Genetic Screening for Aneuploidy in the Second-trimester

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INTRODUCTION

Even though first trimester screening with nuchal translucency, nasal bone and maternal serum PAPP-A and free βhCG detects most of the fetuses with chromosomal abnormalities, ultrasound screening in the second trimester is also very useful in order to detect the majority of affected fetuses in which diagnosis was missed or delayed. The genetic sonogram, during which a thorough search for sonographic signs of aneuploidy is preformed, can be used to identify fetuses at high-risk for aneuploidy. Combining the genetic sonogram with maternal serum screening may be the best method of assessing aneuploidy risk for women who, for various reasons, delayed the assessment for the second trimester. This permits the identification of 60 to 80% of affected fetuses and it applies equally well to women whose fetuses are at high- and low-risk for aneuploidy.[1] Conversely when these sonographic markers are absent, the age-specific risk of aneuploidy can be readjusted in women at increased risk of caring an abnormal fetus because of age.

The first sonographic sign of Down's syndrome, the thickened nuchal fold, was first described in 1985. During the two last decades, a number of sonographically-identified characteristics -called markers- have been described as associated with Down syndrome and other abnormalities of chromosomes.

Genetic sonogram includes evaluation of fetal biometry and anatomy for detection of fetal anomalies which are commonly found in individuals with aneuploides such as congenital heart defects, duodenal atresia, celebrall effects (holoprocengephaly, ventriculomegaly) hypoplasia of the middle phalanx of the fifth digit with clinodactyly, flat face with maxillary hypoplasia, macroglossia, brachycephaly, hydrops and clubbed foot.

Congenital heart disease is the most common anomaly in infants with DS (44% of the infants with DS have CHD). Antiventricular septal defects are the most common cardiac anomaly with a incidence of 45% in abnormal fetuses, verticuloseptal defects in 35%, isolated secundum atrial septal defects in 8%, persistent pattern ductus arteriosus in 7% and isolated tetralogy of Fallot in 4%.[2] The 99% of newborns with trisomy 18, the 90% of newborns with trisomy 13 and 50% of newborns with trisomy 21 have congenital heart disease (CHD) and especially ventricular septal defect. Since heart structural anomalies are such a common problem in fetuses with aneuploidy, the prenatal diagnosis of congenital heart disease (CHD) increases the fetal risk for aneuploidy. When prenatal diagnosis of CHD is found in association with other fetal structural malformations, the incidence of fetal chromosomal abnormalities is 60 to 70% while when isolated CHD is found, the incidence of aneuploidy is 15 to 20%. On the contrary when the cardiac ultrasound examinations with no abnormality, the prior risk for aneuploidy should be decreased significantly.

Non-structural markers (soft markers) include increased nuchal fold thickness, renal pyelectasis, choroid plexus cyst, hyperechogenic bowel, echogenic intracardiac focus and extremity shortening (humerus or femur). These markers are often transient but they can usually be detected during the second trimester. Although soft markers are more common than major or structural abnormalities in fetuses with aneuploidy, they are non-specific since there are also present in fetuses without abnormalities. Major abnormalities are observed in fewer than 25% of affected fetuses whereas one or more soft markers may be observed in 50% of the affected fetuses. Due to their high prevalence soft markers are invaluable for detection of fetal trisomy 21 among high-risk women in whom high sensitivity is desirable. But the false positive rate may be high (13 to 17%) if any one of soft marker is detected among low-risk women, since specificity is quite low. Sonographic markers can be an isolated finding or associated with other structural abnormalities and the risk of aneuploidy such as Down's syndrome increases with the number of markers present. Even though the use of soft markers alone does not hold the ideal characteristic for a screening test, it is a helpful tool in assessing the individual risk for aneuploidy especially in women aged more than 35 years or in correlation with other screening test such as maternal serum biochemistry in the second or the first trimester of pregnancy.

