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ORIGINAL ARTICLE

Clinical article: screening for trisomy 13 using traditional combined screening versus an ultrasound-based protocol

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ABSTRACT

Aims: To compare the screening capability of ultrasonography in detecting trisomy 13 (T13) using a multiparameter sonographic protocol (NT+) with a classical combined screening test (CST) protocol.

Methods: The project was a prospective, multicenter study based on a nonselected mixed-risk population of women referred for a first-trimester screening examination. Each subject was offered a choice between either the gold standard, traditional combined screening test (CSG arm) or the ultrasound-based screening protocol (USG arm). General and MA-based screening performances were checked.

Results: The study population comprised 20,887 pregnancies: 12,933 in the CSG arm, including 27 cases of T13, and 7954 in the USG arm, including 30 cases of T13. The DR for T13 was higher in the CSG arm than in the USG arm for all tested cutoff points: 1/50 (88.5 versus 63.3%, respectively), 1/100 (88.5 versus 70%, respectively) and 1/300 (92.3 versus 83.3%, respectively). Using the ROC curves for fixed FPRs of 3 and 5%, the T13 detection rate in our study reached 90 and 93%, respectively, in the USG arm and 92 and 96%, respectively, in the CSG arm. MA influenced the T13 screening performance in the USG arm and reduced the DR in patients <31 years. Such influence was not detected in the CSG arm.

Conclusions: Classic CST was more effective in detecting T13 than the ultrasound-only approach. However, the recommended cutoff of 1/50 showed unsatisfactory results for both traditional CST and the multiparameter sonographic test we proposed.

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Combined screening; first trimester; free β-hCG; PAPP-A; trisomy 13

Introduction

Trisomy 13 (T13), also called Patau syndrome, is the third most common autosomal trisomy [1]. Fetuses with T13 often experience intrauterine fetal demise, and of those who are born alive, 20% will survive the first month of life, and 5% will survive the first 6 months. The estimated incidence of T13 is 1 in 5000–20,000 live births [2]. T13 is associated with a characteristic pattern of congenital anomalies, including single umbilical artery, prolonged persistence of fetal hemoglobin, microcephaly, microphthalmia, bilateral cleft lip and palate, alobar holoprosencephaly and a wide variety of abnormalities involving the heart and great vessels [3]. First-trimester screening for T13 based on maternal age, fetal nuchal translucency (NT), and biochemical markers in maternal serum has been shown to have a high detection rate (DR) of 90% with a false-positive rate (FPR) of 0.5% [4]; however, in another study, Combined Screening Test (CST) by using the algorithm for trisomy 21 showed DR for T13 at the level of 59%. By adding algorithm for trisomy 18, DR rose to 74%, and by adding T13 algorithm DR increased to only 75%. The same study showed that QUAD and Integrated Screening Test results present even lower DRs for T13 [5]. T13 is associated with a decrease in maternal serum-free β-hCG and PAPP-A and an increase in fetal nuchal translucency [4,6]. However, the use of biochemical markers in maternal serum as a screening tool for T13 seems to be less promising than for other aneuploidies, such as trisomy 21 (T21) and trisomy 18 (T18), for which serum markers are also used. Additionally, noninvasive prenatal screening (NIPT) for T21 showed a detection rate (DR) above 99% with a false-positive rate (FPR) of

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below 1%, while for T13, the DR is only 80–91% with a FPR below 1% [7–10]. In comparison, Benacerraf reported the sensitivity of ultrasonography in diagnosing T13 to be 90–100% when a detailed second-trimester scan was performed [11]. First-trimester sonographic characteristics of T13 are well described and include the following: increased NT, holoprosencephaly, microcephaly, facial abnormalities, cardiac abnormalities, exomphalos and postaxial polydactyly [1,12–15].

Altogether, trisomy 13 has the lowest first and second trimester detection rates among major trisomies basing on various first and second trimester screening policies [5].

