Leaders

Guidelines for investigation of the α and β thalassaemia traits

The Thalassaemia Working Party of the BCSH General Haematology Task Force

Introduction

The thalassaemia syndromes are inherited disorders of haemoglobin (Hb) production, characterised by a reduction in globin chain synthesis leading to an imbalance of the globin chains. There are many different DNA mutations leading to these disorders (see guidelines for the fetal diagnosis of the globin chain disorders[1]) but it is helpful to subdivide them into two groups: α° and β° in which no globin is synthesised and α^+ and β^+ in which some, but a reduced amount, of globin is synthesised. Although most patients have either α or β thalassaemia these may interact with each other ($\alpha\beta$ thalassaemia), with δ thalassaemia ($\delta\beta$ thalassaemia), and with Hb variants such as Hb S, Hb E, Hb O^{arab}, and Hb Lepore. Although most cases of hereditary persistence of fetal Hb (HPFH) have no clinical or genetic implications a few are associated with a β thalassaemia-like phenotype and can occasionally interact with a β thalassaemia trait to produce a β thalassaemia intermedia phenotype. Cases with thalassaemic features may be genetically and phenotypically related to $\delta\beta$ thalassaemia.

Although the carrier states for these conditions are clinically silent, the homozygote and double heterozygote states may have major clinical implications and it is therefore essential to be able to detect and identify the carrier states shown in the table. Knowledge of the carrier state can also be useful in explaining hypochromic, microcytic red cell indices.

The thalassaemia disorders are common in people of Mediterranean, Middle Eastern, African, Pakistani, Indian, and South East Asian ancestry, but occur sporadically in all populations. In our increasingly multicultural society it is becoming important to consider the diagnosis of a thalassaemia trait regardless of the apparent ethnic origin. The universal availability of electronic cell counters provides the haematologist with a simple screening procedure that can be used to select patients for further investigation.

The purposes of these guidelines are to:

(*a*) advise on which people should be tested and on how the diagnosis should be established.

(*b*) Document the routine procedures available in most laboratories which are necessary to make the diagnosis. DNA analysis is covered in separate guidelines.[1]

(*c*) Provide useful addresses for counselling, haemoglobinopathy cards, etc.

Selection of patients to be investigated

Investigation is usually carried out for genetic purposes—that is, to identify those at risk of producing offspring with a clinically significant form of thalassaemia (homozygous or doubly heterozygous) such as β thalassaemia

These Guidelines should be read in conjunction with the Guidelines for Fetal diagnosis of Globin Gene Disorders

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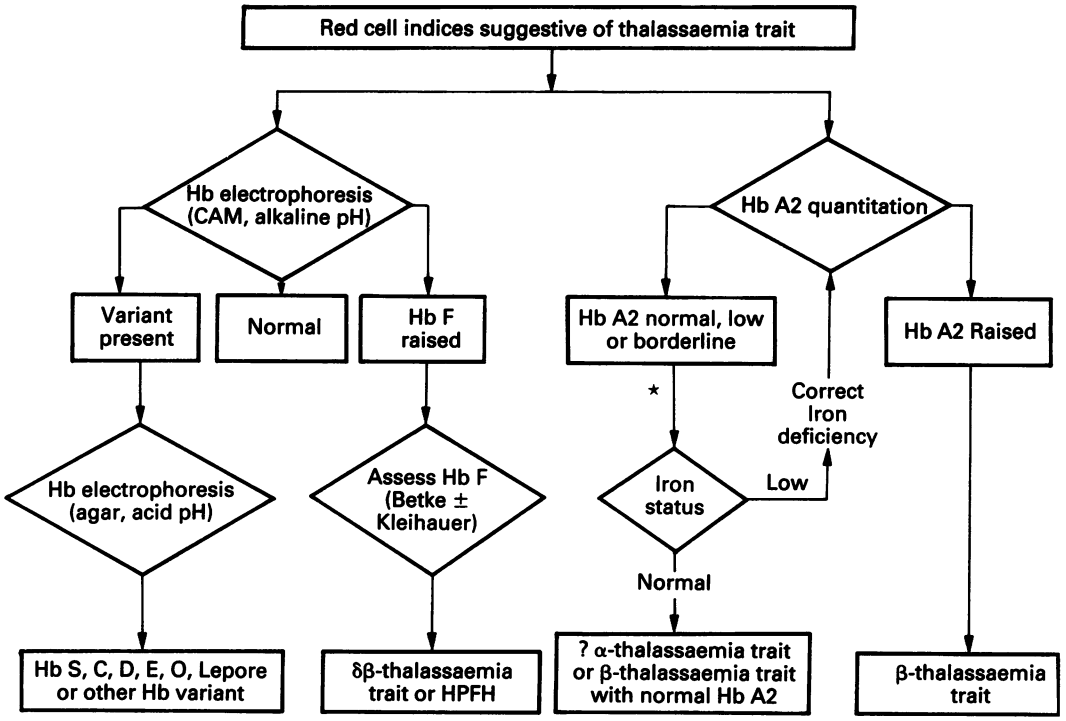
Characteristics of thalassaemia

