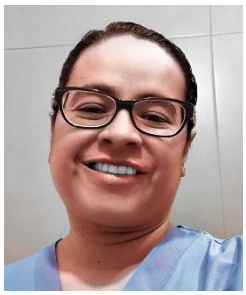
ISSN 2954-467X

The Journal of Reproduction

www.thejournalofreproduction.com

Discordant non-invasive prenatal test, train of thought when having positive ultrasound markers



Fragoso-Benítez Marcela

Fragoso-Benítez Marcela[1], 0000 0002 4218 9951; Noriega Jiménez Miguel Ángel[2], 0000 0002 5746 0150; Ballescá Estrada Adriana[3]; 0000 0003 0365 335X; Espinosa Arce Andrea Fernanda[4], 0000 0002 2584 8900.

ABSTRACT

Here we present a clinical scenario and the approach taken by the laboratory and medical staff in a private Reproductive Genetics laboratory in Mexico City.

We received a maternal blood sample for non-invasive prenatal testing that resulted in high risk for trisomy 18. Confirmatory amniocentesis was performed and amniotic fluid qfPCR showed an euploid fetus. In this case report we discuss our approach to provide accurate genetic testing for the couple.

KEYWORDS: NIPT, Trisomy 18, first trimester.

MANUSCRIPT

A sample from a 10.5 gestational age pregnancy with ultrasound positive markers was sent to our laboratory for non-invasive prenatal testing (NIPT); the sample was processed as usual obtaining a high risk of trisomy 18 in a female fetus. Notification for the requirement of a confirmatory qfPCR testing was done and amniocentesis was accepted by the mother. This confirmatory testing was processed as usual and resulted in 46,XX, confirming the female fetus but discordant for aneuploidies. Because of contradictory results a case study was initiated and an interview with the parents was scheduled.

Parents were a healthy young couple, ages 31-33, first grade cousins. Ultrasound was reported at week 10 with small nasal bone and heart murmur, those were the indications for the NIPT. Genetic counselling was focused on different possibilities:

- First, the possibility of maternal contamination in the amniocentesis sample. We recommended to perform Short Tandem Repeat testing (STR) comparing both samples, fetus versus mother.

[1] Medical Director, GD Technologies.
[2] Medical Geneticist, GD Technologies.
[3] Former Laboratory Director, GD Technologies.
[4] Former Laboratory Supervisor, GD Technologies.

NOTE: The numbers following the affiliation markers are the author's ORCID iD.

ARTICLE HISTORY:
Received September 4, 2022
Revised September 5, 2022
Accepted September 12, 2022
Available online October 1, 2022

CONTACT:
Fragoso Benítez Marcela.
marcela.fragoso@grupo-bio.com.
GD Technologies, San Luis Tlatilco 5,
Fraccionamiento Industrial. Naucalpan, Edo.
Méx. CP53370, México.
Phone: +52 55 1328 1328.

• Then, the possibility of a false positive non-invasive prenatal testing but with an alternative diagnosis occurring on the fetus hence the alterations observed in the ultrasound, and considering monogenic diagnosis that may be or not related to the consanguinity. For this, we recommended follow up and structural second trimester ultrasound, at first.

• And of course, the possibility of placental mosaicism or maternal mosaicism.

Parents decided to follow the pregnancy, regardless the risks addressed during the genetic consultation. STR testing discarded maternal contamination of the amniocentesis sample, therefore the other possibilities increased their chance. Different testing was proposed to the parents, but because of the costs, and because for monogenic recessive disorders where no potential risks were identified during the genetic consultation, the recommendation was following up, second trimester ultrasound and genetic consultation of the new born. A third party, recommended the couple repeating the NIPT which was discouraged out by us, but at the end, the couple decided to follow the third-party recommendations and we lose contact with them.

Prenatal diagnosis is an informative tool to provide patients enough information for their reproductive choices (1), however since in Mexico not all gynaecologists, obstetricians or reproduction health workers have an adequate training in genetic counselling, some patients may be disinformed about the optimal studies, in this case the patients were offered a second non-invasive prenatal testing, which of course, would not be able to address different information.

Current guidelines of most colleges of Genetics and Obstetrics recommend prenatal screening to all women regardless their age (1), however, the selection of the type of screening may represent a great difference in costs, which in low income countries such as Mexico could compromise the reproductive autonomy of the couple. Some studies, for example Gekas et.al and Ohno et.al., (1, 2) address that when NIPT requires confirmatory testing would represent cost effective as compared when use as a diagnostic tool, and is as well cost-effective in high-risk patients due to maternal age 35 or greater (3). In fact, some studies in general population have shown economic justification (4). In low income countries such as ours, these topics should be start to be discussed, and all genetic testing should be accompanied by genetic counselling to determine the optimal follow up. In our laboratory, considering the cost of invasive procedures, the confirmatory testing is offered for free.

Discordant NIPTs is strictly referred to as those being different from a fetal karyotype. False positive cases are more frequent than false negative in a ratio of 27:1 (5). False positive cases main reasons include maternal copy number variation, confined placental mosaicism, positive vanished twin, maternal mosaicism, and maternal malignancy. Whereas false negative requires further investigation, most cases reported are due to complex chromosomal formulas: true fetal mosaicism, discordant abnormal formulas between NIPT and fetus and/or placenta, placental complex mosaicism, etc. (6). However, due to the emotional impact of a positive result, false positives should be addressed differently, especially when ultrasound markers or abnormality are present.

