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REVIEW ARTICLE

Screening for Fetal Aneuploidies in 1st Trimester

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ABSTRACT

Effective screening of fetal aneuploidy started in the early 1960s, initially based on the age of the mother. Recent screening protocols based on various maternal serum factors and on new ultrasound techniques during the 1st and 2nd trimester offered to all pregnant women noninvasive prognostic methods for risk assessment of chromosomal abnormalities and performance-invasive prenatal diagnostic methods only in high-risk cases. In this review, we discuss the ultrasound and biochemical markers of chromosomal abnormalities in the 1st trimester, the evaluation of free fetal deoxyribonucleic acid in the peripheral blood of pregnant women, and different antenatal screening protocols as known today.

Keywords: Aneuploidies, Noninvasive prenatal diagnosis, Nuchal translucency, PAPP-A serum, Trisomy 21, 1st-trimester screening.

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INTRODUCTION

The early detection of fetal chromosomal abnormalities is one of the fundamental goals in prenatal diagnosis. Applying various methods, using chromosomal markers, and defining risk limits, pregnant women can be separated in high- and low-risk groups for chromosomal abnormalities.

Each chromosomal marker should have high sensitivity and specificity in order to detect the majority of embryos with chromosomal abnormalities, using less invasive methods. The cut-off risk is set according to the procedure-related miscarriage rate, such as amniocentesis and chorionic villus sampling.

In the 1960s, the main method of screening for fetal aneuploidies was based on the correlation of trisomy 21 and maternal age and in the 1980s by maternal serum biochemistry and detailed ultrasound examination ("genetic sonogram") in the 2nd trimester. In the 1990s, screening methods shifted to the 1st trimester when it was realized that the great majority of fetuses with major aneuploidies can be identified by a combination of maternal age, fetal nuchal translucency (NT) thickness, maternal serum free β-human chorionic gonadotropin (β-hCG), and pregnancy-associated plasma protein-A (PAPP-A). In the last 10 years, several additional 1st-trimester sonographic markers have been described in order to improve the detection rate of aneuploidies and to reduce the false-positive rate. In the current decade, prenatal diagnostic armamentarium has been enriched by chromosomal microarray (CMA) and a method expected for many years, the evaluation of cell-free fetal deoxyribonucleic acid (cf-DNA) in the peripheral blood of pregnant women.

This review will discuss the sonographic and biochemical markers of chromosomal abnormalities in the 1st trimester, the evaluation of cf-DNA in the peripheral blood of pregnant women in singleton and multiple gestations, and the use of different antenatal protocols prevailing today. To improve understanding of this review, we introduce the definitions of methods which will be mentioned below.

Conventional karyotype: Conventional cytogenetics is the analysis of chromosomes up to 5 MV bases following cell culture of tissue, to detect abnormalities associated with various clinical manifestations.

Low-density CMA: Chromosomal microarray is a revolutionary application that allows study of chromosomes for any nonbalanced chromosomal abnormality, including all numeral (e.g., Down's syndrome) and structural abnormalities up to more than 2 Kb bases. In this manner, it is possible to detect minor rearrangements which may lead to the development of serious clinical symptoms and syndromes. Consequently, CMA can detect about 139 chromosome regions in addition to those of the conventional karyotype.

SCREENING FOR CHROMOSOMAL ABNORMALITIES IN 1ST TRIMESTER

Noninvasive screening methods in singleton and multiple pregnancies during the 1st trimester are based on the

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Table 1: Different methods of prenatal screening for trisomy 21

Method of screening	*Detection rate (%)*	*False-positive rate (%)*
MA	30	5
First trimester		
MA + fetal NT	75–80	5
MA + serum free β-hCG and PAPP-A	60–70	5
MA + NT + free β-hCG and PAPP-A (combined test)	85–95	5
Combined test + nasal bone or tricuspid flow or ductus venosus flow	93–96	2.5
Second trimester		
MA + serum AFP, hCG (double lest)	55–60	5
MA + serum AFP, free β-hCG (double test)	60–65	5
MA + serum AFP, hCG, uE3 (triple test)	60–65	5
MA + serum AFP, free β-hCG, uE3 (triple test)	65–70	5
MA + serum AFP, hCG, uE3, inhibin A (quadruple test)	65–70	5
MA + serum AFP, free β-hCG, uE3, inhibin A (quadruple test)	70–75	5
MA + NT + PAPP-A (11–13 weeks) + quadruple test	90–94	5

MA: Maternal age; NT: Nuchal translucency; β-hCG: β-human chorionic gonadotrophin; PAPP-A: Pregnancy-associated plasma protein-A

measurement of NT of the fetus associated with the measurement of the levels of PAPP-A and free β-hCG. Several additional 1st-trimester sonographic markers have been described which improve the detection rate of aneuploidies and reduce the false-positive rate (Table 1)[1] as well as the evaluation of free fetal DNA in the peripheral blood of women.