Phenotype	Genotype		Usual MCV	Usual MCH	Hb A_2	Hb H bodies
α Thalassaemia trait	$-\alpha/\alpha\alpha$	α^+/α	N	N	N	–
α Thalassaemia trait	$-\alpha/-\alpha$ or $/--/\alpha\alpha$	α^+/α^+ or α°/α	↓	↓	N or ↓	±
Hb H disease (mild)	$--/\alpha-$	α°/α^+	↓	↓	N or ↓	+++
Hb H disease (severe)	$--/\alpha\alpha^T$	α°/α^T	↓	↓	N or ↓	+++
Hb Barts hydrops (α thalassaemia major)	$--/--$	$\alpha^\circ/\alpha^\circ$	↓	↓	–	–
β Thalassaemia trait	β°/β or β^+/β		↓	↓	↑	–
$\delta\beta$ Thalassaemia trait	$\delta\beta^\circ/\beta$		↓	↓	N or ↓	–
β Thalassaemia trait (normal Hb A2)	β^+/β		↓	↓	N	–
Hb Lepore trait	Hb Lepore/β		↓	↓	N	–
β Thalassaemia intermedia	Heterogeneous		↓	↓	↓, N or ↑	–
β Thalassaemia major	β°/β°, β°/β^+, β^+/β^+		↓	↓	↓, N or ↑	–

For α thalassaemia: There are two pairs of allelic structural genes that code for the α globin chains and "classical" α thalassaemia deletion (α-) but non-deletional forms ($\alpha\alpha^T$) also occur that may vary in severity and more than 30 genotypes have been characterised. The above is a classification of α thalassaemia that indicates two ways of expressing the genotype.
For β thalassaemia: There is only one pair of allelic genes that code for the β globin chains but there are more than 100 DNA subtypes of β thalassaemia, most of which are non-deletional.
N = normal.

A simple flow chart for diagnostic tests



**Refer to Estimation of Hb A_2, point 10.*

major, Hb Barts hydrops, or Hb S β thalassaemia; or for clinical purposes, to establish the cause of microcytosis. The methods of selecting patients for investigation depend on which of these circumstances applies and on whether or not the patient is a pregnant woman.

RED CELL INDICES AND THALASSAEMIA TRAITS

Most people with thalassaemia traits have a reduced mean corpuscular volume (MCV) (microcytosis) and a reduced mean corpuscular Hb (MCH) in the presence of a normal or near normal mean corpuscular Hb concentration (MCHC). The Hb concentration is usually normal or only slightly reduced and the red cell count is often raised. Although many people with the α^+ thalassaemia trait have normal red cell indices,[2] (WHO unpublished report HDP/WG/HA 87.5. Alpha thalassaemia) with information available at present, it is highly likely that those with the α° thalassaemia trait will have an MCH below 26 pg. Those with the rare β thalassaemia trait (silent β thalassaemia trait) may also have normal red cell indices.

