Ferritin (serum, plasma)

1 Name and description of analyte

1.1 Name of analyte
Ferritin

1.2 Alternative names
Apoferritin (when no iron is bound)

1.3 NLMC code
To follow

1.4 Description of analyte
Ferritin is a ~450 kDa protein comprising 24 apoferritin monomers that associate to form a hollow spherical particle. Up to 4000 atoms of iron can bind in the sphere where they are stored as Fe^{3+} ions. In human cells, two subunits of ferritin exist; light (L) and heavy (H); most tissue ferritin molecules are a heterogenous mixture varying proportions of the two subunits. Circulating ferritin is normally predominantly in the L form, and is not iron-bearing.

In normal individuals, 50–81% circulating ferritin is glycosylated; glycosylated ferritin has a longer half-life (~50 h) than non-glcosylated ferritin (5 h).

1.5 Function of analyte
Ferritin is the principal storage protein for iron in tissues and is involved in its uptake, accumulation and release in cells. Ferritin sequesters iron in its bio-available form, thus protecting cells from its toxic effects, such as its propensity to form reactive oxygen species. It is found in virtually all cells, although most iron is stored in liver hepatocytes, macrophages in bone marrow and in the spleen, thus providing a readily available supply of iron for haemoglobin and haem protein synthesis.

Only minute amounts of ferritin are present in plasma but in health its concentration is directly proportional to total body iron stores. This relationship makes the serum or plasma assay for ferritin an ideal non invasive test of iron status. When [iron] is low, ferritin synthesis at the translational level is suppressed, and *visa versa*. However, this is only true if the direct relationship between plasma [ferritin] and the iron storage pool is not disturbed by ferritin release from parenchymal cells e.g. the liver, or by a change in plasma ferritin synthesis or metabolism (see 3.2).

About two-thirds of the iron stores in the human body are contained in ferritin. The remaining iron stores are contained in insoluble hemosiderin, which most likely represents a form of denatured ferritin.

2 Sample requirements and precautions

2.1 Medium in which measured
Ferritin can be measured in plasma or serum.

2.2 Precautions re sampling, handling etc.

Ferritin can be measured in serum using serum separator tubes (SST), or plasma using lithium heparin or EDTA tubes. Centrifugation and separation within 24 h of sample collection is required and ferritin is stable for 7 days at 2–8°C. It is recommended that samples are centrifuged to remove precipitates and fibrin before performing the assay. Heat-inactivated samples should not be used. Individual plasma concentrations may differ from corresponding serum values by more than 10%, depending on the assay used. When serial specimens from individual patients are being evaluated, the same sample tube type should be used throughout.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

Ferritin measurements are used:

1. to diagnose iron deficiency
2. in the differential diagnosis of anaemia, including iron deficiency anaemia
3. to monitor the response to iron therapy
4. to monitor iron mobilisation therapy
5. to aid in the diagnosis of iron overload, including the genetic condition hereditary haemochromatosis (HH).

3.2 Limitations

- There is a disproportionate increase in plasma [ferritin] in relation to iron stores in certain conditions:
  - inflammation (acute and chronic)
  - significant tissue destruction
  - liver disease e.g. hepatic cell damage (which make elevated values difficult to interpret in haemochromatosis
  - alcoholic liver disease (as part of acute phase response or due to release of ferritin from damaged hepatocytes)
  - malignancies e.g. acute leukaemias, Hodgkin's disease, carcinoma of the lung, colon, liver and prostate
  - therapy with iron supplements.
- Ferritin concentrations must be interpreted in light of clinical and pathological findings.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

4 Analytical considerations

4.1 Analytical methods

Ferritin can be measured using immunoassays e.g. ELISA, immuno-chemiluminescence and immunoturbidimetric assays.

Immunoradiometric assays are now rarely used, owing to the health and safety risks associated with using radioactively labelled substances. Most immunoassays use antibodies to either spleen or liver ferritin. Because of its variable structure, different antibodies to ferritin may not react equally with all forms, so that results obtained with one manufacturer's method may not be directly comparable with others.

