WHAT IS YOUR DIAGNOSIS?

A three-year old female entire Shar Pei was presented to the R(D)SVS Internal Medicine Service for investigation following a diagnosis of azotaemia and proteinuria. The dog had been recently treated for a urinary tract infection with amoxicillin-clavulanic acid, and the treatment was stopped two weeks prior to referral. She had a 2-year history of intermittent and recurrent episodes of lethargy, pyrexia, lameness of the hind legs, and swollen, hot hock joints. The signs responded to non-steroidal anti-inflammatory drugs. She was up-to-date with vaccinations and deworming, and had no history of travel outside Scotland.

At the time of admission, the dog was bright, alert and responsive with good body condition. Mucous membranes were pink and moist. Cardiac auscultation revealed a heart rate of 100 beats/min and a regular rhythm with strong matching pulses, while no murmur was detected. The respiratory rate was 24 breaths/min, with normal effort and no adventitious sounds. Percussion of the chest and abdominal palpation were unremarkable. Peripheral lymph node, and joint palpation were unremarkable. Rectal temperature was 38.7°C. Rectal, neurological, and orthopaedic examinations were unremarkable. Systemic blood pressure using the Doppler method was mildly elevated (150 mmHg).

The complete blood count was unremarkable and the serum biochemistry revealed the presence of mild hypoproteinaemia (51 g/L, RI: 58-73) due to hypoalbuminaemia (22.6 g/L, RI: 26-35), and azotaemia (creatinine: 212 umol/L, RI:22-115; urea: 15.3 mmol/L, RI: 1.7-7.4). Urinalysis showed low urine specific gravity (1.015), increased urine pH (8), and a strongly positive protein reaction (+4) on dipstick analysis. Urine sediment was unremarkable, and the urine protein:creatinine ratio was markedly increased (UPC = 8.0, RI: <0.2).

1) What are your differential diagnoses for azotaemia, hypoalbuminaemia and proteinuria?

2) How would you evaluate this case further?

3) What are the treatment options for this dog?
1. **Differential diagnosis:**

   a. Selective hypoalbuminemia, may be secondary to inflammation (negative acute phase protein), decreased production (malabsorption/maldigestion, hepatic insufficiency), increased loss (e.g. protein-losing nephropathy [PLN] or enteropathy [PLE], vasculitis), third spacing, or haemodilution.

   b. Increased creatinine and urea concentration with isosthenuria can be related to renal azotemia. Pre-renal and post renal azotemia were considered less likely.

   c. Proteinuria may result from pre-renal, renal or post renal proteinuria. Based on bloodwork and urine analysis a renal proteinuria was considered most likely (protein losing nephropathy). UPC of 8 raised the suspicion of glomerular disease. Differential of protein losing nephropathy includes:

   i. Infectious disease such as leptospirosis, tick borne diseases
   ii. Immune complex glomerulonephritis
   iii. Amyloidosis
   iv. Glomerulosclerosis
   v. Toxic/drug
   vi. Neoplosia
   vii. Metabolic disease such as hyperadrenocorticism

2. **Evaluation**

   Based upon these clinical results, the major findings included the suspicion of protein losing nephropathy. An underlying infectious disease, neoplasia, inflammatory/immune-mediated disease or amyloidosis were suspected most likely. Infection diseases was investigated by testing urine culture, vector borne diseases/ Angiostrongylosis (in house SNAP test) and leptospirosis (microscopic agglutination test and PCR on serum and urine). All infectious disease testing were negative. Thoracic radiographs were unremarkable, no evidence of effusion, infectious or neoplasia were reported. Abdominal ultrasonography revealed a moderately enlarged liver with mildly heterogeneous echotexture, gall bladder sludge, mildly and diffusely stippled splenic echotexture (figure 1). Ultrasound-guided fine needle aspiration of the liver and spleen was performed after testing normal coagulation profile.
The cytology of the spleen revealed a variably-sized aggregates of reticular cells and lymphocytes that appeared embedded in abundant purple, extracellular material arranged in irregular clumps. The same extracellular material was noted on the liver cytology. The cytological diagnosis was suspected amyloid deposition (figures 2.1-2.2).

Figure 1: Abdominal ultrasound of the kidneys, liver and spleen.

Figure 2.1. Cytology from the spleen. May-Grünwald Giemsa. A) x2 objective. B) x40 objective.

Figure 2.2. Cytology from the liver. May-Grünwald Giemsa. A) x10 objective. An abundant, smooth, eosinophilic to purple, extracellular material is noted admixed with the hepatocytes (black arrows). B) x40 objective.
Two of the cytology smears from the spleen and the liver were stained with Congo Red (figure 3). The smooth extracellular material described previously showed positive Congophilic (red) staining when observed by light microscopy in both spleen and liver. The findings on examination of the Congo Red stained smears confirmed the presence of amyloid in both organs.

