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Increased nuchal translucency before 11 weeks of gestation: Reason for referral?

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Abstract

Objectives: In this era of non-invasive-prenatal testing (NIPT), when dating scans are usually performed around 10 weeks of gestation, an increased NT before the official established timeframe (CRL between 45 and 84 mm) may be encountered. Information on management of these pregnancies is limited. Therefore, we evaluated the relationship between an early increased NT and adverse pregnancy outcome. Secondary, we evaluated the rate of chromosomal anomalies that might have been missed in first trimester should solely NIPT be performed as first-tier test, and the rate of adverse pregnancy outcome if NT normalizes before 14 weeks.

Methods: We performed a retrospective cohort study that included all pregnancies between January 1, 2007 and June 1, 2020 in Amsterdam UMC locations AMC and VUmc. We included fetuses with a crown-rump length (CRL) < 45 mm (~11 weeks) and a nuchal translucency (NT) measurement ≥2.5 mm. Fetuses referred with an early increased NT and a major fetal anomaly at the dating scan were excluded, as were cases of parents with a family history of monogenetic disease(s) or recognized carriers of a balanced translocation.

Results: We included 120 fetuses of which 66.7% (80/120) had an adverse pregnancy outcome. Congenital anomalies were present in 56.7% (68/120), 45.8% (55/120) had a chromosomal anomaly. The prevalence of congenital anomalies was 30.3% in fetuses with NT 2.5–3.4 mm compared to 66.7% with NT ≥ 3.5 mm ($p < 0.001$). 16.7% (20/120) had a chromosomal anomaly that might have been missed by conventional NIPT in first trimester. We found an adverse pregnancy outcome of 24% in the group with a normalized NT compared to 78.1% in the group with a persistently increased NT ($p < 0.001$).

Conclusion: An early increased NT should make the sonographer alert. In this selected cohort, an early increased NT was associated with a high probability of having an adverse pregnancy outcome. Regardless of CRL, we deem that an early

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increased NT ≥ 3.5 mm warrants referral to a Fetal Medicine Unit for an extensive work-up. NT normalization seems favorable, but a prospective study should define the appropriate work-up for NT in the lower range (2.5–3.4 mm).

Key points

What's already known about this topic?

- Increased nuchal translucency (NT) is an indisputable marker for chromosomal anomalies and adverse pregnancy outcomes.
- The current age to perform a NT measurement is between 11 and 14 weeks of gestation, corresponding with a crown rump length (CRL) of 45–84 mm.

What does this study add?

- An increased NT with CRL <45 mm, and thus before the official timeframe, could be found when a dating scan is performed around 10 weeks of gestation preceding non-invasive-prenatal testing (NIPT). This early increased NT, with NT ≥ 2.5 mm and CRL <45 mm should make sonographers alert.
- Regardless of CRL, an early increased NT ≥ 3.5 mm warrants referral to a Fetal Medicine Unit for an extensive work-up. In cases with early increased NT 2.5–3.4 mm, the additional value of a systematic follow-up in a Fetal Medicine Unit should be further explored in a prospective study.

1 | INTRODUCTION

Increased nuchal translucency (NT) is an indisputable marker for chromosomal anomalies and adverse pregnancy outcomes. It is associated with an increased chance on miscarriage, congenital heart defects, and numerous other structural defects and genetic syndromes.[1–6] The optimal gestational age to perform NT measurement is between 11 and 14 weeks of gestation, corresponding with a crown rump length (CRL) of 45–84 mm.[2,7] Little is known about the significance of an early increased NT with a CRL <45 mm.

At present, pregnant women in the Netherlands can opt for prenatal screening in the first trimester to test for common aneuploidies, trisomy 21, 18, and 13. They can choose between first-trimester combined testing (FCT) or non-invasive prenatal testing (NIPT) based on cell-free fetal DNA (cfDNA), from 11 weeks onwards. Cases with visible structural anomalies or a NT of ≥3.5 mm (>99th percentile) are not offered NIPT, but referred to a Fetal Medicine Unit for further counseling and offered invasive diagnostics.[8,9] NIPT has revolutionized prenatal screening for common aneuploidies and because of the superior test characteristics of NIPT, the use of FCT has dramatically decreased.[10–12]

