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*OBM Genetics*

Review

# Prenatal Screening for Fetal Aneuploidy

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**Academic Editor:** François Rousseau and Joanne Traeger-Synodinos

*OBM Genetics*
2017, Volume 1, Issue 3
doi:10.21926/obm.genet.1703007

**Received:** August 1, 2017
**Accepted:** September 14, 2017
**Published:** September 27, 2017

**Abstract**

Prenatal genetic aneuploidy screening approaches are designed to identify pregnant patients at increased risk of having a fetus affected. Conventional prenatal screening has consisted in providing women a risk estimate of having a pregnancy affected with trisomy 21 or trisomy 18 based on maternal age and analysis of serum markers and ultrasound nuchal translucency (NT) measurement. In 2011, the introduction of cell-free DNA (cfDNA) based screening into clinical practice has provided new options for aneuploidy screening programs. Different protocols are currently in use, some that perform screening in the first trimester (combined first trimester screening), some in the second trimester (QUAD), some that integrate first and second trimester (serum integrated screening, integrated screening, sequential, or contingent screening). cfDNA screening can be implemented as a second tier / contingent screen or as a first tier screen and has the ability to detect chromosomal anomalies other than the common aneuploidies screened for by conventional screening. The choice of protocol will be based on local expertise and resources. Aneuploidy screening should also be offered to pregnant women with twin gestation although fewer validation studies in twins of the test being offered are available compared to studies done in singleton gestations. Professional societies and expert groups emphasize the need for pre-test and post-test counselling to ensure that women are making an informed decision. The general

counselling points to be covered are similar regardless of the type of prenatal screening being offered. Prenatal screening programs should be implemented with resources that support patient and health care provider education, quality assurance of laboratory and NT services, access to genetic counselling services and diagnostic testing. The rapid pace of new developments in cfDNA screening for chromosomal anomalies brings new opportunities to enhance the performance of the screening but at the same time, challenges as the test menus are expanded and data is lacking as to the cost-effectiveness and clinical utility of implementing expanded panels.

**Keywords**
Aneuploidy; prenatal screening; cell free DNA; nuchal translucency ultrasound

## Introduction

Chromosomal abnormalities in the fetus constitute one of the leading causes of stillbirth and births of infants with malformations [1]. Aneuploidy is defined as having one or more extra or missing chromosomes, leading to an unbalanced chromosome number in a cell [2]. The incidence of aneuploidy increases with maternal age but aneuploidy pregnancies are seen at all reproductive ages. Down syndrome (trisomy 21) is the most common autosomal aneuploidy to affect live born children and is seen in approximately 1:800 newborns [3]. The other two autosomal trisomies seen in livebirths are trisomy 18 and trisomy 13.

Prenatal genetic aneuploidy screening approaches are designed to identify pregnant patients at increased risk of having an affected fetus. Screen positive women are then offered diagnostic testing. Since the 1980s, the focus of prenatal screening has been for the detection of trisomy 21 with the incidental diagnosis of a large proportion of trisomies 18 and 13 [4]. Conventional prenatal screening has consisted in providing women a risk estimate of having a pregnancy affected with trisomy 21 or trisomy 18 based on maternal age and analysis of serum markers and ultrasound nuchal translucency (NT) measurement [4]. In 2011, the introduction of cell-free DNA based screening into clinical practice has provided new options for aneuploidy screening programs, by introducing cfDNA screening as a second tier or contingent screen to conventional screening tests or as a first tier screening option.

Guidelines from numerous professional societies indicate that 'all pregnant women should be offered screening for aneuploidy'. These guidelines stress the need for patient education and pre-test and post-test counselling. This goal can be facilitated by the use of decision support tools. This review will cover the following topics: tests currently available for screening for the common fetal aneuploidies in singletons and twin gestations; considerations related to expanding screening for sub-chromosomal aneuploidy and rare autosomal trisomies using cfDNA; points to discuss in pre-test and post-test counselling; and issues related to quality assurance.

