

Justification for Coverage of Microarray Analysis on Products of Conception

A review of relevant literature and proposal for coverage decisions

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1. Purpose

1.1. Purpose of proposal

The purpose of this document is to initiate a discussion on the use of chromosome microarray technology for the analysis of products of conception (POC) specimens. Multiple pieces of evidence support the use of microarray testing in this setting, and the application of microarray technology to the analysis of POCs is fast becoming a preferred testing strategy for managing patients with multiple miscarriages.

We believe that the currently available data strongly support microarray as a superior technology compared to conventional karyotyping in the setting of recurrent pregnancy loss (RPL).

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3. Executive Summary

Pregnancy loss occurs in approximately 25-30% of all clinically recognized pregnancies, and recurrent pregnancy loss, defined as ≥ 2 unexplained miscarriages, affects approximately 1% of all couples (ASRM, 2012; Ford and Schust, 2009). Evaluation of the miscarriage tissue/products of conception for chromosomal abnormalities can help define the etiology of the loss and thus instruct future pregnancy management and recurrence risk counseling (Carp, 2001; Zhang, 2009). For the past three decades, chromosomal analysis has been performed by karyotyping, however, karyotyping of products of conception (POCs) often proves disappointing because between 25-55% of the time, cell culture fails and no result is obtained (Lomax et al., 2000; Bell et al., 1999; Robberect et al., 2009). Unlike karyotyping, microarray analysis does not rely upon cell culture, which means that microarray analysis provides results in approximately 95% of cases, compared to a 45-75% success rate with conventional karyotyping. In addition, microarray analysis can be performed directly on fresh POC tissue as well as on samples for which a karyotype cannot be performed, such as formalin-fixed, paraffin-embedded (FFPE) POC tissue.

In order to provide optimized, cost-effective care for couples with recurrent pregnancy loss (RPL), it is important to determine whether the pregnancy was miscarried as the result of a chromosomal abnormality or possibly another factor, indicating that additional testing is warranted. Approximately 50-60% of all first trimester pregnancy losses are due to chromosomal abnormalities, regardless of whether they have occurred sporadically or in a couple with RPL (Foyouzi et al., 2012; Shearer et al., 2011). Some of these abnormalities are sporadic, and unlikely to reoccur, while others confer an increased risk of recurrence and will require the offer of prenatal diagnosis in future pregnancies. In cases where the chromosomes are normal, an evidence-based work-up can be initiated to try to identify the etiology of the RPL (ASRM, 2012; Foyouzi et al., 2012). Thus, the results of microarray analysis direct the medical management of couples who have experienced RPL and are attempting to conceive. A positive result has a direct impact on genetic counseling for the couple, and a negative result directs the in-depth evidence based workup for endocrinologic, hematologic, anatomic, or immunologic imbalances in the mother.

The consensus of the data indicates that microarray analysis has a superior success rate, faster turn-around-time, broader ability to analyze multiple sample types, and is cost-effective.

4. Background Information

4.1. Definition and Etiology of Products of Conception (POC)

4.1.1. Definition

Fetal loss is the most common pregnancy complication, occurring in 25-30% of recognized pregnancies. Recurrent pregnancy loss (RPL) affects at least 1% of all couples (Ford and Schust, 2009) and can be defined as two or more failed pregnancies (van den Boogaard, 2010; ASRM, 2012). Pregnancy losses are further subdivided depending on when the loss occurred. Fetal loss prior to 20 weeks of gestation has been termed a “spontaneous abortion”, and the fetal and placental tissue are referred to as products of conception (POC). Fetal loss after 20 weeks of gestation has been termed “stillbirth” (Smith, 2007; Warren, 2008; Reddy, 2012a).

4.1.2. Etiology

Several potential factors have been implicated in recurrent pregnancy loss, including a spontaneous, *de novo* chromosomal abnormality in the fetus, an unbalanced fetal chromosomal complement as the result of a balanced translocation in one of the parents, abnormal uterine anatomy, and endocrinologic, immunologic, metabolic, or thrombotic issues in the mother (reviewed in Stephenson, 2007; ASRM, 2012). The most common etiology of early pregnancy loss is chromosome abnormalities in the fetus, which account for at least 50 - 60% of all first trimester losses, including those in cases of RPL (see below, and Shearer, 2011; Reddy, 2012a; Gao, 2012; Stephenson, 2007). Chromosome abnormalities in stillbirth are found at a rate of 8-10% (see below, Reddy et al., 2012b).