![Figure 3. Cytology from the spleen. A) An abundant Congophilic (red) material is observed. Congo Red, x10 objective. B) When the same field is examined under polarised light, the Congophilic material exhibits apple green birefringence in the thicker areas (yellow arrows). Congo Red under polarised light, x10 objective.](image)

Core biopsies of the renal cortex were performed under general anaesthesia for optical microscopy, immunofluorescence and ultrastructural examination. The morphological diagnosis was moderate to severe glomerular amyloidosis and marked diffuse acute tubular epithelial injury with frequent tubular proteinosis and atrophic tubules associated with chronic inflammation (figure 4). One core of renal tissue, was evaluated using immunofluorescence and found to be negative for IgG, complement component C3, and IgA, while only a diffuse, global, weak, undefined labelling along capillary walls was detected for IgM. In agreement with the histopathological results, tissue
immunofluorescence findings were not consistent with an underlying immune-mediated glomerulonephritis. Ultrastructural evaluation of a section of renal tissue under transmission electron microscopy revealed moderate glomerular, tubular, and interstitial changes consistent with amyloid (figure 5).

Figure 4. Histopathological section of the kidney. Section at the level of a glomerulus with normal cellularity is depicted. A) Amyloid stains pale pink and waxy in the mesangium and capillary loops with PAS (x20 objective). B) Amyloid stains mottled blue to orange in the mesangium and capillary loops with Masson’s Trichrome (x20 objective). C) Amyloid does not take up silver with JMS stain (x40 objective).
Treatment

Treatment included enalapril (0.5mg/kg, PO, q12 hours) and a renal specific diet for the management of the severe proteinuria, and clopidogrel (4mg/kg, PO, q24 hrs) to prevent thromboembolism. After confirmation of renal amyloidosis on histopathology, colchicine (0.03mg/kg, PO, q24 hours) was added to the treatment protocol. Two weeks after treatment initiation, physical examination was unremarkable and the proteinuria was still evident (UPC: 6.4, RI:<0.2). Enalapril was increased to 1.0mg/kg q 12 hours. Four weeks after treatment initiation, the dog remained clinically stable, but azotaemia (urea: 10.8 mmol/L, RI: 2.5-9.6 mmol/L; creatinine: 254 μmol/L, RI: 44-159 μmol/L) and proteinuria (UPC: 5, RI:< 0.2) were persistent.

Discussion

Amyloidosis is a condition characterised by the extracellular deposition of fibrillar proteins in tissues, and it is associated with a variety of disorders (1). These insoluble fibrils are produced by the aggregation of misfolded proteins, which are otherwise soluble when normally-folded, and their accumulation in tissues can cause damage and loss of functionality (1). Several different amyloid fibril proteins and their respective precursors have been identified in both human and veterinary medicine (2). In human medicine, systemic amyloidosis is clinically

Figure 5. Ultrastructural evaluation of a section of renal tissue. Amyloid fibrils are densely packed and organised into small spicules oriented perpendicular to the GBM beneath podocytes and in the mesangium. A) Transmission electron microscopy, x3800. B) Transmission electron microscopy, x6700.
classified as primary (amyloid light chain, AL-amyloidosis) or secondary/reactive (amyloid-associated, AA-amyloidosis), while hereditofamiliar amyloidosis is a collective term for a heterogeneous group of inherited syndromes (1).

Familiar amyloidosis of Shar Pei dogs appears to be linked to familiar Shar Pei fever, an auto-inflammatory disease that clinically resembles a human hereditary syndrome, called familiar Mediterranean fever (3,4,5,6). Briefly, familiar Shar Pei fever (or SPAID - Shar Pei auto-inflammatory disease) is characterised by short, recurrent episodes of pyrexia and localised inflammation, which usually involves the hocks. It is postulated that the episodes of inflammation may lead to a subclinical, chronic auto-inflammatory state that can predispose Shar Pei dogs to the development of reactive systemic AA amyloidosis.

In the present case, the dog had a 2-year history of episodes of presumptive familiar Shar Pei fever and it was eventually referred for further investigation of previously diagnosed azotaemia and proteinuria. After the detection of ultrasonographically abnormal splenic and hepatic echotexture, the cytological examination showed the presence of amyloid deposits in both organs. Although in dogs (including Shar Pei), amyloidosis affects primarily the kidneys, it can also be seen in other organs, such as liver, spleen, pancreas, adrenal and thyroid glands, gastrointestinal submucosa, lung, myocardium, lymph nodes, prostate, and central nervous system (3,4,5,6).

In our case, the initial diagnosis of amyloidosis was confirmed using Congo Red on the cytological samples of the liver and spleen, as well as on the histopathological sample of the kidney. Congo Red is the most frequently used stain for the confirmation of amyloid deposition (7).

The moderate to severe glomerular amyloidosis seen in the present case explains the mild renal azotaemia and marked proteinuria. Excessive loss of albumin through the kidneys is probably the underlying cause of mild hypoalbuminaemia, although decreased albumin production by the liver cannot be completely excluded as a possible minor contributing factor. Hypoproteinemia, azotaemia and isothenuria are commonly reported in dogs with renal amyloidosis (5). The prognosis most of the time is very poor as the disease is progressive, and leads to kidney failure or thromboembolism events. Dogs can be given symptomatic treatment of chronic kidney failure but there is no specific treatment that can prevent the development of amyloidosis or promote amyloid breakdown. Colchicine has been described in Shar-Pei with renal amyloidosis (8). However, no controlled studies have been done to prove the usefulness of colchicine in preventing renal amyloidosis, although anecdotal reports suggested that it did help to retard the progression of renal amyloidosis.

References

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