Estimation of Detection Rates of Aneuploidy in High-Risk Pregnancy Using an Approach Based on Nuchal Translucency and Non-Invasive Prenatal Testing: A Cohort Study

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Key Words

Non-invasive prenatal testing · Karyotype ·
Nuchal translucency · Prenatal diagnosis · Chorionic villus
sampling · Pregnancy

Abstract

Objectives: The aim was to investigate aneuploidy detection using an approach based on nuchal translucency (NT) and non-invasive prenatal testing (NIPT). **Methods:** This was a cohort study including 5,306 high-risk pregnancies with NT measurements and chorionic villus samples (CVS) tested for full karyotype. **Results:** The fetal karyotype was normal in 4,172 (78.6%) cases and abnormal in 1,134 (21.4%), including 1,009 with a likely clinically significant adverse outcome. Universal CVS with full karyotyping would lead to the diagnosis of all clinically significant abnormalities. A policy of relying solely on NIPT would have led to the diagnosis of 88.9% of clinically significant abnormalities. A strategy whereby NIPT is the main method, with CVS reserved for cases with NT ≥ 3.0 mm, would require CVS in 21.7% of cases, identify 94.8% of significant abnormalities and avoid miscarriage in 41 pregnancies compared to CVS for all. **Conclusions:** A policy of NIPT for increased-risk cases and CVS with full karyotype if the NT was ≥ 3.0 mm reduced the risk of miscarriage yet still identified 95% of clinically significant aneuploidy.

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Introduction

Non-invasive prenatal diagnosis using cell-free fetal DNA in the maternal plasma to determine fetal sex has become a part of routine care in pregnancies at risk of sex-linked disorders [1–3]. Non-invasive prenatal testing (NIPT), using directed cfDNA analysis methods, provides a risk assessment for fetal trisomies 21, 18 and 13 [4]. The test requires that the fraction of fetal DNA in the plasma be greater than 4%, which is influenced by maternal and fetal characteristics [5]. There is also an assay failure rate of 1–10%, which increases with the maternal body mass index [5]. NIPT for aneuploidy using cell-free DNA in maternal plasma is increasingly used in clinical practice [6, 7]. Several studies have reported promising results and some have also included sex chromosome aneuploidy analysis [4, 8–29]. Some have reported correct detection of all trisomy 21 fetuses and 98% of trisomy 18 cases, with all euploid fetuses correctly identified and a very low (1%) assay failure rate [8, 10]. The use of NIPT as a universal screening tool is unlikely to be cost effective, but its contingent use in women found by conventional screening to be at moderate or high risk has been predicted to be cost effective [7, 30]. Whether NIPT can replace prenatal invasive testing or whether its use should be limited to selected cases has not been fully elucidated.

Increased fetal nuchal translucency (NT) thickness is associated with many chromosomal abnormalities and genetic syndromes [31–34]. Previous studies have demonstrated that the prevalence of chromosomal defects increases with NT thickness [35]. In a study of over 10,000 pregnancies, the prevalence of aneuploidy increased from approximately 7% in fetuses with NT between the 95th and 99th centiles for crown-rump length to 75% for cases with an NT of 8.5 mm or more [35]. However, half of fetuses with aneuploidy were affected by chromosomal abnormalities other than trisomy 21 [35]. Therefore, first-trimester screening followed by prenatal invasive testing and full karyotyping allows the detection of a broad range of additional chromosomal abnormalities, many of which may not yet be detectable by NIPT [35, 36]. Furthermore, the use of microarrays following invasive testing can detect microdeletion syndromes and pathogenic copy number variants associated with a significant risk of physical or mental disability and this is becoming a popular approach [37–39]. In a study of more than 4,000 pregnancies with samples with a normal karyotype, microarray analysis revealed clinically relevant deletions or duplications in 6.0% of cases with a structural anomaly and in 1.7% of those whose indications were advanced maternal age or positive screening results [40]. The aim of this study was to investigate the detection rates of clinically significant aneuploidy using an approach based on NT and NIPT.

