

Reticulocytes

Differentials

Description: Reticulocytes are immature red blood cells that do not have a nucleus. Their presence in circulation is used to differentiate regenerative from nonregenerative anaemia. Reticulocytosis in the absence of anaemia can be an indication of significant underlying disease accompanied by RBC loss or destruction with an adequate regenerative response (compensated anaemia). Reticulocytes should be evaluated along with the reticulocyte haemoglobin (RETIC-HGB) for early detection of blood loss and inflammatory conditions prior to the development of anaemia.

Increased Reticulocytes

- Regenerative anaemia
 - Blood loss
 - Haemorrhage
 - Parasitism
 - Immune-mediated haemolysis
 - Primary (idiopathic) immune-mediated hemolytic anaemia (IMHA)
 - Secondary IMHA
 - Infection
 - Neoplasia
 - Systemic immune-mediated disease
 - Drugs/vaccines
 - Increased red blood cell destruction (non-immune)
 - Heinz body anaemia
 - Toxins
 - Drugs
 - Dietary
 - Mechanical fragmentation (microangiopathic)
 - Vasculitis
 - Intravascular coagulation, both disseminated and local
 - Haemangiosarcoma
 - Heartworm infection
 - Metabolic
 - Hereditary
 - Envenomation
- Reticulocytosis in the absence of anaemia
 - Physiologic reticulocytosis - splenic contraction
 - Excitement
 - Exercise
 - Drugs: epinephrine
 - Compensated anaemia
 - Blood loss
 - Haemolysis
 - Absolute erythrocytosis
 - Endocrine
 - Secondary polycythaemia
 - Polycythaemia vera

Next Steps and Related Findings for Increased Reticulocytes

- Regenerative Anaemia:
 - Blood loss
 - CBC:
 - Decreased RBC, haematocrit, MCHC, MCH
 - Increased MCV and RDW

- Increased reticulocytes
 - Decreased reticulocyte haemoglobin (RETIC-HGB)
 - Platelets - variably decreased (consumption)
 - Inflammatory leucogram
- Iron panel:
 - Decreasing total serum iron concentration and serum ferritin
 - Normal to increasing total iron binding capacity (transferrin) with progression
- Chemistry:
 - Decreased total protein and/or albumin
- Next steps to evaluate for causes of blood loss:
 - Positive faecal ova and parasite screen, faecal antigen tests, faecal occult blood
 - Urinalysis: haematuria, transitional epithelial cells on urine cytology
 - Abnormal coagulation tests:
 - Increased prothrombin time (PT) and activated partial thromboplastin time (APTT)
 - Prolonged buccal mucosal bleeding time
 - Low von Willebrand factor concentration
- Immune-mediated hemolytic anaemia
 - CBC:
 - Decreased RBC, haemoglobin, MCHC and MCH
 - Increased MCV and RDW
 - Increased reticulocytes +/- nucleated RBCs
 - Platelets decreased if concurrent immune-mediated thrombocytopaenia (Evan's syndrome)
 - Inflammatory leucogram
 - Decreased reticulocyte haemoglobin (RETIC-HGB)
 - Spherocytosis (in dogs) and/or blood parasites may be seen on blood film review
 - Positive Coombs or saline agglutination test
 - Chemistry - increased serum bilirubin
 - Urinalysis - bilirubinuria +/- haemoglobinuria
 - Increased C-reactive protein (CRP)
 - Next steps to evaluate for causes of secondary IMHA:
 - Assess for history of recent vaccination or medication
 - Serology, PCR or antigen testing positive for infectious causes (e.g. anaplasmosis, ehrlichiosis, FeLV, FIV, haemotropic *Mycoplasma*, babesiosis, cytauxzoonosis)
 - Positive antinuclear antibody (ANA) titer
 - Assess thoracic and abdominal imaging for neoplasia
 - Primary IMHA is a diagnosis of exclusion of secondary causes
- Heinz body anaemia (oxidative injury)
 - CBC:
 - Decreased RBC, haemoglobin, MCHC and MCH
 - Increased MCV and RDW
 - Increased reticulocytes
 - Increased nucleated RBCs
 - Decreased reticulocyte haemoglobin (RETIC-HGB)
 - Inflammatory leucogram
 - Blood film review: Heinz bodies, target cells, basophilic stippling, polychromasia +/- mild spherocytosis
 - Chemistry:
 - Increased serum bilirubin
 - Increased ALP, AST, +/- GGT, ALT
 - Intravascular haemolysis noted (red colour to serum)
 - Urinalysis:
 - Haemoglobinuria
 - Bilirubinuria
 - Proteinuria
 - Next steps to evaluate for causes of Heinz body anaemia:
 - Increased zinc concentration
 - Abdominal radiographs may reveal gastrointestinal metallic foreign body
 - History of exposure to drugs or dietary causes of Heinz body anaemia, e.g.:

- Onions
 - Garlic
 - Propylene glycol
 - Acetaminophen (dogs and cats)
 - Phenothiazine (horses)
- Reticulocytosis in absence of anaemia
 - Physiologic reticulocytosis - most often due to splenic contraction (dogs only)
 - CBC:
 - Normal RBC, haematocrit, MCV and RDW
 - Reticulocytosis is generally mild
 - Compensated anaemia - regeneration of RBCs is keeping up with RBC loss or destruction
 - CBC:
 - RBC and haematocrit at low end of normal
 - MCV and RDW increased
 - Decreased MCH and MCHC
 - Decreased reticulocyte haemoglobin (RETIC-HGB)
 - Inflammatory leucogram
 - Absolute erythrocytosis
 - CBC:
 - High normal to increased RBC and haematocrit
 - +/- increased nucleated RBCs
 - Endocrine
 - Increases in RBC, haemoglobin and haematocrit are typically mild
 - Evaluate for endocrine imbalances:
 - Hyperthyroidism
 - Hyperadrenocorticism (Cushing's disease)
 - Acromegaly
 - Secondary polycythaemia
 - CBC - marked increases in RBC and haematocrit (typically > 60%)
 - No evidence of dehydration on physical exam, chemistry or urine specific gravity
 - Erythropoietin (where available) usually increased, but may be normal
 - Systemic hypoxia:
 - Thoracic imaging for evidence of pulmonary disease, cardiac disease, or right-to-left heart defects
 - History of residence in, or travel to, high altitude
 - Decreased PAO₂ and SaO₂
 - Increased Cardiopet proBNP test
 - Inappropriate erythropoietin secretion:
 - Renal disorders causing localized hypoxia or paraneoplastic syndrome
 - Abdominal imaging for evidence of kidney abnormalities (e.g. cyst, tumor, hydronephrosis) or extra-renal neoplasia (e.g. hepatoma)
 - Polycythaemia vera
 - CBC:
 - Marked increases in RBC and haematocrit (typically > 60%)
 - Platelets - variably increased
 - No evidence of dehydration on physical exam, chemistry or urine specific gravity
 - Decreased or normal erythropoietin (where available)
 - No evidence of systemic hypoxaemia, endocrine imbalances, kidney abnormalities or neoplasia
 - Polycythaemia vera is often a diagnosis of exclusion

Normal Reticulocytes

- Normal reticulocytes with anaemia
 - Non-regenerative anaemia
 - Chronic inflammatory disease
 - Infection
 - Immune-mediated
 - Neoplasia

- Chronic blood loss
 - Parasitism
 - Haemorrhage
- Decreased erythropoietin production
 - Chronic kidney disease
 - Hypothyroidism
 - Hypoadrenocorticism (Addison's disease)
- Bone marrow suppression
 - Drugs/toxins
 - Bone marrow disease
- Liver failure or portosystemic shunt (PSS)
- Immune-mediated (targeting RBC precursors)
 - Pure red cell aplasia
- Nutritional deficiencies
 - Vitamin B12 (cobalamin)
 - Folate
- Acute pre-regenerative anaemia
 - Acute blood loss
 - Acute hemolytic anaemia
- Normal reticulocytes in absence of anaemia
 - Normal - a non-anaemic patient typically has low numbers of circulating reticulocytes

