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Research Protocol

Diagnostic Strategies using DNA testing for Hereditary Haemochromatosis in at-risk populations

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1 Project title

Diagnostic Strategies using DNA testing for Hereditary Haemochromatosis in at-risk populations

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3 Planned investigation

3.1 Background

3.1.1 Hereditary Haemochromatosis

Hereditary haemochromatosis (HHC) results from a genetic disorder of iron metabolism which leads to excessive intestinal absorption of iron and a progressive abnormal deposition of iron in the liver, heart, pancreas and other vital organs. Iron levels in the body are usually carefully regulated. Iron is absorbed from dietary sources to maintain iron stores and replace iron that is lost daily, mostly due to the loss of iron containing red blood cells into the gut. In younger women, menstruation also makes an important contribution to iron loss. Haemochromatosis (the clinical condition of iron overload) occurs when this careful balance of iron is gradually lost. In HHC absorption of iron from the gastrointestinal tract continues to occur when bodily iron stores and blood iron levels have reached and then exceeded normal levels and iron therefore continues to accumulate within cells.

The prevalence of haemochromatosis from studies using biochemical assessments of iron overload and from population studies of the genotype frequencies is estimated to be 1 in 300 in Northern European populations,[2] although the prevalence of clinically diagnosed cases is much less. The number of individuals admitted to any NHS provider within England with a diagnosis of haemochromatosis in the year 2002 to 2003 was 2061, approximately 1 in 17000 (based on Hospital Episode Statistics data and adult population estimates).[4;5] This discrepancy may be due to under diagnosis or to that fact that there is considerable variability in the phenotypic severity of the disease and many individuals with the genetic predisposition for HHC do not develop iron overload. Since the discovery of the common gene mutations linked to HHC there

has been a debate in the literature about the phenotypic severity and hence clinical morbidity associated with this condition.[6;7] Some of this debate has centred on the penetrance of the gene mutations, that is, the probability that a person with the gene mutations will develop clinical consequences – disease. Claims have been made stating that the penetrance may be less than one percent.[9;10] This is in contrast to the 40% penetrance in male relatives of affected individuals reported in earlier studies.[11] It is likely that a range of factors, such as other gene abnormalities and environmental effects, and others which are unknown, determine the extent to which the phenotype is observed in a particular individual.

There is agreement that the clinical condition of haemochromatosis is the end result of a combination of genetic and environmental factors, not all of which have been described. The mutations associated with a risk of HHC are very common and therefore whilst they are a good diagnostic indicator in those already suspected of having haemochromatosis or in the context of family testing they are not useful for screening at the population level.

3.1.2 Symptoms of disease

In the early stages HHC is usually asymptomatic but as excess iron continues to be deposited damage begins to occur in a wide range of organs. Initial symptoms are non-specific and may often be ignored or misdiagnosed. In men, clinical signs of HHC usually become overt in the fourth or fifth decade of life. Women may present later in life, because the loss of iron during menstruation and pregnancy confers some degree of protection against the process of iron accumulation over time. Patients may present in middle age with liver disease, diabetes, arthralgia and fatigue when excess iron deposited in the liver, pancreas, joints, anterior pituitary and heart has led to organ damage resulting in morbidity and mortality in middle life. The main signs, symptoms and clinical presentation are shown in the box below.[12;13]

Liver - In reports of case series the liver is one of the most common organs to be affected and hepatomegaly is one of the most frequent findings at clinical presentation.[1] Early signs include abdominal pain and abnormal liver function tests. It is assumed that without treatment progressive iron overload leads to liver fibrosis ultimately progressing to cirrhosis which is a risk factor for both hepatic carcinoma and liver failure. The percentage of patients who are reported to have cirrhosis at the time of presentation varies but there is a suggestion that it is reducing over time, possibly because of earlier referral and diagnosis.[3] The presence of cirrhosis at diagnosis was predictive of poorer survival in these studies. Studies of patients having liver transplants suggest that undiagnosed haemochromatosis is not infrequent, that the occurrence of unsuspected hepatocellular carcinoma in this group is increased, and that life expectancy post transplant for patients with undiagnosed haemochromatosis is significantly reduced.[8]

Joints - Arthropathies are found in 40 to 75 percent of patients[5] but the occurrence may be overestimated since arthritis is a common symptom in the general population, estimates are usually based on patient information and the actual site and severity of the arthropathy is often not characterised. Arthritis as a symptom of haemochromatosis appears to be associated with a reduced quality of life.[2;15]

Endocrine - Diabetes mellitus is the major endocrine disorder associated with HHC.[5] The insulin producing beta cells of the pancreas may be damaged directly by accumulating iron, or indirectly by autoimmune reactions. In addition a secondary consequence of hepatic damage may be insulin resistance. Hypogonadism also occurs and is caused primarily by gonadotropin deficiency resulting from iron deposition in the pituitary or hypothalamus. Sexual dysfunction e.g. loss of libido or impotence may be an early sign of HHC. Other endocrine disorders including impairment of the thyroid, parathyroid, or adrenal glands have been reported but are rarely seen.

