No. 218, October 2008

Carrier Screening for Thalassemia and Hemoglobinopathies in Canada

This clinical practice guideline has been prepared by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists (CCMG) and reviewed and approved by the Executive and Council of the SOGC and the Board of Directors of the CCMG.

PRINCIPAL AUTHORS

Sylvie Langlois, MD, Vancouver BC

Jason C. Ford, MD, Vancouver BC

David Chitayat, MD, Toronto ON

CCMG PRENATAL DIAGNOSIS COMMITTEE

David Chitayat, MD, Toronto ON

Valerie A. Désilets, MD, Montreal QC

Sandra A. Farrell, MD, Mississauga ON

Michael Geraghty, MD, Ottawa ON

Sylvie Langlois (Chair), MD, Vancouver BC

Tanya Nelson, PhD, Vancouver BC

Sarah M. Nikkel, MD, Ottawa ON

Andrea Shugar, MSc, Toronto ON

David Skidmore, MD, Halifax NS

SOGC GENETICS COMMITTEE

Victoria M. Allen, MD, Halifax NS

François Audibert, MD, Montreal QC

Claire Blight, RN, Halifax NS

Valérie A. Désilets, MD, Montreal QC

Alain Gagnon, MD, Vancouver BC

Jo-Ann Johnson, MD, Calgary AB

Sylvie Langlois, MD, Vancouver BC

R. Douglas Wilson (Chair), MD, Philadelphia PA

Philip Wyatt, MD, North York ON

Key Words: Carrier screening, thalassemia, sickle cell disorder, hemoglobinopathies

Abstract

Objective: To provide recommendations to physicians, midwives, genetic counsellors, and clinical laboratory scientists involved in pre-conceptional or prenatal care regarding carrier screening for thalassemia and hemoglobinopathies (e.g., sickle cell anemia and other qualitative hemoglobin disorders).

Outcomes: To determine the populations to be screened and the appropriate tests to offer to minimize practice variations across Canada.

Evidence: The Medline database was searched for relevant articles published between 1986 and 2007 on carrier screening for thalassemia and hemoglobinopathies. Key textbooks were also reviewed. Recommendations were quantified using the Evaluation of Evidence guidelines developed by the Canadian Task Force on Preventive Health Care.

Values: The evidence collected from the Medline search was reviewed by the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists (CCMG) and the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC).

Benefits, Harms, and Costs: Screening of individuals at increased risk of being carriers for thalassemia and hemoglobinopathies can identify couples with a 25% risk of having a pregnancy with a significant genetic disorder for which prenatal diagnosis is possible. Ideally, screening should be done pre-conceptionally. However, for a significant proportion of patients, the screening will occur during the pregnancy, and the time constraint for obtaining screening results may result in psychological distress. This guideline does not include a cost analysis.

Recommendations

1. Carrier screening for thalassemia and hemoglobinopathies should be offered to a woman if she and/or her partner are identified as belonging to an ethnic population whose members are at higher risk of being carriers. Ideally, this screening should be done pre-conceptionally or as early as possible in the pregnancy. (II-2A)
2. Screening should consist of a complete blood count, as well as hemoglobin electrophoresis or hemoglobin high performance liquid chromatography. This investigation should include quantitation of HbA2 and HbF. In addition, if there is microcytosis (mean cellular volume < 80 fL) and/or hypochromia (mean cellular hemoglobin < 27 pg) in the presence of a normal hemoglobin electrophoresis or high performance liquid chromatography the patient should be investigated with a brilliant cresyl blue stained blood smear to identify H bodies. A serum ferritin (to exclude iron deficiency anemia) should be performed simultaneously. (III-A)
3. If a woman's initial screening is abnormal (e.g., showing microcytosis or hypochromia with or without an elevated HbA2, or

a variant Hb on electrophoresis or high performance liquid chromatography) then screening of the partner should be performed. This would include a complete blood count as well as hemoglobin electrophoresis or HPLC, HbA2 and HbF quantitation, and H body staining. (III-A)

4. If both partners are found to be carriers of thalassemia or an Hb variant, or of a combination of thalassemia and a hemoglobin variant, they should be referred for genetic counselling. Ideally, this should be prior to conception, or as early as possible in the pregnancy. Additional molecular studies may be required to clarify the carrier status of the parents and thus the risk to the fetus. (II-3A)
5. Prenatal diagnosis should be offered to the pregnant woman/couple at risk for having a fetus affected with a clinically significant thalassemia or hemoglobinopathy. Prenatal diagnosis should be performed with the patient's informed consent. If prenatal diagnosis is declined, testing of the child should be done to allow early diagnosis and referral to a pediatric hematology centre, if indicated. (II-3A)
6. Prenatal diagnosis by DNA analysis can be performed using cells obtained by chorionic villus sampling or amniocentesis. Alternatively for those who decline invasive testing and are at risk of hemoglobin Bart's hydrops fetalis (four-gene deletion α-thalassemia), serial detailed fetal ultrasound for assessment of the fetal cardiothoracic ratio (normal < 0.5) should be done in a centre that has experience conducting these assessments for early identification of an affected fetus. If an abnormality is detected, a referral to a tertiary care centre is recommended for further assessment and counselling. Confirmatory studies by DNA analysis of amniocytes should be done if a termination of pregnancy is being considered. (II-3A)
7. The finding of hydrops fetalis on ultrasound in the second or third trimester in women with an ethnic background that has an increased risk of α-thalassemia should prompt immediate investigation of the pregnant patient and her partner to determine their carrier status for α-thalassemia. (III-A)

Validation: This guideline has been prepared by the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists (CCMG) and the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) and approved by the Board of Directors of the CCMG and the Executive and Council of the SOGC.

