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# Evaluation of indications for amniocentesis in cases of normal fetal ultrasound results

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## ABSTRACT

**Objectives:** The objective of this study was to analyze indications for amniocentesis in cases of patients with normal fetal ultrasound results between 11+0 and 13+6 weeks of gestation.

**Material and methods:** The results of first-trimester screening tests performed between 2014 and 2018 on 6,863 patients of the Prenatal Testing Outpatient Clinic at the Clinical Department of Obstetrics and Gynecology, Pomeranian Medical University, Szczecin, Poland, were analyzed. The inclusion criteria were a singleton pregnancy and normal results of fetal ultrasound between 11+0- and 13+6-weeks' gestation. Depending on the calculated risk of fetal trisomy 21, the patients were divided into three groups (group A = RS > 1:300, group B = RS 1:300 – 1:999, group C = RS ≤ 1:1000). Subsequently, values such as PAPP-A and fβ-hCG protein levels and maternal age were analyzed for each of the groups.

**Results:** The patients, 6,310 (91.94%) met the inclusion criteria. A high risk of fetal trisomy 21 was identified for 514 women (8.15%). Group B had 733 (11.62%) and group C 5,063 (80.23%) patients. In group A, an fβ-hCG level of ≥ 2.000 MoM was shown for 50.97% of the women. A PAPP-A level ranging from 0.001 to 0.499 MoM was observed for 38.72% of group A patients. The mean maternal age in groups A, B and C was 36.45, 36.08 and 31.64 years, respectively.

**Conclusions:** In the first-trimester, patients with normal ultrasound results obtained during prenatal screening tests, the main cause of an increased risk of trisomy 21 was elevated PAPP-A and fβ-hCG concentrations. According to this paper's authors, in these cases extension of diagnosis to include other gestational complications, *e.g.* preeclampsia, should be considered.

**Key words:** amniocentesis; ultrasonography; prenatal; PAPP-A; HCG-beta

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## INTRODUCTION

Although first-trimester screening tests are recommended to every pregnant patient, the percentage of such tests performed in Poland continues to be low. As an example, 389,455 children were born in 2018, while only 116,079 pregnant women, including 74,543 (64.2%) below the age of 35, took advantage of the screening project funded by the National Health Fund (NFZ). Biochemical tests, together with maternal age and ultrasound results, obtained between 11+0- and 13–6-weeks' gestation allow for detecting almost 95% of all chromosomal aberration cases, with 5% of false positive results [1]. Every result suggesting an increased risk of fetal aneuploidy is an indication for a karyotype test, with the most common invasive method allowing for the test to be carried out being amniocentesis. In 2018, 6,926 such procedures were performed under NFZ financing. The NFZ program is designed to support chromosomal aberration diagnosis, but its enormous potential allowing for the screening of distant obstetric complications is not utilized. World literature reports that false positive results of prenatal tests can be useful in indicating pregnancy complications that are not related to an abnormal number of chromosomes. Trisomy 21 is associated with characteristic values of the proteinsare determined in these tests. For human chorionic gonadotropin (β-hCG), that value is most deemed to be over 2 MoM, and for pregnancy-associated plasma protein A (PAPP-A) — below 0.5 MoM [1]. These values, with a normal ultrasound picture and fetal euploidy indicated by the karyotype test, may be linked to numerous second trimester complications, such as preeclampsia, PIH,

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gestational diabetes mellitus, IUGR, premature rupture of membranes and preterm labor [2, 3].

The aim of this paper was to assess the indications for amniocentesis, accompanied by a detailed analysis of cases with normal ultrasound results between 11 + 0- and 13 + 6-weeks' gestation.

