The Application of Late Amniocentesis: A Retrospective Study in a Tertiary Fetal Medicine Center in China

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Abstract

Background: To assess the indications and complications of late amniocentesis, and the advantage of advanced genetic test results in a tertiary university fetal medical medicine unit.

Methods: In this retrospective study, women that underwent amniocentesis at 24⁺⁰ to 39⁺⁴ weeks, between January 2014 and December 2019, were recruited. Indications, complications, genetic test results, and pregnancy outcomes were reported for each pregnancy. Information was retrieved from patient medical records, checked by research staff, and analyzed.

Results: Of the 1277 women (1311 fetuses) included, late detected sonographic abnormalities (86.2%) were the most common indication. The overall preterm birth and intrauterine demise rate were 2.6% and 1.2%, respectively, after amniocentesis. Sixty-six fetuses with aneuploidy (5.1%) and sixty-seven fetuses with pathogenic copy number variations (5.1%) were identified by chromosomal microarray analysis. One pathogenic copy number variation was detected by whole-exome sequencing. The maximal diagnostic yield (36.1%) was in the subgroup of fetuses with the abnormal noninvasive prenatal test, following by multiple abnormalities (23.8%). And 35.8% of the pregnancies were finally terminated.

Conclusions: Due to the high detection rates of advanced genetic technologies and safety of the invasive procedure, it is reasonable to recommend late amniocentesis as an effective and credible method to detect late-onset fetal abnormalities. However, chromosomal microarray and whole-exome sequencing may result in uncertain results like variants of uncertain significance. Therefore, comprehensive genetic counseling is necessary.

Introduction

Amniocentesis is a procedure in which a small amount of amniotic fluid is withdrawn from the sac surrounding the fetus for testing in prenatal diagnosis. Since the late 1960s, amniocentesis became a widely accepted method of obtaining fetal genetic information(1). It is conventionally performed between 16–24 gestational weeks and it provides pregnant women and their families with an opportunity for early diagnosis of the undesirable findings and appropriate intervention for the pregnancies(2).

Nowadays, clinically available methods for analyzing fetal genetic information from amniotic fluid include traditional karyotyping, chromosomal microarray analysis (CMA), whole-genome sequencing (WGS) and whole-exome sequencing (WES). The detection ranges of them are different, with different detection rates and costs. For example, compared with traditional karyotyping, CMA provides more fetal genomic information and has demonstrated an increase in the diagnostic yield by 5–9%(3, 4). Consistently, 5.3% fetuses with normal karyotype results and late-onset fetal abnormalities ultrasound findings showed pathogenic CMA results. Furthermore, the CMA and WES results are available in a median of 10 and 20 workdays, respectively. Owing to the higher detection rate, shorter turnaround time, and affordable expense, CMA fleetly became the first-tier method in prenatal diagnosis associated with fetal structural anomalies and/or increased nuchal translucency (NT)(1).

Clinical implementation of next-generation sequencing (NGS) in the field of prenatal diagnostics is widely available. Previous studies noted that patients who underwent WES had higher diagnostic yields (25–35%) among fetuses with genetic disorders, while negative findings were observed by either karyotyping or CMA(5, 6). Although both WGS and WES can detect novel pathogenic genes, WGS analyzes the entire genome while WES only focuses on the exons(7, 8). As the exons were demonstrated to have more clinical relevance to human diseases, WES is more frequently used in prenatal diagnosis(7). Because of the different costs and diagnostic yields of WES and CMA, patients can choose according to clinical geneticists' consultation and their financial condition.

Nevertheless, the natural history of several kinds of genetic syndromes necessitates genetic evaluation late in the pregnancy since the evolving abnormal findings can be gradually detected by ultrasound examination(9). The chances of late-onset abnormalities after routine 1st and 2nd trimester ultrasound examinations are estimated at 5.5%-17%(10). A recent study demonstrated that a large number of fetal abnormalities, especially central nervous system (CNS) abnormalities, are detected for the first time during the ultrasound scan at 35–37 gestation(9). Recent studies suggested that late amniocentesis is safe and effective to be performed after 24 gestational weeks onwards(1, 11). However, a large clinical investigation of late amniocentesis is lacking regarding the indications, procedure-related complications, and pregnancy outcomes. In China, termination of major fetal abnormalities after 24 gestational weeks is legal. This retrospective study aimed to provide more comprehensive clinical data regarding late amniocentesis 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.

