FUNdamentals of Hematology:
Diagnosing Anemia

Eric Morissette DVM, Dipl. ACVP (Clinical Pathology)

Introduction

Anemia is one of the most common hematologic abnormalities encountered in veterinary clinical practice. It is the manifestation of an underlying disorder, like a fever, and not a diagnosis. It can be a primary sign of disease (e.g., hemorrhage, immune-mediated hemolytic anemia) or a marker of underlying disease (e.g., cancer, chronic kidney disease). Therefore, even mild, asymptomatic anemia should be investigated thoroughly to diagnose and treat the primary problem.

Anemia is defined as a decrease in red blood cell (RBC) mass and is recognized by a low packed cell volume (PCV), hematocrit (HCT), hemoglobin concentration (HGB), or RBC count below the reference intervals for that species. Anemia can be mild, moderate, or severe and could be caused by an acute disease process or have been ongoing for a long time due to a chronic condition.

> **When evaluating an anemic patient, hematology testing MUST include BOTH quantitative automated cell count + qualitative blood smear evaluation.**

Once receiving abnormal RBC, HCT, HGB, or PCV results, how do we proceed?

1. Evaluate the hematology results considering the patient's clinical status and other diagnostic tests. Consider age, breed, and any potential for laboratory or sampling error. If an automated count is performed, confirm with a PCV and total solids (TS) since this is the measured percent of blood that is occupied by red blood cells.

> **Note:** Anemia can occasionally be masked by concomitant dehydration, since the erythrocytes are relatively concentrated, falsely increasing their measured concentration. In contrast, decreased measured erythrocyte parameters may also be observed when the total-body erythrocyte mass is normal, but there is an expansion of the vascular space faster than the expansion of the total-body erythrocyte mass (relative anemia). This can generally result from overhydration resulting in dilution of erythrocytes, and from splenic sequestration of erythrocytes as occurs with splenic relaxation during anesthesia, and various other causes of splenomegaly.[1,2,3]

2. Perform a blood smear to examine the RBC morphology in addition to confirming the automated cell counts. Remember, a comprehensive, complete blood count (CBC) includes an automated cell count, a blood smear, and a PCV/TS. It is essential, especially with any sick pet, to start with the complete hematologic picture. Often in the case of an anemia, the blood smear can help establish a diagnosis and prognosis.

Classify the Anemia

Two primary laboratory classification schemes are used to describe anemia in veterinary patients: classification by bone marrow responsiveness and classification by RBC indices. These two schemes help the clinician narrow the anemia differential diagnosis list and determine an appropriate therapeutic regimen. The two schemes are often used in tandem.[4]

I. Classification by Bone Marrow Responsiveness (Strength of Erythropoiesis):

Classification of anemia based on bone marrow responsiveness is based on the presence or absence of an increased number of immature erythrocytes in circulation (known as reticulocytosis, polychromasia) or erythroid hyperplasia in the bone marrow. Evaluation must be interpreted relative to the duration and severity of the anemia.[4]

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Reticulocytes (RETIC) vs Polychromatophils (PCM) — Same Cell, Different Stain

In most veterinary species, a RETIC count is considered the easiest, most reliable measure of marrow responsiveness. Note: The horse releases few to no immature RBCs into circulation; therefore a bone marrow sample must be used to determine the erythroid response.

Reticulocytes and polychromatophils are essentially the same cell identified using different stains. Historically, in the absence of a RETIC count, the number of PCM served as an estimate of the bone marrow response.

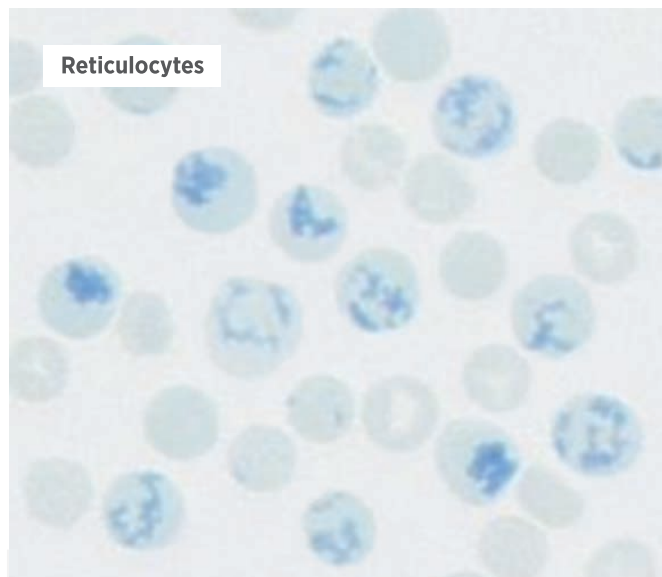**Figure 1.** Example of Reticulocytes (RETIC) vs Polychromatophils (PCM)

