

# Interpreting CSF Lactic Acidosis: Effect of Erythrocytes and Air Exposure

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## ABSTRACT

**Objective:** Elevated cerebrospinal fluid (CSF) lactate concentrations in neurotrauma and sub-arachnoid haemorrhage are associated with a poor prognosis. However, in blood-stained CSF, elevated lactate levels may arise from red cell metabolism, even without ischaemia, potentially reducing specificity. This study was undertaken to quantify the erythrocyte contribution to CSF lactate measurements, with and without, exposure to room air.

**Methods:** Blood was added to CSF to achieve three different red cell concentrations. The CSF was then exposed at 37°C to either room air or 5% CO<sub>2</sub> and 95% oxygen. Vancomycin and gentamycin were added to inhibit bacterial growth. Lactate concentrations and red cell concentrations were measured prior to the addition of blood and 10 minutes, 6 hours and 24 hours later. CSF without the addition of blood was used as a control.

**Results:** In the control specimens there were no increases in CSF lactate concentrations over time, either in air or CO<sub>2</sub>, whereas all specimens with blood added demonstrated significant increases in lactate at 6 and 24 hours ( $P < 0.01$ ). The lactate increases in both air and CO<sub>2</sub> were correlated directly with red cell counts ( $R^2 = 0.62$  to  $0.87$ ). At all red cell concentrations, the mean lactate increase was greater in air.

**Conclusions:** Red cells in CSF cause significant increases in lactate concentrations, more so when exposed to air. This should be considered when interpreting lactate in blood stained CSF. Blood-stained CSF specimens for lactate assay should be collected directly from an external ventricular drain rather than a reservoir bag. (**Critical Care and Resuscitation 2003; 5: 177-181**)

**Key words:** Cerebrospinal fluid, lactate, alkalosis, drain, haemorrhagic, ventricular

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Cerebrospinal fluid (CSF) lactic acidosis is an important predictor of outcome in a variety of neurological and neurosurgical diseases including head injury<sup>1-3</sup> and subarachnoid haemorrhage.<sup>4</sup> In these conditions elevated CSF lactate concentrations have been regarded as arising primarily from central nervous system anaerobic metabolism.<sup>5</sup> The normal lactate concentration in the CSF is about 1 - 1.5 mmol/L. Nearly all of this arises from cerebral metabolism, since normal CSF is relatively acellular with minimal intrinsic metabolic activity. As lactate is an ionized molecule, diffusion across the blood brain

barrier in health is slow.<sup>6</sup>

Elevations in the measured CSF lactate concentration may result from a number of intrinsic and extrinsic processes other than anaerobic metabolism. For example, in subarachnoid haemorrhage, secondary to trauma or aneurysmal rupture, rises in CSF lactate may result from ongoing erythrocyte metabolism in the CSF. Outside the CSF, erythrocyte glycolysis contributes substantially to the total body pool of lactate<sup>7</sup> and this metabolic activity may continue unimpeded in the CSF compartment. Moreover, the frequency and extent of CSF recirculation (normally 4

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- 5 times/day) may be reduced in disease states.<sup>8</sup>

The combination of continued erythrocyte production and impaired lactate clearance from the CSF may elevate CSF lactate even in the absence of cerebral anaerobic metabolism. Froman *et al*,<sup>9</sup> have examined the impact of erythrocyte contamination on CSF lactate concentrations, although the "mock CSF" preparation they used may have biased their results due to the short duration of observations, specimen haemolysis and testing using a single concentration of haemoglobin.

Another possible influence on CSF lactate measurements is the mode of CSF sampling. In many intensive care units, critically ill patients with raised intracranial pressure (ICP) undergo placement of an external ventricular drainage system to monitor the ICP and to sample CSF. An external ventricular drainage system consists of a ventricular catheter connected via sterile tubing to a Y-piece, one limb of which is used to measure the ICP while the other drains into a reservoir bag via a burette. Blood stained CSF collected from reservoir bags may have higher lactate concentrations than CSF collected directly from the ventricular catheter, due to lactate generated by erythrocytes while awaiting sampling. Furthermore, an acquired *in vitro* respiratory alkalosis (as CO<sub>2</sub> is lost to room air) may potentiate erythrocytic phosphofructokinase activity, accelerating glycolysis and lactate production.<sup>10</sup> These factors may have had an impact on previous studies examining the prognostic value of CSF lactate in neurotrauma and subarachnoid haemorrhage as most workers tend not to specify the precise site of sampling.

