# Splenic aspirates from a dog

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## Signalment

Dog, Shar Pei, 3.5 years old, entire female

## History

The dog was referred to the Hospital for Small Animals, Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK for further 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 2 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.

## Physical examination

At the time of admission, the dog was bright, alert and responsive with good body condition. Mucous membranes were pink and moist with a capillary refill time <2 sec. Hydration status was adequate. 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,

limb, 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).

**Clinicopathological evaluation**

The complete blood count (ADVIA 2120, Siemens Medical Solution Diagnostics Ltd., USA) revealed a mild, non-regenerative anaemia (red blood cells: 5.30 x $10^{12}$/L, RI: 5.50-8.50 x $10^{12}$/L; haemoglobin: 10.9 g/dL, RI: 12-18 g/dL; haematocrit: 34.2%, RI: 39-55%, reticulocytes: 23.6 x $10^{9}$/L, RI: <60 x $10^{9}$/L). Moderate numbers of acanthocytes and small numbers of schistocytes were found during the blood smear examination. Serum biochemistry (AU480, Beckman Coulter, Brea, USA) revealed the presence of mild hypoproteinaemia due to hypoalbuminaemia, mild azotaemia, mild hypercholesterolaemia, mild hyperglycaemia, and borderline increase in ALP activity (Table 1). Urinalysis showed low urine specific gravity (1.015), increased urine pH (8), and a strongly positive protein reaction (+4) and weakly positive blood reaction (+1) on dipstick analysis. Urine sediment was unremarkable, while urine protein:creatinine ratio (AU480, Beckman Coulter, Brea, USA) was markedly increased (8.0, RI: <0.2). Point-of-care ELISA (Snap 4Dx Plus Test, IDEXX Laboratories, Westbrook, USA) was negative for *Dirofilaria immitis* antigen and antibodies to *Ehrlichia* spp., *Borrelia burgdorferi*, and *Anaplasma* spp.

**Diagnosting imaging**

Thoracic radiographs were unremarkable, except for a moderately gas-distended stomach, likely due to aerophagia. Abdominal ultrasonography revealed a moderately enlarged liver with mildly heterogeneous echotexture, gall bladder sludge, mildly and diffusely stippled splenic echotexture, bilateral, mild, diffuse adrenomegaly, and mild colonic wall thickening. Ultrasound-guided fine needle aspiration of the liver and spleen was performed.

**Table 1.** Clinical biochemistry results of a 3.5-year-old Shar Pei dog that was referred with a history of azotaemia and proteinuria.

| Analyte | Result | Reference interval | Unit |
|---|---|---|---|
| **Total proteins** | 51 | 58-73 | g/L |
| **Albumin** | 22.6 | 26-35 | g/L |
| **Globulins** | 28.4 | 18-37 | g/L |
| **Glucose** | 6.1 | 3-5 | mmol/L |
| **Cholesterol** | 10.3 | 3.8-7 | mmol/L |
| **Triglycerides** | 0.57 | 0.57-1.14 | mmol/L |
| **Urea** | 15.3 | 1.7-7.4 | mmol/L |
| **Creatinine** | 212 | 22-115 | µmol/L |
| **ALT** | 76 | 21-102 | U/L |
| **ALP** | 61 | 20-60 | U/L |
| **Bilirubin** | 2.4 | 0-6.8 | µmol/L |
| **Bile acids** | 4.5 | 0-10.5 | µmol/L |
| **Total calcium** | 2.4 | 2.3-3 | mmol/L |
| **Ionised calcium** | 1.27 | 1.15-1.5 | mmol/L |
| **Inorganic phosphate** | 1.2 | 0.9-2 | mmol/L |
| **Magnesium** | 0.90 | 0.69-1.18 | mmol/L |
| **Potassium** | 3.7 | 3.6-5.6 | mmol/L |
| **Sodium** | 145 | 139-154 | mmol/L |
| **Chloride** | 115 | 102-118 | mmol/L |
| **CK** | 78 | 50-200 | U/L |
| **C-reactive protein** | <5 | <6.8 | mg/L |