Heart - Cardiac manifestations of haemochromatosis are thought to be associated with iron deposition in the myocardium. Congestive heart failure has been seen in 2 to 35 percent and arrhythmias are present in 7 to 36 percent of HHC patients in various case series.[16]

Skin Bronzing - Untreated patients may appear to have a permanent tan as excessive melanin secretion leads to bronze pigmentation of the skin.

3.1.3 Treatment

Treatment by removing excess iron with regular phlebotomy (blood-letting from a vein) is effective. [14] Phlebotomy involves weekly venesection of 500ml blood. This removes about 250mg of iron (which is in haemoglobin), and it may take up to 2 years to reduce bodily iron to acceptable levels. A normal life expectancy can be achieved[3] if HHC can be diagnosed and treatment started in the pre-cirrhotic phase, before irreversible end organ damage occurs. Unfortunately arthritis is one of the symptoms that is probably not improved by venesection therapy and may in fact deteriorate in some patients.[3;17] Other consequences of iron deposition and organ damage, such as diabetes mellitus are also unlikely to resolve once treatment has commenced therefore late presentation is associated with early mortality.

Some questions remain around when to start treatment, for instance should C282Y homozygotes with raised transferrin saturation and a normal serum ferritin be given treatment. The BSH Guidelines on Haemochromatosis[18] state that treatment would not normally be given at that stage of iron accumulation and some emerging evidence suggests that early venesection may increase iron absorption. For those with normal values of transferrin saturation and serum ferritin concentration no treatment is necessary. The guidelines suggest that it would be reasonable to monitor iron status at yearly intervals to detect when transferrin saturation becomes raised indicating the onset of tissue iron accumulation.

3.1.4 Diagnosis of Haemochromatosis

Different strategies can be employed to confirm diagnosis if a case of haemochromatosis is suspected but the most appropriate strategy is not clear. These include biochemical testing, liver biopsy and genetic testing.

3.1.4.1 Biochemical Testing and Liver biopsy

Prior to the discovery of the common gene mutations, diagnosis of haemochromatosis was based on clinical suspicion including persistently raised transferrin saturation with no other explanation followed by liver biopsy. Both the British Society for Haematology (BSH) (in their guideline on Genetic Haemochromatosis[18]) and the US Centre for Disease Control recommend the transferrin saturation test as the initial diagnostic test for HHC. This test should be carried out on a fasting sample. Elevated fasting transferrin saturation indicates iron accumulation and if this has been demonstrated, the BSH Guidelines recommend that serum ferritin concentration is measured. Serum ferritin concentrations are not usually abnormal in the early stages of iron accumulation but once liver iron concentrations are elevated they rise disproportionately with the degree of liver damage. Liver biopsy with assessment or iron deposition, although previously considered to be the gold standard for diagnosis, is no longer used as a diagnostic tool but is still widely used to confirm cirrhosis and as the only way of determining fibrosis. In the past, the Hepatic Iron Index (HII) was used to quantify the extent of liver iron, with a ratio of hepatic iron concentration/age in years (umol/gm dry wt/age) greater than 1.9 said to be diagnostic of homozygous HCC.[19] It is worth noting that biochemical tests are unable to discriminate between different possible causes of iron overload, so although iron overload may suggest a diagnosis of HHC other possible diagnoses may need to be ruled out.

3.1.4.2 Genetic Testing

The discovery of the HFE gene in 1996[6] has led to increasing interest in haemochromatosis and has introduced DNA based predisposition testing as a possible tool for diagnosis and screening. There are two common mutations which account for the majority of cases. These mutations are a G to A transition at nucleotide 845 on the HFE gene causing tyrosine (Y) to substitute for cysteine (C) at position 282 on the HFE protein (C282Y) and a G to C transition at nucleotide 187 causing a histidine (H) to aspartate (D) substitution at position 63 in the HFE protein (H63D). Over ninety per cent of patients from northern Europe are homozygous for the C282Y mutation and five percent are compound heterozygotes for the C282Y mutation and the H63D mutation.[20] Routine genetic testing is restricted to the two or three most common mutations in the HFE gene which account for most HFE-related HHC. The tests can identify people who are homozygous (or compound heterozygotes) for mutations in the HFE gene before symptoms of iron overload become apparent. Whilst this test identifies people at risk of developing the symptoms of iron overload, due to the low penetrance of the disorder, only a proportion will go on to exhibit the HHC phenotype. This means that both the sensitivity for detecting phenotypic HHC and the positive predictive value of genetic testing is much less than 100%. Other gene mutations have been identified that lead to haemochromatosis, however testing for these is not routinely available outside of the research setting.[21] C282Y frequencies are

significantly greater in Europe than in Africa, Middle East, India, Asia, Australasia and the Americas[20] which means that UK ethnic minorities have reduced rates of homozygosity.

