Marcin Wiechec*, Agnieszka Nocun, Anna Knafel, Ewa Wiercinska, Jiri Sonek, Wioletta Rozmus-Warcholinska, Maciej Orzechowski, Dominika Stettner and Petr Plevak

Combined screening test for trisomy 21 – is it as efficient as we believe?

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Abstract

Objectives: To compare two first-trimester screening strategies: traditional combined screening and the one based on ultrasound markers only. We investigated the effect of maternal age (MA) on the screening performance of both of these strategies.

Methods: This was a prospective observational study based on a non-selected mixed-risk population of 11,653 women referred for first-trimester screening. The study population was divided in two groups: combined screening (CS) and ultrasound-based screening (US). Absolute risk was calculated to determine the influence of MA on screening performance.

Results: The CS arm comprised 5145 subjects including 51 cases of trisomy 21 (T21), and the US arm comprised 5733 subjects including 87 subjects with T21. Seven hundred and seventy-five subjects were excluded from the study. For a false positive rate (FPR) of 3%, the detection rate (DR) of T21 in CS arm was 78% vs. 90% in US arm. For 5% FPR, DR was 84% and 94% in CS and US arm, respectively. MA had an influence on DR positive rates in CS: both DR and FPR for T21 increased with advance in MA.

Conclusions: The US protocol showed higher DR of T21 compared to the CS one. It may be considered as a viable alternative to CS for T21 where access to biochemical testing is limited.

Keywords: First trimester; first-trimester screening; nuchal translucency; trisomy 21.

Introduction

In recent years, screening for trisomy 21 (T21) has undergone an evolution with combined screening test (CST) based on nuchal translucency (NT) measurements and serum biochemistries with a detection rate (DR) of 90%–95% at the false positive rate (FPR) of 5%–7% [1–6], and for non-invasive prenatal testing (NIPT) based on fetal DNA sequencing from maternal blood with a DR above 99% and FPR below 1% [7–9]. However, in the next few years, a wider introduction of NIPT to screening is only possible in countries with high socioeconomic status due to the cost of the test. Even in wealthier countries, NIPT has been adopted as part of a contingent screening strategy so far. For this reason, searching for other screening solutions and policies is still essential, especially for patients with an advanced maternal age (AMA) of 36 years and older presenting high FPRs in CST. In previous extensive research, conducted mainly by the Fetal Medicine Foundation (FMF) and related authors, the aspect of applying ultrasound based on NT and secondary ultrasound markers without the support of biochemical markers was omitted [1–3, 10]. Only a combination of NT with biochemistry and various variations of using secondary ultrasound markers were applied. Our team introduced this concept for the first time in a pilot study basing on a population of 6265 patients, who showed excellent screening performance with a DR of 91.7% at FPR 3% and 95.2% at FPR 5% [11]. Recently, Kagan et al. have presented comparable results [12]. In everyday work, despite maintaining high standards of biochemical

***Corresponding author: Marcin Wiechec,** Chair of Gynecology and Obstetrics, Jagiellonian University in Krakow, 23 Kopernik Street, 31-501 Krakow, Poland, Tel.: +48124248423, Fax: +48124248584, E-mail: marcin_wiechec@su.krakow.pl; and Ultrasound Group Practice "dobreusg", 20A Felinskiego Street, 31-236 Krakow, Poland
Agnieszka Nocun and Anna Knafel: Ultrasound Group Practice "dobreusg", 31-236 Krakow, Poland
Ewa Wiercinska: Voivodeship Sanitary-Epidemiological Station in Krakow. 31-202 Krakow, Poland
Jiri Sonek: Division of Maternal Fetal Medicine, Wright State University, Miami Valley Hospital. 128 Apple St., Ste. 3800 Weber CHE Dayton, OH 45409, USA
Wioletta Rozmus-Warcholinska: Division of Prenatal Diagnostics, St. Lucas Obstetric Center, 42-202, Czestochowa, Poland
Maciej Orzechowski: Profemi Private Practice, 63-700 Krotoszyn, Poland
Dominika Stettner: Chair of Gynecology and Obstetrics, Jagiellonian University in Krakow, 31-501 Krakow, Poland; and Ultrasound Group Practice "dobreusg", 31-236 Krakow, Poland
Petr Plevak: Fetal Medicine Division, Genetika Pilzen Sro, Parková 1254/11a, Plzen-Cernice, Czech Republic

