Research Article

Effect of Sample Storage Temperature and Time Delay on Blood Gases, Bicarbonate and pH in Human Arterial Blood Samples

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Background: Results of arterial blood gas analysis can be biased by pre-analytical factors, such as time interval before analysis, temperature during storage and syringe type.

Objectives: To investigate the effects of samples storage temperature and time delay on blood gases, bicarbonate and PH results in human arterial blood samples.

Patients and Methods: 2.5 mL arterial blood samples were drawn from 45 patients via an indwelling Intraarterial catheter. Each sample was divided into five equal samples and stored in multipurpose tuberculin plastic syringes. Blood gas analysis was performed on one of five samples as soon as possible. Four other samples were divided into two groups stored at 22°C and 0°C. Blood gas analyses were repeated at 30 and 60 minutes after sampling.

Results: PaO_2 of the samples stored at 0°C was increased significantly after 60 minutes (P = 0.007). The $PaCO_2$ of the samples kept for 30 and 60 minutes at 22°C was significantly higher than primary result (P = 0.04, P < 0.001). In samples stored at 22°C, pH decreased significantly after 30 and 60 minutes (P = 0.017, P = 0.001). There were no significant differences in other results of samples stored at 0°C or 22°C after 30 or 60 minutes.

Conclusions: In samples stored in plastic syringes, overestimation of PaO_2 levels should be noted if samples cooled before analysis. In samples stored in plastic syringes, it is not necessary to store samples in iced water when analysis delayed up to one hour.

Keywords: Blood Gas Analysis; Bicarbonate; Temperature

1. Background

Arterial blood gas (ABG) analysis is important in the evaluation of clinical condition of critically ill patients. Results of ABG analysis can be biased by pre-analytical factors, such as time interval before analysis, temperature during storage and the syringe type. However, the acceptable temperature and delay between the time of collection and arterial blood gas analysis remain unknown (1). Based on limited studies, delay in the analysis can decrease arterial oxygen partial pressure (PaO_2) and increase arterial carbon dioxide partial pressure ($PaCO_2$) because of the cell metabolism (2, 3). Ice preservation is recommended; however, there is no reason to keep arterial blood in ice if the blood gas analysis is performed within 30 minutes (4).

Although most studies indicate that glass syringes are superior to plastic syringes in preserving samples, especially for PaO_2 determination (1, 5-7), plastic high density polypropylene syringes are most commonly used for collecting arterial blood samples, at the present time. Pre-heparinized plastic syringes are also available in many countries that make them more popular than glass syringes.

Cooling arterial-blood samples in plastic syringes is still the method of choice for delayed analysis; however, the effects of storage temperature and time on ABG results have not been adequately described (5). To the best of our knowledge, there is no study to determine accepted time delay and temperature for accurate results.

2. Objectives

The purpose of this study was to investigate the effects of sample storage temperature and time delay on blood gases, bicarbonate and pH in human arterial blood samples.

3. Patients and Methods

In a multi-step experimental study, 50 consecutive patients with indwelling Intraarterial catheter were selected from cardiac surgery intensive care unit (ICU). The study performed from January to December 2012 in Imam Khomeini hospital (a referral educational hospital of Teh-

ran University of Medical Sciences, Tehran, Iran). With the confidence level at 95% and margin of error at 5%, at least 35 cases expected for the study. Febrile patients (body temperature > 37.5°C) and patients with hemoglobin below 10 and more than 15 were excluded from the study.

Because of the background disease and cardiac surgery ICU rule, these patients already had arterial line. Therefore, no additional invasive procedure was performed on them to obtain blood samples. The study was approved by Tehran University of Medical Sciences ethical committee (Code: 130/2031) and informed consent was obtained from the patient him or herself (when possible) and in most cases from his or her relatives.

After obtaining the informed constant, 2.5 mL arterial blood samples were drawn from each patient via an indwelling intraarterial catheter. To minimize contamination of blood samples with heparin, due its inappropriate effects on measurements, the first 8 to 10 mL of blood was discarded. Each sample was divided into five equal samples, stored in multipurpose tuberculin plastic syringes. To avoid equilibration with gas bubbles, we tried to remove air bubbles from the samples. Minimal heparin amounts were used to only coat the side of the syringes. For blood gas analysis, the AVL 995 blood gas analyzer (Block Scientific Inc. Bohemia, New York-11716, USA) was used by one experienced technician.

Blood gas analysis was performed on one of five samples as soon as possible after sampling (the time needed for transport to the laboratory was less than three minutes). Four other samples were divided into two groups according to the method of storage, group I was stored at 22°C (room temperature) and group II was stored at 0°C (iced water). Blood gas analyses were repeated at 30 and 60 minutes after sampling. To avoid probable device error, blood gas analyzer was calibrated each time, before primary analysis and before analysis after 30 and 60 minutes. For each sample, arterial pressure of oxygen in arterial blood (PaO_2), partial pressure of carbon dioxide in arterial blood (PaO_2) and pH were measured. HCO_3 was calculated by blood gas analyzer using Henderson-Hasselbalch equation. Results were compared to the samples analyzed immediately.