A wide range of DNA based tests to identify the C282Y and H63D mutations have been described, most commonly polymerase chain reaction (PCR) followed by restriction enzyme digest, amplification refractory mutation system (ARMS) PCR, and PCR with Single-Strand Conformation Polymorphism (SSCP) analysis.

3.1.5 Family screening

HHC is an autosomal recessive condition and as such children and siblings are at increased risk for the disease, with probabilities of 1 in 20 and 1 in 4 respectively. The purpose of testing family members is to detect those individuals at risk who would benefit from treatment, and to detect those at risk who do not currently require treatment but who will be monitored for a suitable period of time until the need for future treatment can be ascertained. Testing can also identify those not at risk who can be excluded from further investigations. Penetrance may be higher in families as they share other genetic and environmental factors.

3.1.6 Psychosocial Aspects of Genetic testing

Concern about genetic testing and screening is evidenced by a number of reports which focus on issues of potential stigmatisation, discrimination, family implications and the possible psychological consequences.[22;23] However, the rationale for genetic exceptionalism may not be well established.[24] In addition concerns relating to unfair discrimination will be influenced by the legal framework and welfare provision of the country of residence. For example the situation regarding coverage and reimbursement for health care is very different in North America to that in the UK. In haemochromatosis the purpose of using the genetic test is to identify individuals who will benefit from treatment or further monitoring and to rule it out. Studies have investigated the psychosocial consequences of using DNA tests for population and targeted screening for haemochromatosis and found few adverse effects.[25-27]

3.1.7 Current UK practice

Many patients are identified incidentally when they are already in the secondary care setting. A serum ferritin is often done routinely and a significant proportion of patients are diagnosed on the basis of abnormalities in liver function tests. Patients attending diabetic clinics may also be tested and identified. Also, increasing numbers are identified because a family member has been diagnosed with haemochromatosis. Relatively few patients are referred on the basis of abnormal liver function tests (LFT) from the primary care setting due perhaps to the low awareness of HHC but also because of its asymptomatic nature or because early symptoms are non-specific. It is also diagnosed following identification of an affected proband in a family.

HHC is managed in many centres often by a dedicated team consisting of a clinician supported by a nurse specialist and possibly junior medical staff who will do most of the phlebotomy and provide a day-to-day contact point for the patients. Haematologists, gastroenterologists and hepatologists provide the medical input, with care pathways following the British Society of Haematology guidelines.[18] Genetic advice may also be provided by the same teams, with some having in-house genotyping services and others using regional centres. Regional genetics centres also provide a service offering advice and family investigation, with referral of patients to the local haematologist/gastroenterologist for phlebotomy.

3.1.8 Rationale for the study

There is broad agreement that early diagnosis of haemochromatosis and treatment with venesection is effective at reducing the risk of complications.[3;21] It is important therefore to identify (i) the best diagnostic strategy for those suspected clinically of having haemochromatosis and (ii) the best testing strategy for family members to identify those who need treatment or monitoring. Biochemical tests will identify the presence of raised iron levels requiring treatment. However when iron levels are not so high as to require treatment, the predictive value of varying levels of transferrin saturation is unclear, as is the additional value of a DNA test. As mentioned before, liver biopsy with assessment of iron deposition, is no longer the gold standard for diagnosis, but is still widely used to confirm cirrhosis and as the only way of determining fibrosis.

From the perspective of the patient and their family the pressing clinical issue is to effectively and efficiently diagnose people early enough in order that they may benefit from treatment and avoid serious complications and in affected families to rule it out. The wider NHS perspective is the most efficient and cost effective use of the tests available and prevention of more costly complications.

3.2 Research Aim

The aim of this project is to evaluate the use of DNA tests for detecting HHC in subgroups of patients suspected of having the disorder on the basis of clinical presentation and disturbed iron parameters, and in family members of those diagnosed with haemochromatosis. A clear distinction will be drawn between diagnostic strategies in those suspected of having haemochromatosis and testing strategies in family members since the consequences are different.

3.3 Objectives

The main objectives will be as follows:

- To determine the clinical validity of DNA tests to diagnose HHC.
- To summarise the evidence on the clinical utility of diagnostic strategies using DNA tests to detect cases for treatment or monitoring in terms of clinical effectiveness and cost effectiveness.
- To compare the costs and consequences by decision analytic modeling of diagnostic algorithms for HHC and family testing strategies with and without DNA testing in terms of cost per case detected.
- To review the psychosocial literature and compare the psychosocial benefits and harms of adding DNA testing to diagnostic algorithms.
- To identify priorities for future primary research.

Existing research

Preliminary scoping searches of key databases (Medline, PubMed, Cochrane, DARE, NHS EED and Embase) have been undertaken which identified over 800 references. However, the results suggest that there are no existing systematic reviews of diagnostic algorithms for case detection in haemochromatosis and no traditional diagnostic test accuracy studies of DNA tests for HHC. A few studies address family testing strategies and although a number of cost effectiveness studies were identified most report on the cost-effectiveness of screening for HHC at a population level; only two or three of these appear to report on the cost-effectiveness of family screening. There are some studies concerning the psychosocial impact of genetic testing in haemochromatosis.

