

CHAPTER

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*Screening for
Hemoglobinopathies
in Canada*

By Richard B. Goldbloom

Screening for Hemoglobinopathies in Canada

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In its 1979 report, the Canadian Task Force on the Periodic Health Examination reviewed the available evidence and concluded that there was no scientific evidence to support screening for thalassemia in the general population, but that there was fair evidence to support screening of people of Asian, African, and Mediterranean ancestry.<1> This chapter updates the earlier report in the light of further publications and technological advances and extends its scope to consider screening for other hemoglobinopathies, including sickle cell disease.

Based on this updated review the Task Force concludes that 1) there is fair evidence to support selective prenatal screening of pregnant women from high risk groups (African, Mediterranean, Middle Eastern, East Indian, Hispanic and Southeast Asian ancestry) (B Recommendation); 2) there is fair evidence to offer DNA analysis of amniotic fluid or chorionic villus samples when both parents have established positive carrier status (B Recommendation); 3) there is good evidence to recommend screening to identify high-risk neonates (A Recommendation). Whether such screening should be applied universally or targeted to identified high risk groups should depend on the demographics of the population being screened; 4) there is insufficient evidence to recommend for or against screening and counselling non-pregnant adolescents and adults for carrier status (C Recommendation). All screening efforts must be accompanied by comprehensive counselling and treatment services.

Burden of Suffering

The Thalassemias

The thalassemias are hereditary conditions due to mutations causing decreased or absent production of the α -globin or β -globin chains of hemoglobin. β -thalassemia major occurs in individuals

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homozygous for a genetic defect in β -globin synthesis. Infants with β -thalassemia are usually born healthy and may remain so for as long as 2-3 years. They then develop severe anemia, requiring regular transfusions and later, iron chelation therapy. Affected individuals usually die in the third decade of life. The cost of treatment is very high, estimated at \$30,000 per year, over 30-35 years or almost \$1 million per patient. Parents of affected children experience considerable stress as a result of this chronic health problem and its treatment. Individuals who are heterozygous for either type of thalassemia may experience mild, hypochromic anemia but are otherwise healthy and asymptomatic.

The β -thalassemias occur among individuals of East Indian, Mediterranean, African, Middle Eastern, Southeast Asian or Hispanic origin, and the proportion of such individuals in the Canadian population is increasing. For example, among Ontario's population of approximately 10 million, about 20% are of African, Southeast Asian, Mediterranean or Middle Eastern ancestry – all groups in which the incidence of hemoglobinopathies is relatively high. Over 130 β -thalassemia mutations have been described.

α -thalassemias result from deletions in 1 or more of the 4 genes responsible for α -globin synthesis. They are common in persons of Southeast Asian descent, but also occur in persons of African and Mediterranean origin. Fetuses with a 4-gene deletion develop hydrops fetalis secondary to severe anemia and die before or soon after birth.

Mothers of these infants are at risk for toxemia during pregnancy, for operative delivery, and for post-partum hemorrhage. The three-gene deletion is referred to as Hemoglobin H disease and affects about 1% of Southeast Asians. Persons with Hemoglobin H disease experience chronic hemolytic anemia that is exacerbated by exposure to oxidants and may require transfusion. Persons with a two-gene deletion have microcytic red blood cells and occasionally mild anemia. The one-gene deletion is a "silent" carrier state. These latter two conditions are often called α -thalassemia trait. The exact prevalence of α -thalassemia is uncertain, but is estimated to be 5-30% among African-Americans, and 15-30% among Southeast Asians.

Hemoglobin E trait is the third most common hemoglobin disorder in the world and the most frequent in Southeast Asia, where its prevalence is estimated to be 30%. Although Hemoglobin E trait is associated with no morbidity, the offspring of individuals who carry this hemoglobin variant may exhibit thalassemia major (hemoglobin E/ β -thalassemia) if the other parent has β -thalassemia trait and contributes that gene. This combination is the most common cause of transfusion-dependent thalassemia in areas of Southeast Asia.