J Obstet Gynaecol Can 2008;30(10):950–959

INTRODUCTION

The current Canadian Guidelines for Prenatal Diagnosis recommend carrier screening be offered to individuals belonging to population groups known to have an increased risk of carrying certain genetic disorders.[1] This population-based genetic screening approach is recommended for the reproductive counselling of healthy couples at risk of having an affected offspring, preferably prior to conception or prior to the birth of such a child. Through genetic counselling, and with the option of prenatal diagnosis, the birth of a severely affected child can be avoided, if desired.

ABBREVIATIONS

CBC	complete blood count
CVS	chorionic villus sampling
Hb	hemoglobin
HPLC	high performance liquid chromatography
MCH	mean cell hemoglobin
MCV	mean cell volume

The thalassemias and the hemoglobinopathies (the most common being sickle cell disorders) are autosomal recessive conditions affecting the quantity and quality, respectively, of hemoglobin molecules within red blood cells. These disorders are found more commonly in certain ethnic groups, lending themselves to effective ethnicity-based population screening.[2,3] The populations at increased risk of thalassemia and/or sickle cell disorders are shown in Table 2.

The thalassemias are distributed across Africa, the Mediterranean region, the Middle East, the Indian subcontinent, and China and throughout southeast Asia in a line stretching from Southern China down the Malaysian peninsula to the Indonesian islands.[4,5] In these populations, the carrier frequency is greater than 1%, in contrast to a carrier frequency of approximately 0.1% in individuals of Northern European ancestry. Table 2 also shows that sickle cell disorders are seen more frequently in populations of African and Caribbean descent and in individuals of Mediterranean, Middle Eastern, and East Indian descent.[6,7] According to the 2001 Canadian Census, over 3.7 million Canadians (approximately 12.5% of the population) identified their ethnic origin as one known to be at increased risk of thalassemia or hemoglobinopathy. Given the proven benefits of carrier screening programs for hemoglobin disorders in the parts of the world where populations are known to be at high risk for these conditions,[8–10] this document was developed to review this group of disorders and provide recommendations to health care providers in Canada for screening pre-conceptionally or early in pregnancy.

EVIDENCE AND OPINION

Accepted criteria for a carrier screening program have been previously published[11] and can be summarized as follows:

- Serious recessive disorder
- Intervention available and impacts outcome
- High frequency of carriers expected
- Availability of inexpensive, reliable test
    - high detection rate
    - low false positive rate
- Access to genetic counselling
- Voluntary participation

The thalassemias and sickle cell disorders meet all the disease-specific criteria for screening: high frequency of carriers, severity of disease, availability of a reliable and inexpensive screening test, and intervention that will affect outcome.

Table 1. Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care

Quality of Evidence Assessment*	Classification of Recommendations†
I: Evidence obtained from at least one properly randomized controlled trial	A. There is good evidence to recommend the clinical preventive action
II-1: Evidence from well-designed controlled trials without randomization	B. There is fair evidence to recommend the clinical preventive action
II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group	C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making
II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category	D. There is fair evidence to recommend against the clinical preventive action
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	E. There is good evidence to recommend against the clinical preventive action
	L. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making

*The quality of evidence reported in these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.[28]
†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the The Canadian Task Force on Preventive Health Care.[28]

THALASSEMIA

The thalassemias are the result of genetic defects that limit the production of specific globin chains of the Hb molecule. Thalassemias are named by reference to the affected globin chain: α-thalassemia involves the α chain, β-thalassemia the β chain. The major adult Hb, HbA, consists of four globin chains (two alpha [α] and two beta [β] chains, represented as $\alpha_2\beta_2$), each linked to a heme molecule. Other minor hemoglobins in adults include HbF (fetal hemoglobin, $\alpha_2\gamma_2$) and HbA2 ($\alpha_2\delta_2$). A normal individual has a total of four α globin genes mapped to the short arm of chromosome 16 (two genes per chromosome, represented as αα/αα) and two β globin genes mapped to the short arm of chromosome 11 (one per chromosome, or β/β). The β-like genes (such as γ and δ) are nearby on chromosome 11.

α-Thalassemia

α-Thalassemia occurs when a genetic mutation leads to reduced synthesis from one or more of the four α-globin genes.[2] α^0-Thalassemia refers to a deletion of both α globin genes on the same chromosome (designated—/), while α^+-thalassemia refers to a deletion of a single α globin gene, leaving the other α globin gene on that chromosome intact (i.e., α-/). In the large majority of cases of α-thalassemia, the α globin chains are structurally normal: they are merely reduced in quantity.

Individuals with only one α globin deletion, that is, heterozygotes for α^+-thalassemia, are known as silent carriers (αα/α-).[12] These individuals are asymptomatic and generally have normal routine hematologic findings: a CBC will usually show normal Hb, MCV, and MCH. Rarely the MCV and/or MCH can be low. Routine laboratory testing for thalassemia (such as Hb electrophoresis, Hb HPLC, and H body staining of a peripheral blood smear—see below) is usually negative outside the newborn period. In general, diagnosis of single gene deletion α-thalassemia can be proven only by molecular (DNA) testing.

