

Detection of Haemoglobins with Abnormal Oxygen Affinity by Single Blood Gas Analysis and 2,3-Diphosphoglycerate Measurement

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The aim is to determine if a single measurement of blood 2,3-diphosphoglycerate combined with gas analysis (pH, PCO₂, PO₂ and saturation) can identify the cause of an altered blood-oxygen affinity: the presence of an abnormal haemoglobin or a red cell disorder. The population (n=94) was divided into healthy controls (A, n=14), carriers of red cell disorders (B, n=72) and carriers of high oxygen affinity haemoglobins (C, n=8). Those variables were measured both in samples equilibrated at selected PCO₂ and PO₂ and in venous blood. In the univariable approach applied to equilibrated samples, we correctly identified C subjects in 93.6% or 96.8% of the cases depending on the selected variable, the standard P₅₀ (PO₂ at which 50% of haemoglobin is oxygenated) or a composite variable calculated from the above measurements. After introducing the haemoglobin concentration as a further discriminating variable, the A and B subjects were correctly identified in 91.9% or 94.2% of the cases, respectively. These figures become 93.0% or 86.1%, and 93.7% or 94.9% of the cases when using direct readings from venous blood, thereby avoiding the blood equilibration step. This test is feasible also in blood samples stored at 4° C for 48 h, or at room temperature for 8 h.

Key words: pH; PCO₂; PO₂; P₅₀; Oxygen dissociation curve.

Abbreviations: BF, Bohr factor; DPG, 2,3-diphosphoglycerate; Hb, haemoglobin; P₅₀, PO₂ at which 50% of haemoglobin is oxygenated; P₅₀std, standard P₅₀; RBC, red blood cell; ROC, receiver operating characteristics; SO₂, haemoglobin-O₂ saturation; ΔPO₂, difference between calculated PO₂ and measured PO₂.

Introduction

The effect of pH, PCO₂, temperature, carbonmonoxyhaemoglobin and 2,3-diphosphoglycerate (DPG) on the blood-O₂ affinity is well known and has already been represented as an empirical algorithm (1). With the exception of heavy smokers and gas poisoning, the role of carbonmonoxy-haemoglobin is not clinically relevant. In addition, the relevance of pH and PCO₂ in

subjects with altered blood-O₂ affinity is relatively low, as powerful metabolic, respiratory and endocrinological mechanisms restore these variables, especially pH, towards normality. Therefore, the factors that are ultimately relevant in the control of the P₅₀, *i.e.*, the PO₂ at which 50% of haemoglobin (Hb) occurs as HbO₂ and regarded as a useful index of the blood-O₂ affinity, are the red blood cell (RBC) content of DPG and the presence of Hb variants with altered O₂ affinity. In practice, this may lead to confusion when first diagnosing an abnormal blood-O₂ affinity. Indeed, the elective marker, the P₅₀, may become useless as its value depends on parameters that may be altered as a part of the physiological response aimed at maintaining adequate tissue oxygenation. In principle, the P₅₀ alteration may even be completely blunted. On the other hand, none of the algorithms proposed to calculate the P₅₀ (2-6) includes the possible role of variant Hbs.

In this study, we assess whether a single measurement of DPG, pH, PCO₂, PO₂, and the haemoglobin-O₂ saturation (SO₂) can be used to quickly recognise the cause of an altered P₅₀ value: the presence of a variant Hb with abnormal O₂ affinity, or RBC with an altered metabolism. The algorithm described here determines in a simple and inexpensive way whether the measured PO₂-SO₂ pair fully depends on the measured values for pH, PCO₂ and DPG, or if it is necessary to postulate the presence of an Hb with abnormal O₂ affinity. This approach was already tried when considering the case of glycated Hb (7): the altered P₅₀ found in diabetic subjects appeared to be due entirely to the altered RBC metabolism, without the need to postulate that the O₂ affinity of glycated Hb is abnormal. In this study, we also evaluate if the above goal is achieved avoiding the tedious and time-consuming tonometry with calibrated gases by using venous blood. Indeed, the relatively low PO₂ in venous blood may be suitable for this purpose because the respective SO₂ values fall in the mid range. This may not be the case when using arterial blood, as usually employed for typical gas analysis. We show that this shortcut is possible with minor loss of precision and accuracy.

Subjects, Materials and Methods

Subjects

The population (n=94, inpatients and outpatients at the Haematology Division of the Ospedale di Careggi, Firenze, Italy) was divided into three groups: no signs of haematological disorders (group A, n=14); RBC disorders (group B, n=72); carriers of Hb variants with altered blood-O₂ affinity (group C, n=8).