Methods

This was a retrospective cohort study including 5,306 high-risk pregnancies from which chorionic villus samples (CVS) had been taken and tested for full karyotype after the fetal NT thickness was measured at 11 + 0 to 13 + 6 weeks of gestation. Cases were identified by searching the genetic laboratory database and the ViewPoint database (ViewPoint 5.6.8.428; ViewPoint Bildverarbeitung GmbH, Wessling, Germany) at St George's Hospital from January 1997 to January 2013. Data collected included: gestational age at CVS, NT measurement, structural malformations, full karyotype and any further investigations, including amniocentesis, detailed ultrasound, disomy studies, parental karyotyping and pregnancy outcome. The indications for CVS included first-trimester high-risk combined screening results, family history, advanced maternal age, increased NT thickness and fetal structural abnormalities. The karyotypes obtained from cultured chorionic villi were classified as normal or abnormal. All abnormal karyotypes were reviewed by a clinical geneticist and grouped according to whether or not the chromosome anomaly might be clinically significant. Cases with clinically significant chromosomal abnormalities were categorized further into those that would be detected using NIPT and those that would not. Currently NIPT can detect fetuses with trisomy 21, 13 or 18 and sex aneuploidy [5–12]. In a recent study,

Nicolaides et al. [41] reported that cfDNA testing by targeted sequencing and allelic ratio analysis of single-nucleotide polymorphisms covering chromosomes 21, 18, 13, X and Y can detect diandric triploidy and raise the suspicion of digynic triploidy. Therefore, we assumed that NIPT is likely to detect, or at least suspect, triploidy and tetraploidy cases as well.

We then examined the distribution of NT thickness in each group and estimated the potential impact, in terms of prenatal diagnosis of clinically significant chromosomal defects, of 3 policies. The first policy reflects the current practice, where all of these cases have a CVS. A second policy would rely entirely on NIPT to replace CVS for all of these high-risk cases. The third policy uses NIPT as the main method of prenatal diagnosis, with CVS followed by full karyotyping reserved for cases with increased NT thickness and a family history of chromosomal abnormalities. We studied the potential impact on the overall miscarriage risk and the cost implications. Assuming a risk of miscarriage associated with CVS of 1%, we calculated the number of miscarriages potentially avoided if NIPT replaced CVS [42–44].

Results

CVS followed by aneuploidy screening (quantitative fluorescent polymerase chain reaction, qf-PCR) and full karyotype was performed in 5,306 cases. The karyotype was normal in 4,172 (78.6%) cases and abnormal in 1,134 (21.4%). The indications of CVS were classified into 5 categories: high-risk screening including those with increased NT with or without biochemistry (44.1%), structural anomalies (3.5%), advanced maternal age or anxiety (36.1%) and family history (16.3%). We divided the cases with an abnormal karyotype into 1,009 with a high risk of a clinically significant adverse outcome and 125 with no risk or a very low risk of an adverse outcome. Therefore, in total, we had 4,297 cases at no or low risk and 1,009 at high risk for a clinically significant adverse outcome (fig. 1). We further subdivided the group at high risk for a clinically significant adverse outcome into those who would be ($n = 897$; 88.9%) and those who would not be ($n = 112$; 11.1%) detected using NIPT. The details of these cases are shown in figure 1.

Table 1 shows the distribution of fetal karyotypes according to fetal NT thickness. A policy of relying entirely on NIPT would lead to the diagnosis of 88.9% of chromosome abnormalities. An alternative strategy whereby NIPT is the main method of analysis for a high first-trimester risk but with fetal NT <3 mm, and CVS with full karyotyping is reserved only for those cases with a minimum fetal NT thickness of 3.0 mm, would require CVS in 1,154 (21.7%) of cases and would identify 957 (94.8%) of the significant abnormalities. In this case, the residual risk of an unidentified clinically sig-

Table 1. Implications of proposed policies in prenatal diagnosis to include CVS only, NIPT only and NIPT with CVS confirmation

