ZOETIS DIAGNOSTICS

FUNdamentals of Haematology:

Diagnosing Anaemia

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Introduction

Anaemia is one of the most common haematologic 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., haemorrhage, immune-mediated haemolytic anaemia) or a marker of underlying disease (e.g., cancer, chronic kidney disease). Therefore, even mild, asymptomatic anaemia should be investigated thoroughly to diagnose and treat the primary problem.

Anaemia is defined as a decrease in red blood cell (RBC) mass and is recognised by a low packed cell volume (PCV), haematocrit (HCT), haemoglobin concentration (HGB), or RBC count below the reference intervals for that species. Anaemia 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 anaemic patient, haematology testing MUST include BOTH quantitative automated cell count and qualitative blood smear evaluation.

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

1. **Evaluate the haematology 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: Anaemia 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 anaemia). This can generally result from overhydration resulting in dilution of erythrocytes, and from splenic sequestration of erythrocytes as occurs with splenic relaxation during anaesthesia, 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 haematologic picture. Often in the case of an anaemia, the blood smear can help establish a diagnosis and prognosis.

Classify the Anaemia

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

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

Classification of anaemia 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 anaemia.[4]

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)

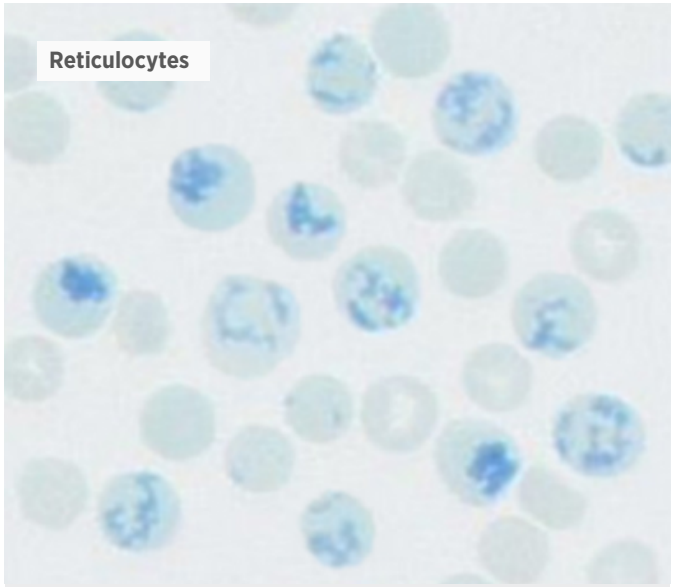


Image: *vetclinpathimages.com/2018/02/26/polychromatophils*

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.

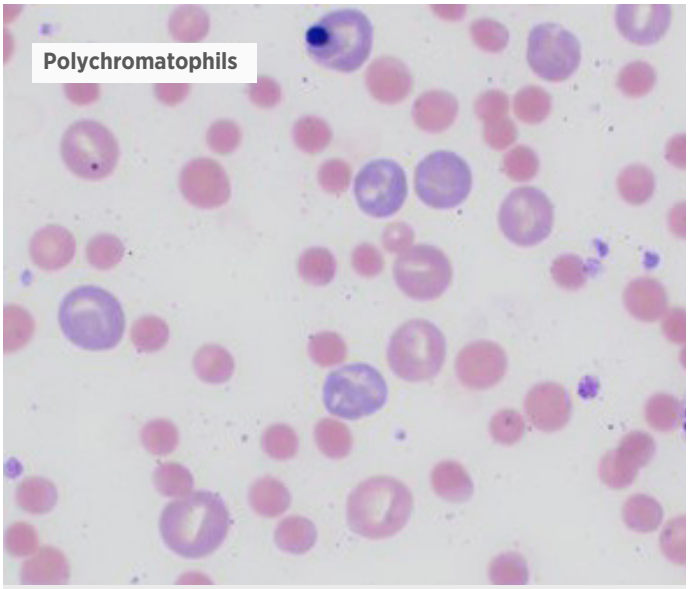


Image: Obtained from the VETSCAN IMAGYST at 400x magnification

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 haemoglobin 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 anaemia. In a study using artificial intelligence (AI) technology on the VETSCAN IMAGYST®, blood samples submitted to a reference laboratory were utilised 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 VETSCAN 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 analysers, which is much less labor-intensive than a traditional manual count, and is generally more precise. Precision is superior to that of the manual count because many more cells are counted, which has allowed reliable flagging of reticulocytosis or reticulocytopaenia.[5]

i Data on file: SR No. DH7MR-US-21-038 Zoetis 2022. Zoetis demonstrating PCM is an estimate for RETIC on the VETSCAN IMAGYST AI.

