Ultrasound Obstet Gynecol 2017; **49**: 465–469
Published online 1 March 2017 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.15995

Utility of ultrasound examination at 10–14 weeks prior to cell-free DNA screening for fetal aneuploidy

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KEYWORDS: advanced maternal age; cell-free DNA screening; first-trimester ultrasound

ABSTRACT

Objective *To estimate the frequency of unexpected first-trimester ultrasound findings that would alter prenatal management in pregnant women eligible for cell-free (cf) DNA screening because of advanced maternal age (AMA).*

Methods *This was a retrospective cohort study of all AMA women at a tertiary care center who had a 10–14-week ultrasound examination between 1 January 2012 and 27 April 2015. Information on pregnancy dating, obstetric ultrasound examination, prenatal screening and genetic testing were collected from a perinatal database. The primary outcome was an unexpected ultrasound finding in the first trimester that would alter the prenatal screening/testing strategy.*

Results *In total, 2337 women met the inclusion criteria, with a total of 2462 fetuses. Sixty-eight (2.9%) women had an anomalous fetus, of which 44 (64.7%) had diagnostic testing. In the entire cohort, a non-viable pregnancy was identified in 153 (6.5%) women. Multiple gestation was identified in 32 (1.4%) women; five had a cotwin demise. Gestational dating was revised for 126 (5.4%) women. Among those who opted for aneuploidy screening (*n = *1806), 68.5% had cfDNA screening and 31.5% had first-trimester screening by analysis of maternal serum biomarkers and nuchal translucency thickness. Among those eligible for cfDNA screening, 16.1% (95% CI, 15.0–18.0%; 377/2337) had an ultrasound finding (anomaly, incorrect dating, multiple gestation, non-viable pregnancy) at the time of testing that would have altered the provider's counseling regarding the prenatal screening/testing strategy.*

Conclusions *A substantial proportion of AMA women eligible for cfDNA screening have fetal ultrasound findings that could alter genetic testing strategy and clinical management. This study recommends ultrasound examination prior to cfDNA screening in AMA women.* *Copyright © 2016 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Non-invasive prenatal screening, using massively parallel sequencing or single-nucleotide polymorphism technology to analyze cell-free (cf) DNA fragments in maternal plasma, was introduced to clinical settings in the USA in 2011[1–3]. At the time of its introduction, the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal–Fetal Medicine recommended cfDNA screening in women at increased risk of fetal aneuploidy[4]. The new screening paradigm was quickly adopted by providers for high-risk patient populations and has significantly reduced the use of alternative screening and diagnostic testing in these populations[5–8].

The revised ACOG committee opinion on cfDNA screening for fetal aneuploidy states that a baseline ultrasound examination should be considered with cfDNA screening[4,9]. An ultrasound examination at the time of cfDNA screening has the potential to change prenatal management and prenatal genetic testing strategies, including the provider's approach to counseling and the patient's preferences and decision to select a screening or diagnostic test[4,10–12]. For instance, diagnostic testing with microarray is recommended preferentially over cfDNA in cases of fetal structural anomalies identified by ultrasound[13,14]. Despite the aforementioned ACOG committee opinion and the potentially important implications of first-trimester ultrasound examination prior to cfDNA screening, there is insufficient information available to determine how often ultrasound findings would result in a change in prenatal counseling and management. Consequently, cfDNA screening has been widely adopted without establishing a standardized or consistent approach to genetic screening and fetal

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Accepted: 5 June 2016

ultrasound. We hypothesize that first-trimester ultrasound findings will change prenatal management in a clinically significant proportion of women of advanced maternal age (AMA).

Our main aim was to identify unexpected first-trimester ultrasound findings in a large cohort of AMA women to determine how frequently a first-trimester ultrasound exam gives results that could change a prenatal genetic testing strategy. Our second aim was to identify a subpopulation of patients who are candidates for cfDNA screening that are at highest risk of having an unanticipated abnormal ultrasound finding.

