

# Quantitative and qualitative Ductus Venosus blood flow evaluation in the screening for Trisomy 18 and 13 — suitability study

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## ABSTRACT

**Objectives:** The objective of the paper is the suitability assessment of screening for Trisomy 18 and 13 on the basis of nuchal translucency (NT) measurement, Fetal Heart Rate (FHR), double test, quantitative [Ductus Venosus (DV) Pulsatility Index for Veins (PIV)] and qualitative (the A-wave assessment) blood flow evaluation in the DV.

**Material and methods:** The study was performed in 7296 singleton pregnancies. In each fetus NT, FHR, DV-PIV were examined. Double test from maternal blood was examined. These ultrasound and biochemical factors were in combined screening investigated. Additional doppler ultrasound markers such as abnormal a-wave in Ductus Venosus and Pusatility Index for Veins of Ductus Venosus were and their impact on Trisomies 18 and 13 screening were examined.

**Results:** Two groups of patients were compared — with chromosomal normal and chromosomal abnormalities — Trisomy 18 and 13. Detection Rate of Trisomies 18 and 13 at the risk cutoff 1/300 using combined screening was 90.2% and FPR was 6%. Detection Rates of examined chromosomal abnormalities using contingent screening were: 92.1% using DV abnormal a-wave and 94.84% using DV-PIV. FPR's for booths parameters 5.8% and 5.4% respectively.

**Conclusions:** Quantitative analysis of the flow — assessment of DV-PIV in the first trimester significantly influences the improvement of screening values focusing on Trisomy 18 and 13 detection.

**Key words:** Combined test trisomy 18 trisomy 13; first trimester nuchal translucency thickness; ductus venosus pulsatility index for veins; serum free  $\beta$ -hcg; pregnancy-associated plasma protein A

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## INTRODUCTION

Congenital anomalies and chromosomal defects are most common causes of neonatal mortality and morbidity. On that account, in the period of the last dozen years we have been observing the development of scientific research that would enable the early diagnosis of defects and would let provide these defects possible treatment [1].

First trimester screening for chromosomal defects is based on ultrasound examination with assessment of NT (Nuchal Translucency) and “double test” based on free  $\beta$ -human Chorionic Gonadotropin (hCG) and Pregnancy Associates Plasma Protein-A (PAPP-A) concentrations in maternal blood expressed in Multiplies of the Median (MoM).

The essential ultrasound screening performed between the 11 + 0 and 13 + 6 weeks. [corresponding to Crown-Rump Length (CRL) between 45–84 mm] is based on the assessment markers of chromosomal defects with NT being the first. Additional markers are Nasal Bone (NB), Tricuspid Regurgitation (TR) and ductus venosus flow assessment (DV). The latter is more and more often used in screening [2–4].

In 65% of fetuses with Down syndrome and 55% of fetuses with Trisomy 18 or 13, the reversed A-wave, corresponding to atrial contraction, is detected in DV (it is the qualitative assessment of the flow) [5]. Additionally, Pulsatility Index for Veins (PIV), which provides the quantitative

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evaluation, is also taken into consideration. The average value of DV-PIV is 0.94 (range from 0,53 to 1.99) [6].

In first trimester screening for the most common chromosomal defects in general population for trisomy 21, 18 and 13, Fetal Medicine Foundation has developed the algorithm based on maternal age, CRL, NT, fetal heart rate (FHR) free  $\beta$ -hCG and PAPP-A. This screening strategy provides the Detection Rate (DR) of approximately 90% with the False Positive Rate (FPR) of approximately 3–5% [7]. If the assessment of DV flow is added, the test DR increases to 96% for Trisomy 21, to 92% for Trisomy 18 and to 100% for Trisomy 13. FPR for all is 3% [8].

Non-invasive prenatal screening focuses on identifying pregnant women who are at high risk of chromosomal defects and in whom invasive testing would be justified. [9]

### Objectives

The objective of the paper is the suitability assessment of screening for Trisomy 18 and 13 on the basis of NT measurement, double test, quantitative (DV-PIV) and qualitative (the A-wave assessment) blood flow evaluation in the DV.

