

# Anaemia in a Cat

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## Case presentation

### Signalment

A 12yr male castrated Domestic Shorthair cat

### History

He was presented to his usual vet with a history of weight loss, polyuria, polydipsia and hiding away from his owners.

### Clinical examination

The veterinarian noted very pale mucous membranes.

The cat was fully vaccinated against feline panleukopaenia (FPV), feline calicivirus (FCV), feline viral rhinotracheitis (FVR), feline leukaemia virus (FeLV) and rabies. The last vaccination had taken place more than a year previously.

An abdominal ultrasound revealed a bilateral nephrosis and mild splenomegaly.

A blood sample was taken and sent to the In Vitro Laboratory for further investigation.

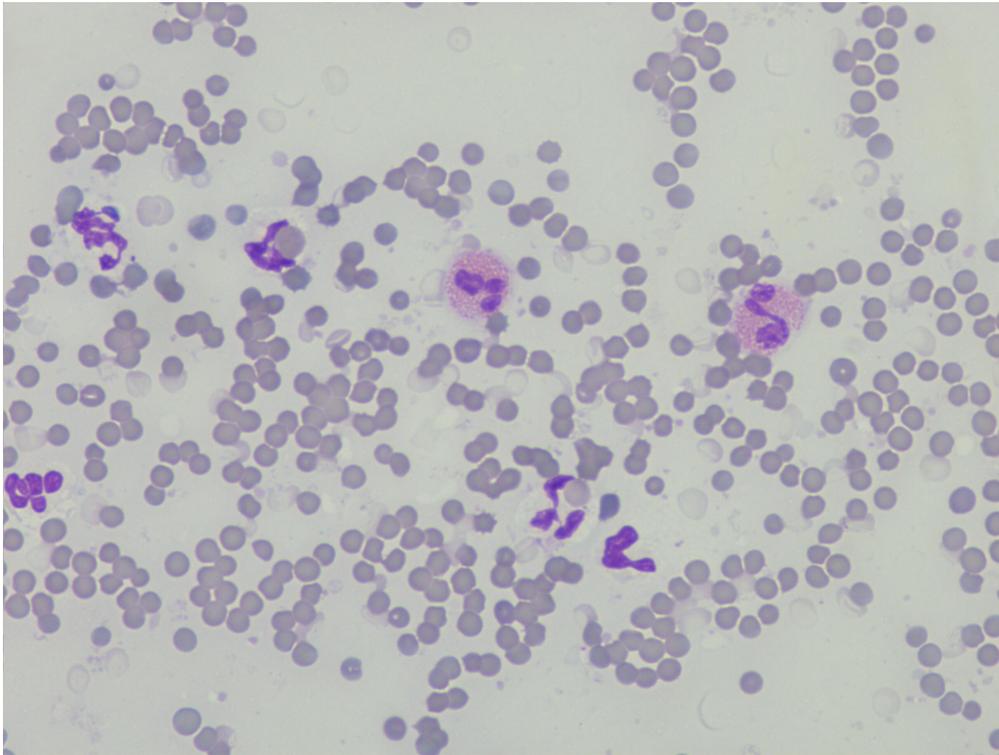
### Laboratory results

The data from the initial and selected subsequent blood samples are presented in Table 1.

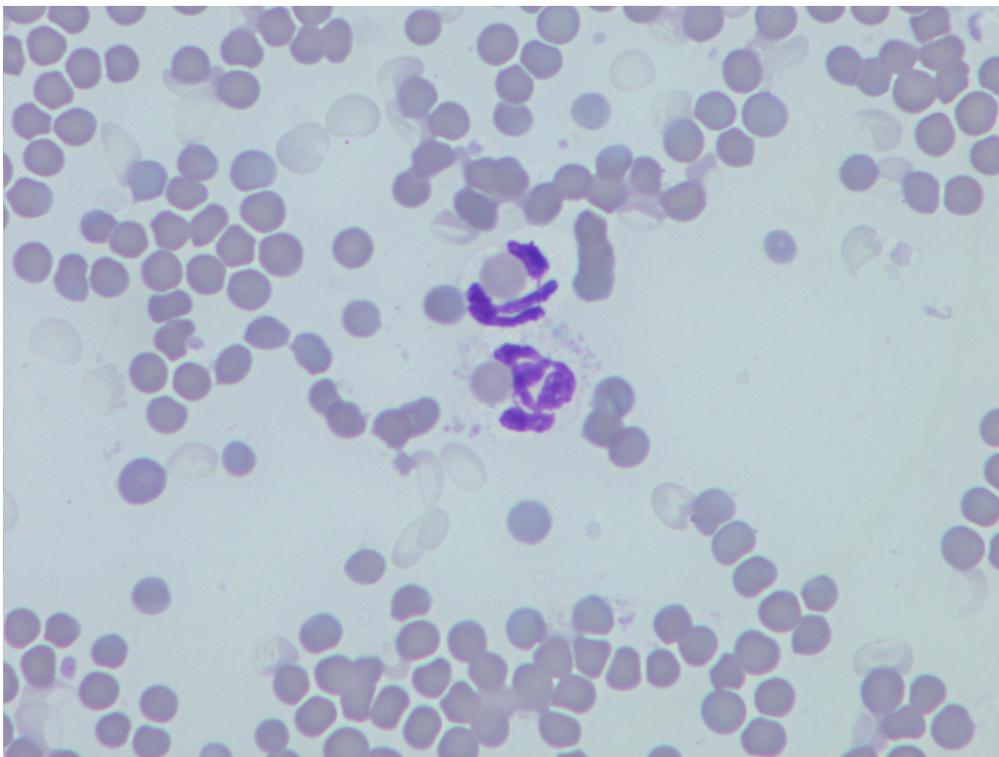
**Table 1:** Sequential laboratory data for the feline patient

