

Objective: Recommendations for preconceptional or antenatal screening, prenatal diagnosis and genetic counselling of haemoglobinopathies

Target population to be aware of: medical staff expert and non-experts in the field

On behalf of ENERCA

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1. INTRODUCTION

The inherited disorders of globin chain synthesis comprise a large group of monogenic disorders that include thalassaemia (reduction of α or β globin chain synthesis), sickle cell disease (resulting from a structural modification of β globin) and other haemoglobinopathies (can involve complex interactions between several different mutant genes). Management of thalassaemic patients is lifelong, complex and expensive, while poorly managed cases have a limited survival.

Haemoglobinopathies, a significant worldwide public health problem, are the most frequent group of monogenic disorders in Southern Europe and especially in the Mediterranean area. Nevertheless, the increasing immigration to Northern and Central Europe has resulted in a significant rise in the prevalence of haemoglobinopathies also in the rest Europe with sickle cell disease being more frequent in the North while β -thalassaemia is more frequent in the South (Modell B, et al. 2007, 2007; Roberts I, de Montalembert M. 2007).

The laboratory preconceptional or antenatal screening and prenatal diagnosis of haemoglobinopathies is of growing importance and has to include different procedures:

- For presumptive and definitive diagnosis i.e. screening procedures to identify couples at risk of conceiving a child with a major haemoglobinopathy, and, if indicated, to offer genetic counselling and reproductive choice

These guidelines review the most important aspects of carrier detection procedures, population screening, genetic counselling and prenatal diagnosis of haemoglobinopathies.

The **responsible** health professionals who should adopt the proposed guidelines and direct people from at-risk groups for screening to specified units or Centres are mainly family doctors, paediatricians, biologists, geneticists, and obstetricians, especially obstetricians, who are responsible for guiding couples at-risk to a team experienced in genetic counselling and prenatal diagnosis.

Role of the family doctor or obstetrician

- ▶ To request identification of a pregnant woman (or ideally a woman who is not yet pregnant) as a carrier of a haemoglobinopathy.
- ▶ To request testing of her partner.
- ▶ If there is a risk of a major haemoglobinopathy in a fetus, either offer genetic counselling or send to a genetic counsellor.

2. SCREENING PROGRAMMES

Screening programmes have already been applied for β thalassaemia and sickle cell disease carrier identification, in a number of at-risk populations (e.g. Greek and Turkish Cypriots, Greeks, Continental Italians and Sardinians), (*Angastiniotis M, Hadjiminis M, 1981; Cao A, et al. 2001; Loukopoulos D., 1996, 1998*) and should be regarded as models for determining recommendations for prenatal diagnosis.

A. PUBLIC AWARENESS

Selection of the appropriate population awareness approach must take into account local socio-economic parameters and additional factors which may influence the acceptance of the prospective parents towards carrier identification. These include the influence of various religious and political views and the national legal framework as well as the availability of prenatal diagnosis.

Such an approach should include information, sensitization and education of the involved population group who must understand what the consequences of the haemoglobin disorders are.

B. CARRIER SCREENING

– THE TARGET POPULATION

The **target group** for screening may include: newborns, adolescents, premarital couples... Preconception and especially premarital screening should be widely applied in those populations at highest risk. *In Belgium, an important target group is comprised of pregnant women whose ancestry is from high risk areas or whom partner is from high risk areas (Figure 1 and Table1).* Identification of the couple should ideally be premarital in order to offer the greatest number of choices or at least it must be completed before 11-12 weeks of pregnancy to be able to offer prenatal diagnosis. 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 and they will benefit from a genetic counselling.

Figure 1. Schematic representation of the geographic origin of several haemoglobinopathies.



In Belgium, carrier screening is not offered in an integrated, national screening programme.

– LABORATORY TECHNIQUES

A full blood count, a separation of the haemoglobin fractions and quantification of Hb A₂ and Hb F are the key parameters in screening for haemoglobinopathies. The possibility of iron deficiency should be taken into account (Ryan K. et al.2010).

More precisely, carrier detection is carried out by:

1) PHENOTYPIC TESTS

Routine haematological tests: measurement of Hb, RBC count, MCV, MCH and RDW. *Cut-off values indicating possible heterozygosity for thalassaemia include MCV and/or MCH under the lower reference values (MCV < 78 fL and MCH < 27 pg are admitted cut-off values for patients older than 18 y.o.)* Sensitivity of the analyser to samples older than 24 hours should be evaluated. (Please specify time and preservation limit for the sample for reliable measurement).

