

Prenatal Screening for Fetal Aneuploidy in Singleton Pregnancies

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). It was approved by both the Executive and Council of the Society of Obstetricians and Gynaecologists of Canada and the Board of Directors of the Canadian College of Medical Geneticists.

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Abstract

Objective: To develop a Canadian consensus document on maternal screening for fetal aneuploidy (e.g., Down syndrome and trisomy 18) in singleton pregnancies.

Options: Pregnancy screening for fetal aneuploidy started in the mid 1960s, using maternal age as the screening test. New developments in maternal serum and ultrasound screening have made it possible to offer all pregnant patients a non-invasive screening test to assess their risk of having a fetus with aneuploidy to determine whether invasive prenatal diagnostic testing is necessary. This document reviews the options available for non-invasive screening and makes recommendations for Canadian patients and health care workers.

Outcomes: To offer non-invasive screening for fetal aneuploidy (trisomy 13, 18, 21) to all pregnant women. Invasive prenatal diagnosis would be offered to women who screen above a set risk cut-off level on non-invasive screening or to pregnant women whose personal, obstetrical, or family history places them at increased risk. Currently available non-invasive screening options include maternal age *combined* with one of the following: (1) first trimester screening (nuchal translucency, maternal age, and maternal serum biochemical markers), (2) second trimester serum screening (maternal age and maternal serum biochemical

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markers), or (3) 2-step integrated screening, which includes first and second trimester serum screening with or without nuchal translucency (integrated prenatal screen, serum integrated prenatal screening, contingent, and sequential). These options are reviewed, and recommendations are made.

Evidence: Studies published between 1982 and 2009 were retrieved through searches of PubMed or Medline and CINAHL and the Cochrane Library, using appropriate controlled vocabulary and key words (aneuploidy, Down syndrome, trisomy, prenatal screening, genetic health risk, genetic health surveillance, prenatal diagnosis). Results were restricted to systematic reviews, randomized controlled trials, and relevant observational studies. There were no language restrictions. Searches were updated on a regular basis and incorporated in the guideline to August 2010. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies. The previous Society of Obstetricians and Gynaecologists of Canada guidelines regarding prenatal screening were also reviewed in developing this clinical practice guideline.

Values: The quality of evidence was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care.

Benefits, harms, and costs: This guideline is intended to reduce the number of prenatal invasive procedures done when maternal age is the only indication. This will have the benefit of reducing the numbers of normal pregnancies lost because of complications of invasive procedures. Any screening test has an inherent false-positive rate, which may result in undue anxiety. It is not possible at this time to undertake a detailed cost-benefit analysis of the implementation of this guideline, since this would require health surveillance and research and health resources not presently available; however, these factors need to be evaluated in a prospective approach by provincial and territorial initiatives.

Recommendations

1. All pregnant women in Canada, regardless of age, should be offered, through an informed counselling process, the option of a prenatal screening test for the most common clinically significant fetal

aneuploidies in addition to a second trimester ultrasound for dating, assessment of fetal anatomy, and detection of multiples. (I-A)

2. Counselling must be non-directive and must respect a woman's right to accept or decline any or all of the testing or options offered at any point in the process. (III-A)
3. Maternal age alone is a poor minimum standard for prenatal screening for aneuploidy, and it should not be used as a basis for recommending invasive testing when non-invasive prenatal screening for aneuploidy is available. (II-2A)
4. Invasive prenatal diagnosis for cytogenetic analysis should not be performed without multiple marker screening results except for women who are at increased risk of fetal aneuploidy (a) because of ultrasound findings, (b) because the pregnancy was conceived by in vitro fertilization with intracytoplasmic sperm injection, or (c) because the woman or her partner has a history of a previous child or fetus with a chromosomal abnormality or is a carrier of a chromosome rearrangement that increases the risk of having a fetus with a chromosomal abnormality. (II-2E)
5. At minimum, any prenatal screen offered to Canadian women who present for care in the first trimester should have a detection rate of 75% with no more than a 3% false-positive rate. The performance of the screen should be substantiated by annual audit. (III-B)
6. The minimum standard for women presenting in the second trimester should be a screen that has a detection rate of 75% with no more than a 5% false-positive rate. The performance of the screen should be substantiated by annual audit. (III-B)
7. First trimester nuchal translucency should be interpreted for risk assessment only when measured by sonographers or sonologists trained and accredited for this service and when there is ongoing quality assurance (II-2A), and it should not be offered as a screen without biochemical markers in singleton pregnancies. (I-E)
8. Evaluation of the fetal nasal bone in the first trimester should not be incorporated as a screen unless it is performed by sonographers or sonologists trained and accredited for this service and there is ongoing quality assurance. (II-2E)
9. For women who undertake first trimester screening, second trimester serum alpha fetoprotein screening and/or ultrasound examination is recommended to screen for open neural tube defects. (II-1A)
10. Timely referral and access is critical for women and should be facilitated to ensure women are able to undergo the type of screening test they have chosen as first trimester screening. The first steps of integrated screening (with or without nuchal translucency), contingent, or sequential screening are performed in an early and relatively narrow time window. (II-1A)
11. Ultrasound dating should be performed if menstrual or conception dating is unreliable. For any abnormal serum screen calculated on the basis of menstrual dating, an ultrasound should be done to confirm gestational age. (II-1A)
12. The presence or absence of soft markers or anomalies in the 18- to 20-week ultrasound can be used to modify the a priori risk of aneuploidy established by age or prior screening. (II-2B)
13. Information such as gestational dating, maternal weight, ethnicity, insulin-dependent diabetes mellitus, and use of assisted reproduction technologies should be provided to the laboratory to improve accuracy of testing. (II-2A)
14. Health care providers should be aware of the screening modalities available in their province or territory. (III-B)
15. A reliable system needs to be in place ensuring timely reporting of results. (III-C)