SOFT MARKERS

Nuchal Fold

Sonographic measurements of nuchal thickness are obtained at the level of the posterior fossa on a modified transverse view of the fetal head which includes the thalami and the cerebellum hemisphere as well as the occipital bone (Figs 1A and B). The soft tissue thickness posterior to the occiput is measured from the outside of the occipital bone to the outer skin edge with measurements of > 6 mm considered abnormal.[3,4] The gestational age at which the nuchal fold is measured is definitely an important issue. The nuchal fold is a transient finding which may fluctuate even within the second trimester and this marker does resolve overtime. A thickened nuchal fold can be appeared one week and gone next with normal follow-up measurements still resulting in a fetus with DS. Nuchal thickness was the first sonographic marker associated with an increased risk for fetal DS and now is accepted as the single most sensitive and specific marker for the detection of DS in the second trimester in high- and low-risk pregnancies. It has being suggested that a nuchal fold of > 6 mm was associated with a marked increase in the risk of DS and several investigators recommended that nuchal fold measurements become a part of every scan performed in this period of the second trimester.[5] It has been shown that a normal nuchal fold in a high-risk patients can decrease the risk of an affected fetus both in patients with abnormal triple test and in patients of advanced maternal age.[6,7] The nuchal fold is the single most sensitive and specific finding leading the list for detecting fetuses at risk for DS although the combination of several markers together has resulted in the most effective method of detecting affected fetuses. Nuchal fold measurement is a useful marker for the detection of fetuses at risk for Down's syndrome.

Echogenic Intracardiac Foci (EIF)

Echogenic intracardiac focus (golf ball) is defined as a small bright area with the same echogenity as bone within the fetal heart ventricles. It is most commonly found in the left ventricle (Figs 2 and 3) but occasionally bilateral or right-sided located below the mitral or tricuspid valves. Echogenic intracardiac foci represent microcalcification in the papillary muscle or chordae tendinae of the fetal heart in the second trimester.[8] During the routine examination of the four chamber view of the fetal heart, these foci are seen to move synchronously with the valve leaflets throughout the cardiac cycle. Recently, it has been confirmed that EIF is caused by mineralization within the papillary muscle but the cause of such changes remains unknown.

Echogenic intracardiac focus is the most recent and the most controversial of the soft markers, because is a subjective finding and its prevalence is influenced by a variety of factors, including
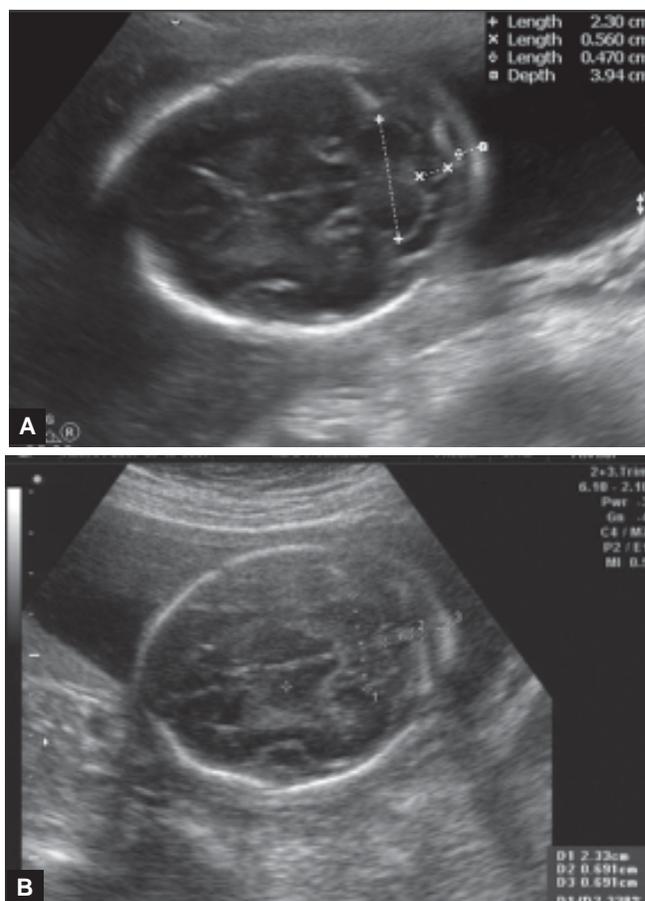
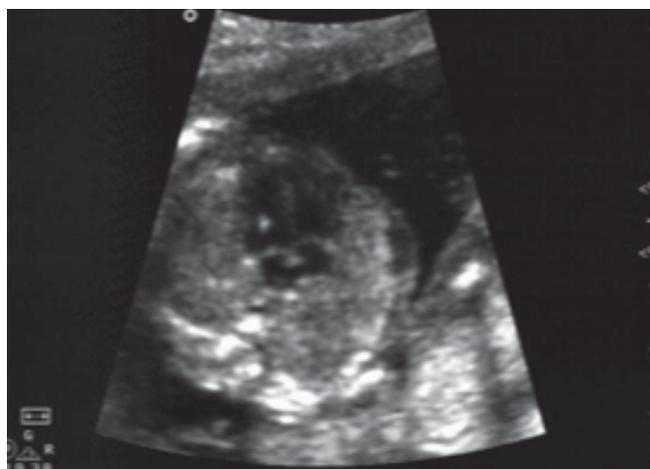**Figs 1A and B:** Measurement of nuchal fold normal (A) and increased (B)

