Authors: Márton Márialigeti, Zsolt Becker and Péter Vajdovich
Affiliation: University of Veterinary Medicine, Budapest, Department of Clinical Pathology and Oncology
Address: H-1078, Istvan str. 2. Budapest, Hungary
E-mail: marialigeti.marton@univet.hu, becker.zsolt@univet.hu, vajdovich.peter@univet.hu

Signalment (fig 1)
Species: dog
Breed: hungarian vizsla
Color: golden rust
Age: 8 years
Gender: male

Fig 1

Histrory
Symptoms hed been noted 4 to 5 weeks before the owners brought the dog to our clinic. The referring veterinarian had removed a spike and put the dog on antibiotics. Shortly after the dog vomited and collapsed. The patients blood test results showed anaemia, hyperproteinaemia, hypoalbuminaemia, elevated fructoseamine and urea. Since then the dog was lethargic showing normal appetite and water intake. The dog did not leave Hungary for any other countries.

Clinical presentation
The dog was mildly lethargic. Temperature, pulse rate, breathing were within phisiologic limits. Peripheral lymph node examination did not reveal alterations. Abdomnal palpation was unremarkable. A IV/VI murmur was ausculted over the heart. Right front (RF) leg showed lamenes was observed along with hyperkeratotic foot pads on all four limbs and a painful RF foot pad.

Hematology (analyzer: Sysmex XT 2000i, fig 2: histograms)

RBC	3,18 x 10^{12}	(5,12 – 7,90)
Ht	20,2 %	(35,0 – 52,0)
Hb	68 g/L	(120 – 190)
MCV	63,5 fL	(62,0 – 60,0)
MCH	21,4 pg	(22,0 – 26,0)
MCHC	337 g/L	(305 – 355)
RDW-CV	22,6 %	(12,9 – 18,2)
Platelet	138 x 10^{9}	(110 – 600)
PCT	0,13 %	(0,14 – 0,50)
MPV	9,5 fL	(9,0 – 14,0)
PDW	11,6 fL	(10,0 – 20,0)
P-LCR	22,2 %	(16,0 – 50,0)
WBC	6,50 x 10^{9}	(5,60 – 14,50)
Neut%	66,6 %	(40,0 – 75,0)
Lymph%	26,0 %	(10,0 – 27,0)
Mono%	5,8 %	(2,0 – 12,0)
Eo%	1,4 %	(2,0 – 12,0)
Baso%	0,2 %	(0,0 – 0,1)
Atyp%	0,3 %	
Retic %	1,51 %	(0,15 – 1,50)

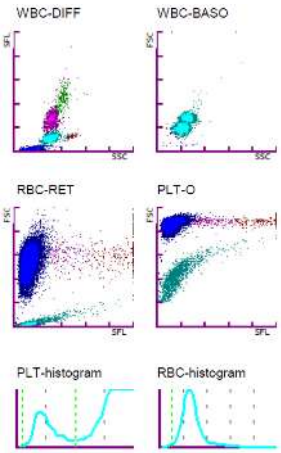


Fig 2

Absolute counts

Neut#	4,33 x 10^9/L	(3,00 – 9,50)
Lymph#	1,69 x 10^9/L	(1,50 – 5,00)
Mono#	0,36 x 10^9/L	(0,10 – 10,40)
Eo#	0,09 x 10^9/L	(0,20 – 1,80)
Baso#	0,01 x 10^9	(0,00 – 0,10)
Atyp#	0,02 x 10^9	
Retic#	48,0 * 10^9	(20,0 – 150,0)

Blood smear examination
Blood smear examination correlated well with the automated cell count. Acanthocytosis was found and several microfilariae were observed without centrifugation.

Findings
Normocytic, mildly hypochromic non-regenerative anaemia, microfilaraemia.

