text{VO}_2$  ( $\Delta\text{VO}_2 \leq 10\%$  after VE (oxygen-supply independency). Twenty-five patients were fluid responders. In the whole population,  $\Delta\text{-}\Delta\text{PCO}_2$  and  $\Delta\text{ScvO}_2$  were significantly correlated with  $\Delta\text{CI}$  after VE ( $r = -0.30, p = 0.03$  and  $r = 0.42, p = 0.003$ , respectively). The AUCs for  $\Delta\text{-}\Delta\text{PCO}_2$  and  $\Delta\text{ScvO}_2$  to define fluid responsiveness (increase in CI > 10% after VE) were 0.76 ( $p < 0.001$ ) and 0.68 ( $p = 0.02$ ), respectively. In patients with  $\Delta\text{VO}_2 \leq 10\%$  ( $n = 36$ ) after VE, the correlation between  $\Delta\text{ScvO}_2$  and  $\Delta\text{CI}$  was 0.62 ( $p < 0.001$ ), and between  $\Delta\text{-}\Delta\text{PCO}_2$  and  $\Delta\text{CI}$  was  $-0.47$  ( $p = 0.004$ ). The AUCs for  $\Delta\text{-}\Delta\text{PCO}_2$  and  $\Delta\text{ScvO}_2$  were 0.83 ( $p < 0.001$ ) and 0.73 ( $p = 0.006$ ), respectively. In these patients,  $\Delta\text{-}\Delta\text{PCO}_2 \leq -37.5\%$  after VE allowed the categorization between responders and non-responders with a positive predictive value of 100% and a negative predictive value of 60%. In sedated and mechanically ventilated septic patients with no signs of tissue hypoxia (oxygen-supply independency),  $\Delta\text{-}\Delta\text{PCO}_2$  is a reliable parameter to define fluid responsiveness.

## Abbreviations

CO <sub>2</sub>	Carbon dioxide
VO <sub>2</sub>	Oxygen consumption
DO <sub>2</sub>	Oxygen delivery
$\Delta\text{PCO}_2$	Venous-to-arterial carbon dioxide tension difference
$\Delta\text{ContO}_2$	Arterial-to-venous oxygen content difference
PaCO <sub>2</sub>	Partial arterial carbon dioxide tension
PcvCO <sub>2</sub>	Central venous carbon dioxide tension
ScvO <sub>2</sub>	Central venous oxygen saturation
SaO <sub>2</sub>	Arterial oxygen saturation
PaO <sub>2</sub>	Partial arterial oxygen tension
PvO <sub>2</sub>	Partial venous oxygen tension
Hb	Hemoglobin
CI	Cardiac index
LSC	Least significant change
AUC	Area under the curve

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ROC	Receiver operating characteristic
PPV	Positive predictive value
NPV	Negative predictive value
LR	Likelihood ratio
VE	Volume expansion
HR	Heart rate
ICU	Intensive care unit

Hemodynamic optimization through fluid resuscitation is commonly used in critically ill patients with tissue hypoperfusion. The goal of volume expansion (VE) is to raise cardiac output and oxygen supply to improve tissue oxygenation. Recognizing patients who would benefit from VE remains challenging<sup>1</sup>. Identifying such patients is often dependent on measuring cardiac output<sup>2,3</sup>. Echocardiography is a skill that has made strides but not fully penetrated all critical care areas; it is limited by ultrasound availability and poor echogenicity, especially in mechanically ventilated patients. Passive leg raising test and end-expiratory occlusion methods also necessitate cardiac output measurement to assess fluid responsiveness<sup>4,5</sup>. Other measurements such as pulse pressure and stroke volume variations require specific technologies<sup>6</sup> or are restricted to certain patient populations<sup>7</sup>. With these limitations, defining fluid responsiveness without cardiac output measurement would be of great help to the clinician at the bedside.

Venous-to-arterial CO<sub>2</sub> tension difference reflects the balance between CO<sub>2</sub> production and CO<sub>2</sub> delivery to the lungs, a surrogate of the cardiac output<sup>8,9</sup>. Opposing changes over time in central venous-to-arterial CO<sub>2</sub> tension difference ( $\Delta$ PCO<sub>2</sub>) and cardiac output were reported in septic shock patients<sup>10–12</sup>. In post-cardiac surgery sedated and mechanically ventilated patients, Yazigi et al. observed a significant inverse correlation between changes in  $\Delta$ PCO<sub>2</sub> ( $\Delta$ - $\Delta$ PCO<sub>2</sub>) and changes in cardiac index ( $\Delta$ CI) induced by VE<sup>13</sup>. Moreover, in the same population, changes in central venous oxygen saturation ( $\Delta$ ScvO<sub>2</sub>) after VE were a reliable parameter to define fluid responsiveness<sup>14</sup>. However, data in the septic population is lacking.

In situations with tissue hypoxia, the increase in cardiac output and oxygen delivery (DO<sub>2</sub>) after VE would result in an increase in CO<sub>2</sub> production (VCO<sub>2</sub>) and oxygen consumption (VO<sub>2</sub>) (oxygen supply dependency). These metabolic changes might confound fluctuations in arteriovenous O<sub>2</sub> and CO<sub>2</sub> parameters attributed solely to circulatory changes. This might reduce the changes in  $\Delta$ PCO<sub>2</sub> and ScvO<sub>2</sub> induced by VE. Clinical studies have shown that the ratio of  $\Delta$ PCO<sub>2</sub> over the arterial-to-venous oxygen content ( $\Delta$ PCO<sub>2</sub>/ $\Delta$ ContO<sub>2</sub>) was a good indicator of oxygen supply dependency (tissue hypoxia) in critically ill patients<sup>15,16</sup>. This indicator could perhaps be used to identify such patients and guide the usage of CO<sub>2</sub> and oxygen gaps.

Therefore, our study aimed to investigate: (1) if  $\Delta$ - $\Delta$ PCO<sub>2</sub> and  $\Delta$ ScvO<sub>2</sub> are reliable parameters to identify fluid responsiveness in overall sedated and mechanically ventilated septic patients; (2) if the reliability of these parameters would be better in the sub-group of patients with no tissue hypoxia, defined as the absence of an increase in VO<sub>2</sub> induced by a rise in DO<sub>2</sub> after VE (oxygen supply independency); (3) if baseline  $\Delta$ PCO<sub>2</sub> /  $\Delta$ ContO<sub>2</sub> ratio is a good predictor of tissue hypoxia. Such measurements are readily available in these patients with central venous and arterial catheters.

## Materials and methods

This prospective and observational study was conducted in a single, mixed medical and surgical adult intensive care unit (ICU) between April and December 2017. The study was approved by the local institutional ethics committee (Comité d’Ethique du centre hospitalier du Dr. Shaffner de Lens). Informed consent was obtained from the next of kin of each patient. All experiments were performed in accordance with relevant guidelines and regulations.

**Patients.** We studied mechanically ventilated patients with sepsis<sup>17</sup> for whom the attending physician decided to give VE due to the presence of at least one clinical sign of tissue hypoperfusion<sup>17</sup> as previously described<sup>15</sup>: (a) systolic arterial pressure < 90 mmHg, mean arterial pressure < 65 mmHg, or the requirement for vasopressor administration; (b) skin mottling; (c) lactate levels > 2 mmol/l; or urinary output < 0.5 ml/kg/h for  $\geq$  2 h. Also, patients had to have a PiCCO device (PiCCO, Pulsion Medical System, Munich, Germany) as part of routine management of persistent signs of inadequate tissue perfusion in our ICU. Exclusion criteria were: pregnancy, age < 18 years old, moribund, and risk of fluid loading-induced pulmonary edema.

**Measurements.** Demographic data, acute circulatory failure etiology, the Simplified Acute Physiology Score (SAPS) II, and the Sequential Organ Failure Assessment (SOFA) scores were obtained on the day of enrollment. CI was obtained with the PiCCO device by central venous injections of 20 ml of iced 0.9% saline solution and recorded as the average of the three measurements.

Arterial and central venous blood gases were measured using the GEM Premier 4000 (Instrumentation Laboratory Co, Paris, France). The central venous blood was collected from a central venous catheter with the tip confirmed to be in the superior vena cava, near or at the right atrium, by radiograph as previously described<sup>15</sup>.  $\Delta$ PCO<sub>2</sub> was calculated as the difference between the central venous carbon dioxide tension and the arterial carbon dioxide tension. The arterial oxygen content was calculated as CaO<sub>2</sub> (ml) = 1.34 × Hb (g/dl) × SaO<sub>2</sub> + 0.003 × PaO<sub>2</sub> (mmHg), where SaO<sub>2</sub> is the oxygen saturation of arterial blood, Hb the hemoglobin concentration, and PaO<sub>2</sub> the arterial oxygen tension. The central venous oxygen content was calculated as CcvO<sub>2</sub> (ml) = 1.34 × Hb (g/dl) × ScvO<sub>2</sub> + 0.003 × PcvO<sub>2</sub> (mmHg), where PcvO<sub>2</sub> is the central venous oxygen tension.  $\Delta$ ContO<sub>2</sub> (ml) was calculated as CaO<sub>2</sub> – CcvO<sub>2</sub>. DO<sub>2</sub> (ml/min/m<sup>2</sup>) was calculated as CaO<sub>2</sub> × CI × 10. VO<sub>2</sub> (ml/m<sup>2</sup>) was calculated as

Variables	All patients (n = 49)	Responders (n = 25)	Non-responders (n = 24)	p-value
Age (years)	67 [60–73]	62 [59–74]	68 [65–71]	0.25
Weight (kg)	79 [67–96]	80 [67–90]	78 [66–100]	0.89
BMI (kg/m <sup>2</sup> )	27.2 [23.6–33.0]	26.5 [22.6–29.2]	27.5 [23.8–32.4]	0.32
Admission SAPS II	62 ± 15	62 ± 17	65 ± 20	0.57
SOFA score	10 [7–14]	10 [7–12]	10 [7–14]	0.54
Male, n (%)	34 (69.4)	19 (76.0)	15 (62.5)	0.30
Mechanical ventilation, n (%)	49 (100)	25 (100)	24 (100)	1.00
<b>Infection source, n (%)</b>				
Pneumonia	27 (55)	14 (56)	13 (54)	0.88
Peritonitis	12 (25)	7 (28)	6 (25)	0.93
Meningitis	3 (6)	2 (8)	1 (4)	0.99
Catheter related infections	2 (4)	0 (0)	2 (8)	0.46
Others	5 (10)	2 (8)	3 (12)	0.96
Norepinephrine, n (%)	37 (75.5)	19 (76)	18 (75)	0.93
Norepinephrine (µg kg min <sup>-1</sup> )	0.14 [0.06–0.45]	0.14 [0.05–0.46]	0.13 [0.06–0.47]	0.99

**Table 1.** Baseline characteristics of the study population. BMI, body mass index; SAPS II, Simplified Acute Physiologic Score; SOFA, Sequential Organ failure Assessment. Data are expressed as mean ± SD, median [25–75 interquartile range], or count.

$CI \times \Delta \text{ContO}_2 \times 10$ . Oxygen extraction was defined as  $OE = \text{VO}_2 / \text{DO}_2$ . We also calculated the  $\Delta \text{PCO}_2 / \Delta \text{ContO}_2$  ratio.

**Study protocol.** A first set of hemodynamic and oxygen-CO<sub>2</sub> derived variables measurements was performed at baseline, including heart rate (HR), systemic arterial pressure, CI (thermodilution), DO<sub>2</sub>, VO<sub>2</sub>, ScvO<sub>2</sub>, arterial lactate level, and  $\Delta \text{PCO}_2$ . A 500 ml of colloid solution (4% Human serum albumin, Vialebex<sup>®</sup>; LFB) was administered to the patient over 15 min via a specific venous line. The same set of measurements was repeated immediately after the end of VE infusion. Ventilation parameters and infusions of norepinephrine and sedation drugs were remained unchanged during the VE.

Changes in hemodynamic and oxygenation variables were expressed as relative changes ( $[(\text{parameter after volume expansion} - \text{parameter before volume expansion}) / \text{parameter before volume expansion}] \times 100$ ).

**Statistical analysis.** Patients in whom 500-ml VE increased thermodilution-derived CI > 10% were defined as responders and the remaining ones as non-responders. Also, patients were divided into two subgroups according to their increases in VO<sub>2</sub> ( $\leq$  or > 10%) induced by VE. All data are expressed as mean ± SD, or as median [25–75%, interquartile range, (IQR)], as appropriate. The normality of data distribution was assessed using the Shapiro–Wilk test. Comparisons of values between responders and non-responders were performed by two-tailed Student's t test, or Wilcoxon rank-sum test, as appropriate. Pairwise comparisons between different study times were assessed using paired Student's t test or Wilcoxon signed-rank test, as appropriate. Analysis of categorical data was performed using the Chi<sup>2</sup> and Fisher's exact tests. Linear correlations were tested by using the Pearson or the Spearman test, as appropriate.

