

**The influence of delayed sample processing time on the pO<sub>2</sub> values in critically ill patients with sepsis-induced leukocytosis**

by

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SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In fulfilment of the requirements for the degree:

**Master of Medicine in Anaesthesiology**

Faculty of Health Sciences

**UNIVERSITY OF CAPE TOWN**

Date of Submission:

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## List of abbreviations

Hb	Haemoglobin
ICU	Intensive Care Unit
O <sub>2</sub>	Oxygen
pCO <sub>2</sub>	Partial pressure of carbon dioxide
pO <sub>2</sub>	Partial pressure of oxygen
pH	Measure of hydrogen ion activity
WCC	White Cell Count
kPa	Kilopascal
FiO <sub>2</sub>	Fractional inspiratory Oxygen concentration
P50	Oxygen tension at which haemoglobin is 50% saturated

## **Chapter 1: Introduction and Literature Review**

## **1. Objectives**

This narrative review aims to discuss the importance of the need for early processing of blood gas samples in the critically ill, especially those with sepsis- induced leucocytosis. The correct handling, type of syringe, temperature and transport medium all play a role.

## **2. Literature Search Strategy**

The full text of relevant publications was obtained online, from the University of Cape Town Health Science Library search facility. The literature search was initiated using the Pubmed Central digital archive and Google scholar. Searching reference lists identified further appropriate papers. Thirty-six relevant research papers were identified.

Literature not published in the English language was excluded.

## **3. Quality criteria**

The keywords used for the search, included each of the following, in various combinations: blood specimen, collection, blood gas analysis, syringes leukocyte count, temperature, pO<sub>2</sub> and hypoxemia.

## **4. Summary of the literature**

### **4.1. Introduction**

The partial pressure of gases and the acid-base balance measured in an arterial blood sample changes over time. The extent of this change depends on the time between taking and analysing the sample. These changes are due to two processes. Firstly, the blood cools. The analyser compensates for loss of heat by warming the sample to 37°C.<sup>1,2</sup> Secondly, white cells in the blood continue to be metabolically active and therefore continue to utilise oxygen and produce carbon dioxide. Reference nomograms<sup>3</sup> designed to compensate for ongoing white cell metabolism are not derived from critically ill patients. "Pseudohypoxaemia"<sup>4-9</sup> has been observed

in patients with myeloproliferative disorders with a white cell count (WCC)  $> 50 \times 10^9$  cells/L.<sup>7</sup> These values are rarely seen in the critically ill, however white cells are often more numerous in critically ill patients with sepsis. Reference nomograms are derived from healthy populations.

## 4.2. Temperature correction

Temperature affects both the solubility and affinity of haemoglobin for oxygen. It therefore makes temperature correction of the measured oxygen tension in blood a complex matter.

Errors therefore originate as a result of any discrepancy between the temperature of the sample and that of the patient at time of analysis. Mathematical equations have been derived to use a correction factor to compensate for these changes. Figure 1 is a graphical representation of Temperature correction coefficient as a function of the oxygen tension.<sup>2</sup>

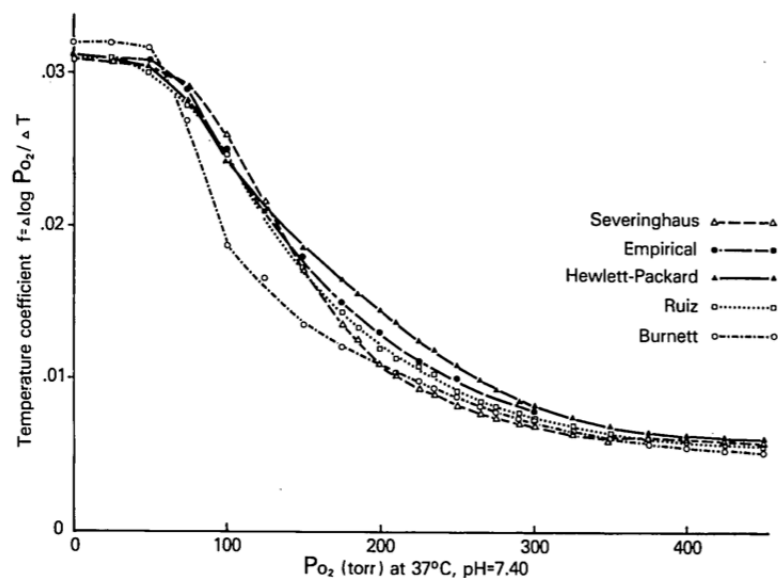
**Figure 1:**

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BLOOD-GAS TEMPERATURE CORRECTION

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Temperature correction coefficient,  $f_{\Delta T}$ , as a function of oxygen tension, estimated by the equations of Severinghaus, Ruiz, Burnett, and Hewlett-Packard. Empirical curve based upon original observations of Severinghaus.



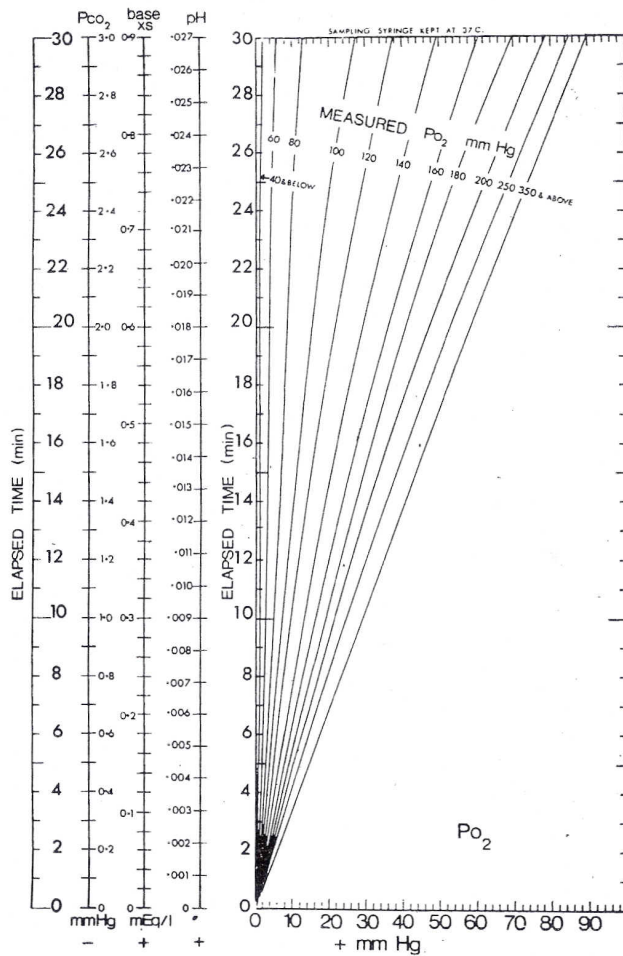
With cooling, pH is increased, and partial pressure of Oxygen ( $pO_2$ ) decreases. The opposite of this is true with warming.<sup>3</sup>

Research has shown that the fall of oxygen content is linear with time. Kelman's nomograms was constructed with a mean value of  $9.0 \times 10^{-5}$  ml / ml per min at  $37^\circ\text{C}$ .

