Stability of blood gases when refrigerated

João Pedro Ferreira, Sara Vieira Silva, Patrícia Rodrigues, Miguel Araújo Abreu, José Miguel Maia, Daniela Carvalho and Luísa Carvalho

Abstract

Background: Blood gas analysis is a widely used procedure. In clinical practice, the physicians may not always have a blood gas analyzer in their proximity. Not infrequently, blood gas samples are stored in a fridge or on ice and read retrospectively. Continued anaerobic and aerobic metabolism in the blood may alter blood gases in the interval between drawing arterial blood and its analysis, which may cause a fall in the PaO_2 and pH and a rise in the $PaCO_2$.

Methods: Two sets of arterial blood samples were obtained from hospitalized patients. After the initial analysis, one sample from each patient was put in raw ice within a specimen bag (0 to +1 °C) and the other in the fridge (+4 to +8 °C). These samples were submitted to serial analysis at 30 minutes, 1 hour and 2 hours after the initial analysis.

Results: Two hundred arterial blood gas results from 25 patients were analysed. The mean values of PaO_2, $PaCO_2$, HCO3-, Na^+, K^+, Ca^{2+} and lactate at 0 minutes, 30 minutes, 1 hour and 2 hours were not significantly different between the two alternatives of storage. However, within each group, significant changes were found over time for PaO_2, K^+, Na^+, Ca^{2+} and lactate.

Conclusions: When using plastic syringes, arterial blood gas analysis should be processed shortly after collecting the sample. Despite the fact that low temperatures can slow down the metabolism, neither the ice nor the fridge preserved all the sample parameters.

Keywords: blood gas analysis, refrigeration, ice, plastic syringes

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Introduction

Blood gas analysis is a widely used procedure; however, in clinical practice, the physicians do not always have a blood gas analyzer in their proximity and, not infrequently, the samples are stored in a fridge or on ice and read retrospectively.

Continued anaerobic and aerobic metabolism in the blood after collection may alter blood gas composition in the interval between drawing arterial blood and its analysis, such as a fall in the PaO_2 and pH and a rise in the $PaCO_2$. These changes are temperature and time dependent (1-3). In addition, oxygen will diffuse into the sample, particularly in plastic syringes (2). However, previous research has indicated that if blood gas analysis is done within 30 minutes, there seems to be no reason to keep it on ice (2), but we found no previous studies comparing longer periods of preservation on ice versus in the fridge.

Based on the limited existing information, we designed this study to answer the following questions:

- How long does it take for blood gas composition to change?
- Which parameters change and when does that change become statistically significant?
- Are there any significant differences between keeping the samples in ice or in the fridge?

Methods

Arterial blood samples were obtained randomly from hospitalized patients as part of their normal clinical management. All patients had an arterial line placed. One milliliter (mL) of blood was collected in 1 mL heparinized (2 IU/mL) plastic syringes (RAPID Lyte® 1 mL L/S Syringe, Siemens).

For each patient, two arterial blood samples were collected at the same time using the arterial line. Samples were mixed and care was taken to ensure no air entered the syringes. If air bubbles were found, these were carefully removed from the syringe after each analysis to avoid equilibration with room air. Samples were analysed immediately. After the initial analysis, one sample was placed on ice (0 to +1 °C) and the other one was placed in the fridge (+4 to +8 °C). The samples were capped using filter caps that came with the syringes. These samples were submitted to serial testing at 30 minutes, 1 hour and 2 hours after the initial analysis. The pH, PaO_2, $PaCO_2$, HCO_3^-, Na^+, K^+, Ca^{2+} and lactate were measured.

All the determinations were performed using a RAPIDLab® 1200 Blood Gas Analyzer (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) according to the manufacturer's instructions. All samples were analysed under a standard temperature (4).

The number of samples needed (n=25) to obtain potentially significant results was determined using Piface software (3). Statistical analysis was performed using Software IBM SPSS Statistics 19® (IBM Corporation, Somers, NY, USA).