3.4 Research Methods

3.4.1 Systematic Review

Systematic reviews will be undertaken in accordance with the NHS Centre for Reviews and Dissemination guidelines,[28] and published criteria for appraising economic evaluations.[29;30] The systematic reviews will cover the following topics:

- Clinical Validity: the performance of DNA tests to detect haemochromatosis.
- Clinical Utility: the clinical and cost effectiveness of different diagnostic strategies using DNA testing for HHC in those suspected of having haemochromatosis on the basis of clinical presentation and disturbed iron parameters, and in family members.
- Psychosocial aspects of DNA testing for haemochromatosis.

Systematic literature searches will also be undertaken to inform the economic model for the following areas:

- Epidemiology of haemochromatosis, including factors such as the prevalence of confirmed HHC in different patient groups and abnormal iron values in family members.
- Clinical validity of biochemical tests used in current UK practice to diagnose haemochromatosis.
- Complication rates of ultrasound-guided liver biopsy.

3.4.1.1 Literature search

Literature will be identified from several sources including electronic databases, bibliographies of articles, grey literature sources and consultation with experts in the area. A comprehensive database of relevant published and unpublished articles will be constructed using the Reference Manager software package. The searches carried out will include:

- General health and biomedical databases: Medline; Embase; PubMed (previous 6 months); Science Citation Index (SCI); BIOSIS, Psychlit
- Specialist electronic databases: Database of Abstracts of Reviews of Effectiveness (DARE); Cochrane Library; MEDION (a database of diagnostic test reviews set up by Dutch and Belgian researchers); Health Technology Assessment Database (HTA); NHS Economic Evaluation Database (NHS EED); EconLit
- Grey literature and Conference Proceedings: NLM (National Library of Medicine); Gateway Databases; Index to Scientific and Technical Proceedings; Conference Proceedings Index; BioIron, PapersFirst; HMIC (Health Management Information Consortium); Index to Theses; Dissertation Abstracts; SIGLE; WorldCat; British Library Public Catalogue; COPAC, HuGENet
- Contact with individual experts and those with an interest in the field
- Checking of reference lists
- Research in Progress: National Research Register (NRR); Current Controlled Trials; Clinical Trials.gov

All databases will be searched from inception to the current date. Searches for literature on DNA tests for C282Y and H63D mutations will be restricted to 1996 onwards because this is when the HFE gene was discovered. In the first instance searches will be conducted in all languages with non-English language articles set to one side in a separate foreign language reference database. The primary focus will be English language articles but the need to include non-English articles will be considered in the light of what is found and within the constraints of available time for translation.

3.4.1.2 Study inclusion

Specific inclusion criteria will be defined and tailored to each of the systematic reviews and systematic searches undertaken. The full literature search results will be screened by one reviewer and checked by a second reviewer to identify all citations that may meet the inclusion criteria. Full manuscripts of all selected citations will be retrieved and assessed by two reviewers against the inclusion criteria. An inclusion flow-chart will be developed and used for each paper assessed. Disagreements over study inclusion will be resolved by consensus or if necessary by arbitration by a third reviewer.

The planned inclusion/exclusion criteria for the systematic reviews are shown in Table 1.

Table 1 Inclusion criteria for systematic reviews

	Systematic Review: Clinical validity	**Systematic Review: Clinical utility**		**Systematic review:**
	DNA tests	**Clinical effectiveness of diagnostic strategies**	**Cost effectiveness of diagnostic strategies**	**Psychosocial aspects of DNA testing**
Patients	Caucasian patients with iron overload, signs and symptoms suggestive of HHC (defined). Emphasis to be UK populations/North European.	Caucasian patients with iron overload, signs and symptoms suggestive of HHC (defined) Relatives of suspected cases. Exclude specialist clinic based patient groups (eg diabetic clinics) and population screening.	Caucasian patients with iron overload, signs and symptoms suggestive of HHC (defined) Relatives of suspected cases. Exclude specialist clinic based patient groups (eg diabetic clinics) and population screening.	At risk individuals ie suspected HHC cases and first degree relatives.
Intervention	DNA tests	DNA tests	DNA tests	DNA tests
Comparator	Control population (eg healthy controls or comparator patient group attending clinic/hospital for non-HHC/iron overload reasons)	Any case identification strategy. May include: Liver biopsy to give HII or Quantitative phlebotomy or other iron studies	Any case identification strategy. May include: Liver biopsy to give HII or Quantitative phlebotomy or other iron studies	n/a
Outcomes	Sensitivity and specificity (reported or calculable).	Treatment, morbidity, mortality, QoL, psychosocial. (patient based outcomes)	Cost per case detected, cost minimisation, cost effectiveness or cost utility	Psychosocial. (treatment compliance, psychological, legal implications, QoL, discrimination/stigmatisation)
Design	Controlled cohort or case control.	RCTs, controlled cohort, case-control. (Highest level of evidence only)	Economic evaluations, modelling studies.	Any primary research. Quantitative and qualitative.