Fig. 2: Echogenic intracardiac focus (golf ball) in the left ventricle

the resolution of the sonographic equipment, the technique and the sonographer's experience and the maternal ethnic background. Fetal position is also important and EIF is best
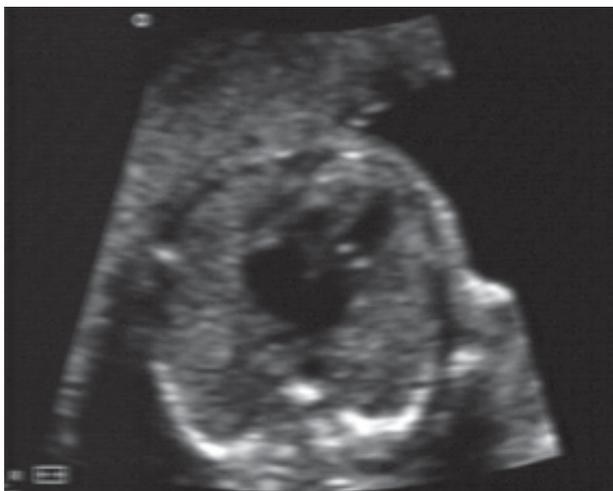
**Fig. 3:** Echogenic intracardiac foci (golf balls) in the left ventricle

visualized when the cardiac apex is oriented towards the transducer. The association between aneuploidy and EIF was first seen in early 90's and now is well established.[9,10] This finding is neither sensitive nor specific since less 20% of aneuploid fetuses as well as 5 to 10% of normal fetuses display this sonographic marker. EIF is not associated with congenital heart defects in chromosomally normal fetuses. The prevalence is influenced by experience and maternal ethnic background.[11,12] Whether or not low-risk woman should undergo invasive testing because of the presence of a fetal EIF, remains controversial.[4] Isolated echogenic intracardiac focus carries a relative risk of two times the prior risk for the presence of DS.[13,14] The prevalence of this marker in high- and low-risk pregnancies is between 3 to 5% making this finding the most commonly detected ultrasound marker.

