Acute phase proteins

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- Ferritin is a circulating iron storage protein that is increased in proportion to body iron stores. However, ferritin is also an acute phase reactant .that can increase independently of iron status in disorders associated with inflammation, infection, liver disease, heart failure, and malignancy . The ferritin concentration that predicts the absence of marrow iron is debated. While many sources use a cutoff level of 12 to 15 ng/mL (99 percent specific but only 57 percent sensitive), our practice is to use a cutoff of 30 ng/mL, which is supported by bone marrow correlations and international guidelines .
- The sensitivity and specificity for a cutoff at 30 ng/mL is estimated to be 92 percent and 98 percent, respectively .
- A 2020 guideline from the American Gastroenterological Association uses a cutoff of <45 ng/mL to define iron deficiency in patients with anemia . A very low ferritin level is diagnostic of iron deficiency, but the ferritin level may be "falsely normal" in individuals with comorbidities, and a ferritin within the normal range cannot be used to eliminate the possibility of iron deficiency in individuals with comorbidities

- The normal range for ferritin in plasma or serum is approximately 40 to 200 ng/mL
- A ferritin level ≥200 to 300 ng/mL (in a male or ≥150 to 200 ng/mL in a female is consistent with iron overload, and a level below these values is good evidence that the patient does not have iron overload.
- Typically, ferritin levels in iron overload are in the range of up to 2000 to 3000 ng/mL (even higher if iron overload is causing severe liver disease or carmcg/L), cardiomyopathy. If the individual has received multiple transfusions, ferritin may also be higher.
- Elevated serum ferritin is a sensitive test for iron overload, but it is not very specific. Numerous conditions other than iron overload can elevate serum ferritin . Ferritin is an acute phase reactant that increases with infection or inflammation. Ferritin can also be elevated in patients with liver disease. These other causes of abnormally high ferritin must be distinguished in order to avoid unnecessary invasive testing and/or delays in treatme

- In individuals with transfusional iron overload, there is some correlation between the ferritin level and the total body iron burden (individuals with a ferritin in the range of 500 to 1000 ng/mL are unlikely to have severe iron overload; those with ferritin in the range of 3000 to 4000 ng/mL may have substantial iron burden),
- but there is variability between individuals and between measurements for the same individual. The correlation between iron stores and ferritin levels may only be reliable at a ferritin below 3000 to 4000 ng/mL . Observational studies have generally found some weak correlations between ferritin and iron burden (assessed by liver biopsy or therapeutic phlebotomy) but results are mixed, and sensitivity and specificity are in the range of 60 to 80 percent .
- The correlation of ferritin and liver iron concentration is not linear in individuals with a component of non-transfusional iron overload from ineffective erythropoiesis. The correlation is generally weak in regularly transfused patients with sickle cell disease (SCD) compared with thalassemia, though high ferritin levels (>3000 ng/mL) and low ferritin levels (<1000 ng/mL) generally correlate with high and low liver iron concentration, respectively.