**Cytological images of the splenic aspirates**

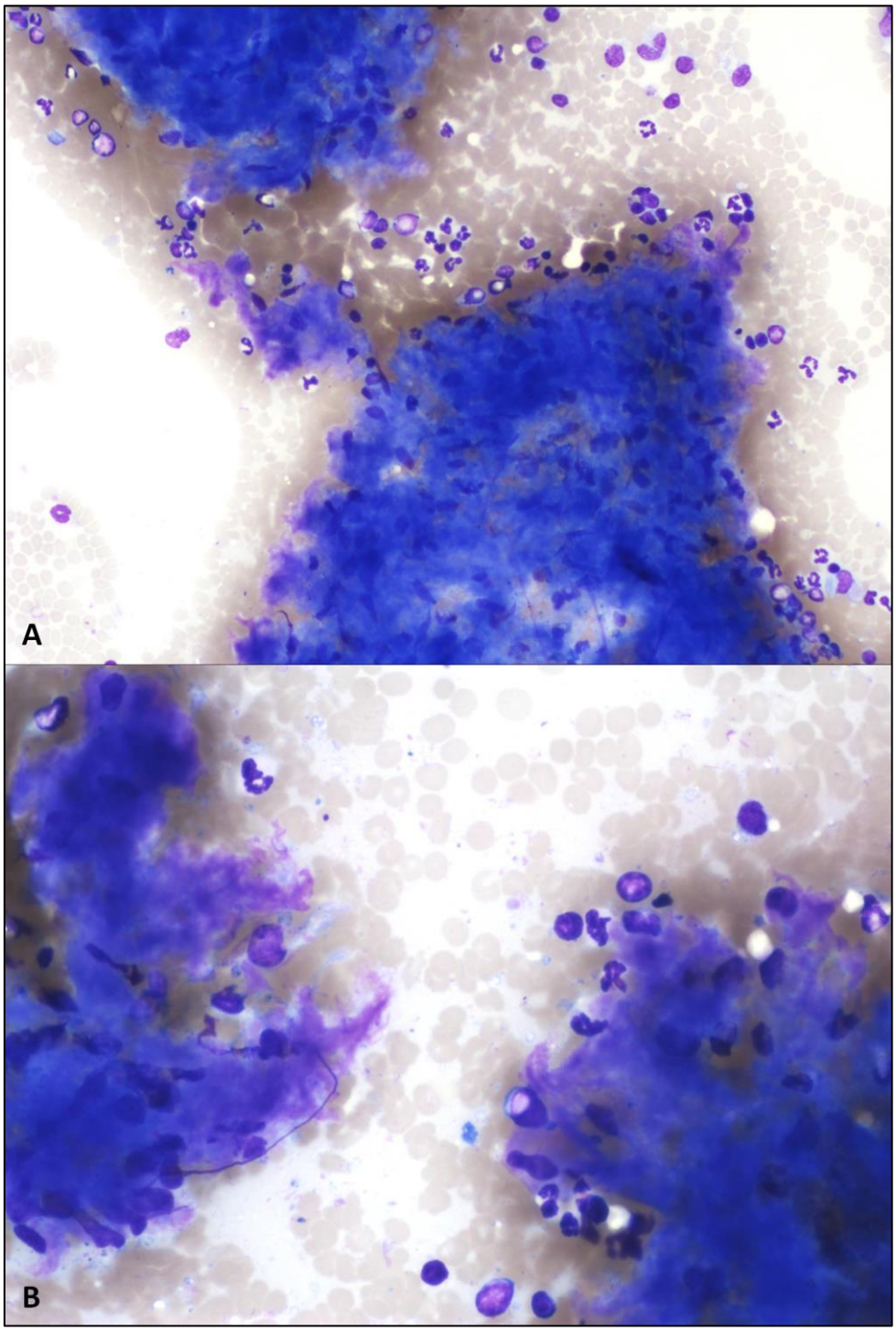


**Figure 1.** Spleen, ultrasound-guided FNA from a 3.5-year-old Shar Pei dog. May-Grünwald Giemsa. **A)** x20 objective. **B)** x40 objective.

**Questions**

What is your cytological description and interpretation of the cytological findings?

What additional tests could be performed to further investigate this case?

