No. 287, February 2013

Current Status in Non-Invasive Prenatal Detection of Down Syndrome, Trisomy 18, and Trisomy 13 Using Cell-Free DNA in Maternal Plasma

This committee opinion has been prepared by the Genetics Committee and approved by the Executive of the Society of Obstetricians and Gynaecologists of Canada.

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Disclosure statements have been received from all members of the committee.

Abstract

Objective: To provide a review of published studies on the use of cell-free fetal DNA in maternal plasma for the non-invasive diagnosis of Down syndrome, trisomy 18, and trisomy 13.

Evidence: PubMed was searched for articles published between 2006 and October 2012, using appropriate key words (e.g., non-invasive prenatal diagnosis, Down syndrome, cell-free fetal DNA, aneuploidy screening). Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies. Searches were updated on a regular basis and incorporated in the guideline to October 31, 2012. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice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.

Values: The studies reviewed were classified according to criteria described by the Canadian Task Force on Preventive Health Care, and the recommendations for practice were ranked according to this classification (Table 1).

Recommendations

1. Non-invasive prenatal testing using massive parallel sequencing of cell-free fetal DNA to test for trisomies 21, 18, and 13 should be an option available to women at increased risk in lieu of amniocentesis. Pretest counselling of these women should include a discussion of the limitations of non-invasive prenatal testing. (II-2A)
2. No irrevocable obstetrical decision should be made in pregnancies with a positive non-invasive prenatal testing result without confirmatory invasive diagnostic testing. (II-2A)
3. Although testing of cell-free fetal DNA in maternal plasma appears very promising as a screening test for Down syndrome and other trisomies, studies in average-risk pregnancies and a significant reduction in the cost of the technology are needed before this can replace the current maternal screening approach using biochemical serum markers with or without fetal nuchal translucency ultrasound. (III-A)

J Obstet Gynaecol Can 2013;35(2):177–181

Key Words: Non-invasive prenatal diagnosis, prenatal screening, Down syndrome, trisomy 18, trisomy 13, cell-free fetal DNA

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
		E.	There is good evidence to recommend against the clinical preventive action
III:	Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	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.[21]

†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the Canadian Task Force on Preventive Health Care.[21]

INTRODUCTION

Until recently, prenatal genetic diagnosis, either for increased risk of chromosome aneuploidy or for specific gene testing, has required a sample of fetal tissue obtained through invasive diagnostic procedures (CVS, amniocentesis, or umbilical blood sampling) that have associated fetal and maternal procedural risks including pregnancy loss. The finding of intact fetal cells and cffDNA in maternal blood has allowed the development of non-invasive prenatal diagnosis of certain fetal conditions. Several studies have validated the use of cffDNA in maternal plasma fetal sex determination[1] and fetal Rh typing.[2,3] Molecular testing of cffDNA has also been carried out for the detection of paternally inherited mutations in single genes.[4]

The presence of cffDNA in maternal plasma in all pregnancies, along with the development of new molecular technologies, has prompted research into the use of maternal plasma testing to detect fetal Down syndrome. As fetal DNA represents only 10% to 20% of the total DNA found in maternal plasma, the primary challenge of developing an accurate assay has been discriminating between a DNA sample with a fetal complement that contributes 3 and one that contributes 2 copies of chromosome 21 to the total amount of plasma cell-free DNA of the mother. A recently developed massive parallel shotgun sequencing technology, in conjunction with sophisticated sequencing data analyses, has been used successfully to detect fetal Down syndrome in women with pregnancies at high risk for chromosomal abnormalities. In essence, millions of small fragments of cffDNA (either random or specific to chromosomes of interest) from maternal plasma (containing both fetal and maternal cell-free DNA) are amplified and sequenced. After the fragments are mapped to the human genome and analyzed for frequency/density along each chromosome, fetal Down syndrome is detected with a high degree of accuracy by observing an over-representation of chromosome 21. The same approach can be taken for the detection of other chromosomal abnormalities.

