NATIONAL LABORATORY HANDBOOK

Laboratory Testing for Hereditary Haemochromatosis

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National Clinical & Integrated Care Programmes
Person-centred, co-ordinated care

PATHOLOGY

ROYAL COLLEGE OF PHYSICIANS OF IRELAND

Contents

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Scope

A National Model of Care for Hereditary Haemochromatosis (HH) was published in July 2016, and provides detailed information on the management of people with this condition, together with a framework for service delivery.[1] The purpose of this guideline is to summarise the laboratory aspects required to support this model of care.

Key Recommendations for Clinical Users

HH should be suspected in patients with

- a family history of HH,
- raised iron studies,
- liver disease,
- diabetes,
- arthritis,
- skin pigmentation,
- erectile dysfunction,
- cardiomyopathy,

together with evidence of iron overload (elevated serum ferritin and fasting transferrin saturation).

Patients with suspected iron overload should have a serum ferritin and fasting transferrin saturation tests performed.

Those with an increased fasting transferrin saturation (evidence of iron overload), should have HFE genetic testing performed.

Patients with HH are monitored using serum ferritin. These measurements should be performed when the patient is well as serum ferritin may increase significantly during an acute phase response. Additional C-reactive protein (CRP) testing may aid interpretation of ferritin levels in this scenario.

Key Recommendations for Laboratories

Serum ferritin and fasting transferrin saturation should be used to test for iron overload.

HFE genetic testing should be performed in those with evidence of iron overload, and in adult first degree relatives (children, siblings, parents) of C282Y homozygotes.

Laboratories should test for the C282Y HFE mutation.

Depending on local practice, the H63D mutation can be considered an optional complementary test that can be performed sequentially or concurrently to C282Y testing.[2]

The S65C variant is not deemed pathogenic, and testing for this variant is not recommended.[2]

- It is not possible to avoid testing for S65C when using some CE marked HFE kits. However, where possible on the analysis platform used, S65C should be selected not to be reported, and where this is not possible reports should contain a general statement of policy regarding the non-reporting of incidental findings / variants not of clinical relevance. [2]
- It is recommended that testing laboratories are accredited according to international standards (ISO15189 or equivalent).[2]

Adult first degree relatives should have serum ferritin/fasting transferrin saturation levels measured in addition to genetic testing. The partner of a C282Y homozygote should be offered genetic testing to determine the risk to their offspring and thereby avoid genetic testing in children before the age of consent.

Background & Epidemiology

Ireland has the highest incidence of HH worldwide. HH is a condition where increased absorption of iron may lead to iron overload and deposition in the liver, joints, heart, pancreas and other organs. HH is caused by mutations in the HFE gene, located on chromosome 6. One in 83 of the Irish population is genetically predisposed to develop HH.[4]

The diagnosis of HH is based on homozygosity for C282Y mutation in the HFE gene, associated with evidence of iron overload, with or without symptoms. Diagnosis should not be made on C282Y homozygosity alone, due to variable penetrance.

C282Y/H63D compound heterozygotes should be tested for iron overload. However other causes of hyperferritinaemia should be considered. Controversy remains around the association of H63D homozygosity with iron overload and other causes should be investigated.[2]

The aim of treatment is to maintain serum ferritin in the normal range using phlebotomy as required. C282Y homozygotes who do not have iron overload should have ferritin measured annually, to ensure treatment is commenced should ferritin levels begin to rise. There is no evidence on which to base a decision to stop or decrease monitoring frequency of ferritin with advancing age. However, given the slow tempo of iron accumulation, clinical judgement should be used to determine when detection of iron overload is no longer relevant.

Rare cases of non-HFE related HH have been described, due to pathogenic variants in other iron-related genes, such as hemojuvelin (HJV), hepcidin (HAMP) and transferrin receptor 2 (TFR2). Non-HFE HH is outside the scope of this guideline.

Testing

Patients presenting with symptoms should be assessed for iron overload (serum ferritin and fasting transferrin saturation), and if iron overload is present, proceed to HFE testing. Where HFE testing excludes C282Y homozygosity, secondary causes of iron overload should be excluded.

Adult siblings of C282Y homozygotes may be tested for HFE mutations and iron studies simultaneously.