1. The Abbott Architect® assay is a two-step chemiluminescent microparticle immunoassay.
2. The ADVIA Centaur® ferritin assay is a chemiluminometric two-site sandwich immunoassay. Two antibodies are used; goat anti-ferritin antibody labelled with acridinium ester and mouse anti-ferritin antibody, which is covalently coupled to paramagnetic particles.
3. The Tinta-quant® ferritin assay is based on the immunological agglutination principle with enhancement of the reaction by latex. Anti-ferritin antibodies bound to latex react with the antigen in the sample to form an antigen-antibody complex. Agglutination is then measured turbidimetrically.
4. The Roche ECLIA® electrochemiluminescence immunoassay uses two monoclonal mouse antibodies, M-4.184 and M-3.170, to form the sandwich complex in the assay.

4.2 Reference method

A reference method has not been defined.

4.3 Reference materials

3rd International Recombinant Standard for Ferritin (NIBSC Code 94/572).

4.4 Interfering substances

No significant interference with conjugated and unconjugated bilirubin concentration ≤500 μmol/L, haemolysis ≤0.5 g/L, lipaemia ≤17μmol/L (triglycyeride), biotin ≤205 nmol/L. Rheumatoid factors ≤100 IU/mL do not interfere with the assays.

4.5 Sources of error

Grossly haemolysed samples should not be analysed because the release of intracellular ferritin can cause an increase in [ferritin] by 60%. In patients receiving therapy with high doses of biotin (i.e. >5 mg/day), no sample should be taken until at least 8 h after the last biotin administration. Erroneous findings may be obtained with samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes. This can also occur in patients who are routinely exposed to animals or animal serum products (see. 3.2)

4. Reference intervals and variance

The normal reference interval has a wide range because of age and gender variations. Intra-individual variation of serum [ferritin] is very low and no

circadian variation has been determined. The reference interval also has considerable variation depending on method used and therefore should be individually determined for each laboratory.

4.1.1 Typical reference interval (adults)
Men, 20–60 years: 30–400 μg/L
Women, 17–60 years: 15-150 μg/L
Men and women, 60–90 years: 15–650 μg/L

4.1.2 Reference intervals (others)
Children, 6 months to 15 years: 7–140 μg/L

5.1.3 Extent of variation

5.1.3.1 Interindividual CV: 15%

5.1.3.2 Intraindividual CV: 14.2%

5.1.3.3 Index of individuality: 0.95

5.1.3.4 CV of method: 4% for [ferritin] 100–300 μg/L
10% for [ferritin] 10–20 μg/L

5.1.3.5 Critical difference:

5.1.4 Sources of variation
Serum [ferritin] in adults ranges from 15–300 μg/L between 20– 50 years. From puberty to middle age (<50 years), men have higher [ferritin] than women of the same age. During the post-menopausal period, the gender difference disappears. Children have typically have lower [ferritin] than adults.

6 Clinical uses of measurement and interpretation of results

6.1. Uses of measurement

1. Depletion of iron stores
   Serum [ferritin] <15 μg/L always indicates depleted iron stores and is evidence of iron depletion with or without anaemia. This cut-off is only valid for patients without co-existent disease that could influence [ferritin]. *A normal [ferritin] does not exclude iron deficiency*.
2. Diagnosis of iron deficiency anaemia
   Serum ferritin measurement is indicated after haematological investigations including full blood count have shown low values for [haemoglobin] and the haematocrit and/or a blood film has demonstrated hypochromic microcytic anaemia. [Ferritin] declines early in the development of iron deficiency, before the decline of [Hb], red cell size, or serum [iron].
3. Differential diagnosis of hypochromic anaemia
   In hypochromic anaemias not caused by or associated with iron deficiency (i.e. thalassemias, sideroblastic, tumour- and infection-related), [ferritin] is normal or elevated, not low.
4. Control of iron therapy over time
   In patients with iron deficiency anaemia, determination of [ferritin] before commencing therapy gives a measure of the body's iron reserves. Ferritin measurement is indicated to confirm improvement of body iron stores to within normal limits. [Ferritin] should be maintained within the reference range. Regular measurements are required to check compliance and to sufficient replacement (see 2.2).