analysis, we observe a higher FPR of CST than expected, arising from biochemistries without any coincidences with positive ultrasound markers among the Polish population. These observations induced us to plan this study. It has also been suggested that the inclusion of maternal age (MA) excessively increases the FPR in the AMA group excessively. Some researchers have even suggested that the elimination of the background risk based on MA actually improves the performance of the CST [13–16].

The aim of the study is to compare two first trimester screening strategies: traditional CST and the strategy that is based on NT, secondary ultrasound markers, and early anomaly findings. We also analyzed how the performance of the two strategies is affected by the inclusion of MA.

Methods

This is a prospective, observational multicenter non-randomized study based on a non-selected mixed-risk population of 11,653 pregnant women referred for first-trimester screening between January 2009 and June 2012. The patients were examined at five centers: Ultrasound Group Practice "dobreusg" (Krakow), Ultrasound Lab at the Department of Gynecology and Obstetrics of Jagiellonian University (Krakow), St. Lukas Obstetric Center (Czestochowa), Profemi Private Practice (Krotoszyn), and Genetika Pilzen (Czech Republic). 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 patients' body mass index (BMI) was calculated in kg/m^2 on the day of the ultrasound evaluation. The presence or absence of chromosomal aberration or congenital defects was recorded for each patient. The pregnant volunteers who agreed to participate in the study were offered a choice between the traditional combined screening test [combined screening group (CSG) arm] and ultrasound-based screening [ultrasound-based screening group (USG) arm]. Each participant signed a written consent, which was approved by the local Ethics Committee.

All patients included in both arms of the study had dates established by an early first trimester viability scan performed by referring obstetricians (completed 7th–9th weeks). All of the operators that performed the combined screening tests in the CSG arm had been accredited by FMF for at least 5 years and had successfully undergone regular audits. The ultrasound examination protocol for the CSG not only included CRL, NT, and fetal heart rate (FHR) measurements, and a basic assessment of the entire fetus based on excluding five major anomalies related to chromosomal aberrations (holoprosencephaly, omphalocele, extensive diaphragmatic hernia, atrio-ventricular septal defect, and megacystis), but also anencephaly and severe limb abnormalities [17, 18]. In the CSG arm, patients' serum samples for free beta subunit of human chorionic gonadotropin (fβhCG) and pregnancy-associated plasma protein A (PAPP-A) analysis were collected between 10 and 11 weeks' gestation in order to optimize sampling time [19]. Biochemistry was evaluated using an accredited and quality-controlled Cobas E4 Analyzer (Roche, Manheim, Germany).

Subjects in the USG arm were examined by operators who had been accredited by the FMF not only in NT but also qualified and highly experienced in evaluating all of the three additional first-trimester ultrasound markers [nasal bone (NB), tricuspid flow, and ductus venosus (DV) velocimetry]. Furthermore, they were highly experienced in first-trimester fetal anatomy evaluation and early echocardiography. Despite parameters used in the CSG arm, the ultrasound examination protocol in the USG arm included three secondary markers of aneuploidy, which were used in the risk calculation. The protocol also included systematic assessment of the early fetal anatomy and detailed examination of the fetal heart (cardiac situs, inflows to the ventricles in color mapping, and three-vessel and trachea view in color mapping).

All ultrasound scans were performed transabdominally using Voluson E6 and Voluson E8 ultrasound systems (General Electric Healthcare, Zipf, Austria). Tansvaginal sonography was employed only if it was essential to complete the examination. Adjusted first-trimester risk for T21 was calculated by entering appropriate history, sonographic, and biochemical data into the FMF 2.3.2 software (Astraia Gmbh, Munich, Germany). Risk assessment used in this study was based on data collected on the day of screening. The details regarding screening tests used in both arms of the study are depicted in Table 1.

