The application of late amniocentesis: a retrospective study in a tertiary fetal medicine center in China

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Abstract

Objective: To assess the indications and complications of late amniocentesis, as well as the advantagement of advanced genetic test results. Design: Retrospective analysis of case notes of women who underwent late amniocentesis. Setting: A tertiary fetal medicine center in China Population or Sample: 1243 pregnant women (1272 fetuses) that underwent amniocentesis at 24+0 to 39+4 weeks, between January 2014 and June 2019 in our hospital. Methods: Indications, complications, genetic test results and pregnancy outcomes were reported for each pregnancy. Information was obtained from case records, validated by research staff and analyzed by SPSS 21. Main Outcome Measures: Indications, complications, genetic test results, and pregnancy outcomes. Results: Of the 1243 women included, late detected abnormal ultrasound finding(s) (88.3%) comes to be the most common indication. PTB rate and IUD rates were 3.1% and 1.7% separately. Sixty-six fetuses with aneuploidy (5.2%) and Sixty-seven others with a pathogenic CNVs (5.3%) were identified by CMA. One pathogenic CNV (8.3%) were reported via WES. The diagnostic yield turned to maximal (31%) in the sub-group of fetuses with suspected prenatal diagnosis results, following by combination of ultrasound findings (23.1%). Conclusions: Since CMA and ES have considerable detection rates, it is reasonable to serve late amniocentesis as an effective and safe method to detect fetal abnormalities or reassure parents following late detected abnormal ultrasound findings. However, A percentage of CMA and ES may expose uncertain results like VOUS. Therefore, comprehensive genetic counseling is necessary. Key words: Prenatal diagnosis; Chromosomal-microarray-analysis; Fetal malformations; Late amniocentesis; Exon sequencing; Third trimester

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Running Title: Yield of genomic technology in late amniocentesis

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Tweetable abstract

Owing to considerable detection rates and safety, it is reasonable to serve late amniocentesis as an effective and safe method.

Introduction

Amniocentesis is a prenatal diagnostic procedure in which a small amount of amniotic fluid is withdrawn from the sac surrounding the fetus for testing. It has been a conventional method of obtaining fetal genetic information since the late 1960s[1]. It is traditionally performed between 16-24 gestational weeks in China, and it provides pregnant women and their families with an opportunity for early diagnosis of and appropriate intervention for the undesirable findings[2]. Nowadays, clinically available methods for analyzing fetal genetic information from amniotic fluid include traditional karyotyping, chromosomal microarray analysis (CMA), medical exome sequencing (MES), whole-genome sequencing (WGS) and whole-exome sequencing (WES). The detection ranges of them are different which result in different detection rates and costs. For example, compared with traditional karyotyping, CMA provides more genetic information of the fetus and has demonstrated to increase the diagnostic yield by 5-9% [3, 4]. Consistently, 5.3% fetuses with late-appearing abnormal ultrasound findings and normal karyotype results showed pathogenic CMA results. Owing to its higher detection rate, shorter turnaround time, and affordable expense, CMA fleetly became the first-tier method in prenatal diagnosis associated with the occurrence of fetal structural anomalies and/or increased nuchal translucency (NT)[1]. Clinical implementation of next-generation sequencing (NGS) in field of prenatal diagnostics are widely available. Petrovski and co-workers noted that patients underwent WES by NGS had higher diagnostic yields (25–35%) among fetuses with genetic disorders, while negative findings were observed by either karyotyping or CMA techniques [5, 6]. Although both WGS and WES can detect novel pathogenic genes, WGS analyzes the entire genome while WES and MES focus only on the exons[7, 8].