In this study, we conducted a cohort analysis of pregnant women who underwent amniocentesis after 24 gestational weeks in our fetal medicine unit from January 2014 to December 2019. Exclusion criteria were multiple gestations that underwent selective termination of pregnancy. Amniocentesis performed for indications such as twin to twin transfusion syndrome (TTTS), abnormal karyotype results and abnormal CMA results were also excluded. (Fig. 1)

Patient records were retrieved from the fetal medicine system (Astraia software gmbh, Munich, Germany), including the indications, genetic test results, complications and pregnancy outcomes (deliveries or terminations). Pregnancy outcomes were obtained by phone contact if the participant did not give birth in our hospital.

All ultrasound scans were performed by FMF certified sonographers, and all patients underwent a pretest and post-test counseling before invasive testing. The procedures were performed by fetal medicine

specialists, and the genetic tests were carried out either within our hospital or at the hospital approved laboratories.

Results

In our center, women were suggested to undergo amniocentesis and prenatal genetic test (CMA or WES) with informed consent when fetal structural anomalies are detected by ultrasound or results of noninvasive prenatal testing (NIPT) indicates high risk for a chromosomal anomaly.

The demographics data are given in Fig. 1. Out of the 1337 pregnancies recruited, 1277 were included for analysis, including 1243 singleton pregnancies, 12 monochorionic diamniotic pregnancies and 22 dichorionic diamniotic twin pregnancies. All the amniocentesis in twin pregnancy were performed with double punctures. Except for 75 women who were lost to follow-up, the pregnancy outcome was available for 1202 pregnancies. The median age of the pregnant woman was 29.83 years, and the median gestational age at amniocentesis was 28.03 weeks. (Table 1)

Table 1
Maternal and fetal characteristics of the study group.

Maternal age (years)		29.83 ± 5.54
Gravidity		2 (1–8)
Parity		1 (0–4)
Gestational age at diagnosis (weeks)		30.04 ± 3.85
Gestational age at amniocentesis (weeks)		28.03 ± 3.43
Pregnancy outcome	Live born	469
	Termination of pregnancy	751
	IUD	16
	Unknown	75
Abbreviations: IUD-Intra uterine death.		

Indications

Table 2 showed the indications. It was observed that the most common cause was late detected abnormalities (or a combination of ultrasound abnormalities with other indications) (1130/1311 fetuses, 86.2%). These abnormalities included CNS malformations (185/1311, 14.1%), cardiovascular malformations (177/1311, 13.5%), urinogenital malformations (170/1311, 13.0%) and others. Another common indication of late amniocentesis was previous suspected prenatal screening results (115/1311 fetuses, 8.8%), including abnormal NIPT results and positive second trimester DS screening results. Other were advanced maternal age, abnormal childbearing history and family history of monogenic disease.

Table 2
Table 2 Indications for late amniocentesis

Of note, every indication was counted once but many cases had more than one indication.

Indication	Number	Percentage (%)	Pathogenic results	Diagnostic yield(%)	VUS
<i>late detected abnormalities</i>	1130	86.2	100	8.8	55
Central nervous system malformations	184	14.0	12	6.5	13
Cardiovascular malformations	177	13.5	23	13.0	7
Urogenital malformations	170	13.0	11	6.5	12
multiple abnormalities	101	7.7	24	23.8	4
Facial malformations	70	5.3	5	7.1	2
Skeletal malformations	68	5.2	4	5.9	3
Ventriculomegaly	66	5.0	3	4.5	1
Polyhydramnios	56	4.3	3	5.4	4
Digestive system malformations	55	4.2	0	0	0
SGA	52	4.0	3	5.8	5
FGR	48	3.7	5	10.4	1
Increased NT	25	1.9	5	20.0	1
Fetal tumor	21	1.6	1	4.8	0
Thoracic abnormalities	15	1.1	1	6.7	0
Ascites	10	0.8	0	0	1
Fetal appendage malformations	7	0.5	0	0	0
Oligohydramnios	5	0.4	0	0	1
<i>Suspected prenatal screening results</i>	115	8.8	32	27.8	6
Abnormal NIPT result	84	6.4	30	35.7	5
positive second trimester DS screening result	31	2.4	2	6.5	1
<i>Others</i>	66	5.0	2	3.0	1
Patient's request	25	1.9	0	0	1
Advanced maternal age	25	1.9	2	8	0

