thermoscientific

Contingent Screening

Study overview on integrating cfDNA/NIPT test in routine first trimester screening

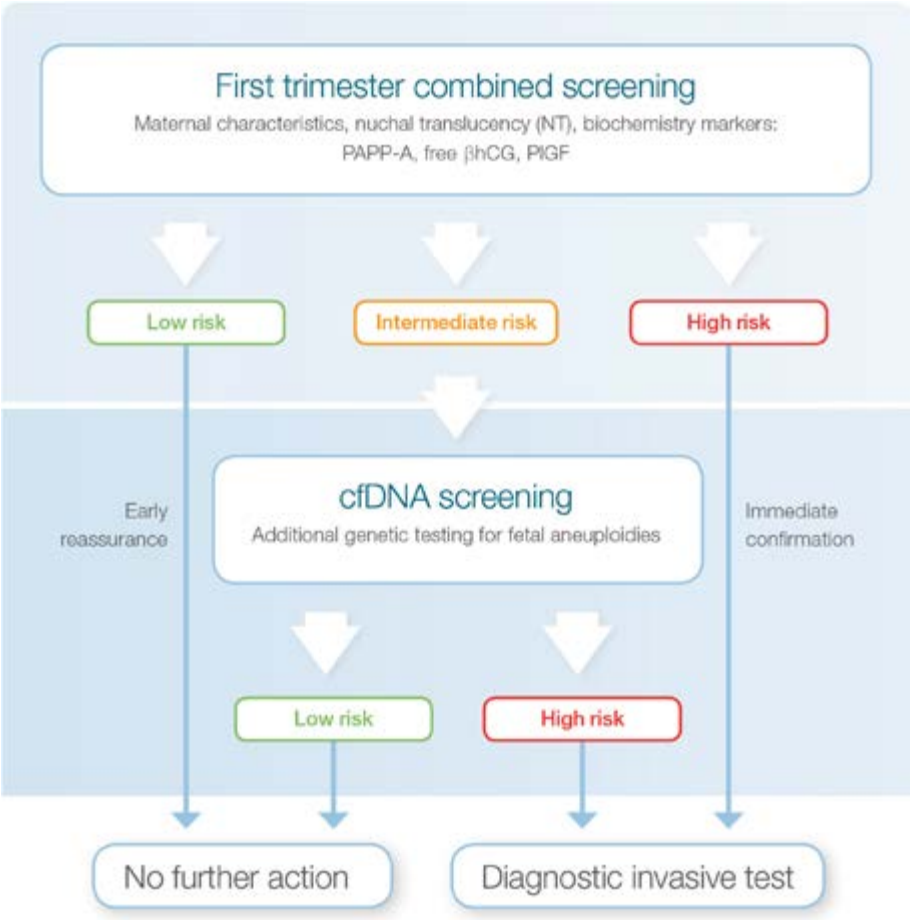
ThermoFisher SCIENTIFIC

Content

3. Contingent screening strategy implementation

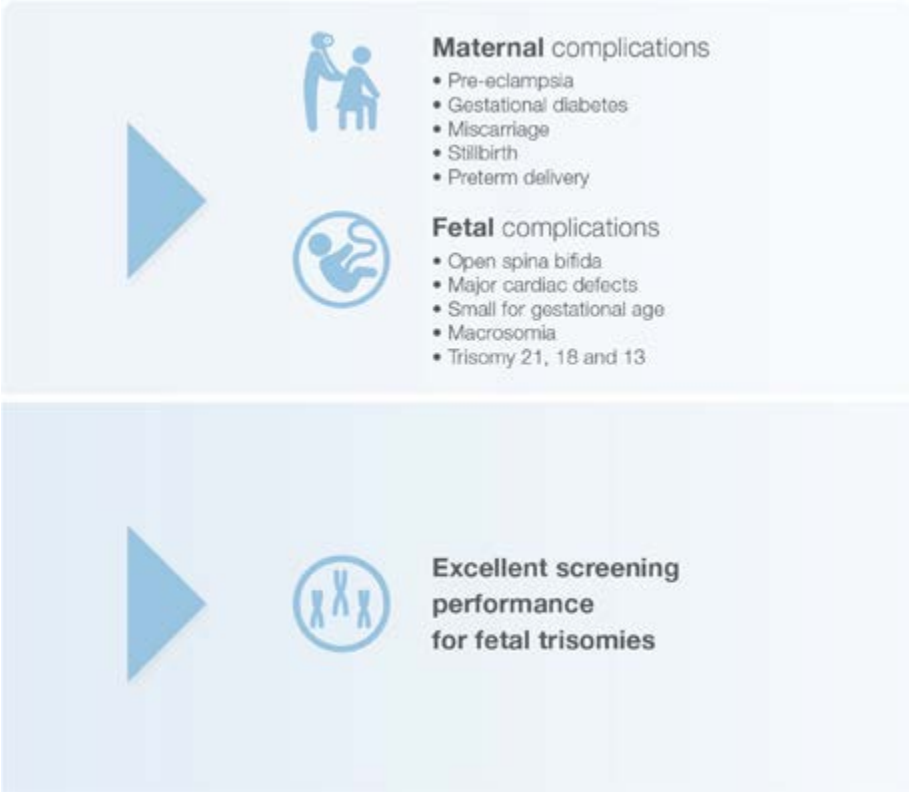
Two screening steps for a most comprehensive pregnancy management

The contingent screening strategy combines routine first trimester screening for multiple pregnancy complications with additional NIPT or cell free DNA screening as a second step for an intermediate risk group of fetal trisomies. Combined screening is routine first trimester screening with maternal history, ultrasound markers and biochemistry markers (PAPP-A, free βhCG, PlGF) to provide the estimation of individual risk of pregnant women for pregnancy related disorders and adverse outcome conditions.
Cell free DNA screening is based upon evaluation of the proportion of placental cfDNA in the blood of pregnant women, thereby estimating the risks for fetal trisomies and other genetic disorders.



ADVANTAGES of the contingent screening model

- **Comprehensive and cost-effective pregnancy management to predict a wide range of pregnancy complications**
- **High detection rates for fetal trisomies with further reduction of unnecessary invasive procedures**



1. Contingent screening model, position of cfDNA testing in prenatal screening

First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing

Nicolaides KH, Syngelaki A, Poon LC, Gil MM, Wright D
Fetal Diagn Ther. 2014;35(3):185-92

Rationale

To examine potential performance of screening for trisomies by cell-free (cf) DNA testing in maternal blood contingent on results of first-line testing by combinations of fetal translucency thickness (NT), fetal heart rate (FHR), ductus venosus pulsatility index (DV PIV), and serum-free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A), placental growth factor (PLGF) and α-feto-protein (AFP).

