CHAPTER 3

Non-invasive prenatal testing for trisomy 13; more harm than good?

INTRODUCTION

Trisomy 13 (T13; Patau syndrome) is a rare and lethal disease. Fetuses with T13 often die in utero, and neonates rarely survive beyond the first few months following birth.[1] The associated structural anomalies can be almost always reliably detected by ultrasound examination. In this paper, we discuss whether early testing for T13 using cell-free DNA (cfDNA) testing in maternal serum has benefits that outweigh the potential harm associated with false positive results, which may lead to unnecessary invasive procedures and anxiety.

Non-invasive prenatal testing (NIPT) using chromosome selective sequencing of cfDNA has been shown to be an excellent test to predict presence or absence of fetal trisomy 21 (T21; Down syndrome) from as early as 10 weeks gestation. The major reason for many pregnant women to request testing for T21 is, apart from being a relatively common genetic condition, the fact that T21 is *not* a lethal disease, but a non-treatable serious handicap associated with a life expectancy of over 50 years. NIPT can also be used to evaluate the risk for other chromosomal anomalies, including T13. Studies published thus far however reported a lower detection rate and, more importantly, higher false positive rate (FPR) and higher false negative rate than reported for T21 and T18.[2-7] Explanations for false positive NIPT results include technical reasons such a relatively high Guanidine-Cytosine (GC) content of chromosome negatively influence sequencing reliability. In addition, the presence of confined placental mosaicism, or a lost, perhaps unrecognized, co-twin may provide an increased amount of DNA fragments in maternal plasma from chromosome 13. Strictly speaking, in such cases the test can be considered true positive on a cfDNA level, however, the fetus itself may have a normal chromosome configuration. Consequently, physicians, professional organizations like ACOG, and companies offering NIPT advocate confirmation of positive NIPT results by amniocentesis, rather than confirmation by chorion villus sampling.

One of the most important advantages of cfDNA testing is the expected significant reduction of invasive diagnostic procedures and the associated iatrogenic miscarriages in healthy pregnancies. For T21, this is undoubtedly true. For a more rare condition such as T13, combined with a higher FPR, there may not be a reduction in invasive procedures. We would like to illustrate this with a case report, followed by a mathematic assessment of the potential harm caused by large scale application of NIPT for T13.

Case

In the fall of 2012, a German 35 year old pregnant women, primigravid, pregnant after IVF, contacted us because of a T13/T18 risk of 1:55 based on the first trimester combined test. After searching the internet she found that the Leiden University Medical Center just started a pilot project sending maternal blood samples for NIPT to the United States (MaterniT21

Plus test, Sequenom, San Diego, CA). At that time, the German Praena NIPT test (Lifecodexx, Konstanz) was only available for T21 testing. She and her partner decided to have blood drawn in Leiden at 14+5 weeks' gestation. Ultrasound to verify the gestational age revealed a CRL on the 2.3 percentile, with no obvious structural anomalies, and no evidence for a second gestational sac. After 13 days we received an email by the head of the laboratory, and a fax the next day, with the result. The test was positive for T13. The fetal fraction was 7%.

We advised her to contact her obstetrician in Germany, and we stressed the fact that before considering any irreversible action, confirmation by amniocentesis was warranted. Alternatively, she could have an advanced ultrasound and refrain from invasive testing if no abnormalities were seen. Obviously, the next days were particularly stressful for the couple. Detailed ultrasound examination revealed no obvious anomalies. After counselling, the patient elected to undergo an amniocentesis 5 days after receiving the NIPT result. The QF-PCR result was available 3 days thereafter revealing no signs of a trisomy, and full karyotyping confirmed a normal 46XY result. The couple still expressed being grateful for the opportunity of NIPT, despite the false positive result and the associated 8 days of significant anxiety.

DISCUSSION

In table 1, the available literature on the diagnostic accuracy of NIPT for T13 is summarised. A total of only 71 T13 cases have been reported thus far. Although we realize that several different laboratory techniques were used in the different studies, and that techniques have been improved over time, we elected to include all published studies in our primary analysis, which gave an overall detection rate of 91.6%, with a 0.097% false positive rate. All of the tested samples thus far were selected from stored samples obtained from high-risk pregnancies; consequently the prevalence of T13 in this cohort was high. Ashoor et al acknowledged that the total number of cases of T13 examined in their series was too small for accurate assessment of the detection rate.[7] Walsh et al evaluated the published evidence for NIPT as either a 'primary' or an 'advanced' screening test for the different trisomies. They concluded that future studies must address its use as a primary screening test for T13 in high-risk women.[10]

We conclude that the performance, thus the benefit of NIPT for the prediction of T13 is not clear yet, even in high-risk populations. Yet benefits in a low risk population are more doubtful.

