Investigation and management of a raised serum ferritin

Dr Jonathan O. Cullis, Dr Edward Fitzsimons, Dr Bill Griffiths, Dr Emmanuel Tsochatzis, Dr D. Wayne Thomas on behalf of the British Society for Haematology

Summary

Serum ferritin is one of the most commonly requested investigations in both primary and secondary care. Whilst low serum ferritin levels invariably indicate reduced iron stores, raised serum ferritin levels can be due to multiple different aetiologies including iron overload, inflammation, liver or renal disease, malignancy, and the recently described metabolic syndrome. A key test in the further investigation of an unexpected raised serum ferritin is the serum transferrin saturation. This guideline reviews the investigation and management of a raised serum ferritin. The investigation and management of genetic haemochromatosis is not dealt with however and is the subject of a separate guideline.

Scope

The objective of this guideline is to provide healthcare professionals with guidance on the management of patients with a raised serum ferritin. The guidance may not be appropriate to every patient and in all cases individual patient circumstances may dictate an alternative approach.

Methodology

This guideline was compiled according to the BSH process at: http://b-s-h.org.uk/guidelines/proposing-and-writing-a-new-bsh-guideline/. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org.

Literature review details

The literature search entailed a systematic search of MEDLINE and PUBMED for publications that included an abstract and were published in English between 1980 and 2017 using the following key words: raised serum ferritin, hyperferritinaemia, as well as specific search terms relevant to each section.

Working group membership

The guideline group was selected to be representative of UK-based experts in the investigation and management of raised serum ferritin levels.

Review

Review of the manuscript was performed by the British Society for Haematology (BSH) Guidelines Committee General Haematology Task Force, the BSH Guidelines Committee and the sounding board of BSH. It was also on the members section of the BSH website for comment.

Introduction

Since the development of a sensitive immunoradiometric assay in 1972 (Addison *et al,* 1972) measurement of serum ferritin (SF) as a surrogate measure of body iron stores has largely replaced laboratory assays of serum iron and transferrin or total iron binding capacity in clinical practice. Its great value to the clinician lies in the finding that in health the SF is directly proportional to the level of iron stores (Worwood 1982). A study of quantitative phlebotomy in normal volunteers showed a correlation between storage iron and SF concentration with 1 µg/l of SF equivalent to approximately 8 mg of storage iron (Walters *et al,* 1973).

Reduced SF levels are only found in patients with reduced body iron stores. There is no other cause and guidelines for the management of patients with low SF and iron deficiency anaemia are well established in medical practice (Goddard *et al,* 2011). In some circumstances, for example in patients with co-existent inflammatory disorders, SF may be within the normal or elevated range even when iron stores are absent and anaemia is due to iron deficiency, and this is discussed further below in the section of Inflammatory and Infective Disorders. The clinical and laboratory management of patients with raised SF values however is not at all well recognised and is the subject of this guideline and the companion updated guideline on Genetic Haemochromatosis (GH).

Structure and Function of Ferritin

Ferritin is a soluble 450 kDa protein. It is found in all cells of the body but in high concentrations in marrow macrophages, the spleen and the liver. It provides intracellular storage of bio-available iron in a safe and readily accessible form. It protects cells from iron-mediated free radical formation and toxicity such as might result from the Fenton reaction between iron and hydrogen peroxide. Ferritin is comprised of 24 monomer subunits that consist of either H type (Heavy, 21 kDa) or L type (Light, 19 kDa) polypeptide chains encoded by 2 different ferritin genes. The 24 monomer subunits associate to form a hollow spherical particle that can store up to 4000 iron atoms as Fe^{3+} ions. The proportion of H and L type chains depends on the tissue of origin: liver and spleen ferritin are rich in L chains whereas ferritin in the heart or red blood cells is rich in H subunits. Haemosiderin, the "stainable iron" found in iron laden macrophages, represents insoluble, denatured ferritin from which iron is less readily available. For a review of the structure and function of ferritin and haemosiderin see Harrison and Arosio (1996).

Ferritin produced by the lens of the eye consists entirely of L chains. This L chain ferritin is capable of forming crystals under certain conditions as seen in the hereditary hyperferritinaemia cataract syndrome (HHCS) (Cazzola *et al,* 1997).

Serum Ferritin

A tiny amount of ferritin is found in the serum. This SF plays no role in iron transport or cellular iron uptake. That is the role of transferrin. Serum ferritin is almost entirely made up of L chains, has a half-life of 30 hours, is not iron bearing and is some 50–80% glycosylated. As glycosylation occurs intracellularly this would indicate that SF is a secretory plasma protein. The cell of origin and the mechanism by which this glycosylated ferritin passes into the serum is not well understood.

The proportion of glycosylated ferritin in serum may alter in certain disease types that allow intracellular (non-glycosylated) tissue ferritin to leak into the plasma with a half-life of about 9 minutes (Worwood 1986). The percentage glycosylation is low in liver necrosis and in Still's disease (Worwood 2012) but is almost 100% in certain hereditary hyperferritinaemic states.

Measurement of Serum Ferritin

Serum ferritin can be measured using immunoassays e.g. enzyme-linked immunosorbent assay (ELISA), immunochemiluminescence (Abbott Architect assay, ADVIA Centaur assay, Roche ECLIA assay) or immunoturbidometric assay (Tinta-quant assay). Immunoradiometric assays are now rarely used owing to the health and safety risks to laboratory personnel associated with using radioactive labelled substances. Most immunoassays use antibodies to either spleen or liver ferritin. Assays should be calibrated against the 3rd International Recombinant Standard for Ferritin (NIBSC Code 94/572).For further information on analysis see Worwood *et al* (2017) and The Association of Clinical Biochemistry (2012).

Raised Serum Ferritin

The recognition of a raised SF is dependent upon the upper limit of the normal range. Thereafter the appropriate action taken depends on the source of the sample, whether it is taken in primary or secondary care and knowledge of the patient's medical history. SF shows an acute phase response such that levels may be raised beyond that appropriate for reticuloendothelial system (RES) iron stores by inflammation or by tissue damage. Levels in serum will also be raised by any condition or treatment (e.g. blood transfusion or iron infusion) that lead to a genuine increase in RES iron stores.