Method

Performance was estimated for firstly, screening by cfDNA in all pregnancies and secondly, cfDNA testing contingent on results of first-line testing by combinations of ultrasound and biochemical markers.

Results

In first-line screening by cfDNA testing, the detection rate for trisomy 21 and trisomies 18 or 13 would be 99 and 96%, respectively, after invasive testing in 1% of the population. In contingent screening, a detection rate of 98% for trisomy 21 and 96% for trisomy 18 or 13, at an invasive testing rate of 0.7%, can be achieved by carrying out cfDNA testing in about 35, 20 and 11% of cases identified by first-line screening with the combined test alone (age, NT, FHR, β-hCG, PAPP-A), the combined test plus PLGF and AFP and the combined test plus PLGF, AFP and DV PIV, respectively.

Conclusion

Effective first-trimester screening for trisomies can be achieved by contingent screening incorporating biomarkers and cfDNA testing.

KEY FACTS

- **Contingent approach retains the major advantages of cfDNA testing in increasing DR and decreasing FPR, but at considerably lower cost than offering cfDNA testing to the whole population.**
- **In contingent screening, detection of 98% of fetuses with trisomy 21 and about 96% of fetuses with trisomies 18 or 13 can be achieved at an overall invasive testing rate of less than 1%.**

First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing

Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM
Ultrasound Obstet Gynecol. 2013 Jul; 42(1):41-50

Objective
To define risk cut-offs with corresponding detection rates (DR) and false-positive rates (FPR) in screening for trisomy 21 using maternal age and combinations of first-trimester biomarkers in order to determine which women should undergo contingent maternal blood cell-free (cf) DNA testing.

Method
From singleton pregnancies undergoing screening for aneuploidies at three UK hospitals between March 2006 and May 2012, we analyzed prospectively collected data on the following biomarkers: fetal nuchal translucency thickness (NT) and ductus venosus pulsatility index for veins (DV-PIV) at 11 + 0 to 13 + 6 weeks' gestation and serum free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A), placental growth factor (PlGF) and alpha-fetoprotein (AFP) at 8 + 0 to 13 + 6 weeks. Estimates of risk cut-offs, DRs and FPRs were derived for combinations of biomarkers and these were used to define the best strategy for contingent cfDNA testing.

Results
In contingent screening, detection of 98% of fetuses with trisomy 21 at an overall invasive testing rate < 0.5% can be potentially achieved by offering cfDNA testing to about 36%, 21% and 11% of cases identified by first-line screening using the combined test alone, using the combined test with the addition of serum PlGF and AFP and using the combined test with the addition of PlGF, AFP and DV-PIV, respectively.

Conclusion
Effective first-trimester screening for trisomy 21, with DR of 98% and invasive testing rate < 0.5%, can be potentially achieved by contingent screening incorporating biomarkers and cfDNA testing.

KEY FACTS

- **Screening for trisomy 21 by cfDNA testing contingent on the results of combined test would retain the advantages of the first trimester combined screening, with increase in DR and decrease in the rate of invasive testing.**
- **Measurement of serum PlGF and AFP can be performed in the same sample and by the same automated machines as those used for free β-hCG and PAPP-A at little extra cost. Those metabolites are useful in first-trimester screening for pre-eclampsia, fetal growth restriction and preterm birth.**

Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis

Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH

Ultrasound Obstet Gynecol. 2015 Mar;45(3):249-66

Objective

To review clinical validation or implementation studies of maternal blood cell-free (cf) DNA analysis and define the performance of screening for fetal trisomies 21, 18 and 13 and sex chromosome aneuploidies.

Method

Searches of PubMed, EMBASE and The Cochrane Library were performed to identify all peer-reviewed articles on cfDNA testing in screening for aneuploidies between January 2011, when the first such study was published, and 4 January 2015.

Results

In total, 37 relevant studies were identified and these were used for the meta-analysis on the performance of cfDNA testing in screening for aneuploidies. These studies reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome. Weighted pooled detection rates (DR) and false-positive rates (FPR) in singleton pregnancies were 99.2% (95% CI, 98.5-99.6%) and 0.09% (95% CI, 0.05-0.14%), respectively, for trisomy 21, 96.3% (95% CI, 94.3-97.9%) and 0.13% (95% CI, 0.07-0.20) for trisomy 18, 91.0% (95% CI, 85.0-95.6%) and 0.13% (95% CI, 0.05-0.26%) for trisomy 13, 90.3% (95% CI, 85.7-94.2%) and 0.23% (95% CI, 0.14-0.34%) for monosomy X and 93.0% (95% CI, 85.8-97.8%) and 0.14% (95% CI, 0.06-0.24%) for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR for trisomy 21 was 93.7% (95% CI, 83.6-99.2%) and the FPR was 0.23% (95% CI, 0.00-0.92%).

Conclusion

Screening for trisomy 21 by analysis of cfDNA in maternal blood is superior to that of all other traditional methods of screening, with higher DR and lower FPR. The performance of screening for trisomies 18 and 13 and sex chromosome aneuploidies is considerably worse than that for trisomy 21.

KEY FACTS

- **cfDNA analysis of maternal blood in screening for Trisomy 21 in singleton pregnancies is superior to all previous methods in screening performance. The weighted pooled DR for T21, T18 and T13 are 99,2%, 96,3% and 91% with respective FPRs of 0,09%, 0,13% and 0,23%.**
- **Expansion of the indications of cfDNA testing to include trisomies 18 and 13 and sex chromosome aneuploidies would increase the cumulative FPR eight-fold, from 0.09% to 0.72%.**

Screening for trisomies by cell-free DNA testing of maternal blood: consequences of a failed result

Revello R, Sarno L, Ispas A, Akolekar R, Nicolaides KH

Ultrasound Obstet Gynecol. 2016 Jun;47(6):698-704

Objective

First, to report the distribution of the fetal fraction of cell-free (cf) DNA and the rate of a failed cfDNA test result in trisomies 21, 18 and 13, by comparison with pregnancies unaffected by these trisomies, second, to examine the possible effects of maternal and fetal characteristics on the fetal fraction, and third, to consider the options for further management of pregnancies with a failed result.

Method

This was a cohort study of 10 698 singleton pregnancies undergoing screening for fetal trisomies 21, 18 and 13 by cfDNA testing at 10-14 weeks' gestation. There were 160 cases of trisomy 21, 50 of trisomy 18, 16 of trisomy 13 and 10 472 were unaffected by these trisomies. Multivariate regression analysis was used to determine significant predictors of fetal fraction and a failed cfDNA test result amongst maternal and fetal characteristics.