**Cytology**

*Spleen, ultrasound-guided FNA, May-Grünwald Giemsa*

The smears were moderately cellular with a heavy background of erythrocytes. There were variably-sized aggregates of reticular cells and lymphocytes that appeared embedded in abundant purple, extracellular material arranged in irregular clumps. That material appeared smooth and eosinophilic at the periphery of the clumps. In small areas the reticular cells and lymphocytes were admixed with light blue material (stroma). The lymphoid population was scant and composed mainly of small lymphocytes with a few medium-sized and large lymphocytes being present and rare plasma cells. The cytological diagnosis was suspected amyloid deposition.

*Liver, ultrasound-guided FNA, May-Grünwald Giemsa (Figure 2)*

The smears were highly cellular and had a moderate background of erythrocytes. The hepatocytes predominated and exfoliated in variably-sized clusters. They often contained small to moderate numbers of discrete to smudged and rarefied cytoplasmic vacuoles (lipids and glycogen accumulation/hydropic degeneration), and a few, small, blue to green cytoplasmic granules (lipofuscin, likely). The hepatocytes showed mild to focally moderate anisocytosis and anisokaryosis, and were often binucleated. A moderate amount of the same extracellular material previously described in the spleen was frequently observed amongst the hepatocytes. The cytological diagnosis was suspected amyloid deposition and mild to moderate vacuolar hepatopathy with mild hepatocyte dysplasia.

*Spleen and liver, ultrasound-guided FNA, Congo Red (Figure 3)*

Two of the cytology smears from the spleen and the liver previously examined were de-stained and stained anew with Congo Red. The smooth extracellular material described previously showed positive Congophilic (red) staining when observed by light microscopy in both spleen and liver. When examined under polarised light, the Congophilic material exhibited apple green birefringence in the thicker areas of the splenic aspirate and in a few areas of the liver aspirate. The findings on examination of the Congo Red stained smears confirmed the presence of amyloid in both organs.