SPSS 16 for window program (SPSS, Inc, Chicago, IL) was used to perform statistical analysis. Results were expressed as mean ± SD. Repeated measures test (One-way ANOVA following Bonferroni post-hoc test) was used to assess the significance of differences between baseline and results obtained at 30 and 60 minutes after storage of samples in iced water or room temperature. The statistical significance was set at P < 0.05.

4. Results

Five samples were discarded because of clot formation. Forty five patients were included in the study (Table 1). These patients admitted in the ICU after cardiac surgery consisted of 32 coronary Artery Bypass Graft, four Heart Transplantation, eight cardiac valve replacements and one Myxoma.

The results of arterial blood gas analysis of samples after 30 and 60 minutes compared with primary results is shown in Tables 2 and 3.

Table 1. Baseline Characteristics of Patients Enrolled in Study [a]

Variable	Value
Age, y	51.28 ± 17.59
Body Temp, °C	37.09 ± 0.32
Hemoglobin, g/dL	11.26 ± 1.85
Gender, M	28 (62.2)

[a] Data are presented as Mean ± SD or No. (%).

Table 2. Arterial Blood Gas Analysis Variables (mean ± SD) After 30 and 60 minutes in Samples Stored at 22°C [a]

Parameter	0	30th minute	60th minute
PO_2, mmHg	85.74 ± 40.86	83.89 ± 33.77	83.40 ± 34.05
PCO_2, mmHg	35.84 ± 5.80 [b,c]	36.78 ± 6.20 [b]	37.27 ± 6.03 [c]
pH	7.413 ± 0.059 [c,d]	7.400 ± 0.049 [d]	7.392 ± 0.049 [d]
HCO_3, mg/L	21.90 ± 3.42	21.98 ± 3.35	21.94 ± 3.48

[a] Data are presented as Mean ± SD.
[b] P = 0.042: 0 minute vs. 30th minute (by ANOVA with post hoc Bonferroni tests).
[c] P < 0.001: 0 minute vs. 60th minute (by ANOVA with post hoc Bonferroni tests).
[d] P < 0.017: 0 minute vs. 30th minute (by ANOVA with post hoc Bonferroni tests).

Table 3. Arterial Blood Gas Analysis Variables After 30 and 60 Minutes in Samples Stored at 0 °C [a]

Parameter	0	30th minute	60th minute
PO_2, mmHg	85.74 ± 40.86 [b]	86.69 ± 39.10	87.93 ± 40.03 [b]
PCO_2, mmHg	35.84 ± 5.80	35.39 ± 5.95	35.54 ± 5.86
pH	7.413 ± 0.059	7.415 ± 0.049	7.413 ± 0.052
HCO_3, mg/L	21.90 ± 3.42	22.00 ± 3.44	22.05 ± 3.40

[a] Data are presented as Mean ± SD.
[b] P = 0.007: 0 minute vs. 60th minute (by ANOVA with post hoc Bonferroni tests).

Compared with samples analyzed immediately, PaO_2 of samples stored for 30 and 60 minutes at 0°C was higher, but it was significantly increased after 60 minutes (P = 0.007). PaO_2 of samples stored for 30 and 60 minutes at 22°C was decreased insignificantly (P = 0.223, P = 0.175).

In 32 of 45 samples stored at 0°C, PaO_2 was increased after 60 minutes; the magnitude of difference was more than 5 mmHg in 7 of 45 samples. Compared with others, no significant difference was present in hemoglobin or body temperature of these patients (hemoglobin 1.49 ± 0.90, body temperature 37.03 ± 0.39).

There was no significant difference in $PaCO_2$ of samples stored at 0°C after 30 or 60 minutes. But $PaCO_2$ of the

samples for 30 and 60 minutes at 22°C was significantly higher than primary results (P = 0.042, P < 0.001). The magnitude of difference was not clinically important after 60 minutes.

There was no statistically significant change in pH of samples stored at 0°C. In samples stored at 22°C, pH was decreased significantly after 30 and 60 minutes (P = 0.017, P = 0.001), although the magnitude of difference (< 0.1) was not clinically important. Values of pH did not show variations more than 0.02 for the first hour, irrespectively of storage condition. HCO_3 results were slightly increased in all samples after 30 and 60 minutes, but it was not statistically significant. Furthermore, the magnitude of difference (< 0.5) was not clinically important.

5. Discussion

Several pre-analytical factors can affect results of arterial blood gas analysis. If air bubbles occupy more than 1% to 2% of the blood volume in the syringe, an artificially higher or lower arterial PaO_2 and an underestimation of the true arterial PaO_2 may result from equilibration of these gases between air bubbles and the specimen (8-10). The magnitude of this error is greatest when the difference in gas tensions between blood and air high, when the surface area of bubbles is maximized by agitation and when the time between specimen collection and analysis is prolonged (11).

