Significant haemoglobinopathies: guidelines for screening and diagnosis

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Summary of key recommendations

Antenatal screening/testing of pregnant women should be carried out according to the guidelines of the NHS Sickle Cell and Thalassaemia Screening programme.

Newborn screening and, when necessary, follow up testing and referral, should be carried out according to the guidelines of the NHS Sickle Cell and Thalassaemia Screening programme. All babies under 1 year of age arriving in the UK should be offered screening for sickle cell disease.

Preoperative screening for sickle cell disease should be carried out in patients from ethnic groups in which there is a significant prevalence of the condition. Emergency screening with sickle solubility tests must always be followed by definitive analysis.

Objectives

Disorders of globin chain synthesis, both thalassaemias and haemoglobin variants, are common in the UK and constitute a significant public health problem. Diagnosis may be required: (i) to confirm a provisional diagnosis, such as sickle cell disease or β-thalassaemia major; (ii) to explain a haematological abnormality, such as anaemia or microcytosis; (iii) to identify an abnormality in the presymptomatic phase, as in neonatal screening; (iv) to identify fetuses at risk of significant haemoglobinopathies and offer the parents informed choice; (v) to permit genetic counselling of prospective parents; (vi) to identify the presence of sickle cell haemoglobin preoperatively.

Improved fully automated systems and reagents for techniques such as high-performance liquid chromatography (HPLC) and isoelectric focusing (IEF) have led to their introduction in many laboratories. There is also increasing use of other methods to identify globin gene abnormalities including DNA analysis, mass spectrometry and immunological methods.

There is therefore a need for an updated guideline defining the role of new techniques and their place in screening and in specific diagnostic settings. The detection of unstable haemoglobins, methaemoglobins and high and low oxygen affinity haemoglobins is not discussed but laboratories should either have methods for detecting these variant haemoglobins or should refer such samples to a reference laboratory.

It should be noted that the identification of haemoglobins is often presumptive, based on electrophoretic mobility or other characteristics in an individual of appropriate family origin. Presumptive identification should be based on a minimum of two techniques based on different principles. Definitive identification usually requires DNA analysis, mass spectrometry or protein sequencing. Family studies are also of considerable importance in elucidating the nature of disorders associated with sickle cell disease and thalassaemia.
of haemoglobin synthesis. As testing for haemoglobinopathies has implications for genetic counselling, informed consent should be obtained from individuals prior to testing.

Throughout these guidelines the term ‘sickle cell disease’ (SCD) encompasses both homozygous and the compound heterozygous states that lead to symptomatic disease as the result of the presence of haemoglobin S. Sickle cell anaemia refers specifically to those homozygous for $\beta^S$. These guidelines are intended for UK Haematologists and the approach to screening is that which is considered practical and feasible for the British population. Different strategies may be required for populations with a different prevalence of haemoglobinopathies.

Methods

These guidelines are an update of previous guidelines [British Committee for Standards in Haematology (BCSH) 1988, BCSH 1994a,b, 1998] and were written by clinical and laboratory experts representing areas of high and low prevalence of haemoglobin disorders. A patient representative was also included. Sections relating to antenatal and newborn screening are based on policies produced by the laboratory subcommittee of the National Health Service (NHS) Sickle Cell and Thalassaemia Screening programme and available in the programme’s Laboratory Handbook (NHS Sickle Cell and Thalassaemia Screening Programme 2009).

For this updated guideline PubMed, MEDLINE and EMBASE were searched systematically for publications in English from July 2005 to March 2008 using key words (see Appendix I). Other publications between 1995 and July 2005 were also considered. The writing group produced a draft guideline, which was subsequently reviewed by consensus by members of the General Haematology Task Force of the British Committee for Standards in Haematology. The guideline was then reviewed by a sounding board of approximately 65 UK haematologists, the BCSH and the British Society for Haematology Committee and comments incorporated as appropriate. Criteria used to quote levels and grades of evidence are as outlined in appendix 3 of the Procedure for Guidelines Commissioned for the BCSH (http://www.bcshguidelines.com/process1.asp#appendix7).

Introduction

Haemoglobin synthesis during development

The normal pattern of haemoglobin synthesis during embryonic, fetal and adult life is summarized in Fig 1.

Fetal haemoglobin, haemoglobin (Hb) F, $(\alpha_2\gamma_2)$ represents 90–95% of all haemoglobin by 34–36 weeks gestation. Adult haemoglobin, Hb A, $(\alpha_2\beta_2)$ accounts for 4–13% of total haemoglobin in the fetus. After 34 weeks gestation, Hb A production increases significantly as Hb F production falls. At term, Hb F represents 53–95% of all haemoglobin with Hb A levels reaching 20–30%. In addition to being increased in some haemoglobinopathies, increased levels of Hb F can be seen in infants who are small for gestational age, who have experienced chronic hypoxia or who have trisomy 13.

Haemoglobin F percentage remains static for the first 2 weeks of life and then decreases by approximately 3% per week when erythropoiesis recommences and is normally <2–3% of total haemoglobin by 6 months of age. Hb A becomes the predominant haemoglobin by 3 months of age, although this switch may be delayed in sick preterm infants.

Haemoglobin A$_2$ $(\alpha_2\delta_2)$ is produced in small amounts from birth and usually reaches adult levels by 6 months of age, although it can rise further for the first 1–2 years of life. Hb A$_2$ and Hb Bart’s $(\gamma_4$ tetramers) may be detected in normal infants born at term.

The pattern of haemoglobin synthesis during development explains why $\alpha$ chain abnormalities cause clinical problems from early fetal life and why $\beta$ chain abnormalities may be difficult to diagnose in the neonatal period.

Indications for screening and testing

Pre-conception testing

Pre-conceptual testing for haemoglobinopathies is recommended in at-risk groups (Table I). Pre-conceptual testing is important because it can be difficult to complete antenatal screening and fetal diagnosis within the first 12 weeks of pregnancy if the couple is unaware of the risk. This is most likely to be feasible in general practice but other medical practitioners should be alert to the possibility of a carrier state for a haemoglobinopathy and should offer screening. The individuals concerned must be informed of the result, whether or not an abnormality is found. If an abnormality is detected (Hb variant or possible/probable thalassaemia), partner testing...
should be offered, according to the antenatal testing algorithm (see below), if appropriate.

Pre-conceptual testing should always be performed in women being investigated for infertility and in those having assisted conception. If a woman is found to have or be a carrier for a significant haemoglobinopathy, the partner or other sperm donor should be tested, if appropriate, and the women given counselling. If a donor ovum is to be used, the donor should be screened for relevant haemoglobinopathies.

Pre-marriage screening for haemoglobinopathies is not usual in the UK but for some religious/ethnic groups pre-marital screening for β thalassaemia heterozygosity may be more acceptable than pre-conceptual or antenatal screening.

Antenatal

National Screening Committee (NSC) policy recommends antenatal screening for haemoglobinopathies (http://sct.screening.nhs.uk/policy). Clinically significant haemoglobinopathies that should be detected are shown in Table II. The recommended procedure differs according to whether the antenatal unit is in a high or low prevalence area for SCD and thalassaemia. For high prevalence areas (fetal prevalence of SCD 1-5 per 10 000 pregnancies or higher), universal laboratory screening and use of the Family Origin Questionnaire (FOQ) (Appendix II) is advised. For low prevalence areas (fetal prevalence of SCD <1.5 per 10 000 pregnancies), screening is based on assessing the individual risk by determining the family origin of the woman and her partner by means of the FOQ (NHS Sickle Cell and Thalassaemia Screening Programme 2009). Whichever screening method is applied, the laboratory must ensure a provisional report is available within three working days from sample receipt.

