



Abnormal laboratory results

Testing for HFE-related haemochromatosis

Andrew T St John, Advanced trainee in Gastroenterology, **Katherine A Stuart**, Gastroenterologist, and **Darrell HG Crawford**, Professor, Department of Medicine, The University of Queensland, and Department of Gastroenterology, Greenslopes Private Hospital, Greenslopes, Queensland

Summary

HFE-haemochromatosis is a genetic disorder resulting from mutations of the HFE gene. It primarily affects people of Northern European descent. Clinical manifestations result from the progressive deposition of iron into various organs including the liver. An elevated serum ferritin concentration greater than 300 microgram/L and a transferrin saturation of greater than 45% will identify almost all patients with HFE-haemochromatosis. HFE genotyping confirms the diagnosis. In some patients, liver biopsy may still be necessary as the degree of hepatic fibrosis has prognostic implications.

Key words: ferritin, iron, liver, transferrin.

(*Aust Prescr* 2011;34:73–6)

Introduction

HFE-haemochromatosis is the most frequent inherited cause of iron overload in humans. The condition is due to an inborn error of iron metabolism leading to inappropriately increased intestinal iron absorption. Its clinical manifestations result from progressive iron deposition in certain organs. It is important to identify individuals with HFE-haemochromatosis early in the course of their disease, as early intervention can prevent the development of complications. These include cirrhosis of the liver, cardiomyopathy, arthropathy and diabetes. Most patients are diagnosed following a presentation with nonspecific symptoms such as lethargy and fatigue, altered liver function tests, or a family history of haemochromatosis.

Genetics

The HFE gene is located on chromosome six. It codes for a cell surface protein which is involved in the regulation of iron metabolism. Mutations of the gene therefore disrupt normal iron metabolism.

HFE-haemochromatosis is the most common autosomal recessive disorder in Northern European populations with

heterozygous carrier rates of 1 in 10 and homozygosity rates of approximately 1 in 300. Homozygosity for the C282Y mutation accounts for 80–90% of cases of HFE-haemochromatosis and compound heterozygosity (C282Y/H63D) is the next most common genotype.¹ Phenotypic expression is highly variable with only a minority of patients developing systemic complications of iron overload.

An Australian study of people of Northern European descent reported symptoms and signs of iron overload in 28.4% of males and 1.2% of females who were C282Y homozygous.² The same study found that symptomatic iron overload was rare among compound heterozygotes (0.2%).

Transferrin saturation

The first approach to diagnosing HFE-haemochromatosis is the assessment of indirect markers of iron stores. Fasting transferrin saturation is considered to be the most sensitive screening test for HFE-haemochromatosis.

An elevated fasting transferrin saturation greater than 50% in women and 60% in men of Northern European descent has a positive predictive value of 86% for the diagnosis of HFE-haemochromatosis.³ Lowering the threshold transferrin saturation to 45% improves the sensitivity and negative predictive value, but reduces the positive predictive value. In an Australian population study, a value of 45% was able to correctly identify 98% of C282Y homozygotes.⁴ However, using this value will also detect heterozygotes who do not need further investigations. Approximately 30% of C282Y heterozygotes have a transferrin saturation greater than 45%.

The combination of an elevated fasting transferrin saturation (greater than 45%) and an elevated serum ferritin has a negative predictive value of 97%. This exceeds the accuracy of either test used alone.³

Serum ferritin concentration

Raised serum concentrations of ferritin occur in a number of different conditions including iron overload. There are also several causes of iron overload (see box). In the setting of iron overload, the serum ferritin tends to reflect total body iron stores and generally increases with progressive iron loading.⁵

An Australian population-based study reported a sensitivity of 50% and specificity of 87% for serum ferritin concentrations greater than 300 microgram/L for the diagnosis of C282Y homozygosity.⁶ Higher serum ferritin thresholds have been studied in an attempt to lower the rate of false positives and increase the positive predictive value for the detection of HFE-haemochromatosis. For example, a population-based study screening 29 699 people identified 59 patients with a serum ferritin concentration greater than 1000 microgram/L, of whom 24 had HFE-haemochromatosis with 20 people being C282Y homozygous.⁷

Serum ferritin concentrations greater than 1000 microgram/L are associated with a higher risk of cirrhosis and may be used as an indication for liver biopsy.⁸ A French study reported a sensitivity of 98%, a specificity of 72% and a positive predictive value of 55% when using a serum ferritin concentration of 1000 microgram/L to predict the presence of severe fibrosis among C282Y homozygotes.⁹ Similar findings have been reported in Australian and Canadian populations.^{10,11} Other factors that increase the clinical probability of severe fibrosis include hepatomegaly, abnormal transaminase levels, age greater than 35 years and a history of excessive alcohol intake.

