Laboratory methods for evaluating canine porto systemic shunts

CM Smuts*[a], MD Bennett[a], M Sharman[ab], JN Mills[a] and T Gaál[a]
*Corresponding author
[a]School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch, Western Australia, 6150; c.smuts@murdoch.edu.au
[b]Faculty of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia;

ABSTRACT

In dogs with reduced liver function (portosystemic shunting, parenchymal liver disease), metabolic functions of the liver are disrupted, resulting in increased concentrations of ammonia, amino acids and poorly water soluble uric acid in the blood. This is associated with hepatoencephalopathy and excretion of ammonium-urate crystals in the urine. The analytes are measured in the blood and serum and urate crystals are easily recognised in the urine sediment under light microscopy.

Changes in liver function can be documented by measurement of pre and post prandial serum bile acid concentrations and, when available, whole blood ammonia concentration. Measurement of serum amino acid concentrations are routinely performed for people but infrequently applied in animals although testing can be available through human laboratories. Energy dispersive analysis is a new, semi-quantitative method for examining urinary crystals and is available to veterinarians.

This report describes the whole blood ammonia, serum aromatic amino acid concentrations and urinary crystal analysis in a dog diagnosed with a portosystemic shunt. The urine crystals were identified as sodium and potassium urate using energy dispersive analysis. Purchasing a portable ammonia-meter for whole blood ammonia measurement and sending serum samples to human or research laboratories for determination of amino acid concentrations could provide useful additional information for clinicians assessing dogs with hepatic dysfunction. Because many urinary crystals are morphologically similar, crystal analysis can be useful to determine their content and hence their pathogenesis. This information is especially useful when the typical ammonium biurate crystals are absent. *Aust Vet Practit* 2012;42(1):xxx-xxx

A 10-month-old spayed female bichon frise presented with a history of intermittent vomiting, periodic disorientation, agitation, circling and collapse noted since purchase at 10-weeks of age. The dog was anorexic for several days at a time and showed the above clinical signs for two to three days after eating. The referring veterinarian performed a complete blood count, serum biochemistry and a gastrointestinal barium study. Findings on the complete blood count and serum biochemistry were consistent with reduced liver function and the major differential diagnosis was a portosystemic shunt. The dog was referred to Murdoch University Veterinary Hospital for further evaluation.

On admission the dog appeared bright and alert with a body condition score of 3.5/9.[1,2] Complete blood count at admission (Siemens ADVIA® 120 multispecies haematology analyser, Bayer, Germany, with manual evaluation of the blood smear) revealed marginal microcytic, normochromic red cells with mild anisocytosis and occasional codocytes. There was a marginal mature neutrophilia and mild eosinophilia with rare immunoreactive lymphocytes. Serum biochemistry using commercial test kits (Randox Laboratories Ltd, UK) showed increased aspartate aminotrasnferase (AST) and alanine aminotransferase (ALT) activities, decreased urea concentration, hypocholesterolaemia and panhypoproteinaemia (Table 1).

The history, clinical signs and laboratory results supported a diagnosis of reduced liver function. Fasting serum bile acid concentration, serum uric acid concentration (Vetpath Laboratories, Perth, Western Australia), whole blood ammonia concentration (Ammonia test kit II strips; benchtop PocketChem BA blood ammonia meter, Arkray Inc, Japan)[3] and serum aromatic amino acid concentration, specifically phenylalanine, tyrosine and methionine (New South Wales Biochemical Genetics Service) were also measured. Since reference intervals for serum amino acid concentrations in dogs are not available, serum from a clinically normal age and sex-matched dog was also submitted. Results showed markedly elevated fasting serum bile acid, whole blood ammonia and serum uric acid concentrations consistent with reduced liver function. Marked elevation in the serum concentration of phenylalanine, tyrosine and methionine was consistent with abnormal amino acid metabolism in this dog (Table 2).

Urinalysis showed minimally concentrated urine (specific gravity 1.014) and mild alkaluria (pH 8). Two types of crystals were identified by light microscopy. The crystals had morphology consistent with tiny sodium urate and tyrosine-like crystals (Figure 1). No ammonium biurate crystals were observed. Urine sediment was dried onto an aluminium stub and scanned by electron microscopy (Philips XL20 scanning electron microscope, Oxford Instruments Pty Ltd, England) followed by energy dispersive analysis (Oxford electron energy dispersive analysis system, Oxford

Table 1. Complete blood count and serum biochemistry analysis for a 10-month-old spayed female bichon frise diagnosed with an intrahepatic portosystemic shunt