FERRITIN

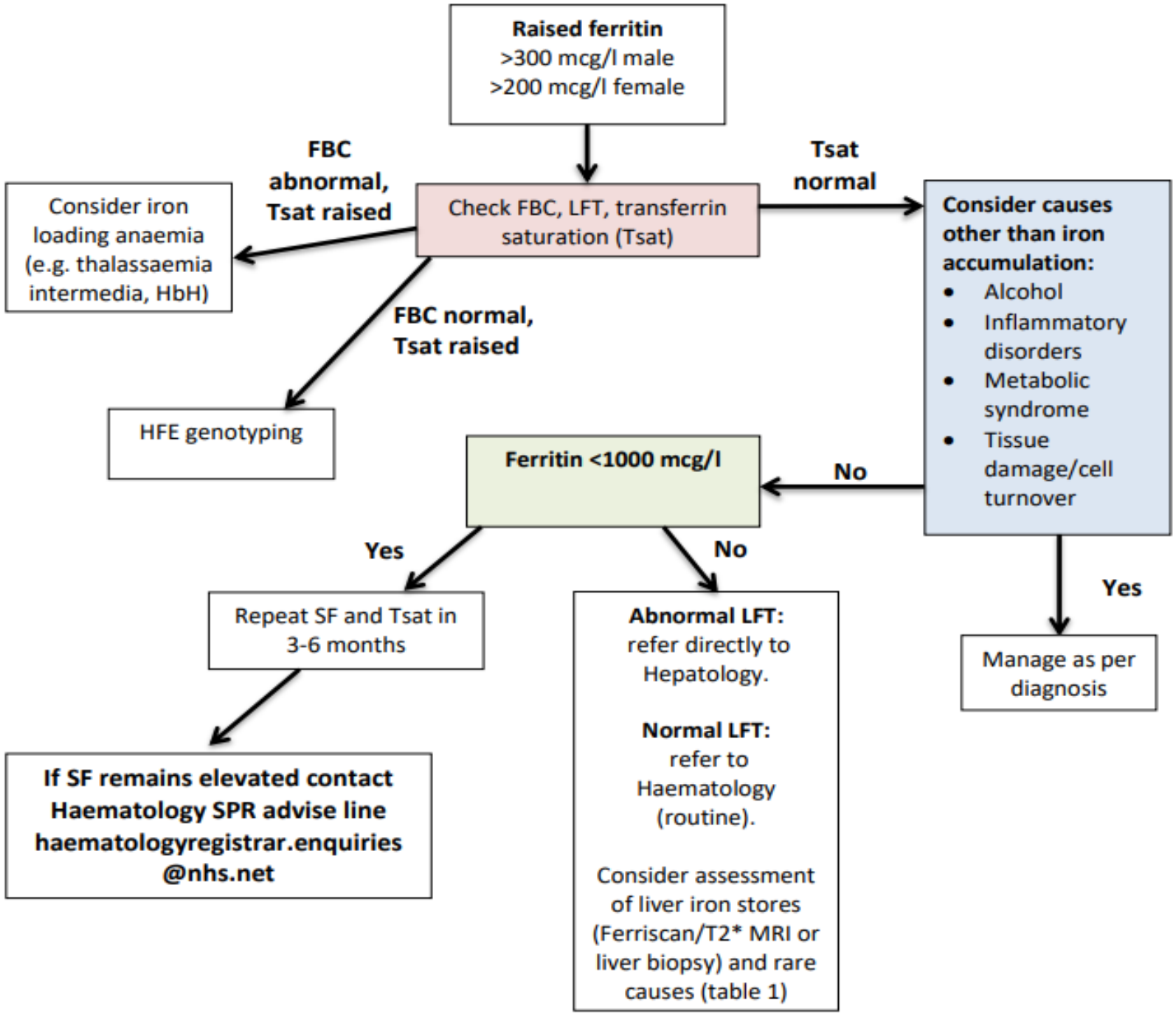
- Low (cutoffs vary)
- <30 ng/mL is often used
- High
- Female: >200 ng/mL
  Male: >300 ng/mL

TSAT

- Low (cutoffs vary)
- ≤19% is often used
- High
- >45%

Causes of increased ferritin

Condition (examples)	Pattern
Iron overload ■ Hereditary hemochromatosis ■ Transfusional iron overload ■ Ineffective erythropoiesis (eg, thalassemia)	Progressive/cumulative increase in ferritin over time, eventually causing organ damage if not treated. TSAT will be high (typical value >45%).
Massive cell/tissue death ■ HLH ■ Cancer ■ Liver failure	Rapid rise in ferritin to very high levels (eg, >3000 ng/mL), usually in the setting of acute illness with immune dysregulation. TSAT will not be increased (typical value <45%).
Inflammatory block ■ Anemia of chronic disease/anemia of inflammation (ACD/AI, as in diabetes, cancer, chronic infection, or autoimmune disorders) ■ Anemia of chronic kidney disease ■ Chronic liver disease	Chronic, modest increase in ferritin (approximately two to three times normal). Ferritin is an acute phase reactant. TSAT will not be increased (typical value <45%).


Raised ferritin
>300 mcg/l male
>200 mcg/l female
Check FBC, LFT, transferrin saturation (Tsat)
FBC abnormal, Tsat raised
Consider iron loading anaemia (e.g. thalassaemia intermedia, HbH)
FBC normal, Tsat raised
HFE genotyping
Tsat normal
Consider causes other than iron accumulation:
Alcohol
Inflammatory disorders
Metabolic syndrome
Tissue damage/cell turnover
No
Ferritin <1000 mcg/l
Yes
Repeat SF and Tsat in 3-6 months
If SF remains elevated contact Haematology SPR advise line haematologyregistrar.enquiries @nhs.net
No
Abnormal LFT: refer directly to Hepatology.
Normal LFT: refer to Haematology (routine).
Consider assessment of liver iron stores (Ferriscan/T2* MRI or liver biopsy) and rare causes (table 1)
Yes
Manage as per diagnosis