We set the cut-off of 1/300 for the CST test as it is recommended by the local society [20] and the cut-off for NT+ test of 1/100, which was estimated by receiver operating characteristic (ROC) curves in our first study [11]. We also decided to validate the cut-off of 1/100 for CST in this study due to the observed high number of FPR results in our population in the pre-study period when we used CST with the cut-off of 1/300. Absolute risk (AR) was calculated for tests: CST and NT+ by dividing the results of adjusted risk by background risk. For AR tests, a cut-off of 1.2

Table 1: Screening tests for trisomy 21 used in the study.

Study arm	Screening test	Components
Combined screening group (CSG)	CST 1/300	MA, NT, FHR, fβhCG, PAPP-A, and major anomaly findings
	CST 1/100	MA, NT, FHR, fβhCG, PAPP-A, and major anomaly findings
	AR CST	NT, FHR, fβhCG, PAPP-A, and major anomaly findings
Ultrasound-based screening group (USG)	NT+	MA, NT, FHR, NB, DV, TR, and major anomaly findings
	AR NT+	NT, FHR, NB, DV, TR, and major anomaly findings

Abbreviations: CST 1/300 and CST 1/100=Combined screening test risk with cut-offs 1/300 and 1/100, AR CST=absolute risk of combined screening test, NT+=adjusted risk by NT and secondary ultrasound markers with the cut-off 1/100, AR NT+=absolute risk of combined screening test, MA=maternal age, NT=nuchal translucency, FHR=fetal heart rate, NB=nasal bone, TR=tricuspid regurgitation, DV=ductus venosus velocimetry, fβhCG=free beta subunit of human chorionic gonadotropin, and PAPP-A=pregnancy-associated plasma protein A.

was used [14]. All patients who were found to be at an increased risk (CSG arm: CST risk above 1/300; USG arm: NT+ risk>1/100) underwent genetic counseling and were counseled according to published DRs and FPRs for particular tests used in our study [1–6, 11]. Irrespective of the screening results, those patients in whom a fetal structural anomaly was detected underwent genetic counseling as well. They were offered fetal karyotyping, and underwent a targeted scan as well as fetal echocardiography between 18–19 weeks' gestation. The examinations were performed according to existing guidelines [21, 22]. The outcome results were collected from medical records and included the karyotype if available, the results of ultrasound examinations at 18–21 and 28–32 weeks' gestation, autopsy examinations, and neonatal findings.

Statistical analysis

In statistical data assessment, the Kolmogorov-Smirnov test was applied for continuous variable distribution. The χ^2 test was used to demonstrate the differences. Groups of independent variables were compared using the Student's *t* -test. Nonparametric test of Mann-Whitney was also utilized. SPSS Statistics v.17 (IBM Co., Armonk, NY, USA) software was applied in this study. The figures of $P<0.05$ were considered significant. Screening performance was assessed by means of standardized parameters including detection rate, false positive rate, screening accuracy, positive predictive value (PPV), and negative predictive value (NPV) as well as by ROC curves. General and MA range-dependent screening performance was estimated for all tests used in this study. Four MA ranges were selected for MA range-dependent screening performance evaluation: 26–30 years, 31–35 years, 36–40 years, and ≥41 years.

Results

Study population

Screening for T21 was carried out in 11,653 singleton pregnancies that 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 postnatal assessment. Seven hundred and seventy-five (6.6%) cases were excluded from further analysis because in 552 (4.7%) cases it was impossible to determine the fetal karyotype due to losing them from the follow-up; 73 (0.6%) cases resulted in miscarriages not-related to invasive testing, 28 (0.4%) had intrauterine fetal demise without subsequent karyotyping, and in 107 (0.9%) cases there was a chromosomal abnormality other than T21 [trisomy 18 (T18) (n=40); trisomy 13 (T13) (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 of 10,878 pregnancies: 5,145 in the CSG arm including 51 cases of T21 and 5733 in the USG arm including 87 cases of T21. These groups were not statistically different according to the prevalence of T21 (P=0.14). The characteristics of the study population are summarized in Figure 1.