Receiver operating characteristic (ROC) curves were constructed to evaluate the ability of each parameter to predict fluid responsiveness after fluid challenge. The AUCs were compared using the nonparametric technique described by DeLong et al.<sup>18</sup>. Previously, we have shown that the upper 95% confidence interval values of the least significant changes (LSC), which are the minimum changes that needed to be measured by a laboratory analyzer in order to recognize a real change of measurement, for  $\Delta \text{PCO}_2$  and ScvO<sub>2</sub> were 36.5% and 5.0% respectively<sup>19</sup>. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR<sup>+</sup>), negative likelihood ratio (LR<sup>-</sup>), and their 95% confidence intervals were calculated for  $\Delta \text{PCO}_2$  and  $\Delta \text{ScvO}_2$ .

Variables are usually considered of good clinical tool (having good discriminative property tests) when the inferior limits of the 95% confidence interval of their AUC are more than 0.75<sup>20</sup>. For this purpose, 43 patients would be sufficient for a power of 80% and an alpha risk of 0.05. Statistical analysis was performed using STATA 14.0 (StataCorp LP, College Station, Texas, USA).  $p < 0.05$  was considered statistically significant. All reported  $p$  values are 2-sided.

## Results

We studied 49 patients whose characteristics are summarized in Table 1. Twenty-five of the 49 patients (51%) were defined as responders because thermodilution CI increased by > 10% after VE of 500-ml.

There were no significant differences in patient characteristics, SOFA score, and norepinephrine between responders and non-responders (Table 1).

	Before volume expansion	After volume expansion
<b>Heart rate (beats min<sup>-1</sup>)</b>		
Responders	101 ± 26	100 ± 26
Non-responders	99 ± 25	97 ± 26
<b>Systolic arterial pressure (mmHg)</b>		
Responders	102 ± 21	119 ± 20 <sup>a</sup>
Non-responders	107 ± 29	116 ± 28 <sup>a</sup>
<b>Diastolic arterial pressure (mmHg)</b>		
Responders	58 ± 12	59 ± 12 <sup>a</sup>
Non-responders	55 ± 12	62 ± 10 <sup>a</sup>
<b>Mean arterial pressure (mmHg)</b>		
Responders	72 ± 13	81 ± 12 <sup>a</sup>
Non-responders	72 ± 17	78 ± 17 <sup>a</sup>
<b>Pulse pressure (mmHg)</b>		
Responders	44 ± 18	57 ± 17 <sup>a</sup>
Non-responders	52 ± 21	57 ± 22 <sup>a</sup>
<b>Central venous pressure (mmHg)</b>		
Responders	13 ± 6	16 ± 6 <sup>a</sup>
Non-responders	16 ± 5	19 ± 6 <sup>a</sup>
<b>Intra-thoracic blood volume index (ml m<sup>-2</sup>)</b>		
Responders	841 ± 188	951 ± 215 <sup>a</sup>
Non-responders	858 ± 236	971 ± 223 <sup>a</sup>
<b>Extravascular lung water index (ml kg<sup>-1</sup>)</b>		
Responders	9.1 ± 4.2	9.2 ± 4.3
Non-responders	8.9 ± 3.6	9.1 ± 3.4
<b>Systemic vascular resistance index (DS m<sup>-2</sup> cm<sup>5</sup>)</b>		
Responders	1702 [1218–2225]	1587 [1220–1990] <sup>a</sup>
Non-responders	1540 [957–1894]	1678 [1123–2096]
<b>Cardiac index (l min<sup>-1</sup> m<sup>-2</sup>)</b>		
Responders	2.6 [2.1–3.5]	3.46 [2.59–4.16] <sup>a</sup>
Non-responders	2.9 [2.2–3.9]	2.78 [2.19–4.07]
<b>Stroke volume index (ml m<sup>-2</sup>)</b>		
Responders	29.6 ± 10.5	36.6 ± 10.7 <sup>a</sup>
Non-responders	33.6 ± 13.6	33.6 ± 12.2
<b>Hemoglobin (g/dL)</b>		
Responders	10.3 ± 1.6	9.5 ± 1.3 <sup>a</sup>
Non-responders	9.6 ± 1.8	9.1 ± 1.6 <sup>a</sup>
<b>Arterial pH</b>		
Responders	7.35 [7.28–7.37]	7.34 [7.26–7.36]
Non-responders	7.34 [7.20–7.39]	7.31 [7.21–7.37]
<b>Central venous pH</b>		
Responders	7.28 [7.22–7.33]	7.30 [7.22–7.33]
Non-responders	7.30 [7.15–7.34]	7.28 [7.16–7.34]
<b>Base excess (mmol L<sup>-1</sup>)</b>		
Responders	−6.5 [−8.9 to −1.7]	−6.7 [−9.5 to −2.1] <sup>a</sup>
Non-responders	−4.7 [−12.4 to −1.1]	−5.1 [−12.1 to −1.7] <sup>a</sup>

**Table 2.** Hemodynamic and acid–base variables before and after 500 ml of volume expansion. Data are expressed as mean (SD) or median [25–75 interquartile range]. Responders n = 25; Non-responders n = 24. <sup>a</sup>*p* < 0.05 comparisons between responders and non-responders. <sup>a</sup>*p* < 0.05 comparisons between before and after volume expansion.

**Effect of volume expansion on hemodynamic variables.** At baseline, all the tested hemodynamic variables were similar between the two groups (Table 2). VE significantly increased arterial pressures, CVP, and intrathoracic blood volume, and decreased hemoglobin in both groups. CI and stroke volume index increased significantly only in responders after VE, whereas HR and extravascular lung water did not change (Table 2). Arterial and venous pH were not significantly different between responders and non-responders' groups at baseline, and did not change significantly after VE (Table 2).

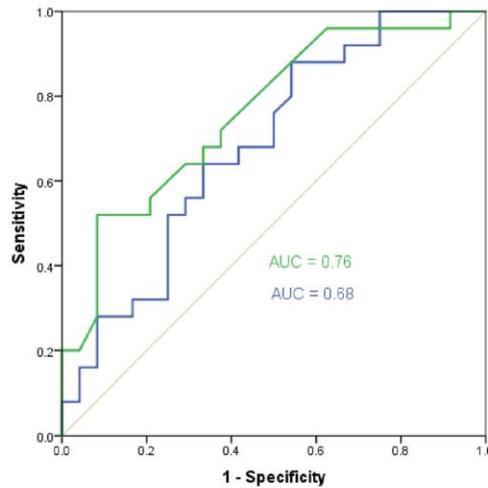
	Before volume expansion	After volume expansion
<b>Central venous oxygen saturation (%)</b>		
Responders	62 ± 13	68 ± 13 <sup>†*</sup>
Non-responders	57 ± 15	56 ± 15
<b>Oxygen delivery (ml min<sup>-1</sup> m<sup>-2</sup>)</b>		
Responders	382 ± 130	441 ± 134 <sup>†</sup>
Non-responders	388 ± 163	368 ± 151
<b>Arterial oxygen content (mL)</b>		
Responders	13.4 ± 2.2	12.5 ± 1.8 <sup>†</sup>
Non-responders	12.3 ± 2.5	11.8 ± 2.2
<b>Central venous oxygen content (mL)</b>		
Responders	8.6 ± 2.2	8.7 ± 2.1 <sup>*</sup>
Non-responders	7.4 ± 2.6	7.1 ± 2.6
<b>Oxygen consumption (mL min<sup>-1</sup> m<sup>-2</sup>)</b>		
Responders	129.6 ± 44.5	125.8 ± 45.2
Non-responders	144.3 ± 49.5	139.3 ± 51.0
<b>Oxygen extraction (%)</b>		
Responders	36.0 ± 12.8	30.4 ± 12.8 <sup>†*</sup>
Non-responders	40.0 ± 15.0	41.1 ± 14.9
<b>PaCO<sub>2</sub> (mmHg)</b>		
Responders	38 [33–41]	39 [32–41]
Non-responders	38 [34–40]	38 [33–41]
<b>PcvCO<sub>2</sub> (mmHg)</b>		
Responders	45 [38–49]	44 [36–48] <sup>†</sup>
Non-responders	45 [42–47]	45 [41–50]
<b>ΔPCO<sub>2</sub> (mmHg)</b>		
Responders	7.0 [5.0–9.0]	5.0 [3.0–6.0] <sup>†</sup>
Non-responders	7.0 [5.0–9.0]	6.5 [5.0–9.0]
<b>ΔPCO<sub>2</sub>/ΔContO<sub>2</sub> (mmHg/mL)</b>		
Responders	1.59 ± 0.53	1.51 ± 0.56
Non-responders	1.60 ± 0.70	1.60 ± 0.49
<b>Lactate (mmol/L)</b>		
Responders	2.0 [1.1–3.6]	1.7 [1.0–3.4] <sup>†</sup>
Non-responders	1.5 [1.3–4.7]	1.6 [1.3–4.2]
<b>Arterial oxygen saturation (%)</b>		
Responders	96 [92–99]	96 [94–98]
Non-responders	96 [93–97]	96 [94–97]
<b>PaO<sub>2</sub>/FiO<sub>2</sub> ratio (mmHg)</b>		
Responders	202 [149–294]	211 [157–295]
Non-responders	197 [124–273]	202 [154–265]

**Table 3.** Oxygenation and CO<sub>2</sub>-derived variables before and after 500 ml of volume expansion. PaCO<sub>2</sub>, arterial CO<sub>2</sub> tension; PcvCO<sub>2</sub>, central venous CO<sub>2</sub> tension; ΔPCO<sub>2</sub>, central venous-to-arterial PCO<sub>2</sub> difference; ΔContO<sub>2</sub>, arterial-to-venous oxygen content difference; PaO<sub>2</sub>, arterial oxygen tension; FiO<sub>2</sub>, inspiratory oxygen fraction. Data are expressed as mean (SD) or median [25–75 interquartile range]. Responders n = 25; Non-responders n = 24. \**p* < 0.05 comparisons between responders and non-responders. <sup>†</sup>*p* < 0.05 comparisons between before and after volume expansion.

**Effect of volume expansion on oxygenation and CO<sub>2</sub>-derived variables.** At baseline, there were no significant differences between the responders and non-responders' groups regarding all the oxygenation and CO<sub>2</sub>-derived variables (Table 3). DO<sub>2</sub> and ScvO<sub>2</sub> increased significantly, and OE decreased only in responders' group after VE. VE significantly reduced ΔPCO<sub>2</sub> and lactate levels only in the responders' group (Table 3). VO<sub>2</sub> and ΔPCO<sub>2</sub>/ΔContO<sub>2</sub> ratio remained unchanged in both groups after VE.

We observed significant correlations between ΔScvO<sub>2</sub> and ΔCI (*r* = 0.42, *p* = 0.003) and between Δ-ΔPCO<sub>2</sub> and ΔCI (*r* = -0.30, *p* = 0.03) after VE.

In patients with an increase in VO<sub>2</sub> ≤ 10% (n = 36) after VE, the correlation between ΔScvO<sub>2</sub> and ΔCI was of 0.62 (*p* < 0.001). Also, in these patients, Δ-ΔPCO<sub>2</sub> was significantly correlated with ΔCI (*r* = -0.47, *p* = 0.004).



**Figure 1.** Receiver operating characteristic (ROC) curves showing the ability of the changes in  $\Delta\text{PCO}_2$  ( $\Delta\text{-}\Delta\text{PCO}_2$ ) (green curve),  $\text{ScvO}_2$  ( $\Delta\text{ScvO}_2$ ) (blue curve) between before and after 500 mL of volume expansion to define fluid responsiveness (increase in cardiac index > 10% after volume expansion).

**Ability of  $\Delta\text{ScvO}_2$  and  $\Delta\text{-}\Delta\text{PCO}_2$  to define fluid responsiveness (increase in CI > 10% after VE).** The AUC for  $\Delta\text{ScvO}_2$  was 0.68 (95% CI: 0.53–0.83) ( $p = 0.02$ ) and for  $\Delta\text{-}\Delta\text{PCO}_2$  was 0.76 (95% CI: 0.63–0.89) ( $p < 0.001$ ) (Fig. 1). There were no significant differences between the AUCs for  $\Delta\text{ScvO}_2$  and  $\Delta\text{-}\Delta\text{PCO}_2$  ( $p = 0.41$ ).

The best cutoff value (according to Youden index) for  $\Delta\text{ScvO}_2$  was  $\geq 3.5\%$  (sensitivity = 64% [95% CI: 42–82%], specificity = 65% [95% CI: 43–84%]), which was lower than its LSC (5%). Taking into account the repeatability (LSC), the best cutoff value was  $\geq 7.4\%$  (sensitivity = 56% [95% CI: 35–76%], specificity = 71% [95% CI: 49–87%], PPV = 63% [95% CI: 39–83%], NPV = 61% [95% CI: 41–78%],  $\text{LR}^+ = 1.8$  [95% CI: 0.9–3.7], and  $\text{LR}^- = 0.7$  [95% CI: 0.4–1.1]).

The best cutoff value (according to Youden index) for  $\Delta\text{-}\Delta\text{PCO}_2$  was  $\leq -23.5\%$  (sensitivity = 52% [95% CI: 31–72%], specificity = 87% [95% CI: 68–97%]), which was lower than its LSC (36.5%). Taking into account the repeatability (LSC), the best cutoff value was  $\leq -37.5\%$  (sensitivity = 32% [95% CI: 15–53%], specificity = 92% [95% CI: 73–99%], PPV = 79% [95% CI: 42–97%], NPV = 58% [95% CI: 42–74%],  $\text{LR}^+ = 3.8$  [95% CI: 0.9–16.3], and  $\text{LR}^- = 0.7$  [95% CI: 0.6–1.0]).

