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Cell-free DNA screening experience for samples received following an initial test failure or atypical result

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1. Introduction

Prenatal cell-free DNA (cfDNA) screening is performed using different technologies, each with their own strengths and weaknesses. Currently, the American College of Obstetricians and Gynecologists (ACOG) states that patients whose cfDNA screening test results are uninterpretable should be offered diagnostic testing.[1] Despite this recommendation, in some circumstances, a second cfDNA specimen may be sent to a different laboratory in an attempt to obtain a screening result for the patient. The current study examines 145 cases submitted for cfDNA rescreening using massively parallel sequencing (MPS) at one clinical laboratory following a failed, uninformative, or atypical cfDNA result from another laboratory that uses a different cfDNA technology.

2. Methods

Maternal blood samples submitted over a period of time for cfDNA rescreening after a failed, uninformative, or atypical cfDNA result from a laboratory using a SNP-based method of cfDNA analysis were identified by the test indication on the requisition form and/or from information provided by the ordering clinician. Rescreening samples were submitted for one of two MPS-based tests: traditional analysis (includes screening for trisomies 21, 18, and 13, with "opt-in" analysis for sex chromosome aneuploidies, select microdeletion syndromes, and trisomies 16 and 22) or genome-wide analysis (includes all content from traditional analysis, plus reporting of rare autosomal aneuploidies and copy number variants ≥7Mb in size). Samples submitted for MPS-based screening were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as described by Jensen et al.[2] For cases in which genome-wide analysis was ordered, sequencing data were analyzed using a novel algorithm to detect aneuploidies and other subchromosomal events as described by Lefkowitz et al.[3] The results of the MPS-based assay were compiled and analyzed for these cases.

For specimens with positive results from MPS-based screening, as per typical laboratory protocol, a mosaicism ratio was calculated. Mosaicism ratio (MR) is a laboratory metric derived by dividing the fraction of cfDNA associated with the abnormal event by the overall fetal fraction of the specimen, as described by Rafalko et al.[4] Cases with an MR <0.7 were assigned a "mosaic" comment from the laboratory director. "Mosaic" sequencing data may result from biological factors such as placental mosaicism or co-twin demise. Cases with a disproportionally high MR, in which the sequencing data was suggestive of a maternal event, were assigned a "maternal" comment by the laboratory director. In these circumstances, the robust signal produced by the suspected maternal abnormality precluded assessment of fetal status for that chromosome region. A maternal event typically does not affect interpretation of the rest of the genome, but if the maternal event is confirmed, the fetus is at typically at 50% risk to inherit the abnormality.

3. Results

145 specimens were submitted for MPS-based cfDNA screening following a failed result from a laboratory using a SNP-based cfDNA screening assay. There were 59 cases referred following an "atypical finding", 47 "uninterpretable DNA pattern", 22 "fetal fraction based risk", and 17 failures (not otherwise specified).

Of 145 cases for which rescreening was ordered, 50% (n=73) had MPS-based testing for traditional content and 50% (n=72) were tested for genome-wide abnormalities.

The average maternal weight of patients submitted for MPS-based rescreening was 168 lbs (median 154 lbs). The average fetal fraction of rescreened cases was 9% (median 8%), and the average gestational age was 16.6 weeks (median 14.9).

Among the 145 rescreened cases, 131 (90.3%) had reportable results using the MPS-based platform, including 57 positive (39%) and 74 negative (51%) results. (**Figure 1**) The positive cases consisted of: 35 aneuploidies with mosaic sequencing data (MR <0.7), 8 aneuploidies with non-mosaic sequencing data (MR ≥0.7), 2 cases with multiple aneuploidies, and 3 copy number variants. There were also 9 cases with suspected maternal events. (**Figure 2**) Of the 74 negative results, 13 included information from the sequencing data which might have explained the initial atypical or uninformative result. Specifically, the majority of these cases involved negative male results with an underrepresentation of X chromosome material, suggestive of low-level maternal or placental mosaicism for monosomy X.

The outcomes of MPS-based screening were analyzed by the type of SNP-based failure. The highest positivity rate (51%) in rescreened cases was observed following an "uninterpretable DNA pattern", followed by a 36% positivity rate in cases that were "high risk" based on a fetal fraction-based risk algorithm, 35% positivity in failures of unspecified type, and 32% in "atypical findings". (**Figure 3**)

4. Conclusions

90% of cases that failed analysis using a SNP-based cfDNA screening approach were reportable using an MPS-based assay

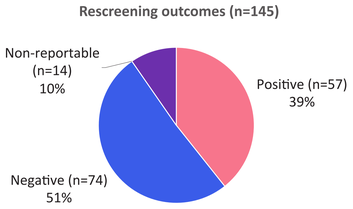
Failed, uninformative, or atypical test results may complicate counseling, increase patient anxiety, and delay appropriate pregnancy management. Diagnostic testing is the clear recommendation in these situations; however, in some cases, after shared decision-making, clinicians and patients have opted for rescreening using an alternate cfDNA technology. The data presented here suggest that cfDNA rescreening using an MPS-based technology can provide discrete results in ~90% of cases that could not be resulted using a SNP-based cfDNA assay. Overall, 39% of rescreened cases were positive for an abnormality from either traditional or genome-wide MPS-based screening.