METHODS

This retrospective cohort study included all AMA women seen at a tertiary care center who had a first-trimester ultrasound examination. Study patients were identified via a prenatal diagnosis and ultrasound database. Patients included in this cohort chose a variety of testing options after counseling, including screening with cfDNA or traditional first-trimester screening of maternal serum analytes and nuchal translucency measurement, diagnostic testing with amniocentesis or chorionic villus sampling or no screening. cfDNA screening, accompanied by a first-trimester ultrasound examination, has been offered to high-risk women at our institution since 2012 as one option among a few testing strategies. We routinely offer an ultrasound exam at the time of first-trimester cfDNA screening to evaluate for fetal anomalies, including increased nuchal translucency (>3 mm), cystic hygroma, multiple gestation, revision of gestational dating and viability. Cystic hygroma is defined as a septate fluid-filled cavity that tends to be largest in the nuchal region but may extend along the length of the fetus. The study was reviewed and deemed exempt from approval by the University of North Carolina at Chapel Hill (UNC-CH) Institutional Review Board because the research involved the study of existing data and the information was recorded in such a way that participants could not be identified, directly or indirectly, through identifiers linked to them. First-trimester ultrasound examinations were performed, using Voluson E8 ultrasound machines (GE Healthcare Ultrasound, Milwaukee, WI, USA) between 1 January 2012 and 27 April 2015 at UNC-CH or Rex Hospital in Raleigh, NC (a UNC-CH affiliate) by certified sonographers in accordance with the American Institute of Ultrasound in Medicine Practice Guidelines for the Performance of Obstetrical Ultrasound Examinations[15]. All ultrasound examinations were interpreted by a board-certified or board-eligible maternal–fetal medicine physician.

We searched the prenatal ultrasound database (R4; Hyland Software, Westlake, OH, USA) to identify all women aged ≥ 35 years who underwent a first-trimester ultrasound at 10–14 weeks' gestation, which is when many women typically consider prenatal genetic testing options, including cfDNA screening. In total, 2337 women were identified as eligible for inclusion in the study. Women who were not AMA and who had not had a first-trimester ultrasound examination were excluded. We included patients who had an ultrasound at 10–14 weeks even if they had had a prior ultrasound, because we were interested in incidental findings at the time of first-trimester cfDNA screening. In the case of a single eligible subject having had two or more pregnancies with first-trimester ultrasound examinations during the inclusion period, the first pregnancy was included and any subsequent pregnancy was excluded to avoid information bias due to clustered data (i.e. observations from two different pregnancies of the same patient are not independent). From the medical records of each subject we abstracted information on demographics, pregnancy dating, obstetric ultrasound, prenatal screening, indication for ultrasound and genetic diagnostic testing. We used this information to determine how often first-trimester ultrasound at the time of cfDNA screening could change prenatal counseling and management by causing a provider to: (1) recommend no screening at all (i.e. non-viable pregnancy); (2) counsel more directly to consider diagnostic testing (i.e. fetal anomaly); (3) recommend an alternative test such as traditional serum screening instead of cfDNA screening (i.e. multiple gestation); or (4) delay ordering the screening test (i.e. if the pregnancy was not within the 9–10-week gestational-age range at which the screening result would provide an informative result). We used descriptive statistics to characterize the study population and to estimate the rate of the primary outcome. We included women who had an ultrasound examination prior to 10 weeks because this was an implementation study, and we were interested in incidental findings at the usual time of cfDNA screening for aneuploidy. We also performed a sensitivity analysis to estimate the rate of the primary outcome when women who had had an ultrasound prior to 10 weeks were excluded.

Statistical analysis

Nested within the cohort, we performed a case–control analysis to identify risk factors for the cohort study's primary outcome. We defined as cases women who had an ultrasound finding that would have changed a prenatal genetic testing strategy and controls as those with an ultrasound finding that would not have changed management. Wilcoxon, Mann–Whitney and chi-square tests, where appropriate, were used to compare values between cases and controls to identify a subpopulation for which the ultrasound examination may be most influential. We used multivariable logistic regression analysis to estimate adjusted odds ratios (aORs) for identified risk factors associated with a case. The aim of this multivariable analysis was to identify a subpopulation of highest-risk patients who would benefit most from an ultrasound examination at the time of fetal aneuploidy screening. We assessed maternal age as a risk factor in the multivariable analysis in two different models: first as a continuous variable and second as a categorical variable, defined as 35–37.9 years, 38–40 years and > 40 years.

Table 1 Clinical and demographic characteristics of women of advanced maternal age with 10–14-week ultrasound examination who were eligible for cell-free DNA testing for fetal aneuploidy, comparing those who had abnormal ultrasound findings that would have changed screening strategy and management (cases) with those who did not have abnormal findings (controls)