Since the majority of pregnant women will undergo a dating scan around 9–10 weeks of gestation preceding NIPT, an early increased NT with CRL <45 mm, may be observed. In 1995, an NT distribution model from 9 to 14 weeks of gestation was constructed with 771 euploid fetuses. At 10 weeks of gestation, NT measurement ≥2.5 mm was only found in 4.6% of euploid fetuses, corresponding with the p95.[13] More recently, reference ranges for NT at 28–44 mm CRL (9 + 4 to 11 + 1 weeks) were established and the 95th percentile ranged from 1.95 to 2.38 mm in a series of 583 chromosomally normal fetuses.[14]

At this moment, counseling expectant parents about an early increased NT is challenging with only scarce information available. Standard practice in the Netherlands is to repeat the NT measurement in the correct timeframe, unless other structural anomalies are suspected. If the NT remains <3.5 mm, NIPT can be offered, otherwise women are referred to a Fetal Medicine Unit. This practice is based on consensus rather than evidence.

Therefore, the aim of this study was to describe the association of an early increased NT (CRL <45 mm and NT measurement ≥2.5 mm) with chromosomal anomalies, structural anomalies, single-gene disorders, perinatal loss, and adverse pregnancy outcome of the above mentioned outcomes. In addition, we evaluated which anomalies could be missed should only NIPT be used as a first-tier screening test and follow-up ultrasound data on NT are not available. Moreover, the outcome of an early increased NT if normalized is studied. The results of this study can be used by sonographers in daily practice to give more specific information to pregnant women about the possible outcomes of an early increased NT.

2 | METHODS

2.1 | Design and participants

We conducted a retrospective cohort study covered by two Fetal Medicine Units in the Amsterdam region: Amsterdam UMC location AMC and VUmc. The study population comprised of pregnant women attending our departments for routine antenatal care (dating scan, prenatal screening) or referred by primary care facilities because of the presence of an early increased NT. An early increased NT was defined

as a NT measurement ≥2.5 mm, with a corresponding CRL <45 mm. This manuscript is reported following Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[15] Following Dutch guidelines, the preferable gestational age to perform a reimbursed dating scan is between 10 and 12 weeks of gestation.[16] If an early increased NT with a CRL <45 mm is visible at the dating scan, the current advice is to perform a second NT measurement in the correct time frame, based on the standards of the Fetal Medicine Foundation (FMF).[2] If the second NT measurement is ≥3.5 mm, patients are referred to a Fetal Medicine Unit for counseling on invasive techniques for detailed chromosome analysis. An NT measurement <3.5 mm allows for prenatal screening (FCT or NIPT), which is voluntary and self-payed. All women are subsequently offered a reimbursed 20-week anomaly scan. In cases with a NT < 3.5 mm, this scan is performed locally in primary care facilities, while cases with a NT > 3.5 mm are scanned in a Fetal Medicine Unit. During the study period, FCT was available since 2007 for trisomy 21, with extended screening for trisomy 13 and trisomy 18 in 2011. The FCT risk cut-off used in the Netherlands is 1:200. NIPT using cfDNA was introduced in 2014, first available for women at high risk (increased risk after FCT or preceding pregnancy with trisomy 21, 18, or 13)[17] and in 2017 for all women. NIPT is offered in two ways: Women can opt for NIPT that solely tests for abnormalities on chromosomes, 21, 18, 13, or for extended NIPT with chromosomal aberrations (~10 MB) on all autosomes.[18] All referred cases with early increased NT were offered a repetitive NT measurement with detailed anomaly scan at 11–14 weeks. Invasive prenatal genetic testing was offered in case of a NT ≥ 3.5 mm (despite CRL), abnormal first trimester screening (increased risk after FCT or abnormal NIPT) or if other structural anomalies were present on ultrasound. In case parents opted for invasive diagnostics immediately, they were not rescanned again between 11 and 14 weeks. All cases (alive) were rescanned at 20 weeks. Comparable to other countries, chorionic villus sampling (CVS) was performed from 11 weeks and amniocentesis from 16 weeks.[19,20] Karyotyping was used before 2011 and Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR) analysis thereafter. From 2012 onwards, chromosome microarray analysis (CMA) was performed if QF-PCR was normal. Whole exome sequencing (WES), the current high standard test, was rarely performed since its introduction for prenatal diagnostics in 2018 and reserved only for cases with normal QF-PCR and array and with persistently increased NT or fetal hydrops, or if anomalies were encountered at the 20 week anomaly scan.

