Prenatal Aneuploidies Computerized Screening (SCA TEST): a pilot study on 1000 women

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Summary

The SCA-TEST, Prenatal Aneuploidies Screening, is an innovating program with very articulated and differentiated calculation potentials. It is a software which allows executing a sequence-like national screening involving the ultrasound study of the first and second trimester. The program enables to execute a complete and different-levels combined screening, through very sophisticated mathematic analysis methods. In particular, it enables to make: a first trimester screening combining it with nuchal translucency and biochemical parameters of free beta-hCG and PAPP-A; a second trimester screening by the evaluation of up to 6 biometric parameters (biparietal diameter, cranial circumferentia, femur, humerus, pyelectasis and plica nucalis), and up to 7 associated morphologic parameters (hyperechogenic bowel, cardiac foci, interventricular defect, pericardial effusion, tricuspid valve regurgitation, right/left heart disproportion, and structural abnormalities).The purpose of the study was to present the performance of the SCA TEST in the second trimester of pregnancy through the evaluation of a prospective study performed in the period between April 2007 and December 2007 on 1000 women who underwent the SCA TEST followed by amniocentesis. Studying all the cardiovascular and non-cardiovascular markers, SCA TEST made it possible to identify 62.5% fetuses affected by Trisomy 21 with a specificity of 94.6%, and a 5.4% of false positive. Considering only women older than 35 years the detection rate reaches 80% with a 7.8% of false positive. The statistical analysis confirmed that the second trimester screening gives essential information regarding the aneuploidia risks in particular in high risk women, and in those who did not perform first trimester screening.

KEY WORDS: second trimester, sonography, ultrasound, Down syndrome, trisomy 21, fetus, SCA TEST.

Introduction

Obstetrician ecography developed during the last ten years in an extraordinary way. In particular the high definition of images that can be obtained today thanks to new technologies, give increased quality and quantity information so to direct many obstetrician ecographistes almost exclusively in this diagnostic field. Ecographic prenatal diagnosis represents an essential method which must be correctly used during the second trimester screening, in particular, in order not to generate anxiety and false reassurances. For the first time the SIDiP (Italian Society of Prenatal Diagnosis and Fetal Maternal Medicine) developed the SCA TEST (Prenatal Aneuploidies Screening Test), a software born to respond to the advanced demand of ecographists that screen the first and second trimester of pregnancy. The software is the result of a multidisciplinary work that involved engineers, obstetricians and statistical mathematicians during those years, having as reference international literature and adapting it to the Italian population. The SCA TEST enables to execute a complete and different-levels combined screening, through very sophisticated mathematic analysis methods. In particular, it enables to do: the morpho-biometric screening of the first trimester of pregnancy through the ultrasound evaluation, up to 3 biometric parameters (crown-rump length, nuchal translucency, fetal heart rate), and up to 2 morphologic parameters (nasal bone and tricuspid valve regurgitation), and to combine the above results with biochemical parameters of free beta-hCG and PAPP-A. In the second trimester the SCA TEST enables to perform: the morpho-biometric evaluation, up to 6 biometric parameters (biparietal diameter, cranial circumferentia, femur, humerus, pyelectasis and plica nucalis), and up to 7 associated morphologic parameters (hyperechogenic bowel, cardiac foci, interventricular defect, pericardial effusion, tricuspid valve regurgitation, right/left heart disproportion, and structural abnormalities). The SCA TEST gets into the specialists laboratory through a precise procedure aimed to ensure its quality. The test which is proposed to patients is under the didactic shield and the scientific control of the SIDiP. The qualifying element of the SCA TEST program is the system of the automatic audit. The specialist who executes it is submitted to periodic checks. Each year, data obtained from the activity of each single operator, will be put together and processed by the SIDiP coordination group to check if the sampling executed by each operator diverges from the natural frequency of the phenomenon. In this case, the abnormality is communicated to the operator. This procedure allows to monitor the results of the tests execution and to enlarge a database with a confident scientific value. In that way, a work network is created at a national level. It is made up by pro-

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fessionals trained by specific training programs which enable to the use of the software, which, in the exercise of their professional specificity, contributes crucially to the scientific research in an extremely delicate sector such as that of the fetal aneuploidies screening.

SCA TEST: morphological detections

PLICA NUCALIS (NP): NP represents a subcutaneous deposit of fluid in the retro-occipital region. In the second trimester of pregnancy, the nuchal translucency disappears and a NP is observed in its place (Fig.s 1, 2). Sometimes, in such an age, an abnormal deposit of retronuchal fluid persists and it can show as: cystic hygroma, cystic, septate bilateral aspect; more often it is associated to the Turner syndrome; nuchal edema: associated with trisomies, cardiovascular and pulmonary defects, congenital infections, genetic syndromes, skeletal dysplasias. The measurement of the NP is considered the most sensitive and specific sign of fetal chromosomal abnormalities in the second trimester increasing the risk for about 11 to 17 times according to the authors (1-7).

