Urinary cytological twist

Contributors

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Specimens

Whole blood-EDTA; heparin-plasma; serum; urine; urinary cytology obtained through cystocentesis; cytocentrifuge preparation; liver and spleen fine needle aspirates (FNA).

Signalment

Eleven-year-old male neutered Rottweiler mixed breed dog.

History

The dog presented to the emergency service at the Hospital Clínic Veterinari (HCV) of the Universitat Autònoma de Barcelona (UAB), with a four-day history of apathy, anorexia, and polyuria/polydipsia.

Two weeks prior to the current episode, the dog developed nonspecific clinical signs such as vocalization. At that time, hematological analyses were performed by the referring veterinarian (data not available), and, according to the owners, there were no abnormalities. Aside from a corneal ulcer, the physical examination was unremarkable.

A nonsteroidal anti-inflammatory drug (NSAID), carprofen, was prescribed to address presumed pain-related signs, along with topical ophthalmic treatment. Clinical signs of pain resolved during the 8-day NSAID course, however, due to unsatisfactory improvement of the corneal ulcer, the dog was referred to the ophthalmology service at the HCV. A corneal ulcer with detached edges was diagnosed, with cytological findings revealing a scarce number of neutrophils with intra- and extracellular cocci. The area was debrided, and ocular treatment with drops was prescribed. After returning home from this consultation, the dog vomited once, followed by additional episodes throughout the night. From that point onwards, he became anorexic.

Clinical findings

On physical examination, the dog presented mentally alert, but markedly apathetic, with pink to slightly congestive mucous membranes, tachycardia (100 bpm), and showed generalized discomfort on abdominal palpation.

Upon admission to the emergency service, an abdominal point-of-care ultrasound was performed, as well as blood and urine analyses in the in-hospital laboratory: a complete blood cell count (ProCyte Dx Hematology Analyzer) with blood smear review, a complete biochemistry panel (Catalyst Dx Chemistry Analyzer and NOVA Stat Profile Prime Plus® Critical Care Blood Gas Analyzer), urinalysis (IDEXX VetLab UA Analyzer), and urine cytology. The latest was subsequently reviewed at the Clinical Pathology Laboratory of the UAB.

Table 1 - Hematology results for the EDTA-blood specimen performed on the ProCyte Dx. Bolded values are outside the reference interval.

Parameter (units)	Result		Reference interval
RBC (x10^{12}/L)	7.49		5.65 - 8.87
HCT (%)	46.4		37.3 - 61.7
Hgb (g/dL)	16.7		13.1 - 20.5
MCV (fL)	61.9		61.6 - 73.5
MCH (pg)	22.3		21.2 - 25.9
MCHC (g/dL)	36		32 - 37.9
RET (x10^{9}/L)	45.7		10 - 110
RET-He (pg)	**21.7**		**22.3 - 29.6**
WBC (x10^{9}/L)	6.38		5.05 - 16.76
	Automated Count	**Manual Count**	
Neutrophils (x10^{9}/L)	3.91	4.85	2.95 - 11.64
Lymphocytes (x10^{9}/L)	1.40	**0.73**	1.05 - 5.10
Monocytes (x10^{9}/L)	0.83	0.73	0.16 - 1.12
Eosinophils (x10^{9}/L)	0.20	0.06	0.06 - 1.23
Basophils (x10^{9}/L)	0.04	0	0 - 0.10
PLT (x10^{9}/L)	**129**	Adequate (platelet clumping)	**148 - 484**

Table 2 - Biochemistry results for the Heparin-plasma specimen performed on the Catalyst Dx and NOVA. Bolded values are outside the reference interval.[a] Parameter obtained with NOVA analyzer.