Image: *vetclinpathimages.com/2018/02/26/polychromatophils*

When using new methylene blue or brilliant cresyl blue stains on immature RBCs, the dye causes clumping and staining of RNA present, creating a blue staining reticulum, hence the name reticulocytes.
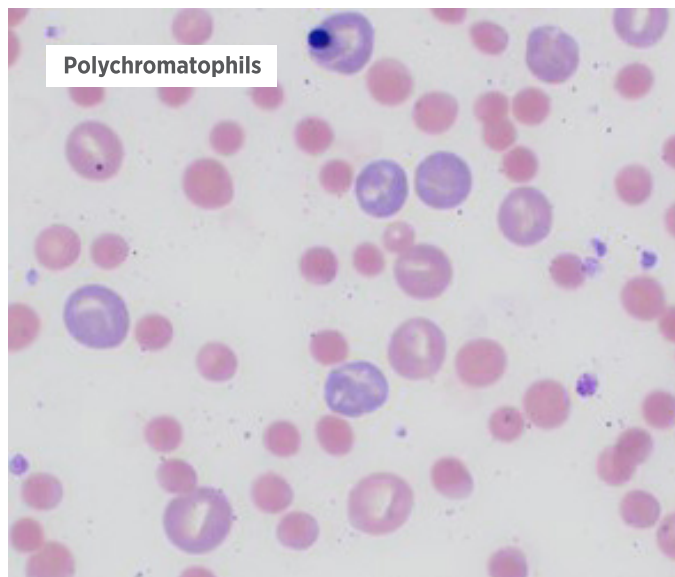

Image: Obtained from the VETSCAN IMAGYST at 400x magnification

On a Wright-stained blood smear, immature RBCs are called polychromatophilic (poly- refers to many, and -chromasia means color) and are usually purple-blue color. A mature RBC generally has an eosinophilic color, since hemoglobin is alkaline, attracting basic stains (red), but since immature RBCs have residual ribonucleic acid (RNA), which is acidic, they attract basic stain (blue), giving the cell a purple-blue color.

New technology supports using a PCM count to classify the bone marrow response to an anemia. In a study using artificial intelligence (AI) technology on the VETSCAN IMAGYST™, blood samples submitted to a reference laboratory were utilized to produce paired slides from a single sample, with one slide stained with new methylene blue (NMB) and the other stained with a Romanowsky-type stain. The paired slides were evaluated by clinical pathologists, who performed RETIC count on the NMB stained slides, and by IMAGYST AI as PCM counts on Romanowsky stained slides. All of the slides had a VETSCAN IMAGYST™ AI Blood Smear PCM percentage within the clinical 95% prediction interval as calculated from RETIC percentage.[i]

A RETIC count is available on some automated analyzers, which is less labor-intensive than a traditional manual count, but still requires a blood smear examination.

> ### An automated RETIC count should always be verified with a blood smear to examine RBC morphology and to confirm the automated RETIC count.

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Interpreting RETIC and PCM Counts

There are several ways to interpret absolute RETIC and PCM counts to determine if RBC regeneration exists, and interpretation must be made relative to duration and severity of the anemia. [This author prefers using the absolute count over the corrected percentage in most cases.] Simply relying on a reference interval may lead to misinterpretation of the erythroid response. To interpret RETIC or PCM counts:

1. Absolute RETIC or PCM count/µL = (% RETIC or % PCM/100) × RBC count/µL
2. Corrected RETIC or PCM % = % RETIC or % PCM × patient's PCV/Average PCV for the species

> **Note:** In cats, only aggregate RETIC that contain large aggregates or large amounts of RNA should be quantified. Punctate RETIC contain less RNA than aggregate RETIC. Punctate RETIC are considered more mature than aggregate RETIC and have a much longer half-life in cats than aggregate RETIC. Therefore, punctate RETIC do not indicate the current response by the bone marrow to an anemia.