COMPLETE BLOOD COUNT			SERUM BIOCHEMISTRY		
Analyte	Results	Reference interval	Analyte	Results	Reference interval
Haemoglobin g/L	125	129-184	CK U/L	182	47 – 228
Haematocrit L/L	0.43	0.37-0.57	AST U/L	203	10 – 60
RBC $x10^{12}$	7.46	5.7-8.8	ALT U/L	158	21 – 142
MCV fL	57.2	58.8-71.2	ALP U/L	182	20 – 184
MCHC g/L	320	310-362	Urea mmol/L	2.3	3.6 – 10
WBC $x10^9$/L	13.0	5.2-13.9	Creatinine µmol/L	57	44 – 132
Neutrophils $x10^9$/L	8.5	3.9-8.0	Cholesterol mmol/L	2.2	3.3 – 6.9
Lymphocytes $x10^9$/L	2.6	1.3-4.1	Bilirubin µmol/L	2	0-8
Eosinophils $x10^9$/L	0.8	0-0.6	Glucose mmol/L	6	3.6-6.8
Monocytes	1.1	0.2-1.1	Protein g/L	46.3	56 – 80
Platelets $x10^9$/L	228	143-400	Albumin g/L	21.8	24 – 38
			Globulins g/L	24.5	28 – 44

Instruments Pty Ltd, England). Tiny, needle-like crystals (Figure 2) were detected with approximate atomic ratios (32% carbon, 28% nitrogen and 23% oxygen) indicative of urate (ratio of C5:N4:O3).

The crystals also contained potassium and sodium ions (potassium and sodium urate crystals) and sulphur. In all spectra studied, a significant chloride-peak (mean of 5.4%) was found which is of unknown origin and significance (Figure 3).

Abdominal ultrasound examination and computed tomography revealed a right divisional intrahepatic portosystemic shunt. The owner declined surgery. The dog was managed with oral lactulose, oral antibiotics and a modified low-protein diet. The owners report the dog is free from clinical signs 18 months after diagnosis.

DISCUSSION

Dogs with reduced liver function due to portosystemic shunting or other causes are unable to convert all the ammonia produced from protein metabolism to urea, resulting in high blood ammonia concentrations, altered amino acid concentrations and hepatic encephalopathy.[4] Uric acid produced from nucleic acid purine metabolism is poorly water soluble and cannot be metabolized to allantoin which is a highly water soluble compound normally excreted in the urine. Consequently, elevated serum concentrations of uric acid and ammonia lead to urate crystalluria which are typically ammonium biurate. Interestingly in this dog, which had no "classic", easily recognizable ammonium biurate crystals, the energy dispersion analysis did identify urate crystals but these contained sodium, potassium and a lower amount of calcium ions, similar to findings in people with uric acid renal calculi.[5] Why ammonium biurate crystals are found in most dogs with portosystemic shunts but not all is unclear and may be due to

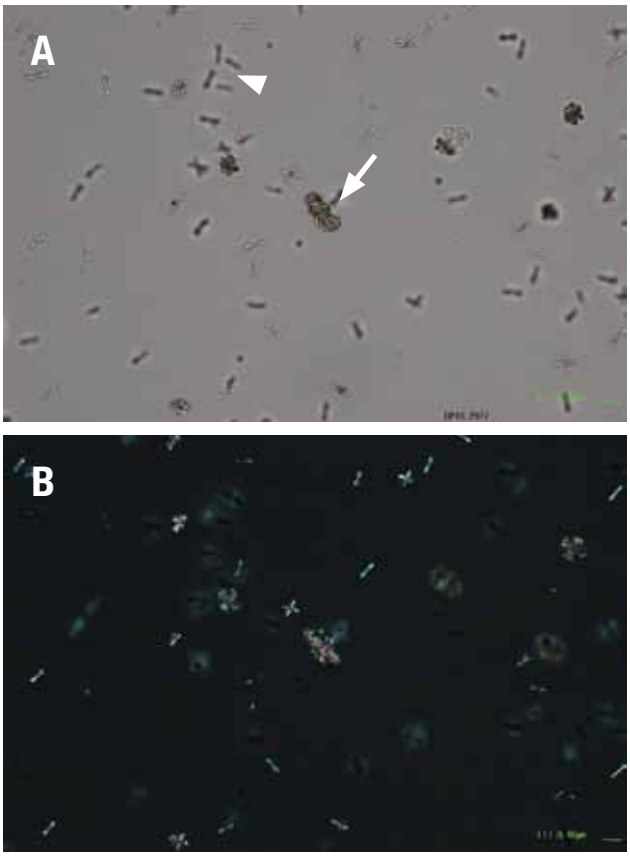

Figure 1. Light microscopy of urate (arrow) and tyrosine-like (arrowhead) urinary crystals in the urine from a 10-month old female spayed bicon frise diagnosed with an intrahepatic portosystemic shunt. x400 magnification. A: bright field. B: polarized light