Who to Test

- Asymptomatic adult siblings of C282Y homozygotes.
- Parents of C282Y homozygotes are obligate carriers. However due to the high frequency of HFE mutations in the Irish population, parents may harbour a second mutation and asymptomatic parents may be offered testing considering age, sex & ferritin levels.
- Genetic testing of adult offspring of C282Y homozygotes is recommended. When there are two or more children it may be more cost effective and clinically appropriate to test the spouse, and then only offer testing to at-risk adult children if the spouse carries C282Y.
- Symptomatic individuals, where serum ferritin and fasting transferrin saturation are consistent with iron overload:
    - Fatigue, lethargy, apathy, weight loss,
    - Hepatomegaly or signs of chronic liver disease,
    - Arthralgia, arthritis, chondrocalcinosis,
    - Diabetes (insulin dependent & early onset type II), hypogonadism, hypothyroidism,
    - Dilated cardiomyopathy, congestive cardiac failure, arrhythmias,
    - Skin pigmentation (bronzing), porphyria cutanea tarda,
    - Loss of libido, erectile dysfunction.

Who Not to Test

- HFE testing of minors is not recommended. There have been no reports of iron overload from C282Y homozygosity occurring before the age of 18 years.[3] International best practice for adult onset genetic disease is not to test minors, or those under 16 years of age.[2]

- Genetic testing of clinically asymptomatic adult first degree relatives of C282Y heterozygous or H63D homozygotes is not recommended. [2]

- In symptomatic individuals, a normal ferritin effectively rules out HH as a cause for symptoms, and further testing in the absence of a family history is not indicated.

- Advice should be sought from the testing laboratory if considering testing after recent transfusion. An alternative (non-blood) sample will be required in patients who have had a haemopoietic stem cell transplant.

Who to Re-Test

- HFE retesting is not indicated.

- C282Y homozygotes who do not have evidence of iron overload should have ferritin measured annually. There is no evidence on which to base a decision to stop or decrease monitoring frequency of ferritin with advancing age. However, given the slow tempo of iron accumulation, clinical judgement should be used to determine when detection of iron overload is no longer relevant.

- For patients undergoing phlebotomy, haematocrit/haemoglobin and serum ferritin should be checked prior to phlebotomy:
    - Every 4-6 phlebotomies during treatment phase,
    - Every / alternative phlebotomies during maintenance phase,
    - Or as clinically indicated e.g. comorbidity such as renal impairment allows haematocrit /haemoglobin to fall by no more than 20% of prior level.

Specimen and Ordering Information

All requests (electronic and paper) and specimens must adhere to the laboratories standard requirements. In order to comply with accreditation standards, laboratories cannot accept or process samples which do not meet minimum standards.

How to Test

For symptomatic individuals, the first line test should be ferritin and fasting transferrin saturation. If there is evidence of iron overload, HFE testing should be undertaken.

In line with the National Model of Care [1], when testing on the basis of family history, a HFE test and iron studies (serum ferritin and fasting transferrin saturation) should be performed. If a clinically significant variant is detected this may be interpreted with knowledge of the individual's iron overload status. The prevalence and impact of detection of non-HH hyperferritinaemia has not been evaluated, and merits future evaluation.

Serum ferritin reflects body iron stores but as an acute phase reactant, can be elevated non-specifically on occasions. While low serum ferritin is a sensitive and specific indicator of low total body iron stores, elevated levels are considered a highly sensitive but non-specific test for iron overload in hemochromatosis.[5, 6] Therefore normal serum concentrations can rule out iron overload. Serum ferritin has a low specificity as raised levels can also result from viral infections, inflammatory conditions, metabolic syndrome, cancer, chronic liver disease, drug toxicity and patients on dialysis. [7, 8, 9]

International guidelines recommend the upper limit of normal for serum ferritin of:

- 200 µg/L in pre-menopausal women,
- 300 µg/L in men and post-menopausal women. [7, 8, 9]

Nevertheless, it is recommended to use local reference values as inter-laboratory coefficient of variance for serum ferritin concentrations is high at 6-13% (source: Foundation of quality control of medical laboratory diagnostics (SKML) data on inter-laboratory variance of serum ferritin from about 270 Dutch laboratories in 2009).[9]

