Understanding Reticulocyte Counts in Cats

Classify your feline anemias correctly

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Overview

One of the most useful first steps in the classification of anemia is to determine if the anemia is regenerative or nonregenerative. The reticulocyte count is the most objective measure of regeneration (appropriate bone marrow response). Mechanisms of regenerative anemia include increased erythrocyte destruction (hemolysis) or blood loss (hemorrhage). A nonregenerative anemia generally indicates that the anemia is the result of decreased or ineffective erythrocyte production; however, caution should be taken in classifying an acute onset anemia. About 3–5 days are required before there is a significant peripheral blood reticulocytosis associated with increased marrow production. Because of the marrow's small storage pool of maturing reticulocytes, a transient slight reticulocytosis may occur due to release of these cells in cases of acute, severe anemia.

Reticulocytes

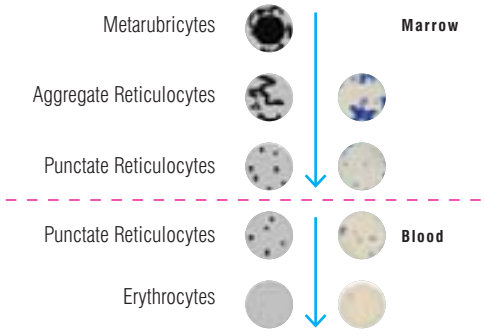
Reticulocytes are immature erythrocytes that form when metarubricytes extrude their nuclei, which mostly occurs in the extravascular space within the marrow. Metarubricytes are released into blood in certain disease states, but should not be used as an indicator of regeneration. Reticulocytes are nonnucleated cells that derive their name from a network or "reticulum" that appears as precipitated ribosomal RNA and proteins when stained with basic dyes such as new methylene blue stain. Two types of reticulocytes are commonly identified; aggregate reticulocytes are less mature and contain large amounts of precipitated material and punctate reticulocytes are more mature and have only few specks of material. With routine blood film staining (Wright's-type stains), aggregate reticulocytes appear bluish red (polychromatophilic) but punctate reticulocytes cannot be differentiated from mature erythrocytes.

Reticulocyte release from bone marrow and maturation in blood

Reticulocyte maturation begins in the bone marrow and is completed in the peripheral blood and spleen of cats and dogs. Therefore, even in health, dogs and cats will have some circulating reticulocytes resulting from normal red blood cell turnover. Aggregate reticulocytes are typically released from canine marrow, but mostly mature punctate reticulocytes are released from feline marrow. Consequently, only a few aggregate reticulocytes, but many punctate reticulocytes, are found in blood from normal adult cats. The high percentage of punctate reticulocytes results from a long maturation time with delayed degradation of ribosomal material.

Aggregate reticulocytes mature to punctate reticulocytes in a day or less, but a week or more is required for maturation of punctate reticulocytes to mature erythrocytes in cat blood.

Pictorial vs. Stained Blood Films of Normal Cats



Reticulocyte counts

Manual reticulocyte counts: Reticulocyte stains are commercially available for manual reticulocyte counts. Equal volumes of blood and stain are mixed together and incubated at room temperature for 10–20 minutes. After incubation, blood films are made and reticulocyte counts are obtained by examining 1,000 nonnucleated erythrocytes microscopically and determining the percentage of reticulocytes. In cats, aggregate and punctate reticulocytes should be reported separately. Based on findings from several authors, normal cats generally have 0%–0.5% aggregate and 1%–10% punctate reticulocytes.[1–4] To reduce the chance that a staining artifact would result in misclassifying a mature erythrocyte as a punctate reticulocyte, the cell being identified as a punctate reticulocyte should have two or more discrete blue granules that are visible without requiring fine focus adjustment of the cell. These inclusions should be away from the cell margin to avoid confusion with erythrocytic *Mycoplasma* organisms or small Heinz bodies.

Corrected reticulocyte counts: A raw (uncorrected) manual reticulocyte count can be misleading when moderate to severe anemia is present because reticulocytes are quantified as a percentage of total erythrocytes (reticulocytes plus mature erythrocytes) counted. The raw manual reticulocyte count would be higher in an anemic animal with a lower number of mature erythrocytes than it would be in a normal animal, even if the actual number of reticulocytes/μL in the circulation were the same in each animal. Therefore, the raw manual reticulocyte count should be corrected for the degree of anemia. To obtain a corrected reticulocyte count, the patient's hematocrit (HCT) is divided by the mean normal hematocrit for the species (42% for cats), and this value is multiplied with the raw manual reticulocyte count.