Policy of intervention	Pregnancies at high risk for an adverse outcome		Residual risk of an adverse outcome, %	CVS, n ^a	Procedure-related miscarriages, n
	detectable	not detectable			
CVS + full karyotype	1,009 (100)	0	0	5,306	53
NIPT in all cases	897 (88.9)	112 (11.1)	2.5	106	1
CVS (full karyotype) for all above the NT threshold + NIPT for the rest					
≥5.5 mm	922 (91.4)	87 (8.6)	2.1	535 (10.1)	5
≥4.5 mm	927 (91.9)	82 (8.1)	1.9	620 (11.7)	6
≥4.0 mm	938 (93.0)	71 (7.0)	1.6	737 (13.9)	7
≥3.5 mm	943 (93.5)	66 (6.5)	1.5	867 (16.3)	9
≥3.0 mm	957 (94.8)	52 (5.2)	0.98	1,154 (21.7)	12
≥2.5 mm	963 (95.4)	46 (4.6)	1.1	1,874 (35.3)	19

Values are given as numbers (%) unless otherwise stated. ^a Without confirming the abnormal NIPT cases.

Fig. 1. Fetal karyotype by CVS in the study population showing the likelihood of an adverse postnatal outcome and detection by NIPT, assuming a 0% failure rate for NIPT.

Table 2. Fetal aneuploidy of clinical significance not identified by a policy whereby NIPT is the main method of analysis and CVS followed by full karyotyping is reserved for those cases with a minimum fetal NT thickness of 3.0 mm

Final karyotype after CVS and further investigation	Total (n = 52), n (%)
Mosaic triploidy, tetraploidy	3 (5.8)
Mosaic trisomies 13, 18 and 21	10 (19.2)
Mosaic sex aneuploidies	3 (5.8)
Trisomies 2, 8, 9, 15, 16, 20 and 22	5 (9.6)
Unbalanced rearrangements	17 (32.7)
Mosaic unbalanced rearrangements	2 (3.8)
Mosaic trisomies 2, 7, 8, 10, 15, 20 and 22	7 (13.5)
Mosaic marker chromosomes (de novo)	5 (9.6)

nificant karyotype abnormality is 0.98%. The latter policy would avoid miscarriage in 41 (78.3%) pregnancies compared to the current practice of CVS for all. The chromosomal abnormalities of clinical significance that would not have been identified (n = 52; 5.1%) are listed in table 2. The largest group was unbalanced rearrangements (n = 17; 32.7%). In 10 cases there were additional abnormalities that were detected prenatally, which would have triggered further testing. The details of these cases are shown in table 3. A similar strategy with fetal NT <3.5 mm instead would require CVS in 867 (16.3%) cases and would identify 943 (93.5%) of the significant abnormalities. In this case the residual risk of an unidentified clinically significant karyotype abnormality is 1.5%. The latter policy would avoid miscarriage in 44 (83.0%) pregnancies compared to the current practice of CVS for all.

When a policy of CVS with full karyotyping reserved only for those cases with a minimum fetal NT thickness of 3.0 mm, with NIPT for the remaining cases, is compared to the current policy in the UK National Health Service (NHS; CVS followed by full karyotyping for cases with a fetal NT thickness greater than 3.0 mm, while the rest of the cases have qf-PCR), 94.8 versus 96.4% of the significant abnormalities are likely to be detected ($p = 0.102$), while 12 pregnancies (vs. 53, $p < 0.001$) would be expected to end in miscarriage. A similar number of pregnancies will have CVS followed by karyotype (21.7 vs. 20.1%, $p = 0.045$), without confirming the abnormal NIPT cases (table 4).

Discussion

Main Findings

We have examined different testing strategies for screen-positive women after first-trimester risk assessment. The findings of this study suggest that a policy of NIPT for first-trimester risk screen-positive women with CVS and karyotyping reserved for those with a minimum fetal NT thickness of 3.0 mm would require invasive testing in 22% of cases, identify 95% of clinically significant abnormalities and avoid the risk of miscarriage in 77% of pregnancies compared to a policy of CVS for all. The main advantage of this policy compared to the current policy in the UK is a potential reduction in the number of CVS-related miscarriages. This does not seem to be associated with a decrease in the detection of clinically significant chromosomal abnormalities.

