Behind the iron panel

By Sunao Fujita

Understanding different forms of iron deficiency comes down to knowing what you want to measure and finding the right test.

Iron metabolism

Iron is an essential nutrient for a variety of metabolic processes, with the most important being transporting oxygen in haemoglobin. Approximately 60–70% of iron in the body is present in the form of haemoglobin. Dietary iron is absorbed mainly in the duodenum in the reduced ferrous ($Fe2^{+}$) state. Ferrous iron is transported across the enterocytes, then exported from the basolateral side to blood vessels by ferroportin (an iron transport protein). The ferrous iron, after uptake into the circulation, is bound to transferrin in the plasma following oxidation to ferric ($Fe3^{+}$) iron. The iron bound to transferrin is called serum iron.

Excessive iron is stored in the hepatocytes, enterocytes and reticuloendothelial system in the form of ferritin, which is soluble and the primary iron storage protein. A portion of ferritin is released into the plasma and is measured as serum ferritin. With iron deficiency, stored iron separates from ferritin and is transported by transferrin. Released iron is transported to bone marrow for use in haemoglobin synthesis (recycling for erythropoiesis).

There is no mechanism to excrete excessive iron in the body. Therefore iron homeostasis is tightly controlled by enteric iron absorption, which is regulated by hepcidin, a hormone produced in the liver. When iron stores are adequate, hepcidin impairs the function of ferroportin and limits iron uptake into circulation. Hepcidin also suppresses the release of stored iron from the hepatocytes and the reticuloendothelial system. The production of the hepcidin is enhanced by excessive iron and chronic inflammation. Conversely, hypoxia, iron deficiency and erythropoiesis suppress synthesis of the hepcidin.

Iron panel tests and erythrocyte alterations

Absolute iron deficiency (AID) is caused by chronic blood loss or nutritional deficiency. Nutritional iron deficiency is not common in dogs and cats fed commercial diets. Instead, AID is mainly seen with chronic bleeding. Functional iron deficiency (FID) occurs due to chronic inflammatory diseases and portosystemic shunts (PSSs).

Serum iron

Serum iron is a measurement of transferrin bound to iron. Note that serum iron is not a measurement of 'free iron', which is significantly harmful for tissues because the free iron is a free radical. Decreased serum iron can typically be seen with AID (chronic blood loss). Serum iron can also be decreased in inflammatory diseases and PSSs in dogs. Increased serum iron can be seen with haemolytic anaemia, glucocorticoid therapy in dogs and horses, hepatocellular necrosis, dyserythropoiesis and aplastic anaemia (Weiss and Wardrop, 2010). Random transient variations can occur in healthy animals.

Total iron binding capacity

Clinically, total iron binding capacity (TIBC) is considered a measure of the total serum transferrin concentration, although this is an indirect measurement. TIBC is increased in AID in horses, cattle and pigs, but is not consistently elevated in dogs, cats and camelids with AID (Weiss and Wardrop, 2010).

Decreased TIBC can be seen with inflammatory disease, as transferrin is a negative acute phase protein. TIBC can also be decreased with hepatic insufficiency, PSSs and protein-losing diseases (protein-losing nephropathy and enteropathy).

Serum ferritin

Serum ferritin is a measurement reflective of tissue iron stores and can be used as a marker for early-stage AID (low ferritin); however, ferritin has not been widely available in veterinary medicine because species-specific reagents are required for an assay (Schaefer and Stokol, 2015). Increased serum ferritin can be seen with inflammatory diseases, hepatic necrosis and hemophagocytic disorders (Weiss and Wardrop, 2010).

Mean corpuscular volume and mean corpuscular haemoglobin concentration

Under the chronic condition of AID, erythrocytes tend to be microcytic (decreased mean corpuscular volume [MCV]) and hypochromic (decreased mean corpuscular haemoglobin concentration [MCHC]). Morphologic erythrocyte alterations (hypochromasia, schistocytosis and keratocytosis) can be seen on blood films, most prominently in dogs and ruminants with chronic iron deficiency anaemia, and less apparent in cats and horses (Weiss and Wardrop, 2010). Unfortunately, the red blood cell (RBC) indices (MCV and MCHC) are not sensitive markers for the detection of early-stage AID, as they are the mean values (they cannot detect small numbers of microcytic and hypochromic RBCs). In addition, the RBC indices do not reflect the very recent status of erythropoiesis, as erythrocytes have a long life-span in circulation. Hereditary microcytosis can also be seen in some Asian dogs, such as Akita, Shiba Inu, Shar Pei and Chow Chow (Schaefer and Stokol, 2015).

Clinical use of iron panel tests

A diagnosis of AID is challenging because there is no true gold-standard test (Schaefer and Stokol, 2015). Low serum iron is neither sensitive nor specific for AID. Most of the haematologic and biochemistry changes listed above can also be seen with FID, especially inflammatory diseases in which hepcidin plays an important role in the suppression of stored iron utilisation. Anaemia of inflammatory diseases is typically normocytic and normochromic, but some patients can have microcytosis, probably due to a prolonged sequestration of storage iron (Schaefer and Stokol, 2015). Furthermore, concurrent inflammatory diseases make the diagnosis of AID more difficult. Therefore the results of the tests listed above need to be interpreted in conjunction with clinical presentation, other clinical tests and trends.

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The RBC indices in mature cells are not sensitive indicators for iron deficiency due to the long life-span of RBCs in circulation. Certain haematology analysers can measure reticulocyte indices, including cell size and haemoglobin content within reticulocytes. The reticulocyte indices are expected to function as complementary markers for iron-limited erythropoiesis in dogs, because normally reticulocytes of dogs only remain in circulation for one to two days before becoming mature, and the reticulocyte indices should reflect very recently produced microcytic and/or hypochromic cells (Schaefer and Stokol, 2015). Some studies have reported the usefulness of the reticulocyte indices in dogs, especially the measurement of haemoglobin content within the reticulocytes, for evaluating iron-limited erythropoiesis (AID and FID) (Fuchs et al., 2017). Routine measurements of the reticulocyte haemoglobin content may be expected as a useful complementary indicator in combination with the iron panels and other clinical examinations (blood film evaluation, CBC, etc).

Sunao Fujita is a clinical pathologist with Gribbles Veterinary.

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