Original Paper

Benefits of Using Sonographic Markers at the Triple Test Ultrasound

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ABSTRACT ***Introduction:*** Part of the SONOSEROSCREEN PROJECT , we study at the end of the first trimester a group of biochemical and ultrasonographic variables. Regardeless the results obtained, all the patients enrolled in this cohort is subject to a second study in the early second trimester that encompases the dosage of AFP(+/- Ia), β-hCG, uE3 and an ultrasonographic exam targeted at precise biometry and fetal morphology. We tried to evaluate the achievable benefits of assesing several markers that are part of the genetic sonogram and the way they change the risk class. ***Methods:*** The markers we chose to pursue in our project were : nuchal fold, nasal bone, cardiac echogenic foci, short femur, short humerus, presence of echogenic bowel. All present results markers were integrated in a formula that allowed reasesment of the risk as published by DeVore and we followed in which way this affected the decision whether to proceed with the amiocentesis or not. Our choice was limited by the fact that this was a retrospective study and so we were forced to choose markers we have routinely looked for in our previous triple test ultrasound exams. ***Results and conclusion:*** Using the recalculation of risk for every single case we would have been able to reduce the amniocentesis rate by 17% and drastically improve the rate of detection for aneuploidies. However the evaluation of all markers and non-automatic recalculation of risk is time consuming and should be used only for cases with intermediate risk at the triple/QUAD test.

KEY WORDS *triple test, sonographic markers, amniocentesis*

Introduction

Part of the SONOSEROSCREEN PROJECT , we study at the end of the first trimester a group of biochemical and ultrasonographic variables: PAPP-A, HCG, NT measurements and spectral Doppler values at the level of ductus venosus (Arantzius) and at the level of the tricuspid valve. Regardeless the results obtained, all the patients enrolled in this cohort is subject to a second study in the early second trimester that encompases the dosage of AFP, (+/-Ia), β-hCG, uE3 an ultrasonographic exam targeted at precise biometry and fetal morphology.

We tried to evaluate the achievable benefits of assesing several markers that are part of the genetic sonogram and the way they change the risk class. The markers we choose to pursue in our project were : nuchal fold, nasal bone, cardiac echogenic foci, short femur, short humerus, presence of echogenic bowel (tabel 1). Our choice was limited by the fact that this was a retrospective study and so we were forced to chose markers we have routinely looked for in our previous triple test ultrasound exams.

Tabel 1. Sonographic markers

nuchal fold	cardiac echogenic foci
nasal bone	short femur
presence of echogenic bowel	short humerus

Nuchal fold - The excess skin in the fetal neck region which is characteristic of Down syndrome individuals can be observed by ultrasound as either increased nuchal translucency (NT) in the first trimester or increased nuchal skin-fold (NF) in the second trimester(6). A nuchal fold is usually considered abnormal if it is greater than 6 mm between 15 and 22 weeks. Although the measurement can be obtained in a sagittal section of the head and neck, it is more typically obtained in the axial section of the head through the cerebellum. Even an isolated nuchal fold is a significant finding, mainly as a predictor for trisomy 21. Cystic hygroma is a very common finding, occurring in 0.5% of all spontaneous abortions. It is also associated with hydrops in 40 to 100% of cases, with congenital heart defect in 0 to 92% of cases, and with aneuploidy in 46 to 90% of cases. Cystic hygroma can be localized in the back of the neck or may extend farther down the back of the embryo or fetus.

Nasal bone - A small nose is a common facial feature of individuals with trisomy 21. Evidence based on radiologic, histomorphologic, and sonographic studies shows that nasal bone abnormalities are significantly more common in trisomy 21 fetuses than in euploid fetuses. These abnormalities, which include both nasal bone absence and short nasal bone length, can be detected by prenatal ultrasound(10). Sensitivity, FPR, and LR of absent nasal bones for detecting Down syndrome according to Goncalves are 34.6% , 3.7% , and 9.0 (95% CI, 1.3–68.7),

respectively. Sensitivity, FPR, and LR of delayed ossification for detecting Down syndrome are 42.3% , 22% and 1.83 (95% CI, 0.8–4.4). We used for reference NB measurements from the fetuses of white women without any chromosomal abnormality(9). NB hypoplasia was defined either as an absent NB or by NB lengths 0.75, 0.5, and 0.25 MoM for gestational age, respectively.