HYPERECHOGENIC BOWEL (HB): HB is present in 0.1-1.8% of fetuses at the second trimester of pregnancy and recognizes different causes among which a previous intraamniotic hemorrhage, a severe uteroplacental insufficiency, the cystic fibrosis. Finally, chromosomal pathologies are found in 7% of cases if the injury is isolated and in 42% of cases if it is associated to other dysmorphologies (8). Following the method proposed by Slotnick (9), a HB is a bowel whose echogenicity, notwithstanding the progressive decrease of the gains of the instrument used, disappears contemporarily or after the echogenicity of the fetal iliac bone taken as reference point (2 or 3 Slotnick degree) (Fig. 3).

HYPERECHOGENIC CARDIAC FOCI (HF): HF is defined as a calcification of the papillary muscle of the left ventriculus (Fig. 4). The most frequent localization is on the papillar muscle of the bicuspid valve (60%), then multiple foci in the left ventriculus (16%), right ventriculus (7%) and in both ventriculi (16%). The data of the international literature are not unequivocal. Indeed, the incidence of isolated HF diagnosed by ultrasonography, varies from 1.1 to 9.6%. A recent work by Anderson shows a prevalence of the isolated hyperechogenic focus equal to 1.6% in women younger than ≤ 35 years and 1.8% in women ≥ than 35 years (10). There is still a controversy also about the capacity of a HF to identify fetuses with Trisomy 21 in high and low-risk patients, notwithstanding the incidence of about 11% of HF in the fetuses affected by Down Syndrome. De Vore asserts that an isolated H.F. is present in 11.9% fetuses affected by Down Syndrome versus 0.88% of healthy fetuses with a likelihood ratio of 1.94 and a risk of Trisomy 21 increased by 1.94 times than the basis risk (11). A meta-analysis by Smith-Bindman [7] has concluded that the

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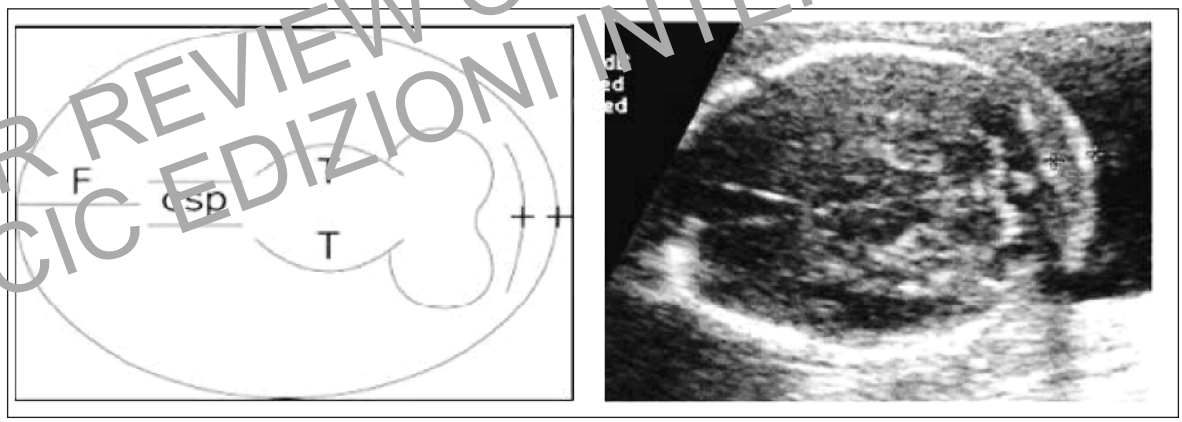

Figures 1 and 2 - Transversal scanning of the fetal cranium.

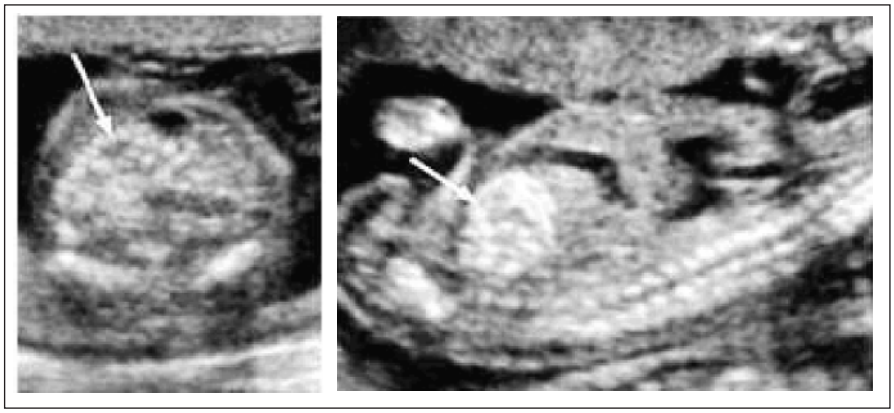

Figure 3 - Intestinal hyperechogenicity: degree 2 (on the left) and degree 3 (on the right).