<i>PARAMETER</i>	<i>Day 1</i>	<i>Day 9</i>	<i>Day 28</i>	<i>Day 51</i>	<i>Reference range</i>
<b>Hct</b>	<b>0.2</b>	<b>0.24</b>	<b>0.16</b>	<b>0.26</b>	0.28-0.47
<b>RBC</b>	<b>3.9</b>	<b>4.4</b>	<b>3.4</b>	<b>5.5</b>	5.5-10.0x10 <sup>12</sup> /L
<b>Hb</b>	<b>63</b>	<b>69</b>	<b>54</b>	<b>76</b>	80-170g/L
<b>MCV</b>	51	54	48	47	40-55fl
<b>MCHC</b>	320	<b>290</b>	330	<b>300</b>	310-340g/L
<b>WBC</b>	6.4	7.1	6.1	8.2	6.0-15.0x10 <sup>9</sup> /L
<b>Reticulocytes</b>	<b>164</b>	31	48	22	28-50x10 <sup>6</sup> /L
<b>Total protein</b>	<b>129</b>	<b>105</b>	<b>107</b>	<b>101</b>	60-75g/L
<b>Urea</b>	<b>32.0</b>	<b>n.d.</b>	<b>n.d.</b>	<b>30.5</b>	3.3-13.7mmol/L
<b>Creatinine</b>	<b>320</b>	<b>n.d.</b>	<b>n.d.</b>	<b>380</b>	0-140µmol/L
<b>Comment</b>	Sample haemolytic, autoagglutination	Sample haemolytic, autoagglutination	Sample haemolytic, autoagglutination	Sample haemolytic, autoagglutination	

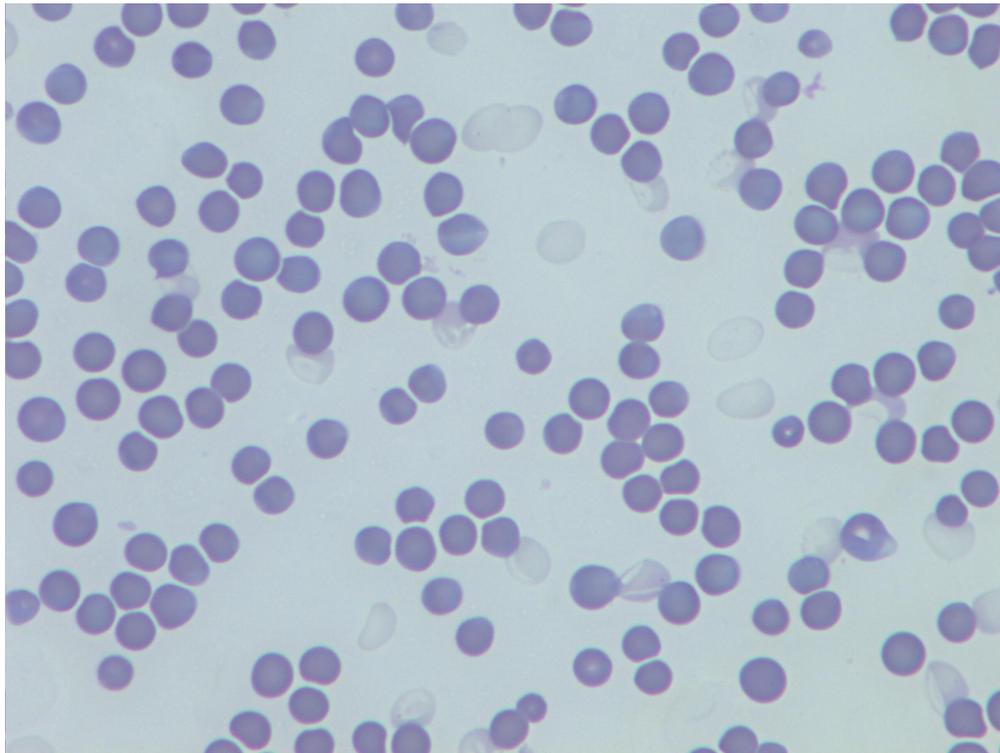
The following photographs are taken from blood smears examined on Day 9 and Day 51.



