The trouble with properly describing the oxygen-transport-related quantities

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There is widespread confusion concerning the quantities to be used in the description of the oxygen-transport properties of human blood [1].

It all began in 1980 with the introduction of a changed (and wrong) definition of oxygen saturation in a paper on a new instrument for multicomponent analysis (MCA) of hemoglobin derivatives [2].

The new definition was implemented in the computer program of the instrument that was introduced into the market.

The consequences of the change were disastrous: it led to numerous diagnostic problems, gave rise to the idea that the definition of oxygen saturation is instrument-dependent, unduly hampered the proper understanding of pulse oximetry, caused many unnecessary experimental studies and led to the introduction of nonsensical terms as "*fractional saturation*", "*functional saturation*" and "*pulse oximeter gap*".

Recently, another confusing new term has been proposed: "*partial oxygen saturation*", symbol pSO_2 [3].

It should be borne in mind that for changing the definition of oxygen saturation no arguments have ever been given. No discussion in any scientific panel had preceded the introduction of the new definition.

It was just announced in a paper and implemented in an instrument. The danger that this would necessarily lead to grave misunderstandings was almost immediately noted and published [4]. This, however, did not provoke a discussion, still less retraction of the new definition.

Now, some 25 years later, the error has become so widespread that it is difficult to eradicate. There is a vast literature in which all kinds of ramifications of the evolved disagreements are discussed, so that the average reader understandably gets confused.

Yet, the correct definition of oxygen saturation is

more than 100 years old, was applied by pioneers such as Christian Bohr and Donald D. VanSlyke, and is indispensable to the description of the oxygen transport by the blood.

The end-capillary oxygen tension is the key factor in the oxygen transport to the tissues, because it determines the driving force for the oxygen diffusion to the most unfavorably situated spots in the tissues.

The end-capillary oxygen tension is dependent on blood-flow rate and capillary density and on the oxygen-carrying properties of the blood, which may be expressed with the help of three quantities: oxygen capacity, oxygen saturation and oxygen affinity.

Although these quantities have been adequately described in many textbooks of physiology, monographs [5] and reviews [6], a concise description in relation to the physiology of blood gas transport may help clarify the definition and physiological significance of each quantity.

Safe and responsible use of the measuring instruments requires knowledge of the measured quantities. Especially the misunderstandings as to what is actually measured by pulse oximetry and by spectrophotometric MCA ("*CO-oximetry*") should be cleared.

Oxygen capacity

The oxygen capacity is the maximum amount of hemoglobin-bound oxygen per unit volume of blood. It is expressed in mmol/L or in mL(STPD)/L or mL(STPD)/dL, where STPD means "*Standard Temperature and Pressure, Dry*".

The oxygen capacity is determined by the concentration of active hemoglobin, i.e., the sum of the concentrations of O_2Hb and HHb. Since the internationally accepted method for the determination of hemoglobin in blood [7] measures the total hemoglobin concentration (*c*tHb), a correction for any dyshemoglobin present in the blood must be made.

Dyshemoglobins are hemoglobin derivatives which have temporarily or permanently lost the capability of reversibly binding oxygen at physiological oxygen tension [8].

Hence, in the calculation of the oxygen capacity (BO_2) from *c*tHb the dyshemoglobin concentration (*c*dysHb) is taken into account. When B and c are expressed in mmol/L and the substance concentration of hemoglobin reflects the monomer:

$$BO_2 = c\text{tHb} - c\text{dysHb} \qquad \text{eq. 1}$$

When *B* is expressed in mL(STPD)/L and *c* in g/L:

$$BO_2 = \beta O_2 (c\text{tHb} - c\text{dysHb}) \qquad \text{eq. 2}$$

where βO_2 is the volume of oxygen in mL(STPD) that can be bound by 1 g of hemoglobin. Theoretically, βO_2 = 22394 / 16114.5 = 1.39 mL/g, where 22394 is the molar volume of oxygen in mL(STPD) and 16114.5 is a quarter of the molar mass of human HbA in g [9].

The experimental value of βO_2 has been found near the theoretical one [8].