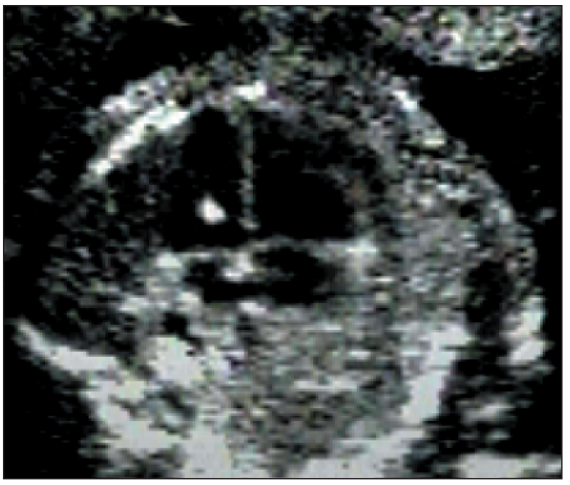

Figure 4 - Heart hyperechogenic focus.

identification of an isolated HF during a sonography of the second trimester, should not been considered as a Trisomy 21 marker , and such an assertion is found also in the work by C. Coco and Jeanty (12). Therefore, the isolated report would not require the execution of an Invasive Prenatal Diagnosis.

VENTRICULAR SEPTAL DEFECTS (VSD): VSD represents 30% of heart congenital dysmorphologies and, with an incidence of 2 to 6 on 1000 newborns, is one of the heart abnormalities most frequently observed (13, 14). The VSD represents the consequence of an incomplete formation or a lack of fusion of the muscular or perimembranous components. The most serious VSDs needed a surgical approach (15, 16). The VSD can be observed alone (70-80%) or in association to congenital heart defects (20-30%) (17). In case of aneuploidies, an increased incidence of cardiopathies (25%) (18) is widely documented in literature (19, 20). In particular, it was observed that the presence of congenital cardiopathies is strictly associated to the increase in the nuchal translucency thickness (NT). The observed incidence was of 55% of cases with a sensitivity for NT values>95° centile of 56% and a specificity for the same NT values of 93.8% (21). In this case, intraventricular defect has been associated to a higher incidence of chromosomopathies and in particular of the trisomy 21 (Down Syndrome), the trisomy 18 (Edwards Syndrome) (22), and the deficiency of the chromosome 22q (23). In Down Syndrome, the incidence of congenital cardiopathies ranges from 40 to 60% according to the different literature studies (19, 20, 24-26), (Fig.s 5-7).

PERICARDIAL EFFUSION (PE): the PE, is a deposit of fluid which takes origin from the atrial-ventricular junction and extends through the apex of the ventriculus, determinating a separation of the pericardium from the epicardium higher than 2 mm during the systole. The PE

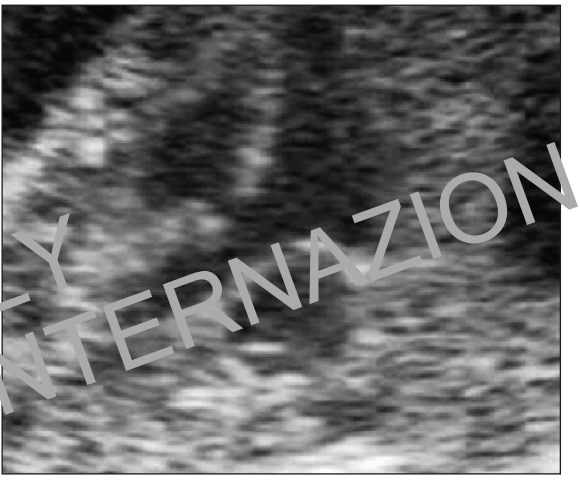

Figure 6 - Left long axe: small defect of the perimembraneous portion, lack of septum-aortal continuity.

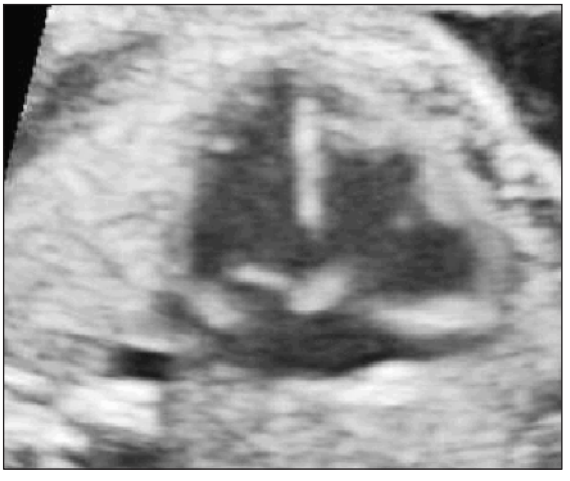

Figure 5 - Four-chambers scanning: small defect of the intraventricular septum of the perimembraneous portion.