Haematologists rely heavily on their automated blood cell counters to select which patients require further investigation for these conditions. Careful attention must therefore be paid to the calibration of the cell counter and its performance should be satisfactory both by local assessment and in the National External Quality Assessment Scheme. Each laboratory should establish its own reference range for the haemoglobin concentration and the red cell indices. Selection of patients for investigation may then be based on the MCH or the MCV or on various formulae. It is still necessary, however, to undertake further testing to distinguish microcytosis due to a thalassaemia trait from that associated with iron deficiency, the anaemia of chronic disorders, or sideroblastic anaemia and to identify which type of thalassaemia trait a person has inherited.

USE OF FORMULAE BASED ON RED CELL INDICES

Various formulae can be used[3,4] to determine whether the blood count is more suggestive of a thalassaemia trait or iron deficiency but these formulae are not applicable to children, pregnant women, or polycythaemic patients with iron deficiency and will only predict the correct diagnosis in 80%–90% of patients.[5] The following formula may be used in which each of the variables included makes a contribution, and the overall function is better than any single measurement:

$$\text{MCV (fl)} - \text{RBC } (\times 10^{12}/\text{l}) - 5 \times \text{Hb (g/dl)} - \text{K}$$

Where K is a constant factor dependent on the instrument calibration and is adjusted by each laboratory so that a negative result with this formula suggests a diagnosis of thalassaemia trait[3] whereas a positive result suggests iron deficiency.

THALASSAEMIA TRAIT WITHOUT MICROCYTOSIS

The red cell indices may also be affected by concomitant B12 or folate deficiency or liver abnormalities and in these situations people with a thalassaemia trait may have normal red cell indices. Those with certain β thalassaemia mutations may also have normal red cell indices.[1]

INVESTIGATION FOR GENETIC PURPOSES

This can be carried out before conception or during pregnancy. In either circumstance it is essential that most cases of the β thalassaemia trait be detected ("silent" β thalassaemia trait cannot be detected from red cell indices) and

that the *α* thalassaemia trait be detected in those ethnic groups where the - -/*αα* (or *α*°) genotype occurs and where the condition Hb Barts hydrops fetalis is therefore possible. Patients may be selected for further testing on the basis of either the MCH or the MCV. Further testing may be carried out on all those who fall below the lower limit of the reference range for the selected variable. Alternatively, a laboratory may investigate a large population of subjects with a suspected thalassaemia trait to establish a satisfactory lower cut off point, thus reducing unnecessary testing.

If the patient is not pregnant, formulae such as that given may be applied to determine whether iron deficiency or a thalassaemia trait is more likely. A provisional diagnosis of iron deficiency should be confirmed and treatment given. If the red cell indices do not return to normal after correction of the iron deficiency investigation for a thalassaemia trait is then indicated. When a patient is already pregnant such formulae become invalid and either the MCH or the MCV should be used to select patients for further investigation. If the woman is pregnant it may be necessary to test for thalassaemia even though she is known to be iron deficient. This is because both thalassaemia and iron deficiency can occur in the same patient and undue delay may occur if thalassaemia testing is not undertaken until the iron deficiency is corrected; this is especially likely if the woman is not of northern European extraction. If the pregnant woman is thought to have the *β* thalassaemia trait or *δβ* thalassaemia trait, or has an interacting Hb variant such as Hb S, C, E, O^{arab}, or Lepore, the putative father should have similar investigations.

At present it seems that Hb Barts hydrops is extremely rare in those of ethnic orgin other than from South East Asia (see also the associated guidelines,[6 7]). If the woman is thought to have the *α* thalassaemia trait and originates from South East Asia she may have the *α*° genotype and the precise genetic diagnosis may therefore be important and the putative father should also be tested for *α* thalassaemia.