Evaluation of the adequacy of the bone marrow regenerative response in the individual patient should also include consideration of severity and chronicity of the anemia, suspected cause of the anemia, and potential for multiple causes contributing to the patient's anemia. Trending the anemia and RETIC/PCM count through sequential CBCs may be helpful.

Since normal bone marrow response to an anemia is generally a vigorous and dynamic, but slightly delayed process, an anemia of acute onset may initially appear "nonregenerative" if the bone marrow has had insufficient time to respond. Since the bone marrow takes approximately 3-5 days to mount a regenerative response, serial RETIC or PCM evaluations may be necessary.

RETIC/PCM counts indicative of regeneration can be seen in a nonanemic patient. This may reflect a normal physiologic response or a response to an increased need. Serial evaluations of the CBC should be done to rule-out an emerging anemia in these patients. Reticulocytosis, or elevated PCM counts, in the absence of anemia (RAA) may indicate recovery from anemia or may be associated with non-anemic chronic hypoxia (e.g., cardiovascular disease, pulmonary disease), or ongoing, subclinical, slow red blood cell destruction. RAA has also been observed in patients with gastrointestinal, inflammatory, and neoplastic disorders, and in dogs with osteoarthritis or receiving osteoarthritis treatments (e.g., anti-inflammatory drugs, nutraceuticals).

Regenerative Anemia: Most regenerative anemias are due to either blood loss **(hemorrhage)** or destruction **(hemolysis)**. The expected bone marrow response depends on the severity of anemia. A robust regenerative response is expected in a moderate to severe anemia, whereas a weaker response would be expected in a mild anemia. The anemia is classified as regenerative if the signs of increased erythropoiesis are considered adequate for the severity of anemia.

Differential Diagnoses — Regenerative Anemia

Hemorrhage/Blood Loss Anemia
- Can be acute or chronic
  - External blood loss
    - Erythrocytes are lost from the body or lost into the alimentary, or urinary tract
  - Internal blood loss
    - Erythrocytes move from the intravascular to the extravascular space (principally peritoneal or pleural cavities)

Hemolytic Anemia
- Extravascular
  - RBCs are lysed outside of blood vessels generally by macrophages within the mononuclear phagocyte system. The mononuclear phagocyte system (formerly known as the reticuloendothelial system) is the portion of the immune system consisting of fixed phagocytic cells found in reticular connective tissue in the spleen, liver, lungs, bone marrow, and lymph node
- Intravascular
  - RBCs are lysed within the blood vessels. It does not include phagocytosis by macrophages in the mononuclear phagocyte system

Nonregenerative Anemia: If the anemia is classified as nonregenerative and the bone marrow has had adequate time to respond, mechanisms of **decreased or ineffective erythropoiesis** are a likely consideration, and further diagnostic testing should focus on identifying the underlying cause(s).

Differential Diagnoses — Nonregenerative Anemia

Reduced Erythropoiesis
- Inflammatory disease (inflammation and neoplasia)
- Chronic renal disease
- Endocrine deficiencies
- Cytotoxic damage to the marrow
- Infectious agents: *Ehrlichia* spp., FeLV, parvovirus (puppies)
- Immune-mediated: Nonregenerative anemia, selective erythroid aplasia, long-term treatment with recombinant human erythropoietin, idiopathic aplastic anemia
- Myelophthisis: Myeloid leukemias, lymphoid leukemias, myelodysplastic syndromes, multiple myeloma, myelofibrosis, osteosclerosis, metastatic lymphomas, metastatic mast cell tumors, other metastatic disease infiltrating the bone marrow

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Defective Erythropoiesis
- Disorders of heme synthesis: Iron, copper, and pyridoxine deficiencies (lead toxicity, drugs)
- Disorders of nucleic acid synthesis: Folate and cobalamin deficiencies
- Abnormal maturation: Erythroleukemia or AML-M6 (primarily cats), myelodysplastic syndromes with erythroid predominance (MDS-Er), inherited dyserythropoiesis of English Springer Spaniels, etc.