Nyberg et al[15] confirmed that EIF is associated with an increased risk of trisomy 21, although as an isolated marker this association just reached statistical significance. Two independent studies in high-risk population showed that the risk of aneuploidy in fetuses with isolated EIF is about four times greater than those with normal scan.[16,17] In a total of 5480 women undergoing amniocentesis, the prevalence of EIF was 4.6% (253 cases) and trisomy 21 was diagnosed in 23 cases (9.1%) of the 253 fetuses with EIF and in 69 (1.3%) of the 5227 without EIF.[9,16-18] In studies of low population such an association was not confirmed. In a study of 9263 low-risk pregnancies, 153 fetuses had EIF and none of them had trisomy 21.[19] Bromley et al[20] have shown that the risk of aneuploidy is also increased when EIF is found in fetuses of low-risk pregnant women. Nevertheless, on the basis of current literature there is a strong evidence to support the fact that EIF is more prevalent in fetuses with chromosomal defects specifically trisomy 13 and trisomy 21. The prenatal detection of EIF might be potentially significant as a marker for aneuploidy. In high-risk population,

an isolated EIF is associated with an increased risk of trisomy 21, whereas in the low-risk population the risk is considerable lower than the risk of fetal loss associated with amniocentesis. When a EIF is identified a detailed scan for other signs of aneuploidy must be undertaken. In the absence of associated sonographic findings suggestive of fetal DS in low-risk women, the risk of an affected fetus when an isolated EIF is found, remains lower than the risk of fetal loss associated with an amniocentesis.

Choroid Plexus Cyst (CPC)

Cysts of the choroids plexus in the fetus usually present in the second trimester as a solitary unilateral and simple round anechoic structure within the choroids plexus of the lateral ventricle (Figs 4A and B). Most of the cases are detected incidentally at routine second trimester anomaly scan. They can be multiple, bilateral or complex. The prevalence of these cysts in the second trimester is approximately 1% and more than 25%
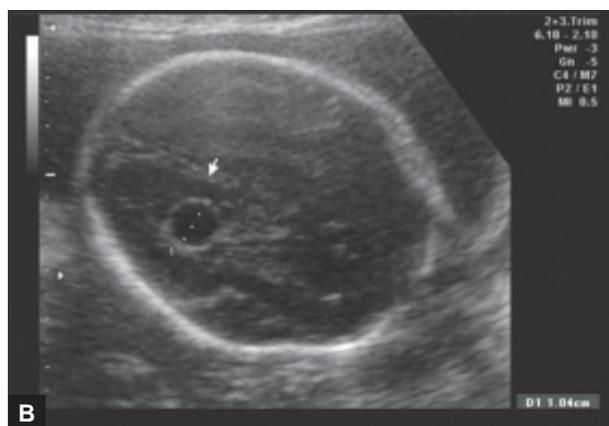
Figs 4A and B: Cysts of the choroids plexus

disappear before 26 weeks. The observation that most of the fetuses with CPC had trisomy 18 demonstrating that CPC can be the only marker in a trisomy 18 fetus.[21,22] Despite the large number of cases of CPC reported, there is no consensus regarding the definitive role of this ultrasound marker in the prenatal detection of aneuploidy. There is agreement that the ultrasound characteristics of CPC such as bilaterality, size, number, complexity and resolution are not related to the risk of aneuploidy. Once a CPC has been identified, a detailed fetal anatomical survey is needed. Concerning the need for amniocentesis in cases which chorionic plexus cysts are found it was concluded that if the cyst is isolated the risk of trisomy 18 is marginally increased and maternal age should be the main factor in deciding whether or not to offer fetal karyotyping.[23, 24] In all cases a detailed ultrasound examination of the fetus should be mandatory to exclude any additional abnormal findings.

