The rate of invasive testing for trisomy 21 is reduced after implementation of NIPT

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ABSTRACT

INTRODUCTION: The non-invasive prenatal test (NIPT) was introduced in the North Denmark Region in March 2013. NIPT is offered as an alternative to invasive tests if the combined first trimester risk of trisomy 21 (T21) is ≥ 1:300. The purpose of this study was to investigate the effect of NIPT implementation among high-risk pregnancies in a region with existing first-trimester combined screening for T21. The primary objective was to examine the effect on the invasive testing rate.

METHODS: This was a retrospective observational study including high-risk singleton pregnancies in the North Denmark Region. The women were included in two periods, i.e. before and after the implementation of NIPT, respectively. Group 1 (before NIPT): n = 253 and Group 2 (after NIPT): n = 302.

RESULTS: After NIPT implementation, the invasive testing rate fell from 70% to 48% ($p < 0.01$), and the number of high-risk women refusing further testing dropped from 26% to 3% ($p < 0.01$). NIPT successfully detected four cases of T21; however, two out of three sex-chromosomal abnormalities were false positives. No false negative NIPT results were revealed in this study.

CONCLUSIONS: In the North Denmark Region, the implementation of NIPT in high-risk pregnancies significantly reduced the rate of invasive testing. However, the proportion of high-risk women who opted for prenatal tests increased as the majority of women who previously refused further testing now opted for the NIPT.

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TRIAL REGISTRATION: The study was approved by the Danish Data Protection Agency (No. 2015-104).

In Denmark, more than 90% of all pregnant women participate in the national first-trimester combined screening programme for Down syndrome [1]. In each pregnancy, risk estimates for trisomy (T) 21, T18 and T13 are calculated based on maternal age, maternal serum levels of pregnancy-associated plasma protein A (PAPP-A) and β-human chorionic gonadotropin (β-hCG)) and the thickness of the nuchal translucency. Using the risk estimate of T21 ≥ 1:300 as a cut-off, the combined screening had a T21 detection rate of approximately 90% at gestational week 12 at a false positive rate of 5% [2]. High-risk women defined as those with a risk of T21 ≥ 1:300 are offered additional invasive diagnostic tests such as chorionic villus sampling (CVS) or amniocentesis. Since March 2013, the non-invasive prenatal test (NIPT) has been offered as an additional test to high-risk women in the North Denmark Region.

NIPT analyses cell-free fetal DNA in maternal plasma [3]. During pregnancy, cell-free DNA is continuously released from the placenta to the maternal circulation because of placental trophoblast cell apoptosis [4]. The majority of cell-free DNA in maternal blood is of maternal origin, and the proportion of fetal DNA (the fetal fraction), should be above 4% to meet the high-quality diagnostic standard [5]. Different techniques are used for the NIPT analysis. The Harmony Prenatal Test (Ariosa Diagnostics, California, USA), which was used in the present study, is based on selective digital analysis of selected fragments (DANSR), which evaluates only selected genomic fragments of cell-free DNA [5].

NIPT performs markedly better than combined screening in the detection of T21 [6]. At a false positive rate of 0.1%, the sensitivity of the NIPT in the detection of T21, T13 and T18 in a routinely screened first trimester population is > 99% , > 96% and > 91%, respectively [7]. However, in the detection of sex chromosomal aneuploidy, NIPT performance is markedly lower with a sensitivity of 93% at a false positive rate of 0.14%, and NIPT performance even lower for detection of monosomy X with a sensitivity of 90% at a false positive rate of 0.24% [7]. The false positive NIPT results are caused mainly by confined placental mosaicism, as cell-free fetal DNA is of placental origin. Therefore, a confirmatory invasive test should always follow a positive NIPT result before termination of pregnancy is considered [8].

NIPT detects only specific chromosomal anomalies such as T21, T13, T18 and sex chromosomal aneuploidy, while other (atypical) chromosomal abnormalities remain undetected. The prevalence of atypical chromosomal abnormalities depends on the study population. In the Danish population, the risk of atypical chromosomal abnormalities among women attending invasive testing was 2.7%, representing 23.4% of the total number of chromosomal abnormalities detected in this population [9]. Specific risk characteristics such as in-

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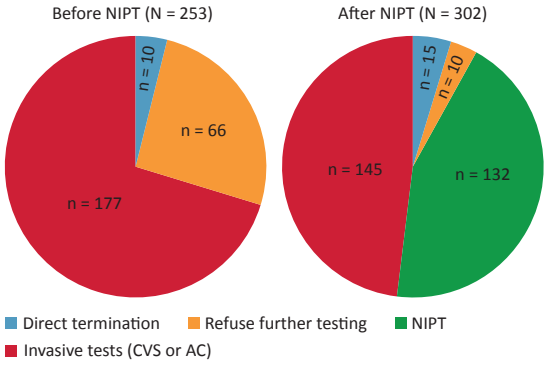
FIGURE 1

Distribution of prenatal tests among high-risk pregnancies in the North Denmark Region. Before (Group 1) and after non-invasive prenatal test (Group 2).