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 anaemia. [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 anaemia.

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

Since normal bone marrow response to an anaemia is generally a vigorous and dynamic, but slightly delayed process, an anaemia 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 nonanaemic 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 anaemia in these patients. Reticulocytosis, or elevated PCM counts, in the absence of anaemia (RAA) may indicate recovery from anaemia or may be associated with non-anaemic 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 Anaemia: Most regenerative anaemias are due to either blood loss (**haemorrhage**) or destruction (**haemolysis**). The expected bone marrow response depends on the severity of anaemia. A robust regenerative response is expected in a moderate to severe anaemia, whereas a weaker response would be expected in a mild anaemia. The anaemia is classified as regenerative if the signs of increased erythropoiesis are considered adequate for the severity of anaemia.

Differential Diagnoses — Regenerative Anaemia

Haemorrhage/Blood Loss Anaemia

- 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)

Haemolytic Anaemia

- 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 Anaemia: If the anaemia 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 Anaemia

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 anaemia, selective erythroid aplasia, long-term treatment with recombinant human erythropoietin, idiopathic aplastic anaemia
- Myelophthisis: Myeloid leukaemias, lymphoid leukaemias, myelodysplastic syndromes, multiple myeloma, myelofibrosis, osteosclerosis, metastatic lymphomas, metastatic mast cell tumors, other metastatic disease infiltrating the bone marrow

Defective Erythropoiesis

- Disorders of haeme synthesis: Iron, copper, and pyridoxine deficiencies (lead toxicity, drugs)
- Disorders of nucleic acid synthesis: Folate and cobalamin deficiencies
- Abnormal maturation: Erythroleukaemia 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 anaemia. These parameters are Mean Cell Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC), respectively.

Table 1. MCV Classification

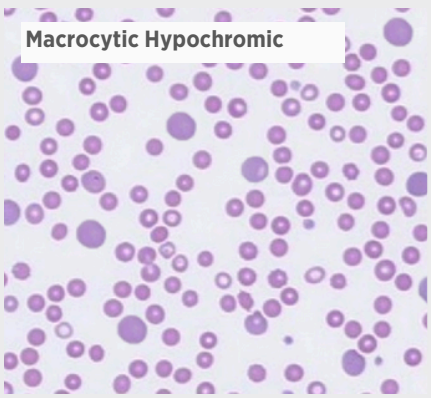
MCV	Description	Common Pathway
Decreases	Microcytic	• Iron Deficiency • Hepatic portocaval vascular shunts • Normal breed variation (ex. Shiba Inu, Akita)
Normal	Normocytic	• Usually a nonregenerative, poorly- or early-regenerative • Early-regenerative refers to blood loss or blood destruction anaemia 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

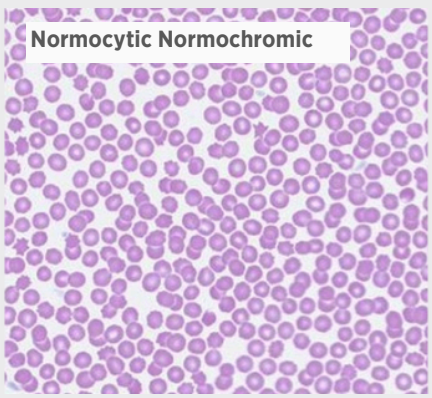
The three most important and relevant anaemia diagnostic patterns using MCV and MCHC indices are:

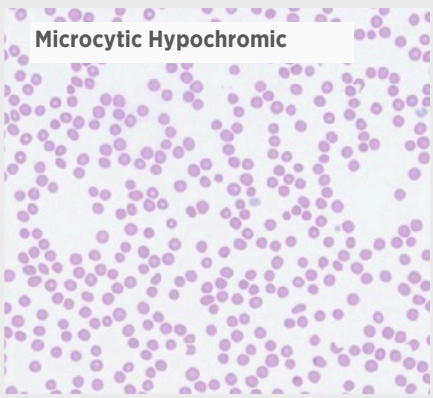
Macrocytic Hypochromic:
Regenerative anaemias with large, young erythrocytes that are not fully haemoglobinised

Normocytic Normochromic:
Nonregenerative anaemias with residual normal erythrocytes

Microcytic Hypochromic:
Usually due to iron deficiency anaemias







Further Diagnostic Testing to Diagnose the Underlying Cause of Anaemia:

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

Figure 2. Image shows anaemia with anisocytosis, polychromasia, schistocytes, and nucleated RBC.