This is based on a haemoglobin concentration of 14.8 g/100ml and a standard oxyhemoglobin dissociation curve, from Severinghaus' work. The rate of decline of  $pO_2$  for a given oxygen consumption varies with the oxyhaemoglobin saturation and with the haemoglobin concentration.<sup>3</sup>

The Nomograms are also based on the assumption that the leucocyte and reticulocyte counts (in the case of  $pO_2$ ) and the haemoglobin concentration, are within the normal range.<sup>3</sup>

Figure 2:



Nomogram for correction of blood  $PCO_2$ , base excess, pH, and  $PO_2$ . Syringe maintained at 37 C between sampling and analysis. The size of the  $PO_2$  correction is greater at higher measured oxygen tensions and is indicated on the abscissa.

In vivo metabolism in a blood specimen slows with decrease in temperature. Halting metabolism is possible by abruptly cooling samples to between 0 and 4 °C.<sup>10</sup>

### 4.3. Effect of syringe material

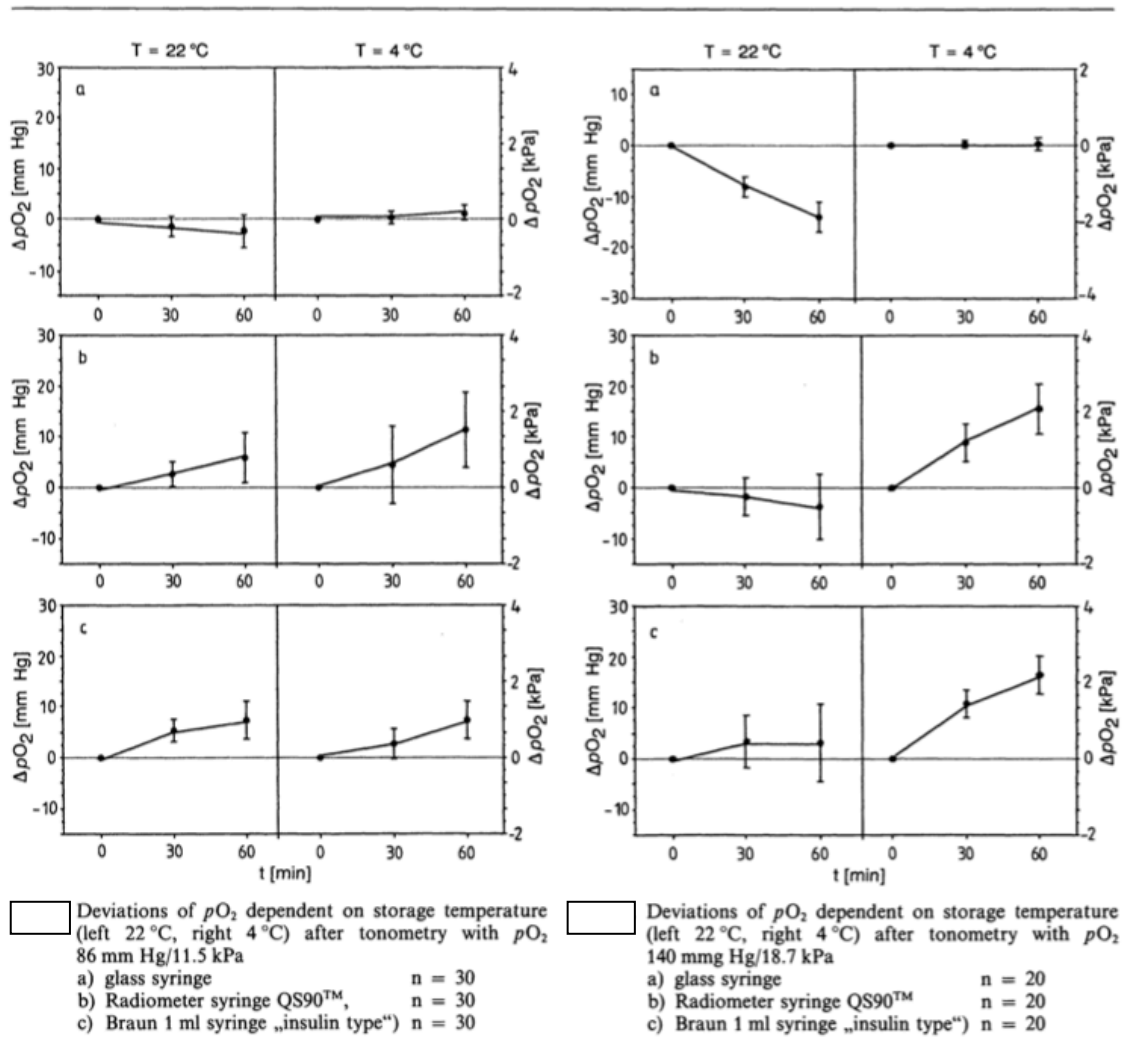
Standard blood gas syringes used in our hospitals are made of plastic and contain dry Heparin. Glass syringes are no longer in use, mainly for safety reasons. Plastic syringes are however not tight to gases. With a reduction in temperature from 37 °C to 4 °C, the solubility coefficient for pO<sub>2</sub> rises from 0.0214 to 0.0395. The affinity of haemoglobin for oxygen also increases with a reduction in P<sub>50</sub>. (The P<sub>50</sub> is the oxygen tension at which haemoglobin is 50% saturated). When whole blood is cooled in this way, the P<sub>50</sub> is reduced from 26.5 mm Hg/3.5 kPa to 4.5 mm Hg/0.61 kPa, at a normal pH. This causes a reduction in the pO<sub>2</sub> and as a consequence a greater gradient between the environment and the sample will develop. This leads to an influx of oxygen, permitting that the pO<sub>2</sub> in the sample is lower than the ambient pO<sub>2</sub> of normally 150 mm Hg/20 kPa. The reverse of this process ensues when the sample is again reheated to 37°C. The p<sub>50</sub> and solubility will return to their original values at 37 °C. This releases the exogenous oxygen, which leads to a falsely increased pO<sub>2</sub>.<sup>10</sup>

Work done by Schmidt, shows a tendency for the pO<sub>2</sub> to increase as a result of this equilibration when plastic syringes are used, especially when it is cooled.<sup>10</sup>

(Figure 3 & 4) When blood is stored in glass syringes, there is very little deviation in the pO<sub>2</sub>.

