Case Report from Turkey

Fetal Aneuploidy Detection by Cell-Free fetal DNA Sequencing For Multiple Pregnancies and Quality Issues with Vanishing Twin

Reference:
Groemminger, S. *et al.* J. Clin. Med. 2014, 3, 679-692; doi:10.3390/jcm3030679

Introduction

In the case of multiple pregnancies, conventional non-invasive examination methods for the determination of fetal trisomies have limitations while invasive methods bear a higher risk for procedure related fetal losses when compared to singleton pregnancies. Therefore, non-invasive prenatal testing (NIPT) by random massively parallel sequencing (rMPS) from maternal blood for multiple pregnancies can be a reliable option in prenatal care. However, NIPT might have quality issues in case of multiple pregnancies. For example, vanishing twins might cause discordant test results, as described in the case report below.

Case report of a discordant NIPT result due to a vanishing twin *(fetus papyraceus)*

An infertile couple (maternal age 39 years) from Turkey underwent assisted reproductive technologies (ART) using ICSI (Fig. 1). Following transfer of two embryos, a twin pregnancy was confirmed with two gestational sacs and heart beats of the two embryos. During an ultrasound scan at week 10 (p.m.), there was just one heartbeat of one fetus detectable. At week 11 the nuchal translucency (NT) was 2.5 mm for the living twin and 3.1 mm for the deceased twin. At week 17+2, the vanishing twin was still visible in ultrasound. The couple then decided to perform NIPT (PraenaTest®), the medical indication was "advanced maternal age". The test result was positive for fetal trisomy 21 with a z-score of 13.5. The follow-up examination (amniocentesis) resulted in a discordant result for the living child, as the karyotype was 46,XY. The NIPT result from the back-up blood sample analyzed after the discordant karyotyping result confirmed the NIPT result of the first blood sample. For the first and the second blood sample, the level of cffDNA measured by QuantYfeX® was 20.7% and 24.8% respectively, while the level of cffDNA based on the Y-chromosomal representation from next generation sequencing was 9.2% and 9.3% respectively.

In the third trimester of the pregnancy (week 38+2) a new blood sample was taken. The NIPT result of the analysis was negative for fetal trisomy 21 and the level of cffDNA measured by Y-chromosomal representation corresponded to the level of cffDNA measured by QuantYfeX® (21.7% versus 21.4%).

The living male twin was born phenotypically normal and a vanishing twin *(fetus papyraceus)* was found in the placental tissue from which a sufficient amount of cells was isolated for cell culture. A trisomy 21 positive, female karyotype was confirmed after GTG banding (47,XX+21).

For further clarification and in order to exclude mosaicism, three different biopsies from the placenta, fetal cord blood of the living child, and peripheral blood from the mother were collected. Various conventional cytogenetic analyses using the collected material confirmed an euploid living male twin (karyotype 46,XY) and a deceased aneuploid female twin (karyotype 47,XX+21). There was no evidence for a trisomy 21 mosaicism, neither in the mother nor in the living child.

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Results

The results show that the first NIPT result positive for fetal trisomy 21was caused by the trisomic cffDNA of the vanishing twin. The difference between the level of cffDNA measured by QuantYfeX® and the Y-chromosomal representation in both blood samples of the first blood draw is due to the presence of the deceased female fetus and the living male fetus, both releasing cffDNA into the mother's blood circulation.

Conclusion

The case report demonstrates that vanishing twins are a limiting factor for NIPT, as undisclosed vanishing twins can contribute a sufficient proportion of cffDNA to the total amount of cffDNA to cause a positive PraenaTest® result being not representative for the continuing singleton pregnancy. Therefore, such pregnancies need to be monitored thoroughly during clinical care in order to be able to interpret NIPT results correctly. So far there have not been any studies which describe in any way whether the size of a vanishing twin or the size of its amniotic cavity correlate with the level of cffDNA in the maternal plasma. It also needs to be investigated whether a vanishing twin might cause a cffDNA flooding into the maternal circulation as a result of dying cells which are increasingly releasing fetal DNA. In the future, for a better understanding and interpretation of NIPT results of such cases a more detailed documentation of the progress of vanishing twins in combination with NIPT results is needed.

Recommendation for the use of PrenaTest®

We recommend responsible physicians to discuss each individual case with us.

We would suggest not to perform the PrenaTest® as long as the vanishing twin and/or the amniotic cavity can still be detected during ultrasound examination, since a positive test result cannot clarify which of the twins is affected with the detected fetal trisomy. On the other side, a negative test result should confirm that the living fetus is not affected, as long as the measured level of cffDNA is at least 8% which is the minimum amount required for a successful PrenaTest® analysis of twin pregnancies.

However, since many vanishing twins remain unrecognized, discordant NIPT results can never be ruled out in general. Therefore, the existence of such cases underpins the recommendation of medical associations that NIPT should be offered only after, or in conjunction with a qualified ultrasound examination.

The full text of the article is freely available:
http://www.mdpi.com/2077-0383/3/3/679

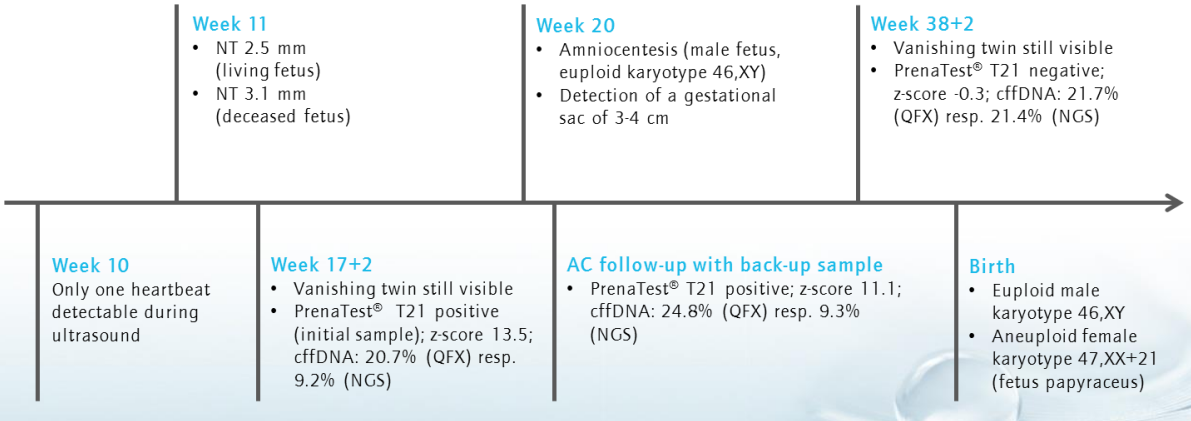

Fig. 1 | Case report from Turkey, Dr. Sanli Erkan, Istanbul | Maternal age 39, ICSI, twin pregnancy

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