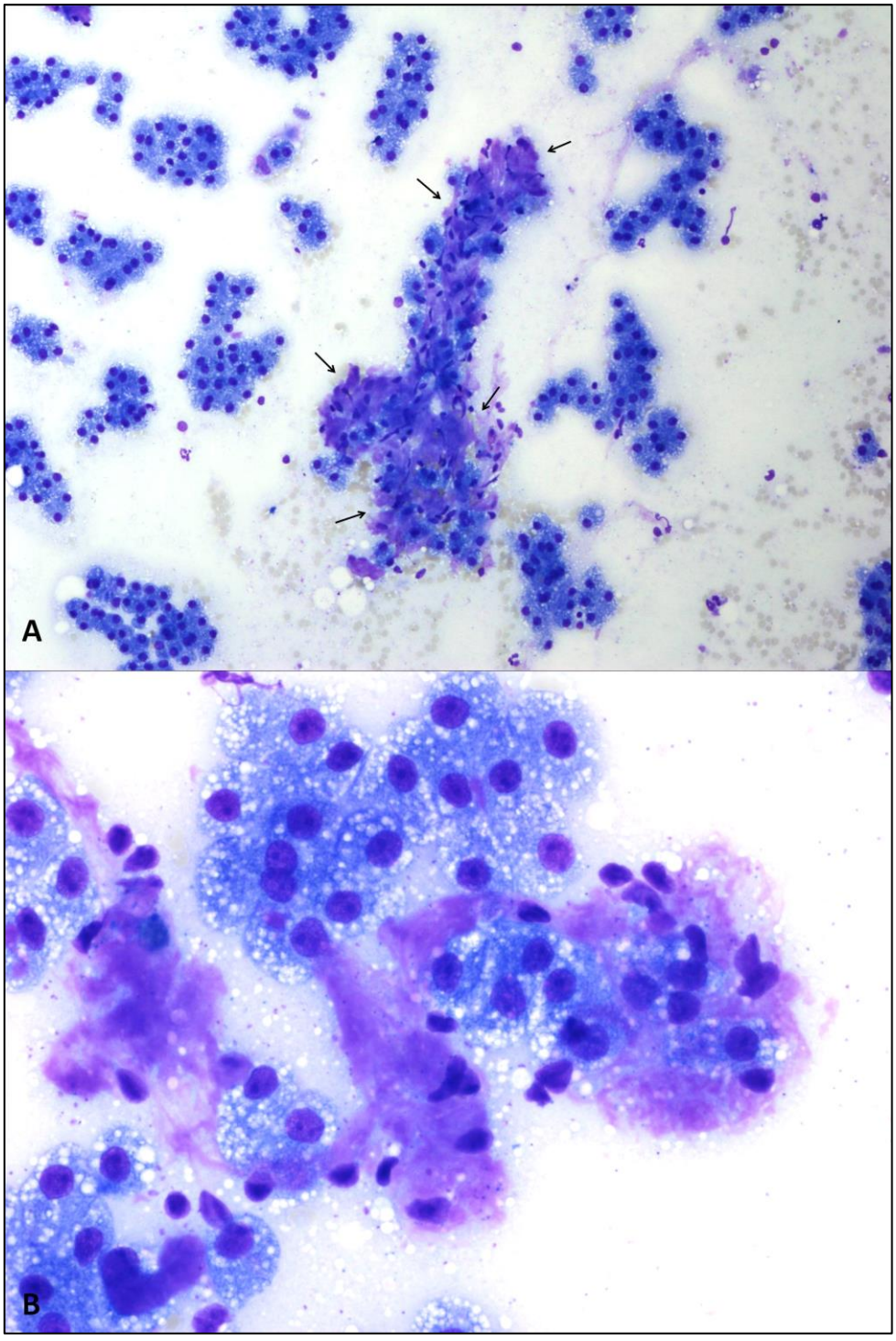


**Figure 2.** Ultrasound-guided FNA from the liver of a 3.5-year-old Shar Pei dog with amyloidosis. **A)** An abundant, smooth, eosinophilic to purple, extracellular material is noted admixed with the hepatocytes in one of the observed clusters (black arrows). May-Grünwald Giemsa, x10 objective. **B)** Higher magnification of the same extracellular material, present amongst the hepatocytes. Note the numerous discrete, small vacuoles that are observed in the cytoplasm of the hepatocytes; they are morphologically consistent with lipids. May-Grünwald Giemsa, x40 objective.