There are several limitations to this study. The specimens submitted for rescreening were, by definition, drawn at a later gestational age than the initial SNP-based analysis (though the specific amount of time that elapsed between the two tests was unknown for the majority of cases). Advancing gestation is typically associated with an increase in fetal fraction which may have facilitated reporting in these cases. However, it should be noted that the average fetal fraction of the rescreened specimens was 9%, which is similar to the average fetal fraction previously documented in general screening populations using traditional and genome-wide MPS testing (8.7% and 9.6%, respectively)[5,6] at earlier average gestational ages (13.4 weeks and 14.8 weeks, respectively)[5,7]. Therefore, the role of enhanced fetal fraction in rescreened cases is expected to be minimal.

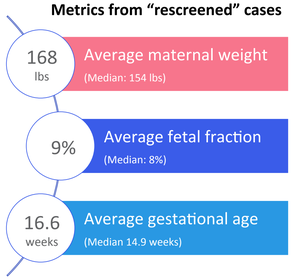
Another limitation was that this study only captured cases in which the ordering provider alerted the MPS laboratory that prior SNP-based screening had failed. Additional cases were likely submitted for rescreening that were not analyzed in this data set. Also, in many cases, the MPS laboratory did not have access to the original report from the patient's prior cfDNA screening, relying on the provider to accurately convey the reason for initial test failure. Lastly, diagnostic testing outcomes were not available as part of this study. Therefore, this authors cannot comment on the positive predictive value (PPV) associated with rescreening results. In general, though, the PPV of the rescreened cases that returned an abnormal result may be somewhat lower than that seen in a general screening population, as over 60% of the positive results showed mosaic sequencing data in the study population. As previously described, cases with depressed mosaicism ratios are associated with lower rates of diagnostic confirmation in the fetus, often due to biological limitations such as placental mosaicism or co-twin demise. Despite the potential for a lower PPV in rescreened cases, the ability to provide a result supports informed patient decision making. Therefore, if a rescreening pathway is being considered, expedited submission of the sample is encouraged to allow maximal time for diagnostic options and to promote appropriate management.

Tables + Figures

Figure 1: Overview of outcomes of MPS-based cfDNA screening in "rescreened" cases

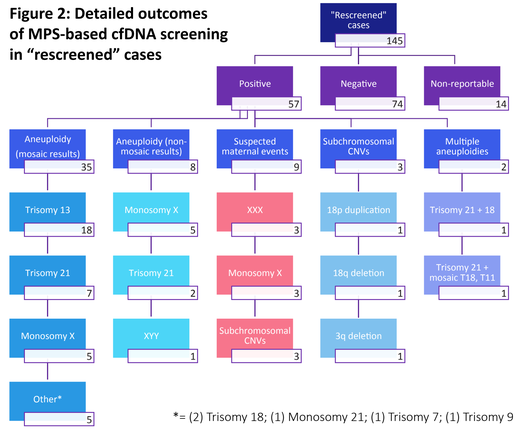


Metrics from "rescreened" cases



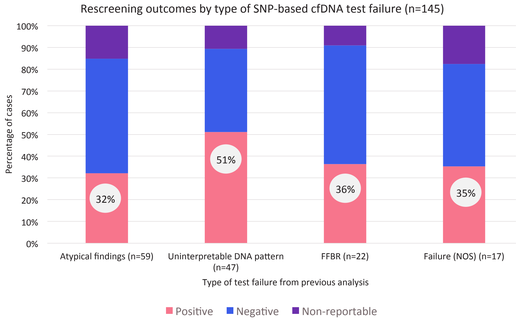
> "Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) should be…offered comprehensive ultrasound evaluation and diagnostic testing."
>
> -ACOG Practice Bulletin 226

Figure 2: Detailed outcomes of MPS-based cfDNA screening in "rescreened" cases



*= (2) Trisomy 18; (1) Monosomy 21; (1) Trisomy 7; (1) Trisomy 9

Figure 3: Outcomes of MPS-based "rescreening" by type of SNP-based test failure



Learning objective

The participant shall be able to analyze the efficacy of utilizing a cfDNA rescreening pathway using massively parallel sequencing following a failed, uninformative, or atypical result using an alternate cfDNA technology.

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