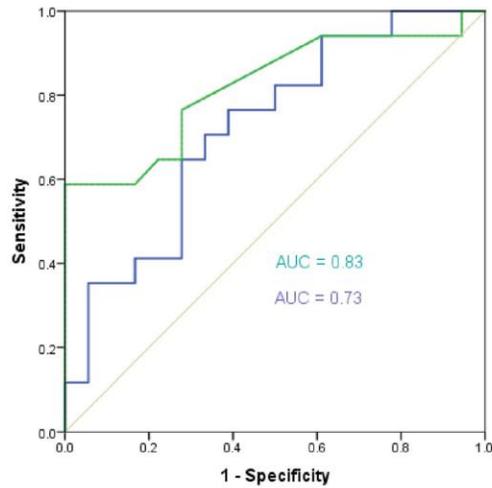
In patients with an increase in  $\text{VO}_2 \leq 10\%$ , the AUC for  $\Delta\text{ScvO}_2$  was 0.73 (95% CI: 0.57–0.90) ( $p = 0.006$ ) and for  $\Delta\text{-}\Delta\text{PCO}_2$  was 0.83 (95% CI: 0.69–0.97) ( $p < 0.001$ ) (Fig. 2). There was no significant difference between the AUCs for  $\Delta\text{ScvO}_2$  and  $\Delta\text{-}\Delta\text{PCO}_2$  ( $p = 0.41$ ).

The best cutoff value (according to Youden index) for  $\Delta\text{ScvO}_2$  was  $\geq 8.1\%$  (sensitivity = 65% [95% CI: 38–86%], specificity = 74% [95% CI: 49–91%]), PPV = 73% [95% CI: 47–91%], NPV = 65% [95% CI: 40–85%],  $\text{LR}^+ = 2.5$  [95% CI: 1.1–5.6], and  $\text{LR}^- = 0.5$  [95% CI: 0.2–1.0]).

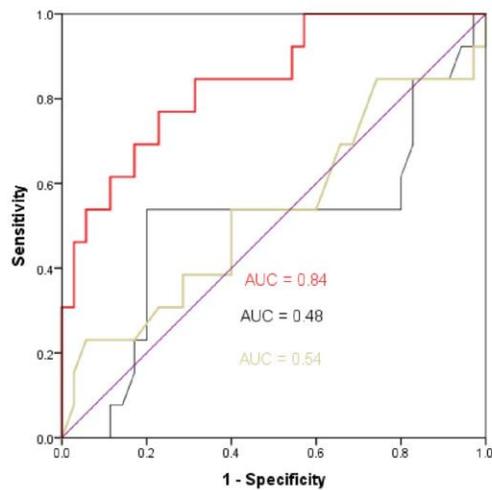
The best cutoff value (according to Youden index) for  $\Delta\text{-}\Delta\text{PCO}_2$  was  $\leq -25\%$  (sensitivity = 59% [95% CI: 33–82%], specificity = 89% [95% CI: 65–99%]), which was lower than its LSC (36.5%). Taking into account the repeatability (LSC), the best cutoff value was  $\leq -37.5\%$  (sensitivity = 41% [95% CI: 18–67%], specificity = 100% [95% CI: 59–100%], PPV = 100% [95% CI: 62–100%], NPV = 60% [95% CI: 40–78%],  $\text{LR}^+ = \infty$ , and  $\text{LR}^- = 0.60$  [95% CI: 0.4–0.9]).

**Characteristics of patients with tissue hypoxia ( $\Delta\text{VO}_2 > 10\%$  after VE).** At baseline,  $\Delta\text{PCO}_2/\Delta\text{ContO}_2$  ratio was significantly higher in patients with  $\Delta\text{VO}_2 > 10\%$  (tissue hypoxia) induced by volume expansion compared to patients with  $\Delta\text{VO}_2 \leq 10\%$  (2.03 [1.73–2.27] vs. 1.39 [1.00–1.71] mmHg/mL,  $p = 0.03$ , respectively). We did not observe significant differences between patients with  $\Delta\text{VO}_2 > 10\%$  and  $\Delta\text{VO}_2 \leq 10\%$  regarding baseline lactate levels (2.35 [1.02–3.97] vs. 1.70 [1.40–3.10] mmol/L,  $p = 0.89$ , respectively) and baseline  $\text{ScvO}_2$  levels (60  $\pm$  17% vs. 59  $\pm$  13%,  $p = 0.78$ , respectively).

The AUCs of baseline lactate and  $\text{ScvO}_2$  values to predict  $\Delta\text{VO}_2 > 10\%$  were 0.48 (95% CI: 0.28–0.69) ( $p = 0.89$ ) and 0.54 (95% CI: 0.33–0.74) ( $p = 0.72$ ), respectively. The AUC of baseline  $\Delta\text{PCO}_2/\Delta\text{ContO}_2$  ratio to predict  $\Delta\text{VO}_2 > 10\%$  after volume expansion was 0.84 (95% CI: 0.71–0.96) ( $p < 0.001$ ) (Fig. 3). The best cutoff value (according to Youden index) for baseline  $\Delta\text{PCO}_2/\Delta\text{ContO}_2$  ratio was  $> 1.70$  (sensitivity = 77% [95% CI: 46–95%], specificity = 77% [95% CI: 60–90%]), PPV = 90% [95% CI: 73–98%], NPV = 55% [95% CI: 31–78%],  $\text{LR}^+ = 3.4$  [95% CI: 1.7–6.6], and  $\text{LR}^- = 0.3$  [95% CI: 0.1–0.8]).



**Figure 2.** Receiver operating characteristic (ROC) curves showing the ability of the changes in  $\Delta\text{PCO}_2$  ( $\Delta\text{-}\Delta\text{PCO}_2$ ) (green curve),  $\text{ScvO}_2$  ( $\Delta\text{ScvO}_2$ ) (blue curve) between before and after 500 mL of volume expansion to define fluid responsiveness (increase in cardiac index > 10% after volume expansion) in the subgroup of patients with an increase in oxygen consumption  $\leq 10\%$ .



**Figure 3.** Receiver operating characteristic (ROC) curves showing the ability of baseline  $\Delta\text{PCO}_2/\Delta\text{ContO}_2$  (red curve),  $\text{ScvO}_2$  (yellow curve), and lactate (black curve) to predict an increase in oxygen consumption > 10% after 500 mL of volume expansion.

### Discussion

The main findings of our study are: (1) in the whole population  $\Delta\text{-}\Delta\text{PCO}_2$  induced by VE has an acceptable ability to define fluid responsiveness, but not  $\Delta\text{ScvO}_2$ ; (2) The abilities of  $\Delta\text{-}\Delta\text{PCO}_2$  and  $\Delta\text{ScvO}_2$  to define fluid responsiveness improved when we considered only patients in whom changes in  $\text{VO}_2$  were minimal ( $\Delta\text{VO}_2 \leq 10\%$ ) after VE, i.e., patients without tissue hypoxia; (3) Baseline  $\Delta\text{PCO}_2/\Delta\text{ContO}_2$  ratio has a good ability to predict the presence of tissue hypoxia (increases in  $\text{VO}_2 > 10\%$  after VE).

Applying the modified Fick method to CO<sub>2</sub>, venous-to-arterial PCO<sub>2</sub> difference reflects cardiac output. Several experimental studies have demonstrated the primary role of decreased tissue blood flow in the increased venous-to-arterial PCO<sub>2</sub> gap<sup>8,21,22</sup>. Similarly, a mathematical model analysis has confirmed that blood flow represents the major determinant in the elevation of venous-to-arterial PCO<sub>2</sub> gap<sup>23</sup>. A rise in mixed venous-to-arterial PCO<sub>2</sub> gap that was directly linked to a decrease in cardiac output has been observed in different types of circulatory failure including septic shock<sup>24,25</sup>. Mecher et al. found a significant negative correlation between the changes in cardiac output and mixed venous-to-arterial PCO<sub>2</sub> gap after fluid resuscitation in septic shock patients ( $r = -0.42$ ,  $p < 0.01$ )<sup>24</sup>. In post-cardiac surgery sedated and mechanically ventilated patients with cardiac index  $< 2.5$  L/min/m<sup>2</sup>,  $\Delta$ CI induced by VE (500-mL bolus of crystalloid given over 30 min) was found to be significantly correlated with  $\Delta$ - $\Delta$ PCO<sub>2</sub> ( $r = -0.53$ ,  $p = 0.001$ )<sup>13</sup>. However, the AUC for  $\Delta$ - $\Delta$ PCO<sub>2</sub> to define fluid responsiveness was not determined in that study.

The correlation between  $\Delta$ - $\Delta$ PCO<sub>2</sub> and  $\Delta$ CI induced by VE was weaker in our septic patients than what was observed in post-cardiac surgery patients<sup>13</sup>. Also, the ability of  $\Delta$ - $\Delta$ PCO<sub>2</sub> to define fluid responsiveness was not robust (AUC = 0.76). These findings could be explained by several factors. First, the relationship between  $\Delta$ PCO<sub>2</sub> and CI is curvilinear<sup>9</sup>, which means that the magnitude of changes in  $\Delta$ PCO<sub>2</sub> is more pronounced at low CI than at normal or high CI. Second, the relationship between CO<sub>2</sub> content and PCO<sub>2</sub>, which is curvilinear rather than linear, is influenced by many factors such as the degree of metabolic acidosis, hematocrit, and oxygen saturation (Haldane effect)<sup>9,26</sup>. However, we believe that this factor is unlikely to have occurred in our patients. Although base excess and hemoglobin significantly decreased in both groups (responders and non-responders) and ScvO<sub>2</sub> increased only in the responders' group (Tables 2, 3) after VE, it is unlikely that these clinically irrelevant changes could have affected the PCO<sub>2</sub>/CO<sub>2</sub> content relationship. If these changes had affected the PCO<sub>2</sub>/CO<sub>2</sub> content relationship, it would have resulted in an increase in  $\Delta$ PCO<sub>2</sub> in both groups. Third, in situations of tissue hypoxia with VO<sub>2</sub>/DO<sub>2</sub> dependency phenomenon and anaerobic CO<sub>2</sub> production, the rise in CI would increase VO<sub>2</sub> and VCO<sub>2</sub>. This would attenuate the decrease in  $\Delta$ PCO<sub>2</sub> related to the increase in blood flow<sup>9,27</sup>. We believe that this factor may have contributed to the reduction in the performance of  $\Delta$ - $\Delta$ PCO<sub>2</sub> (AUC = 0.76) in defining an increase in CI  $> 10\%$  induced by VE (fluid responsiveness) in the overall population. When we excluded patients with tissue hypoxia, patients with an increase in VO<sub>2</sub>  $\leq 10\%$  (VO<sub>2</sub>/DO<sub>2</sub> independency), we observed an improvement in the ability of  $\Delta$ - $\Delta$ PCO<sub>2</sub> to define fluid responsiveness with a very good AUC of 0.83 (Fig. 2). A decrease in  $\Delta$ - $\Delta$ PCO<sub>2</sub> of more or equal than 37.5% induced by VE allowed discrimination between responders and non-responders with a PPV of 100%. Also, the correlation between  $\Delta$ - $\Delta$ PCO<sub>2</sub> and  $\Delta$ CI was higher than in the overall population.

Venous oxygen saturation is a global marker of adequacy between oxygen consumption and oxygen supply<sup>28</sup>. Therefore, changes in venous oxygen saturation reflect changes in the balance between VO<sub>2</sub> and DO<sub>2</sub> and indicate tissue oxygenation. Giraud et al. observed, in 30 cardiogenic shock or postoperative cardiac surgery patients, that  $\Delta$ ScvO<sub>2</sub> was significantly correlated with  $\Delta$ CI induced by a bolus of 500 mL of normal saline administered over 10-min ( $r = 0.67$ ,  $p < 0.001$ )<sup>14</sup>. Also,  $\Delta$ ScvO<sub>2</sub> had an excellent ability to define an increase in CI  $\geq 15\%$  after VE (fluid responsiveness) with an AUC of 0.90. Our findings are different from those reported by Giraud et al.<sup>14</sup>. We observed in our whole septic population a weaker correlation between  $\Delta$ ScvO<sub>2</sub> and  $\Delta$ CI ( $r = 0.42$ ), and  $\Delta$ ScvO<sub>2</sub> had a poor ability to discriminate between responders and non-responders. In the subgroup of patients without tissue hypoxia (no significant increase in VO<sub>2</sub>, or VO<sub>2</sub>/DO<sub>2</sub> independency), even though the correlation between  $\Delta$ ScvO<sub>2</sub> and  $\Delta$ CI improved, the ability of  $\Delta$ ScvO<sub>2</sub> to characterize fluid responsiveness was not good. The main explanation of the discrepancies between our findings and those of Giraud et al.<sup>14</sup> is that the patient populations are different. As has been previously described, venous oxygen saturation may not be a good indicator of tissue oxygenation in the setting of sepsis, due to microcirculatory shunting and mitochondrial dysfunction that can result in oxygen extraction abnormalities<sup>29</sup>.

