Probable systemic lupus erythematosus (SLE) in a dog with polyarthritis

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Signalment:

A 4 years old, neutered female, 27kg, Alaskan malamute.

History:

The dog was referred with a 10 weeks history of reluctance to stand up and walk, which was intermittent and partially responded to NSAIDs and steroids. The dog had no other signs of disease and was current on vaccination (last vaccine 6 months before presentation) and parasite control. The owner had probable Systemic Lupus Erythematosus (SLE) for 8 years.

Physical examination:

The dog was slightly lethargic during consultation. It was reluctant to move and stand up. It was painful when trying to stand up, and walking with a stiff gait. The general physical examination was unremarkable (HR 104 bpm, RR 36 rpm, T 38.5º), except for slightly prominent popliteal lymph nodes. However, the dog showed severe pain in several joints, especially in the carpi.

Tests performed

CBC, biochemistry and urine analysis are listed in **Tables 1 to 4**. Abdominal ultrasound and thoracic radiographs were unremarkable. Carpi radiographs (**Figures 1 and 2**) showed a slight increase of the soft tissue surrounding the carpus with no signs of erosion or other bone abnormalities. Synovial fluid was obtained from multiple joints (both carpi, elbows and stifles) and all samples presented similar findings. Synovial fluid analysis results are shown in **Table 5** and cytological findings are shown in **Figures. 3-5.** Smears from popliteal lymph node FNA (not shown) were poorly diagnostic due to marked hemodilution, but only a mild increase in plasma cells was found suggesting lymph node reactivity.

NOTE: Treatment was initiated in day 3.

Table 1. Complete Blood Count (Advia 120 Hematology Analyzer)

Parameter	Day 1	Day 38	Reference Interval	Units
Hematocrit	39	39	37 – 55	%
Red blood cells	5.8	5.7	5.5 – 8.5	$x10^6/\mu L$
Hemoglobin	13.2	14.1	12 – 18	g/dL
MCV	67.2	68.4	62 – 77	fL
MCH	22.7	24.7	21.5 – 26.5	pg
MCHC	33.8	36.1	33 – 37	g/dL
White blood cells	6370	13330	6000 – 17000	x/µL
Segmented neutrophils	4332	**12264**	3000 – 11500	x/µL
Band neutrophils	0	0	0 – 300	x/µL
Lymphocytes	1401	**267**	1000 – 4800	x/µL
Monocytes	127	800	150 – 1350	x/µL
Eosinophils	510	0	100 – 1500	x/µL
Basophils	0	0	0 – 200	x/µL
Platelets	313	449	200 – 500	$x10^3/\mu L$

On day 38th mild mature neutrophilia with lymphopenia suggested stress leukogram due to endogenous/exogenous corticosteroids.

Table 2. Serum biochemistry (Olympus AU400 Chemistry analyzer)

Parameter	Day 1	Day 38	Reference Interval	Units
Glucose	106.9	-	65 – 118	mg/dL
BUN	23.1	34	21.4 – 59.9	mg/dL
Creatinine	0.89	1.23	0.5 – 1.5	mg/dL
Cholesterol	191.1	-	135 – 270	mg/dL
Total proteins	6.46	-	6 – 8	g/dL
Total bilirubin	0.19	-	0.1 – 0.5	mg/dL
ALP	50.03	**4324**	20 - 156	U/L
ALT	23	**860**	21 – 102	U/L
GGT	1	**366**	1.2 – 6.4	U/L
CK	116.1	-	10 – 150	U/L
Calcium	10	-	9 – 11.3	mg/dL
Phosphorus	3.82	-	2.6 – 6.2	mg/dL
Sodium	141.0	-	141 – 152	mmol/L
Potassium	3.79	-	3.5 – 5.4	mmol/L
Chloride	111	-	105 - 115	mmol/L

Marked increase in liver enzyme activities were detected on day 38. These changes (ALPx27, GGTx57 and ALTx8) suggested cholestasis with secondary hepatocellular damage, independently of ALP and GGT induction due to prednisone administration. Steroid hepatopathy could be present and liver function test were recommended in this patient.

