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How Effective Is First-Trimester Screening for Trisomy 21 Based on Ultrasound Only?

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First-trimester screening · Trisomy 21 · Nuchal translucency · Congenital heart defect

Abstract
Objective: To evaluate the most common first-trimester ultrasound features of fetuses with trisomy 21 (T21) and to examine the screening performance for Down syndrome (DS) using only ultrasound-based protocols. To investigate whether maternal age (MA) has an impact on the efficacy of the ultrasound-based screening methods. ***Methods:*** In a prospective study, 6,265 patients were examined. Two ultrasound-based risk calculation protocols were applied: 'NT' (based on nuchal translucency) and 'NT+' (based on NT and secondary markers). ***Results:*** A total of 5,696 patients were enrolled for analysis; 84 subjects with T21 were identified. Combinations of abnormal ultrasound markers were observed in only 1.2% of euploid fetuses compared to 71.5% of fetuses with T21. Among 17.9% of DS cases with cardiac anomaly, 14.3% comprised atrioventricular septal defects. For a false-positive rate of 3%, the detection rates of T21 were 73.8 and 91.7% for the 'NT' and 'NT+' protocols, respectively. The efficacy of both methods was affected by MA. ***Conclusions:*** Most of the fetuses with DS demonstrate a combination of ultrasound markers of aneuploidy in the first trimester. The 'NT+' protocol is efficient and provides comparable performance as a combined screening test. It is a valuable method, especially when the access to biochemical analysis is restricted.

Introduction

The incidence of trisomy 21 (T21) at birth is estimated at 11.8 per 10,000 and shows an average increase of 0.9% per year due to the gradual rise in mean maternal age (MA) [1]. Since the combined screening test (CST) – which is based on first-trimester nuchal translucency (NT), MA and the serum levels of human chorionic gonadotropin (fβhCG) and pregnancy-associated plasma protein A (PAPP-A) – has been widely introduced in recent years, the detection rate (DR) for Down syndrome (DS) increased significantly to 90%, with a false-positive rate (FPR) of 2–3% [2–5]. Further research to increase screening performance for DS mainly in terms of lowering of the FPR led to the development of various strategies for first-trimester combined screening, such as different timing of ultrasound and blood testing, stepwise and contingent policy [5, 6].

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In further research, CST enhanced with secondary ultrasound markers (CST+) like nasal bone (NB), tricuspid regurgitation (TR) and ductus venosus (DV) flow velocimetry proved to improve the DRs of T21 [5, 7, 8]. Researchers are still skeptical about the routine implementation of these well-described markers in screening for DS; however, their reproducibility after proper training is high [9–11].

Due to the common policy of implementing biochemical testing in the first-trimester screening for DS, the actual effectiveness of a method based on ultrasound only by the use of the complete package of markers ('adjusted risk for T21 by NT+') and detailed sonography remains to be established.

The first goal of this study was to summarize ultrasound findings in a group of DS cases compared to euploid fetuses, detected at our tertiary center in the late first trimester. Secondly, we aimed to examine the screening performance for DS of ultrasound methods based on primary ('adjusted risk for T21 by NT') and primary enhanced with secondary ('adjusted risk for T21 by NT+') markers of aneuploidy (NT, DV, NB, and TR). Finally, we tried to investigate whether the performance of the method depends on the MA ranges.

