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ORIGINAL ARTICLE

Screening for trisomy 18 using traditional combined screening vs. ultrasound-based protocol in tertiary center environment

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Abstract

Objectives: To compare the screening performances of combined screening test risk algorithm for trisomy 18 (T18) using various cutoffs with a multiparameter ultrasound-based method. To compare the general and maternal age (MA)-based screening performances for T18 by means of combined screening and an ultrasound-based method.

Methods: This was a prospective, multicenter study based on a mixed-risk non-selected population of women referred to referral centers for a first-trimester screening. Each subject was offered a choice between either a traditional combined screening (CSG arm) or an ultrasound-based screening (USG arm). General and MA-based screening performances were measured.

Results: The study population comprised 10 820 pregnancies as follows: 5132 in the CSG arm, including 28 cases of T18, and 5688 in the USG arm, including 29 cases of T18. In the CSG arm, the detection rate (DR) for T18 at a false-positive rate (FPR) of 3% was 86%, whereas the DR was 100% for the USG arm. MA influenced the T18 screening performance in the CSG arm and reduced the DR in MA ranges <26 years and 31–35 years. This influence was not observed in the USG arm.

Conclusions: Only, a multiparameter ultrasound-based screening method may be considered an effective alternative to combined screening for T18 screening. The technique exhibits high and stable DRs irrespective of MA.

Keywords

Trisomy 18, first trimester, ultrasound, PAPP-A, combined screening

History

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Introduction

Trisomy 18 (T18) is a lethal condition and is one of the crucial chromosomal aberrations detected in the first trimester. The prevalence of T18 has significantly increased over time from 3.95/10 000 births in 1995–1999 to 6.94/10 000 births in 2005–2009 [1]. In contrast with trisomy 21 (T21), the screening policy for T18 is not completely established due to the lower prevalence, high lethality, and potential underrepresentation of low nuchal translucency (NT) T18 cases in previously conducted studies [2,3]. T18 is recognized as one of the major aneuploidies presenting very low serum pregnancy-associated plasma protein A (PAPP-A) levels, as a consequence of low placental volume, which limits the performance of currently expanding non-invasive prenatal testing (NIPT) producing up to 8% failed cell-free DNA (cfDNA) readings [4]. The earliest studies on the first-trimester screening for T18 showed that 79% of affected cases revealed nuchal translucency (NT) above the 95th percentile, 34.1% exomphalos, and 15.9% fetal edema [5,6]. Later studies concentrated more on the input of first-trimester biochemistries in combined screening testing (CST). They correspond to the observations of extremely low serum values of PAPP-A and free β-HCG (fβhCG) identified in T18 pregnancies [7]. To date, various screening policies for T18 using CST were described in the literature. These policies used cutoffs of 1/300, 1/250, or 1/200 of adjusted risk for T21 and cutoffs of 1/50, 1/100, or 1/200 of adjusted risk for T18. In addition, some of these combinations were used [8–11]. The performance of these screening methods differs among published data, ranging from detection rates (DR) of 64% with a false-positive rate (FPR) of 2% to 100% DR with an FPR of 6.4% [8–13]. In addition, some of the latest papers utilize only DRs and FPRs obtained from receiver operating characteristic (ROC) curves [12,13]. However, from a clinical point of view, the screening performance of risk cutoffs, which are required for screening, is more important. Taking into account these inconsistencies in the literature and results from our recent studies on first-trimester ultrasound-based screening for T18 and T21, we designed this two-arm study. The first goal is to

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compare the screening performances of the traditional CST risk algorithm for T18 using various cutoffs with an ultrasound-based method [14–16]. The second aim of this study is to compare the general and maternal age (MA)-based screening performances for T18 using CST and ultrasound-based methods.