### MATERIAL AND METHODS

In total, 7466 fetuses in singleton pregnancies were examined. It should be stressed that the fetuses with other syndromes (Trisomy 21 — 83, Turner Syndrome — 12, Tetraploidy — 4, Unbalanced translocations — 5 cases respectively) and structural defects with normal karyotype, such as: heart defects — Hypoplastic Left Heart Syndrome — 12, Atrioventricular Septal Defects - 8, Tetralogy of Fallot — 4 and Transposition of the Great Arteries — 2 cases were excluded from the research. Other structural fetal defects excluding fetuses from the study were: Fetal Hydrops — 13, Spina bifida — 10, Hydrocephalus — 6, Palate or upper lips cleft — 6, Omphalocele — 3 and Gastroschisis — 2 cases. The study was performed in 7296 singleton pregnancies scanned in the Department of Obstetrics and Gynecology in Ruda Śląska and in Outpatient Clinic "GENOM" in Ruda Śląska. All ultrasound examinations at 11<sup>0</sup> a 13<sup>+6</sup> weeks were performed according recommendations of Polish Gynecological Society (PTG - Polskie Towarzystwo Ginekologiczne) and Fetal Medicine Foundation (FMF). Assessed parameters were: CRL, FHR, NT and DV-PIV were measured and the qualitative assessment of A-wave in DV was performed according to FMF rules [9]. In all cases CRL was between 45–84 mm.

For the PIV the 95<sup>th</sup> percentile was designated and it was taken into consideration within the quantitative analysis.

Specialists in obstetrics and gynecology with valid of FMF and PTG certificates performed all scans using GE Voluson Expert 730 or GE Voluson E8 ultrasound machines.

Directly after the scan, all patients had their blood taken for double test. Maternal blood was tested using the

FMF-certified Delfia Express Perkin–Elmer and the concentration of the free  $\beta$ -hCG and PAPP-A were converted to MoMs and then the risk of trisomies was calculated by the FMF certified ASTRAIA software.

Patients in high risk for chromosomal defects ( $\geq 1/300$ ), were offered amniocentesis for karyotyping (total amount of amniocentesis were 337 cases, whereas in 100 cases, parents did not consent to invasive test). In patients, who declined invasive testing, postnatal follow up was performed. Pregnancy outcome was collected in all cases by the questionnaires (filled and returned by the patients) and the medical history of newborns. The neonatal phenotype was also evaluated. In the cases with no developmental defects and congenial diseases, the newborns were qualified as healthy. In all cases of increased NT in the first trimester and/or development defects suspected on prenatal ultrasound, a newborn baby underwent a detailed examination by a neonatologist, pediatric cardiologist and a geneticist. Karyotyping was performed in all phenotypically abnormal newborns. For the purpose of this paper, only the phenotypically normal newborns (they were approved as healthy) and fetuses that were detected Trisomy 13 and 18 were calculated. In this way two groups were obtained: 7239 normal neonates and 57 cases of Trisomies 18 and 13 respectively.

Statistical analysis: The Statplus Mac Ver 5 and Wizard Version 1.6.8 statistical packages were used to analyze the data. The Shapiro-Wilk test was applied to assess these results distribution. After ascertaining differences in relation to normal results distribution, the Mann Whitney's U test was applied to conduct further calculations. To analyze qualitative parameters, the Chi<sup>2</sup> test was used. The diagnostic threshold value for particular measurements was determined on the basis of the ROC (Receiver Operating Curve). Sensitivity and specificity were calculated for each threshold value. In all tests, the p level of probability, which was lower than the assumed level of significance ( $p < 0.05$ ), was adopted as the level of differences statistical significance. DR and FPR for separate configurations of markers of the first pregnancy trimester: NT, MA, BC and FHR have been compared.

### RESULTS

Two groups of patients were compared. The group of healthy women was constituted by 7239 patients whose fetuses were not detected chromosomal aberrations or any other development defects. The group of diseased women included 57 patients whose fetuses had Trisomy 13 or 18 confirmed on the basis of the karyotype examination.

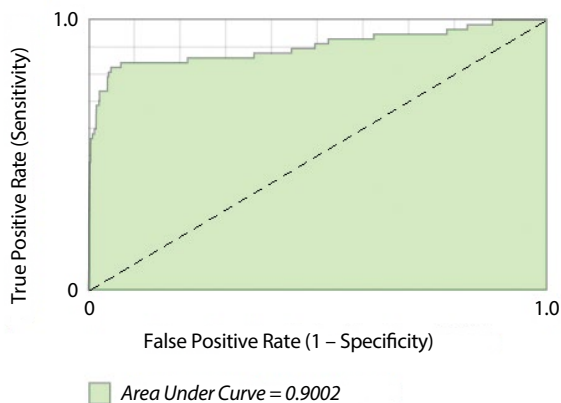
The medians of outcomes of all examined and assessed parameters in both groups of healthy and diseased patients were observed and compared by Mann Whitney's U test (Tab. 1).