In our screening population, we have observed cases of trisomy 13, which were screen negative in CST. Delayed diagnosis of T13 to the time of second trimester enhances parental stress because of fetal-maternal bonding caused by factors like maternal perception of fetal movements.

This is why we decided to conduct a study focused on T13 basing on our previous experiences with screening for trisomy 21, trisomy 18, and Turner syndrome. However, we were aware that in screening for T13, other parameters may play role, and the background risk differs from background risks for other major trisomies.

Despite advances in early diagnostic methods for detecting T13, there is a lack of studies describing the accuracy and reproducibility of screening based solely on ultrasound in comparison to those of screening based on biochemical markers combined with NT.

The aim of our study was to compare the screening capability of ultrasonography in detecting T13 using a multiparameter sonographic protocol (NT+) with a classical combined screening test (CST) protocol. Our sonographic multiparameter protocol was based on NT, nasal bone (NB), tricuspid flow (TF), and ductus venosus velocimetry (DV) enhanced with early anomaly and echocardiography findings.

Materials and methods

The project was a prospective, multicenter observational study that was based on a nonselected mixed-risk population of women referred for a first-trimester screening examination and that spanned from January 2012 to January 2017. The study protocol was approved by the Bioethics Committee at the District Medical Chamber in Krakow (opinion no. 77/KBL/OIL/2012). We recruited patients at the following six referral centers: Ultrasound Group Practice "MWU DOBREUSG" (Krakow), Ultrasound Laboratory at the Department of Gynecology and Obstetrics of Jagiellonian University (Kraków), St. Lukas Obstetric Center (Czestochowa), Opolian Center for Prenatal Diagnostics (Opole), Medical Center Semedica (Krakow), and Medical Center Civis VITA (Toruń). After the purpose of the study was explained, each subject was offered a choice between either a gold standard, traditional combined screening test (CSG arm) or multiparameter ultrasound-based screening (USG arm) performed by highly competent physicians at the first-trimester sonography. All subjects were informed regarding the screening performance of CST and the results of our previous studies showing the high screening performance of multiparameter ultrasound-based screening for T21, trisomy 18 (T18), and Turner syndrome but unknown results for T13. Subjects were also informed that they were allowed to change their study arm until when the crown-rump length (CRL) measurement exceeded 84 mm. Each participant signed a written consent form.

A group of patients was assessed for eligibility based on inclusion and exclusion criteria. The following inclusion criteria were used in this study: singleton pregnancy, crown-rump length (CRL) measurement of 45–84 mm, and known pregnancy outcome. The exclusion criteria included multiple pregnancy, pregnancy over 14 weeks, and intrauterine fetal death.

We applied the same screening methods as described in our study designed for the detection of T18, including using adjusted T13 risks with cutoffs of 1/50, 1/100, and 1/300 in both arms of the study [16].

In the CSG arm, the adjusted risk for T13 was calculated based on the maternal age (MA), fetal nuchal translucency (NT), fetal heart rate (FHR), measurement of the placental products of free β-hCG and PAPP-A (in MoM) in maternal blood samples using accredited and quality controlled Cobas E4 Analyzer (Roche, Manheim, Germany), major anomaly findings with fixed risk values (holoprosencephaly, exomphalos, diaphragmatic hernia, AVSD, and megacystis), and major anomalies without any influence on the risk for aneuploidy (anencephaly and severe limb defects) with the use of Fetal Medicine Foundation (FMF) algorithm software (Astraia GmbH, Munich, Germany). In the USG arm, the adjusted risk for T13 was calculated using FMF software based on MA, NT, FHR, all secondary markers [ductus venosus flow (DV), tricuspid flow (TF), nasal bone (NB)] and major anomaly findings (the same as in the CSG arm). Taking into account the significance of early anomaly findings for T13 screening,

all identified abnormalities at the time of nuchal scan were recorded.