Figure 2. Scanning electron micrograph of the urate crystals in the urine sediment from a 10-month-old spayed female bichon frise diagnosed with an intrahepatic portosystemic shunt

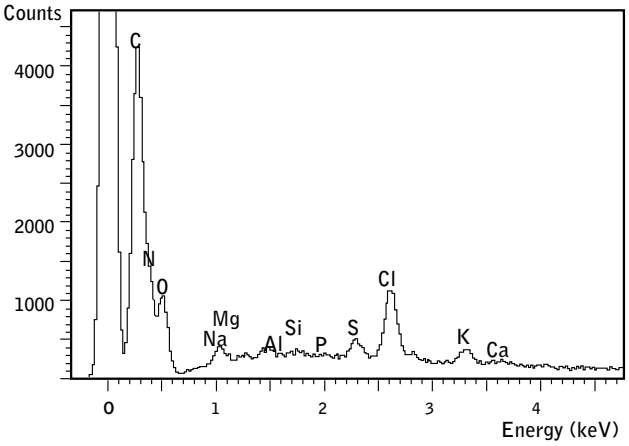

Figure 3. Energy dispersion trace of carbon, nitrogen, oxygen, sodium, sulphur, chloride and potassium present in different proportions in the urate crystal complex from a 10-month-old spayed female bichon frise diagnosed with an intrahepatic portosystemic shunt

Table 2. Serum and whole blood biochemistry analysis of bile acids, ammonia, uric acid and aromatic amino acids concentrations for a 10-month-old spayed female bichon frise diagnosed with an intrahepatic portosystemic shunt

Analyte	Results	Reference interval
Bile acids µmol/L (fasting)	270	<20
Ammonia µmol/L (whole blood)	412	< 60
		Concentration for matched control
Uric acid µmol/L	50	10
Phenylalanine µmol/L	260	73
Tyrosine µmol/L	169	45
Methionine µmol/L	93	52

variations in urine pH or different precipitation characteristics of Na/K urate and ammonium urate.[6,7] Energy dispersive analysis is available in laboratories with electron microscopy, including most veterinary schools and our institution.

Accurate whole blood ammonia measurement requires minimal delay between sampling and analysis. The PocketChem BA analyser for measurement of ammonia on whole blood has been validated for dogs.[3] Assessment of blood ammonia concentrations can provide information on liver function in dogs for which serum bile acid measurements are unreliable such as those with concurrent bilirubinaemia and for Maltese terriers.[8] The ammonia analyser is readily available on the market and would be a useful addition to the biochemistry laboratory of a practice. It may be useful in mixed animal practice with application for ruminants.

Serum concentrations of phenylalanine, tyrosine and methionine in this dog were elevated compared to those of a clinically normal age and sex-matched dog. In people with reduced liver function, amino acid metabolism is adversely affected and there is an increase in serum concentrations of aromatic amino acids with a decrease in concentrations of branched-chain amino acids.[9] Elevated concentrations of aromatic amino acids contribute to clinical signs of hepatic encephalopathy since they act as neurotransmitters or interfere with normal neurotransmitter function.[10,11] Measurement of amino acid concentrations can provide a diagnostic method for investigating animals with central neurologic signs. Analysis is readily available in private and university veterinary clinical pathology laboratories.

The sulphur present in the urine crystals in this dog may be due to the elevated serum concentrations of methionine and other unmeasured sulphur-containing metabolites. Although methionine is readily reabsorbed by the kidney,[12] sulphur-containing amino acids can be metabolised to produce sulphates that are excreted in the urine.[13] The high concentration of chloride in the urine remains unexplained, however may be the result of decreased bicarbonate excretion to maintain electrical neutrality in the presence of ammonium-induced acidosis.[14] Unfortunately acid-base status was not assessed in this dog to verify acidosis.

Measurement of whole blood ammonia concentration, and serum uric acid and amino acid concentrations are additional biochemical analyses available to veterinarians to support the clinical diagnosis of reduced liver function. Their measurement may also be useful in monitoring the response to therapy. Electron energy dispersive analysis of urine sediment can help to semi-quantify crystal composition which can further define the metabolic abnormalities in the dog with reduced liver function.

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