Screening in high prevalence areas. The screening policy for high prevalence areas starts with a full blood count (FBC) and HPLC or suitable alternative technique on a maternal blood sample. The women should also be asked to complete the FOQ or local equivalent. The request form sent to the laboratory should include the period of gestation and the results of the FOQ. Testing of the women should ideally be completed before 11 weeks and the whole process including partner testing, if applicable, should be completed within the first 12 weeks of pregnancy (NHS Sickle Cell and Thalassaemia

Table II. Conditions to be detected as part of the antenatal screening programme (NHS Sickle Cell and Thalassaemia Screening Programme 2009).

<table>
<thead>
<tr>
<th>Category</th>
<th>Conditions</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Significant maternal haemoglobinopathies</td>
<td>SS and other types of sickle cell disease (Hb SC, Hbs/βthalassaemia, etc.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β thalassaemia intermedia</td>
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<tr>
<td></td>
<td>Hb H disease (α−/α−β) (β thalassaemia major will be clinically apparent)</td>
<td></td>
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<tr>
<td>(ii) Maternal conditions requiring partner testing</td>
<td>Significant disorders to be detected in the fetus</td>
<td></td>
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<tr>
<td></td>
<td>a) Conditions in (i)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) Carrier states in mother</td>
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<tr>
<td></td>
<td>AS</td>
<td>SS</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>SC</td>
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<tr>
<td></td>
<td>AD-Punjab</td>
<td>SD-Punjab</td>
</tr>
<tr>
<td></td>
<td>AE</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td>AO-Arab</td>
<td>SO-Arab</td>
</tr>
<tr>
<td></td>
<td>A Lepore</td>
<td>S/Lepore and Hb βthalassaemia/Lepore</td>
</tr>
<tr>
<td></td>
<td>β thalassaemia trait</td>
<td>S/β thalassaemia</td>
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<tr>
<td></td>
<td>δβ thalassaemia trait</td>
<td>S/δβ thalassaemia</td>
</tr>
<tr>
<td></td>
<td>αβ thalassaemia trait (α−/α−β)</td>
<td>Hb Bart’s hydrops fetalis (α−/α−β)</td>
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<tr>
<td></td>
<td>HPFH</td>
<td>β thalassaemia major (except cases with silent or near silent maternal phenotype)</td>
</tr>
<tr>
<td></td>
<td>c) Any compound heterozygote state including one or more of the above conditions</td>
<td>E/β thalassaemia</td>
</tr>
<tr>
<td></td>
<td>d) Any homozygous state of the above conditions</td>
<td></td>
</tr>
</tbody>
</table>

Table I. Ethnic groups with a clinically significant prevalence of haemoglobin S and α and β thalassaemia.

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Specific Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin S</td>
<td>African including North Africans, African-Caribbeans, African-Americans, Black British and any other African ethnicity (e.g. Central and South Americans of partly African ethnicity), Greeks, Southern Italians including Sicilians, Turks, Arabs, Indians</td>
</tr>
<tr>
<td>αβ thalassaemia</td>
<td>Chinese, Taiwanese, South-East Asian (Thai, Laotian, Cambodian, Vietnamese, Burmese, Malaysian, Singaporean, Indonesian, Philippino), Cypriot, Greek, Turkish and Sardinian</td>
</tr>
<tr>
<td>β thalassaemia</td>
<td>All ethnic groups other than Northern Europeans</td>
</tr>
</tbody>
</table>
Guideline

Screening Programme 2006, 2009, National Collaborating Centre for Women’s and Children’s Health 2008). However, even women presenting for the first time late in pregnancy should be offered testing because the results will be relevant both to this and future pregnancies.

Screening in low prevalence areas. For low prevalence areas it is recommended that all women be offered screening and the FOQ used to determine their family origin and that of the baby’s father. All should have an FBC and the red cell indices should be assessed and acted on in a similar manner as for high prevalence areas. Haemoglobin analysis is otherwise confined to those women whose own or the baby’s father’s family origin is not Northern European or is unknown.

Assessment of abnormal antenatal screening results

Detection of a haemoglobin variant. If a significant Hb variant is identified it should be confirmed by a suitable alternative method (e.g. haemoglobin electrophoresis or IEF if the initial method was HPLC) and the baby’s father should be offered screening without waiting for the definitive result if he has not been tested before or his result is not available. Variant haemoglobins of clinical relevance in this context are haemoglobins S, C, D-Punjab, E, H, Lepore and O-Arab.

Raised Hb A2 percentage. If no relevant variant haemoglobin is identified, Hb A2 percentage is assessed, when appropriate. This is essential if the mean cell haemoglobin (MCH) is <27 pg. Hb A2 level of ≥3.5% in the presence of a MCH <27 pg indicates heterozygosity for β thalassaemia. A Hb A2 of >4% with a normal MCH should be assessed further as it may indicate a milder β thalassaemia carrier state that would warrant testing of the baby’s father (see below). If the Hb A2 is apparently >10% on HPLC, a diagnosis of Hb Lepore should be considered while a Hb A2 level apparently >15% may indicate Hb E trait. Other variant haemoglobins also have a retention time similar to that of Hb A2 on HPLC.

Raised Hb F percentage. In the context of an MCH <27 pg, an isolated raised Hb F of >5% identifies possible heterozygosity for δβ thalassaemia and testing of the baby’s father is required. In the presence of a normal MCH, hereditary persistence of fetal haemoglobin (HPFH) should be considered.

Possible α thalassaemia heterozygosity. In the absence of a variant Hb and β or δβ thalassaemia heterozygosity, α thalassaemia carrier states should be considered if the MCH is <27 pg. This should be considered regardless of iron status as there is insufficient time in the antenatal setting to re-assess indices after iron treatment. If the MCH is <25 pg, the individual should be assessed for the possibility of α thalassaemia heterozygosity in the light of his or her family origin. Family origins that indicate that α thalassaemia is possible are shown in Table I. An alternative explanation for these laboratory findings is homozygosity for α thalassaemia or iron deficiency, which may also be seen in these ethnic groups.

α thalassaemia is found in many ethnic groups, with a high (10–30%) carrier frequency in some parts of Africa and South Asia. Even if both partners are carriers, there is no risk to the fetus. Homozygous α thalassaemia is not a clinically significant disorder with respect to genetic or obstetric complications, but can cause diagnostic confusion with α thalassaemia trait or iron deficiency.

• Heterozygotes (carriers) generally have a MCH of 25–28 pg and a normal Hb A2 level. Approximately one-third of cases are silent.

• Homozygotes generally have a MCH <25 pg, as seen in carriers for α thalassaemia.

If the MCH is <25 pg and α thalassaemia is possible, the approach that makes best use of resources is to assess the family origin and red cell indices of the partner and proceed to DNA analysis, simultaneously in the woman and the baby’s father, only if both are at risk of α thalassaemia. However, if there is any delay in obtaining a blood sample from the baby’s father or if he is not available, then it is appropriate to test the mother’s DNA. The partners of women with haemoglobin H disease also require assessment for α thalassaemia. Consent for DNA testing is a legal requirement; this can be obtained at the initial antenatal clinic consultation to avoid any delay later. Ultrasound to detect fetal anaemia may be offered as an alternative to fetal DNA testing from 12 to 16 weeks in cases where the latter is declined.