Statistical analysis

SPSS Statistics v(0).17 software (IBM Co., Armonk, NY, USA) was used for calculations. The results with $p < .05$ were considered significant. The screening performance was measured by the following parameters: DR (detection rate), FPR (false-positive rate), screening accuracy, PPV (positive predictive value), and NPV (negative predictive value) using receiver-operator characteristic (ROC) analysis. Comparison of the means was performed with the nonparametric Mann–Whitney U-test for two independent tests. The sets of independent variables were compared applying Student's *t*-test. The χ^2 test was utilized to check the differences. The Kolmogorov–Smirnov test was used for continuous variable distribution.

Results

Screening for aneuploidy was carried out for 22,240 singleton pregnancies that were not randomly recruited to this study. Fetal karyotyping was obtained by means of amniocentesis (2318 cases). The remainder of the subjects included in the study were considered to be euploid based on postnatal assessment. A total of 1353 (6.1%) cases were excluded from further analysis because in 943 (4.2%) cases, it was impossible to determine the fetal karyotype due to loss before follow-up; in 93 (0.4%) cases, miscarriages not related to invasive testing occurred; in 51 (0.2%) cases, intrauterine fetal demise (IUFD) occurred without subsequent karyotyping, and in 266 (1.2%) cases, there was a chromosomal abnormality other than trisomy 13 (trisomy 21 in 143 cases; trisomy 18 in 52 cases; Turner syndrome in 41 cases; triploidy in 12 cases; Klinefelter syndrome in five cases; 47, XX, +idic(22) in one case; 46, XY, del(4)(q13.3q21.3) in one case; 46, XX, del(22)(q11.2q11.2) in one case; 46, XX, der(4)t(4;6)(p16;q23.3) in one case, 45, XX, der(14;21)(q10;q10) in one case, 47, XY + 18(29)/46, XY(26) in one case, 47, XY + 21(47)/46, XY(3) in one case, 45, X, der/5/t/5;14//5pter->5q35:14q13->14qter in one case, 45, XY, der(4)(4;13)(p15.3;q12) in one case, 46, XX, del(22)(q11.2;q11.2) in one case, 46, XY [10]/46, XY, t(4;14) (q10;q10) [15] in one case, 46, XX, der(9)t(2;9)(q33;p24) in one case, and 46, XY, inv(16)(p12q21) in one case).

Therefore, our study population comprised 20,887 pregnancies: 12,933 in the CSG arm, including 27 cases of T13, and 7954 in the USG arm, including 30 cases of T13 (Figure 1).

The median maternal body mass index was 22.6 kg/m^2 (range 17.3–35.8) in the CSG arm and 22.7 kg/m^2 (range 17.3–35.9) in the USG arm. These differences were not statistically significant ($p > .05$). All women participating in this study were Caucasian.

In the context of T13 and euploidy subjects, the USG arm showed no statistical significance according to CRL ($p = .366$) and maternal age ($p = .530$) but showed a statistical significance between euploidy and trisomy 13 according to NT ($p = .000$) and FHR ($p = .000$). In the context of T13 and euploidy subjects, the CSG arm showed no statistical significance according to CRL ($p = .594$) and maternal age ($p = .165$) but showed statistical significance between euploidy and trisomy 13 according to NT ($p = .000$), FHR ($p = .000$), free β-HCG ($p = .000$), and PAPP-A ($p = .000$). The demographic, sonographic, and biochemical parameters of each study arm are shown in Table 1.

In both arms of the study, the best sensitivity was obtained for 1/300 cutoffs: 92.3 and 83.3% for CST T13 and NT + T13, respectively. The lowest FPR (false-positive rate), on the other hand, was demonstrated for 1/50 cutoffs in both arms of the study. All screening tests in the CSG and USG arms demonstrated very high negative predictive values and high diagnostic accuracy.

The screening performance results for each study arm according to the cutoffs arranged in the protocol are shown in Table 2.

