Clin Chem Lab Med 2000; 38(10):951–954 © 2000 by Walter de Gruyter · Berlin · New York

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

Giovanni Guerrini[1], Alberto Morabito[2] and Michele Samaja[2]

[1]Servizio di Fisiopatologia Toracica, Villa D'Ognissanti, Ospedale di Careggi, Firenze, Italy
[2]Dipartimento di Medicina, Chirurgia ed Odontoiatria, Polo Ospedale San Paolo, Università di Milano, Milano, Italy

The aim is to determine if a single measurement of blood 2,3-diphosphoglycerate combined with gas analysis (pH, PCO_2, PO_2 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 PCO_2 and PO_2 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 P_{50} (PO_2 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; PCO_2; PO_2; P_{50}; Oxygen dissociation curve.

Abbreviations: BF, Bohr factor; DPG, 2,3-diphosphoglycerate; Hb, haemoglobin; P_{50}, PO_2 at which 50% of haemoglobin is oxygenated; P_{50}std, standard P_{50}; RBC, red blood cell; ROC, receiver operating characteristics; SO_2, haemoglobin-O_2 saturation; ΔPO_2, difference between calculated PO_2 and measured PO_2.

Introduction

The effect of pH, PCO_2, temperature, carbonmonoxy-haemoglobin and 2,3-diphosphoglycerate (DPG) on the blood-O_2 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 PCO_2 in subjects with altered blood-O_2 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 P_{50}, *i.e.*, the PO_2 at which 50% of haemoglobin (Hb) occurs as HbO_2 and regarded as a useful index of the blood-O_2 affinity, are the red blood cell (RBC) content of DPG and the presence of Hb variants with altered O_2 affinity. In practice, this may lead to confusion when first diagnosing an abnormal blood-O_2 affinity. Indeed, the elective marker, the P_{50}, 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 P_{50} alteration may even be completely blunted. On the other hand, none of the algorithms proposed to calculate the P_{50} (2-6) includes the possible role of variant Hbs.

In this study, we assess whether a single measurement of DPG, pH, PCO_2, PO_2, and the haemoglobin-O_2 saturation (SO_2) can be used to quickly recognise the cause of an altered P_{50} value: the presence of a variant Hb with abnormal O_2 affinity, or RBC with an altered metabolism. The algorithm described here determines in a simple and inexpensive way whether the measured PO_2–SO_2 pair fully depends on the measured values for pH, PCO_2 and DPG, or if it is necessary to postulate the presence of an Hb with abnormal O_2 affinity. This approach was already tried when considering the case of glycated Hb (7): the altered P_{50} found in diabetic subjects appeared to be due entirely to the altered RBC metabolism, without the need to postulate that the O_2 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 PO_2 in venous blood may be suitable for this purpose because the respective SO_2 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-O_2 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, PCO_2 and PO_2 (ABL500 Radiometer, Copenhagen, Denmark), as well as SO_2, 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% O_2, 5.3–5.4% CO_2, balance N_2, 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 $HClO_4$, centrifugation, neutralisation of the supernatant with 2.5 M K_2CO_3, 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 P_{50} (P_{50}std) was calculated from the PO_2-SO_2 pair by the Hill formula assuming n=2.6 (9), followed by pH correction using a Bohr factor (BF) =–0.4 (8):

$$\text{Log } P_{50}\text{std} = \text{Log}PO_2 - \text{Log}[SO_2/(100-SO_2)]/n + 10^{(7.4-pH)*BF}$$

To extrapolate the Hb-O_2 dissociation curve in the PO_2 range 5-70 mmHg, we employed a procedure that inputs pH, PCO_2 and DPG (10). The software, written both in Turbo Pascal and MSExcel, is available from the authors. From the dissociation curve, we calculated the PO_2 corresponding to the measured SO_2. Then, this PO_2 value was compared with the actually measured PO_2. The difference between the calculated PO_2 and the measured PO_2 is referred to as ΔPO_2.

Stability test

Some samples (n=2-4) were used for testing the stability of ΔPO_2 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_{50}std or ΔPO_2 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 polycythemias (33%). The main variant Hbs were HbF (n=7).

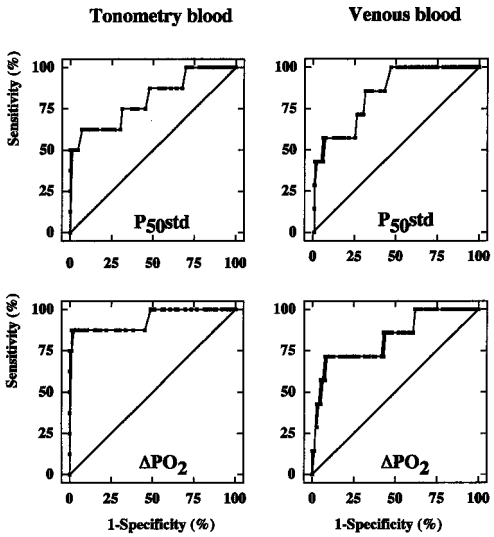

Fig. 1 Receiver operating characteristic (ROC) curves describing the sensitivity *vs.* specificity relationship for discriminating group C (carriers of high O_2 affinity haemoglobins) from groups A (healthy controls) and B (carriers of red cell disorders). The procedure is applied to either the standard P_{50} (P_{50}std, upper panels) or the difference between the calculated and the measured PO_2 (ΔPO_2, 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.

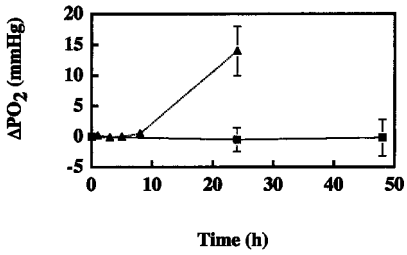

Fig. 2 Change in ΔPO_2 during blood storage at room temperature (triangles) and 4°C (squares). For each group, n=2–4. The vertical bars represent SE.