**Table 1. Medians of outcomes of all examined and assessed parameters in both groups of normal and abnormal karyotypes**

	Healthy	Trisomy 18 or 13	P
n	7239	57	
CRL	62.70	59.50	0.0000
NT	1.70	3.90	0.0001
βHCG MoM	1.05	0.51	0.0000
PAPP-A MoM	0.96	0.34	0.0000
FHR	161.0	159.00	0.0000
MA	34.00	30.00	0.0000
DV PIV	1.00	1.80	0.0001

CRL — crown rump length; NT — nuchal translucency; FHR — fetal heart rate; βhCG — free subunit β of human Chorionic Gonadotropin; PAPP-A — pregnancy associates plasma protein-A; MoM — multiple of median, DV PIV — Ductus Venosus Pulsatility Index for Veins; MA — maternal age; p95 — 95 percentile

Receiver operating characteristic

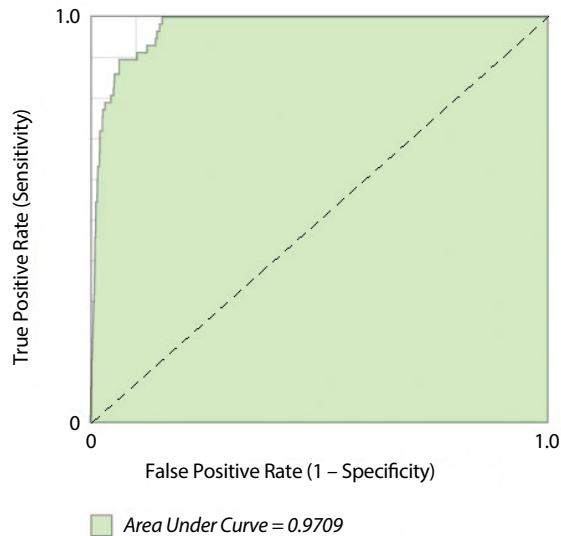


**Figure 1.** ROC for NT + BC + MA

The test with the lowest DR factor was characterized. It took into consideration the combination of the following basic markers: NT, MA, BC and FHR. In this case this factor amounted 90.02%. The test DR increased to 92.10% after attaching the analysis of a flow in Ductus Venosus. However, the highest DR was obtained when, apart from NT, MA, the DV-PIV — Pulsatility Index for Veins in Ductus Venosus was analyzed and then it amounted 81%. FPR amounted respectively: 6.0 for NT + MA + BC, 5.8 for NT + MA + BC + the A-wave and 5.4 for NT+MA+BC+DV-PIV. The above results were obtained for the group risk 1:300.

Comparing mutually all groups of Trisomy 13 and 18 high risk, which is 1:300, 1:200, 1:100 and 1:50, the analysis indicated similar dependencies. In all groups the test diagnostic sensitivity increased after taking into account the quantitative

Receiver operating characteristic



**Figure 2.** ROC for NT + BC + MA + DVPIV

and qualitative assessment of a flow through DV. At the same time the number of FPR results decreased. It appeared that the most advantageous method was the consideration of the flow through a venous ductus together with the qualitative assessment of DV-PIV within the combined test, which was characterized by the highest DR = 89.30%. Additionally, the reduction of the level of Trisomy 18 and 13 risks to the level of 1:200 let maintain similar sensitivity with the decrease of the percentage of FPR results in the analogical group and with the consideration of the qualitative assessment of DV-PIV to 3.5%.

Using statistical analysis sensitivity and specificity for tests that include measurements of NT, biochemistry, mother’s age — NT + BC + MA (Fig. 1) as well as for the test enlarged by the measurement of PIV — Pulsatility Index in a Venous Duct — NT + BC + MA + DV-PIV (Fig. 2), the ROC were designated. Comparing both graphs for NT + BC + MA and NT + BC + MA + DV-PIV tests (Fig. 2), great diagnostic suitability of both tests was stated. However, it was noted that analyzing the Area Under Curve (AUC), the diagnostic suitability was bigger for the test extended by the DV-PIV (AUC respectively 0.9002 and 0.9709). Therefore, the most favorable values were obtained for the test in which the combination of MA, NT, double test and DV-PIV, was taken into consideration. Tables 2–4 shows comparison of Detection and False Positive Rates of Trisomies 18 and 13 using examined parameters.