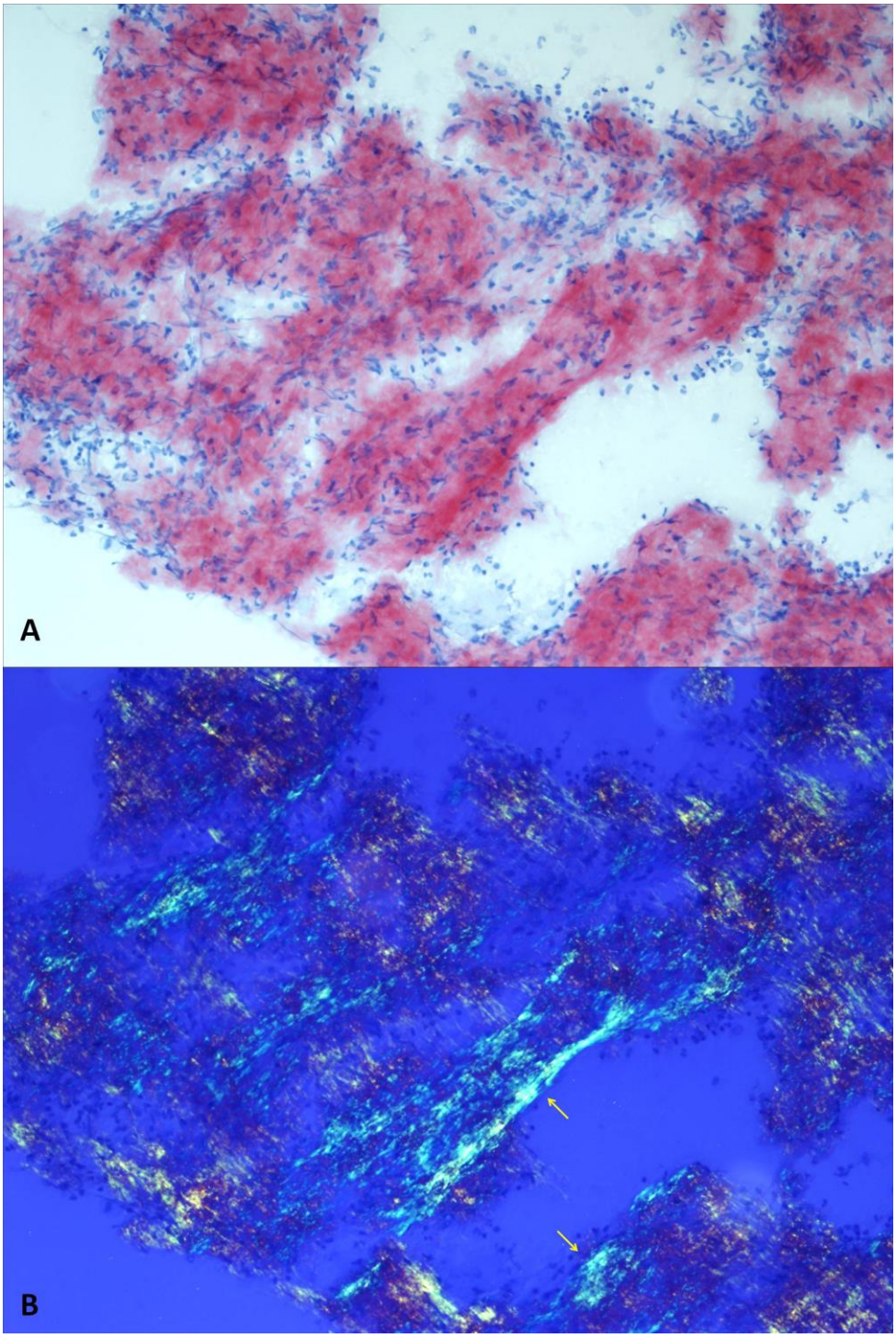


**Figure 3.** Ultrasound-guided FNA from the spleen of a 3.5-year-old Shar Pei dog with amyloidosis. **A)** An abundant Congophilic (red) material is observed. Congo Red, x10 objective. **B)** When the same field is examined under polarised light, the Congophilic material exhibits apple green birefringence in the thicker areas (yellow arrows). Congo Red under polarised light, x10 objective.

**Additional diagnostic tests**

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were within reference intervals, whereas fibrinogen concentration was mildly decreased (1.6 g/L, RI: 2-4 g/L). Serology for *Leptospira bratislava*, *Leptospira canicola*, *Leptospira copenhageni*, and *Leptospira icterohaemorrhagiae*, performed using microscopical agglutination test, was negative (cut-off titre: 1/100). Urine culture and urine PCR for *Leptospira* spp. were negative.

**Histopathology**

Three small cylindrical core biopsies of renal cortex were obtained for optical microscopy, immunofluorescence and ultrastructural examination. For histology, twelve glomeruli were available for evaluation, two of which were globally sclerotic. All glomeruli had moderate mesangial expansion by waxy to fibrillary, Congophilic material (amyloid). The material was pale pink when stained with PAS, mottled blue and pale peach with Masson's Trichrome and did not take up silver when the Jones methenamine silver stain was used (Figure 4). Segmentally, glomerular capillary walls were also expanded by the amyloid, resulting in compression or effacement of peripheral capillary lumens causing ischaemic damage. However, most glomeruli maintained a few open capillary loops. Podocytes were markedly hypertrophied and some contained large cytoplasmic protein reabsorption droplets. Amyloid was not detected in the interstitium or in vasculature. Diffuse acute tubular epithelial injury, characterised by loss of the apical brush border, increased numbers of singly necrotic/apoptotic cells, sloughed cells in the tubular lumens, and large protein casts, was reported. Many tubules were ectatic due to the presence of protein casts. Interstitial fibrosis was mild and patchy and there were a few multifocal atrophic tubules. 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.

**Immunofluoresence test**

One core of renal tissue, containing five glomeruli, 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.

**Transmission electron microscopy**

Ultrastructural evaluation of a section of renal tissue, including two glomeruli, revealed moderate glomerular, tubular, and interstitial changes. Glomeruli showed diffuse areas with visceral epithelial cell foot-process fusion. The filtration membrane and mesangium had confluent areas of thickening associated with aggregates of fine, fibrillary, non-branching structures (measuring 8-15 nm in width). These were morphologically consistent with amyloid (Figure 5). Visceral epithelial and tubular epithelial cells showed mild vacuolar changes. Tubules ranged from unremarkable, to attenuated, to showing expanded lumen with some containing homogenous material.