II. Classification by Red Blood Cell Indices (Automated CBC)

It is important to review the pertinent RBC parameters found on an automated CBC report which describe trends in RBC size and HGB concentration and thus aid in classification of the anemia. These parameters are Mean Cell Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC), respectively.

Table 1. MCV Classification

MCV	Description	Common Pathology
Decreased	Microcytic	• Iron Deficiency • Hepatic portocaval vascular shunts • Normal breed variation (ex. Shiba Inu, Akita)
Normal	Normocytic	• Usually a nonregenerative, poorly- or early-regenerative • Early-regenerative refers to blood loss or blood destruction anemia in which evidence of regeneration is not yet apparent because the bone marrow has not had time to respond to acute loss
Increased	Macrocytic	• Regeneration - Bone marrow is responding and is releasing PCM/RETIC that are larger than normal • Congenital Poodle macrocytosis • Hereditary stomatocytosis • Myelodysplasia • FeLV

The three most important and relevant anemia diagnostic patterns using MCV and MCHC indices are:

Macrocytic Hypochromic:
Regenerative anemias with large, young erythrocytes that are not fully hemoglobinized

Normocytic Normochromic:
Nonregenerative anemias with residual normal erythrocytes

Microcytic Hypochromic:
Usually due to iron deficiency anemias
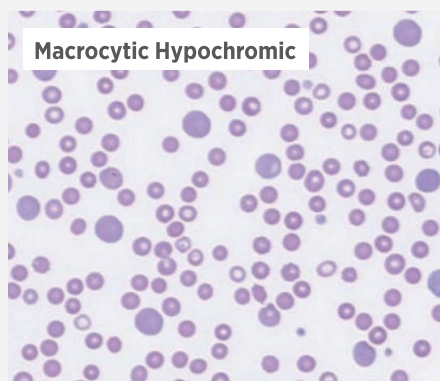
Macrocytic Hypochromic
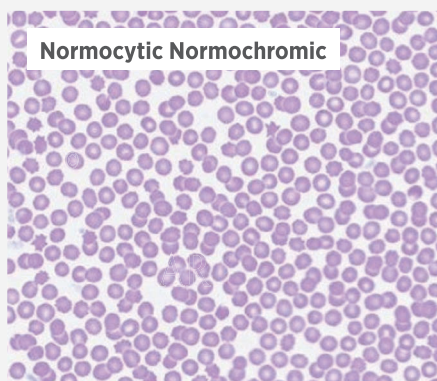
Normocytic Normochromic
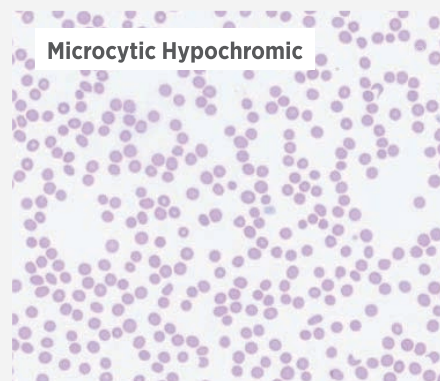
Microcytic Hypochromic

Images from VETSCAN IMAGYST™

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Further Diagnostic Testing to Diagnose the Underlying Cause of Anemia:

Blood smear examination is an essential part of the assessment of an anemic patient because it provides a wealth of information about blood cell pathology and the potential for blood parasites not available with automated analyzers. In addition, evaluation of RBC morphology can help pinpoint a diagnosis, determine the recommended treatment, and monitor the response to treatment for anemia. See **Figure 2** and **Table 2.**