- a) **Separation and quantification of the haemoglobin fractions** can be performed using different techniques.

Commercially available techniques for haemoglobin pattern analysis

- High Performance Liquid Chromatography
- Haemoglobin electrophoresis at pH alkaline pH using cellulose acetate membrane or by capillary electrophoresis

- Haemoglobin electrophoresis at acid pH using citrate agar gel, acid agarose or by capillary electrophoresis
- Isoelectric focusing
- In case of suspicion of haemoglobin S, the solubility test could be used as a second line method in order to obtain a reliable diagnosis of the presence of Hb S

Recommendations

- The screening technique or the combination of techniques used should allow the detection of the more common clinically significant haemoglobin variant.
- When a haemoglobin variant is detected (abnormal fraction), at least a second technique based on a different principle of separation should be used to give a presumptive diagnosis.
- In case of high levels of foetal haemoglobin detection, evaluation of the sensitivity as well as the separation capability of the technique used to detect an abnormal haemoglobin fraction is required.
- The use of a fresh blood sample is required for the detection of a haemoglobin H.
- *The technique should be dedicated to the separation of the haemoglobin fractions.*

Commercially available and recommended techniques for quantification of the haemoglobin fractions

- High Performance Liquid Chromatography (HPLC)
- Capillary electrophoresis
- Microcolumn chromatography for quantisation of Hb A₂ Alkali denaturation for quantification of Hb F

Recommendations

- Hb A₂ and Hb F analysis by electrophoresis using cellulose acetate membranes or agarose gels is appropriate only for qualitative results. Quantitative evaluation by automatic densitometry of these results is inappropriate. Currently most laboratories use HPLC or automated Capillary Electrophoresis and these are the recommended methods for identifying carriers.
- *An external quality control has to be included.*

Interpretation of results

There is no international standardisation for quantification of Hb A₂. The cut-off value indicating suspicion of a β thalassaemia trait is method dependent. Borderline values (close to the upper limit of the reference values) should be

interpreted together with the red blood cell indices results and may require further investigation with other techniques such as family studies or molecular studies.

- In the presence of HbS, derivative products of HbS co-elute with Hb A2. In this case, the screening for β thalassaemia is based on the % ratio between HbA and HbS. A compound heterozygosity for haemoglobin S and beta thalassaemia may be suspected if the ratio $\text{HbS \%}/\text{HbA\%} > 1.0$.
- Cut-off value indicating heterozygosity for $\delta\beta$ -thalassaemia is Hb F $>5\%$,
- Pitfalls in diagnosis include the co-inheritance of alpha thalassaemia with beta thalassaemia and the co-inheritance of delta thalassaemia with beta thalassaemia trait. Most common is the presence of iron deficiency which may modify the picture of thalassaemia traits and must be excluded especially if screening in pregnancy. The presence of iron deficiency does not rule out the possibility of an underlying thalassaemia trait.

2) GENOTYPIC TESTS

Molecular diagnosis is necessary to obtain a definitive diagnosis for α and β thalassaemia when one member of the couple is a carrier and the other has abnormal RBC indices and / or HbA₂ levels.

