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ORIGINAL ARTICLE

Does venous blood gas analysis provide accurate estimates of hemoglobin oxygen affinity?

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Abstract Alterations in hemoglobin oxygen affinity can be detected by exposing blood to different PO_2 and recording oxygen saturation, a method termed tonometry. It is the gold standard to measure the PO_2 associated with 50 % oxygen saturation, the index used to quantify oxygen affinity (P_{50}Tono). P_{50}Tono is used in the evaluation of patients with erythrocytosis suspected to have hemoglobin with abnormal oxygen affinity. Since tonometry is labor intensive and not generally available, we investigated whether accurate estimates of P_{50} could also be obtained by venous blood gas analysis, co-oximetry, and standard equations (P_{50}Ven). In 50 patients referred for evaluation of erythrocytosis, pH, PO_2, and oxygen saturation were measured in venous blood to estimate P_{50}Ven; P_{50}Tono was measured for comparison. Agreement among P_{50}Ven and P_{50}Tono was evaluated (Bland–Altman analysis). Mean P_{50}Tono was 25.8 (range 17.4–34.1) mmHg. The mean difference (bias) of P_{50}Tono-P_{50}Ven was 0.5 mmHg; limits of agreement (95 % confidence limits) were −5.2 to +6.1 mmHg. The sensitivity and specificity of P_{50}Ven to identify the 25 patients with P_{50}Tono outside the normal range of 22.9–26.8 mmHg were 5 and 77 %, respectively. We conclude that estimates of P_{50} based on venous blood gas analysis and standard equations have a low bias compared to tonometry. However, the precision of P_{50}Ven is not sufficiently high to replace P_{50}Tono in the evaluation of individual patients with suspected disturbances of hemoglobin oxygen affinity.

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Introduction

The causes of erythrocytosis, i.e., an increased erythrocyte mass, include either a decreased plasma volume (relative erythrocytosis) or an increase in the erythrocyte mass (absolute erythrocytosis) as determined by radionucleotide techniques. Absolute erythrocytosis may be primary due to myeloproliferative neoplasia (polycythemia vera), congenital due to erythropoietin receptor mutations, or secondary due to various causes including cardiopulmonary or renal disease, congenital due to defects of the oxygen sensing pathway (VHL, *PHD2*, or HIF-2α gene mutations) or other rare congenital defects like hemoglobin variants or bisphosphoglycerate mutase deficiency (Table 1). Guideline for the investigation of erythrocytosis [1, 2] suggest to search for polycythemia vera and other common causes by history including the use of medication, clinical examination, full blood count, renal and liver function tests, serum ferritin, vitamin B_{12}, and erythropoietin measurement, arterial blood gas analysis, chest radiography, and abdominal ultrasonography. If the etiology of the erythrocytosis remains unclear, a more specialized stage 2 evaluation includes whole body CT scan, bone marrow examination, cytogenetics of the bone marrow aspirate, high performance liquid chromatography of the hemoglobin, and determination of the oxygen-hemoglobin dissociation curve. It allows to detect hemoglobin variants with altered oxygen affinity as rare cause of erythrocytosis [3, 4]. The hemoglobin oxygen affinity is quantified during tonometry by exposing blood to changing partial pressures of oxygen (PO_2) while continuously recording oxygen saturation. The P_{50}, i.e., the PO_2 at which 50 % of

Table 1 Differential diagnosis of erythrocytosis

Primary causes of erythrocytosis		
Congenital:	Erythropoietin receptor mutations	
Acquired:	Polycythemia vera	
Secondary causes of erythrocytosis		
Congenital:	Defects of the oxygen sensing pathway:	VHL gene mutations
		PHD2 mutations
		HIF-2α mutations
	Other congenital defects:	High oxygen affinity hemoglobin
		Bisphosphoglycerate mutase deficiency
Acquired:	Central hypoxia:	Smoker's erythrocytosis
		Chronic lung disease
		Hypoventilation syndromes including obstructive sleep apnea
		Right-to-left cardiopulmonary vascular shunts
		High altitude
		Carbon monoxide poisoning
	Local hypoxia:	Renal artery stenosis
		Hydronephrosis
		Renal cysts (polycystic kidney disease)
		Post-renal transplant erythrocytosis
		End-stage renal disease
	Pathologic EPO production	Cerebellar hemangioblastoma
		Meningioma
		Parathyroid carcinoma/adenoma
		Hepatocellular carcinoma
		Renal cell cancer
		Pheochromocytoma
		Uterine leiomyoma
	Drug associated	Erythropoietin administration
		Androgen administration
Idiopathic erythrocytosis		