4.2. Karyotyping of POCs

4.2.1. Rationale for karyotyping

Because chromosomal abnormalities have been observed in over half of early pregnancy losses, and >90% of those represent numeric chromosomal abnormalities (trisomies, monosomies, polyploidies), karyotyping has historically been employed to assess fetal chromosome status. Table 1 summarizes the current literature on the types of chromosomal changes most frequently detected in POC samples.

Table 1. Chromosomal Abnormalities in POC/Spontaneous Abortion Samples

Study	Ljunger E, et al. Acta Obstet Gynecol Scand. Nov 2005;84:1103-7.	Robberecht C, et al. Genet Med. Sept 2009;11(9):646-54.	Shearer BM, et al. Genet Med. Jun 2011;13(6):545-52.
Sample Size	259	103	2040
Notes	Karyotyping only	Karyotyping and array	Karyotyping and FISH
Autosomal Trisomies	88 (34%)	8 (8%)	1175 (58%)
>1 Aneuploidy	7 (3%)	1 (1%)	52 (3%)
Triploidy	0 (0%)	17 (16%)	268 (13%)
Monosomy X	15 (6%)	8 (7%)	303 (15%)
Other SCAs	14 (5%)	2 (2%)	7 (<1%)
Structural	12 (4%)	3 (3%)	105 (5%)

The finding of a chromosomal abnormality provides a diagnosis for the loss, reduces the need for additional testing, and leads to a better prognosis for subsequent pregnancies (Carp et al., 2001).

4.2.2. Issues with karyotyping

Since the majority of chromosomal abnormalities seen in POCs are whole chromosome aneuploidies, the issue of resolution is not as critical in the analysis of POCs as compared to prenatal or pediatric cases. Thus, if the karyotype is successful, the underlying genetics of pregnancy loss are readily identified. However, as karyotyping requires live, growing cells in cell culture, this testing method leads to longer turn-around time for results, has a significant risk of not obtaining any results due to a culture failure rate (20-65%, see Reddy, 2012a; Raca, 2009). In addition, during culture where fetal and maternal cells are mixed, the selective overgrowth of maternally derived cells, which leads to a normal female karyotype despite an underlying fetal abnormality, occurs in 29-58% of cases. (Lomax et al., 2000; Bell et al., 1999; Robberecht et al., 2009).

These frequent failure modes have resulted in many clinicians deciding to forego karyotype analysis on POCs, despite the clinical utility of the data. The frustration of waiting for three weeks only to learn of a failed cell culture or the overgrowth of maternal cells is upsetting to patients and their families, and for this reason, many clinicians have gradually moved away from POC testing altogether (personal communications with OB/GYNs and MFMs).

5. Microarray Use in POC Analysis

5.1. Benefits of using microarray testing in the analysis of POCs

Chromosomal microarray analysis (whether by oligonucleotide or single nucleotide polymorphism array) offers a more complete, more dependable, and faster means of analyzing the fetal chromosomes. Most importantly, the need for cell culture is eliminated, which leads to a significantly improved result yield, regardless of the type of array used (Schaeffer, 2004). However, the use of single nucleotide polymorphism (“SNP”) arrays also provides the additional benefits of the ability to identify polyploidy (which is common cause of spontaneous pregnancy loss) and maternal cell contamination (Scott, 2010).

5.2. Data for utility of microarrays in early pregnancy loss

There have been multiple studies which demonstrate the increased clinical utility of microarray analysis compared to conventional karyotyping in early pregnancy loss/POCs:

- **Schaeffer et al. (2004)** used a targeted BAC array analyze 41 POCs. Microarray identified all 13 abnormalities identified by karyotyping as well as four pathogenic chromosomal imbalances that were not detected by karyotyping.
- **Le Caignec et al. (2005)** used a targeted large-insert clone array to examine 49 fetuses with multiple structural anomalies from pregnancies that either ended in a spontaneous abortion or were medically terminated, the authors found that the detection rate for chromosomal imbalances known to be pathogenic was 8.2% (4 of 49).
- **Zhang et al. (2009)** analyzed 115 first trimester miscarriages using conventional karyotyping versus a 244K oligonucleotide array. Karyotyping was performed in the 92 cases for which cell culture was successful. In the 23 cases (20%) for which cell culture failed, microsatellite analysis was performed. Combining these two methodologies, cytogenetic abnormalities were identified in 57 of 115 cases. Microarray analysis was then performed for the 58 samples in which no chromosomal abnormality had been identified, and identified five clinically significant copy number variants not detected by karyotyping or microsatellite analysis.
- **Warren et al. (2009)** identified a prospective series of 35 women with pregnancy losses occurring between 10 and 20 weeks of gestation who had either had a normal karyotype (N=9) or no cytogenetic testing (N=26) and attempted to analyze the samples using a 244K oligonucleotide array. Microarray analysis identified de novo chromosomal imbalances not previously detected in 13% of the cases.
- **Reddy et al. (2012b)** compared conventional karyotyping to microarray analysis in 532 samples obtained from stillbirths. A single-nucleotide polymorphism (SNP) array was used to detect copy-number variants of at least 500 kb in placental or fetal tissue. The CNV results were grouped into three categories if they were not found in databases of benign polymorphisms: pathogenic, likely benign, or variant of unknown clinical significance.