Upper Limit of the Normal Range for Serum Ferritin

Most UK laboratories simply report 300-400 µg/l as the upper limit of normal for SF in adult males and 150-200 µg/l as the upper limit of normal for adult females (Worwood *et al,* 2017; Association for Clinical Biochemistry, 2012). There is however considerable variation in SF values in response to age, ethnic origin and sex. Mean SF values in neonates are high (around 200 µg/l) and remain so for about 2 months. From 2–12 years mean values approximate 30 µg/l for both boys and girls (Worwood, 1982). Within this age group values >100 µg/l are only seen in the context of inflammatory disease, malignant disease or juvenile hereditary haemochromatosis.

Mean SF values at 18 years are significantly higher in males (60-80 µg/l) than in females (25-30 µg/l) (Wiedemann & Jonetz-Mentzel, 1993; White *et al,* 1993; Custer *et al,* 1995; Milman *et al,* 2003). Thereafter in males SF values rise to plateau with median values of approximately 120 µg/l from the age of 30 years. Irrespective of age, approximately 20% of Caucasian male adults in primary care will have SF values >300 µg/l (Ogilvie *et al,* 2010; Adams *et al*, 2013). As a result of iron loss from menstruation and pregnancies SF values in adult females only start to rise after 50 years of age to plateau with median values of about 100µg/l after 60 years. Values >200 µg/l in adult females show a significant age effect and are seen in 3%, 10% and 17% respectively of women 30–50 yrs, 50–70 yrs and >70 yrs (Ogilvie *et al,* 2010; Adams *et al*, 2013).

Mean SF values are higher at all ages in adult black than in adult white males. In black females higher values are only seen after the menopause. In multi-ethnic population studies in the USA it was found that elevated SF values are found more frequently in Afro-Caribbean and Asian subjects than in whites or Hispanics. Indeed very high SF levels >1000 µg/l are 2–3 times more common in black and Asian volunteers despite an almost total absence of iron loading genotypes in these 2 populations (Adams *et al,* 2005).

Recommendation: The normal ranges for serum ferritin in an individual patient should take the variation due to age, gender and possibly ethnic origin into account (Grade 2A).

Raised serum ferritin levels (hyperferritinaemia)

Raised Serum Ferritin Values for Primary Care

Serum ferritin is the most frequently requested haematinic assay in the UK and some 50% of SF requests are made from primary care. The primary care population is predominantly a well patient population thereby reducing (although not eliminating) the effect of secondary care disorders on SF values. Major studies of raised SF values in primary care have been reported particularly in relation to population screening for GH and iron overload (see BSH Guideline on GH). It is however an acute phase protein and only a minority of subjects with elevated SF levels in the population based Hemochromatosis and Iron Overload Screening study were found to be homozygotes for C282Y in the *HFE* gene, demonstrating that hyperferritinaemia is usually due to causes other than GH (Adams *et al*, 2005).

The commonest causes of hyperferritinaemia without iron overload relate to inflammatory disorders, malignancy, chronic alcohol consumption, liver disease or metabolic abnormalities. A study of patient samples from primary and secondary care in Newcastle with SF levels ≥1500µg/L showed that liver disease, alcohol, inflammatory disorders, malignancy, renal failure and haematological disorders were all commoner causes of raised SF than GH (Hearnshaw *et al*, 2006).

Raised Serum Ferritin Values in Secondary Care

In 627 patients seen in a tertiary academic medical centre with SF≥1000 µg/l (Moore *et al*, 2013), the commonest causes were malignancy, followed by iron overload. Other causes included adult onset Still's disease, systemic juvenile idiopathic arthritis and haemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome: seven patients appeared to have anaemia of chronic disease, and in five the cause of the elevated SF was not defined. In 83 patients identified with SF levels >3000 µg/l at a teaching hospital in Vancouver, 21 cases (25%) were due to transfusional iron overload, 16 (19%) due to liver disease and 15 (18%) due to mixed factors. HLH was diagnosed in 6 patients (7%) (Wormsbecker *et al,* 2015). Finally a study of markedly elevated SF levels ≥10,000 µg/l in patient samples analysed over a 12 month period in a district general hospital biochemistry laboratory revealed 23 cases, with an incidence of 0.08% of SF requests: malignancy accounted for 6/23 cases, liver disease 5, transfusion or thalassaemia 5, and infections 4 (Crook & Walker, 2013).

Overview of causes of raised serum ferritin

Table 1 shows a brief outline of the causes of a raised SF. Further investigations can be tailored to narrow down the possible aetiologies. Conditions associated with primary iron overload such as GH are outside the scope of this guideline, and readers are directed to the recently updated management guideline for this condition (see accompanying BSH Guideline on GH).

Common causes of hyperferritinaemia

For the majority of persons with a raised SF, chronic inflammatory or infective causes as well as liver disease, alcohol and malignancies will be the more likely conditions seen in practice, and if clinically apparent, further investigations of the causes of hyperferritinaemia may not be necessary.

Hepatic disorders

Elevated SF is seen in almost any cause of liver injury, including alcoholic and non-alcoholic steatohepatitis (NASH), and viral hepatitis (Wong & Adams, 2006). SF increases in response to alcohol intake, is affected more by beer than wine or spirit consumption, and is especially increased in subjects with a history of alcohol dependence (Whitfield *et al*, 2001). Thus it is important to document alcohol intake, measure liver function tests and consider abdominal ultrasonography in subjects with unexplained raised SF. The finding of fatty infiltration in the liver on ultrasound may suggest the presence of alcohol-related or non-alcoholic fatty liver disease, while chronic or excessive alcohol consumption will usually cause elevation of liver enzymes, especially the γ-glutamyltransferase (γGT). Hepatitis B and C infection often cause elevated SF with normal transferrin saturation (Tsat), so hepatitis virus serology should be considered as part of work up if liver function tests are abnormal.