ABBREVIATIONS

AFP	alpha fetoprotein
CVS	chorionic villus sampling
DR	detection rate
FPR	false-positive rate
FTS	first trimester screening
hCG	human chorionic gonadotropin
IPS	integrated prenatal screening
MMS	multiple marker screening
MoM	multiples of the median
MSAFP	maternal serum alpha fetoprotein
NT	nuchal translucency
ONTD	open neural tube defect
PAPP-A	pregnancy-associated plasma protein-A
PR	positive rate
SLOS	Smith-Lemli-Opitz Syndrome
uE3	unconjugated estriol

16. Screening programs should be implemented with resources that support audited screening and diagnostic laboratory services, ultrasound, genetic counselling services, patient and health care provider education, and high quality diagnostic testing, as well as resources for administration, annual clinical audit, and data management. In addition, there must be the flexibility and funding to adjust the program to new technology and protocols. (II-3B)

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INTRODUCTION

Screening for chromosomal anomalies and open neural tube defects is part of prenatal care offered to all Canadian women. Since the methods of screening for ONTDs have not changed since the mid-1970s, they are not discussed here. Screening for ONTDs in Canada requires second trimester serum alpha fetoprotein (16 to 20 completed weeks) and/or ultrasound examination done at 18 to 22 weeks of gestation.

Screening for fetal chromosomal anomalies, including Down syndrome, began with amniocentesis in the mid-1960s. At that time, the criterion for screening was maternal age. In Canada, screening was offered only to women ≥ 35 years at the expected date of delivery. This was determined to be the point at which the risk of a pregnancy loss was less than the chance of identifying a pregnancy with a significant chromosomal abnormality. This clinical practice guideline reviews the evolution of screening for fetal aneuploidy from screening using maternal age alone to the many options currently available and makes recommendations regarding the minimum standard of prenatal screening that should be available to all Canadian women. The level of evidence and quality of recommendations are described using the criteria and classifications of the Canadian Task Force on Preventive Health Care¹ (Table 1).

WHAT IS SCREENING?

Screening is the process of surveying a population, using a specific marker or markers and defined screening cut-off levels, to identify the individuals in the population at higher risk for a particular disorder. Screening is applicable to a population; diagnosis is applied at the individual patient level.²

Screening for a disorder should be undertaken only when the disorder is considered to be serious enough to warrant intervention. The marker or markers used in screening must be sufficiently sensitive to identify a significant proportion of affected persons with minimal misidentification of unaffected persons. There must also be both a highly accurate diagnostic test to determine whether the person

with a screen positive result truly has the disorder and an intervention available to all persons who are identified as being affected. The screen, including follow-up testing and intervention, must be affordable. Finally the screen must be acceptable to the population being screened.

The screening procedure should not be merely a test but must be a comprehensive program. The program must include the provision of understandable information for both patients and health care providers to ensure informed decision-making, timely access to the screening test, a system of notification of results and referral to follow-up testing, and access to an intervention. The screening process must allow patients to decline intervention at each step throughout the process. A screening program must include regular clinical audit to evaluate local performance and should have the flexibility to incorporate new technology.

The Appendix provides a glossary of terms commonly used in screening

IMPORTANT CONCEPTS UNDERLYING PRENATAL GENETIC SCREENING

Multiple marker screening uses a combination of maternal age and 2 or more biochemical tests, with or without an ultrasound examination, to produce a single result for risk of Down syndrome, trisomy 18, and ONTDs, which is used to offer options for clinical management. A screen is positive when the risk of one or more of the screened disorders falls above a designated risk cut-off. Counselling and further testing options are offered when a screen is positive. In the discussion of prenatal screening, the terms false-positive rate or positive rate, and detection rate are used (Appendix). As screening performance improves, the FPR decreases and/or the DR increases. A risk cut-off might be chosen based upon the desired DR, FPR, or a combination of both. A risk cut-off is expressed as the risk or likelihood of the condition being present in the fetus at term or at mid-trimester. The risk for the latter will be higher, because 23% of fetuses with Down syndrome are miscarried between mid-trimester and term (risk cut-off of 1:350 at term would be similar to 1:280 at mid-trimester).

The other commonly used term in multiple marker screening is multiples of the median. Each marker result, including both biochemistry and nuchal translucency measurements, can be expressed in MoM. The absolute value of the assayed marker (serum or nuchal translucency) is divided by the gestation-specific median value of the serum marker in the measuring laboratory or by using standard or sonographer-specific curves for nuchal translucency. This allows direct comparison of results between programs.

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.¹

† 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.¹

SCREENING FOR CHROMOSOMAL ANOMALIES

Traditionally, in Canada, the option of invasive testing was recommended when a woman's risk of having a pregnancy with a chromosome anomaly was higher than the risks associated with the common invasive procedures (amniocentesis or CVS). New developments in maternal serum and ultrasound screening have improved the ability to identify pregnancies at increased risk for Down syndrome, trisomy 18, and other chromosomal abnormalities. This allows use of these screening tests to identify pregnancies at high enough risk to warrant invasive diagnostic testing, which has a risk of pregnancy loss.