The median maternal BMI in the CSG arm was 22.7 kg/m^2 (range 17.5–35.7) and in USG 22.8 kg/m^2 (range 17.4–35.9). These differences were not statistically significant (P>0.05). All women participating in this study were Caucasian.

The mean NT thickness in the CSG arm was 1.8 mm (range 0.7–12.7) and in the USG 1.7 mm (range 0.7–28.6), $P<0.05$. NT above the 95th centile was noted in 406 subjects (7.8%) of the CSG arm and in 307 cases (6.5%) of the USG arm. The mean CRL at the time of examination in the CSG was 63.8 mm and in the USG 63.5 mm; the difference was not clinically significant. The subjects in the CSG arm were noted to be older than those in the USG. The mean MA in the CSG was 34.8 years (range 15–48) whereas in the USG it was 30.5 years (range 16–46), $P<0.05$, which may suggest that younger patients were more likely to choose to participate in the USG protocol. Supplementary characteristics of the study arms are shown in Table 2.

Screening performance

Screening tests in the USG arm demonstrated higher specificity, PPV, and diagnostic accuracy in comparison to the tests used in the CSG arm (Table 2). Similar to the CSG arm, all screening tests in the USG arm showed high NPVs. The details are shown in Table 3.

The performance of all tests based on ROC method is summarized in Table 4.

Detection rate of T21 in relation to maternal age

In both the CSG and the USG arms, cases with T21 were divided into groups based on MA. In the CSG arm their prevalence was as follows: 26–30 years (n=8), 31–35 years (n=14), 36–40 years (n=20), and ≥41 years (n=9). In the USG arm, their occurrence was noted in 26–30 years (n=14), 31–35 years (n=20), 36–40 years (n=35), and ≥41 years (n=18). DR and FPR of the tests used in this study according to the MA subgroups are presented in charts in Figures 2 and 3.

Discussion

To our knowledge, this is the first study that prospectively compares the performance of two first trimester screening strategies for T21: the traditional one based on combined

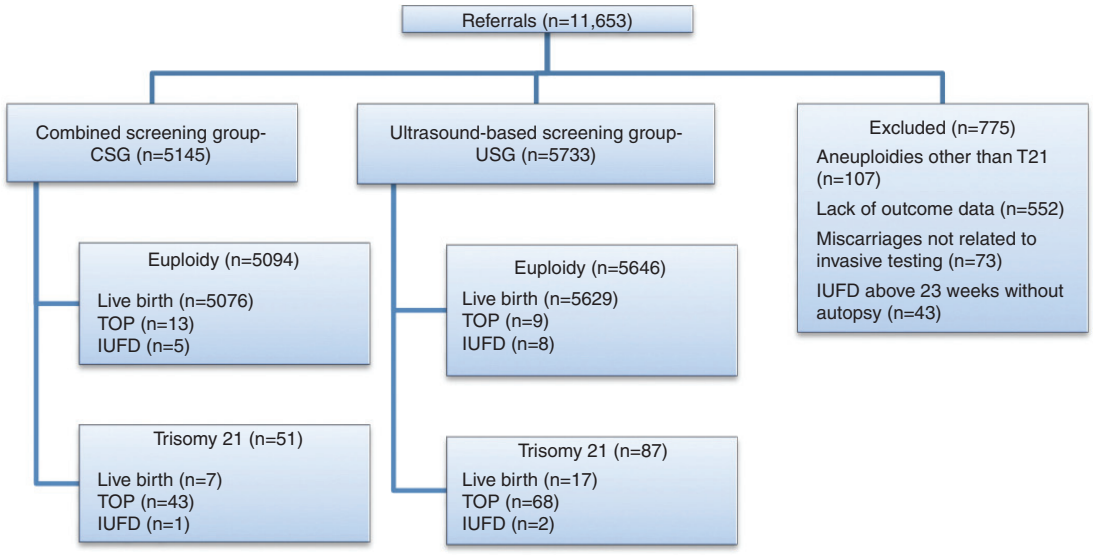

Figure 1: Study population diagram.
Abbreviations: T21=Trisomy 21, IUFD=intrauterine fetal demise, TOP=termination of pregnancy.