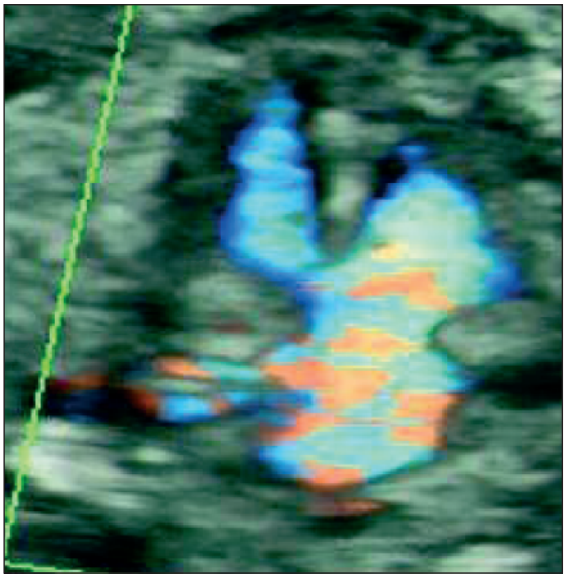

Figure 7 - Intraventricular defect: communication between both ventriculi well highlighted by the color-Doppler.

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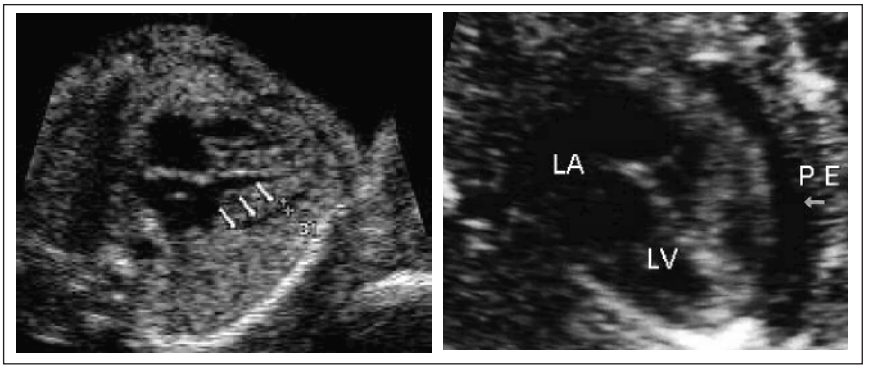

Figure 8 - (D1) normal pericardial fluid; (PE) pericardial effusion.

represents an independent entity in comparison with the rima of pericardial fluid (Figs. 8, 9) which can be normally shown during the fetal echocardiography (27-31). It is also known that in most cases, the pericardial effusion resolves spontaneously (32). The conditions in which the pericardial fluid deposits are: left cardiac hypoplasia, teratoma, rhabdomyoma and hemangioma, tachyarrhythmia, chorioangioma, sacrococcygeal teratoma with hemorrage, valvular defects, cardiomyopathy, Rh immunizzation, pericarditis associated with renal agenesis or posterior urethral valves and finally twin-to-twin transfusion (32). Once excluded the association with heart and/or extra-cardiac abnormalities, including the evaluation of the heart rhythm, the evaluation of the fetal karyotype must be considered (33). The PE is a well-known heart marker, independent and predictive for Trisomy 21. In the fetuses with Down Syndrome, the PE is generally detected along the right ventriculus (26).

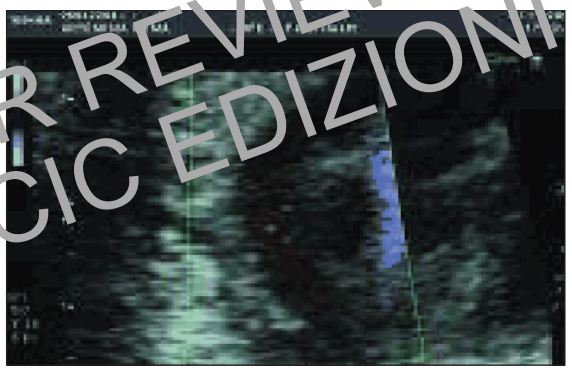

Figure 9 - Schematic representation of the color-Doppler under PE.

TRICUSPID VALVE REGURGITATION (TVR): TVR is based on the presence of a bidirectional flow through the valve, that is a normal flow from the auricle to the ventriculus during the diastole and from a regurgitation in the opposed direction during the systole (from the ventriculus to the auricle), (Figs. 10, 11). The studies by Nyberg and Benacerraf identified a congenital heart dysmorphology only in 5-10% of fetuses affected by trisomy 21 (34, 35). The study by De Vore showed that the support of the Color Doppler increases the detection rate of the structural and functional heart abnormalities in the fetuses affected by Trisomy 21 (26). Other studies in literature showed a low variability intra- and inter-operator in documenting the trans-tricuspidalic flow and a feasibility of the measurement in about 90% of the cases (36, 37).

RIGHT/LEFT HEART DISPROPORTION (RL): the RL shows as an asymmetry between the ventricular or atrial right and left chambers. The RL is a well-known predictive heart marker for the Trisomy 21 (26); the atrial and the ventricular biometrics (38, 39) should be taken respectively during the ventricular systole and at the last phase of the ventricular diastole (Figs. 12, 13).