Figure 3:

Figure 4:



#### 4.4. Effect of raised WCC on $pO_2$

Blood gas analysis in ICU, is one of the most frequently performed tests. It is primarily done to determine the adequacy of gas exchange, by measuring the  $pO_2$ . This measurement can however be affected by metabolically active cells – these include white blood cells and/or platelets.<sup>7</sup>

The consumption of oxygen continues well after blood has been withdrawn from the bloodstream, but this is usually not detectable. This is due to the relatively low amount of these cells in patients without leukocytosis.<sup>11</sup>

No literature regarding the effect of sepsis-induced leukocytosis was found. The only

literature obtained was in regards to patients with very high white cell counts due to a myeloproliferative process, e.g. leukemia. All these studies were retrospective and values were usually obtained from patients records.

As far back as 1979, Hess et al demonstrated, that blood stored in a syringe at room temperature, the  $pO_2$  rate of decline was faster in patients with severe leukocytosis and thrombocytosis, compared to control subjects. The negative deviation could be significant enough to result in the incorrect diagnosis of hypoxaemia. In patients with leukemia, this phenomenon of lowered oxygen tension due to ongoing leucocyte oxygen consumption, carries labels that include: "spurious hypoxaemia"<sup>11-15</sup>, "oxygen steal"<sup>9</sup> and "pseudohypoxaemia"<sup>4-9</sup>.

The decline in  $pO_2$  is evident within seconds in these patients and there is a correlation between the rate of decline with both the number and immaturity of the cells. There is a clear increased rate of metabolism of oxygen, in the more immature cells.<sup>16</sup>

The drop can be so abrupt, that accurate results can only be obtained by point-of-care analyzers or continuous blood gas analysis.<sup>8</sup>

It is thought that abnormally low  $pO_2$  values are seen when the white cell count exceeds 50,000/ L.<sup>7</sup>

It has not however been clarified if this decay, seen over time, is exponential or linear with excessively raised cell counts.<sup>14</sup>

Schmidt did however artificially raise the WCC in specimens with set partial pressure of oxygen and then looked at the change of  $pO_2$  over time. The increments were 20, 40 and 60 x 10<sup>9</sup>/l white cell counts. It again showed that the cooled glass syringes is superior in maintaining the original  $pO_2$ . The authors did however see substantial negative deviations when syringes were stored at room temperature. These varied from -1.46 to - 5.87 kPa after 60min and the magnitude was greater with increasing white cell counts.<sup>10</sup> (Figure 5 & 6)

Figure 5:

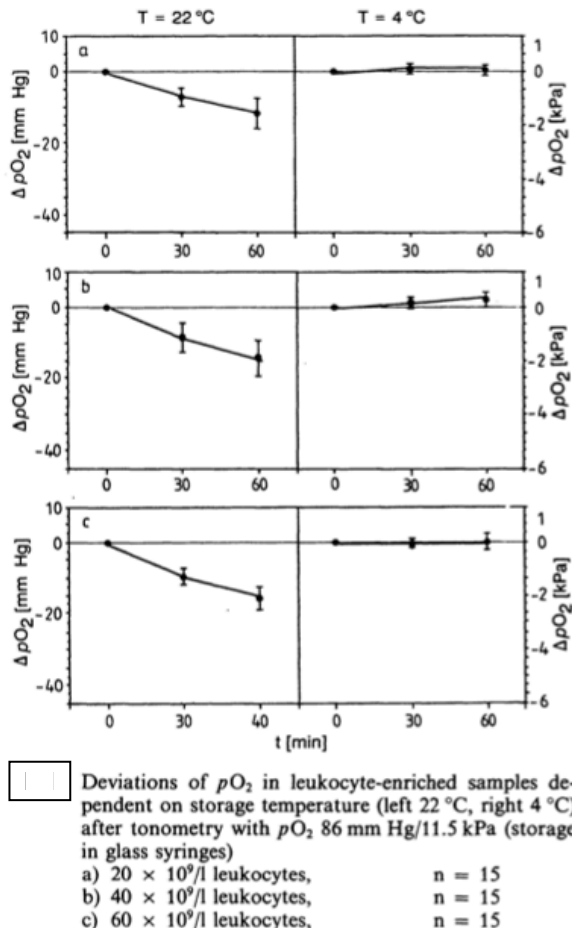
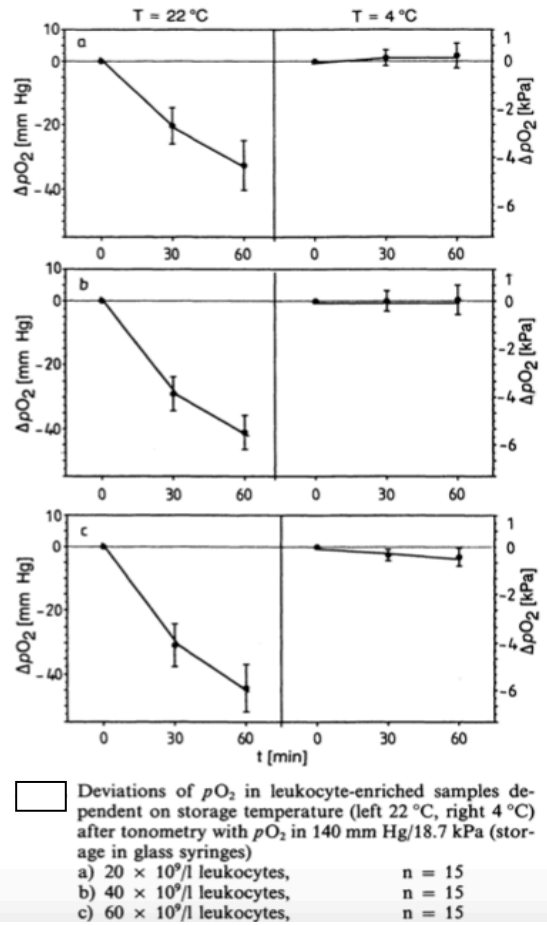


Figure 6:



#### 4.5. Conclusion

From the above literature, it is clear that the correct handling of samples to obtain  $pO_2$  measurement is imperative. Metabolically active cells will continue to consume oxygen after the sample has been collected, as well as equilibration to ambient Oxygen tension if stored in a plastic syringe. Using ice to rapidly cool down a glass syringe would enable one to halt metabolism and use this specimen as the control, therefore isolating the effect that white cell metabolism has on the decline in  $pO_2$ .