Diurnal variation of serum iron will affect the transferrin saturation level which is best measured on a fasting sample. Transferrin saturation is the proportion of the iron transport protein transferrin that is saturated with iron. It is calculated from the ratio of serum iron (µmol/L) to total iron-binding capacity (g/L) [8, 9] and when elevated reflects altered iron metabolism. Increased transferrin saturation reflects increased absorption of iron, the underlying biological defect of this condition. It is more sensitive in detecting early iron overload and is likely to be elevated before serum ferritin increases. Raised levels can also be caused by iron loading anaemias, use of iron supplements, patients with hepatitis and over consumption of alcohol.[9]

Fasting transferrin saturation of ≥45% is strongly suggestive of HH[8, 10] with increasing specificity when the threshold is increased to ≥55%.[6] The cut-off of ≥45% is considered to have a high sensitivity for detecting C282Y homozygotes and will also identify persons with minor secondary iron overload as well as some C282Y/wild-type heterozygotes, and these cases will require further evaluation.[8]

HFE testing should be performed only in those with increased fasting transferrin saturation. [6] Taken together elevated fasting transferrin saturation (≥45%) and ferritin (above upper limit of normal) have a strong positive predictive value, and their absence give an even stronger negative predictive value of HH.[7]

It has been documented that about 25% of C282Y heterozygotes may exhibit mild to moderately raised indices of iron overload.[11]

A systematic review found sensitivity and specificity of C282Y homozygosity to be about 90% and almost 100% respectively, for the presence of an iron overload phenotype in white northern Europeans.[12] The term ‘‘non–HFE-related HH’’ refers to several genetically distinct forms of inherited iron overload affecting individuals without HFE mutations.[13] Non-HFE related HH is rare and accounts for less than 5% of cases of inherited iron overload.

Genes involved in non-HFE forms of inherited iron overload include hemojuvelin (HJV), ferroportin (SLC40A1), transferrin receptor 2 (TFR2), and hepcidin (HAMP). It is possible to diagnostically screen for mutations in 16 iron regulatory genes via next generation sequencing. However, screening for non–HFE-related HH is not recommended [14] except for certain cases such as evidence of iron overload, confirmed on liver biopsy or MRI, negative HFE gene mutations, and other hepatic and haematological disorders have been excluded.[7] It is recommended that genetic testing for suspected rare forms of haemochromatosis should only be considered by specialist centres.[2]

Interpretation of tests

Elevations of serum ferritin in the range of 300–1000 µg/L are common, but may be indicative of secondary iron overload. If serum ferritin is ≥1000µg/L specialist review is required due to the increased risk of fibrosis and cirrhosis above this threshold.[6]

The association with haemochromatosis of HFE variants other than C282Y is still debated and no established consensus has been published.[2] C282Y/H63D compound heterozygotes and H63D homozygotes presenting with increased serum ferritin (>200µg/L in females, >300µg/L in males), increased fasting transferrin saturation (>45% in females, >50% in males) or increased liver iron should first be investigated for other causes of hyperferritinaemia.[7] Table 1 documents the interpretation of HFE gene C282Y and H63D related genotypes.

C282Y/H63D compound heterozygosity may be a risk factor predisposing to mild or moderate forms of iron overload when in association with comorbidity factors, for example, alcohol or metabolic syndrome.[2, 13] The association of homozygosity for H63D with iron overload is debated and requires further clinical research, but it is now recommended that other risk factors or other genetic causes should be sought and investigated in patients with this genotype and demonstrated iron overload.[15]

Table1. Summary of diagnostic and predictive interpretation comments for the HFE gene C282Y and H63D related genotypes. Taken from EMQN Guidelines.[2]