2.2 | Patient selection

We included singleton and multiple pregnancies with a CRL <45 mm and NT measurement ≥2.5 mm in two Fetal Medicine Units, from January 1, 2007, until June 1, 2020. We chose this period, as a nationwide screening program was implemented in the Netherlands from 2007 onwards. In total, 167 fetuses with a CRL <45 mm and a NT ≥ 2.5 mm were identified: 132 at location AMC and 35 at location VUmc. We excluded cases with unknown pregnancy outcome and cases referred because of an early increased NT and major anomalies at the dating scan (anencephaly, holoprosencephaly, omphalocele, multiple congenital anomalies, and conjoined twins). We also excluded cases with a family history of monogenetic disease(s) or recognized carriers of a balanced translocation given the high a priori risk for genetic and chromosomal anomalies.

2.3 | Outcome measures

The primary outcome was adverse pregnancy outcome, defined as chromosomal anomalies, single gene disorders, structural anomalies, or perinatal loss. Perinatal loss was defined as any intrauterine fetal loss and neonatal death until 28 days after birth, including termination of pregnancy. A normal outcome was registered as a live birth with normal physical examination after birth. We evaluated the rate of chromosomal anomalies that might not have been detected if only conventional NIPT was performed and cases were not referred to a Fetal Medicine Unit. In addition, we examined normalization of NT by repetitive measurements in the correct time frame according to the standards of the Fetal Medicine Foundation (FMF).[2] An NT <3.5 mm (<p99) was considered normalized and an NT ≥ 3.5 mm (≥p99) was considered as persistently increased.

2.4 | Data collection

The local obstetric databases and electronic patient records were evaluated to collect information on maternal and fetal characteristics; maternal age, obstetric history, smoking, alcohol use, positive family history of congenital anomalies, multiple pregnancy, gestational age of dating scan, performance of prenatal screening (FCT or NIPT), NT measurements, and structural anomalies on ultrasound. Data on pregnancy and neonatal outcome, such as gestational age at birth, presence of dysmorphic features postnatally and mortality were retrieved from electronic patient records. Data on prenatal and postnatal genetic testing were extracted from clinical genetic reports. We consulted a clinical geneticist to discuss which chromosomal aberrations (size resolution Mb) would be identified by Dutch laboratories based on current test methods.

2.5 | Data analysis

Continuous data were described as mean with standard deviation (SD) or median with interquartile rage (IQR) when appropriate. Categorical data were expressed as number with percentages. A chi-square test was used to test associations between categorical variables. For dichotomous outcomes, differences between the groups were tested using the *z*-test for the difference in two (independent) proportions. Confidence intervals were also calculated. We considered *p*-values <0.05 as statistically significant. Statistical analyses were done using IBM Corp SPSS Statistics version 25.0 (IBM) and RStudio version 1.2.1335.[21]

2.6 | Ethics

The Medical Ethics Committee of the Amsterdam UMC deemed that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study (W21_026) and official approval was not required.

3 | RESULTS

3.1 | Population characteristics

During the study period, 167 fetuses with a CRL <45 mm and an NT ≥ 2.5 mm were identified. We excluded 47 cases: Two cases because of missing data, 23 cases with major congenital anomalies at referral and 22 cases with a family history of monogenetic disease(s) or recognized balanced translocation We included 120 fetuses with a CRL <45 mm and a NT ≥ 2.5 mm in the final analysis Table 1 presents baseline characteristics of the study cohort. Mean maternal age at time of NT measurement was 33.3 years (SD, 5.2) (Table 1). Median CRL was 41.7 mm (range 26.3–44.9 mm) and median NT was 4.3 mm (range 2.5–11.2 mm) (Table 1).

TABLE 1 Baseline characteristics of 120 cases

Parameter	
Maternal characteristics	
Age (years)	33.3 (±5.2)
Weight (kg)	62.5 (58–70.0)
BMI	22.0 (20.3–24.5)
Smoking, *n* (%)	4 (3.1%)
First pregnancy, *n* (%)	49 (40.8%)
Singleton gestation, *n* (%)	111 (92.5%)
Crown-rump length (mm)	41.7 (26.3–44.9)
Nuchal translucency thickness (mm)	4.3 (2.5–11.2)

Note: Data are given as *n* (%), mean (± standard deviation) or median (range).

