

**ASSESSING ANEMIA AND HYPOVOLEMIA RELATED
ALTERED OXYGEN BALANCE**

PhD thesis

Szilvia Kocsi M.D.



Department of Anaesthesiology and Intensive Therapy
Doctoral School of Multidisciplinary Medicine
Faculty of Medicine, University of Szeged

Supervisor:

Prof. Zsolt Molnár, MD, PhD, DEAA

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Introduction

Tissue oxygenation is determined by the balance between the rate of oxygen transport to the tissues (oxygen delivery) and the rate at which the oxygen is used by the tissues (oxygen consumption). In the critically ill there is often an imbalance between oxygen delivery and consumption. Oxygen delivery may be inadequate based on two grounds; **arterial oxygen content** and/or **cardiac output** may be reduced.

One of the most common causes of decreased **arterial oxygen content** in the critically ill is anemia. The prevalence of anemia among critically ill patients could be as high as 95 % by day three. A number of guidelines are of help in transfusion practice, however the criteria for the optimal management of anemia are not clearly defined. In most guidelines the transfusion trigger, i.e. the indication and timing of blood transfusion, is a certain level of hemoglobin, usually 70-100 g/L. It was recently suggested that hemoglobin level should not be the only factor on which the indication of blood transfusion is based. There is increasing evidence that transfusion is a double-edged sword: untreated anemia can be associated with a worse outcome and increased mortality, while transfusion may cause various infectious and non-infectious adverse effects. There is a clear need for additional quantitative parameters that would give information on anemia related altered oxygen extraction and hence the need for blood administration.

A common cause for decreased **cardiac output** in the intensive care unit is hypovolemia. Diagnosing hypovolemia is an everyday challenge in critical care. Clinicians utilize a large array of tools from simple clinical signs to invasive hemodynamic measurements, but a universally accepted gold standard parameter remains elusive. Although diagnosis may prove difficult, early recognition of hypovolemia is of utmost importance. By the time macro-hemodynamic changes manifest, the microcirculation may already be damaged. Furthermore, fluid therapy is ambiguous, on the one hand fluid resuscitation can save lives, but on the other hand a cumulative positive fluid balance is an independent risk factor for mortality. Deciding on the level of monitoring (non-invasive, 'less' invasive, invasive), and which parameter to monitor in order to keep the critically ill patient normovolemic remains uncertain.

Parameters of interest

Central venous oxygen saturation (ScvO₂), the hemoglobin oxygen saturation measured in a central vein, is a potentially useful physiological parameter. It is easily obtained as a blood gas sample from the vena cava superior via the central venous catheter already *in situ* in most critically ill patients. The main factors influencing ScvO₂ are hemoglobin, arterial hemoglobin oxygen saturation, cardiac output and oxygen consumption. Its normal value varies between 73-82 %. Although, the value reflects the oxygen extraction of the brain and the upper extremities, it is considered a reasonable surrogate marker of mixed-venous oxygen saturation (SvO₂) – the parameter representing the whole body oxygen extraction - in the clinical setting. Thence, it is often used as a marker of the balance between oxygen delivery and consumption.

Changes in ScvO₂ reflect systemic oxygen metabolism, but may fail to detect regional hypoxia and may also be false-negative when >70%. Under these conditions the central venous-to-arterial carbon dioxide difference (CO₂-gap) has been proposed as an alternative. It is as easily obtained as ScvO₂, only a simultaneous arterial and central venous blood gas sampling is needed. The physiological threshold of CO₂-gap is <5 mmHg, but this may be higher in low flow states. A concept based on the CO₂ stagnation phenomenon considers the cause of an increased CO₂-gap to be low blood flow, and an inverse correlation has been found between CO₂-gap and CO in non-septic and septic circulatory failures. Moreover, literature findings indicate that the amount of CO₂ produced is negligible when anaerobic respiration is present and therefore CO₂-gap cannot serve as a marker of tissue hypoxia.

