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CLINICAL ARTICLE

Patient decisions regarding prenatal aneuploid fluorescence *in situ* hybridization results

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Abstract

Objective: To determine an appropriate risk cut-off to offer prenatal aneuploid FISH, and if FISH results affect patient decisions regarding pregnancy management. *Method*: Retrospective evaluation of 707 patients presenting for diagnostic prenatal testing. Studied parameters included gestational age, indication for testing, aneuploid risk, procedure performed, FISH (whether offered, requested, and/or performed), result turn-around time, karyotype results, decision after obtaining results, and the timing of that decision. Patients who were offered FISH were compared to those not offered FISH (student *T*-test). *Results*: Twenty-five clinically significant abnormalities were detected by karyotype and/or FISH analysis. Thirteen out of 17 patients electing pregnancy interruption had FISH performed. There were no differences between the group that interrupted following FISH ($n=7$) and the group that interrupted following final karyotype results ($n=6$). Turn-around times for those abnormal samples with FISH testing was significantly shorter than for those without FISH testing ($p=0.02$). Risk thresholds of $\geq 0.5\%$, $\geq 1\%$, $\geq 2\%$, or $\geq 3\%$, would detect 92%, 84%, 48%, and 32% of the clinically significant anomalies with 663, 317, 118, and 66 FISH analyses performed, respectively. *Conclusion*: Acting on FISH results alone afforded a significantly shorter interval between test and pregnancy interruption. A risk cut-off $\geq 1\%$ appears to optimize the detection rate and the yield of abnormal results.

1. Introduction

The American College of Obstetrics and Gynecologists (ACOG) provides recommendations regarding which patients should be offered prenatal diagnostic testing [1]. Prenatal aneuploid fluorescence *in situ* hybridization (FISH) interphase analysis is an adjunct test to standard karyotype analysis. Interphase FISH has the benefit of not requiring cell culture, thereby allowing for a quicker turn-around time. Since the FISH technique involves the testing of only certain regions of specific chromosomes, the process has inherent limitations [2].

Several studies [3–8] have evaluated the detection rate of fetal chromosome abnormalities by prenatal aneuploid FISH. The detection of clinically important chromosomal abnormalities using prenatal aneuploid FISH ranged from approximately 82% [4,6] to 94% [7] with relatively low false-positive

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Table 1 Comparison of patients offered and accepted FISH and those who declined

Characteristic	Declined FISH ($n=10$)	Accepted FISH ($n=101$)	*P*-value
Gestational age (weeks)	19.3 range (16–29)	18.5 range (10–35)	0.56
Aneuploidy risk (%)	1.90 range (1.00–7.50)	4.17 range (0.21–50)	0.26

rates (from 0 [4] to 0.15% [5]), and uninformitivity rates (from 0.6% [8] to 2.3% [4]). Pergament et al. [6] further warn of the possibility for discordant results between FISH and routine karyotyping.

Although there have been several studies assessing the utility of prenatal aneuploid FISH, few studies have evaluated when prenatal aneuploid FISH should be offered to patients pursuing diagnostic testing. Furthermore, little is known about patient decisions made solely based on FISH results. This study sought to determine an appropriate risk cut-off to offer FISH, as well as the impact of the FISH results on patients' decisions regarding pregnancy outcome.

2. Materials and methods

This retrospective analysis evaluated 707 patients who presented between January 1, 2002 and December 31, 2004 at Georgetown University Hospital. All patients who elected diagnostic testing solely for chromosome analysis were included in the study.

Patients were offered prenatal aneuploid FISH when the risk for fetal aneuploidy was 1% or greater or when diagnostic testing took place at a gestational age of 20 weeks or greater. However, when the aneuploidy risk was based solely on maternal age, prenatal aneuploid FISH was not consistently offered. The FISH test used a standard panel of commercially available probes for chromosomes 13, 18, 21, X, and Y (AneuVysion®, Abbott Laboratories, Downers Grove, IL). Patients were counseled regarding the limitations of FISH. The need for routine chromosome analysis was discussed as the gold standard. Patients were also counseled that insurance may or may not cover the cost of FISH testing, approximately $350. No additional sample from the amniocentesis or chorionic villus sampling was required for FISH.