The interpretation of lactate concentrations in blood-stained CSF may require an estimate of the lactate rise attributable to red cells. This estimate should take into account the magnitude and duration of the contamination and the added impact of an *in vitro* respiratory alkalosis. Such information would also help determine optimum sites and timing of sampling from a ventricular drainage system. However, published data are lacking. We undertook a study to:

- 1) quantify the erythrocyte contribution to CSF lactate measurements and,
- 2) assess the impact of room air exposure on CSF lactate levels in blood stained samples.

## METHODS

The hospital Human Research Ethics Committee approved the protocol. CSF used in the study was that drained and discarded from a patient as part of a normal intracranial pressure management protocol. Permission to use the fluid was given by the next of kin. On microscopy the CSF was found to be free of

red cells immediately after collection, with < 5 white cells/mm<sup>3</sup>.

Thirty mL was incubated at 37°C in four incubating chambers, two of which were exposed to room air and the other two to a mixture of 5% CO<sub>2</sub> and 95% oxygen. Vancomycin (100 mg) and gentamicin (40 mg) were added to inhibit bacterial growth. After 20 minutes of incubation, the first samples were collected on ice for analysis (Time point 1). Blood was then added to the residual CSF in the incubating chambers to generate erythrocyte concentrations of 120, 200 and 445 x 10<sup>9</sup>/L, simulating the range of values usually found in subarachnoid haemorrhage. CSF without blood was used as a control. After adding blood, further samples were placed on ice at the following time points: 10 minutes (Time point 2), 6 hours (Time point 3) and 24 hours (Time point 4). Red cell counts, gas tensions, glucose and lactate concentrations were measured and all samples were subjected to microscopy and culture.

For cell count analysis, 2 mL of CSF was collected in a sterile chamber and examined in acrylic graduated counting chambers ("Fast-read 102 New Grid"), which are used to count cells by the Neubauer haemocytometer method.<sup>11</sup> The individual performing the cell count was blinded to the expected red cell concentration.

Two mL samples of CSF were collected anaerobically from each incubating chamber and analysed in triplicate for pH, PCO<sub>2</sub> and lactate concentrations in a blood gas analyser (ABL 625, Radiometer, Copenhagen). This analyser has previously been validated for measurement of PCO<sub>2</sub> and PO<sub>2</sub> in CSF.<sup>12</sup> In preparation for this study, we also determined the bias and precision for lactate measurement in non-blood fluids, and found it to be  $-0.2 \pm 0.5$  mmol/L (range 1 - 10mmol/L).

All specimens were plated on solid media, and simultaneously cultured in broth for 24 hours followed by solid media subculture if broth-positive.

## Data Analysis

Data from the CSF exposed to room air and carbon dioxide are referred to as the 'air arm' and the 'CO<sub>2</sub> arm' of the study respectively. Where appropriate, data are presented as mean  $\pm$  SD. Change in lactate from baseline was termed ' $\Delta$ lactate'. Analysis of variance of repeated measures, accompanied where indicated by paired t-tests, was used to compare mean  $\Delta$ lactate values under different environmental conditions over the 4 time points. The relationship between measured CSF erythrocyte count and  $\Delta$ lactate was examined by linear regression. A p value of < 0.05 (two-tailed) was taken as significant.

## RESULTS

Actual red cell counts ranged from 100 to 512 x 10<sup>9</sup>/L. The overall mean pH in the 'air arms' exceeded that in the 'CO<sub>2</sub> arms' (7.72 ± 0.01 vs 7.28 ± 0.01, p < 0.001). The difference was presumably as a result of differing CO<sub>2</sub> tensions (14 ± 4 mmHg vs 38 ± 11 mmHg, p < 0.001). Glucose concentrations for all specimens ranged from 2.8 mmol/L to 5.2 mmol/L.

Mean baseline lactate concentrations in the specimens with blood added ranged from 1.3 ± 0 mmol/L to 2.1 ± 0.0 mmol/L. In control specimens, there was no increase in CSF lactate concentrations over time, either in the air arm or the CO<sub>2</sub> arm. All specimens with blood demonstrated a statistically significant increase in lactate at Time points 3 and 4 (Figures 1 and 2, p < 0.01). Δlactate values in both air and CO<sub>2</sub> at Time points 3 and 4 were correlated directly with corresponding measured red cell counts (R<sup>2</sup> = 0.62 to 0.87).

At all red cell concentrations the mean Δlactate was greater in the air arm, both at 6 hours and at 24 hours (Time points 3 and 4, Table 1).

Some mixed bacterial growth was identified in both control and CSF samples with blood added. The colony count appeared similar in all samples, although direct quantification was not performed.

## DISCUSSION

The principal finding was that blood contamination elevates CSF lactate concentrations over time, at a rate directly correlated with the degree of contamination and increased by pre-analytic CO<sub>2</sub> loss. Presumably erythrocytic glycolysis was the predominant source of CSF lactate production, as the erythrocyte produces about 300 mmol of lactate per day by glycolysis.<sup>13-15</sup> However, alternative mechanisms may be responsible.