Biochemistry (analyzer: Beckmann-Coulter AU480)

Alb	4,4 g/L	(25 – 45)
TP	144,3 g/L	(55 – 75)
ALT	61 U/L	(< 60)
AP	72 U/L	(30 – 280)
Amy	771 U/L	(< 900)
Urea	8,6 mmol/l	(4,0 – 9,0)
Creatinine	124 µmol/l	(40 – 140)
Phosphate	1,6 mmol/l	(0,8 – 1,8)
TotCalcium	2,50 mmol/l	(2,0 – 3,0)
Ca^{2+}	1,43 mmol/l	(0,50 – 1,50)
K^{+}	5,03 mmol/l	(3,5 – 5,5)
Na^{+}	140,7	(135-155)
LDH	41 U/L	(<200)

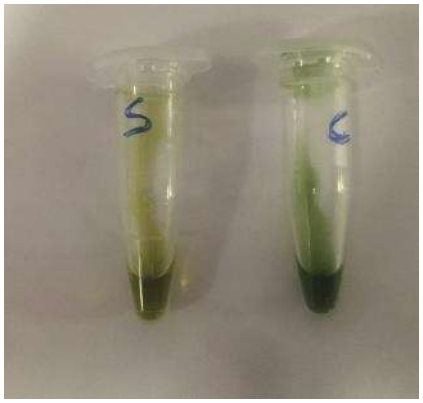


Fig 3

Hemolytic, icteric and lipaemic indices were normal.

Findings
A markedly elevated total protein (TP) and decreased albumin (Alb) was seen. Albumin assay was performed manually on the serum sample as well as on a control sera to inspect for sample turbidity changes (fig 3). There was no turbidity observed.

Serum protein electrophoresis, agarose gel (IDEXX Gmbh, Ludwigsburg, fig 4)

Serum electrophoresis (agarose gel)				
Total protein	**155**	+	54 - 76	g/l
A/G	**0.3**	-	> 0.8	
Albumin (%)	**21.0**	-	44.5 - 62.2	%
alpha-1 globulin (%)	**3.0**		2.3 - 4.2	%
alpha-2 globulin (%)	**3.5**	-	11.4 - 19.0	%
beta-1 globulin (%)	**10.8**	+	3.2 - 8.9	%
beta-2 globulin (%)	**2.8**	-	9.8 - 18.7	%
gamma globulin (%)	**58.9**	+	5.7 - 17.0	%
Albumin (abs.)	**32.6**		24.0 - 47.0	g/l
alpha-1 globulin (abs.)	**4.7**	+	1.3 - 2.8	g/l
alpha-2 globulin (abs.)	**5.4**	-	6.0 - 13.0	g/l
beta-1 globulin (abs.)	**16.8**	+	1.8 - 6.6	g/l
beta-2 globulin (abs.)	**4.3**	-	5.1 - 13.0	g/l
gamma globulin (abs.)	**91.4**	+	3.5 - 9.4	g/l
Leishmania infantum antibodies (ELISA)	**0.1**		< 7.0	TU

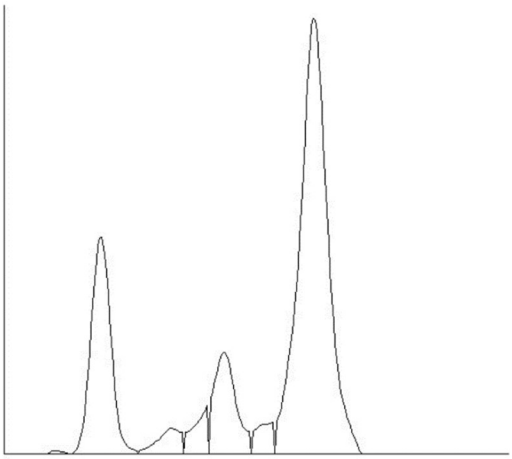


Fig 4

Findings
Serum protein electrophoresis shows a marked elevation of TP. Hypergammaglobulinaemia along with elevated beta-1 globulin levels are readily visible. Absolute values of the other globulin fractions

are decreased. Contrary to the extremely low albumin levels measured by our biochemistry system the electrophoresis indicates albumin levels within the referene interval.

Serology
Negative IDEXX SNAP Heartworm RT
Leishmania infantum ELISA test: 0,1 TU (<7 TU considered negative)

Urine examination
TP (ultrasensitive): 2,25 g/l
Crea: 4425 umol/l
TP/Crea: 4,5

Findings
Elevated TP (ultrasensitive)/creatinine ratio.