ABBREVIATIONS

cffDNA	cell free fetal DNA
CVS	chorionic villus sampling
NIPT	non-invasive prenatal testing

REVIEW OF REPORTED NIPT STUDIES USING MASSIVELY PARALLEL SEQUENCING TO DETECT DOWN SYNDROME, TRISOMY 18, AND TRISOMY 13

Studies comparing the detection rate of Down syndrome by cffDNA testing with the detection rate by cytogenetic analysis of chorionic villus samples or amniocytes have been limited to high-risk pregnancies to enrich the cohort with cases that would indeed have Down syndrome and therefore allow validation of the technology being developed. Although the initial work was done in small cohorts of patients as proof of feasibility of this new technology,[5–7] more recent studies validated its use in larger

Table 2. Results of validation studies for non-invasive detection of fetal trisomy 21

Study	Number samples tested	Failure rate*	Sequencing approach	Detection rate	False-positive rate
Chiu et al. 2011[8]	764	1.4%	8-plex, shotgun	79.1% (68/86)	1.1%
	232	N/A	2-plex shotgun	100% (86/86)	2.1%
Palomaki et al. 2011[9]	1696	0.8%	4-plex shotgun	98.6% (209/212) 95% CI 95.9 to 99.7	0.2% 95% CI < 0.1 to 0.6
Ehrich et al. 2011[10]	467	3.9%	4-plex shotgun	100% (39/39) 95% CI 89 to 100	0.2% 95% CI 0.1 to 1.5
Lau et al. 2011[11]	108	0	12-plex shotgun	100% (11/11)	0
Sehnert et al. 2011[12]	47	0	1-plex shotgun	100% (13/13)	0
Sparks et al. 2012[13]	167	0†	96-plex selective	100% (36/36)	0.8%
Ashoor et al. 2012[14]	400	0.75%	96-plex selective	100% (50/50)	0
Bianchi et al. 2012[15]	532	3%	6-plex	100% (89/89) 95% CI 95.9 to 100	0
Norton et al. 2012[16]	3228	4.5%	96-plex selective	100% (81/81) 95% CI 95.5 to 100	0.03% 95% CI 0.002 to 0.2

*Percentage of samples that did not meet quality control requirements for the sequencing so that no results could be obtained.

†5% failure in their training set.

N/A: not applicable—only samples that passed original sequencing quality control were retested with the 2-plex.

cohorts of pregnant women who had undergone an invasive procedure (CVS or amniocentesis).[8–16] The indications for invasive diagnostic testing included advanced maternal age, abnormal aneuploidy screening results, and/or sonographically diagnosed fetal abnormalities. Results of these clinical trials are summarized in Table 2. Only trials that collected maternal blood samples before invasive procedures are included. While all studies used massive parallel sequencing, there were differences in methodology and sequencing data analysis between studies that prevent summing of the results. However, the detection rate for Down syndrome in all studies was, or approached, 100% with a false-positive rate less than 1%. The focus of studies so far has been the detection of Down syndrome; however, this technology has also been used to detect trisomy 18 and trisomy 13, although initially with less success because of a larger coefficient of variation and therefore lower precision in estimating the proportion of chromosomes 18 or 13[11,17] to the total chromosomes. However, recent publications suggest that this can be overcome by using a targeted sequencing approach[13,14,16] and/or a different sequencing data analysis approach.[15,18] This is leading to the utilization of this technology for the detection of trisomy 18 and trisomy 13 in addition to Down syndrome. Results of those studies are presented in Table 3.