Individuals with two deleted copies of the a-globin gene have α-thalassemia trait. These individuals are either heterozygotes for α^0-thalassemia (αα/—) or homozygotes for α^+-thalassemia (α-/α-). Both of these types of α-thalassemia trait (i.e. αα/—or α-/α-) are essentially identical clinically and on routine hematology testing. Patients are generally asymptomatic. A CBC will typically show microcytosis (low MCV, e.g., < 80 fL) and hypochromia (low MCH, e.g., < 27 pg); the patient may also be mildly anemic. Despite anemia, the red blood cell count is often mildly elevated. Hb electrophoresis and Hb HPLC are normal in α-thalassemia trait after the newborn period, and the HbA2 level is normal, which is not the case in β-thalassemia trait (see below for a discussion of HbA2 in β-thalassemia). α-Thalassemia trait is usually diagnosed by staining a peripheral blood smear with brilliant cresyl blue

to detect abnormal red blood cell inclusions called H bodies. Although not pathognomonic, the finding of H bodies in the right clinical and ethnic context is typically considered diagnostic of α-thalassemia trait. As noted above, patients with a double deletion on one chromosome (αα/—) and patients with single deletions on both chromosomes (α-/α-) have essentially the same results in all these tests, and can be differentiated only by molecular methods. Such molecular distinction is crucial for the identification of a couple at risk for having a fetus with four-gene deletion α-thalassemia (hemoglobin Bart's hydrops fetalis, or Hb Bart's disease,—/—). Hb Bart's disease can occur only when the fetus inherits a double deletion from each parent, that is, when both parents are carriers of α^0-thalassemia, αα/—. In such a situation, the couple has a 25% risk of having a fetus with all four α globin genes deleted.

Hemoglobin Bart's hydrops fetalis is characterized by severe intrauterine anemia resulting in fetal hydrops and, in almost all cases, intrauterine death. There are only rare case reports of infants surviving with this condition. Without any α globin production, these fetuses are unable to make any fetal (HbF) or adult (HbA) hemoglobin. Instead, they can produce only embryonic hemoglobins, which generally cannot support life past the third trimester. Some of these fetuses will also have congenital abnormalities such as terminal limb defects.[13] Pregnancies affected with hydrops fetalis that continue should be monitored carefully for the development in the mother of Ballantyne syndrome (also known as mirror syndrome) as any severe fetal hydrops with massive placental hydrops may produce this potentially lethal maternal complication.[14]

Between the two-gene deletion of a-thalassemia trait and the four-gene deletion in Hb Bart's hydrops fetalis is the three-gene deletion hemoglobin H disease. Patients with Hb H disease have one functioning α globin gene (α-/—): they inherit α^0-thalassemia (—/) from one parent and α^+-thalassemia (α-/) from the other. This condition is in most cases clinically mild: many patients require occasional transfusion of red blood cells, but these patients are usually not considered "transfusion dependent." However, marked phenotypic variability has been noted, and some patients do require regular transfusions to survive. There are even occasional cases of Hb H disease presenting as hydrops fetalis.[15] Patients with Hb H disease are usually anemic, microcytic, and hypochromic on routine hematologic testing. Hb electrophoresis and HPLC may show an abnormal hemoglobin, Hb H, although in some cases this may be difficult to detect or require special techniques. On H-body staining, almost every red blood cell will show Hb H inclusions. Because Hb H disease is generally clinically mild, most prenatal screening programs for α-thalassemia are not designed to prevent this condition; instead, screening programs typically focus on the lethal Hb Bart's hydrops fetalis.

The different types of α-thalassemia are usually the consequence of deletional mutations involving α globin gene loci. There are rare non-deletional mutations affecting α globin production, such as Hb Quong Sze and Hb Constant Spring. In most respects these are similar to the more common deletional forms of α-thalassemia, although some are clinically more severe: for example, a patient with a two-gene α globin deletion and a concomitant Constant Spring α globin mutation (and therefore with the genotype α-/α^{CS}- or $\alpha\alpha^{CS}$/—) will usually be more severely affected and require more transfusion support than a patient with routine three-gene deletion Hb H disease (α-/—). Non-deletional forms of α-thalassemia can often be diagnosed by phenotypic laboratory methods (such as Hb electrophoresis), or they may require molecular testing.

β-Thalassemia

β-Thalassemia arises out of mutations in the β globin genes on chromosome 11. These mutations can result in decreased (β^+) or absent (β^0) β globin production. As with α-thalassemia, in β-thalassemia the β globin chains are structurally normal but quantitatively reduced.

Since normal individuals have two copies of the β gene, individuals with one normal gene and one affected gene (genotypically represented as β/β^+ or β/β^0 depending on the type of β gene mutation) are often asymptomatic. Clinically these patients are described as having β-thalassemia trait or β-thalassemia minor. The CBC will show mild or no anemia, and MCV and MCH are usually low. Like α-thalassemia trait, the RBC count is often high. Because reduced production of β globin means an inability to generate as much normal HbA ($\alpha_2\beta_2$), they compensate by increasing production of other β-like chains, namely δ and γ, leading to increases in the levels of the minor hemoglobins HbA2 ($\alpha_2\delta_2$) and HbF ($\alpha_2\gamma_2$). The routine diagnostic test is Hb electrophoresis or Hb HPLC: these will demonstrate increases in HbA2 (i.e., > 3.5% of total hemoglobin) and usually HbF (i.e., >1%).[12] In the right clinical and ethnic context, an elevated HbA2 is considered diagnostic of β-thalassemia trait.

If two mutated β globin genes are inherited, one from each parent, the patient may be clinically categorized as either β-thalassemia intermedia or β-thalassemia major. In intermedia there is limited β globin production; in contrast, there is either zero or almost zero β globin chain synthesis

in major. Genotypically, intermedia is usually β^+/β^+ or possibly β^+/β^0, while β-thalassemia major is β^+/β^0 or β^0/β^0. The clinical distinction between intermedia and major syndromes is based on what degree of transfusion support is required: intermedia patients may require periodic transfusion, whereas in β-thalassemia major, regular lifelong red blood cell transfusions are necessary. In both of these syndromes, by 6 to 12 months of age, patients will be anemic, microcytic, and hypochromic, and Hb electrophoresis or HPLC will show elevated HbA2. Clinically, most of the problems encountered by these patients are actually complications of transfusion rather than complications of the disease itself, and include iron overload syndromes and infectious complications. Despite therapy, these complications cause death in early adult life in most affected individuals, unless a cure is achieved via successful bone marrow transplantation.