Results

Fetal fraction decreased with increasing body mass index and maternal age, was lower in women of South Asian racial origin than in Caucasians and in assisted compared to natural conceptions. It increased with fetal crown-rump length and higher levels of serum pregnancy-associated plasma protein-A and free β-human chorionic gonadotropin. The median fetal fraction was 11.0% (interquartile range (IQR), 8.3-14.4%) in the unaffected group, 10.7% (IQR, 7.8-14.3%) in trisomy 21, 8.6% (IQR, 5.0-10.2%) in trisomy 18 and 7.0% (IQR, 6.0-9.4%) in trisomy 13. There was a failed result from cfDNA testing after first sampling in 2.9% of the unaffected group, 1.9% of trisomy 21, 8.0% of trisomy 18 and 6.3% of trisomy 13. In the cases with a failed result, 7% of women had invasive testing, mainly because of high risk from the combined test and/or presence of sonographic features suggestive of trisomies 18 and 13. All cases of trisomies were detected prenatally.

Conclusion

In cases of a failed cfDNA test, the rate of trisomies 18 and 13, but not trisomy 21, is higher than in those with a successful test. In the management of such cases, the decision in favor of invasive testing should depend on the risk of prior screening and the results of detailed ultrasound examination.

KEY FACTS

In trisomies 18 and 13, but not in trisomy 21, the fetal fraction is lower and the rate of failed cfDNA test is higher than in unaffected pregnancies.

A unified approach to risk assessment for fetal aneuploidies

Wright D, Wright A, Nicolaides KH

Ultrasound Obstet Gynecol. 2015 Jan;45(1):48-54

Objective

To examine the potential impact of combining measures from cell-free DNA (cfDNA) testing with maternal age and first-trimester biomarkers in screening for fetal trisomies.

Method

This was a theoretical study using Bayes' theorem to combine the a priori risk for fetal trisomy 21 derived from maternal age with likelihoods from nuchal translucency thickness, serum pregnancy-associated plasma protein-A, serum free β-human chorionic gonadotropin and plasma cfDNA. We adopted a binomial counting model for the cfDNA likelihoods and developed a model to account for errors in estimating fetal fraction.

Results

When Bayes' theorem was used to combine the a priori risk for trisomy 21 derived from the first-trimester combined test with likelihoods from the cfDNA test, and when the true fetal fraction was known, the detection rate increased from 62% at a fetal fraction of 4% to 100% at a fetal fraction of ≥ 9%; the positive likelihood ratio (trisomic/euploid) increased from 620 to 1000 and the negative likelihood ratio (euploid/trisomic) increased from 3 to > 10 000. When the fetal fraction is < 4%, the cfDNA test has traditionally been considered to be a failure, but the cfDNA results can be used to improve the performance of screening by the combined test.

Conclusion

In contingent policies that use the first-trimester combined test for first-line screening to select the subgroup for cfDNA testing, the data from the latter should be used to update the risk from the former. Individual patient results from cfDNA testing depend crucially on the fetal fraction and the precision of its measurement.

KEY FACTS

- **The general practice of companies offering the cfDNA test to report results as positive/negative or as risk>99%/<1:10 000 does not reflect the true estimate of individual patient-specific risk for a given trisomy, especially when the fetal fraction is<10%.**
- **In the absence of the necessary data from the suppliers of the cfDNA test, it would be preferable for clinicians managing individual patients to use the risk estimate from the first-line method of screening as the prior risk and modify this by the appropriate positive or negative likelihood ratio for a given fetal fraction from the cfDNA test.**

Screening for chromosomal abnormalities by first trimester combined screening and noninvasive prenatal testing

Kagan KO, Hoopmann M, Hammer R, Stressig R, Kozlowski P
Ultraschall Med. 2015 Feb;36(1):40-6

Objective
To examine combined first trimester screening (FTS), noninvasive prenatal testing (NIPT) and a two-step policy that combines FTS and NIPT in screening for aneuploidy.

Method
Retrospective study involving 21,052 pregnancies where FTS was performed at the Praxis Praenatal.de in Duesseldorf, Germany. In each case, the sum risk of trisomy 21, 18 and 13 was computed. We assumed that NIPT detects 99 %, 98 %, 90 % and 99 % of cases with trisomy 21, 18, 13 and sex chromosomal abnormalities and that the false-positive rate is 0.5 %. The following screening policies were examined: NIPT or FTS with sum risk cut-offs of 1 in 50 and 1 in 250 in all patients or a two-step-policy with FTS in all patients followed by NIPT in the intermediate sum risk group. For the intermediate risk group, sum risk cut-offs of 1 in 50 and 1 in 1000 and 1 in 150 and 1 in 500 were used.

Results
There were 127, 34, 13 and 15 pregnancies with trisomy 21, 18, 13 and sex chromosomal abnormalities. 23 fetuses had other chromosomal abnormalities with an increased risk for adverse outcome that are not detectable by NIPT. 20,840 pregnancies were classified as normal as ante- and postnatal examinations did not show any signs of clinically significant chromosomal abnormalities. FTS with a sum risk cut-off of 1 in 50 and 1 in 250 detects 81 % and 91 % for all aneuploidies. NIPT detects 88 % of the respective pregnancies. The 2-step approach with sum risk cut-offs of 1 in 50 and 1 in 1000 detects 94 % of all aneuploidies. With sum risk cut-offs of 1 in 150 and 1 in 500, the detection rate is 93 %.

Conclusion
A 2-step policy with FTS for all patients and NIPT in the intermediate risk group results in the highest detection rate of all aneuploidies.

KEY FACTS

- **Trisomy 21 accounts for about 60 % of all chromosomal abnormalities and about 10 % of atypical chromosomal abnormalities are associated with an adverse outcome that cannot be identified by common NIPT programs.**
- **The costs of contingent screening approaches were substantially lower than with 1st line NIPT screening and only moderately higher than first trimester screening with a risk cut-off of 1:250.**

2. The value of biomarkers and ultrasound in the first trimester screening

Inverted Pyramid of Care

Sonek JD, Kagan KO, Nicolaides KH
Clin Lab Med. 2016 Jun; 36(2):305-17

First-trimester pregnancy evaluation using fetal and maternal parameters not only allows for diagnoses to be made early in gestation but can also assess the risk of complications that become clinically evident later in pregnancy.

This evaluation makes it possible for pregnancy care to be individualized. In select cases, treatment that reduces the risk of complications can be started early in pregnancy.