ROC analysis for both screening strategies for fixed 3 and 5% FPRs was also performed. The results are presented in Table 3.

In both arms of the study, patients with trisomy 13 were assigned to groups based on MA (maternal age) as follows: below 26 years old (no patients in the CSG and 7 patients in the USG); 26–30 years (7 patients in the CSG and 6 patients in the USG), 31–35 years (5 patients in the CSG and 10 patients in the USG), 36–40 years (11 patients in the CSG and 5 patients in the USG), and over 41 years (5 patients in the CSG and 3 patients in the USG). Detection rates and false-positive rates according to maternal age ranges in the USG arm and the CSG arm are shown in Figures 2 and 3:

Discussion

We present the results of a prospective study in which the traditional CST (CSG arm) was compared with an ultrasound screening protocol (USG arm) for T13. To

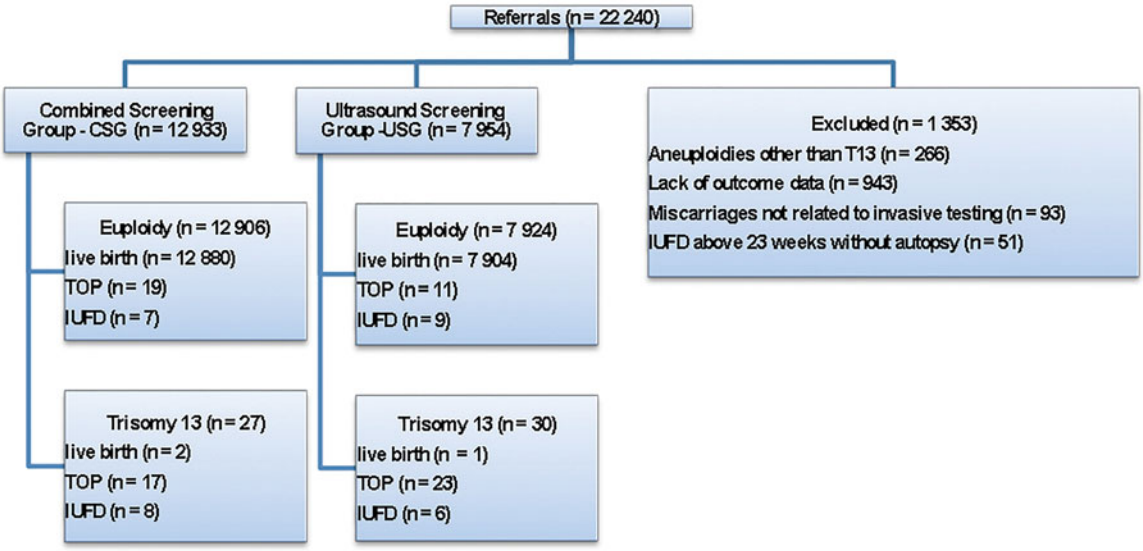

Figure 1. Study population diagram. T13: trisomy 13; IUFD: intrauterine fetal demise; TOP: termination of pregnancy.

Table 1. Demographic and clinical analysis of patients included in the study.