INVESTIGATION FOR CLINICAL PURPOSES

This is most commonly undertaken in people with microcytosis or a low MCH in the absence of iron deficiency or after correction of iron deficiency. Except in the special circumstances of pregnancy it is not generally useful to investigate for a thalassaemia trait in the presence of iron deficiency and moderately severe anaemia (Hb < 8 g/dl).

DIAGNOSIS IN SPECIAL GROUPS

Infants less than 1 year old

The presence of Hb Barts hydrops at birth indicates *α* thalassaemia trait and if it is noticeably raised the baby may have Hb H disease[8 9] and may need specialist follow up. *β* Thalassaemia heterozygotes will develop thalassaemic indices and increased concentrations of Hb A_2 between 3 and 6 months of age. At present the diagnosis of *β* thalassaemia trait cannot be made reliably in the neonatal period, but if clinically indicated, studies can be carried out when the baby is over 6 months old.

Thalassaemia interactions with structural variants

In families that carry certain structural Hb variants (for example, Hb S, C, E, O^{arab}, and some unstable Hbs), it is necessary to investigate for the presence or absence of *β* thalassaemia genes that may interact with these structural variants.

How to establish the diagnosis

In most clinical situations the first step in the diagnosis of the thalassaemia traits and the phenotypically similar syndromes such as Hb Lepore trait is the measurement of the red cell indices. If the cells are normocytic, the diagnosis can be excluded with sufficient accuracy for clinical purposes as long as there is no coexisting disorder such as B12 or folate deficiency, or liver disease which may raise the MCV and MCH into the normal range. Once the possibility of a thalassaemia trait has been identified specific tests are required to confirm or exclude the diagnosis of thalassaemia, and if present, to determine whether it is *α*, *β*, or *δβ* thalassaemia, Hb E trait, or Hb Lepore trait. The table summarises the general classification of the thalassaemia syndromes together with the typical haematological features for those more than 1 year of age who have the common genotypes discussed in these guidelines. More precise diagnoses require the measurement of globin chain synthesis ratios and/or DNA analysis.

Specific tests are an essential step in the diagnosis of thalassaemia trait and include the following: (*a*) electrophoresis of Hb on cellulose acetate membrane at alkaline pH; (*b*) measurement of Hb A_2; (*c*) assessment of Hb F; (*d*) looking for Hb H inclusion bodies; (*e*) assessment of iron status.

Additional tests are sometimes required such as isoelectric focusing, globin chain synthesis, or DNA analysis.

ELECTROPHORESIS OF HB

Electrophoresis of Hb at alkaline pH on cellulose acetate membrane should be carried out on all samples tested for thalassaemia because this procedure will detect the Hb variants, Hb Lepore, Hb S, Hb C, Hb E, and Hb O^{arab}, together with Hb H, Hb Barts, and most Hb A_2 variants, all of which have medical implications when associated with a thalassaemia trait. The technique used is based on that described by Schneider *et al*[10] and given in detail by the International Committee for Standardization in Haematology (ICSH).[9]

(1) Tetrasodium EDTA (1 g/l in 100 mg/l potassium cyanide) should be used as the haemolysing reagent.[5] The haemolysate is liable to oxidation and should be used within seven days.

(2) Strong detergents such as Triton X-100 should not be used with adult specimens as they are likely to denature Hb H and if this happens Hb H disease may be missed; however, they do not seem to harm Hb Barts and so can be used for neonatal specimens.

(3) If a variant Hb is detected the sample should also be examined by Hb electrophoresis on citrate agar at acid pH[9] or by a similar technique on commercial precast agar or agarose gels. Some workers find commercial gels easier to use than gels prepared in house but they are expensive and some agarose gels do not give as much information as a well run citrate agar gel.

