Fetal Diagnosis and Therapy

Prenatal Diagnosis

Fetal Diagn Ther 2024;51:453–462
DOI: 10.1159/000539463

Received: May 26, 2023
Accepted: April 15, 2024
Published online: May 31, 2024

Is Nuchal Translucency of 3.0–3.4 mm an Indication for cfDNA Testing or Microarray? – A Multicenter Retrospective Clinical Cohort Study

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Keywords
Increased nuchal translucency · Chromosomal microarray · Noninvasive prenatal testing · Prenatal diagnosis

Abstract

Introduction: This study aimed to evaluate the occurrence of clinically relevant (sub)microscopic chromosomal aberrations in fetuses with the nuchal translucency (NT) range from 3.0 to 3.4 mm, which would be potentially missed by cfDNA testing. ***Methods:*** A retrospective data analysis of 271 fetuses with NT between 3.0 and 3.4 mm and increased first trimester combined test (CT) risk in five cohorts of pregnant women referred for invasive testing and chromosomal microarray was performed. ***Results:*** A chromosomal aberration was identified in 18.8% fetuses (1:5; 51/271). In 15% (41/271) of cases, trisomy 21, 18, or 13 were found. In 0.7% (2/271) of cases, sex chromosome aneuploidy was found. In 1.1% (3/271) of cases, CNV >10 Mb was detected, which would potentially also be detected by genome-wide cfDNA testing. The residual risk for missing a submicroscopic chromosome aberration in the presented cohorts is 1.8% (1:54; 5/271). ***Conclusion:*** Our results indicate that a significant number of fetuses with increased CT risk and presenting NT of 3.0–3.4 mm carry a clinically relevant chromosomal abnormality other than common trisomy. Invasive testing should be offered, and counseling on NIPT should include the test limitations that may result in NIPT false-negative results in a substantial percentage of fetuses.

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Introduction

Chromosomal microarrays (CMAs) have replaced classical cytogenetic examination because of the potential for detecting submicroscopic aberrations [1]. Using CMA is not dependent on the cell culture, which also reduces turnaround time that is of great importance in prenatal settings. Moreover, single nucleotide polymorphism arrays allow the detection of genetic disorders resulting from uniparental disomy, mosaicism or triploidy [2]. The use of CMA in prenatal diagnosis of fetal structural abnormalities is widely recognized, which is reflected in the recommendation for the genetic diagnosis of fetal structural abnormalities by many scientific societies [3–5]. However, pregnant women with a high risk of the most common trisomies 21, 18, and 13 calculated based on the FMF algorithm without concomitant ultrasound abnormalities are still offered classical cytogenetic examination or assessment using cfDNA testing [6]. Depending on the nuchal translucency (NT) value, the microarray method is recommended as a reference. Most societies tend to consider an NT value of ≥3.5 mm (≥99 percentile) as abnormal and in these situations recommend an invasive diagnosis using microarrays [6–8]. However, ACOG/SMFM, in an update to their guidelines, advised that an NT value of 3.0 mm should be considered abnormal rather than the current 3.5 mm [5].

Still, the role of the use of the microarray in cases of isolated slightly elevated NT in the range of 3.0–3.4 mm (95–99th percentile) needs to be investigated. In these clinical situations, in the absence of other anatomical abnormalities in the fetus, noninvasive prenatal testing is often considered due to its high sensitivity in detecting the three most common aneuploidies [5]. Some authors do not recommend NT measurement when cfDNA testing had previously been performed [9]. However, recent work as well as a meta-analysis indicate an increased prevalence of submicroscopic aberrations in fetuses with NT ≥3.5 mm with a significant impact on the future development and function of their children [10]. The detection of variants of unknown significance, copy number variant (CNV) for late-onset aberrations, or susceptibility CNVs for neurodevelopmental disorders (NNDs) (such as autism) is often blamed for the adverse effects of microarray testing. However, before each decision to perform invasive diagnostics during genetic pretest counseling, parents can decide whether they want such aberrations to be reported in the result [11]. The aim of our study was to evaluate the occurrence of (sub) microscopic CNVs <10 Mb that cannot be detected by cfDNA testing but have or could have an impact on parental decisions or early child development requirements in fetuses with apparently isolated increased NT (3.0–3.4 mm) and increased CT risk.