	CSG		USG	
Study arm	Euploid $n = 12{,}906$	Trisomy 13 $n = 27$	Euploid $n = 7924$	Trisomy 13 $n = 30$
Median maternal age (IQR)	34 (15–48)	36 (26–42)	30 (16–46)	32 (17–42)
Maternal age > 35 (%)	5766 (44.7)	18 (44.4)	2628 (33.0)	23 (76.7)
Median crown-rump length (IQR)	65.5 (45.0–84.0)	62.4 (47.0–81.0)	63.5 (45.0–84.0)	60.2 (45.0–84.0)
Median NT (IQR)	1.8 (0.1–4.9)	2.6 (1.4–6.4)	1.7 (0.1–4.9)	4.9 (1.4 1.3–11.5)
NT >95 percentile	874 (6.8)	18 (64.3)	5 (16.6)	14 (46.7)
Median FHR (IQR)	159 (101–207)	171 (149–201)	160 (109–191)	169 (148–189)
Absent NB (%)	NA	NA	107 (1.4)	11 (26.7)
TR (%)	NA	NA	98 (1.2)	6 (20.0)
Reverse DV (%)	NA	NA	116 (1.4)	9 (39.0)
>1 structural defect, *n* (%)	34 (0.7)	6 (21.4)	51 (0.9)	12 (41.4)
CNS anomaly, *n* (%)	43 (0.3)	6 (19.4)	43 (0.3)	6 (19.4)
Facial	7 (0.1)	4 (12.9)	7 (0.1)	6 (14.6)
Abdominal anomaly, *n* (%)	11 (0.1)	4 (12.9)	15 (0.2)	9 (22.0)
Limb anomaly, *n* (%)	7 (0.1)	3 (9.7)	10 (0.1)	3 (7.3)
Heart defects, *n* (%)	87 (0.7)	20 (74.1)	56 (0.8)	20 (66.6)
Megacystis, *n* (%)	2 (0.0)	2 (7.4)	5 (0.1)	1 (3.3)
Median PAPP-A (IQR)	1 (0.1–9.6)	0.2 (01-0.8)	n/a	n/a
Median β-HCG (IQR)	0.9 (0.1–9.5)	0.6 (0.2–1.9)	n/a	n/a

our knowledge, no other studies have made this type of comparison. Our results show that the DR for T13 is higher in the CSG arm than in the USG arm for all tested cutoff points: 1/50 (88.5 versus 63.3%, respectively), 1/100 (88.5 versus 70%, respectively) and 1/300 (92.3 versus 83.3%, respectively). In our study, the 1/50 cutoff shows unsatisfactory results for both the traditional CST test and the multiparameter sonographic test that we proposed (USG arm). However, it should be noted that the risk calculation program, FMF First Trimester Screening (Astraia GmbH, Munich, Germany), recommends a cutoff of 1/50 for T13 as well as for T18.

The most effective cutoff point for both methods in the screening for T13 seemed to be 1/300. In this case, we obtained satisfactory results and the smallest difference in screening performance between the

Table 2. Screening performance with fixed risks of 1/50, 1/100, and 1/300:.

	Combined Screening Group (CSG)			Ultrasound-based Screening Group (USG)		
Study arm Test	CST T13 with a cutoff of 1/50	CST T13 with a cutoff of 1/100	CST T13 with a cutoff of 1/300	NT + T13 with a cutoff of 1/50	NT + T13 with a cutoff of 1/100	NT + T13with a cutoff of1/300
Euploidy high risk (FPR)	124 (0.9%)	175 (1.4%)	323 (2.5%)	40 (0.7%)	48 (0.8%)	72 (1.8%)
T13 high risk (DR)	23 (88.5%)	23 (88.5%)	24 (92.3%)	19 (63.3%)	21 (70%)	25 (83.3%)
Diagnostic accuracy	99.0 (98.8–99.2)	98.6 (98.4–98.8)	97.5 (97.2–97.7)	99.2 (98.9–99.4)	99.1 (98.8–99.3)	98.7 (98.4–98.9)
PPV	15.6 (10.7–22.4)	11.6 (7.9–16.8)	6.9 (4.7–10.1)	32.2 (21.7–44.9)	30.4 (20.8–42.1)	25.8 (18.1–35.3)
NPV	99.9 (99.9–100.0)	99.9 (99.9–100.0)	99.9 (99.9–100.0)	99.8 (99.7–99.9)	99.8 (99.7–99.9)	99.9 (99.8–100.0)

Table 3. Screening performance from ROC analysis:.

