Value of increased nuchal translucency in the era of noninvasive prenatal testing with cell-free DNA

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Abstract
Objective: To assess the value of increased nuchal translucency (NT) at first-trimester screening (FTS) despite the superiority of noninvasive prenatal testing with cell-free DNA (cfDNA) for the detection of fetal aneuploidies.
Methods: Retrospective analysis of all FTS data from 2005 to 2015 in our department. Only cases with increased NT and euploid karyotype were considered eligible for inclusion. Abnormal findings, diagnostic work-up, and perinatal outcomes were assessed.
Results: Of 18,084 FTS results, 460 (2.5%) showed increased fetal NT, of which 242 (52.6%) underwent invasive karyotyping and 179 (74.0%) had an aneuploidy. Of the remaining 63 cases, 61 (96.8%) showed an additional sonographic finding at FTS and 25 (78.1%) had a major anomaly at the second trimester organ scan. The outcome was termination of pregnancy in 28 (44.4%) cases, fetal demise in 5 (7.9%), delivery of an infant with malformation in 21 (33.3%), and delivery of a healthy infant in 7 (11.1%) cases.
Conclusion: All cases with increased NT would have been detected by cfDNA or by a major sonographic anomaly not later than the second trimester. Routine use of cfDNA, a basic sonogram, and an organ scan could reduce unnecessary work-up and anxiety.

KEYWORDS
Amniocentesis; Cell-free DNA; Chorionic villus sampling; Noninvasive prenatal testing; Nuchal translucency; Trisomy

1 | INTRODUCTION

Measurement of fetal nuchal translucency (NT) during the first trimester enables identification of 90% of fetuses with trisomy 21 and other major aneuploidies, with a false-positive rate of 5% in combination with maternal serum markers. In the absence of aneuploidies, fetal NT above the 99th percentile of the normal range for crown-rump length (CRL) is associated with a wide spectrum of structural anomalies, malformations, and syndromes, as well as with a higher risk of spontaneous abortion and fetal demise.

The conventional algorithm for prenatal diagnosis was revolutionized when noninvasive prenatal testing (NIPT) with cell-free DNA (cfDNA) was introduced for the detection of fetal aneuploidies. cfDNA was shown to be superior in detecting the most frequent aneuploidies, while generating fewer false-positive results. First-trimester screening (FTS) with measurement of NT is still considered standard care worldwide, despite an increasing trend toward replacement with NIPT using cfDNA.

In making the transition to cfDNA, it is important to evaluate whether there is any added value using ultrasonography-based...
detection methods after normal cfDNA results. This question has been investigated by Lichtenbelt et al., who reported that in only 3 of 25,057 (0.01%) cases, the combination of cfDNA (for the detection of trisomies 21, 18, and 13) and the second-trimester organ scan would have missed a chromosomal anomaly that could have been identified by FTS; one of the three anomalies was triple X and two were identified by array. In their study, most of the chromosomally abnormal cases experienced fetal demise or showed major sonographic anomalies at the second trimester scan, and 85 of their 225 cases with an abnormal FTS resulted in live-born children without anomalies. These false-positive results regularly lead to additional and unnecessary diagnostic work-up, parental anxiety, or even pregnancy loss as a complication of invasive testing.

The aim of the present study was to review the outcomes of cases with increased NT and abnormal FTS. We aimed to determine whether conventional screening with measurement of NT offers a benefit for cases that are not detectable by cfDNA.

2 | MATERIALS AND METHODS

We retrospectively reviewed data from all women who had undergone FTS and measurement of NT between January 1, 2005 and January 1, 2015, at the Department of Obstetrics and Gynecology, Medical University of Vienna, Austria. Only cases with increased NT that underwent fetal karyotyping by amniocentesis and/or chorionic villus sampling (CVS) were eligible for the analyses. As part of our routine protocol, all women who underwent FTS registered for a planned delivery at our institution and were screened by a detailed organ scan during the second trimester of pregnancy.

FTS included measurement of the following parameters: CRL, NT, fetal heart rate (FHR), and (facultative) nasal bone and tricuspid valve regurgitation. The angiogenic factors pregnancy-associated plasma protein A (PAPP-A) and beta-human chorionic gonadotropin (beta-hCG), which are known to increase the detection rate of chromosomal abnormalities and predict placental complications, are not routinely used at our institution. Transabdominal or vaginal screening was performed by sonographers, certified by the Federal Medicine Foundation, between 11 + 0 and 13 + 6 weeks of pregnancy. Only women with singleton pregnancies identified to have fetal NT exceeding 3.5 mm in thickness were considered eligible for inclusion. Infants were considered “healthy” in cases where there were no malformations and no circumstances requiring pediatric follow-up, or “with malformations” when any circumstances (e.g., structural defects) required postnatal interventions.

All relevant data were collected from obstetric databases and patient charts, and were electronically reviewed using PIA Fetal Database software, version 5.6.16.917 (General Electric Company, GE-Viewpoint, Munich, Germany). Demographic information was summarized and displayed using descriptive statistics. Discrete data are presented as number (%) and continuous data are given as mean ± standard deviation (SD). Statistical calculations were performed using SPSS, version 23.0 (IBM, Armonk, NY, USA).