Materials and Methods

Cohort Selection

We present a retrospective multicenter study, which included 271 fetuses with apparently isolated, increased value of NT (3.0–3.4 mm) and increased CT risk (1:250–300), who were referred for invasive sampling followed by CMA. Diagnostic CMA was performed in 5 genetic laboratories and in 4 countries: Czech Republic (Prague [PRG] n = 152), Poland (Krakow [KRA] n = 48, Łódź [LOD] n = 7), Portugal (Lisbon [LIS] n = 46), and Spain (Madrid [MAD] n = 18). Laboratory databases were searched to find patients who meet the inclusion criteria. In total, 271 pregnant women met the inclusion criteria. The number of cases in individual laboratories and the aberrant subgroups are shown in the online supplementary Table 1 (for all online suppl. material, see https://doi.org/10.1159/000539463). The particular subcohort descriptions, CT risk cut-offs and diagnostic procedures are presented in the Supplementary material. All cases underwent CMA testing unless rapid aneuploidy test identified an aberration. In some cases with pathogenic CNVs follow-up was available if patients did not terminate the pregnancy, additional phenotypic features included anomalies that were diagnosed later in pregnancy (the second and third trimester) and after birth.

Statistical Analysis

Data were presented as number of cases with percentage for qualitative variables and as median with quartile range for quantitative variables due to significant deviations from normal distribution and high disparity of cases. Pearson's χ^2 test was used to compare qualitative variables, while for quantitative variables, the Kruskal-Wallis test with a post hoc multiple comparisons test was used. The analysis was performed in R in the RStudio environment. Values of $p < 0.05$ were considered statistically significant.

To compare the data of the current cohort, we performed a literature review and the meta-analysis was conducted in R in the RStudio environment but using the metaprop package. Fixed effect analysis and, due to suspected high heterogeneity, random-effect analysis was used. The papers used in the meta-analysis were ordered by year of publication.

Results

The study cohort included 271 fetuses with NT values in the range of 3.0–3.4 mm with no apparent ultrasound anomalies at the 1st trimester examination and at the moment of invasive testing, which was extracted from a larger cohort of 650 fetuses with isolated NT ≥3.0 mm and/or abnormal combined screening. Because the study involved multicenter retrospective analysis with known differences, such as cut-off for invasive testing, cultural

Table 1. Risk for chromosomal aberrations in fetuses with NT 3.0–3.4 mm and an increased risk after first trimester combined test

	Total number and percentage of abnormal cases, *n* (%)	Risk in our cohort
Total number of abnormal results in our cohort of 271	51 (18.8)	1:5
Trisomy 21, 18, 13	41 (15)	1:7
SCA	2 (0.7)	1:135
CNV >10 Mb (all syndromic)	3 (1.1)	1:90
CNV <10 Mb included	5 (1.8)	1:54
a) Early-onset syndromic CNV <10 Mb	1 (0.4)	1:271
b) Late-onset syndromic CNV <10 Mb	1 (0.4)	1:271
c) Number of susceptibility CNV for NDD (<10 Mb)	3 (1.1)	1:90
Residual risks after different cfDNA tests		
Residual risk of early-onset chromosomal aberrations not detectable by cfDNA testing for trisomy 21, 18, 13 and X/Y aneuploidy (excluding susceptibility CNV for NDD and a late-onset disorder)[a]	5 (1.8)	1:54
Residual risk of early-onset chromosomal aberrations not detectable by cfDNA testing for trisomy 21, 18, 13 (excluding susceptibility CNV for NDD and a late-onset disorder)[a]	7 (2.6)	1:38
Residual risk of early-onset chromosomal aberrations not detectable by genome-wide cfDNA testing (excluding susceptibility CNV for NDD and a late-onset disorder)[a]	1 (0.4)	1:271

SCA, sex chromosome aberration; CNV, copy number variant; NDD, neurodevelopmental disorder. [a]Additionally to aberrations that are not in the scope of the particular cfDNA test, the risk for false-negative cfDNA results need to be discussed if fetus has NT 3.0–3.4 mm and an increased CT risk, but cfDNA test presented no chromosomal aberrations.