Parameter (units)	Result	Reference Interval
Glucose (mg/dL)	94	70 - 143
Creatinine (mg/dL)	**2.8**	**0.5 - 1.8**
BUN (mg/dL)	**51**	**7 - 27**
Phosphorus (mg/dL)	6	2.5 - 6.8
Calcium (mg/dL)	**14.9**	**7.9 - 12**

Sodium (mmol/L)	152	144 - 160
Potassium (mmol/L)	4.1	3.5 - 5.8
Chloride (mmol/L)	110	109 - 122
Total Protein (g/dL)	**8.3**	**5.2 - 8.2**
Albumin (g/dL)	3.5	2.2 - 3.9
Globulins (g/dL)	**4.8**	**2.5 - 4.5**
ALT (U/L)	82	10 - 125
ALP ((U/L)	76	23 - 212
GGT (U/L)	0	0 - 11
Cholesterol (mg/dL)	156	110 - 320
Free calcium (fCa)[a] (mmol/L)	**1.74**	**1.25 - 1.5**

Table 3 - Urinalysis results for the urine specimen performed on the IDEXX VetLab UA Analyzer. Bolded values are considered abnormal.

Parameter (units)	Result
Color	Straw
Turbidity	Very Cloudy
Specific gravity	1.030
pH	8
Protein	**3+**
Glucose	**1+**
Ketones	Negative
Blood/ Hemoglobin	**4+**
Bilirubin	Negative
Urobilinogen	Normal
Leukocyte esterase	2+

Urine cytological preparations were obtained by cystocentesis, centrifuged and stained with Wright-Giemsa, using Hematek 2000, for cytologic examination.