An isolated elevated serum ferritin is often seen with acute or chronic inflammation. Patients with an isolated elevated serum ferritin should therefore be evaluated for other causes before genetic testing is considered.¹² More than 90% of people in the general community who have an elevated serum ferritin will have one of the following diagnoses:

- systemic inflammation
- chronic alcohol consumption
- non-alcoholic fatty liver disease
- hepatocellular necrosis
- malignancy.

In these clinical conditions, serum ferritin concentration is usually less than 1000 microgram/L and is often accompanied by a normal transferrin saturation. This is a common clinical scenario and interpretation of these patients' iron studies is often compounded by the presence of heterozygosity for the HFE mutations. Despite the elevated serum ferritin concentration, the vast majority of these patients do not have significant iron overload and treatment of the underlying condition usually results in a decrease in the serum ferritin concentration. Moreover, serum ferritin concentration increases with age and is influenced by gender and physiological blood loss. Interpretation of serum ferritin concentration requires careful consideration of these characteristics in each patient.

Measuring C-reactive protein may help to exclude systemic inflammation if it is not clinically evident. Other tests such as serum aspartate transaminase, alanine transaminase, creatinine

Box

Causes of iron overload

Hereditary haemochromatosis

- HFE-haemochromatosis
- non-HFE-haemochromatosis

Secondary iron overload

- multiple blood transfusions
- iron-loading anaemia (β -thalassaemia and sideroblastic anaemia)

Chronic liver disease

- hepatitis C infection
- alcohol-related liver disease
- non-alcoholic fatty liver disease
- porphyria cutanea tarda

kinase, erythrocyte sedimentation rate, fasting glucose, and the lipid profile may also help to exclude other causes of an isolated elevated serum ferritin.

HFE genotyping

The diagnostic evaluation of people with suspected HFE-haemochromatosis changed following the discovery of the HFE gene in 1996.¹ Blood tests for HFE genotyping should be considered in people with suspected iron overload, patients with a family history of HFE-haemochromatosis and cases of unexplained chronic liver disease with an increased transferrin saturation¹² (see Fig. 1). Genetic screening for HFE-haemochromatosis in the general population is not recommended because the disease penetrance is low.¹²