In addition, the results of systematic literature searches to identify relevant studies in the areas of the epidemiology of haemochromatosis, performance of biochemical tests and complications of liver biopsy will be assessed against inclusion criteria and used to inform the decision models. (see Table 2).

Table 2: Inclusion criteria for systematic searches

	Epidemiology	**Biochemical Tests**	**Complications of liver biopsy**
Patients	Population is Caucasian patients with iron overload, signs and symptoms suggestive of HHC (defined) Relatives of suspected cases. Emphasis to be UK populations/North European.	Caucasian patients with iron overload, signs and symptoms suggestive of HHC (defined)	Caucasian patients with iron overload, signs and symptoms suggestive of HHC (defined). If no data, extend to other patients having elective biopsy without decompensated liver disease.
Intervention	n/a	Transferrin saturation (TS) and serum ferritin (SF) reporting cut off values	Ultrasound guided liver biopsy
Comparator	n/a	Liver biopsy to give HII or Quantitative phlebotomy or DNA. (to confirm diagnosis)	Liver biopsy without ultrasound
Outcomes	Incidence, prevalence, natural history,	Sensitivity and specificity PPV, NPV Reported and/or calculable	Adverse events/complications reported as frequencies/probabilities.
Design	Observational studies	RCTs, cohorts, case control (highest level of evidence only).	RCTs

3.4.1.3 Data extraction

The extraction of studies' findings will be conducted by two reviewers using a pre-designed and piloted data extraction form to avoid any errors. Any disagreements between reviewers will be resolved by consensus or if necessary by arbitration by a third reviewer.

3.4.1.4 Quality assessment

The methodological quality of included studies will be assessed using formal tools specific to the design of the study and focusing on possible sources of bias. Quality assessment of RCTs will be conducted using criteria developed by NHS Centre for Reviews and Dissemination[28] and observational studies will be assessed using criteria developed by Spitzer[31] (Appendix 1). For diagnostic test studies quality assessment will be conducted using a tool such as the QUADAS where appropriate (Quality Assessment of Diagnostic Accuracy Studies, Appendix 1).[32] Quality assessment of economic evaluations will be conducted using a checklist adapted from those developed by Drummond et al[29] and Philips et al.[30] Study quality will be assessed by two reviewers. Any disagreements between reviewers will be resolved by consensus or if necessary by arbitration involving a third reviewer.

3.4.1.5 Data synthesis

The methods of data synthesis will be determined by the nature of the studies identified through searches and included in the review. Quantitative synthesis of results e.g. meta-analysis will be considered if there are several high quality studies of the same design and sources of heterogeneity will be investigated by subgroup analyses if applicable. The results of any included studies suitable for quantitative synthesis will also be summarised in a narrative form along with a narrative synthesis of the results from studies for which quantitative synthesis is not possible. All results will also be tabulated (see Appendix 2).

3.4.1.6 Evaluation of genetic tests

Various authors have raised issues concerning the methods for assessing diagnostic tests and there is a consensus that explicit frameworks should be developed analogous to those used in studies of clinical effectiveness.[33;34] The ACCE model has been developed by the Office of Genomics and Disease Prevention (Center for Disease Control, Atlanta, USA), working with the Foundation for Blood Research to evaluate

DNA-based genetic tests.[12] This model takes its name from the four components of the evaluation: Analytic Validity, Clinical Validity, Clinical Utility and Ethical, Legal and Social Issues. This model is still in its development stage; however it provides a useful framework to inform the evaluation of genetic tests.

Analytical validity is the ability of the test to accurately and reliably measure the genotype of interest and is concerned with assessing test performance in the laboratory and is closely related to quality assurance of the laboratory processes surrounding the test. Clinical validity is defined as the ability of the test to detect or predict the phenotype (disorder) of interest. Elements of clinical validity include clinical sensitivity, clinical specificity, and positive and negative predictive values of the test (See Table 3). The clinical sensitivity measures the proportion of individuals with the defined disorder, or who will get the disorder in the future, and whose test results are positive, whilst clinical specificity measures the proportion of individuals who do not have the defined clinical disorder and whose test results are negative.

Table 3: Calculation of components of clinical validity

	Participants		
Test	With disease	Without disease	Total
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	A+B+C+D

Sensitivity = A/A+C Positive predictive value = A/A+ B
Specificity = D/B+D Negative predictive value = D/C+D
PPV and NPV vary with disease prevalence but are useful clinically for ruling the condition in or out.
The ideal study to determine these parameters in the case of HHC is a population-based genotyped cohort of young adults followed through life; as this is not possible, a pragmatic approach is to use controlled cohort studies.

Clinical utility is defined as the likelihood that the test will lead to an improved outcome, and incorporates assessment of the risk and benefits of genetic testing, as well as economic evaluation. This is perhaps the most important aspect of the evaluation in that it assesses whether testing will alter clinical management, benefit those tested and at what cost.