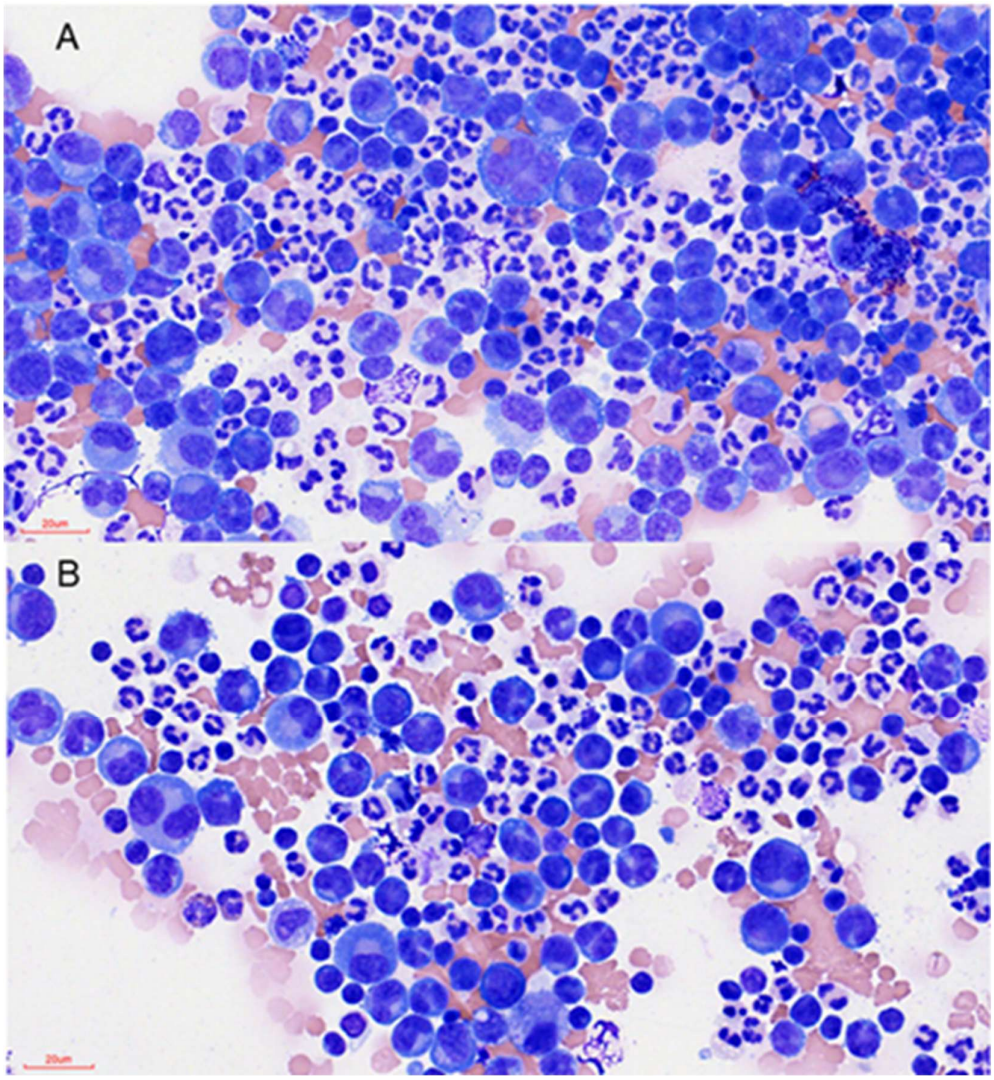

Figure 1 – A and B: urine cytological preparations of the dog from this report. Wright-Giemsa stain, images acquired using a digital slide scanner (Motic EasyScan One, MoticEurope SLU, Barcelona, Spain).

Questions

1. Considering the laboratory findings, what are your main differential diagnoses? Considering the presence of a round cell population in the urine, morphologically compatible with plasma cells, and the main biochemical alterations, a myeloma related disorder, most likely, a multiple myeloma (MM) was considered as the main differential diagnosis. A lymphoma with plasmacytoid differentiation could be considered a differential diagnosis, due to the morphological characteristics of the neoplastic population and tissue involvement.

2. What other diagnostic tests would you consider performing? Abdominal ultrasound with FNA from possible organ lesions; serum electrophoresis with immunofixation, determination of urinary Bence-Jones proteinuria, imaging exams (namely body computed tomography or conventional radiographs of vertebrae and long bones), bone marrow cytology and biopsy, immunocyto(histo)chemistry, and flow cytometry.

Interpretation / Diagnosis

Biochemistry: Free hypercalcemia, azotemia and mild hyperproteinemia due to hyperglobulinemia.

Urinalysis: Proteinuria, glycosuria without hyperglycemia, hematuria.

Cytology: Round cell neoplasia compatible with plasma cell neoplasia in the urine, with associated neutrophilic inflammation and hematuria.

Presumptive diagnosis: Round cell neoplasia, with plasmacytoid morphology, compatible with a myeloma related disorder (MRD).

Additional information

The dog was hospitalized overnight. On the following day, a complete abdominal ultrasound was performed, revealing heterogeneous hepatic parenchyma with multiple hypoechoic lesions, hypoechoic splenic lesions, hepatic and pancreaticoduodenal lymphadenomegaly, and a hypoechoic prostatic parenchyma. No additional

abnormalities were observed (Figure 2). Ultrasound-guided FNA of the hepatic and splenic lesions were only obtained at that time.