Measurements

Venous blood was withdrawn without stasis, stored in heparinized tubes, and subdivided into three aliquots. In the first, we immediately measured the venous blood values: pH, PCO₂ and PO₂ (ABL500 Radiometer, Copenhagen, Denmark), as well as SO₂, carbonmonoxy-haemoglobin, methaemoglobin and total Hb concentration (CO-Oxymeter IL282, Instrumentation Laboratory, Milan, Italy). The second aliquot (2 ml), was equilibrated at 37° C for 20 min with ternary gas mixtures with known compositions (3.1–3.8% O₂, 5.3–5.4% CO₂, balance N₂, Rivoira, Milan, Italy) in a tonometer (IL237, Instrumentation Laboratory, Milan, Italy). After tonometry, we measured the same parameters to obtain the tonometry blood values. In the third aliquot (1 ml), we measured the DPG content following a standard procedure (8): extraction with 5 ml 0.6 M HClO₄, centrifugation, neutralisation of the supernatant with 2.5 M K₂CO₃, followed by DPG assay by an enzymatic method (Boehringer Mannheim Biochemia, Mannheim, Germany). The level of DPG is expressed as molar ratio over Hb tetramers ([DPG]/[Hb]).

Calculations

The standard P₅₀ (P_{50std}) was calculated from the PO₂-SO₂ pair by the Hill formula assuming n=2.6 (9), followed by pH correction using a Bohr factor (BF) = -0.4 (8):

$$\text{Log } P_{50\text{std}} = \text{LogPO}_2 - \text{Log}[\text{SO}_2 / (100 - \text{SO}_2)] / n + 10^{(7.4 - \text{pH}) * \text{BF}}$$

To extrapolate the Hb-O₂ dissociation curve in the PO₂ range 5–70 mmHg, we employed a procedure that inputs pH, PCO₂ and DPG (10). The software, written both in Turbo Pascal and MSExcel, is available from the authors. From the dissociation curve, we calculated the PO₂ corresponding to the measured SO₂. Then, this PO₂ value was compared with the actually measured PO₂. The difference between the calculated PO₂ and the measured PO₂ is referred to as ΔPO₂.

Stability test

Some samples (n=2–4) were used for testing the stability of PO₂ following blood storage at either room temperature (up to 24 h) or 4° C (up to 48 h).

Data analysis

Data are expressed as mean±SE. To compare three groups, we first ran one-way Analysis of Variance. If significant (p<0.05), the difference between selected pairs of groups was tested with the Fisher post-test. To evaluate the diagnostic value of the test, we performed statistical analysis of the receiver operating characteristic (ROC) curve (11). For this purpose, we assessed the discrimination of the variables by estimating sensitivity, specificity, and the likelihood ratio of a positive test result [sensitivity/(1-specificity)] as the area under the ROC curve (Stata Statistical Software, Stata Corporation, College Station, TX, USA). We attempted two distinct approaches. The aim of the first was to discriminate group C from groups A and B by dichotomising either P_{50std} or ΔPO₂ at selected values in a univariable, single-test approach. The aim of the second was to discriminate between groups A and B by introducing other parameters in a multivariate approach that made use of logistic regression modelling (Stata Technical Bulletin STB-52, Nov 1999).

Results

The main haematological disorders associated with group B were polycythemia vera (22%), familial polycythemia (7%) and non-myeloproliferative polycythemia (33%). The main variant Hbs were HbF (n=7).

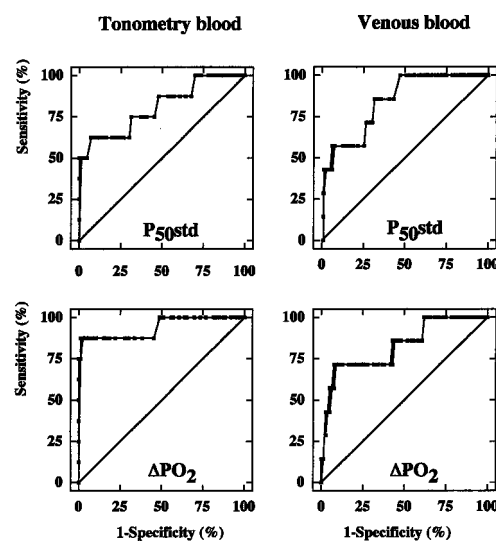


Fig. 1 Receiver operating characteristic (ROC) curves describing the sensitivity vs. specificity relationship for discriminating group C (carriers of high O₂ affinity haemoglobins) from groups A (healthy controls) and B (carriers of red cell disorders). The procedure is applied to either the standard P₅₀ (P_{50std}, upper panels) or the difference between the calculated and the measured PO₂ (ΔPO₂, lower panels) as obtained from either tonometry (left panels) or venous (right panels) blood. The area under the ROC curves is reported in Table 2.