PYELECTASIS: is the dilatation of the renal pelvi higher than 4 mm in the anteroposterior diameter (Fig. 14). It is defined as a variant and rarely has a pathologic meaning for the renal function both fetal and post-natal. Since 1990, Benacerraf (40) suggested the association of pyelectasis with chromosomal abnormalities, then widely confirmed by

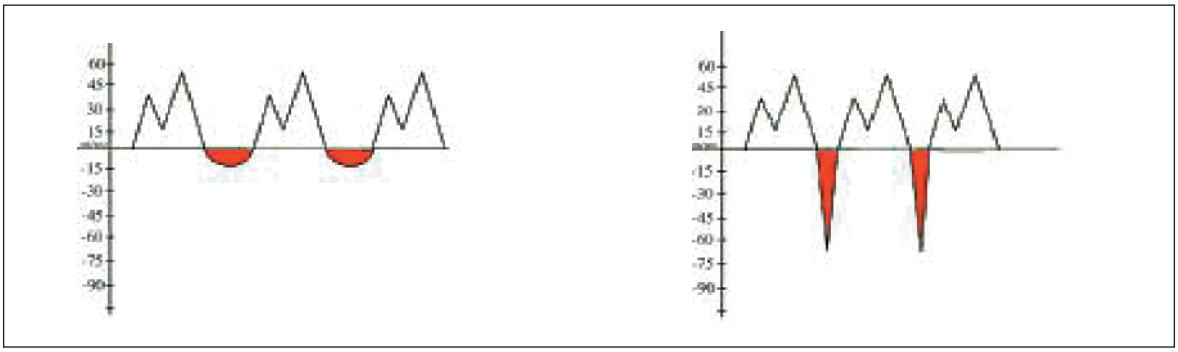

Figure 10 - Normal outline with no regurgitation on the left and spike due to tricuspid valve clausura (< 60 cm/sec) on the right.

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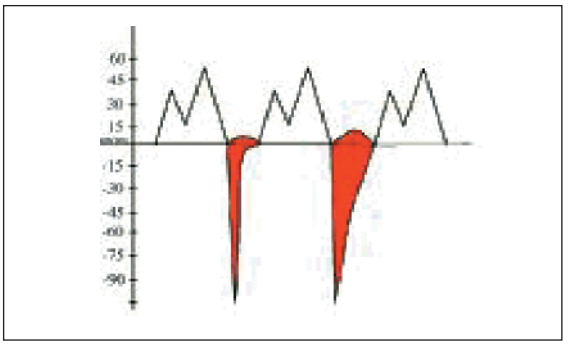

Figure 11 - Pathologic outline: regurgitation for almost 1/2 of the systole and speed ≥ 60 cm/sec.

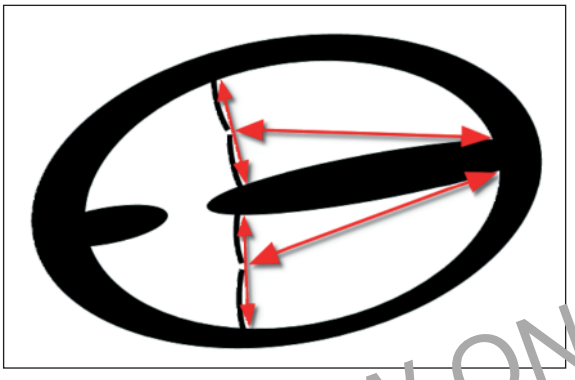

Figure 12 - Short axe and long axe of ventricular at the end of the ventricular diastole. Measure: from the coaptation point of the valvular flaps to the side wall.

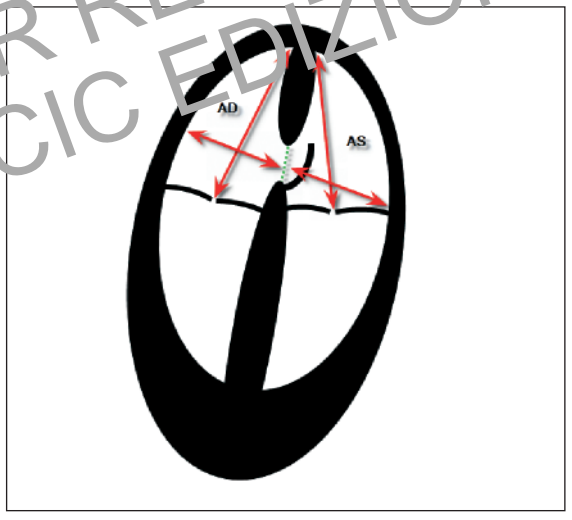

Figure 13 - Longitudinal axe and transversal axe of the atria at the end of the ventricular systole. Measure: from the apex of the ventricular chambers to the coaptation point of the valvular flaps.

subsequent studies (41-43). The prevalence of pyelectasis in the unselected population varies according to the studies from 0.73 to 5.5% (Table I) with a net prevalence in males (males/females ratio 1,9:1) (44).