Table 2. Nasal bone(NB) multiples of the median (www.ajog.org)

GA(week)	NB-Regressed media (cm)	0.75 MoM	0.5 MoM	0.25 MoM
15	0.40	0.30	0.20	0.10
16	0.41	0.31	0.21	0.11
17	0.45	0.34	0.23	0.11
18	0.48	0.34	0.24	0.12
19	0.51	0.38	0.26	0.13
20	0.53	0.40	0.27	0.13

Echogenic bowel - This is the most common echogenic mass in the fetal abdomen. The bowel is considered echogenic only if it is as bright as or brighter than the adjacent bone, namely, the iliac crest. The commonest cause for echogenic bowel is fetal ingestion of blood. This is usually identified as 'floating flakes' in the amniotic fluid and is invariably associated with a history of vaginal bleeding. Some studies have shown association with trisomy 21, especially when associated with absent nasal bone or increased nuchal fold. In some euploid cases it can be associated with cystic fibrosis.

Cardiac echogenic foci - The reported prevalence of EF has been described at 0.5 – 20.3 % , depending on the population and the methodology. Brown et al. were the first to describe the association between echogenic foci and trisomy 21(5). In low-risk populations, EF have been described in 3 – 5 % of cases. 63 , 64 According to a review article (3), in a total of 13 493 pregnancies screened, 334 (2.5 %) of fetuses with EF had been described until that time. .According to a study by Nicoloso&co in 456 (93.8), no association with structural or functional heart abnormalities was seen, and only two fetuses (0.4) were shown to have trisomy 21 (4).

Short femur/ short humerus - It has long been known that children with Down's syndrome have shorter limbs than do normal children. In their study population, Benacceraf et al. observed a cutoff limit of 91% of the expected size of the femur in relation to the BPD, a sensitivity of 68% and a specificity of 98% in detecting fetuses with trisomy 21. These rates were not confirmed in other studies, although the trend clearly exists and has been extended to the finding of a short humerus.

Materials and Methods

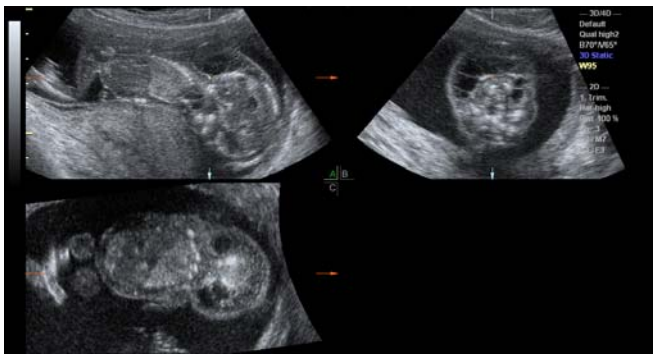
We tried to evaluate the achievable benefits of assesing several markers that are part of the genetic sonogram and the way they change the risk class. The markers we choose to pursue in our project were : nuchal fold, nasal bone, cardiac echogenic foci, short femur, short humerus, presence of echogenic bowel. All present results markers were integrated in a formula that allowed reasesment of the risk as published by DeVore (13) and we followed in which way this affected the decidion whether to proceed with the amiocentesis or not. Our choice was limited by the fact that this was a retrospective study and so we were forced to chose markers we have routinely looked for in our previous triple test ultrasound exams.