**DISCUSSION**

During the diagnostic test planning, the highest DR, with a simultaneous reduction of FPR, is tried to be achieved.

**Table 2. Analysis of DR and FPR using NT + MA + BC**

Risk	1/300	1/200	1/100	1/50
DR [%]	90.02	76.80	66.10	51.80
FPR [%]	6.00	4.70	2.6	1.30

DR — detection rate; FPR — false positive rate

**Table 3. Analysis of DR and FPR using NT + MA + BC + A-wave**

Risk	1/300	1/200	1/100	1/50
DR [%]	92.10	85.00	80.30	76.00
FPR [%]	5.80	4.10	2.80	2.10

DR — detection rate; FPR — false positive rate

**Table 4. Analysis of DR and FPR using NT + MA + BC + DVPIV**

Risk	1/300	1/200	1/100	1/50
DR [%]	94.81	89.30	83.90	80.40
FPR [%]	5.40	3.50	2.50	1.40

DR — detection rate; FPR — false positive rate

Therefore, it is aimed at making the noninvasive tests sensitive enough in order to provide the lowest number of patients selected to invasive examinations. The analysis of the blood flow in DV (as Trisomy 13 and 18 additional marker analyzed after obtaining the risk outcomes of a disease calculated according to NT and the double test) lets provide more precise selection of the chromosomal aberration high risk group in the comparison with a method that is based only on the NT measurement and the double test analysis. It should be added that the flow assessment in DV includes two methods: the qualitative method — assessment of the A wave shape (an atrial component) and the semi-quantitative method providing the PIV [3]. In the case of the qualitative assessment, the existence of the retrograde wave in DV is checked. In a situation when the A retrograde wave occurs during a heart contraction, in 80% of cases the fetus's development can be normal but there exists an increased risk of the occurrence of chromosomal disorders, isolated heart defects or even death of a fetus [10]. Literature data show the 60% dependence between the occurrence of the A wave in DV and fetus's heart defects [11]. In their scientific work Mainz and Nicolaidis present even 68% of such dependencies [12].

Effectiveness of Trisomy 13 and 18 screening calculated only on the basis of the NT measurement and the double test components lets detect about 91.8% of cases (DR = 91.8%) with the FPR about 2.2% [13]. Within our research in such a case we obtained DR — 90.02% and FPR — 6.0%. After considering the flow in DV, DR increased to 95.4% and FPR

decreased to 1.3%. In the case of our results, DR and FPR amounted respectively: 94.81% and 5.4%. Similar outcomes for the assessment of NT and the double test were obtained by Kagan et al [14]: DR 91% for Trisomy 18 and 87% for Trisomy 13 but his FPR was significantly lower and it amounted 0.2% for both aberrations.

The qualitative assessment of the flow (assessment of the A-wave) lets obtain DRT13, 18 at the level of 92.10% with FPR amounting 5.8%. So, slightly better indicators than the Trisomy 13 and 18 risks assessment without the evaluation of the A wave in DV (DR 90.02% and FPR 6.0%) have been obtained. Results of our paper show that it is possible to gain even greater increase of DR to the amount of 94.81% with a simultaneous decrease of FPR to the value of 5.4% when PIV in DV included to the analysis. Similar results — the increase of DR and the decrease of FPR are also gained in the Trisomy 21 screening after including the assessment of the flow in DV. In such a case, Mainz and his partners obtained the following results: DR — 96%, FPR — 3%.

## CONCLUSIONS

Concluding, it should be noted that the quantitative analysis of the flow — assessment of DV-PIV in the first trimester significantly influences the improvement of screening values focusing on Trisomy detection. This thesis is confirmed also by Zimmer and his partners' [15] examinations, which indicate that for the test including NT + MA + BC + DV-PIV, the DR increased to 92% whereas for the test without DVPIV analysis of the factor amounted only 84%. Summing up, in comparison with the qualitative assessment of the flow in DV, our research indicates obtaining more effective type of Trisomy 13 and 18 screening with the usage of the assessment of NT, double test and the quantitative analysis of DV flow.

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