PAPER

Co-oximetry in clinically healthy dogs and effects of time of post sampling on measurements

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Objectives: **Co-oximetry is a complex and valuable laboratory method that measures haemoglobin species and oxygenation status by multi-wavelength spectrophotometry. The purpose of this study was to establish reference intervals for clinically healthy dogs and to determine the effect of time of analyses and sex of animals on the accuracy of results.**

Methods: **Blood was collected from 27 healthy adult dogs of various breeds and sex. Co-oximetry was performed on a CCX co-oximeter that measures eight haemoglobin and oxygen transport related parameters: carboxyhaemoglobin (COHb), deoxyhaemoglobin (HHb), oxyhaemoglobin (O_2Hb), methaemoglobin (MetHb), total haemoglobin (tHb), oxygen saturation (SO_2%), oxygen content (O_2Ct) and oxygen capacity (O_2Cap).**

Results: **Results obtained after 2 and 4 hours were not significantly different from those obtained immediately after sampling. But after 48 hours, the results for total haemoglobin, oxygen saturation, oxyhaemoglobin, oxygen content and oxygen capacity were significantly lower, and carboxyhaemoglobin and deoxyhaemoglobin values were significantly higher than determination immediately after sampling. Gender had no significant impact on co-oximetry values.**

Clinical Significance: **Co-oximetry offers several advantages compared with other methods, including ease of use, increased accuracy and greater differentiation among haemoglobin species.**

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INTRODUCTION

The ability of red blood cells to attract, carry and release the proper amounts of oxygen at the correct location and time is determined by many factors. In these determinations co-oximetry plays a central and vital role. The total amount of haemoglobin in the blood, its ability to bind and carry oxygen and the amount of haemoglobin species present in circulation are some of the most important factors responsible for the development of hypoxaemia. Based on the ability to bind oxygen molecules, haemoglobins are divided into two classes: normal haemoglobins and dyshaemoglobins. The normal haemoglobins are those capable of binding O_2 and they include oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb). The dyshaemoglobins are haemoglobin derivatives incapable of binding O_2, such as carboxyhaemoglobin (COHb), methaemoglobin (MetHb) and sulfhaemoglobin (SHb) (Haymond and others 2005). Normally, there are four species of haemoglobin typically circulating in animal blood: oxyhaemoglobin, deoxyhaemoglobin, methaemoglobin and carboxyhaemoglobin.

Examination of the oxygen transport status of animals is still based largely on clinical signs, such as cyanosis. With the standardisation of methods and insight into haemoglobin molecules the accurate evaluation of oxygen carrying capacity is available. The methodology for measuring oxygen saturation in the clinical laboratory has progressed through the development of numerous (spectro)photometric methods that use the Beer-Lambert law. Arterial blood gas analysis, pulse oximetry, end-tidal capnometry,

transcutaneous oximetry and capnometry all can provide accurate and valuable insights into the gas distribution process. However, these techniques have some fundamental weaknesses: first, they are measuring only the dissolved portion of the oxygen being carried in the blood which represents only a small fraction of the total oxygen-carrying capacity of the blood (with the exception of blood gas analysis and pulse oximetry), and none of these techniques can accurately differentiate among the various species of haemoglobin (Mathews 1995, Ehrmeyer and Shrout 1996).

Today, the most precise method of determining arterial blood saturation is via analysis with a co-oximeter (Mathews 1995, Rausch-Madison and Mohsenifar 1997, Kress and others 1999, Ali and others 2001). Co-oximeters are dedicated multi-wavelength spectrophotometers that automate the analyses of blood samples. For all co-oximeters, the determination of each haemoglobin species is dependent on the difference between the derivative's light absorption and the total light absorption for all the other haemoglobin species that might be present in various amounts in the blood sample. Certain wavelengths are absorbed to a greater degree by different types of haemoglobin such that each type of haemoglobin has a unique pattern of absorption (Brunelle and others 1996).

Literature concerning co-oximetry determinations in veterinary medicine is very limited and suffers from a lack of uniform methodology (different sex and age groups, reference values, different diseases). The purpose of this study was to establish reference intervals for clinically healthy dogs and to determine the effect of time of analysis and sex of animals on the accuracy of results.