α thalassaemia occurs, rarely, in other ethnic groups, e.g. Pakistanis, Indians, some Middle Eastern populations (from United Arab Emirates, Iran, Yemen, Kuwait, Syria, Jordan) and in individuals originating in North-West England (Wigan and other parts of Lancashire). In the Middle East there is also a significant prevalence of non-deletional α thalassaemia, which can give rise to severe haemoglobin H disease in homozygotes. Individuals from such areas should be assessed individually but in general DNA analysis and partner testing is not recommended. The rare cases of Bart’s hydrops fetalis should be detected on ultrasound screening.

It should be noted that a diagnosis of β thalassaemia heterozygosity does not exclude co-existing α thalassaemia heterozygosity and, in ethnic groups with a significant prevalence of the latter, DNA analysis is indicated when relevant to reproductive choice. For example, if one partner has β thalassaemia heterozygosity and the other possible α thalassaemia heterozygosity, both partners should be offered testing for α thalassaemia.

Newborn screening

National Screening Committee and NHS Policy is that all newborn babies should be screened for SCD. Such screening
should also be extended to babies under the age of 1 year newly arrived in the UK (NHS Sickle Cell and Thalassaemia Screening Programme 2009). The main objective of the newborn screening programme is to improve outcomes in SCD through early treatment and care. The screening programme will also detect certain other variant haemoglobins by virtue of the analytical methods currently used. Additionally, the finding of Hb F only or of a very low percentage of haemoglobin A (<1.5%) on the newborn screen will identify the majority of babies with β thalassaemia major. Neonatal screening is based on the mother’s place of residence and is done at the age of 5–8 days as part of the newborn dried blood spot screening programme (http://www.newbornscreening-bloodspot.org). Further testing of samples that show a significant abnormality is required. Informed parental consent is required and parents have the right to opt out of testing although the programme is recommended. Opting out should be documented.

Clinically significant conditions that should be detected are shown in Table III. In addition, certain conditions that are asymptomatic or have a mild phenotype will be identified and need to be subsequently distinguished from clinically significant abnormalities. For example, sickle cell/HPFH needs to be distinguished from severe forms of SCD and Hb E homozygosity needs to be distinguished from Hb E/β thalassaemia. Clinical follow-up, counselling and repeat testing is arranged for all babies in whom there is the possibility of a clinically significant abnormality and results are notified to the parents, general practitioner and responsible health care consultant at the place of the baby’s birth. In the case of suspected SCD, confirmatory testing and clinical follow-up should be performed in a timely manner so that penicillin prophylaxis can be started by 3 months of age; conjugate pneumococcal vaccine is now given to all babies from 8 weeks of age but is particularly important if the child has SCD (http://sct.screening.nhs.uk/cms.php?folder=2465). Clinical follow-up is also necessary for all babies with Hb F only. Babies who have been transfused in utero or in the early neonatal period are now tested using DNA techniques for the presence of the sickle gene as part the NHS Sickle Cell and Thalassaemia Screening programme. If this is not available or is declined, repeat testing should be performed 4 months from the date of transfusion. Other variant haemoglobins of potential clinical or genetic significance (e.g. haemoglobins C, D, E, O-Arab) will also be detected and in this case the parents, general practitioner and responsible health care practitioner at the place of the baby’s birth should be informed, and parents offered counselling.

General testing

Opportunistic testing may be initiated by a general practitioner or other medical practitioner, with the informed consent of the patient, or by a haematology laboratory, where an abnormality that requires explanation is detected, e.g. individuals found to have red cell indices or morphological appearances suggestive of a haemoglobinopathy. Opportunistic testing for sickle cell heterozygosity is best initiated in general practice. Reflex testing by the laboratory will depend on local policy.

Pre-operative/pre-anaesthesia

It is important to detect SCD prior to anaesthesia because its presence will influence clinical management. Testing should be initiated by clinical staff on the basis of a clinical history and assessment of family origin. All patients from groups with a high prevalence of Hb S (Table I) should be offered testing as some cases of milder disease may be unrecognized and the presence of Hb S heterozygosity may also influence perioperative techniques. For example, cell salvage techniques carry a theoretical risk of red cell sickling and are probably contraindicated, and the use of limb tourniquets should be considered carefully although there is little evidence on which to base recommendations. Appropriate counselling should be given before testing so that patients are able to give their informed consent as there may be implications for patients who discover they are carriers of the sickle cell gene. The patient/parent or guardian (for children) should be informed of the results of testing, even when negative and the result documented in the patient’s medical record to avoid unnecessary repeat testing. Counselling should be offered if the result of the test is positive [National Institute for Clinical Excellence (NICE) 2003].

For routine operations, FBC and haemoglobin analysis using HPLC or a suitable alternative diagnostic method should be performed at the pre-assessment visit. In an emergency, an FBC and a sickle solubility test should be performed. Results in this situation should be evaluated clinically and must be followed by definitive testing (see below).

Table III. Haemoglobinopathies likely to be detected in the neonatal screening programme (NHS Sickle Cell and Thalassaemia Screening Programme 2009).

<table>
<thead>
<tr>
<th>Sickle cell disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell anaemia (SS)</td>
</tr>
<tr>
<td>Sickle cell/β thalassaemia</td>
</tr>
<tr>
<td>Sickle cell/hemoglobin C disease</td>
</tr>
<tr>
<td>Sickle cell/hemoglobin D-Punjab</td>
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<tr>
<td>Sickle cell/hemoglobin O-Arab</td>
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<tr>
<td>Sickle cell/hemoglobin Lepore</td>
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<tr>
<td>Sickle cell/β thalassaemia</td>
</tr>
<tr>
<td>Sickle cell/hemoglobin E</td>
</tr>
<tr>
<td>Sickle cell/hereditary persistence of fetal haemoglobin</td>
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</tbody>
</table>

Other clinically significant haemoglobinopathies

Most cases of β thalassaemia major
Most cases of β thalassaemia intermedia
Most cases of haemoglobin H disease
Haemoglobin E/β thalassaemia
Guideline

Investigation of microcytosis outside the antenatal situation

Patients who present for diagnosis should be distinguished from individuals, often healthy, who are being screened for haemoglobinopathies. Such patients require a clinical history and physical examination and the FBC should be assessed, together with other laboratory tests, in the light of the clinical context and family origin. If there is microcytosis, appropriate tests for iron deficiency and anaemia of chronic disease should be performed and testing for thalassaemia considered in patients of appropriate family origin. Depending on agreed local policies, such tests may be initiated by the laboratory. Some laboratories use various published formulae to decide when to initiate such investigations but it should be noted that such formulae are not likely to be reliable in children or pregnant women or in sick patients who may have multiple medical problems influencing the Hb and red cell indices. Haemoglobinopathy investigations should therefore be considered in any unexplained microcytosis, even if the red cell indices are not typical of thalassaemia or another haemoglobinopathy.

Diagnosis and testing in the management of clinical disorders

Haemoglobinopathy investigations may be indicated in the following circumstances:

- In new arrivals in the UK with a history of SCD or significant thalassaemia
- In patients with SCD receiving blood transfusion, including exchange transfusion, with the aim of lowering the percentage of Hb S
- In patients with SCD or β thalassaemia intermedia being administered hydroxycarbamide or other agents to raise the Hb F percentage. HPLC or Hb electrophoresis is satisfactory but it should be noted that some programmes on some HPLC instruments underestimate Hb F levels (see below).
- In the investigation of other unexplained microcytosis or laboratory findings (see Table IV)

Recommendation

General practitioners should consider pre-conceptual screening/testing of their patients of child-bearing age.

General practitioners should consider and discuss pre-marital screening/testing of patients of child-bearing age when prenatal testing is unacceptable to the individuals.