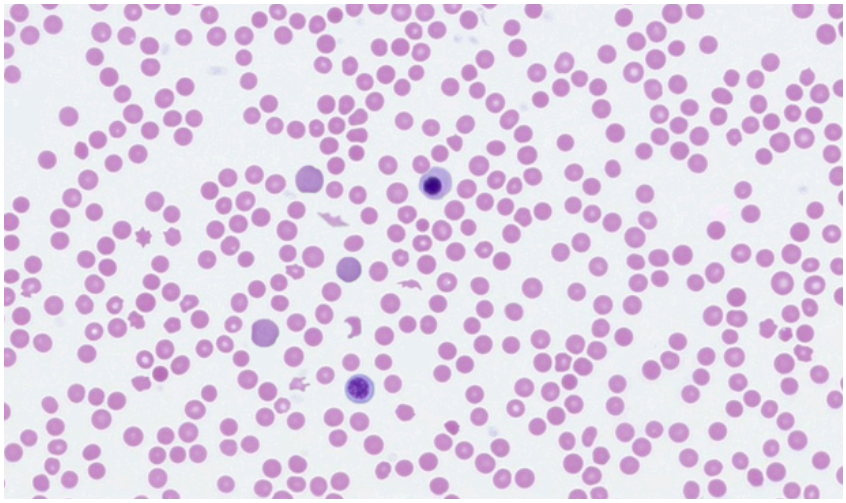


Table 2. Common RBC Morphologies (not exhaustive)

IMHA	Regenerative Anaemia	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

Anaemia 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 utilised based on the differential diagnoses suggested by the classification of anaemia:

A. Clinical chemistry profile/urinalysis
   I. Evidence of renal or hepatic failure; causes secondary anaemia
   II. Evidence of systemic diseases; variable causes of anaemia

B. Virology, serology if infection is likely (e.g., fever, lymphadenopathy, etc.)

c. Endocrinologic examination; hypothyroidism or other dysfunction (e.g., mild, normocytic normochromic anaemia)

D. Toxicity
   I. Check for ovarian neoplasm or access to exogenous estrogen
   II. Withhold any current drug therapy and monitor for haematocrit recovery
   III. Check for environmental toxicants

E. Bone marrow examination may reveal many diagnoses:
   I. Myelofibrosis, aplastic anaemia, bone marrow necrosis/inflammation, dyserythropoiesis, leukaemia, metastatic neoplasia, myelodysplastic syndromes, etc.

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Anaemia cases can be challenging and determining the best treatment and monitoring plan can be time-intensive. Using the Vetscan OptiCell,™ 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.

Anaemia Classification Tree

Anaemia Present

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

Yes

Regenerative Anaemia
- 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]

External Blood Loss
- External Haemorrhage
- Parasites
- GI Ulcers
- Intestinal Neoplasia
- Haematuria

Internal Blood Loss
- Internal Haemorrhage
- Haemopertoneum
- Haemothrax
- Haematoma

TP Normal → **Haemolysis**

Immune Mediated
- Primary: idiopathoc IMHA, transfusion reaction
- Secondary: Infectious, neoplasia, drugs, toxins, transfusion reaction

Infectious
- Haemotropic mycoplasmosis, Ehrlichiosis, Babesiosis, Cytauxzoonosis, FeLV, septicaemia

Fragmentation/Physical Damage to RBC Membrane
- DIC, caval syndrome, glomerulonephritis, haemolytic uraemic syndrome, haemangiosarcoma, vasculitis, heatstroke, splenic torsion, hepatic disease, severe burns

Oxidative Damage
- Drugs, toxins, chemical damage, zinc, snake envenomation, hypophosphataemia, 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

Abbreviations:

PCM: polychromatophil
DIC: disseminated intravascular coagulopathy

No

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

Pre-Regenerative Anaemia[10, 11]

Following the onset of haemolysis 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 Anaemia**[13]
- **Myelodysplastic Syndrome**[13]
- **Myeloproliferative Diseases**[13]
- **Myelophthisis**[13, 14]
- **Myelonecrosis**[7]
- **Viral Infection**[16, 13, 14]
  - Parvovirus (Dog/Cat)
  - FeLV (Feline
  - FIV (Feline)
- **Drug-Induced Haematologic Dyscrasia**[14, 15]

