Experimental determination of A_{tot} and pK_a of whole blood of healthy volunteers, patients with sepsis and post-operative patients: an *in vitro* study

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INTRODUCTION

During acute respiratory acid-base perturbations, pH changes are limited by the non-carbonic buffers, mainly consisting of proteins and phosphates in plasma, plus hemoglobin in whole blood. According to Stewart's physicochemical approach, the total weak acid concentration (A_{tot}) is one of the three independent variables determining pH [1]. The amount of dissociated A_{tot} (A^-) depends on their acidic dissociation constant (K_a), also known in its logarithmic form: $pK_a = -\log_{10} K_a$. Experimental estimates for A_{tot} and K_a were previously obtained for human plasma of healthy volunteers (17.2 ± 3.5 mmol/L and 0.80 ± 0.60 x 10^{-7}, pK_a = 7.10, respectively) [2]. Of note, while in clinical practice acid-base measurements are performed on whole blood, no data regarding A_{tot} and K_a of blood are currently available.

Objective

To compute pK_a and A_{tot} for whole blood of healthy subjects and two different populations of ICU patients, *i.e.* patients with sepsis and post-operative patients.

METHODS

Blood was collected from 30 volunteers, 30 patients with sepsis [3] and 27 post-operative patients (ICU "Vecla", Ospedale Maggiore Policlinico, Milan and 2 ICUs of FNKV University Hospital, Prague). Hemoglobin, albumin, total proteins and phosphates concentrations were measured. Blood was equilibrated with different gas mixtures to obtain 20 experimental points with PCO_2 ranging between 20 and 120 mmHg. For each subject, the variation of Strong Ion Difference (SID) over PCO_2 was modeled, and the normal value of SID at PCO_2 of 40 mmHg (SID_{40}) was computed. Measured pH and PCO_2, SID_{40} and the simplified strong ion electroneutrality equation were used to calculate A_{tot} and K_a through the Marquardt nonlinear regression procedure:

$$0.0307 \cdot PCO_2 \cdot 10^{pH-6.120} = SID_{40} - \frac{A_{tot} \cdot K_a}{K_a + 10^{-pH}}$$

T-test and Mann-Whitney rank sum test were used for analysis.

RESULTS

Age [54 ± 15 *vs.* 61 ± 16 *vs.* 57 ± 18 yr, p = 0.3] and gender [14 (47%) *vs.* 9 (33%) *vs.* 12 (40%) of females, p = 0.6] did not differ among volunteers, post-operative and septic patients. Both populations of ICU patients had lower hemoglobin, albumin and total proteins concentrations as compared to healthy volunteers (**Table 1**), while phosphates were similar. Septic patients had lower values of A_{tot} as compared to post-operative patients, which had lower values as compared to healthy controls. Also, pK_a showed a decreasing trend going from controls to post-operative and septic patients.

	Controls (N=30)	Post-operative patients (N=27)	Septic patients (N=30)	p
Hemoglobin (g/dL)	14.4 ± 1.0	10.9 ± 1.8	9.6 ± 1.3	<0.001
Albumin (g/dL)	4.8 ± 0.3	3.2 ± 0.4	2.5 ± 0.4	<0.001
Total protein (g/dL)	7.2 (6.7 – 7.6)	4.8 (4.6 – 5.1)	4.8 (4.1 – 5.1)	<0.001
A_{tot} (mmol/L)	94 (72 – 132)	53 (43 – 62)	39 (37 – 46)	<0.001
pK_a	7.91 (7.84 – 8.19)	7.74 (7.52 – 7.82)	7.35 (7.11 – 7.46)	<0.001

Table 1. Comparison between nonvolatile buffers concentration and the estimated A_{tot} and pK_a values among groups.

CONCLUSIONS

Healthy volunteers, septic patients and post-operative patients had different values of both A_{tot} and pK_a of whole blood. Of note, both estimates performed through whole blood equilibration were remarkably different as compared to the ones previously obtained on plasma. Interestingly, the values of pK_a we computed were higher than 7.40, suggesting a higher non-carbonic buffer power of blood against acute respiratory alkalosis rather than acidosis.

REFERENCES

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