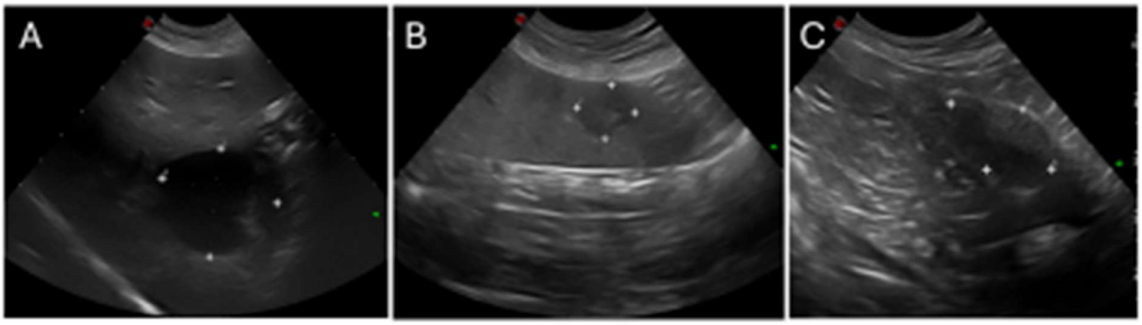

Figure 2 – (A) Liver longitudinal scan showing a 3.4×3.7 cm well-defined hypo/anechoic hepatic lesion (+) near the diaphragm (echoic line). (B) Splenic tail with a 1×1 cm hypoechoic, mildly heterogeneous lesion with ill-defined margins. (C) Prostate longitudinal scan (+), measuring 2.1x1.2cm, with well-defined margins, hypoechoic, considered normal for a neutered male.

Urine microscopic analysis revealed numerous round cells arranged individually. These cells were generally large, with round, oval, kidney-shaped, or lobulated nuclei, sometimes with a flower-like appearance. The chromatin pattern was irregular to finely reticulated. The cells had scant to moderate amount of cytoplasm, intensely basophilic, and often showed a perinuclear clear zone. Some of these cells were bi- or multinucleated, showing marked anisocytosis and anisokaryosis. Numerous well-preserved, non-degenerated neutrophils and, to a lesser extent, small lymphocytes were also present. No infectious agents were identified. Mild to moderate numbers of erythrocytes were present.

Cytology of both liver and spleen revealed a round cell population with similar cytomorphology as described in the urinary cytology. Liver samples also showed mild cholestasis, and apparently normal hepatocytes, and splenic samples showed evidence of erythroid extramedullary hematopoiesis (Figure 3).

Immunocytochemistry with a double staining technique was performed on one of the urine sample slides using PAX5 and CD3 antibodies. CD3 identified only few infiltrating small T-cell lymphocytes, but the neoplastic cells did not show immunoreactivity to either marker (Figure 4).

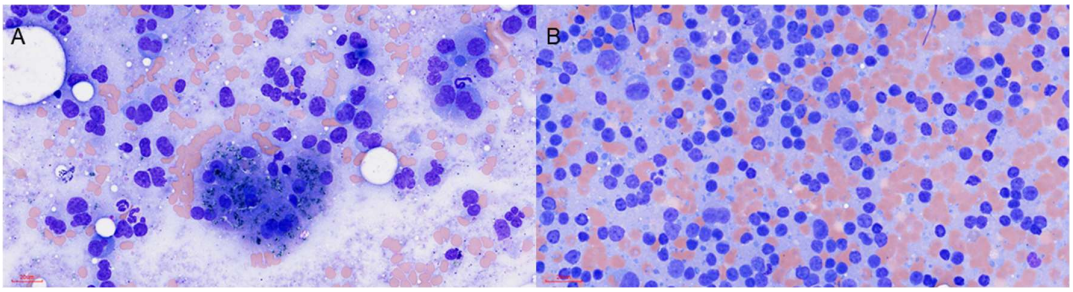

Figure 3 – Hepatic (A) and splenic (B) FNA of the dog from this report. Wright-Giemsa stain, images acquired using a digital slide scanner (Motic EasyScan One, MoticEurope SLU, Barcelona, Spain).