Clinics carrying out assisted conception should always carry out relevant screening and, when necessary, testing of both the egg donor and/or the sperm donor.

Antenatal screening/testing of pregnant women should be carried out according to the guidelines of the NHS Sickle Cell and Thalassaemia Screening programme.

Newborn screening and, when necessary, follow up testing and referral, should be carried out according to the guidelines of the NHS Sickle Cell and Thalassaemia Screening programme. Babies under 1 year of age arriving in the UK should be offered screening for SCD as part of the blood spot screening programme.

Preoperative testing should be carried out in patients from ethnic groups in which there is a significant prevalence of sickle cell haemoglobin. Emergency screening with a sickle solubility test and full blood count must always be followed by definitive analysis.

The need to investigate for thalassaemia and haemoglobinopathies should be considered in patients with unexplained microcytosis.

Patients with SCD who are being treated by blood transfusion or with hydroxycarbamide should be appropriately monitored.

Laboratory methods

The availability of fully automated systems and reagents for techniques, such as cation-exchange HPLC and IEF, have led to their introduction in a large proportion of laboratories, replacing cellulose acetate electrophoresis (CAE) as a first-line screening method. The use of mass spectrometry is becoming more widespread for variant identification and may have potential for screening also.

The choice of methodology and equipment will be based on volume of workload, sample material (liquid blood or dried blood spots), ease of handling, reproducibility, local availability and expertise and cost. Although the consumables for CAE are inexpensive, the labour costs are relatively high. Therefore, a switch to HPLC may be cost-neutral when many samples are to be analysed (Phelan et al, 1999). Principles and methodology of the techniques used for haemoglobin analysis are outside the scope of this guideline but are available in standard text books.

Cellulose acetate electrophoresis

Haemoglobin electrophoresis at pH 8.4–8.6 using a cellulose acetate membrane is simple, reliable and rapid. It enables the provisional identification of haemoglobins A, F, S/G/D, C/E/O-Arab, H and a number of less common variant haemoglobins. Differentiation between haemoglobins migrating to a similar position can be obtained by using electrophoresis on acid (agarose) gels, HPLC or IEF. The provisional identification of any variant haemoglobin should be supported by at least one further unrelated method. Application of an alternative technique will exclude the possibility that a single band in either the S or C position represents a compound heterozygous state such as SD or SG and CE or CO-Arab respectively. If a patient has microcytosis the possibility that a single band represents compound heterozygosity for a variant haemoglobin and β0 thalassaemia must also be considered.
Variant haemoglobins, such as Hb S can be quantified by scanning densitometry after electrophoresis/staining; however quantification of haemoglobin A2 by this method is not recommended as the precision is not good enough for the diagnosis of β-thalassaemia trait (BCSH 1998). CAE is time consuming when a large number of samples are to be analysed.

High-performance liquid chromatography (HPLC)

High-performance liquid chromatography can be used for the quantification of haemoglobins S, A2 and F and for the detection, provisional identification and quantification of many variant haemoglobins. HPLC usually provides accurate quantification of Hb A2 and is therefore suitable for the diagnosis of β-thalassaemia trait. Automated HPLC systems are being used increasingly as the initial diagnostic method in laboratories with a high workload. In comparison with haemoglobin electrophoresis, HPLC has the following advantages:

1. The analysers are automated, therefore require less staff time and permit processing of large batches.
2. Very small sample volumes (5 μl) are sufficient for analysis.
3. Quantification of normal and separated variant haemoglobins is available on every sample.
4. Provisional identification of a larger proportion of variant haemoglobins can be made.
5. δ chain variants (recognition of which is important in the diagnosis of β-thalassaemia heterozygosity) are more easily detected.

High-performance liquid chromatography usually separates haemoglobins A, A2, F, S, C, D-Punjab and G-Philadelphia from each other. However, both Hb E and Hb Lepore often co-elute with A2 (as other haemoglobins co-elute with A, S and F) but may be recognized by alternative techniques. HPLC has the disadvantage that it also separates glycosylated and other derivative forms of haemoglobin, which can make interpretation more difficult. For example, derivatives of haemoglobin S co-elute with haemoglobin A2, rendering its quantification inaccurate. Careful examination of every chromatogram is essential. As with every method of haemoglobin analysis, controls should be run with every batch. Identification of variants is only provisional, and unrelated second-line methods should be used for confirmation.

If HPLC is used as the screening technique, it is essential to check and maintain the positions of the windows, which are used as the first stage identification of any variants found. This is generally done by adjusting the column temperature or the flow rate so that the Hb A2 peak appears at a standard time. This is just as important as the calibration of the Hb A2 and Hb F levels and should be checked daily. Appropriate controls should be included wherever possible.

Isoelectric focusing (IEF)

Isoelectric focusing is satisfactory for the analysis of whole blood samples, haemolysates or dried blood spots. IEF gives good separation of Hb F from Hb A and clinically significant variant haemoglobins (S, C, D-Punjab, E and O-Arab). IEF can be semi-automated, rendering the technique suitable for screening large numbers of samples. However, this technique has not been validated for HbA2 quantification.

Although IEF has better resolution and the advantage that it separates more variants than CAE, it also has the disadvantage that it separates haemoglobin into its

| Table IV. Indications for haemoglobinopathy investigations in investigation of clinical disorders. |
|-----------------------------------------------|-----------------------------------------------|
| Indication | Abnormality tested for |
| Hydropic fetus | Haemoglobin Bart’s hydrops fetalis |
| Neonate or infant with anaemia and either haemoglobin F only or an unexpectedly low percentage of haemoglobin A | β thalassaemia major |
| Unexplained anaemia and splenomegaly | β thalassaemia major or intermedia, haemoglobin H disease, unstable haemoglobin |
| Suspected thalassaemia or unexplained microcytosis | Thalassaemias including haemoglobin H disease |
| Clinical and haematological features suggestive of sickle cell disease | Haemoglobin S and interacting haemoglobins (C, D-Punjab, O-Arab) or β thalassaemia, Haemoglobin H disease, unstable haemoglobin |
| Unexplained haemolysis | Thalassaemia, variant haemoglobin |
| Unexplained target cells | Variant haemoglobin, particularly haemoglobin C or an unstable haemoglobin |
| Unexplained irregularly contracted cells | High affinity haemoglobin |
| Unexplained polycythaemia | Haemoglobin M |
| Unexplained cyanosis with normal oxygen saturation | |

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post-translational derivatives e.g. Hb F separates into F\(_1\) (acetylated) and F\(_{1s}\); Hb A can separate into A\(_0\), A\(_1\), A(α\(_{\text{met}}\)), A(β\(_{\text{met}}\)) and A(αβ\(_{\text{met}}\)) – and similarly for other haemoglobins. This makes interpretation more difficult. Identification of variants is still only provisional, and second-line methods should be used for further analysis.

Sickle solubility test

The kits for sickle cell solubility tests that are predominantly used in the UK will detect haemoglobin S down to a concentration of 20% (and sometimes below; in some cases as low as 8%) (BCSH 1998). The method of Lewis et al (2006), although less sensitive than some commercial kits, can detect Hb S down to a concentration of 20%. The methods are therefore capable of detecting all cases of sickle cell trait beyond the period of infancy, even when there is coexisting \(\alpha\) thalassaemia trait (but possibly not when there is coexisting Hb H disease). False positives have been described in patients with high plasma protein levels (Canning & Huntsman, 1970) and in anaemic patients when double the volume of blood is used in the test (Arras & Perry, 1972; Lilleyman et al, 1972). The latter problem can be avoided, however, by using a more concentrated sample of blood or washing the red cells.