Table 3. Serum protein electrophoresis (day 1) (Hydrasys Analyzer)

Parameter	Value	Reference Interval	Units
Albumin	2.65	2.6 – 3.3	g/dL
α1 Globulins	0.33	0.2 – 0.5	g/dL
α2 Globulins	0.97	0.3 – 1.1	g/dL
β Globulins	1.67	0.9 – 1.6	g/dL
γ Globulins	0.75	0.3 – 0.8	g/dL

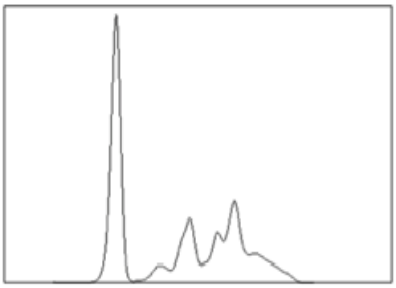
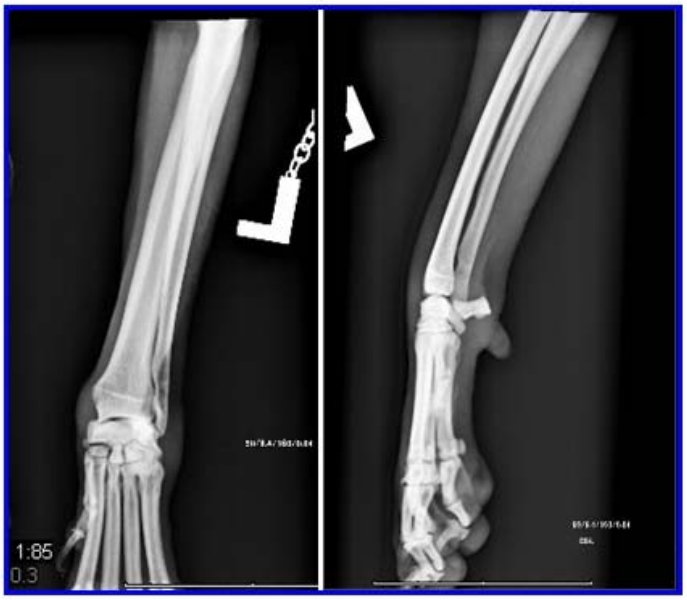


Table 4. Urine analysis.

Method of collection: Cystocentesis.

Parameter	Day 1
Specific gravity	1.030
pH	5.0
Glucose	Negative
Ketones	Negative
Bilirrubin	Negative
Proteins	1+
Blood	Negative
Leukocytes	Negative
Sediment	Normal

