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Nonregenerative Anemia:  
Recent Advances in Understanding Mechanisms of Disease

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INTRODUCTION

Classifying anemia as regenerative or nonregenerative is clinically useful because it provides information about the mechanism of disease.

The CBC hallmark of regenerative anemias (except in horses) is reticulocytosis – i.e., increased concentration of immature erythrocytes in circulation.

Reticulocytosis indicates an appropriate compensatory response to anemia, characterized by erythroid hyperplasia in the bone marrow, and increased release of erythrocytes into the circulation before they are fully mature. These immature RBCs are polychromatophilic on a routinely stained blood smear. Reticulocytosis and polychromasia do not occur in horses, even in the context of regeneration.

The term “preregenerative” is sometimes used to describe anemia with a regenerative response that is impending, but not yet apparent on the CBC. In a case of acute blood loss or hemolysis, it typically takes 3 to 4 days until reticulocytosis is evident on the CBC, and several more days until the regenerative response peaks.

The CBC hallmark of nonregenerative anemias is absence of reticulocytosis – i.e., the lack of an appropriate compensatory response to anemia – which indicates that erythropoiesis is being inhibited in some way. Nonregenerative anemias typically develop slowly because erythrocytes already in circulation have a long life span. Most
nonregenerative anemias are normocytic and normochromic (i.e., MCV and MCHC are within normal limits) – but, as discussed below, there are exceptions.

There are many potential underlying causes of nonregenerative anemia, as will be discussed further below.

MECHANISMS OF DISEASE

Anemia of inflammation

The most common form of nonregenerative anemia is known as anemia of inflammation or anemia of chronic disease.

For many years physicians and veterinarians have recognized that patients with infectious or inflammatory disease frequently develop anemia, and that this phenomenon is associated with altered iron homeostasis. Affected individuals have laboratory features of iron deficiency – hypoferremia (low serum iron concentration) and decreased total iron binding capacity (low serum transferrin concentration) – but have increased iron stores in the reticuloendothelial system. These seemingly paradoxical findings are consistent with functional iron deficiency.

Of course, iron is an essential constituent of hemoglobin:

![Hemoglobin Synthesis in Erythrocyte Precursors](image)

The veterinary clinical literature contains many references to anemia of chronic disease or anemia of inflammation, and it is generally accepted to be a common clinical entity.

Patients with anemia of inflammation typically have nonregenerative anemia that is normocytic and normochromic (i.e., normal MCV and MCHC, respectively). This is in contrast to classical iron deficiency anemia, which in advanced cases is microcytic and hypochromic.
A proposed evolutionary rationale for anemia of inflammation is that it is a side-effect of an immunologic adaptation to limit microbial access to iron. The last decade or so has seen breakthroughs in the understanding of molecular mechanisms of anemia of inflammation, including new insights into the relationship between innate immunity and iron regulation.

Many of the new insights involve a hormone called **hepcidin**, which is now generally accepted as the key mediator of anemia of inflammation.

Some facts about hepcidin:

- Synthesized mainly in the liver.
- First recognized for its direct antimicrobial effects
- Bioactive form is a highly conserved 25-amino acid peptide:

![Hepcidin Structure](image)

- Expression increases with inflammation (it is an acute phase protein) or iron overload, and decreases with anemia or hypoxia.

Hepcidin exerts its effects by binding to the cell surface iron efflux protein, ferroportin, and inducing its internalization and degradation:

![Hepcidin Effect](image)
This interaction inhibits absorption of dietary iron (from intestinal epithelium) and inhibits release of storage iron (from macrophages and hepatocytes), thus resulting functional iron deficiency.

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Inflammation can also contribute to anemia via other mechanisms.

For example:

- Inflammation has been shown to cause decreased erythrocyte survival in some species.

- Studies have implicated inflammatory cytokines as inhibitors of erythropoiesis via:
  - Direct toxic effects on erythroid precursors (e.g., via free radical formation or induction of apoptosis).
  - Decreased expression of hematopoietic factors including Epo and stem-cell factor.
  - Decreased expression of Epo receptors.