3.2 | Adverse pregnancy outcome

Figure 1 shows a flowchart of the outcomes. Invasive diagnostics was performed in 79.2% (95/120), 57.9% (55/95) had a chromosome aberration, and 42.1% (40/95) had a normal chromosome pattern. In 21% (25/120) invasive diagnostics was not performed, of which 7.5% (n = 9) resulted in early perinatal loss. Five resulted in early intrauterine fetal death (IUFD) and four in termination of pregnancy. A normal chromosome pattern was found in three of the 12 fetuses with perinatal loss, 2 resulted in intrauterine fetal death (IUFD) (NT 2.6 and 4.8 mm) and 1 in termination of pregnancy (NT 3.9 mm). In total, we found a normal outcome in 33.3% (40/120) and an adverse pregnancy outcome in 66.7% (80/120) (Figure 1).

3.3 | Congenital anomalies

Table 2 presents congenital anomalies in relation to the degree of NT enlargement. Congenital anomalies occurred in 56.7% (68/120). The prevalence of congenital anomalies increased proportionally to

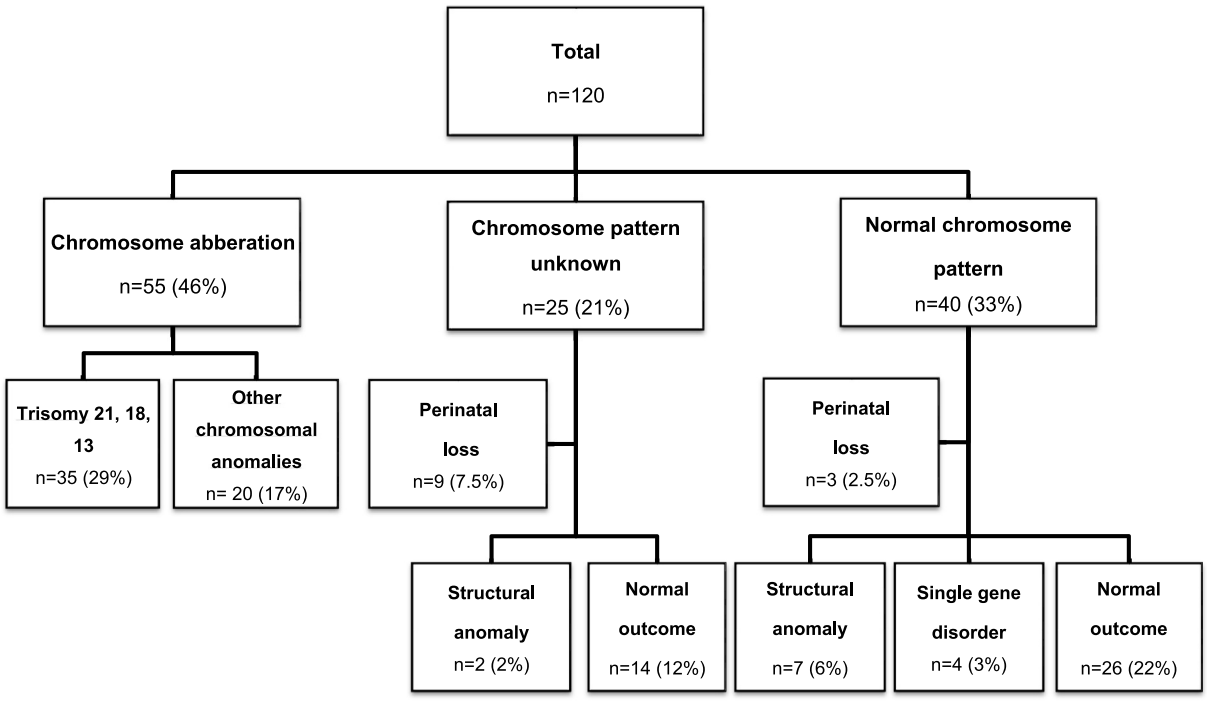

FIGURE 1 Normal outcome and adverse pregnancy outcome of 120 cases

the degree of NT enlargement, ranging from 30.3% (10/33) in cases with NT 2.5–.4 mm, to 66.7% (58/87) in cases with NT ≥ 3.5 mm (difference: 36.4, 95% CI 17.8–54.9, $p < 0.001$). An abnormal genetic outcome was present in 49.1% ($n = 59$), 45.8% ($n = 55$) with a chromosomal anomaly and 3.3% ($n = 4$), with a single gene disorder (not previously known to the parents).