Of particular relevance to this project are questions of clinical validity and clinical utility. Additionally, the last component of the ACCE framework will be covered by considering psychosocial aspects of using genetic testing for HHC in terms of psychological issues, quality of life, discrimination and stigmatisation and legal implications.

3.4.2 Economic evaluation

A comparison of the costs and consequences of the diagnostic testing strategies with and without DNA testing will be made using decision analytic models (see Appendix 3). These will be populated with data from systematic reviews and systematic searches of the literature and, where necessary, using Guidelines and expert opinion. Costs will be derived from primary data from previous studies, and national and local NHS unit costs. The outcome will be reported as cost per case detected.

The structure and data inputs of all the decision trees will be informed by systematic literature reviews and the results of systematic searches [section 3.4.3] and discussion with experts.

3.4.2.1 The addition of DNA testing to diagnostic algorithms in people suspected of having haemochromatosis

Prior to the discovery of the common gene mutations, diagnosis of haemochromatosis was based on clinical suspicion including persistently raised transferrin saturation with no other explanation followed by liver biopsy. Since the identification of the gene it has been possible to use DNA testing to confirm the diagnosis in those in whom it is suspected. Liver biopsy then becomes a prognostic test in those suspected of having liver damage and can be avoided in those with moderately raised iron levels and no biochemical evidence of liver damage (consistent with figure 1).

Decision models will be constructed in DATA Treeage to compare the costs and consequences of two diagnostic algorithms in patients suspected of having haemochromatosis on the basis of persistently raised transferrin saturation. The end point of both algorithms is detection of a case requiring treatment according to current clinical guidelines and the reported outcome will be cost per case detected. The algorithms for people with suspected HHC on the basis of symptoms/signs and then raised transferrin saturation and serum ferritin are as follows:

- Liver biopsy in all.
- Genetic testing for all (with liver biopsy only for prognostic reasons in those with very high SF or signs of liver damage).

The goal of a diagnostic strategy is to improve patient management and ultimately patient outcomes. These outcomes will be incorporated into the model by considering:

- avoidance of liver biopsy

3.4.2.2 DNA testing for family members of patients diagnosed with haemochromatosis

Separate decision models will be constructed to compare the costs and consequences of two testing algorithms in family members of patients diagnosed with haemochromatosis. The end point of the algorithms are, detection of a case requiring treatment according to current clinical guidelines, identification of family members at risk who need to be monitored and identification of family members who are not at risk for HHC. The reported outcome will be cost per case detected. The algorithms for testing family members are as follows:

- Biochemical testing for all
- Genetic testing for all

The goal of family testing is to identify family members who need treatment, identify family members at risk of HHC so that they can be monitored and treatment started if this becomes necessary, identify family members who are not at risk who will not need to be monitored or treated in the future. These outcomes will be incorporated into the model by considering:

- Unnecessary monitoring investigation of those with false-positive diagnoses (eg gene positive but never penetrant) or where the initial test is not informative (eg negative biochemical test)
- missed diagnoses

3.4.3 Ethical arrangements

No specific ethical arrangements necessary.

3.4.4 Outputs of the review

In addition to the preparation of the HTA monograph, the findings of this project will be presented at clinical meetings and meetings of patient support groups. Papers will be submitted to relevant peer reviewed journals. The findings will also be presented to expert clinical groups in order to develop evidence based guidelines for the identification of patients who would benefit from treatment.

4 Project management and milestones

Project management and milestones

Major Milestones	Date
Development of protocol	May 2006 – December 06
Drafting of final report	May 2007 – June 2007
Submission and dissemination of report	August 2007

Competing Interests: No member of the team has registered any competing interests.

5 Advisory Group

Representatives and other potential users of the review from different professional backgrounds and opinions, including academics, clinicians, health economists, patient groups, professional organizations, will be invited to provide expert advice to support the project. Experts will be asked to provide comments on a version of the protocol and of the final report, as well as advising on the identification of relevant evidence. All experts will be asked to register competing interests and to keep the details of the report confidential.

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Appendix 1: Quality assessment

a. Quality criteria for assessment of experimental studies (NHS CRD)[28]

Item	Judgement*
1. Was the assignment to the treatment groups really random?	
2. Was the treatment allocation concealed?	
3. Were the groups similar at baseline in terms of prognostic factors?	
4. Were the eligibility criteria specified?	
5. Were outcome assessors blinded to the treatment allocation?	
6. Was the care provider blinded?	
7. Was the patient blinded?	
8. Were the point estimates and measure of variability presented for the primary outcome measure?	
9. Did the analyses include an intention to treat analysis?	
10. Were withdrawals and dropouts completely described?	