Characteristic	*Total cohort* (n = *2337*)	*Cases* (n = *377*)	*Controls* (n = *1960*)	P
Maternal age (years)	37.5 (36–39)	37.91 ± 2.8	37.49 ± 2.25	0.04
Ethnicity				< 0.001
Caucasian	1398 (59.8)	134 (35.5)	1264 (64.5)	
African American	243 (10.4)	19 (5.0)	224 (11.4)	
Hispanic	157 (6.7)	32 (8.5)	125 (6.4)	
Asian	153 (6.5)	20 (5.3)	133 (6.8)	
Other	108 (4.6)	16 (4.2)	92 (4.7)	
No ethnicity recorded	278 (11.9)	156 (41.4)*	122 (6.1)	
In-vitro fertilization	134 (5.7)	16 (4.2)	118 (6.0)	0.18
GA at first ultrasound (weeks)	12 + 1 (11 + 4 to 12 + 6)	12 + 0 ± 0.99	12 + 2 ± 1.20	0.01

Data are given as median (interquartile range), mean ± SD or *n* (%). *153/156 cases of unknown ethnicity were miscarriages; ethnicity not documented because genetic counselor did not see patient. GA, gestational age.

Table 2 First-trimester ultrasound findings with potential to change genetic testing strategy in 2337 women of advanced maternal age who were eligible for cell-free DNA testing for fetal aneuploidy

Ultrasound finding	n *(%)*
Non-viable fetus	153 (6.5)
Revision of gestational dating	126 (5.4)
Fetal anomaly	68 (2.9)
Multiple gestation (new finding)	32 (1.4)
Cotwin demise	5 (0.2)
Total	377 (16.1)*

*There were 384 findings in 377 pregnancies.

Table 3 Fetal anomalies identified at first-trimester ultrasound in 68 women of advanced maternal age who were eligible for cell-free DNA testing for fetal aneuploidy

Fetal anomaly	n *(%)*
Cystic hygroma only	41 (60.3)
Increased NT only*	11 (16.2)
Cystic hygroma + omphalocele	7 (10.3)
Omphalocele only	3 (4.4)
Acrania/anencephaly	3 (4.4)
Cystic hygroma + limb anomaly	1 (1.5)
Cystic hygroma + cardiac anomaly	1 (1.5)
Multiple anomalies	1 (1.5)

*Median nuchal translucency (NT), 3.3 (range, 3.0–4.5) mm.

RESULTS

There were 2337 AMA women who met the inclusion criteria, with a total of 2462 fetuses. Among those who opted to have prenatal genetic screening ($n = 1806$), 68.5% had cfDNA screening and 31.5% had traditional first-trimester screening. All women had a 10–14-week ultrasound examination. Clinical and demographic characteristics of the cohort are shown in Table 1. Within the cohort of 2337 women, the incidence of fetal anomalies was 2.9% (68/2337) (Tables 2 and 3 and Figure 1). Of these 68 women, 64.7% (44/68) had diagnostic testing (Figure 1).

The following outcome and complication rates were estimated as they could influence prenatal management or screening effectiveness: 153 (6.5%) women had a non-viable pregnancy identified incidentally on ultrasound, consistent with a missed miscarriage. A multiple gestation was found incidentally in 32 (1.4%) women and five had a cotwin demise. Gestational dating was revised for 126 (5.4%) women. In total, 16.1% (95% CI, 15.0–18.0%; 377/2337) had a first-trimester ultrasound finding (fetal anomaly, incorrect dating, multiple gestation, non-viable pregnancy) that would have altered the counseling for a screening or diagnostic testing strategy for fetal aneuploidy. A small subgroup of patients ($n = 155$; 6.6%) had an ultrasound scan prior to 10 gestational weeks. To assess the effect of having an early ultrasound, we performed a sensitivity analysis excluding these patients and found that 15.7% (95% CI, 15.0–18.0%; 342/2182) of women had an ultrasound finding that resulted in a change in prenatal genetic testing strategy or counseling at the 10–14-week ultrasound. Of note, 21.9% (34/155) of the women who had an ultrasound examination prior to 10 weeks had a subsequent unexpected finding at 10–14 weeks (anomaly or missed miscarriage) that would have altered the genetic counseling or prenatal screening/diagnosis strategy.

Of note, 35.3% (24/68) of women with a fetal anomaly on first-trimester ultrasound did not choose to have diagnostic testing, and 17 instead had cfDNA screening (Figure 1). Of these, eight (47.1%) had a negative result and nine (52.9%) had a positive result. Of those with a positive result, six were confirmed after birth and the remaining three resulted in miscarriage and the providers did not obtain confirmatory genetic testing on the products of conception. Of those with a negative result, five had a normal newborn examination, two were lost to follow-up and one had a postnatal diagnosis of a genetic syndrome, oculodentodigital dysplasia, confirmed by molecular testing.