5. Iron overload
   Ferritin concentrations >400 μg/L in the absence of a distribution disorder are suggestive of iron overload. Further investigations are then required to confirm this and determine its cause.
   - Hereditary haemochromatosis (HH)
     In the early stages of disease, serum [ferritin] may be normal in the presence of increased iron liver content. The diagnosis is suggested by a transferrin saturation >60% and elevated plasma [ferritin] (typically >700 μg/L in symptomatic patients).
   - Secondary iron overload (e.g. secondary to repeated blood transfusion)
     Serum [ferritin] is always elevated. In patients with chronic kidney disease, very high ferritin concentrations (>800 μg/L) suggest iron overload due to excessive iron supplementation.
6. Monitoring of iron mobilisation therapy ('deironing')
   In monitoring the treatment of HH, (i.e. chelation or repeated venesection), [ferritin] should decrease in response to the reduction in iron stores.

6.2. Confounding factors
See 3.2. On further investigation, by no means all individuals with plasma [ferritin] >400 μg/L will be found to have iron overload.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes (assuming patient is not receiving blood transfusions, oral/parental iron therapy):
- hereditary haemochromatosis (HH)
- unrelated to iron stores
  - tissue necrosis
  - iron accumulation within RES due to disease
  - alteration of hepatic ferritin clearance
  - blocked erythropoiesis
  - increased synthesis in tumour tissue
  - hereditary hyperferritinaemia cataract syndrome (HHCS)
  - benign hyperferritinaemia (due to mutations in the ferritin L-chain with apparent hyperglycosylation)
  - ferroportin disease.

7.1.2 Investigation (see also 6.1(5))
In HH, [ferritin] is less sensitive than measurement of serum iron, TIBC and % transferrin saturation, which provide the earliest indication of HH. Measurement of iron in a liver biopsy is the gold standard for diagnosis, and is recommended if there is biochemical evidence of liver damage (deranged liver function tests). Diagnosis can be confirmed by molecular genetic analysis.
In disease states with an inflammatory component, [ferritin] must be interpreted with caution and in the light of the clinical situation because it is a positive acute phase protein.

7.2 Low values

7.2.1 Causes:

- acute blood loss: concentrations fall within 1–2 weeks depending on its extent
- chronic blood loss e.g. menorrhagia excessive blood donation, gastrointestinal haemorrhage, blood vessel defects
- malabsorption syndromes
- increased requirement (in presence of inadequate intake) e.g. pregnancy, menstruating females, adolescence
- nutritional iron deficiency (with normal requirements).

In patients with co-existing disease (chronic conditions with an inflammatory component resulting in iron release and impaired utilisation of stored iron), a cut-off value of <50 μg/L is consistent with iron deficiency. Chronic anaemia can develop in these conditions, but iron deficiency is unlikely if plasma ferritin concentration is >60 μg/L.

In patients with chronic kidney disease (CKD), [ferritin] <100 μg/L indicates true iron deficiency; [ferritin] between 100–200 μg/L with a transferrin saturation of <20% indicates 'functional' iron deficiency. Successful treatment of anaemia in CKD requires adequate iron stores.

7.2.1 Investigation

Ferritin should only be measured if there is a specific indication, and the cause of a low value should then often be readily apparent. If not already measured, a full blood count is required to determine if iron deficiency anaemia is present. Measurement of serum iron and transferrin saturation are required if not already performed.

7.3 Notes

1. A combination of chronic disease and iron deficiency may result in a normal [ferritin].
2. In iron mobilisation therapy, it should be considered that [ferritin] may be disproportionally high compared to iron stores owing to intravascular haemolysis and increases in liver enzymes.
3. Parental (i.v.) iron therapy results in induction of ferritin synthesis and [ferritin] is often disproportionately high compared to iron stores.
4. Measurement of ferritin has been shown to be of use in patients with suspected liver metastases, with 76% of such patients having [ferritin] >400 μg/L.

8 Performance

8.1 Sensitivity and specificity for individual conditions

1. In iron deficiency determined by bone marrow aspirate from 54 patients:
   - cut-off ≤12 μg/L: sensitivity 25%, specificity 98%
   - cut-off ≤30 μg/L: sensitivity 100 %, specificity 98%.

