SOGC CLINICAL PRACTICE GUIDELINE

No. 456, November 2024 (Replaces No. 348, September 2017)

Guideline No. 456: Prenatal Screening for Fetal Chromosomal Anomalies

(En français : Dépistage prénatal des anomalies chromosomiques fœtales)

The English document is the original version; translation may introduce small differences in the French version.

This clinical practice guideline was prepared by the authors, reviewed by the Clinical Obstetrics Committee (2024), and approved by the SOGC Guideline Management and Oversight Committee.
This clinical practice guideline supersedes No. 348, published in September 2017.

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J Obstet Gynaecol Can 2024;■(■):102694

https://doi.org/10.1016/j.jogc.2024.102694

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Acknowledgement: The authors would like to acknowledge and thank Jo-Ann. Johnson, MD, Calgary, AB, for contributions to the previous version of this guideline.

Disclosures: Statements were received from all authors. Dr. R. Douglas Wilson disclosed his involvement with Vitara and the Children's Hospital of Philadelphia on an upcoming study, as well as the position he held on the SOGC Board of Directors until June 2024. All authors have indicated that they meet the journal's requirements for authorship.

Subject Categories: maternal fetal medicine, diagnostic imaging

Keywords : prenatal diagnosis; cell-free nucleic acids; genetic counselling; chromosome aberrations; congenital abnormalities

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This document reflects emerging clinical and scientific advances as of the publication date and is subject to change. The information is not meant to dictate an exclusive course of treatment or procedure. Institutions are free to amend the recommendations. The SOGC suggests, however, that they adequately document any such amendments.

Informed consent: Patients have the right and responsibility to make informed decisions about their care, in partnership with their health care provider. To facilitate informed choice, patients should be provided with information and support that is evidence-based, culturally appropriate, and personalized. The values, beliefs, and individual needs of each patient in the context of their personal circumstances should be considered and the final decision about care and treatment options chosen by the patient should be respected.

Language and inclusivity: The SOGC recognizes the importance to be fully inclusive and when context is appropriate, gender-neutral language will be used. In other circumstances, we continue to use gendered language because of our mission to advance women's health. The SOGC recognizes and respects the rights of all people for whom the information in this document may apply, including but not limited to transgender, non-binary, and intersex people. The SOGC encourages health care providers to engage in respectful conversation with their patients about their gender identity and preferred gender pronouns and to apply these guidelines in a way that is sensitive to each person's needs.

Weeks Gestation Notation: The authors follow the World Health Organization's notation on gestational age: the first day of the last menstrual period is day 0 (of week 0); therefore, days 0 to 6 correspond to completed week 0, days 7 to 13 correspond to completed week 1, etc.

RECOMMENDED CHANGES IN PRACTICE

1. Regardless of aneuploidy screening choice, all pregnant persons should be offered a fetal ultrasound, optimally between 11 and 14 weeks, to confirm viability, gestational age, number of fetuses, chorionicity in multiples, early anatomic assessment, and nuchal translucency evaluation.
2. Maternal circulating cell-free DNA is the most accurate method of early prenatal screening for common trisomies and should be discussed as an option with all pregnant persons.
3. The diagnostic role of fetal exome/genome sequencing is a rapidly evolving area and maternity care providers should be aware that the technology may become available and be prepared to provide additional information and counselling to patients on fetal anomalies

KEY MESSAGES

1. Because of its superior performance, circulating cell-free DNA screening for common trisomies should be discussed as an option with all pregnant persons.
2. Regardless of aneuploidy screening choices, good quality first- and second-trimester ultrasound examinations are important for the detection of many structural and genetic anomalies.
3. The presence of an isolated fetal soft ultrasound marker in the second trimester should not be used to adjust the a priori risk for aneuploidy in pregnancies at low risk based on previous screening.

ABSTRACT

Objective: To review the available prenatal aneuploidy screening options and to provide updated clinical guidelines for reproductive care providers.

Target Population: All pregnant persons receiving counselling and providing informed consent for prenatal screening.

Benefits, Harms, and Costs: Implementation of the recommendations in this guideline should increase clinician competency to offer counselling for prenatal screening options and provide appropriate interventions. Given the variety of available options for prenatal screening with different performance, cost, and availability across Canada, appropriate counselling is of paramount importance to offer the best individual choice to Canadian pregnant persons. Prenatal screening may cause anxiety, and the decisions about prenatal diagnostic procedures are complex given the potential risk of fetal loss.

Evidence: Published literature was retrieved through searches of Medline, PubMed, and the Cochrane Library in and prior to July 2023, using an appropriate controlled vocabulary (prenatal diagnosis, amniocentesis, chorionic villi sampling, non-invasive prenatal screening) and key words (prenatal screening, prenatal genetic counselling). Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies written in English and published from January 1995 to July 2023.

Validation Methods: The authors rated the quality of evidence and strength of recommendations using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. See Appendix A (Tables A1 for definitions and A2 for interpretations).

Intended Audience: Health care providers involved in prenatal screening, including general practitioners, obstetricians, midwives, maternal–fetal medicine specialists, geneticists, and radiologists.

Social Media Abstract: Non-invasive prenatal screening is the most accurate method for detecting major aneuploidies. It is not universally available in the public health system and has some limitations.

SUMMARY STATEMENTS:

1. First-trimester ultrasound (at 11–14 weeks gestation) offers many advantages for prenatal screening and pregnancy management, including accurate dating, determination of twin chorionicity, and early detection of some major structural abnormalities, regardless of the aneuploidy screening options *(high).*
2. The risk of fetal loss after prenatal invasive testing is lower than the rates currently cited and appears to be negligible when these interventions are compared with control groups of patients with the same risk profiles. Second-trimester amniocentesis may increase the risk of pregnancy loss, but it is not possible to quantify this increase precisely. Transcervical chorionic villous sampling may be associated with a higher risk of pregnancy loss than transabdominal chorionic villous sampling and second-trimester amniocentesis *(low).*
3. Maternal circulating cell-free DNA is the most accurate method of early prenatal screening for common trisomies (21, 18, 13). Due to its superior performance, circulating cell-free DNA screening for common trisomies should be discussed as an option with all pregnant persons *(high).*
4. In most provinces in Canada, first-tier cell-free DNA screening is not currently offered to all pregnant persons by the public health care system owing to cost and resource issues. Some provinces offer public cell-free DNA screening for some indications. Offering cell-free DNA in a contingent model (after conventional screening) is a less costly option that has the potential to achieve a detection rate above 90% with an invasive testing rate below 3%. First-tier screening by cell-free DNA could become the first-line option in the future if costs decrease and if technical resources are made available *(moderate).*
5. In twin pregnancies, the most accurate screening for aneuploidy is achieved with cell-free DNA screening, although with weaker evidence and a higher rate of test failure due to lower fetal /placental fraction. First-trimester serum screening combined with nuchal translucency may also be considered in twin pregnancies *(moderate).*
6. The presence of specific ultrasound *soft markers* associated with fetal trisomy 21 (echogenic intracardiac focus) or trisomy 18 (choroid plexus cysts) at the time of the second-trimester ultrasound is not clinically relevant because of its poor predictive value, and such findings do not warrant further testing. The value of other soft markers, including mild ventriculomegaly, absent nasal bone, renal pyelectasis, thickened nuchal fold, and echogenic bowel, is weak in pregnancies at low risk for aneuploidy based on previous screening (*moderate*).

RECOMMENDATIONS:

1. All pregnant persons in Canada, regardless of age, should be offered, through an informed counselling process with shared decision-making, the option of a prenatal screening test for the most common fetal aneuploidies and for major fetal anomalies (*strong, high*).
2. Health care providers should be aware of the screening modalities available in their province or territory. A reliable provincial system should ensure timely reporting of screening results. Prenatal

screening programs should be implemented with resources that support audited screening and laboratory services, ultrasound services, genetic counselling, and patient and health care provider education. In addition, there must be flexibility and funding to adjust the program based on new technology and protocols (*strong, high*).

3. A discussion of the risks, benefits, expectations, and alternatives of the various prenatal screening and diagnostic options should be undertaken with all patients prior to prenatal aneuploidy screening. Following this, patients should be offered the choice of a) no aneuploidy screening; b) standard aneuploidy prenatal screening based on locally offered programs, with various combinations of serum screening and nuchal translucency; c) maternal plasma cell-free DNA screening, where available, with the understanding that it may not be publicly funded; or d) invasive diagnostic testing using chorionic villus sampling or amniocentesis with the understanding that it may not be publicly funded or even offered as a first-tier test (*strong, high*).
4. Regardless of aneuploidy screening choice, all pregnant persons should be offered a first-trimester fetal ultrasound, (optimally between 11- and 14-weeks gestation), to assess viability, gestational age, number of fetuses, chorionicity in multiples, early fetal anatomy, and nuchal translucency. Maternal serum screening (with or without nuchal translucency measurement for aneuploidy risk estimation) should not be performed if cell-free DNA screening is performed or planned (*strong, high*).
5. A high nuchal translucency measurement (above 3.5 mm) is a marker for fetal cardiac and other structural anomalies, as well as genetic conditions such as RASopathies (including Noonan syndrome). Genetic counselling and invasive testing are strongly recommended for diagnosis, followed by advanced genetic testing and ultrasound follow-up (*strong, high*).
6. Training of ultrasound providers, including maternal–fetal medicine specialists, radiologists, and sonographers, should be promoted to improve access to high-quality first-trimester ultrasound services for all Canadians. (*strong, moderate*).
7. Persons considering maternal plasma cell-free DNA screening should be informed that a) it is a highly effective screening test for the common fetal trisomies (21, 18, 13), when performed after 10 weeks gestation; b) there is a possibility of a failed ('no-call') test, false-negative or false-positive fetal aneuploidy result, or unexpected fetal or maternal result; c) all positive maternal cell-free DNA screening results should be confirmed with diagnostic fetal testing; d) routine cell-free DNA screening for fetal microdeletions is not currently recommended; e) routine cell-free DNA screening for sex chromosome abnormalities is debated and not currently recommended (*strong, high*).
8. If a fetal structural abnormality (not a soft marker) is identified during the first- or second-trimester ultrasound, regardless of previous screening test results, genetic counselling and invasive diagnostic testing should be offered, with rapid aneuploidy detection and reflex microarray analysis or exome/genome sequencing, if rapid aneuploidy detection is normal or inconclusive. The diagnostic role of fetal exome/genome sequencing is a rapidly evolving area, and maternity care providers should be aware of this technology (*strong, high*).
9. The presence of an isolated fetal soft marker in the second trimester should not be used to adjust the a priori risk for aneuploidy in persons at low risk based on previous screening (*strong, high*).

INTRODUCTION

The landscape of prenatal screening and diagnosis has evolved considerably in the last decade, with the rapid development of new technologies, particularly the introduction of next-generation sequencing, allowing non-invasive prenatal screening (NIPS) using circulating maternal cell-free DNA (cfDNA) and more in-depth diagnostic analysis of the fetal genome with techniques such as chromosomal microarray and exome/genome sequencing. These new tools have added complexity with respect to pretest patient counselling and post-test counselling and interpretation of conventional tests such as ultrasound examination, maternal serum screening, and invasive fetal testing.

The objective of this guideline is to: 1) update the advances in this area since the last version of this document and summarize them for maternity care providers[1]; 2) provide guidance for prenatal screening programs across Canada; and 3) discuss the impact of new technologies on prenatal counselling, screening, and diagnosis.

The scope of this guideline is limited to prenatal screening for common trisomies (trisomy 21, 18, and 13) and other chromosomal conditions. It excludes screening for fetal genetic anomalies and major structural defects, as well as for adverse pregnancy outcomes.

GENERAL CONSIDERATIONS FOR PRENATAL GENETIC SCREENING

Over the last few decades, most high-resource countries have implemented prenatal genetic screening programs with the objective to detect fetal anomalies and provide reproductive options to pregnant persons. Most efforts have focused on the detection of common aneuploidies (trisomy 21, 18, and 13) because they represent the most frequent chromosomal anomalies, affecting about 0.2% of pregnancies. However, it must be noted that collectively there is a much higher incidence of other fetal anomalies, both chromosomal (other than the common trisomies) and genetic abnormalities and structural malformations. Together, these anomalies affect about 2%–3% of pregnancies.[2]

It is important that patients, care providers, institutions, and societies have a clear understanding of the objectives and limitations of prenatal screening programs and the principle of patient autonomy. Clear information should be given about screening for common aneuploidies only versus screening for other chromosomal abnormalities and fetal malformations. Because most malformations are only detectable by ultrasound (in the first, second, or third trimester), ultrasound should remain the cornerstone of prenatal genetic screening strategies, whatever option is chosen for common aneuploidy screening.