Sickle Cell Disease

The Sickle Cell Association of Ontario estimates the black population of Canada at about 700,000, and growing. The carrier frequency of the sickle gene is cited at 1 in 10 in the U.S. The carrier rate may be higher in Canada, where the black population is composed largely of individuals of Caribbean (carrier rate 10-14%) and African origin (carrier rate 20-25% in West Africa). Based on various assumptions, it has been estimated that as many as 67 black infants affected with sickle cell disease may be born annually in Canada. This figure does not take into account other population groups, e.g. East Indian, Middle Eastern and Mediterranean in which the sickle gene is also represented with considerable frequency. In the United States, 1 of every 150 African-American families is at risk of giving birth to a child with sickle cell disease (about 3,000 pregnancies per year).

Mortality in patients with sickle cell disease peaks between 1 and 3 years of age, chiefly due to sepsis caused by *Streptococcus pneumoniae*, estimated to occur in a frequency of 8 episodes per 100 person-years of observation in affected children under 3 years of age.

After infancy, patients with sickle cell disease are usually anemic and may experience painful crises and other complications, including acute chest syndrome, strokes, splenic and renal dysfunction, bone and joint symptoms, priapism, ischemic ulcers, cholecystitis and hepatic dysfunction associated with cholelithiasis.

Less severe but similar symptoms may be experienced by persons heterozygous for hemoglobin-S and hemoglobin-C (Hb SC) and those heterozygous for hemoglobin-S and β -thalassemia (HbS/ β -thal). It has recently been reported that individuals with sickle cell trait have increased susceptibility to death from exertional heat illness during military training. Otherwise, morbidity for such individuals has been considered to be negligible.

Maneuver

Determination of the mean corpuscular volume (MCV) as part of a complete blood count (CBC) provides a primary indicator for the presence of α - or β -thalassemia trait (carrier state). Carriers of either trait have microcytosis (MCV <80 fL) and hypochromia. Carriers of β -thalassemia usually have an elevated concentration of HbA₂ (>3.5%), with or without an elevated concentration of HbF (>1.5%), as determined by hemoglobin electrophoresis. By contrast, α -thalassemia carriers have normal hemoglobin electrophoresis.

Blood for screening for carrier states is collected in heparinized tubes. For newborn screening, capillary blood is collected on filter paper (Guthrie paper blotter). Cellulose acetate electrophoresis, or thin layer isoelectric focusing are the preferred screening tests for



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hemoglobin disorders. Cellulose acetate electrophoresis is not specific for HbS if used alone. Citrate agar electrophoresis is used by many laboratories to confirm the presence of abnormal hemoglobins detected by another technique. High-performance liquid chromatography (HPLC) is a newer technique that offers higher resolution than 2-tier electrophoresis.

In over two million automated HPLC screening tests carried out in California between 1990 and 1993, only 1 false positive and 1 false negative test have been recorded (unpublished report). Newer techniques, employing monoclonal antibodies and recombinant DNA technology may be used more widely in the future.

Electrophoresis is highly specific in the detection of certain hemoglobin disorders, such as sickle cell disease. In one study, all 138 children with hemoglobin S identified in screening 2,976 African-american newborns were found to have a sickling disorder when retested at age 3-5 years.<3> Another study of 131 infants detected by screening found only nine instances in which the sickling disorder required reclassification and no instance in which a child originally diagnosed as having sickle cell disease was found to have sickle cell trait.<4> Ten years' experience with universal screening of Colorado newborns (528,711) using filter paper specimens and two-tier hemoglobin electrophoresis was recently reported.<5> Fifty infants with sickle cell diseases (HbSS, HbSC, HbS/ α -thal) and 27 infants with other hemoglobin disorders were identified. Initial screening failed to identify 4 infants with sickle cell disease, but three of these were diagnosed on routine follow-up testing of infants suspected of having sickle cell trait. There were 32 false positive results, 27 of whom were confirmed to have a hemoglobinopathy trait on follow-up testing. The remaining 5 had normal hemoglobin.<5>



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The yield in screening pregnant women for hemoglobin disorders depends on the risk profile of the population being tested. In one study, electrophoresis in combination with a complete blood count was performed on 298 African-American and Southeast Asian prenatal patients. Ninety-four women (31.5%) had a hemoglobin disorder (including sickle cell disease, sickle cell trait, hemoglobin E, α -thalassemia trait, β -thalassemia trait, hemoglobin H, and hemoglobin C).<6> In a larger study in a different community, similar tests were performed on 6,641 prenatal patients selected without regard to race or ethnic origin.<7> One hundred eighty-five women (3%) had sickle cell trait, 68 (1%) had hemoglobin C, 30 (0.5%) had β -thalassemia trait, and 17 (0.3%) had other disorders (hemoglobin E, α -thalassemia trait, hemoglobin H, hemoglobin E/ β -thalassemia disease). These results were obtained by combining electrophoresis with red cell indices. When low mean corpuscular volume (MCV) has been used as the only screening test to detect thalassemia, the yield has been 0.3-0.5%.