The most common chromosome conditions associated with advanced maternal age involve the presence of an additional chromosome (21, 18, 13, or X). Down syndrome, trisomy 18, and trisomy 13 are associated with congenital anomalies and intellectual disability. With ultrasound and maternal serum screening, pregnancies affected by these conditions can now be recognized with a significant degree of reliability. The practice of using solely maternal age at the estimated date of delivery to identify at-risk pregnancies *should be abandoned*. The maternal age-related risk should be modified by additional non-invasive markers, which consist of maternal serum markers and ultrasound assessment. The approach to screening and diagnosis may vary between provinces. It is the responsibility of each health care provider to be aware of what is available to his or her patients so that appropriate counselling is provided.

INVASIVE PRENATAL DIAGNOSIS TO BE LIMITED TO WOMEN AT INCREASED RISK OF FETAL ANEUPLOIDY

The probability of conceiving a fetus with a trisomy increases with maternal age. Prenatal screening for chromosome anomalies starts with a discussion of the maternal age-related risk of having a baby with a chromosomal abnormality. However, maternal age screening is inferior to newer screening approaches that use multiple biochemical markers with or without a first trimester ultrasound assessment of nuchal translucency. These strategies provide a greatly reduced FPR with a substantially improved DR when applied across all age groups, and they provide evidence that the practice of offering invasive prenatal diagnosis for age alone as an indication should be abandoned.³ Women ≥ 40 years should not be offered an amniocentesis without prior screening, because with a negative screening result, their risk of a clinically significant chromosomal abnormality remains $< 1/200$. Invasive prenatal diagnosis for cytogenetic analysis should be offered only to women who are considered to be at increased risk of fetal aneuploidy on the basis of a non-invasive screen result above the risk cut-off, because of ultrasound findings, because the pregnancy was conceived by IVF with intracytoplasmic sperm injection,⁴ or because the woman or her partner has a history of a previous child or fetus with a chromosomal abnormality or is a carrier of a chromosome rearrangement that increases the risk of having a fetus with a chromosomal abnormality.

In these scenarios, the risk of a chromosomal abnormality, including chromosomal anomalies not detected by screening, is high enough to offer invasive testing without prior screening.

Recommendations

1. All pregnant women in Canada, regardless of age, should be offered, through an informed counselling process, the option of a prenatal screening test for the most common clinically significant fetal aneuploidies in addition to a second trimester ultrasound for dating, assessment of fetal anatomy, and detection of multiples (I-A)
2. Counselling must be non-directive and must respect a woman's right to accept or decline any or all of the testing or options offered at any point in the process. (III-A)
3. Maternal age alone is a poor minimum standard for prenatal screening for aneuploidy, and it should not be used a basis for recommending invasive testing when non-invasive prenatal screening for aneuploidy is available. (II-2A)
4. Invasive prenatal diagnosis for cytogenetic analysis should not be performed without multiple marker screening results except for women who are at increased risk of fetal aneuploidy (a) because of ultrasound findings, (b) because the pregnancy was conceived by in vitro fertilization with intracytoplasmic sperm injection, or (c) because the woman or her partner has a history of a previous child or fetus with a chromosomal abnormality or is a carrier of a chromosome rearrangement that increases the risk of having a fetus with a chromosomal abnormality. (II-2E)

CHOOSING A SCREEN

The most appropriate screening test for Down syndrome would have the lowest FPR and the highest DR. Cost and logistics should also be considered. Generally, the costs associated with screening are measured by the cost per Down syndrome pregnancy diagnosed. This has been estimated using different screening options in several studies.⁵⁻⁹ One of the difficulties with cost analyses is that the expenses associated with the ultrasound and serum sample analyses vary greatly from one jurisdiction to another. In addition, cost has not been estimated for many screening options, including the second trimester ultrasound. Consequently, a comprehensive cost comparison remains to be undertaken.

Given geographic limitations and resource differences, it is unlikely that a single screening protocol can be endorsed or practically applied for all women across Canada; however, screening options should meet acceptable performance characteristics. Considering the tests currently available and the risk and benefit ratio, it is believed that at a minimum, screening programs should provide a screen that offers women who present in the first trimester a DR for Down syndrome of 75% with no more than a 3% FPR.^{10,11} For women presenting in the second trimester, the screen offered should have a minimum DR of 75% with no more than a 5% FPR. Table 2 provides details of currently available screening options and their screening performance. Table 3 details timing of results for options that meet the minimum standard. These include first trimester screening, quad screening in second trimester, 2-step integrated first and second trimester prenatal serum screening with or without nuchal translucency (IPS and serum IPS).¹² IPS can be offered as full integrated screening for all women or as contingent or sequential screening. Access to follow-up services should also be ensured. Finally, prenatal screening programs must balance minimizing the FPR (which minimizes the number of invasive procedures needed and thus the number of normal pregnancies lost to chorionic villus sampling or amniocentesis) against the desire to detect as many cases as possible as early in gestation as possible. Some studies suggest women prefer a lower positive rate,¹³⁻¹⁵ while others suggest that women want early diagnosis.^{16,17} Individual programs should determine what is appropriate for their jurisdiction.