## MATERIAL AND METHODS

The results of first-trimester screening tests performed between 2014 and 2018 on 6,863 patients of the Prenatal Testing Outpatient Clinic at the Clinical Department of Obstetrics and Gynecology, Pomeranian Medical University, Szczecin, Poland, were analyzed retrospectively. The patients were aged between 14 and 46 and had singleton pregnancies. The analysis included patients whose ultrasound scans made between 11 and 13+6 weeks' gestation showed normal fetal anatomy and for whom all ultrasound markers for chromosomal abnormalities were within normal ranges, according to the Recommendations of the Ultrasound Section of the Polish Society of Gynecologists and Obstetricians. Subsequently, the patients were divided into three groups according to their test results:

- group A — an increased risk of fetal trisomy 21 (RS > 1:300);
- group B — a moderate risk of fetal trisomy 21 (RS 1:300–1:999);
- group C — a low risk of fetal trisomy 21 (RS ≤ 1:1000).
- Additionally, group A was divided into two subgroups:
- subgroup A1 — an extremely high risk (RS > 1:100);
- subgroup A2 — a high risk (RS 1:100–1:299).

Within these groups, PAPP-A MoM and free β-hCG MoM were analyzed to establish their correlations with the risk of trisomy 21. The ranges determined for PAPP-A MoM were 0.001–0.499 and ≥ 0.500, while those for free β-hCG MoM were of 0.001–1.999 and ≥ 2.000.

The results were then analyzed using the Statistica software (ver. 13.1). The statistical analysis was performed using the Mann-Whitney U test and Pearson's $\chi^2$ test, assuming the significance level of $p < 0.05$. The obtained results are shown in the tables and figures below.

## RESULTS

Out of the whole study population, 6,310 (91.94%) patients satisfied the inclusion criteria. A result indicating an increased risk of fetal trisomy 21 was obtained in 514 cases (8.15%). Out of those, 222 (3.52%) showed a value of > 1:100, while for 292 (4.63%) the risk ranged between 1:100 and 1:299.

The mean PAPP-A MoM values for groups A, B and C differed in a statistically significant manner, and were 0.65 ± 0.36, 0.80 ± 0.43 and 1.08 ± 0.60 ($p_{AB}$, $p_{AC}$, $p_{BC} < 0.001$), respectively.

The mean fβ-hCG MoM values for groups A1 and A2 differed in a statistically significant manner and were 2.70 ± 1.81 and 2.14 ± 1.32 ($p < 0.001$), respectively.

The mean PAPP-A MoM values for groups A1 and A2 differed in a statistically significant manner and were 0.55 ± 0.28 and 0.73 ± 0.39 ($p < 0.001$), respectively.

An fβ-hCG level of ≥ 2,000 MoM was shown in 50.97% of group A patients. This figure was 56.31% for subgroup A1 and 46.92% for A2. For group B, such levels were determined for 26.06%, and for group C for 10.21% of the patients.

A PAPP-A level ranging from 0.001 to 0.499 MoM was determined for 38.72% of group A patients. This figure was 49.55% for subgroup A1 and 30.48% for subgroup A2. For group B, such levels were determined for 24.56%, and for group C for 9.54% of the patients.

**Table 1.** Descriptive statistics of the variables analyzed for groups A, B and C.

| | | n | M | Me | SD | Min. | Max. |
|---|---|---|---|---|---|---|---|
| Group A | Age | 514 | 36.45 | 37.00 | 4.75 | 16.00 | 46.00 |
| | CRL [mm] | 514 | 64.67 | 64.40 | 7.63 | 45.00 | 84.00 |
| | β-hCG MoM | 514 | 2.38 | 2.05 | 1.57 | 0.22 | 13.14 |
| | PAPP-A MoM | 514 | 0.65 | 0.58 | 0.36 | 0.12 | 3.65 |
| Group B | Age | 733 | 36.08 | 36.00 | 4.85 | 20.00 | 45.00 |
| | CRL [mm] | 733 | 64.92 | 64.50 | 7.66 | 45.20 | 84.00 |
| | β-hCG MoM | 733 | 1.64 | 1.40 | 1.02 | 0.06 | 6.89 |
| | PAPP-A MoM | 733 | 0.80 | 0.69 | 0.43 | 0.07 | 3.07 |
| Group C | Age | 5,063 | 31.64 | 33.00 | 5.41 | 14.00 | 41.00 |
| | CRL [mm] | 5,063 | 63.78 | 64.40 | 7.83 | 43.00 | 84.00 |
| | β-hCG MoM | 5,063 | 1.15 | 0.96 | 0.74 | 0.005 | 7.94 |
| | PAPP-A MoM | 5,063 | 1.08 | 0.95 | 0.60 | 0.002 | 16.73 |