(4) It is important to detect the presence of Hb A_2 variants during the Hb electrophoretic run as it is the sum of the "normal" Hb A_2 and the Hb A_2 variant that must be assessed in the diagnosis of the β thalassaemia trait (see later). If the presence of an Hb A_2 variant is missed it may mean that any coexistent β thalassaemia trait is also missed.

ESTIMATION OF HB A_2

A raised Hb A_2 is the hallmark of the classic β thalassaemia trait. It should be measured whenever a thalassaemia trait is suspected. Details of techniques are to be found in ICSH recommended methods[11] but the following points should be observed:

(1) Preparation and storage of haemolysate: the haemolysate for Hb A_2 and Hb F determinations should be prepared by washing packed red cells with three volumes of isotonic saline (0·15 M). Mix the washed, packed red cells with an equal volume of distilled water and a half volume of carbon tetrachloride and mix well for 10 minutes. Centrifuge and remove the supernatant Hb solution, check the Hb concentration and adjust to 10 ± 2 g/dl. The Hb solution may then be stored in a stoppered tube at 4°C for up to two weeks.

(2) Both electrophoresis and elution from cellulose acetate and microcolumn chromatography are recommended by ICSH[11] but the precision and accuracy of automated scanning densitometry are inadequate for Hb A_2 estimations (see (8)). Routine techniques cannot be used for the determination of Hb A_2 in the presence of Hb C, Hb E, and Hb O (and some other Hbs) because of their similar mobility to Hb A_2 on cellulose acetate and these microcolumns. Family studies and/or DNA analysis may be helpful in these situations.

(3) When preparing TRIS buffers it is essential to use a pH electrode that is known to be suitable for use with TRIS because TRIS interferes with the function of some electrodes. If an electrode can be calibrated with two standard buffers after immersion in TRIS buffer for at least 15 minutes it may be considered suitable for use with TRIS.

(4) Improved results may be obtained with microcolumn chromatography when a 10 mM rather than a 50 mM TRIS buffer is used.[12]

(5) If a glycine "developer" is used the pH must be adjusted very slowly as the molarity of the developer is an important factor in the elution of the Hb fractions and if the developer is made too acidic it must be discarded and not made alkaline again because this would increase the total ionic concentration and therefore alter the elution pattern.

(6) Commercial microcolumn kits for the estimation of Hb A_2 are available. It is important to follow the manufacturer's instructions carefully regarding the preparation, equilibration (especially temperature), and the use of the columns.

(7) Automated high performance liquid chromatography can save time but is expensive in both capital and revenue costs. If used, it is important to examine the peak shape, baseline, and resolution and to ensure that they are satisfactory before using the computed peak areas.

(8) Reference materials have been developed by the ICSH for use in Hb A_2 measurement and will be available from the National Institute of Biological Standards and Controls (NIBSC).

(9) If an Hb A_2 variant is present it is essential to measure both the normal Hb A_2 and the variant Hb A_2 because it is the total Hb A_2 which indicates whether or not a person has the β thalassaemia trait. If an Hb A_2 variant is present, electrophoresis and elution of the Hb A_2 and the Hb A_2 variant bands is usually the best technique to use for measurement.

(10) Interpretation: values below 3·3% are usually normal and over 3·7% usually indicate the β thalassaemia trait. Some overlap in Hb A_2 values between normal and β thalassaemia trait has been reported[12] and values between 3·3 and 3·7% need to be interpreted with care. Knowledge of the iron state of the individual patient is important as iron deficiency sufficient to cause moderately severe anaemia (Hb < 8 g/dl) can reduce the Hb A_2 level and lead to people with the β thalassaemia trait being classified as not having this condition.[13–15] Normal Hb A_2 concentrations can be found in the $\delta\beta$ thalassaemia trait and in the Hb Lepore trait (table) although both these conditions have the same clinical significance as the β thalassaemia trait. Raised levels have been described in the presence of some unstable Hb β globin variants and in some cases of pernicious anaemia. A value above 7% suggests either an analytical error or the presence of another Hb that is not Hb A_2.