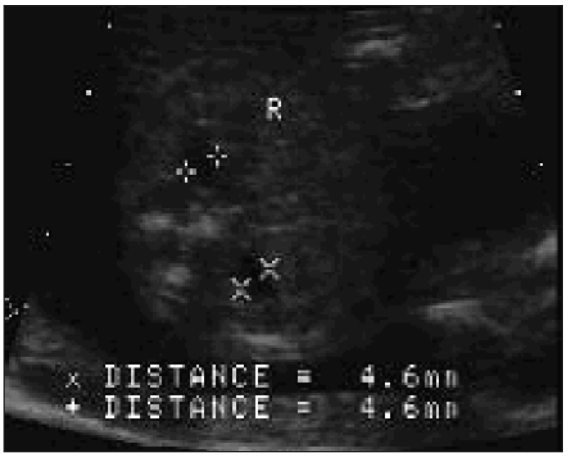

Figure 14 - Measurement of the renal pelvi in a foetus with bilateral pyelectasis.

Methods

During the period between April and December 2007 a prospective study was performed on a thousand women who underwent second trimester screening with SCA TEST followed by amniocentesis. All women were screened at the Fetal-Maternal Medical Centre ARTEMISIA, in Rome, Italy. All ecographic procedures were performed by four of the authors (C.C, P.C, L.M and A.S) who have a similar background of experience in prenatal diagnosis and certified for using SCA TEST. All amniocentesis were performed by C.G. Biometric measurements of head, abdomen, femur, homerus, pyelectasis and plica nucalis were obtained. These were followed by an anatomical survey of the head, chest, abdomen, pelvis and limbs and up to seven associated morphologic parameters were obtained: hyperechogenic bowel, cardiac foci, interventricular defect, pericardial effusion, tricuspid valve regurgitation, right/left heart disproportion and structural abnormalities. The heart examination was performed with color Doppler in order to seek for: asymmetry between the ventricular or atrial right and left chambers, abnormal fluid in the pericardial space, tricuspid regurgitation, and interventricular septal defect (Table II). All these markers obtained for each patient through ultrasound examination have been included in the SCA TEST

Table I - Prevalence of pyelectasis in unselected population.

Author	Prevalence of pyelectasis
Persutte et al.	5.5%
Coco et al.	2.9%
Benacerraf et al	2.84%
Corteville et al.	2.1%
Sairam et al.	2.34%
Havutcu et al.	1.25%
Whitlow	0.8%
Wickstrom	0.72%
Chudleigh et al.	0.73%

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software (Fig. 15) which provides through its algorithms automatically to calculate the background risk and the post test integrated risk (taking into account all previous markers) for aneuploidia after the screening (Fig. 16). Those pregnancies that have a risk greater than a predetermined cut-off of 1:250 are identified by the SCA TEST as the high-risk, screen-positive, group. All fetal karyotypes were analyzed by a single reference laboratory ("ARTEMISIA" Fetal-Maternal Medical Centre, Department of Genetics and Molecular Biology, Rome). Normal variants, common inversions, balanced translocation and pseudomosaicisms were classified as normal for this study. A Voluson 730 Pro and a Voluson 730 Expert (General Electric) with a 5.0 and/or 7.0 MHz curvilinear transducer was used for real time and color Doppler ultrasound examination. Statistical analysis was performed with SPSS software. For each ultrasound marker and for the integrated test, sensitivity, specificity, positive and negative predictive value was calculated. Fisher test has been used to evaluate the association between ultrasound markers and Trisomy 21.

Result

From April 2007 to December 2007 a thousand women underwent second trimester screening with SCA TEST followed by amniocentesis. The median age was 34

Table II - Ultrasound diagnostic criteria.

	Real time ultrasound criteria	Color-Doppler ultrasound criteria
Head		
Choroid plexus cyst	Hypoechoic structures(s), unilateral or bilateral, any size	NA
Central nervous system	Any malformation other than choroid plexus cyst	NA
Nuchal skin fold	Nuchal skin fold ≥ 6 mm	NA
Chest		
Ventricular septal defect	Hypoechoic dropout of the ventricular septum imaged in a plane parallel or tangential to the ultrasound beam	Color observed to fill or cross the ventricular septum corresponding to the hypoechoic dropout observed with real time ultrasound
Cardiac foci	Hyperechogenic heart focus equal to the bone echogenicity of the thoracic spine	NA
Right to left chamber disproportion	Asymmetry between the right and/or left atrial or ventricular chambers	Color observed within the atrial and/or ventricular chambers in which disproportion in present
Tricuspid regurgitation	NA	Flow from the right ventricle to their respective atrium during ventricular systole, confirmed with pulsed Doppler ultrasound
Pericardial effusion	Separation ≥ 2 mm of the epicardium and pericardium by fluid during the ventricular systole	Color filling the pericardial space, opposite in direction to the flow of blood entering or exiting the ventricles
Abdomen		
Pyelectasis	Hypoechoic spherical or elliptical space within the renal pelvis ≥ 4 mm. Measured in the anterior-posterior plane when the kidneys were imaged in a trasverseplane	NA
Hyperechoic bowel	Increased echoes within the bowel that are 2 or 3 Slotnick degree	NA