Next Steps and Related Findings for Anemia without Reticulocytosis

- Non-regenerative anaemia
 - Anaemia of chronic inflammatory disease (causing iron sequestration)
 - CBC:
 - Mild to moderate, stable anaemia
 - +/- microcytosis
 - Hypochromasia (uncommon)
 - Decreased reticulocyte haemoglobin (RETIC-HGB)
 - Inflammatory leucogram
 - Chemistry:
 - Increased total protein and globulins
 - Decreased albumin
 - Increased C-reactive protein (CRP)
 - Iron Panel:
 - Decreased total serum iron concentration and iron binding capacity (transferrin)
 - Increased serum ferritin
 - Next steps to evaluate for evidence of underlying causes of inflammation:
 - Immune-mediated diseases:
 - CBC:
 - Spherocytosis (if IMHA)
 - RBC agglutination (if IMHA)
 - Severe thrombocytopenia (if ITP)
 - Coombs - positive if IMHA
 - Anti-nuclear antibody (ANA) tests - variably positive
 - Joint fluid cytology - neutrophilic inflammation if immune-mediated polyarthritis
 - Systemic infection:
 - PCR, serology or antigen tests - positive for infectious diseases (e.g. Lyme, ehrlichiosis, leptospirosis, systemic fungal, heartworm, etc.)
 - Chronic localised inflammation or infection:
 - Diagnostic imaging (radiographs and/or ultrasound) to assess for localised inflammation or infection
 - Pancreatitis:
 - Spec cPL or Spec fPL Test increased
 - Amylase and lipase variably increased

- Pyelonephritis:
 - Chemistry - increased SDMA, creatinine and urea
 - Urinalysis - active sediment (pyuria, haematuria, bacteriuria, casts)
 - Urine culture - positive
 - Neoplasia:
 - Diagnostic imaging (radiographs and/or ultrasound) to assess for neoplasia
- Chronic blood Loss - causing iron deficiency
 - CBC:
 - Microcytosis (decreased MCV) and hypochromasia (decreased MCHC)
 - Decreased reticulocyte haemoglobin (RETIC-HGB)
 - Iron panel:
 - Decreased total serum iron concentration and serum ferritin
 - Normal to increased total iron binding capacity (transferrin)
 - Chemistry:
 - Decreased total protein and/or albumin
 - Next steps to evaluate for causes of blood loss:
 - Positive faecal ova and parasite screen, faecal antigen tests, faecal occult blood
 - Urinalysis: haematuria, transitional epithelial cells on urine cytology
 - Abnormal coagulation tests:
 - Increased prothrombin time (PT) and activated partial thromboplastin time (APTT)
 - Prolonged buccal mucosal bleeding time
 - Low von Willebrand factor concentration
- Decreased erythropoietin production
 - CBC:
 - Normocytic, normochromic anaemia
 - Decreased RBC and haematocrit
 - Normal RDW
 - Evaluate for underlying causes:
 - Chronic kidney disease:
 - Degree of anaemia is relative to severity and chronicity of kidney disease
 - Chemistry - increased SDMA, creatinine, urea, phosphorus
 - Urinalysis:
 - Inappropriate concentration (urine specific gravity < 1.030 in dogs; < 1.035 in cats; < 1.025 in horses)
 - +/- proteinuria
 - Urine protein: creatinine ratio - variably increased
 - Evaluate blood pressure for systemic hypertension
 - Hypothyroidism:
 - Chemistry - increased fasting serum cholesterol and triglycerides
 - Decreased T4 and free T4, increased cTSH
 - Hypoadrenocorticism (Addison's disease):
 - CBC - normal to increased lymphocytes and eosinophils
 - Chemistry:
 - Variably decreased sodium and chloride
 - Variably increased potassium and calcium
 - Decreased cholesterol and albumin
 - Variably increased SDMA, creatinine and urea with secondary decreased renal perfusion and GFR
 - Adrenal function tests:
 - Decreased resting serum cortisol
 - Abnormal ACTH stimulation
- Bone marrow suppression:
 - Assess history for exposure to drugs, e.g.:
 - Chemotherapy
 - Estrogen (endogenous or exogenous)
 - Phenylbutazone (dogs and horses)
 - Trimethoprim sulfadiazine/sulfonamide
 - Griseofulvin (cats)