When comparing cases and controls, women with ultrasound findings that would have changed their management were significantly older ($P = 0.04$), had earlier ultrasound examinations ($P = 0.014$) and were more likely to have missing data on ethnicity ($P < 0.001$) (Table 1). Missing ethnicity data for women with an

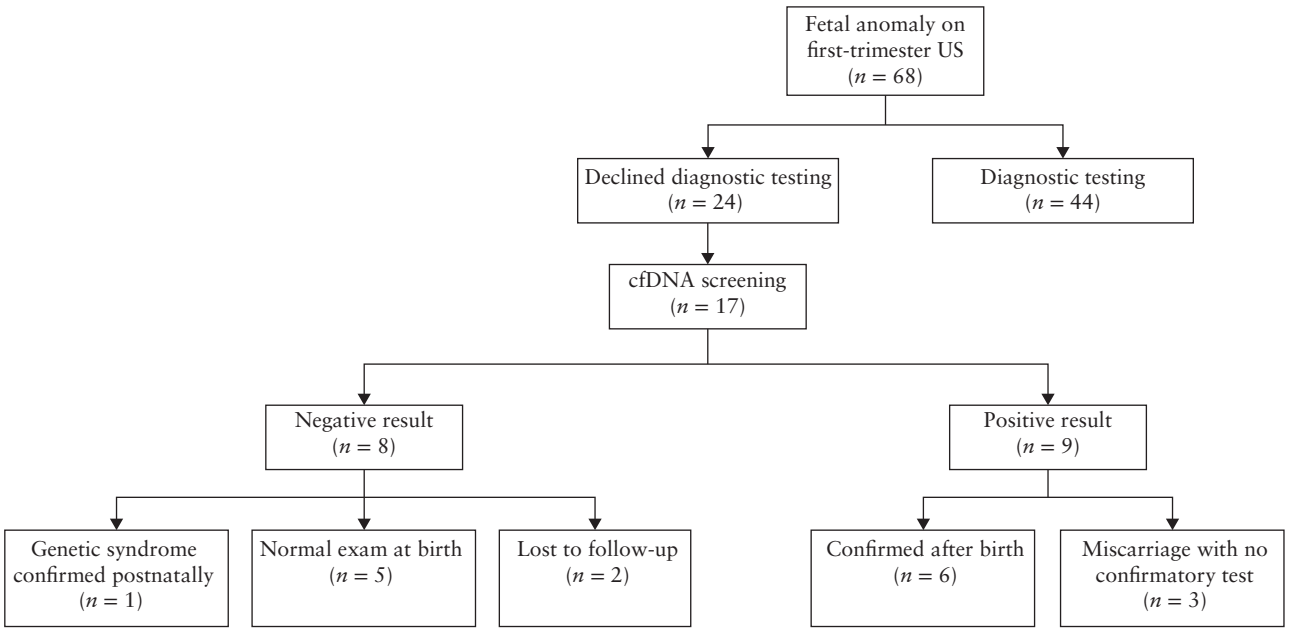

Figure 1 Flowchart of screening and diagnostic choices of women of advanced maternal age after diagnosis of fetal anomaly on first-trimester ultrasound examination (US). cfDNA, cell-free DNA.

abnormal ultrasound finding is explained by 153/156 (98.1%) resulting in miscarriage, therefore they were classified as of unknown race because the genetic counselor did not meet the patient and race was not documented in the ultrasound database. There was no difference between cases and controls with regard to the rate of *in-vitro* fertilization (IVF) ($P = 0.18$). African Americans were least likely to have ultrasound findings that changed management compared with other ethnicities (Table 1). The final explanatory regression model for cases included maternal age, race and IVF conception. In logistic regression analysis, we found that maternal age and race were significantly associated with cases. In the multivariable model, cases with management-changing ultrasound findings were more likely to be over 40 years of age (aOR, 1.5 (95% CI, 1.1–2.0)) and were more likely to be Asian/Pacific Islander (aOR, 1.9 (95% CI, 1.1–3.5)) or Hispanic (aOR, 2.9 (95% CI, 1.6–5.4)).

DISCUSSION

Initial studies on cfDNA that led to the introduction of cfDNA fetal aneuploidy screening in clinical practice were performed in high-risk populations as defined by ACOG[4]. However, cfDNA screening has been widely adopted in clinical practice without establishing a standardized approach to pretest prerequisites. In our cohort of AMA women, we observed that 16.1% of patients who were candidates for cfDNA screening had unexpected abnormal fetal ultrasound results that would impact on pretest counseling and consequently could impact on the screening approach. Our study highlights the importance of first-trimester ultrasound examination at the time of cfDNA screening.