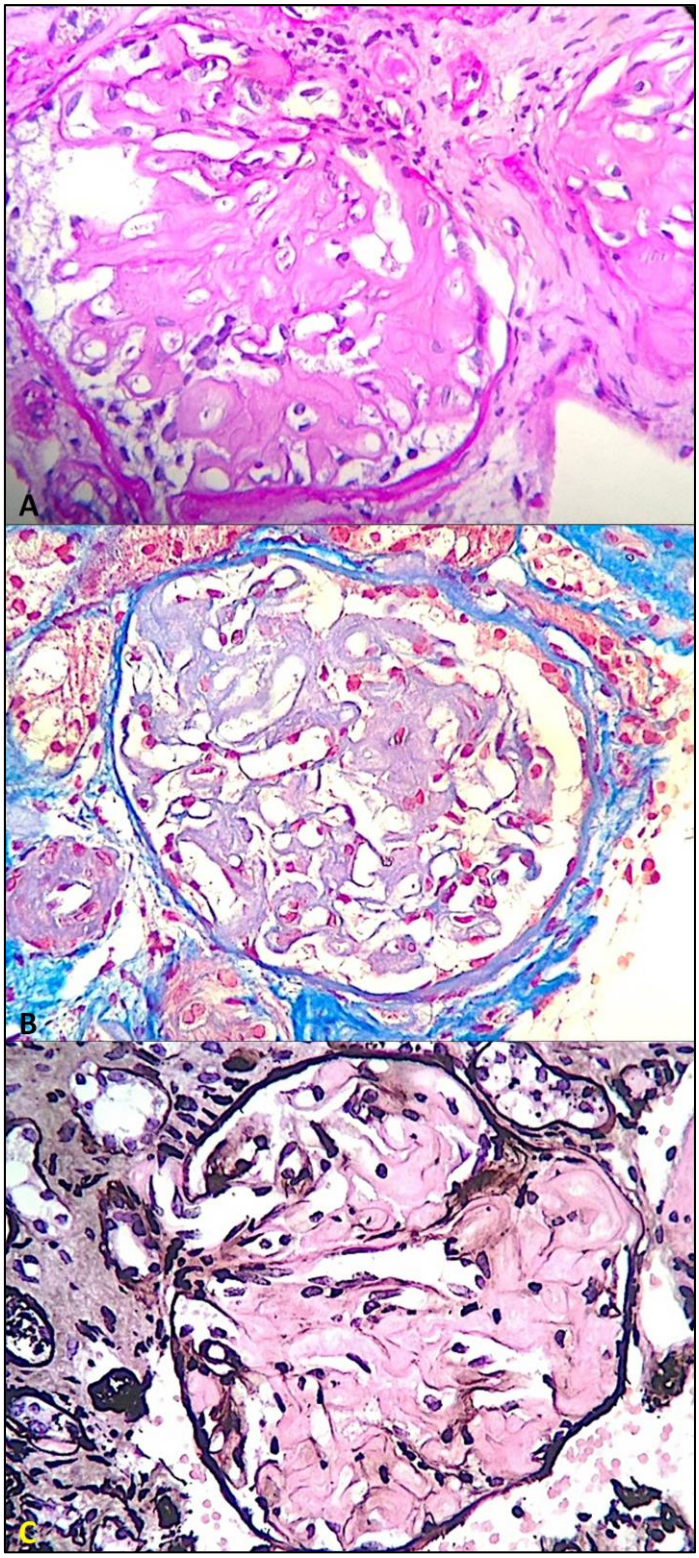


**Figure 4.** Histopathological section of the kidney of a 3.5-year-old Shar Pei dog with amyloidosis. A glomerulus with normal cellularity is depicted. **A)** Amyloid stains pale pink and waxy in the mesangium and capillary loops with PAS (x20 objective). **B)** Amyloid stains mottled blue to orange in the mesangium and capillary loops with Masson's Trichrome (x20 objective). **C)** Amyloid does not take up silver with JMS stain (x40 objective).