Singleton Pregnancies

Fetal NT

Nuchal translucency refers to the subcutaneous layer of clear liquid present in the neck of the embryos which can be demonstrated sonographically. In 1992, Nicolaides et al[2] described the correlation between increased NT of the fetus and the likelihood of Down's syndrome (T21), Edwards syndrome (T18), and Patau syndrome (T13). The relationship between increased NT and congenital heart diseases and various anatomical and genetic defects was later confirmed as well.[3]

The criteria for correct measurement of NT are well-established by the Fetal Medicine Foundation and have been accepted by the Hellenic Society of Ultrasound in Obstetrics and Gynecology.

The risk assessment for chromosomal abnormalities can be calculated in three ways:

- Risk assessment based on the age of the pregnant woman and the measurement of fetal NT
- Consideration of risk based on age of the pregnant woman and the plasma levels of the pregnant woman of PAPP-A and free β-hCG
- Consideration of risk by maternal age, measurement of NT, and levels of biochemical markers PAPP-A and free β-hCG.

The vast majority of the studies indicated that NT has sensitivity for Down's syndrome ranging from 70 to 80%, with a false-positive rate from 5 to 8.1%.[4] In fetuses with fetal NT measurement below the 95th centile, the probability of a healthy fetus in birth is up to 97%. In such cases, the probability of chromosomal abnormalities is 0.2%, for intrauterine death before 20 weeks of gestation is up to 1%, for congenital anomalies up to 1.5% and for congenital heart disease up to 0.3%. Conversely, increased NT is a common phenotypic expression of trisomy 21 and other chromosomal defects and can be associated with increased likelihood for fetal death, fetal malformations, congenital heart diseases, and genetic syndromes. In cases of increased NT up to 99th percentile (up to 3.5 mm) the probability of chromosomal abnormalities increases up to 3.5% and for major fetal abnormalities up to 2.5%. However, in these cases we can assure parents that the probability of delivering a healthy fetus is up to 93%. If NT is above the 99th percentile (> 3.5 mm), the probability of chromosomal defects is very high and increases about 20% for NT up to 4.0 mm, 33% for NT up to 5.0 mm, 50% for NT up to 6.0 mm and 65% for NT of 6.5 mm or more.

The decision for fetal karyotyping will be based on the estimated risk for chromosomal abnormalities, as resulting from the combination of maternal age, sonographic findings, and biochemical effects of β-hCG and PAPP-A in maternal serum. Cut-off risk for performing an invasive diagnostic procedure, in our country, is 1/300, which is considered to be the procedure-related miscarriage risk following chorionic villus sampling or amniocentesis.

Additional 1st Trimester Ultrasound Chromosomal Markers

Absence of the nasal bone, increased impedance to blood flow in the ductus venosus, and tricuspid regurgitation in the 1st trimester have been proposed as additional ultrasound markers for fetal aneuploidies.[5-12] The

evaluation of the nasal bone of the fetus during the 1st trimester involves the magnification of the image, at a level in which only the fetal head and the upper thorax are included in the display. In the correct section, three different lines are observed. The first two, which are horizontal and parallel to each other, represent the skin and the nasal bone (which is thicker and more echogenic than the overlying skin). Recent studies have shown sensitivity of 68.8%, with false-positive results depending on the nationality of pregnant women (9% Afro-Caribbean, 5% Asians, and 2.2% Caucasians).[5,6]