Methods

This was a prospective, multicenter study based on non-selected mixed-risk population of women referred for a first-trimester screening examination between January 2009 and June 2012. The patients were examined at the following four referral centers: Ultrasound Group Practice ''dobreusg'' (Krakow), Ultrasound Lab at the Department of Gynecology and Obstetrics of Jagiellonian University (Krakow), St. Lukas Obstetric Center (Czestochowa), Opolian Center for Prenatal Diagnostics (Opole), and Medical Center Semedica (Krakow). The following inclusion criteria were used in this study: singleton pregnancy, crown-rump length (CRL) measurement of 45 to 84 mm, and known pregnancy outcome. After explaining the purpose of the study, each subject was offered a choice between either a gold standard, traditional combined screening test (CSG arm) or an ultrasound-based screening (USG arm). Each participant signed a written consent form, which was approved by the local ethics committee. We have applied the same screening methods as described in our study designed for the detection of T21 [16]. The only difference was that in T18 protocol we used adjusted T18 risks with cutoffs of 1/50, 1/100, and 1/300 in both arms of the study because these values were previously reported in the literature [8–13]. In the CSG arm, the adjusted risk for T18 was computed based on the maternal age (MA), fetal NT, fetal heart rate (FHR), major anomaly findings with fixed risk values (holoprosencephaly, omphalocele, extensive diaphragmatic hernia, atrioventricular septal defect, and megacystis), major anomalies without any influence on risk for aneuploidy (anencephaly and severe limb defects), and maternal serum fβhCG and PAPP-A levels (in MoM) with the use of the Fetal Medicine Foundation (FMF) algorithm (Astaia Gmbh, Munich, Germany). In the USG arm, the adjusted risk for T18 was calculated using FMF software based on MA, NT, FHR, all secondary markers [(ductus venosus velocimetry (DV), tricuspid flow (TF), nasal bone (NB)], and major anomaly findings (same as in the CSG arm). Taking into account the significance of early anomaly findings for T18 screening, all identified abnormalities at the time of nuchal scan were recorded.

Statistical analysis

The Kolmogorov–Smirnov test was utilized for continuous variable distribution. The χ^2 test was applied to assess the differences. The sets of independent variables were compared using Student's *t*-test. The Mann–Whitney *U* test was also utilized as a non-parametric measure. The calculations were performed with SPSS Statistics v(0).17 software environment (IBM Co., Armonk, NY). The results with $p < 0.05$ were considered significant. The screening performance was measured with the use of receiver operating characteristic (ROC) and traditional parameters, including DR, FPR, screening accuracy, positive predictive value (PPV), and negative predictive value (NPV).

Results

Screening examination was performed in 11 678 singleton pregnancies who were recruited for this study. Fetal karyotyping was obtained by means of amniocentesis in 1325 cases. The remainder of the subjects in the study was considered to be euploid based on the postnatal assessment. In total, 858 (7.3%) cases were excluded from further analysis because it was impossible to determine the fetal karyotype due to losing them from the follow-up in 552 (4.7%) cases, 73 (0.6%) cases resulted in miscarriages not related to invasive testing, 28 (0.2%) had intrauterine fetal demise without subsequent karyotyping, and a chromosomal abnormality other than trisomy 18 was noted in 205 (1.7%) cases (trisomy 21 ($n = 138$); trisomy 13 ($n = 17$); Turner syndrome ($n = 33$); triploidy ($n = 10$); Klinefelter syndrome ($n = 4$); 47,XX, +idic(22) ($n = 1$); 46,XY, del(4)(q13.3q21.3) ($n = 1$); and 46, XX del(22)(q11.2q11.2) ($n = 1$).

Therefore, our study population comprised 10 820 pregnancies as follows: 5132 in the CSG arm, including 28 cases of T18 and 5688 in the USG arm, including 29 cases of T18. These groups were not significantly different according to the prevalence of trisomy T18 ($p = 0.798$). Median maternal age (MA) at the time of examination was 36 (15–48) years in the CSG arm and 30 (16–46) years in the USG arm. The characteristics of the study population in both arms of the study are summarized in Figure 1 and Table 1.

The general performance of adjusted T18 risk cutoffs for screening tests used in this study is presented in Table 2.