The charts of eligible patients were reviewed for: year of counseling and testing, indication, gestational age, cited risk for chromosome abnormality, and procedure performed. Regarding FISH analysis, charts were reviewed for whether the test was offered or requested by patient, whether FISH was performed, turn-around time for FISH and routine cytogenetic results, and FISH results. Karyotype results, discrepancies between karyotype and FISH results, decision to interrupt the pregnancy, the timing of that decision (whether decision was made based on FISH results or if patient waited for karyotype analysis), and which laboratory performed the analysis were also evaluated. Three of the four laboratories used perform prenatal FISH. If FISH was desired, the specimen was sent to one of the three. The laboratory used was dependent upon the patient's insurance.

The turn-around time for FISH and routine cytogenetic results were compared using the Student's *t*-test. A *p*-value of <0.05 was considered significant. Those offered and accepting FISH were compared to those declining the test for gestational age and risk quoted; those offered FISH were compared to those requesting FISH for these same parameters. The subgroup of those with clinically significant chromosome abnormalities was further evaluated. Gestational age, risk, maternal age, gravidity, parity, and decision regarding whether to continue or interrupt the pregnancy were compared in those who were offered FISH and those who were not offered FISH using the Student's *t*-test and χ^2 analysis. For those electing not to continue the pregnancy, the timing of that decision (whether following the FISH results alone or following the karyotype results) was recorded. As a final outcome, the detection rate compared to the number of studies performed for FISH-detectable aneuploidies was calculated using risk cut-offs of $>0.5\%$, $>1\%$, $>2\%$, and $>3\%$.

3. Results

Between January 1, 2002, and December 31, 2004, 707 diagnostic procedures (653 amniocenteses and 54 chorionic villus sampling [CVS] procedures) were performed. FISH was offered to 111 patients; of these, 10 declined, electing only routine karyotype studies. An additional 49 patients requested FISH for a total of 150 FISH tests. There were no inconsistencies between the FISH results and final karyotype results.

The gestational age and risk for chromosome abnormality between those accepting FISH and those declining FISH, and those offered FISH and those requesting FISH are compared in Tables 1 and 2, respectively. No statistically significant differences were found between those accepting and those declining FISH. Additionally, there was no statistically significant difference between those requesting FISH and those offered FISH because of advanced gestational age. Statistically significant differences were noted for the aneuploidy risk between those requesting FISH and those offered FISH ($p=0.001$) (Table 2), with those being offered FISH having a greater risk. Turn-around time was evaluated for those with abnormal results comparing the patients having FISH and those not having FISH. Seventeen of the 19 abnormal FISH results were performed by a single laboratory; therefore, inter-laboratory comparisons could not be made. Statistical significance was achieved between these two groups regarding time for final results ($p=0.02$) with the karyotype results from those having FISH available in 8.6 days