All positive and equivocal sickle solubility tests should be confirmed by HPLC or an alternative technique both for confirmation of the presence of Hb S and to distinguish sickle cell trait from sickle cell anaemia and from compound heterozygous states. In an emergency, e.g. pre-anaesthesia, this distinction can be made with reasonable accuracy with a sickle solubility test combined with a blood film and a blood count. It is also recommended that all negative sickle solubility tests be confirmed by HPLC or an alternative technique. Conversely, sickle solubility testing should be employed whenever an unknown haemoglobin is encountered that elutes in the position of the ‘Hb S Window’ by HPLC or is in the position of Hb S on CAE or IEF.

In general, a sickle solubility test is not indicated in an infant before the age of 6 months because a negative result may be misleading. However, a sickle solubility test can sensibly be performed in an emergency, prior to anaesthesia, as if it is negative it is unlikely that anaesthesia will cause any clinical problems because the Hb S % will be too low. The wording of the report on such a test must state that a negative test does not exclude the presence of a low percentage of haemoglobin S and that further testing is necessary and will follow.

Detection of haemoglobin H bodies (Hb H bodies)

Haemoglobin H bodies are intracellular precipitates of β\(_4\) tetramers seen in red cells following supravital staining with brilliant cresyl blue. They are seen in conditions where there is an excess of β globin chains, which nearly always results from a deficiency of α globin. They can be detected in carriers of \(\alpha\) thalassaemia and in Hb H disease as well as in \(\alpha\) thalassaemia and mental retardation (ATRX syndrome) and acquired Hb H disease.

Detection of Hb H bodies has been used to try and distinguish between homozygous \(\alpha^+\) (\(\alpha/\alpha\)) and heterozygous \(\alpha^0\) (\(\alpha/\alpha^+\)). These two conditions are haematologically indistinguishable, although only the latter can lead to a fetus with Hb Bart’s hydrops fetalis syndrome in the homozygous state. Although Hb H bodies are much easier to find in \(\alpha^0\) heterozygosity this is an unreliable test which is very time consuming, and has been superseded by DNA analysis. Detection of Hb H bodies is useful to confirm the diagnosis of Hb H disease, in which golf-ball like inclusions are seen in about 5% of red cells following supravital staining. It is also useful to look for Hb H bodies in cases of suspected ATRX syndrome or acquired Hb H disease. Both of these are typically suspected when there is otherwise unexplained hypochromia in the presence of characteristic clinical features, although confirming the diagnosis is difficult. Detection of Hb H bodies in these circumstances can be diagnostic. The number of Hb H bodies varies from very occasional in some cases of ATRX to more than 5% in acquired Hb H disease.

Hb A\(_2\) measurement

Microcolumn chromatography or HPLC may be used to quantify Hb A\(_2\). Electrophoresis with elution is acceptable but only in a laboratory performing the technique regularly. IEF and scanning densitometry are not acceptable. No confirmatory test is necessary if the Hb A\(_2\) is raised (but <10%) and the red cell indices are typical of \(\beta\) thalassaemia trait. Hb Lepore and Hb E may appear in the A\(_2\) window and should be considered if the level is >10%. Occasional other haemoglobin variants appear in the A\(_2\) window so any value above 10% should be assessed further.

Quantification of Hb F

Quantification of Hb F is indicated if raised Hb F is detected beyond infancy, e.g. in SCD, thalassaemia major or intermedia, suspected HPHF, suspected \(\delta\beta\) thalassaemia and bone marrow failure syndromes, such as juvenile myelomonocytic leukaemia and Diamond–Blackfan anaemia. A Kleihauer test may assist in differentiation of these conditions although DNA testing has superseded its use in the UK. However a Kleihauer test can still be used to confirm that Hb in the Fwindow on HPLCis actually Hb F.

The 2-min alkali denaturation test is suitable for the quantification of Hb F levels <15%, but significantly underestimates higher levels. Quantification by HPLC has the disadvantage of separating, and thus excluding the adducted (post-translational) derivatives of Hb F, which can lead to a significant underestimate at high levels. Additionally, there can be interference from Hb A\(_1c\), and some variant haemoglobins that co-elute with Hb F by HPLC.
Selection of laboratory methods

Screening for abnormal variants. The analytical procedures employed must be capable of detecting all the common clinically significant haemoglobin variants, i.e. S, C, D-Punjab, E, O-Arab and Lepore, and must be suitable for screening for thalassaemias. The following techniques can be used in antenatal screening for haemoglobin variants:

- High performance liquid chromatography (HPLC)
- Isoelectric focusing (IEF)
- Cellulose acetate electrophoresis (CAE)

Abnormal results should be confirmed by a different technique that is appropriate for the likely variant. Another technique that can be used for confirmation, besides those listed above, is acid agar or acid agarose electrophoresis although this is not suitable as a screening technique.

Sickle solubility testing can be used as confirmation of an initial screen that suggests the presence of sickle haemoglobin (see above).

Screening for thalassaemia. Methods used are red cell indices in conjunction with measurement of Hb A2 levels. Routine measurement of blood indices includes measurements of MCH and mean cell volume (MCV); it is recommended that MCH is used to screen for thalassaemia as this parameter is more stable than MCV. These measurements are usually reported for all routine blood counts. Hb A2 is quantified by HPLC or microcolumn chromatography.

Selecting an HPLC system. When choosing an HPLC system for use in antenatal screening for sickle cell and thalassaemia, some general considerations need to be borne in mind.

A national recommended cut-off Hb A2 of 3.5% or above has been set as the action point in the diagnosis of carriers of β thalassaemia. A value of 5% for Hb F has been set for the investigation of a raised fetal haemoglobin in pregnancy. The chosen system must therefore be able to measure Hb A2 and Hb F with accuracy and precision at these cut-offs and detect the haemoglobin variants as specified by the antenatal screening programme. Peaks should be clearly separated for accurate quantification. Laboratories should understand how integration takes place and be aware that peaks measured on sloping baselines or on shoulders of adjacent peaks are likely to be less reliable (NHS Sickle Cell and Thalassaemia Screening Programme 2009).

High-performance liquid chromatography systems must be able to detect Hb A2 variant peaks, due to α or δ chain variants, and quantify them accurately. These should be added into the total Hb A2 percentage. In a patient with an MCH below the cut-off point (<27 pg), further investigation will be required if the total Hb A2 is above 3.5%.

If using equipment or an elution programme for more than one analyte, e.g. Hb A2 and Hb A1c, laboratories should ensure that the quantification of Hb A2 and Hb F is not compromised. This may require a different column/buffer system.

Problems with the measurement and interpretation of Hb A2

Besides the difficulties associated with the separation and quantification of small peaks or bands in any chromatographic or electrophoretic system, some other factors need to be considered when interpreting Hb A2 results.

With many HPLC systems, Hb A2 is overestimated in the presence of Hb S. However, this is not a problem because in sickle cell trait the percentage of Hb A is greater than Hb S and the reverse is true in Hb S/β thalassaemia. The Hb A2 may be lowered by up to 0.5% in cases of severe iron deficiency anaemia.

Hb A2 values >4.0% with normal indices may indicate β thalassaemia trait with or without co-existing α thalassaemia. In this case:

- Re-analyse FBC
- Repeat Hb A2

In the case of preconceptual or antenatal testing, consider the results in the partner and arrange DNA analysis when appropriate. The α thalassaemia risk needs to be considered in the light of the family origin of the patient. The major risk is for β thalassaemia, but the risk of Hb Bart’s hydrops fetalis should not be overlooked.