All pregnant persons should be offered screening for fetal aneuploidy and major congenital anomalies. The following factors can modify the risk of fetal aneuploidy, genetic disorders, and/or malformations, and should be interpreted together, rather than separately, for individual risk estimation and counselling:

- Maternal history: maternal age, previous pregnancy affected with aneuploidy, maternal or paternal chromosome rearrangements with an increased risk for chromosomal imbalance.
- First-trimester (11–14-week) ultrasound evaluation[3–6]: where available with documented expertise, offers many advantages, including accurate dating; determination of twin chorionicity; early detection of major structural abnormalities such as anencephaly, omphalocele, or megacystis; and aneuploidy screening using nuchal translucency (NT). A high NT measurement (>3.5 mm) is associated with an increased risk of fetal conditions, in particular congenital heart disease, fetal akinesia, structural malformations, some single-gene disorders (such as Noonan syndrome[7]), chromosome abnormalities, and poor pregnancy outcomes, including fetal death. The American Institute of Ultrasound in Medicine (AIUM)[8] and the International Society of Ultrasound Obstetrics and Gynecology (ISUOG)[9–13] support the continued measurement of NT in the first trimester, even if not used in the context of aneuploidy screening. The International Society for Prenatal Diagnosis (ISPD) notes that, if non-invasive prenatal testing replaces the NT

ABBREVIATIONS

AIUM	American Institute of Ultrasound in Medicine
cfDNA	cell-free DNA
CNV	copy number variant
CVS	chorionic villous sampling
IPS	integrated prenatal screening
ISPD	International Society for Prenatal Diagnosis
ISUOG	International Society of Ultrasound Obstetrics and Gynecology
NIPT	non-invasive prenatal testing
NIPS	non-invasive prenatal screening
NT	nuchal translucency
PPV	positive predictive value
SIPS	serum integrated screening
WES/WGS	whole exome sequencing / whole genome sequencing

ultrasound at 11–13 weeks, its implementation as a primary screen would also reduce opportunities for early ultrasound detection of fetal structural anomalies.[14,15] In a recent study, detailed first-trimester ultrasound identified more fetuses with a potential abnormality than did non-invasive prenatal testing alone.[16] The SOGC encourages NT measurement but with adequate and monitored quality control. Every effort should be made to improve access to high-quality first-trimester ultrasound for all Canadian pregnant persons. In areas where NT ultrasound is not available, a first-trimester dating ultrasound improves the accuracy of maternal serum screening and the management of pregnancy.[17]

- Second-trimester (18–22-week) ultrasound evaluation for malformation by a provider with expertise in fetal ultrasound.[12,18]
- Maternal serum aneuploidy screening: first- and/or second-trimester screening using placental and fetal biochemical analytes with or without NT, as part of first-trimester screening, second-trimester quadruple serum (QUAD) screening, integrated screening (IPS), or serum integrated screening (SIPS).
- NIPS using circulating maternal cfDNA.

Summary Statement 1 and Recommendations 1, 2, 3, 4, 5, and 6

PRENATAL SCREENING AND DIAGNOSIS FOR COMMON ANEUPLOIDIES: TRADITIONAL METHODS

Prenatal Screening

Traditional prenatal screening protocols for fetal trisomies 21, 18, and 13 are based on combinations of maternal serum biochemical markers with or without an NT measurement.[1,19] The addition of NT measurement greatly improves screening performance compared to biochemical screening alone, and has the major advantage of providing the additional benefits of a detailed first-trimester ultrasound, including screening for a large number of fetal defects and genetic conditions.[3] However, adequate resources and quality control are not universally available.

The various combinations include:

- First-trimester screening combining assessment of NT and biomarkers at 11–14 weeks, including free β–human chorionic gonadotropin (β-hCG) and pregnancy associated plasma protein A (PAPP-A), with the option of adding α-fetoprotein (AFP) and placental growth factor (PlGF);

1. Second-trimester (QUAD) screening,

- SIPS;
- IPS, combining SIPS and NT; and
- Contingent screening, with first line, first-trimester screening, followed by second test only if the risk is above a certain threshold. The second test can be quadruple serum screening, or cfDNA where available.

These combinations offer a detection rate for trisomy 21 of 64%–95%, with a screen-positive rate of 1%–5%. (See table 1 in ACOG Practice Bulletin Summary, Number 226).[20,21]

Choice of screening and strategy depends on locally available resources, which vary by province and territory. Where NT ultrasound is available, first-trimester screening has the advantage of a one-step approach with earlier results. First-trimester screening can also be enhanced with biomarkers, such as PlGF, which provide additional screening for preeclampsia and placenta-mediated pregnancy complications.

Table 1. Prenatal screening options using combinations of maternal serum markers with or without NT

Screening combination	Timing, wk	Markers used	NT*	Detection rate for a 5% screen-positive rate, %
First-trimester screening	11–14	Free β-hCG, PAPP-A, with or without AFP, PlGF (optional, "enhanced" screening with 4 markers)	Yes	82–95
Second-trimester QUAD screening	14–20	β-hCG, AFP, estriol, inhibin A	No	72–82
SIPS	11–14; then 14–20	FTS (no NT) + QUAD	No	85–87
IPS	11–14; then 14–20	NT + FTS + QUAD	Yes	90–96

*Where available, provides the advantage of additional early screening for fetal defects.

AFP: α-fetoprotein; β-hCG: β–human chorionic gonadotropin; FTS: first-trimester screening; NT: nuchal translucency; IPS: integrated prenatal screening; PAPP-A: pregnancy associated plasma protein A; PlGF: placental growth factor; SIPS; serum integrated prenatal screening; QUAD quadruple serum screening.

In pregnancies achieved following preimplantation genetic testing, refer to SOGC committee opinion No. 406, for specific recommendations.[22]

Invasive Testing and the Estimated Risk of Procedure-Related Fetal Loss

If prenatal screening identifies a high risk of aneuploidy, invasive testing is advised to confirm the diagnosis. Testing uses mostly second-trimester amniocentesis or first-trimester chorionic villous sampling (CVS).