ISSUES FOR CONSIDERATION

The development of new screening methods to detect Down syndrome through testing of cffDNA in maternal plasma offers promising opportunities to improve prenatal screening. Before these methods can replace current prenatal screening options, validation studies using them in average-risk pregnancies need to be done. Only one cohort study of 2049 women has so far been done in women at average risk.[19] As the cost of the technology is high compared with current screening methods, cost-effectiveness studies are also needed. Given the demonstrated value of NIPT in high risk pregnancies, this testing should be an option available to pregnant women found to be at increased risk of fetal Down syndrome, trisomy 18, and trisomy 13 on the basis of currently available screening tests or ultrasound findings. It is important for these women to receive detailed pretest counselling that explains the benefits and limitations of the test.[20] Counselling should include the following points:

- Non-invasive cffDNA testing should not be considered equivalent to conventional cytogenetic analysis of CVS or amniocytes.
- Cytogenetic testing of CVS or amniocytes detects 100% of cases of Down syndrome, trisomy 18, and trisomy 13 (thus is diagnostic), whereas the test done on cffDNA does miss some cases (see Table 2 and Table 3, detection rate).
- The currently available tests screen only for Down syndrome, trisomy 18, and trisomy 13. Other trisomies, triploidy, and structural chromosomal abnormalities would not be detected by the commercially available ccfDNA test.

Table 3. Results of validation studies for non-invasive detection of fetal trisomy 18 and trisomy 13

Study	Sequencing approach	Trisomy 18 detection rate	Trisomy 18 false-positive rate	Trisomy 13 detection rate	Trisomy 13 false-positive rate
Lau et al. 2011[11]	12-plex shotgun	90% (9/10)	0	100% (2/2)	0
Sehnert et al. 2011[12]	1-plex shotgun	100% (8/8)	0	No data	No data
Sparks et al. 2012[13]	96-plex selective	100% (8/8)	0.8%	No data	No data
Ashoor et al. 2012[14]	96-plex selective	98% (49/50)	0	No data	No data
Bianchi et al. 2012[15]	6-plex	97.2% (35/36)	0	78.6% (11/14)	0
Norton et al. 2012[16]	96-plex selective	97.4% (37/38)	0.07%	No data	No data
Palomaki et al. 2012[18]	4-plex shotgun	100% (59/59)	0.28%	91.7% (11/12)	0.97%

- cffDNA testing has a higher rate of false-positive results than current diagnostic tests based on cytogenetic analysis of amniocytes or chorionic villi. (Tables 2 and 3, false positive rate).
- Some women will have a cffDNA positive result and not carry a fetus with Down syndrome, trisomy 18, or trisomy 13 (false positive). No irrevocable obstetrical decision should be made in pregnancies with a positive cffDNA test for Down syndrome without confirmatory invasive diagnostic testing.
- cffDNA testing fails to provide a result in a small percentage of women.

CONCLUSION

NIPT using massive parallel sequencing for the detection of Down syndrome, trisomy 18, and trisomy 13 has shown promising results in clinical trials of women identified by screening as having a high-risk pregnancy. NIPT should be an option as a second-level contingent screening test (after a positive result from currently used serum and ultrasound screening techniques) for women wishing to avoid invasive testing. Further studies are needed to determine if this approach can be reliably used as a first-tier screening test in average-risk pregnancies. Finally, cost-effectiveness studies are needed before this cffDNA approach can replace current screening options.

Recommendations

1. Non-invasive prenatal testing using massive parallel sequencing of cell-free fetal DNA to test for trisomies 21, 18, and 13 should be an option available to women at increased risk in lieu of amniocentesis. Pretest counselling of these women should include a discussion of the limitations of non-invasive prenatal testing. (II-2A)
2. No irrevocable obstetrical decision should be made in pregnancies with a positive non-invasive prenatal testing result without confirmatory invasive diagnostic testing. (II-2A)
3. Although testing of cell-free fetal DNA in maternal plasma appears very promising as a screening test for Down syndrome and other trisomies, studies in average-risk pregnancies and a significant reduction in the cost of the technology are needed before this can replace the current maternal screening approach using biochemical serum markers with or without fetal nuchal translucency ultrasound. (III-A)

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