Renal disorders

SF is not a useful marker of iron stores in patients with chronic kidney disease (CKD), and is elevated in almost half of all patients on maintenance haemodialysis (Kalantar-Zadeh *et al*, 2006) but the raised SF does not represent iron that is available for erythropoiesis. Current NICE guidelines (2015) advise against the use of SF and Tsat alone (unless thalassaemia or thalassaemia trait is present) to assess need for iron replacement in CKD patients. Novel markers for functional iron deficiency such as percentage hypochromic red cells (%HYPO) or reticulocyte haemoglobin concentration (CHr) (reported by Bayer Advia 120 haematology analyser, Siemens Healthcare Diagnostics, Deerfield, IL, USA) have improved clinical utility (NICE guidelines, 2015) and should be used if available in UK laboratories. For CKD patients on treatment with erythropoietic stimulating agents (ESA), iron supplementation should routinely be offered to patients to keep their %HYPO<6% or CHr >29 pg or Tsat >20% unless their SF is >800 µg/l, with markers checked every 1–3 months in patients on haemodialysis, or every 3 months in patients who are pre-dialysis or on peritoneal dialysis. Current

Renal Association guidelines (2017) recommend that SF should not exceed 800 µg/l in patients treated with iron, and to achieve this iron management should be reviewed when SF is >500 µg/L.

Malignancy

SF is frequently elevated in the setting of malignancy, and in some studies of the causes of hyperferritinaemia cancer has been the most frequent association (Moore *et al,* 2013): ferritin is variably overexpressed by various tumours (Alkhateeb & Connor, 2013), including hepatocellular carcinoma, haematological malignancies (Matzner *et al*, 1980) and breast and pancreatic tumours.

Inflammatory and infective disorders

SF may be elevated in a variety of inflammatory and infective conditions. SF levels may correlate with disease activity in systemic lupus erythematosus (SLE) and rheumatoid arthritis (Zandman-Goddard & Shoenfeld, 2007; Tripathy *et al*, 2015; Yildirim *et al*, 2004). The pathogenesis of hyperferritinaemia is thought to be cytokine-mediated, with interleukin (IL)1α, IL1β, IL6, IL18, tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and macrophage-colony stimulating factor (M-CSF) all implicated. Other inflammatory conditions and acute or chronic infections will also produce elevations in SF, usually with elevated levels of C-reactive protein, but normal Tsat.

Mention should be made of anaemia of chronic disease (ACD), also termed anaemia of inflammation, the pathogenesis of which includes IL6-mediated increased levels of hepcidin, which produces a state of functional iron deficiency, in which iron absorption from the intestine and release from macrophages is inhibited making it unavailable for haemopoiesis (reviewed in Cullis, 2011). Identifying accompanying iron deficiency in patients with ACD can be difficult as SF levels will frequently be normal or raised due to circulating inflammatory cytokines. Typically Tsat is low: algorithms using the ratio of the serum transferrin receptor to log SF concentration may help distinguish ACD from ACD with accompanying iron deficiency (Skikne 2008), but guidelines now support the use of novel red cell parameters such as CHr and %HYPO (Thomas *et al*, 2013).

Measurement of serum transferrin receptor (sTfR) levels have been advocated in distinguishing iron deficiency anaemia from ACD, levels being elevated in the former but normal in ACD (Ferguson *et al,* 1992; Cazzola *et al,* 1996; Berlin *et al,* 2011), but other studies have suggested no advantage over conventional indicators of iron stores (Mast *et al,* 1998; Wians *et al,* 2001; Lee *et al,* 2002) and the test has not been widely adopted.

Emerging Disorders

A more recently described cause of raised SF is the metabolic syndrome, sometimes referred to as dysmetabolic hyperferritinaemia, first described in France (Mendler *et al*, 1999; Moirand *et al*, 1997), and increasingly recognised in western society: cardinal features include hyperglycaemia, dyslipidaemia, obesity and hypertension (Ford *et al*, 2002; Alberti *et al*, 2009): patients typically demonstrate elevated SF levels with normal Tsat (Chen *et al*, 2011). In some, but not all studies, hepatic iron stores are increased (Chen *et al*, 2011; Castiella *et al*, 2016). The increase in SF levels correlates with increased hepcidin production, as well as levels of other inflammatory cytokines in these patients (Andrews *et al*, 2015).

Haematological causes

A variety of red cell disorders, characterised by ineffective erythropoiesis or haemolysis, are associated with increased iron absorption from the gastrointestinal tract and resultant increased SF, even in the absence of red cell transfusion therapy (Porter *et al,* 2017); these include thalassaemic disorders, such as thalassaemia intermedia, pyruvate kinase deficiency, hereditary spherocytosis (Bolton-Maggs *et al,* 2012) and inherited or acquired sideroblastic anaemias. Prolonged or chronic transfusion therapy, for example in patients with major haemoglobinopathies, myelodysplastic syndromes, or during treatment for haematological malignancies, will also cause transfusional iron overload.

There is a well recognised correlation between SF and hepatic iron concentration in transfused patients with beta thalassaemia major and sickle cell disease (Brittenham *et al,* 1993; Pakbaz *et al,* 2007) but the relationship between SF and hepatic iron concentration is different for patients with non-transfusion dependent, but iron loading, anaemias such as thalassaemia intermedia and haemoglobin H disease, in which SF levels may be lower despite comparable degrees of hepatic iron overload (Taher *et al,* 2008; Taher *et al,* 2015; Ang *et al,* 2017). This is important to recognise in parts of the world where methods of assessing hepatic iron concentration, such as MRI, are unavailable and SF is therefore the only available means of assessing iron stores: in these non-transfusion dependent thalassaemic disorders lower SF thresholds may need to employed in decisions about iron chelation (Taher *et al,* 2015).

Recommendation: Reactive causes of raised serum ferritin levels, including malignancy, inflammatory disorders, renal failure, liver disease, and metabolic syndrome should always be considered as they are all considerably more common than true iron overload (Grade 1B).

