Introduction

Genetic screening on chromosomopathy is performed in the first trimester of pregnancy, between the 11th and 14th week, by determining the nuchal translucency of a fetus (of the cervical crease – NT) with the ultrasound and the fetoplacental Pregnancy associated plasma protein-A (PAPP-A) and free human chorionic gonadotropin (free-beta HCG) biomarkers in maternal serum. PAPP-A is a glycoprotein synthesized in the trophoblastic placental tissue. In
trisomy 21, 18 and 13, concentrations in maternal serum are more than 40% lower than in normal pregnancy. Free-beta HCG is a hormone which produces syncytiotrophoblast. Its increased level in pregnancy is considered the most sensitive marker in detecting trisomy 21, while in trisomy 18 and 13 the values are much lower. The value of each biomarker is calculated in conventional units and in relation to a median (MOM – multiple of median). The sensitivity of genetic screening has been improved with the use of software which adapts the values of fetal biomarkers in the serum according to the mother’s age, weight, ethnic group, diabetic status, the number of smoked cigarettes and gestational age calculated with the ultrasound by measuring the crown-rump length (CRL). By combining the biomarkers with the ultrasound marker NT, an individual risk expressed as 1:n can be obtained. As a cut-off risk indicating prenatal karyotyping, 1:270 is used which corresponds to a pregnant woman aged 35.

It has been estimated that the rate of false positive values of genetic screening is about 5%, which has resulted in the increased number of invasive diagnostic procedures of prenatal karyotyping in risk free pregnant women with respect to age, but also in the negative implications on the psychological condition of a pregnant woman (12–14). The causes of the false positive results of genetic screening have lately been increasingly investigated and the model for the reading of fetal biomarkers as well as for the interpretation of pathological values has been sought for.

Through the prospective and partly retrospective analysis of genetic screening on chromosomopathy in the first trimester of pregnancy, the frequency of false positive results has been tested. In normal fetal karyotyping, the correlation between the pathological values of biomarkers and other pregnancy disorders has been sought out. Is false genetic screening worthless after the prenatal karyotyping and normal fetal karyotyping? Are the pathological values of the biomarkers indicators of other possible risks in pregnancy?

**Material and Methods**

2000 pregnant women have been tested. In the first trimester of their pregnancy the genetic screening on chromosomopathy has been performed, the nuchal translucency of the fetus (NT) has been measured, and the PAPP-A and free-beta HCG biomarkers in maternal serum have been determined. From every pregnant woman a detailed personal, gynecological, hereditary and teratogenic anamnesis has been taken, while monitoring their respective health status during pregnancy, the outcome of the pregnancy and the health status of the neonate. PAPP-A and free-beta HCG biomarkers have been read with IMMULITE 2000 SIEMENS which operates on the principle of chemiluminescence, using the original reagents (15). The processing of data and determination of the risk of trisomy 21 and 18 have been done with PRISCA 4 SOFTWARE (16).

For the statistical processing of the data in a dichotomous manner, i.e. to establish whether a marker is normal or pathological, whether there is a risk or not, the table of contingency (chi-quadrant test) has been used.

### Results

Out of 2000 pregnant women tested, there were 138 (6.9%) false positive screening results with the final risk in PRISCA software higher than 1:250 indicating prenatal karyotyping, while 202 pregnant women (10.1%) had a final risk higher than the initial age risk. 340 pregnant women with bad genetic screening results were submitted to the fetal karyotyping test and a normal karyotype of the fetus was obtained. In pregnancies terminated in miscarriages the cytogenetic analysis of the miscarried sample of the chorion and fetal tissue has been performed and a normal karyotype has been obtained which proved the false positive result of genetic screening.

Only 5.3% of the false positive results had a pathological NT marker, while in 94.7% false results were caused by the pathological values of PAPP-A and free-beta HCG biomarkers. Increased free-beta HCG had the largest influence on false positive results (48.24%).

The connection between the increased value of free-beta HCG over 2 MOM and the maintained pregnancy and the health status of the neonate. 33.53% of pregnancies that ended in labour (48.24%) produced syncytiotrophoblast. Its increased level in pregnancy is considered the most sensitive marker in detecting trisomy 21, while in trisomy 18 and 13 the values are much lower.

The sensitivity of genetic screening has been improved with the use of software which adapts the values of fetal biomarkers in the serum according to the mother’s age, weight, ethnic group, diabetic status, the number of smoked cigarettes and gestational age calculated with the ultrasound by measuring the crown-rump length (CRL). By combining the biomarkers with the ultrasound marker NT, an individual risk expressed as 1:n can be obtained. As a cut-off risk indicating prenatal karyotyping, 1:270 is used which corresponds to a pregnant woman aged 35.