Fetal Echogenic Bowel

Fetal echogenic bowel is not a truly pathological condition, but rather a non-specific ultrasound feature associated with a number of fetal pathological conditions of which chromosomal defects and cystic fibrosis (CF) most well-known. Fetal echogenic bowel is diagnosed when the fetal small bowel seems as echogenic as the surrounding bone in the second trimester fetus (Fig. 5). The main limitation to the use of FEB as an ultrasound marker is the fact that the diagnosis is highly subjective and attempt to grade the echogenicity of fetal bowel have abandoned due to the subjective nature of the findings. Different pathological conditions such as trisomy 21, cystic fibrosis, severe early onset of IUGR, thalassemia, intramniotic bleeding, gastrointestinal obstruction, mesenteric ischemic, perinatal death and cytomegalovirus infections have been associated with second trimester hyperchoid bowel.[25-27] Poor perinatal outcome has been noted in 1/3 of pregnancies with FEB including chromosomal defect in 9% of the cases, cystic fibrosis in 2%, congenital infection in 3% and IUGR in 8%.

Once the diagnosis of FEB is made, a detailed ultrasound examination of the fetus is warranted for other markers for aneuploidy to excluded intramniotic bleeding. If no associated findings are detected the option of perinatal karyotyping should be discussed with the parents.[27] While this is not a particularly sensitive marker for DS, it is present in only 0.6% of the second trimester fetal population, hence the identification of this marker should lead to consideration of karyotyping particularly if accompanied by any other sonographic findings.[8] If prenatal karyotyping is performed, fetal DNA testing for cystic fibrosis mutations should be considered. Routine screening for infection has not been advocated in view of the low yield and because most affected fetuses have additional findings suggestive of congenital infections.

Fig. 5: Fetal echogenic bowel

Mild Ventriculomegaly

Mild Ventriculomegaly (MV) is defined as a ventricular width of 10 mm or greater[28] at the level of the atrium (Fig. 6). A practical decision was made to include this as an anomaly rather than as a marker. Mild Ventriculomegaly including Isolated Mild Ventriculomegaly (IMV) is the most common brain abnormality found on prenatal ultrasound. The incidence of chromosomal abnormalities varied from 3 to 12.6% (mean 9%) in cases with MV and 40% have other findings while in cases with IMV the incidence is decreased to 4%. It has been suggested that the majority of fetuses with MV and abnormal karyotyping will have additional sonographic abnormalities.[29]

Fig. 6: Mild pyelektasis

Mild Pyelectasis

Dilatation of the fetal renal pelvis (pyelectasis), was defined as an anterioposterior diameter > 4 mm of fetal renal pelvis at 16 to 20 weeks, > 5 mm at 20 to 30 weeks, > 7 mm at 30 to 40 weeks of gestation.[30] Pyelectasis may be unilateral of bilateral, but is more frequently reported as bilateral. There is also a marked sex difference with a male: female ratio of around 2:1.[31] Mild pyelectasis is commonly detected in the second trimester fetus and is associated both with an increased risk of chromosomal abnormalities and of urinary tract pathologies. The association with aneuploidy remains nuclear but it seems likely that mild pyelectasis is a marker which will alter prior risks. Pyelectasis was described in association with trisomy 21, 13 and 18 and Benacerraf reported seven fetuses (3.3%) with DS from a selected population of 211 fetuses with hydronephrosis. It also has been shown that the risk is higher when pyelectasis is associated by other abnormalities.[30]

Chudleigh et al[32] has shown in a study of 737 fetuses with mild pyelectasis from an unselected population of 101,600 births, that when pyelectasis was present as an isolated finding, in woman < 36 years of age, the risk of aneuploidy was 0.33% and 2.2% in women > 36 years. Another approach has been to estimate the increased risk due to mild pyelectasis and adjust the prior risk by this factor. It has been suggested that isolated mild pyelectasis confers a risk of trisomy 21 which is 1.5 times the background risk.[33]

Mild pyelectasis is a soft marker to be used only in combination with other markers. Isolated pyelectasis in a low-risk patient should not warrant an amniocentesis.[34] The detection of perinatal pyelectasis is an indication for detailed genetic sonogram looking for extra renal anomalies, other markers of aneuploidy and the urogenital system should be examined carefully and care should be taken to exclude duplex system, multicystic dysplastic kidney, and to examine the bladder.

