

# Implementing a Protocol to Optimize Detection of Chromosome Abnormalities in Cases of Miscarriage or Stillbirth at a Midwestern Teaching Hospital

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## ABSTRACT

**Context:** Results from chromosome testing after spontaneous abortion (SAB) or intrauterine fetal demise (IUFD) are useful in patient counseling; however, results can be inconclusive when cell cultures for chromosomes are unable to grow from products of conception. Chromosomal microarray analysis (CMA) can analyze DNA from nonviable fetal tissue.

**Objective:** To examine whether establishing a genetic testing protocol for karyotype and CMA on SAB and IUFD tissues increases diagnostic yield.

**Design:** A retrospective chart review was conducted in cases of SAB or IUFD when karyotyping and/or CMA was requested, comparing two periods: Preprotocol and postprotocol implementation.

**Main Outcome Measures:** Diagnostic yield was compared by using the number of determinate test results in the preprotocol and postprotocol study periods. A case was considered to have indeterminate results when the final genetic test results reported no fetal tissue or no cell culture growth.

**Results:** A total of 55 preprotocol and 52 postprotocol patients were analyzed. Diagnostic yield increased from 72.7% to 94.2% after implementation of the genetic testing protocol ( $p = 0.0004$ ). Indeterminate results occurred more frequently before compared with after implementation of the protocol.

**Conclusion:** A protocol of reflexing to CMA or proceeding directly with CMA gives a higher diagnostic yield in the genetic evaluation of SAB or IUFD. Institutions should consider implementing a genetic testing protocol to improve diagnostic yield. Our study results emphasize the importance of proceeding directly to microarray analysis and give support for current clinical recommendations for genetic testing after fetal demise.

## INTRODUCTION

Of clinically recognized pregnancies, 10% to 15% result in a spontaneous abortion (SAB, defined as a fetal loss at < 14 weeks).<sup>1-3</sup> Of these, approximately 65% to 70% of SAB cases and 8% to 13% of cases of intrauterine fetal demise (IUFD, defined as a fetal loss at > 14 weeks 0 days), also called stillbirth, are caused by chromosome aneuploidy.<sup>4-7</sup> Confirmation of a chromosomal cause, such as unbalanced translocations, deletions, and duplications, can be used to counsel patients regarding recurrence risk for future pregnancy losses.<sup>8,9</sup> Identification of aneuploidy as

the cause of SAB or IUFD can provide closure to the loss, can inform whether parental karyotype testing is indicated, and eliminates the need for other diagnostic evaluation.<sup>10</sup> However, culture failure compromises the diagnostic yield in 25% to 50% of SAB and IUFD cases.<sup>11,12</sup>

Chromosomal microarray analysis (CMA) has an advantage over karyotyping because it can analyze DNA from nonviable fetal tissue.<sup>13-15</sup> In a series of stillbirths that had both CMA and karyotyping, CMA provided a result in 87.4% cases vs 70.5% results with karyotyping, which demonstrated CMA to be sensitive in the

detection of chromosome anomalies.<sup>15</sup> Wapner et al<sup>16</sup> found that among fetuses with suspected growth or structural anomalies, 6.0% had clinically relevant findings using CMA that were not discovered with karyotyping.

Typically, chromosome analysis on SAB and IUFD tissues is offered in recurrent pregnancy loss, or if the fetal ultrasound scan or postnatal examination of the fetus detects anomalies suggestive of aneuploidy.<sup>10,17</sup> The American College of Obstetricians and Gynecologists (ACOG) recommends that patients with an IUFD be offered karyotype analysis and that amniocentesis for fetal karyotyping has the highest yield.<sup>10</sup> The Society for Maternal Fetal Medicine (SMFM) recommends that microarray analysis be offered for patients with IUFD.<sup>18</sup> Variations in professional recommendations make genetic testing after pregnancy loss unclear to clinicians and may potentially limit the diagnostic yield or results.<sup>10,19</sup>

The purpose of this study is to report the diagnostic yield after implementation of a genetic testing protocol that uses karyotyping with reflex to CMA when culture failure occurs. The primary outcome for this study was diagnostic yield, which is based on the proportion of determinate test results.

## METHODS

This project was a retrospective cohort study evaluating genetic testing results during two periods: Preprotocol and postprotocol. A local institutional review board approved this study.