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#### Table I

|                  | Free beta HCG | Normal  | Pathological | Total |
|------------------|--------------|--------|--------------|-------|
|                  |              | < 2 MOM | > 2 MOM      |       |
| **Maintained**   |              |        |              |       |
| **pregnancy**    |              |        |              |       |
| No               | 96           | 104    |              | 200   |
| Yes              | 80           | 60     |              | 140   |
| **Total**        | 176          | 164    |              | 340   |

\[ \chi^2 = 2.7570 \quad \text{d.f.} = 1 \quad p = 0.0968 \]
The connection between increased free-beta HCG over 2 MOM and fetal hypoxia has been analysed. A significant connection was established between the increased value of free-beta HCG over 2 MOM and fetal hypoxia (Table IV).

**Discussion**

The free-beta HCG biomarker is the most frequent cause of false positive results of genetic screening with values higher than 2 MOM, thus its correct interpretation and correlation with the pregnancy pathology is necessary.

The increased free-beta HCG over 2 MOM is particularly noticeable in pregnant women who have suffered from threatened miscarriage, bleeding in the early pregnancy and the maintained pregnancy supported by progesterone pills. Of 140 maintained pregnancies with bleeding, the increased free-beta HCG over 2 MOM was noticed in 60 (47.14%). Free-beta HCG is the product of placental syncytiotrophoblast. It is considered that the level of the HCG serum biomarker can also represent a placental marker. Retrospectively, the correlation between beta HCG and placental vascular disruption has been observed, so that the high level of beta HCG markers over 2.5 MOM can indicate a risk of preeclampsia (17, 18). The increased level of free-beta HCG biomarkers is the result of placental trophoblast hyperplasia. In these conditions, the mechanism for the rise of free-beta HCG lies in the focal necrosis of syncytiotrophoblast and the rise of mitotic activity with cellular cytotrophoblast proliferation which is transformed into syncytiotrophoblast. In the transformed syncytiotrophoblast there is an increased production of free-beta HCG.

Out of 340 pregnancies with the false positive genetic screening, 47 (13.82%) ended in MISSed, 37 (10.88%) in miscarriage, 30 (8.82%) by induced pregnancy termination. Among the indicated pregnancy terminations there were 3 cases of anencephaly, 8 of cystic hygroma, 3 of omphalocele, 4 of heart defect, 1 of a diaphragmatic hernia, 3 of Dandy-Walker Syndrome, 2 of polycystic kidney disease, 1 of spina bifida, 3 of multiple anomaly and 2 pregnancies terminated because of teratogenic effects. Fetal karyotyping has been done in all pregnancies before or after the termination and there were no chromosomal aberrations. 226 (66.47%) pregnancies ended in labour, 184 (54.12%) in spontaneous labour, 38 (11.18%) in a Caesarean section and 4 (1.17%) in stillbirth.

Based on the health status of neonates, two groups were formed: healthy and vital babies made up one group, diseased and non-vital babies with normal karyotypes formed the other group. Among the diseased babies were the ones with fetal respiratory distress, asphyxiation, intracranial hemorrhage and convulsions, all resulting from hypoxia. The connection between the level of HCG markers in maternal serum on the one hand and complications in pregnancy on the other has been mentioned in various reference books (19–22). In this research, the condition of fetal hypoxia has been statistically much more often observed in pregnancies with the increased level of HCG markers over 2 MOM than in the control group (p > 0.01). Inadequate perfusion leads to reduced placental oxygenation. The condition of hypoxia leads to syncytiotrophoblastic hyperplasia which produces increased HCG and these changes in the placenta are connected to the fatal outcome of pregnancy.

Although the primary risk was low, complications occurred in 78.52% of pregnancies with pathological genetic screening, MISSed in 13.82%, miscarriages in 10.88%, induced pregnancy termination in 8.82% and births with disturbed fetal vitality in 45%.

The results of this study have contributed to the elucidation of the false positive results of genetic screening.
screening and to the determination of the level of risk in a pregnancy. Pathological genetic screening with normal fetal karyotyping can indicate placental dysfunction and fetal vitality disruption. When the performed prenatal cytogenetic analysis has confirmed the fetal karyotype as normal and the genetic screen as falsely positive, it should not be forgotten that the fetoplacental biomarker values were pathological; instead we should look for the cause of their abnormal production. Such pregnancies should be treated as risky and should be clinically and laboratory controlled. In the third trimester there is the possibility of determining other biomarkers indicating fetoplacental vitality, such as human placental lactogen (HPL), glucose tolerance tests, preeclampsia risks, regular ultrasound controls of fetal biometry, estimation of biophysical profile, doppler test of uteroplacental and fetoplacental circulation.

**Conflict of interest statement**

The authors stated that there are no conflicts of interest regarding the publication of this article.

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