Methods

This was a prospective observational study for T21 in singleton pregnancies carried out in a tertiary center setting at 11^{+0}–13^{+6} weeks' gestation. For the purpose of this study, we offered ultrasound-based screening for T21 without the addition of biochemistry to pregnant volunteers. All potential candidates had the option to undergo traditional combined screening and were assured that well-trained personnel perform the ultrasound scans. Maternal demographic characteristics and ultrasound measurements were recorded in a computer database. Karyotype results and postnatal outcome were added to the database as soon as they were available. The local Ethics Committee approved the study protocol and all participating subjects gave their written consent. A search of the database was done to identify all singleton pregnancies in which first-trimester screening was carried out between January 2009 and June 2012. The digital sonograms and reports were evaluated according to the following inclusion criteria: singleton pregnancy, crown-rump length (CRL) measurement of 45–84 mm, and known pregnancy outcome. The patients' body mass index (BMI) was calculated on the day of the ultrasound evaluation. Evidence of chromosomal aberration or congenital defects was recorded for each patient. Three examiners certified for the complete package of ultrasound markers by the Fetal Medicine Foundation (FMF) were employed for the study conduction. One examiner presented a 1-year experience in first-trimester screening (M.J.) and 2 others an 8-year expertise (M.W. and A.N.). All ultrasound scans were performed transabdominally using the Voluson E6 ultrasound system (GE Healthcare, Zipf, Austria). Transvaginal sonography was employed only if needed to complete fetal evaluation. Our first-trimester ultrasound scan protocol covered a systematic assessment of the entire fetus, also including the following early fetal echocardiography parameters: visceral situs, 4-chamber view, outflow tracts, three-vessel and trachea view in B-mode, and color mapping. The ultrasound markers of aneuploidy (NT, NB, TR, and DV) were evaluated following the FMF guidelines. DV was assessed by a qualitative method (reverse a-wave was considered abnormal) because at the beginning of the study, a quantitative technique by measuring DV pulsatility index for veins was not utilized. The required history and ultrasound data were employed for T21 first-trimester risk calculations by using FMF 2.3.2 software (Astraia GmbH, Munich, Germany). In all cases, two methods of risk calculation based on ultrasound only were applied: 'adjusted risk for T21 by NT' and 'adjusted risk for T21 by NT+' enhanced with all secondary ultrasound markers (NB, TR, and DV). If the adjusted risk for T21 was >1/100, independent of the method used at the time of the scan, it was defined as a high risk. All high-risk patients, including those with detected structural anomalies but presenting low-risk results, underwent genetic counseling, were qualified for karyotyping, and were scanned between 18 and 19 weeks according to the second-trimester and fetal echocardiography guidelines of the American Institute of Ultrasound in Medicine [12, 13]. The outcome records were collected from medical documentations and included karyotyping, 18–21 and 28–32 weeks' sonography, autopsy examinations, and neonatal findings.

Statistical Analysis

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 Student's t test. Nonparametric tests were also utilized. SPSS Statistics v.17 (IBM Co., Armonk, N.Y., USA) software was applied in this study. $p < 0.05$ was considered as significant.

Results

Study Population

Ultrasound screening was carried out in 6,265 singleton pregnancies. The majority of referrals were low-risk patients (n = 4,777), but due to the tertiary center setting, a significant group of high-risk subjects (n = 1,488) was also enrolled in this study. Among the high-risk-patients, the following were included: those with an MA >35 years (n = 783) and those with suspicious ultrasound findings on the initial scan performed by nonqualified and nontrained antenatal care services for first-trimester screening (n = 705). In our study, 569 (9.08%) cases were excluded from further analyses due to the following reasons: (a) in 416 (6.6%) patients, it was impossible to establish a fetal karyotype, since they were lost to follow-up, (b) 58 (0.93%) cases had miscarriages unrelated to invasive testing, (c) 28 (0.45%) patients had intrauterine fetal demise without subsequent karyotyping, and (d) in

67 (1%) cases, a chromosomal abnormality other than T21 was found. The last mentioned subgroup presented a different ultrasound picture, which would have potentially disturbed our analysis, including trisomy 18 (33 cases), trisomy 13 (14 cases), Turner syndrome (16 cases), triploidy (2 cases), and Klinefelter syndrome (2 cases). Fetal karyotyping was obtained by means of amniocentesis (652 cases). Finally, our study population was comprised of 5,696 pregnancies: 5,612 with a normal karyotype or delivery of a normal baby (euploidy group) and 84 cases of T21. The characteristic of the study population is summarized in figure 1. The median maternal BMI was 22.2 (range 17.6–35.2). All women participating in this study were Caucasian. In order to complete the scan, transvaginal sonography was applied in 5.6% of the cases.

Euploid and T21 Fetal Characteristics

The mean NT thickness in the euploidy subgroup was 1.7 mm (range 0.7–4.9) and in the T21 subgroup it was 4.1 mm (range 1.4–12.9; $p < 0.05$). The mean MA in the euploidy group was 30.5 years (range 25–42) compared to 34 years (range 26–43) in the T21 group ($p < 0.05$). The mean CRL at the time of examination was 63.3 mm in the euploidy group versus 64.9 mm in the T21 group. No statistically significant differences were found between the euploidy and T21 groups in terms of CRL and fetal heart rate (table 1).

NT thickness above the 95th percentile was observed in 228 euploid fetuses (4.06%) and in 67 fetuses (79.76%) affected by T21 (fig. 2).