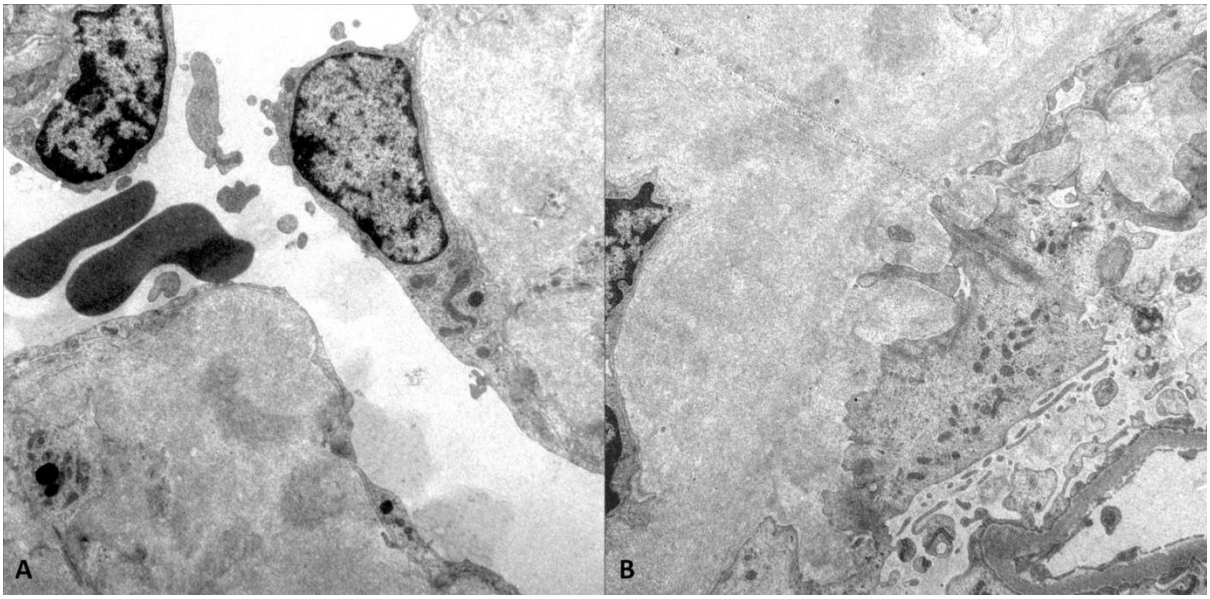


**Figure 5.** Ultrastructural evaluation of a section of renal tissue of a 3.5-year-old Shar Pei dog with amyloidosis. Amyloid fibrils are densely packed and organised into small spicules oriented perpendicular to the GBM beneath podocytes and in the mesangium. **A)** Transmission electron microscopy, x3800. **B)** Transmission electron microscopy, x6700.

**Treatment and clinical outcome**

Treatment included enalapril (0.5mg/kg, PO, b.i.d.) and a renal specific diet for the management of the severe proteinuria, and clopidogrel (4mg/kg, PO, q.d.) to prevent thromboembolism. After confirmation of renal amyloidosis on histopathology, colchicine (0.03mg/kg, PO, q.d.) was added to the treatment protocol.

Two weeks after treatment initiation, physical examination was unremarkable. A mild increase in serum creatinine concentration (240 μmol/L, previous result: 212 μmol/L, RI: 22-115 μmol/L) was observed, probably due to decrease in glomerular filtration rate secondary to enalapril administration. Marked proteinuria was still evident (urine protein:creatinine ratio: 6.4, RI: <0.2). Enalapril was increased to 1.0mg/kg b.i.d. 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 (urine protein:creatinine ratio: 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] Amyloid deposits can be localised or generalised and can potentially affect any tissue or organ.[3] In human medicine, systemic amyloidosis is clinically classified as primary (amyloid light chain, AL-amyloidosis) or secondary/reactive (amyloid-associated, AA-amyloidosis), while heredofamiliar amyloidosis is a collective term for a heterogeneous group of inherited syndromes.[1] However, according to the latest guidelines of the International Society of Amyloidosis, apart from the clinical classification, amyloidosis should also be classified based on the implicated protein.[2]

AL-amyloidosis is associated with overproduction of monoclonal light chains by neoplastic plasma cells and, in contrast to humans, it is very rare in domestic animals.[4] AA-amyloidosis is by far the most common form of amyloidosis in veterinary medicine and is associated with deposition of amyloid A, a N-terminal fragment of the acute-phase protein serum amyloid A (SAA).[5] AA-amyloidosis is closely related to chronic inflammatory diseases or neoplasia.[4] Familiar amyloidosis is reported in Shar-Pei dogs and in Siamese and Abyssinian cats, but it is still considered a type of AA-amyloidosis, for which the aforementioned breeds have genetic predisposition.[4,6]