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### Study Population

This study included a consecutive series of patients with SAB or IUFD between March 21, 2012, and April 1, 2014, for whom karyotyping, CMA, or both was requested. Patients were included in the preprotocol cohort if testing was ordered between March 21, 2012, and March 21, 2013. Patients were included in the post-protocol cohort if testing was ordered between April 1, 2013, and April 1, 2014. All testing results had been completed and results were obtained before initiation of this research study. Study personnel and the genetic counselor reviewed genetic test results and collected the following data: Clinical history, laboratory results, outcomes of SAB or fetal demise, and further information as necessary to complete the clinical history. All samples in the study consisted of tissue from SAB and IUFD. Amniotic fluid was used in 2 cases.

### Genetic Testing Protocol for Miscarriage and IUFD (Stillbirth)

In March 2013, our institution implemented a genetic testing protocol to optimize diagnostic yield for SAB and IUFD. The genetic testing protocol allowed physicians to use 2 genetic testing options: Chromosome testing with reflex to CMA if there is no cell culture growth, or proceeding with CMA directly. The physician completed the genetic testing order form and an intake form detailing maternal and fetal history. At our institution, the genetic counselor is available to discuss the case with the ordering physician to determine if proceeding directly to CMA would be more conclusive or if maternal blood should be sent along with the SAB or IUFD sample. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes. Tissues from the cord insertion site, fetus, and/or placenta were collected in 1 cm × 1 cm blocks of tissue and sent to the laboratory in lactated Ringer's solution at room temperature. All genetic testing was performed by LabCorp (Burlington, NC). The CMA platform used was single nucleotide polymorphism-based microarray.

The indications for testing and maternal cell contamination (MCC) studies along with sample collection methods were as follows. Blood for MCC study

Characteristic	Pre (n = 55)	Post (n = 52)	p value
<b>Genetic test performed, no. (%)</b>			
Karyotyping reflex to chromosome microarray analysis	1 (1.8)	12 (23.1)	< 0.0001
Karyotyping only	53 (96.4)	19 (36.5)	
Chromosome microarray analysis only	1 (1.8)	21 (40.4)	
<b>Fetal sex, no. (%)</b>			
Male	18 (35.3)	13 (27.7)	0.3923
Female	15 (29.4)	11 (23.4)	
Indeterminate	18 (35.3)	23 (48.9)	
Missing records <sup>a</sup>	4	5	
<b>Other characteristics</b>			
Maternal age at delivery, years, mean (SD)	31.3 (6.4)	28 (5.7)	0.0076
Median no. of prior spontaneous abortions <sup>b</sup>	0 (0-1)	1 (0-2)	0.1813
Median no. of prior stillbirths <sup>b</sup>	0 (0-0)	0 (0-0)	0.1778
Gestational age in weeks, mean (SD)	24.2 (9.8)	19.4 (10.3)	0.0261

<sup>a</sup> Outcome could not be determined because of missing records and thus were excluded.

<sup>b</sup> Values are presented as the median (first quartile-third quartile).

Post = postprotocol; Pre = preprotocol; SD = standard deviation.

was requested and sent with the SAB or IUFD specimen if the fetal demise was less than 12 weeks of gestation, sex was not determined before demise, or there was concern about the source of the tissue collected after dilation and curettage. In such cases, MCC studies were done as reflex to karyotype analysis or concurrent with microarray testing. Blood for MCC study was not collected at the time of fetal specimen collection if the demise was greater than 12 weeks of gestation because the source of the tissue was clear (cord insertion site or tissue directly from the fetus) and the chance for MCC was low. In such cases, MCC was requested after receipt of karyotype and/or microarray results if results of testing were discrepant from the sex noted on the ultrasonogram or post-delivery physical examination. Blood for MCC study was not routinely collected on all cases because of the laboratory costs that could be avoided in cases where it would not be as informative (specimen directly from the fetus or cord insertion site). MCC testing was performed by the laboratory that analyzes short tandem repeat markers by polymerase chain reaction and capillary electrophoresis. In cases of recurrent miscarriage (defined as 2 or more unexplained instances of SAB) or fetal demise before 12 weeks' gestation, the protocol suggested directly performing CMA because the chance of a chromosome deletion or duplication is

higher and miscarriage specimens before 12 weeks are frequently contaminated with maternal cells.<sup>20,21</sup> Each patient consented to genetic testing.

