3. Screening and Making the Diagnosis

Principles

- To promptly establish the correct diagnosis for a neonate or infant with a thalassemia syndrome (transfusion dependent or non-transfusion dependent thalassemia).
- To correctly diagnosis patients with clinical suspicion of thalassemia syndrome.
- To identify prospective parents who are thalassemia heterozygotes and are at risk for having a child with transfusion dependent thalassemia.

Recommendation

- Prospective parents from high risk areas should be screened before conception.
- Pre-natal diagnois should be offered to prospective parents from high risk areas who did not receive pre-conception testing.
- Universal newborn screening should include testing for thalassemia syndromes.
- Patients with a clinical thalassemia syndrome should be offered testing.

Background

Thalassemia encompasses a wide spectrum of clinical diseases, from asymptomatic carrier to transfusion-dependent thalassemia. Some forms of thalassemias can be detected at birth through sickle cell disease (SCD) newborn screening, where available.

In Canada, high performance liquid chromatography (HPLC and/or and hemoglobin electrophoresis) are the methods most often used by SCD newborn screening program. Neonatal screening for SCD may identify serious thalassemias syndromes such as beta thalassemia major, hemoglobin H disease and Eβ thalassemias. When detected, these infants are referred to specialist centre where diagnosis will be confirmed and delivery of medical care will be ensured. Carriers of the sickle gene and other hemoglobinopathies can also be identified through newborn screening. These patients are usually clinically asymptomatic, but the information may be useful for purposes of genetic counseling.

Screening and confirmatory tests for thalassemia syndromes should be done for patients with a clinical suspicion of thalassemia; or the parents of a suspected child, or prospective/expectant parents where there may be a risk of having a child with a transfusion dependent thalassemia. Common scenarios include preconception screening, prenatal testing in pregnant mothers, newborn screening and clinical situations in which thalassemia syndromes need to be considered.

Further, preconception testing allows prospective parents to make informed reproductive decisions. The most common cause of hemoglobinopathy-related non-immune hydrops fetalis (NIHF) is alpha-thalassemia. It is most common in Southeast Asian populations accounting for 28-55% of NIHF.[3–6] Incidence is lower in other populations (approximately 10%).3,4 Case reports exist of infants with Hemoglobin Bart's surviving to delivery and beyond with use of aggressive intrauterine transfusion and lifelong chronic transfusion similar to individuals with beta-thalassemia major.[8–15]

Several provinces and territories (Ontario, Quebec, British Columbia, PEI, Nova Scotia, New Brunswick, Yukon, Nunavut) have newborn screening available which can detect significant thalassemia syndromes (beta-thalassemia major, Hemoglobin H, Hemoglobin E/beta-thalassemia) in addition to other hemoglobinopathies.

Not all provinces in Canada offer newborn screening for thalassemia. Additionally, Canada welcomes large numbers of immigrants from all over the world every year. These new Canadians may not have had the same access to medical care prior to coming to Canada. Offering testing to all patients with a clinical suspicion of a thalassemia syndrome will ensure appropriate care for the patient and their affected family members.

Pre-conception testing of prospective parents

- Screen parents with ethnic background from high risk areas (Table 2) with: complete blood count with red blood cell indices, serum ferritin and hemoglobinopathy investigation e.g. electrophoresis, high-performance liquid chromatography (HPLC) or Capillary Zone Electrophresis (CZE)[1,2]
- If both parents are found to be heterozygotes of a thalassemia mutation, hemoglobin variant, or combination of the two, referral for genetic counselling is recommended. Ideally, this should be done prior to conception or as early as possible in pregnancy. Additional molecular DNA analysis may be required to clarify the carrier status of the parents and thus the risk to the fetus. Further consultation with specialists in Hematology or Genetics is recommended.[2]

Prenatal Diagnosis

The Society of Obstetricians and Gynecologists of Canada published guidelines for pre-natal screening of thalassemia syndromes in 2008. Their recommendations are summarized below.[2]

1. Prenatal diagnosis should be offered to the pregnant woman/couple at risk for having a fetus affected with a clinically significant thalassemia. Prenatal diagnosis should be performed with the patient's informed consent.

2. Prenatal diagnosis by DNA analysis can be performed using cells obtained by chorionic villus sampling or amniocentesis. Testing of fetal DNA after isolation from maternal blood is a new technique currently under development and is not yet widely available as routing diagnostic testing.