Familiar amyloidosis of Shar Pei dogs appears to be linked to familiar Shar Pei fever, an autoinflammatory disease that clinically resembles a human hereditary syndrome, called familiar Mediterranean fever.[7] Briefly, familiar Shar Pei fever (or SPAID - Shar Pei autoinflammatroy 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 autoinflammatory state that can predispose Shar Pei dogs to the development of reactive systemic AA amyloidosis.[8]

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 grands, gastrointestinal submucosa, lung, myocardium, lymph nodes, prostate, and central nervous system.[3,9,10] It is interesting to note that splenic amyloidosis has been recognised as a cause of atraumatic splenic rupture in humans.[11] Nonetheless, amyloid is rarely detected in cytological samples and can be easily overlooked. In fact, in the majority of

the reported human cases of amyloidosis, amyloid was initially missed during cytological evaluation and it was recognised only retrospectively in the cytological specimens.[12] Additionally and depending on the examined tissue, amyloid can be potentially misdiagnosed as basement membrane-associated material, osteoid, colloid, or inspissated mucin.[12]

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.[13] When viewed under polarised light, amyloid shows apple green birefringence due to the parallel alignment of the amyloid fibrils and dye molecules.[12] However, the described feature is dependent on section thickness, which ideally should be 8-10 μm; in thinner samples (as may happen in some areas of cytological preparations) the characteristic green birefringence may not be evident, and this could be the reason why this finding was present only in a few small areas of the liver smear in our case.[14] Conversely, the retention of excess dye in the tissue may results to false positive results.[12] Transmission electron microscopy is not usually required to establish a definitive diagnosis of renal amyloidosis; nevertheless, it can demonstrate the presence of the characteristic fibrillary material in the mesangium and glomerular basement membrane, as in our case.[13]

The histopathological examination of the dog's kidney revealed the presence of glomerular amyloid deposition. In contrast to the rest of the dog breeds, renal amyloidosis in Shar Pei dogs was initially reported to involve mainly the medullary interstitium.[10] However, severe, diffuse glomerular amyloid deposition was a consistent histopathological finding in Shar Pei dogs in a recent retrospective study of renal amyloidosis.[3]

The moderate to severe glomerular amyloidosis seen in the present case explains the mild renal azotaemia and marked proteinuria. The latter usually accompanies immune-complex glomerulonephritis, but it can also be seen in cases of renal amyloidosis, especially when deposition of amyloid is glomerular.[3,15] In this case, an immune-complex glomerulonephritis was further excluded on the basis of renal tissue immunofluorescence.[13] On these grounds, 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.

A mild increase in serum cholesterol concentration was also found in the present case; hypercholesterolaemia is a common finding in dogs with renal amyloidosis[3,9,10] and is postulated to be associated with high concentration of SAA, which is an apolipoprotein of high-density lipoproteins (HDL) and a transporter of cholesterol in the circulation.[16] In

addition, the dog also presented mild hyperglycaemia and mild non-regenerative anaemia. The former is likely attributed to stress, while the latter could be due to the diminished erythropoietin production by the affected kidneys, or to inflammation. Moderate acanthocytosis and mild schistocytosis were also observed during blood smear examination. Acanthocytes and schistocytes are associated with erythrocyte fragmentation and can be seen in cases of glomerulopathies; in our patient, it can be attributed to amyloid deposition in the glomeruli.[17] Additionally, acanthocytes can be associated with alterations in erythrocyte membrane lipids and, in our case, this could be due to the hypercholesterolaemia.[17]

In conclusion, we reported a case of a Shar Pei dog with amyloidosis, which was initially diagnosed on cytological examination of spleen and liver aspirates. Although in dogs amyloidosis primarily affects the kidneys, it can also be seen in a variety of organs. However, amyloid deposits may be difficult to recognise and can be misdiagnosed in cytological specimens. The examination of cytological or histopathological preparations stained with Congo red under polarised light is recommended to confirm the diagnosis of amyloidosis, while transmission electron microscopy can also be used as additional test.

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