As the common dyshemoglobins present in human blood are carboxyhemoglobin (COHb) and methemoglobin (MetHb), the oxygen capacity may be approximated by

$$BO_2 = \beta O_2 \times (c\text{tHb} - c\text{COHb} - c\text{MetHb}) \qquad \text{eq. 3}$$

Originally, the oxygen capacity was determined by measuring the concentration of total oxygen in a known volume of blood and correcting for the freely dissolved oxygen.

Soon after its introduction, the Beckman DU spectrophotometer enabled COHb and MetHb to be measured in the clinical chemical laboratory [10]. Determination of *c*tHb by the standard method [7] and correction for the fractions of MetHb and COHb thus made a simple determination of the oxygen capacity possible.

The validity of this procedure has been experimentally confirmed [5, 8].

Oxygen saturation

The oxygen saturation of the blood (sO_2) is defined as the concentration of hemoglobin-bound oxygen divided by the oxygen capacity. This is equivalent to the concentration of O_2Hb divided by the sum of the concentrations of O_2Hb and HHb:

$$sO_2 = cO_2(Hb) / BO_2 \qquad \text{eq. 4}$$

$$sO_2 = [ctO_2 - cO_2(free)] / BO_2 \qquad \text{eq. 5}$$

$$sO_2 = cO_2Hb / (cO_2Hb + cHHb) \qquad \text{eq. 6}$$

where $cO_2(Hb)$ is the concentration of oxygen bound to hemoglobin, $cO_2(free)$ is the concentration of oxygen dissolved in blood but not bound to any other substance, and ctO_2 is the concentration of total oxygen in blood.

It should be noted that there is a conceptual difference between $cO_2(Hb)$ and cO_2Hb, although the two quantities are numerically equal, when both are expressed in mmol/L.

The classical procedure for measuring sO_2 was the determination of ctO_2 of a blood sample by means of VanSlyke's manometric method and repeating the measurement after equilibration of the remaining part of the sample with air at room temperature.

After calculation of $cO_2(free)$ for the two measurements, sO_2 was determined using eq. 5. In the development of photometric procedures for measuring sO_2, the manometric method long remained the gold standard.

Among the numerous photometric procedures for measuring sO_2, there were many two-wavelength methods using various combinations of wavelengths and multiple types of cuvettes with different light path lengths. These methods were quite accurate and rather easy to carry out with general purpose spectrophotometers.

An other approach was the construction of instruments for the continuous measurement of sO_2 *in vivo*. Since the early German devices of the 1930s and Millikan's first oximeter of 1942 [11], several oximeters using either transmitted or reflected light [5] have been constructed and used in (patho)physiological research and for many special clinical purposes. These instruments were quite accurate, but the procedures were too complicated for their routine use in clinical conditions.

Oxygen affinity

The oxygen affinity of the blood is usually described with the help of a graph showing the relationship between oxygen saturation and oxygen tension: the oxygen dissociation curve (ODC).

The influence of various factors on the oxygen affinity is shown by changes in the position and/or the shape of the ODC. The oxygen affinity is influenced by various factors: pH, pCO_2, temperature (T), 2,3-diphosphoglycerate (2,3-DPG), COHb, MetHb and possibly some other ones. The standard ODC is the ODC at pH = 7.40, pCO_2 = 5.33 kPa (40 mmHg), T = 37 °C.

Since at high sO_2 the ODC is flat, nearly full saturation is reached in the lung capillaries over a rather wide pO_2 range. The standard ODC of human blood [5, 16] shows that even at pO_2 = 10 kPa (75 mmHg), sO_2 is still > 95 %.

In the pO_2 range prevailing in the tissue capillaries, the ODC is steep, so that much oxygen can be released as a result of only a slight fall in pO_2. The normal mixed venous sO_2 (approx. 70 %) shows that at rest most oxygen is released at a pO_2 around 5 kPa (38 mmHg).

This is the driving pressure for oxygen diffusion to the tissue cells. In some tissues, such as the heart and skeletal muscle during heavy exercise, the high blood-flow rate and high capillary density allow the release of much more oxygen per unit volume of blood. The

driving pressure is then lower, but it remains the limiting factor.