Table 2: Additional characteristics of both study arms.

Parameter arm	MA in euploidy [years]	MA in T21 cases [years]	AMA n (%)	AMA in T21 cases n (%)	NT>95th centile in T21 cases n (%)	Median and range of fβhCG in euploidy [MoM]	Median and range of PAPP-A in euploidy [MoM]	Median and range of fβhCG in T21 [MoM]	Median and range of PAPP-A in T21 [MoM]
CSG	34.9 (15–48)	35.6 (25–45)	3538 (78.6%)	34 (66.7%)	35 (68.6%)	1.04 (0.01–9.24)	0.88 (0.02–9.58)	2.12 (0.37–8.2)	0.51 (0.06–2.54)
USG	30.4 (16–46)	34.2 (22–44)	966 (21.4%)	42 (48.3%)	71 (81.6%)	–	–	–	–

Abbreviations: MA=Maternal age, T21=trisomy 21, AMA=advanced maternal age cases of 36 years and above, NT=nuchal translucency, fβhCG=free beta subunit of human chorionic gonadotropin, and PAPP-A=pregnancy-associated plasma protein A.

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

Arm	Combined screening group (CSG)			Ultrasound-based screening group (USG)	
Screening method	CST 1/300	CST 1/100	AR CST	NT+	AR NT+
Sensitivity	92.16% (81.5–96.9)	86.27% (74.3–93.2)	90.2% (79.0–95.7)	90.8% (82.9–95.3)	90.8% (82.9–95.3)
Specificity	80.86% (79.7–81.9)	90.57% (89.7–91.3)	86.94% (86.0–87.8)	96.31% (95.8–96.8)	95.51% (94.9–96.0)
PPV	4.603% (3.5–6.1)	8.397% (6.3–11.1)	6.479% (4.9–8.5)	27.53% (22.68–32.96)	23.8% (19.5–28.7)
NPV	99.9% (99.7–100.0)	99.85% (99.7–99.9)	99.89% (99.7–99.9)	99.85% (99.7–99.9)	99.85% (99.7–99.9)
Diagnostic accuracy	80.97% (79.9–82.0)	90.53% (89.7–91.3)	86.98% (86.0–87.9)	96.23% (95.7–96.7)	95.44% (94.9–95.9)

Abbreviations: CST=Adjusted risk by combined screening test, AR=absolute risk, NT+=adjusted risk by nuchal translucency and secondary ultrasound markers, PPV=positive predictive value, and NPV=negative predictive value. The estimates of lower to upper 95% confidence intervals (CIs) are shown in brackets.

screening test (CSG arm) and the one that is based on ultrasound alone (USG arm). In the USG arm, for fixed 3% and 5% FPRs, DRs using the NT+ approach were 90% and 94%, respectively. By comparison, with the addition of maternal serum biochemistries but exclusion of the additional ultrasound markers (CST arm), the detection rates for the same FPRs were only 78% and 84%, respectively.

Lowering of the CST cut-off risk from 1/100 to 1/300 in the advanced MA population increased the DR. However, it also involved doubling the FPR (from 7.9% to 16% for

Table 4: Detection rates (DR) for trisomy 21 and 95% confidence intervals (CI) at fixed false positive rates (FPR) of 3% and 5% in adjusted risk and absolute risk screening methods.

Screening test		AUC	DR% (95% CI) at 3% FPR	DR% (95% CI) at 5% FPR
Combined	DR of "CST"	0.946	78% (72.6–83.4)	84% (78.4–89.6)
screening group	DR of "AR CST"	0.944	78% (72.6–83.4)	84% (74.8–89.6)
Ultrasound-based	DR of "NT+"	0.980	90% (85.6–94.4)	94% (85.4–98.6)
screening group	DR of "AR NT+"	0.963	89% (84.6–93.4)	93% (88.5–97.5)

Abbreviations: AUC=Area under the curve, CST=adjusted risk by combined screening test, AR=absolute risk, and NT+=adjusted risk by nuchal translucency and secondary ultrasound markers. The estimates of lower to upper 95% confidence intervals (CIs) are shown in brackets.