The procedure-related risk of prenatal invasive procedures is a subject of long-standing debate.

According to the most recent systematic review and meta-analysis, the procedure-related risks of miscarriage following amniocentesis and CVS are lower than currently quoted. The risk appears to be negligible when these interventions were compared with control groups of patients with the same risk profiles.[23] The procedure-related risk of miscarriage is 0.30% following amniocentesis, whereas there is no significant procedure-related risk associated with transabdominal CVS, which may be a safer procedure than amniocentesis. When the analysis is restricted to studies in which the control population has a similar risk profile for chromosomal abnormalities as the population who underwent invasive prenatal testing, the point estimates for miscarriage are even lower, with no significant increase in risk of miscarriage, for either amniocentesis or CVS.

In the most recent Cochrane systematic review, the authors concluded that second-trimester amniocentesis increases the risk of pregnancy loss, but it is not possible to quantify this increase precisely from only one randomized study, carried out more than 30 years ago. Early amniocentesis (before 15 weeks gestation) is not as safe as second-trimester amniocentesis, as illustrated by increased incidences pregnancy loss and congenital anomalies (talipes). Transcervical CVS may be associated with a higher risk of pregnancy loss than transabdominal CVS and second-trimester amniocentesis, but these findings were heterogeneous.[24]

Summary Statement 2

PRENATAL SCREENING FOR COMMON ANEUPLOIDIES: NON-INVASIVE SCREENING USING CELL-FREE DNA

The introduction of maternal plasma cfDNA-based technology has both superior performance to the traditional serum analyte screening approach, and has been able to dramatically enhance the traditional approach. Cell-free DNA screening also has the ability to determine fetal sex and blood type and to detect single-gene syndromes, including the possibility of identifying paternally derived genetic abnormalities.[25]

Widely referred to as NIPT or NIPS, maternal plasma cfDNA screening (the preferred nomenclature) is based on genomic sequencing of maternal plasma cfDNA fragments (of both maternal and placental origin) using either *massively parallel* sequencing or targeted-sequencing methods (either chromosome selective or single nucleotide polymorphism (SNP)—based) combined with advanced bioinformatic analysis. Data from recent meta-analyses of published clinical validation and implementation studies show high sensitivity and specificity for fetal trisomies 21, 18, and 13, regardless of the method used.[26–29] Table 2 shows the screening performance of NIPS based on recent meta-analyses.

Cell-free DNA screening can also be used to determine fetal sex, fetal blood type, and to detect certain paternally derived autosomal genetic abnormalities although not as widely used as for screening for common aneuploidies.

This section discusses the risks, benefits, and limitations of cfDNA screening identified through its clinical use in average and high-risk populations and provides an updated implementation model and counselling considerations.[30]

Interpretation of Circulating cfDNA Screening Results

The average turn-around time for cfDNA screening results is currently 4—10 days. Report formats vary from a simple positive or negative screening result to a numerical risk (e.g., >99% (high risk) or less than 1/10 000 (low risk). The American College of Obstetricians and Gynecologists (ACOG)[28] and Society for Maternal-Fetal Medicine (SMFM)[31] recommend that positive predictive value (PPV) and residual risk be included in cfDNA screening reports. While the sensitivity and specificity of cfDNA screening has been shown to be similar in the general and high-risk obstetric populations, PPV is lower in the general population, given the lower prevalence of fetal aneuploidy. Thus, fewer people with a positive result in the general obstetric population will have an affected fetus, and there will be more false-positive results. Other factors influencing PPV include previous serum screening results, ultrasound findings, incidence of aneuploidy, and gestational age. For persons who receive a negative result, the likelihood that the fetus does not have one of the common aneuploidies (negative predictive value) also depends on

Table 2. Non-invasive prenatal testing performance.

Test Statistic	No. of Studies	Result, % (95% CI)*	I^2, %
Trisomy 21		$P < .0001$	
Sensitivity	17	98.80 (97.81–99.34)	0.0
Specificity	14	99.96 (99.92–99.98)	75.9
PPV	28	91.78 (88.43–94.23)	68.3
NPV	14	100 (99.99–100)	0.0
FPR	14	0.04 (0.02–0.08)	75.9
Accuracy	14	99.94 (99.91–99.96)	80.2
DOR, OR (95% CI)	14	110 000 (44 000–260 000)	55.7
Trisomy 18		$P < .0001$	
Sensitivity	6	98.83 (95.45–99.71)	0.0
Specificity	7	99.93 (99.83–99.97)	94.9
PPV	17	65.77 (45.29–81.68)	88.5
NPV	7	100 (100–100)	0.0
FPR	7	0.07 (0.03–0.17)	75.9
Accuracy	6	99.91 (99.73–99.97)	95.7
DOR, OR (95% CI)	6	29 000 (4800–180 000)	94.9
Trisomy 13		$P < .0001$	
Sensitivity	7	100 (0–100)	0.0
Specificity	8	99.96 (99.92–99.98)	81.5
PPV	18	37.23 (26.08–49.93)	71.9
NPV	8	100 (100–100)	0.0
FPR	8	0.04 (0.02–0.08)	81.5
Accuracy	8	99.95 (99.90–99.97)	82.2
DOR, OR (95% CI)	7	29 000 (8900–94 000)	0

Results do not include studies without adequate data to include in meta-analyses.

Adapted from a systematic review by Rose et al.26 This article was published in Genetics in Medicine, 24(3), Rose, N. C., Barrie, E. S., Malinowski, J., Jenkins, G. P., McClain, M. R., LaGrave, D., … & Guidelines Committee, Systematic evidence-based review: The application of noninvasive prenatal screening using cell-free DNA in general-risk pregnancies, p. 1379, Copyright Elsevier.

*Unless otherwise specified.

DOR: diagnostic odds ratio; FPR: false-positive result rate; NIPS: non-invasive prenatal screening; NPV: negative predictive value; OR: odds ratio.

multiple factors but is overall very high (>99%). This information is important for health care providers and patients to understand, to enable more accurate and informative counselling regarding screening results.