AC = amniocentesis; CVS = chorionic villus sampling; NIPT = non-invasive prenatal test.

creased nuchal translucency ≥ 3.5 mm [10], a combined risk of T21 ≥ 1:100, maternal age above 45 years, β-hCG ≤ 0.2 or ≥ 5.0 multiple of the median (MoM) and PAPP-A ≤ 0.2 MoM [11] significantly increase the risk of atypical chromosomal abnormalities [9].

The purpose of this study was to investigate the effect of the implementation of NIPT among high-risk pregnancies in a region with existing first-trimester screening for T21. The primary objective of this study was to examine the effect on the invasive testing rate among high-risk pregnant women.

METHODS

This was a retrospective observational study including all singleton high-risk pregnancies (combined risk of T21 ≥ 1:300) in the North Denmark Region. Patients were included according to the time of their 12-week scan and the implementation of NIPT. Group 1, before NIPT (n = 253): from 1 March 2011 to 1 February 2013. Group 2, after NIPT (n = 302): from 1 March 2013 to 1 February 2015. Prenatal data according to the combined screening were extracted from the local Fetal Medicine Database (Astraia Software gmbh, Munich, Germany), while postnatal data were found in local electronic health records (Clinical Suite and AS400).

The NIPT analysis was performed by The Harmony Prenatal Test (Ariosa Diagnostics, California, USA).

In each group, the invasive testing rate was calculated as a mean rate across the entire inclusion period. The rates were compared by a chi-squared test, and $p < 0.05$ was considered significant.

For the high-risk women included after the implementation of NIPT (Group 2), the risk characteristics are compared between the women opting for the invasive test and the women opting for NIPT. We used the Mann-Whitney U-test as data were not normally distributed.

All neonates in this study had a standard neonatal examination at birth. All autosomal and sex chromosomal aneuploidy detected by NIPT was confirmed by prenatal invasive tests or postnatal karyotyping.

Trial registration: This study was approved by the Danish Data Protection Agency (No. 2015-104).

RESULTS

The distribution of prenatal tests in the two groups is presented in **Figure 1**. The implementation of NIPT was associated with a significant reduction in the invasive testing rate from 70% to 48% ($p < 0.001$). Furthermore, the number of women refusing further testing was significantly reduced from 26% to 3% ($p < 0.001$).

All positive NIPT findings are presented in **Table 1**. NIPT successfully detected four cases of T21. However, two out of three cases with sex chromosomal aneuploidy were false positives. The majority of positive NIPT findings were confirmed prenatally by amniocentesis, but two cases (one case of T21 and one case of monosomy X) were confirmed by postnatal karyotyping according to the parents' wish.

A total of 132 NIPT were performed in the study period. In 125 cases, NIPT revealed a normal test result. Despite a normal NIPT, two cases had additional invasive testing in the second trimester because small fetal biometries had been detected at the 20-week scan. In both cases, the fetal karyotype was normal. NIPT test failure due to low fetal fraction occurred in one case (0.8%). A repeat NIPT was performed yielding a normal test result.

After the implementation of NIPT, the high-risk women (Group 2) were divided into two groups according to their choice of prenatal test: the NIPT group and the invasive group, respectively. The risk characteristics of each group are presented in **Table 2**. In the NIPT group, the risk estimate and the nuchal translucency were significantly reduced and the maternal age was significantly increased compared with the invasive group. However, the serum concentration of β-hCG and PAPP-A was not significantly different between the two groups.

DISCUSSION

In this study, we demonstrated that in a region with existing first-trimester combined screening, the implementation of NIPT significantly reduced the invasive testing rate among high-risk women. The majority of women opting for NIPT would previously have asked for the CVS. However, in addition hereto, a rather large share of women who previously refused further testing now opted for NIPT.

This study is limited by the small number of pregnant women included, and therefore inappropriate for

estimation of NIPT performance in the detection of T13, T18 and T21. However, our data are in accordance with previous large-scale studies reporting that NIPT has a high sensitivity for detection of T21 [7]. The high number of false positives in relation to sex chromosomal abnormalities seen in our data was also reported by larger studies [7].

The reduction in the invasive testing rate among high-risk women from 70% to 48% demonstrated in the present study is in accordance with a recent review from 2015 [12]. The review reports reductions in the invasive testing rate ranging from 17% to 76% depending on the country and the screening population.