3.3.1 | Chromosomal anomalies and small structural chromosomal aberrations

Chromosomal anomalies were present in 45.8% (55/120), with increasing prevalence to 56.3% (49/87) for fetuses with NT ≥ 3.5 mm. The majority, 63.6% (35/55), were diagnosed with trisomy 21, 18, or 13. Trisomy 18 was the most common chromosomal anomaly ($n = 24$, 43.6%) (Table 3). Frequent other chromosomal anomalies identified were monosomy X ($n = 11$) and triploidy ($n = 5$). Structural chromosomal aberrations were identified in 7.2% (4/55): double duplication ($n = 1$), unbalanced submicroscopic translocation ($n = 1$) and submicroscopic microdeletion ($n = 2$) (Table 3).

3.3.2 | Single-gene disorders

Single-gene disorders were diagnosed in 3.3% (4/120). These included medium-chain acyl-CoA dehydrogenase deficiency (MCAD) and three RASopathies including Noonan syndrome (RIT1 gene), cardio-facial-cutaneous (CFC) syndrome (MAP2K1 gene), and one case with another heterozygous DNA variant in the KRAS gene.

3.3.3 | Isolated fetal structural anomalies

We found isolated fetal structural anomalies on ultrasound in 7.5% (9/120). 33.3% ($n = 3$) were cardiac anomalies, including pulmonary atresia ($n = 1$), hypoplastic left heart syndrome ($n = 1$), and Tetralogy of Fallot ($n = 1$). One case presented with congenital diaphragmatic hernia ($n = 1$, 10%). The remaining five fetuses had multiple congenital anomalies (50%), four with a normal chromosome pattern (80%), and one with unknown chromosome pattern (20%). Of the structural anomalies, 66.7% (6/9) were detected before 14 weeks of gestation, 33.3% (3/9) after 18 weeks of gestation with targeted follow-up ultrasound.

3.4 | Cases potentially missed by conventional NIPT

In total, 16.7% (20/120) of cases had a chromosomal anomaly that would have remained undetected if only NIPT was performed in first trimester (Table 2). These comprised 36.3% (20/55) of the total number of chromosomal anomalies found in this cohort (Table 2). The chromosomal anomalies that would probably remain undetected in first trimester included monosomy X ($n = 11$), triploidy ($n = 5$) and small structural chromosomal aberrations ($n = 4$). Consequently, all single gene disorders ($n = 4$) in this cohort would not have been detected by NIPT, neither would the structural anomalies ($n = 10$) (Table 2).

In the subgroup with NT 2.5–3.4 mm, 12.1% (4/33) had a chromosomal anomaly that would have remained undetected by NIPT alone (Table 2). These comprised 66.7% (4/6) of all chromosomal anomalies found in this group. Potentially missed chromosomal anomalies included one case of triploidy, unbalanced submicroscopic translocation (2; 8), double duplication (6; 15) and a microdeletion (22q11). Consequently, the two single gene disorders in this group, MCAD deficiency and CFC syndrome, would remain undetected. Scan findings of the chromosomal anomalies that would not have been dectected by NIPT and with a NT between 2.5 and 3.4 mm are listed below. Triploidy presented with a cystic placenta partial mole on ultrasound at 11-14 weeks. No structural anomalies were seen in the case with the microdeletion (22q11). An absent nasal bone and reversed a-wave in ductus venosus were seen in the case with the double duplication (6; 15) at 11–14 weeks. The case with an unbalanced submicroscopic translocation presented with a SUA, fetal tachycardia and an omphalocele at 11–14 weeks.

TABLE 2 Degree of early NT enlargement and relationship with the prevalence of congenital anomalies

		Adverse pregnancy outcome						
		Congenital anomalies						
Early NT (mm)	Total *n*	Trisomy 21/18/13	Other chromosomal anomalies	Single gene disorders	Structural anomalies	Total	Perinatal loss	Live birth, no defects
2.5–3.4	33	2 (6.1)	4 (12.1)	2 (6.1)	2 (6.1)	**10 (30.3)**	4 (12.1)	18 (54.5)
3.5–4.4	34	13 (38.2)	4 (11.7)	0 (0.0)	0 (0.0)	**17 (50.0)**	4 (11.8)	13 (38.2)
4.5–5.4	24	8 (33.3)	5 (20.8)	0 (0.0)	3 (12.5)	**16 (66.7)**	2 (8.3)	6 (25.0)
≥5.5	29	12 (41.3)	7 (24.1)	2 (6.9)	4 (13.7)	**25 (86.2)**	2 (6.9)	2 (6.9)
Total	120	35 (29.1)	20 (16.7)	4 (3.3)	9 (7.5)	**68 (56.7)**	12 (10.0)	39 (32.5)

Note: Data are given as *n* (%).

