CELL-FREE DNA PRENATAL SCREENING

(ALSO KNOWN AS NON-INVASIVE PRENATAL TESTING)

Prenatal screening using cell-free DNA (cfDNA), also known as Non-Invasive Prenatal Testing (NIPT), is a test to prenatally detect Down syndrome and other common aneuploidies. This test assesses fragments of cfDNA derived from the placenta that are circulating in maternal blood to determine if there is an increased chance that the fetus has an aneuploidy. cfDNA screening should be considered in pregnancies at increased risk of aneuploidy. cfDNA screening has **higher sensitivity and specificity** for trisomy 21 (Down syndrome) and trisomy 18 than conventional screening tests – First Trimester Screening (FTS)/Integrated Prenatal Screening (IPS)/ Maternal Serum Screening (MSS) - however **it is not considered diagnostic.** Positive results should be confirmed by diagnostic testing (chorionic villus sampling or amniocentesis) prior to any irrevocable action. Negative results are reassuring but additional follow-up testing and consultation may still be indicated. Some provinces fund cfDNA screening for women who meet certain high risk criteria. Those who do not meet criteria can pay for cfDNA screening themselves. Price varies by company (~500$).

WHAT IS CELL-FREE DNA PRENATAL SCREENING?

Cell-free DNA (cfDNA) prenatal screening, also known as non-invasive prenatal testing (NIPT) is a **highly sensitive and specific** way to **screen** for chromosome aneuploidies (an abnormal chromosome number (extra or missing)), in particular, trisomies 21, 13 and 18. cfDNA screening can also be used for sex chromosome identification for the purpose of fetal sex determination where there is increased risk for an X-linked disorder or a sex chromosome abnormality.

This screening assesses fragments of cfDNA derived from the placenta that are circulating in maternal blood and represent the fetal genetic profile. cfDNA from the pregnancy comprises a small percentage (<10%) of DNA in maternal blood and the amount increases with gestational age. Private companies offer cfDNA testing and use various technologies and proprietary algorithms for analysis. Prenatal screening using cfDNA is a non-invasive test performed on a maternal blood sample that poses no risk to pregnancy. A dating ultrasound is recommended prior to drawing the blood sample to ensure viability, obtain an accurate gestational age, and to exclude multiple pregnancies.

cfDNA (NIPT) is not a replacement for diagnostic prenatal testing. A positive/high risk cfDNA result should be confirmed by diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) prior to any irrevocable action.

WHAT ARE THE CURRENT CANADIAN RECOMMENDATIONS ON PRENATAL SCREENING?

Canadian clinical practice guidelines by the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG) state that **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 fetal aneuploidies. All women should be offered the options of:[1]

1. no aneuploidy screening
2. standard prenatal screening based on locally available programs e.g. First Trimester Screening (FTS)
3. invasive testing (e.g. CVS or amniocentesis) when appropriate indications are present e.g. a fetal nuchal translucency (NT) measurement of 3.5mm or greater or a congenital anomaly
4. cfDNA screening where available, with the understanding that it may not be provincially funded

Additionally, where available, women should be offered a first trimester ultrasound (11 to 14 weeks gestation) for accurate dating, determination of twin chorionicity, early anatomy assessment and NT measurement. A second trimester (18 to 22 weeks gestation) ultrasound should be offered for evaluation of structural anomalies. Ultrasounds should be performed at centres with expertise in fetal ultrasound.[1]

Note: The primary screening test for the detection of fetal structural abnormalities including open/closed neural tube defects (O/CNTDs) is the second trimester ultrasound. The primary use of maternal serum alpha fetoprotein for O/CNTDs screening should be discontinued with the limited exceptions of pregnant women with a pre-pregnant BMI ≥ 35 kg/m2 or where access to timely and good quality ultrasound is limited.[2]

RED FLAGS TO CONSIDER OFFERING CFDNA SCREENING AND/OR GENETIC CONSULTATION

Evidence supports screening by cfDNA in women determined to be at high risk of having a fetus with certain aneuploidies (trisomies 21, 13 and 18, and X and Y detection). Some provinces and territories offer funded cfDNA screening, in a contingent model, to women deemed to be at increased risk to have a baby with one of the specified aneuploidies. Eligibility criteria vary by province. See www.geneticseducation.ca or contact your local genetics centre for updates on provincial criteria. In general, red flags for women considered to be at increased risk are those who:

- Are of advanced maternal age, defined as 40 years of age or older at EDB
- Have an abnormal serum screen i.e. FTS/IPS/MSS
- Have a fetal nuchal translucency (NT) measurement of 3.5mm or greater
- Have had a previous pregnancy or child with trisomy 21, 13 or 18
- Have fetal congenital anomalies on ultrasound highly suggestive of trisomy 21, 13 or 18
- Have multiple soft markers[1,3] on ultrasound which are highly suggestive of a specific aneuploidy
- Are at risk of carrying a male fetus with an X-linked condition (cfDNA screening would be used for sex determination)

As each prenatal genetics centre has variable referral criteria and practice, abnormalities seen on ultrasound (e.g. congenital anomalies, NT ≥ 3.5mm or other soft markers) should be discussed with your local genetics centre to decide whether a referral is appropriate, whether cfDNA screening could be offered first, or if additional testing should be considered.

WHAT DOES THE TEST RESULT MEAN?

Depending on the company, results may be worded as: positive/negative; aneuploidy detected/no aneuploidy detected/aneuploidy suspected/borderline value; or high risk/low risk.

