CASE REPORT FROM HUNGARY

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

Due to an increased risk for chromosomal aneuploidy determined during the first trimester screening in January 2014, a PrenaTest® analysis was performed in gestational week 13+2 for a singleton pregnancy. The test result of the initial blood sample was positive for fetal trisomy 21 with a z-score of 3.4. As this z-score lies within the borderline range of 2.5 to 3.5 (cut-off z-score ≥ 3.0), the analysis was repeated using the back-up blood sample, resulting again in a borderline z-score of 2.6 for fetal trisomy 21 (Fig. 1).

For both blood samples, the Y-chromosomal representation (measured by next generation sequencing; NGS) indicated male specific cffDNA of 3.0% and 2.7% respectively. Furthermore, since the level of cffDNA measured by QuantYfeX® (QFX) was 13.4% for the initial sample and 10.0% for the back-up sample, i.e. quite higher than the level of cffDNA measured by NGS, we assumed that two fetuses with discordant sex contributed to the fetal fraction and that the male fetus would be affected with trisomy 21. The reason for this assumption was the clear correlation of the level of cffDNA measured by NGS (3.0% and 2.7%) with the borderline z-score of 2.6 being close to the cut-off z-score of 3.0. We observe a correlation of z-scores positive for trisomy 21 with the measured level of cffDNA during laboratory routine, which has already been described by Palomaki *et al* (2011)[1].

As the PrenaTest® analysis had originally been requested for a singleton and not for a twin pregnancy, we assumed that the male specific cffDNA derived from a vanishing twin affected with trisomy 21 and that the deceased fetus had either not been recognized during ultrasound examination or had just not been noted on the test request form. Therefore, we reported to the responsible physician an indecisive result for chromosome 21 and also informed him about our findings. He confirmed that the pregnancy had indeed started as a twin pregnancy after the application of assisted reproductive technologies (ART) and that the sex of the living fetus was female. Therefore the portion of cffDNA that resulted in the positive z-score for trisomy 21 originated from a deceased male fetus.

The living female twin was born phenotypically normal and the vanishing male twin had been completely absorbed during the course of the pregnancy.

Results

This case report clearly demonstrates that NIPT results need to be interpreted carefully and that all available NIPT analysis data must be examined in correlation in order to be able to detect potential result distortions which might be caused by a vanishing twin.

Also, the absorption procedure of the deceased male twin seemed to have nearly been completed at the point of blood draw at gestational week 13+2, since only a small proportion of the total cffDNA could be assigned to the vanishing twin (i.e. approximately 25% of the total cffDNA). Further studies are required for a detailed understanding of the dynamics of the vanishing or absorption process and how various levels of cffDNA can have an impact on NIPT results.

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 PrenaTest® 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

Pregnancy	PrenaTest®	LifeCodexx' assumption	Physician's confirmation
• Singleton pregnancy from Hungary • Increased risk for aneuploidy	**Initial sample:** • Borderline z-score 3.4 (trisomy 21) • level of cffDNA: 13.4% (QFX) resp. 3.0% (NGS) **Back-up sample:** • borderline z-score 2.6 (trisomy 21) • level of cffDNA: 10.0% (QFX) resp. 2.7% (NGS) **Test result:** • indecisive for fetal trisomy 21	Two fetuses with discordant sex (living female twin and vanishing male twin) due to a) Difference between levels of cffDNA measured with QFX and NGS b) Correlation between level of cffDNA (NGS) and borderline z-score (Palomaki et al 2011)	• Singleton pregnancy started as twin pregnancy (ART) • Female twin born phenotypically normal • Vanishing male twin absorbed

Fig. 1 | Case report from Hungary

[1] Palomaki, G.E.; Kloza, E.M.; Lambert-Messerlian, G.M.; Haddow, J.E.; Neveux, L.M.; Ehrich, M.; van den Boom, D.; Bombard, A.T.; Deciu, C.; Grody, W.W.; et al. DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study. Genet. Med. 2011,13, 913–920.

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