- Some investigators have suggested that oxidants produced by activated neutrophils alter erythrocyte surface antigenicity, which could in turn lead to accelerated destruction of erythrocytes by immune-mediated mechanisms.

**Absolute iron deficiency**

True iron deficiency (aka absolute iron deficiency) has long been recognized as a cause of anemia. Iron deficiency in domestic animals occurs most commonly because of chronic blood loss, and thus loss of iron-rich hemoglobin, and occurs much less frequently due to nutritional deficiency.

There are many underlying conditions that involve hemorrhage-induced iron deficiency anemia, including primary or secondary gastrointestinal disease (e.g., hookworms, neoplasia, ulceration) and severe ectoparasitism.

Although iron deficiency often results in nonregenerative anemia, it is not always so, especially if caused by chronic hemorrhage and when nutritional iron is not a limiting factor. In other words, iron deficiency anemia can be regenerative or nonregenerative.

The classic hematologic picture with iron deficiency is microcytic (subnormal MCV), hypochromic (subnormal MCHC) anemia. Microcytosis typically develops before hypochromasia.
In severe cases, microcytosis and hypochromasia may be discernible on review of a blood smear as RBCs that are abnormally small, or paler staining because of their subnormal hemoglobin concentration, respectively. However, microscopic examination is not a reliable means of detection, especially in the case of mild abnormalities.

**Other mechanisms of nonregenerative anemia**

**Chronic renal failure**

Nonregenerative anemia is an expected complication of chronic renal failure (CRF) in humans and in domestic animals, and is recognized to contribute to overall morbidity. The major cause of anemia in patients with CRF is decreased production of erythropoietin (Epo).

Species-specific recombinant canine (rcEpo) and feline Epo (rfEpo) have been developed, and experimental work has shown them to effective in stimulating erythropoiesis in their respective species. However, rfEpo resulted in development of red cell aplasia in some cats. Neither rfEpo nor rcEpo is commercially available at the time of this writing.

Other contributing factors recognized in people include inflammation, hemorrhage, iron deficiency, decreased RBC survival due to metabolic insult or mechanical damage, and the anti-proliferative effects of uremic toxins.

Concurrent disease may increase the risk of anemia in patients with renal disease (e.g. diabetes mellitus is a risk factor for development of anemia in human beings with impaired renal function).

**Neoplasia**

The prevalence of anemia in the general population of animals with cancer is not well-characterized, but anemia certainly is recognized as a complication of cancer in veterinary medicine and is documented to be common in some types of cancer. Anemia occuring secondary to neoplasia is often, but not necessarily, nonregenerative.

As in people, recognized causes of anemia in animals with cancer include both disease- and treatment-related factors. The deleterious effects of anemia on quality of life in veterinary patients with cancer have received little attention and needs further investigation.

Disease-related mechanisms of anemia include hemorrhage, hemolysis, and decreased or ineffective erythropoiesis.
Bleeding may occur directly from a neoplastic lesion, or elsewhere due to defective hemostasis secondary to an underlying neoplasm. Hemolysis may occur via phagocytosis of erythrocytes by neoplastic cells, secondary immune-mediated destruction, oxidative insult, or mechanical damage (e.g., microangiopathic hemolysis). Decreased erythropoiesis may occur due to inflammation; space occupation of the bone marrow by neoplastic infiltrates or fibrosis; elaboration of myelotoxic factors (e.g., estrogen); or increased apoptosis.

Often the cause of anemia in animals with cancer is multifactorial. In principle, anemia can occur as a complication of virtually any type of neoplasia, but the risk is higher in certain types of malignancies.

**Endocrinopathies**

Some endocrine diseases that are well characterized in veterinary medicine—including hypothyroidism, diabetes mellitus, and hypoadrenocorticism—may be associated with anemia.

**Hypothyroidism**

Nonregenerative anemia is a recognized complication of hypothyroidism in people and animals, occurring in approximately 1/3 of cases in dogs.