Hb A2 values ≤4.0% with normal red cell indices and a normal Hb F level can usually be regarded as normal, although some mild β thalassaemia alleles (mainly in subjects of Mediterranean origin) are associated with an A2 of 3.5–4.0%.

Interpretation of results in the presence of iron deficiency

Severe iron deficiency anaemia (Hb<80 g/l) can reduce the Hb A2 level slightly (by up to 0.5%). Outside of pregnancy, anaemia should be treated and the haemoglobin analysis repeated when the patient is iron replete. In pregnant women there is no justification for delaying the investigation for haemoglobinopathies whilst treating iron deficiency presumptively, as this will delay the process of identifying at-risk carrier couples, who could be offered prenatal diagnosis. It may be appropriate to simultaneously investigate pregnant women for iron deficiency, using ferritin or zinc protoporphyrin (ZPP), but this is not specifically part of haemoglobinopathy screening.

Newborn screening/testing

The NHS Sickle Cell and Thalassaemia Screening Programme advises that all neonates be offered screening for variant haemoglobin (http://sct.screening.nhs.uk/cms.php?folder=2425). Previous recommendations and guidelines have been published elsewhere, e.g. (BCSH 1988, 1994a,b).

The dried blood spot card used for sickle screening is the same as used for the other newborn screening programmes and
samples are taken at 5–8 days. When clinically indicated, such as in the situation of a high-risk pregnancy, testing in advance of screening can be carried out on the baby using a liquid sample direct from the baby. This must be done in a laboratory experienced in the interpretation of newborn samples. Two methods of analysis are currently recommended for newborn screening for sickle cell disorders from dried blood spot samples: HPLC and IEF. Either of these is suitable for first-line screening, the alternative procedure being used for second-line testing to validate the presumed identity of the variant. Newer analytical methods using tandem mass-spectrometry, DNA analysis, immunological reagents or capillary electrophoresis may be suitable for newborn screening but have not been fully evaluated.

The application of HPLC and IEF for newborn screening has the disadvantage that the process also separates the normally occurring adducted fractions, i.e. acetylated Hb F (Hb F1) and glycated haemoglobin, which can make interpretation difficult. When using the percentages of the haemoglobin fractions to interpret the results, the possibility of the presence of transfused blood should also be considered.

The analytical procedures employed must be capable of detecting all the common clinically significant haemoglobin variants, i.e. S, C, D-Punjab, E, Lepore and O-Arab, in addition to Hb F and Hb A. Neonatal samples are typically composed of mostly Hb F (approximately 75%) with approximately 25% Hb A and small quantities of acetylated Hb F and sometimes Hb Bart’s. The procedures used must therefore be sensitive, reliable and reproducible in terms of detecting small quantities of Hb A and the abnormal haemoglobin fractions listed, in the presence of large amounts of Hb F.

It is also important to realize that occasionally the presumptive identification of a haemoglobin variant using screening methods is incorrect, because some variants give exactly the same results using current screening techniques. The sensitivity and specificity are approximately 99% for the methods used. Unequivocal identification of haemoglobin variants can only be achieved by either protein analysis (e.g. mass spectrometry) or DNA analysis.

**Recommendation**

Abnormal laboratory screening results should be confirmed by a different technique that is appropriate for the likely abnormality.

Laboratories performing antenatal screening should utilize methods capable of detecting significant variants and be capable of measuring Hb A2 and Hb F at the cut-off points required by the national antenatal screening programme.

Quantification of Hb A2 by CAE plus scanning densitometry is not recommended.

A sickle cell solubility test is not generally indicated in infants below the age of 6 months and is not recommended as a primary screening tool except in an emergency situation. All sickle solubility tests should be confirmed by HPLC or an alternative method.

Assessment of iron status may be useful in the interpretation of laboratory tests but should not delay partner testing in the antenatal screening programme.

Examination for Hb H bodies cannot reliably distinguish between α thalassaemia traits and should not be used for screening.

Laboratories should be aware of the conditions that may not be detected when using the Antenatal and Newborn Screening Programmes’ algorithms and also of the effect of blood transfusion on the interpretation of results.

**Quality assurance and laboratory standards**

**External Quality Assessment**

External Quality Assessment (EQA) provides a long-term, retrospective assessment of laboratory performance, allowing laboratories to demonstrate consensus with their peers and providing information on inter-method comparability. Participation in EQA is required for laboratory accreditation and is an essential part of clinical governance.

National Health Service policy requires antenatal and newborn screening laboratories both to participate in an accredited EQA scheme and to demonstrate satisfactory performance. Within the UK, participants in the UK National External Quality Assessment Scheme (UK NEQAS) for General Haematology’s haemoglobinopathy suite of schemes are requested to give written permission for their identity to be disclosed to the Director of the National Programme for Sickle and Thalassaemia Screening in the event of persistent unsatisfactory performance.

Laboratory standards for antenatal and newborn screening have been defined by the NHS Sickle Cell and Thalassaemia Screening Programme and are available in their laboratory handbook (NHS Sickle Cell and Thalassaemia Screening Programme 2009).

**Indications for DNA referral**

The policy guidance developed by the National Screening Programme, which is subject to review, should mean that DNA analysis is performed only in a limited number of cases and not usually in those where α+ thalassaemia is suspected. The majority of couples at risk of having a child affected with β thalassaemia or SCD should be identified initially by routine laboratory techniques through the antenatal screening programme.

The diagnosis of α thalassaemia is more complicated because DNA analysis is the only accurate way to distinguish between α+ and α0 thalassaemia. However it is not practical to seek to confirm all potential cases of α thalassaemia by DNA analysis because the α+ form is too common and not usually clinically important; it is not cost-effective for DNA laboratories to perform analysis on all such cases. Furthermore, non-dele-
thought and rapid methods for their detection are not available.

Table V (NHS Sickle Cell and Thalassaemia Screening Programme 2009) summarizes the main genetic risk combinations that require antenatal screening actions, according to the antenatal screening recommendations, and indicates which cases require referral of samples for further studies by DNA analysis. For other haemoglobinopathy combinations, results should be assessed by a consultant expert in the field.

**Standardized reporting**

Testing for haemoglobinopathies is a complex area because of the number of haemoglobin disorders that are detectable. The National Screening Programme has produced a set of report formats for both newborn and antenatal screening that should allow all normal and 95% of abnormal results to be reported in a standardized manner (NHS Sickle Cell and Thalassaemia Screening Programme 2009). However, because of the diversity of haemoglobin variants and thalassaemia syndromes, there

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**Table V. Referral guidelines for antenatal screening specimens (NHS Sickle Cell and Thalassaemia Screening Programme 2009).**