and financial differences, which may influence the decisions on prenatal testing, we performed homogeneity analysis on the whole cohort of 650 fetuses to assess, whether the study group is homogenous enough to be presented as one study cohort. The analysis of the homogeneity showed that the groups from each center are homogeneous in terms of bHCG and PAPP-A (multiple of medians) values, NT values distribution, and estimated risk of T13, T18, and T21 as shown in online supplemental Table 2. In terms of GA (gestational age) at the time of invasive procedures, statistically significant differences were seen. The Polish cohort was tested statistically significantly later than other countries, which reflects the preferred sampling method (amniocentesis). Amniocentesis was performed in 44% (119/271) cases and chorionic villi biopsy in 56% (152/271). Based on these results, we concluded that although the cohorts are multinational, the statistical homogeneity is achieved and allows further data extraction (271 fetuses with NT 3.0–3.4 mm out of 650 with NT ≥3 mm) and data analysis.

The risk of an abnormal result in our cohort is 18.8% (51/271); 1 in 5 pregnant women received a result classified as abnormal. One in 7 pregnant women had common trisomies (21, 13, 18), which represents 15% of the study population. In two cases, numerical aberrations of sex chromosomes were found (monosomy X and XYY). Structural chromosomal abnormalities were found in 2.95% cases (8/271) (Table 1): 3 cases of aberrations >10 Mb (Table 2) and 5 instances <10 Mb (Table 3). Three of the submicroscopic aberrations were susceptibility CNVs for NND (Table 3).

If targeted cfDNA testing for T21, T18, and T13 had been offered in this cohort, the residual risk of 2.6% (7/271; 1:38) for the abnormal array result should have been counseled during pretest counseling. If whole genome cfDNA testing including SGA had been offered, the residual chance for submicroscopic CNV would have been 0.4% (1/271; 1:271). The risk for submicroscopic CNV associated with early-onset syndromic disorder would be 1:270, but the risk associated with NNDs would be 1.1% (3/271; 1:90).

Additionally, to compare our results to the literature data, we calculated pooled prevalence of chromosomal aberrations (event rates) including 95% CI similarly in available cohorts: current study and published before

Table 2. Microscopic CNV findings (larger than 10 Mb) in fetuses with NT 3.0–3.4 mm and an increased first trimester combined test risk, but no structural anomalies at the time of first trimester screening or invasive testing

NT, mm (risk for T21 in CT)	Array result	Size, Mb	Interpretation	Usefulness of US to detect anomalies later in pregnancy	Pregnancy outcome and anomalies diagnosed later in pregnancy or after birth
3.1 (KRA) (1:19)	arr[hg19] 9q33.2q34.3(124205191_139389803) x3	15.2 Mb gain	Interstitial duplication of chromosome 9q	Not all patients present structural anomalies detectable on ultrasound	Live birth – anomalies: FGR, CHD, duodenal atresia, pACC, polyhydramnion, facial dysmorphic features, hypotonia, seizures
3.4 (KRA) (1:69)	arr[hg19] 5p15.33p15.31(22149_7449397)x1, 5p15.31p12(7506131_44341490)x3	7.43 Mb loss 36.84 Mb gain	Cri-Du-Chat syndrome (OMIM # 123450)	Not all patients present structural anomalies detectable on ultrasound	Live birth – anomalies: FGR, CHD, ACC, hydronephrosis, died within first week
3.4 (LIS) (no data)	arr[hg19] 18p11.32p11.22(136228_10801332)x1	10.7 Mb loss	18p deletion (OMIM # 146390)	Unlikely detectable	Data not available

arr, array, CT, first trimester combined test; US, ultrasound anomalies; FGR, fetal growth restriction; CHD, congenital heart defect; ACC, agenesis of corpus callosum; pACC, partial agenesis of corpus callosum.

[12–19]. The meta-analysis was performed for data including all abnormal results (typical trisomies, sex chromosome aberrations, CNVs both larger and smaller than 10 Mb were included) as shown in Figure 1.

To facilitate counseling and comparing our results to the literature, we present the analysis for different CT cut-off risks for the occurrence of the most common three trisomies in cases where the risk for T21 was specified. Cases where risk for given approximately (e.g., <300 or >300) were excluded. Table 4 presents the frequency of the common trisomies in the selected risk cut-offs.