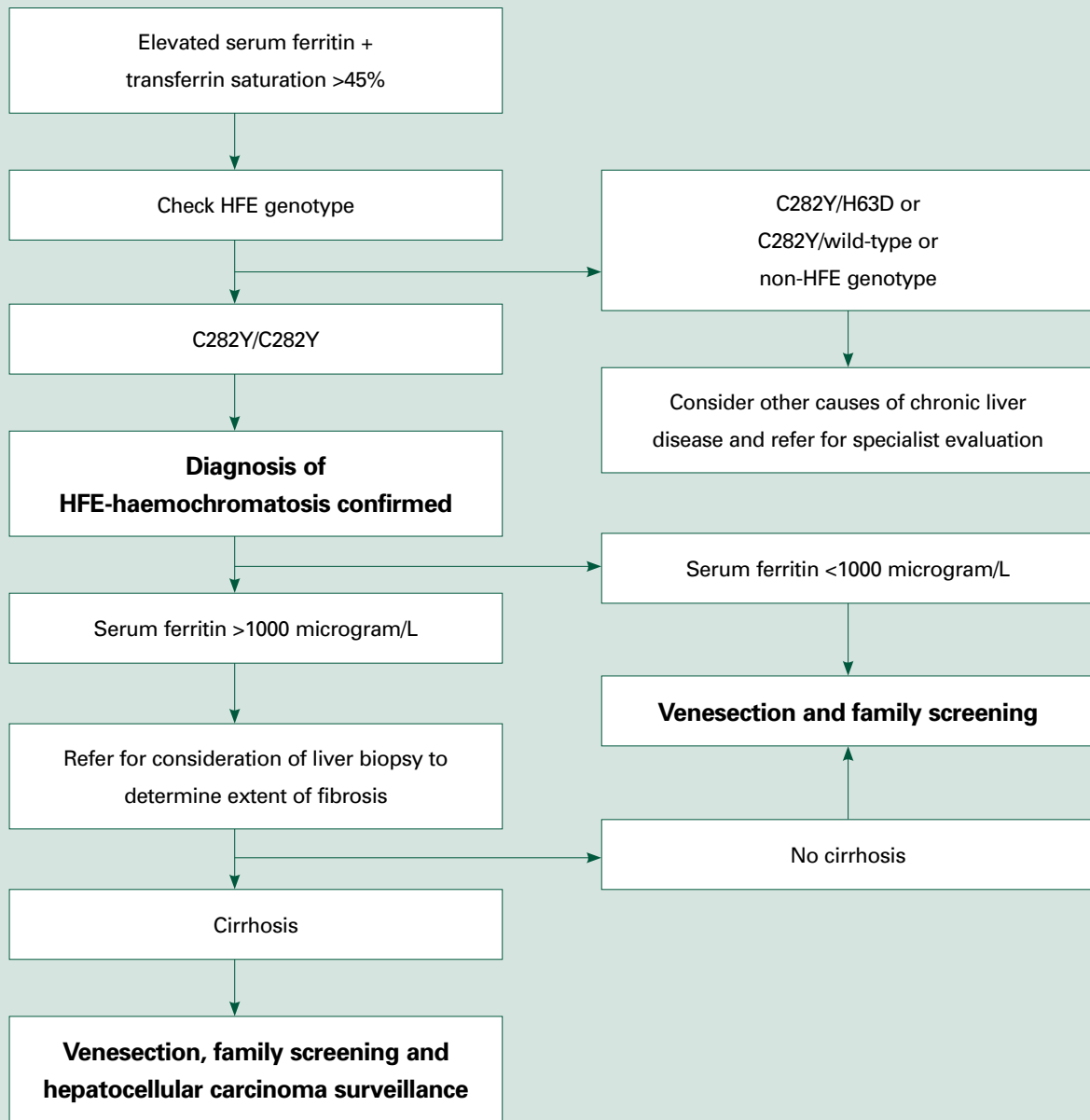
Most patients with HFE-haemochromatosis are C282Y homozygotes and the majority of the remaining cases are compound heterozygotes (C282Y/H63D). H63D homozygosity does not result in significant hepatic iron overload and an elevated serum ferritin in these patients is usually the result of hepatic steatosis or excess alcohol consumption.¹³

Other tests

Following the advent of HFE genotyping, liver biopsy is no longer necessary to make a diagnosis of HFE-haemochromatosis. However, liver biopsy is still required to stage hepatic fibrosis, especially as patients with serum ferritin concentrations greater than 1000 microgram/L are more likely to have cirrhosis.⁹ Diagnosing the presence of cirrhosis in HFE-haemochromatosis is clinically important as affected patients have a significant risk of hepatocellular carcinoma and should enter a surveillance program. When the diagnosis of

Fig. 1

Algorithm for the diagnosis and management of HFE-haemochromatosis



iron overload remains unclear, liver biopsy or MRI may still be required to assess for hepatic iron overload.

The special form of MRI is a non-invasive method of directly assessing hepatic iron concentration. There is an excellent inverse correlation between the signalling and hepatic iron concentration.¹⁴ The main limitation of this method is its inability to stage hepatic fibrosis. Transient elastography, a special form of ultrasound, may have a role in staging fibrosis as an alternative to liver biopsy.¹⁵

Family screening

Siblings of patients with HFE-haemochromatosis should undergo HFE genotyping as they have a 25% chance of being affected.¹² In clinical practice, most family members also have serum iron indices measured to assess their body iron stores. Whether individuals are screened depends upon several factors including their age and health status, and the attitudes of the family.¹² In the case of children who have a parent with HFE-haemochromatosis, HFE genotyping of the unaffected

parent may be of value.¹⁶ In such cases, the likelihood of genetic susceptibility and the need for testing of children later in life can be established.