In the evaluation of sonographic markers we registered the folowing results:

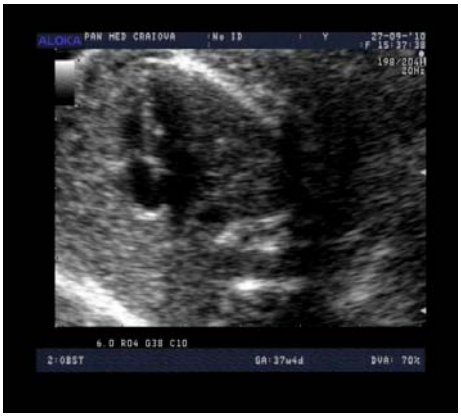
Table 3 . Sonographic markers results

nuchal fold	23
cardiac echogenic foci	103
presence of echogenic bowel	20
nasal bone	43
short femur	150
short humerus	48

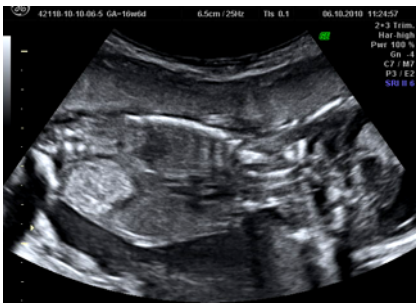
Fig 1. Images of sonographic markers assesments



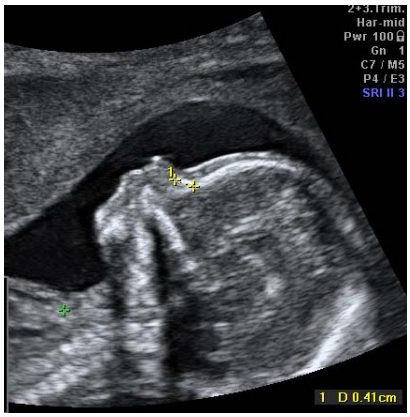
a. Cystic hygroma



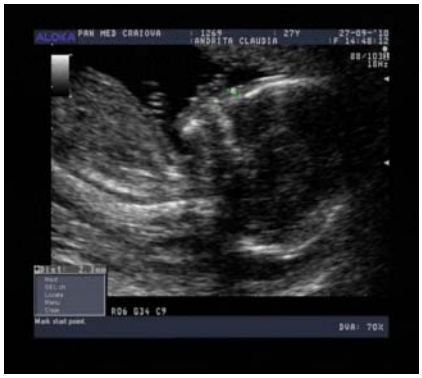
b. cardiac echogenic foci



c. echocenig bowel



d. nasal bone normal



e. hypoplastic

The LR (likelihood ratio) for our markers were provided by studies of Nicolaides(8) and Goncalves(9).

Table 4 . Sonographic markers likelihood ratio

Nuchal fold	9.8
Absent nasal bone	9.0
Hipoplastic nasal bone	1.83
Presence of echogenic bowel	3.0
Cardiac echogenic foci	1.1
Short femur	1.6
Short humerus	4.1

The triple test (TT) risk value was retrospectively reassesed as follows according to markers likelihood ratio. For example:

Single abnormal ultrasound finding

Situation : TT risk 1/300

Abnormal marker: echogenic bowel

Calculation of risk:

1. Divide 1/(300-1) = 0.0033
2. Multiply the TT risk with the markers LR (3.0)
3. Calculation: 0.0033*3.0=0.01
4. Divide 1/0.01= 100
5. The new risk for T21 is 1 in 100

Two independent ultrasound findings

Situation : TT risk 1/300

Abnormal markers: hypoplastic nasal bone, short femur

Calculation of risk:

1. Divide 1/(300-1) = 0.0033
2. Multiply the TT risk with the markers LR (1.83*1.6)
3. Calculation: 0.0033*1.83*1.86 =0.0096
4. Divide 1/0.0096= 104
5. The new risk for T21 is 1in 104

No abnormal ultrasound finding

Situation : TT risk 1/300

Abnormal markers: none – the LR following a normal study was 0.22 (modified after DeVore LR with exclusion of cardiac anomalies assesment)

Calculation of risk:

1. Divide 1/(300-1) = 0.0033
2. Multiply the TT risk with the normal LR (0.11)
3. Calculation: 0.0033*0.22 =0.0007
4. Divide 1/0.0007= 1428
5. The new risk for T21 is 1in 1428

Results

The triple test was done in 2787 cases either as a second net in adjuction to the combined test or for a specific reason (high risk class in the combined test, Down syndrome in the family or a previous child with Down's, age over 37 or at the patient's request).The cut-off for amniocentesis was established at 1/250. The results were as follows:

Table 5.Triple test results

	Triple test
Positive Test	266 (9,54%)
Negative Test	2521(90,46%)
Total	2787

All subjects were reasessed after amniocentesis or fetal evaluation at birth.