Prenatal diagnosis of sickle cell disease and other hemoglobinopathies in the fetus has been aided by advances in

techniques of obtaining and analyzing specimens. Early tests involved the analysis of fetal blood obtained by fetoscopy or placental aspiration.<8> Recent genetic advances, however, have provided a safer<9> and more practical method in which amniocytes are obtained by amniocentesis and chromosomal mutations are identified directly through recombinant DNA technology. These techniques are highly accurate in detecting sickle cell disease and certain forms of thalassemia.<8-12> Their principal disadvantage, however, is that amniocentesis cannot be performed safely until about 16 weeks' gestation, thus delaying diagnosis and potential intervention until late in the second trimester. Chorionic villus sampling (CVS) is a means of obtaining tissue for DNA analysis as early as 8-10 weeks of gestation and is an established technique for prenatal diagnosis.<13,14> Several centers now offer the option of "early amniocentesis" (done several weeks earlier than conventional amniocentesis) as an alternative to CVS. Amniocentesis or CVS are part of the screening protocols for Down Syndrome (Chapter 8) and neural tube defects (Chapter 8).

Effectiveness of Early Detection and Treatment



Newborns with sickle cell disease benefit from early detection through early institution of penicillin prophylaxis to prevent pneumococcal sepsis

Screening for hemoglobin disorders is usually considered for two target populations: neonates, and adults of reproductive age. Newborns with sickle cell disease benefit from early detection through early institution of penicillin prophylaxis to prevent pneumococcal sepsis. A multi-center, randomized, double-blind, placebo-controlled trial demonstrated that the administration of prophylactic oral penicillin to infants and young children with sickle cell disease reduced the incidence of pneumococcal septicemia by 84%.<15> Other benefits of identifying newborns with sickle cell disease include prompt clinical intervention for infection or splenic sequestration crises and education of caretakers about the signs and symptoms of illness in these children. A seven-year longitudinal study reported lower mortality in children with sickle cell disease identified in the newborn period than in children diagnosed after 3 months of age (2% vs. 8%), but the investigators did not account for confounding variables in the control group.<16> A briefer longitudinal study (8-20 months) reported no deaths in 131 newborns detected through screening.<4> In the experience described above, 47 of the 50 newborns with sickle cell disease identified through screening remained in the study area beyond 6 months of age. None of the 47 died during the period of observation.<5> In addition to the health benefits to affected infants, neonatal screening carries the added benefit of identifying at-risk couples, thereby providing the opportunity for genetic counselling regarding options for future pregnancies. Screening of older children and adolescents is designed to detect carriers with sickle cell trait, β -thalassemia trait, and other hemoglobin disorders that often escape

detection during the first years of life. Although heterozygotes rarely suffer clinically significant effects, their carrier status has direct implications for their offspring. Identification of carriers before childbearing permits genetic counselling about partner selection and the availability of diagnostic tests in the event of pregnancy. There is some evidence that individuals who receive certain forms of counselling retain this information and may encourage other individuals, such as their partners, to be tested.<7,17-19> A prospective study of 142 persons screened for β -thalassemia trait found that 62 (43%) encouraged other persons to be screened.<17> Compared with controls, those who had received counselling demonstrated significantly better understanding of thalassemia when tested immediately after the session. There is no direct evidence, however, that individual genetic counselling by itself significantly alters reproductive behavior or the incidence of births of infants with hemoglobin disorders.<20>