VARIANT HEMOGLOBINS WITH SIMULTANEOUS β-THALASSEMIA

Clinically significant disease can occur from the co-inheritance of a β-thalassemia mutation (β^+ or β^0) from one parent, and a mutation that results in production of a mutant ("variant") β globin chain from the other parent.

The most common variant hemoglobins that may be co-inherited with β-thalassemia are Hemoglobin S, Hemoglobin E, and Hemoglobin C. HbS/β-thalassemia can produce a sickling syndrome of variable severity (see below). Hemoglobin E is sometimes referred to as a "thalassemic hemoglobinopathy," because in addition to being structurally abnormal it is produced in reduced quantities. HbE is extremely common in some Southeast Asian populations, reaching a gene frequency of up to 70% in northern Thailand.[16] A patient who inherits a HbE mutation from one parent and a β-thalassemia mutation from another (HbE/β-thalassemia) will clinically be similar to a patient with β-thalassemia intermedia or major. In fact, this combination (HbE/β-thalassemia) is one of the most important causes of clinically severe thalassemia worldwide. Hemoglobin C/β-thalassemia is clinically and hematologically very heterogeneous, ranging from very mild to very severe.

SICKLE HEMOGLOBINOPATHIES

Whereas the thalassemias are caused by gene mutations resulting in decreased production of α or β globin chains, the sickle cell disorders are hemoglobinopathies caused by mutations in the β globin gene resulting in the production of a structurally abnormal β globin chain. This abnormal hemoglobin, HbS, forms a gel polymer at conditions of low oxygen tension. Collectively, the polymerized Hb molecules force the red cell into a sickled shape, which may become trapped in small blood vessels causing a wide variety of clinically severe complications and increased mortality in affected individuals.

Sickle Cell Anemia (HbSS)

Homozygous HbS disease, sickle cell anemia, affects individuals who have inherited from both parents the specific HbS mutation in the β globin gene. In this condition, no normal HbA is produced: instead, red cells contain primarily HbS (with small amounts of HbA2 and HbF). In HbSS, sickled RBCs have a considerably shorter life span than normal RBCs (due to extravascular hemolysis) and cause intermittent episodes of vascular occlusion under conditions of decreased oxygen tension. This causes tissue ischemia, and acute and chronic organ dysfunction involving the spleen, brain, lungs, and kidneys. Pain and swelling of hands and feet (hand-foot disease) is a frequent early presentation of this disease in infants and young children, and occurs as a result of aseptic necrosis of the small carpal and tarsal bones. The hemolysis leads to chronic anemia and predisposes the patient to aplastic crises.[17]

In contrast to the generally severe manifestation of homozygous HbS, heterozygous carriers (i.e., HbS heterozygotes, designated HbAS) are asymptomatic and have normal red cell indices on CBC, with normal RBC morphology on peripheral blood smear. Both HbSS and HbAS are routinely diagnosed by Hb electrophoresis or HPLC.

Other Sickle Cell Disorders

The clinical picture of sickle cell anemia is also seen in individuals with genetic mutations other than HbSS. The two most common other situations are co-inheritance of HbS and HbC (also known as SC disease), and the co-inheritance of HbS with β-thalassemia (i.e., S/β-thalassemia).[17] Sickling syndromes also occur in individuals who co-inherit HbS with other hemoglobinopathies, such as HbD.

Individuals with SC disease inherit the β gene mutation responsible for HbS from one parent, and the β gene mutation for HbC from the other parent. These individuals usually are less severely affected than individuals with HbSS. SC can generally be diagnosed with Hb electrophoresis or HPLC, as can other double heterozygote conditions like SD.

The clinical severity of S/β-thalassemia depends on the type of β-thalassemia mutation. If the patient is S/β^0, then no normal β globin chains are produced: one gene produces only the S version of the β chain, and the other gene produces nothing. Like patients with HbSS, these patients have "only" HbS (plus HbA2 and HbF), and no HbA. The clinical and hematological findings are comparable to sickle cell

anemia (with some differences, such as microcytosis in S/β-thalassemia but not in HbSS). If the patient is instead S/β^+, then some functioning β globin is produced, and therefore some HbA is present. These patients have a milder sickling syndrome than HbSS or S/β^0. Differentiating S/β^0-thalassemia from HbSS requires not only Hb HPLC but also additional information such as CBC results, physical examination findings, and occasionally family or molecular studies.

SCREENING FOR THALASSEMIA AND HEMOGLOBINOPATHIES

Populations at increased risk for thalassemia or sickle cell disorders are listed in Table 2. Of note, Japanese, Koreans, Caucasians of Northern European ancestry, Native Americans (First Nations in Canada), and Inuit are not at increased risk of hemoglobinopathies.[18] From a practical point of view, one can take the approach that any patient who is not Japanese, Korean, Caucasian of Northern European ancestry, First Nations, or Inuit should be screened.

There are a variety of approaches worldwide to screen for α - and β-thalassemia that are based on the clinical presentation of these disorders. A relatively common approach has consisted of a complete blood count to assess the mean cell volume and the mean cell hemoglobin.[19] The finding of a normal MCV (i.e., ≥ 80 fL) in combination with a normal MCH (i.e., ≥ 27 pg) would rule out most cases of thalassemia and would require no additional thalassemia testing. For individuals with MCV < 80 fL or MCH < 27 pg, the next step is hemoglobin electrophoresis or HPLC, quantitation of HbA2 and HbF, and a blood smear stained for H bodies. This approach is generally successful in women and couples at risk of having children with severe thalassemic syndromes. However, it may fail to detect carriers of hemoglobinopathies such as HbS, HbC, or HbD, because hemoglobinopathy heterozygotes may have a normal CBC (normal MCV and normal MCH). Given that homozygosity and compound heterozygosity for a hemoglobinopathy thalassemia can produce very significant morbidity and early mortality, hemoglobinopathy screening may be as important as screening for thalassemias.[20] Detection of hemoglobinopathy carriers cannot be reliably performed by CBC alone and requires hemoglobin HPLC or electrophoresis.[21] Therefore it is appropriate to recommend that all pregnant women from an ethnic background at increased risk of hemoglobinopathy and/or thalassemia be screened by both CBC, to assess the MCV and MCH, and a hemoglobin electrophoresis or HPLC.