It has been suggested that  $\Delta$ PCO<sub>2</sub>/ $\Delta$ ContO<sub>2</sub> ratio, considered as a surrogate of the respiratory quotient, can be used as a marker of global tissue hypoxia in critically ill patients<sup>15,16,30</sup>. In our study, we found that baseline  $\Delta$ PCO<sub>2</sub>/ $\Delta$ ContO<sub>2</sub> value was significantly higher in patients with global tissue hypoxia (defined as an increase in VO<sub>2</sub>  $> 10\%$  after VE) than those without global tissue hypoxia. Also, baseline  $\Delta$ PCO<sub>2</sub>/ $\Delta$ ContO<sub>2</sub> ratio had a very good ability to predict the presence of VO<sub>2</sub>/DO<sub>2</sub> dependency (global tissue hypoxia). Our results confirmed our previous findings<sup>15</sup> and those reported by Monnet et al.<sup>16</sup>, who observed excellent predictability of baseline  $\Delta$ PCO<sub>2</sub>/ $\Delta$ ContO<sub>2</sub> value for tissue hypoxia (AUCs = 0.96 and 0.94, respectively). Baseline lactate and ScvO<sub>2</sub> levels had poor ability to predict VO<sub>2</sub>/DO<sub>2</sub> dependency (global tissue hypoxia) in our study. This finding is in line of what we observed previously in septic shock patients<sup>15</sup>.

To the best of our knowledge, our study is the first to investigate the role of  $\Delta$ - $\Delta$ PCO<sub>2</sub> and  $\Delta$ ScvO<sub>2</sub> in defining fluid responsiveness in septic patients. Our findings are valuable as they can be integrated in a clinical algorithm and used by the bedside provider as part of the assessment of fluid responsiveness. These values are readily available as patients in septic shock usually have arterial and central venous catheters inserted. After measuring O<sub>2</sub> content and CO<sub>2</sub> partial pressures, the  $\Delta$ PCO<sub>2</sub>/ $\Delta$ ContO<sub>2</sub> ratio can help the provider recognize patients without tissue hypoxia. In these patients,  $\Delta$ PCO<sub>2</sub> can be measured before and after VE and be used to appreciate if the latter has resulted in a significant increase in CI and to guide further fluid resuscitation when cardiac output monitoring is not available.

Our study presents several limitations. First, it is a single-center study, so the results might not universally apply. Second, we used central venous samples instead of mixed venous to assess oxygen and CO<sub>2</sub>-derived variables, limiting its accuracy. However, we were interested in the changes in these variables induced by fluid challenge rather than their absolute values. Moreover, it has been shown that calculating the oxygen and CO<sub>2</sub>-derived variables from the central venous blood permitted the detection of global tissue hypoxia in critically ill patients<sup>15,16</sup>. Third, our patients were sedated and mechanically ventilated with stable oxygen consumption; thus, our findings might not apply to spontaneously breathing patients with varying oxygen demands. Finally,

our study was not sufficiently powered for subgroup analyses; thus, our findings need to be replicated in a future study with larger sample size.

### Conclusions

In sedated and mechanically ventilated septic patients with no signs of tissue hypoxia,  $\Delta$ - $\Delta$ PCO<sub>2</sub> is a reliable parameter to define fluid responsiveness and can be used in the absence of CI measurement. Baseline  $\Delta$ PCO<sub>2</sub>/ $\Delta$ ContO<sub>2</sub> ratio could help the physician recognize the presence of tissue hypoxia.

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### References

1. Michard, F. & Teboul, J. L. Predicting fluid responsiveness in ICU patients: a critical analysis of the evidence. *Chest* **121**, 2000–2008 (2002).
2. Vincent, J. L. & Weil, M. H. Fluid challenge revisited. *Crit. Care Med.* **34**, 1333–1337 (2006).
3. Cecconi, M., Parsons, A. K. & Rhodes, A. What is a fluid challenge?. *Curr. Opin. Crit. Care* **17**, 290–295 (2011).
4. Monnet, X. *et al.* Passive leg raising predicts fluid responsiveness in the critically ill. *Crit. Care Med.* **34**, 1402–1407 (2006).
5. Monnet, X. *et al.* Predicting volume responsiveness by using the end-expiratory occlusion in mechanically ventilated intensive care unit patients. *Crit. Care Med.* **37**, 951–956 (2009).
6. Mallat, J. *et al.* Decrease in pulse pressure and stroke volume variations after mini-fluid challenge accurately predicts fluid responsiveness. *Br. J. Anaesth.* **115**, 449–456 (2015).
7. Mahjoub, Y. *et al.* Evaluation of pulse pressure variation validity criteria in critically ill patients: a prospective observational multicentre point-prevalence study. *Br. J. Anaesth.* **112**, 681–685 (2014).
8. Vallet, B., Teboul, J. L., Cain, S. & Curtis, S. Venous-arterial CO<sub>2</sub> difference during regional ischemic or hypoxic hypoxia. *J. Appl. Physiol.* **89**, 1317–1321 (2000).
9. Mallat, J., Lemyze, M., Tronchon, L., Vallet, B. & Thevenin, D. Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock. *World J. Crit. Care Med.* **5**, 47–56 (2016).
10. Mallat, J. *et al.* Central venous-to-arterial carbon dioxide partial pressure difference in early resuscitation from septic shock: a prospective observational study. *Eur. J. Anaesthesiol.* **31**, 371–380 (2014).
11. Vallée, F. *et al.* Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock?. *Intensive Care Med.* **34**, 2218–2225 (2008).
12. Cuschieri, J. *et al.* Central venous-arterial carbon dioxide difference as an indicator of cardiac index. *Intensive Care Med.* **31**, 818–822 (2005).
13. Yazigi, A. *et al.* Correlation between central venous-arterial carbon dioxide tension gradient and cardiac index changes following fluid therapy. *Ann. Card Anaesth.* **13**, 269–271 (2010).
14. Giraud, R. *et al.* ScvO<sub>2</sub> as a marker to define fluid responsiveness. *J. Trauma* **70**(4), 802–807 (2011).
15. Mallat, J. *et al.* Ratios of central venous-to-arterial carbon dioxide content or tension to arteriovenous oxygen content are better markers of global anaerobic metabolism than lactate in septic shock patients. *Ann. Intensive Care.* **6**, 10 (2016).
16. Monnet, X. *et al.* Lactate and venous-arterial 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 (2013).
17. Dellinger, R. P. *et al.* Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit. Care Med.* **41**, 580–637 (2013).
18. DeLong, E. R., DeLong, D. M. & Clarke-Pearson, D. L. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* **44**, 837–845 (1988).
19. Mallat, J. *et al.* Repeatability of blood gas parameters, PCO<sub>2</sub> gap, and PCO<sub>2</sub> gap to arterial-to-venous oxygen content difference in critically ill adult patients. *Medicine (Baltimore)* **94**, e415 (2015).
20. Ray, P., Le Manach, Y., Riou, B. & Houle, T. T. Statistical evaluation of a biomarker. *Anesthesiology* **112**, 1023–1040 (2010).
21. Nevière, R., Chagnon, J. L., Teboul, J. L., Vallet, B. & Wattel, F. Small intestine intramucosal PCO<sub>2</sub> and microvascular blood flow during hypoxic and ischemic hypoxia. *Crit. Care Med.* **30**, 379–384 (2002).
22. Dubin, A. *et al.* Intramucosal-arterial PCO<sub>2</sub> gap fails to reflect intestinal dysoxia in hypoxic hypoxia. *Crit. Care* **6**, 514–520 (2002).
23. Gutierrez, G. A mathematical model of tissue-blood carbon dioxide exchange during hypoxia. *Am. J. Respir. Crit. Care Med.* **169**, 525–533 (2004).
24. Mecher, C. E., Rackow, E. C., Astiz, M. E. & Weil, M. H. Venous hypercarbia associated with severe sepsis and systemic hypoperfusion. *Crit. Care Med.* **18**, 585–589 (1990).
25. Bakker, J. *et al.* Venous-arterial carbon dioxide gradient in human septic shock. *Chest* **101**, 509–515 (1992).
26. Teboul, J. L. & Scheeren, T. Understanding the Haldane effect. *Intensive Care Med.* **43**, 91–93 (2017).
27. Lamia, B., Monnet, X. & Teboul, J. L. Meaning of arterio-venous PCO<sub>2</sub> difference in circulatory shock. *Minerva Anesthesiol.* **72**, 597–604 (2006).
28. Squara, P. Central venous oxygenation: when physiology explains apparent discrepancies. *Crit. Care* **18**, 579 (2014).
29. Vallet, B., Pinsky, M. R. & Cecconi, M. Resuscitation of patients with septic shock: please “mind the gap”. *Intensive Care Med.* **39**, 1653–1655 (2013).
30. Mekontso-Dessap, A. *et al.* Combination of venous-arterial PCO<sub>2</sub> difference with arteriovenous O<sub>2</sub> content difference to detect anaerobic metabolism in patients. *Intensive Care Med.* **28**, 272–277 (2002).

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### Author contributions

J.M., B.N., and M.B. designed the study. J.M. conducted statistical analyses. N.V., G.G., F.P., and J.T. collected data. J.M., B.N., L.T., and D.T. participated in manuscript writing and reviewing. All authors read and approved the final manuscript.

### Competing interests

The authors declare no competing interests.

### Additional information

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## IV. Limitations

### 1. Limitations related to the PCO<sub>2</sub>/CCO<sub>2</sub> relationship

$\Delta\text{PCO}_2_{\text{Mix}}$  or  $\Delta\text{PCO}_2_{\text{Cent}}$  and  $\Delta\text{PCO}_2_{\text{Mix}}/\Delta\text{O}_2_{\text{Mix}}$  or  $\Delta\text{PCO}_2_{\text{Cent}}/\Delta\text{O}_2_{\text{Cent}}$  ratio might be affected by other factors than blood flow or anaerobic metabolism by influencing the relationship between CCO<sub>2</sub> and PCO<sub>2</sub>, which is quasilinear over the physiological range of PCO<sub>2</sub> [120], but it becomes curvilinear if these factors change [106]. These factors are the degree of metabolic acidosis [121] (Figure 7), hematocrit [122], and oxygen saturation (Haldane effect) [119, 122] (Figure 8). Indeed, severe metabolic acidosis, low hematocrit, and high oxygen saturation can increase PCO<sub>2</sub> for a given CCO<sub>2</sub> since less CO<sub>2</sub> is bound to hemoglobin.

In conditions of very low venous oxygen saturation (< 30%), Jakob et al. [124] found that changes in  $\Delta\text{PCO}_2$  might not parallel changes in CCO<sub>2</sub> differences or blood flow due in part to the Haldane effect. However, in an experimental study of vascularly isolated, innervated, and perfused dog hindlimb, Mallat et Vallet [127] observed that the Haldane effect did not influence the PCO<sub>2</sub>/CCO<sub>2</sub> relationship as the latter was the same, accounting or not for the changes in venous oxygen saturation even when the latter decreased far below 30%. In healthy subjects, during heavy exercise, Sun et al. [174] also reported that Haldane effect (changes in venous oxygen saturation) had a minor impact on the PCO<sub>2</sub>/CCO<sub>2</sub> relationship. Furthermore, in septic shock patients, Mesquida et al. [175] found that venous oxygen saturation (Haldane effect) had a minimal impact on the PCO<sub>2</sub>/CCO<sub>2</sub> relationship.

In the experimental study of perfused dog hindlimb [127], the degree of metabolic acidosis impacted the PCO<sub>2</sub>/CCO<sub>2</sub> relationship significantly. Indeed, when the changes in venous pH were ignored, the PCO<sub>2</sub>/CCO<sub>2</sub> relationship was almost linear. However, CCO<sub>2</sub> was not linearly related

to  $PCO_2$  when the changes in pH were acknowledged. In fact,  $PCO_2$  and  $CCO_2$  changed in opposite directions as metabolic acid was added to the blood by the hypoxic cells. That is because metabolic acidosis causes plasma and red blood cell  $CCO_2$  and bicarbonates to decrease. Also, changes in pH had a significant influence on the  $PCO_2/CCO_2$  relationship with  $CCO_2$  not linearly related to  $PCO_2$  and even varied in opposite directions after the lactic acidosis threshold was reached during heavy exercise in healthy subjects [174]. In addition, metabolic acidosis (pH) was the only best predictor of the discrepancy found between  $\Delta PCO_2\_Mix/\Delta O_2\_Mix$  and  $\Delta CCO_2/C(a-v)O_2$  in septic shock patients [175]. Thus, in situations with moderate/severe metabolic acidosis, an elevated  $\Delta PCO_2$  might not reflect only low or inadequate blood flow but could also be ascribed to modifications of the  $CO_2$ –hemoglobin dissociation curve.

The impact of hemoglobin on the  $PCO_2/CCO_2$  relationship was less studied. In surgical patients with sepsis and different degrees of illness, Chiarla et al. [122] observed that the decrease in hemoglobin increased  $\Delta PCO_2$  for any given  $\Delta CCO_2$ . However, the impact was less evident in normal conditions, and it becomes relevant only in extreme conditions where several combinations of low hemoglobin concentration, with low-flow state, acidosis, and hypercapnia, may synergistically act to disturb the physiological balance.