We found that women with ultrasound findings that would change prenatal management were more likely to have had an earlier ultrasound and, on average, were older than women whose ultrasound would not have changed management. Women aged over 40 years were at the highest risk of having an abnormal or management-changing ultrasound finding, with a 1.5-fold increase compared with other patients in the cohort. Other subpopulations in our cohort who had increased rates of abnormal or unexpected ultrasound findings were Asians and Hispanics. While age and ethnicity are associated with the likelihood of an abnormal ultrasound finding, neither factor's association was strong enough for use in clinical decision-making or for clinical prediction to select a subgroup of women to target for pretest sonography. A previous study found differences in birth defects among ethnicities in the USA using a twelve-state-based birth defects tracking system[16].

Among the minority of women who had an early ultrasound scan prior to 10 weeks, 22% were subsequently found to have a fetal anomaly or demise at the 10–14-week ultrasound, indicating that ultrasound before 10 weeks does not predict reliably the absence of fetal abnormalities that could adversely impact on cfDNA aneuploidy screening. This finding supports the idea of delaying sonography, if possible, or performing a second ultrasound examination nearer the time at which fetal aneuploidy screening is performed.

In a recent retrospective cohort study, Reiff *et al.*[17] described the 11–14-week ultrasound findings in women with negative cfDNA screening results for aneuploidy. In their cohort of high-risk women, unexpected ultrasound findings were seen in 3.5% of patients with a negative cfDNA aneuploidy screen result. Combined with the findings of Reiff *et al.*, our study helps highlight the importance of first-trimester ultrasound at the time of cfDNA screening. However, there is an important

difference between their study and ours; our study population included all AMA candidates eligible for cfDNA screening before a decision about testing was made while the study population of Reiff *et al.* included patients who had already decided to undergo screening, with a negative result. Thus, our study assesses the pretest utility while that of Reiff *et al.* assesses the post-test utility of fetal ultrasound in a testing strategy that employs cfDNA screening. Additionally, it is difficult to assess the effect of ultrasound in the implementation and performance of cfDNA screening in the cohort of Reiff *et al.* as the timing of ultrasound examination with respect to cfDNA screening was variable; the majority of patients had cfDNA screening prior to (18%) or on the day of (65%) prenatal ultrasound examination, with only 17% having the examination prior to cfDNA screening. Thus the two studies complement each other in estimating the usefulness of first-trimester ultrasound in implementing cfDNA screening. Importantly, even women with a negative cfDNA test result could have ultrasound findings that decrease the accuracy or undermine the validity of cfDNA screening.

Our study is not without limitations. The retrospective observational design allows potential for information or misclassification bias. However, we minimized this possibility of bias by using a standardized data-collection process and by validating database diagnostic and outcome information with electronic medical record review, including postnatal records. Owing to the inclusion criterion of AMA, our findings may not be generalizable to other low-risk populations. However, this does not invalidate our findings since they can be applied to the most prevalent high-risk population (AMA) currently recommended for cfDNA screening. There was potential for selection bias as we did not include other high-risk populations such as women aged < 35 years at delivery with either a prior fetus with a trisomy, abnormal serum screening result, fetal cystic hygroma or who was a translocation carrier. However, these women would have a higher likelihood of having an ultrasound abnormality than AMA women, and bias would probably result in our study underestimating the clinical utility of ultrasound examination prior to genetic screening.

Our study provides important and novel information to help guide the implementation of fetal aneuploidy screening with cfDNA. Specifically, a considerable proportion of patients (16%) had a first-trimester ultrasound finding that could alter decisions about, or performance of, the genetic testing strategy and clinical management. Our findings indicate that first-trimester ultrasound provides important information in a substantial proportion of AMA patients eligible for cfDNA screening. The absence of a fetal ultrasound examination prior to cfDNA screening has the potential to increase screening costs, as well as the rate of duplicative screening or diagnostic testing, as a direct result of undetected abnormalities or errors. Prospective studies and studies assessing cost-effectiveness of an ultrasound examination at the time of cfDNA screening would add to our knowledge on how to employ optimal cfDNA screening in high-risk and general populations. However, until more data are available, we believe our study indicates that it is reasonable and prudent to offer a pretest first-trimester ultrasound examination to all high-risk women considering cfDNA screening in centers in which this resource is available.

ACKNOWLEDGMENTS

This study was supported by a grant from the National Institute of Health Building Interdisciplinary Research Careers in Women's Health K12 HD001441-16; Carolina Summer Research Medical Student Program; NIH 5T35DK738635. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health.

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