Genotype	Interpretation (diagnostic test)	Interpretation (predictive test)
Homozygous C282Y	Consistent with the diagnosis of HFE-related HH in the presence of documented evidence of iron overload.	At risk of developing HFE-related HH. Prompt assessment of iron parameters indicated.
Compound heterozygous C282Y/H63D	Excludes the diagnosis of the most common form of HFE-related HH; genotype consistent with mild to moderate iron overload; prompt the search for other causes (e.g. alcohol consumption, fatty liver disease and/or metabolic syndrome).	At low risk for development of significant iron overload. May be at-risk of developing mild to moderate iron overload in association with comorbid factors.
Heterozygous C282Y	Excludes the diagnosis of the most common HFE-related HH. Other causes of iron overload should be considered.	Carrier for HFE-related HH. Is at no increased risk of developing HFE-related HH.
Homozygous H63D	Excludes the diagnosis of the most common HFE-related HH. Other causes of iron overload should be considered.	May develop iron-overload in association with other risk factors or other genetic causes.
Heterozygous H63D	Excludes the diagnosis of the most common HFE-related HH. Other causes of iron overload should be considered.	At no increased risk of developing HFE-related HH.
S65C detected	In the absence of supporting evidence for a role in HH, testing for the S65C variant is not recommended for diagnostic purposes.	

Recommendations for National Laboratory Information System (MedLIS)

A mechanism should be developed to ensure that HFE tests are available on MedLIS to prevent tests being reordered. If this cannot be achieved by straightforward data migration, mechanisms for importing PDFs of previous reports need to be evaluated.

The use of an order entry form indicating if testing is requested because of family history (including relation to proband and genotype if known) or based on evidence of iron overload will aid future audit.

An interpretive comment when the fasting transferrin saturation >45% should indicate probable iron overload, and suggest HFE test, if not previously performed.

The Model of Care refers to a future National Register for HH. Consideration should be given to such a register having MedLIS connectivity, and development of MedLIS analytic reports to support data collection.

Information for Patients

A comprehensive guide for patients explaining the diagnosis and management of HH is included in the Model of Care document. [1]

Consultation Plan and History

This guideline is extracted from the Model of Care for HH, which has been through extensive consultation under the governance of the Haemochromatosis Working Group.

The purpose of this guideline is to extract the information of relevance to laboratories underpinning the HH Model of Care.

References

1. Hereditary Hemochromatosis. Model of Care. HSE, 2016.

2. Porto G, Brissot P, Swinkels DW, Zoller H, Kamarainen O, Patton S, et al. EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis (HH). Eur J Hum Genet. England; 2016 Apr; 24 (4): 479–95.

3. Delatycki MB, Powell LW, Allen KJ. Hereditary hemochromatosis genetic testing of at- risk children: what is the appropriate age? Genet Test. United States;2004;8(2):98–103.

4. Ryan E, Byrnes V, Coughlan B et al. Under-diagnosis of hereditary haemochromatosis: lack of presentation or lack of penetration? Gut. 2002 Jul;51(1):108-12.

5. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G-->A(C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002 Jan;359 (9302): 211–8.

6. Goot K, Hazeldine S, Bentley P, Olynyk J, Crawford D. Elevated serum ferritin- what should GPs know? Aust Fam Physician.2012 Dec;41 (12): 945–9.

7. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol.2010 Jul;53(1):3–22.

8. Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS. Diagnosis and management of hemochromatosis: 2011 Practice Guideline; American Association for the Study of Liver Diseases. Hepatology. 2011; 54(1): 328–43.

9. Bokhoven MA, van Deursen CTBM, van Swinkels DW. Diagnosis and managementof hereditaryhaemochromatosis.BMJ[Internet].BMJPublishingGroupLtd;2011;342.

10. Swinkels DW, Jorna ATM, Raymakers RAP. Synopsis of the Dutch multidisciplinary guideline for the diagnosis and treatment of hereditary haemochromatosis. Neth J Med. 2007Dec;65(11):452–5.

11. Worwood M. Inherited iron loading: genetic testing in diagnosis and management. Blood Rev. 2005;19:69–88. doi: 10.1016/j.blre.2004.03.003.