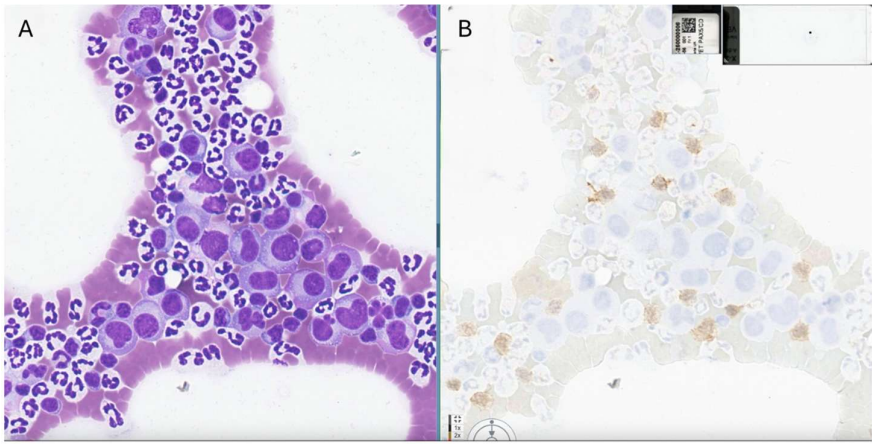

Figure 4 – Urine cytological preparation from the dog in this report. (A) Wright-Giemsa stain; (B) double immunocytochemical staining for Pax5 and CD3. Only small lymphocytes show CD3 immunoreactivity. Images captured via Leica WebViewer.

Due to the strong suspicion of a MM, to better characterize the hyperproteinemia and hyperglobulinemia, a serum electrophoresis with immunofixation was performed. The results revealed two monoclonal bands, one major in the beta region, and a faint one in the gamma region, both identified as complete IgA (heavy chain and bound light chain). These findings are compatible with a paraproteinemia and could represent a true biclonal gammopathy with two distinct M-components of IgA isotype, or a monoclonal gammopathy involving dimer and trimer or tetramer of IgA, supporting the presence of an immunoglobulin secreting neoplasia.

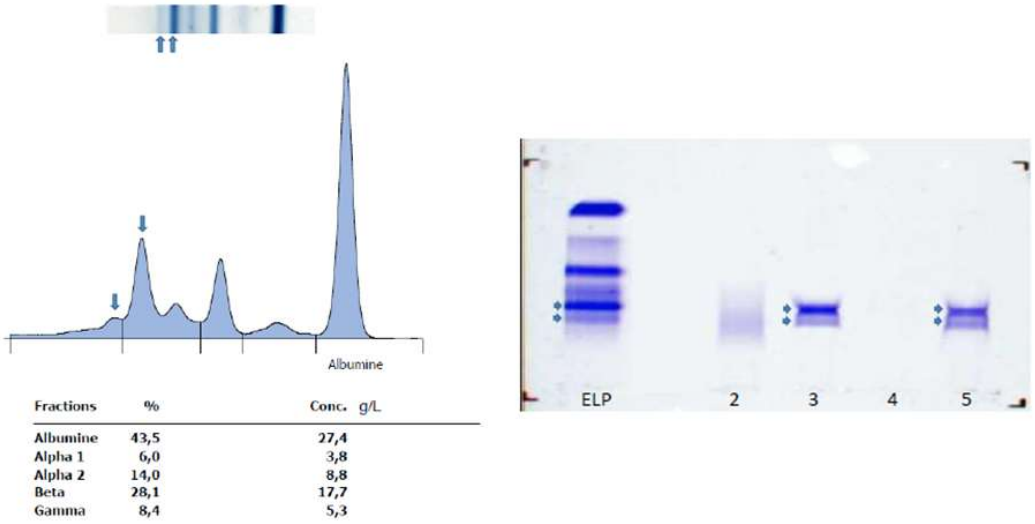

Figure 5 - Serum protein electrophoresis with immunofixation from the dog of this report. Arrows indicate the restricted bands, one major in the beta region, and a faint one in the gamma region. Abbreviations: ELP, electrophoresis trace, no antiserum, unspecific protein staining; 2, IgG_{FC} heavy chains; 3, IgA heavy chains; 4, IgM heavy chains; 5, bound light chains.

Follow up and clinical outcome

Due to the unfavorable clinical progression and the poor prognosis, the owners did not want to pursue further investigation and elected humane euthanasia a few days later.

