CASE REPORT FROM GERMANY

Influence of a *missed abortion* of a fetus on the result of a non-invasive prenatal test (NIPT) in the case of a multiple pregnancy

Introduction

In the case of multiple pregnancies, conventional non-invasive examination methods for the determination of fetal trisomies have limitations while invasive methods bear an elevated 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 twin pregnancies can be a reliable option in prenatal care. However, the result of a NIPT can be influenced by a *missed abortion* of a fetus, as described in the below case report.

Case report

Week 12+6 | Detection of missed abortion

A 42-year old pregnant woman with a dichorial twin pregnancy after ICSI presented to a specialized prenatal practice for further diagnosis. The pregnancy was inconspicuous until then. During the examination the missed abortion of a fetus was determined (CRL 42.9 mm, fig. 1). The other fetus (CRL 67.7 mm, NT 1.7 mm, nasal bone was representable) showed no abnormalities. There was a dichorial placentation with respect to the chorion ratios. After detailed counseling, the patient did not want an invasive diagnosis (CVS) at first, but decided to await the further course of the pregnancy. A new appointment in four weeks time was agreed.

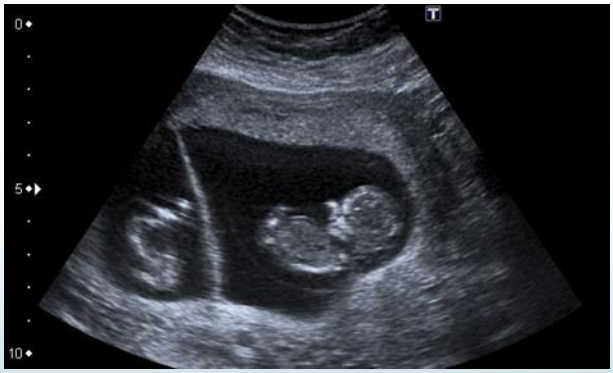

Fig. 1: Detection of a deceased fetus in week 12+6

Week 17+0 | Positive PrenaTest® result for trisomy 21

In gestational week 17+0 the vital fetus still did not show any indication for a trisomy 21. The deceased fetus and the gestational sac were still detectable (fig. 2). Following genetic counseling and weighing all options, the patient rejected an amniocentesis and opted for the PrenaTest® instead, accepting that the test result might be inconclusive. It was mutually decided to perform the PrenaTest® analysis as a project of research & development. The test result of the initial blood sample was positive for fetal trisomy 21 with a z-score of 3.1. Measured by QuantYfeX® (QFX), the level of cffDNA was 16.27% and the sex of both fetuses was determined to be female as no Y chromosomal reads were detected by next generation sequencing (NGS). Due to the strong correlation between z-score and level of cffDNA it was concluded that one of the fetuses would be affected with trisomy 21. The patient still rejected an amnio-centesis and decided to wait for the result of the second PrenaTest® analysis.

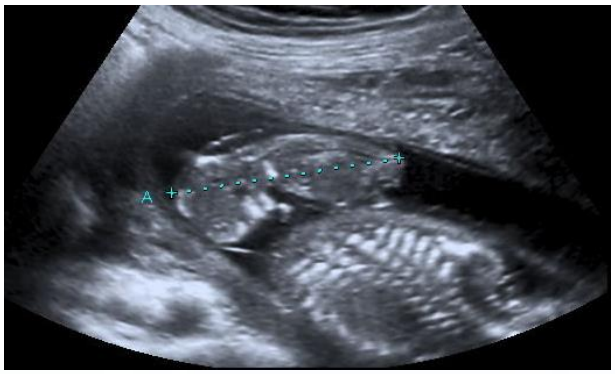

Fig. 2: The deceased fetus in week 17+0

WM-1185-EN-001 / May 2017

Week 19+2 | Second positive PrenaTest® result for trisomy 21

A second blood sample was taken and analyzed in gestational week 19+2. The test result was positive for fetal trisomy 21 with a z-score of 11.6 and a level of cffDNA (QFX) of 16.92%. As before, there was no Y chromosomal representation. The patient still did not wish to have an amniocentesis performed.

Week 21+6 | Vital fetus without indication for trisomy 21

In gestational week 21+6 the vital fetus still did not show any indication for a trisomy 21. The deceased fetus as well as the gestational sac continued to be representable (fig. 3). As before, the patient rejected an amniocentesis due to the presumably elevated risk for a procedure related miscarriage. An abortion would have only been an option for her in case of a severe malformation.