In our study, test failure due to low fetal fraction was seen in one case out of 132 NIPT tests (0.8%), which is in line with the previous literature [13]. The fetal fraction is strongly related to maternal body mass index. At gestational week 12 + 0, the estimated proportion of pregnant women with test failure due to fetal a fraction below 4% is 0.7% at 60 kg, but it increases to 51.1% at 160 kg [13].

A major concern regarding NIPT is the risk that atypical chromosomal abnormalities remain undetected. In our study, no atypical chromosomal abnormalities were detected in the NIPT group either pre- or postnatally. However, the majority of women who opted for NIPT did not have additional invasive testing or a neonatal karyotyping. Therefore, less significant atypical chromosomal abnormalities may have remained undetected in the present study. The prevalence of atypical chromosomal abnormalities among women opting for invasive testing in Denmark is 2.7% [9]. Because of the small number of women included in the present study, we could not expect to find cases of atypical chromosomal abnormalities in any of the study groups.

In the North Denmark Region, experienced doctors in fetal medicine counsel all high-risk women prior to their decision regarding additional prenatal testing. Additional markers of T21 such as absent nasal bone, an abnormal ductus venous Doppler flow and tricuspid valve regurgitation are specifically investigated in order to further address the risk of trisomy. Furthermore, it is specifically explained that potential atypical chromosomal abnormalities will not be detected by NIPT. In case of an increased risk of atypical chromosomal abnormalities (a risk of T21 ≥ 1:100, maternal age ≥ 45 years, nuchal translucency ≥ 3.5 mm and extreme maternal serum levels of β-hCG and PAPP-A), the woman is advised to have an invasive test for a full fetal karyotype. The counseling doctors remained the same during the entire study period, and counseling followed local guidelines.

Before the implementation of NIPT, 26% of the high-risk women in the North Denmark Region refused further testing. This is a rather large proportion compared with other regions of Denmark. According to the national FØTO database of 2014, a total of 2,315 women had an increased risk estimate of T21 following their first trimester scan. In the same period, a total of 2,305 CVSs were performed. These numbers indicate that most high-risk women opted for the invasive testing. The main indication for CVS is increased risk for T21 at the first-trimester scan, but other indication, such as a family history of chromosomal abnormalities or anxiety may also contribute to this number. Cultural differences as well as the individual counseling including additional markers of T21, which is not included as a standard in the national screening programme, may further explain this regional difference.

As demonstrated by the distribution of risk characteristics in the two groups, women with an increased risk of atypical chromosomal abnormalities still opt for the NIPT. However, as indicated by the difference in the distribution of risk characteristics, the majority of women opted for invasive testing whenever the risk of atypical chromosomal abnormalities was increased.

This study demonstrates that in a region with existing first-trimester combined screening, the implementation of NIPT reduces the invasive testing rate among women with an increased risk of T21. This finding indicates that the small risk of miscarriage associated with invasive testing is a major concern for the pregnant

TABLE 1

Chromosomal diagnosis	NIPT result	Confirmatory test	False positive
Normal	125	0	0
Trisomy 21	4	4	0
Monosomi X	2	1	1
Triple X	1	0	1
Total	132	5	2

All positive non-invasive prenatal test (NIPT) findings and the confirmatory tests. The values are n.

TABLE 2

A comparison of risk characteristics between high-risk women opting for a non-invasive prenatal test and invasive testing. The values are median (range).

Risk characteristic	NIPT group	Invasive group	p-value[a]
Nuchal fold, mm	2.0 (1.6-2.7)	2.9 (1.9-3.7)	< 0.01
Risk estimate	1:176 (1:121-1:236)	1:71 (1:31-1:159)	< 0.01
Age, yrs	33 (28-38)	31 (28-35)	0.04
PAPP-A concentration, MoM	0.48 (0.36-0.85)	0.56 (0.33-1.02)	0.09
β-hCG concentration, MoM	1.10 (0.75-1.75)	1.11 (0.71-1.67)	0.99

hCG = human chorionic gonadotropin; MoM = multiple of the median; NIPT = non-invasive prenatal test; PAPP-A = pregnancy-associated plasma protein A.
a) Man-Whitney U-test.

Non-invasive prenatal testing for trisomy 13, 18 and 21. A new method to be applied in the Danish national screening programme for trisomy 21.

women. Also, for a proportion of women this concern exceeds the small risk of atypical chromosomal abnormalities not detected by NIPT. Furthermore, the proportion of high-risk women opting for prenatal tests was increased as the majority of women previously refusing further testing now opted for NIPT.

This study underlines the demand of NIPT among high-risk women, which should be noticed in the national debate on how to implement NIPT in the national screening programme for T21.

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