- **Haptoglobin** – The normal range for serum haptoglobin is wide. A low haptoglobin is likely to be due to hemolysis, and an undetectable haptoglobin level is almost always due to hemolysis. In a series of 100 patients with various medical conditions, a haptoglobin level of 25 mg/dL provided the best cutoff between hemolytic and non-hemolytic disorders . The sensitivity and specificity of a haptoglobin ≤25 mg/dL were 83 and 96 percent. However, a normal or increased haptoglobin does not eliminate the possibility of hemolysis because haptoglobin is an acute phase reactant that can be increased in the setting of inflammation

- Other causes of low haptoglobin include :
- hepatic insufficiency, abdominal trauma, and congenital ahaptoglobinemia

- **Hepcidin (primary regulator of iron homeostasis)** — Hepcidin is a small peptide produced by the liver in response to cytokines or exposure to bacterial antigens, as a component of the innate immune response to infection .
- Hepcidin binds to the plasma membrane channel ferroportin, blocking iron export ; this leads to internalization and degradation of ferroportin . Removal of the ferroportin channel in turn prevents iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages.
- Increased iron released from macrophages is the major source of iron for the heme synthesis required for erythropoiesis. Macrophages scavenge and ingest senescent RBCs; iron recycling from these RBCs accounts for 90 to 95 percent of the daily total body iron requirement . Hepcidin also reduces the transfer of dietary iron or oral iron supplements from duodenal enterocytes into the circulation by the same actions on enterocyte ferroportin .

- Production of hepcidin by other cells such as monocytes has also been reported; this is thought to form an autocrine mechanism to increase macrophage iron sequestration
- As an antimicrobial peptide, hepcidin produced by the intestine dendritic cells might protect the local mucosa by sequestering iron from the local microbiome, favoring mucosal healing in inflammatory bowel disease (IBD)
- Hepcidin production, decreased urinary excretion of hepcidin, and increased serum hepcidin levels have been seen in patients with infection, malignancy, or an inflammatory state (as evidenced by C-reactive protein [CRP] levels >10 mg/dL). The complex role of hepcidin in different infections may go beyond iron homeostasis regulation . As example:
- ●In certain skin infections such as necrotizing fasciitis caused by group A *Streptococcus*, the role of hepcidin in the skin is to induce expression of CXCL1 to recruit neutrophils.
- ●In hospitalized patients with COVID-19, increased hepcidin production and iron dyshomeostasis, indicated by changes in serum ferritin levels, has been linked to a poor clinical course and outcome .

- **Increased hepcidin** – Hepcidin is the primary controller of iron availability to developing RBCs, as described above (see 'Hepcidin (primary regulator of iron homeostasis)' above). Cytokines including interleukin (IL)-1, IL-6, and IL-22 induce hepcidin production, as evidenced by numerous preclinical and clinical studies:
- •In a mouse model, knockout of IL-6 completely blunted the induction of hepcidin in response to inflammation [14].
- [28].

- •In a series of 92 consecutive patients admitted for sepsis, hepcidin levels at admission were high, increased with the number of systemic inflammatory response syndrome (SIRS) criteria, and correlated with both IL-6 levels and the subsequent decrease in hemoglobin over the following days [27]. In a series of 150 patients with severe trauma, urinary hepcidin levels were extremely high on admission; hepcidin was positively correlated with the Injury Severity Score (ISS) and the duration of anemia, and negatively correlated with hypoxia

- •In patients with ACD/AI, hepcidin mRNA levels in monocytes were significantly correlated with serum IL-6 concentrations [15,19].
- •Patients with inflammatory conditions who are treated with an anti-tumor necrosis factor (TNF) antibody or an anti-IL-6 antibody have reductions in inflammatory markers such as IL-6, hepcidin, and/or CRP, which correlates with improvement in anemia [20,29-32].
- •Emerging evidence suggests that vitamin D may suppress hepcidin [33]. Vitamin D deficiency and anemia sometimes coexist, and correction of vitamin D deficiency can improve anemia in a certain percentage of patients; this is believed to result from direct inhibition of hepcidin formation by the active vitamin

- C-reactive protein — Elevations of CRP occur in association with acute and chronic inflammation due to a range of causes, including infectious diseases and noninfectious inflammatory disorders.
- A rough correction of the CRP for age can be made by using the following formulas: the upper limit of the reference range (mg/dL) equals (age in years)/50 for men and (age in years/50) + 0.6 for women