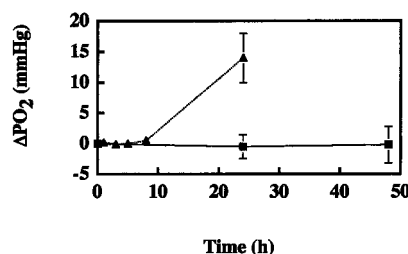


Fig. 2 Change in ΔPO₂ during blood storage at room temperature (triangles) and 4° C (squares). For each group, n=2–4. The vertical bars represent SE.

The results are shown in Table 1. Figure 1 shows the ROC curves employed for discriminating group C from groups A and B by using the univariable, single-test approach applied to the values for either P_{50std} or ΔPO₂ as obtained from either venous or tonometry blood. Table 2 shows the results of the univariable ROC curve analysis associated with the therein proposed set of cut-off values. In the multivariate approach, we found that the haemoglobin concentration ([Hb]) could be successfully used not only to discriminate group C with a higher likelihood ratio than in the univariable approach, but also between groups A and B (Table 3).

Figure 2 shows that blood storage does not significantly affect ΔPO₂ for up to 8 h at room temperature and 48 h at 4° C.

Tab. 1 Subjects data.

	Group A	Group B	Group C
n, males/females	6 / 8	63 / 9	5 / 3
Age (years)	41.8 ± 4.9	46.4 ± 2.1	33.3 ± 3.3
Hb (g/l)	13.2 ± 0.5	16.5 ± 0.2 ^a	12.1 ± 1.8 ^b
[DPG]/[Hb]	0.97 ± 0.05	0.90 ± 0.02	1.13 ± 0.09 ^{ab}
metHb (%)	0.4 ± 0.0	0.5 ± 0.0	1.9 ± 0.5 ^{ab}
HbCO (%)	1.4 ± 0.2	2.2 ± 0.2	3.3 ± 0.8 ^a
Tonometry			
PO ₂ (mmHg)	24.5 ± 0.6	24.0 ± 0.2	26.2 ± 1.6 ^b
PCO ₂ (mmHg)	39.9 ± 0.2	39.8 ± 0.1	40.0 ± 0.2
pH	7.44 ± 0.01	7.44 ± 0.00	7.39 ± 0.03 ^{ab}
SO ₂ (%)	41.4 ± 1.8	42.9 ± 0.4	54.9 ± 3.5 ^{ab}
P ₅₀ std (mmHg)	29.1 ± 0.4	27.8 ± 0.2 ^a	24.1 ± 1.5 ^{ab}
PO ₂ (mmHg)	0.3 ± 0.3	0.9 ± 0.1	7.6 ± 1.8 ^{ab}
Venous blood			
PO ₂ (mmHg)	32.8 ± 4.6	35.3 ± 1.6	21.9 ± 2.1 ^b
PCO ₂ (mmHg)	47.2 ± 2.2	46.0 ± 0.9	46.8 ± 1.9
pH	7.37 ± 0.01	7.38 ± 0.01	36 ± 0.02
SO ₂ (%)	49.8 ± 6.7	61.0 ± 2.4	48.7 ± 5.8
P ₅₀ std (mmHg)	28.6 ± 0.6	26.2 ± 0.3 ^a	21.5 ± 2.0 ^{ab}
PO ₂ (mmHg)	1.3 ± 0.9	3.6 ± 0.5	10.9 ± 2.8 ^{ab}

^a Significant difference ($p < 0.05$) vs. group A; ^b significant difference ($p < 0.05$) vs. group B (Analysis of Variance and Fisher post-test).

Tab. 2 Proposed cut-off values for discriminating group C (Hb with abnormal O₂ affinity) from groups A (healthy controls) and B (red cell disorders). The univariable receiver operating characteristic (ROC) curve analysis was applied to either the stan-

Parameter	Area under the ROC curve ±SE	Cut-off value (mmHg)	Sensitivity (%)	Specificity (%)	(%) correctly classified
P ₅₀ std, tonometry blood	0.810±0.096	25.4	50.0	97.7	93.6
P ₅₀ std, venous blood	0.844±0.073	21.0	42.9	97.4	93.0
PO ₂ , tonometry blood	0.940±0.059	3.0	87.5	97.7	96.8
PO ₂ , venous blood	0.826±0.095	6.6	71.4	87.3	86.1