2. Hereditary haemochromatosis. Liver biopsy with quantitation of hepatic iron is considered the gold standard. Cut-off >200 µg/L (women), >300 ug/L (men): sensitivity 50%, specificity 87%.

9 Systematic reviews and guidelines

9.1 Systematic reviews

Wang W, Knovich MA, Coffman LG *et al.* Serum ferritin: Past, present and future. Biochim Biophys Acta. 2010;1800**:** 760-769. This r*eview discusses the role of serum ferritin in physiological and pathological processes and its use as a clinical tool*.

Schmitt B, Golub RM, Green R. Screening Primary Care Patients for Hereditary Hemochromatosis with Transferrin Saturation and Serum Ferritin Level: Systematic Review for the American College of Physicians. *Ann Intern Med.* 2005;143**:**522-536. *The authors concluded that benefits of screening for haemochromatosis do not outweigh the risks and costs*.

9.2 Guidelines

1. NICE guidelines CG114 February 2011: Anaemia management in people with chronic kidney disease.

- Diagnosis: serum [ferritin] <100 µg/L in stage 5 CKD, considered when serum [ferritin] <100 µg/L in stage 3 and 4 CKD.
- Treatment: iron correction should maintain serum [ferritin] >200 µg/L. Review iron dose when serum [ferritin] reaches 500 µg/L (should not be >800 µg/L).
- Maintenance: maintain serum [iron] when [ferritin] 200–500 µg/L in both haemodialysis and non-haemodialysis patients. Iron dosing regimen will depend on modality, for example haemodialysis patients will require the equivalent of 50–60 mg i.v. iron per week. People receiving erythropoiesis-stimulating agent maintenance therapy should be given iron supplements to keep [ferritin] between 200–500 µg/L in both haemodialysis and non-haemodialysis patients.
  http://guidance.nice.org.uk/CG114
  http://www.nice.org.uk/nicemedia/live/13329/52857/52857.pdf

2. The American Haemochromatosis Society has released guidelines to educate the medical community and general public on HH.
   http://www.americanhs.org/guidelines.htm
3. The British Society of Gastroenterology has issued guidelines for the management of iron deficiency anaemia. For management, ferritin measurement can be reserved for cases where haemoglobin or red cell indices have not been restored by therapy.
   http://www.bsg.org.uk/pdf_word_docs/iron_def.pdf
4. The UK Renal Association have also release clinical practice guidelines for Anaemia of CKD. Recommendations include baseline investigations of serum ferritin to assess iron stores. They recommend that ESA therapy should not be initiated in the presence of absolute iron deficiency ([ferritin] <100µg/L). For patients treated with iron, [ferritin] should not exceed 800µg/L and to achieve this iron management should be reviewed when the ferritin is > 500µg/L.

http://www.renal.org/Clinical/GuidelinesSection/AnaemiaInCKD.aspx
5. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease. Guidelines recommend that serum [ferritin] be kept at 200 µg/L or higher in patients receiving erythropoiesis-stimulating agents (ESAs). http://www.kidney.org/professionals/kdoqi/guidelines_anemia/cpr32.htm

9.3 Recommendations
See 9.2.

10. Links

10.1 Related analytes
None

10.2 Related tests

1. Serum [iron]: amount of circulating iron bound to transferrin. Units of measurement: µmol/L.
2. Transferrin: the main transport protein of iron in the blood and is normally 33% saturated. Units of measurement: g/L.
3. Percent transferrin saturation: the amount of transferrin saturated by iron (serum [iron]/ TIBC x 100), which gives an indication of how much plasma iron is bound. Transferrin is normally about one-third saturated with iron.
4. Total iron binding capacity (TIBC): a functional measurement of transferrin concentration. Transferrin can be measured by immunoassay and the iron-binding capacity calculated from it. The theoretical ratio of TIBC (in µmol/L) to TRF (in g/L) is 25.1, i.e. TIBC (µmol/L) = 25.1 × TRF (g/L).

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