3.5 | Subgroup analysis: second NT measurement in correct time frame

A subgroup analysis was performed in cases with a second NT measurement ($n = 57$), either normalized NT (<p99) or persistently increased NT (≥p99) (Table 4). Median NT was 3.7 mm (IQR, 3.1–5 mm) with an NT range from 2.5 to 10 mm. A normalized NT was found in 43.9% (25/57) and 56.1% (32/57) had a persistently increased NT. Among fetuses with NT in the lower range (2.5–4.5 mm), normalization of NT was common (63%, 24/38), while in the higher range (>4.5 mm) it was rarely observed (5%, 1/19) (Table 4). The incidence of an adverse pregnancy outcome was 24% (6/25) in the group with a normalized NT compared to 78.1% (25/32) in the group with a persistently increased NT (difference: 54.1, 95% CI 28.5–79.7, $p < 0.001$). Four cases with adverse pregnancy outcome and normalized NT occurred in the group with an early increased NT 2.5–3.4 mm and two cases in the group with early increased NT 3.5–4.4 mm (Table 4). Two cases resulted in neonatal death (trisomy 13 mosaicism and congenital diaphragmatic hernia), two were live born (22q11 deletion and one with dysmorphic features and congenital anomalies, but no definitive genetic diagnosis), and in two cases, the pregnancy was terminated (one case with pulmonary atresia and one case with multiple congenital anomalies).

TABLE 3 Chromosomal and structural chromosomal aberrations

Chromosomal anomalies	Total $n = 55$
Trisomy 18	24 (43.6)
Monosomy X	11 (20.0)
Trisomy 21	7 (12.7)
Triploidy	5 (9.1)
Trisomy 13[a]	4 (7.3)
Double duplication • 6q25.3q27 duplication (11.2 Mb) and 15q11.1q15.3 duplication (24.6 Mb)	1 (1.8)
Unbalanced submicroscopic translocation • 2p25.3 duplication (4.1 Mb) and 8p23.3p23.1 deletion (6.2 Mb)	1 (1.8)
Submicroscopic microdeletion • 22q11.21 deletion (2.8 Mb) • 2p16.1 (1 Mb)	2 (3.7)

Note: Data are given as *n* (%).

[a]Three full Trisomy 13 and one Trisomy 13 mosaicism.

4 | DISCUSSION

4.1 | Main findings

We demonstrated that an early increased NT is associated with a high probability of having an abnormal outcome. The incidence of congenital anomalies rises significantly with increasing NT: 30.3% for NT 2.5–3.4 mm to 66.7% for NT ≥ 3.5 mm. If only conventional NIPT was used as a first-tier test, more than one-third of the chromosomal anomalies in our cohort would not have been detected. Besides, normalization at the established time frame carries a lower risk of an adverse pregnancy outcome, although this does not apply for the lower range NT 2.5–3.4 mm. Therefore, sonographers should be alert if they encounter an early increased NT and refer cases with NT ≥ 3.5 mm to a Fetal Medicine Unit for an extensive work-up

TABLE 4 Analysis of early NT measurement and relation between normalized (<p99) or persistently increased NT (≥p99) at second NT measurement and outcome

Parameter	Normal outcome (%)	Adverse pregnancy outcome (%)	Total (*n*)	*p*-Value
Early NT 2.5–3.4 mm			19	
Second NT < p99	8 (66.7)	4 (33.3)	12	1.000
Second NT ≥ p99	4 (57.1)	3 (42.9)	7	
Early NT 3.5–4.4 mm			19	
Second NT < p99	10 (83.3)	2 (16.7)	12	<0.001
Second NT ≥ p99	0 (0.0)	7 (100.0)	7	
Early NT 4.5–5.4 mm			11	
Second NT < p99	1 (100.0)	0 (0.0)	1	0.1
Second NT ≥ p99	1 (10.0)	9 (90.0)	10	
Early NT ≥ 5.5 mm			8	
Second NT < p99	0 (0.0)	0 (0.0)	0	NA
Second NT ≥ p99	2 (25.0)	6 (75.0)	8	
Total	26 (45.6)	31 (54.4)	57 (100.0)	

Note: Data are given as *n* (%). Normal outcome = live birth, no defects.

including a scan at 11–14 weeks and should be counseled about the limitations of NIPT.