<table>
<thead>
<tr>
<th>Maternal carrier state</th>
<th>Paternal carrier state</th>
<th>Further studies by DNA analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No abnormalities detected</td>
<td>Partner testing not required</td>
<td>None required</td>
</tr>
<tr>
<td>Any abnormal Hb</td>
<td>No abnormality detected</td>
<td>None required</td>
</tr>
<tr>
<td>Hb S</td>
<td>Hb S or Hb C</td>
<td>None required until PND</td>
</tr>
<tr>
<td>Hb S</td>
<td>Hb O-Arab, D-Punjab, Lepore,</td>
<td>Send bloods for mutation confirmation of paternal carrier</td>
</tr>
<tr>
<td></td>
<td>β thalassaemia</td>
<td></td>
</tr>
<tr>
<td>Hb S</td>
<td>HPFH</td>
<td>Send bloods for mutation confirmation of paternal carrier</td>
</tr>
<tr>
<td>Hb C</td>
<td>Hb S</td>
<td>None required until PND</td>
</tr>
<tr>
<td>Hb D-Punjab</td>
<td>Hb S</td>
<td>Send bloods for mutation confirmation for suspected D-Punjab</td>
</tr>
<tr>
<td>Hb O-Arab</td>
<td>Hb S</td>
<td>Send bloods for mutation confirmation of Hb O-Arab</td>
</tr>
<tr>
<td>Hb Lepore</td>
<td>Hb S, E, O-Arab, Lepore,</td>
<td>Send bloods for mutation confirmation of Hb Lepore or Hb O-Arab</td>
</tr>
<tr>
<td></td>
<td>β thalassaemia</td>
<td></td>
</tr>
<tr>
<td>Hb E*</td>
<td>β thalassaemia, Hb Lepore</td>
<td>Send bloods for mutation confirmation of β thalassaemia, Hb Lepore</td>
</tr>
<tr>
<td>β thalassaemia</td>
<td>Hb S, E, O-Arab, Lepore,</td>
<td>Send bloods for mutation confirmation of β thalassaemia, Hb Lepore</td>
</tr>
<tr>
<td></td>
<td>β thalassaemia</td>
<td></td>
</tr>
<tr>
<td>β thalassaemia*</td>
<td>Suspected α thalassaemia (MCH &lt;25 pg)</td>
<td>Send bloods for mutation confirmation if of appropriate Mediterranean or SE Asian origin</td>
</tr>
<tr>
<td>Suspected α⁺ thalassaemia (MCH of 25–27 pg)</td>
<td>Partner testing not required</td>
<td>None required</td>
</tr>
<tr>
<td>Suspected heterozygous α⁰ or homozygous α⁺ thalassaemia (MCH &lt;25 pg)</td>
<td>Partner testing not required</td>
<td>None required</td>
</tr>
<tr>
<td>1. Indian, Pakistani, African or North European, Middle Eastern</td>
<td>Test partner and if MCH &lt;25 pg and from high risk area</td>
<td>Send maternal and paternal bloods for mutation confirmation</td>
</tr>
<tr>
<td>2. South-East Asian, Eastern Mediterranean (Cyprus, Greece, Turkey, Sardinia), British† or unknown</td>
<td>MCH ≥25 pg and/or low risk area</td>
<td>None required</td>
</tr>
</tbody>
</table>

PND, prenatal diagnosis; MCH, mean cell haemoglobin.

*Hidden risk of α thalassaemia.

†The National Screening Committee guidelines indicate that the partner need not be tested unless the patient is from a local high risk area (e.g. North-West England).
will always be some situations that require further tests on different specimens or family studies before a conclusive diagnosis can be achieved.

General notes on reporting screening results

- The sample date must be given (this can be extremely important if a person has had a recent blood transfusion).
- If a blood transfusion has been received within 4 months, misleading data and conclusions may result. This includes in utero transfusions. This possibility should normally be mentioned when haemoglobinopathy results are reported.
- Analytical fact should be separated from interpretative opinion. The factual results should be given first and should be followed by a clear conclusion, which may include recommendations. If there is likely to be a delay in producing a final result, an interim result should be issued which may be sufficient for the clinician to move forwards with that patient’s clinical care.
- Consideration should also be given to adding the comment on all newborn reports where there is nothing abnormal detected that the presence of Hb F is normal in newborns.
- If information from the blood count is used in coming to a conclusion about the significance of the analytical data (as in probable \( \beta \) thalassaemia) then those aspects of the blood count used (such as haemoglobin concentration, red cell count, MCH, MCV) must be included in the haemoglobinopathy report.
- Similarly, if information on ethnicity/family origin is used, it should be stated in the report.
- Results of the sickle solubility test, in the absence of results from an unrelated confirmatory method, should only be reported as an ‘interim’ report. The final report with information from the blood film, HPLC and/or electrophoresis and any other appropriate tests should follow as soon as possible.
- As it improves clarity, the conclusion should always be given both in full text and in standard abbreviation form in parentheses. For example: Sickle Cell Carrier (AS) or Sickle Cell Anaemia (SS). The convention recommended is for the Hb initials to be reported in the order of greatest to least percentage.
- If no further action is required it may be helpful to say so or to state that partner testing is not indicated (for antenatal samples).

Limitations of haemoglobinopathy diagnosis

Sensitivity/specificty. No technique can identify all abnormalities but the combined sensitivity/specificity of the HPLC and IEF techniques for haemoglobins present at the time of screening is approximately 99%. The pattern of haemoglobin variants is not unique however and whilst some will be clarified by using the second technique, unequivocal identification can usually only be made by DNA analysis or mass spectrometry. For example, Hb D will only be of significance if found to be Hb D-Punjab, but for the purposes of the screening programmes, further testing is only required if Hb D occurs in possible combination with HbS in an individual or in a woman and her partner. Nevertheless, since Hb D-Punjab may be relevant to other family members, offering definitive identification should be considered.

Recent blood transfusion. Haemoglobin interpretation is misleading after a recent blood transfusion and necessitates repeat testing after 4 months if a pre-transfusion sample has not been analysed or the baby was too premature for the reliable interpretation of results (see below). DNA testing for the sickle gene is now recommended for transfused neonates to avoid the need for a repeat specimen at 4 months post-transfusion, although a pre-transfusion specimen is still the preferred specimen. Laboratory staff undertaking testing should utilize information from their laboratory computer records where a possible recent blood transfusion may have occurred.

Conditions not detected by the newborn screening programme. The following conditions can only be detected once the mature haemoglobin pattern has developed:

- \( \beta \) thalassaemia carriers, as HbA2 levels in the neonate are below the levels of reliable detection/quantification.
- Hereditary persistence of fetal haemoglobin, as neonates all have large amounts of Hb F with considerable variability in the levels.

Conditions not differentiated by the newborn screening programme. The following conditions cannot be clearly differentiated until the mature haemoglobin pattern develops and, in some instances, until red cell indices are available

- Homozygous Hb S versus Hbs/\( \beta^+ \) thalassaemia versus Hbs/HbH, as all will produce only FS in the neonate; further diagnostic testing is required.
- HbE/\( \beta^0 \) thalassaemia versus Hb E/E, as only FE will be present
- Other possible compound heterozygous or homozygous states of less or no clinical significance.

Premature babies. Haemoglobin A is usually detectable at 30 weeks gestation and is sometimes detectable at 24 weeks. Very premature babies may not have any Hb A present and therefore will need repeat testing to ensure Hb A does develop and that they do not have \( \beta \) thalassaemia major. Hbs and other \( \beta \) chain variants are generally present at lower levels than Hb A so may not be apparent in a very premature baby. Prematurity may also add to the difficulty in differentiating a sickle cell carrier from a baby with Hbs/\( \beta^+ \) thalassaemia when the HbS level is greater than the Hb A level. Family studies and DNA testing may be helpful to make a diagnosis.
Combined low prevalence area laboratories. The following conditions will not be detected when following the algorithms for both low and high prevalence areas:

- silent/near silent \( \beta \) thalassaemia carriers
- \( \alpha^0 \) thalassaemia carriers in non-high risk groups
- dominant haemoglobinopathies in partners of women with negative screens
- any silent haemoglobinopathy e.g. unstable or altered affinity haemoglobins

The following conditions are additional risks when using the algorithm for low prevalence areas:

- haemoglobin variants in northern European families
- thalassaemia carriers obscured by vitamin \( \text{B}_{12} \)/folate deficiency, liver disease or other causes of a rise in the MCV/MCH
- combined \( \beta \) thalassaemia carrier and \( \alpha \) thalassaemia carrier in low risk groups, because a normal MCH would mean no HPLC analysis is performed

The programme is not designed to detect couples at risk of a child with Hb H disease. Babies with Hb H may however be detected by the newborn screening programme and may require medical follow-up.