## Established screening protocols

### *Conventional screening:*

Pregnancy risk screening for trisomy 21 and trisomy 18 is carried out using multiple pregnancy related markers: maternal serum human chorionic gonadotropin (hCG), the free-β subunit of hCG, pregnancy-associated plasma protein (PAPP)-A, unconjugated estriol (uE3), inhibin A and AFP, and ultrasound for fetal nuchal translucency (NT) measurement [4]. PAPP-A is a first trimester marker whereas Inhibin A is a second trimester marker. Free β-hcg performs better in the second trimester than the first trimester and is general better than hCG. Alpha fetoprotein (AFP) and unconjugated estriol (uE3) have similar performance in the first and second trimester but are usually used in the latter [4]. The NT measurement is done by ultrasound imaging between 11 and 13 weeks 6 days gestation [5].

Based on the available described markers, a number of different screening protocols have been adopted by either performing the test in the first trimester (first trimester combined screening cFTS), second trimester (Quad) or by combining markers from the first and second trimester (serum integrated screening, integrated screening, sequential screening or contingent screening):

1. First trimester combined screening (cFTS): involves the first trimester measurement of serum PAPP-A and hCG in addition to an NT measurement. The performance of cFTS can be improved by adding additional maternal serum markers such as placental growth factor (PlGF) and AFP [6] or ultrasound markers [7].
2. Quad screening: involves the second trimester measurement (14-20 weeks 6 days) of maternal serum AFP, uE3, Inhibin A and hCG.
3. Serum integrated screening: involves the combination of first and second trimester serum markers (PAPP-A, AFP, uE3, Inhibin A and hCG) with one blood draw in the first trimester, a second blood draw in the second trimester, and one risk calculation after all markers have been analyzed.
4. Integrated screening: involves an NT measurement in addition to serum integrated screening described above. In this protocol, risk estimate and results are provided after analysis of the second trimester analytes.
5. Sequential screening: involves performing the same tests as integrated screening but a result is provided after the first trimester screen. If the risk is high, invasive testing or cfDNA testing is offered. For women opting to do both the first trimester and second trimester blood tests, a second integrated result which takes into considerations all markers is issued after the second blood draw.
6. Contingent screening: involves first trimester combined screening with recommendations based on the first trimester screening results. Women at high risk are offered diagnostic testing or cfDNA screening. Women at low risk are reassured and women at intermediate risk are offered second trimester testing for an integrated risk assessment.

Risk cut-offs vary between different screening programs and the choice of any given cut-off will influence detection rates (DR) and false positive rates (FPR). A cut-off that results in a higher DR will be associated with a higher FPR. The relative performance of the different screening tests however, can be assessed by comparing the performance of the different tests for a fixed FPR.

Table 1 provides DR and positive predictive values (PPVs) for the different screening tests for a fixed FPR of 5%.

**Table 1** Comparison of model predicted DR and PPV at term for different screening tests for Down syndrome according to a fixed 5% FPR.

| Screening Test | DR (%) | PPV |
|---|---|---|
| **First trimester** | | |
| **cFTS** | 85% | 1 in 46 |
| **Second trimester** | | |
| **Quad** | 71% | 1 in 54 |
| **Both trimesters** | | |
| **Serum Integrated** | 76% | 1 in 51 |
| **Integrated** | 92% | 1 in 42 |
| **Contingent^** | 91% | 1 in 42 |

^First-stage cutoff risks 1 in 50 and 1 in 2000 at term

Table adapted from Cuckle H and Maymon R [4]

***cfDNA based screening:***

A new approach for screening for common aneuploidy is based on the analysis of cell free DNA in maternal plasma (which includes maternal and fetal cell free DNA) as opposed to serum markers. This approach was first suggested in 1997 by Lo et al who reported that cell-free DNA from the fetus could be detected in maternal plasma [8]. Fetal cfDNA is present in maternal plasma as early as 7 weeks and at 10 weeks, the median percent fetal cfDNA has been shown to be 10.2% of total cfDNA increasing by 0.1% per week between 10 and 21 weeks gestation with 2% of pregnancies having less than 4% fetal cfDNA in maternal plasma [9]. This biological finding has led to the development of different approaches for non-invasive prenatal testing (NIPT) using cfDNA in maternal blood for screening for common aneuploidies.