- An MCV ≥ 80 fL and an MCH ≥ 27 pg, with a normal electrophoresis or HPLC, requires no further testing.
- Individuals with an MCV < 80 fL or MCH < 27 pg can have α- and/or β-thalassemia and/or iron deficiency anemia.
- In general, β-thalassemia trait can be reliably diagnosed by hemoglobin electrophoresis or HPLC, with HbA2 and HbF quantitation. Patients with β-thalassemia trait have an elevated HbA2, i.e., > 3.5%.
- In patients with a low MCV, but with a normal Hb electrophoresis/HPLC and HbA2 and HbF quantitation, the differential diagnosis includes iron deficiency anemia and α-thalassemia. A serum ferritin (to exclude iron deficiency anemia) and a smear to screen for the H bodies of α-thalassemia are therefore required. For pregnant patients, these tests (ferritin and H body stain) should be ordered concurrently. In the right clinical context (e.g., microcytic anemia in an "ethnically appropriate" patient), the presence of H bodies can identify the patient as a carrier of α-thalassemia.
- H body testing is not 100% sensitive (see below), and therefore the absence of H bodies does not completely exclude α-thalassemia carrier status in an ethnically at-risk patient. If iron deficiency is ruled out in a pregnant woman with a negative H body test, testing of the partner remains crucial to determine the risk of having an affected fetus with Hemoglobin Bart's hydrops fetalis. Molecular studies may also be done to confirm or exclude carrier status for α-thalassemia.
- Hemoglobin electrophoresis or HPLC will allow identification of Hb variants, such as HbS, C, D, and E. A phenotypic sickle cell preparation (such as a slide or tube sickling test) is not helpful in identifying other types of β globin variants besides HbS, so should not be used as a carrier screen for hemoglobinopathies.
- The finding of any abnormality (e.g., low MCV or MCH, or abnormal hemoglobin electrophoresis or HPLC) requires screening of the partner, which entails CBC, smear for H bodies, hemoglobin electrophoresis or HPLC, and HbA2 and HbF quantitation. If the patient is pregnant, testing of the partner should be done promptly.
- If both partners are found to be carriers of hemoglobin mutations (i.e., any combination of thalassemias and Hb variants), they should be referred for genetic counselling, ideally in the pre-conception period, or as early as possible in the pregnancy. Additional molecular studies may be required to clarify the risk to the fetus.

Please note that the foregoing paragraphs make repeated reference to the use of an H body stain on peripheral blood to test for α-thalassemia. Although this is a very useful test

Table 2. Geographic distribution of ethnic populations at increased risk for thalassemia or sickle cell disorders

Regions of Origin	Thalassemia	Sickle Cell Disease
Africa	↑	↑
Mediterranean region e.g., Sardinia, Corsica, Sicily, Italy, Spain, Portugal, Greece, Cyprus, Turkey, Egypt, Algeria, Libya, Tunisia, Morocco, Malta	↑	↑
Middle East e.g., Iran, Iraq, Syria, Jordan, Saudi Arabia and other Arabian peninsula countries, Qatar, Lebanon, Palestine, Israel (both Arabs and Sephardic Jews affected), Kuwait	↑	↑
South East Asia e.g., India, Afghanistan, Pakistan, Indonesia, Bangladesh, Thailand, Myanmar	↑	↑ in parts of India
Western Pacific region e.g., China, Vietnam, Philippines, Malaysia, Cambodia, Laos	↑	–
Caribbean countries	↑	↑
South American countries	↑	↑

if performed in a laboratory that has experience in performing these tests, its sensitivity has been called into question. For example, a 1992 study[22] found that the sensitivity of the H body stain in patients with a double α deletion (i.e., αα/—) was approximately 90%; in other words, almost 10% of patients with an αα/—genotype had a false negative H body stain. Given this limitation, in women found to have unexplained low MCV or MCH whose partner is shown to be or suspected to be a carrier of α-thalassemia, molecular analysis for α-thalassemia should be done even if the H body screen is negative. As molecular analysis for α-thalassemia (e.g., multiplex PCR) becomes faster and more inexpensive, some laboratories may wish to omit the H body stain and proceed directly to molecular screening in all cases with unexplained low MCV or MCH.

The Figure illustrates the approach to screening. Given the autosomal recessive nature of thalassemia and sickle cell disorders, the identification of any individual as a carrier indicates that the mutation is segregating in the family. Carriers need to be informed that their siblings, their children, and other family members are at increased risk of being carriers and should discuss the matter with their health care providers.

INDICATIONS FOR ADDITIONAL MOLECULAR TESTING AND REFERRAL TO A GENETIC CENTRE

A number of clinical scenarios arising from screening will require molecular (DNA) testing to clarify the carrier status, and hence the risk of having an affected child, and/or to provide the information required for prenatal diagnosis. These studies can be facilitated through referral to a clinical genetics unit. Table 3 provides the most common indications for molecular testing in the context of screening for hemoglobinopathies.