Cell-free DNA Test Failures and the Importance of Fetal Fraction

To obtain a cfDNA screening result, the maternal plasma DNA sample must be of sufficient quality and the fetal fraction (%) adequate to differentiate between a normal and abnormal result. The fetal fraction is the percentage of fetal cfDNA in the maternal sample, which consists of maternal and placental (fetal surrogate) cfDNA.

Factors affecting the fetal fraction include earlier gestational age, maternal obesity, a multiple gestation pregnancy, the use of assisted reproductive technologies, and the presence of an aneuploidy in either the placenta or the mother. The median fetal fraction between 11–14 weeks gestation is 10%, and failure rates are low at this gestational age (1%–6%). At earlier gestational ages, the fetal fraction is not consistently adequate, and therefore screening prior to 10–11 weeks gestation is not recommended. Maternal obesity is inversely related to fetal fraction, and for persons over 110 kg, the failure rate of cfDNA screening is over 10%.[32–35] The likely mechanism is a dilutional effect, combined with increased adipocyte turnover, resulting in increased maternal relative to fetal serum cfDNA.[36] Cell-free DNA samples with a low fetal fraction (<4%) may not produce an interpretable result and should be reported as a "no-call" result, although the determination for reporting results should be a laboratory standard, which is beyond the scope of this document.

The overall probability of a failed (no-call) result ranges from 1% to 6%, depending on the laboratory and method used.[37] Patients whose first screening results are inconclusive owing to low fetal fraction, should be counselled and be offered a redraw, which has a 50%–60% likelihood of getting an interpretable result; however, these patients must be informed that this repeat process may significantly delay diagnosis. Given that test failure is associated with an increased risk of fetal aneuploidy (as high as 5%),[20] persons with a no-call result should also be offered genetic counselling to discuss invasive fetal chromosome investigations. Follow-up should include an ultrasound examination (if not recently done), as the presence of fetal abnormalities would further guide management. In a recent cohort of more than 17 000 patients, a nonreportable result was found in 3.4%.[38] Trisomy 13, 18, or 21 was confirmed in 1.6% of cases with nonreportable tests versus 0.7% of those with reported results. Patients with nonreportable cfDNA results were also at increased risk for a number of adverse outcomes, including aneuploidy, preeclampsia, and preterm birth.

False-Positivity Rate and Confirmation of Abnormal Results

Cell-free DNA screening is associated with an overall false-positive rate of about 1% for the common aneuploidies.[26,27,29,37] The specificity for each screening condition is reported separately, so false-positive rates are cumulative.[39] There are several biological and non-biological explanations for false-positive NIPS results other than fetal aneuploidy, including confined placental mosaicism,[40] maternal aneuploidy, maternal copy number

variants (CNVs),[41] maternal malignancy,[42,43] or co-twin demise (i.e., vanishing twin syndrome).[44]

Invasive diagnostic testing, either through CVS or amniocentesis, is thus recommended after a positive cfDNA fetal aneuploidy screening result, and no irrevocable pregnancy decision or procedure should be taken solely based on a positive cfDNA screening result.

Trisomies 21 and 18 have a low probability of CVS mosaicism; therefore, CVS may be appropriate as a confirmatory diagnostic procedure. Conversely, trisomy 13 and monosomy X have higher incidences of placental mosaicism on CVS, waiting for an amniocentesis would appear to be the most appropriate step.[45] However, because cfDNA screening is frequently performed in the first trimester, CVS may offer an earlier definitive diagnosis. If mosaicism is identified on CVS, confirmatory amniocentesis is recommended because of the possibility of discordance based on confined placental mosaicism.

Confined placental mosaicism refers to the presence of a chromosomal abnormality in the placenta with a normal fetal karyotype and occurs in 1%–2% of placental samples obtained by CVS.[46,47] Since fetal cfDNA originates mainly from the trophoblast layer of the chorionic villi and not the fetus, cfDNA screening can be considered equivalent to a non-invasive CVS, hence a similar incidence of confined placental mosaicism is expected.

Role of cfDNA Screening in Twins

While cfDNA screening is available for trisomies 21, 18, and 13 in twins, there is less large cohort validation data supporting its use in this population than in singleton pregnancies; however, two recent meta-analyses suggest that the performance cfDNA for identifying trisomy 21 in twin pregnancies is similar to that achieved in singleton pregnancies.[48,49]

The main challenge of screening twin pregnancies is that the cfDNA in the maternal circulation is derived from both fetal placentas. Therefore, the results are reported for the entire pregnancy, not for each individual fetus. Invasive testing is required to determine which fetus, if any, is affected. In addition, multiple gestation results in a lower per fetus fetal fraction than a singleton pregnancy. One approach, therefore, is to base the assessment of risk in dichorionic twins on the lower fetal fraction of the twins, rather than on the total fetal fraction. While this improves performance, it is also associated with higher failure rates.[48] There is little data validating the performance of cfDNA screening in high-order multiple pregnancies.[50,51]

Chromosomal Microdeletion Syndromes

Commercial companies offer screening for specific chromosome microdeletion syndromes through maternal plasma cfDNA analysis, in addition to the common aneuploidy screening. Peer reviewed data validating the performance of these investigations are few, and given the low incidence of each of these microdeletions, the PPV is very low.[52,53] Fetal submicroscopic chromosomal changes are individually rare but are reported to have an estimated cumulative incidence of about 1%–1.5%. Unlike fetal trisomies, the risk for these chromosomal microdeletions/duplications is independent of maternal or paternal age.

The National Institute of Child Health and Human Development study evaluated more than 4400 patients who had an invasive diagnostic karyotype.[54] Approximately 1.7% of pregnancies with advanced maternal age or a positive prenatal screen and a normal standard karyotype had a pathogenic or likely pathogenic CNV detected by the microarray. Among cases with abnormal ultrasound and a normal standard karyotype, additional microarray testing identified a CNV pathogenic result in 2.8% and a possible clinically significant result in 3.2% (total 6.0%).