**Table 2.** Descriptive statistics of the variables analyzed for subgroups A1 and A2.

| | | n | M | Me | SD | Min. | Max. |
|---|---|---|---|---|---|---|---|
| Group A1 | Age | 222 | 36.83 | 38.00 | 4.64 | 19.00 | 45.00 |
| | CRL [mm] | 222 | 64.65 | 65.15 | 7.28 | 48.30 | 80.70 |
| | β-hCG MoM | 222 | 2.70 | 2.31 | 1.81 | 0.26 | 13.14 |
| | PAPP-A MoM | 222 | 0.55 | 0.50 | 0.28 | 0.12 | 1.65 |
| Group A2 | Age | 292 | 36.16 | 37.00 | 4.82 | 16.00 | 46.00 |
| | CRL [mm] | 292 | 64.68 | 64.20 | 7.90 | 45.00 | 84.00 |
| | β-hCG MoM | 292 | 2.14 | 1.87 | 1.32 | 0.22 | 8.64 |
| | PAPP-A MoM | 292 | 0.73 | 0.65 | 0.39 | 0.16 | 3.65 |

The mean fβ-hCG MoM values for groups A, B and C differed in a statistically significant manner, and were 2.38 ± 1.57, 1.64 ± 1.02 and 1.15 ± 0.74 ($p_{AB}$, $p_{AC}$, $p_{BC}$ < 0.001), respectively