Recommendations

5. At minimum, any prenatal screen offered to Canadian women who present for care in the first trimester should have a detection rate of 75% with no more than a 3% false-positive rate. The performance of the screen should be substantiated by annual audit. (III-B)
6. The minimum standard for women presenting in the second trimester should be a screen that has a detection rate of 75% with no more than a 5% false-positive rate. The performance of the screen should be substantiated by annual audit. (III-B)

REVIEW OF SCREENING OPTIONS

First Trimester Screening: Nuchal Translucency Combined With Biochemical Markers

Nuchal translucency refers to the subcutaneous layer of fluid behind the fetal neck and lower cranium, which can be visualized on ultrasound. In 1992, Nicolaides et al.¹⁸

Table 2. Current available screening options and their screening performance*

Screening option	Markers	Trimester	Term risk cut-off	DR, %	FPR, %	OAPR
Options that meet the minimum standard						
FTS ^{5,10}	NT, free β -hCG, PAPP-A, MA	1st	1 in 325	83	5.0	1:27
Quad screening ¹¹	AFP, uE3, free β -hCG, inhibin A, MA	2nd	1 in 385	77	5.2	1:50
IPS ^{5,10}	NT, PAPP-A, AFP, uE3, free β -hCG/total hCG, inhibin A, MA	1st & 2nd	1 in 200	87	1.9	1:10
IPS without inhibin A ⁵	NT, PAPP-A, AFP, uE3, total hCG, MA	1st & 2nd	1 in 200	88	3.0	1:20
Serum IPS ^{5,10}	PAPP-A, AFP, uE3, free β -hCG/total hCG, inhibin A	1st & 2nd	1 in 200	85	4.4	1:26
Options that do not meet the minimum standard:						
Maternal age ¹²	MA	1st & 2nd	1 in 385	44	16	1:218
Triple screening ¹²	AFP, uE3, total hCG, MA	2nd	1 in 385	71	7.2	1:59

MA: Maternal age; OAPR: Odds of being affected given a positive result.

* Some centres in Canada may offer variation on IPS (sequential screening or contingent screening) with cut-offs set that achieve at least the minimum standard.

Table 3. Available screening options that meet minimum standard

Screening methods that meet guideline minimal standard of 75% DR with 3% FPR	Timing of results	Is 2nd trimester ultrasound still recommended?
1st trimester screen	1st trimester	Yes
2nd trimester quad screen	2nd trimester	Yes
Two-step screens		
Contingent	For most patients, result available in 1st trimester; small proportion of patients require second trimester testing	Yes
Integrated	Single result in 2nd trimester	Yes
Serum integrated	Single result in 2nd trimester	Yes
Sequential	Results in 1st and 2nd trimester for the same patient	Yes

described an association between an increased size of the nuchal translucency on the 11- to 14-week fetal ultrasound scan and an increased risk of fetal Down syndrome.¹⁸ Several large studies have shown that NT has a DR for Down syndrome ranging from 69% to 75%, with an FPR of 5% to 8.1%.^{5,10,19} This marker is associated with other numeric chromosome abnormalities, other fetal anomalies such as cardiac defects or diaphragmatic hernia, and a number of single gene disorders, particularly those associated with decreased fetal movement. An NT above the 99th percentile has a sensitivity of 31% and specificity of 98.7% for major congenital heart defects when the fetal karyotype is normal. One in 33 fetuses with an NT above the 95th percentile (above 2.2 to 2.8 mm depending on gestational age) and 1 in 16 with an NT above the 99th percentile (≥ 3.5 mm regardless of gestational age) have a major cardiac defect detected.²⁰ Finding an increased NT at 11 to 14 weeks' gestation with a normal fetal karyotype warrants offering a detailed ultrasound examination at

18 to 20 weeks, with an assessment of the fetal heart, including a 4-chamber view and view of the outflow tracts as a minimum²¹ or a fetal echocardiogram if available.

Two first trimester maternal serum biochemical markers emerged at the same time as NT was being investigated. These are PAPP-A and hCG (free beta or total). PAPP-A is lower in Down syndrome pregnancies and hCG is higher.^{22,23} In a study by Wald and Hackshaw that used a combination of the maternal age-related risk, maternal serum PAPP-A and free β -hCG, the DR of Down syndrome was 61%, with a 5% false-positive rate.²⁴ The first trimester biochemical markers alone were not as efficacious as second trimester screening; however, a combination of the 2 first trimester biochemical markers with NT, demonstrated a significant improvement over second trimester triple and quadruple screening. FTS using maternal age, NT plus PAPP-A, and free β -hCG will detect 83% of cases of Down syndrome, with a 5% FPR,

using a term risk cut-off for Down syndrome of 1:300 (or will detect 78% of cases of Down syndrome with a 3% FPR) and thus fulfills the guideline recommendation.⁵ FTS also has the ability to screen for trisomies 13 and 18.

Limitations on using FTS include the availability and reproducibility of NT as well as the availability of chorionic villus sampling as a diagnostic testing option for those with a screen positive result. Guidelines for measuring NT to maximize reproducibility and accuracy have been developed by the Fetal Medicine Foundation in the United Kingdom.²⁵ The Royal College of Obstetricians and Gynaecologists (United Kingdom) study group on first trimester assessment of Down syndrome recommended that NT measurement should be implemented only in centres with appropriately trained sonographers using high-quality equipment, and that the results should be subject to regular audit by an external agency.²⁶ The use of NT in a clinical setting requires a program of quality control and maintenance of skills through an ongoing audit of NT measurements to achieve standardization and maintain the quality essential to obtain the desired DR and FPR of the screening tests.²⁷

Finally, if local ultrasound services are unable to provide a comprehensive screen for neural tube defects at 18 to 20 weeks' gestation, patients undergoing first trimester screening for aneuploidy should be offered MSAFP in the second trimester to screen for open neural tube defects.