Discussion

The presence of neoplastic cells in canine urine is usually associated with lower urinary tract or prostatic carcinomas. Very rarely, other neoplastic cells could be present, with only three cases found by the authors, describing the identification of neoplastic lymphocytes on urine cytologic evaluation from canine bladder and renal lymphomas.[1–3] In the present case, the round cells observed in the urine cytology are compatible with plasma cells, which immediately raised the suspicion of a MRD. This was considered the main differential diagnosis because the presence of such cells in urine was deemed to be associated with an aggressive and metastatic neoplasm. Their observation in the urine was interpreted to be due to possible renal or prostatic infiltration by neoplastic

cells, a finding that may occur in more aggressive neoplasms, such as MM, other presentations of plasma cell tumors or lymphoma.[2,4–6]

At the time of the urinary cytology, the dog had already undergone a complete abdominal ultrasound. The FNA of the spleen and liver were performed due to the presence of parenchymal lesions. Considering the results of Di Donato and colleagues, describing hypoechoic tissue changes in the prostate of dogs with prostatic lymphoma, in the present case, since the dog also had hypoechoic prostatic parenchyma, this origin was considered a possible source of the neoplastic cells visualized in the urine.[7] However, it is well established that the absence of morphological changes on ultrasound does not rule out tissue infiltration by neoplastic cells.[8] Therefore, even without ultrasonographic evidence of renal abnormalities, kidney infiltration could not be ruled out.

The additional evaluation of liver and spleen FNA, showing the presence of a round cell population with similar cytomorphological characteristics as the ones observed in the urine, and the biochemistry abnormalities registered, further supported the possibility of a MM over other MRD.

Multiple myeloma is estimated to account for <1% of all malignant tumors occurring in domestic animals and approximately 8% of all hematopoietic malignancies in dogs.[9] In veterinary medicine, traditional diagnosis of MM involves documenting two of four criteria: (1) plasma cell neoplasia within the bone marrow (≥20% plasma cells) (2) lytic bone lesions, (3) serum monoclonal gammopathy, that is, M-Ig or heavy chain, and (4) Bence-Jones proteinuria.[10]

The diagnostic criteria for MM in humans have recently been revised.[11] According to the current guidelines, diagnosis begins with the identification of a plasma cell neoplasm, either by detecting ≥10% clonal plasma cells in the bone marrow or confirming a plasma cell tumor through biopsy, whether bone-related or extramedullary. In addition to this, at least one “myeloma-defining event” must be present. These defining events are grouped into two categories: CRAB features (hypercalcemia, renal dysfunction, anemia, or bone lesions), which indicate end-organ damage and specific biomarkers of malignancy, such as more than 60% bone marrow plasma cells, a significantly abnormal kappa-to-lambda light chain ratio, or at least one focal lesion detected by magnetic resonance imaging.

In the present case, it was not possible to meet the minimal criteria in either of the classification schemes to be able to achieve the criteria necessary to diagnose this case as MM. However, the presence of plasma cells confirmed by cytology in the liver, spleen and urine is considered to constitute extramedullary progression of disease. Also, the occurrence of hypercalcemia constitutes CRAB features, and the serum electrophoresis paired with immunofixation findings, demonstrated a paraproteinemia. Taken together, these findings are strongly suggestive of MM.

Sixty-four percent of dogs with MM present with bone osteolytic lesions and 25% with hypercalcemia, a biochemistry alteration that is suspected to result from the release of an osteoclast activating factor by either the bone marrow microenvironment or by cancer cells locally in bone.[9,10] In the present case, in spite of not being possible to perform other imaging exams, namely radiographs, computed tomography or magnetic resonance imaging, to look for bony lesions, the presence of hypercalcemia (of both total calcium concentration and fCa) suggests its occurrence, which further supported the suspicion of a MM. Other differential diagnoses for hypercalcemia include malignancy-associated hypercalcemia (commonly due to lymphoma or carcinomas), primary hyperparathyroidism (e.g., parathyroid neoplasia), or reduced urinary calcium excretion (e.g. renal failure).