Our aims were the following:

- 1.** To evaluate the change in ScvO₂ before and after transfusion in a retrospective study and to test whether the combination of hemoglobin and ScvO₂ may reflect anemia caused altered oxygen balance better than hemoglobin alone.
- 2.** To monitor the changes of ScvO₂ in isovolemic anemia using a large animal model, and test whether an altered oxygen extraction balance, which is due solely to a decreased hemoglobin level, can be detected by ScvO₂.
- 3.** To investigate how CO₂-gap changes during experimental isovolemic anemia and how it contributes to ScvO₂.
- 4.** To determine the effect of hypovolemia on ScvO₂ and CO₂-gap and the association between ScvO₂, CO₂-gap and indicators of microcirculatory blood flow.

Materials and Methods

1.1. Changes in ScvO₂ before and after transfusion

The retrospective study was conducted in four intensive care units of the University of Szeged over a six month period. All the patients who received blood transfusions were searched via the medical network system. Only those patients were included in the study who had arterial and central venous blood gas analysis before and after transfusion. The parameters of interest were: hemoglobin, central venous oxygen saturation, arterial oxygen saturation of hemoglobin, mean arterial pressure, heart rate, central venous pressure, lactate and the simplified oxygen extraction ratio.

For between-groups analysis the Mann-Whitney test and for before-after comparison the Wilcoxon test were used.

1.2. Changes of ScvO₂ in isovolemic anemia – an animal experiment

The study was carried out in the research laboratory of the Institute of Surgical Research. Vietnamese mini-pigs were anaesthetised, intubated, mechanically ventilated and after splenectomy they were bled 10% of their estimated blood volume over 5 minutes interval and the blood loss was replaced by the same volume of colloid. After each bleeding period (T₁-T₅) haemodynamic measurements and blood gas analysis were performed. Isovolemic anemia was achieved in five intervals.

For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter, a central venous line and a pulmonary artery catheter were placed in the central artery and veins. They were also used to draw blood gas samples.

Cardiac output, global end-diastolic volume, extravascular lung water, stroke volume, stroke volume variation, index of left ventricular contractility, heart rate, and mean arterial pressure were measured by transpulmonary thermodilution and pulse contour analysis at baseline and at the end of each interval.

Arterial, central venous and mixed venous blood gas samples were drawn and analyzed by cooximetry simultaneously at baseline and at the end of each cycle.

The changes in all parameters throughout the experiment were tested by repeated measures analysis of variance (ANOVA); and the number of degrees of freedom was adjusted to Greenhouse-Geisser epsilon when needed. For pairwise comparisons, Pearson's correlation was used. To evaluate the performance of central venous oxygen saturation in the detection of altered oxygen extraction receiver operating characteristics curve analysis was performed, and sensitivity, specificity, and positive predictive and negative predictive values were also determined. To model the linear relationship between oxygen extraction and the possible indicator of altered oxygen extraction, linear regression model was used.

1.3. Changes of CO₂-gap in isovolemic anemia – animal experiment

The study protocol was approved by the local ethics committee at the University of Szeged and the study was carried out in the research laboratory of the Institute of Surgical Research. This experiment complements the previously described data on the relationship of ScvO₂ and isovolemic anemia. The animals and instrumentation, the experimental protocol and the hemodynamic measurements are detailed above. Changes in all parameters throughout the experiment were tested by Friedman test and repeated measures analysis of variance (RM ANOVA), and the number of degrees of freedom was adjusted to Greenhouse-Geisser epsilon when needed. For pairwise comparisons Pearson's correlation was used. To evaluate the performance in detecting altered oxygen extraction of >30% (considered as the "physiological threshold"), receiver operating characteristics (ROC) curve analysis was performed.

1.4. ScvO₂ and CO₂-gap in moderate hypovolemia – animal experiment

The study was conducted in the research laboratory of the Institute of Surgical Research. Anesthetized, mechanically ventilated vietnamese mini-pigs underwent forced diuresis (HG – hypovolemic group). The animals were given a bolus furosemid - 5mg/kg - and then a continuous infusion - 5mg/kg/h - for two hours. Every 20 minutes invasive hemodynamic measurements, blood gas analysis and orthogonal polarization spectral imaging were performed (T₁-T₅). There were 5 animals in the sham group (SG), who did not receive any furosemide, but maintenance infusion of lactated Ringer (4mL/kg/h) and invasive hemodynamic measurements,

blood gas analysis and orthogonal polarization spectral imaging were performed in the same fashion.