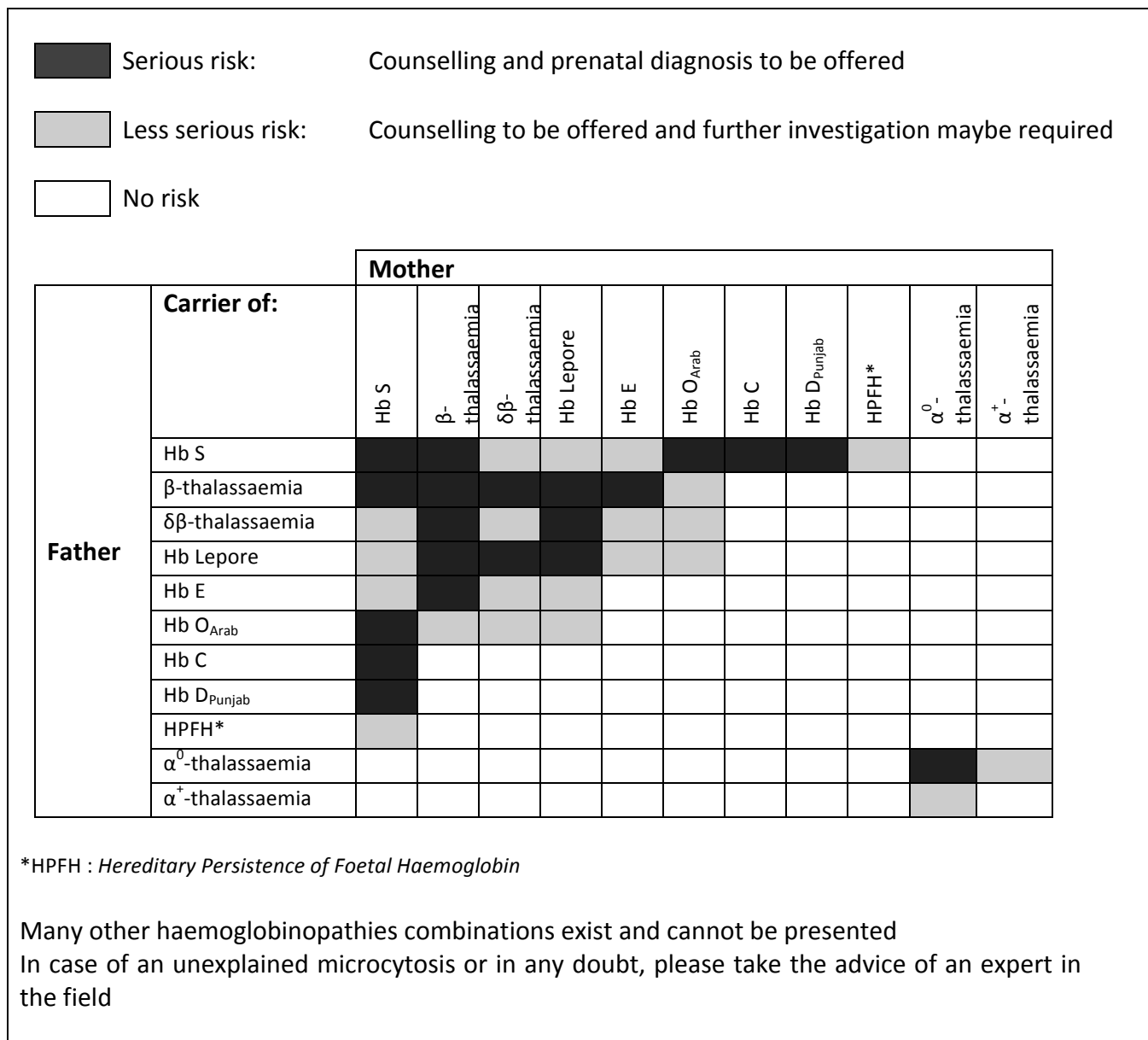
Confirmation by a molecular method is not mandatory for a haemoglobin variant which has been demonstrated in a consistent way by at least two methods with two different principles of separation and one of these is quantitative.

Table 1. Recommendations for preconception or antenatal screening for haemoglobinopathies

SCREENING (At distance of a transfusion)	
All women	<ul style="list-style-type: none"> – Full blood count – Ferritin – <i>CRP</i>
Women with one of these risk factors <ul style="list-style-type: none"> – MCH < 27 pg without iron deficiency – Clinical signs of a haemoglobinopathy – High risk ancestry (Mediterranean Basin, Middle-East, Asia, Africa) – Partner of high risk ancestry 	<ul style="list-style-type: none"> – Full blood count – Ferritin – <i>CRP</i> – Separation of the haemoglobin fractions, HbA₂ and HbF
PARTNER TESTING	
If maternal screening is positive, test the partner	<ul style="list-style-type: none"> – Full blood count – Ferritin – <i>CRP</i> – Separation of the haemoglobin fractions, HbA₂ and HbF
COUPLE AT RISK	
If both partners are at-risk and if not already performed (figure 2)	<ul style="list-style-type: none"> – Genetic counselling – Molecular diagnosis: identification of the mutation involved *

* Not always mandatory for a haemoglobin variant

Figure 2. Antenatal screening: (combinations that give rise to the risk of a foetus affected by a severe haemoglobinopathy (adapted from the work of Prof. B. Modell and published by the UK National Screening Committee)



3. PRENATAL DIAGNOSIS

Prenatal diagnosis along with genetic counselling, are significant aspects of prevention.