In the CSG arm, the ''CST T18 1/300'' test had the best sensitivity. The highest specificity in this arm was observed with the ''CST T18 1/50'' test, which was statistically significant ($p = 0.000$). All screening tests in the CSG arm demonstrated very high negative predictive values (range: 99.7–99.9%). The highest diagnostic accuracy (92.7%) in this arm was observed with ''CST T18 1/50.''

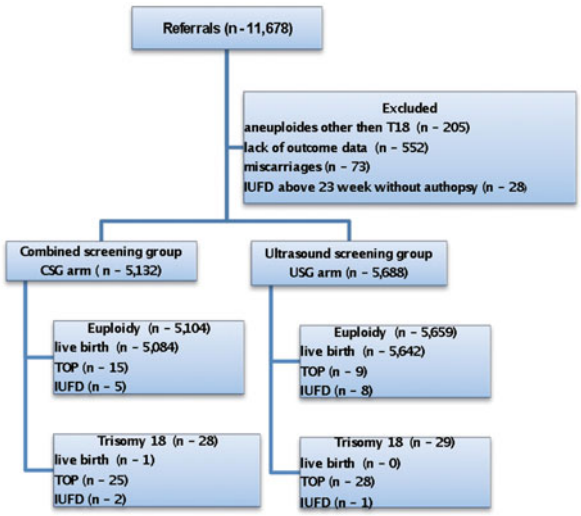

Figure 1. Study population diagram. Abbreviations: T18: trisomy 18; IUFD: intrauterine fetal demise; TOP: termination of pregnancy.

Table 1. Comparison of population characteristics, ultrasound findings, and biochemistry readings (CSG arm) at the first-trimester screening in two arms of the study.

	CSG		USG	
	Euploid, $n = 5104$	Trisomy 18, $n = 28$	Euploid, $n = 5659$	Trisomy 18, $n = 29$
Maternal age median (IQR)	36 (25–41)	35 (22.9–45.0)	30 (24–38)	34 (21–43)
Maternal age >35 (%)	2656 (52.0)	18 (64.3)	680 (12)	11 (37.9)
Crown-rump length median (IQR)	63.6 (50.0–78.9)	60.5 (49.3–78.7)	62.9 (48.6–79.7)	60.6 (45.2–78.8)
NT median (IQR)	1.7 (1.2–2.7)	2.8 (1.5–7.8)	1.6 (1.1–2.5)	5.1 (1.4–11.0)
NT >95th percentile	312 (6.1)	18 (64.3)	242 (4.3)	22 (75.9)
FHR median (IQR)	160 (148–172)	157 (135.5–171.7)	160 (148–172)	160 (139–177.5)
Absent NB (%)	NA	NA	85 (1.5)	11 (37.9)
TR (%)	NA	NA	100 (1.8)	14 (48.3)
Reverse DV (%)	NA	NA	119 (2.1)	11 (37.9)
>1 structural defect, *n* (%)	34 (0.7)	6 (21.4)	51 (0.9)	12 (41.4)
CNS anomaly, *n* (%)	22 (0.4)	2 (7.1)	15 (0.3)	2 (6.9)
Facial	2 (0.0)	1 (3.6)	4 (0.1)	2 (6.9)
Abdominal anomaly, *n* (%)	0 (0.0)	4 (14.3)	4 (0.1)	2 (6.9)
Limb anomaly, *n* (%)	4 (0.1)	1 (3.6)	7 (0.1)	2 (6.9)
Heart defects, *n* (%)	26 (0.5)	6 (21.4)	51 (0.9)	12 (41.4)
Megacystis, *n* (%)	1 (0.0)	2 (7.1)	5 (0.1)	2 (6.9)
PAPP-A median (IQR)	0.99 (0.02–9.58)	0.4 (0.04–2.16)	n/a	n/a
fb-HCG median (IQR)	1.31 (0.01–9.24)	0.39 (0.06–2.16)	n/a	n/a

Table 2. Performance of screening tests for trisomy 18 with fixed cutoffs evaluated in this study in both arms of the study.

