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Unrecognized Preanalytical Problem with the Spectrophotometric Analysis of Cerebrospinal Fluid for Xanthochromia

To the Editor:

The primary diagnostic test for subarachnoid hemorrhage (SAH)[1] is a computed tomography scan, but because SAH is found in a small percentage of patients with negative computed tomography results, lumbar puncture (LP) and spectrophotometric analysis of cerebrospinal fluid (CSF) for xanthochromia are still needed to make decisions about angiography *(1)*. Oxyhemoglobin (O_2Hb) is released from erythrocytes (RBCs) in the CSF in vivo and converted to bilirubin in the leptomeninges in a time-dependent process. Although the trauma of LP frequently produces RBCs in CSF and RBC lysis leads to the presence of O_2Hb, no bilirubin is formed in such cases. The presence of bilirubin is considered the most specific sign of SAH, and a LP is recommended 12 h after the appearance of symptoms to test for the presence of bilirubin *(2, 3)*. O_2Hb and bilirubin have absorbance peaks at 413–415 nm and 450–460 nm, respectively, but because of the overlap in absorbance wavelengths with O_2Hb, bilirubin is preferentially measured at 476 nm *(2–4)*. Nevertheless, avoidance of hemolysis ex vivo will improve the analysis of bilirubin in CSF and may make O_2Hb a more reliable marker of bleeding. We conducted experiments to elucidate why RBCs lyse in CSF.

A CSF pool was prepared from nonpathologic CSF samples and stored at −80 °C (approval from regional ethics committee not required). After thawing, RBCs that had been obtained from volunteers with informed consent were added to the CSF pool and a physiological saline solution to final RBC concentrations of 20–100 × 10^9/L. We then centrifuged vials with the solutions at 2000*g* for 5 min immediately and at hourly intervals up to 4 h after RBC addition and measured hemolysis by monitoring the absorbance at 415 nm (Thermo Spectronic Unicam UV300). Fig. 1 shows the considerable RBC- and time-dependent hemolysis values we obtained. In physiological saline, only slight hemolysis was observed, even after 4 h (maximum, 0.039 absorbance units; data not shown). Interestingly, the pH of the CSF

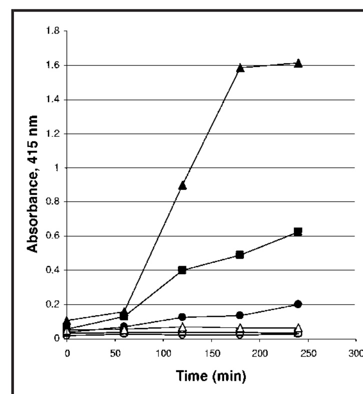

Fig. 1. Hemolysis of RBCs added to a pool of CSF during a 4-h incubation at ambient temperature.

Hemolysis was measured by monitoring the supernatant absorbance at 415 nm. The 3 upper curves are results for the original CSF pool (pH 8.8), and the 3 lower curves are results obtained after preincubating the CSF pool in a 5% CO_2 atmosphere, producing an approximate pH of 7.4. RBCs were added to final concentrations of 20 × 10^9/L (●), 50 × 10^9/L (■), and 100 × 10^9/L (▲) in the CSF pool at pH 8.8, and to final concentrations of 20 × 10^9/L (○), 50 × 10^9/L (□), and 100 × 10^9/L (△) in the CSF pool at pH 7.4.

[1] Nonstandard abbreviations: SAH, subarachnoid hemorrhage; LP, lumbar puncture, CSF, cerebrospinal fluid; O_2HB, oxyhemoglobin; RBC, erythrocyte (red blood cell).

pool was 8.8 (Radiometer ABL725), and HCO_3^- and the P_{CO_2} were undetectable. Thus, the pH increase was likely caused by the evaporation of CO_2 during handling and storage. After we subsequently incubated the CSF pool in a CO_2 chamber containing 5% CO_2, the pH decreased to approximately 7.4, reflecting physiological pH. When we repeated the experiment described above at this pH, we found almost no hemolysis (Fig. 1), even at the highest RBC concentration.

We then obtained 53 consecutive routine CSF samples from neurosurgical patients suspected of SAH and analyzed the samples within 1–2 h of collection. The mean (SD) pH was 8.07 (0.12). Hemolysis is also considerable in CSF at pH 8.0, approximately 20%–40% of the hemolysis observed at pH 8.8 during the first hour, although the increase is less at subsequent hours. Thus, routine samples obtained for spectrophotometric analysis most often have a pH at which preanalytical hemolysis takes place.

When 1 mL CSF (equilibrated to pH 7.4) was added to 6- to 10-mL vials usually used for LP, the pH increased at a rate of 0.01 pH units/min during the first hour (slightly faster during the first 30 min), which is equivalent to an increase in the pH to 7.8–8.0 after 1 h. Agitation of the vial speeds up this process. The pH changes were much less in a 2-mL vial containing 1 mL CSF, and the physiological pH is conserved if the vial is completely filled.

The results show that the amount of hemolysis depends at least partly on the pH of the CSF sample and that the pH of CSF increases ex vivo upon exposure to atmospheric air owing to the evaporation of CO_2. If the volume of air above the CSF in the vial is small and the vial is capped quickly, changes in pH are reduced substantially, with almost negligible hemolysis ex vivo. Surprisingly, no publications have described this preanalytical problem, although several have shown that the presence of RBCs after a traumatic tap may cause a falsely increased O_2Hb in the CSF sample *(3, 5)*.

Evaporation of CO_2 is well known to lead to a pH increase in fluids containing HCO_3^-. The nonbicarbonate buffering capacity in CSF is low because of the low protein concentration, and the pH increases quickly if the sample is exposed to air. The use of a small vial completely filled with CSF and capping the vial immediately after the LP will minimize the problem and improve the analysis, because the presence of O_2Hb may affect the ability to accurately detect bilirubin. This simple step may even improve the reliability of detecting O_2Hb as a marker of SAH sooner than the prescribed 12 h, but the impact of this approach needs to be shown in a clinical setting.

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Diagnosis of Metachromatic Leukodystrophy by Immune Quantification of Arylsulphatase A Protein and Activity in Dried Blood Spots

To the Editor:

Metachromatic leukodystrophy (MLD),[1] an autosomal recessive neurodegenerative disease resulting from a deficiency of arylsulfatase A (ASA), results in the lysosomal accumulation of sulfatide in several peripheral organs, notably the central nervous system. The

[1] Nonstandard abbreviations: MLD, metachromatic leukodystrophy; ASA, arylsulfatase A; ASA-PD, ASA pseudo-deficiency; DBS, dried blood spots.