Schmidt concluded that when plastic syringes are used, the analysis should be done within 15 minutes. When analysis is likely to fall outside this time reference, glass

syringes should be used and cooled. In severe leukocytosis  $> 40 \times 10^9/l$ , blood should be stored in glass syringes and iced until analysis can be performed.<sup>10</sup>

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## 1: Title Page

# **The influence of delayed sample processing time on the pO<sub>2</sub> values in critically ill patients with sepsis-induced leukocytosis**

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## **Acknowledgements**

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The ICU staff and ICU technologists at Groote Schuur Hospital

There are no conflicts of interest to declare

## 2. Structured Abstract

**Background:** The ability to correctly measure the partial pressure of Oxygen is one of the fundamental tests that influence clinical decision making in septic intensive care unit (ICU) patients. The study examined the extent of error over time, from collection to processing, when measuring blood gas samples for pO<sub>2</sub>, pCO<sub>2</sub>, pH, in critically-ill patients with sepsis<sup>1</sup> and metabolically active leucocytosis > 12 000/mm<sup>3</sup> and compared it with a control, where immersing it in ice has stopped metabolism.

**Methods:** Thirty septic ICU patients with confirmed leucocytosis > 12 000/mm<sup>3</sup>, who had routine arterial blood analysed were included in the study. Blood from the standard *PICO50* radiometer arterial blood sampler (2ml) syringe was decanted into two 1ml Glass syringes that was pre-heparinised with 1ml Heparin 1000U – all excess Heparin removed. One syringe was cooled with ice slurry and tested as a control at 60 minutes. The other syringe was used to repeatedly analyse the sample at 0, 10, 30 and 60 minutes. The syringes were sealed with plastecine and a glass capillary tube was used to decant the sample just prior to analysis to fit the analyser. Samples were processed using an *ABL 800* blood gas analyser.

**Results:** The mean absolute difference in pO<sub>2</sub> at 10 minutes was -0.94 kPa (95% CI: -1.48 to -0.4 kPa), at 30 minutes -2.42 kPa (95% CI: -3.10 to -1.75 kPa) and at 60 minutes -4.44 kPa (95% CI: -5.54 to -3.34 kPa). The relative difference in pO<sub>2</sub> at 10 minutes was -4.98% (95% CI: -8.12 to -1.84%), at 30 minutes -13.79% (95% CI: -17.40 to -10.17%) and 60 minutes -25.46% (95% CI: -30.97 to -19.95%)

The absolute difference in pO<sub>2</sub> between 0 and 60 minutes on ice was -0.32 kPa (95% CI: - 0.95 to 0.31 kPa).

**Conclusion:** Delayed blood gas analysis in Septic ICU patients with a raised WCC > 12 000/mm<sup>3</sup>, results in a statistical and possible clinical significant abnormality in the pO<sub>2</sub>, that progressively worsens with time. After 10 minutes there was a 5% change, at 30 minutes a 14% change and a 25% change from baseline pO<sub>2</sub> at 60 minutes. The magnitude of change with statistical mixed

linear models, shows the rate of decline to be of the magnitude of 1% per minute. So at 60 minutes, the ratio change is 0.7313859 (0.9948 to the power of 60). This deviation may alter clinical decision making.

**Keywords:** Sepsis, Hypoxaemia, delayed blood gas analysis, pO<sub>2</sub>, leukocytosis

### 3. Main text

#### Introduction

The partial pressure of gases and the acid-base balance measured in an arterial blood sample changes over time. The extent of this change depends on the time between taking and analysing the sample.<sup>2</sup> These changes are due to two processes. Firstly, the blood cools. The analyser compensates for loss of heat by warming the sample to 37°C.<sup>3,4</sup> Secondly, white cells in the blood continue to use oxygen and produce carbon dioxide and acid. Reference nomograms<sup>5</sup> designed to compensate for ongoing white cell metabolism are not derived from critically ill patients.

White cells are often more numerous and metabolically active in critically ill patients with sepsis.<sup>1</sup> Reference nomograms are derived from healthy populations.

The early detection of Respiratory compromise in septic patients is very important. A clear distinction between low PaO<sub>2</sub> values due to sampling error versus low values due to early onset respiratory compromise, (prior to clinical signs) is a very important distinction.

The authors aimed to set out to investigate the extent of decline in pO<sub>2</sub> over time in samples taken from septic patients with a white cell count (WCC) >12000/mm<sup>3</sup> and compare it to halting white cell metabolism by placing the sample in ice.

## Methods

Descriptive laboratory study that was done from September 2015 to March 2016 at Groote Schuur Hospital, Cape Town, South Africa. It included patients from the Medical and Surgical ICUs. The protocol was approved by the Human Research Ethics Committee of the University of Cape Town, HREC REF 534/2015.

Deferred consent was sought from participants.

On given study days, all ICU patients with sepsis, as defined by the *International Sepsis Definition Conference*<sup>1</sup>, with a raised white cell count of  $>12000/\text{mm}^3$ , on the basis of their routine early morning laboratory white cell count, and who had arterial blood analysed, were included.

Once routine samples were drawn for blood gas analysis, the study samples were obtained with the remaining amount of blood. One study subject's sample was tested at a time.

Blood from the standard *PICO50* radiometer arterial blood sampler (2ml) syringe was decanted into two 1ml Glass syringes that were pre-heparinised with 1ml Heparin 1000U – all excess Heparin removed. All air was removed and the syringes capped with plasticine.

One syringe was cooled with ice slurry and tested as a control at 60 minutes. The other syringe was used to repeatedly analyse the sample at 0,10,30 and 60 minutes. Glass syringes are incompatible with the machine; therefore to enable analysis, the sample was decanted into a glass capillary.

Samples were processed using the same *ABL800* blood gas analyser.

## Statistical analysis

A power calculation was done, based on data and results from previous studies. The mean  $\text{pO}_2$  decreases by 1.33 kPa in 30 minutes with a standard deviation of 0.67 kPa. A normal distribution of samples using a dependent sample model, with each subject being its own control, required a number of 23 samples in each group. Due to the great variation in the numbers in previous studies, the recruitment included 30 patients in each group.

Analysis focussed on exploring the change in arterial blood gas over time – where for each subject, one sample is on ice and one is not.

Time points were set at: 0, 10, 30, 60 minutes.

Objectives:

- Compare 0 minute sample with iced sample at 60 minutes.
- Model a pattern of degradation of samples that were not on ice, focusing on relative and absolute changes over time.
- Control for WCC and FiO<sub>2</sub>; where WCC is expected to impact the relative change over time.

T-tests (sign tests) were used for assessing means and medians. Linear mixed models were used for analysing profiles over time.

As the primary outcome is the change in arterial blood gas pO<sub>2</sub> over time, this was the focus for modeling.

For secondary outcomes, the changes in pH and pCO<sub>2</sub>, only descriptive statistics was done.