- Moderate to marked elevation of CRP — In most inflammatory conditions, the CRP, like the ESR, becomes elevated as part of the acute phase response. Markedly elevated levels of CRP are strongly associated with infection. Infections, most often bacterial, were found in approximately 80 percent of patients with values in excess of 10 mg/dL (100 mg/L) and in 88 to 94 percent of patients with values over 50 mg/dL (500 mg/L) [92,93]. Levels of CRP may also be elevated in patients with viral infections, although usually not to the degree seen in patients with bacterial infection

- Although CRP levels do not correlate with gestational age in pregnancy, median CRP levels are marginally higher during pregnancy when compared with nonpregnant persons [87,88]. Also, CRP levels increase during labor [88]. In addition to usual metabolic stressors, this low level of subclinical inflammation has been associated with preeclampsia and gestational diabetes

- Minor CRP elevation (concentrations between 3 and 10 mg/L) is regarded as a marker of low-grade inflammation. Inflammation, one of the first responses of the activated innate immune system, has long been defined as the response to infection and tissue injury

crp

- Roles of CRP — CRP and many other APR can influence multiple stages of inflammation, and CRP has both proinflammatory and antiinflammatory actions, although the primary effect may be antiinflammatory [35,36]. CRP can promote the recognition and elimination of pathogens and enhance the clearance of necrotic and apoptotic cells [37-43]. The protein consists of five identical, non-covalently associated subunits, each with a molecular weight of approximately 23 kD, which are arranged symmetrically around a central pore

- A major function of CRP is its ability to bind phosphocholine, thereby permitting recognition both of foreign pathogens that display this moiety and phospholipid constituents of damaged cells [37]. CRP can also activate the complement system and bind to phagocytic cells via Fc receptors, suggesting that it can initiate elimination of pathogens and targeted cells by interaction with both humoral and cellular effector systems of inflammation [36]. These functions of CRP may have negative effects in some settings. As an example, CRP levels are increased in patients with immune thrombocytopenia (ITP), where CRP may amplify antibody-mediated platelet destruction upon binding to phosphocholine that is exposed after oxidation triggered by antiplatelet antibodies

- However, APR measurements in clinical use are not specific to any particular disease, nor can they distinguish infection from other causes of acute and chronic inflammation. The most widely used indicators of the acute phase response are the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels

esr

- Marked elevations in the ESR are more often due to infection than other causes, but noninfectious disorders are also a common etiology. In a retrospective study of 1006 consecutive outpatients, ESR values of over 100 mm/hour were most commonly due to infection (33 percent), with malignant neoplasms and renal disease responsible for 17 percent each and a variety of inflammatory disorders responsible for 14 percent

- Conditions or factors unrelated to acute or chronic inflammation that may also increase the ESR include:

- ●Increased age and female sex – ESR values increase markedly with age [62] and are slightly higher among women than men. As a result, any single set of normal values will not be valid for the population at large. One can roughly correct ESR for age by using the following formulas: the upper limit of the reference range equals (age in years)/2 for men and (age in years + 10)/2 for women [63].

- ●Anemia – It has long been known that anemia increases the sedimentation rate [64]. The sedimentation of red blood cells is presumably impeded by other red blood cells; sedimentation is thus more rapid in anemia, in which this retardation is lessened, thus increasing the ESR. Macrocytosis may also increase ESR [65].

- ●Pregnancy – The pregnant state includes hemodilution because of increase in plasma volume; corrected for this anemia, ESR increases with gestational age of the pregnancy

- Renal disease – The ESR is elevated (greater than 25 mm/hour by the Westergren method) in almost all patients with end-stage kidney disease (ESKD) or the nephrotic syndrome, and is unaffected by hemodialysis [67-69]. Nearly 60 percent of patients with ESKD have an ESR above 60 mm/hour, while 20 percent have extreme elevations above 100 mm/hour. Thus, an isolated ESR elevation in a patient with renal disease, without other systemic signs or symptoms, does not necessarily indicate the presence of infection, disease activity, or an underlying malignancy (see "Membranous nephropathy: Pathogenesis and etiology"). Compared with hemodialysis patients, patients utilizing peritoneal dialysis have a higher ESR

- Obesity – Both ESR and CRP can be elevated in obesity [72]; this is due at least in part to interleukin (IL) 6 secretion by adipose tissue [73]. In fact, metabolic syndrome is associated with an increase in ESR [74].