Since the exons generally have greater clinical relevance and applicability to human diseases, WES and MES were more frequently used in prenatal diagnosis. The chances of late-appearing abnormal ultrasound findings after a normal fetal-targeted organ scan before 24 gestational weeks are estimated at 5.5%-17% [9, 10]. Late amniocentesis can be performed after 24 gestational weeks onwards which is considered to be safe and effective[1]. However, though it is associated with a higher risk of miscarriage than amniocentesis[11], cordocentesis is still widely employed as the method of choice for prenatal diagnosis after 24 weeks in China. Clinical data remains lacking regarding the incidence, indications, complications, and outcomes associated with late amniocentesis. The aim of this retrospective study was to provide more detailed clinical data associated with late amniocentesis procedures in prenatal diagnosis.

Materials and Methods

Data collection This study was approved by the Research Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University. When fetal structural anomalies are detected by ultrasound or results of noninvasive prenatal testing (NIPT) indicates high risk for a chromosomal anomaly, pregnant women were suggested to undergo amniocentesis and prenatal genetic test (CMA, MES or WES) with informed consents. Since 2014, CMA was adopted as the first-tier test for patients with above indications in our center. Subsequently, at the beginning of 2017, MES and WES became available in our clinical setting which allowed further definite diagnoses in patients with complex phenotypes. In China, termination of pregnancies after 24 gestational weeks is legal in cases where the continuation of the pregnancy constitutes a danger to the mother's physical or mental health or life, or major fetal abnormalities (based on ultrasound findings, genetic testing, or both). The fetal medicine system (Astraia software gmbh, Munich, Germany) was used to analyze the appropriateness of fetal growth. From January 2014 to June 2019, except for 75 women who were lost to follow-up, we record the indications, genetic test results, complications and pregnancy outcomes for all target women who underwent late amniocentesis ([?]24 gestational weeks) in the Third Affiliated Hospital of Guangzhou Medical University. Pregnancy outcomes (deliveries or terminations) were obtained from medical records or by phone contact if the participant did not give birth in our hospital.**CMA**Genomic DNA was obtained from amniotic fluid (10 ml) collected by amniocentesis using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA (50 ng) was labeled using Affymetrix Cytogenetics Reagent Kit, and the labeled DNA was applied to an Affymetrix Cytoscan 750K array (Affymetrix Inc., Santa Clara, CA). The platform contains 550,000 non-polymorphic Copy Number Variation (CNV) probes and more than 200,000 Single Nucleotide polymorphism (SNP) probes with an average resolution of 100 kb. Practical procedures were carried out according to the manufacturer's instructions. The data files generated for each sample were analyzed using Chromosome Analysis Suite (ChAS) Software. The characteristics and spectrum of CNV including the type of aberrations (gains/duplications or losses/deletions), genomic loci, sizes, and the mode of inheritance (familial or de-novo) were studied. The data were interpreted by using information available in the scientific literature and public databases (CLINVAR, Database of Genomic Variants, etc.). These information were used to classify detected CNVs based on their expected clinical significance as benign, likely benign, variants of uncertain significance (VOUS), likely pathogenic or pathogenic [11], in accordance with the recommended guidelines from the International Standard Cytogenomic Array and the American College of Medical Genetics (ACMG). Quantitative Fluorescence Polymerase Chain Reaction and multiplex probe ligation assay (MLPA) for common aneuploidies (chromosomes 21, 18, 13, X, and Y) were performed when a rapid result was required. In some cases, with pathognostic ultrasound findings or known family history, targeted fetal molecular diagnosis for specific single gene mutations was also made.**ES (MES/WES)**

Parental blood samples were collected for DNA extraction using the SolPure Blood DNA kit (Magen, Guangzhou, China) according to the manufacturer's instructions. Genomic DNA of the fetuses was obtained from amniotic fluid as described above. The genomic DNA was fragmented by a Q800R Sonicator (Qsonica, Newtown, USA) to generate 300-500 bp DNA fragments. The paired-end libraries were prepared using the library preparation protocol (Illumina, San Diego, CA). Custom designed NimbleGen SeqCap probes (Roche NimbleGen, Madison, WI) were used for in-solution hybridization to enrich target sequences.

