pool was 8.8 (Radiometer ABL725), and \(\text{HCO}_3^-\) and the \(\text{PCO}_2\) were undetectable. Thus, the pH increase was likely caused by the evaporation of \(\text{CO}_2\) during handling and storage. After we subsequently incubated the CSF pool in a \(\text{CO}_2\) chamber containing 5\% \(\text{CO}_2\), 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 neu-rosurgical 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 \(\text{CO}_2\). 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 \(\text{O}_2\text{Hb}\) in the CSF sample (3, 5).

Evaporation of \(\text{CO}_2\) is well known to lead to a pH increase in fluids containing \(\text{HCO}_3^-\). The non-bicarbonate 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 \(\text{O}_2\text{Hb}\) may affect the ability to accurately detect bilirubin. This simple step may even improve the reliability of detecting \(\text{O}_2\text{Hb}\) 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.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References


Søren Risom Kristensen* Anna Maria Salling Susanne Tofting Kristensen Annebirthe Bo Hansen

* Department of Clinical Biochemistry Cardiovascular Research Center Aalborg Hospital Aarhus University Hospital Aalborg, Denmark
3 Department of Biochemistry Genetics and Pharmacology Odense University Hospital Odense, Denmark

* Address correspondence to this author at: Department of Clinical Biochemistry Cardiovascular Research Center Aalborg Hospital Aarhus University Hospital DK-9000 Aalborg, Denmark
Fax +45 9813 1196 E-mail srk@rn.dk

DOI: 10.1373/clinchem.2008.107367

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 arylsulfa-tase 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.
carrier frequency of MLD at 1:152, this frequency will lead to a prevalence of compound heterozygotes as high as 1:2300. Diagnostic laboratories are often required to perform additional complex sulfatide assays or molecular analysis to obtain a definitive diagnosis.

We have developed and evaluated 2 immune-based assays that enable the differentiation of MLD individuals from individuals with ASA-PD and unaffected controls. These assays use an immune-quan-
tification assay to quantify the amount of ASA protein and an immune-capture activity assay to determine enzyme activity. Both assays are performed on 3-mm dried blood spots (DBS) collected on filter paper.

Affinity-purified sheep anti-ASA polyclonal antibody was produced and Eu³⁺-labeled as previ-
uously described (3). Recombinant human ASA protein was expressed in a CHO-K1 expression system from a full-length ASA cDNA clone in HT 14/CP 8 Bluescript® vector. Calibrators and quality control materials were prepared by diluting recombinant human ASA in working buffer (0.1 mol/L sodium acetate/acetic acid, 0.1% heat-treated BSA, pH 5.0) or Delfia® assay buffer (Perkin-Elmer Life Sciences) to measure ASA activity and protein, respectively.

All DBS were stored at −20 °C before analysis. The institute’s ethics committee approved use of the patient samples. ASA protein from DBS was assayed using a single 3-mm disk as described previously (3). A calibration curve (2.0–1000 pg/well) was included in each assay. ASA activity in DBS was determined as described previously (4), using two 3-mm disks per assay, 0.1 mol/L sodium acetate/acetic acid, 0.1% heat-treated BSA, pH 5.0, as elution buffer; 20 mmol/L sodium acetate/acetic acid, pH 5.0, as wash buffer; and 5 mmol/L 4-methylumbelliferyl sulfate in 0.2 mol/L sodium acetate/acetic acid, pH 5.0, 0.1% BSA (100 μL/well) as substrate. A calibration curve using recombinant human ASA (2.0 –800 pmol/h/well) was included in each assay.