the hemoglobin is saturated with oxygen, is used as the numeric value characterizing hemoglobin oxygen affinity (Fig. 1). Unfortunately, tonometry is time consuming and requires expensive equipment. The technique is therefore not widely available. Estimation of the P_{50} from venous blood gas analysis and co-oximetry using standard equations according to Severinghaus [5] has been suggested as a simpler alternative to tonometry. However, this approach of determining the P_{50} has not been well validated [6, 7]. The purpose of the current study is therefore to evaluate the accuracy of P_{50} estimated from venous blood gas analysis in comparison to tonometry, the gold standard for measurement of P_{50}, in patients referred for evaluation of erythrocytosis.

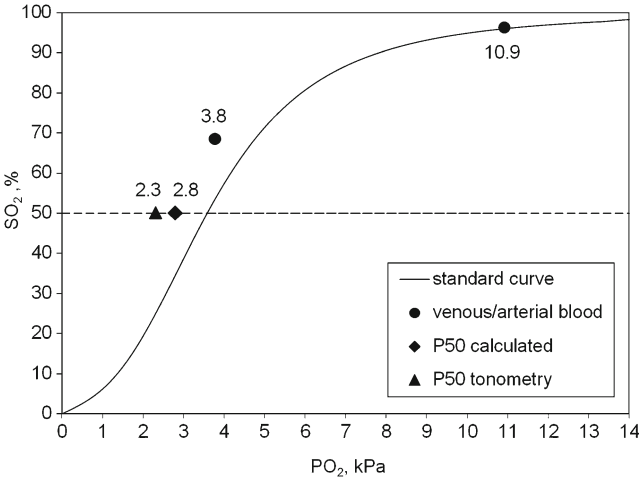

Fig. 1 Standard oxygen dissociation curve according to the Severinghaus equation. The arterial PO_2 (10.9 kPa), the venous PO_2 (3.8 kPa), and the P_{50} measured by tonometry (2.3 kPa) in a patient with erythrocytosis due to a high-affinity hemoglobin variant (heterozygous mutation for hemoglobin Cutlerville) are indicated. The P_{50} estimated from venous blood gas analysis and co-oximetry (2.8 kPa) has been calculated by linear extrapolation of the venous PO_2 to a saturation of 50 %. The values of P_{50} are situated to the left of the standard curve indicating an increased oxygen affinity. kPa can be converted to mmHg by multiplication by 7.5

Methods

Patients

Data from all patients referred to the Hematology and Pulmonary Division, University Hospital of Zurich, from 2007 to 2009 for arterial and venous blood gas analysis and tonometry as part of the diagnostic evaluation of erythrocytosis, were included. The protocol was in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the University Hospital of Zurich.

Measurements

Blood analysis

Venous and arterial blood samples were obtained from a cubital vein and radial artery, respectively, and analyzed immediately. Blood gas analysis and co-oximetry were performed by an ABL (700 Series, Radiometer Copenhagen) device. P_{50} was measured by tonometry (P_{50}Tono) (Hemox-Analyser, model B, TCS, Medical Products Division, Southampton, PA). The tonometer measures hemoglobin fractions by two-wavelength spectrophotometry and PO_2 by a Clark electrode during exposure of the blood sample to increasing O_2 fractions from 0.21 to 1.0. Validation studies and the normal range for the P_{50} measured with this device (22.9-26.8 mmHg) have been published [8]. P_{50} was also calculated from the oxygen saturation and PO_2

measured in the venous blood sample incorporating pH adjustments according to Severinghaus (P_{50}Ven). [5]:

$$P_{50} = 26.7 \times P_{O_2}(\text{obs})/P_{O_2}(\text{std}) \quad (1)$$

Where

$$P_{O_2}(\text{obs}), \text{pH } 7.4 = P_{O_2} \times e^{1.1(\text{pH}-7.4)} \quad (2)$$

And

$$P_{O_2}(\text{std}) : \exp\left(0.385 \ln\left(S^{-1}-1\right)^{-1} + 3.32 - (72S)^{-1} - S^6/6\right) \quad (3)$$

The normal range for the P_{50} calculated with this formula is 25.3–30.7 mmHg [9].