### Evaluation

Each case was reviewed, and genetic testing results were designated either as determinate or indeterminate on the basis of the final result received. Diagnostic yield was based on the number of determinate test results, or the number of cases per period when testing was desired and results were obtained. A case was considered to have determinate results when one of the following genetic testing outcomes resulted: Normal female karyotype, normal male karyotype, or abnormal. A case was considered to have indeterminate results when the final genetic testing results reported no fetal tissue or no cell culture growth. In cases in which karyotyping occurred with reflex to CMA, the CMA result was the final outcome and was designated as determinate (definite result reported) or indeterminate (no definitive result reported). Results of the testing, including MCC studies, were not the focus of this project and are not included in this article.

### Statistical Analysis

All statistical analyses were conducted using SAS for Windows Version 9.3 (SAS Institute, Cary, NC). Descriptive statistics are presented as means and standard

deviations (SDs) for continuous variables, and frequencies and proportions for categorical variables. A baseline proportion of having a determinate diagnostic yield before the protocol was calculated. A post-protocol proportion of having a determinate diagnostic yield was also calculated. A comparison between the preprotocol and postprotocol proportions was conducted using a 1-sided Z test. A power analysis revealed 64 cases per group was required to achieve at least 80% of power. The assumption was 20% having determinate findings during the preprotocol period and 40% of cases having determinate findings in the postprotocol period. The statistical test was 1-sided on the basis of the theory that the implementation of the standardized protocol will improve the percentage of cases having determinate findings. A p value less than 0.05 was considered to be statistically significant.

**RESULTS**

A total of 107 patients were included in the final analysis, with 51.4% (n = 55) in the preprotocol cohort and the remaining 48.6% (n = 52) in the postprotocol cohort. Postprotocol patients were younger in age than preprotocol patients, with a mean age of 28 (SD = 5.7) years compared with age 31.3 (SD = 6.4) years (p = 0.008). Gestational age at fetal demise was also significantly different, with a mean of 19.4 (SD = 10.3) weeks in the postprotocol cohort compared with 24.2 (SD = 9.8) weeks in the preprotocol cohort (p = 0.026). In the preprotocol cohort, the majority (96.4%, n = 53) underwent karyotyping only. In the postprotocol cohort, 36.5% (n = 19) underwent karyotyping only, and the remaining 63.5% of patients (n = 33) proceeded directly to CMA or reflexed to CMA after karyotyping (Table 1).

Diagnostic yield increased from 72.7% to 94.2% after implementation of the testing protocol (p = 0.0004). In the preprotocol cohort, 27.3% (n = 15) had indeterminate results owing to no fetal tissue growth (Figure 1). In comparison, in the postprotocol cohort, results were not obtained in 5.7%, or 3 cases (Figure 2). In those 3 cases, results were not obtained because no fetal tissue was available to attempt DNA extraction for CMA. In the preprotocol group, 1 case underwent

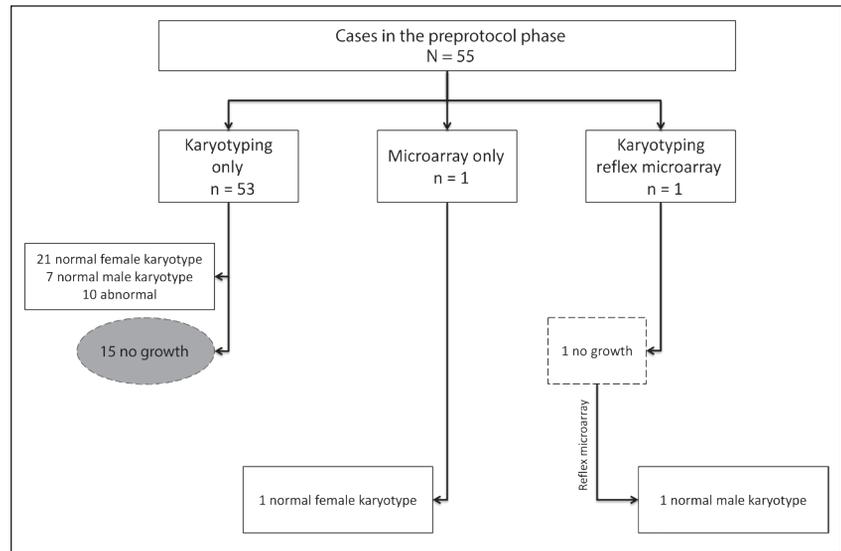


Figure 1. During the preprotocol phase, most women underwent karyotyping, with only one case that yielded no cell culture growth proceeding to chromosomal microarray analysis.