Regarding ductus venosus the incidence of abnormal blood flow is higher in fetuses with chromosomal abnormalities. An analysis of eight different studies reported abnormal flow in 3.7% of euploid fetuses and 69.1, 71.3, 64.8, and 76.2% of fetuses with trisomy 21, 18 and 13 and Turner syndrome respectively.[13] Furthermore, the abnormal flow in ductus venosus seems to be detected more frequently in fetuses with congenital heart diseases compared with normal fetuses.[13] Recent studies have reported that abnormal blood flow in the ductus venosus is an independent factor for congenital heart disease increasing by 11% the possibility of early diagnosis.[14]

Tricuspid regurgitation between 11 and 13^{+6} weeks occurs in 1% of euploid fetuses, in 56% of fetuses with trisomy 21 and in about 1/3 of those with trisomy 18, 13 and Turner syndrome.[11,12]

The disadvantage of screening with additional chromosomal markers is that assessment of them can be time consuming and requires appropriately trained sonographers. However, guidelines for ultrasound evaluation have been proposed by the Fetal Medicine Foundation and endorsed by the Greek Society of Ultrasound in Obstetrics and Gynecology.

Prognostic Maternal Serum Markers

Biochemical markers in 1st trimester. Pregnancies with fetal aneuploidies are associated with increased maternal serum concentrations of various feto-placental products, including free β-hCG and PAPP-A.[15-17] In various screening methods using biochemical markers, the measured concentrations are expressed as multiples of average (MoM) of normal pregnancies for the given week of pregnancy. Therefore, in euploid fetuses, concentrations in maternal serum of free β-hCG and of PAPP-A is expected to be 1.0 MoM.[16,18] In trisomy 21, free β-hCG concentration in maternal serum is higher (about 2 MoM) than in chromosomally normal fetuses, whereas PAPP-A is lower (about 0.5 MoM). Although the difference in free β-hCG between trisomic and euploid pregnancies increases with gestation, the magnitude of the difference is smaller than that of the opposite relation of PAPP-A. In trisomy 18 and 13, the free β-hCG and PAPP-A levels are decreased. In sex chromosomal abnormalities, β-hCG level is normal and PAPP-A concentration is low. In triploidy of parental origin, β-hCG level is greatly increased, whereas PAPP-A is mildly decreased. On the contrary, triploidy of maternal origin is associated with a significant reduction of β-hCG and PAPP-A levels.[19] The screening in the 1st trimester by a combination of maternal age and serum free β-hCG and PAPP-A identifies about 65% of affected pregnancies with a false-positive rate of 5%, while the combined 1st trimester test (NT plus serum free β-hCG and PAPP-A) has detection rate for Down's syndrome up to 91% and for trisomy 13 and 18 up to 91 and 96% respectively.[20-24]

Evaluation of free fetal DNA in the peripheral blood of pregnant women (cf-DNA—noninvasive prenatal testing (NIPT)). Noninvasive prenatal testing is a new method based on the next-generation sequencing of cf-DNA presented in peripheral blood of pregnant women and resulting in evaluation of the most common chromosomal abnormalities, such as Down's syndrome. However, it is not a diagnostic method.

Cell-free DNA in the blood of pregnant women is in the most part maternal in origin. Only a small proportion (about 10%) derives from the fetus (more precisely from the placenta). Noninvasive prenatal testing requires this "fetal fraction" of cell-free DNA in maternal blood to be above a minimum level for adequate analysis, for which most laboratories set a limit at 4%.[25] Although cell-free fetal DNA can be found in maternal blood very early, the fetal fraction may not be large enough yet, if testing is done prior to 9 or 10 weeks. However, fetal fraction may still be too low, due to maternal factors, like high maternal body weight in obese women.[26,27] Reported failure rates vary considerably between laboratories, ranging from 0 to 5%.[28] So, in false-positive results a rate of about 5 to 8% in singleton and over a 10% in twins must be added, due to the method's failure.[29]

In a very recent meta-analysis of 37 studies,[30] the cf-DNA was found to have a sensitivity of 99% and a specificity of 99.92% for Down's syndrome, sensitivity of 96.8% and specificity of 99.85% for trisomy 18, and 92.1% and 99.80% for trisomy 13 respectively.[30] Only few of these studies have been conducted in lower risk populations. However, there is growing evidence that good results can also be achieved in general obstetrical populations, making NIPT an alternative to current 1st-trimester screening protocols.[29] A major reason why NIPT for common autosomal aneuploidies is not diagnostic is because the DNA sequence represents a combination of maternal and fetal cell-free DNA, with the latter actually deriving from the placenta.[31] A positive result may be generated by factors other than an aneuploid fetal karyotype, including placental mosaicism, a vanishing twin, or a maternal tumor.