Ultrasound

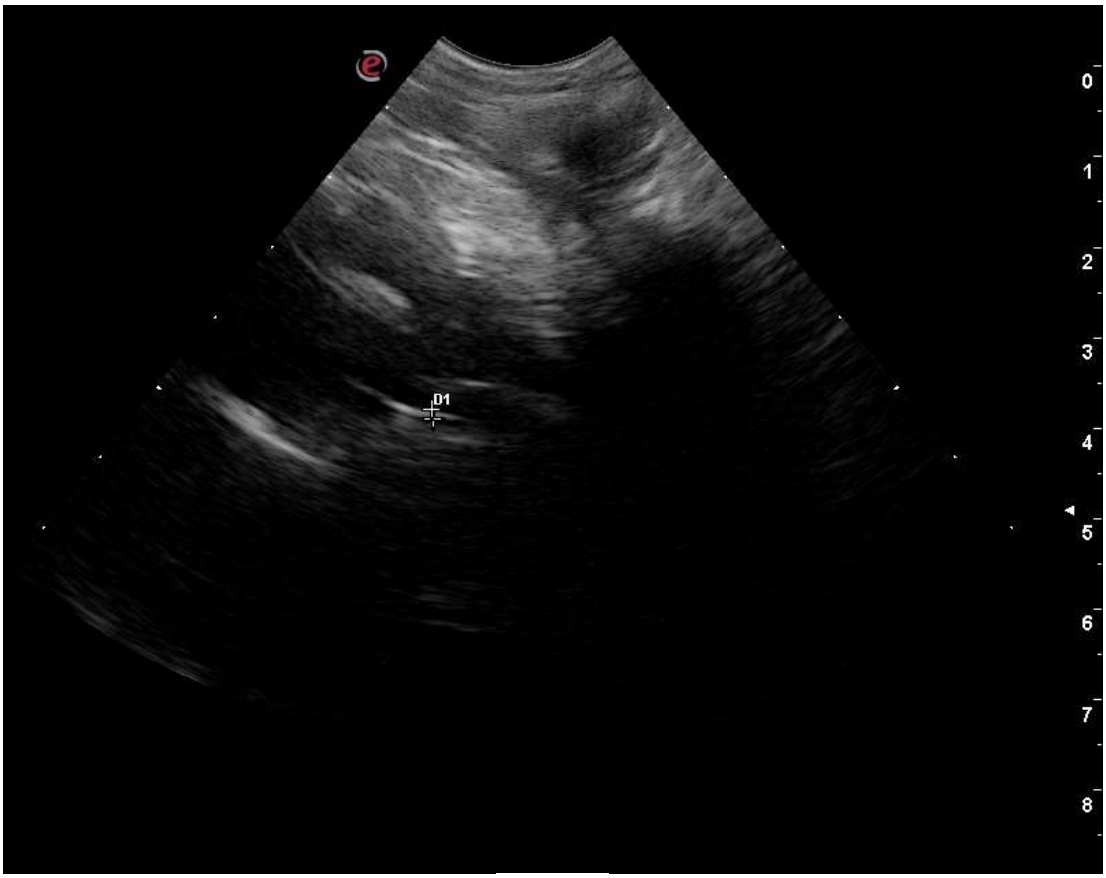


Fig 5

Findings
A hyperechoic shadow appears in the pulmonary artery consistent with D. immitis infection (fig 5). Abdominal scan revealed hepatomegaly and splenomegaly. Renal corticomedullary echogenicity was increased on both sides.