For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter, a central venous line and a pulmonary artery catheter were placed in the central artery and veins. They were also used to draw blood gas samples.

Arterial, central venous and mixed venous blood gas samples were drawn and analyzed by cooximetry simultaneously at baseline and at the end of each cycle.

For continuous noninvasive visualization of the microcirculation in the sublingual region an intravital orthogonal polarization spectral (OPS) imaging technique was used. A 10x objective was introduced onto the sublingual serosa, and microscopic images were recorded with an S-VHS video recorder.

For the tonometry special probes were used and monitoring was performed with a Sidestream Microcap Handheld Capnograph instrument.

To assess further biochemical changes in the microcirculation, plasma big-endothelin-1 (BigET) levels were determined. For measurements of BigET, blood samples of 2 ml were drawn from the jugular vein into chilled polypropylene tubes containing EDTA (1 mg/mL). The samples were centrifuged at 1200g for 10 min at 4°C. The plasma samples were then collected and stored at -70°C until assay.

Changes in all parameters throughout the experiment were tested by repeated measures analysis of variance (RM ANOVA); and the number of degrees of freedom was adjusted to Greenhouse-Geisser epsilon when needed. Mann-Whitney U-test with Bonferroni correction was used for between-groups analysis. For pairwise comparisons Pearson's correlation was used. To evaluate the performance of central venous oxygen saturation,

central venous-to-arterial carbon dioxide difference and microcirculatory parameters in detecting altered oxygen extraction receiver operating characteristics (ROC) curve analysis was performed, and sensitivity, specificity, positive predictive and negative predictive values were also determined.

Results

2.1. Changes in ScvO₂ before and after transfusion

After transfusion hemoglobin levels increased significantly, which was accompanied by significant changes in all the other parameters but arterial oxygen saturation of hemoglobin. The median ScvO₂ was 71%, therefore we divided the patients into two groups: low group (LG, ScvO₂<70%), n=27; and high group (HG, ScvO₂>70%), n=23. There were no significant changes in ScvO₂ and the simplified oxygen extraction ratio in the HG, while on the contrary in the LG ScvO₂ and simplified oxygen extraction ratio improved significantly after transfusion.

2.2. Changes of ScvO₂ in isovolemic anemia – an animal experiment

The bleeding caused a gradual decrease in hemoglobin level after each phase and by the end of the experiment it had fallen by 61% of the baseline value. The preload as indicated by global end-diastolic volume value did not change significantly. Oxygen delivery fell significantly from T₂, oxygen consumption at T₄, oxygen extraction increased significantly from T₃, and exceeded the physiologic threshold of 30%. The change in ScvO₂ displayed a similar pattern as oxygen extraction and changed significantly and also fell below 70% only at T₄. The other parameters did not change significantly. We determined the association between oxygen extraction and ScvO₂, and found a strong, negative correlation. Furthermore, linear regression revealed a significant relationship between ScvO₂ and oxygen extraction.

2.3. Changes of CO₂-gap in isovolemic anemia – animal experiment

The bleeding caused a gradual decrease in hemoglobin level after each phase. The change in ScvO₂ displayed a similar pattern as oxygen extraction and changed significantly and also fell below 70% only at T₄. There was strong negative correlation between oxygen extraction and ScvO₂. The CO₂-gap was calculated for both, central venous and mixed venous blood. By T₄ central venous-to-arterial CO₂-gap increased significantly, however mixed venous-to-arterial CO₂-gap did not change. The correlations of oxygen extraction and ScvO₂ were significant with central venous CO₂-gap, while there were only weak correlations with mixed venous CO₂-gap. ROC analysis revealed the same tendency as the correlation.