By using the Pearson χ^2 test, statistical differences were found in the presence of TR [euploidy group: n = 116 (2.1%) vs. T21 group: n = 34 (40.5%); p = 0.000] and reverse a-wave in DV [122 (2.2%) vs. 28 (33.3%); p = 0.000] between the euploidy and T21 groups. Similarly, a statistically significant difference was observed in the absent NB between the euploidy (n = 93; 1.7%) and T21 groups (n = 34; 40.6%; $p < 0.05$).

A total of 5,118 (91.2%) euploid fetuses did not reveal any of the abnormal first-trimester markers. Isolated markers were identified in this group in 433 cases (7.7%), with the largest fraction in this subgroup of NT above the 95th percentile [181 cases (41.8%)]. Markers observed in combination were found only in 61 cases (1.1%) of euploid fetuses and in 60 fetuses (71.4%) with T21. The configuration of ultrasound markers in the groups of euploidy and DS is summarized in table 2.

The analysis of extracardiac anatomy demonstrated a higher prevalence of structural anomalies in the group of DS [4 cases (4.76%)] compared with the group of euploidy [48 cases (0.9%)], which was statistically significant ($p < 0.05$). The incidence and type of abnormalities are shown in detail in table 3. Extracardiac and cardiac anomalies were analyzed as independent variables. The majority of euploidy and T21 cases demonstrated isolated cardiac or extracardiac malformations. Among the euploid subjects, 2 cases of hydrops were combined with congenital heart defects (CHDs; 1 pulmonary stenosis and 1 atrioventricular septal defect) and 2 cases of brain anomalies (aqueductal stenosis) were combined with tetralogy of Fallot. Of 2 hydropic T21 cases, the first one presented atrioventricular septal defects and the second tetralogy of

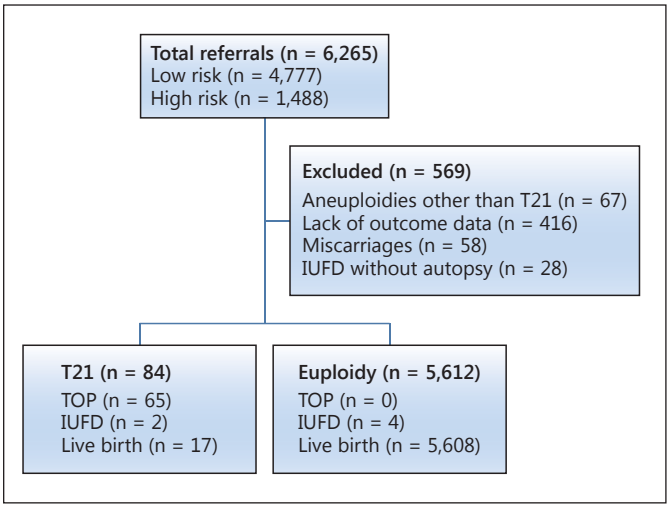
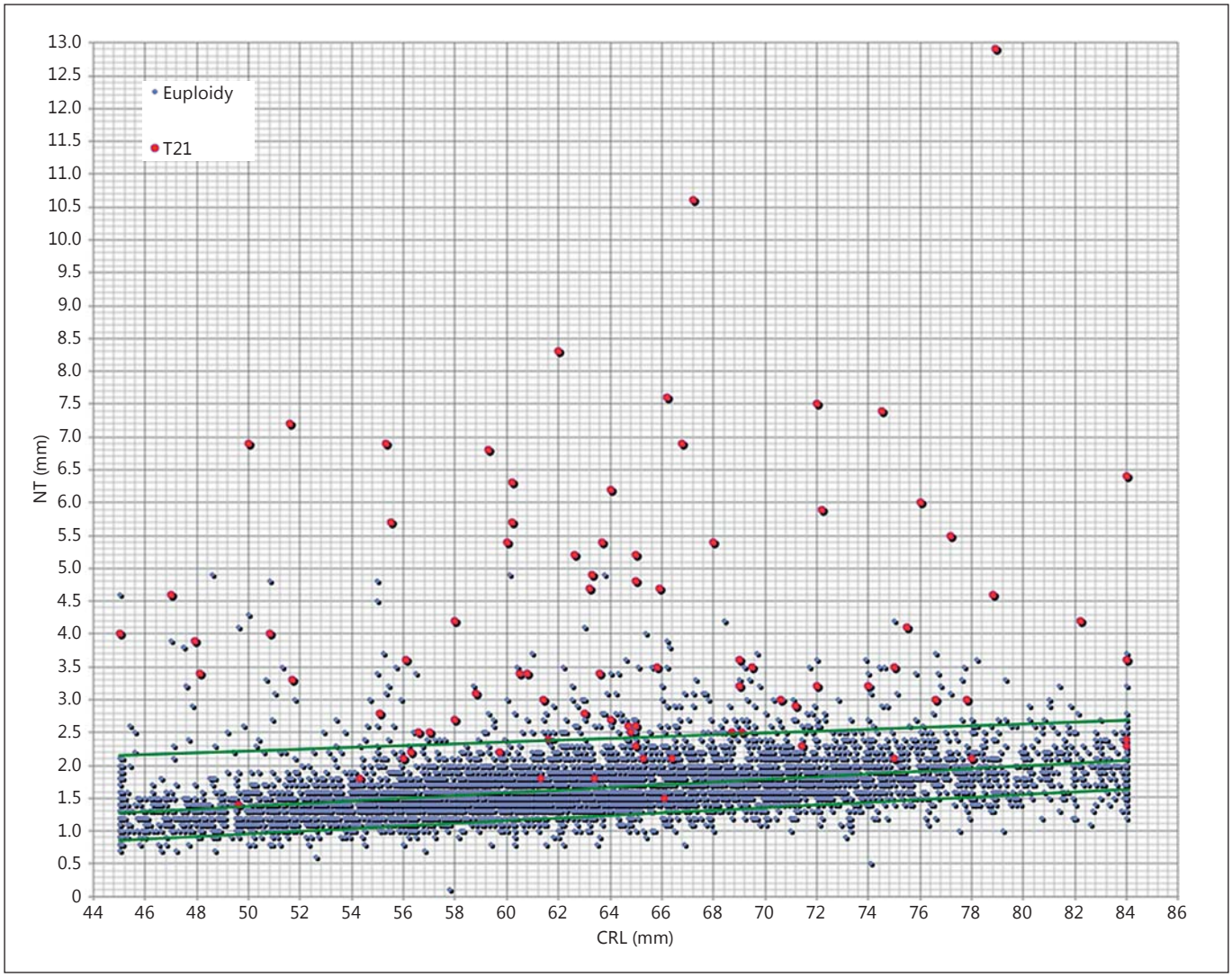