Tab. 3 Analysis of the receiver operating characteristics (ROC) curves for discriminating between groups A and B using either the univariable approach or the multivariate approach that includes the haemoglobin concentration ([Hb]). In

Parameter	Area under the ROC curve, univariable approach	x ₁	x ₂	x ₃	Area under the ROC curve, multivariate approach	Cut-off value for y	(%) correctly classified
P ₅₀ std, tonometry blood	0.7153	0.473	1.83	-39.1	0.9504	0.12	91.9
P ₅₀ std, venous blood	0.8047	-0.091	1.32	-15.7	0.9502	1.01	93.7
PO ₂ , tonometry blood	0.6404	0.20	1.47	-20.49	0.9494	0.60	94.2
PO ₂ , venous blood	0.6841	-0.17	1.38	-19.47	0.9565	0.25	94.9

Discussion

Both RBC disorders and Hb variants affect to varying extents the [DPG]/[Hb] molar ratio and hence the P₅₀std. Consequently, it is often difficult to distinguish the primary cause from P₅₀std alone. This study was designed to assess whether PO₂ helps to distinguish if an altered P₅₀std is attributable to the presence of a Hb variant or to altered RBC metabolism.

Criticism of the method

The extrapolation of P₅₀std from the PO₂-SO₂ pair assumes the validity of the employed Hill and Bohr coefficients (12). The Hill transform is valid only if 40% < SO₂ < 60%, as from Table 1. An error of ±0.1 in the Hill coefficient value leads to an error of ±0.16 mmHg in P₅₀std (8). The accuracy of the empirical procedure to simulate the blood-O₂ dissociation curve (10) is subject to several assumptions: no factors besides pH, PCO₂ and DPG alters position and shape of the blood-O₂ dissociation curve; the Adair equation fully represents the dissociation curve despite the known oversimplification of the Hb-ligands interactions; the temperature is strictly controlled at 37° C.

standard P₅₀ (P₅₀std) or the difference between the calculated and the measured PO₂ (PO₂) as obtained from either venous or tonometry blood. A dichotomising cut-off value is proposed in order to maximise the specificity and the likelihood ratio.

the latter case, a temporary variable (y) is calculated from the equation $y = x_1 * \text{parameter} + x_2 * [\text{Hb}] + x_3$. As in Table 2, the proposed cut-off for y maximises specificity and the likelihood ratio.

Prediction of variant haemoglobins and RBC disorders

Table 3 shows that a single measurement of blood gases and the [DPG]/[Hb] ratio may provide enough information to discriminate among the three groups under study. The proposed approach involves a two-step procedure. The first step discriminates the presence of Hbs with abnormal O₂ affinity. The second step, that is applied if the first step yields a negative result, discriminates the presence of RBC disorders leading to abnormal blood-O₂ affinity. Both the P_{50std} and the PO₂ can be used as parameters, but the latter yields a slightly better likelihood ratio. Of interest, the tonometry step can be avoided if venous blood with relatively low PO₂ is available. We expect that high-PO₂ arterial blood may not be as useful because of the high SO₂ that prevents meaningful calculation of P_{50std} and the PO₂. Also, we found that blood storage for up to 8 h at room temperature or 24 h at ice temperature, while affecting the apparent P_{50std}, does not significantly affect PO₂ provided that the DPG content is measured concomitantly with the blood gas analysis. In this study, we encountered only cases with high-O₂ affinity Hbs, but there are no reasons to believe that low-O₂ affinity Hbs cannot be recognised with this approach. Nevertheless, it is crucial that each laboratory defines its own standards and cut-off values.

The greatest limitation in this approach is the need to measure the blood DPG content. This operation may not be easily feasible in routine laboratories, as it requires experienced personnel, relatively expensive enzymatic kits, and is not easily automated. On the other hand, we successfully explored the possibility to use venous blood in the place of tonometered blood, thereby avoiding the tedious and time-consuming tonometry. In addition, the maintenance of PO₂ during blood storage for an extended period of time allows rather flexible organisation and the possibility to test the sample rather far from the patient.

Conclusions

The described test allows transferring the determination of the blood-O₂ affinity from the research to the routine laboratory. When diagnosing an altered P_{50std}, this test would help to quickly address the patient towards the screening of variant Hbs or the assessment of RBC disorders. As this test is aimed at relatively rare situations (13/94 cases in this study), it can be justified in association with major haematological centres interested in high specialisation programmes, or in centres where the incidence of variant Hbs or altered RBC disorders is relatively high.

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