Discussion

In the cfDNA testing era, a fetus with increased NT without visible structural abnormalities at the first trimester screening may present difficulties in genetic counseling and in choosing the optimal management model. The current contingent screening strategy is coming into use in many countries, replacing the previous conventional approach using an invasive diagnosis in the high-risk group. The cut-off level for the high-, intermediate- and low-risk groups are defined differently in different countries and has changed over the years with the entry into the use of cfDNA testing and the change in patients' care providers' approach to invasive diagnosis [6, 20].

The availability of cfDNA testing can identify fetuses with common trisomies. However, the sensitivity of cfDNA testing for other chromosome aberrations is still lower than CMA. Moreover, a recent statement from the International Society for Prenatal Diagnosis (ISPD) emphasized that there is no consensus to use alternative NT cut-off points (3.0 mm or 99th percentile) to define the target population for offering invasive diagnostics [21].

Nevertheless, our study shows that fetuses with NT of 3.0–3.4 mm (and an increased risk after combined test) are at very high risk for chromosomal aberrations (1:5), which justifies offering invasive diagnostic testing in order to prevent diagnostic delay. Additional information on the CT risk is of significant value for counseling. However, we have shown (Table 4) that even in the group of the patients with intermediate risk (CT: 1:51–1:250), the incidence of trisomy 21 was higher than 1:50.

Both our study and literature data show that a significant number of such fetuses carry a clinically relevant chromosomal abnormality other than common trisomy, which potentially can be missed by cfDNA testing targeting only common trisomies [22]. This information should be included in the pretest counseling for cfDNA testing and invasive testing to assure informed individual choices. Our data are in

Table 3. Submicroscopic CNV findings (smaller than 10 Mb) in fetuses with NT 3.0–3.4 mm and an increased risk after combined test, but no structural anomalies at the time of first trimester screening or invasive testing

	NT, mm, (risk for T21 in CT)	Array result	Size, Mb	Interpretation	The usefulness of the US to detect anomalies later in pregnancy	Pregnancy outcome and anomalies diagnosed later in pregnancy or after birth
Susceptibility CNVs	3.0 (LIS) (no data)	arr[hg19] 1q21.1q21.2(146023922_147398268) x3	1.44 Mb gain	1q21.1 microduplication	Not all patients present structural anomalies detectable on ultrasound	Data not available
	3.4 (LIS) (no data)	arr[hg19] 16p11.2(29638641_30190029)x3	0.55 Mb gain	16p11.2 microduplication (OMIM # 614671)	Undetectable	Live birth – normal
	3.4 (MAD) (no data)	arr[hg19] 15q11.2(22880274_23179948)x1 pat	0.3 Mb loss	15q11.2 microdeletion	Not all patients present structural anomalies detectable on ultrasound	Live birth – normal
Syndromic CNV	3.1 (KRA) (1:111)	arr[hg19] 17p12(14111772_15442066)x1	1.33 Mb loss	Neuropathy, hereditary with liability to pressure palsies; HNPP (OMIM #162500)	Undetectable	Live birth – normal (late-onset, incidental finding)
	3.4 (KRA) (1:69)	arr[hg19] 12p13.33p13.32(230421_3358023)x1	3.12 Mb loss	12p13.33 microdeletion syndrome (ORPHA: 280325)	Not all patients present structural anomalies detectable on ultrasound	TOP – anomalies: cerebral hypoplasia, ventriculomegaly, hemivertebra, bilateral talipes

arr, array, Mb, megabase pair; CT, first trimester combined screening test; US, ultrasound anomalies; TOP, termination of pregnancy.

concordance with the recent updated ACOG/SMFM guidelines [5] which advise that an NT value of 3.0 mm should be considered abnormal rather than the current 3.5 mm. Our results are consistent with those recently published papers [12–19] and further point out that the risk for chromosomal aberrations in this group is significant, as presented in Figure 1.

If the pregnant woman opted for the cfDNA testing analyzing only trisomy 21, 18, and 13, in 1:38 fetuses in our cohort, a chromosome aberration would have been missed. However, if genome-wide cfDNA testing, including screening for sex chromosome aneuploidies, was performed, one case of early-onset syndromic disorder would have been missed among 271 fetuses. This finding aligns with existing literature data [23]. A recent paper by Maya et al. [24] on the residual risk of clinically significant CNV in early gestation pregnancies indicated a residual risk of 0.5% (1:199). Current results and previous