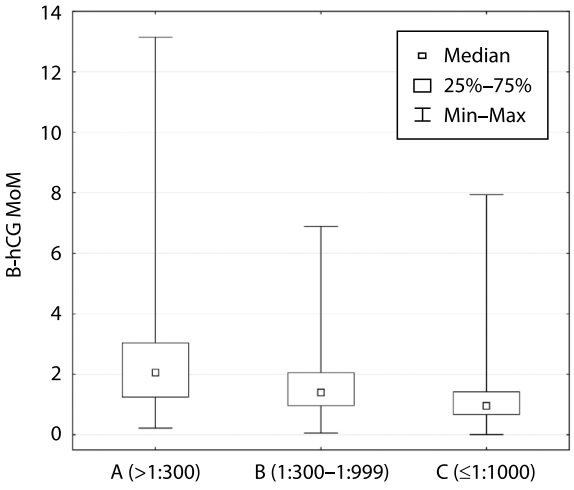


**Figure 1.** A comparison of fβ-hCG MoM distributions for groups A, B and C

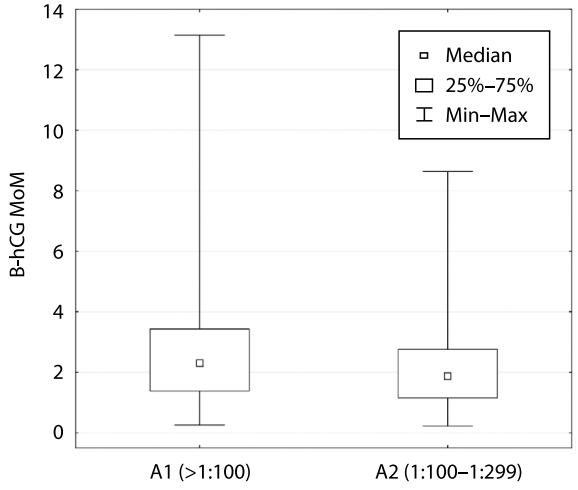


**Figure 3.** A comparison of fβ-hCG MoM distributions for groups A1 and A2

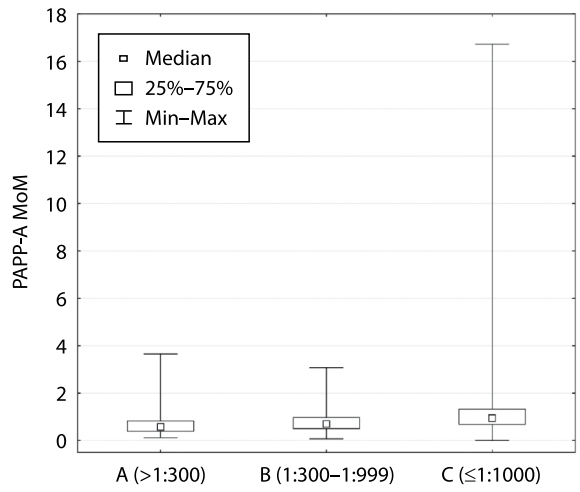


**Figure 2.** A comparison of PAPP-A MoM distributions for groups A, B and C

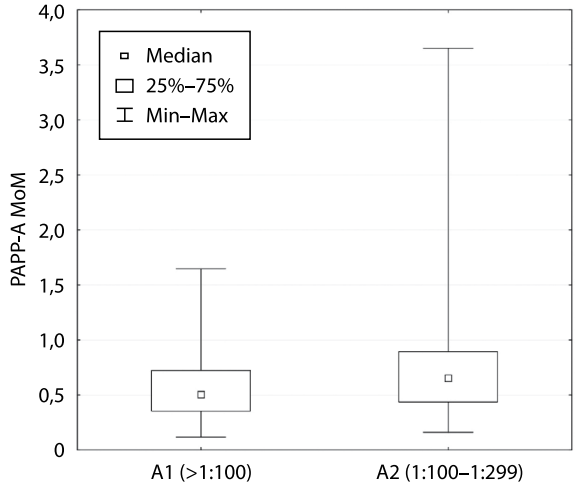


**Figure 4.** A comparison of PAPP-A MoM distributions for groups A1 and A2

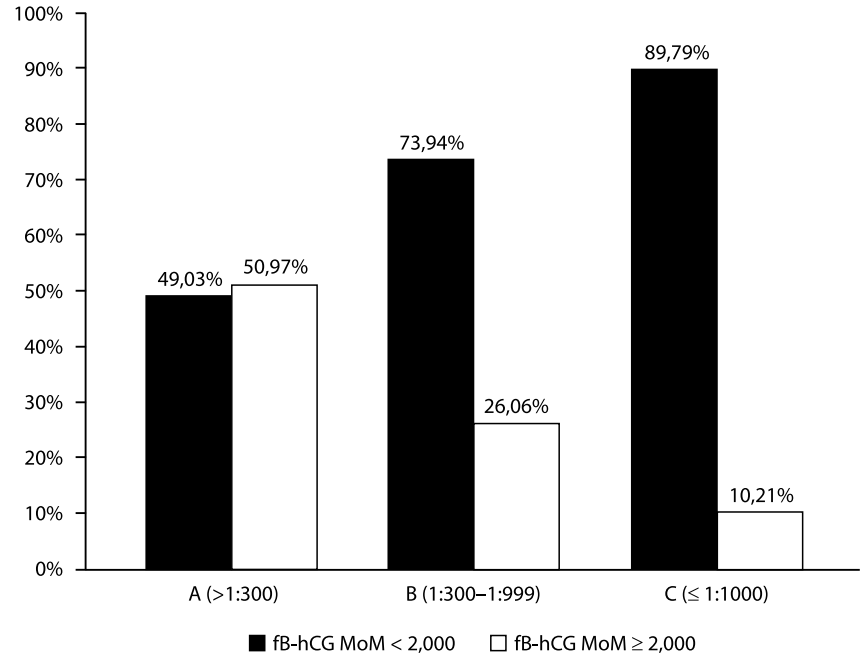


**Figure 5.** Correlations between fβ-hCG levels and the adjusted risk of fetal trisomy 21 for groups A, B and C

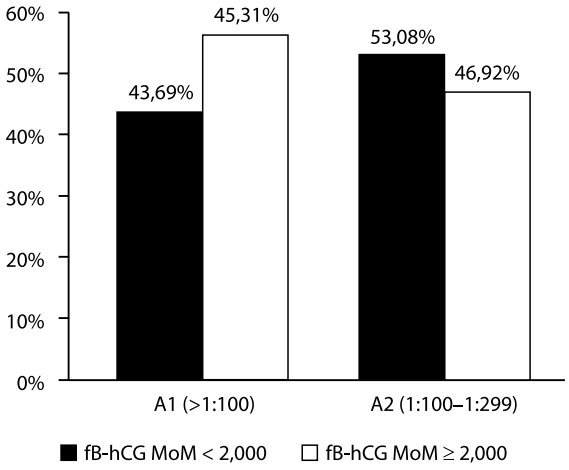


**Figure 6.** Correlations between fβ-hCG levels and the adjusted risk of fetal trisomy 21 for subgroupsA1 and A2

## DISCUSSION

The Polish National Health Fund introduced a first-trimester screening program in 2004. Since then, the percentage distribution of the most common indications for genetic amniocentesis has changed [4, 5]. Similarly, to any other invasive procedure, amniocentesis carries the risk of such complications as pregnancy loss (0.1–1%), rupture of membranes after the procedure (1–2%) and chorioamnionitis (0.01%) [6]. According to research, the prevalence of detected karyotype irregularities ranges between 11.7% and 33.9%, while an abnormal ultrasound result is the most frequent indication for amniocentesis in such cases [4, 7]. The calculated risk of trisomy 21 may be affected by three main groups of parameters, namely maternal age, maternal