Clinical diagnosis

A detailed medical history, clinical examination, and laboratory tests were obtained in all patients according to published guidelines for evaluation of erythrocytosis. [1, 2]

Data analysis and statistics

Data are summarized by counts, means, and SD. The accuracy of the P_{50} calculated from the venous blood using the Severinghaus equation was compared to corresponding values measured by tonometry by a Bland–Altman analysis [10]. Diagnostic accuracy of P_{50}Ven was also determined in terms of sensitivity and specificity to detect an abnormal P_{50}Tono.

Results

Data from 50 patients (13 women) with mean age 46.1 years, SD 15.5, and a range from 20 to 76 years were available. Their clinical diagnoses along with the number of patients with a P_{50}Tono outside the normal range of 22.9 to 26.8 mmHg are listed in Table 2. In 14 patients, the cause of erythrocytosis could not be definitively determined despite extensive evaluations. Tonometry revealed a mean P_{50}Tono of 25.8 mmHg (range 17.4 to 34.1 mmHg) with 25 of the 50 values outside the normal range. Only seven of the 25 abnormal results were below the lower limit of 22.9 mmHg, in one of these patients abnormal hemoglobin with high oxygen affinity could be identified (Hemoglobin Cutlerville). The majority of patients with myeloproliferative disorder showed an elevated P_{50}Tono (>26.8 mmHg), probably due to secondary regulatory mechanisms.

The corresponding P_{50}Ven was 26.3 mmHg (range 20.9 to 29.4 mmHg). Figure 2 shows the Bland–Altman analysis evaluating the accuracy of P_{50}Ven compared to P_{50}Tono. The bias was +0.5 mmHg with limits of agreement (95 % confidence interval) from −5.2 to +6.1 mmHg. Table 3 shows the diagnostic performance of the P_{50}Ven compared to P_{50}Tono. The sensitivity was 5 %, the specificity was 77 %, and the positive and the negative predictive values were 12 and 57 %, respectively.

Discussion

The current study is the largest comparison of P_{50} estimated by blood gas analysis and co-oximetry of venous blood

Table 2 Diagnoses of patients with normal and abnormal P_{50} by tonometry

Diagnosis	No. patients	P_{50} by tonometry, mmHg			Suspected etiology of abnormal P_{50}
		Low <22.9	Normal range 22.9–26.8	High >26.8	
Myeloproliferative neoplasia	15	2	6	7	JAK-2 mutation was positive in 7, negative in 2, and not analyzed in 6 patients
Smokers erythrocytosis	11	0	8	3	Elevated COHb >3 %
Relative erythrocytosis	9	1	6	2	Gaisböck syndrome
High-affinity hemoglobin	1	1	0	0	Hemoglobin Cutlerville
Post-renal transplant erythrocytosis	1	0	0	1	Erythropoietin elevation
Cardiovascular disease	1	0	0	1	Congenital pulmonary stenosis
Pulmonary disease	1	0	1	0	COPD
Hereditary spherocytosis	1	0	1	0	Erythrocyte membrane disorder
Idiopathic/unclear	14	3	6	5	etiology not identified
Total[a]	54	7	28	19	

[a] The total number exceeds 50 because four smokers had a combined etiology of erythrocytosis (one patient had chronic obstructive pulmonary disease (COPD), one had a myeloproliferative neoplasia, and two had Gaisböck syndrome)

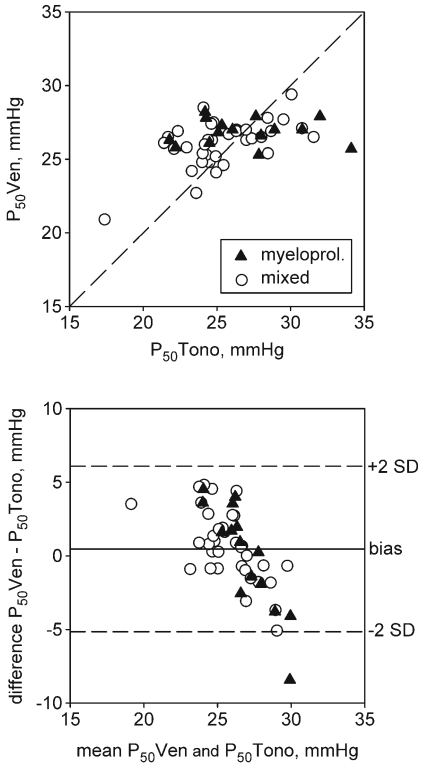

Fig. 2 Bland–Altman analysis of P_{50} estimated from a venous blood gas analysis using the Severinghaus equation vs. corresponding values measured by tonometry. *Upper panel* identity plot; *lower panel* differences vs. mean values. *Different symbols* reflect the etiology of the erythrocytosis (myeloproliferative vs. other etiologies)

using standard equations with tonometry, the gold standard for P_{50}, in patients with erythrocytosis. We found that despite a negligible bias, the precision of the values of P_{50}Ven was not sufficiently high to allow their use as substitutes for P_{50}Tono in the clinical evaluation of polycythemia in individual patients.