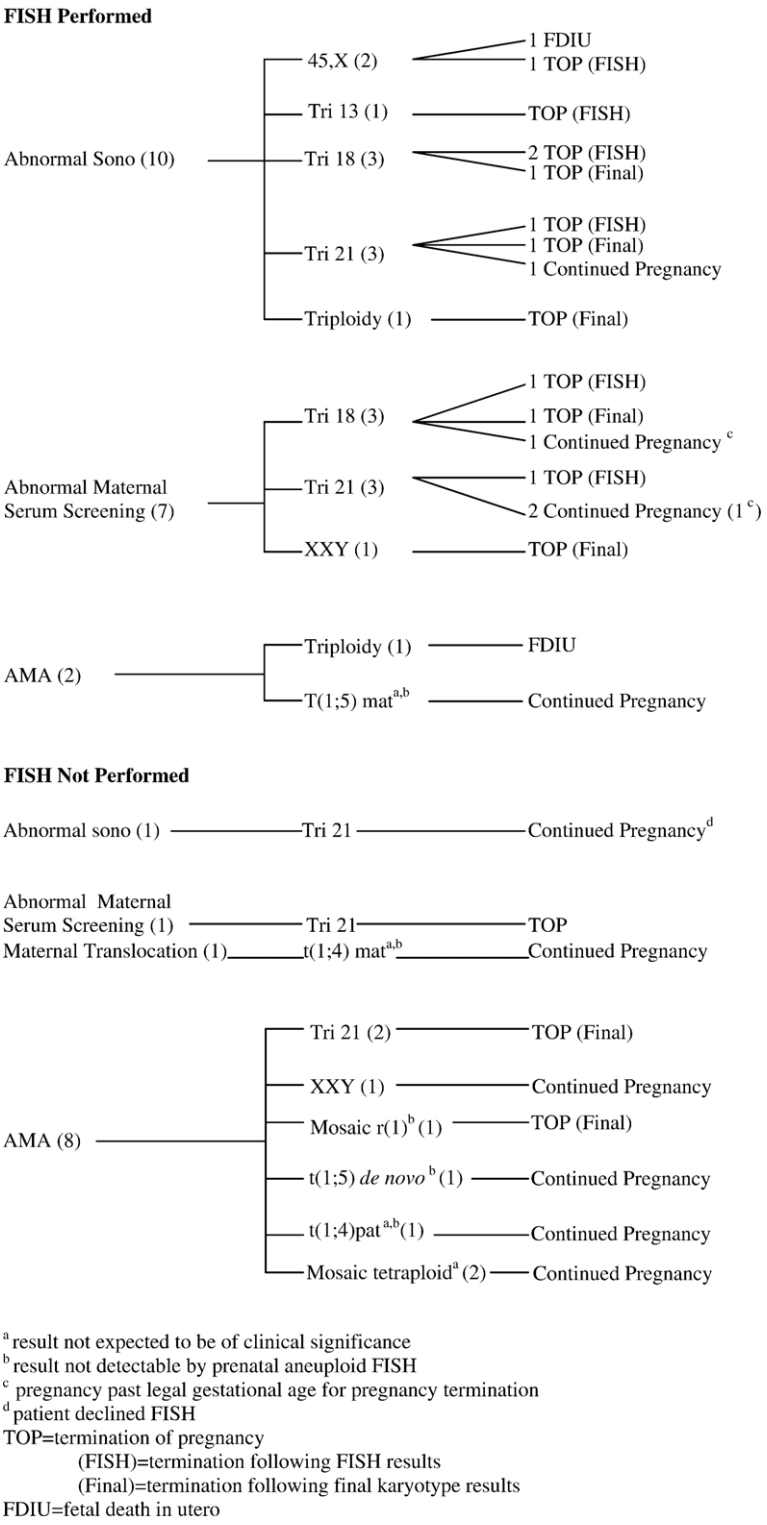
Table 2 Comparison of patients requesting FISH to those offered FISH

Characteristic	Requested FISH ($n=49$)	Offered FISH ($n=111$)	*P*-value
Gestational age (weeks)	17.3 range (11–34)	18.6 range (10–35)	0.06
Chromosome risk (%)	0.98 range (0.05–3.23)	3.96 range (0.21–50)	0.001*

* Denotes statistical significance.

(range 6–11 days) and from those not having FISH available in 11.2 days (range 8–14 days).

Thirty cytogenetically abnormal results were detected, 25 of which would be expected to have clinical consequence. Fig. 1 summarizes the test results and patient decisions. Of the clinically significant abnormal results, two (8%) were not detectable by prenatal aneuploid FISH. Two of the pregnancies (one with 45, X and one with triploidy) resulted in fetal death prior to final karyotype results; another 2 pregnancies underwent testing at a gestational age beyond the legal option for pregnancy interruption. Of the remaining 21 affected pregnancies,



[a] result not expected to be of clinical significance
[b] result not detectable by prenatal aneuploid FISH
[c] pregnancy past legal gestational age for pregnancy termination
[d] patient declined FISH
TOP=termination of pregnancy
(FISH)=termination following FISH results
(Final)=termination following final karyotype results
FDIU=fetal death in utero

Figure 1 Abnormal karyotype results, patient decisions, and timing of decision categorized by reason for prenatal diagnosis.

pregnancy interruption was elected in 17, while 4 continued the pregnancy.

In those with clinically significant abnormal results ($n=25$), statistically significant differences were detected only for gestational age when comparing the FISH offered and the FISH not offered ($p=0.03$) with those offered FISH having a greater gestational age. There were no statistically significant differences between the two groups regarding risk ($p=0.18$), maternal age ($p=0.42$), gravidity ($p=0.73$) and parity ($p=0.75$).