* adequate, inadequate, not reported, unclear

b. Quality criteria for assessment of diagnostic studies[32]

Item		Judgement*
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	
2	Were selection criteria clearly described?	
3	Is the reference standard likely to correctly classify the target condition?	
4	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	
5	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	
6	Did patients receive the same reference standard regardless of the index test result?	
7	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	
8	Was the execution of the index test described in sufficient detail to permit replication of the test?	
9	Was the execution of the reference standard described in sufficient detail to permit its replication?	
10	Were the index test results interpreted without knowledge of the results of the reference standard?	
11	Were the reference standard results interpreted without knowledge of the results of the index test?	
12	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	
13	Were uninterpretable/ intermediate test results reported?	
14	Were withdrawals from the study explained?	

* yes, no, unclear

c. Quality criteria for assessment of observational studies[31]

These quality criteria were adapted from Spitzer and colleagues.[31] The original checklist was modified to include items of particular relevance to assessing observational studies.

1. Does the trial use proper random assignment?

A study with proper random assignment would include multiple conditions with random assignment and would use an appropriate method for the assignment (e.g., random numbers table, computer generated, etc.) with allocation concealment.

2. Did the study use proper sampling?
   A study with proper sampling would allow for all patients to be equally likely to enter the study (e.g., patients selected consecutively or randomly sampled).
3. Was the sample size adequate?
   Proper sample size enables adequately precise estimates of priority variables found to be significant (e.g., can compute CI within relatively small range or relatively small SEM).
4. Were the criteria for definition or measurement of outcomes objective or verifiable?
   Good outcome measures would be defined by clear methods for measuring outcomes (i.e., an operational definition) that are public, verifiable and repeatable.
5. Were outcomes measured with blind assessment?
   In studies with blind assessment those evaluating outcomes are unaware of the treatment status of those being evaluated.
6. Were objective criteria used for the eligibility of subjects?
   Good eligibility criteria would use clear, public, verifiable characteristics that are applied for inclusion and exclusion.
7. Were attrition rates (%) provided?
   A study should report the number of patients who could not be contacted for outcome measures or later, e.g., drop-outs or withdrawals due to treatment toxicity.
8. Were groups under comparison comparable?
   Comparable groups show similar results across a reasonable range of baseline characteristics that could be expected to affect results.
9. Are the results generalisable?
   Generalisable results come from a sample population that is representative of the population to which results would be applied.

Appendix 2: Data extraction form – Generic Sample

Reference and Design	Intervention	Participants	Outcome measures
Author: Year: Country: Study design: RCT Number of centres: ? Funding:	Intervention: Control: Other interventions used:	Number of Participants: Intervention: Control: Sample attrition/dropout: Sample crossovers: Inclusion criteria for study entry: Exclusion criteria for study entry: Characteristics of participants:	Primary outcomes: Secondary outcomes: Method of assessing outcomes: Adverse symptoms: Length of follow-up: Recruitment dates:

Results

Primary Outcomes	Intervention	Control	P Value

Comments:

Secondary outcomes	Intervention	Control	P value
			P=0.87

Comments:

Note: If reviewer calculates a summary measure or confidence interval PLEASE INDICATE

Methodological comments
Allocation to treatment groups:
Blinding:
Comparability of treatment groups:
Method of data analysis:
Sample size/power calculation:
Attrition/drop-out:

General comments
Generalisability:
Outcome measures:
Inter-centre variability:
Conflict of interests:

Appendix 3: Proposed diagnostic strategies using DNA testing for HHC

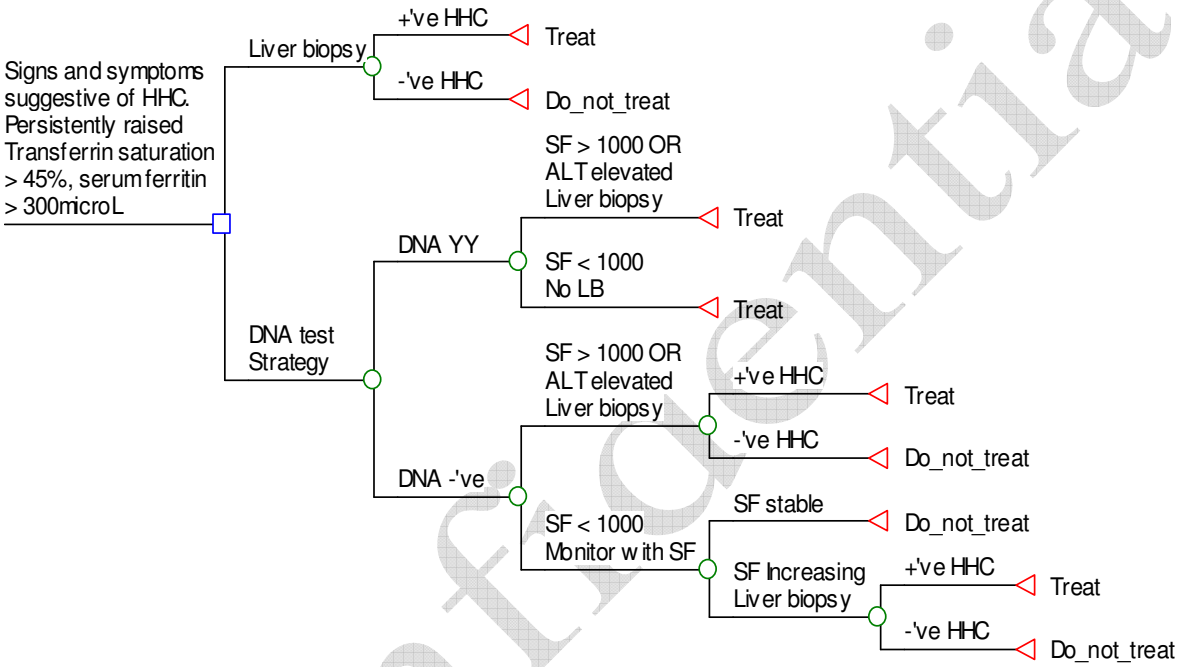
1.Decision tree using DNA testing in people suspected of having HHC (see figure 1).