Genes with the phenotype-causing mutation were identified from Online Mendelian Inheritance in Man (OMIM). Subsequent sequencing of the enriched DNA was performed on a NextSeq500 sequencer (Illumina, San Diego, CA).

Sequencing reads from the fetal DNA were mapped to the reference human genome version hg19 (http://genome.ucsc.edu/). Variants were called and reviewed by NextGENe software (SoftGenetics, State College, PA) and in-house annotation pipeline. Literature, mutation and population databases were used for variant annotation, including 1000 Genomes, dbSNP, GnomAD, Clinvar, HGMD, and OMIM. The synonymous and common SNPs (MAF>0.1%) were filtered out, and rare variants with high confidence were considered as a disease-causing candidate for further genetic evaluation. Multiple computational algorithms were applied to assist the genetic evaluation of pathogenicity, including SIFT (https://sift.bii.a-star.edu.sg/, Craig Venter Institute), Polyphen-2 (https://genetics.bwh.harvard.edu/pph2/, Harvard University), and Mutation Taster (https://www.mutationtaster.org, NeuroCure Cluster of Excellence). The interpretation of variants was performed according to the ACMG guidelines.

Results:

From January 2014 to June 2019, 1243 pregnant women between 24^{+0} and 39^{+4} gestational weeks underwent late amniocentesis in our hospital. They carried 1272 fetuses including 1154 singleton pregnancies, 5 monochorionic pregnancies with single puncture (monochorionic monoamniotic, MCMA), 44 monochorionic pregnancies with double puncture (monochorionic diamniotic, MCDA), 36 dichorionic twin pregnancies with double puncture (dichorionic diamniotic, DCDA) and 2 triplet pregnancies with triple punctures (trichorionic triamniotic, TCTA). Among them, 7 MCDA and 5 DCDA chose to conduct amniocentesis on the fetuses with abnormal ultrasound finding; 24 MCDA, 11 DCDA and 2 TCTA chose to conduct amniocentesis on the healthy fetuses because the abnormal ones had been terminated already.**Indications**Table 1 showed the late amniocentesis indications among women studied. It was observed that the most common cause was late detected aberrant ultrasound findings (or a combination of ultrasound findings with other earlier indications) (1123/1272 fetuses, 88.3%). These abnormal ultrasound findings included Central nervous system anomalies (183/1272, 14.4%), cardiovascular defects (161/1272, 12.7%) and urinogenital defects (157/1272, 12.3%). Another common cause of late amniocentesis was suspected prenatal diagnosis results (113/1272 fetuses, 8.9%). Other causes were advanced maternal age, abnormal childbearing history and monogenic disease in the family.**Complications**Common complications of amniocentesis include chorioamnionitis, preterm birth (PTB), intra-uterine demise (IUD) and trauma [12]. In our study, only one chorioamnionitis was identified on the third day following the amniocentesis. A total of 38 PTBs (38/1243, 3.1%) and 21 IUDs (21/1272, 1.7%) were identified in our study. Among these PTB and IUD cases, 6 PTBs (15.8%) and 4 IUDs (19%) developed within the first week after amniocentesis. 8 PTBs (21.1%) and 5 IUDs (23.8%) developed within one month but before 37 gestational weeks after amniocentesis. The remaining PTBs (63.2%) and IUDs (57.1%) cases took place after one month of the amniocentesis. 89.7% (104/116) complication is associated with fetal abnormalities, and 7.8% (9/116) complication took place in fetuses with suspected prenatal screening tests. (Table S1)

PTBs occurred in 0.3% (4/1155) and 0.5% (6/1155) in singleton pregnancies, while that occurred in 2.4% (2/84) and 2.4% (2/84) in twin pregnancies (one week and one-month post-procedure, respectively). No PTB was observed in triplet pregnancies. The earliest PTB occurred in a singleton pregnancy at the third day after the operation. 89.5% PTBs (34/38) took place in fetuses with ultrasound anomalies. Near one-third (12/38,31.6%) of PTBs among our cases happened to twin pregnancies, as twin pregnancies are faced with a higher risk (56.6%) for PTB than singleton pregnancies (9.7 %) even without late amniocentesis[13]. Of note, all 12 twin pregnancies suffering PTBs have double punctures, including 8 MCDA and 4 DCDA.