**Fig. 1:** Blood smear; Day 9, 400x magnification.



**Fig. 2:** Blood smear; Day 9, 1000x magnification.



**Fig. 3:** Blood smear; Day 51, 1000x magnification.

**Additional tests**

**Table 2:** Serological tests

FIV	negative (IC)
FeLV	negative (ELISA)
Feline corona virus	1:10 (IFAT)
<i>Mycoplasma haemofelis</i>	negative (PCR)
<i>Candidatus Mycoplasma haemominutum</i> ,	negative (PCR)
<i>Candidatus Mycoplasma turicensis</i>	negative (PCR)
<i>Ehrlichia canis</i> , <i>Babesia canis</i>	negative (IFAT)
<i>Anaplasma phagocytophilum</i>	negative (IFAT)

Erythrocyte osmotic fragility (day 140): 0.70% (normal range 0.48-0.58%)

Coombs' test: 1:64 positive at 37°C and 4°C.

ANA (IFAT): negative

The cat's veterinarian reported a 4+ positive reaction for haeme on an in-house urine dipstick; sediment examination was negative for red blood cells, leading to a diagnosis of haemoglobinuria. Unfortunately no further data regarding urinalysis (e.g., specific gravity or urine protein) could be obtained.

**Blood smear evaluation**

Day 9: Numerous ghost red blood cells present. Approximately 10% of the neutrophils contained phagocytosed red blood cells.

Day 51: Numerous ghost red blood cells present

## **Diagnosis**

Persistent primary immune-mediated haemolytic anaemia (IMHA) with intravascular haemolysis.

## **Description of results**

A diagnosis of primary IMHA was made based on an initially regenerative anaemia with hyperproteinaemia, autoagglutination and exclusion of other causes of IMHA. The azotaemia present may have been pre-existing to the onset of the anaemia or may be secondary to haemoglobin nephropathy.

Treatment was started with prednisolone at a dose of 2mg/kg oid reduced to eod after 7days, clindamycin 5mg/kg bid, benazepril 0,125mg/kg oid and a renal diet.

A repeat blood sample taken on day 9 revealed an improvement in the degree of the anaemia although the reticulocyte count was reduced. Many lytic red blood cells, also known as red blood cell “ghosts” were seen on the blood smear, and approximately 10% of the neutrophils contained phagocytosed red blood cells.

The anaemia had worsened again by day 28, spontaneous agglutination was noted in the sample and red blood cell ghosts were again present. No phagocytosis of red blood cells by neutrophils was noted. One neutrophil containing a phagocytosed red blood cell, as well as numerous red blood cell ghost cells were observed in the smear from day 51.

## **Discussion**

Immune-mediated haemolytic anaemia is a process characterised by a reduced red blood cell lifespan related to the presence of autoantibodies directed against erythrocytes. The haemolysis can occur secondarily to concurrent conditions which include non-infectious and infectious diseases. Non-infectious diseases include toxic causes like drugs, neoplasia, blood transfusions and systemic lupus erythematosus (SLE). None of these were present in the history in our case; ANAs were negative. Infectious causes in cats include FIV, FeLV and haemoplasmosis<sup>2</sup> – these were excluded here through serological testing and PCR. If no cause can be found for the haemolytic anaemia it is referred to as primary. Diagnosis of primary immune-mediated destruction of the red blood cells is made by excluding the above causes and when evidence of an immune reaction against the red blood cells – i.e. agglutination or a positive Coombs’ test, is present.

The pathogenesis of IMHA involves the attachment of autoantibodies to red blood cell membrane glycoproteins called glycophorins. These antibodies can be of the IgA, IgG and IgM classes . Complement factor 3 (C3) also plays a role<sup>6</sup>. Haemolysis can be extravascular, intravascular or a combination of both.