Screening in Multiple Pregnancies

In twin pregnancies, screening for chromosomal abnormalities is provided by the combined method of the 1st trimester including maternal age and fetal NT thickness and serum biochemistry free β-hCG and PAPP-A.[32-35] In twins at 11 to 13 weeks, the level of free β-hCG and PAPP-A is about twice that in singleton pregnancies. In contrast, in monochorionic twins, levels of free β-hCG and PAPP-A are lower than in dichorionic twins.[36,37]

In case of dichorionic twins, the modified risk is calculated for each fetus separately and the detection rate is similar to that of singletons.[33] This provides great advantages in prenatal testing of twin pregnancies, because already since the 1st trimester, there is a possibility for early diagnosis of chromosomal abnormalities and safe intervention with selective reduction of affected fetus.[33,38,39]

In monozygotic twins, for the calculation of the modified risk for trisomy 21, fetal NT should be measured in both fetuses and the average of the two should be used.[40] The false-positive rate, which may result from the increased NT in one of two embryos, is higher in monozygotic twins. It should be noted that in monochorionic twins, increased NT in at least one of the embryos may be a chromosomal marker, as well as an early onset of twin to twin transfusion syndrome.[41]

The accuracy of screening for aneuploidy with cf-DNA test is limited in multiple gestations. With any method based on maternal blood (serum markers or DNA), only a single composite result for the entire gestation is provided, with no ability to distinguish a differential risk between fetuses. Preliminary findings suggest that this screening is accurate. However, larger prospective studies and published data are needed before this method can be recommended for multiple gestations.[42,43]

DISCUSSION—SUGGESTED PROTOCOLS

Fetal aneuploidy is one of the major cause of perinatal deaths, neonatal and infant complications. Therefore, detection of chromosomal aberrations is a major indication for invasive prenatal diagnosis. Nevertheless, the risk of miscarriage after invasive methods imposed limitation of these methods only in high-risk pregnancies, for aneuploidy.

Chorionic villus sampling is used for the detection of fetal aneuploidy in the 1st trimester, while amniocentesis is used during the 2nd trimester of pregnancy.[44,45] The accuracy of the detection of fetal chromosomal abnormalities is approximately 99,9%,[46] with a possibility of pseudo-cell mosaicism under 1% respectively.[47]

From earlier studies it was estimated that the risk of miscarriage after chorionic villus sampling or amniocentesis was 0.5 to 1%.[48-51] However, recent data have shown that these two methods have almost similar procedure-related miscarriage risk.[46] Furthermore, we believe that risk rates now are significantly lower, as reported by a very recent study and counted to 0.22% for chorionic villus sampling and 0.11% for amniocentesis.[52] In addition, a very recent study reported that neither chorionic villus sampling nor amniocentesis was associated with increased risk of miscarriage or stillbirth, suggesting that the procedure-related risk of chorionic villus sampling and amniocentesis is very low.[53]

It is well-known that the probability of fetal aneuploidy is associated with maternal age. The estimated risks for fetal trisomies 21, 18 and 13 for a woman aged 20 years at 12 weeks of gestation are about 1 in 1000, 1 in 2500 and 1 in 8000 respectively. Instead, at the age of 35 years, the probability is calculated as 1/250, 1/600 and 1/1800 respectively.[1]

Based on these data, in the early 1970s, women aged 35 years or older were defined as "high-risk" group and represented the 5% of all pregnant women. In the subsequent years, in developed countries, the percentage of pregnant women older than 35 years exceeded more than 20%, and this group contains about 50% of the total number of fetuses with trisomy 21.