Screening test		AUC	DR % (95% CI) with a 3% FPR	DR % (95% CI) with a 5% FPR
Combined screening group	CST T13 Cutoff	0.970	92% (83.7–100.3) 1/399	96% (87.6–104.4) 1/983
Ultrasound-based screening group	NT + T13 Cutoff	0.977	90% (82.4–97.6) 1/1512	93% (85.3-100.7-108.1) 1/3427

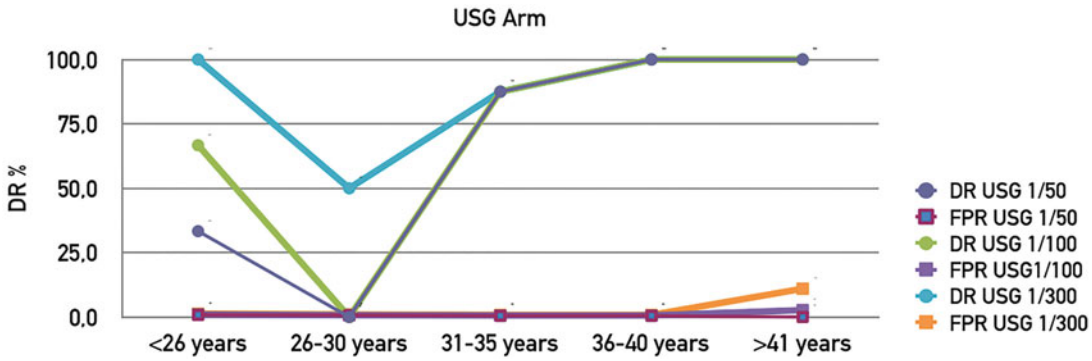

Figure 2. Detection rates (DRs) and false-positive rates (FPRs) of the tests used in the USG arm depending on maternal age ranges.

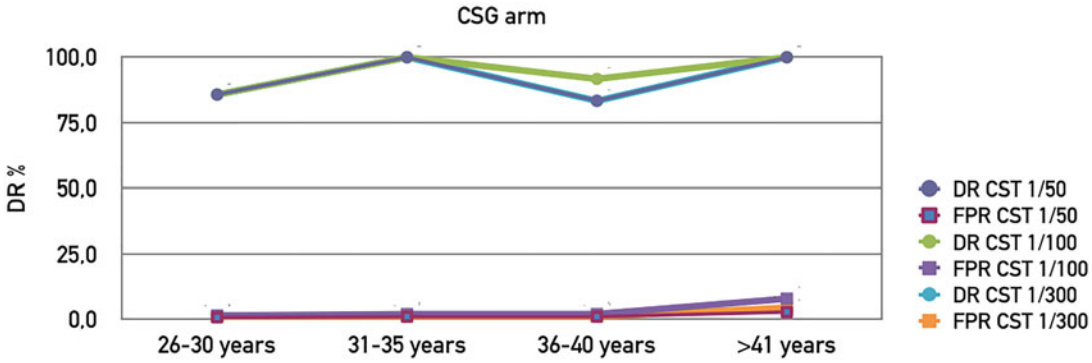

Figure 3. Detection rates (DRs) and false-positive rates (FPRs) of the tests used in the CSG arm depending on maternal age ranges.

tests. In the USG arm, we obtained a DR of 83.3% with a FPR of 1.8%, and for the same cutoff point in the CSG arm, we obtained a DR of 92.3% with a FPR of 2.5%. However, better PPV (25.8 versus 6.9, respectively) and diagnostic accuracy (98.7 versus 97.5, respectively) were obtained with the NT + test.

Even though our results showed the highest DR for T13 in CST with the cutoff of 1/300, it should be discussed and evaluated, if the cost of 2.5% FPR coming with this cutoff can be acceptable.