When the autoantibodies are of the IgG class, they also attach to specific receptors on mononuclear-phagocytic cells, specifically splenic macrophages. The red blood cell is then phagocytosed. This process is accelerated if complement is fixed by the autoantibodies bound to the red blood cell membrane: the opsonin complement factor 3b (C<sub>3</sub>b) is subsequently deposited on the cell membrane and interacts with macrophage receptors leading to phagocytosis<sup>1</sup>. Sometimes only partial phagocytosis takes place and the semi-intact red blood cells are released

back onto circulation as spherocytes. This process of red blood cell phagocytosis by macrophages is known as extravascular haemolysis.

Spherocytes are difficult to detect in feline blood smears as normal feline red blood cells are smaller than canine red blood cells and lack central pallor. It has been suggested that an increased osmotic fragility (OF) of red blood cells may be used to determine if spherocytes and therefore IMHA is present – spherocytes have a reduced membrane surface area to cell volume and are thus more osmotically fragile than normal red blood cells<sup>2, 12</sup>. The OF carried out in this cat was increased (0,70%, reference range 0,48-0,58%) suggesting the presence of spherocytosis. The OF was carried out on day 140, indicating that despite glucocorticoid treatment, an element of extravascular haemolysis was still present in this case.

Agglutination of red blood cells takes place when IgM antibodies are attached to the red blood cells<sup>6</sup>. In this case strong autoagglutination was observed on multiple occasions, indicating the presence of IgM autoantibodies.

In some cases, IgM can trigger the entire complement cascade and cause the formation of membrane attack complexes (MACs) in the red cell membrane. These MACs form pores in the cell membrane, resulting in the leakage of haemoglobin out of the cell. This is manifested as a haemoglobinaemia and haemoglobinuria and results in the presence of ghost cells on blood smears. This process is known as intravascular haemolysis. The presence of red blood cell ghosts, persistent haemoglobinaemia (serum/plasma haemolytic) and haemoglobinuria supports the presence of intravascular haemolysis in this cat.

The Coombs' test performed here was polyvalent and thus did not differentiate between IgG, IgM and complement. It was positive at a dilution of 1:64 at 4°C and 37°C, which would indicate the presence of at least cold-agglutinin IgM. It can also be assumed that at least body temperature-active IgM and complement were present here, based on the autoagglutination and intravascular haemolysis. In a study by Kohn *et al*, two out of 19 cats were positive for IgM and none for C<sub>3</sub>b (at 37°C)<sup>2</sup>.

Although secondary IMHA is the most common cause of haemolytic anaemia in felines<sup>6</sup>, primary IMHA is uncommon in cats<sup>1</sup>. This is in contrast to dogs, where 60-75% of IMHA cases are primary<sup>7</sup>. Reported average ages for cats with primary IMHA in previous studies are 3 years (5 cats)<sup>4</sup>, 3.2 years (19 cats)<sup>2</sup> and 6.2 years (25 cats)<sup>3</sup>, our cat is therefore relatively old for this disease at an age of 12 years. A higher incidence in male cats has been reported<sup>2,3</sup>. These studies also report that autoagglutination is fairly common, with 15 out of a total of 49 cats exhibiting this phenomenon, so the autoagglutination seen here is not unusual. Intravascular haemolysis has however not previously been documented in studies of feline primary IMHA<sup>2,3,4</sup>.

Another unusual feature of this case was the erythrophagocytosis displayed by neutrophils in the blood smear examined on day 9 and day 28. As previously mentioned, RBC phagocytosis in IMHA is usually performed by macrophages. Phagocytosis by neutrophils depends on the binding of an opsonin to the material to be phagocytosed. Opsonins can be antibodies, complement factors (C<sub>3</sub>b) or alpha or beta globulins. Neutrophils have specific receptors which bind to these proteins; once this has occurred phagocytosis takes place. In this case the opsonins could be IgM antibodies or complement factors<sup>8</sup>, both of which can be assumed to be present at fairly high levels based on the high numbers of ghost cells, and persistent hemolysis and autoagglutination.

Destruction of RBCs by feline neutrophils has been shown in vivo, however the invitro significance is unknown<sup>5,9</sup>. Erythrophagocytosis by neutrophils in blood has been reported in humans in cases of sickle cell anaemia<sup>10</sup> and paroxysmal cold haemoglobinuria (PCH)<sup>11</sup>. (PCH is

caused by an IgG autoantibody which binds to red blood cells at low temperatures and then induces haemolysis via complement when the red blood cells are warmed).

This is an unusual case of IMHA in a cat exhibiting intravascular haemolysis and neutrophil erythrophagocytosis .

## References

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