Although proof of concept studies and case reports have conveyed the capacity of cfDNA to detect fetal microdeletions and CNVs in maternal plasma, there are currently few studies to support the use of cfDNA for expanded fetal genetic screening.[52,55,56] In a large study of more than 8000 pregnancies with NIPS to calculate the PPV of common aneuploidies and subchromosomal microdeletions and microduplications, 51 (0.63%) positive cases for chromosomal microdeletions or microduplications were found, but only 13 (36%) were true-positive cases.[36] Individually, any given CNV is rare; however, 22q11.2 deletion syndrome, the most common pathogenic CNV identified prenatally, has been estimated to have a prevalence range of 1 in 990 to 1 in 2148.[29] There is no known maternal age impact on the incidence of fetal CNVs.[57] A study assessed the performance of SNP-based NIPS for the 22q11.2 microdeletion in a cohort of more than 18 000 pregnancies.[58] Ten out of 12 cases of 22q11.2 deletion syndrome were detected. Using a risk cut-off of 1 in 100, there were 19 screen-positive cases for a false-positive result rate of 0.05%. The PPV with this approach was 52.6%. The 2022 guidelines from the American College of Medical Geneticists (ACMG) suggest that NIPS for 22q11.2 deletion syndrome be offered to all patients in addition to NIPS for common trisomies (conditional recommendation, based on moderate certainty of the evidence). The same document suggests that,

at this time, there is insufficient evidence to recommend routine screening for CNVs other than 22q11.2 deletions.

Given the low incidence of each individual submicroscopic chromosome change, the PPV is expected to be low in pregnancies without fetal anomalies and will increase the risk of false-positive results. Screening for microdeletions involves complex issues of pre- and post-test counselling that are currently unresolved. For these reasons, routine cfDNA screening for fetal microdeletions, including 22q11.2, is currently not recommended in Canada.

Fetal Sex Determination

Several common DNA sequences specific to the Y chromosome allow the determination of fetal sex as early as 7 weeks gestation, with nearly 100% determination by 10 weeks.[59] The only clinical indication for prenatal fetal sex determination is to assess the risk of transmission of an X-linked genetic disorder, or to determine the potential fetal risk of virilization of a female fetus at risk of congenital adrenal hyperplasia. Cell-free DNA analysis for the purposes of sex determination alone is not recommended, even with patient autonomy considerations.

Sex Chromosome Aneuploidy

Testing for fetal sex determination will result in the potential identification of fetal sex chromosome aneuploidy, therefore couples will need to decide whether they wish to receive this information. Genetic counselling following the prenatal discovery of 47,XXX, 47,XXY, or 47,XYY is complex, and the identification of these conditions through cfDNA screening is particularly challenging, as placental mosaicism remains possible. Although the sensitivity and specificity of cfDNA for sex chromosome abnormalities are high, it is lower than for common aneuploidies, and the PPV is approximately only 50%.[60] The PPV is higher for sex chromosome abnormalities with a supernumerary Y chromosome (71%) and lower for monosomy X (32%). Pretest counselling and patient consent for fetal sex determination and sex chromosome aneuploidy screening is recommended. The 2022 guidelines from the ACMG recommend that NIPS be offered to patients with singleton gestations to screen for fetal sex chromosome aneuploidy (strong recommendation, based on high certainty of evidence).[29] However, this screening is debated in Canada.[1] Except for monosomy X and mosaicism for monosomy X, none of the sex chromosome aneuploidies are expected to have a major clinical impact on a future child's health and well-being. The ISPD, in its 2023 position statement, notes that due to the lower PPV of NIPS and the ethical debate on offering sex chromosome aneuploidy screening, there is more variation in public policy and patient choice surrounding NIPS for these conditions, with several countries and regions choosing not to offer sex chromosome aneuploidy screening at all.[14]

Models for Clinical Implementation of cfDNA Genetic Screening

Contingent cfDNA Screening

Based on available data, considering the current cost of cfDNA testing to a publicly funded health care system, a contingent cfDNA screening model would seem to be the most appropriate strategy at present.[61] Contingent cfDNA screening refers to the use of cfDNA screening as a follow-up test for persons with a positive or "high risk" conventional screening result prior to the use of an invasive diagnostic test. Cost and performance modelling studies[61–63] have demonstrated that contingent screening with cut-off adjustment can approach the same detection rates and false-positive rates as primary cfDNA screening (especially when considering the test-failure rate of cfDNA), while maintaining the benefits of the 11–14-week fetal ultrasound within a multiple marker screening system. In this approach, the primary test should preferably be the first-trimester combined test. Using IPS followed by cfDNA contingent screening has not been tested in clinical studies and would lead to a three-step screening process with delayed results.

While the sensitivity of cfDNA for trisomy 21 is 99.3%, the overall detection rate using a contingent protocol will only be as good as the primary screening test (80%–90%). A recommended strategy is to adjust the initial screen cut-off upward (i.e., to 1/500 or 1/1000) to create an intermediate risk category eligible for cfDNA screening. Modelling data show this would result in 15%–20% of cases being eligible for cfDNA, and overall detection would approach that of primary screening (96%–98%).

Funding for contingent cfDNA screening has been approved in some provinces in Canada (British Columbia, Ontario, Nova Scotia, New Brunswick, Newfoundland and Labrador, Manitoba, and Québec) and should be considered by the provincial governments of the remaining provinces and territories.

Primary cfDNA screening

A study in Ontario has shown that a primary cfDNA screening model with 100% uptake would approximately quadruple the total program cost for aneuploidy screening.[62] In 2014, the predicted price of cfDNA screening for use in a cost-neutral universal screening program was approximately CAD $226. Universal access to cfDNA as a first-tier screening is currently not feasible but can be offered based on provincial screening funding

arrangements, via patient private insurers, or with a voluntary self-pay process.