MATERIALS AND METHODS

Blood was collected from 27 healthy adult dogs of various breeds (German shepherd, Belgian sheepdog, golden retriever and sharplaninac) and sex (11 females and 16 males) from six months to eight years old (average age 2·5 years) into a commercial preheparinised syringe. Assessment of health status was determined by physical examination and laboratory findings (haematology and biochemical results). None of the animals were neutered. Samples of blood were taken from the femoral artery under anaerobic conditions. After collection, the closed syringe was mixed and immersed in a mixture of ice and water. Co-oximetry was performed on a CCX co-oximeter, CCX-13 model (Nova Biomedical, Waltham, MA, USA) that measures eight haemoglobin and oxygen transport related parameters: carboxyhaemoglobin (COHb), deoxyhaemoglobin (HHb), oxyhaemoglobin (O_2Hb), methaemoglobin (MetHb), total haemoglobin (tHb), oxygen saturation (SO_2%), oxygen content (O_2Ct) and oxygen capacity (O_2Cap). The instrument was operated and maintained according to the manufacturer's instructions. The CCX co-oximeter aspirates approximately 115 microlitres of whole blood. The lysed sample is drawn into an optical cuvette, and the absorbance of light is measured at seven predefined wavelengths. The co-oximetry was determined immediately after sampling, then at 2, 4 and 48 hours after blood sampling. Samples were stored in ice water at 2 and 4 hours after collection, and at +4°C 48 hours after collection (temperature variation was within 2°). The study protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Zagreb, Croatia.

All data were reported as mean ±standard deviation (sd). The Mann-Whitney test was used to determine statistical differences, and significance was set at $P<0·05$. Reference intervals were calculated using nonparametric statistical methods (the interquartile range) considering the low number of animals. All statistical analyses were performed with the statistical software program Statistica (Statistica 8 for Windows; StatSoft Inc.).

RESULTS

Results obtained from blood analysis on the co-oximeter immediately after sampling are shown in Table 1, as mean ±sd and the corresponding minimum and maximum values, respectively, together with their 95% confidence level.

Measurements were repeated after 2, 4 and 48 hours, and compared results. No statistical differences for any parameter after 2 and 4 hours was found. But after 48 hours results for total haemoglobin, oxygen saturation, oxyhaemoglobin, oxygen content and oxygen capacity were significantly lower than measurements made immediately after sampling. Carboxyhaemoglobin and deoxyhaemoglobin values were significantly higher. Only methaemoglobin did not change significantly. Data for mean ±sd for every time frame are shown in Table 2.

Co-oximetry values were also determined comparing the gender of animals and found no significantly different results for any parameter (values not shown).

Reference intervals were calculated using nonparametric statistical methods as a range between the 25th and 75th percentiles, using the values obtained from the first measurements on the samples immediately post collection. Data are shown in Table 3. The reference intervals and 95% confidence levels are based on the same analytical data obtained on the first measurements on the collected blood samples of healthy animals of mixed breed, age and sex.

DISCUSSION

Determination of the haemoglobin species in total haemoglobin is necessary in order to get correct information about the cause of hypoxia, oxygen transport disorders, anaemias and other clinical problems. The dyshaemoglobins (COHb, methaemoglobin, sulfhaemoglobin) are present in a very small amount in healthy individuals, so they do not cause a change in oxygen saturation, but in pathological processes their presence is clinically significant (Haymond and others 2005).

COHb is formed when carbon monoxide is bound to the haemoglobin. Small amounts of carbon monoxide are present as a metabolic end product in all animal blood. Environmental exposure, however, can raise this concentration. An interval for COHb was established as 1·30 to 2·70% with a mean of 2·1%,

Table 1. Co-oximetry values determined immediately after blood collection in healthy dogs