Mean values of all parameters at 0 min, 30 min, 1 hr and 2 hr were compared between groups by an independent two-tailed *t* test within each group, and the magnitude of change was examined by comparing the differences in the measurements for each value at 0, 30 min, 1 hr, and 2 hr using a paired two-tailed *t* test. A p value of ≤0.05 was defined as significant.

Ethics approval was obtained from the local ethics committee. Informed consent was obtained from the patients.

Results

Two hundred arterial blood determinations from 25 patients were entered in the study - 100 measurements for each of the two experimental conditions. The mean values of PaO_2, $PaCO_2$, HCO_3^-, Na^+, K^+, Ca^{2+} and lactate at 0 min, 30 min, 1 hr and 2 hr were not significantly different between the two groups (Table 1).

Table 1. Blood gas values at 0, 30 min, 1 hr and 2 hr. Comparisons between groups.

Blood gas parameter	Time	Ice	Fridge	p
pH	0	7.39 ±0.09	7.40 ±0.09	NS
	30 min	7.40 ±0.09	7.41 ±0.09	NS
	1 hr	7.40 ±0.09	7.40 ±0.09	NS
	2 hr	7.39 ±0.10	7.40 ±0.10	NS
PaO_2 mm hg	0	71.96 ±14.0	72.25 ±12.90	NS
	30 min	77.09 ±16.22	78.75 ±17.07	NS
	1 hr	84.62 ±21.29	84.84 ±21.55	NS
	2 hr	92.49 ±25.16	95.39 ±27.15	NS
$PaCO_2$ mm hg	0	42.25 ±25.54	43.92 ±25.26	NS
	30 min	44.66 ±24.86	43.07 ±24.05	NS
	1 hr	44.56 ±25.36	43.84 ±24.99	NS
	2 hr	44.44 ±25.31	42.96 ±24.64	NS
HCO_3^- mmol/L	0	25.99 ±8.05	25.10 ±7.47	NS
	30 min	25.84 ±8.13	24.87 ±7.10	NS
	1 hr	25.92 ±8.02	24.99 ±7.62	NS
	2 hr	25.54 ±7.99	24.32 ±7.04	NS
K^+ mmol/L	0	4.17 ±0.91	4.03 ±1.00	NS
	30 min	4.28 ±0.94	3.98 ±0.99	NS
	1 hr	4.41 ±0.93	4.04 ±1.01	NS
	2 hr	4.53 ±0.94	4.37 ±1.19	NS
Na^+ mmol/L	0	138.08 ±5.96	138.58 ±6.00	NS
	30 min	138.90 ±5.58	139.42 ±6.49	NS
	1 hr	139.95 ±6.24	140.34 ±6.75	NS
	2 hr	139.02 ±5.24	141.84 ±6.34	NS
$Ca2^+$ mmol/L	0	1.13 ±0.09	1.11 ±0.09	NS
	30 min	1.10 ±0.10	1.06 ±0.11	NS
	1 hr	1.07 ±0.10	1.06 ±0.12	NS
	2 hr	1.01 ±0.10	0.99 ±0.18	NS
Lactate mmol/L	0	1.31 ±0.60	1.32 ±0.59	NS
	30 min	1.47 ±0.61	1.47 ±0.64	NS
	1 hr	1.59 ±0.60	1.64 ±0.62	NS
	2 hr	1.83 ±0.52	1.93 ±0.67	NS

NS=not significant

However, within each group, significant changes were found over time in the blood gases and electrolytes. The PaO_2, K^+, Na^+ and lactate increased; $PaCO_2$ and Ca^{2+} decreased (Table 2). The increase in the PaO_2 was significant in both groups, compared with baseline values. These changes were noticed at 30 min in both groups, increasing even further afterwards. The decrease in $PaCO_2$ was statistically significant at 30 min and 2 hr in the fridge, compared to baseline. No significant differences were found when these samples were kept on ice. The pH remained stable in both groups; however the lactate levels increased significantly in both groups after 30 min. The decrease in HCO_3^- was statistically significant only after 2 hr storage in the fridge.