Unfortunately, determination of P_{50} by tonometry is not feasible in many institutions because it is labor intensive, time consuming, and requires specialized equipment. Replacement of this technique by a simpler method based on venous blood gas analysis would therefore be desirable. However, P_{50}Ven has not been well established as a means to assess hemoglobin oxygen affinity, possibly due to the lack of appropriate validation. We identified only two studies involving less than 30 patients in whom P_{50}Ven was evaluated in comparison to tonometry. The first one has been published by Kohzuki et al. [7]. In this study, the P_{50}Ven of 25 normal Japanese adults has been compared to the P_{50} obtained by microtonometry. With this technique, 6 discrete points on the dissociation curve at different PO_2 were determined by a tonometer instead of the complete dissociation curve recorded in the current study. The mean ±SD deviation of the P_{50} derived from venous blood and the 6-point technique in the cited study was 0.4±2.5 Torr (at pH 7.4, PCO_2 40 Torr and 37 °C); similar results were obtained for pH 7.2 and 7.6. In the second study by Aberman et al. [6], the P_{50}Ven at various SO_2 (between 20 and 90 %) were determined in 135 blood samples from 21 healthy nonsmokers and in eight patients. The standard deviation of P_{50}Ven on the same sample of blood at different SO_2 was ±1.0 Torr. A validation of the P_{50}Ven comparison to P_{50}Tono has not been performed in this study. Based on their results, the authors of both studies suggested that P_{50}Ven could serve as a simple surrogate for assessment of the hemoglobin oxygen affinity. However, our data obtained in a larger group of 50 patients do not support these expectations because of the imprecision of P_{50}Ven values in comparison to tonometry. The scatter of P_{50}Ven resulted in a low diagnostic accuracy in detecting patients with an abnormal P_{50}Tono with positive and negative predictive values of only 12 and 57 %, respectively, which is inappropriate for clinical use.

Based on our data, we are unable to differentiate whether the imprecision of P_{50}Ven was mainly related to instrument variability or to physiological reasons. However, the measurement techniques used for blood gas analysis and co-oximetry are the same as for tonometry and we therefore speculate that deviations in the shape of the individual hemoglobin dissociation curve from the standard curve explains some of the discrepancy between P_{50}Ven and P_{50}Tono. The narrow normal range of P_{50} (3.9 mmHg, i.e., from 22.9 to 26.8 mmHg) together with the only slight deviation of P_{50} that is associated with abnormal hemoglobin variants (see example in Fig. 1) suggests that measurement of P_{50} should be performed with a greater precision than what can be expected from P_{50}Ven. According to a study by Guarnone et al. [8], the P_{50}Tono measured by the tonometer used in the current study has a small intra-assay variability with a standard deviation of 0.39 mmHg suggesting that relatively small deviations of P_{50} from the normal range can be detected.

Guidelines for investigation of erythrocytosis by McMullin et al. [1, 2] suggest that a genetic analysis of a potential hemoglobin variant should only be performed if other more common causes of erythrocytosis have been evaluated. Our study indicates that determination of P_{50} by tonometry but not P_{50}Ven may help to further guide clinical

Table 3 Diagnostic performance of P_{50} estimated from venous blood gas analysis

		P_{50} calculated from venous blood gas analysis (P_{50}Ven)	
		Abnormal	normal
P_{50} measured by tonometry (P_{50}Tono)	Abnormal	1 (low)	24 (P_{50}Tono low in 6, high in 18)
	Normal	7 (P_{50}Ven low)	18

investigations directed at identifying rare causes of polycythemia. This is also emphasized by a recent study by Rumi et al. [11], where tonometry, besides other clinical tests, illustrated the utility of P_{50} measurement in the diagnostic process of isolated erythrocytosis in 102 patients with erythrocytosis.

Conflict of interest The authors declare that they have no conflict of interest.

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