The ASA protein and activity assays gave linear responses over the biological range ($R^2 > 0.999$). The detection limits were 0.8 pg/well and 1.5 pmol/h/well. The intra- and interassay CVs were 9% and 10%, respectively, for the protein assay and 6% and 13% for the activity assay. The median amount of ASA protein in whole blood from unaffected, ASA-PD, and heterozygote individuals was 31.9 μg/L (range 21.0–46.3 μg/L), 13.0 μg/L (range 8.3–17.0 μg/L), and 12.5 μg/L (range 12.0–13.0 μg/L), respectively; no detectable ASA protein was observed in MLD individuals (Fig. 1A). The median ASA activity in unaffected and hetero-
zygote individuals was 4.16 μmol/min/L (range = 1.48–7.72 μmol/min/L) and 2.62 μmol/min/L (range = 2.44–2.80 μmol/min/L); no detectable activity was observed in ASA-PD or MLD individuals (Fig. 1B).

ASA activity did not enable differentiation of ASA-PD individuals from MLD patients; however, the ASA protein quantification assay enabled clear differentiation between controls and ASA-PD and MLD individuals. To further investi-
gate why ASA-PD showed no ASA activity, we analyzed DBS from an unaffected individual. This DBS was stored at room temperature for up to 30 days, and during this period ASA activity decreased such that only 39% of activity remained after 30 days; in contrast, no decrease was observed in the ASA protein. Measured ASA protein and activity in heterozy-
gotes were both in the lower end of the reference interval.
The ability to use DBS to measure ASA protein and activity will simplify procedures for collection, handling, and storage of patient samples. The ease of transporting DBS, combined with the diagnostic sensitivity and specificity of these assays, provide a powerful approach to the diagnosis of MLD. After further investigation and validation, these assays may be used for newborn screening for MLD and other lysosomal storage disorders. Importantly, the inability to differentiate ASA-PD from ASA by the activity assay alone suggests that ASA protein determination from DBS may be the most appropriate screening approach for newborns, as has recently been proposed in a report of a multiplex immune-quantification assay (5).

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the manuscript.

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Research Funding: This work was supported by the National Health and Medical Research Council (Australia). Expert Testimony: None declared.
Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References


Mohd. A. F. Tan2,3,4
Caroline J. Dean2
John J. Hopwood2,3
Peter J. Meikle2,3,5*

2 Lysosomal Diseases Research Unit
Department of Genetic Medicine
Children Youth and Women’s Health Service
North Adelaide, South Australia, Australia
3 Department of Paediatrics
University of Adelaide
Adelaide, South Australia, Australia
4 Department of Chemical Pathology
Universiti Sains Malaysia
Health Campus
Malaysia
5 Present address: Baker IDI Heart and Diabetes Institute
Melbourne, Victoria 3004, Australia

* Address correspondence to this author at: Baker IDI Heart and Diabetes Institute
PO Box 6492, St Kilda Road Central
Melbourne, Victoria 8008, Australia
Fax (613) 8532 1100
E-mail peter.meikle@baker.edu.au

Circulating Matrix Metalloproteinase-7: An Early or Metastatic Marker for Renal Cell Carcinoma?

To the Editor:

We read with great interest the article by Sarkissian et al. (1) reporting the identification of serum matrix metalloproteinase-7 (MMP-7) as a marker for renal cell carcinoma (RCC). For the purpose of identification, the authors used sera from healthy individuals and RCC patients and combined 2-dimensional electrophoresis of an RCC cell-line supernatant and an immunoblotting procedure. Subsequently, the authors measured serum MMP-7 concentrations with a self-developed immunoassay. Higher concentrations were found in RCC patients than in healthy individuals (7.56 vs 2.13 μg/L), whereas serum MMP-7 concentrations in patients with localized RCC did not differ from concentrations in patients with metastatic RCC (7.26 vs 7.87 μg/L). These results suggest that MMP-7 could be a useful marker for detecting RCC during the early stages of the disease. These findings are of utmost importance because at present no blood marker is known for the early detection of RCC.

Continuing our studies on the diagnostic performance of MMPs, we investigated circulating MMP-7 in RCC patients, but our results (2) differed from those of Sarkissian et al. We measured MMP-7 in heparin-plasma samples using Fluorokine multianalyte profiling assays (R&D Systems). In our study MMP-7 concentrations (Fig. 1) in healthy controls were not found to be different from those in RCC patients with localized cancer.