Cases of termination of pregnancy after a positive NIPT result but without a confirmation test have been reported up to 6.2-19.6% of cases (6), raising up the need of having a genetic counselling before the, as sensitivity rates are known to be 99% with false positive below 1%, but positive predictive value is limited to 40-90% (6), and the knowledge of the mechanisms involved in discordant NIPTs should be brought to the table on priority, to address the need of further testing and/or follow up. Pre and post-test genetic counselling should be mandatory, to all women choosing NIPT.

Unfortunately, further studies of placenta, maternal chromosomal abnormalities, and/or fetal mosaicism in false positive cases are limited, not only because these tests are often invasive, but because the normal outcome of the baby diminishes the need of answers and the need to pay for further testing; this type of practices have also hind to establish biostatistical values for NIPTs in aneuploidies not concerning 13, 18, 21, X and Y chromosomes, as well for CNVs.

Beulen et al. (7) have questioned the clinical utility of non-invasive prenatal testing in ultrasound anomalies, they reported normal NIPT results in 89.2% of performed tests with ultrasound abnormalities, however their population included cases in which whole single aneuploidies may not be the main diagnostic consideration or where NIPT would indeed not be recommended by a geneticist, for example, ultrasound with multiple important abnormalities such as holoprosencephaly, multiple pregnancies, etc. It is of enormous importance that although NIPT has demonstrated to be a good test for single whole chromosomal aneuploidies, NGS is not intended to be a substitute neither for karyotype, especially in the case of rearrangements with minimal gain/loss nor NIPT is at this point available to discard other genetic abnormalities that may be implicated in an abnormal ultrasound. Guidelines upon how to test pregnancies with ultrasound abnormalities should be agreed by colleges but should not discard NIPT.

It is very important to remember that teratogenic and monogenic disorders are responsible of most part of the congenital diseases/complexes and/or syndromes and there is no yet a protocol or tests to address them all (5); indeed, chromosomal abnormalities are age-related in a population that has an increasing age of maternity and therefore, NIPT as well as other screening tests are available for this population, there is still no test 100% reliable.

CONCLUSIONS

Though NIPT is a reliable test for prenatal diagnoses, certain cases require not only consolidation of guidelines but also specialized counselling with a geneticist. Cases in which NIPT has a discordant result but in the presence of ultrasound abnormalities should be of special interest of looking for optimal testing, follow up with the consideration of cost-effectiveness and securement of reproductive autonomy.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

REFERENCES

[1]. Gekas J, Langlois S, Ravitsky V, Audibert F, van den Berg DG, Haidar H, Rousseau F. Non-invasive prenatal testing for fetal chromosome abnormalities: review of clinical and ethical issues. Appl Clin Genet. 2016 Feb 4;9:15-26. doi: 10.2147/TACG.S85361. PMID: 26893576; PMCID: PMC4745955.

[2]. Ohno M, Caughey A. The role of noninvasive prenatal testing as a diagnostic versus a screening tool--a cost-effectiveness analysis. Prenat Diagn. 2013 Jul;33(7):630-5. doi: 10.1002/pd.4156. Epub 2013 Jun 17. PMID: 23674316.

[3]. Song K, Musci TJ, Caughey AB. Clinical utility and cost of non-invasive prenatal testing with cfDNA analysis in high-risk women based on a US population. J Matern Fetal Neonatal Med. 2013 Aug;26(12):1180-5. doi: 10.3109/14767058.2013.770464. Epub 2013 Mar 6. PMID: 23356557; PMCID: PMC3741020.

[4]. Fairbrother G, Burigo J, Sharon T, Song K. Prenatal screening for fetal aneuploidies with cell-free DNA in the general pregnancy population: a cost-effectiveness analysis. J Matern Fetal Neonatal Med. 2016;29(7):1160-4. doi: 10.3109/14767058.2015.1038703. Epub 2015 May 22. PMID: 26000626; PMCID: PMC4776726.

[5]. Liehr T. False-positives and false-negatives in non-invasive prenatal testing (NIPT): what can we learn from a meta-analyses on > 750,000 tests? Mol Cytogenet. 2022 Aug 19;15(1):36. doi: 10.1186/s13039-022-00612-2. PMID: 35986330; PMCID: PMC9392255.

[6]. Hartwig TS, Ambye L, Sørensen S, Jørgensen FS. Discordant non-invasive prenatal testing (NIPT) - a systematic review. Prenat Diagn. 2017 Jun;37(6):527-539. doi: 10.1002/pd.5049. Epub 2017 Jun 1. PMID: 28382695.

[7]. Beulen L, Faas BHW, Feenstra I, van Vugt JMG, Bekker MN. Clinical utility of non-invasive prenatal testing in pregnancies with ultrasound anomalies. Ultrasound Obstet Gynecol. 2017 Jun;49(6):721-728. doi: 10.1002/uog.17228. PMID: 27515011; PMCID: PMC5488200.