- Scientific personnel involved: haematologists, molecular geneticists, biologists in Prevention/Prenatal Units, genetic counsellors, obstetricians in public and private sectors, paediatricians in treatment Units
- Target population: Couples at risk of having an affected foetus (both parents are carriers).

A. GENERAL PRINCIPLES

- All couples considering prenatal diagnosis should have access to professionals who are knowledgeable in the field and skilled in the procedures.
- Each partner of the couple should have an appropriate assessment of family history and genetic counselling before invasive prenatal diagnosis is carried out.
- Counselling should be given in a non-directive manner in order to allow an informed choice by the couple.
- The distinction between screening and diagnostic investigations should be clarified, including the frequency of abnormal results, false-positive, and false-negative tests. Accuracy of results, frequency of need for repeat testing, and risk of pregnancy loss are of particular relevance with invasive prenatal diagnosis procedures. The couple should be reminded that normal test results do not rule out every genetic or structural abnormality in their foetus.
- Introduction of any new prenatal diagnostic investigation, or alteration of previously established approaches, requires careful follow-up and audit to assess risk, accuracy, and impact.

Given the risk of foetal loss and morbidity related to the sampling procedure, prenatal screening should be offered for serious clinical conditions. It is offered to couples at risk of an affected pregnancy and specifically (see Figure 2):

- Serious sickle cell disorders (Hb SS; Hb SC; Hb SO_{Arab}; Hb SD_{Punjab}, Hb S/thalassaemia (β^+ , β^0 , or $\delta\beta$ -thalassaemia, haemoglobin Lepore, haemoglobin E), homozygous β -thalassaemia
- Hb Bart's hydrops fetalis

Given the advances in treatment, these indications must be reviewed regularly. For example in the case of patients with a HbSC disease living in an industrialized country, their life expectancy is now more than 60 years, the indication is thus subject of debate.

B. GENETIC COUNSELLING

Genetic counselling - Objectives

- ▶ To provide the information required in a simple, clear and non-directive manner following well-established recommendations
- ▶ To offer the screening and diagnostic procedures in a timely manner to ensure that the overall process is given at least by the end of 11 weeks of pregnancy
- ▶ To offer at couple at high risk of an affected pregnancy the possibility of informed decision-making
- ▶ To offer support to women or couples whether prenatal diagnosis is accepted or refused

Following counselling, most parents accept prenatal diagnosis, although not in all cultural or ethnic groups (Modell B. et al. 1997; Modell B. et al. 2000).

Under ideal conditions, the counselling session must be personal, confidential, adequate and friendly, thus enabling both partners to understand in a satisfactory manner the probabilities of having an affected child, the limitations and the potential consequences of the procedure. Counselling should be non-directive and should leave the final decision entirely within the responsibility of the involved individuals.

There are both theoretical and practical reasons to evaluate genetic counselling. Theoretical reasons include analysing factors affecting comprehension of genetic information, determining how genetic risks influence decision making and characterizing patterns of adjustments to genetic burdens. Practical reasons include improving the quality of services to patients and both improving the training and evaluating the performance of genetic counsellors.

Genetic counsellors should be trained, qualified and experienced personnel ideally experienced in counselling for haemoglobinopathies. The cultural background of the person provided genetic counselling should always taken into consideration.

Prior to embarking on prenatal diagnosis testing, couples should be made aware of the full range of options when confronted with an abnormal test result. Prior commitment to termination of pregnancy following the diagnosis of foetal abnormality is not a prerequisite for prenatal diagnosis.

C. PRENATAL DIAGNOSIS APPLICATION?

Prenatal / foetal diagnosis is carried out for couples at risk of an affected foetus (both parents are carriers). Most of the mutations involved in inherited haemoglobinopathies can be detected by DNA analysis of the foetus at risk. It is therefore vital to determine accurately the parental genotypes, **preferably before**

foetal diagnosis in order to avoid mistakes if mutations are missed because of an incorrect diagnosis of the carrier state. Due to the large number of mutations and the complexity of evaluation of results, it is recommended that foetal diagnosis by DNA analysis is only undertaken in reference centres.