Even though cell free DNA is a significant advance in diagnosing fetal aneuploidy, the combination of first-trimester ultrasound and maternal serum biochemistries casts a much wider diagnostic net; therefore, the 2 technologies are best used in combination.

KEY FACTS

- **Most fetal chromosomal and structural anomalies can be diagnosed by the end of the first trimester of pregnancy.**
- **Cell free fetal DNA is a significant advance in screening for fetal aneuploidy; however, its use is limited and is best used in combination with first-trimester ultrasound and maternal serum screening.**
- **The risk of some pregnancy complications that become clinically evident only later in pregnancy can be established in the first trimester; the incidence of some of these disorders, such as preeclampsia, can be reduced if treatment is instituted early in pregnancy.**
- **First-trimester screening also shows some promise in other pregnancy-related problems (e.g, spontaneous preterm birth, small for gestational age without preeclampsia, macrosomia, gestational diabetes) and represents a fertile field for future research.**

Accuracy of first trimester combined test in screening for trisomies 21, 18 and 13

Santorum M, Wright D, Syngelaki A, Karagioti N, Nicolaides KH
Ultrasound Obstet Gynecol. 2016 Aug 23

Objective
To examine the diagnostic accuracy of a previously developed model for the first-trimester combined test in screening for trisomies 21, 18 and 13.

Method
This was a prospective validation study of screening for trisomies 21, 18 and 13 by a combination of maternal age, fetal nuchal translucency, fetal heart rate and serum free ß-hCG and PAPP-A at 11+0 -13+6 weeks' gestation in 108,982 singleton pregnancies undergoing routine care in three maternity hospitals. A previously published algorithm was used for the calculation of patient-specific risk of trisomy 21, trisomy 18 and trisomy 13 in each patient. The detection rates (DR) and false positive rates (FPR) at estimated risk cut-offs from 1 in 2 to 1 in 1000 were determined. The proportions of trisomies were compared to their expected values in different risk groups.

Results
In the study population there were 108,112 (99.2%) cases with normal fetal karyotype or the birth of a phenotypically normal neonate and 870 (0.8%) cases with abnormal karyotype including trisomy 21 (n = 432), trisomy 18 (n = 166), trisomy 13 (n = 56), mosomy X (n = 63), triploidy (n = 35) or other aneuploidy (n = 118). The screen positive rates, standardized according to the maternal age distribution of England and Wales in 2011, of fetuses with abnormal and normal karyotype were compatible with those predicted from the previous model; at risk cut-off of 1 in 100, the FPR was about 4% and the DRs for trisomies 21, 18 and 13 were 90, 97 and 92%, respectively. There was evidence that the algorithm over-estimated risks. This could to some degree reflect under ascertainment in pregnancies ending in miscarriage or stillbirth.

Conclusion
In a prospective validation study the first-trimester combined test detected 90, 97 and 92% of trisomies 21, 18 and 13, respectively, as well as >90% of cases of monosomy X, >85% of triploidies and >30% of other chromosomal abnormalities, at FPR of 4%.

KEY FACTS

The combined test provides effective screening for trisomies 21, 18 and 13 (DR of 90%, 97%, 92%) and helps identify a high proportion of other chromosomal abnormalities, at FPR of 4%.

Replacing the combined test by cell-free DNA testing in screening for trisomies 21, 18 and 13: impact on the diagnosis of other chromosomal abnormalities

Syngelaki A, Pergament E, Homfray T, Akolekar R, Nicolaides KH
Fetal Diagn Ther. 2014;35(3):174-84

Objective
To estimate the proportion of other chromosomal abnormalities that could be missed if combined testing was replaced by cell-free (cf) DNA testing as the method of screening for trisomies 21, 18 and 13.

Method
The prevalence of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other chromosomal abnormalities was examined in pregnancies undergoing first-trimester combined screening and chorionic villus sampling (CVS).

Results
In 1,831 clinically significant chromosomal abnormalities in pregnancies with combined risk for trisomies 21, 18 and 13≥1:100, the contribution of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other chromosomal abnormalities at high risk of adverse outcome was 82.9, 8.2, 3.9 and 5.0%, respectively. Combined screening followed by CVS for risk≥1:10 and cfDNA testing for risk 1:11-1:2,500 could detect 97% of trisomy 21 and 98% of trisomies 18 and 13. Additionally, 86% of monosomy X, half of 47,XXY, 47,XYY or 47,XXX, half of other chromosomal abnormalities and one third of triploidies, which are currently detected by combined screening and CVS for risk≥1:100, could be detected.

Conclusion
Screening by cfDNA testing, contingent on results of combined testing, improves detection of trisomies, but misses a few of the other chromosomal abnormalities detected by screening with the combined test.

KEY FACTS

- **Trisomies 21, 18, 13 account for about 80% of detected clinically significant chromosomal abnormalities**
- **Contingent screening in the intermediate risk group (1:11-1:1000, 15% of the population) would potentially detect most of the cases of monosomy X and between half and one third of the few other clinically significant chromosomal abnormalities that are currently detected by invasive testing with 1:100 cut-off in combined screening**

Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10-11 weeks' gestation and the combined test at 11-13 weeks

Quezada MS, Gil MM, Francisco C, Oròsz G, Nicolaides KH

Ultrasound Obstet Gynecol. 2015 Jan;45(1):36-41

Objective

To examine in a general population the performance of cell-free DNA (cfDNA) testing for trisomies 21, 18 and 13 at 10-11 weeks' gestation and compare it to that of the combined test at 11-13 weeks.

Method

In 2905 singleton pregnancies, prospective screening for trisomies was performed by chromosome-selective sequencing of cfDNA in maternal blood at 10-11 weeks' gestation and by the combined test at 11-13 weeks' gestation.

Results

Median maternal age of the study population was 36.9 (range, 20.4-51.9) years. Results from cfDNA analysis were provided for 2851 (98.1%) cases and these were available within 14 days from sampling in 2848 (98.0%) cases. The trisomic status of the pregnancies was determined by prenatal or postnatal karyotyping or clinical examination of the neonates. Of the 2785 pregnancies with a cfDNA result and known trisomic status, cfDNA testing correctly identified all 32 cases with trisomy 21, nine of 10 with trisomy 18 and two of five with trisomy 13, with false-positive rates of 0.04%, 0.19% and 0.07%, respectively. In cases with discordant results between cfDNA testing and fetal karyotype, the median fetal fraction was lower than in those with concordant results (6% vs 11%). Using the combined test, the estimated risk for trisomy 21 was ≥ 1/100 in all trisomic cases and in 4.4% of the non-trisomic pregnancies.