There are currently three broad cfDNA testing methods available:

1. a shotgun (genome wide) massively parallel sequencing (s-MPS) approach that relies on the identification and counting of large numbers of DNA fragments in plasma specimens
2. a targeted analysis of cfDNA that focuses on specific chromosomes of interest and assesses the count of these regions by either MPS or microarray
3. an approach that takes advantage of single nucleotide polymorphism (SNP) differences between mother and fetus and, after multiplex PCR amplification of nearly 20,000 SNP sequences, each product is evaluated based on the hypothesis that the fetus has trisomy, monosomy or is euploid and a final risk score is calculated [10].

For any given cfDNA testing approach, a bioinformatics platform is developed to classify results as screen negative (low risk) or screen positive with or without a specific risk estimate given.

Screening for the most common autosomal aneuploidies by analysis of cfDNA in maternal plasma in singleton pregnancies has been shown to be highly effective with sensitivities greater than 99% for trisomy 21, 98% for trisomy 18 and 99% for trisomy 13, and a combined false positive rate (FPR) of 0.13% [11]. Although the initial studies that established these performances were

done in high risk pregnancies, a recent meta-analysis aimed at determining the performance of cfDNA in a general pregnant population has shown that the cfDNA screen performs as well for trisomy 21, whatever the a priori risk with a pooled sensitivity of 0.993 (95% CI 0.955-0.999) and specificity of 0.999 (95% CI 0.998-0.999) [12].

Although all cfDNA methods are associated with high DRs and low FPRs, the positive predictive value (PPV) in high risk women has been shown to be 94% [13], while two prospective studies in average risk women showed PPVs of 45.5% to 80.9% [14, 15]. These PPVs indicate that NIPT by cfDNA is not a diagnostic test and that false positive results are possible. Reasons for the discordancies include true fetal mosaicism, confined placental mosaicism, maternal chromosomal abnormality, unrecognized vanishing twin, low fetal fraction and/or insufficient depth of DNA sequencing [10].

An important metric that is often overlooked in the evaluation of cfDNA NIPT performance is the rate of test failures or no-result rate [16]. An analysis of no-result rates in all studies on NIPT that included more than 1000 samples concluded that the magnitude of no-result rate is dependent on the testing methodology: MPS-based methods have the lowest failure rate (1.58%), followed by chromosome specific sequencing (3.56%) with the SNP-based approach having the highest rate (6.39%) [16]. Recent publications suggest that patients who receive a “no result” are at increased risk of aneuploidy, in particular, trisomies 13, 18 and triploidy [15, 17]. This finding has led some professional societies to recommend that women with a “no result” be offered counselling and an invasive diagnostic test [18, 19]. A redraw for a second cfDNA screening attempt is another option. A test result is expected in approximately 50% of patients unless the maternal weight is greater than 120kg which is associated with a decreased the fetal fraction and higher no-result rate [9].

In many countries, genomics-based non-invasive prenatal screening (NIPS using cfDNA) has been adopted or is being implemented as a second-tier or contingent prenatal screening test for the common aneuploidies. In that context, women with a positive conventional screen are offered the option of cfDNA testing as a more accurate screen in lieu of going directly to invasive diagnostic testing with its associated risk of pregnancy loss. In this model, the risk cut-off to offer NIPT can be lowered compared to current risk cut-offs used to offer invasive diagnostic testing[20, 21]. In addition, cfDNA testing can be used as a first tier screen. This strategy would maximize detection while ensuring that relatively few women have invasive prenatal diagnosis. However, at current costs for cfDNA testing, it would be a significantly more expensive screening approach [10].

**Choice of protocol**

Availability of screening resources (such as availability of certified sonographers for NT measurements) and National Health economic considerations are likely to result in geographic differences of screening protocols. The American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM) have stated that given the performance of conventional screening methods, the limitations of cell-free DNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional aneuploidy screening methods remain the most appropriate choice for first–line screening for most women in the general obstetric population [18]. However, numerous professional societies including ACOG and SMFM do support the use of cfDNA screening as a first

tier option for women in the general obstetric population, provided women receive pre-test counselling that includes the benefits and limitations of this screening approach [18, 19, 22, 23].