Strengths and Limitations

The strengths of this study include the large number of cases and the ascertainment of the clinical significance of abnormal karyotypes, especially those that might not be detected by NIPT. An attempt to investigate the implications, including the miscarriage risk and the cost, is likely to be important especially in countries where there is a national healthcare system. The limitations of this study include its retrospective design and the fact that it focuses on a high-risk population. The latter issue has been a common limitation in most studies investigating NIPT so far. This study was of all women having CVS for different reasons, reflecting the nature of cases in a tertiary referral centre and not only those at high risk resulting from the routine screening programme. The constitution of this group might limit the generalizability of the findings. We did not include data on the results of the first-trimester screening using the combined test as routine implementation of first trimester serum biochemistry, as part of the combined test, started in 2007. The high chromosomal rate found in our study might skew the analysis. Therefore, we would recommend a similar analysis in a different population to replicate our findings. Furthermore, we assumed that NIPT would detect all cases with trisomy 21, 18, 13, sex aneuploidy, triploidy and tetraploidy, which is unlikely as the detection rate is not 100%. However, as many of these cases, in particular trisomy 18, 13, triploidy and tetraploidy, have structural abnormalities that are easily detected using ultrasound, most of these cases are likely to undergo invasive testing or termination of the pregnancy.

Table 3. Fetuses with a clinically significant abnormal karyotype, an NT thickness <3.0 mm and structural abnormalities (n = 10)

Fetal karyotype	Fetal NT thickness, mm	Fetal abnormalities
46,XX,rec(5)dup(5q)inv(5)(p15q35)mat	1	small cerebellum
46,XY,add(8)(q24.3)[4]/46,XY[10]	1.3	bilateral talipes
47,XY,+6	1.3	Ebstein's anomaly
47,XX,+18[28]/46,XX[6]	1.4	exomphalos
46,XX,t(5;9)(q?14;q34),del(13)(q22)[5]/46,XX[30]	1.6	severe fetal growth restriction
mos 46,XY[24]/48,XY,+7,+15[6]	1.6	severe fetal growth restriction
45,X[22]/46,XX[8]	1.8	hydronephrosis
92,XXYY/46,XY	2.1	bilateral talipes
47,XX,+16	2.3	exomphalos
46,XX,der(13)t(13;18)(q32;q23)	2.6	holoprosencephaly

Table 4. Implications of proposed policies in prenatal diagnosis to include CVS with qf-PCR only or CVS with full karyotype

Policy of intervention	Pregnancies at high risk for an adverse outcome		Residual risk of an adverse outcome, %	CVS, n	Procedure-related miscarriages, n	CVS + full karyotype, n
	detectable	not detectable				
CVS + full karyotype	1,009 (100)	0	0	5,306	53	5,306
CVS + qfPCR in all cases	934 (92.6)	75 (7.4)	1.7	5,306	53	0
CVS (full karyotype) for all above the NT threshold + qf-PCR for the rest						
≥4.0 mm	958 (94.9)	51 (5.1)	1.2	5,306	53	643 (12.1)
≥3.5 mm	962 (95.3)	47 (4.7)	1.1	5,306	53	776 (14.6)
≥3.0 mm ^a	973 (96.4)	36 (3.6)	0.8	5,306	53	1,069 (20.1)
≥2.5 mm	979 (97.0)	30 (3.0)	0.7	5,306	53	1,804 (34.0)

Values are given as numbers (%) unless otherwise stated. ^a This is the current policy at the NHS in the UK.

Interpretation

As NIPT improves, its diagnostic accuracy is likely to improve and more chromosomal abnormalities might be detected, especially with the validation of genomic sequencing from NIPT samples. Furthermore, the role of the first-trimester scan has expanded to include the detection of many fetal structural malformations, which will be followed by CVS and full karyotype, improving the detection rate of atypical chromosomal abnormalities. Despite the promising results of NIPT to date, the assay failure rate remains a limitation, ranging between 0 and 4.9% [4, 8, 10, 13, 14, 17, 23, 26–28, 45]. The fetal fraction in maternal plasma cfDNA is affected by maternal and fetal characteristics [5]. In a study where the authors extracted cfDNA from the maternal plasma at 11–13 weeks' gestation in 1,949 singleton pregnancies, the fetal fraction decreased with increased maternal weight, advancing gestation and smoking. The estimated proportion with a fetal

fraction below 4%, when a reliable result is unlikely to be possible, increased with maternal weight from 0.7% at 60 kg to 7.1% at 100 kg and 51.1% at 160 kg [5].