For IUD, it took place in 1.1% (13/1154) singleton pregnancies and 5.9% (5/85) twin pregnancies. No IUD was observed in triplet pregnancies. The earliest IUD occurred in a singleton pregnancy with CNS anomalies on the next day after the operation. Only one of the 21 IUDs cases reported no fetal malformations and all 5 twin pregnancies suffering IUD have double punctures. It should be noted that among 5 IUD cases in twin pregnancies, 2 cases show 50% mortality rate, with one case having oligohydramnios and the other

with hydramnios.**Pathogenic findingsCMA**In our cohort, chromosomal disorders were identified in 133 (133/1272, 10.5%) fetuses. Sixty-six of them were aneuploidies (66/1272, 5.2%), including 35 trisomy 21, 9 trisomy 18 and 6 trisomy 13. Other aneuploidies included sex chromosomal abnormality (like XXX, XXY), trisomy 8 and trisomy 9. (Table S2) Pathogenic copy number abnormality were identified in 67 (67/1272, 5.3%) fetuses by CMA, while only two could be detected by karyotyping (a deletion of on chr18q22.3q23[14] and a duplication of on 5q21.1q22.2). The other 24 CNVs (24/26, 92.3%), two trisomy 21 and one trisomy 18 from CMA could not be identified via karyotyping.**ES (MES and WES)**MES and WES were carried out in five and seven fetuses (parents and fetus) respectively.

Chromosomal disorders were identified in one fetus (1/12, 8.3%) whose CMA result was negative. Two copy number changes were considered likely pathogenic (2/12, 16.7%), while another two cases were likely benign (2/12, 16.7%).

However, 6 cases tested by exome sequencing showed different genetic results compared with tests by CMA. 4 cases tested by exome sequencing (1 case with pathogenic result, 2 cases with likely pathogenic result and 1 with likely benign result) were reported positive by CMA. 2 cases tested by exome sequencing (1 case positive and 1 likely benign) were reported aneuploidies by CMA. (Table 2)

Two ES reports were supported by the ultrasound findings. In the first case, the pregnant woman with pathogenic WES findings underwent amniocentesis due to FGR identified by ultrasound. Trio-exome sequencing showed a mutation c.625+1G>A in the SLC7A7 gene compatible with fetal Lysinuric protein intolerance (LPI) [15]. The couple chose to continue the pregnancy, but IUD took place 2 months after the amniocentesis. In the second case, the couple was referred for genetic counseling during their fourth pregnancy due to fetal cenencephalocele, hydrocephalus and cerebellum dysplasia. They decided to terminate the pregnancy, although CMA showed normal result. The muscle tissue of the aborted fetus was then isolated for WES. ISPD gene mutations, c.674delC(p.A225Dfs*21) and c.1106T>G(p.V369G), associated with Walker-Warburg syndrome were identified[16] which was consistent with the ultrasound findings

Pregnancy outcome In our cohort, 725 (57.0%) pregnancies resulted in live births, 451 (35.5%) pregnancies were terminated, 21 (1.7%) fetuses died in utero, and 75 (5.9%) fetuses were lost to follow up. (Table 3) Among the 451 terminated pregnancies, pathogenic gene was identified in 114 cases by one of CMA and ES (114/132, 86.4%). 310 couples terminated the pregnancy despite normal results of CMA and ES (310/1272, 24.4%) owing to the fetal abnormalities detected by ultrasound, especially urinogenital and cardiovascular malformations. 27 of 65 (41.5%) fetuses that have been diagnosed with a VOUS, likely pathogenic or likely benign in the report, decided to terminate the pregnancy owing to the abnormal finding on ultrasound examination. Others were live birth, except one was lost to follow up and the other two were IUD. In 66 fetuses with aneuploidy identified by CMA, 31/35 pregnancies with trisomy 21 (include the two pregnancies whose ES results showed normal and likely benign), 9/9 pregnancies with trisomy 18, 6/6 pregnancies with trisomy 13 and 13/16 other aneuploidy were terminated after receiving the genetic reports. Two fetuses with trisomy 21 had IUD and one had PTB took place before receiving the genetic result. The other 4 among the 66 women finally decided to give birth to the babies. 60 out of 67 pregnancies with pathogenic CNV identified by CMA were finally terminated. A woman with a CNV was IUD and another three was PTB before receiving the genetic result. The other 3 women chose to continue the pregnancy and gave birth to the fetuses finally.