Prenatal diagnosis includes:

- **Parental mutation identification** (Ideally before pregnancy; see table 1). With the parent’s samples, information should be provided according to the laboratory protocol or a form such as in Figure 3.

D. Foetal sampling

Invasive prenatal diagnosis techniques include chorionic villus sampling (CVS), amniocentesis and under certain circumstances cordocentesis or percutaneous umbilical blood sampling (PUBS). See Table 2 (*JOGC Clinical Practice guidelines*).

Table 2. Summary of amniocentesis and chorionic villus sampling information

	Amniocentesis	CVS
Procedure	Amniotic fluid removed by needle and syringe	Chorionic villi removed by transcervical (TC) catheter and syringe or transabdominal (TA) needle insertion
Timing	15 to 17 weeks	10 to 11-6/7 weeks (greater than 12 weeks TA CVS only)
Added risk of miscarriage due to procedure	+/- 0.5%	+/- 1%
Fetal malformation risks	-	1 in 3,000 vascular limb malformation (suggested but not proven)
Chance of successful sampling	Approximately 99%	Approximately 99%. If unsuccessful, can follow with amniocentesis
Time required for prenatal diagnosis	3 to 7 working days (if cultured amniotic fluid cells: 2 to 3 weeks)	3 to 7 working days

Special attention: Contamination by maternal residual tissue has to be checked, although this potential problem should be minimized with very careful attention to cleaning or stripping of the chorionic villi of maternal residual cells under the dissecting microscope prior to DNA isolation. This has not been a significant problem in most laboratories with long term experience in CVS (Rudd N. 1989; Ledbetter DH, Martin A. et al. 1990).

E. Foetal DNA analysis (*can be performed as a preimplantation diagnosis*)

Foetal DNA can be isolated from CVS or amniotic fluid foetal cells (cultured or not) manually.

Genetic analysis approaches are direct mutation analysis or indirect mutation analysis.

Results should be verified by newborn umbilical cord blood haemoglobin analysis or on a newborn blood sample obtained during the first days of life. In a normal sample HbA₂ should be absent and HbA should be approximately between 6-25% of the total haemoglobin.

SUMMARY : PRENATAL DIAGNOSIS FOR HAEMOGLOBINOPATHIES

The diagnosis is made by molecular analysis

BUT the phenotype and/or the genotype of the parents has to be done before any CVS or amniotic fluid sampling. These results have to accompany the prenatal sample.

BUT, a prenatal diagnosis should not be conceived without an informed choice. The information should be given by a genetic counsellor.

Search for a sickle cell disorder (Hb SS, Hb SC, S β -thalassaemia, Hb SD_{Punjab}, ...)

Search for a β -thalassaemia major

Search for an α -thalassaemia major (HbH hydrops fetalis, rare cases of Hb H disease)

Type of samples (depending on gestational age) :

- ❑ *CHORIONIC VILLOUS SAMPLING (CVS): sterile T25 bottle + 25 ml BME Basal Medium*
- ❑ *AMNIOTIC FLUID (amniocentesis) : 20 - 30 ml sterile bottle*
- ❑ *FOETAL BLOOD (cordocentesis) : minimum 1 mL whole blood on EDTA*

Room temperature storage, same day shipping.

+ both parents – sample on EDTA (purple cap) – 5 cc

Figure 3. Information that has to be provided with the foetal sampling for prenatal diagnosis

HAEMOGLOBINOPATHIES : PRENATAL DIAGNOSIS

Previous prenatal diagnosis ?

Yes
Where and when?Same partners? YES/NO

If NO :
Maternal screening (or add the protocol)
Las name, first name, birth date.....

Date of screening / / 20.....

In Belgium, protocol available
 In another country, protocol available YES NO

Results

Hb AS
 Hb AC
 Beta-thalassaemia trait – Hb A2 (%): **MCH** :
 Alpha- thalassaemia trait – Hb A2 (%): **MCH** :
 Other

Paternal screening (or add the protocol)
Las name, first name, birth date.....

Screening / / 20.....

In Belgium, protocol available
 In another country, protocol available YES NO

Results

Hb AS
 Hb AC
 Beta-thalassaemia trait – Hb A2 (%): **MCH** :
 Alpha- thalassaemia triat – Hb A2 (%): **MCH** :
 Other

Genetic counselling by a genetic counsellor? **YES NO**

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