- ●Other lifestyle characteristics – Smoking has been associated with elevated ESR [74].

- ●Technical factors – Tilting of the ESR tube or high room temperature may increase the ESR.

- Decreased ESR — A number of factors may spuriously result in a very low ESR or ESR that is less than the expected level in a patient with acute or chronic inflammation [75,76]. These include:

- ●Abnormalities of erythrocytes – Changes in red cell shape or number may reduce the ESR, including sickle cell disease, anisocytosis, spherocytosis, and acanthocytosis, as well as microcytosis and polycythemia. This can be confusing, for example, for patients with sickle cell disease, who may have a spuriously low ESR [77].

- ●Extreme leukocytosis

- ●Extremely high serum bile salt levels [76]

- ●Heart failure

- ●Hypofibrinogenemia

- ●Cachexia

- Other lifestyle characteristics – Moderate and high regular physical activity has been associated with decreased ESR, as has light alcohol consumption (ie, one to four drinks weekly) compared with nonconsumers [74].

- •Technical factors, including:

- •Clotting of the blood sample or delay in testing of greater than two hours

- •Low room temperature

- •Short ESR tube

Chronic elevation of ESR	Chronic depression of ESR
RBC abnormalities (anemia and macrocytosis)	RBC abnormalities (sickle cell, microcytosis, polycythemia)
Age	Other laboratory abnormalities (extreme leukocytosis or bile acid salts) and technical factors of assay performance
Female sex	Heart failure
Pregnancy	Cachexia
Obesity, metabolic syndrome	Hypofibrinogenemia (eg, chronic liver failure)
Renal disease (even on RRT)	High and moderate regular physical activity
Smoking	Light alcohol consumption

Subacute elevation of ESR	Subacute depression of ESR
Inflammatory conditions and infections (PMR, rheumatoid arthritis osteomyelitis, etc)	
Malignancy	Other laboratory abnormalities (extreme leukocytosis or bile acid salts) and technical factors of assay performance
Tissue injury or ischemia (gangrene)	Heart failure
Trauma	
	Hypofibrinogenemia (eg, macrophage activation syndrome)

- Discrepancies between ESR and CRP are found with some frequency. An elevated ESR observed together with a normal CRP is often a misleading result that may, for example, reflect the effects of blood constituents, such as monoclonal immunoglobulins, that are not related to inflammation but that can influence the ESR. It should not be routine practice to order serum protein electrophoresis (SPEP) and urine protein electrophoresis (UPEP) in such instances, unless the clinical presentation suggests that a plasma cell dyscrasia may be present.

- Systemic lupus erythematosus (SLE) represents an exception to the generalization that CRP concentrations correlate with the extent and severity of inflammation in patients with rheumatic disorders [96]. The ESR may be elevated, sometimes markedly, in patients with active SLE, while the CRP response is muted. The muted CRP response in SLE appears to result from the ability of type I interferons, which are highly expressed in most lupus patients, to inhibit CRP induction in hepatocytes

- While many patients with active SLE do not have significantly elevated CRP concentrations [105], CRP concentrations may be quite elevated in patients with active lupus serositis [106] or with chronic synovitis [107]. In a febrile lupus patient, marked CRP elevation (greater than 6 mg/dL) favors the diagnosis of bacterial infection

- Another more common example reflects the acuity of the acute phase response. CRP levels measure a single molecule, and one will note acute and rapid rise and fall with an insult. By contrast, because ESR is the reflection of numerous factors and the interaction of these elements (ie, long half-life of some plasma proteins), ESR levels do not rapidly rise at the beginning of an inflammatory insult; similarly, normalization is slower. This difference between ESR and CRP can help clinicians distinguish between acute processes and a more chronic process (for example, high CRP and normal ESR may suggest an acute paronychia; by contrast, elevated CRP and ESR may suggest osteomyelitis).

- In patients with active rheumatoid arthritis, the ESR and CRP generally tend to be parallel (ie, both are elevated or not elevated in a single patient). However, one study found that results for the two tests were discordant (ESR >28 mm/hr with CRP ≤0.8 mg/dL or ESR ≤28 mm/hr with CRP >0.8 mg/dL) in about one-quarter of patients with active rheumatoid arthritis in a large practice-based registry

- Several studies have suggested that elevations of the acute phase protein procalcitonin are highly specific for infection [109-111]; thus, procalcitonin may prove useful in differentiating infections from other inflammatory stimuli in autoimmune disease patients