The corrected reticulocyte response to blood-loss anemia in the cat is shown in figure 1. As noted above, 3–5 days are required for maximal aggregate reticulocyte response to anemia. The maximal punctate reticulocyte response occurs considerably later, primarily because of the long time required for punctate reticulocytes to mature to erythrocytes. As can be seen in figure 1, punctate reticulocyte release from bone marrow continues after the aggregate reticulocyte release has ceased and note the parallel increases in packed cell volume (PCV) and punctate reticulocyte counts as well as a parallel return to normal for both. Consequently, cats with mild anemia may have increased punctate reticulocyte counts and normal aggregate reticulocyte counts. Since punctate reticulocyte counts are so dramatic, a rapid scan of a reticulocyte-stained blood film is generally acceptable to properly classify this type of anemia (see figure 2).

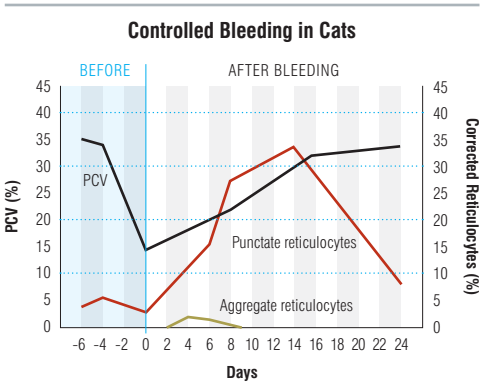


Figure 1. Corrected reticulocyte response following controlled bleeding in cats. Corrected reticulocyte counts have been calculated using packed cell volume (PCV) values from data published by Alsaker RD, Laber J, Stevens JB, Perman V: A comparison of polychromasia and reticulocyte counts in assessing erythrocyte regenerative response in the cat. *J Am Vet Med Assoc.* 1977;170:39. (Reproduced with permission from: Meyer DJ, Harvey JW. *Veterinary Laboratory Medicine: Interpretation and Diagnosis.* Philadelphia: Saunders; 2004.)

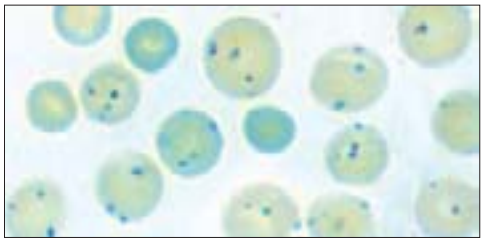


Figure 2. Peripheral blood film from a cat with mild regenerative anemia with a marked punctate reticulocytosis and normal aggregate reticulocyte count, new methylene blue stain, 100x oil objective field of view.

Absolute reticulocyte counts: If the total erythrocyte count is known, an absolute reticulocyte count (reticulocytes/μL) can be determined. This is done by multiplying the percentage of reticulocytes counted (expressed as a fraction) times the total erythrocyte count. Normal adult cats have up to approximately 60,000/μL aggregate reticulocytes, but punctate reticulocytes may be as high as 650,000/μL. Absolute aggregate reticulocyte counts can also be determined directly by flow cytometry with some in-house and reference laboratory automated hematology analyzers. These instruments provide more rapid results with better precision than the manual method. However, it is essential that even these automated counts be validated by blood film inspection for polychromasia, which correlates to aggregate reticulocytosis. In the cat, it is especially important to examine blood stained with reticulocyte stains since automated counts do not currently enumerate punctuated forms.

Interpretation of Reticulocyte Counts

The presence of a substantial absolute reticulocytosis in blood indicates that either hemorrhage or increased erythrocyte destruction is the cause of the anemia. In feline anemias where the HCT decrease is moderate to severe (HCT less than approximately 20%), the aggregate count is used to characterize the regenerative status. However, in cats with a mild anemia, aggregate reticulocyte counts are often low and there may be little to no polychromasia on routinely stained blood films even when there is a bone marrow response. A simple scan of a reticulocyte-stained blood film for punctate reticulocytes can be performed to accurately characterize bone marrow responsiveness. Absolute reticulocyte counts are generally higher in response to a hemolytic anemia compared to a blood-loss anemia and absolute reticulocyte counts should increase as the severity of anemia worsens. The absence of a reticulocytosis indicates that decreased erythrocyte production is the cause of the anemia, so long as sufficient time has passed for increased reticulocyte production to occur. |DX|