Pre- and Post-screen Counselling in the Era of cfDNA

1. A discussion of the risks, benefits, and alternatives of the various prenatal diagnosis and screening options, including the option of no testing, should be undertaken with all patients prior to any prenatal genetic screening. People should be informed of the local and provincial options available. Following this, they should be offered a) no aneuploidy screening; b) standard prenatal aneuploidy screening based on locally offered programs; c) ultrasound-guided invasive diagnostic testing, with the understanding that it may not be provincially funded or even offered in all settings; or d) maternal plasma cfDNA screening, with the understanding that it may not be provincially funded.
2. Regardless of aneuploidy screening choice, all persons should be offered an initial fetal ultrasound, (optimally between 11 and 14 weeks), to confirm viability, gestational age, number of fetuses, and chorionicity in multiples; assess early fetal anatomy; and measure NT. Aneuploidy risk estimation with NT (combined with maternal serum) should not be performed if cfDNA screening is used. However, NT evaluation is part of the first-trimester ultrasound, and an increased NT measurement of 3.5 mm and above should prompt further assessment, even if cfDNA screening is used. The management of increased NT is detailed above in this document.
3. Persons who are considering having maternal plasma cfDNA screening should be informed that:
    a. It is a highly effective screening test for the common fetal trisomies (21, 18, 13), has similar detection rates in both persons at increased aneuploidy risk and at low risk, and should be initiated after 10 weeks gestation.
    b. There is a possibility that the test will return a failed (no-call), false-negative or false-positive, or unexpected fetal or maternal result. The chance of a false-positive result is higher in persons with an "average or no increased risk" because of the lower maternal age—related prevalence of aneuploidy.
    c. All positive cfDNA screening results should be confirmed with diagnostic fetal or newborn testing. In screened cases with high-risk cfDNA results (positive for trisomy 21, 18, or 13, or monosomy X), amniocentesis is the most appropriate diagnostic follow-up procedure, but CVS can be considered for urgent, high-risk trisomy 21 and 18 results. Persons should be aware there is a risk of detecting placental mosaicism (2%—4%) and further testing (amniocentesis) would be required for confirmation, thus delaying the diagnostic process.
    d. Management decisions including termination of pregnancy should be based on diagnostic testing and not maternal plasma cfDNA results alone. Cell-free DNA screening for aneuploidy is not a diagnostic test.
    e. If a fetal structural abnormality is identified regardless of previous genetic screening test results, genetic counselling and invasive diagnostic testing should be offered with rapid aneuploidy detection and reflex to microarray analysis or exome/genome sequencing if rapid aneuploidy detection is normal or inconclusive.
    f. Cell-free DNA screening for aneuploidy in twin pregnancy is available and has similar accuracy as in singleton pregnancies but has a higher no-call rate.
    g. Routine cfDNA screening for fetal microdeletion syndromes and sex chromosome aneuploidy is not currently recommended.

Summary

Maternal plasma cfDNA is a highly effective form of prenatal aneuploidy screening that can facilitate early detection of common trisomies (21, 18, 13) and provide early reassurance when a pregnancy is considered at increased risk. Implementation of maternal plasma cfDNA screening in clinical practice requires changes in patient referral patterns, pre-screen counselling and post-test management of persons with positive results. Offering cfDNA in a contingent model with cut-offs set to optimize detection is an affordable option that has the potential to achieve improved test performance while maintaining the benefits of conventional screening through early ultrasound, which has applications beyond age-based aneuploidy detection. Currently, universal cfDNA analysis as a primary screening method is not offered in most provinces owing to cost issues.

Summary Statements 3, 4, and 5 and Recommendation 7

PRENATAL GENETIC SCREENING: ROLE OF SECOND-TRIMESTER ULTRASOUND AND "SOFT MARKERS"

All pregnant persons are offered an ultrasound evaluation in the second trimester, and the *genetic ultrasound* was developed as an additive screening option for

Table 3. Interpretation of second-trimester ultrasound soft markers for trisomy 21

Marker	DR, % (95% CI)	FPR, % (95% CI)	LR+ (95% CI)	LR− (95% CI)	LR isolated marker
Intracardiac echogenic focus	24.4 (20.9–28.2)	3.9 (3.4–4.5)	5.83 (5.02–6.77)	0.80 (0.75–0.86)	0.95
Ventriculomegaly	7.5 (4.2–12.9)	0.2 (0.1–0.4)	27.52 (13.61–55.68)	0.94 (0.91–0.98)	3.81
Increased nuchal fold	26.0 (20.3–32.9)	1.0 (0.5–1.9)	23.30 (14.35–37.83)	0.80 (0.74–0.85)	3.79
Echogenic bowel	16.7 (13.4–20.7)	1.1 (0.8–1.5)	11.44 (9.05–14.47)	0.90 (0.86–0.94)	1.65
Mild hydronephrosis	13.9 (11.2–17.2)	1.7 (1.4–2.0)	7.63 (6.11–9.51)	0.92 (0.89–0.96)	1.08
Short humerus	30.3 (17.1–47.9)	4.6 (2.8–7.4)	4.81 (3.49–6.62)	0.74 (0.63–0.88)	0.78
Short femur	27.7 (19.3–38.1)	6.4 (4.7–8.8)	3.72 (2.79–4.97)	0.80 (0.73–0.88)	0.61
ARSA	30.7 (17.8–47.4)	1.5 (1.0–2.1)	21.48 (11.48–40.19)	0.71 (0.57–0.88)	3.94
Absent or hypoplastic nasal bone	59.8 (48.9–69.9)	2.8 (1.9–4.0)	23.27 (14.23–38.06)	0.46 (0.36–0.58)	6.58

Adapted from Agathokleous M, Chaveeva P, Poon LC, et al. Meta-analysis of second-trimester markers for trisomy 21. Ultrasound Obstet Gynecol. 2013;41:247-61. Available at http://www.ncbi.nlm.nih.gov/pubmed/23208748. Copyright John Wiley and Sons.

ARSA: aberrant right subclavian artery; DR: detection rate; FPR: false-positive result rate; LR: likelihood ratio.

aneuploidy.[64,65] As prenatal genetic screening strategies have greatly evolved in the last two decades, the relative importance of soft markers has shifted. Several ultrasound soft markers were previously recommended for aneuploidy screening: enlarged nuchal fold, absent nasal bone, echogenic bowel, mild ventriculomegaly, echogenic heart focus, urinary tract dilatation, and choroid plexus cysts. While none of these ultrasound-identified features are considered malformations, all were shown to be associated with an increased relative risk of trisomy 21 or 18 (see Table 3). If a marker is present, the patient's a priori risk of aneuploidy is increased by a specific likelihood ratio; conversely, the risk may be decreased if no markers are present. The negative likelihood ratio after a normal comprehensive genetic ultrasound has been estimated between 0.13[65] and 0.52.[66] In clinical practice, using a negative likelihood ratio of 0.3 is suggested. Of these markers, increased nuchal fold thickness and absent nasal bone are the most powerful with a likelihood ratio of 23 (but only 3.8 and 6.6, respectively, if isolated) for trisomy 21 while choroid plexus cysts are only associated with a minimally increased risk of trisomy 18 only. Echogenic bowel is associated with a slightly increased risk for trisomy 21 but has additional implications including an increased risk of cystic fibrosis (2%), fetal infection (3%), and gastrointestinal malformation (6%). Mild fetal ventriculomegaly is associated with an increased risk of trisomy 21, but also central nervous system malformations or intracranial infection, as well as some other inherited conditions. Hypoplastic or absent nasal bone in the second trimester has a relatively high likelihood ratio, but the reproducibility of this marker has not been adequately studied.