This demonstrates that, at least at sea level, an increase in oxygen affinity, resulting in a shift of the ODC to the left, endangers the transport of oxygen to the tissues more than a decrease in affinity, resulting in a shift to the right. Therefore, pO_2 at sO_2 = 50 % (p50) is used as an indicator of the oxygen affinity.

The normal value of standard p50) (p50) at pH = 7.40, pCO_2 = 5.33 kPa (40 mmHg) and T = 37 °C is 3.55 kPa (27 mmHg).

Recent developments

In the last decades of the 20th century, two technical developments brought the determination of almost all oxygen-transport-related quantities within reach of daily clinical practice: spectrophotometric multicomponent analysis (MCA) of hemoglobin derivatives [12, 13] and pulse oximetry.

For routine MCA of hemoglobin, dedicated multiwavelength photometers were developed that measure the concentration of the various hemoglobin derivatives present in a blood sample, from which total hemoglobin concentration, oxygen saturation and dyshemoglobin fractions (cdysHb/ctHb) can be calculated.

Introduction of the pulse principle by the Japanese engineer Takuo Aoyagi [14], made oximetry in vivo suitable for routine clinical application. A pulse oximeter is a two-wavelength photometer that determines arterial sO_2 through measuring light absorption in a piece of well-perfused tissue. In this respect it is similar to a conventional oximeter; the difference is in the data processing [14].

Taking into account the varying part of the transmitted light, resulting from the cyclic expansion of the arterial bed, as well as the non-varying part that interacted with tissue components, with non-pulsatile arterial blood and with venous blood, a ratio is obtained of the relative changes of the transmitted light intensities in the two wavelengths.

This quantity depends on the absorptivity of O_2Hb and HHb at the two wavelengths and the arterial sO_2. The relationship with sO_2 is determined by measurements in healthy volunteers. Therefore, it can only be ascertained in the higher oxygen saturation range.

For values lower than 70 %, the relationship is determined by extrapolation. This makes pulse oximeters less accurate at low values of sO_2.

Through proper wavelength selection, photometric interference by other hemoglobin derivatives can be minimized. When using 660 and 940 nm, COHb causes only a slight underestimation of sO_2, MetHb causes a moderate underestimation in the higher and some overestimation in the lower sO_2 range; around sO_2 = 70 % the error is negligible [15].

A parallel development was the addition to blood gas analyzers of a computer program for calculating sO_2 from pO_2, pCO_2 and pH with the help of the standard oxygen dissociation curve (see above).

The method is theoretically sound and works reasonably well in normal blood. In patients, however, the standard ODC may be different because of changes in the oxygen affinity of the blood due to other factors. This gives erroneous results, since these factors can hardly be accounted for in the computer program.

The three methods for the determination of sO_2 in clinical conditions (MCA, pulse oximetry and calculation from pO_2) thus provide the same quantity (eq. 6). Because of the differences in technique, there are differences in the precision and accuracy that can be attained, and in the sources of error that may confound the results.

Dyshemoglobin

An increased fraction of dyshemoglobin decreases the oxygen capacity (eq. 1). The common dyshemoglobins, COHb and MetHb, also affect the oxygen affinity; both

displace the ODC to the left. This effect is quantitatively expressed by the relationship of standard *p*50 and the dyshemoglobins as given for COHb in equations 7 [17]:

$$p50 = -3.6 \times FCOHb + 3.4 \qquad \text{eq. 7}$$

where

$$FCOHb = cCOHb / ctHb \qquad \text{eq. 8}$$

It follows from equation 7 that at FCOHb = 0.35, standard *p*50 = 2.14 kPa (16 mmHg).

Consequently, in the presence of COHb, pO_2 must fall to a considerably lower level for the release of the amount of oxygen needed by the tissue.

This causes a corresponding fall in driving pressure for oxygen diffusion to the tissues, which, in turn, may cause tissue hypoxia, especially in the cells dependent on the end-capillary pO_2. MetHb has a similar effect, though to a lesser extent.

It is through their effect on the oxygen affinity that some dyshemoglobins, especially COHb, impair the oxygen transport to the tissues so much more than a corresponding fall in oxygen capacity through a decrease in total hemoglobin concentration [17].