Table 2. Changes in blood gas values from 0 min to 30 min, 1 hr and 2 hr. Comparisons within groups.

Blood gas parameter	Values in comparison	Ice	p	Fridge	p
pH	0 and 30 min	0.01 ±0.03	NS	0.02 ±0.04	NS
	0 min and 1hr	0.01 ±0.03	NS	0.01 ±0.03	NS
	0 min and 2 hr	0.01 ±0.03	NS	0.01 ±0.04	NS
PaO_2 mm hg	0 and 30 min	5.02 ±4.31	<0.001	6.28 ±7.10	<0.001
	0 min and 1hr	12.39 ±13.21	<0.001	12.34 ±11.19	<0.001
	0 min and 2hr	20.53 ±15.71	<0.001	23.27 ±17.01	<0.001
$PaCO_2$ mm hg	0 and 30 min	-0.58 ±2.16	NS	-0.84 ±2.09	0.05
	0 min and 1hr	-0.68 ±2.23	NS	-0.07 ±3.86	NS
	0 min and 2hr	-0.80 ±2.65	NS	-1.04 ±2.27	=0.03
HCO_3^- mmol/L	0 and 30 min	-0.14 ±0.93	NS	-0.26 ±0.89	NS
	0 min and 1hr	-0.07 ±1.12	NS	-0.09 ±1.78	NS
	0 min and 2hr	-0.45 ±1.21	NS	-0.80 ±0.74	<0.001
K^+ mmol/L	0 and 30 min	0.11 ±0.26	=0.05	-0.07 ±0.18	NS
	0 min and 1hr	0.24 ±0.28	<0.001	0.01 ±0.19	NS
	0 min and 2hr	0.36 ±0.34	<0.001	0.32 ±0.77	0.05
Na^+ mmol/L	0 and 30 min	0.84 ±1.00	<0.001	0.83 ±1.90	0.04
	0 min and 1hr	1.86 ±1.97	<0.001	1.71 ±2.58	0.003
	0 min and 2hr	0.94 ±5.41	NS	3.28 ±2.90	<0.001
Ca^{2+} mmol/L	0 and 30min	-0.03 ±0.06	NS	-0.05 ±0.05	<0.001
	0 min and 1hr	-0.06 ±0.05	<0.001	-0.06 ±0.05	<0.001
	0 min and 2hr	-0.12 ±0.07	<0.001	-0.15 ±0.12	<0.001
Lactate mmol/L	0 and 30min	0.15 ±0.13	<0.001	0.15 ±0.12	<0.001
	0 min and 1hr	0.27 ±0.18	<0.001	0.31 ±0.17	<0.001
	0 min and 2hr	0.46 ±0.22	<0.001	0.55 ±0.29	<0.001

NS=not significant

The K^+ started to increase significantly after 30 min on ice, but when kept in the fridge significant changes were observed only after 2 hr. Na^+ increased in samples stored on ice at 30 min and 1hr, whereas in the fridge Na^+ increased significantly throughout the three time periods. The decrease in ionized Ca^{2+} was sustained in both groups, but this change appeared to begin later in the samples kept on ice.

Discussion

Refrigerating the blood may delay changes in arterial blood gas composition (5,6). A robust and well conducted study by Liss and Payne analysed the changes in PaO_2, $PaCO_2$ and pH at 0, 15 and 30 min (7). They found a statistically significant increase in the PaO_2 at 15 and 30 min in both groups, and a statistically significant decrease in the $PaCO_2$ at 15 min in both groups. There was also a statistically significant decrease in the pH at 15 min in both groups. There were no differences between the samples stored at room temperature or in ice. In our study the changes in the blood gas composition over time were not in the direction as previously found (5, 8-9). The authors of those studies attributed their finding to the use of plastic syringes. Plastic syringes can act as semipermeable membranes and allow diffusion of gases (10). This was corroborated by another study (12).

According to Fletcher et al, the PaO_2 of oxygenated water stored in glass syringes remains stable for 1 hr if kept on ice or at room temperature, but not in plastic syringes (8). Another study comparing plastic versus glass syringes regarding blood gas tensions in samples with high oxygen partial pressures concluded that glass syringes are superior to plastic syringes in preserving samples with a high PaO2, and prompt and adequate cooling of such samples is essential for accurate blood gas analysis (12).