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3. Harvey JW. *Atlas of Veterinary Hematology.* Blood and Bone Marrow of Domestic Animals. Philadelphia, Pa: WB Saunders; 2001.
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CASE STUDY

Feline Anemia

Taking the guesswork out of diagnosing feline hemotropic mycoplasmosis

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Alex

Patient: Alex, 5-week-old, intact male domestic shorthair kitten

Presenting complaint: Found laterally recumbent by good samaritan in parking lot

History: Previous history unknown

Physical examination: Alex was laterally recumbent and nonresponsive. His mucous membranes were markedly pale. He was dehydrated and hypothermic with a temperature of 94°F. He was bradycardic with a heart rate of 50 beats per minute. Thoracic auscultation and abdominal palpation were unremarkable. He had a severe flea infestation. Alex was in good body condition and weighed 1 lb.

Preliminary laboratory data: In-house packed cell volume (PCV) was 5%; total solids (TS) were 3.2 mg/dL and results of SNAP® FIV/FeLV Combo Test were negative.

Assessment: Alex had a severe life-threatening anemia. To help determine the cause of an anemia, it should be characterized as regenerative or nonregenerative. This is based upon the presence or absence of reticulocytes ("immature" erythrocytes) in the peripheral circulation. There are two main causes of a regenerative anemia: blood loss or hemolytic disease. Caution should be taken to avoid misinterpretation of reticulocyte counts because there is a lag of at least 3–5 days before a regenerative response is seen in the peripheral blood following acute blood loss or hemolysis.

In Alex's situation, there was no historic information to allow identifying a timeline for this clinical presentation. The observed clinical signs and degree of anemia and decrease of total solids, especially in light of the dehydration observed was most supportive of a blood-loss anemia secondary to flea infestation. Contribution to the anemia by endoparasites was not ruled out.

Therapeutic plan: 20-mL fresh whole blood transfusion from in-house blood donor cat. Posttransfusion PCV was 20%. Alex became much more responsive, alert and was eating posttransfusion. Alex was then given a flea bath and dewormed.

Diagnostic plan: Ideally a complete blood count (CBC) with blood film review and reticulocyte panel as well as a complete biochemical profile would have been performed; however, Alex's veterinarian was dealing with a critical patient and felt that it was best to institute emergency intervention. Alex was scheduled for a recheck examination and CBC in one week.

Steps to Determining Cause of Anemia in Cats

1. Perform comprehensive CBC with blood film review and retroviral (FeLV/FIV) testing.
2. Characterize severity of anemia and correlation to clinical picture.
3. Determine if anemia is regenerative or nonregenerative.
4. If regenerative, look for bleeding (external and internal).
5. If regenerative and no obvious bleeding, consider hemolytic disease.
6. If hemolytic disease is suspected, review retrovirus status history for possible toxin exposure and blood film morphology, and perform FHM PCR test.
7. If nonregenerative, review retroviral status, assess renal function, investigate for evidence of chronic/inflammatory illness, perform FHM PCR test and consider bone marrow aspirate/biopsy.

One-week recheck

History: Alex had been doing well during the week posttransfusion. He had a reasonable appetite and was somewhat playful.

Physical examination: Alex was bright, alert, responsive and adequately hydrated. Mucous membranes were pale pink. Rest of the physical examination was within normal limits. No fleas were seen. Weight was 1.4 lb.

Laboratory results: In-house PCV was 18%. Comprehensive CBC results from IDEXX Reference Laboratories revealed a moderate macrocytic, normochromic anemia with a moderate to marked reticulocytosis supportive of a strongly regenerative anemia.