***First trimester versus Second trimester screening:***

Protocols that provide results in the first trimester (cFTS, contingent or sequential screening, first tier cfDNA screening) have the advantage of allowing women with a negative screen earlier reassurance and those with a positive screen earlier diagnostic testing. For patients with a confirmed diagnosis of an abnormal karyotype who decide to terminate their pregnancies, first trimester abortion is safer, more readily available and more acceptable to women [24]. However, to truly benefit from earlier screening results other than early reassurance, screening programs must ensure the availability of chorionic villus sampling (CVS) which is typically performed between 10 and 13 weeks gestation. Although it can be performed later, some CVS providers are unwilling to perform the test much after 13 weeks or will limit later procedures to a transabdominal approach which is not feasible in all cases [25]. Therefore, for a significant proportion of women undergoing first trimester screening, the small gestational age window for CVS precludes them from first trimester diagnostic testing as they must resort to invasive diagnostic testing by amniocentesis typically done from 15 weeks of gestation. It has also been argued that first trimester screening identifies affected fetuses that may have been miscarried between the first and second trimesters thus early detection of those pregnancies leads to surgical terminations of pregnancies that may have aborted spontaneously [26]. The other impact noted from shifting Down syndrome screening from the second trimester to the first has been the loss of MSAFP screening for spina bifida. A review of prenatally diagnosed cases of spina bifida over three years (first year with second trimester screening, second year of transition and third year where majority of pregnant women had first trimester screening) showed a tangible effect on the gestational age at diagnosis of spina bifida, and resulted in a decrease of 25% of cases detected before 20 weeks gestation [27].

***Choosing only one screening test:***

Any screening test has a FPR and therefore, doing more than one screening test has a cumulative effect on the FPR. Given this, numerous professional societies have made the clear recommendation that women should only undergo one aneuploidy screening test [18, 22, 23]. At a time where multiple aneuploidy screening options are available, pregnant women who chose first tier cfDNA screening and obtain a successful and unambiguous result should be counselled against undergoing subsequent conventional screening tests for trisomies.

**Screening in twin pregnancies**

Screening for aneuploidy in twin pregnancies and higher multiples should be offered to pregnant patients with the following considerations reflected in the counselling of the patients.

Approximately one third of twins are monozygotic and ‘almost’ always genetically identical meaning that if one fetus has Down syndrome, the other twin will also be affected. In contrast, two thirds of twins are dizygotic and in such instance, if one fetus has Down syndrome, the other will almost always be unaffected. Prenatally, zygosity cannot always be determined even though it

does affect the performance of the screening tests. While all monochorionic pregnancies are monozygotic, and all dizygotic pregnancies are dichorionic, it is important to understand that dichorionic pregnancies are found in approximately 33% of monozygotic twins [28]. As such it is important to determine chorionicity by first trimester ultrasound but zygosity can not always be inferred by the chorionicity.

Prenatal screening for Down syndrome calculates a risk based on maternal serum markers and NT measurement but also incorporates maternal age-specific risk for Down syndrome in the risk estimate. One may have assumed that the maternal age specific risk for Down syndrome at any given maternal age would be the same for monochorionic twin as singletons and the risk for dichorionic twins, closed to double the risk in singletons. These assumptions used to calculate the risk of Down syndrome in twin gestations have been shown in two large studies to be inaccurate. A population-based prevalence study based on EUROCAT congenital anomaly registries comprising a population of 14.8 million births between 1990 and 2009 with 2.89% of those births being multiple, found that the adjusted relative risk of Down syndrome for monozygotic versus singleton pregnancies was 0.34 (95% CI 0.25 – 0.44) and for dizygotic versus singleton pregnancies 1.34 (95% CI 1.23-1.46) [29]. A retrospective review of California Prenatal Screening Program participants between 1995 and 2012 including 77,279 twin pregnancies with 182 (0.2%) with had at least one fetus with Down syndrome found a ratio of observed to expected Down syndrome incidence per pregnancy of 33.6%, 75.2% and 70% for monozygotic, dizygotic and all twins respectively ($P<0.001$ for all comparisons) [30]. Both studies establish that the observed incidence of Down syndrome in twin pregnancies is lower than expected, most notably in monozygotic twin pregnancies.