Conclusion

HFE-haemochromatosis is a common genetic disorder primarily affecting people of North European descent. Early diagnosis and treatment prevent progressive disease. It is important that people with characteristics associated with severe hepatic fibrosis or cirrhosis are identified and managed appropriately.

Recommendations

- Patients with suspected iron overload should first have their serum ferritin and fasting transferrin saturation measured.
- HFE genotyping should be carried out in all patients with an elevated serum ferritin and transferrin saturation.
- Diagnosis of HFE-haemochromatosis should not be based on C282Y homozygosity alone, but requires evidence of increased hepatic iron stores. People who are C282Y homozygotes with normal iron stores should undergo regular testing.
- Compound heterozygotes (C282Y/H63D) and H63D homozygotes presenting with an elevated serum ferritin should first be investigated for other causes of an elevated serum ferritin, in particular alcohol and non-alcoholic fatty liver disease.
- Liver biopsy should be offered to C282Y homozygotes with a serum ferritin greater than 1000 microgram/L as these patients are at risk of cirrhosis.
- As HFE-haemochromatosis is an autosomal recessive disease, genetic testing of siblings and other first degree family members is recommended.

References

1. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399-408.
2. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med* 2008;358:221-30.
3. Bassett ML, Halliday JW, Ferris RA, Powell LW. Diagnosis of hemochromatosis in young subjects: predictive accuracy of biochemical screening tests. *Gastroenterology* 1984;87:628-33.
4. McLaren CE, McLachlan GJ, Halliday JW, Webb SI, Leggett BA, Jazwinska EC, et al. Distribution of transferrin saturation in an Australian population: relevance to the early diagnosis of hemochromatosis. *Gastroenterology* 1998;114:543-9.
5. O'Neil J, Powell L. Clinical aspects of hemochromatosis. *Semin Liver Dis* 2005;25:381-91.
6. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. *N Engl J Med* 1999;341:718-24.
7. Waalen J, Felitti VJ, Gelbart T, Beutler E. Screening for hemochromatosis by measuring ferritin levels: a more effective approach. *Blood* 2008;111:3373-6.
8. Tavill AS, American Association for the Study of Liver Diseases, American College of Gastroenterology, American Gastroenterological Association. Diagnosis and management of hemochromatosis. *Hepatology* 2001;33:1321-8.
9. Guyader D, Jacquelinet C, Moirand R, Turlin B, Mendler MH, Chaperon J, et al. Non-invasive prediction of fibrosis in C282Y homozygous hemochromatosis. *Gastroenterology* 1998;115:929-36.
10. Crawford DH, Murphy TL, Ramm LE, Fletcher LM, Clouston AD, Anderson GJ, et al. Serum hyaluronic acid with serum ferritin accurately predicts cirrhosis and reduces the need for liver biopsy in C282Y hemochromatosis. *Hepatology* 2009;49:418-25.
11. Beaton M, Guyader D, Deugnier Y, Moirand R, Chakrabarti S, Adams P. Noninvasive prediction of cirrhosis in C282Y-linked hemochromatosis. *Hepatology* 2002;36:673-8.
12. European Association for the Study of the Liver. EASL clinical practice guidelines for HFE hemochromatosis. *J Hepatol* 2010;53:3-22.
13. Sebastiani G, Wallace DF, Davies SE, Kulhali V, Walker AP, Dooley JS. Fatty liver in H63D homozygotes with hyperferritinaemia. *World J Gastroenterol* 2006;12:1788-92.
14. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005;105:855-61.
15. Adhoute X, Foucher J, Laharie D, Terrebbonne E, Vergniol J, Castéra L, et al. Diagnosis of liver fibrosis using FibroScan and other non-invasive methods in patients with hemochromatosis: a prospective study. *Gastroenterol Clin Biol* 2008;32:180-7.
16. El Serag HB, Inadomi JM, Kowdley KV. Screening for hereditary hemochromatosis in siblings and children of affected patients. A cost-effectiveness analysis. *Ann Intern Med* 2000;132:261-9.

Conflict of interest: none declared

Self-test questions

The following statements are either true or false (answers on page 91)

3. The diagnosis of HFE-haemochromatosis requires a liver biopsy.
4. HFE-haemochromatosis is the most common cause of an isolated elevation of serum ferritin.