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

Anaemia of Chronic Inflammatory Disease
- Infection
- Inflammation
- Neoplasia

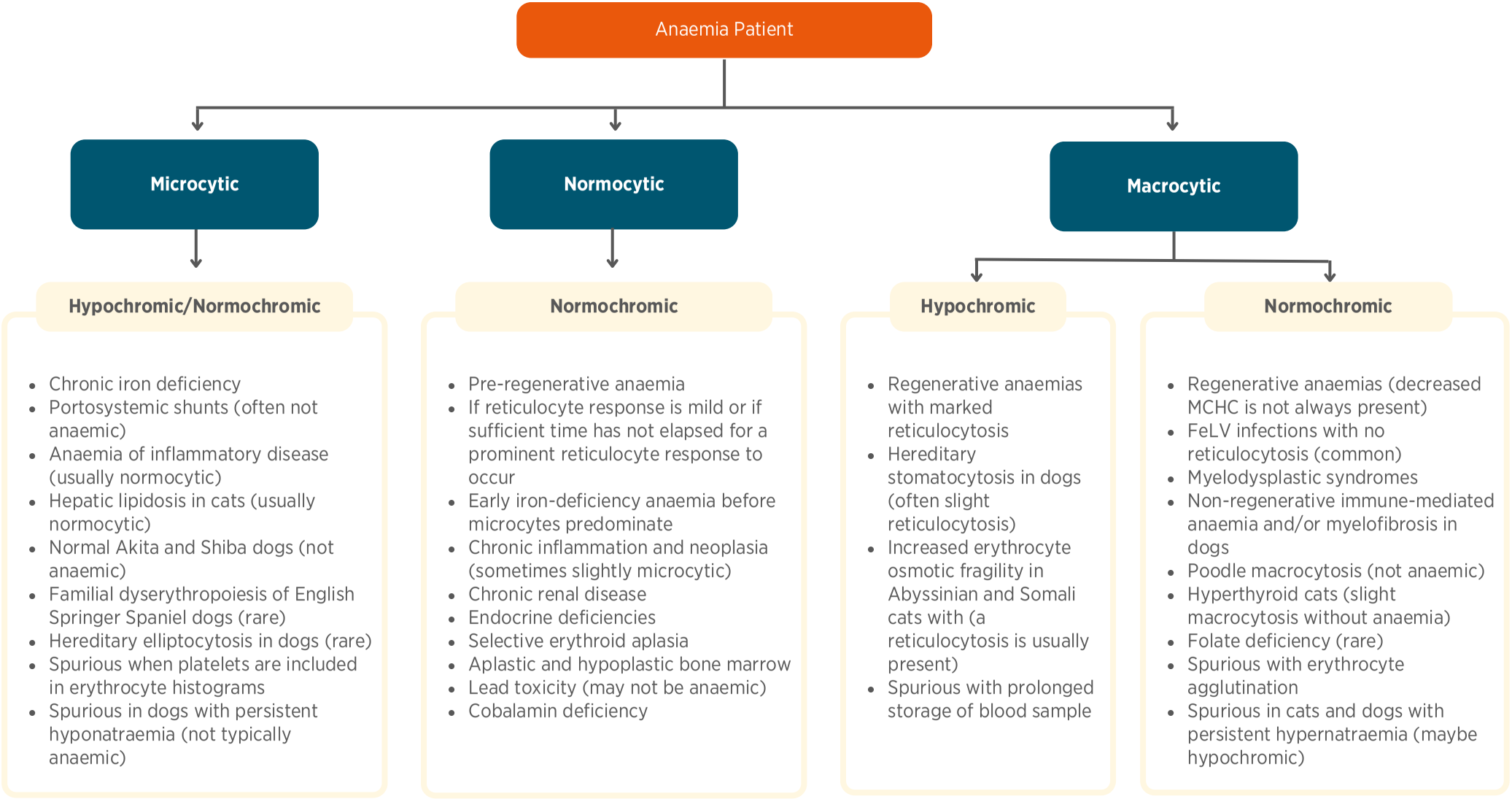
Chronic Renal Failure

Endocrine Disorder
- Hypothyroidism
- Hypoadrenocorticism
- Oestrogen 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 anaemia, suspected cause of the anaemia, and potential for multiple causes contributing to the patient's anaemia. Trending the anaemia 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-anaemic 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 anaemia in these patients. RETIC or elevated PCM counts in the absence of anaemia (RAA) may indicate recovery from anaemia or may be associated with non-anaemic 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).

Anaemia Classification by Red Blood Cell Indices



Additional Anaemia Information:

Macrocytic hypochromic: Regenerative anaemias with large, young erythrocytes that are not fully haemoglobinised

Normocytic normochromic: Non-regenerative anaemias with residual normal erythrocytes

Microcytic hypochromic: Usually due to iron deficiency anaemias

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