- CBC - variable decreases in other cell lines
 - Thrombocytopaenia
 - Leukopaenia
 - Absence of regenerative response (normal reticulocytes)
- Bone marrow cytology or biopsy to assess for decreased cellularity, neoplastic infiltration, fibrosis, erythrophagocytosis, immune-mediated destruction of erythrocyte precursors, megakaryocytes or other changes
- Liver failure or portosystemic shunt (PSS)
 - CBC:
 - Decreased reticulocyte haemoglobin (RETIC-HGB) with PSS
 - Blood film review - variably abnormal RBC morphology (poikilocytosis) e.g. target cells in dogs and keratocytes in cats, ovalocytes, acathocytes
 - Chemistry:
 - Increased (or decreased) ALT, ALP
 - Variably increased bilirubin (not seen with PSS)
 - Decreased albumin, urea, cholesterol, glucose
 - Urinalysis:
 - Urine specific gravity is variable, may be inappropriate (USG < 1.030 in dogs; < 1.035 in cats) due to altered thirst and/or low BUN
 - Ammonium biurate crystalluria
 - Bilirubinuria (not seen with PSS)
 - Increased bile acids
 - Increased ammonia
- Acute pre-regenerative anaemia
 - Regenerative response can take 3-4 days to become apparent. Decreased reticulocyte hemoglobin (RETIC-HGB) may be an early indicator
 - Acute blood loss
 - Chemistry - low serum total protein
 - Evaluate for evidence of blood loss
 - Haemolytic disease
 - CBC - RBC agglutination, spherocytes
 - Coombs or saline agglutination tests - variably positive
 - Chemistry - increased bilirubin
 - Urinalysis - bilirubinuria +/- haemoglobinuria

Additional Information

Physiology

- Reticulocytes are immature red blood cells that do not have a nucleus. Reticulocytes have stainable cytoplasmic RNA which differentiates them from mature red blood cells.
- Reticulocytes are released into circulation as part of a regenerative response to anaemia (except in the horse).
- Circulating reticulocytes have a 2-3 day life span in dogs. Reticulocyte life span is more variable in cats.
 - In dogs with an acute anaemia, reticulocytosis is noted in about 3-4 days, peak reticulocyte production occurs at about 7 days
 - In cats, peak reticulocyte production is expected 7-14 days after an acute anaemia
- For all species except the horse, anaemia can be classified as non-regenerative or regenerative based on the bone marrow response assessed by the number of reticulocytes in circulation. Reticulocytes are rare in the horse.
 - Non-regenerative anaemias have low reticulocyte counts.
 - Note that some non-regenerative anaemias may be pre-regenerative (the anaemia has developed too recently for the regenerative response to have started; typically <3-4 days).
 - Regenerative anaemias have increased reticulocyte counts.
- Reticulocytosis can be found in non-anaemic animals due to either splenic contraction or a compensated anaemia (the RBC production is greater than RBC loss or destruction)
 - Splenic contraction usually results in a mild reticulocytosis. Moderate to severe reticulocytosis is concerning for significant underlying disease with compensated anaemia.
 - Monitoring CBCs over time can help to determine if the reticulocytosis is persistent and progressive.

- Concurrent decreased reticulocyte haemoglobin (RETIC-HGB) suggests compensated anaemia

Diagnostic Methodology

- Incubation of RBCs with new methylene blue causes the stain to precipitate and allows detection of the RNA and mitochondria on the blood film.
- Some reticulocytes can be seen on a Wright-stained blood film. The cells have a blue tinge (polychromatophils) to them but if there is not enough RNA in the cell it may not be detected by this method.
- Cats have two types of reticulocytes (punctate and aggregate) that can be detected when evaluating reticulocytes on a new methylene blue stained blood film.
 - Aggregate reticulocytes have large aggregates of RNA and are a better indicator of active marrow release
 - Punctate reticulocytes have 2-6 small granules
- Reticulocytes can be expressed as a reticulocyte concentration or reticulocyte percentage
 - Absolute reticulocyte count (reticulocyte concentration) – the number of reticulocytes per μL . This is the preferred method for reporting reticulocytes
 - Reticulocyte percentage- the percent RBC that are reticulocytes. For example – if you have 5 reticulocytes per 1000 RBC, the reticulocyte percentage would be 0.5%
- Reticulocytes can be evaluated by two techniques
 - Manual – A new methylene blue stained blood smear is evaluated – the number of reticulocytes per 1000 nonnucleated RBCs is evaluated
 - Automated – CBC analysers either use a light scatter method or fluorescence detection to identify reticulocytes.

References

- Fuchs J, Moritz A, et al. Reticulocytosis in non-anaemic cats and dogs. *J Small An Practice* 2019 *in press*.
- Harvey JW. *Atlas of Veterinary Hematology: Blood and Bone Marrow of Domestic Animals*. Philadelphia, PA: WB Saunders; 2001.
- Latimer KS, Mahaffey EA, Prasse KW, eds. *Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology*, 4th ed. Ames, IA: Blackwell; 2003.
- Stockham SL, Scott MA. *Fundamentals of Veterinary Clinical Pathology*, 2nd ed. Ames, IA: Blackwell; 2008.

Last updated 1/7/2018