<u>Causes of a significantly elevated serum ferritin</u>

Markedly elevated SF levels (>10,000 µg/l) may be seen in adult onset Still's disease, a rare, immune-mediated, multisystem inflammatory disorder characterized by fever, rash and arthritis, typically affecting young individuals (75% cases are between 16 and 35 years of age) and frequently presenting as pyrexia of unknown origin (PUO). 89% of cases in a recent series demonstrated elevated SF levels, with over half having levels five times the upper limit of normal (Uppal *et al*, 2007): levels may reach 50,000 µg/l.

Haemophagocytic lymphohistiocytosis is another condition associated with markedly elevated SF levels. This heterogeneous group of disorders share clinical features of pancytopenia,

hypertriglyceridaemia, hyperferritinaemia and multiorgan failure, and often have a fatal outcome. The condition may be familial but can also develop in the setting of Still's disease or other autoimmune conditions, including SLE, as well as in lymphoproliferative disorders and following viral infections, particularly Epstein–Barr virus and cytomegalovirus. It should be considered in the differential diagnosis of any critically ill patient with evidence of systemic inflammation or multiple organ involvement with multiple cytopenias. Frequently ferritin levels are >10,000 µg/l, and associated rises in other markers of the disease, such as serum IL2 receptor-α, may support the diagnosis (Filipovich, 2009).

Marked hyperferritinaemia has often been uniquely ascribed to such rare rheumatological and inflammatory disorders (Rosário *et al*, 2013), and may be very specific for them. A retrospective study by Allen *et al* at Texas Children's Hospital (2008) reported that levels of >10,000 µg/l had 90% sensitivity and 98% specificity for HLH, but another study (Schram *et al*, 2015) in adults in three large US hospitals identified over 800 patients with SF>10,000 µg/l, of whom 113 had levels >50,000 µg/l: the most frequently observed conditions in this adult population with marked hyperferritinaemia included renal failure (65%), hepatocellular injury (54%), infection (46%) and haematological malignancy (32%). Rheumatological conditions and HLH accounted for 18% and 17% respectively, suggesting that marked hyperferritinaemia in adults is associated with a variety of disorders and is not uniquely predictive of HLH.

Recommendation: Markedly elevated serum ferritin levels (>10,000 µg/l) should prompt consideration of rare conditions such as adult onset Still's disease or HLH, but may also be seen in commoner conditions such as renal or liver disease, infections and malignancies (Grade 2B).

<u>Rare disorders associated with raised SF</u>

Porphyria cutanea tarda is the commonest human porphyria, and is characterised by photosensitive dermatosis with blistering skin lesions. It is caused by reduced levels of uroporphyrinogen decarboxylase, and exists in both familial and non-familial forms, the latter frequently associated with inheritance of GH mutations, alcoholic liver disease, hepatitis C infection or oestrogen usage (Roberts *et al,* 1997; Elder, 1998). SF and Tsat are frequently both increased (Bulaj *et al*, 2000). The condition should be considered in patients with increased SF in the presence of a photosensitive rash.

Hereditary hyperferritinaemia cataract syndrome (HHCS) is a rare autosomal dominant condition due to various mutations in the iron responsive element of the gene encoding L-ferritin (Bonneau, *et al*, 1995). SF levels are increased, but Tsat is not raised. L-ferritin deposition in the ocular lens results in bilateral cataract formation at an early age.

Another rare genetic disorder associated with elevated SF levels but normal Tsat is Gaucher disease (Stein *et al*, 2010; Mekinian *et al*, 2012). Inherited in autosomal recessive fashion and caused by deficiency in glucocerebrosidase, Gaucher disease presents with hepatosplenomegaly, painful bone lesions, anaemia and thrombocytopenia, and some correlation is seen between SF levels and disease severity, particularly anaemia (Stein *et al*, 2010).

Acaeruloplasminaemia is caused by a mutation in the *CP* gene that encodes caeruloplasmin, and results in raised SF with normal Tsat (Nittis & Gitlin, 2002). Iron overload is present and clinical manifestations include retinal problems and neurological abnormalities. Microcytic anaemia may be present.

Loss-of- function mutations in the *FPN1* gene encoding ferroportin, an iron transport protein that acts as a receptor for hepcidin, result in a rare iron overload disorder known as FPN1 haemochromatosis characterised by raised SF, normal Tsat and hepatic iron overload. This is discussed further in the GH guideline.

Atransferrinaemia, caused by congenital deficiency due to autosomal recessive mutations in the *TF* gene, is also extremely rare, and usually presents at birth with severe hypochromic, microcytic anaemia requiring red cell transfusion, but paradoxical iron overload in tissues. SF is very high, but iron and transferrin levels will be very low. Infusion of fresh frozen plasma combined with phlebotomy or iron chelation may be indicated (Beutler *et al*, 2000).

A recently described condition is benign hyperferritinaemia (Kannengiesser *et al*, 2009) in which a novel mutation has been found in the coding sequence of the *FTL* gene encoding L ferritin: subjects carrying this mutation had SF levels ranging from 400-6000 µg/l but did not have raised Tsat levels nor increased liver iron.

How to investigate raised serum ferritin

When SF is raised the most crucial questions to ask are a) is it secondary to a known clinical condition and b) is it associated with iron overload? A suggested algorithm for the investigation of a patient with a finding of raised SF is shown in Figure 1. The understanding that many chronic inflammatory and hepatic disorders can raise SF as outlined in the previous section means that the potential cause of hyperferritinaemia may be clear from the outset, while measurement of Tsat will identify whether iron stores are increased and is therefore a key investigation.

A clinical history and examination, together with a few simple investigations, will often reveal the likely underlying cause. In particular, patients should be questioned about alcohol intake and other risk factors for liver disease, transfusion history or oral iron supplementation, family history of iron overload and the presence or absence of diabetes mellitus, obesity and hypertension, history of early cataracts, as well as for symptoms and signs that may point to an underlying inflammatory or malignant disorder. Initial investigations should include a full blood count, repeat SF, Tsat, renal and liver function tests (LFT) (with viral hepatitis serology if LFTs are abnormal) and inflammatory

markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) or plasma viscosity. Marked fluctuations in SF values and elevated aspartate aminotransferase (AST) rather than alanine aminotransferase (ALT), with increased γGT, are more typical of alcohol-induced liver damage than GH (Adams & Barton, 2011). Glycosylated haemoglobin levels may indicate impaired glucose tolerance, and raised serum lipids, body mass index and hypertension may point to underlying metabolic syndrome. Abdominal ultrasonography may demonstrate an echogenic liver suggesting alcohol- or non-alcohol-related fatty liver disease. In such cases non-invasive fibrosis assessment is indicated using transient elastography (Fibroscan). Iron overload is more likely to be present if the SF has risen progressively or the SF is >1000 µg/l: in an otherwise well patient with SF >1000 µg/l or abnormal LFTs proceeding directly to screen for GH is therefore indicated (Adams 2011; Beaton & Adams, 2012), whereas secondary causes are more likely with more modest increases in SF.