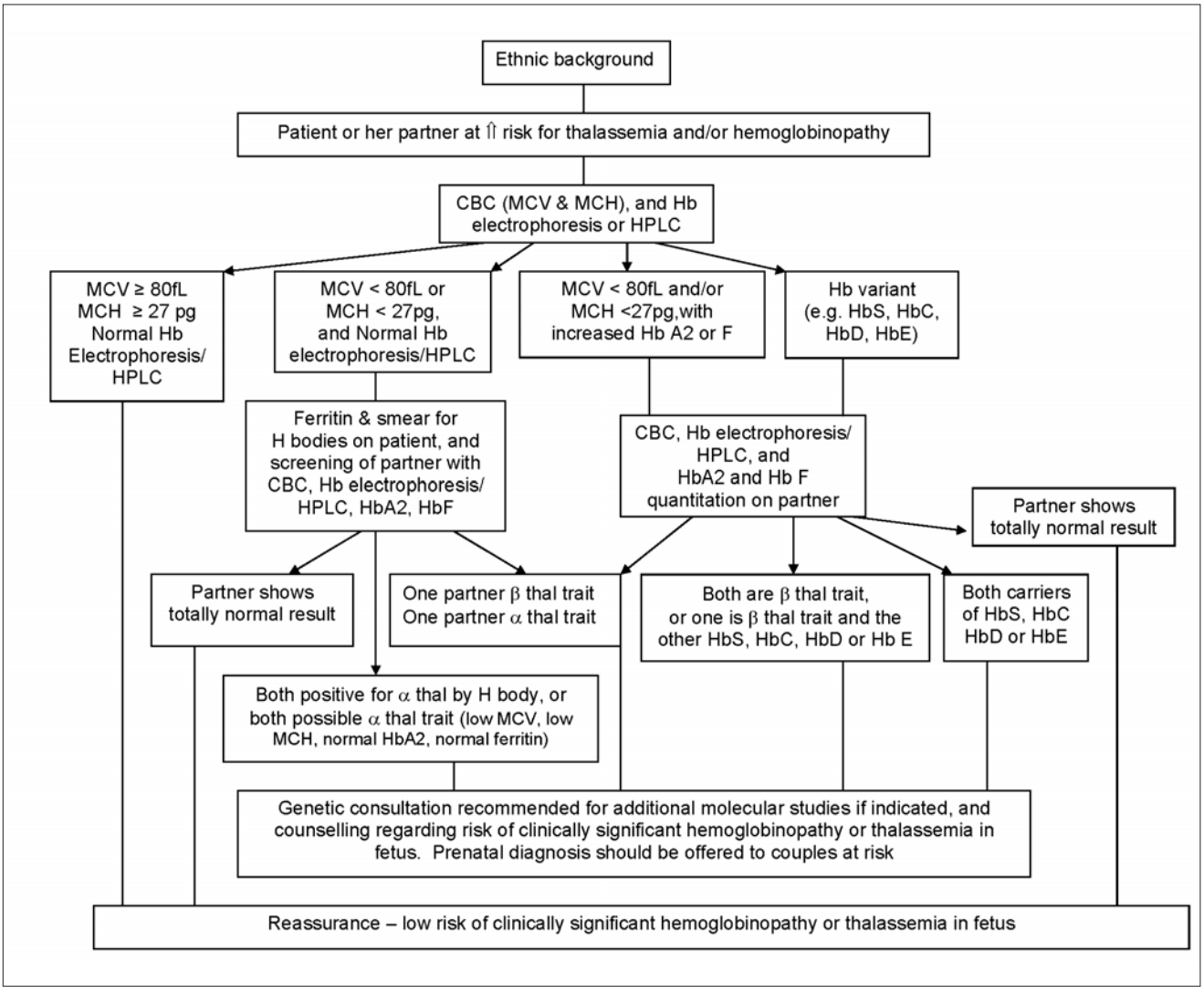
Prenatal Diagnosis

Prenatal diagnosis by DNA analysis of chorionic villi or amniocytes should be offered to all carrier couples at risk of having a child with a clinically significant thalassemia or sickle cell disorder, and should be performed with the informed consent of the woman or couple.

For couples at risk of severe α-thalassemia who decline invasive prenatal diagnosis, or who present after 20 weeks of gestation, ultrasound assessments in a tertiary centre to determine the fetal cardiothoracic ratio could be offered. This has been shown to be a reliable mean of identification of a fetus with Hb Bart's hydrops fetalis.[23] This can be done at 18 and 22 weeks' gestation. A four chamber cardiac view is obtained, and the fetal transverse cardiac diameter at the level of the atrioventricular valves between the epicardial surfaces is measured and expressed as a ratio against the transverse thoracic diameter.[23,24] A cardiothoracic ratio level equal to or greater than 0.5 identifies all affected pregnancies and has a false positive rate of 8%. No false positives were noted if the cardiothoracic ratio was ≥ 0.53.[23] An abnormal finding on ultrasound examination should lead to confirmatory studies by DNA analysis of amniocytes or chorionic villi, if termination of pregnancy is being considered.

Doppler measurement of the middle cerebral artery peak systolic velocity (MCA-PSV) may be another approach to assess pregnancies at risk of severe α-thalassemia when the

Approach to screening



woman or couple decline invasive prenatal diagnosis. This approach has, however, not been validated in the second trimester for this indication. A number of studies have demonstrated the use of MCA-PSV to estimate hemoglobin concentration in fetuses at risk of anemia, but most of those fetuses were at risk because of maternal red cell alloimmunization or congenital infection.[25,26] One study reports on MCA-PSV in the investigation of non-immune hydrops, with only one case in their series due to α-thalassemia.[27] In that one case, there was a correlation between the severity of the anemia present and increased MCA-PSV. The only study that has investigated the use of MCA-PSV in a series of 19 affected fetuses homozygous for α^0-thalassemia (i.e., genotype—/—) did so at 12 to 13 weeks' gestation. Although at that gestational age the affected fetuses had significantly higher MCA-PSV, extensive overlap was noted between affected and unaffected fetuses, precluding the use of this method in predicting anemia at that gestational age.[28] No similar study has been done at 18 to 20 weeks' gestation.