The dog presented with azotemia and a urinalysis revealing marked proteinuria (3+), glucosuria (1+), and hematuria (4+ RBCs/hemoglobin). The azotemia, in the context of a urine specific gravity of 1.030 and polyuria, likely reflects an appropriate renal response to conserve water, rather than true renal failure. However, the presence of glucosuria despite normoglycemia indicates proximal tubular dysfunction, most likely due to impaired reabsorption mechanisms.

Serum electrophoresis coupled with immunofixation was considered essential to support a diagnosis of an immunoglobulin secreting neoplasm. Due to the mild hyperproteinemia and hyperglobulinemia, and albumin concentration within reference interval, it was expected that the traditional monoclonal gammopathy on the serum electrophoresis would be absent, making essential to couple an immunofixation technique to confirm the occurrence of a paraproteinemia. The serum electrophoresis revealed two monoclonal bands, one major in the beta region, and a faint one in the gamma region, both identified as complete IgA (heavy chain and bound light chain). The present case highlights, as previously described, that the shorter peaks on both beta and gamma region make it difficult to diagnose paraproteinemia solely on the basis of a serum electrophoresis evaluation.[12] Also, as described by Seelig and colleagues, as the IgA fraction represents a very small amount of the total Ig component in the normal dog, possibly even a massive IgA increase might not be sufficient to increase the overall globulin concentration.[13] This would help to explain the concentrations of total protein and globulins, in the present case, as well as the absence of a marked globulin peak in the electrophoresis. The presence of a biclonal gammopathy of IgA, could result from several dimerization states of a single immunoglobulin or be the result of the production of two different clones, i.e. two different IgA heavy chains or one heavy chain and two different light chains.[12] In order to differentiate both of these possibilities, a serum protein electrophoresis using 2-mercaptoethanol could rule out dimerization of IgA, as a cause of the biclonal

gammopathy. This reagent can reduce the IgA bonds leading to dimerization, resolving these cases into a single peak.[12,14] However, in the present case, there was not the possibility to perform this test, making it impossible to differentiate both entities.

It is also important to highlight that despite neoplastic infiltration on liver parenchyma, all the transaminase's activity measurements were within reference intervals. These findings highlight the low sensitivity of these enzymes activities to predict neoplasia infiltration.

Despite the presence of plasma cells on urine cytological evaluation, additional diagnostic methods are necessary to reach a definitive diagnosis, particularly to distinguish between a B-cell immunoglobulin-producing lymphoma and MM, as treatment strategies differ between these entities. In this case, both conditions could exhibit clonal rearrangement on PCR for antigen receptor rearrangement, making immunocytochemistry a potentially useful tool for further characterization. Immunocytochemistry was therefore performed on a urine cytology slide using PAX5 and CD3 antibodies. The atypical cells were negative for both markers. The lack of PAX5 expression, a B-cell lineage marker, supports the suspicion of MM over B-cell lymphoma. However, due to the low sensitivity of the primary plasma cell nuclear marker MUM1 in aged cytology slides, this more specific confirmatory staining could not be performed.

The presence of urinary plasma cells confirmed by cytology, and supported by the results of the immunocytochemistry, associated with an IgA paraproteinemia represents a novel finding and, to the authors' knowledge, this is the first time being reported in a canine patient. In human medicine, the identification of plasma cells in the urine of MM patients has been described as well as in patients with plasma cell tumors of the urinary bladder and urethra.[15, 16,17] In human medicine, the presence of plasma cell-like morphology cells in urinary cytology could also be related with plasmacytoid urothelial carcinoma (PUC) of the urinary bladder.[18,19] This tumor has a low incidence in humans, and only two cases in dogs were found, reporting bladder lesions histologically compatible with PUC.[20] In the present case, this was not considered a differential diagnosis, due to the absence of urinary bladder lesions on ultrasonography.

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