Commonly advocated to be performed on a fasting sample, a raised Tsat indicates increased trafficking of iron through the body. Given the issues of patient compliance, potential negative impact of abnormal results upon the patient and the lack of evidence to the contrary, Tsat need not necessarily be measured on a fasting sample provided borderline results are either repeated or checked on fasting if desired (Adams & Barton, 2011). It is worth noting that acute infections, menstrual bleeding and recent blood donation can temporarily reduce Tsat to within the normal range in patients with iron overload (Barton *et al*, 1991), indicating that normal Tsat does not completely exclude iron overload. In the setting of persistent borderline results, genotyping for GH should be performed.

Recommendation: Patients found to have raised serum ferritin should be questioned about alcohol intake and other risk factors for liver disease, transfusion history, family history of iron overload and the presence or absence of type 2 diabetes mellitus, obesity and hypertension, as

well as for symptoms and signs that may point to an underlying inflammatory or malignant disorder (Grade 1C).

Recommendation: In patients with a finding of elevated serum ferritin levels first line investigations should include full blood count and film, repeat serum ferritin, transferrin saturation, inflammatory markers (C-reactive protein, erythrocyte sedimentation rate or plasma viscosity) to detect occult inflammatory disorders, serum creatinine and electrolytes for renal function, liver function tests with consideration of viral hepatitis screening and abdominal ultrasonography (if abnormal liver function), and blood glucose and lipid studies (Grade 1C).

Males with SF > 300µg/l and Tsat>50% and females with SF >200µg/l and Tsat>40% will usually have iron overload and have a 19% and 16% likelihood of being C282Y homozygotes (Ogilvie *et al,* 2015), and genotyping for GH is indicated: this is not dealt with further in this guideline (see BSH Guideline on GH). In unresolved cases quantitation of liver iron, using either liver biopsy or newer MRI techniques, along with more detailed genetic testing, may be useful in distinguishing some of the rarer causes of hyperferritinaemia (Figure 1), but further discussion of these techniques is outside the scope of this guideline and should be discussed with a hepatologist.

How to manage raised serum ferritin without elevated transferrin saturation

In otherwise well patients with unexplained elevated SF and Tsat <40% (female) or 50% (male), a period of observation may be informative: stable, moderately increased levels may not require further investigation, whereas fluctuating levels are typically seen in hepatic steatosis or alcohol

excess. Persistent unexplained hyperferritinaemia, especially at levels >1000 µg/l, merits consideration of onward referral to a specialist, usually a hepatologist.

Recommendation: In otherwise well patients with unexplained and moderately elevated serum ferritin levels (<1000 µg/l) and normal transferrin saturation, a period of observation, with lifestyle adjustment if appropriate, may be reasonable with repeat assessment after 3–6 months (Grade 2C).

Recommendation: Patients with unexplained persistent hyperferritinaemia (especially >1000 µg/l) require referral to a hepatologist (Grade 2C).

In most cases of hyperferritinaemia secondary to inflammatory or other conditions, management of the underlying condition will lead to reduction in SF levels. Alcohol abstinence for example will usually lead to improvement in SF within weeks to months; and weight loss and improved control of diabetes and blood pressure will usually lead to lowering of levels in patients with metabolic syndrome.

The role of phlebotomy in patients with increased SF associated with liver disease other than GH is most likely of little benefit. Although the practice of phlebotomy in patients with non-alcoholic fatty liver disease (NAFLD), with the aims of reducing liver iron stores, is quite common on the basis that elevated SF levels in NAFLD are an independent predictor of the presence of non-alcoholic steatohepatitis (NASH) and hepatic fibrosis (Kowdley *et al,* 2012), randomised trials of venesection in NAFLD patients did not show improvement in prognostic markers with venesection (Adams *et al*, 2015).

Recommendation: There is no evidence to support venesection therapy to reduce serum ferritin levels in patients with non-alcoholic fatty liver disease (Grade 1B).

Conclusions

The finding of a raised serum ferritin is a common conundrum in modern day clinical practice, both in primary and secondary care. Iron overload is a relatively uncommon cause of this picture and can be excluded by the finding of a normal transferrin saturation, so consideration of the many reactive (hepatic, malignant, renal, haematological and metabolic) causes is important: many cases will not require further investigation if a few simple investigations are performed. Our understanding of rarer causes of hyperferritinaemia is expanding but will require specialist molecular genetics for diagnosis.

Acknowledgements

The authors wish to thank Professor Mark Worwood for reviewing and commenting on the initial drafts of this Guideline. The BSH General Haematology Task Force member at the time of writing this guideline was Dr D. Wayne Thomas. The authors would like to thank them, the BSH sounding board, and the BSH guidelines committee for their support in preparing this guideline.

Author contributions

All authors were involved in formulation, writing and approval of the guidelines. All authors approved the final version of the manuscript. The authors would like to thank the BSH General

Haematology Task Force, the BSH sounding board and the BSH executive committee for their support in preparing these guidelines.

Declaration of interests

All authors have made a full declaration of interests to the BSH and Task Force Chairs, which may be reviewed on request.

Review process

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BSH guidelines website at http://b-s-h.org.uk/guidelines if minor changes are required due to changes in level of evidence or significant additional evidence supporting current recommendations a new version of the current guidance will be issued on the BSH website.