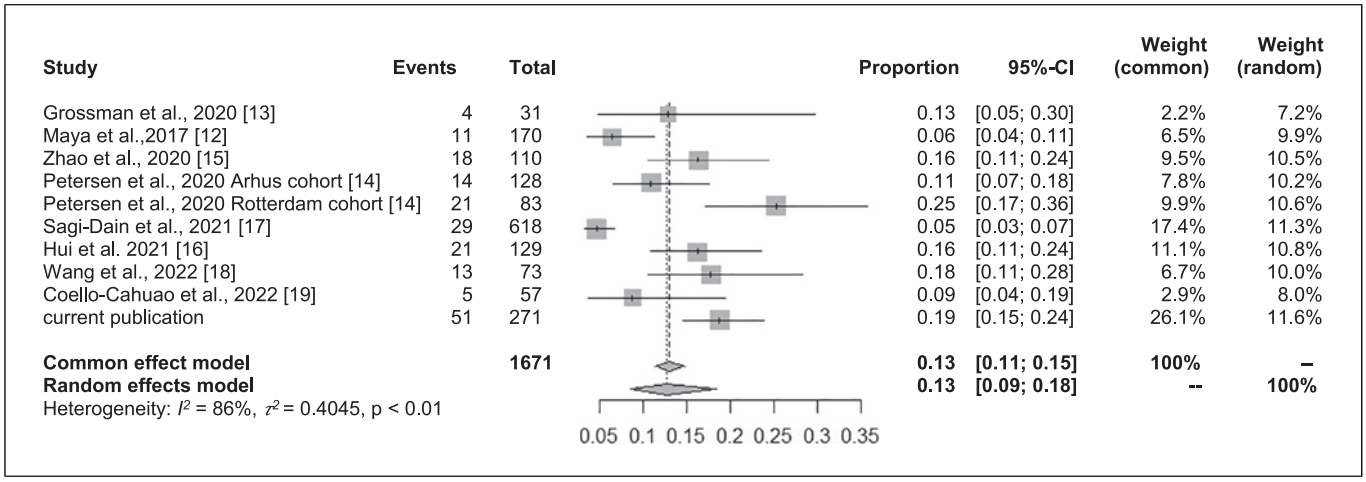

Fig. 1. Forest plot presenting event rate of all abnormal cases based on cytogenomic results in our cohort of fetuses NT 3–3.49 mm in selected sources representing the risk for overall risk for chromosomal aberrations in the combined cohort (13%, 95% CI: 9%–18% equivalent to a risk of about 1:9). Only the first author is given for each study. Boxes represent event rate per source, their size is proportional to their weight in the analysis, and lines represent 95% CI. Diamond represents pooled estimate, and its width represents the 95% CI.

Table 4. First trimester combined screening test risk groups – risk groups risk stratification for trisomy 21, 18, and 13

Risk group	*N*	T21	T18	T13	Risk for common trisomies T21/T18/T13 (%)	Submicroscopic pathogenic syndromic CNVs <10 Mb
Very high-risk group CT ≥1/10	38	22	2	0	1:2 (58%)	
High-risk group CT >1/11–1/50	40	7	0	0	1:6 (17.5%)	
Intermediate-risk group CT ≥1/51–1/250	73	4	0	0	1:18 (5.5%)	17p12 deletion 12p13 deletion
Intermediate-risk group CT ≥1/250–1/1,000	42	0	0	0	–	
Low-risk group CT >1/1,000	26	0	0	0	–	
Total	219	33	2	0	1:7 (16%)	

CT, first trimester combined test; *N*, total patient number (including all patients for whom data from the first trimester combined screening test were available in the laboratory database); T21/T18/T13, number of patients with trisomy 21/trisomy 18/trisomy 13 detected by invasive testing (including all patients for whom data form the first trimester combined screening test were available in laboratory database).

literature support the hypothesis that the risk for submicroscopic CNVs associated with syndromic disorders is not notably increased in fetuses with NT 3.0–3.4 mm.