If the result is negative/low risk, this is reassuring. Your patient should still be offered:

- fetal ultrasounds, optimally one between 11 and 14 weeks and one between 18 and 22 weeks as described in *WHAT ARE THE CURRENT CANADIAN RECOMMENDATIONS PRENATAL SCREENING?*
- depending on the reason your patient had cfDNA screening, in some circumstances a referral for genetic and/or maternal fetal medicine consultation may still be offered for discussion of additional testing and/or follow up (e.g. a detailed ultrasound, fetal echocardiogram and additional genetic investigations would be offered for an ultrasound finding of an NT ≥ 3.5mm)

If the result is positive/high risk, follow-up genetic counselling is indicated and confirmation by diagnostic testing should be offered. The SOGC/CCMG recommendations are that **no irrevocable obstetrical decisions should be made in pregnancies with abnormal cfDNA results without confirmatory invasive testing**[1] (CVS or amniocentesis) as false positive results do occur.

If there is no result, the laboratory will often request that a second blood sample is drawn and the test repeated at no extra cost. This may occur in about 1-5% of cases[4]. Possible reasons are technical issues e.g. a poor sample or quality assessment failure, and most often low fetal fraction (*amount of fetal cfDNA relative to mother's)* which is influenced by a number of factors such as early gestational age, high maternal BMI, or fetal aneuploidy[4,5,6]. If the result is still not interpretable, due to an increased aneuploidy risk, genetic counselling may be considered and invasive testing may be offered.

WHAT ARE THE BENEFITS OF CFDNA SCREENING?

- **Increased accuracy:** With higher sensitivity and lower false positive rates as compared to conventional prenatal screening, fewer women are expected to undergo invasive diagnostic testing associated with risk of miscarriage. A meta-analysis reviewing clinical validation and implementation studies found that in singleton pregnancies the detection rate (DR) and false positive rate (FPR) were[5]:
  - DR: 99% and FPR: 0.1% for trisomy 21
  - DR: 96% and FPR: 0.1% for trisomy 18
  - DR: 91% and FPR: 0.1% for trisomy 13
  - DR: 90% and FPR: 0.2% for Turner syndrome (monosomy X)

- **Increased positive predictive value** (PPV) over conventional screening: Early studies suggest that the PPV of cfDNA screening in a low risk, general obstetrical population is about 10 times higher for trisomy 21 (45% versus ~4% for standard screening)[7]. The PPV appears to be significantly higher in high risk populations, as PPV is dependent upon prevalence of the condition in a given population. Note: *The PPV is not 100% in any population. cfDNA is a screening test and is not diagnostic.*
- **Earlier timing**: Screening by cfDNA is a single blood test that is available as early as 9-10 weeks. Results are available within 1-2 weeks. Earlier screening results allow expectant parents more time for decision-making and potentially offer more options e.g. CVS at 11-13 weeks versus amniocentesis after 15 weeks or early reassurance.

WHAT ARE THE LIMITATIONS OF CFDNA SCREENING?

- **cfDNA screening is not a diagnostic test.** Although the sensitivity is high and the false positive rate is low, in the event of a positive/high risk result no irrevocable obstetrical decisions should be made in pregnancies with abnormal results without confirmatory invasive diagnostic testing.
- **No result:** This may occur in about 1-5% of cases[4]. This does not occur with conventional screening. This may delay diagnosis. Possible reasons are described in *WHAT DOES THE TEST RESULT MEAN?*
- **cfDNA screening does not screen for all possible conditions.** cfDNA cannot:
  - detect aneuploidy of chromosomes other than 21, 13, 18, X and Y
  - completely rule out aneuploidy
  - detect single gene conditions
  - detect congenital anomalies
  - guarantee a healthy baby with a negative cfDNA result
- **Incidental findings:** Although marketed to consumers as a screening test for Down syndrome, cfDNA screens for additional conditions. For example, the test could be ordered because of an increased fetal risk for trisomy 21 and the report could indicate high risk of another chromosome abnormality, e.g. sex chromosome aneuploidy, like Turner syndrome (45,X) or Klinefelter syndrome (47,XXY). Additionally cfDNA analysis does not differentiate between maternal and fetal DNA and reports of maternal aneuploidy have occurred[5]. Pre-test counselling and appropriate follow up are important.
- **Twins:** cfDNA screening is available in twin pregnancies, however less data are available and testing appears to be less accurate as compared to singleton pregnancies[6]. cfDNA screening should be undertaken with caution in these pregnancies.

WHAT ABOUT ADDITIONAL SCREENING USING CFDNA?

Some companies offer cfDNA screening to test for other genetic conditions, such as *microdeletion* and *microduplication* syndromes. These are rare genetic conditions, occurring in about 1 in 5,000 to 1 in 50,000 pregnancies. They are caused by very tiny extra or missing pieces of chromosomes.

The addition of these rare conditions to cfDNA screening increases the false positive rate and decreases the positive predictive value. This would result in more women having diagnostic tests, with associated risk of miscarriage. Current recommendations do not support the routine inclusion of screening for microdeletion and microduplication syndromes in cfDNA screening.[1]

Check online for updates as prenatal genetics is a rapidly evolving area. References and specifics about provincial availability can also be found online.

See Public Resources for the updated A guide to understanding prenatal screening tests to help expectant parents and their healthcare providers choose the right testing option, including cfDNA.

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