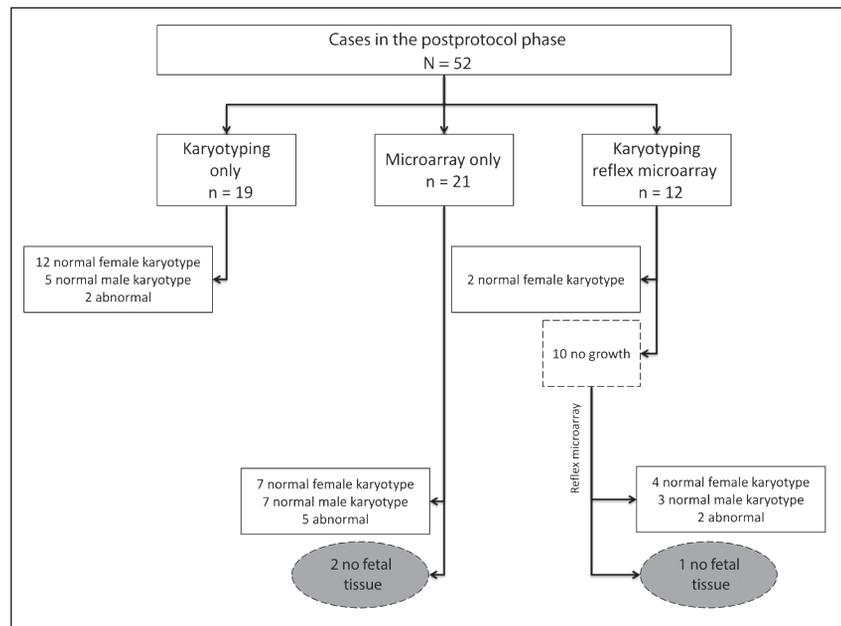


Figure 2. During the postprotocol phase, reflex to chromosomal microarray analysis after karyotyping yielded no cell culture growth eventually led to determining fetal karyotype in most cases.

karyotyping, with an indeterminate result (no growth), and then reflexed to CMA and yielded a determinate result (normal male karyotype). In the postprotocol cohort, among 10 patients who underwent CMA after karyotype yielded no growth, 9 received a determinate result and 1 case resulted in no fetal tissue growth.

**DISCUSSION**

Our study evaluated a genetic testing protocol that was implemented with the goal of improving the likelihood of a determinate result. Our results demonstrate an improved rate of obtaining determinate results using a protocol in which CMA was performed by default if there was no

growth of cell culture after karyotyping. Our findings from this study are consistent with previous reports in which CMA improves diagnostic yield<sup>19</sup>; however, our study findings demonstrate the utility of karyotyping, with reflexing to CMA when there is no growth.

The genetic testing protocol at our institution permits karyotyping with reflex to microarray or direct microarray analysis, and this reflects the practice suggested by the recent SMFM guidelines and has a higher diagnostic yield than just karyotyping alone.<sup>18</sup> However, ACOG guidelines on evaluation after stillbirth recommend karyotype testing.<sup>10</sup> This lack of consistency between ACOG and SMFM guidelines regarding genetic evaluation after pregnancy loss has the potential to be unclear to clinicians and may potentially limit diagnostic yield or results. There are limitations to CMA such as inability to detect balanced translocations. However, in the context of evaluation of SAB and IUFD, this limitation is mitigated by the fact that unlike unbalanced translocations, a balanced translocation in the fetus typically does not lead to SAB or IUFD. Therefore, parental karyotypes are indicated only if fetal CMA indicates a chromosome abnormality that could be inherited from a parent. Tetraploidy is a rare condition and is not detected with CMA, which is a limitation. Unlike karyotyping, interpretation of microarray can be complicated when it detects variants of uncertain clinical significance or clinically significant deletions and duplications that are not associated with SAB or IUFD. Our genetic testing protocol suggests ordering a karyotype test with reflex to microarray analysis when prenatal findings are suggestive of common chromosome aneuploidy consistent with the recommendations from SMFM.<sup>18,19</sup>