The study was approved by the Ethics Committee of the Medical University of Vienna in accordance with the Declaration of Helsinki and the guidelines of Good Clinical Practice (Registration 1421/2017). Due to the retrospective study design, written informed consent was not obtained. All records were anonymized and de-identified prior to the analyses.

3 | RESULTS

During the 10-year study period, a total of 18,084 first-trimester screenings were performed at our institution. Of this cohort, 17,624 (97.5%) had a fetal NT measurement less than 3.5 mm, whereas 460 (2.5%) had increased NT at or above 3.5 mm. Of these, 242 (52.6%) underwent invasive fetal karyotyping. In 179 (74.0%) cases, fetal trisomy 21, 18, or 13 was identified. The remaining 63 (26.0%) cases had no fetal trisomies and were therefore considered as the study population, which could not have been detected by cfDNA (Fig. 1).

The maternal characteristics of the cases in the study population (n=63) are given in Table 1. Mean fetal NT was 4.9 ± 3.1 mm. All 63 cases presented with a visible nasal bone. Apart from increased NT, 61 (96.8%) cases showed one or more of the following additional abnormal sonographic findings: megacystis, abnormal tricuspid valve regurgitation, (persistent) omphalocele/gastroschisis, exencephaly/anencephaly, heart defect, hydrops fetalis, extremity anomalies, generalized skin edema, and cystic hygroma coli (Table 2).
All 63 cases underwent fetal karyotyping: 52 (82.5%) by CVS and 14 (22.2%) by amniocentesis (multiple procedures were allowed). A second-trimester organ scan was performed in pregnancies that reached 20 weeks (n=32; 50.8%). In 25 (78.1%) of these cases, a major sonographic anomaly was identified at the organ scan; for all these cases the organ scan would have detected the underlying pathology of the increased NT. The remaining 7 (21.9%) cases without a major sonographic anomaly resulted in live births of healthy infants. Of these 7 healthy infants, 5 cases had suspected anomalies at the FTS; however, no anomalies were detected later on. NIPT with cfDNA was performed in 1 (1.6%) case.

Of the study population, 5 (7.9%) cases led to fetal demise during the second or third trimester of pregnancy. Termination of pregnancy was performed in 28 (44.4%) cases. No anomalies were found in the additional diagnostic assessment of 7 (11.1%) cases, which all resulted in live births of healthy infants. Another 21 (33.3%) cases resulted in live births of infants with malformations that required postnatal interventions. Two (3.2%) cases were lost to follow-up.

Overall, the mean gestational age at delivery was 28 weeks: 27 (42.9%) infants were delivered prior to 23*0 gestational weeks and 21 (33.3%) were delivered at term. Of 28 (44.4%) live-born infants, 21 (75.0%) were born with malformations and 7 (25.0%) were healthy. Of the infants with malformations, 7 (33.3%) died postpartum.

A total of 5 (7.9%) cases resulted in fetal demise. One fetus died as a result of hydrops fetalis from a parvovirus B19 infection at 21 gestational weeks and another fetus died from pulmonary hypoplasia at 29 gestational weeks. Seven of 63 (11.1%) infants died postpartum. In 5 (71.4%) of these cases, potentially lethal malformations were detected at FTS. Termination of pregnancy was offered to these women, but all of them declined. In 1 (14.3%) case, the parents opted for a termination because of the serious malformations that were detected at the organ scan. One (14.3%) infant died shortly after birth from sepsis and renal failure following cardiac surgery. The perinatal outcomes are shown in Table 3.

### 4 DISCUSSION

The introduction of cfDNA into clinical routine has been the target of many studies to help understand the best way to incorporate this
practice without losing the benefits of an early sonogram. This paper assesses whether determining increased NT has additional value in the era of cfDNA, by evaluating cases that would not have benefited from its high sensitivity and specificity to detect fetal trisomies.

Lichtenbelt et al.⁵ reported that there was a benefit of FTS over cfDNA in only 0.01% of cases. More recently, Huang et al.¹⁴ were unable to identify additional aneuploidies using cfDNA, concluding that the percentage of chromosomal aberration that would be missed in cases with increased NT might be equal to those with a normal NT. Our results stand in accordance with these studies, as we did not find a single case that benefited from FTS. Cases with increased NT would have been detected by cfDNA (in the case of a trisomy), by the organ scan, or they would have led to healthy infants.

In times of sparse human and financial resources, the conventional algorithm—which might trigger unnecessary work-up—is debatable. Our data showed that 7 cases ultimately resulted in live-born and healthy infants after they had undergone expensive and time-consuming diagnostic assessments. In the Lichtenbelt study,⁵ 38% (85/225) of the cases with increased NT but without trisomy 21, 18, or 13, resulted in the live birth of a healthy infant. Another study reported term birth of infants without malformations in 72% of euploid fetuses with increased NT.¹⁵ According to Mula et al.,¹⁶ 63% of infants with increased NT are born without malformations. In comparison to these studies, the 11.1% rate of live-born healthy infants in our study seems relatively low. This is because we did not include cases with an unknown fetal karyotype; if we had included these, we would have been able to report even more cases of healthy infants.