The decision for pregnancy interruption was not influenced by whether or not the patient had FISH testing ($p \leq 1$). In those electing pregnancy interruption, a history of a previous spontaneous abortion/termination did not differ between those who had FISH and those who did not ($p \leq 0.20$ and $p \leq 1$, respectively). Of those with FISH who interrupted the pregnancy ($n=13$), 7 (53.8%) elected to do so following the FISH results, not waiting for confirmation on routine studies. In comparing this group to those with FISH who waited for the final results, there were no statistically significant differences in gestational age ($p=0.13$), risk ($p=0.65$), maternal age ($p=0.68$), gravidity ($p=0.25$), history of miscarriage ($p=0.54$), or history of prior termination ($p=0.30$). Data analyses indicated that both groups had nearly identical indications for diagnostic testing (see Table 3).

Using a $\geq 1\%$ risk cut-off, though not consistently enforced in practice, 19 of the 25 (76%) affected pregnancies were offered FISH studies. If this practice had been more consistent, 21 of the 25 (84%) affected pregnancies would have been offered FISH, resulting in a total of 317 FISH procedures performed. If a 0.5% risk cut-off for offering FISH was used, all of the FISH-detectable clinically significant abnormalities would have been detected. However, 663 FISH procedures would have needed to be performed. Using 2% and 3% risk cut-offs would have yielded 48% and 32% detections, with 118 and 66 FISH procedures performed, respectively.

Table 3 Indications for diagnostic testing among those not continuing pregnancy

Termination following FISH	Termination following routine analysis
Cystic hygroma	Cystic hygroma
Increased trisomy 18 risk on second trimester maternal serum screening	Increased trisomy 18 risk on second trimester maternal serum screening
Multiple ultrasound anomalies	Multiple ultrasound anomalies
Hydrocephalus	Ventriculomegaly
Increased Down syndrome risk on second trimester maternal serum screening	Increased Down syndrome risk on second trimester maternal serum screening
Advanced maternal age and fetal omphalocele	Fetal intracardiac echogenic focus and intrauterine growth restriction
Increased nuchal translucency	

4. Discussion

Using a $\geq 1\%$ risk cut-off to begin offering FISH studies appears to have the highest utility regarding abnormalities detected per number of studies performed. Assuming an additional cost of $350/FISH test, at the 1% risk cut-off, 21 abnormalities detected per 317 FISH tests yield a cost of $5283/abnormality detected. At the 0.5% risk cut-off, 23 abnormalities detected per 663 FISH tests nearly double the cost at $10,089/abnormality detected. Using the 2% and 3% risk cut-offs lowers the costs ($3441/abnormality and $2887/abnormality, respectively), but at the trade of detecting fewer than half of the abnormal fetuses.

A considered benefit of FISH has been a quicker turn-around-time for final karyotype results. This study found a statistically significant quicker turn-around time for final karyotype results when comparing the clinically significant abnormal results for those who had FISH performed to those who did not have FISH performed. Therefore, even those patients who elect to wait for confirmation of the abnormal FISH findings receive the routine karyotype results quicker than those patients who do not have FISH performed.

As all patients in this study underwent formal genetic counseling prior to diagnostic testing, it is of interest to note that only 10 of the 110 (9.1%) patients in our study declined this testing. It is speculated that high (91%) utilization of FISH is related to the fact that the testing posed no further risk, a larger amniotic fluid or chorionic villus sampling was not required, and it could alleviate some anxiety by providing some information in a shorter time than waiting for karyotype results.

It would be interesting to determine the factors women take into account when making a decision regarding pregnancy management. Women in our study group who elected not to continue the pregnancy following the FISH results did so 1 week earlier than if FISH had not been offered. Follow-up studies on the patients who chose to interrupt the pregnancy would be useful in determining if this reduction in waiting time for results has a significant impact on the patient's perceived level of stress and anxiety. Another interesting aspect would be to know, retrospectively, if patients, with both normal and abnormal results, were satisfied with their decision to have the FISH procedure.

According to ACMG/ASHG [2], decisions to act on laboratory results should be supported by at least 2 of 3 possible pieces of information (FISH results, karyotype results, and clinical information). In this study, there was only one abnormal FISH result without a clinical indication other than maternal age. That pregnancy resulted in FDIU. Therefore, all of the patients who acted on results from FISH met this ACMG/ASHG guideline.

This study demonstrates that prenatal FISH for the detection of aneuploidy is a beneficial adjunct to routine karyotyping. The use of a $\geq 1\%$ risk is recommended as a reasonable threshold in offering FISH to patients undergoing invasive prenatal diagnosis for increased aneuploidy risk.

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