Detection of carrier status during pregnancy can provide prospective parents with the option of testing the fetus for a hemoglobinopathy. If the test is positive, they have the time to discuss continuation of the pregnancy and to plan optimal care for their newborn. Parents appear to act on this genetic information. About 70% of pregnant women who were identified as β -thalassemia carriers and received counselling referred their partners for testing. Among couples at risk for sickle cell disease, about 60% consent to amniocentesis.<7> If sickle cell disease is diagnosed in the fetus, about 50% of parents elect therapeutic abortion.<11,21> In a recent study, in Rochester, N.Y., 18,907 samples from pregnant women were screened for abnormal hemoglobin including thalassemia and hemoglobin S. In 810 (4.3%), an abnormal hemoglobin was identified. Sixty-six percent occurred in mothers unaware that they carried an abnormal hemoglobin, and 80% occurred in mothers unaware that they were at risk for giving birth to a child with a serious hematologic disorder. Eighty-six percent of mothers who received counselling said they wanted their partner tested and 55% of partners were tested. Seventy-seven pregnancies were identified as being at high risk because the partner also was a carrier of an abnormal hemoglobin. Of these 77 pregnancies, the gestation was too advanced for prenatal diagnosis in 12 cases and the condition for which the pregnancy was at risk was too mild for this service to be offered in 12 others. Prenatal diagnosis was offered in the remaining 53 pregnancies and accepted by 25 couples (47%). Of 18 amniocenteses performed, 14 were at risk for sickling disorders and the remaining 4 for the Hb H disease or Hb H with Hb E trait. Five fetuses were found to have clinically significant hemoglobinopathies and one of these pregnancies was terminated.<22> A comparison of the distribution of hemoglobinopathies detected in the Rochester, N.Y. study with screening results reported from Hamilton, Ontario<23> shows significant differences in the spectrum of abnormalities detected.

Those differences may reflect different ethnic mixes in Canada and the U.S. or may be partly due to ascertainment bias since most referrals in the Hamilton study were for investigation of low MCV.

Hemoglobinopathy	Rochester (Rowley 1991)<7>	Hamilton (Ali & Lafferty 1992)<23>
Hb S trait	474 (58.5%)	847 (10.7%)
Hb C trait	150 (18.5%)	230 (2.9%)
β -thalassemia trait	92 (11.4%)	4,497 (56.7%)
Hb E trait	37 (4.6%)	149 (1.9%)
Hb D or G trait	17 (2.1%)	49 (0.6%)
$\delta\beta$ -thal trait	6 (0.7%)	191 (2.4%)
α -thalassemia trait	3 (0.4%)	1,248 (15.7%)
Others	31 (3.8%)	724 (9.1%)
TOTALS	810 (100%)	7,935 (100%)

There is evidence from some European communities with a high prevalence of β -thalassemia that the birth rate of affected infants declined significantly following the implementation of routine prenatal screening,<8,24,25> and other data suggest a similar trend in some North American communities that have introduced community education and testing for thalassemia. This decline may reflect more than one factor, possibly including 1) a general decline in birth rate; 2) termination of pregnancies with affected fetuses; and 3) "at risk" couples choosing not to have children.

Since hemoglobinopathies occur among all ethnic and racial groups, efforts at targeting specific high-risk groups for newborn screening inevitably miss some affected individuals due to difficulties in properly assigning race or ethnic origin in the newborn nursery. In one study of 528,711 newborns, parental race, as requested on a screening form, was found to be inaccurate or incomplete in 30% of cases.<5> Proponents of selective screening of high-risk populations emphasize that, especially in geographic areas with a small population at risk, cost effectiveness is compromised and considerable expense incurred in screening large numbers of low-risk newborns to identify the rare individuals with sickle cell disease or other uncommon hemoglobin disorders. Studies supporting this argument have compared universal screening to no screening, not to targeted screening. Recent research that accounts for the additional procedural and administrative costs of targeted screening suggests that universal screening may be the more cost effective alternative to targeted screening.

There has been considerable debate over the value of sickle screening and screening for other hemoglobinopathies in persons of reproductive age. Critics cite evidence that sickle cell screening programs in the past have failed to educate patients and the public adequately about the significant differences between sickle cell trait and sickle cell disease. This has resulted in unnecessary anxiety for carriers and inappropriate labelling by insurers and employers. In addition, there is no evidence that counselling, however comprehensive, will be remembered throughout the individual's reproductive life, influence partner selection, alter use of prenatal testing, or ultimately reduce the rate of births of affected children. Proponents argue that these outcomes should not be used as measures of effectiveness since the goal of genetic counselling is to facilitate informed decision making by prospective parents. In this regard, clinicians are responsible for making the individual aware of the diagnosis, the risk to future offspring, and the recommended methods to reduce that risk, regardless of the strength of the evidence that such counselling reduces the number of affected offspring.