Fig. 1. Study population diagram. IUFD = Intrauterine fetal demise; TOP = termination of pregnancy.

Table 1. Comparison of fetuses with euploidy and T21 according to four parameters

	NT, mm	MA, years	CRL, mm	FHR, bpm
Euploidy (n = 5,612)				
Mean	1.7	30.5	63.3	160.3
Median	1.6	30.0	62.8	160.0
SD	0.5	4.2	9.1	7.3
T21 (n = 84)				
Mean	4.1	34.0	64.9	160.4
Median	3.4	34.0	64.7	161.0
SD	2.1	4.9	9.3	8.7
Statistical significance (p value)	0.101	0.000	0.000	0.101

FHR = Fetal heart rate; bpm = beats per minute.



Color version available online

Fig. 2. The distribution of fetal NT thickness according to CRL in euploid fetuses (blue dots) and in cases with T21 (red dots). For colors, see online version.

Fallot. Additionally, 1 case of T21 with enlarged cervical lymphatic sacs showed double outlet right ventricle.

Abnormal cardiac findings were more frequent in the T21 group [15 cases (17.9%)] compared to the euploidy group [27 cases (0.5%)], which was statistically significant ($p < 0.05$). Particular abnormalities and cardiac findings are summarized in table 4.

Screening Performance

The receiver operating characteristic curve of 'adjusted risk for T21 by NT' and 'adjusted risk for T21 by NT+' methods is depicted in figure 3. In table 5, DRs are shown for arbitrary FPR at the level of 3 and 5%.

DR of T21 in Relation to MA

We identified 22 cases with DS (26.2%) at an MA range between 26 and 30 years, 31 (36.9%) cases at an MA range between 31 and 35 years, 21 (25%) cases at an MA range between 36 and 40 years, and 10 (11.9%) cases at an MA >40 years. The results of the analysis of DRs and FPRs depending on MA ranges are presented in figure 4. There was a tendency of an increase in the DR and FPR with the advance of the MA in two analyzed risk calculation methods; however, both models demonstrated the lowest DR in the MA range between 31 and 35 years. The differences in the DRs and FPRs between the MA range groups were statistically significant ($p < 0.05$).