Ultrasound screening for delayed ossification of the fetal nasal bone can be done in the first or second trimester. The first trimester ultrasound, which determines the presence or absence of the nasal bone between 11 and 14 weeks of gestation, may be more likely to be incorporated into other screening modalities. First trimester assessment of the fetal nasal bone was described by Cicero et al.²⁸ and detected 77% of Down syndrome cases. Subsequent work has shown a DR of 68.8% and that the FPR depends upon maternal ethnicity (9% in Afro-Caribbeans, 5% in Asians, and 2.2% in Caucasians).²⁹ The FPR also varied with crown-rump length (increasing with decreasing crown-rump length) and NT (increasing with increasing NT).²⁹ The difficulty in performing first trimester nasal bone sonography consistently in the general population might limit the usefulness of this screening technique.³⁰ A study of intra- and interoperator variability in fetal nasal bone assessment during the first trimester showed that the assessment was only fairly reproducible.³¹ Guidelines for the ultrasound assessment of the nasal bone have been developed by the Fetal Medicine Foundation.²⁵ As with the use of NT, ultrasound assessment of the nasal bone in a clinical setting requires a program of training, quality control, and maintenance of skills through annual audit of nasal bone images.

Recommendations

7. First trimester nuchal translucency should be interpreted for risk assessment only when measured by sonographers or sonologists trained and accredited for this service and when there is ongoing quality assurance (II-2A), and it should not be offered as a screen without biochemical markers in singleton pregnancies. (I-E)
8. Evaluation of the fetal nasal bone in the first trimester should not be incorporated as a screen unless it is performed by sonographers or sonologists trained and accredited for this service and there is ongoing quality assurance. (II-2E)
9. For women who undertake first trimester screening, second trimester serum alpha fetoprotein screening and/or ultrasound examination is recommended to screen for open neural tube defects. (II-1A)

Second Trimester Screening

In the 1970s, alpha fetoprotein was identified as a second trimester marker for open neural tube defects. MSAFP continues to be used as part of multiple marker screening for this purpose, but is also effective as a screen for other open fetal defects such as gastroschisis and omphalocele.

In 1983, low MSAFP was noted in a patient who had a baby with trisomy 18.³² Further investigation showed this marker was low in Down syndrome as well,³² and for a few years, MSAFP combined with maternal age was used as a marker for Down syndrome. In 1988, Wald et al.³³ demonstrated that the combination of maternal age and MSAFP with two other maternal serum markers, unconjugated estriol (MSuE3) and human chorionic gonadotrophin (MShCG), measured between 15 and 20 weeks' gestation, would detect 65% of fetuses with Down syndrome using a 5% FPR.³³ These predictions were confirmed in several subsequent studies.^{12,34} Triple marker screening has been available in Canada since 1991. Using a term risk cut-off of 1:385, the triple marker screening detects 72% of fetuses with Down syndrome with a 7% FPR.¹² The triple marker screening also screens for ONTDs, other open fetal defects (e.g., gastroschisis, omphalocele), placental dysfunction, Smith-Lemli-Opitz syndrome, and trisomy 18. The triple screen does not fulfill the guideline recommendation.

Inhibin A is a fourth marker that can be added in the second trimester, resulting in the quad screen. Inhibin A will increase the DR of Down syndrome by 10%.^{35,36} With a risk cut-off of 1:230 at term, the DR is 75% to 80%, and the FPR is lowered to 3% to 5%, thus meeting the minimal standard set by this guideline.^{5,10}

Combined First and Second Trimester Options Integrated prenatal screening

In an effort to further improve performance, the first and second trimester screening tests have been combined into a process called integrated prenatal screening. Wald et al.³⁷ predicted that integrating first and second trimester screening would result in an 83% DR for Down syndrome, with a 2.1% FPR at a term risk cut-off of 1:200. IPS was based on the use of PAPP-A and NT in the first trimester and the quad screen in the second trimester, with results released when all the testing was completed.³⁷ This approach has been controversial, with some authors suggesting women had the right to know their results early and that it was unethical to withhold the first trimester results.³⁸ However, when IPS utilizes a quad screen in the second trimester, studies have shown a detection rate of 85% to 87% with an FPR of 0.8% to 1.5%.^{5,10} When Inhibin A is excluded from the IPS, the FPR increases to ~2.5% when the first trimester markers are performed at 12 weeks. Full integrated screening meets the guideline minimal standard. The benefit of IPS over FTS is the achievement of a lower FPR and reduction of the number of invasive diagnostic procedures needed.

The optimal time for the PAPP-A measurement is 9 to 10 weeks' gestation with the performance of PAPP-A decreasing between 10 and 13 weeks. The proportion of pregnancies in which a satisfactory NT measurement can be obtained is the highest at 11 to 13 weeks' gestation. First trimester measurements are usually carried out between 11 and 14 weeks' gestation as a compromise to make the timing favourable for NT and PAPP-A.⁵ IPS also screens for open fetal neural tube defects and trisomy 18.