Our concerns about the use of NIPT for T13 are clarified with a hypothetical calculation. When we extrapolate the test characteristics to a general average-risk pregnant population of 1.000.000 women, with an expected prevalence in the first trimester, of 1 case of T13 per10.000, and all women would be tested using NIPT, 1059 women would receive a positive

test result for T13 (92% detection rate of 100 T13 cases = 92 true positives, 0.097% false positive rate of 1,000,000 women = 967 false positives.) The positive predictive value will be 92/1059 = 8.7%. If all these women would elect further testing by amniocentesis, with a one percent additional risk for miscarriage, 10 (0.913x0.01x1059) healthy fetuses would be lost for 92 T13 cases detected. In addition, the other 957 women not losing the pregnancy but experiencing serious stress for at least some days to even a few weeks, represent another disadvantage.
In contrast, we can extrapolate the test characteristics to a high-risk pregnant population of 1,000,000 women, with an expected prevalence in the first trimester, of T13 of 1:200, or a 5000 fetuses with T13. If all women would be tested using NIPT, 5540 women would receive a positive test result for T13 (91.6% detection rate of 5000 T13 cases = 4578 true positives, 0.097% false positive rate of 1,000,000 women = 962 false positives.) The positive predictive value will be 4578/5540 = 82.6%. If all these women would elect further testing by amniocentesis, with a one percent additional risk for miscarriage, 10 healthy fetuses would be lost for 4578 T13 cases detected.

These hypothetical calculations show that although the test performance is similar, the positive predictive value is highly depending on the prevalence of the disease. Before introducing NIPT in a screening and diagnosis program we should balance harm and benefits.
Especially in a low risk population the only benefit for the few women that have the misfortune to carry a fetus with T13 is earlier detection, thus the option for earlier termination, when NIPT is offered at 10 weeks' gestation. The end result, losing the fetus, is the same for all. Given the issue of confined placental mosaïcism, a chorionic villus sampling is not recommended. Amniocentesis can be done from 15 weeks' gestation onwards, providing a rapid aneuploidy detection results close to 16 weeks. Since all T13 cases are associated with multiple anomalies that are hard to miss on detailed ultrasound examination, T13 will be detected at the routine 20-week scan, and often even earlier. Papageorghiou et al described that >90% of T13 cases are identified at the 11 to 14-week scan.[11] Given the unfavourable balance between benefit and harm related to using NIPT to test for T13, we suggest reconsidering its use especially in a general population. We lose health children for a few week earlier detection.
One option, likely the most practical, would be to discuss these issues in the pre-test counselling for both high and general risk women, and to arrange a detailed ultrasound as soon as possible after a positive T13 result. Refraining from invasive testing in the absence of any ultrasound abnormality would seem justifiable, however, there may be residual anxiety experienced by the parents until birth. A second option would be to design the NIPT in such a way that only those abnormalities are tested that, after careful consideration by professional bodies and policy makers, are regarded clinically relevant and for which the test has acceptable false positive rates. When introducing NIPT for low risk populations we suggest to consider

not including T13 testing or rather not reporting this result, although we understand the difficulties of this concept.

CONCLUSION

NIPT is a revolutionary improvement of prenatal care, undoubtedly soon finding its way into routine practice. Importantly however, irrespective of what policy will eventually be implemented, all obstetricians and genetic counsellors should be fully aware of their responsibility to properly counsel women both before and after NIPT, including the consequences of the less than perfect test characteristics, in particular for T13. Screening for diseases that are lethal in the fetal or early neonatal period, at the expense of serious anxiety and iatrogenic miscarriages of healthy fetuses may do more harm than good.

Finally, clinicians in our view could reassure the patient and refrain from invasive testing in case of a positive NIPT result for T13 and a completely normal detailed ultrasound examination.

	Method	*Detection rate*	*False pos. rate*	*PPV**	*NPV**
Chen et al (2011)[2]	MPS	100% (25/25)	1.1% (3/264)	89.3% (25/28)	100% (261/261)
Lau et al (2012)[3]	MPS	100 % (2/2)	0%	100% (2/2)	100% (106/106)
Bianchi et al (2012)[4]**	MPS	78.6 % (11/14)	0%	100% (11/11)	99.4% (485/488)
Palomaki et al (2012)[5]	MPS	91.7 % (11/12)	0.1% (1/1959)	91.7% (11/12)	99.9% (1958/1959)
Jiang et al (2012)[6]	MPS	100% (2/2)	0%	100% (2/2)	100% (901/901)
Zimmerman et al (2012)[7]	Selective	100% (2/2)	0%	100% (2/2)	100% (143/143)
Liang et al (2013)[8]	MPS	100 % (4/4)	0.3% (1/408)	80.0% (4/5)	100% (407/407)
Ashoor et al (2013)[9]	Selective	80 % (8/10)	(0.1%) (1/1938)	88.9% (8/9)	99.9% (1937/1939)
Total sample		**91.6% (65/71)**	**0.097% (6/6204)**	**91.6% (65/71)**	**99.9% (6198/6204)**

Table 1. *literature published until April 1, 2013 on the diagnostic accuracy of NIPT for T13*
**PPV: positive predictive value, NPV: negative predictive value*
*** 2/2 unclassified samples were both positive for T13 by karyotyping*

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