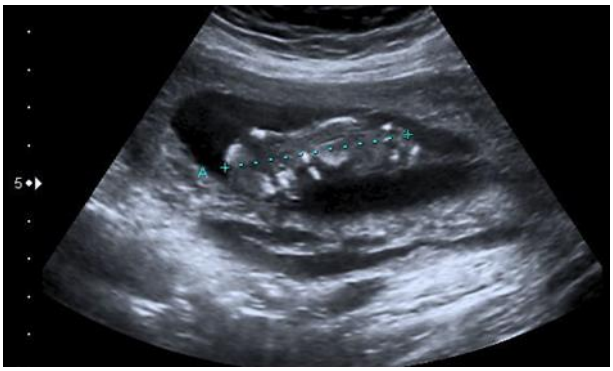

Fig. 3: Deceased fetus with gestational sac in week 21+6

Week 22+1 | Negative PrenaTest® result for trisomy 21

A third blood sample was taken and analyzed in gestational week 22+1. This time, the test result was negative for fetal trisomy 21 with a z-score of 1.6 and a level of cffDNA (QFX) of 20.44%. Again, no Y chromosomal representation could be measured, re-confirming the pregnancy with a female fetus.

Week 26+5 | Deceased fetus still representable

In gestational week 26+5 the vital fetus was still without indication for a trisomy 21. Remaining structures of the deceased fetus continued to be representable though the gestational sac had been resorbed completely (fig. 4).

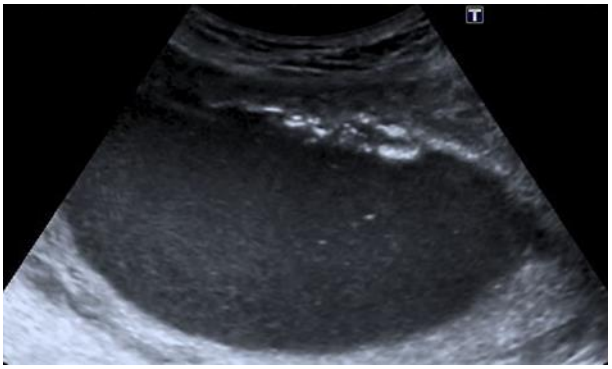

Fig. 4: Remaining structures of the deceased fetus in week 26+5

Week 31+3 | Deceased fetus not representable anymore

A final examination was performed in gestational week 31+3. The living fetus developed appropriately without any indication for trisomy 21. Now, the deceased fetus was not representable anymore.

Week 36+2 | Birth of a healthy female child

In gestational week 36+2 a healthy female child was born (via forceps, 2700g, 48 cm).

Table 1: Results of the PrenaTest® in correlation with the resorption process

Week	cffDNA (QFX)	z-score	Test result
17+0	16.27%	3.1	positive
19+2	16.92%	11.6	positive
22+1	20.44%	1.6	negative

WM-1185-EN-001 / May 2017

Results

The case illustrates in detail the resorption process of the placenta of a deceased fetus during a time span of about five gestational weeks and its influence on the NIPT results. In gestational week 17+0 the resorption had just begun, as can clearly be seen from the rather high level of cffDNA compared to the borderline z-score for fetal trisomy 21. Though merely two weeks later the level of cffDNA had not changed much, the z-score jumped to 11.6, clearly positive for fetal trisomy 21. This might indicate that the peak of the resorption process had been reached and that the placenta of the deceased fetus might have caused cffDNA increasingly pouring into the maternal blood circulation. Three weeks later a new sample from the third blood draw showed an increased level of cffDNA with a z-score negative for fetal trisomy 21 (table 1).

To our knowledge this is the first time that such a resorption process has been described in such detail using NIPT. Further studies are required to understand in greater detail the dynamics of the resorption process and its influence on NIPT results.

Conclusion

The case report demonstrates that a missed abortion of a fetus from a multiple pregnancy can be a limiting factor for NIPT, as the placenta of the deceased twin can contribute a sufficient proportion of cffDNA to the total amount of cffDNA to cause a positive PrenaTest® result not being representative for the vital singleton pregnancy. A similar case has already been described in Groemminger et al 2014.[1] Therefore, such pregnancies need to be monitored closely during clinical care in order to be able to interpret NIPT results correctly. To our knowledge, so far there have not been any studies which describe in any way to which extent the size of a deceased twin or the time of its death correlate with the level of cffDNA in the maternal plasma, since in these cases – should the parents wish to draw any conclusions –invasive diagnostics are usually performed. However, in our described case the patient rejected an invasive examination.

Recommendation in the use of PrenaTest®

We recommend the responsible physician to discuss each individual case with us. We advise not to perform the PrenaTest® as long as the deceased twin and/or the gestational sac is representable 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% being the minimum fetal fraction required for a successful PrenaTest® analysis of twin pregnancies. However, since a deceased twins often remains unrecognized in particular in early pregnancy (*vanishing twin* phenomenon), discordant NIPT results can never be ruled out in general, especially if a NIPT is performed early, e.g. in gestational week 10 or 11. 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, preferably in gestational week 12 to 14.[2]

Acknowledgements

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1 S. Groemminger et al 2014. Fetal Aneuploidy Detection by Cell-Free DNA Sequencing for Multiple Pregnancies and Quality Issues with Vanishing Twins. J. Clin. Med. 2014, 3, 679-692; doi:10.3390/jcm3030679

2 Recommendations of medical associations are available at auf www.lifecodexx.com

WM-1185-EN-001 / May 2017