Fig. 1 and 2. Dorsopalmar (left) and mediolateral (right) views of left carpus



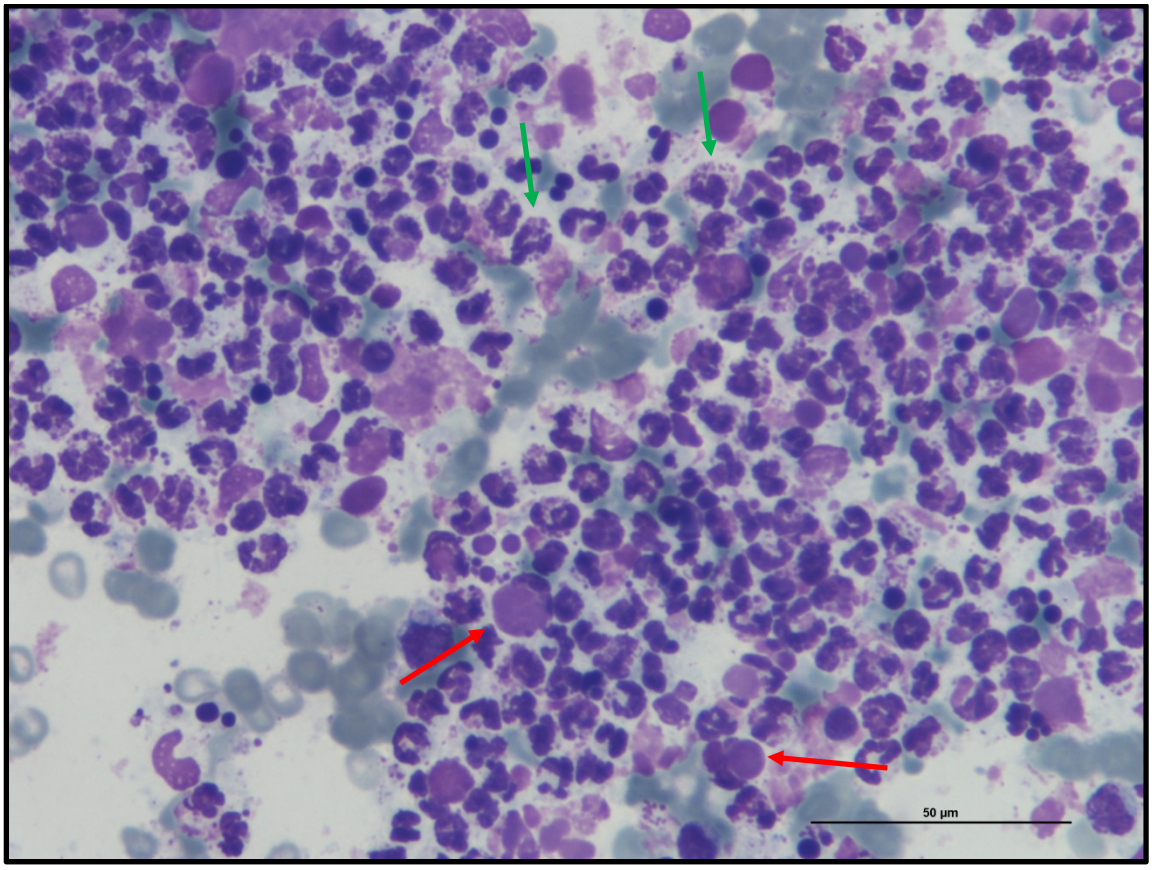
Slight increase of the soft tissue surrounding the carpus. No signs of erosion or other bone abnormalities are present.

Table 5. Synovial fluid analysis.

Parameter	
Appearance	Cloudy
Protein (ref)	3.7 to 4.1 mg/dL
Viscosity	Decreased
Mucin clot	Poor
Cell Count (automatic)	Over 4000/µL
Neutrophils	>90%
Mononuclear cells	<10%
Microorganisms	Not found