When CMA is chosen as a follow-up test, a significant pool of aberrations represented recurrent susceptibility CNVs for NDD. These CNVs are associated with a spectrum of NNDs including autism spectrum disorders, intellectual disability, communication disorders, attention deficit and hyperactivity disorders, specific learning and motor disorders, and schizophrenia. These

are disorders that may affect the neurodevelopment of the child, even if some of them are inherited from a healthy parent [25]. There is no doubt that genetic counseling after such a result is necessary and may be challenging for both the counselor and the prospective parents [26]. Ideally, prospective parents should be informed of the possibility of such a result and have a choice about its reporting. Previous research has shown that when an increased NT is truly isolated, the risk for developing NND is not significantly increased, indirectly reassuring about an excess of carriers of a susceptibility CNV fetuses with increased NT [27, 28]. To answer the question, whether the fetuses with both increased NT and a susceptibility CNV have an increased risk for an NND, long-term follow-up research in large populations is needed. Nevertheless, even if the individual risk is challenging to assess, some parents are interested in such information [29, 30]. Reporting susceptibility CNVs may influence the implementation of early programs to support the psychomotor and neurodevelopment of children with prenatally diagnosed susceptibility CNV for NDD. The detected CNV may have an impact on prognosis, psychomotor development of the child, and social functioning. Knowledge of these chromosomal aberrations may allow better organization of care and early support in child development [31], but it may also increase uncertainty in the prospective parents [11]. Although it seems that there is no notably increased risk for submicroscopic CNVs associated with syndromic disorders in the studied cohort, one cannot underestimate invasive testing providing a rapid final diagnosis in high-risk pregnancies.

If the risks for chromosomal aberrations are higher than 1:10, one should carefully consider whether the second screening test, such as cfDNA testing, is the most suitable option for pregnant women who wish to receive a rapid and final diagnosis. Choosing cfDNA testing, primarily targeted for trisomy 21, 18, 13, may bring false reassurance as 30% of chromosomal aberrations were other than common trisomies, and the second screening will lead to a delayed final diagnosis in a group of pregnant women with very high risks >1:10 [32].

In case a woman has already normal cfDNA testing results as a first-tier screening, and the subsequent ultrasound examination shows increased NT of 3.0–3.4 mm, the residual risk for chromosomal aberrations should be discussed. Based on the current cohort, the residual risk for other chromosomal aberrations than trisomies 21, 18, and 13 is 1:38 (2.6%) in the presented cohort. However, one has to keep in mind that the cohort presented in the current study was referred for CMA due to both increased CT risk calculations and NT 3.0–3.4 mm. The residual risk is therefore highly dependent on the cohort selection. Bardi et al. [33] recently reported an additional risk of chromosomal aberrations up to 0.9% after low-risk genome-wide cfDNA testing. Kelley and colleagues showed previously that when including all pregnancies with NT 3.0–3.4 mm, and not just the subgroup that undergoes prenatal diagnosis, the risk for chromosomal aberrations is notably lower (0.37%, 1:270). However, for risk >1:300, in some countries, diagnostic testing is still conventionally offered after first trimester screening. Therefore, the authors suggested that the option of diagnostic testing may be discussed with parents [34]. Both the residual risks, pros and cons of different screening strategies need to be discussed with patients. However, if genome-wide cfDNA testing including sex chromosome aberration was performed, the risk for submicroscopic early-onset syndromic disorders seemed to be comparable to general population (1:270) [23]. On the other hand, because the presented cohort and literature show an increased prevalence of common trisomies the false-negative rate of cfDNA testing in this group of patients might be potentially elevated, when compared to pregnancies with NT <95th percentile.

Our results confirm the literature data and underline the significant role of pretest counseling in achieving informed choices in our patients' diagnostic path in prenatal settings. Understanding the limitations of the methods used by the medical team caring for the pregnant women and the patients themselves is essential for informed consent that should agree with the pregnant women's attitude toward the genetic testing. In our opinion, we are obliged to present diagnostic and screening possibilities to pregnant women, discuss the limitations, and respect their individual choices [14].

Study Limitations

A primary limitation of the study is that it mainly includes CMA data from fetuses with an NT 3.0–3.4 mm undergoing invasive testing because of increased CT risk and not only because of an apparently isolated NT of 3.0–3.4 mm. Although our analysis showed that the cohort was homogenous, reimbursement differences, different cut-offs and indications for invasive testing, and different attitudes toward prenatal testing may influence cohort selections in other counties. The study reflects the actual clinical situation and therefore is limited to a biased population and is not fully representative of the entire population of fetuses with NT between 3.0 and 3.4 mm.