If Hb A_2 concentrations in the "borderline" area (3·3%–3·7%) in the absence of Hb S (see below), the estimation should be repeated on another blood sample and the iron state assessed. If the patient is iron deficient the iron deficiency should be corrected before the analysis is repeated. If repeat analysis again gives a borderline result globin chain synthesis or DNA analysis should be considered if the diagnosis of the β thalassaemia trait, or its exclusion, would alter the clinical management of the patient or the genetic advice given to a couple. An example of such a situation would be if a pregnant

woman had the β thalassaemia trait and her partner had borderline results.

If a person has both a sickle cell trait and the α thalassaemia trait the Hb A_2 may be slightly raised (3·5%–4·0%) but this does not of itself indicate a coexistent β thalassaemia trait. The simplest way of obtaining a correct diagnosis in this situation is to quantify the Hb S. In the uncomplicated sickle cell trait (Hb A + Hb S) the Hb S comprises less than 40% of the total Hb. If the α thalassaemia trait is also present the Hb S will be lower[16] whereas the combination of sickle cell trait and β thalassaemia trait results in an Hb S concentration of more than 60% and usually 80%–90%.

HB F

The Hb F is raised (by about 1%–3%) in one third to half of the people with the β thalassaemia trait. A raised Hb F (5%–20%) in the presence of hypochromic, microcytic, red cell indices, however, is the hallmark of the δβ thalassaemia trait and it should be measured whenever Hb F is detected on Hb electrophoresis on cellulose acetate membrane. It may also be raised in other conditions such as in pregnancy and in the myeloproliferative disorders.

(1) Hb F should be measured by the two minute alkali denaturation technique described by Betke *et al*[7] and in detail by Pembrey *et al*.[8] Attention to detail is extremely important when undertaking this assay. In particular, the concentration of the haemolysate (10 ± 2 g/dl), the temperature of the alkali denaturation mixture (20 ±2°C), and the time that the denaturation is allowed to proceed (two minutes) must all be carefully controlled. If the Hb F is above 50% a more accurate result may be obtained by the technique of Jonxis and Visser[19] but the increased accuracy will rarely, if ever, alter the clinical management of the patient.

(2) Reference materials developed by ICSH for use in Hb F measurement will be available from NIBSC.

(3) If the Hb F is 5%–20%, the patient may have δβ thalassaemia or HPFH. Pronounced microcytosis favours the first. If the Hb F is more than 20% it is likely that the patient has HPFH and in this condition the Kleihauer cytochemical test for Hb F will show a pancellular distribution. If the patient is pregnant the partner should be tested and if his tests are abnormal a definitive diagnosis by DNA analysis should be considered.

HB H INCLUSIONS

(1) Hb H inclusions are due to the oxidation and precipitation of Hb H within the red cell and they can be generated in vitro by the redox action of certain dyes. They are found in some forms of α thalassaemia. Unfortunately there is batch to batch variation with the dye and any new batch should be checked with a known positive sample.

(2) Blood (preferably anticoagulated with EDTA and used within 24 hours of venesection) is incubated with 1% brilliant cresyl blue or new methylene blue at room temperature (18%–25°C) for four hours; a normal control should always be included (CSR Hatton and DR Higgs, personal communication). Blood films are made and examined. Hb H inclusions form a "golf ball" appearance inside the cells.

(3) In people with the α thalassaemia trait there are usually a few red cells (1/1000 to 1/10 000) that contain typical inclusions (table)[20] but in Hb H disease they are found in 30%–100% of the red cells. The absence of Hb H inclusions does not, however, exclude an α thalassaemia trait.