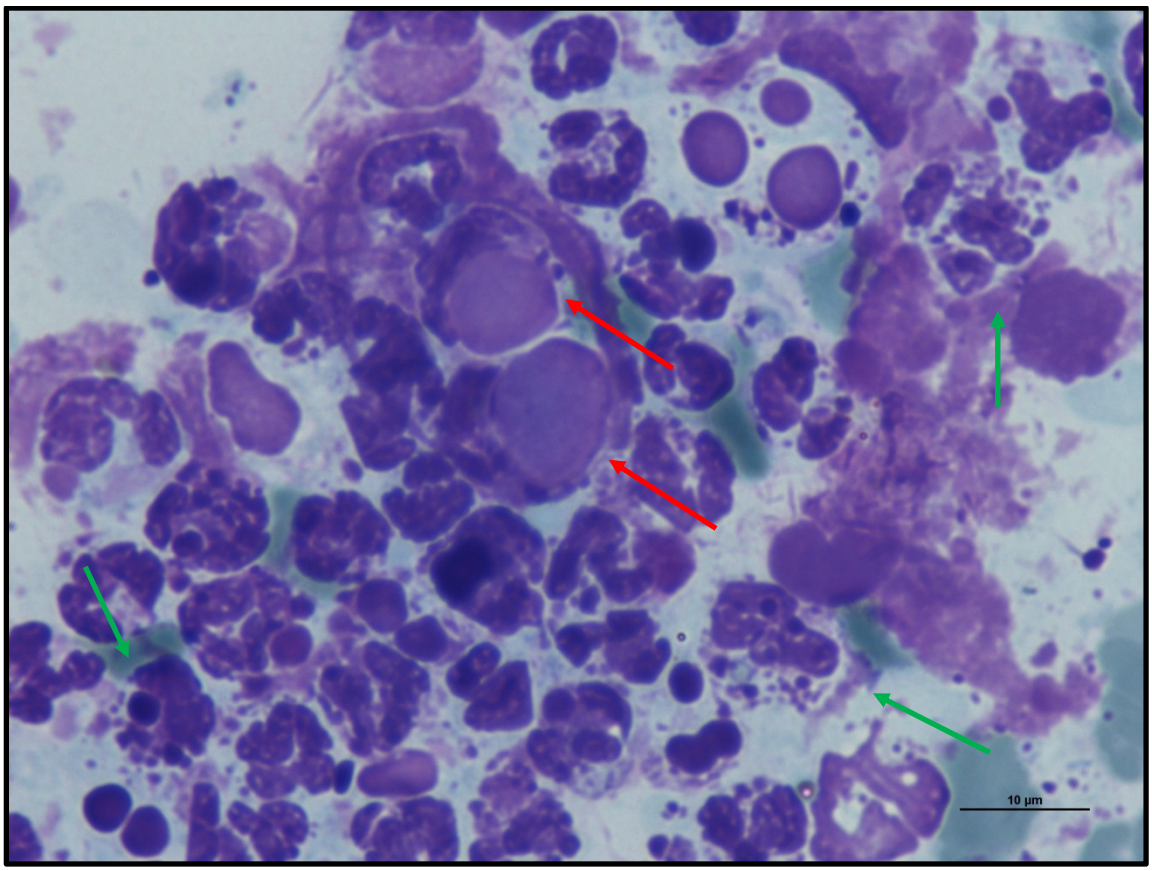
Synovial fluid was classified as inflammatory (inflammatory arthropathy)[1].

Figure 3. Cytospin preparation from left carpal synovial fluid FNA.200x (Quick Panoptic Stain)



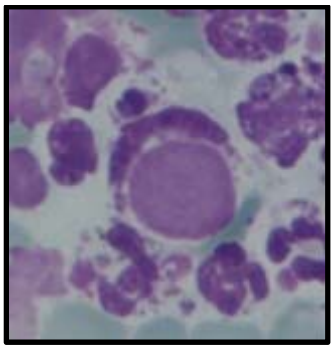
The total cell number is markedly increased and composed of predominantly poorly preserved neutrophils with low numbers of lymphocytes and monocytes. Some pyknotic cells are observed. Moderate amount of eosinophilic amorphous material is present in the hemodiluted background, and it appears frequently forming dense and homogeneous, round to oval inclusions. These inclusions are occasionally seen within neutrophils (red arrows), displacing the nucleus to the periphery of the cell membrane and forming lupus erythematosus (LE) cells. Several ragocytes (also called rheumatoid arthritis [RA] cells) are seen (green arrows). Ragocytes are neutrophils with multiple small, variably sized, purple cytoplasmic inclusions.

Figure 4. Cytospin preparation from left carpal synovial fluid FNA.400x. (Quick Panoptic Stain)



Two large LE cells and some ragocytes.

Figure 5. Cytospin preparation from left carpal synovial fluid FNA.1000x – Detail. (Quick Panoptic Stain)



Detail of LE cell (center) and ragocytes around it.

Following the cytological findings of synovial fluid, antinuclear antibodies (ANA) titers and UPCR were performed. Serologies for *Leishmania infantum* (ELISA) *and Ehrlichia canis* (IFI) antigens, as well as culture of synovial fluid and urine were also carried out. Results of these tests are shown in **Table 6.**

Table 6. Additional tests.

Parameter	Day	Result	Reference Interval
***Leishmaniainfantum* serology**	1	Negative	Negative
***Ehrlichiacanis* serology**	1	Negative	Negative
Urine culture	3	Negative	Negative
ANA titer	3	1/1280	<1/40
Synovial fluid culture	3	Negative	Negative
UPCR*	3	0,2	<0.5

*Urine Protein-to-Creatinine Ratio

Immune mediated polyarthritis (IMPA) caused by a probable SLE was therefore diagnosed.