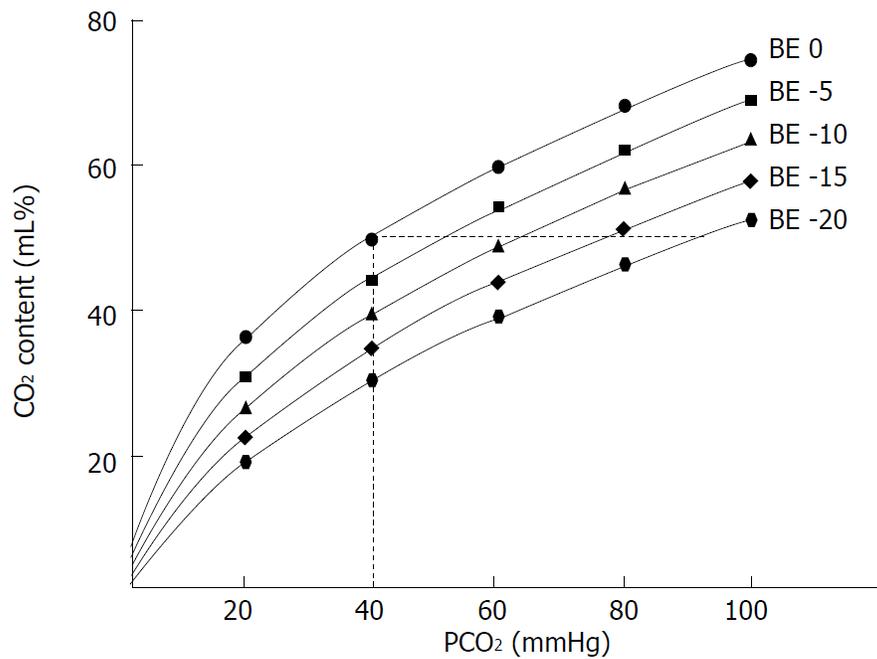
## 2. Limitations related to acute changes in $PCO_2$

Acute changes in  $PCO_2$  induced by changes in ventilator settings might influence  $\Delta PCO_2$ . In a small study that included 10 mechanically ventilated postoperative patients, Morel et al. [176] found that acute hyperventilation significantly increased in  $\Delta PCO_2\_Cent$  without changes in cardiac index. In septic shock patients, Mallat et al. [177] showed that acute hyperventilation induced an increase in  $\Delta PCO_2\_Cent$  independently of the cardiac index. The rise in  $\Delta PCO_2\_Cent$  was related

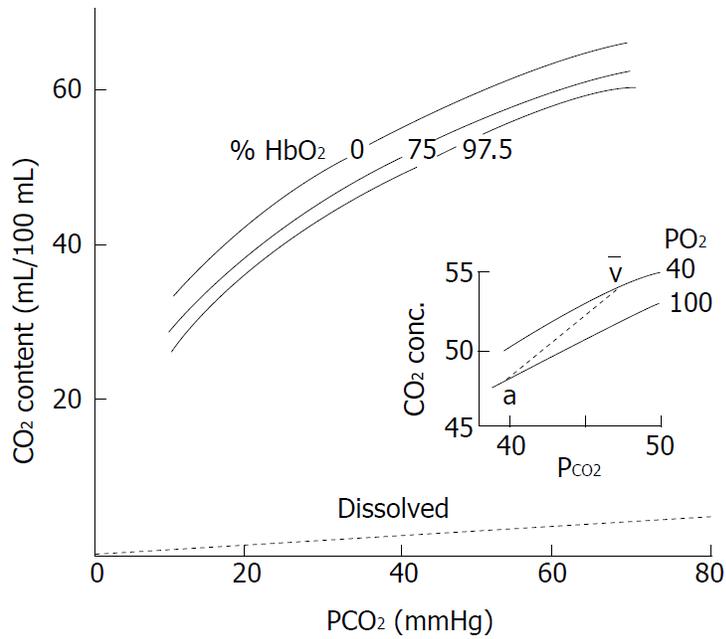
to the increase in oxygen consumption caused by acute respiratory alkalosis. The clinician should be aware of the effects of acute elevation of alveolar ventilation on  $\Delta\text{PCO}_2$  when interpreting  $\Delta\text{PCO}_2$  at the bedside.

### 3. Limitations related to errors in $\text{PCO}_2$ measurement

There are many pre-analytical sources of errors in  $\text{PCO}_2$  measurement that should be avoided to interpret  $\Delta\text{PCO}_2$  correctly: inappropriate sample container, insufficient sample volume compared to anticoagulant volume, and contaminated sample with resident fluid in the line or with air or venous blood, etc. Even after having taken all precautions to minimize the pre-analytical and analytical errors, Mallat et al. found, in a prospective study [178], that the measurement error for  $\Delta\text{PCO}_2_{\text{Cent}}$  was  $\pm 1.4$  mmHg and the smallest detectable difference, which is the least change that requires to be measured by a laboratory analyzer to identify a genuine change of measurement, was  $\pm 2$  mmHg. This means that the changes in  $\Delta\text{PCO}_2_{\text{Cent}}$  should be more than  $\pm 2$  mmHg to be considered as real changes and not due to natural variation [178].



**Figure 7.** CO<sub>2</sub> dissociation curve. CO<sub>2</sub> content (mL/100 mL) vs CO<sub>2</sub> partial tension (PCO<sub>2</sub>). Each curve is described at constant base excess (BE). As displayed, for the same CO<sub>2</sub> content, changing the BE results in a great change in PCO<sub>2</sub> [6].



**Figure 8.** CO<sub>2</sub> dissociation curve. CO<sub>2</sub> content (mL/100 mL) vs CO<sub>2</sub> partial tension (PCO<sub>2</sub>). Differences between the curves result in higher CO<sub>2</sub> content in the blood, and smaller PCO<sub>2</sub> differences between arterial and venous blood. Hemoglobin-O<sub>2</sub> saturation affects the position of the CO<sub>2</sub> dissociation curve (Haldane effect) [6].

## V. Conclusions

Acute circulatory failure leads to tissue hypoperfusion, which, if unrecognized and treated rapidly, will result in tissue hypoxia and eventually death. Indeed, if oxygen availability is limited or oxygen utilization by the cell is altered, cellular oxygen consumption may fall, leading to organ dysfunction. Vital cellular functions can no longer be sustained, and irreversible impairments may develop if the situation persists. Moreover, tissue hypoxia occurs when tissue cells have abnormal oxygen utilization, resulting in anaerobic metabolism. Thus, rapid recognition of tissue hypoperfusion and tissue hypoxia is of utmost importance to avoid the progression to a dreadful outcome. Many tools exist to detect and monitor the presence of tissue hypoperfusion/tissue hypoxia. Many of these require using specific devices that are usually unavailable in many ICUs or used for research purposes. The metabolic approach based on  $\text{CO}_2$  and  $\text{O}_2$ -derived variables is attractive as it necessitates the presence of only a central venous catheter and an arterial line that many critically ill patients are usually equipped with these catheters. Many experimental and clinical observational studies demonstrated the reliability of  $\Delta\text{PCO}_2$  and  $\Delta\text{PCO}_2/\Delta\text{O}_2$  ratio in detecting tissue hypoperfusion and tissue hypoxia, mainly in septic shock or postoperative non-cardiac surgery patients, and their association with outcomes. However, multicenter RCTs are required to demonstrate the efficacy of the metabolic approach compared to the standard of care in the hemodynamic management of patients with acute circulatory failure.

## Abbreviations

DO<sub>2</sub>, oxygen delivery; VO<sub>2</sub>, oxygen consumption; CO<sub>2</sub>, carbon dioxide; O<sub>2</sub>, oxygen; PCO<sub>2</sub>, partial pressure in CO<sub>2</sub>; ΔPCO<sub>2</sub>, venous-to-arterial PCO<sub>2</sub> difference; ΔO<sub>2</sub>, arterial-to-venous oxygen content difference; DNA, deoxyribonucleic acid; ATP, adenosine triphosphate; ERO<sub>2</sub>, oxygen extraction; NIRS, Near-Infrared Spectroscopy; StO<sub>2</sub>, tissue oxygen saturation; VOT, vascular occlusion test; DeO<sub>2</sub>, de-oxygenation slope; ReO<sub>2</sub>, re-oxygenation slope; ICU, intensive care unit; LDF, Laser Doppler Flowmetry; OPS, Orthogonal polarization spectral; SDF, sidestream darkfield; MFI, microvascular flow index; PPV, proportion of perfused vessels; FCD, functional capillary density; TVD, total vessel density; PVD, perfused vessel density; POEM, point of care microcirculation; tPO<sub>2</sub>, tissue oxygen tension; OCT, oxygen challenge test; Tskin-diff, gradient between the forearm and fingertip temperature; CRT, capillary refill time; PDH, pyruvate dehydrogenase; ATP, adenosine triphosphate; RCT, randomized clinical trial; SvO<sub>2</sub>, mixed venous oxygen saturation; ScvO<sub>2</sub>, central venous oxygen saturation; PsICO<sub>2</sub>, sublingual mucosal PCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, bicarbonate ion; H<sub>2</sub>O, water; H<sub>2</sub>CO<sub>3</sub>, carbonic acid; H<sup>+</sup>, hydrogen ion; PvCO<sub>2</sub>, mixed venous blood PCO<sub>2</sub>; PcvCO<sub>2</sub>, central venous blood PCO<sub>2</sub>; PaCO<sub>2</sub>, arterial blood PCO<sub>2</sub>; CvCO<sub>2</sub>, mixed venous blood CO<sub>2</sub> content; CaCO<sub>2</sub>, arterial blood CO<sub>2</sub> content; VCO<sub>2</sub>, total CO<sub>2</sub> production; ΔPCO<sub>2</sub>\_Mix, ΔPCO<sub>2</sub> calculated from mixed venous blood PCO<sub>2</sub>; ΔPCO<sub>2</sub>\_Cent, ΔPCO<sub>2</sub> calculated from central venous blood PCO<sub>2</sub>; ΔO<sub>2</sub>\_Mix, ΔO<sub>2</sub> calculated from mixed venous blood PO<sub>2</sub>; ΔO<sub>2</sub>\_Cent, ΔO<sub>2</sub> calculated from central venous blood PO<sub>2</sub>; CCO<sub>2</sub>, CO<sub>2</sub> content; AUROC, area under the receiving operating characteristic curve; SBT, spontaneous breathing trial.

## REFERENCES

1. Cecconi M, De Backer D, Antonelli M, Beale R, Bakker J, Hofer C, Jaeschke R, Mebazaa A, Pinsky MR, Teboul JL *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. Vincent JL, De Backer D: **Oxygen transport-the oxygen delivery controversy.** *Intensive Care Med* 2004, **30**(11):1990-1996.
3. Legrand M, Payen D: **Understanding urine output in critically ill patients.** *Ann Intensive Care* 2011, **1**(1):13.
4. Zhang H, Vincent JL: **Arteriovenous differences in PCO<sub>2</sub> and pH are good indicators of critical hypoperfusion.** *Am Rev Respir Dis* 1993, **148**(4 Pt 1):867-871.
5. Mekontso-Dessap A, Castelain V, Anguel N, Bahloul M, Schauvliege F, Richard C, Teboul JL: **Combination of venoarterial PCO<sub>2</sub> difference with arteriovenous O<sub>2</sub> content difference to detect anaerobic metabolism in patients.** *Intensive Care Med* 2002, **28**(3):272-277.
6. Mallat J, Lemyze M, Tronchon L, Vallet B, Thevenin D: **Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock.** *World J Crit Care Med* 2016, **5**(1):47-56.
7. Astiz M, Rackow EC, Weil MH, Schumer W: **Early impairment of oxidative metabolism and energy production in severe sepsis.** *Circ Shock* 1988, **26**(3):311-320.

8. Bertram R, Gram Pedersen M, Luciani DS, Sherman A: **A simplified model for mitochondrial ATP production.** *J Theor Biol* 2006, **243**(4):575-586.
9. Mallat J, Rahman N, Hamed F, Hernandez G, Fischer MO: **Pathophysiology, mechanisms, and managements of tissue hypoxia.** *Anaesth Crit Care Pain Med* 2022, **41**(4):101087.
10. De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, Vincent JL: **Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome.** *Crit Care Med* 2013, **41**(3):791-799.
11. 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.
12. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE, Hollenberg SM: **Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis.** *Intensive Care Med* 2008, **34**(12):2210-2217.
13. Tachon G, Harrois A, Tanaka S, Kato H, Huet O, Pottecher J, Vicaut E, Duranteau J: **Microcirculatory alterations in traumatic hemorrhagic shock.** *Crit Care Med* 2014, **42**(6):1433-1441.
14. Lima A, Jansen TC, van Bommel J, Ince C, Bakker J: **The prognostic value of the subjective assessment of peripheral perfusion in critically ill patients.** *Crit Care Med* 2009, **37**(3):934-938.