Table 2. Configuration and prevalence of isolated and combined markers of aneuploidy in euploid and T21 fetuses

Karyotype	Configuration	Number	%
Euploidy	no markers	5,118	91.2
	NT	181	3.2
	NB(–)	63	1.1
	TR	91	1.6
	revDV	94	1.7
	noDV	4	0.1
	NT + NB(–)	7	0.1
	NT + TR	10	0.2
	NT + revDV	14	0.2
	NT + NB(–) + TR	5	0.1
	NT + NB(–) + revDV	3	0.1
	NT + NB(–) + TR + revDV	2	0.0
	NB(–) + revDV	7	0.1
	NT + noDV	2	0.0
	NB(–) + TR	6	0.2
	TR + revDV	1	0.0
	NT + TR + revDV	1	0.0
	NT + SUA	3	0.1
T21	NT	16	19.0
	NB(–)	2	2.4
	TR	1	1.2
	revDV	5	6.0
	NT + NB(–)	10	11.9
	NT + TR	12	14.3
	NT + revDV	6	7.1
	NT + NB(–) + TR	8	9.5
	NT + NB(–) + revDV	3	3.6
	NT + NB(–) + TR + revDV	3	3.6
	NB(–) + revDV	4	4.8
	NT + noDV	2	2.4
	NB(–) + TR	4	4.8
	TR + revDV	1	1.2
	NT + TR + revDV	6	7.1
	NT + SUA	1	1.2

NB(–) = Absent nasal bone; revDV = reversed a-wave in ductus venosus; noDV = absent a-wave in ductus venosus; SUA = single umbilical artery.

Table 3. Extracardiac structural abnormalities summarized in terms of chromosomal status

	Karyotype	
	euploidy	T21
No ECM	5,564 (98.9)	80 (95.2)
Hydrops	5 (0.1)	2 (2.4)
Brain anomalies	12 (0.2)	0 (0.0)
Abdominal anomalies	9 (0.3)	1 (1.2)
Urinary tract anomalies	6 (0.2)	0 (0.0)
Limb anomalies	8 (0.1)	0 (0.0)
Facial/neck anomalies	5 (0.2)	1 (1.2)
Thoracic anomalies	3 (0.1)	0 (0.0)

Figures are numbers with percentages in parentheses. ECM = Extracardiac anomaly.

Table 4. Summarized cardiac anomalies in the euploidy and T21 groups

Karyotype	Cardiac anatomy	
Euploidy	normal	5,585 (99.5)
	septal defects	2 (0.0)
	conotruncal anomalies	10 (0.2)
	left heart defects	8 (0.1)
	right heart defects	6 (0.1)
	aortic arch anomalies	1 (0.0)
T21	normal	69 (82.1)
	septal defects	10 (11.9)
	conotruncal anomalies	2 (2.4)
	right heart defects	1 (1.2)
	AVSD + TOF	2 (2.4)

Figures are numbers with percentages in parentheses. AVSD + TOF = Atrioventricular septal defect and tetralogy of Fallot.

Table 5. DR (95% CI) at fixed FPRs of 3 and 5% in two adjusted risk screening methods

Screening test	AUC	DR at 3% FPR	DR at 5% FPR
'Adjusted risk for T21 by NT'	0.954	73.8% (63.51–82.0)	82.1% (77.7–86.3)
'Adjusted risk for T21 by NT+'	0.984	91.7% (83.78–95.9)	95.2% (90.3–99.7)

AUC = Area under the curve.