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_{50}std or ΔPO_2 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 ΔPO_2 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]
ΔPO_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]
ΔPO_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).

Discussion

Both RBC disorders and Hb variants affect to varying extents the [DPG]/[Hb] molar ratio and hence the P_{50}std. Consequently, it is often difficult to distinguish the primary cause from P_{50}std alone. This study was designed to assess whether ΔPO_2 helps to distinguish if an altered P_{50}std is attributable to the presence of a Hb variant or to altered RBC metabolism.

Criticism of the method

The extrapolation of P_{50}std from the PO_2-SO_2 pair assumes the validity of the employed Hill and Bohr coefficients (12). The Hill transform is valid only if $40\%<SO_2<60\%$, as from Table 1. An error of ±0.1 in the Hill coefficient value leads to an error of ±0.16 mmHg in P_{50}std (8). The accuracy of the empirical procedure to simulate the blood-O_2 dissociation curve (10) is subject to several assumptions: no factors besides pH, PCO_2 and DPG alters position and shape of the blood-O_2 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.

Tab. 2 Proposed cut-off values for discriminating group C (Hb with abnormal O_2 affinity) from groups A (healthy controls) and B (red cell disorders). The univariable receiver operating characteristics (ROC) curve analysis was applied to either the standard P_{50} (P_{50}std) or the difference between the calculated and the measured PO_2 (ΔPO_2) 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.

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
ΔPO_2, tonometry blood	0.940±0.059	3.0	87.5	97.7	96.8
ΔPO_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 the latter case, a temporary variable (y) is calculated from the equation $y=x_1$*parameter + x_2*[Hb] + x_3. As in Table 2, the proposed cut-off for y maximises specificity and the likelihood ratio.

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
ΔPO_2, tonometry blood	0.6404	0.20	1.47	–20.49	0.9494	0.60	94.2
ΔPO_2, venous blood	0.6841	–0.17	1.38	–19.47	0.9565	0.25	94.9

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 O_2 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-O_2 affinity. Both the P_{50}std and the ΔPO_2 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 PO_2 is available. We expect that high-PO_2 arterial blood may not be as useful because of the high SO_2 that prevents meaningful calculation of P_{50}std and the ΔPO_2. 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_{50}std, does not significantly affect ΔPO_2 provided that the DPG content is measured concomitantly with the blood gas analysis. In this study, we encountered only cases with high-O_2 affinity Hbs, but there are no reasons to believe that low-O_2 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 ΔPO_2 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-O_2 affinity from the research to the routine laboratory. When diagnosing an altered P_{50}std, 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.

Acknowledgements

The authors thank Dr. Giacomo Targioni, Laboratorio Centrale of the Careggi Hospital, for the valuable availability and skilfulness in the DPG measurements; Drs. Alberto Grossi and Giovanni Longo, U.O. of Haematology, San Luca (Careggi Hospital) for selecting the subjects and their valuable advice.

References

1. Rovida E, Niggeler M, Carlone S, Samaja M. Carboxyhemoglobin and oxygen affinity of human blood. Clin Chem 1984; 30:1250–1.
2. Siggaard-Andersen O, Wimberley PD, Gothgen I, Siggaard-Andersen M. A mathematical model of the hemoglobin-oxygen dissociation curve of human blood and of the oxygen partial pressure as a function of temperature. Clin Chem 1984; 30:1646–51.
3. Severinghaus JW. Blood gas calculator. J Appl Physiol 1966; 21:1108–16.
4. Siggaard-Andersen O. An acid-base chart for arterial blood with normal and pathophysiological reference areas. Scand J Clin Lab Invest 1971; 27:239–45.
5. Thomas LJ. Algorithms for selected blood acid-base and blood gas calculations. J Appl Physiol 1972; 33:154–8.
6. Severinghaus JW. Simple, accurate equations from human blood O2 dissociation computations. J Appl Physiol 1979; 46:599–602.
7. Samaja M, Melotti D, Carenini A, Pozza G. Glycosylated hemoglobins and the oxygen affinity in whole blood. Diabetologia 1982; 23:399–402.
8. Samaja M, Mosca A, Luzzana M, Rossi Bernardi L, Winslow RM. Equations and nomogram for the relationship of human blood p_{50} to 2,3-diphosphoglycerate, CO_2, and H^+. Clin Chem 1981; 27:1856–61.
9. Samaja M, Winslow RM. The separate effects of H^+ and 2,3-DPG on the oxygen equilibrium curve of human blood. Br J Haematol 1979; 41:373–81.
10. Winslow RM, Samaja M, Winslow NJ, Rossi Bernardi L, Shrager RI. Simulation of continuous blood O_2 equilibrium curve over the physiologic pH, DPG and pCO_2 range. J Appl Physiol 1983; 54:524–9.
11. Thompson ML, Zucchini W. On the statistical analysis of ROC curves. Stat Med 1989; 8:1277–90.
12. Winslow RM, Swenberg ML, Berger RL, Shrager RI, Luzzana M, Samaja M, *et al.* Oxygen equilibrium curve of normal human blood and its evaluation by Adair's equation. J Biol Chem 1977; 252:2331–7.

Received 18 January 2000; revised 26 June 2000; accepted 27 June 2000

Corresponding Author: Dr. Michele Samaja, Dipartimento di Medicina, Chirurgia e Odontoiatria – DiMCO, Polo Ospedale San Paolo, Universita' di Milano, via di Rudinì 8, I-20142 Milano, Italy
Tel.: +39-0289179250/2, Fax: +39-0289179251
E-mail Michele.Samaja@unimi.it