Serum Integrated prenatal screening

When NT is not available, IPS still can be offered, using PAPP-A in the first trimester and triple or quad screening in the second trimester. This approach has an 83% DR for a 4% FPR.⁵ Alternatively, PAPP-A and free β -hCG can be offered in the first trimester, followed by AFP and uE3 in the second with virtually the same performance. The FPR is 4.2% if PAPP-A is measured at 10 completed weeks, and the FPR is doubled (8.5%) if it is measured at 13 completed weeks.⁵ In the FASTER trial, serum IPS showed a 4.4% FPR for an 85% DR.¹⁰ Serum IPS is a practical option for areas of Canada where there is limited or no access to NT screening.

Given that timing is critical for serum analysis, accurate dating of the pregnancy is very important. Ultrasound dating should be performed if menstrual or conception dating is unreliable. For any abnormal serum screen (serum IPS, quad) calculated using menstrual dating, an ultrasound should be done to confirm gestational age.

Recommendations

10. Timely referral and access is critical for women and should be facilitated to ensure women are able to undergo the type of screening test they have chosen as first trimester screening. The first steps of integrated screening (with or without nuchal translucency), contingent, or sequential screening are performed in an early and relatively narrow time window. (II-1A)
11. Ultrasound dating should be performed if menstrual or conception dating is unreliable. For any abnormal serum screen calculated on the basis of menstrual dating, an ultrasound should be done to confirm gestational age. (II-1A)

Contingent screening

The concept of contingent screening has been suggested by Wright et al.³⁹ as an alternative to IPS. In contingent screening, the majority of women receive their result after FTS. Women at high risk (risk >1/50) are offered invasive testing, and women at low risk (risk <1/1500) require no further testing. A proportion of women with a risk between two cut-offs (1/50 and 1/1500) will go on to have second trimester screening and will receive a combined result. Benn et al.⁴⁰ reported the expected screening performance of the contingent strategy by modelling on different risk cut-offs and maternal age distributions of the United Kingdom and the United States. Performance of contingent screening was comparable with IPS if total hCG and/or free β -hCG was measured in both trimesters.⁴⁰ It is possible to select risk cut-offs that achieve performances similar to IPS, thus meeting the guideline recommendation, while achieving detection of a significant proportion of abnormal pregnancies by the end of the first trimester.^{41,42} A study of computer simulations to compare integrated, sequential, and contingent screening strategies with various cut-offs leading to 19 potential screening algorithms showed that the contingent screening strategy had the best cost-effectiveness ratio, with fewer procedure-related euploid miscarriages and unnecessary terminations.⁴³ However, in contingent screening, a proportion of women are identified as having an intermediate risk and asked to have the second trimester serum to modify their risk. Using a prospective first trimester cohort of 18 901 pregnancies and a contingent protocol, Cocciolone et al. reported that 15.8% of cases had first trimester combined risk odds between 1 per 51 and 1 per 1500, thus requiring second trimester serum marker analysis.⁴⁴ The women in this intermediate risk group are likely to experience raised anxiety, and a proportion of them might wish to have invasive testing immediately, thus increasing the FPR.^{42,45}

Sequential screening

Sequential screening selects women for second trimester testing on the basis of their first trimester screening results. Women found to be at high risk on the basis of the FTS (e.g., risk ≥ 1 in 50) are offered invasive testing. Those with a risk lower than the cut-off are offered additional serum screening in the second trimester. The removal of screen positive affected cases in the first trimester decreases the prevalence of Down syndrome in the second trimester and consequently lowers the PPV of second trimester serum screening.^{46,47} As a result the overall cut-off is adjusted to take this into consideration. With appropriate cut-offs, sequential screening has been shown to perform equivalently to full integrated and contingent screening and meets the guideline recommendation.⁴²

Sequential screening that does not incorporate the results of the first trimester testing into the second trimester risk analysis is associated with a significant increased FPR.⁵⁶ Given this high FPR, sequential screening *should not be offered unless the second trimester risk incorporates the first trimester results.*

POTENTIAL OF SCREENING OPTIONS TO DETECT CHROMOSOMAL ANOMALIES OTHER THAN DOWN SYNDROME AND OTHER GENETIC CONDITIONS

In pregnancies with trisomy 18, first trimester PAPP-A is decreased, NT is enlarged, and second trimester serum levels of AFP, uE3, hCG, and inhibin A are significantly reduced.⁴⁸⁻⁵¹ Many centres are now routinely screening for trisomy 18, using protocols designed for this anomaly. With second trimester triple marker screening, at a term risk cut-off of $\geq 1:100$, 60% of trisomy 18 pregnancies can be detected for a FPR of 0.2%.⁵² With serum IPS, using the same cut-off, the DR is 90% for a FPR of 0.1%.⁵³ A protocol for the detection of trisomies 13 and 18 has been developed for FTS.⁵⁴

Studies show a large proportion of fetuses with triploidy can be detected with the current MSS or FTS protocols.^{55,56} Second trimester uE3 is decreased and inhibin A is elevated in pregnancies with trisomy 13.^{57,58} Turner syndrome is associated with a lower uE3. Higher hCG and inhibin A levels also are seen in cases where there is fetal hydrops.⁵⁹⁻⁶¹ Increased NT and a lower PAPP-A have been reported in pregnancies with triploidy of paternal origin, trisomy 13, Turner syndrome, and other sex chromosome aneuploidies.⁶²⁻⁶⁴ Trisomy 13 and 18, Turner syndrome, and triploidy also are associated with anomalies and markers that allow the majority to be detected during the 18- to 20-week ultrasound.