Table 6. Triple test results clasification after amniocentesis or neonatal evaluation

	Triple Test positive	Triple Test negative	TOTAL
T21 confirmed	19	3	22
T21 negative	247	2518	2765

The distribution of markers according to the triple test results and final diagnosis of Down was as follows:

Table 7 . Sonographic markers incidence in euploid and aneuploid fetuses

MARKER	Trisomy 21	Normal
nuchal fold	7/22	16/2765
cardiac echogenic foci	7/22	96/2765
presence of echogenic bowel	4/22	16/2765
nasal bone	14/22	27/2765
short femur	9/22	141/2765
short humerus	7/22	41/2765

The modification of cutt-off for every case would have resulted in the following change of results : 2 more cases of trisomy 21 would have been confirmed and the number of amniocentesis done on euploid fetuses would have been reduced by 42 (17% reduction).

Table 8. Corrected triple test results clasification

	T21 confirmed	**Normal**
TT+&sonographic markers(Triple Test positive)	21(19)	205
TT- &sonographic markers(Triple Test negative)	1(3)	2560
TOTAL	**22**	**2765**

Discussions

Medical studies in which ultrasound evaluation of fetal anatomy has been used to identify fetuses with Down syndrome have focused on individual features of Down syndrome. Individual ultrasound markers which have been studied including those in our report (nuchal fold, nasal bone, cardiac echogenic foci, short femur, short humerus, presence of echogenic bowel) did not demonstrate a detection rate for Down syndrome greater than maternal triple marker serum screening, however providing a supplemental information in managing an informed decision regarding the need for an amniocentesis. The modification of cutt-off for every case would have resulted in reducing the number of amniocentesis done on euploid fetuses by 42 (17% reduction).

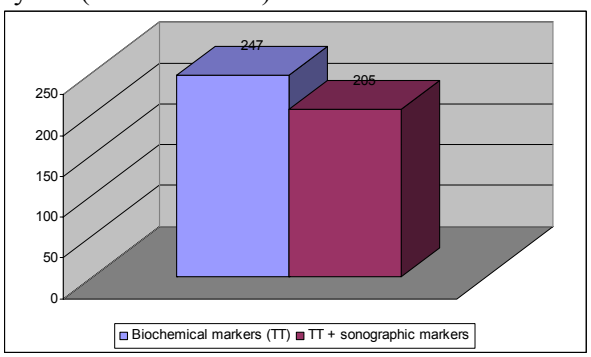


Fig 2. Amniocentesis indication with and without sonographic markers

A study done by DeVore showed that using a B-Mode ultrasound, without examining the fetal heart, detected 60% of fetuses with Down syndrome. However using B-Mode ultrasound plus color Doppler ultrasound to examine the fetal heart in greater detail identified 91% of fetuses with Down syndrome (12). Due to the nature of our study (a retrospective analysis) only certain markers could be included so a complete evaluation of the fetal heart was not part of the corrected score. This was probably the main reason for which our results had a less dramatic effect on the reduction of the number of amniocentesis. At the present time Genetic Ultrasound is an accepted methodology that can be used to determine the risk for Down syndrome. A recent publication from the American College of Obstetricians and Gynecologists entitled the ACOG Practice Bulletin, "Ultrasound in Pregnancy" (Num 58, December 2004)(11), states: "A second-trimester specialized ultrasound examination may be targeted to detect fetal aneuploidy (Down syndrome). This type of examination has been offered in some centers for the past several years and is aimed at the detection of a range of minor anatomic features associated with an increased risk for fetal aneuploidy…. The standard examination is less likely to detect the minor anatomic features associated with aneuploidy."

Conclusion

Using the recalculation of risk for every single case, we would have been able to reduce the amniocentesis rate by seventeen percent and drastically improve the rate of detection for aneuploidies. However the evaluation of all markers and non-automatic recalculation of risk is time consuming and should be used only for cases with intermediate risk at the triple/QUAD test.

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