serum concentrations of free β-hCG and PAPP-A, and fetal ultrasound results. The woman's age, if considered alone, renders a detection rate of 30%. If biochemical markers (PAPP-A and β-hCG) are added to the test, the rate rises to almost 65%. Only after the third element, namely ultrasound parameters, is included does the detection rate reach its highest value of 90–95% of chromosomal aberration cases [8].

The risk of trisomy 21 increases with age. At the same time, women in developed countries are choosing to have offspring increasingly later. Nevertheless, there is credible evidence that the age of over 35 is a poor factor determining the development of fetal chromosomal abnormalities. In our study, the mean age in the increased risk group was 36.4 years with an SD ± 4.75, whereas women aged over 35 accounted for 66.73% of the group. By way of comparison, the mean age in the low risk group was 32.2 years with an SD ± 5.54, and the share of women aged over 35 was 32.13%.

Abnormal fetal nuchal translucency (NT) values are claimed to be especially useful in diagnosing chromosomal abnormalities, although not all reports seem to confirm that. For instance, Maket et al. studied women bearing an increased risk of trisomy 21 for correlations between PAPP-A and β-hCG on the one hand and the NT measurements on the other. They established that although the mean NT was significantly higher in fetuses with confirmed trisomy 21 than in euploid fetuses, a considerable proportion of the former group showed normal NT values. For