Anemia develops primarily as a result of decreased erythropoiesis, with possible mechanisms including:

- Decreased Epo production.
- Decreased responsiveness of developing erythroid cells to Epo.
- Diminished direct effects of thyroid hormone on hematopoietic stem cells and erythroid progenitors.

**Diabetes mellitus**

The etiology is multifactorial, but development of anemia in patients with DM is closely linked with renal disease. DM is a risk factor for development of anemia in people with renal disease. In people with DM, anemia occurs mainly due to secondary renal disease and functional Epo deficiency resulting from renal tubulointerstitial pathology and uncoupling of hemoglobin concentration from Epo synthesis.

DM has been shown to cause increased Heinz body formation in cats, but it is not clear exactly how this relates to development of anemia.
**Hypoadrenocorticism**

People and animals with hypoadrenocorticism are often anemic – however, as with any polyuric condition, the anemia may be masked by decreased plasma volume.

The pathophysiology of anemia in hypoadrenocorticism is not well understood, but most likely involves decreased erythropoiesis, since glucocorticoids have been shown to interact with Epo and stem cell factor to promote erythropoiesis.

Blood loss may also contribute to development of anemia in dogs with hypoadrenocorticism, since affected animals may have gastrointestinal bleeding (sometimes severe).

**Nonregenerative IMHA and other forms of ineffective erythropoiesis**

IMHA is usually regenerative, often strongly regenerative. However, some forms of IMHA – diagnosed by excluding other conditions and by response to immunosuppressive therapy – are nonregenerative.

By the same token, some forms of nonregenerative anemia are not characterized by decreased erythropoiesis. The condition in which blood cells are being produced at normal or increased levels, but are destroyed before they enter the circulation, is called ineffective hematopoiesis.

One study of bone marrow findings in dogs with nonregenerative IMHA found erythroid hyperplasia in a majority of patients. In these patients, erythrocyte production was increased, but the erythrocytes were destroyed via immunologic mechanisms before they entered the circulation. Lower numbers of dogs in that study had erythroid hypoplasia or aplasia.

One variable likely to affect bone marrow findings in patients with nonregenerative IMHA is the stage at which developing erythrocytes express the antigen(s) being targeted immunologically. If a target antigen is expressed on early precursor cells, erythroid hypoplasia is likely. If the target antigen is not expressed until the reticulocyte stage, then erythroid hyperplasia is likely.

Other causes of ineffective hematopoiesis include FeLV infection and myelodysplastic syndrome (MDS). MDS is a neoplastic condition characterized by bone marrow hyperplasia and peripheral cytopenias. In people with MDS, developing blood cells undergo increased apoptosis.

**Liver Disease**

Anemia secondary to liver disease is most likely in cases of chronic liver disease or portosystemic shunts. Proposed mechanisms include:
• Anemia of inflammation.
• Abnormal nutrient metabolism (amino acids, lipids),
• Defective iron handling.

PSS dogs are often microcytic (possibly due to defective iron metabolism), even if they are not anemic.

**Infection of erythropoietic cells**

Some viruses – notably feline leukemia virus and equine infectious anemia virus – can infect hematopoietic precursors. Multiple mechanisms probably contribute to anemia in affected animals, including:

• Direct suppression of early-stage erythropoietic cells.
• Anemia inflammation.
• Immune-mediated hemolysis.

**Toxic insult to the bone marrow**

Toxic injury to the bone marrow is likely to result in pancytopenia. Examples include:

• Anti-cancer therapy, as discussed above.
• Estrogen excess (e.g., Sertoli cell tumor or exogenous administration).
• Bracken fern toxicosis.

**Space-occupying disease in the bone marrow**

Replacement of hematopoietic tissue by abnormal tissue (myelophthisic) is likely to result in pancytopenia. Examples include:

• Fibrosis.
• Neoplasia (primary bone marrow neoplasia or metastatic lesions).

**Other rare conditions**

• **Nutritional deficiencies** (besides iron deficiency) – such as copper deficiency (disrupts iron metabolism), folate deficiency, or cobalamin (B₁₂) deficiency.
• **Inherited conditions** – such as congenital dyserythropoiesis in English springer spaniels and polled Herefords.