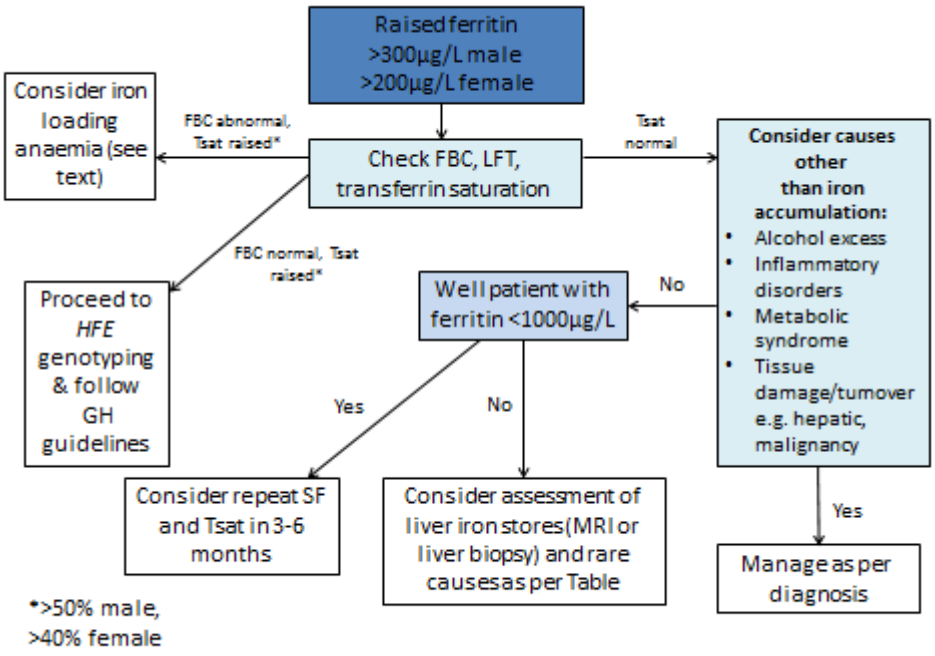
Disclaimer

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, neither the authors, the British Society for Haematology (BSH) nor the publishers accept any legal responsibility for the content of these guidelines.

Table 1: Causes of raised serum ferritin

Increased ferritin synthesis due to iron accumulation	Increase in ferritin synthesis not associated with significant iron accumulation	Increased ferritin as a result of cellular damage
Hereditary (genetic) haemochromatosis Hereditary acaeruloplasminaemia Secondary iron overload from blood transfusion or excessive iron intake/administration Ineffective erythropoiesis: sideroblastic anaemia, some myelodysplastic syndromes (e.g. refractory anaemia with ring sideroblasts) Thalassaemias Atransferrinaemia *FPN1* haemochromatosis	Malignancies Malignant or reactive histiocytosis Hereditary hyperferritinaemia with and without cataracts Gaucher disease Acute and chronic infections Chronic inflammatory disorders Autoimmune disorders	Liver diseases including: liver necrosis, chronic viral hepatitis, alcoholic and non-alcoholic steatohepatitis* Chronic excess alcohol consumption ** May also have iron overloading*

Figure 1: Suggested algorithm for investigation of isolated elevated serum ferritin levels in patients without known secondary iron overload



Abbreviations: FBC, full blood count; GH genetic haemochromatosis; LFT, liver function tests; SF, serum ferritin; Tsat, transferrin saturation.

References

Adams LA, Crawford DH, Stuart K, House MJ, St Pierre TG, Webb M, Ching HLI, Kava J, Bynevelt M, MacQuillan GC, Garas G, Ayonrinde OT, Mori TA, Croft KD, Niu X, Jeffery GP, Olynyk JK (2015) The impact of phlebotomy on nonalcoholic fatty liver disease: a prospective, randomized, controlled trial. Hepatology 61:1555-1564

Adams PC, Raboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, Dawkins FW, Acton RT, Harris EL, Gordeuk VR, Leiendecker-Foster C, Speechley M, Snively BM, Holup JL, Thomson E, Sholinsky P (2005) Hemochromatosis and iron-overload screening in a racially diverse population. New England Journal of Medicine 352:1769-1778

Adams PC, Barton JC (2011) A diagnostic approach to hyperferritinemia with a non-elevated transferrin saturation. Journal of Hepatology 55:453-458

Adams PC, McLaren CE, Speechley M, McLaren GD, Barton JC, Eckfeldt JH (2013) HFE mutations in Caucasian participants of the Hemochromatosis and Iron Overload Screening study with serum ferritin level <1000 µg/L. Canadian Journal of Gastroenterology 27:390-392

Addison GM, Beamish MR, Hales CN, Hodgkins M, Jacobs A, Llewellin P (1972) An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. Journal of Clinical Pathology 25:326-329

Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, et al. (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120:1640–1645

Alkhateeb AA, Connor JR (2013) The significance of ferritin in cancer: anti-oxidation, inflammation and tumorigenesis. Biochimica et BiophysicaActa 1836:245-254

Allen CE, Yu X, Kozinetz CA, McClain KL (2008) Highly elevated ferritin levels and the diagnosis of hemphagocytic lymphohistiocytosis. Pediatric Blood Cancer 50:1227-35

Andrews M, Soto N, Arrendondo-Olquín M (2015). Association between ferritin and hepcidin levels and inflammatory status in patients with type 2 diabetes mellitus and obesity. Nutrition 31:51-7

Ang AL, Le TT, Tan RS (2017) HbH Constant Spring disease has lower serum ferritin relative to liver iron concentration (LIC): importance of LIC measurement and potential impact on serum ferritin thresholds for iron chelation. British Journal of Haematology 176:986-988

Association of Biochemists (2012) Analyte Monographs alongside the National Laboratory Medicine Catalogue: Ferritinhttp://www.acb.org.uk/Nat%20Lab%20Med%20Hbk/Ferritin.pdf

Barton JC, Bertoli LF, Janich MR, Arthur MW, Alford TJ (1991) Normal transferrin saturation in hemochromatosis. Hospital Practice (Office edition) 26 (supplement 3):46-48

Beaton MD, Adams PL (2012) Treatment of hyperferritinemia. Annals of Hepatology 11:294-300