Other Sonographic Markers

There are some other features of fetuses with Down's syndrome. However, these may not be either sensitive or specific enough to be helpful for sonographic screening and they are difficult technically to evaluate accurately. Individuals with Down's syndrome have short ears but the measurement is difficult to accomplish accurately and there is an overlap between normal and abnormal. Hypoplastic or absence of the middle phalanx of the fifth digit is known as a finding among neonates with DS and together with clinodactyly they can be demonstrated in early second trimester. This marker is difficult to measure and the false positive rate is high as 18% for using this finding as a screening tool for DS.[35] Recently there is evidence to suggest that delayed fusion of the amnion and chorion is associated with an increase incidence of DS.[36] This finding is rare, occurring only occasionally in fetuses with DS and in normal fetuses. The separation of the great toe (sandal gap foot) is occasionally detected in utero and to date is not useful in the screening for fetal DS. Some other fetal biometric parameters have been investigated as potential indicators of fetal trisomy 21. They not have the same efficacy for detecting DS. Femur was considered shortened when the measured to expected ratio was 0.91 or less. The overall sensitivity was 29% and specificity 92 %.[37] The short humerus (measured to expected ratio 0.89 or less) has sensitivity 31% and specificity 95% while short femur and humerus has 33% sensitivity and 95% specificity.

Scoring Index

It has been proved that combination of multiple sonographic markers has a better detection rate in terms of risk of aneuploidy than any single marker and that this is the most sensitive method for screening low-risk populations. Several authors have presented different sonographic scores in order to achieve the higher detection rate in combination with an acceptable false positive rate.

In 1993, Benacerraf et al tried to evaluate the ability to identify fetuses with autosomal trisomy using certain second trimester sonographic features in the form of a scoring system.[38] In this system major abnormalities and thickened nuchal fold both had a score of 2 and even when they are found isolated there is a strong indication for amniocentesis. Other soft markers such as short femur and humerus, pyelectasis, echogenic fetal bowel, echogenic intracardiac focus, choroid plexus cyst had a score of 1, since they can be commonly found in euploid fetuses. Using this scoring system and defining as cut off point for amniocentesis any score equal or higher than 2, 33 fetuses with Down's syndrome (73%), 11 (85%) with trisomy 18, two (100%) with trisomy 13, and four control fetuses with abnormality (4%) were identified. The positive predictive value in patients in 1/250, 1/500, and 1/1,000 risk groups was 7.2%, 3.7%, and 1.9% for identification of a fetus with Down's syndrome. The authors concluded that this sonographic markers' scoring system seem to be sensitive for the detection of chromosomal abnormalities in younger women to whom amniocentesis was not routinely offered. They also added that it may be possible to combine the scoring system with maternal age and the triple panel marker serum evaluation to refine further the prediction of risk of aneuploidy for both young and middle-aged women and thereby to perform fewer amniocenteses with a much higher sensitivity and positive predictive value in detection of aneuploidy. Two years later (1995) the same group in another paper[39] concluded that the use of ultrasound-adjusted risks for fetal trisomy 21, is expected to decrease the number of amniocenteses in older women when a genetic ultrasound examination does not demonstrate any abnormal ultrasound markers while still identifying the majority of fetuses with Down's syndrome. In women over 35 years of age, a threshold

of 1 was used as a positive criterion to optimize the identification of affected fetuses. This results in a sensitivity of 83% and a false positive rate of 13% respectively. The same scoring system can be used to decrease the risk of trisomy 21 in patients at risk for an affected fetus either because of advanced maternal age or abnormal triple screening. Women of 40 years of age or older with a genetic sonogram score of 0 were advised to undergo amniocentesis since the score 0 could not reduce the patients age-specific risk below the commonly accepted threshold for offering amniocentesis. Women between the age of 35 and 39 however can benefit from the genetic sonography. It has been suggested, to offer genetic sonogram as an option for women who were at increased risk for having fetuses with aneuploidy but who did not wish to undergo invasive testing without additional information.