X-ray:

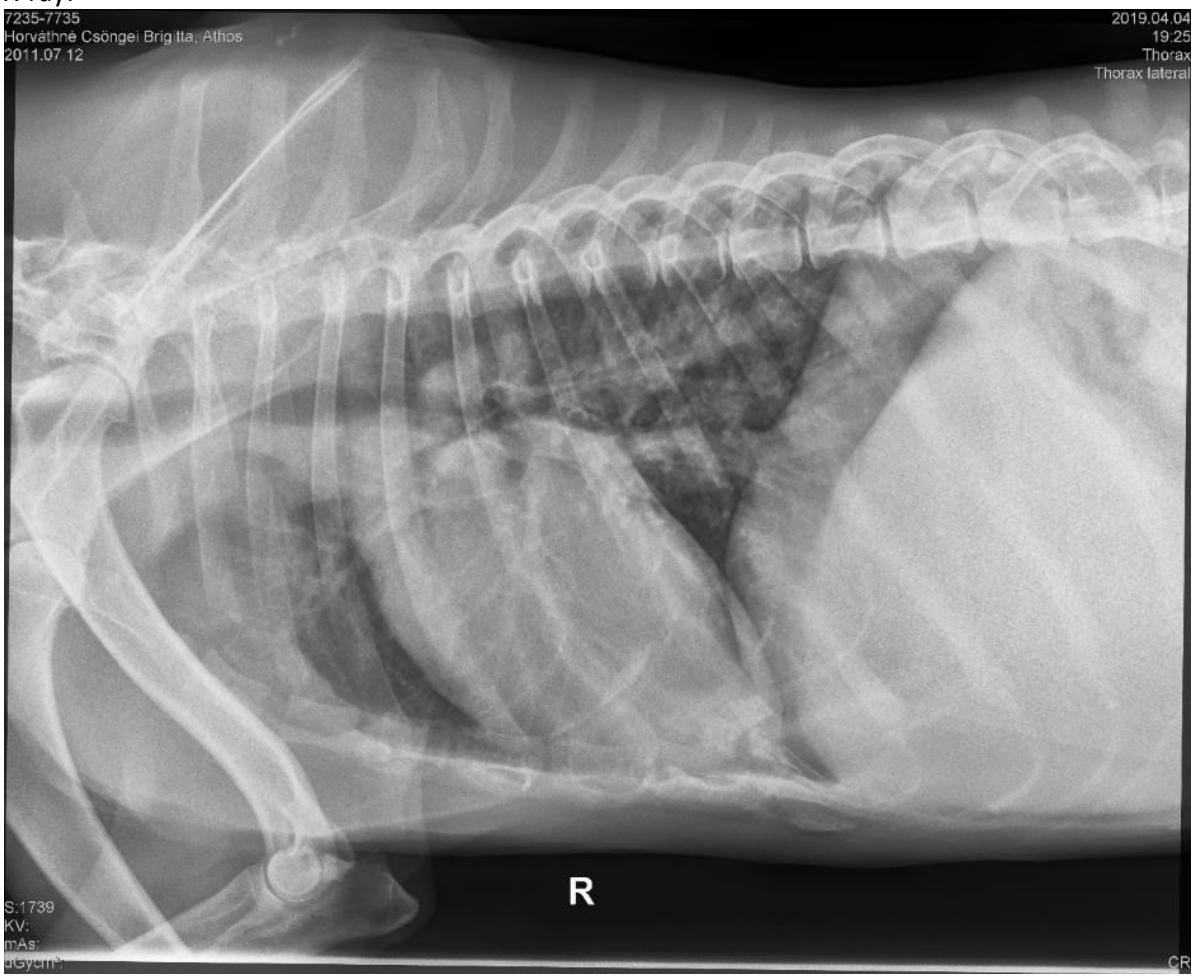


Fig 6

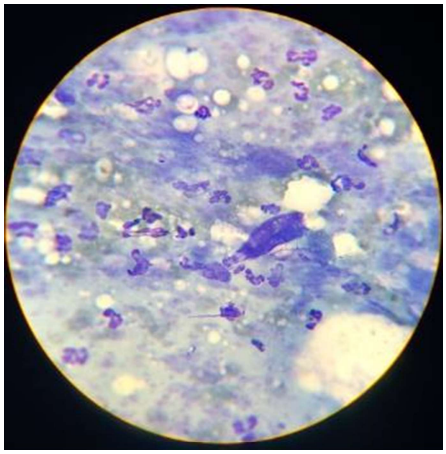
Findings

Right lateral (fig 6), left lateral and ventrodorsal projections were obtained. Osseous pathology has not been observed. Dilatation of the pulmonary veins is be seen. Lytic lesions were not observed.