4.2 | Strengths and limitations

We have retrieved a broad range of data concerning fetuses with an early increased NT in the Amsterdam region, due to experience sonographers. Although our results involved a limited number of cases, the collected data is highly reliable since information came from two Fetal Medicine Units. Besides its clinical relevance, this is the first study providing data on adverse pregnancy outcome in fetuses with an early increased NT. Where other studies solely focused on the relationship with chromosomal anomalies,[13,14] we report on congenital anomalies including structural anomalies and the presence of structural chromosomal aberrations. The current model for NT measurement is only validated for CRL between 45 and 84 mm.[2] With the introduction of NIPT, the unexpected finding of an enlarged NT prior to the correct time frame poses a clinical dilemma. This study provides information for sonographers and clinicians in daily practice when an early increased NT is encountered. Most importantly, with these findings we are able to create awareness and alertness in primary care facilities.

Our study has several limitations. It was subjected to referral bias since data came from highly specialized Fetal Medicine Units, and therefore, this population of patients had a high a priori risk for adverse outcomes. It is very likely that the women referred with an early increased NT were the more serious cases giving the distribution between the groups. Only 20% of cases had an early NT 2.5–3.4 mm compared to 80% with an early NT ≥ 3.5 mm. Based on literature and the currently used NT distribution model, expected percentages in normal population are 4% for NT < 3.5 mm (p95) compared to 1% for NT ≥ 3.5 mm (p99).[13,21] Presumably, the majority of cases with NT < 3.5 mm were not included in our study because NT is not routinely measured. Sonographers tend to only measure NT when it seems to be substantially enlarged. In addition, clinicians may not refer for further assessment if parents opt for NIPT if they are not aware of the general increased risk of poor outcome. However, it should be the largest and it is certainly the most important group, giving the cut-off for NIPT and the remaining chance for adverse pregnancy outcome if NT normalizes. An overestimation of the prevalence of congenital anomalies and adverse pregnancy outcome is inevitable. We tried to minimize this effect by excluding fetuses with major congenital anomalies at referral and excluding cases with a family history of monogenetic disease(s) or known carriers of structural chromosomal aberrations.

Another limitation is the retrospective design of the study. Despite the current advice to perform a second NT measurement in the correct time frame with a CRL >45 mm, in clinical practice referral of an early increased NT is rather seen than performance of a second NT measurement. Therefore, less than half of the fetuses included in our cohort had a second NT measurement. Another explanation could be that parents decided they did not want to wait for the second NT measurement and wished to be referred directly to a Fetal Medicine Unit. Likely, some fetuses with an early increased NT resulted in fetal loss in first trimester. Although this leads to numbers with conservative estimates, we expect that we can extrapolate our findings to an unselected population, since disappearance of an increased NT is studied before and seems to be a favorable prognostic sign.[22]

4.3 | Interpretation

The incidence of congenital anomalies in fetuses with CRL <45 mm has only been studied by a few groups. A large prospective cohort demonstrated that NT is a useful marker for the early detection of aneuploidies in fetuses with CRL 28–44 mm.[14] The majority of other published studies solely report on fetuses with CRL from 45 mm up to 84 mm. By comparing these studies to our results, we came across a number of differences. The rate of chromosomal anomalies was higher in this study (45.8%) compared to those studies (19.2%–35%).[3–5,23–25] In cases with NT > 3.5 mm we also reported higher percentages of chromosomal anomalies: 59.7% in cases with NT > 3.5 mm compared to 38%–48.1% in fetuses with a CRL up to 84 mm.[23,26,27] A possible explanation is the selection bias in our study, leading to an overestimation of the actual incidence. Nevertheless, the percentage of chromosomal anomalies in cases with NT 2.5–3.4 mm (18%) is in accordance with a recent study (23%).[23]

In literature, the prevalence of single gene disorders ranges between 2% and 5%[5,23] with Noonan syndrome as the most frequently described RASopathy related to increased nuchal translucency.[28] Since single gene testing was not performed systematically, the incidence of single gene disorders in our study is not comparable to other studies. Due to differences in cut-off for increased NT, large differences are reported between studies in prevalence of structural anomalies, ranging from 3% to 50%.[29] Our observed incidence of isolated structural anomalies (7.5%) was comparable to the incidence of a recently published large retrospective cohort (9.3%)[23]