While offering screening for Down syndrome is recommended in twin gestations[22, 28], fewer validation studies of the tests being offered in twin gestations are available compared to studies done in singleton pregnancies. Screening was first offered in twin gestations based on theoretical estimates of screening performance according to the test used, maternal age and chorionicity. The estimated detection rate for a false positive rate of 5% was 84% for monochorionic twins and 70% for dichorionic twins for cFTS and 93% for monochorionic and 78% for dichorionic with the integrated test (NT with first and second trimester serum markers) [31]. A systematic review of screening for trisomy 21 in twin pregnancies using first trimester combined screening showed that the estimated performance in dichorionic twins was underestimated as pooled sensitivity and specificity in dichorionic twins was shown to be 0.862 (95% CI 0.728-0.936) and 0.952 (95% CI 0.942-0.96), respectively. In monochorionic twins, the pooled sensitivity and specificity were 0.874 (95% CI 0.526-0.977) and 0.954 (95% CI 0.943-0.963) [32]. To date, there are no prospective studies of the performance of integrated screening in twins [28]. A prospective study of first trimester screening for trisomies by cfDNA testing of maternal blood which included a total of 438 twin pregnancies(85.2% dichorionic and 14.8% monochorionic) tested by the Harmony$^{TM}$ prenatal test included 8 cases of trisomy 21, four of trisomy 18 and one of trisomy 13. The no-result rate after the first blood sampling was 9.4%. In the 417 twin pregnancies with a cfDNA result after first or second sampling, the detection rate was 100% (8/8) for trisomy 21, 75% (3/4) for trisomy 18 and 0% (0/1) for trisomy 13, at a FPR of 0.25% (1/404) [33]. The two main factors contributing to the higher failure rate in twins compared to singletons with this assay were the consequence of selecting the lower fetal fraction of the two fetuses rather than the total fetal fraction and a considerably higher rate of conception by IFV in twin which is known to be a risk factor for lower fetal fraction [33]. Studies of shotgun sequencing (MPSS) to screen for common aneuploidies in

prospectively collected twin pregnancies provided results in all cases as no attempt to determine fetal fraction was made and the DR was 100% (17 /17 ) for trisomy 21, 50% for trisomy 18 (1/2) and 0% FPR [34-36]. Based on these studies, screening by cfDNA testing of maternal blood in twin pregnancies has similar high DR for trisomy 21 and low FPR as singleton pregnancies by the number of cases of trisomy 18 and trisomy 13 are too small to draw conclusions [33].

**Chromosomal abnormalities other than common aneuploidies**

Although conventional screening estimates the risk of trisomy 21 and trisomy 18 (with some also estimating the risk of trisomy 13), studies have demonstrated that these conventional screens lead to the diagnosis of other chromosomal abnormalities. In a retrospective cohort of 1, 324,607 women who had traditional screening through the California Prenatal Screening Program from April 2009 through December 2012, 68,990 (5.2%) were screen-positive. Of those undergoing invasive testing, excluding cases of confined placental mosaicism, 2,948 were found to have a chromosomal abnormality and only 77% of those were trisomies 21, 18 or 13. The other 23% represented other trisomies, sex chromosomal aneuploidy, balanced and unbalanced rearrangements, insertions and deletions, triploidy, tetraploidy and marker chromosomes [37]. The authors estimated that the conventional screening process identified an atypical chromosomal abnormality likely to result in an abnormal phenotype and not likely to be detectable by cfDNA screening in 1.4% of the 68,990 pregnancies with a positive screen. This suggests that some abnormal pregnancies would not be detected if cfDNA screening were to replace the conventional standard screening. Similar findings were noted in a retrospective population-based analysis of 193,638 singleton pregnancies who had a trisomy 21 risk calculation performed, with 10,205 women (5.3%) having a karyotype analysis done with the following findings: 689 cases of trisomies 21, 18 or 13; 262 other chromosomal anomalies likely to be of phenotypic importance and undetectable by NIPT; 45 other chromosomal anomalies unlikely to be of phenotypic importance [38]. Therefore in that cohort, 27.5% of significant chromosomal abnormalities were not trisomies 21, 18 or 13. Considering the entire cohort of 193,638 pregnancies, the incidence of atypical chromosomal abnormalities not detectable by NIPT was 0.14% with 32% of those detected by cFTS. A systematic review of large prospective screening studies that reported diagnoses other than trisomies 21 and 18, identified 10 informative studies with a total of 1,500,999 women screened. 3689 aneuploidy cases were detected in the screen positive women. Trisomies 21 18 and 13 comprised 76% of the cases of aneuploidy and sex chromosomal anomalies, 11% of anomalies, suggesting that 13% of anomalies would not be detected by the fetal cfDNA tests that include only trisomies 21, 18, 13 and sex aneuploidies [39]. The proportion of common trisomies and sex chromosomal abnormalities amongst all cases of aneuploidies detected in the screen positive women was higher for second trimester protocols compared with those carried out completely or partly in the first trimester.