Parameter	Valid n	Mean	±sd	Min	Max	95% confidence interval
tHb (g/L)	26	165·50	38·59	70·0	235·0	149·9 to 181·1
SO_2 (%)	26	98·81	0·54	97·60	99·4	98·59 to 99·02
O_2Hb (%)	27	96·47	1·29	93·5	98·8	95·96 to 96·98
COHb (%)	27	2·10	0·98	0·1	3·9	1·71 to 2·49
MetHb (%)	27	0·25	0·13	0	0·5	0·20 to 0·30
HHb (%)	27	1·15	0·49	0·6	2·3	0·96 to 1·34
O_2Ct (vol%)	27	22·06	4·81	9·6	30·6	20·16 to 23·97
O_2Cap (vol%)	27	22·38	4·99	9·7	31·2	20·40 to 24·35

tHb Total haemoglobin, SO_2 Oxygen saturation, O_2Hb Oxyhaemoglobin, COHb Carboxyhaemoglobin, MetHb Methaemoglobin, HHb Deoxyhaemoglobin, O_2Ct Oxygen content, O_2Cap Oxygen capacity

Table 2. Comparison of values obtained at different analysis time after taking a sample

Time of analyses/hours	0	2	4	48
Parameter	Mean ±sd	Mean ±sd	Mean ±sd	Mean ±sd
tHb (g/L)	165·50 ±38·59	146·60 ±27·75	157·25 ±7·17	139·67 ±20·93*
SO_2 (%)	98·81 ±0·54	99·07 ±0·13	99·11 ±0·15	92·45 ±2·88*
O_2Hb (%)	96·47 ±1·29	97·15 ±0·72	96·84 ±0·89	86·73 ±3·75*
COHb (%)	2·10 ±0·98	1·65 ±0·73	2·06 ±0·80	5·88 ±2·24*
MetHb (%)	0·25 ±0·13	0·30 ±0·13	0·26 ±0·15	0·32 ±0·16
HHb (%)	1·15 ±0·49	0·93 ±0·13	0·89 ±0·15	7·06 ±2·67*
O_2Ct (vol%)	22·06 ±4·81	19·78 ±3·62	21·18 ±1·01	15·98 ±3·78*
O_2Cap (vol%)	22·38 ±4·99	19·97 ±3·64	21·36 ±1·00	17·34 ±4·10*

tHb total haemoglobin, SO_2 oxygen saturation, O_2Hb oxyhaemoglobin, COHb carboxyhaemoglobin, MetHb methaemoglobin, HHb deoxyhaemoglobin, O_2Ct oxygen content, O_2Cap oxygen capacity
*Statistically different (P<0·05)

Table 3. Reference co-oximetry intervals obtained from values determined immediately after blood collection for clinically healthy dogs

Parameter	Median	Reference interval
tHb (g/L)	170·00	150·00 to 183·00
SO_2 (%)	99·00	98·50 to 99·20
O_2Hb (%)	96·5	95·90 to 97·50
COHb (%)	2·30	1·30 to 2·70
MetHb (%)	0·20	0·10 to 0·40
HHb (%)	1·00	0·80 to 1·50
O_2Ct (vol%)	22·80	20·00 to 24·60
O_2Cap (vol%)	23·00	20·30 to 24·80

ftHb Total haemoglobin, SO_2 Oxygen saturation, O_2Hb Oxyhaemoglobin, COHb Carboxyhaemoglobin, MetHb Methaemoglobin, HHb Deoxyhaemoglobin, O_2Ct Oxygen content, O_2Cap Oxygen capacity

due to the endogenous catabolism of heme and the inhalation of ambient CO. Determination of COHb is useful for differentiating haemolytic anaemias, effects of CO exposure on development of cardiovascular diseases and neurotoxicity (Mahoney and others 1993). COHb is also increased in haemolytic diseases due to the increased production following haemolysis, as in babesiosis (Taylor and others 1991). Carboxyhaemoglobinaemia connected with CO poisoning is also one of the conditions in which standard pulse oxymetry is not reliable because it falsely increases SO_2 values. In this case co-oximetry is the method of choice (Mahoney and others 1993, Mack 2007).

Another clinical condition where co-oximetry is considered as a valuable diagnostic method is methaemoglobinaemia. MetHb represents the oxidized, deoxy form [Fe(III)-Hb] of haemoglobin, to which O_2 cannot bind. An interval for MetHb was established as 0·10 to 0·40%, with mean of 0·25%, an amount from physiological formation as a result of endogenous oxidation. Concentrations around 80% are life-threatening (Bradberry 2003).