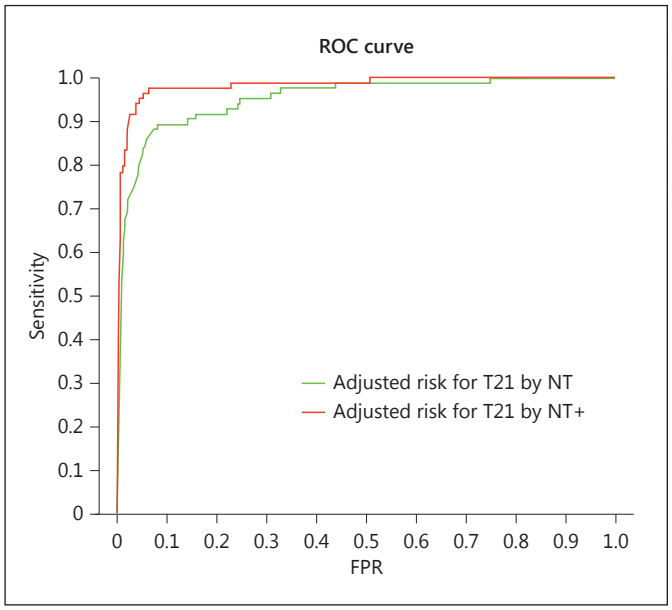


Fig. 3. The effectiveness of screening for T21 by using receiver operating characteristic (ROC) curves. The area under the curve for the 'adjusted risk for T21 by NT' method (green line) was 0.954, and the area under the curve for the 'adjusted risk for T21 by NT+' method (red line) was 0.984. For colors, see online version.

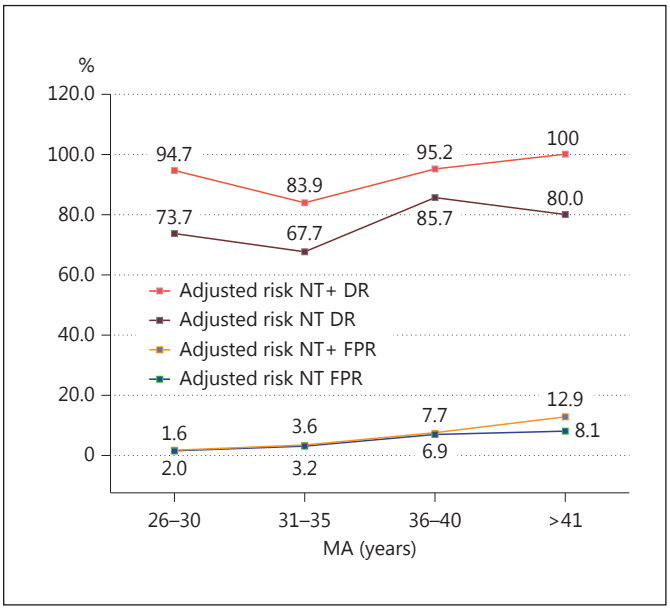


Fig. 4. DRs and FPRs of 'adjusted risk for T21 by NT' and 'adjusted risk for T21 by NT+' depending on the MA ranges.

Discussion

As already mentioned by previous investigators, it was shown in our study that ultrasound markers, extracardiac malformations and CHDs are more frequent in T21 than in euploidy. In this series, we confirmed the importance of the analysis of all first-trimester ultrasound markers of aneuploidy, as they were observed in combination in only 1.2% of euploid fetuses compared to 71.5% of fetuses with T21. Increased NT alone was the most common abnormal isolated finding, found in about 20% of fetuses with DS and in 3.2% cases of euploidy. Increased NT combined with TR was found in 63.5% of T21 cases compared to 0.3% of euploid fetuses. The coincidence of thickened NT and delayed nasal ossification was reported in 28.6% of T21 subjects compared to 0.3% of euploid cases. 17.8% of DS cases presented a combination of absent NB and TR, while in the euploidy group this coincidence was only found in 0.2% of cases. A detailed analysis of the anatomy, with a cardiac evaluation in particular, is crucial when abnormal markers are detected, as almost 20% of the affected fetuses presented CHD. In this group, atrioventricular septal defects were most commonly encountered. This observation implies that an early cardiac examination should be considered as a routine part of the fetal evaluation at the time of the first-trimester scan. An early cardiac scan has been proven to be feasible and seems not to be affected by the CRL and BMI of the mother [14].

The findings of this study demonstrate that the DR of T21 at 11–13 weeks' gestation by a complete combination of ultrasound markers ('adjusted risk for T21 by NT+') identifies about 92% of all affected pregnancies at an FPR of 3%. With an increase of the FPR to 5%, the overall DR increases to 95%. These results prove that the application of all ultrasound markers without the addition of biochemistry shows comparable screening performance to the published data, in which the secondary ultrasound markers were assessed additionally to CST ('adjusted risk for T21 by CST+') [7, 8]. For instance, Ghaffari et al. [7] reached a DR for T21 of 90% for a fixed FPR of 3%, while Karadzov-Orlic et al. [8] achieved a sensitivity of 93% at an FPR of 4.8%.