Conclusion

The performance of first-trimester cfDNA testing for trisomies 21 and 18 in the general population is similar to that in high-risk pregnancies. Most false-positive and false-negative results from cfDNA testing could be avoided if the a priori risk from the combined test is taken into account in the interpretation of individual risk.

KEY FACTS

- **The performance of first-trimester cfDNA testing for trisomies 21 and 18 in the general population is similar to that in high-risk pregnancies.**
- **Most false positive and false negative results from cfDNA testing can be avoided when a priori risk from combined screening is taken into account.**

The value of the first trimester ultrasound in the era of cell free DNA screening

Rao RR, Valderramos SG, Silverman NS, Han CS, Platt LD
Prenat Diagn. 2016 Dec;36(13):1192-1198

Objective
To describe the clinically relevant findings detected by the first trimester ultrasound (FTU) and to determine the additional value of the FTU compared to cell free DNA (cfDNA) alone.

Method
Retrospective cohort study of patients undergoing a FTU at a maternal-fetal medicine referral practice. Fetal, gynecologic, and placental findings detected by ultrasound were analyzed with available cfDNA and diagnostic testing results. A subgroup analysis of positive ultrasound findings and cfDNA results was performed to assess the additional benefit of ultrasound evaluation in FT prenatal screening.

Results
There were 1906 FTU between 1 October 2013 and 1 October 2014. CfDNA results were available for 959 (50%) patients. FTU detected: 42 fetal (2.2%), 286 gynecologic (15.0%), and 317 placental (16.6%) findings. CfDNA results were discordant with invasive testing results in 8/61 cases (13%) and with ultrasound findings in 18/42 (42%) cases. There were six false positive and two false negative cfDNA results confirmed by diagnostic testing. Subgroup analysis revealed that cfDNA as the sole method of prenatal screening in the FT would miss 95% of the fetal findings detected with ultrasound.

Conclusion
The comprehensive FTU provides valuable clinical information about fetal and maternal anatomy that cannot be detected with cfDNA alone.

KEY FACTS

- **While cfDNA testing is an effective screening test for aneuploidy, it is not effective in detecting fetal anomalies that are not associated with aneuploidy or certain genetic derangements.**
- **If cfDNA was used as the only method of evaluating for a normally progressing pregnancy, it would have missed 95%, 99%, and 99% of the fetal, gynecologic, and placental findings that were detected with the use of ultrasound.**

First-trimester detection of structural abnormalities and the role of aneuploidy markers

Grande M, Arigita M, Borobio V, Jimenez JM, Fernandez S, Borrell A
Ultrasound Obstet Gynecol. 2012 Feb;39(2):157-63

Objective
To determine the sensitivity of first-trimester ultrasound for diagnosing different structural anomalies in chromosomally normal pregnancies, and to establish the role of aneuploidy markers in the detection of abnormalities.

Method
This was a retrospective study of chromosomally normal singleton pregnancies with an 11-14-week scan performed in our center during 2002-2009. The ultrasound examination included an early fetal anatomy survey and assessment of nuchal translucency, ductus venosus blood flow and nasal bone.

Results
Among 13 723 scanned first-trimester pregnancies with no genetic anomalies and complete follow-up, 439 fetuses (3.2%) were found to present with structural anomalies (194 with major anomalies and 245 with only minor anomalies). Forty-nine per cent of major structural anomalies were detected during the first-trimester scan, the highest rates corresponding to acrania (17/17), holoprosencephaly (three of three), hypoplastic left heart syndrome (10/10), omphalocele (six of six), megacystis (seven of eight) and hydrops (eight of nine). Higher than expected detection rates were obtained for skeletal (69%) and cardiac (57%) defects, coincidentally showing the highest presence of an increased nuchal translucency or abnormal ductus venosus blood flow (38% and 52%, respectively). The finding of an absent nasal bone did not appear to be associated with structural defects.

Conclusion
About half of major structural abnormalities can be diagnosed in the first trimester. Increased nuchal translucency or abnormal ductus venosus blood flow appear to be associated with cardiac and skeletal defects and may facilitate early detection.

KEY FACTS

First trimester ultrasound can detect 49% of major structural anomalies, including skeletal and cardiac defect, in fetus.

Enlarged NT (≥3.5 mm) in the first trimester - not all chromosome aberrations can be detected by NIPT

Srebniak MI, de Wit MC, Diderich KE, Govaerts LC, Joosten M, Knapen MF, Bos MJ, Looye-Bruinsma GA, Koningen M, Go AT, Galjaard RJ, Van Opstal D
Mol Cytogenet. 2016 Sep 7;9(1):69

Background
Since non-invasive prenatal testing (NIPT) in maternal blood became available, we evaluated which chromosome aberrations found in our cohort of fetuses with an enlarged NT in the first trimester of pregnancy (tested with SNP microarray) could be detected by NIPT as well.

Method
362 fetuses were referred for cytogenetic testing due to an enlarged NT (≥3.5 mm). Chromosome aberrations were investigated using QF-PCR, karyotyping and whole genome SNP array.

Results
After invasive testing a chromosomal abnormality was detected in 137/362 (38 %) fetuses. 100/362 (28 %) cases concerned trisomy 21, 18 or 13, 25/362 (7 %) an aneuploidy of sex chromosomes and 3/362 (0.8 %) triploidy. In 6/362 (1.6 %) a pathogenic structural unbalanced chromosome aberration was seen and in 3/362 (0.8 %) a susceptibility locus for neurodevelopmental disorders was found. We estimated that in 2-10 % of fetuses with enlarged NT a chromosome aberration would be missed by current NIPT approaches.

Conclusion
Based on our cohort of fetuses with enlarged NT we may conclude that NIPT, depending on the approach, will miss chromosome aberrations in a significant percentage of pregnancies. Moreover all abnormal NIPT results require confirmatory studies with invasive testing, which will delay definitive diagnosis in ca. 30 % of patients. These figures are important for pretest counseling enabling pregnant women to make informed choices on the prenatal test. Larger cohorts of fetuses with an enlarged NT should be investigated to assess the additional diagnostic value of high resolution array testing for this indication.

KEY FACTS

In a group of fetuses with enlarged NT trisomies constitute 73%, others include monosomies, triploidies, a pathogenic structural unbalanced chromosome aberrations, etc. Therefore in 2-10 % of fetuses with enlarged NT a chromosome aberration would be missed by current NIPT approaches.