15. Myers DE, Anderson LD, Seifert RP, Ortner JP, Cooper CE, Beilman GJ, Mowlem JD: **Noninvasive method for measuring local hemoglobin oxygen saturation in tissue using wide gap second derivative near-infrared spectroscopy.** *J Biomed Opt* 2005, **10**(3):034017.
16. Mesquida J, Gruartmoner G, Espinal C: **Skeletal muscle oxygen saturation (StO<sub>2</sub>) measured by near-infrared spectroscopy in the critically ill patients.** *Biomed Res Int* 2013, **2013**:502194.
17. Creteur J, Carollo T, Soldati G, Buchele G, De Backer D, Vincent JL: **The prognostic value of muscle StO<sub>2</sub> in septic patients.** *Intensive Care Med* 2007, **33**(9):1549-1556.
18. Mulier KE, Skarda DE, Taylor JH, Myers DE, McGraw MK, Gallea BL, Beilman GJ: **Near-infrared spectroscopy in patients with severe sepsis: correlation with invasive hemodynamic measurements.** *Surg Infect (Larchmt)* 2008, **9**(5):515-519.
19. Lima A, van Bommel J, Jansen TC, Ince C, Bakker J: **Low tissue oxygen saturation at the end of early goal-directed therapy is associated with worse outcome in critically ill patients.** *Crit Care* 2009, **13 Suppl 5**(Suppl 5):S13.
20. Leone M, Blidi S, Antonini F, Meyssignac B, Bordon S, Garcin F, Charvet A, Blasco V, Albanèse J, Martin C: **Oxygen tissue saturation is lower in nonsurvivors than in survivors after early resuscitation of septic shock.** *Anesthesiology* 2009, **111**(2):366-371.

21. Gómez H, Mesquida J, Simon P, Kim HK, Puyana JC, Ince C, Pinsky MR: **Characterization of tissue oxygen saturation and the vascular occlusion test: influence of measurement sites, probe sizes and deflation thresholds.** *Crit Care* 2009, **13 Suppl 5**(Suppl 5):S3.
22. Skarda DE, Mulier KE, Myers DE, Taylor JH, Beilman GJ: **Dynamic near-infrared spectroscopy measurements in patients with severe sepsis.** *Shock* 2007, **27**(4):348-353.
23. Mesquida J, Espinal C, Gruartmoner G, Masip J, Sabatier C, Baigorri F, Pinsky MR, Artigas A: **Prognostic implications of tissue oxygen saturation in human septic shock.** *Intensive Care Med* 2012, **38**(4):592-597.
24. Donati A, Damiani E, Domizi R, Scorcella C, Carsetti A, Tondi S, Monaldi V, Adrario E, Romano R, Pelaia P *et al*: **Near-infrared spectroscopy for assessing tissue oxygenation and microvascular reactivity in critically ill patients: a prospective observational study.** *Crit Care* 2016, **20**(1):311.
25. Gruartmoner G, Mesquida J, Ince C: **Microcirculatory monitoring in septic patients: Where do we stand?** *Med Intensiva* 2017, **41**(1):44-52.
26. Payen D, Luengo C, Heyer L, Resche-Rigon M, Kerever S, Damoiseil C, Lossner MR: **Is thenar tissue hemoglobin oxygen saturation in septic shock related to macrohemodynamic variables and outcome?** *Crit Care* 2009, **13 Suppl 5**(Suppl 5):S6.
27. Schabauer AM, Rooke TW: **Cutaneous laser Doppler flowmetry: applications and findings.** *Mayo Clin Proc* 1994, **69**(6):564-574.

28. Kiessling AH, Reyher C, Philipp M, Beiras-Fernandez A, Moritz A: **Real-time measurement of rectal mucosal microcirculation during cardiopulmonary bypass.** *J Cardiothorac Vasc Anesth* 2015, **29**(1):89-94.
29. Salgado MA, Salgado-Filho MF, Reis-Brito JO, Lessa MA, Tibirica E: **Effectiveness of laser Doppler perfusion monitoring in the assessment of microvascular function in patients undergoing on-pump coronary artery bypass grafting.** *J Cardiothorac Vasc Anesth* 2014, **28**(5):1211-1216.
30. Koller A, Kaley G: **Role of endothelium in reactive dilation of skeletal muscle arterioles.** *Am J Physiol* 1990, **259**(5 Pt 2):H1313-1316.
31. Lamblin V, Favory R, Boulo M, Mathieu D: **Microcirculatory alterations induced by sedation in intensive care patients. Effects of midazolam alone and in association with sufentanil.** *Crit Care* 2006, **10**(6):R176.
32. 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.
33. Hartl WH, Günther B, Inthorn D, Heberer G: **Reactive hyperemia in patients with septic conditions.** *Surgery* 1988, **103**(4):440-444.
34. Young JD, Cameron EM: **Dynamics of skin blood flow in human sepsis.** *Intensive Care Med* 1995, **21**(8):669-674.

35. Sair M, Etherington PJ, Peter Winlove C, Evans TW: **Tissue oxygenation and perfusion in patients with systemic sepsis.** *Crit Care Med* 2001, **29**(7):1343-1349.
36. Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG: **Orthogonal polarization spectral imaging: a new method for study of the microcirculation.** *Nat Med* 1999, **5**(10):1209-1212.
37. Slaaf DW, Tangelder GJ, Reneman RS, Jäger K, Bollinger A: **A versatile incident illuminator for intravital microscopy.** *Int J Microcirc Clin Exp* 1987, **6**(4):391-397.
38. Goedhart PT, Khalilzada M, Bezemer R, Merza J, Ince C: **Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation.** *Opt Express* 2007, **15**(23):15101-15114.
39. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, Pinsky MR, Artigas A, Teboul JL, Reiss IKM *et al*: **Second consensus on the assessment of sublingual microcirculation in critically ill patients: results from a task force of the European Society of Intensive Care Medicine.** *Intensive Care Med* 2018, **44**(3):281-299.
40. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R: **Effect of a maldistribution of microvascular blood flow on capillary O<sub>2</sub> extraction in sepsis.** *Am J Physiol Heart Circ Physiol* 2002, **282**(1):H156-164.
41. 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.

42. Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, Arnold RC, Colilla S, Zanotti S, Hollenberg SM: **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.
43. 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.
44. Aykut G, Veenstra G, Scorcella C, Ince C, Boerma C: **Cytocam-IDF (incident dark field illumination) imaging for bedside monitoring of the microcirculation.** *Intensive Care Med Exp* 2015, **3**(1):40.
45. Tanaka S, Harrois A, Nicolai C, Flores M, Hamada S, Vicaut E, Duranteau J: **Qualitative real-time analysis by nurses of sublingual microcirculation in intensive care unit: the MICRONURSE study.** *Crit Care* 2015, **19**:388.
46. Naumann DN, Mellis C, Husheer SL, Hopkins P, Bishop J, Midwinter MJ, Hutchings SD: **Real-time point of care microcirculatory assessment of shock: design, rationale and application of the point of care microcirculation (POEM) tool.** *Crit Care* 2016, **20**(1):310.
47. Vallet B, Lund N, Curtis SE, Kelly D, Cain SM: **Gut and muscle tissue PO<sub>2</sub> in endotoxemic dogs during shock and resuscitation.** *J Appl Physiol (1985)* 1994, **76**(2):793-800.
48. Dyson A, Stidwill R, Taylor V, Singer M: **Tissue oxygen monitoring in rodent models of shock.** *Am J Physiol Heart Circ Physiol* 2007, **293**(1):H526-533.

49. Dyson A, Simon F, Seifritz A, Zimmerling O, Matallo J, Calzia E, Radermacher P, Singer M: **Bladder tissue oxygen tension monitoring in pigs subjected to a range of cardiorespiratory and pharmacological challenges.** *Intensive Care Med* 2012, **38**(11):1868-1876.
50. Tremper KK, Waxman K, Shoemaker WC: **Effects of hypoxia and shock on transcutaneous PO2 values in dogs.** *Crit Care Med* 1979, **7**(12):526-531.
51. Yu M, Morita SY, Daniel SR, Chapital A, Waxman K, Severino R: **Transcutaneous pressure of oxygen: a noninvasive and early detector of peripheral shock and outcome.** *Shock* 2006, **26**(5):450-456.
52. Yu M, Chapital A, Ho HC, Wang J, Takanishi D, Jr.: **A prospective randomized trial comparing oxygen delivery versus transcutaneous pressure of oxygen values as resuscitative goals.** *Shock* 2007, **27**(6):615-622.
53. Dyson A, Stidwill R, Taylor V, Singer M: **The impact of inspired oxygen concentration on tissue oxygenation during progressive haemorrhage.** *Intensive Care Med* 2009, **35**(10):1783-1791.
54. Kaplan LJ, McPartland K, Santora TA, Trooskin SZ: **Start with a subjective assessment of skin temperature to identify hypoperfusion in intensive care unit patients.** *J Trauma* 2001, **50**(4):620-627; discussion 627-628.
55. Ait-Oufella H, Bakker J: **Understanding clinical signs of poor tissue perfusion during septic shock.** *Intensive Care Med* 2016, **42**(12):2070-2072.

56. van Genderen ME, Paauwe J, de Jonge J, van der Valk RJ, Lima A, Bakker J, van Bommel J: **Clinical assessment of peripheral perfusion to predict postoperative complications after major abdominal surgery early: a prospective observational study in adults.** *Crit Care* 2014, **18**(3):R114.
57. van Genderen ME, Engels N, van der Valk RJ, Lima A, Klijn E, Bakker J, van Bommel J: **Early peripheral perfusion-guided fluid therapy in patients with septic shock.** *Am J Respir Crit Care Med* 2015, **191**(4):477-480.
58. Ait-Oufella H, Bige N, Boelle PY, Pichereau C, Alves M, Bertinchamp R, Baudel JL, Galbois A, Maury E, Guidet B: **Capillary refill time exploration during septic shock.** *Intensive Care Med* 2014, **40**(7):958-964.
59. Hernández G, Ospina-Tascón GA, Damiani LP, Estenssoro E, Dubin A, Hurtado J, Friedman G, Castro R, Alegría L, Teboul JL *et al*: **Effect of a Resuscitation Strategy Targeting Peripheral Perfusion Status vs Serum Lactate Levels on 28-Day Mortality Among Patients With Septic Shock: The ANDROMEDA-SHOCK Randomized Clinical Trial.** *Jama* 2019, **321**(7):654-664.
60. Zampieri FG, Damiani LP, Bakker J, Ospina-Tascón GA, Castro R, Cavalcanti AB, Hernandez G: **Effects of a Resuscitation Strategy Targeting Peripheral Perfusion Status versus Serum Lactate Levels among Patients with Septic Shock. A Bayesian Reanalysis of the ANDROMEDA-SHOCK Trial.** *Am J Respir Crit Care Med* 2020, **201**(4):423-429.

61. Lara B, Enberg L, Ortega M, Leon P, Kripper C, Aguilera P, Kattan E, Castro R, Bakker J, Hernandez G: **Capillary refill time during fluid resuscitation in patients with sepsis-related hyperlactatemia at the emergency department is related to mortality.** *PLoS One* 2017, **12**(11):e0188548.
62. Ait-Oufella H, Bourcier S, Alves M, Galbois A, Baudel JL, Margetis D, Bige N, Offenstadt G, Maury E, Guidet B: **Alteration of skin perfusion in mottling area during septic shock.** *Ann Intensive Care* 2013, **3**(1):31.
63. Ait-Oufella H, Lemoine S, Boelle PY, Galbois A, Baudel JL, Lemant J, Joffre J, Margetis D, Guidet B, Maury E *et al*: **Mottling score predicts survival in septic shock.** *Intensive Care Med* 2011, **37**(5):801-807.
64. Coudroy R, Jamet A, Frat JP, Veinstein A, Chatellier D, Goudet V, Cabasson S, Thille AW, Robert R: **Incidence and impact of skin mottling over the knee and its duration on outcome in critically ill patients.** *Intensive Care Med* 2015, **41**(3):452-459.
65. de Moura EB, Amorim FF, da Cruz Santana AN, Kanhouche G, de Souza Godoy LG, de Jesus Almeida L, Rodrigues TA, da Silveira CDG, de Oliveira Maia M: **Skin mottling score as a predictor of 28-day mortality in patients with septic shock.** *Intensive Care Med* 2016, **42**(3):479-480.
66. Dumas G, Lavillegrand JR, Joffre J, Bigé N, de-Moura EB, Baudel JL, Chevret S, Guidet B, Maury E, Amorim F *et al*: **Mottling score is a strong predictor of 14-day mortality in septic**

- patients whatever vasopressor doses and other tissue perfusion parameters. *Crit Care* 2019, **23**(1):211.
67. Jan Bakker J-LV: **The oxygen supply dependency phenomenon is associated with increased blood lactate levels.** *J Crit Care* 1991, **6**(3):152-159.
68. Zhang H, Vincent JL: **Oxygen extraction is altered by endotoxin during tamponade-induced stagnant hypoxia in the dog.** *Circ Shock* 1993, **40**(3):168-176.
69. Hernandez G, Boerma EC, Dubin A, Bruhn A, Koopmans M, Edul VK, Ruiz C, Castro R, Pozo MO, Pedreros C *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.
70. De Backer D, Creteur J, Dubois MJ, Sakr Y, Koch M, Verdant C, Vincent JL: **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.
71. Alegría L, Vera M, Dreyse J, Castro R, Carpio D, Henriquez C, Gajardo D, Bravo S, Araneda F, Kattan E *et al*: **A hypoperfusion context may aid to interpret hyperlactatemia in sepsis-3 septic shock patients: a proof-of-concept study.** *Ann Intensive Care* 2017, **7**(1):29.
72. Zhang Z, Chen K, Ni H, Fan H: **Predictive value of lactate in unselected critically ill patients: an analysis using fractional polynomials.** *J Thorac Dis* 2014, **6**(7):995-1003.