Prenatal diagnosis is fundamental to enable parents to make an informed decision about whether to accept the fetal condition or terminate the pregnancy. In our study, 44.4% opted to terminate the pregnancy owing to the poor prognosis, which is in accordance with the literature.⁵ However, the question arises: what would have happened if the decision to terminate a pregnancy was made only on the basis of increased NT? Available data reports a 98% rate of favorable pregnancy outcomes among cases with increased NT but with a normal karyotype and anomaly scan.¹⁷

In our study, we found 5 cases with increased NT and suspected anomalies at the time of FTS (i.e. heart defect, persistent omphalocele/gastrochisis) that did not show any anomalies at the organ scan and finally resulted in live-born healthy infants. All women in our study population had a 0.5–1% risk of spontaneous abortion by undergoing the invasive procedures (as well as, for example, fetal MRIs), which may have made their pregnancy psychologically painful.⁹ Although maternal anxiety will decline after receiving a normal test result, the stress caused by this assessment is enormous for parents.¹⁸ In addition, a false-positive test result can cause anxiety and might ultimately result in a termination and/or rejection of the pregnancy.¹⁹

Our findings lead us to propose a revised algorithm for prenatal diagnosis, involving: (1) the routine analysis of cfDNA for the detection of trisomies 21, 18, and 13; (2) a quick and basic sonogram at between 11 and 14 gestational weeks for the detection of major anomalies, missed abortions, and multiple pregnancies; and (3) an elaborate organ scan between 19 and 22 gestational weeks (Fig. 2). This algorithm would require fewer human resources and less expensive equipment; invasive diagnostics would only be necessary for confirming cfDNA-detected trisomies in the case of major anomalies or in high-risk patients.

In case of a failure of NIPT, we would recommend an early organ scan with optional karyotyping. The known test failure rate is relatively low, ranging between 1.6% for methods based on massive parallel sequencing and 6.4% for tests based on single-nucleotide polymorphism analysis.²⁰ We are aware that the clinical utility and accuracy of our revised algorithm should be proven in prospective studies, but we consider it a thought-provoking proposition. Future studies should evaluate the cost-effectiveness of our proposed algorithm, in particular the replacement of NT measurement by cfDNA in routine prenatal care.

The proposal for a quick, basic sonogram instead of a detailed FTS with NT measurement is supported by Kenhkuis et al.,²¹ who demonstrated that all major anomalies were detected at early gestation, and that those detected at early gestation were more severe than those detected in the second trimester. This seems to be feasible, as the detection rate of fetal anomalies is rising with the increasing quality of ultrasound techniques. Indeed, the major sonographic anomalies that form part of the basic sonogram still need to be established, as clear standards are highly warranted.

A significant drawback of the proposed algorithm is the delay in parental decision-making. Major anomalies that usually result in termination of pregnancy, such as anencephaly, are detectable at early gestation and would therefore be detected by the basic sonogram. Anomalies such as omphaloceles or extremity malformations would not be detected later than during the second trimester organ scan, but it is unlikely that these anomalies would result in pregnancy termination. Identification of increased NT and abnormal FTS might provide earlier information on minor fetal anomalies, but we believe that this short timeframe provides a clinical benefit to neither the patient nor the treating physician.

According to our data there was not a single case where FTS provided a benefit for the detection of congenital heart disease, which
is best diagnosed during the organ scan between 18 and 21 gestational weeks. We found 87 abnormal sonographic findings in 61 of 63 patients, all detected at early gestation. In some of these cases, termination of pregnancy could have been performed earlier when following the conventional algorithm, accepting false-positive results that cause additional work-up and parental anxiety. Nevertheless, earlier diagnosis of chromosomal or structural defects offers more possibilities at an earlier gestational stage, which could be beneficial for the affected families.

Our study is one of a few that challenge the conventional system of prenatal diagnosis. The predominant strength of the study is the large number of first-trimester screenings that were included. However, some of the cases with an unknown fetal karyotype (that were not eligible for the study) would have been of interest. Furthermore, we were unable to provide information on postnatal genetic testing results, or on cytogenetic and molecular genetic diagnosis, or atypical chromosomal abnormalities. Finally, there are no long-term outcomes available for the infants in our study, which might be of interest in the light of intellectual disability and autistic spectrum disorders among euploid infants with increased NT.

In conclusion, cases with increased NT are associated either with fetal trisomies that could be detected by cfDNA or with a major sonographic anomaly that could be detected during an early basic sonogram or the second trimester organ scan. We did not find a single case that had a definitive benefit from FTS with NT measurement, but many cases underwent additional diagnostic work-up. Our findings challenge the additional value of conventional FTS for detection of fetal anomalies. A revised algorithm for prenatal diagnosis could involve the routine use of cfDNA and this could help reduce unnecessary diagnostic procedures and parental anxiety.

AUTHOR CONTRIBUTIONS

AF, IH, and PWH contributed to study conception and design. AF, DB, HK, IH, and JS had contributed to the acquisition, analysis, or interpretation of data. All authors contributed to drafting the paper or revising it critically.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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