For each subject, the absolute and relative differences were calculated at each time point, relative to the starting point.

For example, at 30 minutes, the absolute difference is the 30-min measurement minus the 0-min-measurement. The relative difference is this absolute difference divided by the 0-min-measurement, expressed as a percentage.

There was further formal modeling done on the pO<sub>2</sub> samples, which were not kept on ice, over time.

Linear mixed models were applied. In these models, random effects account for the multiple observations per subject. Results are expressed in terms of multiplicative/ratio changes in the mean response, given a change in a predictor. Model fitting was done using the function 'lme' in R, in package nlme. The model fit was assessed through visual inspection of plots.

## Results

The mean absolute difference in pO<sub>2</sub> at 10 minutes was -0.94 kPa (95% CI: -1.48 to -0.4 kPa), at 30 minutes -2.42 kPa (95% CI: -3.10 to -1.75 kPa) and at 60 minutes -4.44 kPa (95% CI: -5.54 to -3.34 kPa). (Figure: 1) The relative difference in pO<sub>2</sub> at 10 minutes was -4.98% (95% CI: -8.12 to -1.84%), at 30 minutes -13.79% (95% CI: -17.40 to -10.17%) and 60 minutes -25.46% (95% CI: -30.97 to -19.95%) (Figure: 2)

The absolute difference in pO<sub>2</sub> between 0 minutes and 60 minutes on ice was -0.32 kPa (95% CI: -0.95 to 0.31 kPa).

Secondary outcomes:

Mean Absolute difference in pH at 10 minutes was 0.02 (95% CI: 0.01 to 0.03), at 30 minutes 0.01 (95% CI: 0.00 to 0.02) and at 60 minutes -0.02 (95% CI: -0.03 to 0.00) (Figure: 3)

Relative difference in pH as a percentage from starting value at 10 minutes 0.3% (95% CI 0.17 to 0.42%), at 30 minutes 0.15% (95% CI: -0.02 to 0.31%) and at 60 minutes -0.22% (95% CI: -0.41 to -0.04%). (Figure: 4)

Mean absolute difference in CO<sub>2</sub> at 10 minutes -0.11 (95% CI: -0.19 to -0.03), at 30 minutes 0.06 (95% CI: -0.04 to 0.17) and at 60 minutes 0.24 (95% CI: 0.12 to 0.37). (Figure: 5)

Relative difference in CO<sub>2</sub> as a percentage from starting value at 10 minutes -2.34% (95% CI -3.98 to 0.69%), 30 minutes 1.74% (95% CI -0.48 to 3.95%) and at 60 minutes 5.93% (95% CI: 3.14 to 8.71%) (Figure: 6)

## Discussion

The descriptive laboratory study shows that delayed sample processing of arterial blood pO<sub>2</sub> measurement, in the critically ill patient in ICU, with sepsis-induced leucocytosis (WCC >12000/mm<sup>3</sup>), may have both clinical and statistical significance.

The main findings indicate a near linear decline in pO<sub>2</sub> over time. (Figure: 1) It equates to an absolute reduction error of roughly 1kPa (5% relative) at 10 minutes, 2.4kPa (14%) at 30 minutes and 4.4kPa (25%) at 60 minutes.

This result is primarily due to the effect of the metabolically active leukocytes present in septic patients that continue their metabolism after the sample has been taken.

To isolate this effect, the samples were kept in glass syringes (due to plastic syringes not being tight to gases).<sup>2, 6, 7</sup> Previous work showed deviations at 30 minutes ranging from -0.8 kPa to + 1.07 kPa when stored at ambient temperatures and an exaggerated response when plastic syringes are stored in ice-slurry with deviations between +0.8 kPa to + 1.8 kPa.<sup>2</sup>

This response can be explained as follows:

With a reduction in temperature from 37 °C to 4 °C, the solubility coefficient for pO<sub>2</sub> rises from 0.0214 to 0.0395.<sup>2</sup> The affinity of haemoglobin for oxygen also increases with a reduction in P50. (The P50 is the oxygen tension at which haemoglobin is 50% saturated). When whole blood is cooled in this way, the P50 is reduced from 3.5 kPa to 0.61 kPa, at a normal pH. This causes a reduction in the pO<sub>2</sub> and as a consequence a greater gradient between the environment and the sample will develop. This leads to an influx of oxygen, permitting that the pO<sub>2</sub> in the sample is lower than the ambient pO<sub>2</sub> of normally 150 mm Hg/20 kPa. The reverse of this process ensues when the sample is again reheated to 37°C. The P50 and solubility will return to their original values at 37 °C. This releases the exogenous oxygen, which leads to a falsely increased pO<sub>2</sub>.<sup>2</sup>

There is between a 4 and 150 times greater chance for Oxygen, under standard temperature and controlled environmental factors, to diffuse across a plastic syringe in comparison to glass. This is mainly due to the difference in pore density of the two substances and the size of the Oxygen molecule.<sup>8</sup>

When blood is stored in glass syringes, there is very little deviation in the pO<sub>2</sub>; it has been shown to negate the effect that plastic could have on the results, as there is very little equilibration to ambient pO<sub>2</sub> values over time.<sup>2</sup>

When storing blood samples, there naturally is a tendency to equilibrate with the ambient temperature. Temperature affects both the solubility and affinity of haemoglobin for oxygen. It therefore makes temperature correction of the measured oxygen tension in blood a complex matter.

Errors therefore originate as a result of any discrepancy between the temperature of the sample and that of the patient at time of analysis. Mathematical equations have been derived to use a correction factor to

compensate for these changes.<sup>4</sup> Research has shown that the fall of oxygen content is linear with time. Kelman's nomograms were constructed with a mean value of  $9.0 \times 10^{-5}$  ml / ml per min at 37°C. It was based on a haemoglobin concentration of 14.8 g/100ml and a standard oxyhaemoglobin dissociation curve, from Servinghaus' work.<sup>5</sup> The rate of decline of pO<sub>2</sub> for a given oxygen consumption varies with the oxyhaemoglobin saturation and with the haemoglobin concentration. The nomograms are also based on the assumption that the leucocyte and reticulocyte counts (in the case of pO<sub>2</sub>) and the haemoglobin concentration, are within the normal range. These nomograms were not derived for septic patients.

The aim of the study was to look at the effect of metabolically active white cells, in septic ICU patients, on the rate of decline of pO<sub>2</sub> over time. Work done in leukemic patients with excessively raised white cell counts, shows "pseudohypoxaemia".<sup>9-15</sup> This is due to white blood cells continuing their metabolism despite being removed from the body. In sepsis, white cells increase in number, not to the extent as with a leukemic process, but are likely more metabolically active due to their activation as part of the host's defence or immune system.