The key advantage of NIPT is avoiding the risk of miscarriage associated with CVS. In this study we assumed a miscarriage rate of 1% for CVS; however, this risk might be lower, especially in experienced centres. Furthermore, a recent meta-analysis reported a 5 times lower procedure-related risk of miscarriage (0.2%) [46]. However, as this was a meta-analysis which included observational studies, and the authors included only studies reporting data on more than 1,000 invasive procedures, the risk of bias including underestimation of the risk of miscarriage should not be ignored. For this reason, we used a miscarriage rate of 1% in the analysis. Moreover, the risk of miscarriage is likely to differ in low-risk compared to high-risk pregnancies, e.g. those affected by aneuploidy. Of concern is the possibility of false-positive results using

NIPT, which could occur in cases of confined placental mosaicism [47, 48]. This has not been demonstrated in studies to date; however, until larger studies in low-risk populations are reported, this risk might remain a concern. So far, pregnant women are advised to confirm a positive (abnormal) NIPT result with invasive prenatal testing to exclude the possibility of a false-positive result.

A prime concern that this study illustrates is the potential failure of NIPT to detect clinically significant aneuploidy that is associated with the risk of physical or mental handicap. However, NIPT technology is likely to be refined to detect other major trisomies. The question remains, though, whether NIPT can detect other chromosomal abnormalities, e.g. mosaicism or unbalanced rearrangement, or whether it would be possible to adapt the technology to perform detailed molecular karyotyping using MLPA or microarray on fetal DNA in the maternal blood. However, we can argue that the screening is offered for Down's syndrome, not other chromosomal abnormalities. Furthermore, failure to detect some of these aneuploidy cases should be weighed against the risk of miscarriage or the anxiety caused by copy number variants of unknown significance, poorly defined penetrance and/or variable expressivity [6]. Future directions might support the option of invasive prenatal diagnosis in cases where the NIPT was normal but the fetus has increased NT or structural abnormalities detected prenatally, as this is the group most likely to have other chromosomal rearrangements [49, 50]. Chitty et al. [51] reported that MLPA is able to detect 94.7%, compared to 68.4% by traditional karyotyping, of pathogenic rearrangements conferring a significant risk of adverse outcome. In a meta-analysis comparing array comparative genomic hybridization technology versus conventional karyotyping in prenatal diagnosis, Hillman et al. [52] demonstrated that up to 5% additional genomic imbalances can be detected using array-comparative genomic hybridization technology in

cases where there is a structural malformation on ultrasound.

One disadvantage of NIPT is the 12 days needed for a result (vs. 2–3 for qf-PCR) and the resulting need for medical (vs. surgical) termination in affected cases. It is also expected that, as public knowledge about this test grows, there will be more demand for it by pregnant women, adding to the cost burden. In a recent study involving more than 1,000 women and their partners, Lewis et al. [53] investigated the users' views on NIPT. Interestingly, only 50% thought that NIPT should be routinely offered to all women, with the remainder favouring contingent NIPT for women at high risk following routine screening [53]. This new technology remains controversial not only among healthcare professionals but also among the public.

Conclusions

This study suggests that a policy of NIPT in all cases with an increased risk of aneuploidy following first-trimester screening with CVS followed by full karyotype only if the fetal NT thickness is increased would reduce the risk of a procedure-related miscarriage and identify 95% of clinically significant chromosomal abnormalities.

Disclosure Statement

B.T. and A.P. are partners at Ultrasound Diagnostic Services, a private women's ultrasound service in London that currently provides both Harmony™ and NIFTY™ NIPT at a non-profit cost to clients. A.K. and B.T. are local PIs of a multicentre national study investigating NIPT in the UK (funded by the NIHR). A.P. is the local PI of the IONA study (an NIPT study which is commercially funded).

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