Finally, the finding of hydrops fetalis on ultrasound in the second or third trimester in a pregnant patient with an ethnic background giving an increased risk of α-thalassemia should prompt immediate investigation of the pregnant woman and her partner to determine their carrier status for α-thalassemia.

Postnatal Diagnosis

If prenatal testing is not conducted in a pregnancy at risk of thalassemia or a sickle cell disorder, testing of the child should be performed to allow early diagnosis and referral to a pediatric hematology centre, if indicated. Although molecular testing can be done at any age, the timing of hematological testing (should molecular testing not be available) depends on the type of hemoglobin abnormality in question.

Table 3. Indications for referral to medical genetics for additional molecular studies and counselling

Clinical scenario	Testing required
Both members of the couple are found to be β-thalassemia carriers	DNA analysis of the β globin gene
One partner is a β-thalassemia carrier and the other partner is a carrier of an Hb variant (e.g., HbS, HbE)	
Both partners are carriers of HbS	
One partner is a carrier of HbS and the other is a carrier of HbC or HbD	
One partner is a β-thalassemia carrier and the other is an α thalassemia carrier.	DNA analysis of the α globin locus
Both partners are α-thalassemia carriers	
Both partners have low MCV or low MCH, with normal iron studies and normal hemoglobin electrophoresis/HPLC	

Homozygous and compound heterozygote hemoglobinopathies (e.g., HbSS and HbSC, respectively), as well as the most severe β-thalassemic syndromes (e.g., β^0-thalassemia) can be diagnosed in infancy with Hb HPLC. The first week of life is also an excellent time to diagnose α-thalassemia by Hb HPLC.

In contrast, making a conclusive diagnosis of some heterozygous hemoglobinopathies or thalassemias (e.g., Hb E trait, or β-thalassemia trait) can be more difficult before six months of age, unless molecular methods are used. Fortunately there are few clinical reasons to seek out most "trait" diagnoses in infancy, so investigation can be easily deferred for most of these patients until they are at least six months old. There are in general good reasons to identify pediatric "trait" patients (e.g., to explain a persistent microcytosis in a child), but these conditions do not usually have to be identified in infancy.

If there is any confusion about the timing or nature of appropriate testing for these disorders in infants or children, consultation with a hematopathologist or pediatric hematologist may be useful.

Recommendations

1. Carrier screening for thalassemia and hemoglobinopathies should be offered to a woman if she and/or her partner are identified as belonging to an ethnic population whose members are at higher risk of being carriers. Ideally, this screening should be done pre-conceptionally or as early as possible in the pregnancy. (II-2A)

2. Screening should consist of a complete blood count, as well as hemoglobin electrophoresis or hemoglobin high performance liquid chromatography. This investigation should include quantitation of HbA2 and HbF. In addition, if there is microcytosis (mean cellular volume < 80 fL) and/or hypochromia (mean cellular hemoglobin < 27 pg) in the presence of a normal hemoglobin electrophoresis or high performance liquid chromatography the patient should be investigated with a brilliant cresyl blue stained blood smear to identify H bodies. A serum ferritin (to exclude iron deficiency anemia) should be performed simultaneously. (III-A)

3. If a woman's initial screening is abnormal (e.g., showing microcytosis or hypochromia with or without an elevated HbA2, or a variant Hb on electrophoresis or high performance liquid chromatography) then screening of the partner should be performed. This would include a complete blood count as well as hemoglobin electrophoresis or HPLC, HbA2 and HbF quantitation, and H body staining. (III-A)

4. If both partners are found to be carriers of thalassemia or an Hb variant, or of a combination of thalassemia and a hemoglobin variant, they should be referred for genetic counselling. Ideally, this should be prior to conception, or as early as possible in the pregnancy. Additional molecular studies may be required to clarify the carrier status of the parents and thus the risk to the fetus. (II-3A)

5. Prenatal diagnosis should be offered to the pregnant woman/couple at risk for having a fetus affected with a clinically significant thalassemia or hemoglobinopathy. Prenatal diagnosis should be performed with the patient's informed consent. If prenatal diagnosis is declined, testing of the child should be done to allow early diagnosis and referral to a pediatric hematology centre, if indicated. (II-3A)

6. Prenatal diagnosis by DNA analysis can be performed using cells obtained by chorionic villus sampling or amniocentesis. Alternatively for those who decline invasive testing and are at risk of hemoglobin Bart's hydrops fetalis (four-gene deletion α-thalassemia), serial detailed fetal ultrasound for assessment of the fetal

cardiothoracic ratio (normal < 0.5) should be done in a centre that has experience conducting these assessments for early identification of an affected fetus. If an abnormality is detected, a referral to a tertiary care centre is recommended for further assessment and counselling. Confirmatory studies by DNA analysis of amniocytes should be done if a termination of pregnancy is being considered. (II-3A)

7. The finding of hydrops fetalis on ultrasound in the second or third trimester in women with an ethnic background that has an increased risk of α-thalassemia should prompt immediate investigation of the pregnant patient and her partner to determine their carrier status for α-thalassemia. (III-A)

ACKNOWLEDGEMENTS

This guideline is dedicated to the memory of Dr Carl Nimrod, who requested this new guideline when he was a member of SOGC Council. Dr Nimrod is missed by the SOGC community, friends, and faculty and students at the University of Ottawa.

SOGC and CCMG thank Dr Isaac Odame from the division of Hematology and Oncology, department of Pediatrics of the University of Toronto for critically reviewing the manuscript.