**Formal modelling examining the rate of decline of pO<sub>2</sub> over time. This model aims to predict the rate of decline in any patient that fits the inclusion criteria used in the study population.**

Linear mixed models were applied using statistical methods. In these models, random effects account for the multiple observations per subject.

It is also hypothesised that WCC and FiO<sub>2</sub> are confounders in this process, and that WCC impacts the growth rate (not only the starting point). Therefore, in the model, WCC and FiO<sub>2</sub> were allowed to impact a subject's starting point, and WCC was additionally allowed to impact the rate of change of pO<sub>2</sub> (through an interaction term).

A likelihood ratio test was used to compare a smaller nested model to a larger model. Large p-values suggest the simpler model is sufficient.

Results are expressed in terms of **multiplicative / ratio changes in the mean response**, given a change in a predictor. Normal approximations of estimated model parameters are used to obtain 95% confidence intervals and p-values. A

ratio change of 1 would correspond with no impact by the predictor (null hypothesis). Small p-values indicate that there is evidence against the null hypothesis (i.e. there seems to be relationship between predictor and response).

### Fitted model

	Exp beta	CI Lower	CI Upper	p-value
Time	0.9948	0.9938	0.9958	< <b>0.0001</b>
Time:WCC_cent	0.9989	0.9983	0.9996	<b>0.0024</b>
WCC_cent	1.0391	0.9687	1.1146	0.2724
FiO <sub>2</sub> _cent	0.9560	0.9151	0.9986	0.0436

#### 1: Time:

When the WCC is at its mean value, for every additional minute, the pO<sub>2</sub> value drops to 0.99 from what it was - i.e a 1% reduction (95% CI: 0.9938 to 0.9958, P-value < 0.0001)

The small p value is evidence of this relationship.

Therefore for an additional 60 minutes, the ratio change is 0.7313 (0.9948<sup>60</sup>) (or a 27% reduction)

This fits with the measured values of pO<sub>2</sub> at 60 minutes, which showed a mean reduction of 25% at 60 minutes.

#### 2: Time: WCC\_cent:

For every 5 units increase in WCC, there is a further ratio change in the mean pO<sub>2</sub> value of 0.9989 (95% CI: 0.9983 to 0.9996, P-value 0.0024)

For example a further decrease of about 0.11%

#### 3: WCC\_cent:

There is little evidence that WCC influences the starting value of pO<sub>2</sub> – which fits in with clinical physiology.

#### 4: $\text{FiO}_2$ \_cent:

All else being equal, for every 5 unit increase in  $\text{FiO}_2$ , the mean response is 0.96 times what it was (i.e. 4% reduction). (95% CI: 0.9151 to 0.9986, P-value 0.0436) – When putting this contradiction into a clinical context; with increasingly diseased lungs, gas exchange is compromised; the need arises to increase the  $\text{FiO}_2$  to maintain  $\text{PO}_2$ . It therefore explains this association that the increase in  $\text{FiO}_2$  was associated with a reduction in  $\text{PO}_2$  rather than the expected increase.

#### Model Fit

To predict the rate of decline in any patient that fits the inclusion criteria used in the study population.

**Figure 7 & 8** Shows the plots of the fitted model and data for each subject.

**X-axis** represents **time** and the **Y-axis** represents the **log measurement**.

- The black line is the fitted population-level trajectory - which is based on time, the WCC and  $\text{FiO}_2$  of each subject.
- The red line is the fitted subject-specific trajectory after taking fitted random effects into account. There is deviation from population-level trajectory because of the subject - specific intercept and slope.
- The black dots shows the data points.

**The plots show that the fitted model trajectories closely align with the data.**

The model fit was further assessed through visual inspection of plots looking at **random effects-intercept and slope to frequency** as well as **plots of residuals by fitted values** looking for any patterns in mean or variability of residuals. (Figure 9 & 10) The plots ideally should be approximately normally distributed. It does show that the modelling of inter-subject variability is not ideal. A plot of residuals by fitted values, should not show any patterns in mean or variability of residuals. (Figure 11) This seems to fit in with the model.

The model would therefore enable one to predict the rate of decline, due to leukocyte metabolism of oxygen in ICU patients with sepsis, when blood gas analysis is delayed.

The limitations of this study, is mainly related to the small sample size. A potential source of error<sup>16</sup> during the study was due to the fact that samples had to be decanted from the *PICO50 radiometer* arterial blood sampler (plastic syringe) into glass syringes after collection in ICU. Although care was taken with speedily decanting the sample and immediately capping it with plasticine, exposure to air was unavoidable. Prior to processing, each sample again had to be decanted into a glass capillary, as the machine was not compatible with glass syringes. Equilibration with ambient PO<sub>2</sub>, although for only a few seconds, could have altered some of the values. The process was uniform for every set of samples.

Each patient acted, as his or her own control. This was achieved by placing a second decanted glass syringe sample, in ice slurry. By cooling the sample, metabolism comes to a halt,<sup>17</sup> and negates the effect of on-going leucocyte metabolism.

Therefore we examined the differences between measurements at the start (0 minutes) and the iced values at 60 minutes. The mean absolute difference between 0 and 60 minutes on ice was -0.32 kPa (95% CI: - 0.95 to 0.31 kPa). (Figure 12)

A histogram was used to assess the distributions of differences. Small p-values indicate evidence against this null hypothesis.

The p-value = 0.3449. (95% CI: 0.215 to 0.594). The probability of success, which is the estimate for the probability of a difference being greater than 0, was 0.392. (Figure 13)

This proves the difference between the starting values and that of the sample kept on ice, is centred around zero. There is therefore no trend towards either a higher or lower measurement than the starting value. Although the two measurements were not equal, the small deviation from zero in the iced sample, is in stark contrast with the substantial deviation in the non-iced values.

It reinforces the superiority of glass syringes, when storing blood on ice to enable the cessation of metabolism and maintaining the values close to its starting values.

When assessing results of the secondary outcomes, there was very little change in pH over the 60 minutes period.

The CO<sub>2</sub> however, although initially decreasing at 10 minutes showed a nearly 2% increase at 30 minutes and 6% increase by 60 minutes. This fits with the production of CO<sub>2</sub> with on-going metabolism. The clinical relevance of this, is negligible.

### **Conclusion**

This clinical laboratory study demonstrates that there is a steady rate of decline of pO<sub>2</sub> when blood gas analysis is delayed in septic ICU patients due to on-going white cell metabolism. Care should be taken, in resource-constrained environments, in the correct handling of blood samples. If a delay is expected, thought should be given to storage of the sample in either a glass syringe or capillary and appropriately capped. It should be cooled in ice slurry, only when glass storage is available, to halt on-going white cell metabolism.