Treatment

Prednisone (2 mg/Kg/BID/PO) was initiated and sun avoidance recommended if photosensitization occurred[4]. The dog improved rapidly. Attempts to reduce prednisone dosage resulted in recurrence of clinical signs. On day 38th a programmed blood work was performed and the owners reported severe polyuria and polydipsia (results shown in **Table 1 and 2**). Ursodeoxycholic acid (10mg/kg/SID) and SAMe (20mg/kg/SID) were initiated. In addition, azathioprine(1mg/kg/SID) was instituted.

Follow up

The dog did well clinically on the new drugs and doses. Controls were established every 1-2 months, including clinical exam, CBC, biochemistry and UPCR but the dog

remained stable. The dose of steroids was tapered down slowly and the dog is currently on 0.3 mg/kg/d and 1 mg/kg/48h of azathioprine, 6 months after diagnosis.

Discussion

Polyarthritis can be degenerative, septic or immune-mediated in origin. Immune-mediated polyarthritis (IMPA) are classified in erosive and non-erosive, being the latter the most common. In the non-erosive polyarthritis group, several causes are included: infectious diseases such as *Ehrlichia spp., Leishmania infantum, Borrelia burgdorferi, Bartonella spp.* and occult bacterial infections (diskospondilits, endocarditis, pyometra, pyelonephritis); vaccinations and drugs such as trimetoprim-sulfonamides, cephalosporines and penicillins; immune-mediated syndromes such as systemic lupus erythematosus (SLE), polyarthritis/polymyositis syndrome, polyarthritis / meningitis syndrome and other breed specific syndromes; and idiopathic. Idiopathic IMPA are classified in 4 types: type I (no underlying disease), type II (associated with infections distant to the joint), type III (associated with GI disorders) and type IV (associated with occult neoplasia)[2].

In this case, the dog lived in an endemic area for *Leishmania infantum* and *Ehrlichia canis*, and therefore these infectious diseases were tested with negative results. *Borrelia spp*. has not been reported in the area and therefore it was considered unlikely.

The possible infectious origin for polyarthritis was unlikely due to the negative results ofurine and synovial fluid culture. Although negative culture in synovial fluid can not exclude the existence of multiple septic arthritis, it was considered less likely because of the lack of fever and swollen joints. The anamnesis did not reveal the administration of any drugs that could have induced the polyarthritis. Occult infections, neoplasia or other immune-mediated diseases could not be completely ruled out.

With all the above findings, an IMPA was diagnosed and SLE suspected. Glomerulonephritis was ruled out due to the absence of azotemia and a UPCR within normal limits. The response to treatment confirmed the immune-mediated ethiopathogenesis. Unfortunately, this dog did not tolerate a dose reduction of prednisone and developed a probable steroid hepatopathy secondary to glucocorticoid treatment.

SLE is an autoimmune disorder that can affect several systems. Pathogenesis is unknown. It is thought that immune system dysregulation leads to immune complex formation that induces tissue damage, but also direct antibody-mediated cytotoxicity and cell-mediated autoimmunity may occur[4].

Diagnosis for systemic lupus erythematosus (SLE) is still controversial. There is not a test to make a definitive diagnose of SLE, therefore, 4 out of 11 diagnostic criteria are suggested to be indicative of the disease in human medicine[3]. In canine SLE, some authors use the same but adapted criteria[4]. The other criteria most commonly used consist on major clinicopathological findings (skin lesions, polyarthritis, hemolytic anemia, glomerulonephritis, polymyositis, leucopenia, thrombocytopenia), minor clinicopathological findings (fever, CNS signs, oral ulceration, lymphadenomegaly, pericarditis, pleuritis) and positive ANA titer. A definitive SLE is reached with 2 major signs + positive ANA or 1 major sign + 2 minor signs + positive ANA. A probable SLE is reached with 1 major sign + positive ANA or 2 major signs + negative ANA[5]. The LE cells are usually not considered in the above-mentioned scheme, although some authors place them at the same level than the ANA test[4,5].