For people with suspected HHC on the basis of raised TS and SF, the two comparative strategies are

a) Liver biopsy in all
b) DNA testing for all

a) Those who have biopsy will either be confirmed positive or negative phenotypic HHC. Those who are positive will be treated and those who are negative will not be.

Figure 1: Decision tree for DNA testing in the diagnosis of those suspected as having haemochromatosis



b) Those who have the DNA test will either be positive YY homozygous, YD compound heterozygous or negative. Those with SF over 1000µL will have a liver biopsy for prognostic purposes. All those with positive DNA will be treated. Those who have negative DNA (ie not at risk of HHC) will be tested using liver biopsy if they have elevated SF or ALT, otherwise they will be monitored by further biochemical tests. Those with stable or decreasing SF will not be treated, those with increasing SF will have a liver biopsy. We assume that all those with raised iron studies and positive DNA test have HHC and will be treated.

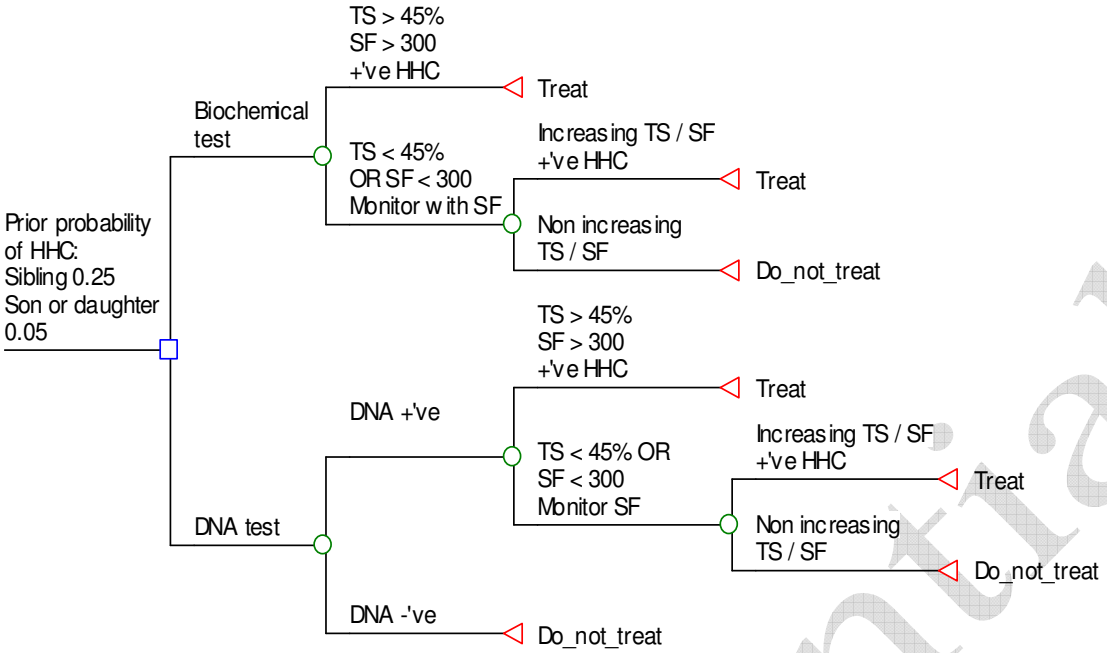
Different strategies can be run for different TS and SF levels.

2. Decision tree for the use of DNA testing in family members (see figure 2).

For family members the two strategies are

a) Biochemical test
b) DNA test

Figure 2 Decision tree for the use of DNA testing in family members



a) Relatives have serum ferritin and transferrin saturation tests. If they have raised iron levels, ie TS > 45% and SF > 300, they will be treated, if not they will be monitored. Those not treated will be monitored (ie retested) for a number of years to see if their iron level increases.

b) Relatives have a DNA test. Those with negative DNA test are discharged. Those with positive YY homozygous have biochemical tests. Those with raised iron levels are treated, those without are monitored.