Over the last few years, cfDNA testing has been developed to detect more than the common autosomal trisomies initially targeted. Screening for sex chromosome aneuploidies (SCAs) was the first addition but the performance of cfDNA for the X and Y chromosomes has been shown to not be as accurate as for chromosome 21. A recent meta-analysis estimated the DR for monosomy X at 95.8% (95% CI 70.3-99.5) with a FPR of 0.14% (95% CI 0.05-0.38) [11]. Limited data is available on the performance of cfDNA testing for sex chromosomal anomalies other than monosomy X.

In addition, targeted SNP based cfDNA testing is offered to screen for five clinically significant microdeletion syndromes: 22q11.2, Prader-Willi, Angelman, cri-du-chat and 1p36. The proof of principle study, prior to clinical offering of the testing, was carried out on a combination of a small number of pregnant women plasma samples and artificially created plasma samples from mixtures of cleaved DNA from affected and unaffected individuals [40].These experiments demonstrated a 97% DR and a FPR of less 0.1% for Prader-Willi, Angelman, cri-du-chat and 1p36 whereas 22q11.2 microdeletion syndrome had a DR of 96% and a FPR of 0.7%. However, given the low prevalence of these microdeletion syndromes, based on the performance of the test, the estimated PPVs for Angelman, Prader Willi, 22q11.2 and cri-du-chat, 1p36 were 3.8%, 4,6%, 5.3% and 17% respectively. The only reported clinical experience of SNP-based cfDNA screening for microdeletion syndromes is limited to screening for 22q11.2 syndrome. In that study, 21,948 samples were submitted for screening for 22q11.2 syndrome and follow-up was conducted for all cases with a high-risk result. As such, the DR of this test cannot be established but information can be gained about the PPV. In the entire cohort including cases with ultrasound anomalies, the PPV was 18%. For positive cases with no abnormal ultrasound findings prior to NIPT, the PPV was 4.9% [41].

cfDNA testing that is based on genome-wide (shot-gun) MPS has the potential to detect subchromosomal segmental aneuploidies and rare autosomal trisomies [42-45]. Non mosaic autosomal trisomies should be easier to detect than trisomy 21 because these other chromosomes are larger and the relative differences in counts between euploid and aneuploidy cases should therefore be greater [46]. Detection of segmental chromosomal imbalances (subchromosomal abnormalities or copy number variants) will be dependent on their size, the DNA composition of the chromosomal region, sufficient fetal fraction and the depth of sequencing [46]. There is a debate regarding the appropriateness of expanding cfDNA screening to include rare autosomal trisomies and subchromosomal abnormalities. Cases of rare autosomal trisomies are likely to result in spontaneous fetal loss if the abnormality is indeed present in the fetus. More often, the rare autosomal trisomy will be confined to the placenta and it is unclear if their detection would impact pregnancy outcome. In NIPT cases positive for trisomy for an imprinted chromosome (chromosomes 6, 7, 11, 14, 15), one could argue that this would allow uniparental disomy studies to be performed to exclude uniparental disomy which would be associated with an abnormal fetal outcome.

There are arguments against screening for subchromosomal abnormalities related to the inability to validate accurately the performance of these tests (DR, FPR and PPV) given the low incidence of these events in the general population. In addition, in the presence of fetal structural anomalies on ultrasound, an invasive diagnostic test with microarray analysis still offers the most accurate and timely diagnosis because it is not possible to reliably exclude the possibility of a false-negative cfDNA result even with deeper sequencing [44]. The International Society for Prenatal Diagnosis' guidelines recommends that when cfDNA screening is extended to microdeletion and microduplication syndromes or rare trisomies, the testing should be limited to clinically significant disorders or well defined severe conditions and there should be defined estimates for the DRs, FPRs and information about the clinical significance of a positive test for each disorder being screened [22]. In contrast, the ACOG, SMFM, SOGC and CCMG recommend against cfDNA screening for microdeletions and micro-duplications [18, 23] and the ACMG has a clear statement against cfDNA testing for CNVs and rare autosomal trisomies [19]. The relentless progress toward

the comprehensive, non-invasive assessment of the fetal genome provides many exciting opportunities and simultaneous challenges for the field of prenatal screening [47].