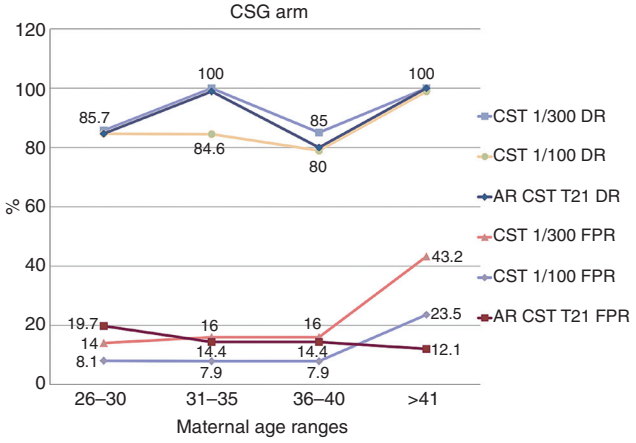

Figure 2: Detection rates (DR) and false positive rates (FPR) of the "adjusted risk for trisomy 21 by Combined Screening Test (CST) with the cut-off 1/100"; "adjusted risk for trisomy 21 by CST with the cut-off 1/300", and "absolute risk (AR) of CST for trisomy 21" depending on maternal age ranges in the Combined Screening Group.

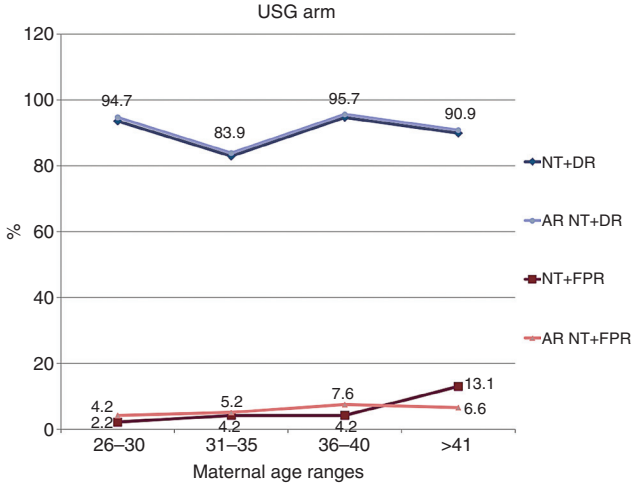

Figure 3: Detection rates (DR) and false positive rates (FPR) of the "adjusted risk for trisomy 21 by nuchal translucency plus (NT+)", and "absolute risk (AR) of nuchal translucency plus (NT+) for trisomy 21" depending on maternal age ranges in the Ultrasound-based Screening Group. Detection rate values of tests "adjusted risk for trisomy 21 by NT+" and "AR NT+ for trisomy 21" are the same.

36–40 year olds and from 23.5% to 43.2% for ≥41 year olds, respectively), which in turn, would lead to a significant increase in the number of unnecessary invasive testing.

We acknowledge that the protocols, which included maternal serum biochemistries in this study, showed higher FPRs than what appeared in the literature [2, 3, 23–26]. This situation occurred despite stringent quality control of the biochemical testing. One potential reason of these results may be related to the fact that our PAPP-A levels were lower than those reported in literature: 0.88 MoM [2, 3]. The second possible factor may be a relatively higher MA in the CSG population. The median age was 34.9 years, which is significantly higher than the average Polish obstetric population (29.0 years) according to the Statistical Report (http://stat.gov.pl/cps/rde/xbcr/gus/RS_rocznik_statystyczny_rp_2012.pdf). This inevitably leads to an increased FPR. Not only is this pattern seen in our study but it is also well-documented in the literature. In one study in which the median MA was 34 years, CST had a FPR of 7.5% at a cut-off risk of 1/300 [27]. The biochemistry, ultrasound, and nuchal translucency (BUN) study analyzed a population of women with a median age of 34.5 years, which is similar to ours. Using a 1/270 cut-off, the DR and FPR were 85.2% and 9.4%, respectively [28]. In another study, the probability of a screen positive result for T21 increases to approximately 30% at the age of 40 by using CST cut-off 1/300 [29].