Figure 2. Image shows anemia with anisocytosis, polychromasia, schistocytes, and nucleated RBC.
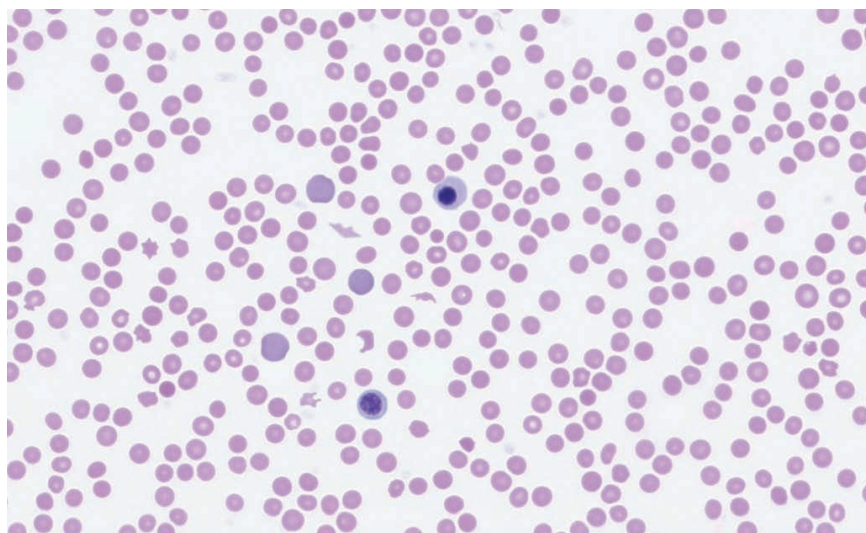
Image from VETSCAN IMAGYST™

Table 2. Common RBC Morphologies (not exhaustive)

IMHA	Regenerative Anemia	RBC Damage Due to Microangiopathy*	Oxidative Damage	Iron Deficiency
Spherocytes	Anisocytosis	Schistocytes	Eccentrocytes	Schistocytes
Agglutination	Howell-Jolly bodies	Acanthocytes	Heinz Bodies	Microcytes
Ghost Cells	Polychromasia	Keratocytes	Spherocytes	Leptocytes

*Associated with neoplasia, DIC, glomerulonephritis, vasculitis

III. Consider the Whole Patient

Anemia is a manifestation of an underlying disorder, not a diagnosis. Further diagnostic testing will likely be necessary to determine the underlying cause. Additional diagnostic tests listed below should be utilized based on the differential diagnoses suggested by the classification of anemia:

A. Clinical chemistry profile/urinalysis
   I.   Evidence of renal or hepatic failure; causes secondary anemia
   II. Evidence of systemic diseases; variable causes of anemia
B. Virology, serology if infection is likely (e.g., fever, lymphadenopathy, etc.)
C. Endocrinologic examination; hypothyroidism or other dysfunction (e.g., mild, normocytic normochromic anemia)

D. Toxicity
   I.   Check for ovarian neoplasm or access to exogenous estrogen
   II. Withhold any current drug therapy and monitor for hematocrit recovery
   III. Check for environmental toxicants
E. Bone marrow examination may reveal many diagnoses:
   I.   Myelofibrosis, aplastic anemia, bone marrow necrosis/inflammation, dyserythropoiesis, leukemia, metastatic neoplasia, myelodysplastic syndromes, etc.

Anemia cases can be challenging and determining the best treatment and monitoring plan can be time-intensive. Using the VETSCAN IMAGYST™ AI Blood Smear and Digital Cytology platforms, coupled with Zoetis Clinical Consultation Service, challenges regarding diagnosis, treatment, and monitoring decisions can be alleviated for the veterinary practitioner.

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Anemia
Classification Tree

Anemia Present

Evidence of Regeneration* with presence of Reticulocytosis/Elevated PCM Count**

Yes

Regenerative Anemia
- Absolute reticulocyte/PCM count:[2]
  - > = 100,000/ul (dog)[1]
  - > = 60,000/ul (cat)[1]

Measure Total Protein (TP)[5]

TP Low → **Blood Loss[4,9]**

TP Normal → **Hemolysis[3,8]**

External Blood Loss
- External Hemorrhage
- Parasites
- GI Ulcers
- Intestinal Neoplasia
- Hematuria

Internal Blood Loss
- Internal Hemorrhage
- Hemoperitoneum
- Hemothorax
- Hematoma