3.Contingent screening strategy implementation

UK NHS pilot study on cell-free DNA testing in screening for fetal trisomies: factors affecting uptake

Gil MM, Giunta G, Macalli EA, Poon LC, Nicolaides KH
Ultrasound Obstet Gynecol. 2015 Jan;45(1):67-73

Objective
This study reports on the clinical implementation of cell-free DNA (cfDNA) testing, contingent on the results of the combined test, in screening for fetal trisomies 21, 18 and 13 in two UK National Health Service hospitals. Women with a combined-test risk of ≥ 1:100 (high risk) were offered the options of chorionic villus sampling (CVS), cfDNA testing or no further testing and those with a risk of 1:101 to 1:2500 (intermediate risk) were offered cfDNA or no further testing. The objective of the study was to examine the factors affecting patient decisions concerning their options.

Method
Combined screening was performed in 6651 singleton pregnancies in which the risk for trisomies was high in 260 (3.9%), intermediate in 2017 (30.3%) and low in 4374 (65.8%). Logistic regression analysis was used to determine which factors among maternal characteristics, fetal nuchal translucency thickness (NT) and risk for trisomies were significant predictors of opting for CVS in the high-risk group and opting for cfDNA testing in the intermediate-risk group.

Results
In the high-risk group, 104 (40.0%) women opted for CVS; predictors for CVS were increasing fetal NT and increasing risk for trisomies, while the predictor against CVS was being of Afro-Caribbean racial origin (r = 0.366). In the intermediate-risk group, 1850 (91.7%) women opted for cfDNA testing; predictors for cfDNA testing were increasing maternal age, increasing risk for trisomies and university education, while predictors against cfDNA testing were being of Afro-Caribbean racial origin, smoking and being parous (r = 0.105).

Conclusion
This study has identified factors that can influence the decision of women undergoing combined screening in favor of or against CVS and in favor of or against cfDNA testing.

KEY FACTS

Contingent screening can be incorporated easily into routine antenatal care within NHS hospitals. Offering cfDNA testing to those with a risk of>1:2500 after first-trimester combined testing would substantially improve the DR to about 97% and reduce the FPR to less than 0.5%, without the major increase in cost.

Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test

Gil MM, Revello R, Poon LC, Akolekar R, Nicolaides KH

Ultrasound Obstet Gynecol. 2016 Jan;47(1):45-52

Objective

Cell-free DNA (cfDNA) analysis of maternal blood for detection of trisomies 21, 18 and 13 is superior to other methods of screening but is expensive. One strategy to maximize performance at reduced cost is to offer cfDNA testing contingent on the results of the first-trimester combined test that is used currently. The objectives of this study were to report the feasibility of implementing such screening, to examine the factors affecting patient decisions concerning their options for screening and decisions on the management of affected pregnancies and to report the prenatal diagnosis of fetal trisomies and outcome of affected pregnancies following the introduction of contingent screening.

Method

We examined routine clinical implementation of contingent screening in 11,692 singleton pregnancies in two National Health Service (NHS) hospitals in the UK. Women with a risk ≥ 1 in 100 (high-risk group) were offered options of invasive testing, cfDNA testing or no further testing, and those with a risk between 1 in 101 and 1 in 2500 (intermediate-risk group) were offered cfDNA testing or no further testing. The trisomic status of the pregnancies was determined by prenatal or postnatal karyotyping or by examination of the neonates.

Results

In the study population of 11,692 pregnancies, there were 47 cases of trisomy 21 and 28 of trisomies 18 or 13. Screening with the combined test followed by invasive testing for all patients in the high-risk group potentially could have detected 87% of trisomy 21 and 93% of trisomies 18 or 13, at a false-positive rate of 3.4%; the respective values for cfDNA testing in the high- and intermediate-risk groups were 98%, 82% and 0.25%. However, in the high-risk group, 38% of women chose invasive testing and 60% chose cfDNA testing; in the intermediate-risk group 92% opted for cfDNA testing. A prenatal diagnosis was made in 43 (91.5%) pregnancies with trisomy 21 and all pregnancies with trisomies 18 or 13. In many affected pregnancies the parents chose to avoid testing or termination and 32% of pregnancies with trisomy 21 resulted in live births.

Conclusion

Screening for fetal trisomies by cfDNA analysis of maternal blood, contingent on the results of the combined test, can be implemented easily in routine clinical practice. In the high-risk group from the combined test, most but not all women chose cfDNA testing rather than invasive testing. Performance of screening for trisomy 21 was superior by the cfDNA test than by the combined test. However, prenatal detection of trisomies and pregnancy outcome depend not only on performance of screening tests but also on parental choice.

Effect of non-invasive prenatal testing as a contingent approach on the indications for invasive prenatal diagnosis and prenatal detection rate of Down's syndrome

Kou KO, Poon CF, Kwok SL, Chan KY, Tang MH, Kan AS, Leung KY
Hong Kong Med J. 2016 Jun;22(3):223-30

Introduction
In Hong Kong, universal combined first-trimester screening for Down's syndrome was started as a 'free service' in July 2010. Non-invasive prenatal testing was available as a self-financed item in August 2011. This study aimed to determine whether the introduction of non-invasive prenatal testing as a contingent approach influenced the indications for invasive prenatal diagnosis and the consequent prenatal detection of Down's syndrome.

Method
This historical cohort study was conducted at the Prenatal Diagnosis Clinic of Queen Elizabeth Hospital in Hong Kong. We compared the indications for invasive prenatal diagnosis and prenatal detection of Down's syndrome in singleton pregnancies 1 year before and 2 years following the availability of non-invasive prenatal testing as a contingent test after a positive aneuploidy test. All pregnant women who attended our hospital for counselling about universal Down's syndrome screening between August 2010 and July 2013 were recruited.

Results
A total of 16 098 women were counselled. After the introduction of non-invasive prenatal testing, the invasive prenatal diagnosis rate for a positive aneuploidy screening reduced from 77.7% in 2010-11 to 68.8% in 2012-13. The new combined conventional plus non-invasive prenatal testing strategy was associated with a lower false-positive rate (6.9% in 2010-11 vs 5.2% in 2011-12 and 4.9% in 2012-13). There was no significant increase in invasive prenatal diagnosis for structural anomalies over the years. There was no significant trend in the overall prenatal detection rate of Down's syndrome (100% 1 year before vs 89.1% 2 years after introduction of non-invasive prenatal testing). Four (2.6%) of 156 women who underwent non-invasive prenatal testing for a screen-positive result had a high-risk result for trisomy 21, which was subsequently confirmed by invasive prenatal diagnosis. There were no false-negative cases.