Discussion:

Main Findings In this retrospective analysis, we have the following findings. Firstly, late detected abnormal ultrasound finding(s) becomes the most common indication of late amniocentesis. Secondly, compared to cordocentesis, complication rate of late amniocentesis is also competitive, while 88.7% of complications took place in fetuses with abnormal ultrasound findings. Thirdly, compared to traditional karyotyping, both CMA and ES achieve better diagnostic yield. Lastly, 86.4% of women who received pathogenic genetic results terminated the pregnancies, and 24% of women with normal genetic results still terminate the pregnancies owing to the severe ultrasound findings.

Strengths and limitations

Our study has some limitations. Although we have spent time in clinical follow-up, there were still 75 women who were lost. Our study started in 2014. However, ES was underdeveloped and unavailable at that time and cause why all our cases of ES were collected in the last two years. Furthermore, we did not analyze all kinds of complications but only focused on PTB and IUD, which were most common. Neither did we have our own data of cordocentesis. The assessment of complication rate and diagnostic yield could be deficient. This study also has several strengths. The sample size in our cohort is the most abundant than previous ones so that our data could be more persuasive. Our study contributes unique and complete data since there are few previous larger studies analyzing late amniocentesis in the era of advanced technology which can improve prenatal and postnatal care and foster physical fitness among the population.

Interpretation

It has been indicated that the commonest indications of routine amniocentesis (between 16-24 gestational weeks) in China were the increased risk at maternal serum screening and advanced maternal age (35 years at the expected time of delivery) [17]. However, late detected aberrant ultrasound findings (or a combination of ultrasound findings with other earlier indications) was the most frequent indication of late amniocentesis and has accounted for 88.3% in our cohort. Among the 67 cases, 13 cases with ultrasound findings show the same results with CMA reports. For instance, a fetus presenting with cardiac anomalies had a microdeletion of chr22q11.2 compatible with DiGeorge syndrome[18]. In another case, a fetus with the absence of cauda cerebelli was found to have a microdeletion on chr7p22.3p21.1 and chr8p23.3p23.2, which is associated with Dandy-Walker syndrome(type II)[19]. Compared to routine amniocentesis, the complication rate of late amniocentesis within one week and one month are 9.6% (11/115) and 11.3% (13/115), respectively. The overall complication rate was 9.0% (115/1272). As Daum and coworkers reported that only 18 complications were identified among their 291 patients undergoing late amniocentesis in Israel[1], while the complication rate reported by Liao and coworkers was 1.9% (only two complications were found among 108 patients) after third-trimester amniocentesis[20]. Owing to the sample size is much more abundant in our cohort, the complication rates of our study are higher than theirs, but similar to that reported by Gabbay et al. [21] and consistent with a recent meta-analysis[22]. Although our complication rate is higher than that of routine amniocentesis[23], it is reasonable to speculate that at least some of the complications are unlikely to have a direct association with amniocentesis owing to 88.7% (102/115) complications took place in fetuses with abnormal ultrasound findings. The main predictor of PTB in singletons was fetal malformations (24/26, 92.3%), mainly CNS anomalies (4/24, 16.7%) and FGR (4/24, 16.7%). The presence of fetal malformations obviously increases the risk of both PTB and IUD, compared to others without fetal malformations. And pregnancies with double puncture are more likely to suffer from PTB after late amniocentesis. This result may offer opportunities for doctors to consider both the indication for late amniocentesis and the risks of PTB comprehensively. On the other hand, the rate of detection of chromosomal aberrations via CMA and ES are 10.5% and 8.3% respectively, while 92.3% of pathogenic CNVs failed to be detected by karyotyping, suggesting that both CMA and ES achieve better diagnostic yield than traditional karyotyping does. Thus, advanced gene diagnosis technologies have a positive role in late amniocentesis. Not only in late amniocentesis but also in routine amniocentesis, the detection rate of CMA and ES, is higher than karyotyping obviously[3, 24]. In our cohort, the diagnostic rate reaches the highest (31%) when suspected prenatal diagnosis results become the indication of amniocentesis, following by Combination of ultrasound findings (23.1%). It is comparable with the diagnostic rate of routine amniocentesis following abnormal ultrasound findings[24]. The vast majority of women (86.4%) finally decide to terminate the pregnancies after receiving pathogenic genetic results. 24.4% of women with normal genetic results still terminate the pregnancies owing to the severe ultrasound findings.