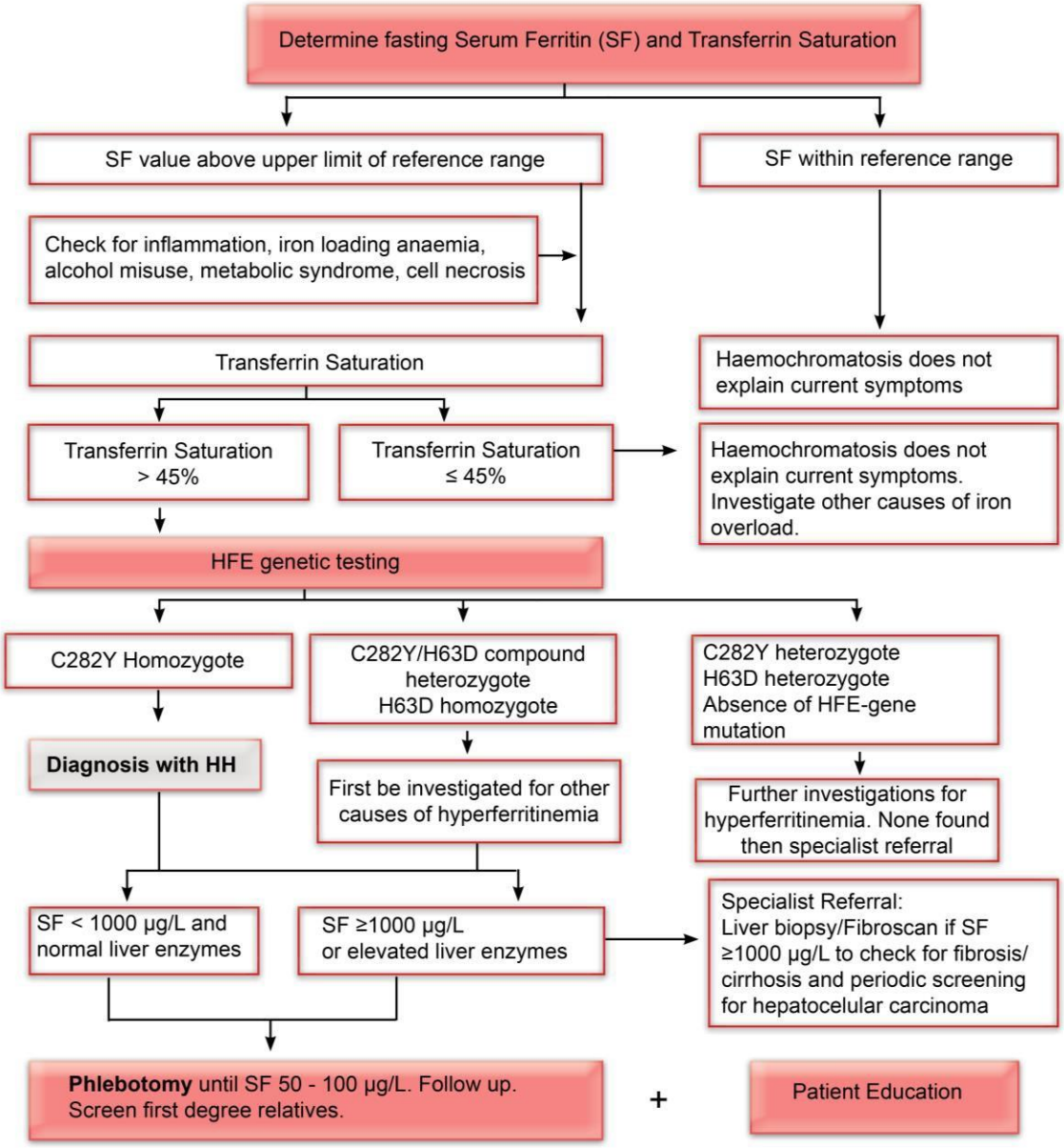
12. Bryant J, Cooper K, Picot J, Clegg A, Roderick P, Rosenberg W, et al. A systematic review of the clinical validity and clinical utility of DNA testing for hereditary haemochromatosis type 1 in at-risk populations. J Med Genet. 2008 Aug; 45(8): 513–8.

13. Pietrangelo A. Non-HFE hemochromatosis. Semin Liver Dis.;2005 Nov; 25(4): 450–60.

14. Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with Hemochromatosis and other liver diseases. Ann Intern Med. 1999 Jun;130(12):953–62.

15. Gurrin LC, Bertalli NA, Dalton GW, Osborne NJ, Constantine CC, McLaren CE, et al. HFE C282Y/H63D compound heterozygotes are at low risk of hemochromatosis-related morbidity. Hepatology. United States; 2009Jul;50(1):94–101.

Appendix 1. Approach to Testing



Diagnostic flow chart for patients with suspected Hereditary Haemochromatosis.[8,12]