ISOELECTRIC FOCUSING

As a general rule the separation of Hbs on isoelectric focusing is similar to that obtained by electrophoresis on cellulose acetate membrane but the bands are sharper and the resolution of some Hbs is better. Isoelectric focusing is not usually indicated in district service laboratories but can be very useful in reference centres and also in regional laboratories where large numbers of samples are processed. This is a useful technique to differentiate Hb Barts from "fast" variants such as Hb J and Hb N in anticoagulated umbilical cord blood samples. It is also useful in laboratories undertaking neonatal screening on dried blood spots from Guthrie cards because it reduces the interference from met Hb which usually prevents adequate resolution of Hb bands when using electrophoresis to analyse Hb eluted from such dried blood spots.

GLOBIN CHAIN SYNTHESIS

This technique is valuable in detecting the presence of the α or β thalassaemia trait in couples considering prenatal diagnosis when the usual tests give equivocal results; it may sometimes be also useful in fetal diagnosis (see associated guidelines[1]). The technique is time consuming and involves culturing the blood in the presence of tritiated leucine and therefore facilities for the use and disposal of radioactive material must be available. For these reasons this test is usually only undertaken in reference centres. If it is undertaken, it is essential that adequate numbers of normal and abnormal controls are analysed regularly.

DNA ANALYSIS

DNA can be analysed from white blood cells, or obtained from amniocytes or chorionic tissue obtained by transabdominal or transvaginal sampling methods. These techniques are usually undertaken in specialist referral centres, and the laboratory techniques are discussed in the associated guidelines.[1]

QUALITY CONTROL

As these procedures are all used to make, or exclude, the diagnosis of an inherited condition, it is essential to ensure that adequate quality control is undertaken for the determination of the red cell indices and all the other tests described in these guidelines. In house

protocols should clearly elucidate all aspects of the procedures to be undertaken. In particular quantitative diagnostic analyses such as Hb A_2 and Hb F should be undertaken in such a way that duplicates agree to within 0·2% of each other (SD < 0·05%). All laboratories should check their reference ranges with each method they use to ensure that their results are accurate as well as precise. The Hb concentration of the haemolysate used for the Hb A_2 and Hb F determinations should be recorded, as should the temperature of the reagents for the Hb F. Laboratories should participate in the appropriate National and Regional Quality Assessment schemes.

Interpretation of results

Although a definitive diagnosis of one of the thalassaemia traits requires detailed DNA analysis (see associated guidelines[1], a diagnosis that is accurate for most clinical purposes except for fetal diagnosis can usually be obtained by assessing the results of a group of laboratory tests. The presence of one of these traits or one of the phenotypically similar variant haemoglobins such as Hb Lepore, Hb Constant Spring, or Hb E trait may have been suspected because of the presence of red cells with reduced MCH and MCV or the measurement of these indices may be used as the first of a series of investigations.

β Thalassaemia trait is typically associated with a raised Hb A_2 but the Hb A_2 is not raised in the $\delta\beta$ thalassaemia trait and it may not be raised if there is coexistent severe iron deficiency anaemia. Hb Lepore, which is phenotypically similar to the β thalassaemia trait, can be detected by Hb electrophoresis.

α Thalassaemia is not associated with a consistent marker such as the raised Hb A_2 in the β thalassaemia trait but Hb H bodies may sometimes be detected. The diagnosis of α thalassaemia trait must usually be made by the exclusion of iron deficiency and other forms of thalassaemia in a person who has hypochromic, microcytic red cells. Hb Constant Spring (and other variant Hbs with globin chain elongation that are phenotypically similar to α thalassaemia) may be detected by Hb electrophoresis, but may be confused with an Hb A_2 variant or a carbonic anhydrase variant. Hb A_2 variants can be differentiated from carbonic anhydrase variants by haem staining with dyes such as o-dianisidine.[9]

The typical haematological features of the common thalassaemia traits are given next and in the table. The subtypes of α thalassaemia are classified according to the number of deleted genes that cause each type as gene deletion is the cause of classical α thalassaemia. Non-deletional forms also occur, however, and are clinically important in that they can cause a severe form of Hb H disease.