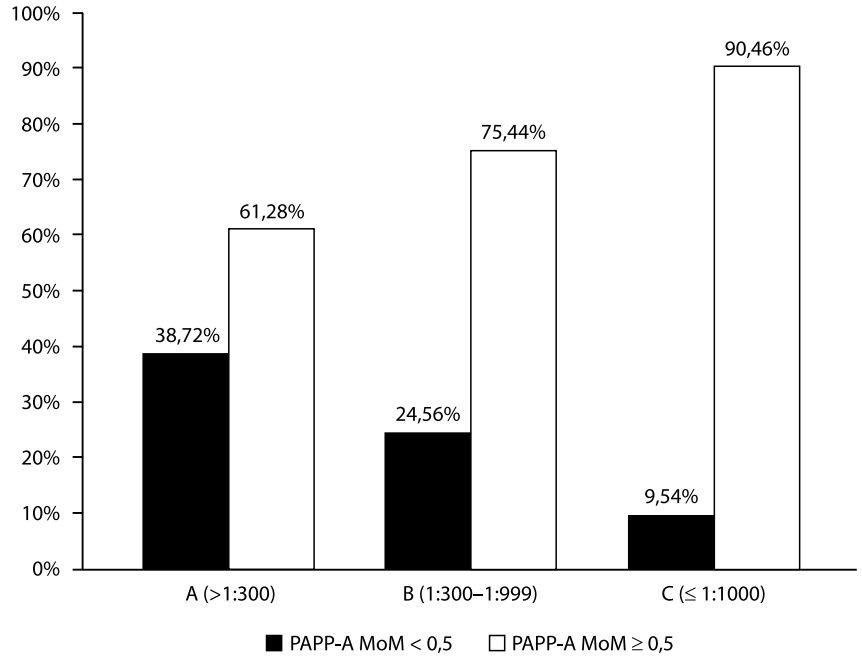


**Figure 7.** Correlations between PAPP-A levels and the adjusted risk of fetal trisomy 21 for groups A, B and C

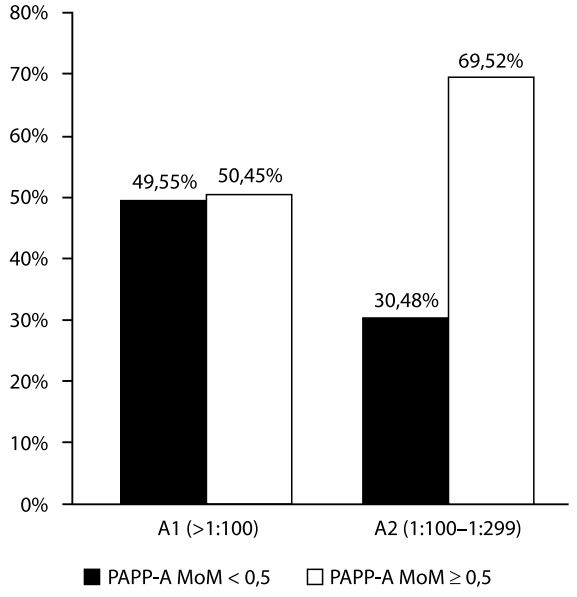


**Figure 8.** Correlations between PAPP-A levels and the adjusted risk of fetal trisomy 21 for subgroups A1 and A2

cases with PAPP-A concentrations of less than 0.5 and β-hCG levels of over 1.5 MoM, the figure was 44.15% and 26.5%, respectively [9].

Another element taken into account is nasal bone imaging. Wojda et al. [10] examined 941 fetuses, with trisomy 21 diagnosed in 45 of them. In their study, absent nasal bone demonstrated a mere 27% sensitivity and a 97% specificity as a marker for trisomy 21. As for its positive predictive value, it was estimated to be 35%. In their conclusions they found that nasal bone imaging was such a poor marker for aneuploidy that it should not be accounted for in risk algorithms [10].

In our study, although 6,310 patients satisfied the condition that all the ultrasound parameters should be normal, yet 514 (8.15%) of them were found to carry an increased risk of trisomy 21.

PAPP-A is a macromolecular protein, produced mainly by the syncytiotrophoblast. Due to the increase in its levels from as early as 6 weeks' gestation, the woman's system does not recognize the trophoblast as foreign tissue and, thus, allows the pregnancy to develop. Another role of PAPP-A is that of a growth-stimulating enzyme, as it releases bioactive insulin-like growth factors IGF-I and IGF-II of the insulin-like growth factor-binding protein (IGFBP) subgroup. Both these factors stimulate normal development of the placenta and, therefore, the embryo. Low PAPP-A concentrations are responsible for low IGF-1 and IGF-II expression, which may lead to impaired trophoblast invasion and, consequently, placental insufficiency. This mechanism is associated with numerous gestational complications, such as preeclampsia, PIH, gestational diabetes mellitus, IUGR, premature rupture of membranes and preterm labor [2, 3].