Conclusion
The introduction of non-invasive prenatal testing as a contingent approach reduced the invasive prenatal diagnosis rate for a positive aneuploidy screening without affecting the invasive prenatal diagnosis rate for structural anomalies or the overall detection rate of fetal Down's syndrome.

Screening for trisomies 21 and 18 in a Spanish public hospital: from the combined test to the cell-free DNA test

Gil MM, Brik M, Casanova C, Martin-Alonso R, Verdejo M, Ramírez E, Santacruz B
J Matern Fetal Neonatal Med. 2016 Nov 22:1-7

Objective
To describe our experience in first-trimester screening for trisomies 21 and 18 firstly by the combined test alone and secondly by cell-free (cf) DNA testing contingent on the results from a previously performed combined test.

Method
Women with singleton pregnancies attending Torrejon University Hospital in Madrid, Spain, from November 2011 to January 2016, were screened for trisomy (T)21 and T18 by the combined test at 11-13 weeks. Before the introduction of cfDNA testing, women at high risk (>1 in 250) were offered invasive testing (IT) and from January 2015 they were offered cfDNA test as well as IT.

Results
Combined test was performed in 6011 pregnancies. The risk was high in 202 (3.4%) cases. There was complete follow-up for 5507 (91.6%) pregnancies. Detection rate (DR) for T21 was 83.3% (15/18) and 100% (4/4) for T18. Additionally, 2/2 (100%) cases of T13 and 2/2 (100%) cases of triploidy were also detected. False positive rate (FPR) was 3.2% (174/5488). The introduction of this contingent model was followed by a 73% reduction on the IT rate in the high-risk group, from 76.3% to 20.8%.

Conclusion
Contingent screening for trisomies 21 and 18 by cfDNA testing at 11-13 weeks is feasible and has a lower IT rate than combined testing alone.

KEY FACTS

In Spain, the first-trimester combined test is well established and recommended by SEGO. cfDNA screening for trisomies contingent on the results from the combined test at 11–13 weeks is feasible and has a lower IT rate than combined testing alone. This model also provides early reassurance to the parents when the screening is normal.

First trimester contingent screening for trisomies 21,18,13: is this model cost efficient and feasible in public health system?

Colosi E, D'Ambrosio V, Periti E

J Matern Fetal Neonatal Med. 2017 Jan 4:1-6

Purpose

To evaluate the effectiveness of three different first trimester screening models for trisomies 21, 18 and 13, in terms of detection rate, invasive test rate and final costs.

Method

We analyzed the distribution of risk for trisomies 21, 18 and 13 in a population of 20,831 singleton pregnancies based on maternal age, fetal heart rate, nuchal translucency, free beta human chorionic gonadotropin and pregnancy-associated plasma protein A (Combined test). On the basis of our data, we estimated the performance and cost of screening for trisomies using three different models at specific cut-offs: Combined test; Cell free DNA test and Contingent screening test.

Results

Using Combined test, DR for major trisomies was estimated to be 94.92%, invasive test rate was 6.3%. cfDNA would result in a DR of 97.92%, with an invasive test rate of 3.64%. Contingent screening approach would result in an overall DR of 97.82, with a rate for invasive procedure of 1.36% and a final cost lower than other screening policies (2,338,433 euro vs 5,796,060 of cfDNA and 2,385,473 of Combined test).

Conclusion

Contingent screening test could be a cost-efficient and feasible first trimester screening test for aneuploidies in public health system.

KEY FACTS

From comparison of three policies, combined test alone, cfDNA test alone and contingent screening (1:10-1:1000), contingent test has confirmed to be a cost-efficient and feasible first trimester screening test for aneuploidies in public health system (in Italy).

Applicability of first-trimester combined screening for fetal trisomy 21 in a resource-limited setting in mainland China

Li B, Sahota DS, Lao TT, Xu J, Hu SQ, Zhang L, Liu QY, Sun Q, Tang D, Ma RM

BJOG. 2016 Sep;123 Suppl 3:23-9

Objective
To assess the feasibility and performance of the first-trimester combined screening test for trisomy 21 in a resource-limited setting in mainland China.

Design
Prospective observational cohort study.

Setting
First Affiliated Hospital of Kunming Medical University, China.

Population
Ten thousand four hundred and forty-two pregnant women requesting first-trimester screening.

Method
The combined screening test was performed from May 2012 to December 2014. Women with a high-risk result (≥1:600) were offered further confirmatory tests after counselling. The threshold for high risk was determined by Monte Carlo simulation to achieve a 5% false-positive rate according to the local age distribution. Pregnancy outcome and screening results were recorded for all women and monthly audits were conducted.

Main Outcome Measures
Sensitivity, screen positive rate, cost per case of Down syndrome detected.

Results
Six hundred and ten women (5.8% of the total screened) had a high-risk screening test, of whom 274 (44.9%) underwent a diagnostic test and 169 (27.7%) opted for a noninvasive prenatal screening test (NIPT); 160 (26.2%) declined further testing after counselling. The pregnancy outcome was available for 10 174 (97.4%) of the women. The observed incidence of Down syndrome was 0.13% (1/750). All 14 women with a trisomy 21 pregnancy had a high-risk screening test result. The cost per Down syndrome detected was RMB596 686 compared with RMB1.79 million if all had been screened by NIPT.

Conclusion
The combined screening test appears to be a more cost-effective strategy in mainland China. Screening performance in China would be improved by adopting Chinese-specific models, external quality control and assurance, and establishing risk thresholds appropriate for the age distribution of the population.

Combined first trimester screening and cell-free fetal DNA - “next generation screening”

Kagan KO, Eiben B, Kozlowski P
Ultraschall Med. 2014 Jun;35(3):229-36
[Article in German]

Abstract

In the last decades, prenatal screening for aneuploidy has become increasingly effective. While first trimester combined screening is considered to be the current gold standard, the use of cell-free fetal DNA (cffDNA), which is also called noninvasive prenatal testing (NIPT), will result in a change of paradigm.

Respective studies indicate that in screening for trisomy 21, the detection and false-positive rates are 99 % and 0.1 %, respectively. For trisomies 18 and 13, there is less evidence but recent studies report detection rates of 98 % and 86 %.

Despite the excellent results in screening for trisomy 21, NIPT should not be considered as a diagnostic test. Due to the costs of NIPT, it is unlikely that NIPT will be applied in the near future in population-based screening for trisomy. In addition, the scope of the current approach in first trimester screening exceeds the screening for aneuploidy as it is possible to assess the risk for various pregnancy complications.

Therefore, a combination of both NIPT and first trimester combined screening seems reasonable. Both examinations could be applied in a contingent model where the latter is offered to everyone and NIPT is restricted to women with an intermediate risk after first trimester combined screening. Such a policy would result in a detection rate of about 97 % for a false-positive rate of about 1 %.