PCR

Positive for both D. immitis and D. repens.

Doxycyclin, prednisolone and clopidogrel were prescribed for the patient. At the time of the initial presentation anasthesia was considered risky. After a month of treatment the patients condition improved considerably. FNA samples from the liver and spleen were obtained as well as bone marrow for smear examination.



FNA and bone marrow aspirate

Bone marrow (fig 7 and 8)

Basophilic metamyelocyte	0	0.00%
Band neutrophil	22	15.94%
Band eosinophil	0	0.00%
Band basophil	0	0.00%
Segmented neutrophil	69	50.00%
Segmented eosinophil	0	0.00%
Segmented basophil	0	0.00%
ERYTHROID SERIES	23	16.67%
Proerythroblast	1	0.72%
Basophilic erythroblast	4	2.90%
Polychromatophilic erythroblast	1	0.72%
Orthochromic erythroblast	17	12.32%
LYMPHOPLASMACYTIC SERIES	11	7.97%
Lymphoblast	0	0.00%
Prolymphocyte	1	0.72%
Lymphocyte	8	5.80%
Plasma cells	2	1.45%
MONOCYTIC SERIES	6	4.35%
Monoblast	0	0.00%
Promonocyte	2	1.45%
Monocyte	4	2.90%
OTHER	0	0.00%
Mast cells	0	0.00%
Other cells	0	0.00%
MITOTIC FIGURES		
Myeloid cell mitosis	0	---
Erythroid cell mitosis	0	---

The predominant cell types are the segmented neutrophhil granulocytes. Monocytes and promonocytes as well as myelocytes were seen, but no promyelocytes or myeloblasts. Some erythropoietic precursor cells were also seen. Many of them were denudated, or showed a very thin cytoplasm. There was a cytoplasmic shirnkage.

Spleen FNA smear (fig 9 and 10):
The splenic sample contained large numbers of plasma cells (51 %) along with splenic stromal cells (33 %) and a small portion of lymphocytes (8,8 %), segmented neutrophils (5,1 %) and monocytes (2,1 %).

Liver FNA smear:
The sample obtained from the liver was very much acellular. It contained some damaged, atypical liver cells with cytoplasmic shrinkage. Occasionally plasma cell and macrophages were also seen.