73. Trzeciak S, Dellinger RP, Chansky ME, Arnold RC, Schorr C, Milcarek B, Hollenberg SM, Parrillo JE: **Serum lactate as a predictor of mortality in patients with infection.** *Intensive Care Med* 2007, **33**(6):970-977.
74. Mikkelsen ME, Miltiades AN, Gaieski DF, Goyal M, Fuchs BD, Shah CV, Bellamy SL, Christie JD: **Serum lactate is associated with mortality in severe sepsis independent of organ failure and shock.** *Crit Care Med* 2009, **37**(5):1670-1677.
75. Jansen TC, van Bommel J, Schoonderbeek FJ, Sleswijk Visser SJ, van der Klooster JM, Lima AP, Willemsen SP, Bakker J: **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.
76. Jones AE, Shapiro NI, Trzeciak S, Arnold RC, Claremont HA, Kline JA: **Lactate clearance vs central venous oxygen saturation as goals of early sepsis therapy: a randomized clinical trial.** *Jama* 2010, **303**(8):739-746.
77. Gu WJ, Zhang Z, Bakker J: **Early lactate clearance-guided therapy in patients with sepsis: a meta-analysis with trial sequential analysis of randomized controlled trials.** *Intensive Care Med* 2015, **41**(10):1862-1863.
78. Ding XF, Yang ZY, Xu ZT, Li LF, Yuan B, Guo LN, Wang LX, Zhu X, Sun TW: **Early goal-directed and lactate-guided therapy in adult patients with severe sepsis and septic shock: a meta-analysis of randomized controlled trials.** *J Transl Med* 2018, **16**(1):331.

79. Garcia-Alvarez M, Marik P, Bellomo R: **Sepsis-associated hyperlactatemia**. *Crit Care* 2014, **18**(5):503.
80. Hernandez G, Luengo C, Bruhn A, Kattan E, Friedman G, Ospina-Tascon GA, Fuentealba A, Castro R, Regueira T, Romero C *et al*: **When to stop septic shock resuscitation: clues from a dynamic perfusion monitoring**. *Ann Intensive Care* 2014, **4**:30.
81. Hernandez G, Bellomo R, Bakker J: **The ten pitfalls of lactate clearance in sepsis**. *Intensive Care Med* 2019, **45**(1):82-85.
82. Krafft P, Steltzer H, Hiesmayr M, Klimscha W, Hammerle AF: **Mixed venous oxygen saturation in critically ill septic shock patients. The role of defined events**. *Chest* 1993, **103**(3):900-906.
83. Schumacker PT, Cain SM: **The concept of a critical oxygen delivery**. *Intensive Care Med* 1987, **13**(4):223-229.
84. Nelson DP, Samsel RW, Wood LD, Schumacker PT: **Pathological supply dependence of systemic and intestinal O<sub>2</sub> uptake during endotoxemia**. *J Appl Physiol (1985)* 1988, **64**(6):2410-2419.
85. Bloos F, Reinhart K: **Venous oximetry**. *Intensive Care Med* 2005, **31**(7):911-913.
86. Chawla LS, Zia H, Gutierrez G, Katz NM, Seneff MG, Shah M: **Lack of equivalence between central and mixed venous oxygen saturation**. *Chest* 2004, **126**(6):1891-1896.

87. Reinhart K, Kuhn HJ, Hartog C, Bredle DL: **Continuous central venous and pulmonary artery oxygen saturation monitoring in the critically ill.** *Intensive Care Med* 2004, **30**(8):1572-1578.
88. Varpula M, Karlsson S, Ruokonen E, Pettilä V: **Mixed venous oxygen saturation cannot be estimated by central venous oxygen saturation in septic shock.** *Intensive Care Med* 2006, **32**(9):1336-1343.
89. 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* 2010, **14**(6):R219.
90. Martin C, Auffray JP, Badetti C, Perrin G, Papazian L, Gouin F: **Monitoring of central venous oxygen saturation versus mixed venous oxygen saturation in critically ill patients.** *Intensive Care Med* 1992, **18**(2):101-104.
91. Edwards JD, Mayall RM: **Importance of the sampling site for measurement of mixed venous oxygen saturation in shock.** *Crit Care Med* 1998, **26**(8):1356-1360.
92. Dueck MH, Klimek M, Appenrodt S, Weigand C, Boerner U: **Trends but not individual values of central venous oxygen saturation agree with mixed venous oxygen saturation during varying hemodynamic conditions.** *Anesthesiology* 2005, **103**(2):249-257.
93. 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 Engl J Med* 2001, **345**(19):1368-1377.

94. Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F *et al*: **A randomized trial of protocol-based care for early septic shock.** *N Engl J Med* 2014, **370**(18):1683-1693.
95. Peake SL, Delaney A, Bailey M, Bellomo R, Cameron PA, Cooper DJ, Higgins AM, Holdgate A, Howe BD, Webb SA *et al*: **Goal-directed resuscitation for patients with early septic shock.** *N Engl J Med* 2014, **371**(16):1496-1506.
96. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, Jahan R, Harvey SE, Bell D, Bion JF *et al*: **Trial of early, goal-directed resuscitation for septic shock.** *N Engl J Med* 2015, **372**(14):1301-1311.
97. Boulain T, Garot D, Vignon P, Lascarrou JB, Desachy A, Botoc V, Follin A, Frat JP, Bellec F, Quenot JP *et al*: **Prevalence of low central venous oxygen saturation in the first hours of intensive care unit admission and associated mortality in septic shock patients: a prospective multicentre study.** *Crit Care* 2014, **18**(6):609.
98. Puskarich MA, Trzeciak S, Shapiro NI, Heffner AC, Kline JA, Jones AE: **Outcomes of patients undergoing early sepsis resuscitation for cryptic shock compared with overt shock.** *Resuscitation* 2011, **82**(10):1289-1293.
99. Pope JV, Jones AE, Gaieski DF, Arnold RC, Trzeciak S, Shapiro NI: **Multicenter study of central venous oxygen saturation (ScvO<sub>2</sub>) as a predictor of mortality in patients with sepsis.** *Ann Emerg Med* 2010, **55**(1):40-46.e41.

100. Textoris J, Fouché L, Wiramus S, Antonini F, Tho S, Martin C, Leone M: **High central venous oxygen saturation in the latter stages of septic shock is associated with increased mortality.** *Crit Care* 2011, **15**(4):R176.
101. Marik PE: **Regional carbon dioxide monitoring to assess the adequacy of tissue perfusion.** *Curr Opin Crit Care* 2005, **11**(3):245-251.
102. Mallat J, Vallet B: **Mucosal and cutaneous capnometry for the assessment of tissue hypoperfusion.** *Minerva Anesthesiol* 2018, **84**(1):68-80.
103. West JB: **Gas transport to the periphery: how gases are moved to the peripheral tissues?:** Williams & Wilkins; 1990.
104. Lamia B, Monnet X, Teboul JL: **Meaning of arterio-venous PCO<sub>2</sub> difference in circulatory shock.** *Minerva Anesthesiol* 2006, **72**(6):597-604.
105. Giovannini I, Chiarla C, Boldrini G, Castagneto M: **Calculation of venoarterial CO<sub>2</sub> concentration difference.** *J Appl Physiol (1985)* 1993, **74**(2):959-964.
106. McHardy GJ: **The relationship between the differences in pressure and content of carbon dioxide in arterial and venous blood.** *Clin Sci* 1967, **32**(2):299-309.
107. Groeneveld AB: **Interpreting the venous-arterial PCO<sub>2</sub> difference.** *Crit Care Med* 1998, **26**(6):979-980.

108. Van der Linden P, Rausin I, Deltell A, Bekrar Y, Gilbert E, Bakker J, Vincent JL: **Detection of tissue hypoxia by arteriovenous gradient for PCO<sub>2</sub> and pH in anesthetized dogs during progressive hemorrhage.** *Anesth Analg* 1995, **80**(2):269-275.
109. 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.
110. Rackow EC, Astiz ME, Mecher CE, Weil MH: **Increased venous-arterial carbon dioxide tension difference during severe sepsis in rats.** *Crit Care Med* 1994, **22**(1):121-125.
111. Benjamin E: **Venous hypercarbia: a nonspecific marker of hypoperfusion.** *Crit Care Med* 1994, **22**(1):9-10.
112. Teboul JL, Mercat A, Lenique F, Berton C, Richard C: **Value of the venous-arterial PCO<sub>2</sub> gradient to reflect the oxygen supply to demand in humans: effects of dobutamine.** *Crit Care Med* 1998, **26**(6):1007-1010.
113. Vallet B, Teboul JL, Cain S, Curtis S: **Venoarterial CO<sub>2</sub> difference during regional ischemic or hypoxic hypoxia.** *J Appl Physiol (1985)* 2000, **89**(4):1317-1321.
114. 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.

115. Nevière R, Chagnon JL, Teboul JL, Vallet B, Wattel F: **Small intestine intramucosal PCO<sub>2</sub> and microvascular blood flow during hypoxic and ischemic hypoxia.** *Crit Care Med* 2002, **30**(2):379-384.
116. Gutierrez G: **A mathematical model of tissue-blood carbon dioxide exchange during hypoxia.** *Am J Respir Crit Care Med* 2004, **169**(4):525-533.
117. 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.
118. Ferrara G, Edul VSK, Canales HS, Martins E, Canullán C, Murias G, Pozo MO, Caminos Eguillor JF, Buscetti MG, Ince C *et al*: **Systemic and microcirculatory effects of blood transfusion in experimental hemorrhagic shock.** *Intensive Care Med Exp* 2017, **5**(1):24.
119. Dubin A, Ferrara G, Kanoore Edul VS, Martins E, Canales HS, Canullan C, Murias G, Pozo MO, Estenssoro E: **Venoarterial PCO<sub>2</sub>-to-arteriovenous oxygen content difference ratio is a poor surrogate for anaerobic metabolism in hemodilution: an experimental study.** *Ann Intensive Care* 2017, **7**(1):65.
120. Cavaliere F, Giovannini I, Chiarla C, Conti G, Pennisi MA, Montini L, Gaspari R, Proietti R: **Comparison of two methods to assess blood CO<sub>2</sub> equilibration curve in mechanically ventilated patients.** *Respir Physiol Neurobiol* 2005, **146**(1):77-83.
121. Jakob SM, Groeneveld AB, Teboul JL: **Venous-arterial CO<sub>2</sub> to arterial-venous O<sub>2</sub> difference ratio as a resuscitation target in shock states?** *Intensive Care Med* 2015, **41**(5):936-938.

122. Chiarla C, Giovannini I, Giuliante F, Vellone M, Ardito F, Tenhunen J, Nuzzo G: **Significance of hemoglobin concentration in determining blood CO<sub>2</sub> binding capacity in critical illness.** *Respir Physiol Neurobiol* 2010, **172**(1-2):32-36.
123. Teboul JL, Scheeren T: **Understanding the Haldane effect.** *Intensive Care Med* 2017, **43**(1):91-93.
124. Jakob SM, Kosonen P, Ruokonen E, Parviainen I, Takala J: **The Haldane effect--an alternative explanation for increasing gastric mucosal PCO<sub>2</sub> gradients?** *Br J Anaesth* 1999, **83**(5):740-746.
125. Gump FE, Butler H, Kinney JM: **Oxygen transport and consumption during acute hemodilution.** *Ann Surg* 1968, **168**(1):54-60.
126. Ospina-Tascón GA, Umaña M, Bermúdez W, Bautista-Rincón DF, Hernandez G, Bruhn A, Granados M, Salazar B, Arango-Dávila C, De Backer D: **Combination of arterial lactate levels and venous-arterial CO<sub>2</sub> to arterial-venous O<sub>2</sub> content difference ratio as markers of resuscitation in patients with septic shock.** *Intensive Care Med* 2015, **41**(5):796-805.
127. Mallat J, Vallet B: **Ratio of venous-to-arterial PCO<sub>2</sub> to arteriovenous oxygen content difference during regional ischemic or hypoxic hypoxia.** *Sci Rep* 2021, **11**(1):10172.
128. Protti A, Fortunato F, Monti M, Vecchio S, Gatti S, Comi GP, De Giuseppe R, Gattinoni L: **Metformin overdose, but not lactic acidosis per se, inhibits oxygen consumption in pigs.** *Crit Care* 2012, **16**(3):R75.

129. Protti A, Russo R, Tagliabue P, Vecchio S, Singer M, Rudiger A, Foti G, Rossi A, Mistraletti G, Gattinoni L: **Oxygen consumption is depressed in patients with lactic acidosis due to biguanide intoxication.** *Crit Care* 2010, **14**(1):R22.
130. Andreis DT, Mallat J, Tettamanti M, Chiarla C, Giovannini I, Gatti S, Protti A: **Increased ratio of P[v-a]CO(2) to C[a-v]O(2) without global hypoxia: the case of metformin-induced lactic acidosis.** *Respir Physiol Neurobiol* 2021, **285**:103586.
131. 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.
132. 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.
133. 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.
134. Ospina-Tascón GA, Umaña M, Bermúdez WF, Bautista-Rincón DF, Valencia JD, Madriñán HJ, Hernandez G, Bruhn A, Arango-Dávila C, De Backer D: **Can venous-to-arterial carbon dioxide differences reflect microcirculatory alterations in patients with septic shock?** *Intensive Care Med* 2016, **42**(2):211-221.
135. Ospina-Tascón GA, Bautista-Rincón DF, Umaña M, Tafur JD, Gutiérrez A, García AF, Bermúdez W, Granados M, Arango-Dávila C, Hernández G: **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.