Nyberg et al in 1998 developed another scoring system based on maternal age and the likelihood ratios of the various sonographic markers.[34] Major structural anomalies, nuchal thickening echogenic bowel, short humerus, short femur, echogenic intracardiac focus and pyelectasis have likelihood ratio of 25, 18.6, 5.5, 2.5, 2.2, 2.0 and 1.5 respectively. Each likelihood ratio can be multiplied by the maternal age-based risk to revise the individual risk of the woman. They found that using one or more sonographic markers allowed identification of 68% of fetuses with DS with false positive rate of 12.5%.

Vintzileos et al[40] determined that the best combination of sonographic markers for detecting affected fetuses, were the thickened nuchal fold, pyelectasis and short humerus, resulting in a sensitivity 89% with false positive rate 6.7%. It also has been demonstrated that a normal ultrasound could reduce the risk of DS by a likelihood ration of 0.4. They reported that the yearly utilization of the genetic sonogram rather that amniocentesis were 0.4% in 1993, 16.6% in 1994, 48% in 1995 and 55% in 1996. They showed that women are more accepting of the genetic sonogram and over half of women presenting to the ultrasound laboratory because of advanced maternal age or abnormal triple test screen, choose to have the genetic sonogram before decide whether or not to have amniocentesis. In addition to the combination of sonographic markers with maternal age, the triple biochemical screen may also add information to the specific risk that a woman has a fetus with DS before a woman makes a decision to undergo amniocentesis. In a study by Yagel et al (1998) evaluating women less than 35 years of age with abnormal triple screen results, the sensitivity for detection of DS using sonographic markers was 80%.[41]

A multicenter study was undertaken to evaluate the diagnostic efficacy of the genetic sonogram.[42] Of a total of 176 fetuses with DS, 125 (71%) had either an abnormal long bone length (femur, humerus) a major structural abnormality of a DS soft marker. The combined diagnostic sensitivity was 71.6%. The sensitivity of individual markers varies between 3% (sandal gap) and 46.5% (nuchal fold thickness). The authors concluded that the genetic sonogram can be used to better adjust the Down's syndrome risk for high-risk patients.

In a recent study Cicero et al[43] demonstrated the methodology of calculating the likelihood ratio for trisomy 21 for some of the ultrasound markers and the process of sequential screening in the interpretation of results. The authors noted that the most rational approach is to carry out a screening test at 11 to 14 weeks by combining maternal age with sonographic measurement of fetal NT, nasal bone and maternal serum measurement of free β-hCG and PAPP-A. A detection rate of 95% can potentially be achieved with an invasive testing rate of about 2%. Since all women should be offered a second trimester ultrasound scan to identify major fetal abnormalities, such as spina bifida and cardiac defects, the diagnosis of major and or minor defects, including nasal bone hypoplasia, will potentially lead to the detection of more than 70% of the remaining per cent cases of trisomy 21. They also added than it is likely that only nasal bone hypoplasia, nuchal edema and the presence of multiple other second trimester sonographic markers will be associated with sufficiently high likelihood ratios to reverse a low background risk after first trimester screening.

CONCLUSION

Even though first trimester screening with nuchal translucency, nasal bone and maternal serum PAPP-A and free βhCG detects most of the fetuses with chromosomal abnormalities, ultrasound screening in the second trimester is also very useful in order to detect the majority of affected fetuses in which diagnosis was missed or delayed. The genetic sonogram, during which a thorough search for sonographic signs of aneuploidy is preformed, can be used to identify fetuses at high-risk for aneuploidy. It has been proved that combination of multiple sonographic markers has a better detection rate in terms of risk of aneuploidy than any single marker and that this is the most sensitive method for screening low-risk populations.

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