Platelet metabolism is negligible, and unlikely to elevate CSF lactate. On the other hand, leukocytes are known to generate lactate and on a weight for weight basis are the most potent source of lactate in the normal human. However because of small numbers,

their contribution to the total lactate pool is negligible. The mean resting leucocyte production of lactate is 0.2 fmol/cell/min.<sup>16</sup> At this level of metabolic activity, based on the expected numbers of CSF white cells introduced with the addition of blood, the lactate generated would have been approximately 35 - 125 micromoles per day, resulting in concentrations 100 x lower than those detected in the present study.

Haemolysis, rather than glycolysis, is unlikely to have caused an increase in CSF lactate, as the intra-erythrocytic lactate content in 1 mL of red cells is 0.9 micromoles.<sup>17</sup> Therefore the maximum increase in CSF lactate due to haemolysis (at the highest introduced red cell concentration) would have been 0.04 mmol/L, and substantially less than that measured. A final potential source of lactate in this study is bacterial growth and metabolism. Bacterial growth was identified in both control and blood CSF samples, and the colony count appeared to be similar in all samples. However, lactate levels rose only in the blood stained samples, suggesting that blood contamination was the dominant stimulus.

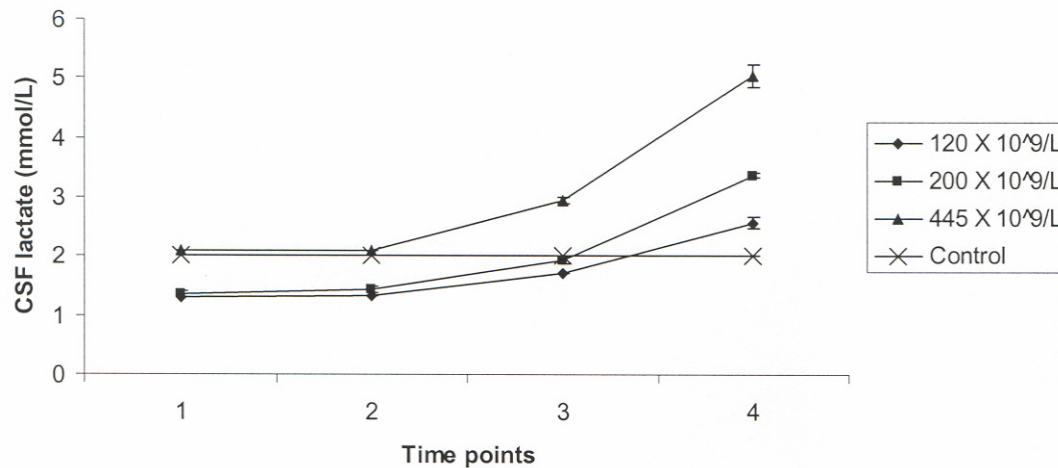
Froman *et al*,<sup>9</sup> documented erythrocytic lactate production in a mock CSF solution. However, bacterial contamination was a more prominent influence than in our experiment, and no data were presented on serial measurements of lactate. Shannon and co-workers confirmed CSF erythrocyte lactate production in a canine model.<sup>18</sup> However, they injected blood with a haematocrit as high as 70%, and did not replicate physiological conditions. Moreover serial measurements were curtailed at 6 hours. Granholm examined the effect of blood admixture with CSF on the lactate/pyruvate ratio and confirmed that despite elevations in lactate and pyruvate, there was no change in the ratio. However, in that study, measures to limit microbiological contamination of the samples were not undertaken, and no data on bacterial growth were presented. Furthermore, neither the effect of storage nor the effect of CO<sub>2</sub> loss on CSF lactate production were studied, both of which are important considerat-