In our study, the measured mean PAPP-A concentration differed significantly between all the compared groups. Its value grew as the risk of trisomy 21 fell, and was 0.65 ± 0.36 MoM for group A, 0.80 ± 0.43 MoM for group B and 1.08 ± 0.60 MoM for group C.

Staboulidou et al. [11] compared PAPP-A concentrations at 11 to 13 weeks' gestation in 165 patients with preeclamp-

sia and 301 patients with parameters indicating fetal aneuploidy, including 200 cases of fetal trisomy 21. The levels of PAPP-A were similar for the trisomy 21 group (0.54 MoM) and the early-onset preeclampsia group (0.58 MoM). Significantly higher values were observed in women with the late-onset variant of preeclampsia (0.9 MoM) [11].

Spencer et al. [12] carried out a retrospective study of PAPP-A levels in 5,867 pregnant patients at 11 to 13 weeks' gestation diagnosed with preeclampsia. They established that low PAPP-A values were associated with a more severe course of the condition.

Similar conclusions were drawn by Odibo AO et al. [13] They found that PAPP-A concentrations, in conjunction with uterine artery PI and PP-13 protein (placental protein 13), may be reasonable individual predictors in women at risk of developing preeclampsia.

The second component of the so-called double marker test is human chorionic gonadotropin (β-hCG). Its production commences as early as approx. 7 days after conception, during the blastocyst stage [14]. In a physiological pregnancy, its concentration increases until approx. 10 weeks' of gestation, and subsequently falls to reach 10–20% of its peak value at 13 to 15 weeks' gestation. In trisomy 21, on the contrary, its concentration remains increased throughout the whole period (> 2.0 MoM) [2]. β-hCG is a hormone influencing a number of processes related to implantation, being also a key factor regulating angiogenesis in the chorion and the placenta by affecting angiogenic factors, e.g. vascular endothelial growth factor (VEGF) and angiopoietin (Ang-1) [14]. A high level of human chorionic gonadotropin in the second trimester of pregnancy is associated with impaired trophoblast invasion of the uterine spiral arteries, which is already observed in the first weeks after fertilization. This mechanism leads to chronic hypoxia and may be related to numerous gestational complications, such as preeclampsia and gestational hypertension [2, 15].

In our study, the mean β-hCG values for groups A, B and C were 2.38 ± 1.57 MoM, 1.64 ± 1.02 MoM and 1.15 ± 0.74 MoM, respectively.

According to the Fetal Medicine Foundation, β-hCG values exceeding 2.0 MoM may be indicative of, *inter alia*, trisomy 21, which is concordant with results reported by numerous researchers [16]. Its high concentrations are also associated with many other gestational complications. Revankar et al. [17] came to the conclusion that a high serum β-hCG level may be a predictor of gestational hypertension. When analyzing the results of tests on 7,754 women, Norwegian researchers observed a positive correlation between the total hCG content in early pregnancy and the risk of preeclampsia [18].

In most cases, positive biochemical parameters entail the application of invasive procedures. All of them, however, carry the risk of pregnancy loss. Therefore, it is essential that the results be correctly interpreted and the patients appropriately qualified for further diagnosis. On the one hand, it must be remembered that a normal ultrasound picture alone provides insufficient evidence of absent chromosomal abnormalities, on the other hand, however, it must be stressed that biochemical tests can be very useful in detecting not only genetic defects, but also other conditions of the fetus.

## CONCLUSIONS

In patients with normal fetal ultrasound parameters obtained in first-trimester screening tests and an increased risk of trisomy 21, the main causes of the increased risk are abnormal PAPP-A and β-hCG concentrations. In such cases, extension of diagnosis to include other gestational complications, e.g. preeclampsia, should be considered.

It is particularly important that patients who receive their first-trimester screening results should be made aware that normal ultrasound scan parameters do not guarantee they will deliver a healthy child, but also that a positive result does not necessarily imply aneuploidy.

In a woman with a singleton physiological pregnancy, β-hCG MoM shows a positive correlation, and PAPP-A MoMa negative correlation with the adjusted risk of fetal trisomy 21.

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