HEMATOLOGY	VALUE	UNITS		REF INTERVAL
RBC	2.99	M/µL	Low	(6.00 - 10.00)
HCT	18.7	%	Low	(29.0 - 45.0)
HGB	5.4	g/dL	Low	(9.5 - 15.0)
MCV	63.0	fL	High	(41 - 58)
MCH	18.2	pg	High	(11 - 17.5)
MCHC	29.1	g/dL		(29 - 36)
nRBC	10/100	WBC	High	(0 - 2/100)
POLYCHROMASIA			Moderate	
Remarks		Slide review microscopically. No feline hemotropic mycoplasma seen.		
Reticulocyte Count	3.9	%	High	(0.00 - 1.0)
Absolute Reticulocyte	116610	mm³	High	(0.00 - 50000)

An absolute reticulocyte count of greater than 50,000/mm³ is considered evidence of regenerative anemia.
Degree of regeneration: /mm³ (Aggregate reticulocytes)
None: <15,000 Slight: 50,000 Moderate: 100,000 Marked: >200,000

Number of nucleated red blood cells (nRBCs) were much fewer than the polychromatophils on the blood film review and this was considered acceptable for degree of regeneration. nRBCs may be seen in cases of strongly regenerative anemia, but they should never be used as the primary indication of regeneration. No feline hemotropic mycoplasma (FHM; formerly *Haemobartonella*) were seen on slide review.

Assessment: Alex's anemia had improved dramatically from presentation and was relatively stable posttransfusion. Alex had no evidence of fleas and had been previously dewormed; therefore, no ongoing blood loss was suspected. Given there was a good regenerative response on the CBC, Alex's veterinarian expected the anemia to resolve over roughly the next week.

Plan: Recheck in one week

Three days later

History: Alex had become quieter and he had not eaten breakfast.

Physical examination: Alex was quiet, alert, responsive and hydrated. His mucous membranes were pale. No other physical examination abnormalities were noted. Weight was 1.6 lb.

Laboratory results: Comprehensive CBC revealed a marked macrocytic, normochromic and markedly regenerative anemia. The hematocrit (HCT) had dropped from 18.7% to 8.3% and the mean corpuscular hemoglobin concentration (MCHC) increased from 29.1 g/dL to 34.3 g/dL in three days.

HEMATOLOGY	VALUE	UNITS		REF INTERVAL
RBC	1.22	M/μL	Low	(6.00 - 10.00)
HCT	8.3	%	Low	(29.0 - 45.0)
HGB	2.8	g/dL	Low	(9.5 - 15.0)
MCV	68.0	fL	High	(41 - 58)
MCH	23.2	pg	High	(11 - 17.5)
MCHC	34.3	g/dL		(29 - 36)
nRBC	35/100	WBC	High	(0 - 2/100)
POLYCHROMASIA			Marked	
Remarks		Slide review microscopically. No feline hemotropic mycoplasma seen.		
Reticulocyte Count	15.0	%	High	(0.00 - 1.0)
Absolute Reticulocyte	183000	mm^3	High	(0.00 - 50000)

An absolute reticulocyte count of greater than 50,000/mm^3 is considered evidence of regenerative anemia.
Degree of regeneration: /mm^3 (Aggregate reticulocytes)
None: <15,000 Slight: 50,000 Moderate: 100,000 Marked: >200,000

The degree of reticulocytosis and the presence of macrocytosis are strongly supportive of a hemolytic mechanism to the anemia. Blood-loss anemias typically have lower bone marrow responsiveness and do not show a macrocytosis due to loss of a critical component of hemoglobin, iron. The increase in MCHC over this same time period suggests the presence of cell-free hemoglobin in the current specimen associated with either in vitro or in vivo hemolysis. As with the previous CBC, the number of nRBCs seemed appropriate for the degree of regeneration and no erythrocyte-associated infectious agents were identified.

Indications for Performing a Feline Hemotropic Mycoplasma PCR Test

- All cats with regenerative anemia where there is no obvious bleeding
- FeLV-positive cats with anemia
- Cats with other chronic illnesses where anemia is worse than expected
- Cats with nonregenerative anemia where there is no apparent cause
- All blood-donor cats

An IDEXX RealPCR™ FHM Test was positive for *Mycoplasma haemofelis.*

Assessment: Alex's anemia had significantly worsened over the previous three days. Based on the CBC findings, a hemolytic anemia was suspected. Upon reevaluation of the previous CBC, it was noted that some of these findings (e.g., strongly regenerative response and macrocytosis) were present and may have given an earlier indication that this was in fact a hemolytic anemia.

Although, no FHM were seen on slide review on the previous or current CBC, Alex's veterinarian was aware that these organisms can come off the surface of affected erythrocytes during EDTA-anticoagulated blood transportation to the laboratory. The veterinarian had also recently attended an IDEXX seminar where data was presented showing the increased sensitivity of the IDEXX RealPCR FHM Test compared to slide review (see table below). Alex tested positive for *Mycoplasma haemofelis*, which is the most pathogenic FHM species and can cause a hemolytic anemia in an immunocompetent cat. Therefore, a diagnosis of feline hemotropic mycoplasmosis (formerly hemobartonellosis) was made.