Since the 1990s, ultrasound measurement of fetal NT shifted screening methods for fetal aneuploidies to the 1st trimester of pregnancy. Nuchal translucency detects 75 to 80% of fetuses with chromosomal abnormalities, with a 5% false-positive rate,[54] while the combined 1st trimester test (NT plus serum free β-hCG and PAPP-A) has detection rate for Down's syndrome up to 91% and for trisomy 13 and 18, up to 91 and 96% respectively.[20-24] In addition to NT, other highly sensitive and specific 1st-trimester markers of trisomy 21 are absence of the nasal bone, increased impedance to flow in the ductus venosus, and tricuspid regurgitation. Detection of these markers between 11 and 13^{+6} weeks of gestation increases the sensitivity rate up to 95% with a false-positive rate of 2%.[55]

Today the introduction into clinical practice of cf-DNA testing has increased detection rate of trisomies 21, 18 and 13 up to 99.5, 95 and 93%, respectively, and decreased false-positive rate and negative results at a level less than 1%. Our view is that the false-positive rate should be added to the above figure, as the method cannot provide results in about 4 to 5% in singleton and around 15% in twins, implying that these women are led to invasive testing. In any case, parents should be fully informed that cf-DNA test is not diagnostic and confirmatory invasive testing is required in the presence of any abnormal results. Furthermore, in the presence of a fetal structural anomaly, the indications for fetal karyotyping and/or microarray

testing should not be modified by a normal NIPT result obtained previously.

With the advent of NIPT, different scenarios for improving prenatal screening offered noninvasive cf-DNA test, either as an alternative to invasive proce dures in high-risk pregnant women or as a screening method in low-risk pregnant women. The following scenarios represent the main options for using NIPT in practice:[56]

- Noninvasive cf-DNA following combined 1st trimester test with cut-off risk 1/200.

Based on this protocol, cf-DNA is offered only to high-risk pregnant women, either due to maternal age, or following combined 1st trimester test and modified risk higher than 1/200. Inserting NIPT as a second test dramatically reduces the need for invasive follow-up testing, thus, making prenatal screening considerably safer. However, with this approach the detection rate will not improve beyond that of combined first-trimester screening, as cases that are initially screen negative will also not be found, as they only could be revealed after an invasive diagnostic procedure and CMA. In addition, the endorsement of safer approach appears to be disputed, as recent studies indicated extremely low procedure-related miscarriage rates (0.22–0.11%).

- Exclusive use of cf-DNA in the prenatal diagnosis.

Following this protocol gives the advantage of detecting more pregnancies with aneuploidy and practically eliminating false reassurance. Furthermore, using NIPT as a first-line prenatal test significantly reduces the number of women who will receive a false alarm.

Major drawbacks of this approach are the failure of early diagnosis of fetal malformations (anencephaly, congenital heart disease, etc.), the relative increase of invasive diagnostic procedures due to increased false-positive results from the implementation of cf-DNA in low-risk pregnancies, and finally, the higher cost than combined test.

- Noninvasive cf-DNA test based on the result of the combined 1st trimester test.

Following this scenario, after a combined 1st trimester test, depending on the results we offer invasive diagnostic procedures if cut-off risk is above 1/150 or cf-DNA if cut-off risk is between 1/150 and 1/1000. Advantages include improvement in the detection rate of fetal aneuploidies, the cost reduction, in addition to reducing the need for invasive follow-up testing.[57] The last protocol was piloted by the British National Health Service and is considered to be more appropriate for the Greek population and recent clinical practice.

In addition, the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)[42] has published a consensus statement, which states that various options should be explained clearly to women, discussing the pros and cons of each, including the expected test performance and the potential adverse effects. If there is a suggestion for further assessment of risk of trisomy 21 and trisomy 13 and 18, the options are:

- Screening methods based on individual risk calculated from maternal age and NT measurement and maternal serum markers (free β-hCG and PAPP-A)
- Invasive testing based on background risk (e.g., maternal age and history of aneuploidy)
- cf-DNA as a first-line screening test.

At the moment, ISUOG proposes screening by combined 1st trimester test. Following this, women can be offered a choice, according to their calculated individual risk of having no further testing, undergoing cf-DNA test, or having an invasive test. Therefore, a proposed protocol can be as follows:

- Cut-off risk above 1/300: Recommended invasive testing (amniocentesis or chorionic villus sampling)
- Cut-off risk between 1/300 and 1/1,000: recommended cf-DNA testing
- Cut-off risk less than 1/1,000: no further action is needed. Inform about cf-DNA test.

The perspectives of new noninvasive methods are immense and the information detected from screening test are changing rapidly. Today and till the cost of cf-DNA would be decreased, the medical community has to address all prenatal screening methods, in the best interests of society.

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