Consequences

Thus, a multiwavelength photometer programmed for MCA of hemoglobin ("*CO-oximeter*") should display:

- Total hemoglobin concentration, ctHb
- Oxygen saturation, sO_2 (eq. 6)
- Carboxyhemoglobin fraction, FCOHb (eq. 8)
- Methemoglobin fraction, FMetHb
- Other dyshemoglobin fractions, if measured

Since in most patients the dyshemoglobin fractions are within the reference range and thus pathophysiologically insignificant [18], it may be agreed to that only increased dyshemoglobin fractions are reported.

Discussion

The quantities mentioned above will in most patients provide adequate information concerning the oxygen supply to the tissues. In more complicated cases, pO_2 and *p*50 will be required. No other quantities are necessary nor desirable for properly describing the oxygen-transport properties of the blood.

Therefore, within the scope of this paper, no further elaboration is appropriate. However, a few remarks seem to be worthwhile on the misunderstandings prevailing in the literature and the failed attempts to clear them away, which only contributed to the confusion.

As mentioned above, the problems began with a change in the definition of oxygen saturation [3]. The fraction of O_2Hb:

$$FO_2Hb = cO_2Hb / ctHb \qquad \text{eq. 9}$$

was substituted for sO_2 (eq. 6), but this quantity was still called oxygen saturation. This led to the "*discovery*" by clinicians that there apparently are two kinds of oxygen saturation.

In patients with an increased dyshemoglobin fraction, they observed a difference between oxygen saturation by pulse oximetry and oxygen saturation by spectrophotometric MCA ("*CO-oximetry*").

Oxygen saturation as measured by "*CO-oximetry*" (actually FO_2Hb) was subsequently called "*fractional saturation*", oxygen saturation as measured by pulse oximetry (actually sO_2) was called "*functional saturation*".

The term "*fractional saturation*" is inadequate, because the concept of "*saturation*" cannot be applied to FO_2Hb. Use of the term "*saturation*" demands that the system can be fully saturated, even in the presence of other ligands.

The presence of COHb for instance, does not prevent the remaining hemoglobin from being fully saturated

with oxygen [19]. The concept "*oxygen saturation*" thus can be applied to the remaining hemoglobin and not to total hemoglobin. A "*saturation scale*" by definition runs from 0 to 1, which is the same as 0 to 100 % [19]. The addition "*functional*" to "*saturation*" for designating sO_2 is redundant.

The new terminology fostered the wrong idea that "*CO-oximeters*" necessarily measure FO_2Hb or anyhow display "*fractional saturation*", and that this is the crucial quantity. Thus, in case of carbon monoxide poisoning, a pulse oximeter is thought to be misleading, because it (correctly) shows a normal oxygen saturation in the presence of a lethal concentration of COHb in the blood.

There is no point in giving a further account of the misconceptions ensuing from the tacit substitution of FO_2Hb for sO_2. However, the suitability of FO_2Hb per se, not as a substitute for sO_2 but as an addition to it, should be discussed, because this has been advocated recently [20].

It should be noted that FO_2Hb per se has no physiological meaning; it depends on pO_2 as well as on the dyshemoglobin fractions. So a low FO_2Hb may signal a decreased pO_2 or an increased dyshemoglobin fraction or both.

A decrease in FO_2Hb through the presence COHb is not a measure of the concomitant impairment of oxygen transport, because it does not reflect the effect of COHb on the oxygen affinity. There is no place for FO_2Hb.

There is no need for it, it does not add anything to reporting the dyshemoglobin fractions, and it is asking for trouble, because it is easily confused with sO_2 [1].

It should be realized that we have run into the paradoxical situation that the introduction of instruments for the clinical examination of vital functions in patients has been associated with a loss of physiological knowledge of the very system for the evaluation of which the measurements are made.

Medical technology has dissociated from pathophysiology. There is no doubt that this leads to clinical errors [21]. It needs a strong effort to reverse this situation.

In my opinion this can only be accomplished with the persistent participation of international organizations, such as the IFCC, and the unselfish cooperation of the companies that produce and sell the measuring instruments.