Using the ROC curves for fixed FPRs of 3 and 5%, the T13 detection rates in our study reached 90 and

93%, respectively, in the USG arm and 92 and 96%, respectively, in the CSG arm. It should be noted, however, that these results were obtained by reading from the ROC curve with cutoffs of 1/1512 and 1/3427 for NT + and 1/399 and 1/983 for CST, respectively. It is difficult to say why the authors in the literature published so far do not provide cutoff points read from the ROC curves, providing the results in only the "DR at fixed FPR" format [17,18]. In our opinion, from a clinical point of view, the effectiveness of the cutoff point is of fundamental importance for the screening practitioner.

Analyzing the dependence of DR on maternal age, we showed a significant decrease in the DR in pregnant women <26 years and in the age range of 26–30 years in the USG arm. The results obtained in the maternal age group range of <26 could not be compared with the CSG arm in our study because there were no patients of this maternal age in that arm (Figure 3). In the case of pregnant women >30 years, we obtained a stable, high DR. The results of DRs in younger women influenced the generally weaker screening performance in the USG arm (Figure 2).

In the CSG arm, a small decrease in the DR occurred in the case of pregnant women aged 36–40; however, in this group, the maternal age did not seem to have such a significant impact on the DR. In agreement with the literature, we showed that in T13, fetal structural abnormalities were identified more often than euploidy [16,19]. The most frequently reported abnormalities in this group are heart defects [11]. Similar to previous studies performed by other researchers, we showed an increased incidence of secondary sonographic markers of aneuploidy in fetuses with T13. In our study, the following markers were significantly more frequent in T13 than in euploidy: absent nasal bone was observed in 26.7% of T13 cases compared with 1.4% of euploidy cases (similar to the study performed by Cicero et al.: 31.8% for T13 versus 2.8% for euploidy [20]), tricuspid regurgitation in 20.0% compared to 1.2% (much less than described in the literature by Faiola et al.: 46.6% for T13 versus 8.5% for euploidy: [21]) and reverse ductus venosus flow in 39.0% for T13 versus 1.4% for euploidy (less than shown in the literature, Maiz et al.: 55% for T13 versus 3.2% for euploidy [22], but more than in our previous studies: 25% for T13 versus 2.46% for euploidy [19]). At least one structural abnormality is more frequently identified in T13 than in euploidy (in the CSG arm, 21.4% compared to 0.7%, and in the USG group, 41.4% compared to 0.9%) [11–15].

We also observed significantly higher fetal heart rate values in the T13 cases than in the euploidy cases ($p = .000$), which is in line with observations of other authors [17,23].

T13, similar to T18, is characterized by a high incidence of structural abnormalities that can be diagnosed during screening tests in the first trimester, but in T13, unlike in T18, the detection rate is higher when using classic CST than when using multiparameter ultrasound only (NT + test) [16]. This finding can be explained by the fact that the T13 risk calculation protocol according to the FMF does not include cleft lip/palate, the presence of a cardiac defect other than AVSD, or abnormalities of upper limbs, such as polydactyly [24,25].

The strengths of our study are the large number of patients enrolled in both the USG and CSG arms and the fact that the study was conducted prospectively. In the USG arm, the study was conducted by physicians who have been audited for over 10 years in the field of all first-trimester aneuploidy markers. The material was collected in large part from reference centers where patients include populations at risk; hence, there was a higher incidence of T13 than the general population incidence, with 1/479 in the CSG arm and 1/265 in the USG arm [1]. In addition, considering our homogeneous Caucasian population, the study may not be applicable to other ethnic groups. Additionally, the lack of patients below 26 years in the CSG arm may cause difficulties in comparing both arms of our study in terms of younger mothers.

In conclusion, the classic CST was more effective in detecting T13 than the NT + approach. In our opinion, the main reason for this fact is the ineffective influence of the maternal age factor in women aged below 30 and the lack of application of anomalies more typical for trisomy 13 in the risk calculation algorithm. However, the recommended cutoff of 1/50 showed unsatisfactory results for both the traditional CST and the multiparameter sonographic test we proposed. Using the 1/300 cutoff significantly increases the detection rate for a minimal increase in the false positive rate.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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