According to these diagnostic criteria for SLE, this dog had 1 major criteria (polyarthritis) and positive ANA titers, which would be considered a probable SLE. Lymphadenomegaly, which is a minor criteria, was not considered as it was very mild. Moreover, although not considered in diagnostic criteria mentioned above, the

presence of LE cells supports the diagnosis as well as the response to immunosuppressive treatment.

The ANA test is considered the most sensitive serological test for SLE. However, it is not the most specific of tests, and can be positive in over 20% of dogs with infectious diseases, particularly leishmaniosis[5,6].

LE cells are neutrophilic phagocytes that contain intracytoplasmatic hematoxylin bodies. The hematoxylin bodies are thought to be formed by the opsonization of cells by ANA typically found in SLE patients. These antibodies lead to the denaturation of dead injured cells, forming homogeneous oval-shaped bodies that are referred to as “hematoxylin bodies” because they stain blue with common cytological stains such as Wright-Giemsa, Papanicolau, and hematoxylin and eosin stains. The hematoxylin bodies are engulfed by neutrophils, creating LE cells[7].

LE cells have been found in synovial fluid; bone marrow aspirates; peripheral blood; cerebrospinal fluid; pericardial, pleural and peritoneal fluids and also in blister fluid from human patients with SLE[4,7] and when present are highly suggestive of SLE[4].

Ragocytes (also referred as RA cells) are neutrophils with multiple small variably sized, purple cytoplasmic inclusions. They are thought to represent remnants or phagocytosed immune complex and they should be distinguished from bacteria. Observations suggest that these cells are seen more commonly in association with immune-mediated polyarthropaties but are not considered diagnostic[1].

In cytological preparations, LE cells must be distinguished from 'tart cells' or 'pseudo-LE cells', which result from the phagocytosis of nuclear debris by macrophages, rather than neutrophils, and are generally seen in effusion-fluid independent of the cause of the effusion. The phagocytosed debris within the tart cell is smaller, and has a non-homogenous (clumped) appearance in contrast to the smooth homogenous character

of the hematoxylin bodies in true LE cells. The incubation of the pleural fluid at room temperature for several hours may enhance the LE cell phenomenon[7].

In 1949, Haserick and Bortz addressed the important question of whether the LE cell phenomenon was a primary cytological alteration or secondary to a constituent of the plasma of patients with SLE. They added plasma from patients with SLE to bone marrow preparations from normal subjects and compared the results with control preparations from the same subjects. Plasma from patients with SLE induced the LE cell phenomenon in these marrows, with the formation of clumps of polymorphs around amorphous masses of nuclear material. The highest number of LE cells developed when plasma from the sickest patient was used. Thus, the formation of LE cells appeared to be secondary to a factor in the plasma of patients with SLE[8].

The LE cell preparation test can be performed as it remains in the American College of Rheumatology's criteria for the classification of SLE[3]. It consists on an in vitro immunologic reaction between the patient's autoantibodies to nuclear antigens and damaged nuclei in the testing medium. It is subject to numerous experimental variables and dependent on subjective interpretation so it is recommended to be abandoned in favor of more definitive, quantitative immunologic tests for this condition[9].

Recently, it has been suggested that LE cells may not be involved in pathogenicity of SLE, so the LE cell can be reconceptualized as a beneficial response, in which the binding of the nucleus facilitates neutralization or removal of a source of damage associated molecules[10].

Risk factors have been described for SLE. A recent study showed that the relative risk ratio for SLE development among pet dogs owned by human patients with SLE was near infinity compared with pet dogs owned by non-SLE households. The authors hypothesized that there may be an environmental or zoonotic factor responsible for the development of human and canine SLE[11]. Interestingly, the owner of the dog reported

here had SLE as well. Another study also hypothesized of an undiagnosed etiologic agent based on the finding of a seasonal pattern. Most cases were presented in summer or fall[12]. In our case, the dog was referred to us in June, but clinical signs started in March.

In conclusion, when LE cells are found in synovial fluid of a dog with polyarthritis, they support the diagnosis of SLE although fulfillment of multiple criteria is required for definitive diagnosis of this not yet completely understood disease.

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