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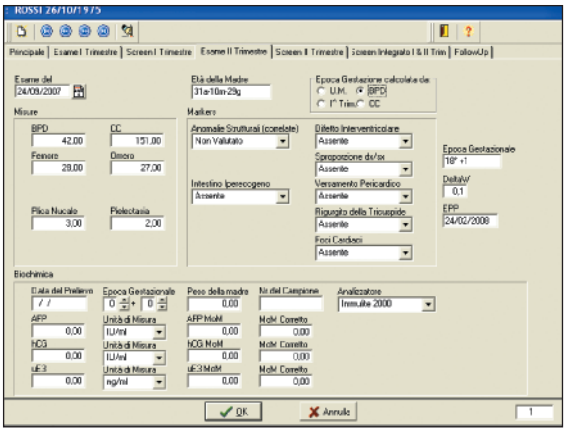

Figure 15 - SCA TEST: ultrasound markers.

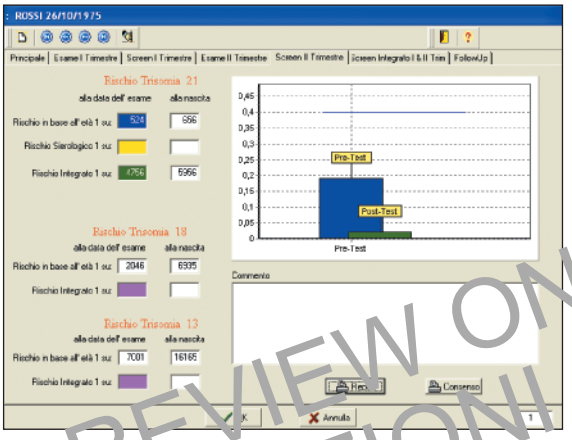

Figure 16 - SCA TEST: current risk (prior risk) and new risk (posterior risk) for Trisomy 21.

years (range, 19-46 years). The percentage of women older than 35 years was 43%. Out of 1000 women, 23 were excluded for inadequate ultrasound screening secondary to maternal obesity, 3 were excluded for previously known structural or other karyotype abnormalities like Trisomy 18, and 10 for pathological first trimester screening. In the study group of 974 women a total of 9 fetuses with Trisomy 21 were identified following by amniocentesis. The mean gestational age at the time of ultrasound examination was 17 weeks (range, 15-19 weeks). Abnormal ultrasound markers were present in 77.8% (n=7/9) of fetuses. Cardiac markers were present in 66.7% (n=6/9) of fetuses: 33.3% (n=2/6) presented structural defects (chamber disproportion and interventricular septal defect), 16.7% (n=1/6) presented functional defects (tricuspid regurgitation), and in 66.7% (n=4/6) showed hyperechogenic heart focus. Non cardiac markers were present in 55.5% (n=5/9) of fetuses, of which 80% (n=4/5) were a nuchal skin fold ≥ 6 mm, and 40%(n=2/5) were a renal pelvis ≥ 4 mm. In 22.2% (n=2/9) of fetuses with Trisomy 21 were not possible to recognize any ultrasound marker (Table III). Using the data from 9 fetuses with Trisomy 21 and 965 controls, the sensitivity, specificity, positive and negative predictive value and false positive were computed for each individual marker and for the SCA TEST integrated test (Table IV). The detection rate of the SCA TEST integrate test was 66.7% with a specificity of 94.8% and a percentage of false positive of 5.1%. Studying women older than 35 years the sensitivity reached 80% with a percentage of false positive of 7.2%. In foetuses with Trisomy 21 the most frequent non cardiovascular marker were abnormal nuchal skin fold (44.4%) and renal pelvis ≥ 4 mm (22.2%). The most frequent cardiovascular marker was ventricular septal defect (22.2%), followed by chamber disproportion (11.1%) and tricuspid regurgitation (11.1%). Hyperechogenic heart focus was present in (44.4%) of the foetuses.

Conclusion

The number of potential sonographic markers of aneuploidy reported in the literature appears to be exponentially increasing. Many, however, would not be classified as part of the routine sonographic assessment, so their application in the low risk population is in some doubt. As it appears to be no real consensus on selecting a reduced number of markers, except for perhaps nuchal fold thickening, other investigators have assigned individual risk to most of the known markers and developed scoring indices or likelihood ratios for revised risk esti-

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Table III - Ultrasound markers in 9 fetuses with trisomy 21: (+) marker present, (–) marker absent.