Other conditions. The following conditions may not be detected by routine laboratory techniques:

- Altered affinity haemoglobins
- Unstable haemoglobins

Accurate information of family origin. The antenatal programme is very reliant on the quality of information supplied from the family origin questionnaire. Incorrect information in the high prevalence areas may result in incorrect interpretation and subsequent inappropriate action, particularly with respect to potential \( \alpha^0 \) thalassaemia carriers. In low prevalence areas, incorrect information could mean that women miss screening completely. Correct detailed family origin information is even more important if DNA testing is required to ensure that tests for the most appropriate genotypes are included.

The quality of this information is dependent on midwife knowledge and training to ensure that women understand the importance of revealing this information, and considering ethnic/family origin, not country of birth and not just of themselves and their partners, but also of earlier generations when known. Where the family origin is unknown or not revealed by a woman, patients are treated as high-risk, which potentially creates a significant extra workload, particularly in low prevalence area laboratories.

Linkage of neonatal and antenatal screening programmes

The original commitment in the White Paper was to establish ‘a linked antenatal and newborn screening programme for haemoglobinopathies and SCD’ (NHS 2000). The current national policy requires that users should experience one service, not separate disjointed services, despite the fact that this must be delivered by separate regional newborn and locally provided antenatal programmes. A key objective for the linkage is to minimize the adverse effects of screening – anxiety, misunderstanding, inaccurate information, unnecessary investigation and irrelevant follow-up – and this can only be achieved by good communication between the two screening services.

Method of linkage

All units are required to have a local policy applying national guidance on the integration of antenatal and newborn screening programmes. Different regions will develop different systems for linkage, which should include some form of ‘at risk pregnancy’ alert notification. Local agreement is required between screening laboratories as to which pregnancies should be notified to the newborn screening laboratory and by whom: in high prevalence areas without good electronic links it may only be practical to ensure that couples found to be at risk of a significantly affected child are notified whereas in low prevalence areas it may also be possible to also notify pregnancies that are not at risk.

It is equally important that a method is established for communicating positive results back to the relevant antenatal services and laboratories, particularly in low prevalence areas where specialist haemoglobinopathy nurses may not be available to ensure that results are reviewed.

Recommendation

A local written policy for linkage is required, including some form of ‘at risk pregnancy’ alert mechanism.

Good communication may help to reduce unnecessary anxiety, possible misunderstandings and missed diagnoses and allow detection and investigation of possible errors.

Haemoglobinopathy cards

National haemoglobinopathy cards are available for affected, carrier and normal individuals following haemoglobinopathy screening. It is considered good practice to issue haemoglobinopathy cards to those individuals with a major haemoglobinopathy and also to carriers where a definitive diagnosis can be made.

The issue of ‘normal’ cards to individuals with no evidence of a significant haemoglobinopathy should be risk assessed by each centre. The national screening programmes have been developed specifically to detect major clinically significant haemoglobinopathies; exclusion of these on such screening programmes does not necessarily equate to normal.

In the absence of confirmatory DNA testing, patients should not be issued with haemoglobinopathy cards stating that they are carriers of \( \alpha \) thalassaemia or probable carriers of \( \alpha \)
thalassaemia. Units may wish to consider issuing explanatory letters or leaflets for this group of patients.

Recommendation

Haemoglobinopathy cards should be issued to individuals with a major haemoglobinopathy.

Haemoglobinopathy cards should be issued to carriers where a definitive diagnosis can be made.

Haemoglobinopathy cards should not be issued to individuals with α thalassaemia unless confirmed by DNA testing.

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 of Haematology nor the publishers can accept any legal responsibility for the content of these guidelines.

References


Appendix I. Search terms

Sources searched: Medline, Embassy, Cochrane Library, National Library for Health

Search strategy

Ovid Medline
1. (Sickle ad cell1).tw.
2. (sickle adj trait1).tw.
3. (haemoglobin adj s).tw.
4. (haemoglobin adj s).tw.
5. (sickle adj disease).tw.
6. exp Anemia, Sickle Cell/
7. (haemoglobinopath or haemoglobinopathy).tw.
8. (thalassaemia or thalassemia).tw.
9. exp Haemoglobinopathies/
10. exp Thalassemia/
11. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
12. screen3.tw.
13. (test or tests or testing).tw.
14. detect3.tw.
15. opportunistic.tw.
16. targeted.tw.
17. universal.tw.
18. selective.tw.
19. mass screening/or genetic screening/or neonatal screening/
20. exp Genetic Counseling/
21. (counseling or counselling3).tw.
22. prevalence/
23. exp Sensitivity/ and Specificity/
24. nanotechnology.tw.
25. exp Nanotechnology/
26. exp Spectrum Analysis, Mass/
27. (tandem M5 or tandem mass spectrometry).tw.
28. exp Follow-Up Studies/
Appendix II. Family origin questionnaire

Family Origin Questionnaire

If using a preprinted form, please attach one to each copy.

Screening Programmes

Sickle Cell & Thalassemia

What are your family origins?

Please tick all boxes in all sections that apply to the woman and the baby’s father.

A. AFRICAN OR AFRICAN-CARIBBEAN (BLACK)
   Caribbean Islands
   Africa excluding North Africa
   Any other African or African-Caribbean family origins (please write in...)

B. SOUTH ASIAN (ASIAN)
   India
   Pakistan
   Bangladish

C. SOUTH EAST ASIAN (ASIAN)
   China including Hong Kong, Taiwan, Singapore
   Thailand, Indonesia, Burma
   Malaysia, Vietnam, Philippines, Cambodia, Laos
   Any other Asian family origins (please write in...)

D. OTHER NON-EUROPEAN (OTHER)
   North Africa, South America etc
   Middle East (Saudi Arabia, Iran etc)
   Any other Non-European family origins (please write in...)

E. SOUTHERN & OTHER EUROPEAN (WHITE)
   Sardinia
   Greece, Turkey, Cyprus
   Italy, Portugal, Spain
   Any other Mediterranean country
   Albania, Czech Republic, Poland, Romania, Russia etc

F. UNITED KINGDOM (WHITE) refer to chart at the back
   England, Scotland, N Island, Wales

G. NORTHERN EUROPEAN (WHITE) refer to chart at the back
   Austria, Belgium, Ireland, France, Germany, Netherlands
   Scandinavia, Switzerland etc

Any other European family origins, refer to chart (please write in the box... (e.g., Australia, N America, S Africa)

# Hb Variant Screening Requested by (D) and/or (E)
# Higher risk for alpha zero thalassemia

H. DON’T KNOW (incl. pregnancies with donor egg/sperm)

I. DECLINED TO ANSWER

J. ESTIMATED DELIVERY DATE (please write in if not known)

K. GESTATION AT TIME OF TEST

All women need to be informed that prenatal analysis of blood may identify them as a thalassemia carrier. In this programme, women with confirmatory carrier screening and who have a positive result for 

don’t need to be informed of the carrier condition. All women should be informed of the implications of a positive result and should be counselled accordingly. The completion of this form is necessary for the purpose of analysing the background genetics and the risk of thalassemia in the offspring. If you have any questions, please contact the family origin team at the hospital.

Guideline

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