We understand that in the current climate, increased health care costs associated with additional testing is a concern. Although we are unable to comment on the cost-effectiveness of our protocol, we believe that further research on this topic is warranted. Harper et al<sup>22</sup> conducted an economic analysis on prenatal cytogenetic technologies for fetal anomalies and determined that CMA alone was the

most effective strategy. A similar study on cost-efficacy and diagnostic yield of karyotyping vs CMA in the evaluation of SAB and stillbirth has not been published, to our knowledge. Shah et al,<sup>23</sup> in their study of miscarriage specimens, comment that out-of-pocket costs for chromosomal and microarray analysis varied widely but was covered by most insurance providers at 90% to 100% of the total cost. Similarly, at our institution, the out-of-pocket cost to the patient varies depending on the insurance provider. It is expected that if karyotyping did not reveal results, CMA would be covered by the insurance company in order to yield a result that is meaningful regardless of whether it is positive or negative. Again, further research regarding a cost-effective testing protocol in cases of SAB and IUFD is needed to inform development of guidelines.

In our cohort, two patients had testing on amniotic fluid. In both cases, there was no growth on cell culture. Our small sample size precludes assessment of diagnostic yield on amniotic fluid as a sample type. Current ACOG guidelines recommend obtaining an amniotic fluid specimen for genetic testing before delivery of the IUFD (termed stillbirth by ACOG).<sup>10</sup> Using a protocol of proceeding directly to CMA is likely to increase diagnostic yield without subjecting the patient to an invasive procedure, such as amniocentesis, that has suboptimal diagnostic yield. However, further research is warranted to assist with creating appropriate clinical guidelines.

Strengths of our study include minimal risk of selection bias because cases were included in the review regardless of presence of anomalies and gestational age at fetal demise. Results from our study are limited by a small sample size that precludes analysis of correlation of diagnostic yield with timing of the demise or correlation of diagnostic yield with the presence or absence of fetal anomalies. Selection bias is also a limitation of this study, which is evidenced by the difference in maternal age and weeks of gestation at demise between the two cohorts. Another limitation is the inconsistency in documentation in electronic medical records, from which information about

timing of demise or ultrasonographic anomalies can be gathered reliably and consistently. With this considered, the main goal of our institution's genetic testing protocol was to improve diagnostic yield regardless of the presence or absence of apparent anomalies or gestational age at fetal demise. We were able to achieve this by having a standardized order set that facilitated the physician when ordering genetic testing on cases of SAB or IUFD. Further research is needed to assess whether proceeding directly to CMA in all cases is more cost-effective in assessing for chromosomal defects as a cause of fetal demise. Such an analysis should take into account charges for karyotyping with reflex to microarray, costs for direct microarray, and the utility of MCC on all cases vs selecting those in which there is higher possibility of MCC. Ultimately, use of a test that consistently obtains results may portend that CMA alone is the best test in cases of SAB or IUFD.

## CONCLUSION

Our study results suggest that a protocol with defined indications for chromosomal testing with reflex to CMA or direct CMA improved the diagnostic yield in a community-based hospital setting. Many genetic testing laboratories offer the option of chromosome tests with reflex to CMA. Institutions should consider using this option in a SAB or IUFD testing protocol that also integrates review of the indication for testing and sample type with a genetic counselor to maximize diagnostic yield. ❖

## Disclosure Statement

*The author(s) have no conflicts of interest to disclose.*

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## Author Contributions

*Shobana Kubendran, MBBS, MS, CGC, contributed to the design and implementation of the study, interpretation of results, drafting of manuscript, and critical revision. Jennifer Duong, MPH, contributed to the design and implementation of the study, interpretation of results, drafting of manuscript, and critical revision. Fanglong Dong, PhD, performed data analysis, interpretation of results, drafting of manuscript, and critical revision. Amy Lueking, MD, contributed to the design*

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## Hypothesis

It is difficult to resist the fascinating assumption that the gene is constant because it represents an organic chemical entity. This is the simplest assumption that one can make at present, and since this view is consistent with all that is known about the stability of the gene, it seems, at least, a good working hypothesis.

— Thomas Hunt Morgan, 1866-1945, American revolutionary biologist, geneticist, embryologist, and science author; 1933 Nobel Prize winner in Physiology or Medicine