Study arm	Combined Screening Group (CSG)			Ultrasound-based Screening Group (USG)		
Test	CST T18 with cutoff 1/50	CST T18 with cutoff 1/100	CST T18 with cutoff 1/300	NT + T18 with cutoff 1/50	NT + T18 with cutoff 1/100	NT + T18 with cutoff 1/300
Euploidy high risk (= FPR)	87 (1.7%)	124 (2.4%)	214 (4.2%)	76 (1.3%)	91 (1.6%)	148 (2.6%)
T18 high risk (= DR)	20 (71.4%)	23 (82.1%)	25 (89.3%)	26 (89.7%)	29 (100%)	29 (100%)

CST: adjusted risk by combined screening test; NT+: adjusted risk by nuchal translucency and secondary ultrasound markers; FPR: false-positive rate; DR: detection rate.

In the USG arm, the highest sensitivity was obtained with the ''NT + T18 1/100'' test. Screening methods in the USG arm demonstrated higher specificity, positive predictive value, and diagnostic accuracy compared with the tests used in the CSG arm. Similar to the CSG arm, all screening tests in the USG arm showed high negative predictive values. The details are provided in Table 3.

The ROC method was also used to compare the screening performances in both arms of the study for fixed FPR values. In the CSG arm, the DR for T18 at FPR of 3% was 86%, whereas the DR for the USG arm was 100% (Table 4).

In the CSG arm and the USG arm, the cases with trisomy 18 were divided into groups based on maternal age as follows: <26 years (4 cases in CSG and 2 cases in USG); 26–30 years (2 cases in CSG and 10 cases in USG), 31–35 years (8 cases in CSG and 6 cases in USG), 36–40 years (9 cases in CSG and 10 cases in USG), and ≥ 41 years (5 cases in CSG and 1 case in USG). Maternal age-dependent screening performance in both arms of the study was assessed by charts and is presented in Figure 2.

Discussion

To our knowledge, this is the first large population-based, prospective study comparing traditional CST (CSG arm) with ultrasound-based screening (USG arm) for T18. Our results indicate that the ultrasound approach with the cutoff of 1/100 presented excellent DR for T18 at the level of 100% with an FPR of 1.6% compared with 82.1% DR and 2.4% FPR for CST at the same cutoff. By reducing the CST cutoff to 1/300, DR increased to 89% with a 2-fold increase in FPR to 4.2%. The results of this study confirmed our pilot results from the report focused on ultrasound-based screening for T18 [14]. Our findings disclosed better screening performance of CST with the cutoff 1/100 compared with the FASTER study, which demonstrated 60% of DR for T18 with 0.1% FPR based on a larger population of 36 171 patients including 28 cases of T18. With the use of ROC curves for fixed FPRs of 3%, the detection rate of T18 in our study was 100% in USG arm and 86% in the CSG arm. Our results confirmed observations of previous researchers that at least one structural abnormality in T18 is identified more often compared with euploidy (in CSG arm: 21.4% vs. 0.7%, and in USG arm 41.4% vs. 0.9%). Of the structural defects, cardiac anomalies are the most common defects that can be depicted. Early anomaly findings and the addition of secondary markers of aneuploidy increase the sensitivity of screening for T18 [17–19]. In our study, these markers were observed significantly more often in T18 compared with euploidy as follows: absent NB was observed in 37.9% of T18 cases vs. 1.5% of euploidy, tricuspid regurgitation in 48.3% vs. 1.8%, and reverse ductus venosus flow in 37.9% vs. 1.4%. Other authors observed these markers

Table 3. Screening performance summary of the methods used in both arms of this study.