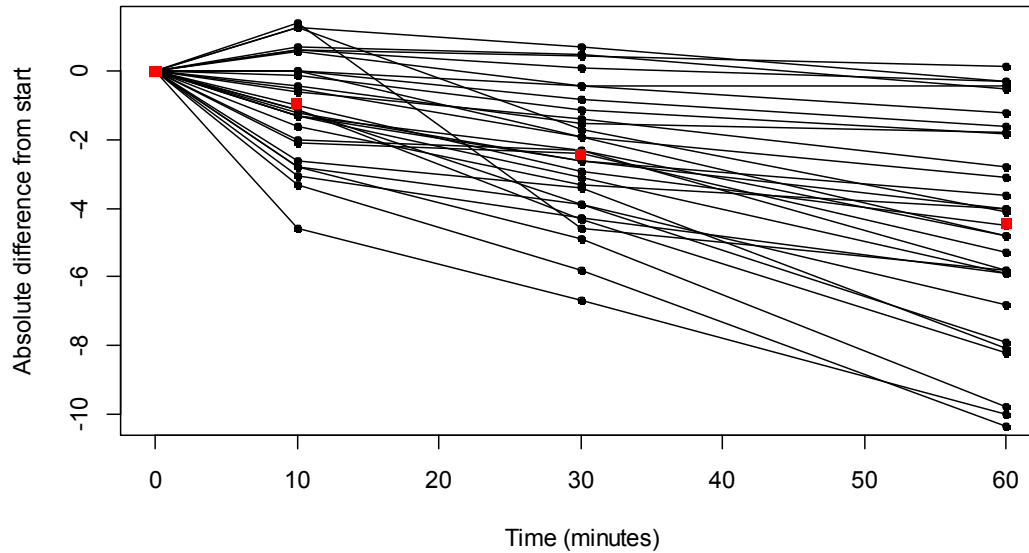
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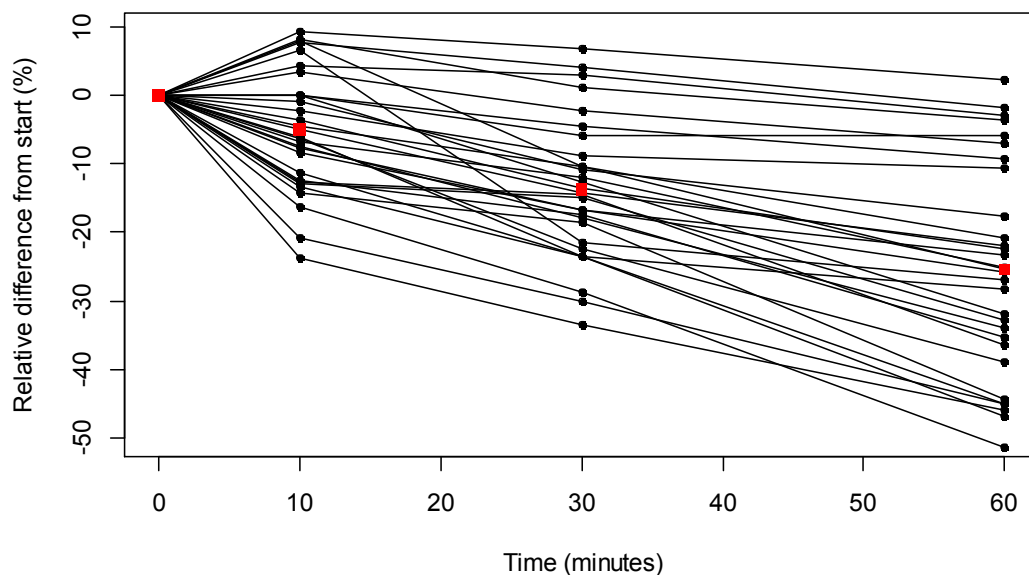
#### 4. Figures and tables

**Figure: 1**  
pO<sub>2</sub>



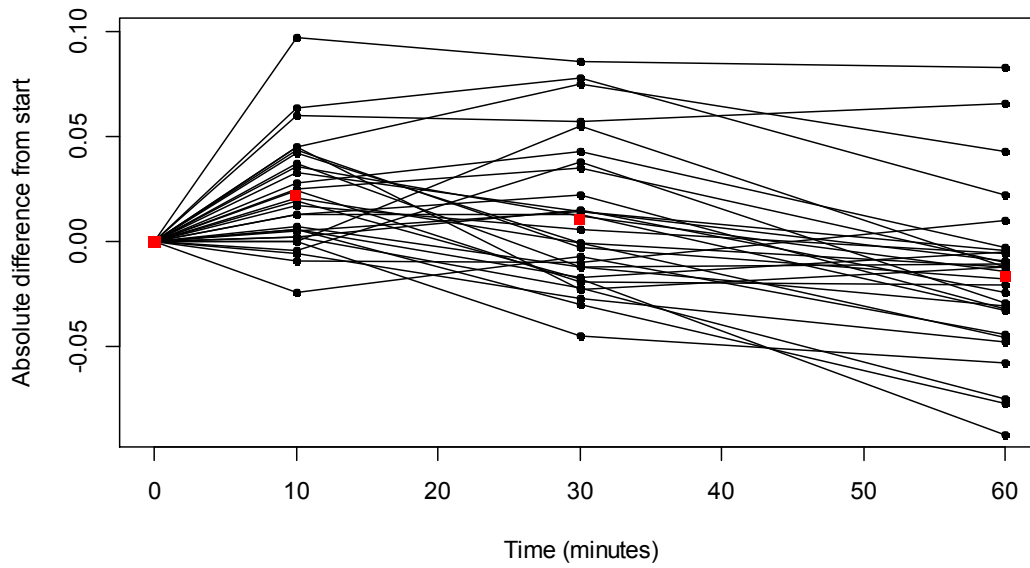
Group	Mean	Std dev	N	95% CI lower limit	95% CI upper limit
1	0	0.00	30	0.00	0.00
2	10	-0.94	30	-1.48	-0.40
3	30	-2.42	30	-3.10	-1.75
4	60	-4.44	30	-5.54	-3.34