In pregnancies without previous aneuploidy screening, soft markers may be used to adjust the a priori risk based on maternal age. However, likelihood ratios should be used with caution, especially for multiple markers, given the inherent biases of retrospective studies tending to overestimate the risk of aneuploidy. In the case of multiple soft markers or structural abnormalities, the approach should be individualized.

Fetal soft marker screening in the second trimester should not be used in isolation if effective first- or second-trimester aneuploidy screening has been provided, and not at all, if maternal cfDNA screening has been performed. This recommendation is also supported by ISUOG which states that the so-called genetic sonogram "should not be performed in pregnancies with a normal NIPS result due to its high false-positive rate and poor positive predictive value."[10] The SMFM also updated its recommendations on the use of soft markers for aneuploidy screening in 2021.[64] The SMFM recommends against testing for aneuploidy solely for the evaluation of an isolated soft marker following a negative serum or cfDNA screening result. We endorse their recommendations, which are summarized in Table 4, including the antenatal management and follow-up imaging recommended for various soft markers.

PRENATAL EXOME VERSUS GENOME SEQUENCING

This guideline focuses on prenatal screening for fetal aneuploidy/chromosomal anomaly, but the basic process of screening is to ultimately identify the presence of any clinically significant fetal anomaly in the screened

Table 4. Recommended management for isolated soft markers

Soft marker	Aneuploidy evaluation	Antenatal management	Follow-up imaging
Echogenic intracardiac focus	• cfDNA or serum screen negative: none • No previous screening: counselling for NIPS	Routine care	N/A
Echogenic bowel	• cfDNA or serum screen negative: none • No previous screening: counselling for NIPS	Evaluation for cystic fibrosis, congenital viral infection, intra-amniotic bleeding, fetal growth restriction	Third-trimester ultrasound examination for reassessment and evaluation of growth
Choroid plexus cyst	• cfDNA or serum screen negative: none • No previous screening: counselling for NIPS	Routine care	N/A
Single umbilical artery	• cfDNA or serum screen negative or no previous screening: none	Routine care	Third-trimester ultrasound examination for evaluation of growth
Urinary tract dilation	• cfDNA or serum screen negative: none • No previous screening: counselling for NIPS	Evaluation for persistence, with frequency of evaluation dependent on initial findings	Third-trimester ultrasound examination to determine whether postnatal pediatric urology or nephrology follow-up is needed
Shortened humerus, femur, or both	• cfDNA or serum screen negative: none • No previous screening: counselling for NIPS vs. invasive testing for aneuploidy	Evaluation for skeletal dysplasias	Third-trimester ultrasound examination for reassessment and evaluation of growth
Thickened nuchal fold	• cfDNA negative: none • Serum screen negative: counselling for no further testing vs. non-invasive vs. invasive testing for aneuploidy • No previous screening: counselling for NIPS vs. invasive testing for aneuploidy	Routine care	N/A
Absent or hypoplastic nasal bone	• cfDNA or serum screen negative: none • No previous screening: counselling for NIPS vs. invasive testing for aneuploidy	Routine care	N/A

Adapted from: Society for Maternal-Fetal Medicine. SMFM Consult Series #57: Evaluation and management of isolated soft ultrasound markers for aneuploidy in the second trimester. Am J Obstet Gynecol 2021. Copyright Elsevier.

cfDNA: cell-free DNA; NIPS: non-invasive prenatal screening; N/A: not applicable.

population. Recommendation 8 briefly touches on the diagnostic use of a rapid aneuploidy detection process and the focused genomic technology of microarray analysis for chromosome abnormalities. Whole exome or genome sequencing (WES/WGS) increases the diagnostic yield compared with chromosomal microarray analysis alone.[67] Placental cytogenetics has long recognized the presence of confined placental mosaicism (1%–2%), which can impact WES/WGS results if CVS is used compared with amniocentesis with amniocytes for the diagnostic tissue source. WES/WGS can be a stand-alone prenatal diagnostic test for identifying DNA sequence variants and CNVs as well as chromosomal aneuploidies. WES usually outperforms WGS in identifying mosaic sequence variants prenatally, with high accuracy due to the use of a higher sequencing depth.[67] Other supporting evidence for increasing the use of WES/WGS technology in selected 'fetal anomaly patterning' is provided in the ISPD updated position statement on the use of genome-wide sequencing for prenatal diagnosis.[68] ISPD provides clinical and laboratory recommendations for WES/WGS. These technologies utilize 'trios' (samples from fetal, maternal, and paternal serum) to be sequenced at the same time for the best genomic analysis and comparison. In addition, it is important to recognize that the genotype-phenotype correlations for the many potential genetic diagnoses are still limited and require genomic team-based experience for interpretation. Single major fetal anomalies and single major fetal with additional minor fetal anomalies are creating better phenotype-genotype correlations for pretest counselling and, ultimately, post-test result sharing.[67–70]

Summary Statement 6 and Recommendations 8 and 9

CONCLUSION

Regardless of age, all pregnant persons in Canada should be offered, through an informed counselling process with shared decision-making, the option of a prenatal screening test for the most common fetal aneuploidies and for major fetal anomalies. First-trimester ultrasound (at 11—14 weeks gestation) offers many advantages for prenatal screening and pregnancy management, including accurate dating, determination of twin chorionicity, and early detection of some major structural abnormalities, regardless of the aneuploidy screening option undertaken. Routine first-trimester ultrasound may not yet be universally available across Canada, but efforts should be made to increase its availability to all pregnant persons. Maternal circulating cfDNA is the most accurate method of early prenatal screening for common trisomies (21, 18, 13), but other screening options based on maternal serum screening and/or ultrasound assessment are still valid and available.

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