REFERENCES

1. Chodirker BN, Cadrin C, Davies GAL, Summers AM, Wilson RD, Winsor EJT, Young D, et al. Canadian guidelines for prenatal diagnosis. Genetic indications for prenatal diagnosis. SOGC Clinical Practice Guideline No. 105, June 2001. J Soc Obstet Gynaecol Can 2001;23:525–31.
2. Weatherall DJ. The thalassaemias. BMJ 1997;314:1675–8.
3. Davies SC. Cronin E. Gill M. Greengross P. Hickman M. Normand C. 1Screening for 1sickle 1cell disease and thalassaemia: a systematic review with supplementary research. Health Technol Assesstles-source1 2000;4:1–99.
4. Lookopoulos D, Kollia P. Worldwide distribution of áThalassemia: In: Steinberg MH, Forget B, Higgs DR, Nagel RL, eds. Disorders of hemoglobin. Genetics, pathophysiology, and clinical management. Cambridge: Cambridge University Press; 2001:861–77.
5. Bernini LF. Geographic distribution of á thalassemia: In Steinberg MH, Forget B, Higgs DR, Nagel RL, eds. Disorders of hemoglobin. Genetics, pathophysiology, and clinical management. Cambridge: Cambridge University Press;2001:878–94.
6. Weatherall DJ. Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bulletin of the World Heath Organization 2001;79:704–11.
7. Steinberg MH. Sickle Trait: In: Steinberg MH, Forget B, Higgs DR, Nagel RL, eds. Disorders of hemoglobin. Genetics, pathophysiology, and clinical management. Cambridge: Cambridge University Press;2001:811–30.
8. Cao A, Rosatelli MC, Monni G, Galanello R. Screening for thalassemia. A model of success. Obstet Gynecol Clin N Am 2002;29:305–28.
9. Leung KY, Lee CP, Tang HY, Lau ET, Ng LKL, Lee YP, et al. Cost-effectiveness of prenatal screening for thalassaemia in Hong Kong. Prenat Diagn 2004;24:899–907.
10. Fessas P. Prevention of thalassaemia and haemoglobin S syndromes in Greece. Acta Haematologica 1987;78:168–72.
11. Khoury MJ, McCabe LL, McCabe ERB. Population screening in the age of genomic medicine. New Engl J Med 2003;348:50–8.
12. Leung, TN, Lau TK, Chung TKH. Thalassaemia screening in pregnancy. Curr Opin Obstet Gynecol 2005;17:129–34.
13. Chitayat D, Silver MM, O'Brien K, Wyatt P, Waye JS, Chiu DH, et al. Limb defects in homozygous alpha-thalassemia: report of three cases. Am J Med Genet 1997;68:162–7.
14. Carbillon L, Oury JF, Guerin JM, Zancot A, Blot P. Clinical biological features of Ballantyne Syndrome and the role of placental hydrops. Obstet Gynecol Surv 1997;52:310–4.
15. Chui DH, Fucharoen S, Chan V. Hemoglobin H disease: not necessarily a benign disorder. Blood 2003;101:791–800.
16. Bain B. Haemoglobinopathy diagnosis. Oxford: Blackwell Science Ltd.;2001:163. Redding-Lallinger R. Knoll C. Sickle cell disease—pathophysiology and treatment. Curr Probl Pediatr Adolesc Health Care 2006:36:346–76.
17. ACOG Committee on Obstetrics. ACOG Practice Bulletin No. 78: hemoglobinopathies in pregnancy. tles-sourceObstet Gynecol 2007;109:229–37.
18. Dumars KW, Boehm C, Eckman JR, Giardina PJ, Lane PA, Shafer FE, for the Council of Regional Networks for Genetic Services (CORN). Practical guide to the diagnosis of thalassemia. Am J Med Genet 1996;62:29–37.
19. Weatherall DJ, Clegg JB, Higgs DR, Wood WG. Hemoglobin E. In: Scriver CR, Beaudet A, Sly WS, Valle D, eds. The metabolic and molecular basis of inherited disease. New York: McGraw-Hill;2001:4592.
20. Old JM. Screening and genetic diagnosis of hemoglobin disorders. Blood Rev 2003;17:43–53.
21. Skogerboe KJ, West SF, Smith C, Terashita ST, LeCrone CN, Detter JC, et al. Screening for α-thalassemia. Correlation of hemoglobin H inclusion bodies with DNA-determined genotype. Arch Pathol Lab Med 1992;116:1012–8.
22. Lam YH, Ghosh A, Tang MHY, Lee CP, Sin SY. Early ultrasound prediction of pregnancies affected by homozygous α-thalassaemia-1. Prenat Diagn 1997;17:327–32.
23. Tongsong T, Wanapirak C, Sirichotiyakul S, Chanprapaph P. Sonographic markers of hemoglobin Bart disease at midpregnancy. J Ultrasound Med 2004;23:49–55.
24. Mari G, Detti L, Oz U, Zimmerman R, Duerig P, Stefos T. Accurate prediction of fetal hemoglobin by Doppler ultrasonography. Obstet Gynecol 2002;99:589–93.
25. Imbar T, Lev-Sagie A, Cohen S, Yanai N, Yagel S. Diagnosis, surveillance and treatment of the anemic fetus using middle cerebral artery peak systolic velocity measurement. Prenat Diagn 2006;26:45–51.
26. Hernandez-Andrade E, Scheier M, Dezerega V, Carmo A, Nicolaides KH. Fetal middle cerebral artery peak systolic velocity in the investigation of non-immune hydrops. Ultrasound Obstet Gynecol 2004;23:442–5.
27. Lam YH, Tang MHY. Middle cerebral artery Doppler study in fetuses with homozygous α-thalassaemia-1 at 12–13 weeks of gestation. Prenat Diagn 2002;22:56–8.
28. Woolf SH, Battista RN, Angerson GM, Logan AG, Eel W. Canadian Task Force on Preventive Health Care. New grades for recommendations from the Canadian Task Force on Preventive Health Care. CMAJ 2003;169(3):207–8.