Recommendations of Others

The U.S. Preventive Services Task Force recommendations are currently under review. Universal screening of newborns for sickle cell disease, regardless of race or ethnic origin, has been recommended in the U.S. by the National Institutes of Health Consensus Development Conference on Newborn Screening for Sickle Cell Disease and other hemoglobinopathies. In April, 1993, the Agency for Health Care Policy and Research (a division of the U.S. Department of Health and Human Services) published its Clinical Practice Guidelines on screening, diagnosis and management of sickle cell disease in newborns and infants, recommending universal screening of newborns for sickle cell disease. Screening of infants from high-risk groups has been recommended by the World Health Organization and the British Society of Haematology. Newborn screening for sickle cell disease, coupled with comprehensive counselling, is advocated in the medical literature³ and is currently universal in 34 states.⁴

Screening of older children and young adults is not universally recommended. Some U.S. states require sickle cell screening of school children, but many medical authorities have advised against this practice.

In Canada, thalassemia screening programs for carrier detection and prenatal diagnosis targeted at known high-risk groups, are currently available in Montreal, Quebec and in Hamilton, Ontario, though large communities at risk are present elsewhere in Canada. Hemoglobinopathy DNA referral diagnostic laboratories are available in Calgary, Hamilton and Montreal, where prenatal diagnosis from chorionic villus sampling or amniocentesis is also available. In Hamilton,

Ontario, the Regional Hemoglobinopathy Reference Laboratory investigates several thousand cases each year. Over a 20-year period, this laboratory has tested over 38,000 samples, referred because of an abnormal CBC (hypochromia, microcytosis or mild anemia). Of these 38,000 referrals, more than 7,300 were carriers of hemoglobin variants or thalassemia, showing that the spectrum of hemoglobinopathies in Canada differs significantly from that of the U.S.

Conclusions and Recommendations

A family and genetic history should be obtained from all patients of Mediterranean, African, Middle Eastern, East Indian, Hispanic or Asian ancestry who may become parents (B Recommendation). Screening for sickle cell hemoglobin and other hemoglobin variants should be performed at the first prenatal visit for all pregnant women from racial and ethnic groups known to be at increased risk for hemoglobinopathies (Asian, African and Mediterranean).

In all neonates from high risk ethnic groups, newborn screening for hemoglobinopathies is recommended, using dried filter paper blood spots (A Recommendation). Cellulose acetate electrophoresis or thin layer isoelectric focusing are currently the preferred screening tests, with citrate agar electrophoresis or high-performance liquid chromatography in a reference laboratory for confirmation. These methods may be superseded by more rapid and accurate techniques in future.

Unanswered Questions (Research Agenda)

1. Further studies are needed to determine the effectiveness and cost-effectiveness of screening non-pregnant adolescents and adults for carrier status.
2. The impact of individual genetic counselling on reproductive behavior requires further study.
3. The criteria for universal as opposed to selective screening for hemoglobinopathies need further definition.

Evidence

The literature was identified with a MEDLINE search in the English language literature for the years 1989 to 1993, using the following key words: anemia, hemoglobinopathies, sickle cell, thalassemia, ethnic groups (Ep). This review was initiated in January 1993 and approved by the Task Force in March 1994.

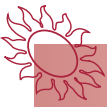
Acknowledgements

The Task Force wishes to thank Dr. John S. Wayne, Co-Director, Provincial Hemoglobinopathy DNA Diagnostic Laboratory, McMaster University Medical Centre, Hamilton, Ontario for his assistance and valuable review of this document.