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References

1. Zijlstra WG. Clinical assessment of oxygen transport-related quantities. Clin Chem 2005; 51: 291-92.

2. Brown LJ. A new instrument for the simultaneous measurement of total hemoglobin, % oxyhemoglobin, % carboxyhemoglobin, % methemoglobin, and oxygen content in whole blood. IEEE Trans Biomed Engin 1980; 27: 132-38.

3. Patientennahe Sofortdiagnostik (Point of Care). Vereinheitlichung von Nomenklatur und Symbolen. Qualitest 8, April 2005.

4. Zwart A, Buursma A, Oeseburg B, Zijlstra WG. Determination of hemoglobin derivatives with the IL282 CO-Oximeter as compared with a manual spectrophotometric five-wavelength method. Clin Chem 1981; 27: 1903-07.

5. Zijlstra WG, Buursma A, van Assendelft OW. Visible and near infrared absorption spectra of human and animal haemoglobin: determination and application. Utrecht: VSP, 2000; pp 368.

6. Zijlstra WG, Maas AHJ, Moran RF. Definition, significance and measurement of quantities pertaining to the oxygen carrying properties of human blood. Scand J Clin Lab Invest 1996; 56. Suppl 224: 27-45.

7. International Committee for Standardisation in Haematology (ICSH). Recommendations for haemoglobinometry in human blood. Brit J Haematol 1967; 13(Suppl): 71-75.

8. Dijkhuizen P, Buursma A, Fongers TME, Gerding AM, Oeseburg B, Zijlstra WG. The oxygen binding capacity of human haemoglobin. Hüfner's factor redetermined. Plügers Arch 1977; 369: 223-31.

9. Braunitzer G, Gehring-Müller R, Hilschmann N, Hilse K, Rudolf V, Wittmann-Liebold B. Die Konstitutions des normalen adulten Humanhämoglobins. Hoppe-Seylers Z Physiol Chemie 1961; 325: 283-88.

10. van Kampen EJ, Zijlstra WG. Determination of hemoglobin and its derivatives. Adv Clin Chem 1965; 8: 141-87.

11. Millikan GA. The oximeter, an instrument for measuring continuously the oxygen saturation of the arterial blood in man. Rev Sc Instr 1942; 13: 434-44.

12. Zwart A, Buursma A, van Kampen EJ, Oeseburg B, van der Ploeg PHW, Zijlstra WG. A multiwavelength spectrophotometric method for the simultaneous determination of five haemoglobin derivatives. J Clin Chem Clin Biochem 1981; 19: 457-63.

13. Zwart A, Buursma A, van Kampen EJ, Zijlstra WG. Multicomponent analysis of hemoglobin derivatives with a reversed optics spectrophotometer. Clin Chem 1984; 30: 373-79.

14. Severinghaus JW, Honda Y. History of blood gas analysis. VII. Pulse oximetry. J Clin Monit 1987; 3: 135-38.

15. Zijlstra WG, Buursma A, Meeuwsen-van der Roest WP. Absorption spectra of human fetal and adult oxyhemoglobin, de-oxyhemoglobin, carboxyhemoglobin, and methemoglobin. Clin Chem 1991; 37: 1633-38.

16. Severinghaus JW. Blood gas calculator. J Appl Physiol 1966; 21: 1108-16.

17. Zwart A, Kwant G, Oeseburg B, Zijlstra WG. Human whole blood affinity: effect of carbon monoxide. J Appl Physiol 1984; 57: 14-20.

18. Zwart A, van Kampen EJ, Zijlstra WG. Results of routine determination of clinically significant hemoglobin derivatives by multicomponent analysis. Clin Chem 1986; 32: 972-78.

19. Wimberley PD, Siggaard-Andersen O, Fogh-Andersen N, Zijlstra WG, Severinghaus JW. Haemoglobin oxygen saturation and related quantities: definitions, symbols and clinical use. Scand J Clin Lab Invest 1990; 50: 455-59.

20. Haymond S, Cariappa R, Eby CS, Scott MG. Laboratory assessment of oxygenation in methemoglobinemia. Clin Chem 2005; 51: 434-44.

21. Hurford WE, Kratz A. Case 23-2004: a 50-year old woman with low oxygen saturation. New Engl J Med 2004; 351: 380-87.