Methaemoglobinaemia can be induced by multiple pharmacological and chemical exposures, including nitrites, benzocaine, lidocaine, sulfonamides, quinones and dye derivatives (Haymond and others 2005). MetHb may also arise from genetic or idiopathic etiologies (NADH-MetHb reductase deficiency) (Wright and others 1999). Methaemoglobinaemia developed in dogs after use of benzocaine-containing products for topical treatment of pruritic skin conditions (Harvey and others 1979). Because in patients with sepsis, large amounts of nitric oxide (NO) are released and converted to methaemoglobin and nitrate, methaemoglobin concentrations may be a useful marker of the onset of sepsis or septic shock (Ohashi and others 1998). Another cause of methaemoglobinaemia is onion poisoning. Dogs are highly susceptible to onion toxicosis. The result of the oxidative haemolytic process induced by *Allium* species consumption is the onset of anaemia, methaemoglobinaemia and impaired oxygen transportation (Harvey and Kaneko 1976, Lee and others 2000, Tang and others 2008, Botha and Penrith 2009).

Lee and others (2006) found that methaemoglobin concentrations can increase with storage, so fresh samples are required for analysis. This is contradictory to our finding that methaemoglobin is stable even 48 hours after blood sampling.

For determining the oxygenation status of blood different approaches were used, which are often misunderstood, because in healthy animals without dyshaemoglobins the values are

virtually identical. It is important to understand that they are not equivalent measurements and their clinical relevance is evident in cases of increased COHb or MetHb. Oxygen saturation (SO_2) represents the percent of haemoglobin bound to oxygen, expressed as a fraction of the amount of haemoglobin capable of binding to oxygen (oxyhaemoglobin and deoxyhaemoglobin) (Galli and Colombo 2004). An interval for SO_2 was established as 98·50-99·20% with a mean of 98·81%.

Oxygen content (O_2Ct) is a calculated value, based on the total haemoglobin and oxyhaemoglobin concentration, so it expresses the oxygen content of haemoglobin. Oxygen capacity (O_2Cap) expresses the volume of oxygen capable of being reversibly bound and transported by haemoglobin. The equation for oxygen capacity includes COHb and MetHb concentrations, so it can express the capacity based on available haemoglobin. O_2Ct and O_2Cap are strongly related values, demonstrating similar values, 20 to 24·6% for O_2Ct and 20·3 to 24·8% for O_2Cap. Assessment of these parameters are important in detecting the cause of hypoxia, oxygen transport disorders, degenerative diseases and others (Galli and Colombo 2004).

The effect of time of analysis was also determined on the accuracy of obtained results. Results obtained after 2 and 4 hours were not significantly different from those obtained immediately after sampling. But after 48 hours, results for total haemoglobin, oxygen saturation, oxyhaemoglobin, oxygen content and oxygen capacity were significantly lower, and COHb and deoxyhaemoglobin values were significantly higher than determination immediately after sampling. The significant decrease in total haemoglobin at 48 hours can be explained due to the breakdown of the haemoglobin. These findings are extremely important, because they allow short-term delay of analysis with appropriate storage. Methaemoglobin values did not change, so this allows determination even 48 hours after sampling, which is important in cases of poisoning, a very common cause of methaemoglobinaemia in dogs. As most veterinary units are still lacking high-performance equipment, including co-oximeters, it would enable transport of samples to an equipped laboratory.

Gender did not have a significant impact on co-oximetry values. Reference intervals generated for 27 dogs of mixed breed, gender and age on the samples immediately post collection, can provide an approximate reference interval only. This is due to the small sample size and variation between subjects.

In general, co-oximetry offers several advantages, including ease of use, increased accuracy and greater differentiation among haemoglobin species. Of importance in today's veterinary medicine is that co-oximetry has a greater availability at lower cost than alternative methods. The ability to quickly, accurately and economically provide diagnostic data related to oxygen transport capacity can provide valuable information that may significantly impact patient care, as all the mentioned conditions are clinically very dramatic and hard to diagnose by standard clinical procedures.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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