Selected References

1. Canadian Task Force on the Periodic Health Examination: The periodic health examination. *Can Med Assoc J* 1979; 121: 1193-1254
2. Chui DHK, Wayne JS, Chitayat, *et al*: Screening for thalassemia and sickle hemoglobin. *Can J Ob Gyn Wom Hlth Care* 1993; 5(3): 453-457
3. Kramer MS, Rooks Y, Johnston D, *et al*: Accuracy of cord blood screening for sickle hemoglobinopathies: three- to five-year follow-up. *JAMA* 1979; 241: 485-486
4. Grover R, Shahidi S, Fisher B, *et al*: Current sickle cell screening program for newborns in New York City, 1979-1980. *Am J Public Health* 1983; 73: 249-251
5. Githens JH, Lane PA, McCurdy RS, *et al*: Newborn screening in Colorado: the first ten years. *AJDC* 1990; 144: 466-470
6. Stein J, Berg C, Jones JA, *et al*: A screening protocol for a prenatal population at risk for inherited hemoglobin disorders: results of its application to a group of Southeast Asians and blacks. *Am J Obstet Gynecol* 1984; 150: 333-341
7. Rowley PT, Loader S, Walden ME: Toward providing parents the option of avoiding the birth of the first child with Cooley's anemia: response to hemoglobinopathy screening and counseling during pregnancy. *Ann NY Acad Sci* 1986; 445: 408-416
8. Alter BP: Advances in the prenatal diagnosis of hematologic diseases. *Blood* 1984; 64: 329-340
9. Kazazian HH Jr, Boehm CD, Dowling CE: Prenatal diagnosis of hemoglobinopathies by DNA analysis. *Ann NY Acad Sci* 1985; 445: 337-348
10. Weatherall DJ, Mold J, Thein SL, *et al*: Prenatal diagnosis of the common hemoglobin disorders. *J Med Genet* 1985; 22: 422-430
11. Boehm CD, Antonarakis SE, Phillips JA III, *et al*: Prenatal diagnosis using DNA polymorphisms: report on 95 pregnancies at risk for sickle-cell disease or beta-thalassemia. *N Engl J Med* 1983; 308: 1054-1058
12. Orkin SH: Prenatal diagnosis of hemoglobin disorders by DNA analysis. *Blood* 1984; 63: 249-253

13. Goosens M, Dumez Y, Kaplan L, *et al*: Prenatal diagnosis of sickle-cell anemia in the first trimester of pregnancy. *N Engl J Med* 1983; 309: 831-833
14. Old JM, Fitches A, Heath C, *et al*: First-trimester fetal diagnosis for hemoglobinopathies: report on 200 cases. *Lancet* 1986; 2: 763-767
15. Gaston MH, Verter JI, Woods G, *et al*: Prophylaxis with oral penicillin in children with sickle cell anemia: a randomized trial. *N Engl J Med* 1986; 314: 1593-1599
16. Vichinsky E, Hurst D, Earles A, *et al*: Newborn screening for sickle cell disease: effect on mortality. *Pediatrics* 1988; 81: 749-755
17. Lipkin M, Fisher L, Rowley PT, *et al*: Genetic counseling of asymptomatic carriers in a primary care setting: the effectiveness of screening and counseling for beta-thalassemia trait. *Ann Intern Med* 1986; 105: 115-123
18. Whitten CF, Thomas JF, Nishiura EN: Sickle cell trait counseling: evaluation of counselors and counselees. *Am J Hum Genet* 1981; 33: 802-816
19. Scriver CR, Bardanis M, Cartier L, *et al*: Beta-thalassemia disease prevention: genetic medicine applied. *Am J Hum Genet* 1984; 36: 1024-1038
20. Rucknagel DL: A decade of screening in the hemoglobinopathies: is a national program to prevent sickle cell anemia possible? *Am J Ped Hem Onc* 1983; 5: 373-377
21. Driscoll MC, Lerner N, Anyane-Yeboah K, *et al*: Prenatal diagnosis of sickle hemoglobinopathies: the experience of the Columbia University Comprehensive Center for Sickle Cell Disease. *Am J Hum Genet* 1987; 40: 548-558
22. Rowley PT, Loader S, Sutera CJ, *et al*: Prenatal screening for hemoglobinopathies: I. A prospective regional trial. *Am J Hum Genet* 1991; 48: 439-446
23. Ali M, Lafferty J: The clinical significance of hemoglobinopathies in the Hamilton region: a twenty-year review. *Clin Invest Med* 1992; 15(5): 401-405
24. Cao A, Rosatelli C, Galanello R, *et al*: The prevention of thalassemia in Sardinia. *Clin Gen* 1989; 36: 277-285
25. Cao A, Rosatelli C, Galanello R: Population-based genetic screening. *Curr Opin Gen Dev* 1991; 1: 48-53