Berlin T, Meyer A, Rotman-Pikielny P, Natur A, Levy Y (2011) Soluble transferrin receptor as a diagnostic laboratory test for detection of iron deficiency anemia in acute illness of hospitalized patients. Israel Medical Association Journal 13:96-8

Beutler E, Gelbart T, Lee P, Trevino R, Fernandez MA, Fairbanks VF (2000) Molecular characterization of a case of atransferrinemia. Blood 96:4071-4074

Bolton-Maggs PH, Langer JC, Iolascon A, Tittensor P, King MJ for the General Haematology Task Force of the British Committee for Standards in Haematology (2012) Guidelines for the diagnosis and management of hereditary spherocytosis – 2011 update. British Journal of Haematology 156:37-49

Bonneau D, Winter-Fuseau I, Loiseau MN, Amati P, Berthier M, Oriot D, Beaumont C (1995) Bilateral cataract and high serum ferritin: a new dominant genetic disorder? Journal of Medical Genetics 32:778-779

Brittenham GM, Cohen AR, McLaren CE, Martin MB, Griffith PM, Nienhuis AW, Young NS, Allen CJ, Farrell DE, Harris JW (1993) Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. American Journal of Hematology 42:81-85

Bulaj ZJ, Phillips JD, Ajioka RS, Franklin MR, Griffen LMR, Guinee DJ, Edwards CQ, Kushner JP (2000) Hemochromatosis genes and other factors contributing to the pathogenesis of porphyria cutanea tarda. Blood 95:1565-1571

Castiella A, Zapata E, Zubiaurre L, Iribarren A, Alústiza JM, Otazua P, Salvador E, Emparanza JI (2016) Liver iron concentration is not raised in patients with dysmetabolic hyperferritinemia. Annals of Hepatology 15:540-4

Cazzola M, Ponchio L, de Benedetti F, Ravelli A, Rosti V, Beguin Y, Invernizzi R, Barosi G, Martini A (1996) Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis. Blood 87:4824-30

Cazzola M, Bergamaschi G, Tonon L, Arbustini E, Grasso M, Vercesi E, Barosi G, Bianchi P E, Cairo G, Arosio P(1997) Hereditary hyperferritinemia-cataract syndrome: relationship between phenotypes and specific mutations in the iron-responsive element of ferritin light-chain mRNA. Blood 90:814-821

Chen LY, Chang SD, Sreenivasan GM, Tsang PW, Broady RC, Zypchen LN (2011) Dysmetabolic hyperferritinemia is associated with normal transferrin saturation, mild hepatic iron overload, and elevated hepcidin. Annals of Hepatology 90:139-43

Crook MA & Walker PLC (2013) Extreme hyperferritinaemia: clinical causes. Journal of Clinical Pathology 66:438-40

Cullis JO (2011) Diagnosis and management of anaemia of chronic disease: current status. British Journal of Haematology 154:289-300

Custer EM, Finch CA, Sobel RE, Zettner A (1995) Population norms for serum ferritin. Journal of Laboratory and Clinical Medicine 126:88–94.

Elder GH (1998) Porphyria Cutanea Tarda. Seminars in Liver Disease 18: 67-75

Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD (1992) Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. Journal of Laboratory and Clinical Medicine 119:385-90

Filipovich AH (2009) Hemophagocytic lymphohistiocytosis (HLH) and related disorders. Hematology 2009:127-31

Ford ES, Giles WH, Dietz WH (2002) Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. Journal of the American Medical Association 287:356–359

Goddard AF, James MW, McIntyre AS, Scott BB (2011) Guidelines for the management of iron deficiency anaemia. Gut 60:1309-1316

Harrison PM, Arosio P (1996) The ferritins: molecular properties, iron storage function and cellular regulation. Biochimica et BiophysicaActa 1275: 161-203

Hearnshaw S, Thompson NP, McGill A (2006) The epidemiology of hyperferritinaemia. World Journal of Gastroenterology 12:5866-5869

Kalantar-Zadeh K, Kalantar-Zaheh K, Lee GH (2006) The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease. Clinical Journal of the American Society of Nephrology 1:S9-18

Kannengiesser C, Jouanolle A-M, Hetet G, Mosser A, Muzeau F, Henry D, Bardou-Jacquet E, Mornet M, Brissot P, Deugnier Y, Grandchamp B, Beaumont C (2009) A new missense mutation in the L ferritin coding sequence associated with elevated levels of glycosylated ferritin in serum and absence of iron overload. Haematologica 94:335-339

Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, Sanyal AJ, Nelson JE (2012) Elevated serum ferritin is an independent predictor of histologic severity and advanced fibrosis among patients with nonalcoholic fatty liver disease. Hepatology 55:77-85

Lee EJ, Oh EJ, Park YJ, Kim BK (2002) Soluble transferrin receptor (sTfR), ferritin, and sTfR/log ferritin index in anemic patients with nonhematologic malignancy and chronic inflammation. Clinical Chemistry 48:1118-21

Mast AE, Blinder MA, Gronowski AM, Chumley C, Scott MG (1998) Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. Clinical Chemistry 44:45-51

Matzner Y, Konijn AM, Hershko C (1980) Serum ferritin in hematologic malignancies. American Journal of Hematology 9:13-22

Mekinian A, Stirnemann J, Belmatoug N, Heraoui D, Fantin B, Fain O, Charpentier A, Rose C (2012) Ferritinemia during type I Gaucher disease: mechanisms and progression under treatment. Blood Cells, Molecules and Diseases 49:53-57

Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, le Gall J-Y, Brissot P, David V, Deugnier Y (1999) Insulin resistance-associated hepatic iron overload. Gastroenterology 117:1155-1163

Milman M, Byg K-E, Ovesen L, Kirchhoff M, Jürgensen KS-L (2003) Iron status in Danish women, 1984–1994: a cohort comparison of changes in iron stores and the prevalence of iron deficiency and iron overload. European Journal of Haematology 71:51-61

Moirand R, Mortaji A, Loreal O, Paillard F, Brissot P, Deugnier Y (1997) A new syndrome of liver iron overload with normal transferrin saturation. Lancet 349:95-97