Comparable to a large retrospective cohort of fetuses with NT > p95, potentially missed chromosomal anomalies by NIPT were small structural chromosomal aberrations (<10 Mb), sex chromosome aberrations, triploidy and single gene disorders.[23,27] If a structured first trimester anomaly scan between 11^{+0} and 13^{+6} weeks of gestation, (preferably at 12–13 weeks of gestation) was added to NIPT as first trimester screening, we hypothesize that less cases would remain undetected. Monosomy X is mostly presented with a very large nuchal fluid accumulation on ultrasound and triploidy of paternal origin mainly presents with increased NT and placental molar changes in first trimester.[30]

Despite the importance of a first trimester anomaly scan, less women undergo such a scan with the increasing number of women choosing NIPT over FCT.[18] This may result in a delay of diagnosis of structural anomalies, which will be detected no earlier than at the second trimester anomaly. An avoidable event, giving the high detection rates of structural anomalies in the first trimester ranging

from 32% in low-risk groups to 60% in high-risk groups.[31] More than half of the cases in our study with an isolated structural anomaly would not have been detected in first trimester, if they were not referred to a Fetal Medicine Unit.

We retrospectively viewed, with consultation of a clinical geneticist and the Dutch laboratory, which structural chromosomal aberrations would not have been detected by conventional NIPT. Likely, some structural chromosomal aberrations between 10 and 20 Mb and/or sex chromosome abnormalities would have been exposed by NIPT in other countries, giving the wide variation in detecting genetic anomalies with NIPT.[32] The proportion of detected genetic anomalies will likely increase in the future due to further development of NIPT for these conditions.[33–35] Recent literature showed a distinctive positive predictive value of NIPT for sex chromosome abnormalities.[36] Although NIPT has allowed accurate prenatal diagnosis of aneuploidy and other chromosomal anomalies, structural anomalies remain undiagnosed without ultrasound, making sonographic screening in first and second trimester crucial.

Decrease and normalization of an enlarged NT is not uncommon.[22,37] The present study indicates that fetuses with a normalized NT thickness have a three times lower chance on having an adverse pregnancy outcome than those with persistently increased NT. We hypothesized that the magnitude of early NT enlargement was inversely correlated with the chance on normalization, a lower first NT measurement would have a higher chance on normalization. However, in the subgroup of fetuses with an NT between 2.5 and 3.4 mm, an adverse pregnancy outcome would still be present in 37% of cases, with no significant changes when it is confirmed (33% vs. 43%). The highest chance on normalization and normal outcome was among cases with an early NT 3.5–4.4 mm, most likely because of the limited number of cases with a second NT measurement.

Counseling patients with an early increased NT before 11 weeks of gestation can be challenging, given the uncertainty about the clinical implications of this sonographic finding. This study creates awareness in case of an early increased NT and shows a high chance of having an adverse pregnancy outcome. However, we are aware that our study is subjected to selection bias, due to our small number of cases, and the retrospective design of the study with the lack of a denominator. To identify the correct prevalence of an increased NT before 11 weeks and to confirm our findings before we change practice and causing unnecessary anxiety to expectant parents, a prospective study should be conducted. We propose a prospective study in an unselected population that includes all fetuses with a NT ≥ 2.5 mm and CRL <45 mm and performance of a second NT measurement with first trimester anomaly scan when CRL measures >45 mm. The incidence of congenital anomalies in the normalized NT group should be compared with the incidence in the persistently increased NT group. This unselected prospective cohort should define the correct early increased NT cut-off value to find out if an early increased NT < 3.5 mm and CRL <45 mm is an indication for referral to a Fetal Medicine Unit for a detailed anomaly scan between 11 and 14 weeks. Until then, an early increased NT ≥ 3.5 mm with CRL <45 mm should receive the same work-up as an increased NT with CRL >45 mm, since the frequency of congenital anomalies is high, irrespectively of subsequent confirmation. Therefore, regardless of CRL, al fetuses with an early increased NT > 3.5 mm should be referred to a Fetal Medicine Unit for an extensive work-up. They should receive a scan at 11–14 weeks by a trained specialist and should be counseled about the limitations of NIPT.

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Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ETHICS STATEMENT

The Medical Ethics Committee of the Amsterdam UMC deemed that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study (W21_026) and official approval was not required.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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