3. For those who decline invasive testing or present after 20 weeks gestational age, the SOGC-CCMG recommend the following:

- Testing of the child should be done as soon as possible to allow for early diagnosis and referral to a pediatric hematology centre if indicated
- For those at risk of Hemoglobin Bart's hydrops fetalis, serial detailed fetal ultrasound for assessment of the fetal C-T ratio (normal <0.5) should be done at a centre with experience conducting these assessments for early identification of an affected fetus.

4. If an abnormality is detected, 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. Any fetus found to have hydrops fetalis on prenatal ultrasound in the second or third trimester, or any infant born with hydrops fetalis needs to have alpha-thalassemia/Hemoglobin Bart's considered as a possible diagnosis.[7]

- This finding during pregnancy in women with an ethnic background that has an increased risk of alpha-thalassemia should prompt immediate investigation of the pregnant woman and her partner to determine if alpha-thalassemia carrier states are present.[7]
- These investigations should also be done in parents of any infant/stillbirth found to have NIHF to allow for further counselling regarding risks alpha-thalassemia NIHF in future pregnancies.[7]

Newborn Screening

- Newborn screening, by HPLC, is recommended. The finding of a Hemoglobin F only or a Hemoglobin A2 less than 1.5% will identify the majority of babies with beta-thalassemia major. The finding of greater than 25% Hemoglobin Bart's is suggestive of Hemoglobin H.[1,16]. If using HPLC only, this is limited as beta thalassemia cannot be distinguished from more benign conditions (eg. Hemoglobin E/beta thalassemia, Hemoglobin EE - both show FE pattern on HPLC) or detect unusual hemoglobin variants.[17]

- A positive screen result requires confirmatory testing as early as possible to ensure proper clinical follow up of affected infants.
- Recommended confirmatory testing is molecular analysis of globin genes.
- Referral to a pediatric hematology centre and/or genetics is recommended to assist with confirmatory testing and interpretation of results.
- Each provincial newborn screening program has its own policy regarding the notification of asymptomatic carriers.
- However, it is worth remembering that some NTDT are not captured by newborn screening and these children may present later in life when a microcytic anemia is diagnosed.

Clinical Suspicion

The following clinical scenarios should prompt clinicians to consider investigations for thalassemia syndromes:

1. Pallor, hepatosplenomegaly, poor growth/anorexia with a microcytic, hypochromic anemia with normal ferritin and iron studies

- Directly from birth in alpha thalassemia,
- Age 3 to 6 months in beta thalassemia,

These indicate the possibility a transfusion dependent thalassemia. Additional confirmatory testing should be performed immediately to allow for close clinical monitoring and therapeutic intervention. Testing should include haematological and genotype analysis. These tests should be collected before the child receives a blood transfusion.

2. Pallor, hepatosplenomegaly, +/- jaundice, +/- over expansion of skull and facial bones with a hypochromic, microcytic anemia and normal ferritin and iron studies in an older infant or child raise suspicion for a non-transfusion dependent thalassemia, such as beta thalassemia intermedia. Additional confirmatory testing should be performed immediately to allow for close clinical monitoring and therapeutic intervention. Testing should include haematological and genotype analysis. These tests should be collected before the child receives a blood transfusion. Refer to table 3.[1,18]

3. In any individual with a hypochromic, microcytic anemia, splenomegaly and an elevated reticulocyte count, a non-transfusion dependent thalassemia such as Hemoglobin H Disease, should be suspected. Individuals may have symptomatic anemia triggered by illness or pregnancy. Some individuals may present with unexplained hemolytic and microcytic anemia (jaundice, dark urine, +/- gallstones). In others, it may be an incidental finding.[1,18–20]

4.Unexplained microcytosis without anemia, or hypochromic, microcytic anemia in the absence of iron deficiency warrants further investigation for the presence of presence of a carrier (heterozygous) state for alpha or beta thalassemia.[18]

Regions High Risk for Thalassemia
North Africa
Mediterranean
Middle East
India
Southeast Asia
China

Table 2: Regions of the World at High Risk for Thalassemia Syndromes (Adapted from Reference 2)

Stages	Recommended Testing
Initial	Complete blood count with red blood cell indices
	Reticulocyte count
	Serum Ferritin
	Hemoglobinopathy Investigations (Hemoglobin
	Electrophoresis and HPLC)
Confirmatory	Genotype analysis of globin gene mutations

Table 3: Recommended Testing for Investigation of Thalassemia Syndromes