**Counselling required for all tests**

Professional societies and expert groups emphasize the need for pre-test counselling to ensure that pregnant women are making an informed decision about whether or not to have fetal aneuploidy screening [2, 19, 22, 23, 48]. The general counselling points to be covered are similar regardless of the type of prenatal screening being offered.

Pre-test counselling should include clinical information about the chromosomal disorders being screened for, the woman's risk of having a baby affected with one of these disorders, and a review of the available options for screening [2]. Discussion of the different tests available should cover the performance of each test in terms of their ability to detect an affected pregnancy and the false positive rates. Other factors that should be discussed and contrasted between the different tests are timing of the tests and results, the chance of a no-result call, the next steps if a screen is positive and the limitations of each test (what it does not detect). Women should gain a clear understanding that whatever test is chosen, it is still a screening test and not a diagnostic test. A negative result is not a guarantee of an unaffected pregnancy and a positive result does not equate to an affected pregnancy. Finally, pre-test counselling should emphasize that every patient has the right to choose or decline aneuploidy screening. Counselling of pregnant women should be non-directive and respect ethical, cultural, moral and religious values.

In addition, pre-test counselling for NIPT requires a discussion with the patient that in rare circumstances, NIPT may raise suspicion for maternal or fetal conditions other than the fetal aneuploidies for which the test is being performed [49]. Reported incidental findings include maternal mosaicism for a chromosomal abnormality, in particular sex chromosomal aneuploidy, maternal malignancies, and fetal or placental chromosomal abnormalities other than the common aneuploidies the patient was being screened for [49].

Post-test counselling, with both positive and negative results, should take place in a timely manner and provide the patient the adjusted likelihood of carrying a fetus with the evaluated aneuploidies [2]:

- In the case of a negative screen, counselling should include a reminder to patient of the conditions being screened, of the potential the fetus may have a condition that is not covered by the screen, and the purpose and benefits of a detailed second trimester ultrasound.
- In the case of a positive screen, counselling should include a discussion of the options for further testing. If the initial screen was a conventional screen based on serum markers and NT ultrasound, both cfDNA testing and invasive diagnostic testing options should be discussed. If the first tier screen was cfDNA, the PPV of the cfDNA test result should be provided to the patient and invasive diagnostic testing offered to confirm the diagnosis. The PPV of the result can be calculated based on the patient's a priori risk and the test performance for the specific chromosome in question. On line applications have been developed to facilitate this process (https://www.perinatalquality.org/vendors/nsgc/nipt/).

It is extremely important for patients to recognize that although cfDNA testing is an excellent screen, it is only a screening test, not a diagnostic test. Invasive diagnostic testing is recommended to confirm the diagnosis. For patients with a confirmed diagnosis of chromosomal aneuploidy

based on cytogenetic analysis of amniocytes or chorionic villi, counselling should include balanced information about the condition and options for management of the pregnancy including termination of pregnancy, and referral to a genetics professional for further counselling. Health care professionals informing patients of the diagnosis of a chromosomal abnormality should be knowledgeable about the condition and be careful to use sensitive language that does not proscribe value on people with a chromosomal abnormality [50]. Patients should be directed to additional resources available through Local and National Societies and Foundations dedicated specifically to support parents of children with the condition diagnosed, as well as offered referral for additional genetic counselling if needed.