While NIPT currently focuses on screening for trisomy 21, 18, 13 and sex chromosomal abnormalities, the scope of NIPT will soon become broader. In this respect, some study groups have managed to examine the whole fetal genome within the course of the pregnancy. However, moral and ethical considerations need to be taken into account.

Models of clinical implementation of cell free DNA in the maternal serum screening test-analysis

Yankova M, Chaveeva P, Stratieva V
Akush Ginekol (Sofiia). 2015;54(7):15-21
[Article in Bulgarian]

Abstract

Prenatal screening by definition is a way of identifying pregnancies, with a high enough risk to specific fetal damage as to justify the subsequent invasive diagnosis among the seemingly normal pregnancies. [1] The aim of the prenatal screening test is to reach the high diagnostic frequency (DR > 95%), with low false-positive rate (FPR < 1%). Several non-invasive prenatal tests (NIPT) are widely adopted and use in clinical practice: 1st Trimester Combined screening (First trimester Combined Screening) and 2nd trimester biochemical screening (Second trimester biochemical screening) and in the last few years through screening Fetal DNA in Maternal serum (cfDNA screening). Since the introduction of the sfDNA test were examined and discussed the results of several ways of application: (1) as a primary screening method without preceding the result of 1st trimester combined screening for chromosomal abnormalities, (2) as a contingent test after 1st trimester combined screening in high risk pregnancies (> 1:100), (3) as a contingent test after 1st trimester combined screening, when the calculated risk is between (1:10 to 1:2500). The purpose of the study: to compare the results of different ways of application screening through cfDNA: detection rate (DR) for Tri21, Tri18 and Tri13, procentage of invasive diagnostics and cost-effectiveness ratio of cfDNA test in comparison with the 1st trimester combined screening. To establish the most suitable algorithm for application of cfDNA test.

Method

Analyzed were the results of several randomized multi-center clinical studies whose data are processed through a meta-analysis.

Results

cfDNA-test has a higher DR for Tri21 for lower FPR, compared to the combined screening in 1st trimester (cfDNA-DR 99%, 1st trimester screening-DR 96% and 0.4%FPR, respectively FPR 5%), but although it is with better results and reduces the incidence of invasive tests, does not justify the significant difference in price-performance ratio. On the other hand cfDNA-test is with a lower detection rate for Tri 18 or 13 (93-95%), which makes it worse for a primary screening test instead of combined screening in the 1st trimester.

Conclusion

The performance of cfDNA-test in terms of the three most common Trisomies: 21,18 and 13 is highest when used after (contingent to) 1st trimester screening and only for patients with an intermediate risk from 1-st trimester screening (risk > 1:10 and 1:2500, around 27% of all pregnancies), as it increases the diagnostic rate of combined screening for Down syndrome (from 90% to 98%), and significantly reduces the percentage of invasive diagnostics (from 3% to 0.7-1%) and that way we are able to achieve optimal result in price-performance result.

The assessment of combined first trimester screening in women of advanced maternal age in an Asian cohort

Li SW, Barrett AN, Gole L, Tan WC, Biswas A, Tan HK, Choolani M
Singapore Med J. 2015 Jan;56(1):47-52

Introduction
First trimester screening (FTS) is a validated screening tool that has been shown to achieve detection rates of 84%-90% for trisomies 21, 18 and 13. However, its effectiveness for different maternal ages has not been assessed. The present study aimed to assess the performance of FTS in an Asian population, and to compare its effectiveness in older (≥ 35 years) and younger (< 35 years) women. The potential use of noninvasive prenatal test (NIPT) as a contingent screening test is also examined.

Method
Data on cases of FTS performed on singleton pregnancies over a six-year period was collated from two Singapore maternal centres, National University Hospital and Singapore General Hospital. Cases that had a 1:250 risk of trisomy were considered to be screen-positive. Pregnancy outcomes were obtained from birth records or karyotype test results.

Results
From 10,289 FTS cases, we obtained a sensitivity of 87.8%, a specificity of 97.6%, a false positive rate of 2.4% and a false negative rate of 0.06% for the detection of aneuploidy. The overall detection rate for trisomy 21 was 86.5%-85.7% for older women and 87.5% for younger women. The mean number of invasive tests required per case of trisomy 21 was 9.3 in younger women, 8.6 in older women and 13.5 in women with intermediate risk (1:250-1,000).

Conclusion
While the performance of FTS was similar in younger and older women, more invasive procedures were required to diagnose trisomy 21 in women with intermediate risk. It may be advantageous to offer contingent NIPT to this group of women to reduce the risk of iatrogenic fetal loss.

KEY FACTS

The results of the present study provide supporting evidence that clinicians in Singapore should recommend FTS as a first-line screening for trisomy 21, regardless of maternal age. It may be advantageous to offer contingent NIPT to this group of women to reduce the risk of iatrogenic fetal loss.

thermoscientific

Thermo Scientific™ B·R·A·H·M·S™ KRYPTOR™

Automated immunofluorescent assays for quantitative determination of pregnancy associated proteins

B·R·A·H·M·S PAPP-A KRYPTOR for determination of pregnancy-associated plasma protein A in human serum

- Outstanding precision: UK NEQAS data prove 16 years of mean CV of 3.1%
- Low detection limit: 0.004 IU/L
- CE mark for first trimester trisomy and pre-eclampsia screening
- In routine use by FMF since 1999

B·R·A·H·M·S Free βhCG KRYPTOR for determination of free beta subunit of human chorionic gonadotropin in human serum

- Outstanding precision: UK NEQAS data prove 16 years of mean CV of 3.0%
- Low detection limit: 0.16 IU/L
- CE mark for first and second trimester trisomy screening
- In routine use by FMF since 1999

B·R·A·H·M·S PlGF plus KRYPTOR for determination of placental growth factor in human serum

- Excellent precision
- Best functional assay sensitivity (FAS): 6.7 pg/mL
- Very low detection limit: 3.6 pg/mL
- CE mark for first trimester trisomy and pre-eclampsia screening

► Fast and reliable detection of clinical values throughout pregnancy

---

Clinical Diagnostics

Thermo Fisher Scientific
B·R·A·H·M·S GmbH
Neuendorfstr. 25
16761 Hennigsdorf
Germany

+49 (0)3302 883 0
+49 (0)3302 883 100 fax
info.brahms@thermofisher.com
www.thermoscientific.com/brahms

Find out more at **thermoscientific.com/brahms**

ThermoFisher SCIENTIFIC

107576.1