Moore C Jr, Ormseth M, Fuchs H (2013). Causes and significance of markedly elevated serum ferritin levels in an academic medical center. Journal of Clinical Rheumatology 19:324-8

NICE guidelines (2015) Chronic kidney disease: managing anaemia. https://www.nice.org.uk/guidance/ng8/resources/chronic-kidney-disease-managing-anaemia-51046844101

Nittis T, Gitlin JD (2002) The copper-iron connection: hereditary aceruloplasminemia. Seminars in Hematology 39:282-289

Ogilvie C, Fitzsimons K, Fitzsimons EJ (2010) Serum ferritin values in primary care: are high values overlooked? Journal of Clinical Pathology 63:1124-1126

Ogilvie C, Gaffney D, Murray H, Kerry A, Haig C, Spooner R, Fitzsimons EJ (2015) Improved detection of hereditary haemochromatosis. Journal of Clinical Pathology 68:218-221

Pakbaz Z, Fischer R, Fung E, Nielsen P, Harmatz P, Vichinsky E (2007) Serum ferritin underestimates liver iron concentration in transfusion independent thalassemia patients as compared to regularly transfused thalassemia and sickle cell patients. Pediatric Blood & Cancer 49:329-332

Porter JB, Cappellini MD, Kattamis A, Viprakasit V, Musallam KM, Zhu Z, Taher AT (2017) Iron overload across the spectrum of non-transfusion-dependent thalassaemias: role of erythropoiesis, splenectomy and transfusions. British Journal of Haematology 176:288-299

The Renal Association (2017) Clinical Practice Guideline Anaemia of Chronic Kidney Disease. https://renal.org/wp-content/uploads/2017/06/anaemia-of-chronic-kidney-disease5d84a231181561659443ff000014d4d8.pdf

Roberts AG, Whatley SD, Morgan RR, Worwood M, Elder GH (1997) Increased frequency of the haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. Lancet 349:321-323

Rosário C, Zandman-Goddard G, Meyron-Holtz EG, d'Cruz DP, Shoenfeld Y (2013) The hyperferritinemic syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. BMC Medicine 11:185

Schram AM, Campigotto F, Mullally A, Fogerty A, Massarotti E, Neuberg D, Berliner N (2015). Marked hyperferritinemia does not predict for HLH in the adult population. Blood 125:1548-52

Skikne BS (2008) Serum transferrin receptor. American Journal of Hematology 83:872-875

Stein P, Yu H, Jain D, Mistry PK (2010) Hyperferritinemia and iron overload in type I Gaucher disease. American Journal of Hematology 85:472-476

Taher A, El Rassi F, Isma'eel H, Koussa S, Inati A, Cappellini MD (2008) Correlation of liver iron concentration determined by R2 magnetic resonance imaging with serum ferritin in patients with thalassemia intermedia. Haematologica 93, 1584-1586

Taher AT, Porter JB, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, Siritanaratkul N, Origa R, Karakas Z, Habr D, Zhu Z, Cappellini MD (2015) Defining serum ferritin thresholds to predict clinically relevant liver iron concentrations for guiding deferasirox therapy when MRI is unavailable in patients with non-transfusion-dependent thalassaemia. British Journal of Haematology 168:284-290

Thomas DW, Hinchcliffe RF, Briggs C, MacDougall IC, Littlewood T, Cavill I (2013) Guideline for the laboratory diagnosis of functional iron deficiency. British Journal of Haematology 161:639-648

Tripathy R, Panda AK, Das BK (2015) Serum ferritin level correlates with SLEDAI scores and renal involvement in SLE. Lupus 24:82-89

Uppal SS, Al-Mutairi M, Hayat S, Abraham M, Malayiya A (2007) Ten years of clinical experience with adult onset Still's disease: is the outcome improving? Clinics in Rheumatology 26:1055–60

Walters GO, Miller FM, Worwood M (1973) Serum ferritin concentration and iron stores in normal subjects. Journal of Clinical Pathology 26:770-772

White A, Nicolas G, Foster K (1993) Health Survey for England 1991. Her Majesty's Stationary Office, 1993.

Whitfield JB, Zhu G, Heath AC, Powell LW, Martin NG (2001) Effects of alcohol on indices of iron stores and of iron stores on alcohol intake markers. Alcoholism: Clinical and Experimental Research 25:1037-1045

Wians FH, Urban JE, Keffer JH, Kroft SH (2001) Discriminating between iron deficiency anemia and anemia of chronic disease using traditional indices of iron status vs transferrin receptor concentration. American Journal of Clinical Pathology 115:112-118

Wiedemann G, Jonetz-Mentzel L (1993) Establishment of reference ranges for ferritin in neonates, infants, children and adolescents. European Journal of Clinical Chemistry and Clinical Biochemistry 31:453-457

Wong K, Adams PC (2006) The diversity of liver diseases among outpatient referrals for elevated serum ferritin. Canadian Journal of Gastroenterology 20:467-470

Wormsbecker AJ, Sweet DD, Mann SL, Wang SY, Pudek MR, Chen LY (2015) Conditions associated with extreme hyperferritinaemia (>3000 µg/L) in adults. Internal Medicine Journal 45:828-833

Worwood M (1982) Ferritin in human tissues and serum. Clinics in Haematology 11:275-307

Worwood M (2012) Estimation of body iron stores. In Iron Physiology and Pathophysiology in Humans Edited by Anderson GJ and McLaren GD. Humana Press, New York

Worwood M, May AM and Bain BJ (2017) Iron deficiency anaemia and iron overload. In Dacie and Lewis Practical Haematology 12th edition. Edited by Bain B, Bates I, Laffan MA . Elsevier

Yildirim K, Karatay S, Melikoglu MA, Gureser G, Ugur M, Senel K (2004) Associations between acute phase reactant levels and disease activity score (DAS28) in patients with rheumatoid arthritis. Annals of Clinical and Laboratory Science 34:423–426

Zandman-Goddard G, Shoenfeld Y (2007) Ferritin in autoimmune diseases. Autoimmunity Reviews 6: 457–463