α Thalassaemia trait (one gene deletion) can often be detected at birth by the presence of Hb Barts. This condition is often haematologically silent in later life although it may be associated with some of the changes found in the variety of α thalassaemia trait caused by two gene deletions (see below).

α Thalassaemia trait (two gene deletion) is characterised by red cells with a reduced MCH (usually less than 26 pg) and MCV in the absence of iron deficiency and no evidence of β or $\delta\beta$ thalassaemia trait (a normal or reduced Hb A_2—less than 3·5%). Hb H bodies may be detected, but their absence does not exclude α thalassaemia. Hb electrophoresis will be normal unless a variant Hb is also present.

Hb H disease (three gene deletion) is characterised by anaemia and by red cells with a reduced MCH and MCV in the absence of iron deficiency and with no evidence of β or $\delta\beta$ thalassaemia trait (a normal or reduced Hb A_2—less than 3·5%). Hb H bodies are found in 30%–100% of the red cells. Electrophoresis of Hb shows the presence of an abnormal band anodal to Hb A comprising about 1%–40% of the total Hb.

β Thalassaemia trait is characterised by red cells with a reduced MCH and MCV in the absence of iron deficiency. The Hb A_2 is raised (3·5–7·0%) and the Hb electrophoresis is otherwise normal unless a variant Hb is also present.

α Thalassaemia trait combined with β thalassaemia trait is characterised by red cells with a normal or reduced MCH and MCV in the absence of iron deficiency. The Hb A_2 is usually raised and Hb electrophoresis is otherwise normal unless a variant Hb is also present.

$\delta\beta$ Thalassaemia trait is characterised by red cells with a reduced MCH and MCV in the absence of iron deficiency. The Hb A_2 is normal or slightly reduced and the Hb F is raised (5%–20%). Electrophoresis of Hb is otherwise normal unless a variant Hb is also present.

Hb E trait is characterised by red cells with a slightly reduced MCH and MCV in the absence of iron deficiency. Electrophoresis of Hb shows a variant band (usually 20%–30%) in the position of Hb A_2 on cellulose acetate membrane at alkaline pH which does not separate from Hb A on citrate agar at acid pH. The Hb stability is usually slightly abnormal.

Hb Lepore trait is characterised by red cells with a reduced MCH and MCV in the absence of iron deficiency and a normal Hb A_2 (less than 3·5%). Electrophoresis of Hb shows a variant band (7%–15%) in the position of Hb S on cellulose acetate membrane at alkaline pH which does not separate from Hb A on citrate agar at acid pH.

Note: iron deficiency can occur in conjunction with any of these genotypes and if it is moderately severe (Hb < 8 g/dl) it may reduce the Hb A_2. For this reason it may be necessary to retest a patient after correction of any iron deficiency.

Conclusions

No guidelines can be all inclusive—there will always be exceptions and cases must be con-

sidered individually. Patients diagnosed as having one of the thalassaemia traits should be counselled and given an explanation of the results of the tests. A haemoglobinopathy card or other written record of the results should be made available to the patients and their doctors. Special counselling and follow up arrangements must be established for couples considering prenatal diagnosis.

Useful addresses

(1) Prenatal diagnosis by DNA analysis and other information on the thalassaemias is available as a national service from: Dr John Old, National Haemoglobinopathy Reference Service, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU Telephone 0865-222449 Fax 0865-222500

(2) Haemoglobinopathy cards and leaflet can be obtained from: DSS Leaflets Unit, PO Box 21, Stanmore, Middlesex HA7 1AY Telephone 081-952-2311

(3) Multilingual explanatory booklets on some of these conditions are available from: The UK Thalassaemia Society, 107 Nightingale Lane, London N8 7QY. Telephone 081-348-0437

(4) The names and addresses of NHS Counselling Centres can be obtained from the UK Thalassaemia Society

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