This study was not designed to evaluate the accuracy of CT, and it is based on retrospective multicenter routine clinical data, and corrections for multiple of medians of serum markers, maternal age, or other demographic factors in the whole studied cohort were not possible. We are aware that it is a subgroup of the general population who opts for invasive testing and most likely represents an upper-bound risk estimate. These data also show higher risk than presented before by Petersen and colleagues, probably because most tested fetuses not only showed NT >3.0 mm but increased risk after combined test as well.

Most laboratories worked on oligonucleotide array platforms, which do not allow to exclude uniparental disomy disorders, and long-term clinical follow-up was unavailable for the laboratories. Therefore, we did not assess the number of fetuses with normal CMA results presenting abnormal phenotype after birth.

The cfDNA testing detection was simplified as we assumed that all aneuploidies as well as structural unbalanced aberrations larger than 10 Mb would be detectable. In clinical pretest counseling for cfDNA testing, one should include the whole test characteristics, especially aspects of confined placental mosaicism the risks for false-negative results and in cases with high risk the fact that additional screening (after CT) may delay final diagnosis of aberrant fetus.

Conclusions

Fetuses with NT of 3.0–3.4 mm and increased CT risk are at very high risk for chromosomal aberrations, which justifies offering invasive diagnostic testing. A significant number of such fetuses carry a clinically relevant chromosomal abnormality other than common trisomy. Counseling on cfDNA testing should include the resolution/scope limitation that may cause false-negative cfDNA testing results in a substantial percentage of fetuses as well as the possibility of diagnostic delays. Both cfDNA testing and invasive testing can be offered together with a proper pretest counseling that ensures individual informed choices.

Statement of Ethics

Our research represents a retrospective multicenter anonymized patient data study that does not fall under the scope of the Dutch WMO (The Medical Scientific Research with Humans Act); therefore, it did not need to be assessed by an accredited Medical Ethical Committee or the CCMO (Central Committee on Research Involving Human Subjects). According to the FMWV Code of Conduct for Health Research, the data that cannot be traced to an individual may be used for research. All presented (online suppl. File) data are anonymous and do not allow identification of the individual patients and were obtained during routine diagnostic procedures, so ethical approval is not required for this study in accordance with local or national guidelines. According to Polish legislation, the consent of the Bioethics Committee is not required for retrospective studies on anonymized data. The need for informed consent for retrospective research based on anonymized data was waived by the Medical Ethical Commission Erasmus MC (MEC-2012-387).

Conflict of Interest Statement

D. Stejskal, M. Trková, and D. Smetanová are employees of GENNET, s.r.o., providing prenatal testing. The remaining authors have no conflicts of interest to declare.

Funding Sources

This research received no external funding.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception (M.R.K. and M.I.S.), study design (M.R.-K., D.S., S.S., J.N., A.G., and M.I.S.), execution (M.R.-K., A.M.-T., K.S., A.S., D.S., S.S., J.N., A.G., and M.I.S.), acquisition of data (M.R.-K., A.M.-T., K.S., M.B.-M., A.S., D.S., M.T., D.S., S.S., H.C., J.N., M.A.M., E.M., L.R., A.K., A.G., H.H., M.K., and M.I.S.), analysis and interpretation (M.R.-K., A.M.-T., K.S., M.B.-M., A.S., D.S., M.T., D.S., S.S., H.C., J.N., M.A.M., E.M., L.R., A.K., A.G., H.H., M.K., and M.I.S.), or in all these areas; took part in drafting (M.R.-K., A.M.-T., A.S., D.S., S.S., J.N., A.G., and M.I.S.), revising or critically reviewing the article (M.R.-K., A.M.-T., K.S., M.B.-M., A.S., D.S., M.T., D.S., S.S., H.C., J.N., M.A.M., E.M., L.R., A.K., A.G., H.H., M.K., M.I.S.), gave final approval of the version to be published (M.R.-K., A.M.-T., K.S., M.B.-M., A.S., D.S., M.T., D.S., S.S., H.C., J.N., M.A.M., E.M., L.R., A.K., A.G., H.H., M.K., M.I.S.), have agreed on the journal to which the article has been submitted, and agreed to be accountable for all aspects of the work (M.R.-K., A.M.-T., K.S., M.B.-M., A.S., D.S., M.T., D.S., S.S., H.C., J.N., M.A.M., E.M., L.R., A.K., A.G., H.H., M.K., M.I.S.).

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

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