Identification of FHM (formerly *Haemobartonella*)—IDEXX RealPCR™ vs. Slide Review by Trained Technicians
(Total samples = 303*)

	Positives found by IDEXX RealPCR FHM Test	Positives found by slide review
Positive for any species	88 (29.0%)	10 (3.3%)
Positives by species or species combination	Mhf: 21 (6.9%) CaMh: 41 (13.5%) CaMt: 5 (1.7%) Mhf & CaMh: 8 (2.6%) Mhf & CaMt: 3 (1.0%) CaMh & CaMt: 3 (1.0%) Mhf & CaMh & CaMt: 7 (2.3%)	Could not distinguish species

Mhf = *Mycoplasma haemofelis*
CaMh = *Candidatus* Mycoplasma haemominutum
CaMt = *Candidatus* Mycoplasma turicensis
*All samples were whole blood from cats with hematocrit less than 25%.

(continued on page 50)

Feline Anemia Case Study *(continued from page 19)*

Plan: Alex was given one dose of 5 mg/kg of doxycycline prior to receiving CBC and IDEXX RealPCR FHM Test results. He showed marked improvement the following day and was prescribed doxycycline at a dose of 5 mg/kg twice daily for three weeks. A recheck was scheduled for one week after the beginning of therapy.

Clinical case outcome

Alex was rechecked one week after initiation of doxycycline therapy. He was active, eating and appeared to be a healthy kitten. Repeat CBC revealed near complete resolution of his anemia. His HCT was 28.3%. A high but slightly decreased MCV (66 fL) resulting from immature RBCs in the circulation persisted; a reticulocyte count was not performed. Alex continued to do well on doxycycline therapy and a second recheck one week later revealed he had gained a pound and had a completely normal CBC. |DX|

Feline Pancreatitis Case Study *(continued from page 23)*

Case management

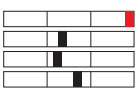
Intravenous fluids were administered for the noted dehydration. After attaining proper fluid balance, the HCT was 27.2% revealing the suspected mild anemia. Antibiotic therapy (intravenous ampicillin) was initiated and intravenous histamine-2 blocker (ranitidine) was administered as well. The owners declined further diagnostics, including biopsy of liver and possible other organs such as intestines and pancreas, because Thebe did not handle anesthesia well during biopsy of liver as the result of an episode of hepatic lipidosis 4–5 years previously.

Due to decreasing liver enzymes over several days of hospitalization, bacterial cholangiohepatitis was suspected and a decision was made to continue antibiotic therapy. Thebe was released from the hospital with oral antibiotics (Clavamox®) and gastrointestinal protectants (famotidine). Instructions for treatment with Otomax® ear ointment following ear cleaning in the hospital were included also.

Additional pending diagnostics

Specialized testing (five days after presentation):

TEST	VALUE	UNITS		REF INTERVAL
fPLI	12.4	µg/L	High	(2.0 – 6.8)
Folate	12.3	µg/L		(9.7 – 21.6)
Cobalamin	440	ng/L		(290 – 1499)
TLI Fasting	65.5	µg/L		(12 – 82)



Microbiology (ten days after presentation):

Urine culture: Negative

Final diagnosis

1. Pancreatitis

2. Probable secondary cholangiohepatitis

3. Possible chronic gastrointestinal disease such as IBD

4. Probable early renal disease

5. Inflammation and superimposed stress—potential triaditis

Clinical case outcome

Over the next year, Thebe had multiple recurring episodes of vomiting with assumed recurring episodes of pancreatitis. Within a year from first presentation, renal failure had developed with increased creatinine in the face of a nonconcentrated urine specific gravity. There was continued weight loss and periods of inappetence. Thebe presented with vomiting while extremely weak, lethargic and anorectic approximately 1.5 years from the original presentation. She died the following day and a complete necropsy was allowed. Necropsy findings included pancreatitis associated with pancreatic adenocarcinoma and metastatic disease, cholangiohepatitis, cholecystitis and interstitial nephritis. A comment was included suggesting the possibility of primary pancreatic adenocarcinoma resulting in secondary recurring episodes of pancreatitis and cholangiohepatitis. |DX|