The third reason may be the relatively high percentage of cases with NT above the 95th centile in both arms of our study (7.8% in the CSG arm and 6.5% in the USG arm). Furthermore, in our study, the range of NT measurements in the euploid groups was quite wide (CSG arm: 0.7–12.7 mm; USG arm: 0.7–28.6 mm) in comparison to those that appear in the literature. For example, Nicolaides et al. demonstrated a mean NT of 1.8 mm in euploid fetuses, which is comparable to our results. However, in his study the range of NT measurements was only 1.5–2.1 mm [10].

There is a scarcity of published data regarding the performance of screening protocols that use a combination of first-trimester markers without the addition of maternal serum biochemistries [11, 12]. In the study of Abele H et al., the DR of T21 by the use of NT and three secondary ultrasound markers was 94% at the 3% FPR basing on the population of 1916 fetuses, including 93 cases of T21 [12].

The high DR of this approach is especially impressive given the fact that the average age of subjects enrolled into the USG arm was only 30.5 years as compared to the CSG arm, in which the average age was 34.8 years. On the other hand, this fact contributed to the relatively low FPRs seen in the USG arm.

In an earlier series, the use of additional (secondary) ultrasound markers was employed only on a contingent basis; their use as a primary method of screening was not recommended [2].

It is recognized that MA has an influence on the detection and false positive rates when CST is used for screening: both the DRs for T21 and the FPRs generally increase with increasing MA. This pattern is especially prominent in the very AMA group (≥41 years). This has even led some investigators to propose AR by not using MA as a component of first trimester screening to reduce the FPRs. To our knowledge, this pattern has not been evaluated in a prospective fashion in first trimester screening using multiple ultrasound markers only. We noted a similar pattern here, but in general, the increase in FPR was less prominent than in the CST group. Considering identical DRs of the tests NT+ and AR NT+, the emphasis should be put on the decrease of the FPR. For AR NT+, FPR was lower compared to NT+ with almost double reduction (6.6% vs. 13%) in the group above 41 years. The same trend in reduction of FPR was observed in the CSG arm, the most substantial in women aged above 41 years, in whom FPR for CST with a cut-off 1/300 was over 40% compared to 12% for AR CST. Our observations are in line with Schmidt et al. who suggested that eliminating MA results in comparable sensitivity with a lower FPR [15]. Combining the screening risk with the age-related background risk greatly increases FPR, which reaches 50% for a 40-year-old woman [30]. We did not find a trend with the AR method toward better detection of affected pregnancies in younger patients as it was observed in the study by Gebb et al [14]. In this group of patients, our detection rates remained unchanged after the exclusion of MA from the calculation.

Our findings are important for several reasons. They suggest that first-trimester screening using multiple fetal markers is associated with high DRs of T21 and low FPRs. In our study, this approach outperformed the CST approach. This is important, as most of the recent series in this field have been on developing contingent strategies using additional biomarkers rather than investigating the power of ultrasound parameters [31]. Our findings are especially important for patients from countries with lower socioeconomic status, who cannot afford NIPT and for populations like ours, which demonstrate suboptimal screening performance of CST. The NT+ test, if positively tested by other groups, may be recognized as an alternative to the traditional CST in mentioned circumstances, but it may also constitute a first step in contingent policy with the use of NIPT.

There are several limitations to the study. One is that the population of fetuses included in our study had a higher prevalence of T21 than in the general population. This is due to the fact that the testing units involved in this study are referral centers. Therefore, many of the cases evaluated here had risk factors based either on ultrasound findings or maternal history that had already been identified by the referring physician. Another limitation of the study is the under-representation of patients below the age of 25; therefore, conclusions regarding the performance of AR method in this group could not be drawn.

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