A considerable disadvantage of late amniocentesis, especially late in pregnancy, was the identification of uncertain results like VOUS, likely pathogenic and likely benign. 41.5% fetuses with uncertain results were terminated. The reason of 85.2% of them was fetal abnormalities, while the other four were even without abnormal sonographic findings, suggesting that the unclear genetic results might have reinforced

their decisions. Of note, except one patient was lost to follow up and the other one was IUD, Three out of the five fetuses whose one of the CMA and ES result showed pathogenic or likely pathogenic were terminated no matter with or without abnormal ultrasound finding. For instance, the couple was referred for genetic counseling during their second pregnancy due to the NIPT result showed trisomy 21 high risk. After amniocentesis, the CMA result showed trisomy 21, but no abnormal finding could be identified through ultrasound. Then the couple chose to have a WES test, and it showed normal afterward. The couple finally terminated the pregnancy.

Worth mentioning, all of the women pay close attention to their genetic test results, which made it reasonable to cogitate the importance and necessity of genetic information even if ultrasound findings are severely abnormal. Moreover, genetic information is also important for future pregnancies without any doubt. In order to increase the accuracy of diagnosis and save time, we also support the idea of offering CMA and ES simultaneously while indeterminate ultrasound findings were identified. However, the economic problem should be taken into account.

Conclusion:

Within the context of modern genetic technologies, late amniocentesis is a reasonable procedure, while late ultrasound findings are identified. The following reasons support it to be regarded as a quick and helpful tool to help pregnant women after 24 gestational weeks. Firstly, patients can gain their genetic results in a median of 10 workdays, and enough time would be provided for them to make a decision of the pregnancy. Secondly, the diagnostic yield of modern genetic technologies (CMA and ES) is much higher than that of traditional karyotype. In cases with normal CMA results and abnormal ultrasound findings, exome sequencing should be considered.

Alternatively, the diagnostic yield turns to maximal when suspected prenatal diagnosis results become the indication of late amniocentesis, following by Combination of ultrasound findings. The risk of PTB and IUD should be taken into account with the presence of abnormal ultrasound findings. Before late amniocentesis, comprehensive genetic counseling is necessary to consist of the patients' expectations from the genetic test, its potential risk and limitations, as well as probable outcomes such as results that are uninterpretable or received post-delivery.**Acknowledgments:**

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Disclosure of Interests: The authors report no conflicts of interest.

Contribution to authorship

CM and LY conceived and designed the study. CM, CJ, CF, JW, WJ, YX and LN was responsible for ultrasound examination and late amniocentesis. YH, CA and ZV was responsible for modern genetic technologies. LY was responsible for data management and statistical analysis. CM and LY drafted the paper, which was revised and approved by all authors. CM and PC contributed by revising the manuscript and providing important input.

Details of ethics approval

This study was approved by the Research Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University.

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