According to the literature, there are scarce data regarding the possible influence of the MA on the performance of the first-trimester screening method. Interestingly, we found that the efficacy of the method, irrespectively of whether the 'adjusted risk for T21 by NT' or the 'adjusted risk for T21 by NT+' protocol was applied, decreases in women aged 31–35 years. With an MA above 35 years, DRs of both methods improved but with a parallel increase in

the FPR. We consider this topic very important as the timing of childbearing has changed over the years. Although the DRs of T21 increased in MA ranges between 36 and 40 years and over, the FPRs increased as well. This would imply a risk of overestimation and unnecessary invasive procedures in older women who get pregnant significantly more often as a result of in vitro conception. With the advent of noninvasive prenatal screening, the issue of increased invasive procedures becomes less problematic; however, this costly method is not widely utilized yet.

For a proper counseling, our findings concerning the influence of MA on the screening performance for T21 should be interpreted with caution. Further studies based on a larger population are required to produce more precise data regarding this subject.

This is the first first-trimester screening study for DS based on ultrasound only. Although we utilized the ultrasound FMF risk calculation algorithm, the applied screening concept differed from the FMF policy.

One of the advantages of this single-center study is the fact that a relatively high number of patients was included. Another benefit was that examiners with different levels of experience performed the ultrasound scans.

We are aware that screening providers from other countries representing a traditional policy by means of a CST may question ultrasound-based aneuploidy screening from an ethical point of view. At this point, it is important to mention that in Poland, CST screening is paid for by a National Healthcare Fund only for patients aged >35 years, and/or for those with a history of chromosomal aberration, and/or if one or both parents demonstrate congenital anomaly or a genetic disorder, and/or if they are at a high risk for single-gene or multifactorial disorder, and/or if abnormal ultrasound or biochemistry findings are detected at a routine antenatal care examination [15]. On the other hand, the average MA in the general Polish obstetric population is 29 years [16], and the majority of patients do not fulfill the criteria for coverage. They undergo first-trimester screening mainly at private diagnostic centers and because of the costs they do not opt for a combined screening and choose detailed first-trimester ultrasound only.

Due to these facts, we gained expertise in ultrasound-based screening on a large group of patients who did not undergo biochemistry analysis. Additionally, in our country, many antenatal care providers as well as patients experienced a high number of FPRs of combined screening because of nonaccredited biochemistry analyzers, which is another reason of the local skepticism towards implementing biochemistry in all patients. The screening ability and the skepticism towards maternal serum biochemistries forced Polish physicians involved in first-trimester screening to improve their ultrasound skills. This reflects the data published on the FMF website regarding Polish physicians who have a certificate for TR (69%) and DV (78%). In contrast, in the UK, only 48% have a certificate for TR and 46% for DV. All in all, we disagree with the opinion that the assessment of secondary markers is time-consuming and requires a long learning curve [2, 11, 17]. All our trainees obtain high competence in this area after a minimum of 3 months of extensive training. In our study, we aimed at simulating the common trend of screening used in Poland. It also has to be mentioned that the mixed risk population of our study is a result of the referrals from local obstetricians who perform elementary sonography focused on viability, fetal growth, and general anatomic impression before the official first-trimester screening. As a result, tertiary screening centers and examiners with a better expertise register a greater number of high-risk patients, i.e. with a suspicion of increased NT, than referring doctors who are not certified for screening. In our opinion, this may only increase the FPR without having an impact on the DR.

PAPP-A and fβhCG have been found to be affected by numerous factors, e.g. gestational age, maternal weight, smoking, ethnicity, and sex of the fetus [18–20]. It is also important to mention that the results of the latest multicenter NEXT study based on a sample of >15,000 patients who were recruited from an unselected screening population demonstrated lower DRs of CST at the level of 79% with a 5% FPR compared to earlier published data [21]. Therefore, our proposed ultrasound-based method of screening for T21 could be considered as an alternative to CST, either as routine antenatal care or under special circumstances, e.g. in centers without certified biochemical analyzers or in patients after assisted reproductive techniques who demonstrate higher FPRs from CST at a level of 10.7% due to the significantly lower serum levels of PAPP-A [22]. It can also be considered in all women suffering from chronic diseases, e.g. renal diseases [20] or insulin-dependent diabetes mellitus [19]. We strongly believe that nowadays, in the era of transition from aneuploidy screening to noninvasive prenatal testing, ultrasound-based methods will also play an important role in the diagnostic process as specific methods for detecting structural and functional abnormalities.

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