Arm	Combined Screening Group			Ultrasound-based Screening Group		
Screening method	CST T18 1/50	CST T18 1/100	CST T18 1/300	NT + T18 1/50	NT + T18 1/100	NT + T18 1/300
Sensitivity	71.4% (52.94–84.75)	82.1% (64.41–92.12)	89.3% (72.8–96.29)	89.7% (73.61–96.42)	100% (88.3–100)	100% (88.3–100)
Specificity	98.3% (97.9–98.61)	97.6% (97.11–97.96)	95.8% (95.22–96.32)	98.77% (98.32–98.92)	98.4% (98.03–98.69)	97.38% (96.93–97.77)
PPV	18.7% (12.44–27.12)	15.7% (10.66–22.38)	10.5% (7.19–14.99)	25.5% (18.03–34.73)	24.2% (17.39–32.55)	16.38% (11.66–22.54)
NPV	99.8% (99.69–99.92)	99.9% (99.77–9.96)	99.9 (99.82–99.98)	99.95% (99.84–99.98)	100% (99.93–100)	100.0% (99.93–100)
Diagnostic accuracy	98.2% (97.74–98.48)	97.5% (97.02–97.88)	95.8% (95.18–96.28)	98.6% (98.27–98.88)	98.4% (98.04–98.69)	97.39% (96.95–97.79)

CST: adjusted risk by combined screening test; NT: adjusted risk by nuchal translucency; NT+: adjusted risk by nuchal translucency and secondary ultrasound markers; PPV: positive predictive value; NPV: negative predictive value; T18: trisomy 18.
The estimates of lower to upper 95% confidence intervals (CIs) are indicated in brackets.

Table 4. Screening for trisomy 18 by different policies based on MA, NT, FHR, fβhCG, PAPP-A, and major anomaly findings (CST) as well as MA, NT, FHR, NB, DV, TR, and major anomaly findings (USG).

Screening test		AUC	DR% (95% CI) at 3% FPR
Combined Screening Group	CST T18	0.949	86% (78.0–93.4)
Ultrasound-based Screening Group	NT + T18	0.994	100% (91.9–108.1)

AUC: area under the curve; CST: adjusted risk by combined screening test; NT+: adjusted risk by nuchal translucency and secondary ultrasound markers; T18: trisomy 18; MA: maternal age; NT: nuchal translucency; FHR: fetal heart rate; NB: nasal bone; TR: tricuspid regurgitation; DV: ductus venosus velocimetry.
The estimates of lower to upper 95% confidence intervals (CIs) are indicated in brackets.

in the following prevalence rates: 57.1% vs. 2.8%, 53% vs. 8.5%, and 82.9% vs. 10.7% [17–19].

The impact of maternal age on the screening performance for T18 is demonstrated for the first time in this study. This effect is mainly observed for the CST test in MA ranges less than 26 years and between 31 and 35 years, which exhibit a reduction in DR to the level of 75% for the cutoff 1/300. For the cutoff 1/100, the DR is reduced to 25% in the youngest MA range. In comparison, in the USG arm, DR is stable at 100% for both cutoffs of 1/100 and 1/300 for all MA ranges. In patients greater than 41 years of age, the FPR increases in both arms of the study to 10.1% for CST 1/300 and 6.6% for NT+ 1/100 and 1/300.

Trisomy 18 is characterized by a high prevalence of abnormal ultrasound findings that can be diagnosed at the time of the first-trimester scan [14,20,21]. For this reason, this scan serves as a perfect tool for the detection of this chromosomal abnormality. In 37 fetuses with trisomy 18 examined by Wiechec et al., 97% of cases presented major anomalies, with cardiac defects observed in approximately 70% of cases and extracardiac anomalies in 35% of cases [14]. In a recent study by Wagner et al., at least one structural defect was diagnosed in 82.5% of affected fetuses, with multiple defects found in 40% of cases [20]. These results are consistent with our findings and confirm that the majority of fetuses with T18 show structural defects that are feasible for diagnosis at the time of first-trimester scan.