Ultrasound markers	Fetus I	Fetus II	Fetus III	Fetus IV	Fetus V	Fetus VI	Fetus VII	Fetus VII	Fetus IX
Nuchal skin fond ≥ 6 mm	–	+	+	–	–	–	–	+	+
Renal pelvis ≥ 4 mm	–	–	–	–	–	–	+	–	+
Hyperechoic bowel 2-3 Slotnick degree	–	–	–	–	–	–	–	–	–
Chamber disproportion	–	–	+	–	–	–	–	–	–
Ventricular septal defect	–	–	+	+	–	–	–	–	–
Pericardial effusion	–	–	–	–	–	–	–	–	–
Tricuspid regurgitation	–	–	+	–	–	–	–	–	–
Hyperechogenic heart focus	–	+	–	–	–	+	+	–	+

Table IV - Ultrasound marker identified in second trimester foetuses with Trisomy 21.

Ultrasound markers	FN	FP	TN	TP	Sensibility (%)	Specificity (%)	PPV (%)	NPV (%)	False positive (%)
Omerus	9	5	960	0	–	99.48	–	99.07	0.51
Nuchal skin fold ≥ 6 mm	5	7	958	4	44.44	99.27	36.36	99.48	0.72
Renal pelvis ≥ 4 mm	7	19	946	2	22.22	98.03	9.52	99.26	1.95
Hyperechoic bowel	8	12	954	0	–	98.75	–	99.16	1.23
Chamber disproportion	7	0	965	2	22.22	100.00	100.00	99.27	–
Ventricular septal defect	7	1	963	3	30.00	99.89	75.00	99.27	0.10
Pericardial effusion	9	1	964	0	–	99.89	–	99.07	0.10
tricuspid regurgitation	8	8	956	2	20.00	99.17	20.00	99.17	0.82
Hyperechogenic heart focus	6	45	920	3	33.33	95.33	6.25	99.35	4.62
SCA TEST integrated test	3	50	915	6	66.66	94.81	10.71	99.67	5.13

FN: false negative, FP: false positive, TN: true negative, TP: true positive, PPV: positive predictive value, NPV: negative predictive value.

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mation, Benacerraf (45, 46) has developed a scoring index for individual abnormalities; Nyberg et al (42) developed an age-adjusted ultrasound risk assessment for Down Syndrome by multiplying the a priori risk based on maternal age, with likelihood ratios resulting from the presence or absence of specific ultrasound findings. Risk adjustment using the principle of Bayes' theorem is commonly utilized in clinical medicine (47). The current risk (prior risk) of disease is known and the new risk (posterior risk) can be computed following the result of the screening test (48).This is accomplished by computing the likelihood or odds ratio and multiplying it by the prior risk (48). Studies from tertiary referral centres show that almost all fetuses with Trisomy 13, 77-100% of fetuses with Trisomy 18 and 33-50% of fetuses with Down Syndrome have sonographic signs which can be seen in the second trimester scan (1, 26, 34, 49-55). The risk of chromosomal abnormality increases with the number of sonographic abnormalities seen (56) and the overall risk may be as high as 35% (57) when multiple abnormalities are present. The prior risk based on maternal age and serum biochemical screening may be modifed by the presence or absence of particular sonographic markers (24, 26, 58-60), with studies reporting detection rates ranging from 59.2-92.8% and with a false positive rates ranging from 5.3% to 14% (26, 34, 42, 46, 61-64). The literature reported incidence of structural abnormalities of the heart in the newborn population with Down syndrome of 46-65.4%(58-60, 65). The majority of fetuses with Trisomy 21 present with a variety of combination of ultrasound findings (66-69). In this study, the incidence of structural abnormalities of the heart was 33,3%. The difference in incidence of congenital heart defects may be accounted for by closure of ventricular septal defect prior to birth (15). In this prospective study using cardiovascular and non cardiovascular markers (SCA TEST integrated screening) the detection rate for Trisomy 21 was 66.7% with a specificity of 94.8% and a false positive rate of 5.1%, while considering women older than 35 years the sensitivity reaches 80% with a false positive rate of 7.2%. Although a high false positive rate may be unacceptable for a screening program, it is quite acceptable if used in high risk population for advanced maternal age or abnormal maternal serum screening (intermediate screening: 1:191 a 1:1000) (70, 71). In conclusion the use of second trimester markers of aneuploidy as a screening tool will remain controversial for application to the low risk population. These markers are common, and potentially cause greater anxiety to parents who ultimately are likely to have a normal baby and may pose some risk to the pregnancy by invasive testing. In addition early screening programmes such as first trimester nuchal translucency and first trimester serum biochemical screening, have undoubtedly the international consensus of scientists. On the other hand, further training in cardiovascular assessment is necessary and using the ultrasound marker listed in table II, the physician can compute the risk for Trisomy 21 with the SCA TEST integrated test based upon the results showed in this paper. Therefore, an extensive counseling of patients and experience in prenatal ultrasound screening is mandatory. Another important aspect which will be part of a successive study is the performance evaluation of the software, integrating it with the maternal sierology (Triple Test) which SCA TEST allows to perform anyway.

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