136. Richard C, Monnet X, Teboul JL: **Pulmonary artery catheter monitoring in 2011.** *Curr Opin Crit Care* 2011, **17**(3):296-302.
137. Cuschieri J, Rivers EP, Donnino MW, Katilius M, Jacobsen G, Nguyen HB, Pamukov N, Horst HM: **Central venous-arterial carbon dioxide difference as an indicator of cardiac index.** *Intensive Care Med* 2005, **31**(6):818-822.
138. van Beest PA, Lont MC, Holman ND, Loef B, Kuiper MA, Boerma EC: **Central venous-arterial pCO<sub>2</sub> difference as a tool in resuscitation of septic patients.** *Intensive Care Med* 2013, **39**(6):1034-1039.
139. Vallet B, Pinsky MR, Cecconi M: **Resuscitation of patients with septic shock: please "mind the gap"!** *Intensive Care Med* 2013, **39**(9):1653-1655.
140. 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**(12):2218-2225.
141. Mallat J, Pepy F, Lemyze M, Gasan G, Vangrunderbeeck N, Tronchon L, Vallet B, Thevenin D: **Central venous-to-arterial carbon dioxide partial pressure difference in early resuscitation from septic shock: a prospective observational study.** *Eur J Anaesthesiol* 2014, **31**(7):371-380.
142. Du W, Liu DW, Wang XT, Long Y, Chai WZ, Zhou X, Rui X: **Combining central venous-to-arterial partial pressure of carbon dioxide difference and central venous oxygen**

- saturation to guide resuscitation in septic shock. *J Crit Care* 2013, **28**(6):1110.e1111-1115.
143. Wang Z, Wei X, Qin T, Chen S, Liao X, Guo W, Hu P, Wu Y, Li J, Liao Y *et al*: **Prognostic value of central venous-to-arterial carbon dioxide difference in patients with bloodstream infection.** *Int J Med Sci* 2021, **18**(4):929-935.
144. Futier E, Robin E, Jabaudon M, Guerin R, Petit A, Bazin JE, Constantin JM, Vallet B: **Central venous O<sub>2</sub> saturation and venous-to-arterial CO<sub>2</sub> difference as complementary tools for goal-directed therapy during high-risk surgery.** *Crit Care* 2010, **14**(5):R193.
145. Silva JM, Jr., Oliveira AM, Segura JL, Ribeiro MH, Sposito CN, Toledo DO, Rezende E, Malbouisson LM: **A large Venous-Arterial PCO<sub>2</sub> Is Associated with Poor Outcomes in Surgical Patients.** *Anesthesiol Res Pract* 2011, **2011**:759792.
146. Robin E, Futier E, Pires O, Fleyfel M, Tavernier B, Lebuffe G, Vallet B: **Central venous-to-arterial carbon dioxide difference as a prognostic tool in high-risk surgical patients.** *Crit Care* 2015, **19**(1):227.
147. Habicher M, von Heymann C, Spies CD, Wernecke KD, Sander M: **Central Venous-Arterial pCO<sub>2</sub> Difference Identifies Microcirculatory Hypoperfusion in Cardiac Surgical Patients With Normal Central Venous Oxygen Saturation: A Retrospective Analysis.** *J Cardiothorac Vasc Anesth* 2015, **29**(3):646-655.
148. Morel J, Grand N, Axiotis G, Bouchet JB, Faure M, Auboyer C, Vola M, Molliex S: **High veno-arterial carbon dioxide gradient is not predictive of worst outcome after an**

- elective cardiac surgery: a retrospective cohort study.** *J Clin Monit Comput* 2016, **30(6):783-789.**
149. Guinot PG, Badoux L, Bernard E, Abou-Arab O, Lorne E, Dupont H: **Central Venous-to-Arterial Carbon Dioxide Partial Pressure Difference in Patients Undergoing Cardiac Surgery is Not Related to Postoperative Outcomes.** *J Cardiothorac Vasc Anesth* 2017, **31(4):1190-1196.**
150. Moussa MD, Durand A, Leroy G, Vincent L, Lamer A, Gantois G, Joulin O, Ait-Ouarab S, Deblauwe D, Caroline B *et al*: **Central venous-to-arterial PCO<sub>2</sub> difference, arteriovenous oxygen content and outcome after adult cardiac surgery with cardiopulmonary bypass: A prospective observational study.** *Eur J Anaesthesiol* 2019, **36(4):279-289.**
151. Zante B, Reichenspurner H, Kubik M, Schefold JC, Kluge S: **Increased admission central venous-arterial CO<sub>2</sub> difference predicts ICU-mortality in adult cardiac surgery patients.** *Heart Lung* 2019, **48(5):421-427.**
152. Mukai A, Suehiro K, Kimura A, Funai Y, Matsuura T, Tanaka K, Yamada T, Mori T, Nishikawa K: **Comparison of the venous-arterial CO<sub>2</sub> to arterial-venous O<sub>2</sub> content difference ratio with the venous-arterial CO<sub>2</sub> gradient for the predictability of adverse outcomes after cardiac surgery.** *J Clin Monit Comput* 2020, **34(1):41-53.**
153. Chen T, Pan T, Luo X, Wang D: **High Central Venous-to-Arterial CO<sub>2</sub> Difference is Associated With Poor Outcomes in Patients After Cardiac Surgery: A Propensity Score Analysis.** *Shock* 2019, **52(6):583-589.**

154. Huette P, Beyls C, Mallat J, Martineau L, Besserve P, Haye G, Guilbart M, Dupont H, Guinot PG, Diouf M *et al*: **Central venous-to-arterial CO(2) difference is a poor tool to predict adverse outcomes after cardiac surgery: a retrospective study.** *Can J Anaesth* 2021, **68**(4):467-476.
155. Al Duhailib Z, Hegazy AF, Lalli R, Fiorini K, Priestap F, Iansavichene A, Slessarev M: **The Use of Central Venous to Arterial Carbon Dioxide Tension Gap for Outcome Prediction in Critically Ill Patients: A Systematic Review and Meta-Analysis.** *Crit Care Med* 2020, **48**(12):1855-1861.
156. Ruffolo RR, Jr.: **The pharmacology of dobutamine.** *Am J Med Sci* 1987, **294**(4):244-248.
157. Bhatt SB, Hutchinson RC, Tomlinson B, Oh TE, Mak M: **Effect of dobutamine on oxygen supply and uptake in healthy volunteers.** *Br J Anaesth* 1992, **69**(3):298-303.
158. Mallat J, Benzidi Y, Salleron J, Lemyze M, Gasan G, Vangrunderbeeck N, Pepy F, Tronchon L, Vallet B, Thevenin D: **Time course of central venous-to-arterial carbon dioxide tension difference in septic shock patients receiving incremental doses of dobutamine.** *Intensive Care Med* 2014, **40**(3):404-411.
159. Nassar B, Mallat J: **Usefulness of venous-to-arterial partial pressure of CO(2) difference to assess oxygen supply to demand adequacy: effects of dobutamine.** *J Thorac Dis* 2019, **11**(Suppl 11):S1574-s1578.
160. Monnet X, Julien F, Ait-Hamou N, Lequoy M, Gosset C, Jozwiak M, Persichini R, Anguel N, Richard C, Teboul JL: **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.
161. Mallat J, Lemyze M, Meddour M, Pepy F, Gasan G, Barrailler S, Durville E, Temime J, Vangrunderbeeck N, Tronchon L *et al*: **Ratios of central venous-to-arterial carbon dioxide content or tension to arteriovenous oxygen content are better markers of global anaerobic metabolism than lactate in septic shock patients.** *Ann Intensive Care* 2016, **6**(1):10.
162. Mesquida J, Saludes P, Gruartmoner G, Espinal C, Torrents E, Baigorri F, Artigas A: **Central venous-to-arterial carbon dioxide difference combined with arterial-to-venous oxygen content difference is associated with lactate evolution in the hemodynamic resuscitation process in early septic shock.** *Crit Care* 2015, **19**(1):126.
163. Du W, Long Y, Wang XT, Liu DW: **The Use of the Ratio between the Veno-arterial Carbon Dioxide Difference and the Arterial-venous Oxygen Difference to Guide Resuscitation in Cardiac Surgery Patients with Hyperlactatemia and Normal Central Venous Oxygen Saturation.** *Chin Med J (Engl)* 2015, **128**(10):1306-1313.
164. Abou-Arab O, Braik R, Huetten P, Bouhemad B, Lorne E, Guinot PG: **The ratios of central venous to arterial carbon dioxide content and tension to arteriovenous oxygen content are not associated with overall anaerobic metabolism in postoperative cardiac surgery patients.** *PLoS One* 2018, **13**(10):e0205950.

165. Zhang S, Zheng D, Chu XQ, Jiang YP, Wang CG, Zhang QM, Qian LZ, Yang WY, Zhang WY, Tung TH *et al*:  **$\Delta$ PCO(2) and  $\Delta$ PCO(2)/C((a-cv))O(2) Are Not Predictive of Organ Dysfunction After Cardiopulmonary Bypass.** *Front Cardiovasc Med* 2021, **8**:759826.
166. Hubmayr RD, Loosbrock LM, Gillespie DJ, Rodarte JR: **Oxygen uptake during weaning from mechanical ventilation.** *Chest* 1988, **94**(6):1148-1155.
167. Pinsky MR: **Cardiovascular effects of ventilatory support and withdrawal.** *Anesth Analg* 1994, **79**(3):567-576.
168. Jubran A, Mathru M, Dries D, Tobin MJ: **Continuous recordings of mixed venous oxygen saturation during weaning from mechanical ventilation and the ramifications thereof.** *Am J Respir Crit Care Med* 1998, **158**(6):1763-1769.
169. Teixeira C, da Silva NB, Savi A, Vieira SR, Nasi LA, Friedman G, Oliveira RP, Cremonese RV, Tonietto TF, Bressel MA *et al*: **Central venous saturation is a predictor of reintubation in difficult-to-wean patients.** *Crit Care Med* 2010, **38**(2):491-496.
170. Mallat J, Baghdadi FA, Mohammad U, Lemyze M, Temime J, Tronchon L, Thevenin D, Fischer MO: **Central Venous-to-Arterial PCO2 Difference and Central Venous Oxygen Saturation in the Detection of Extubation Failure in Critically Ill Patients.** *Crit Care Med* 2020, **48**(10):1454-1461.
171. Yazigi A, Abou-Zeid H, Haddad F, Madi-Jebara S, Hayeck G, Jabbour K: **Correlation between central venous-arterial carbon dioxide tension gradient and cardiac index changes following fluid therapy.** *Ann Card Anaesth* 2010, **13**(3):269-271.

172. Giraud R, Siegenthaler N, Gayet-Ageron A, Combescure C, Romand JA, Bendjelid K: **ScvO(2) as a marker to define fluid responsiveness.** *J Trauma* 2011, **70**(4):802-807.
173. Nassar B, Badr M, Van Grunderbeeck N, Temime J, Pepy F, Gasan G, Tronchon L, Thevenin D, Mallat J: **Central venous-to-arterial PCO(2) difference as a marker to identify fluid responsiveness in septic shock.** *Sci Rep* 2021, **11**(1):17256.
174. Sun XG, Hansen JE, Stringer WW, Ting H, Wasserman K: **Carbon dioxide pressure-concentration relationship in arterial and mixed venous blood during exercise.** *J Appl Physiol (1985)* 2001, **90**(5):1798-1810.
175. Mesquida J, Saludes P, Pérez-Madrigal A, Proença L, Cortes E, Enseñat L, Espinal C, Gruartmoner G: **Respiratory quotient estimations as additional prognostic tools in early septic shock.** *J Clin Monit Comput* 2018, **32**(6):1065-1072.
176. Morel J, Gergele L, Verveche D, Costes F, Auboyer C, Molliex S: **Do fluctuations of PaCO2 impact on the venous-arterial carbon dioxide gradient?** *Crit Care* 2011, **15**(6):456.
177. Mallat J, Mohammad U, Lemyze M, Meddour M, Jonard M, Pepy F, Gasan G, Barrailler S, Temime J, Vangrunderbeeck N *et al*: **Acute hyperventilation increases the central venous-to-arterial PCO(2) difference in stable septic shock patients.** *Ann Intensive Care* 2017, **7**(1):31.
178. Mallat J, Lazkani A, Lemyze M, Pepy F, Meddour M, Gasan G, Temime J, Vangrunderbeeck N, Tronchon L, Thevenin D: **Repeatability of blood gas parameters, PCO2 gap, and PCO2**

**gap to arterial-to-venous oxygen content difference in critically ill adult patients.**

*Medicine (Baltimore)* 2015, **94**(3):e415.