2.4. ScvO₂ and CO₂-gap in moderate hypovolemia – animal experiment

Preload decreased significantly after each phase compared to baseline in HG. In HG oxygen delivery fell significantly from T₁ and remained so for the rest of the experiment. The oxygen extraction increased significantly over 30% from T₁, while ScvO₂ and CO₂-gap followed this change only after T₂. Lactate changed significantly from T₃. There were no significant changes in the sham group throughout the experiment. In HG there was a significant correlation between oxygen extraction and ScvO₂, and CO₂-gap; lactate also showed a significant, but weak correlation. With Receiver Operator Characteristic curves for ScvO₂, CO₂-gap and lactate to detect altered oxygen extraction the area under the curves was significant for ScvO₂, CO₂-gap, while lactate did not reach statistical significance.

The microcirculatory parameters in HG; tonometry increased significantly only when measured in the intestines, capillary perfusion rate and red blood cell velocity gradually and significantly decreased over time. There was a significant difference in capillary perfusion rate, red blood cell velocity and BigET between HG and SG. The ROC curves for predicting altered oxygen extraction proved to be significant for capillary perfusion rate and red blood cell velocity in HG. The correlation between ScvO₂ and the microcirculatory parameters proved to be significant.

Discussion

- 3.1.** The main finding of this retrospective study is that patients with low hemoglobin but normal central venous oxygen saturation levels may have received unnecessary blood transfusion, as transfusion was not accompanied by any significant changes in central venous oxygen saturation and the simplified oxygen extraction ratio.
- 3.2.** In our experiment, despite a continuous and significant drop in hemoglobin levels, the value of oxygen extraction – the parameter indicating altered balance of oxygen delivery and consumption - increased significantly only from the third bleeding interval, when the level of hemoglobin was far below the transfusion threshold. The change in central venous oxygen saturation displayed a similar pattern as oxygen extraction and fell below 70% only at the fourth bleeding interval. If we translate that into clinical practice, the reduced hemoglobin concentrations could have indicated blood transfusion from the second bleeding period, however, we found no evidence of impaired oxygen extraction until the hemoglobin was well below the current recommended transfusion threshold.
- 3.3.** Our results show that besides ScvO₂, only central venous-to-arterial CO₂-gap correlated well with changes in anemia caused increase in oxygen extraction. Furthermore, mixed venous blood driven indices, such as mixed venous-to-arterial CO₂-gap failed to indicate changes in oxygen extraction. Although isovolemia was maintained as indicated by the stable global end diastolic volume index values and

in fact, cardiac output and stroke volume both increased, we observed a rise in central venous-to-arterial CO₂-gap.

- 3.4.** Our study provides evidence that low or decreasing ScvO₂, as well as high or increasing CO₂-gap can reflect changes in hypovolemia and may be complementary in global oxygen balance and altered microcirculatory blood flow. Our goal of achieving hypovolemia was reached, which resulted in a significant drop in cardiac index in the hypovolemic group. Due to this change oxygen extraction increased significantly and this change accompanied by a fall in ScvO₂ and an increase in CO₂-gap. According to the microcirculatory parameters measured in this experiment, the inflicted hypovolemia resulted in significant changes to the microcirculation. Tonometry showed a significant increase, indicating decreased blood flow in the intestines.

New observations

- i. Central venous oxygen saturation is a sensitive indicator of oxygen balance in anemia and may thus serve as a rational additional guide to transfusion therapy.
- ii. A rise observed in central venous-to-arterial CO₂-gap may indicate not only low flow but also isovolemic anemia.
- iii. Low or decreasing central venous oxygen saturation can reflect changes and may be complementary in global oxygen balance and altered microcirculatory blood flow in hypovolemia.
- iv. An altered oxygen extraction caused by hypovolemia is reflected by an increase in central venous-to-arterial carbon dioxide difference.
- v. The conjoint value of central venous oxygen saturation and central venous-to-arterial carbon dioxide difference may be an important alarm signal for the clinician in the decision making at the bedside when considering a fluid challenge, commencing advanced hemodynamic monitoring or administering blood transfusion.

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Publications related to the thesis

- I. Kocsi S,** Demeter G, Fogas J, Erces D, Kaszaki J, Molnár Z. Central venous oxygen saturation is a good indicator of altered oxygen balance in isovolemic anemia. *Acta Anaesthesiol Scand* 2012; 56(3):291-297.
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