Smith-Lemli-Opitz Syndrome is an autosomal recessive disorder associated with intellectual disability and multiple congenital anomalies. The minimum incidence is estimated to be 1 in 60 000.⁶⁵ SLOS is due to an abnormality in cholesterol synthesis resulting in a low cholesterol concentration and accumulation of its precursors in blood and tissue.⁶⁶ SLOS can be diagnosed prenatally by the presence of abnormally elevated amniotic fluid 7-dehydrocholesterol concentration.⁶⁷ In pregnancies with SLOS, maternal serum uE3, AFP, and hCG are reduced.⁶⁸ A screening protocol has been developed for this syndrome that provides a DR of 62% for a FPR of 0.33%.⁶⁹ However, the screen is not specific for SLOS since it detects a number of rare disorders of cholesterol and estriol biosynthesis, such as congenital adrenal hypoplasia and Zellweger syndrome, as well as a relatively common and mild disorder, X-linked steroid sulfatase deficiency (X-linked ichthyosis).⁷⁰

THE USE OF ULTRASOUND IN SCREENING FOR CHROMOSOMAL ANOMALIES

At 18 to 20 weeks' gestation, all pregnant women should be offered a detailed ultrasound that meets previously established minimum standards.⁷¹ Most major fetal anatomic abnormalities should be detected by this screen. In particular, the majority of open neural tube defects should be detected by this ultrasound.⁷² In addition, ultrasound can detect "soft markers," which are features that increase the a priori risk of fetal aneuploidy but can also be variations of normal. When used alone, second trimester ultrasound soft markers do not effectively discriminate between unaffected fetuses and fetuses with Down syndrome, because of the high positive rate from the large number of potential markers.⁷³⁻⁷⁶ Ultrasound soft markers were comprehensively reviewed in a 2007 SOGC guideline,⁷⁷ and both soft markers and anomalies identified in the 18- to 20-week ultrasound can be used to modify any a priori risk established by age or prior screening. In the absence of soft markers and anomalies, a reduction of risk can be applied. In this circumstance, a conservative negative likelihood ratio of 0.5 is often used, based on studies that have shown an odds ratio, in the presence of a normal fetal ultrasound, ranging from 0.2 to 0.55⁷⁸⁻⁸⁶ unless centre-specific levels are determined through clinical audit. However, this should be done only in an established centre performing tertiary level scans.

Recommendation

12. The presence or absence of soft markers or anomalies in the 18- to 20-week ultrasound can be used to modify the a priori risk of aneuploidy established by age or prior screening. (II-2B)

FACTORS POTENTIALLY AFFECTING SCREENING PERFORMANCE

A number of factors may affect screening performance. These include accuracy of gestational dating, maternal weight, ethnicity, insulin-dependent diabetes mellitus, accuracy of NT and serum marker measurements, and the use of assisted reproduction technologies.

Gestational Dating

Accurate dating is important. Ultrasound improves the precision of gestational age estimation, and hence reduces the standard deviation of each screening marker. This effect is greater for markers whose concentrations change most with gestational age. For all marker combinations, the FPR is lower by about 2% when gestational age is estimated using a scan. For example, for a DR of 85%, scan dating could reduce the FPR of serum IPS from 4.2% to 2.7%.⁵

Maternal Weight

There is a negative association between the levels of maternal serum markers and maternal weight, which is due to the dilution effect produced by the physiologic increase in blood volume.⁸⁴ The trend with first trimester markers is similar to that seen with second trimester markers.⁸⁵ With second trimester screening, maternal weight adjustment increases DR by about 1% for a given FPR, or reduces FPR by 0.2% for a given DR.¹¹ Weight adjustment is beneficial if there is a marginally elevated AFP when screening for ONTD. When interpreting measurements of serum markers, many screening centres routinely adjust for maternal weight. It has been suggested that published weight correction formulas may not be optimal because of differences in mean weight between the population served and the populations used to derive the formulas. Each laboratory should calculate its own weight adjustment formulas.⁸⁴

Weight adjustment does not appear to be necessary for NT risk adjustment, because it increases by only a clinically insignificant amount with increasing maternal weight.⁸⁶

Ethnic Origin

There are differences in the levels of screening markers between women of different ethnic origins after accounting for maternal weight. Maternal serum AFP is 15% higher, total hCG is 18% higher, inhibin A is 8% lower, and PAPP-A is 35% higher in Black women than in Caucasian women. AFP is 6% lower, uE3 is 7% higher, total hCG is 6% higher and PAPP-A is 17% higher in South Asian women. Higher levels of first trimester PAPP-A and β -hCG are seen in Asian women, and higher uE3 is seen in Aboriginal Canadian women.^{11,86-90} Adjusting for ethnic

origin slightly increases the DR for a given FPR, but, more importantly, it tends to equalize the FPR among women of different ethnic groups.¹¹

Statistically significant differences in NT measurement have been found between ethnic groups.⁹⁰⁻⁹² However, it seems these differences may be too small to warrant correction.⁸⁸

Insulin-Dependent Diabetes Mellitus

Some second trimester serum markers tend to be lower in women with insulin-dependent diabetes mellitus. After weight correction, AFP is ~10% lower and uE3 is ~5% lower in diabetic women. No change in other markers in diabetic women has been demonstrated.^{11,93,94} To allow for the difference, the observed MoM for a woman with diabetes is divided by the corresponding median MoM in diabetic women without Down syndrome pregnancies. Because of the lack of data in diabetic women who have a Down syndrome pregnancy, a “pseudo risk” can be calculated for diabetic women.¹¹

It appears that NT measurement, free β -hCG, and PAPP-A in women with and without insulin-dependent diabetes are not significantly different.⁹⁵

Assisted Reproduction

When a pregnancy is a result of IVF, the maternal age used for the determination of the risk of Down syndrome is the age of the donor at the time the egg was harvested.