Microarray analysis yielded results more often than karyotype analysis (87.4% vs. 70.5%) and provided improved detection of genetic abnormalities which included aneuploidy or pathogenic copy-number variants (8.3% vs. 5.8%). Microarray analysis also identified more cytogenetic abnormalities among 443 antepartum stillbirths (8.8% vs. 6.5%) and 67 stillbirths with congenital anomalies (29.9% vs. 19.4%). As compared with karyotype analysis, microarray analysis provided a relative increase in the diagnosis of cytogenetic abnormalities of 41.9% in all stillbirths, 34.5% in antepartum stillbirths, and 53.8% in stillbirths with anomalies. The study concluded that microarray analysis is more likely than karyotype analysis to provide a cytogenetic diagnosis, primarily because of its success with nonviable tissue, and is especially valuable in analyses of stillbirths with congenital anomalies or in cases in which karyotype results cannot be obtained.

6. Clinical Practice and the Genetics of POCs

6.1. Handling of recurrent pregnancy loss couples

6.1.1. Identification of etiologic factors contributing to recurrent pregnancy loss

The clinical and laboratory evaluations recommended for couples experiencing RPL include: genetic counseling and assessment of parental karyotypes, imaging of the uterine anatomy through radiographic studies, and blood work to evaluate for endocrine, metabolic, thrombophilic, immunologic and genetic factors (ASRM, 2012; ACOG, 2002; Stephenson, 2007). Given the complex nature of identifying the etiology of RPL, recent decision-analytic model studies have proposed that RPL workup should begin with chromosomal analysis of the POCs with a reflex to the full evaluation if the fetal chromosomes are normal. These studies have shown that a reflex method of care would be cost effective and would increase the diagnostic yield (see below, Bernardi, 2012; Foyouzi, 2012).

6.2. Analysis of cost savings for utilization of microarrays in POCs

Foyouzi et al. (2012) performed a cost analysis to compare the cost of obtaining an evidence-based workup (EBW) for RPL versus obtaining a karyotype of a POC sample and proceeding with an EBW only in the setting of a normal fetal karyotype. Their analysis determined that there is a significant economic advantage to performing a karyotype after the second pregnancy loss versus automatically initiating the EBW. In addition, evidence has shown that cell culture failure plays a major role in the decreased result rate of karyotyping. Because microarray testing does not require cell culture, the possibility of achieving a result from a POC sample is significantly higher than with karyotyping. Based on the reasonable cost estimates and the higher success rate for obtaining a result, it can be concluded that performing microarray testing on a POC after a second pregnancy loss has a significant cost advantage over obtaining an EBW.

7. Proposal for Coverage Decision

The proposal for coverage for use of microarray analysis of POC in RPL would consist of adopting microarrays as the first line technology. For this purpose, RPL would be defined as the second or greater pregnancy loss occurring prior to 20 weeks of gestation.

Multiple lines of evidence support this concept:

1. Microarray analysis of POCs has a demonstrated success rate which is superior to conventional karyotyping (95% success rate, compared to 45 – 65% success rate).
2. Microarrays can be performed on formalin-fixed, paraffin embedded samples (FFPE). Because karyotyping requires growing cells, testing of FFPE samples is not possible.
3. Microarrays have a faster turn-around-time. Microarray analysis can be performed in 5-7 days, while culturing for karyotyping takes 12-15 days. Before a culture is considered a failure, the sample must be grown for 21 days before the culture is discontinued.
4. Two independent studies support the cost-effective approach of utilizing chromosome analysis in RPL. Thus, the overall cost of utilizing microarrays is favorable in this setting.

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