Because CST is the method of choice in the screening of aneuploidies during the first trimester, data regarding the diagnostic accuracy of ultrasound methods in the detection of T18 are hardly available. Wiechec et al. tested protocols dedicated to T21 and T18 in T18 screening using ultrasound markers of aneuploidy (NT, NB, TR, and DV) enhanced with early anomaly and early echocardiography findings [14]. The protocols reported DRs of 92 to 95% with a 3% FPR and 95 to 100% with a 5% FPR. A comparable, high DR of ultrasound-based approach was recently confirmed by other researchers [20]. In screening for T18, trisomy 13, triploidy, and Turner syndrome, a DR of approximately 90% was reported if the calculated risk was based on MA, NT, additional ultrasound markers (NB, TF, DV), and fetal anomalies. Interestingly, such high DRs were not achieved with the use of NT and secondary ultrasound markers without the addition of

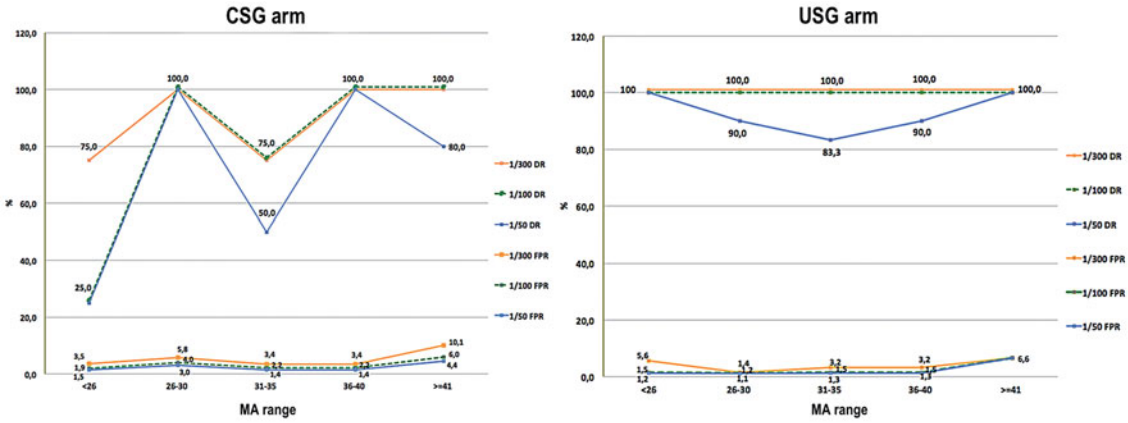

Figure 2. On the left: detection rates (DR) and false-positive rates (FPR) of the tests used in the CSG arm based on maternal age ranges. On the right: detection rates (DR) and false-positive rates (FPR) of the tests used in the USG arm based on maternal age ranges.

structural findings, which confirms the need for detailed anatomy evaluation.

The first arm of our study based on gold standard CST was applied to compare the DR of T18 in the same, unselected mixed-risk population of women. In the study of Kagan et al., approximately 82% of fetuses with T18 were identified with a 3% FPR [12]. In comparison, in our study, we identified 86% of fetuses with T18 with an FPR of 3%.

The lower performance of CST for T18 in our study may be potentially explained by the better specificity of the ultrasound findings in T18 compared with biochemical readings, which reflect the general placental function and are not highly specific for this aneuploidy. Furthermore, there are known factors that affect biochemistry results, such as gestational age, maternal weight, ethnicity, fetal gender, presence of renal insufficiency, and diabetes, which do not affect ultrasound-based protocols [22–24].

The main advantage of the study is the relatively high number of subjects enrolled in both arms and its prospective nature. However, the collection of a representative number of affected cases in this study was only possible due to the referrals to tertiary centers. Hence, numerous cases screened here had risk factors based either on sonographic suspicion or a history that was previously noted by the referring obstetrician. Our study population does not reflect the screening environment given the high prevalence of T18 (1/182 in CSG arm and 1/195 in USG arm). Additionally, given our homogenous Caucasian study population, our findings are potentially not applicable to other ethnic origins.

Conclusions

Ultrasound-based screening is an effective alternative for T18 screening using traditional CST. It must be emphasized that ultrasound-based screening exhibits high and stable DRs regardless of maternal age. Although this method requires a learning curve and quality assurance protocols dedicated for the ultrasound parameters, this method is not affected by disturbing factors, which increase FPRs that are described for serum biochemistries in CST. Therefore, an ultrasound-based approach may be proposed as the first step of a contingent policy using cfDNA testing.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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