Our findings are similar to those described by Liss and Payne (7). However, instead of comparing ice with room temperature, we compared ice with refrigeration using baseline measurements as standard. We also extended the time length, and analyzed changes in the ions and lactate.

Comparisons between groups (ice vs fridge) did not show statistically significant differences. The most significant changes in both groups were a rise in lactate levels and PaO_2 and a decrease in Ca^{2+} over 2 hr. As in another study, no changes in pH, pCO_2 and HCO_3^- were found in samples stored on ice (13).

The changes in PaO_2 are consistent and significant in both groups, i.e. independently of storing blood gas samples on ice or in the fridge, the PaO_2 rose significantly at 30 min, 1 hr and at 2 hr. As other authors have already observed, when plastic syringes are placed on ice there is an inflow of oxygen into the samples, because iced water exhibits a very high oxygen concentration (13). Besides, as the temperature decreases, there is a shift in the oxygen-hemoglobin dissociation curve towards the left and an increase in the solubility of oxygen in the plasma, resulting in an increase of the measured PaO_2. A limitation is that we cannot exclude the possibility that sample manipulation and homogenization may have contributed to air entry into the sample.

The decrease in $PaCO_2$ could be explained using the same principle of gas equilibration between the syringe and the environment. However, the decrease in PaCO2 and HCO3- was significant only after 2 hr in the fridge, explaining why there were no major changes in pH.

The increase in K^+ and decrease in ionized Ca^{2+} can be explained by cell lysis that occurs over time, releasing K^+ and PO_4^-. PO_4^- binds to Ca^{2+}, decreasing ionized Ca^{2+}. In addition K^+ leaks out of red blood cells at temperatures around 4ºC due to the inability of the membrane Na^+K^+-ATPase pump to work correctly and this could also be an explanation for the K^+ results.

The increase in lactate was also expected because of anaerobic metabolism, particularly by red blood cells that utilize anaerobic pathways preferentially in their metabolism (11). Unexpectedly, a decrease in both the HCO_3^- and pH was not observed and this interesting finding may be explained by the release of intracellular HCO_3^- as cell lysis occurs.

Conclusions

When using plastic syringes, the arterial blood gas analysis should be processed shortly after the sample is collected. Despite the fact that low temperatures can slow down metabolism, neither the ice nor the fridge preserved all the sample characteristics. If however, the analysis of the blood gases needs to be postponed, the clinician should be aware that storage in ice or in the fridge are not very different (even though the former is possibly slightly better) and that the levels of PaO_2 and lactate are probably overestimated.

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Author information

João Pedro Ferreira, MD, Internal Medicine Resident[1]
Sara Vieira Silva, MD, Internal Medicine Resident[1]
Patrícia Rodrigues, MD, Cardiology Resident[1,2]
Miguel Araújo Abreu, MD, Infectious Diseases Resident[1,3]
José Miguel Maia, MD, Internal Medicine Resident[1]
Daniela Carvalho, MD, Internal Medicine Resident[1]
Luísa Carvalho, MD, Internal Medicine Hospital Assistant[1]

[1]Internal Medicine Department, [2]Cardiology Department and [3]Infectious Diseases Department, Centro Hospitalar do Porto, Porto, Portugal.

Author contributions

JPF conceived the study, contributed to the analytic work, data acquisition and data analysis, and substantially drafted the main article. SVS contributed to the analytic work, data acquisition and data analysis, and added critical content to the article. PR contributed to data acquisition and added critical content to the article. MAA conceived the study and added critical content to the article. JMM, DC and LC added critical content to the article. The authors declare no conflicts of interest.

Author for correspondence

Dr João Pedro Ferreira, Internal Medicine Department, Centro Hospitalar do Porto, Largo Prof. Abel Salazar, 4099-001 Porto, Portugal. Email: jp7ferreira@hotmail.com

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