Immune Mediated
- Primary: Idiopathic IMHA, transfusion reaction
- Secondary: Infectious, neoplasia, drugs, toxins, transfusion reaction
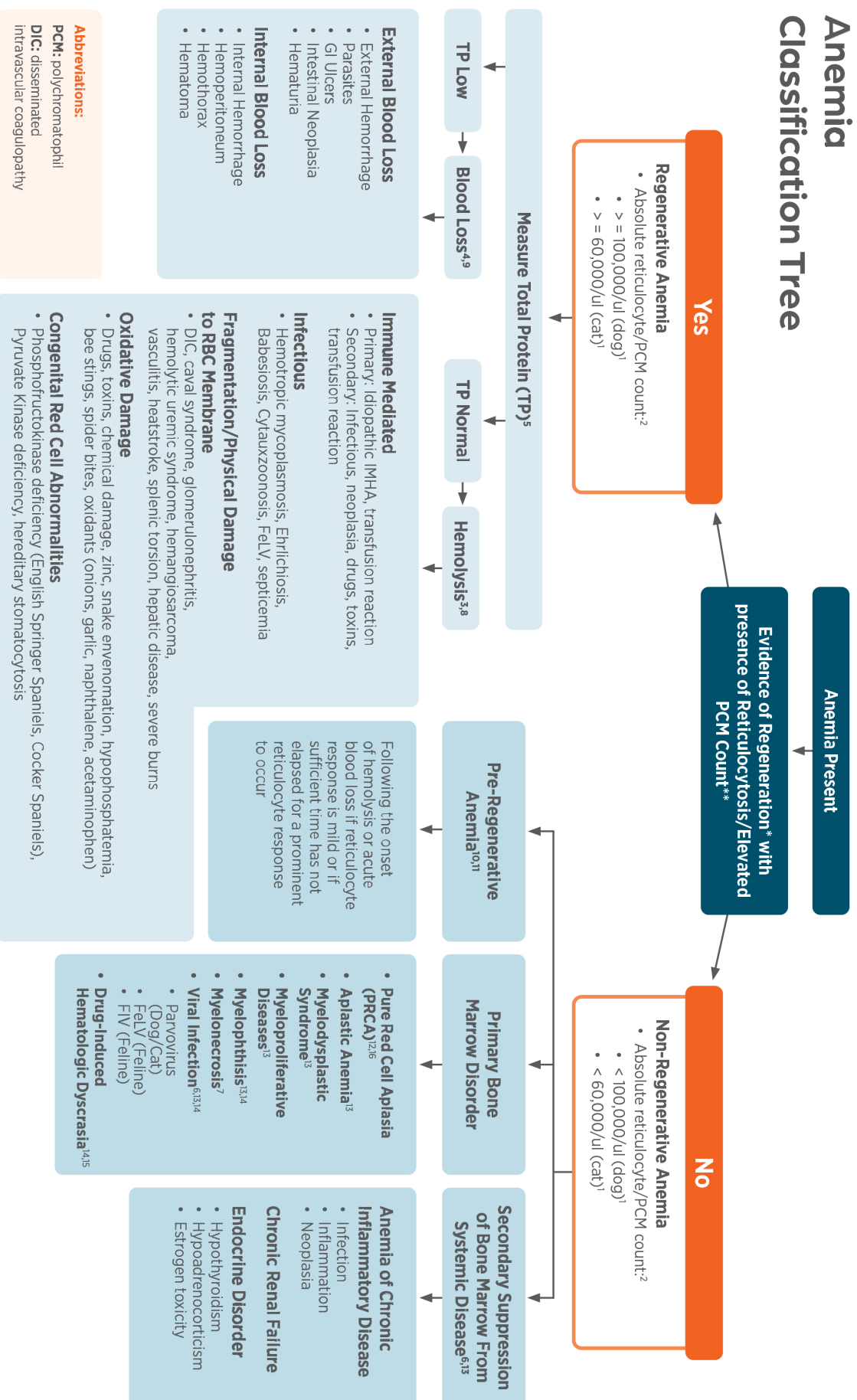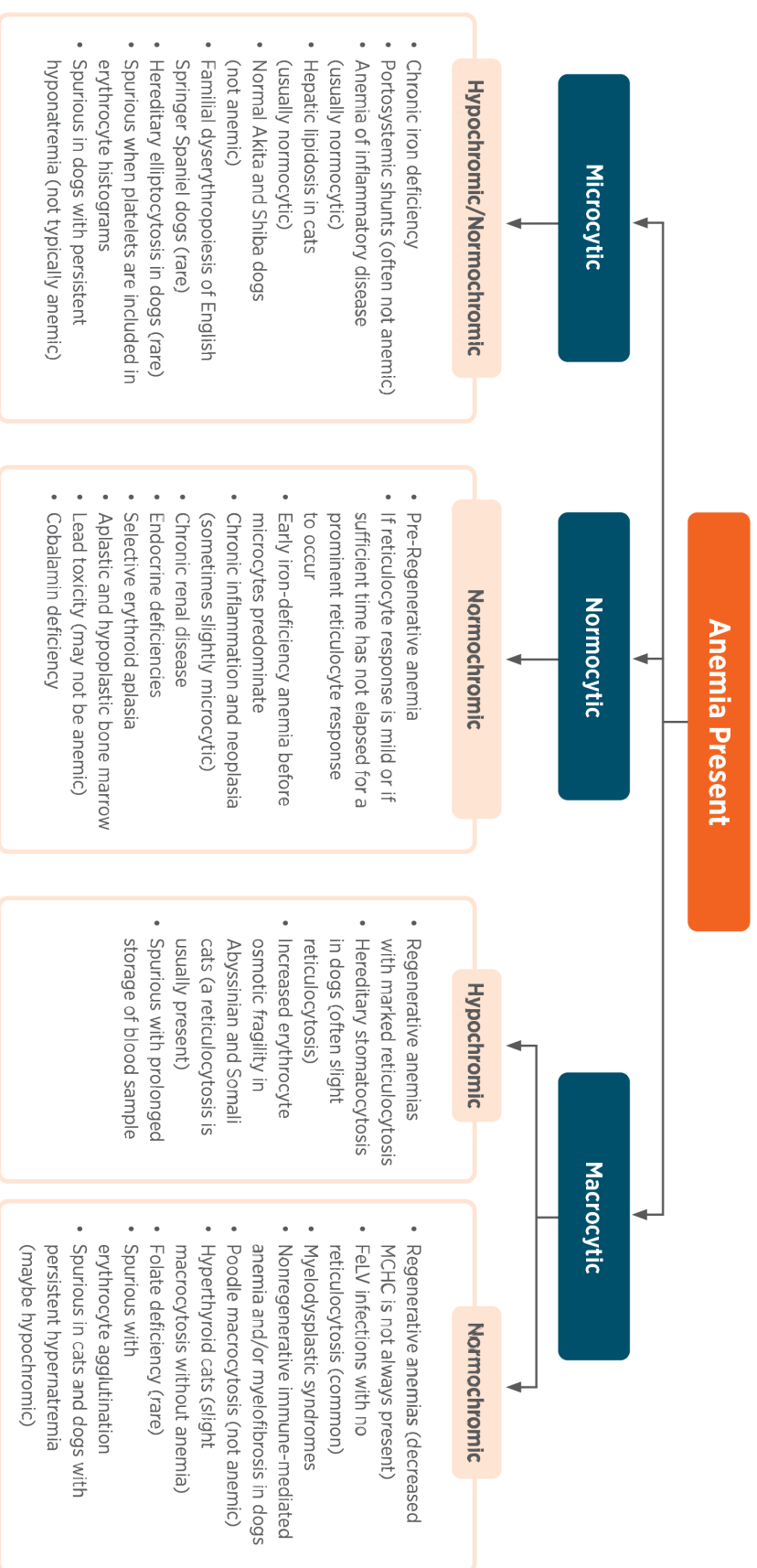
Infectious
- Hemotropic mycoplasmosis, Ehrlichiosis, Babesiosis, Cytauxzoonosis, FeLV, septicemia