**Facilitating informed decision making**

Health care providers counselling pregnant women regarding prenatal screening and diagnostic options have a lot of information to convey and little time to do so in the context of all other pregnancy related information they must cover in a first trimester prenatal visit. Research in the area of informed decision making is needed to allow the development of tools that will facilitate this process. Patient decision aids (PtDAS) help people made difficult, values-sensitive decisions, yet they are rarely used to support decision making in prenatal genetic screening [51]. To analyze the effect of a decision-support guide and elimination of financial barriers to testing, a randomized trial was conducted with women being randomized to usual care as per current guidelines or a computerized, interactive decision-support guide as per current guidelines and access to prenatal testing with no out-of-pocket expense [52]. The group assigned to the intervention with full implementation of the prenatal testing guidelines using the computerized, interactive decision-support guide were less likely to have diagnostic testing suggesting that tools that support informed decision making are needed to ensure women make a more informed and preference-based decision regarding prenatal testing.

**Need for quality assurance**

Prenatal screening for aneuploidy is best implemented in the context of a comprehensive program that coordinates preanalytic, analytic and postanalytic components of the process [53]. Technical standards and guidelines have been developed by the ACMG to provide the laboratories the necessary information to ensure accurate and reliable Down syndrome screening results given a screening protocol [53, 54]. These documents include requirements for patient and health care providers' education and address all components of the testing from sample collection to processing, assay methodologies and results reporting. It emphasizes the need for normative data review, evaluation of medians with new reagent lots, and long term monitoring with collection of pregnancy outcome information as much as possible.

For screening protocols that include an NT measurement, the quality of the NT measurement must be ensured so that the DR and FPR predicted for the test are achieved. The need for such a quality assurance process was demonstrated by a meta-analysis of studies of nuchal translucency-based screening for Down syndrome which suggested an overall Down syndrome DR of 77% at a 6% FPR. However, when the 34 studies were evaluated separately, the Down syndrome DR varied from a low of 29% (4% FPR) to a high of 100% (FPR 5%), a variation best explained by a significant variation in sonographer training and quality of nuchal translucency images obtained [5]. It is

important that individuals performing NT measurements have gone through a training program and a successful submission of NT images that qualifies them to perform NT measurements [53]. Annual assessment of NT images and associated data will ensure on going quality of NTs performed and contribute to the expected performance of the screening tests.

Specific guidelines for quality control and quality assurance for cfDNA screening have not yet been developed. A recent on line survey of laboratories registered in the three European quality assurance schemes for molecular and cytogenetics had responses from 50 laboratories that issued reports for NIPT. Considerable variation in reporting NIPT results were noted [55]. This study led to the development of minimum guidelines for reporting laboratory results for NIPT for aneuploidy and the development of an external quality assessment process. Although the International Society for Prenatal Diagnosis recognizes that specific guidelines for quality control and quality assurance for cfDNA screening have not yet been developed, it recommends that providers utilize laboratory services that meet national guidelines for quality control and proficiency testing consistent with that available for other molecular test [22].

## Conclusion

Prenatal screening programs should be implemented with resources that support patient and health care provider education, quality assurance of laboratory and NT services, access to genetic counselling services and diagnostic testing. The rapid pace of new developments in cfDNA screening for chromosomal anomalies brings new opportunities to enhance the performance of the screening but at the same time, challenges as the test menus are expanded with limited information on the clinical utility of some of those tests. At the present cost of cfDNA screening, most cost-effective studies of the implementation of cfDNA for common aneuploidies conclude that its implementation is cost-effective as a second tier or contingent test [44, 56, 57]. However, expected decreasing costs will allow programs to consider implementing cfDNA as a first tier screen raising debates as to the role of NT measurement and serum analytes in this context. Professional societies are divided in this regard with the International Society of Ultrasound in Obstetrics and Gynecology recommending that nuchal translucency thickness should continue to be measured and reported as a raw value and centile in all women including those with negative cfDNA results, as it has the potential to detect fetuses with cardiac defects, rare genetic syndromes and other chromosomal anomalies [58]. This recommendation is endorsed in a joint guideline of the Society of Obstetricians and Gynaecologists of Canada (SOGC) Genetics Committee and the Canadian College of Medical Geneticists (CCMG) Clinical Practice Committee [23], but the Society for Maternal Fetal Medicine states that for women with a negative cfDNA result, an NT measurement is not recommended [59]. This exemplifies the need for further research studies to provide screening programs that want to integrate new technologies, the data needed to support evidenced based implementation of new screening protocols that will be based on well validated screening performances, clinical utility and cost-effectiveness.

## Funding

The authors have no support or funding to report.

## Competing Interests

The authors have declared that no competing interests exist.

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