Data from most published studies show second trimester serum levels of β -hCG and total hCG are higher, and uE3 is lower in pregnancies conceived through IVF.⁹⁶⁻⁹⁹ There were no significant differences in the levels of AFP and inhibin A between IVF and non-IVF pregnancies.⁹⁷ The variation in hCG is said to be driven by the continuing high progesterone concentrations following hormonal treatment.⁹⁷ Because of the higher hCG and lower uE3 levels, the FPR of second trimester screening is nearly doubled in IVF pregnancies.^{97,100,101} In 1999, Wald et al.⁹⁷ suggested that adjustments for IVF pregnancies could avoid this high FPR. However, results from a recent study in France based on ~1000 IVF pregnancies found no differences in the values of maternal serum AFP, uE3, and hCG between IVF pregnancies and controls. The FPR was similar in the 2 groups.¹⁰²

In the first trimester, a lower value of PAPP-A has been reported in IVF pregnancies, but data on NT and first trimester free β -hCG remain inconsistent.^{101,103-106} Many screening programs routinely collect information on IVF; however, whether adjustment is necessary needs further investigation.

Recommendation

13. Information such as gestational dating, maternal weight, ethnicity, insulin-dependent diabetes mellitus, and use of assisted reproduction technologies should be provided to the laboratory to improve accuracy of testing. (II-2A)

GENERAL CONSIDERATIONS

Screening practice differs across Canada and will also change over time. Practitioners should stay updated on the screening modalities available in their areas.

Recommendations

14. Health care providers should be aware of the screening modalities available in their province or territory. (III-B)
15. A reliable system needs to be in place ensuring timely reporting of results. (III-C)
16. Screening programs should be implemented with resources that support audited screening and diagnostic laboratory services, ultrasound, genetic counselling services, patient and health care provider education, and high quality diagnostic testing, as well as resources for administration, annual clinical audit, and data management. In addition, there must be the flexibility and funding to adjust the program to new technology and protocols. (II-3B)

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APPENDIX. SCREENING TERMINOLOGY

Affected individuals: Individuals who have the disorder for which the screen is being performed.

Cut-off level: The value of a test variable that distinguishes screen positive from screen negative results. The screening cut-off will affect both the detection and false-positive rates: the higher the cut-off, the lower the false-positive rate and the lower the detection rate.

Detection rate (DR) or sensitivity: The proportion of affected individuals with positive screening results (usually expressed as a percentage).

False-positive rate (FPR): The proportion of unaffected individuals with positive screening results (usually expressed as a percentage). It is the complement of the specificity.

First trimester window: The period between 11+0 weeks and 13+6 weeks of gestation.

Incidence: The number of new cases of a disorder that arise during a specific period of time, such as a year. This is usually expressed as a rate per 1000.

Integrated prenatal screen (IPS): The use of markers in the first and second trimester to calculate the overall risk for fetal aneuploidy.

Likelihood ratio (LR): The likelihood that a given test result would be expected in a patient with the target disorder compared with the likelihood that that same result would be expected in a patient without the target disorder. The likelihood ratio for a population is the detection rate divided by the false-positive rate.

Multiple of the median (MoM): The observed value of a specific marker divided by the median value for that marker in a specified population (in prenatal screening, usually pregnancies of the same gestational age).

Marker: A biological measurement that when present at an abnormal level may indicate the presence of disease.

Negative predictive value: The number of unaffected individuals with negative results (true negatives) divided by the total number of individuals with a negative result, both affected and unaffected.

Odds of being affected given a positive result (OAPR): The ratio of the number of affected individuals with positive test results to the number of unaffected individuals with positive results.

Positive predictive value (PPV): The number of affected individuals with positive results (true positives) divided by the total number of individuals with positive results, both affected and unaffected. It is the odds of being affected given a positive result expressed as a proportion or percentage.

Positive rate: The sum of true and false positives. For most screens, the positive rate is virtually equal to the false-positive rate but as the FPR decreases, this becomes a less reliable approximation. The screen positive rate is a useful parameter for the estimation of resource requirements for follow-up services.

Prevalence: The number of cases of a disorder present at a point in time or during a specified period. This is usually expressed as a rate per 1000.

Quality assurance: The policy, procedures, and systematic actions established in an enterprise for the purpose of providing and maintaining a specified degree of confidence of a screening test.

Receiver operator curve (ROC): It is a plot of the true positive rate against the false-positive rate for the different possible cut points of a test. An ROC curve demonstrates the trade-off between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity). Accuracy of the test is measured by the area under the ROC curve.

Second trimester window: The period between 15+0 weeks and 20+6 weeks of gestation.

Specificity: The proportion of unaffected individuals with negative results.

Unaffected individuals: Individuals who do not have the disorder for which the screen is being performed.