Fragmentation/Physical Damage to RBC Membrane
- DIC, caval syndrome, glomerulonephritis, hemolytic uremic syndrome, hemangiosarcoma, vasculitis, heatstroke, splenic torsion, hepatic disease, severe burns

Oxidative Damage
- Drugs, toxins, chemical damage, zinc, snake envenomation, hypophosphatemia, bee stings, spider bites, oxidants (onions, garlic, naphthalene, acetaminophen)

Congenital Red Cell Abnormalities
- Phosphofructokinase deficiency (English Springer Spaniels, Cocker Spaniels), Pyruvate Kinase deficiency, hereditary stomatocytosis

No

Non-Regenerative Anemia
- Absolute reticulocyte/PCM count:[2]
  - < 100,000/ul (dog)[1]
  - < 60,000/ul (cat)[1]

Pre-Regenerative Anemia[10,11]

Following the onset of hemolysis or acute blood loss if reticulocyte response is mild or if sufficient time has not elapsed for a prominent reticulocyte response to occur

Primary Bone Marrow Disorder

- **Pure Red Cell Aplasia (PRCA)[12,16]**
- **Aplastic Anemia[13]**
- **Myelodysplastic Syndrome[13]**
- **Myeloproliferative Diseases[13]**
- **Myelophthisis[13,14]**
- **Myelonecrosis[7]**
- **Viral Infection[6,13,14]**
  - Parvovirus (Dog/Cat)
  - FeLV (Feline)
  - FIV (Feline)
- **Drug-Induced Hematologic Dyscrasia[14,15]**

Secondary Suppression of Bone Marrow From Systemic Disease[6,13]

Anemia of Chronic Inflammatory Disease
- Infection
- Inflammation
- Neoplasia

Chronic Renal Failure

Endocrine Disorder
- Hypothyroidism
- Hypoadrenocorticism
- Estrogen toxicity

*Evaluation of the adequacy of the bone marrow regenerative response in the individual patient should also include consideration of severity and chronicity of the anemia, and potential for multiple causes contributing to the patient's anemia. Trending the anemia and reticulocyte (RETIC)/polychromatophils (PCM) count through sequential Complete Blood Counts (CBC) may be helpful.

****Note:** RETIC/PCM counts supporting regeneration can be seen in non-anemic patient. This may reflect a normal physiologic response or a response to an increased need. Serial evaluations of the CBC should be done to rule-out an emerging anemia in these patients. RETIC or elevated PCM counts in the absence of anemia (RAA) may indicate recovery from anemia or may be associated with non-anemic chronic hypoxia (e.g., cardiovascular disease, pulmonary disease). RAA has also been observed in patients with gastrointestinal, inflammatory, and neoplastic disorders, and in dogs with osteoarthritis or receiving osteoarthritis treatments (e.g., anti-inflammatory drugs, nutraceuticals).

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Anemia Classification by Red Blood Cell Indices

Anemia Present

Microcytic

Hypochromic/Normochromic

- Chronic iron deficiency
- Portosystemic shunts (often not anemic)
- Anemia of inflammatory disease (usually normocytic)
- Hepatic lipidosis in cats (usually normocytic)
- Normal Akita and Shiba dogs (not anemic)
- Familial dyserythropoiesis of English Springer Spaniel dogs (rare)
- Hereditary elliptocytosis in dogs (rare)
- Spurious when platelets are included in erythrocyte histograms
- Spurious in dogs with persistent hyponatremia (not typically anemic)

Normocytic

Normochromic

- Pre-Regenerative anemia
- If reticulocyte response is mild or if sufficient time has not elapsed for a prominent reticulocyte response to occur
- Early iron-deficiency anemia before microcytes predominate
- Chronic inflammation and neoplasia (sometimes slightly microcytic)
- Chronic renal disease
- Endocrine deficiencies
- Selective erythroid aplasia
- Aplastic and hypoplastic bone marrow
- Lead toxicity (may not be anemic)
- Cobalamin deficiency

Macrocytic

Hypochromic

- Regenerative anemias with marked reticulocytosis in dogs (often slight reticulocytosis)
- Hereditary stomatocytosis
- Increased erythrocyte osmotic fragility in Abyssinian and Somali cats (a reticulocytosis is usually present)
- Spurious with prolonged storage of blood sample

Normochromic

- Regenerative anemias (decreased MCHC is not always present)
- FeLV infections with no reticulocytosis (common)
- Myelodysplastic syndromes
- Nonregenerative immune-mediated anemia and/or myelofibrosis in dogs
- Poodle macrocytosis (not anemic)
- Hyperthyroid cats (slight macrocytosis without anemia)
- Folate deficiency (rare)
- Spurious with erythrocyte agglutination
- Spurious in cats and dogs with persistent hypernatremia (maybe hypochromic)

Additional Anemia Information

Macrocytic hypochromic: Regenerative anemias with large, young erythrocytes that are not fully hemoglobinized.

Normocytic normochromic: Non-regenerative anemias with residual normal erythrocytes.

Microcytic hypochromic: Usually due to iron deficiency anemias.

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