**Table 1. Δlactate values (mmol/L) at various red cell concentrations in CSF exposed to air or CO<sub>2</sub>**

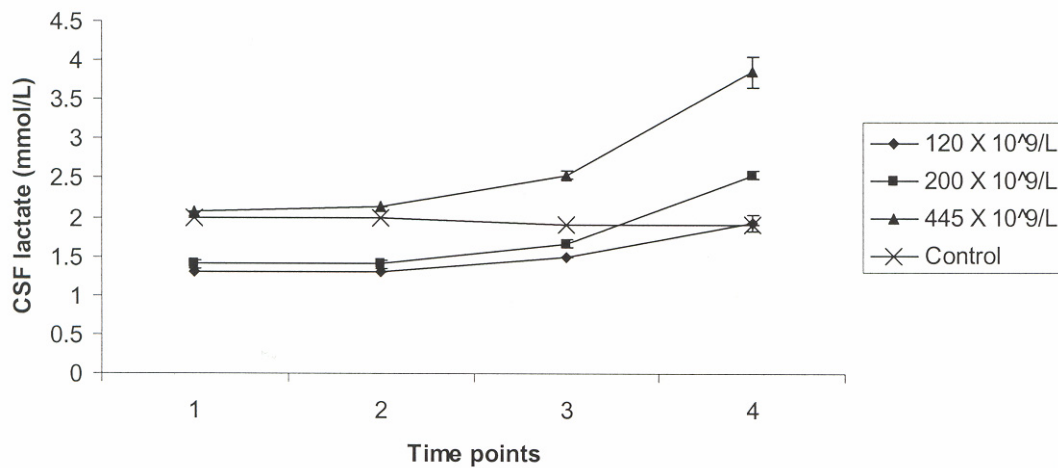
RBC count	Time point 2		Time point 3		Time point 4	
	Air	CO <sub>2</sub>	Air	CO <sub>2</sub>	Air	CO <sub>2</sub>
120 x 10 <sup>9</sup> /L	0.02 ± 0.04	0	0.4 ± 0*	0.2 ± 0	1.3 ± 0.1*	0.6 ± 0.2
200 x 10 <sup>9</sup> /L	0.1 ± 0.1	0	0.6 ± 0.1*	0.3 ± 0.1	2 ± 0.1*	1.1 ± 0.1
440 x 10 <sup>9</sup> /L	0	0.02 ± 0.04	0.8 ± 0.1*	0.5 ± 0.1	2.9 ± 0.2*	1.8 ± 0.5

RBC = red blood cell, Time point 1 = before addition of RBC, Time point 2 = 10 minutes following addition of RBC, Time point 3 = 6 hours following addition of RBC, Time point 4 = 24 hours following addition of RBC

\*Δlactate greater in air than CO<sub>2</sub> (P < 0.05)



**Figure 1.** Changes in cerebrospinal fluid lactate concentration at different red cell counts following exposure to air. Triangles, squares and diamonds represent CSF red cell concentrations of  $445 \times 10^9$ ,  $200 \times 10^9$  and  $120 \times 10^9$  respectively.



**Figure 2.** Changes in cerebrospinal fluid lactate concentration at different red cell counts following exposure to CO<sub>2</sub>. Triangles, squares and diamonds represent CSF red cell concentrations of  $445 \times 10^9$ ,  $200 \times 10^9$  and  $120 \times 10^9$  respectively.

ions when examining samples from ventricular drains or reservoir bags

#### Clinical implications

Before applying the results to clinical practice, it is important to put these data into perspective. Lactate production by erythrocytes is influenced by ambient glucose concentrations. Diffusion across the blood brain barrier *in vivo* maintains equilibration with plasma glucose, whereas in our *in vitro* model no diffusible source of glucose was available, potentially limiting the rise in lactate. In fact there was a tendency for glucose to decrease over time, with the lowest measured glucose concentration being 2.58 mmol/L at Time point 4. As a result, our data may underestimate the lactate increment occurring *in vivo* on introduction of erythrocytes into the CSF.

There is also a potential for gravitational red cell sludging *in vivo*, with pockets of high concentrations of red cells. Sampling such an area might reveal higher lactate concentrations. In all other respects, care was taken in our study to simulate physiological conditions. The erythrocyte concentrations mimicked those seen in patients with subarachnoid haemorrhage and the temperature was maintained at 37°C. Furthermore, the 'air arm' of the study simulated atmospheric conditions which would prevail in the reservoir bag of an external ventricular drainage system.

An elevation in brain extracellular lactate concentrations have been reported to be a marker of ischaemia and secondary brain injury<sup>1</sup> and prognosticate a poor outcome.<sup>2</sup> It might therefore be logical to titrate therapies aimed at reducing cerebral metabolism (such as induced hypothermia and pentobarbital

coma<sup>19</sup>) using brain extracellular lactate to monitor the effect. Although our data relate to lactate measurements in CSF specifically rather than extracellular fluid, the results do have implications for lactate analyses on brain extracellular fluid examined via microdialysis. Both techniques have potential for blood contamination and exposure to air.

Data from our study suggest that the erythrocytic contribution to CSF lactate becomes significant at 6 hours, with progressive increases up to 24 hours later. The study has also shown that pre-analytic exposure of CSF to air doubles the lactate production, presumably due to CO<sub>2</sub> loss. This means that lactate concentrations in blood-stained CSF collected fresh from an external ventricular drain are likely to reflect *in vivo* levels more accurately than CSF collected from reservoirs. These caveats need to be considered when interpreting lactate concentrations in haemorrhagic CSF.

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