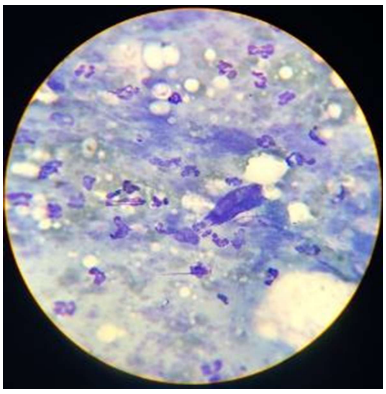


Fig 7

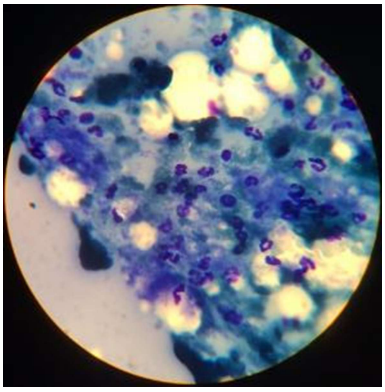


Fig 8

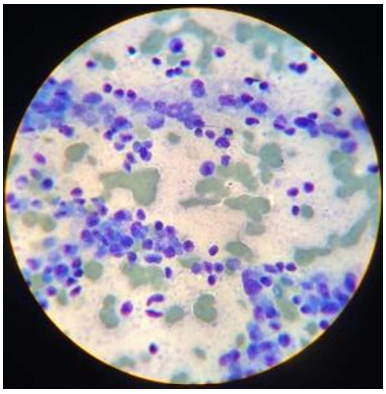


Fig 9

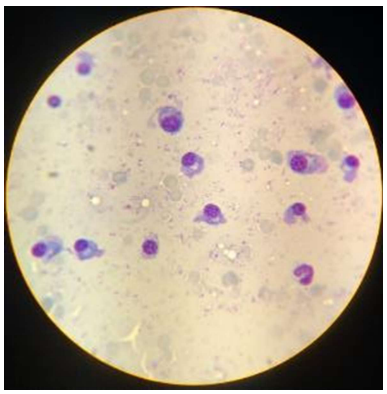


Fig 10

Interpretation

The hematology results indicate a normocytic hypochromic non-regenerative anaemia. Electrolyte results are within the RI. Albumin measurement by the Beckmann-Coulter AU480 system with the bromocresol green method gave an extremely low result with high total protein. Serum electrophoresis results indicated albumin within the RI with monoclonal gammopathy in the gamma fraction and other mild alterations in all other globulin fractions.

Microfilaraemia was observed. SNAP heartworm ELISA as well as Leishmania AB ELISA were negative. PCR results confirmed D. immitis and D. repens coinfection, the former being reinforced by the detection of signs consistent with D. immitis infection on cardiac ultrasound.

Smears prepared from the bone marrow did not show high number of plasma cells.

Diagnosis:

D. immitis and D. repens coinfection with mixed gammopathy and monoclonal gamma fraction peak. Myeloma is highly suggested, but it could not be ruled out entirely.

Discussion

Conflicting results represent a diagnostic challange in this case, in particular various albumin measurement methods gave different results. Pseudohypoalbuminaemia seems to be a rare phenomenon with the bromocresol green method. There is one report, however, of a human case where IgM paraproteinaemia resultes in factitiously low albumin results (Reed, 1987). Ramery and Bureau compared the bromocresol green method and protein electrophoresis for the measurement of serum albumin in canine patinets. Their results indicated that the former method overestimates albumin concentration especially in hypoalbuminaemic patients.

Multiple myeloma (MM) was considered in this case. Based on the diagnostic criteria for MM (2 of the following: markedly increased numbers of plasma cells in the bone marrow, monoclonal gammopathy, radiographic evidence of osteolysis, light chain proteinuria, Thrall et al, 2012, Zachary 2017) it is uncertain at the time of the case submission whether this possibility can be ruled out at all. Some of the findings support multiple myeloma (high number of plasma cells in the spleen, monoclonal gammopathy, proteinuria), others are opposing (lytic bone lesions were not observed, plasma cells were almost absent in the bone marrow sample, calcium levels were within the reference interval /RI/).

It is known that hyperglobulinaemia is a frequent finding in dogs with heartworm disease albeit mostly it is polyclonal. De caprariis et al reported a case of D. immitis related monoclonal gammopathy in a dog whose dysproteinaemia resolved after clearing the infection.

Geigy et al. (2013) reported a case of multiple myeloma with persistent polyclonal gammopathy with a monoclonal spike in a dog with multiple comorbidities including D. immitis infection. In that case factitious albumin results were not found.

Our case points out the possibility of pseudohypoalbuminaemia in dogs with monoclonal gammopathy.

References

D. de Caprariis, M. Sasanelli, P. Paradies, D. Otranto, R. Lia: Monoclonal Gammopathy Associated with Heartworm Disease in a Dog, Journal of the American Animal Hospital Association, Vol 45 No 6, 2009

C. Geigy, B. Riond, C. R. Bley, P. Grest, P. Kircher, H. Lutz: Multiple Myeloma in a Dog with Multiple Concurrent Infectious Diseases and Persistent Policlonal Gammopathy, Veterinary Clinical Pathology, Vol 42 No 1, 2013

E. Ramery, F. Bureau: Measurement of Serum Albumin by Protein Electrophoresis and Bromocresol Green Methods in Canine and Equine Patients, Veterinary Clinical Pathology, Vol 41 No 4, 2012

R. G. Reed: Interference by an IgM Paraprotein in the Bromocresol Green Method for Determination of Serum Albumin. Clinical Chemistry, Vol. 33 No 6, 1987

M. A. Thrall, G. Wieser, R. W. Allison. T. W. Campbell: Veterinary Hematology and Clinical Chemistry, 2nd Ed, Wiley-Blackwell, 2012

J. F. Zachary: Pathologic Basis of Veterinary Disease, 6th ed, Elsevier, 2017