Another potential problem is dilution of blood specimen with heparin. It has been suggested that during sampling from an indwelling intraarterial catheter, to minimize contamination of blood samples with heparin, the first 8 to 10 mL of blood should be discarded (10, 12). A similar error can occur with the use of a heparinized syringe with anticoagulant solution volumes greater than 5% of the volume of the blood sample; it is still crucial that heparin should be less than 200 IU/mL blood (13).

As pointed in published studies, blood gas tensions can alter during storage, especially if blood is collected in plastic syringes. It was believed that oxygen tension of the arterial blood (PaO_2) in these syringes might fall. The continuing metabolism of blood cells and the ability of gases to diffuse through the wall of the syringe have been suggested as probable factors responsible for these alterations (14). Most published studies indicated that glass syringes are superior to plastic syringes in preserving samples, especially for PaO_2 determination (1, 5-7).

Based on nanomaterial composition (calculating the size of O_2 molecule and estimated pore size and pore density of the glass and plastic material), Wiwanitkit concluded that O_2 seems to have a greater chance (4 - 150 times) to diffuse across plastic than glass, which could be a good explanation why a glass syringe can better preserve oxygen in a blood samples for blood gas analysis (6). On the other hand, the plastic syringe is safer and more convenient to be used in daily practice.

From a clinical perspective in our study, there were no clinically important differences in the results of HCO_3, PH,

$PaCO_2$ and PaO_2 of samples stored at 0°C or 22°C, at least up to 60 minutes, regardless of storage condition. Although from a statistical point of view, variation of results stored in iced water was lower compared with samples stored in ambient temperature. However, an interesting phenomenon occurred against our predictions was an increase in PaO_2 of the samples stored at 0°C.

It has been believed that the effect of leukocytes metabolism is more important than gas diffusion through the wall of the syringes, thus cooling of blood samples has been suggested by previous studies.

Cooling of blood could minimize leukocyte metabolism. But However, cooling of blood has two other important effects; first, the solubility coefficient of oxygen in blood doubles (the solubility of oxygen increases from 21.4 mL O2/L plasma at 37°C to 39.5 mL O_2/L plasma at 4°C (4)) and second, there is an increase in the oxygen-hemoglobin affinity, which leads to an initial decrease in sample PaO_2; this phenomenon would increase the atmosphere-to-sample oxygen gradient. If the sample is stored in a semipermeable container such as a plastic syringe, this gradient may cause an increased flux of oxygen into the sample.

The sample is reheated to 37°C in the blood gas analyzer, thus the solubility and oxygen-hemoglobin affinity return to their original values. Any added oxygen while the blood was cooled would release and results in elevated PaO_2 levels during analysis.

It has been a common practice to store arterial blood gas samples in ice while waiting for analysis to minimize leukocyte metabolism. However, based on this study, especially when a plastic syringe is used for sample storage, likelihood of overestimation of PaO_2 should be noted if samples cooled before analysis.

On the other hand, there was no significant difference in $PaCO_2$ of samples stored at 0°C after 30 or 60 minutes, but $PaCO_2$ of samples after 30 and 60 minutes at 22°C were significantly higher than primary results. Metabolism of leukocytes in room temperature could explain this phenomenon.

Additionally, because of greater size of CO_2 molecule, the likelihood of diffusion through the syringes wall is lower compared with O_2 molecule. Thus, it seems that there is no difference in plastic or glass syringes in $PaCO_2$ alterations after delayed analysis.

There is no doubt that the blood gasses sample should be sent for analysis as soon as possible. This study performed during a narrow period by limited cases. Although the results of study were surprising in some points, its confirmation needs more investigations. On the other hand, in practical point of view, there is still a question whether these statistically significant changes really have clinical importance.

Immediate analysis of arterial blood samples should be noted to avoid alterations. In delayed analysis, practitioners should be aware of gas change patterns, especially if a plastic is used for storage of blood samples. In samples stored in glass syringes, cooling samples to decrease the

metabolism of leukocytes is a logical decision, because of low permeability of the glass syringes wall for the O_2 molecule. However, in samples stored in plastic syringes, overestimation of PaO_2 levels should be noted if samples cooled before analysis. Although mild increase in $PaCO_2$ could occur during storage in room temperature, alterations in PaO_2 in cooled samples are greater compared with PaO_2 alterations in samples stored at room temperature. Thus, in samples stored in plastic syringes, it is not necessary to store samples in iced water when analysis delayed up to one hour. Studies involving a larger sample size should be performed to confirm these hypotheses.

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Author's Contributions

Study concept and design: Safavi, Mohammadhoseini; Analysis and interpretation of data: Safavi, Mohammadhoseini, Seifirad; Drafting of the manuscript: Seifirad, Peiman; Critical revision of the manuscript for important intellectual content: Safavi, Firoozbakhsh, Peiman, Seifi; Statistical analysis: Seifirad.

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