**Screening for Hemoglobinopathies
in Canada**

MANEUVER	EFFECTIVENESS	LEVEL OF EVIDENCE <REF>	RECOMMENDATION
Screening for Carrier Status - Pregnant Women*			
<p>Complete blood count (CBC) for identification of hypochromia and microcytosis (MCV <80 fL) followed by hemoglobin electrophoresis when iron deficiency ruled out; cellulose acetate electrophoresis or thin layer isoelectric focusing of blood sample with citrate agar electrophoresis for confirmation (High performance liquid chromatography (HPLC) offers higher resolution)</p>	<p>Tests sensitive and highly specific but yield depends on risk profile.</p> <p>50-55% refer partners for testing and 60% subsequently consent to amniocentesis for sickle cell disease. Uptake rates are higher for the thalassemas.</p>	<p>Cohort and cross-sectional studies <6,7,17> (II-2) Expert opinion<2> (III)</p> <p>Cross-sectional studies<6,22> (II-2); expert opinion <23,24> (III)</p>	<p>Fair evidence to recommend screening for hemoglobinopathies in high-risk** pregnant women (B)</p>
Prenatal Screening and Counselling*			
<p>DNA analysis of tissue sample (amniocentesis or chorionic villus sampling) after confirming positive carrier status of both partners</p>	<p>Technology is highly accurate and available through referral to diagnostic centers.</p> <p>47-60% of parents consent to procedure and 20-50% consent to therapeutic abortion of affected fetus with sickle cell. Rates are higher for the thalassemas.</p> <p>Decline in prevalence of β-thalassemia in European communities with screening programs.</p>	<p>Case-series and expert opinion<2,8-12> (III)</p> <p>Cross-sectional studies<7,22> (II-2); case-series <17-21> (III)</p> <p>Comparison of times and places <8,23,24> (II-3)</p>	<p>Fair evidence to offer prenatal screening and counselling to families with positive carrier status (B)</p>

* All screening must be accompanied by counselling.

** High-risk individuals include all patients of Mediterranean, African, East Indian, Middle Eastern, Hispanic or Asian ancestry and those with a family history of disease.



Screening for Hemoglobinopathies in Canada (concl'd)

MANEUVER	EFFECTIVENESS	LEVEL OF EVIDENCE <REF>	RECOMMENDATION
Neonatal Screening			
Testing of dried capillary blood samples collected on filter paper: Cellulose acetate electrophoresis or thin layer isoelectric focusing, citrate agar electrophoresis liquid chromatography for confirmation (high performance liquid chromatography (HPLC) offers higher resolution)	<p>Tests sensitive and highly specific for Hb Sickle.</p> <p>Prophylactic oral penicillin to infants and young children with sickle cell anemia reduced pneumococcal septicemia 84%. Mortality may also be reduced.</p>	<p>Cohort studies <3-5,16> (II-2)</p> <p>Randomized controlled trials<15> (I)</p>	Good evidence to recommend for high-risk** neonates (A)
Screening for Carrier Status - Non-Pregnant Adolescents and Adults and Counselling*			
CBC for identification of hypochromia and microcytosis (MCV <80 fL) followed by hemoglobin electrophoresis (as described above) when iron deficiency ruled out	<p>Tests sensitive and highly specific.</p> <p>Individuals who receive counselling may encourage partners to be treated; no evidence regarding reproductive behavior or use of prenatal testing. Potential for labelling by insurers and employers and unnecessary anxiety for carriers.</p>	<p>Cohort and cross-sectional studies <6,7> (II-2); expert opinion<2> (III)</p> <p>Controlled trial <17> (II-1); case-series <7,18,19,23,24> (III)</p>	Insufficient evidence to recommend for or against universal screening for non-pregnant adolescents and adults for carrier status (C)

* All screening must be accompanied by counselling.

** High-risk individuals for sickle cell disease include all patients of African ancestry.