**Figure: 2**  
pO<sub>2</sub>



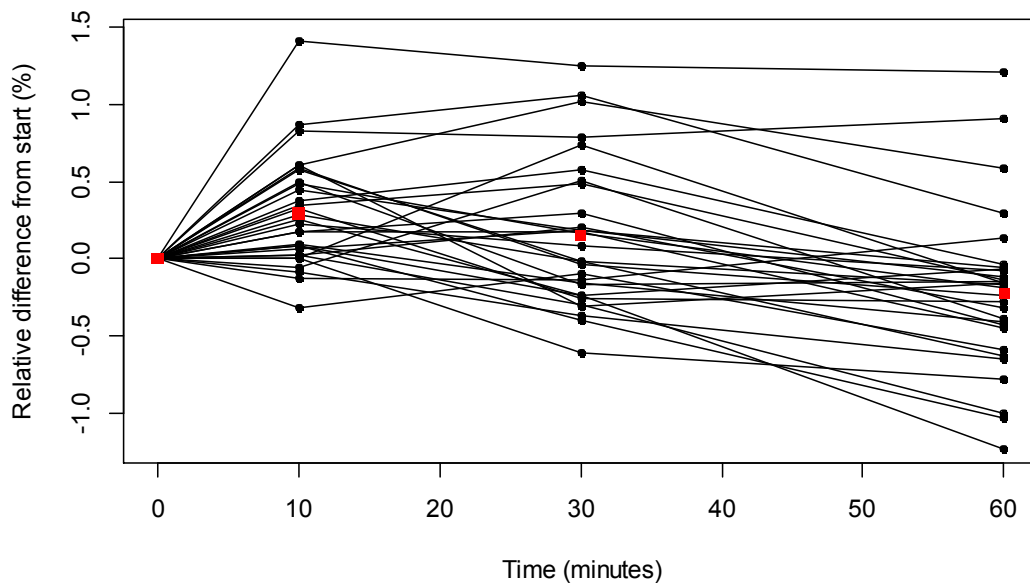
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1	0	0.00	30	0.00	0.00
2	10	-4.98	30	-8.12	-1.84
3	30	-13.79	30	-17.40	-10.17
4	60	-25.46	30	-30.97	-19.95

**Figure: 3**  
pH



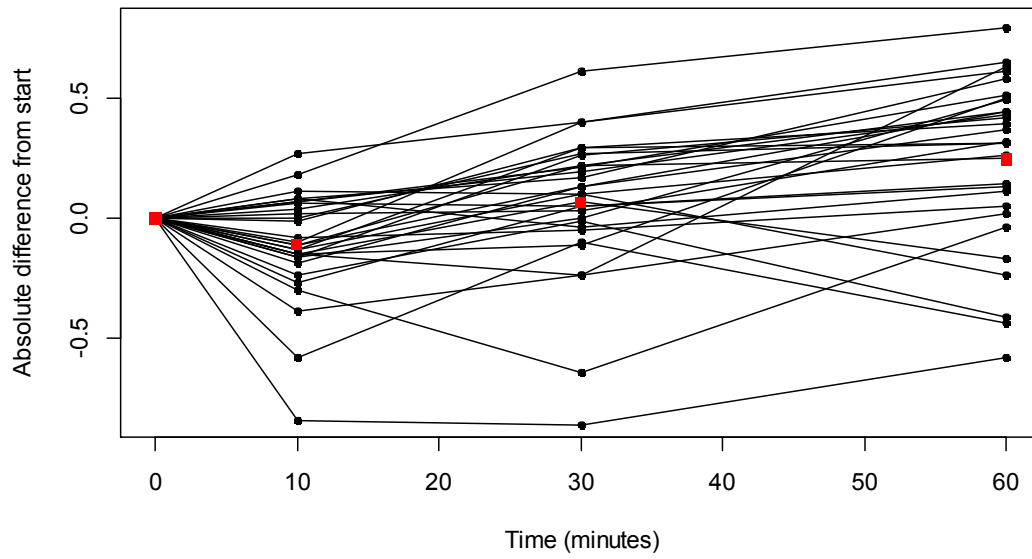
Group	Mean	Std dev	N	95% CI lower limit	95% CI upper limit
1	0	0.00	30	0.00	0.00
2	10	0.02	30	0.01	0.03
3	30	0.01	30	0.00	0.02
4	60	-0.02	30	-0.03	0.00

**Figure: 4**  
pH



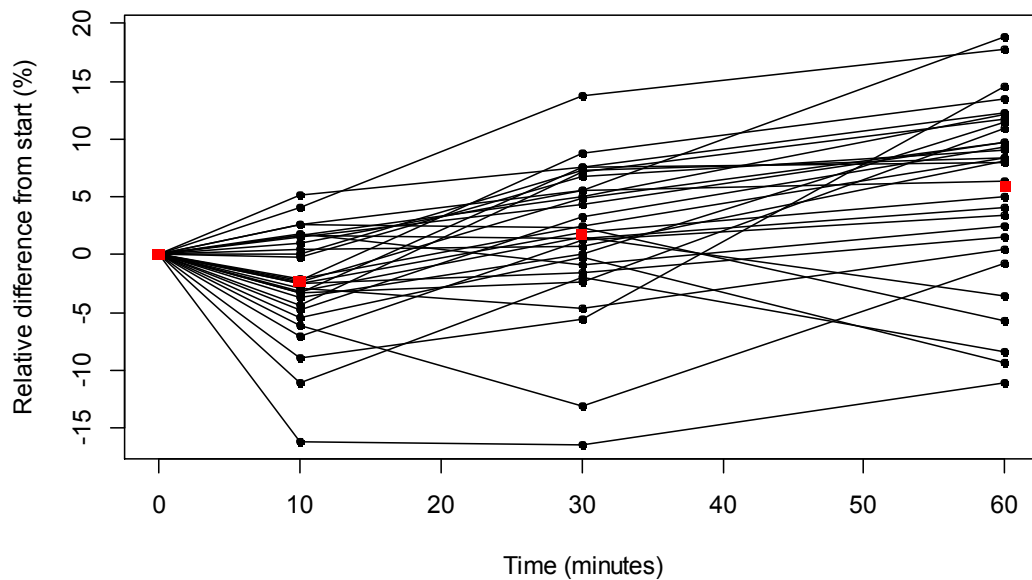
Group	Mean	std dev	N	95% CI lower limit	95% CI upper limit
1	0	0.00	30	0.00	0.00
2	10	0.30	30	0.17	0.42
3	30	0.15	30	-0.02	0.31
4	60	-0.22	30	-0.41	-0.04

**Figure: 5**  
pCO<sub>2</sub>



Group	Mean	Std dev	N	95% CI lower limit	95% CI upper limit
1	0	0.00	30	0.00	0.00
2	10	-0.11	30	-0.19	-0.03
3	30	0.06	30	-0.04	0.17
4	60	0.24	30	0.12	0.37

**Figure: 6**  
pCO<sub>2</sub>



Group	Mean	Std dev	N	95% CI lower limit	95% CI upper limit
1	0	0.00	30	0.00	0.00
2	10	-2.34	30	-3.98	-0.69
3	30	1.74	30	-0.48	3.95
4	60	5.93	30	3.14	8.71