Abnormal childbearing history	9	0.7	0	0	0
Family history of monogenic	7	0.5	0	0	0
Total	1311	100	134	10.2	62
Abbreviations: SGA-Small for gestational age infant, defined as a SFH <10 th centile(19); SFH-Symphysis fundal height; FGR-Fetal growth restriction; NT-Nuchal translucency; NIPT-Noninvasive prenatal testing.					

Complications

In our study, only one chorioamnionitis was identified on the third day following the amniocentesis. A total of 33 PTBs and 16 IUDs were identified. Six PTBs (6/33, 18.2%) and 2 IUDs (2/16, 25%) developed within the first week after amniocentesis. Five PTBs (5/33, 15.2%) and 5 IUDs (5/16, 31.3%) developed after the first week but within one month after amniocentesis. The remaining PTBs (22/33, 66.7%) and IUDs (9/16, 56.3%) cases occurred after one month of the amniocentesis. In our cohort, 86.3% (88/102) of the complications are associated with fetal abnormalities, and 6.9% (7/102) of them occurred in fetuses with abnormal NIPT results. (Table S1)

PTBs occurred in 0.3% (4/1243) and 0.4% (5/1243) in singleton pregnancies, while that occurred in 5.9% (2/34) and 0% in twin pregnancies (within one week and one-month post-procedure, respectively). The earliest PTB occurred in a singleton pregnancy at the third day after the procedure. Ninety-one percentage of PTBs (30/33) occurred in fetuses with ultrasound anomalies. Women with age < 35 years and ≥ 35 years had a PTB rate of 2.4% (25/1021) and 3.1% (8/256), respectively.

IUD occurred in 0.9% (11/1243) singleton pregnancies and 8.8% (3/34) twin pregnancies. Twenty-five percentage (4/16) of the IUDs are with pathogenic chromosomal disorders. The earliest IUD occurred in a singleton pregnancy with CNS malformations on a day after the procedure. Out of the 16 IUDs, there were four fetal growth restriction (FGR), three CNS malformations, two cardiovascular malformations, two skeletal malformations, two multiple malformations, one urogenital malformation and two structurally normal fetuses. It should be noted that the two of the 16 IUDs cases without fetal malformations are twins with abnormal NIPT results. In addition, two DCDA suffered from IUD within one month after the procedure, and no fetuses survived. One of the MCDA suffered from IUD after one month, and another was livebirth.

Pathogenic findings

CMA

CMA was performed in 1301 fetuses in our cohort, and chromosomal disorders were identified in 133 (133/1301, 10.2%) of them. Sixty-six were aneuploidies (66/1301, 5.1%), including thirty-six trisomy 21, nine trisomy 18 and five trisomy 13. Other aneuploidies included sex chromosomal abnormality (like XXX,

XXY, XYY), trisomy 8, trisomy 9 and trisomy 12. (Table S2) Pathogenic copy number abnormalities were identified in 67 (67/1301, 5.1%) fetuses by CMA. Karyotyping was performed in 22 of them. Only three CNVs (3/22, 13.6%) could be correctly detected via karyotyping (a deletion on chr18q22.3q23, a deletion on chr4p16.3p15.2 and a duplication on 5q21.1q22.2). The other 19 CNVs (19/22, 86.4%), two trisomy 21 and one trisomy 18 detected by CMA could not be identified via karyotyping. Uncertain results were reported in sixty-two cases (62/1301, 4.7%), including 50 VUS results, 7 likely pathogenic results and 5 likely benign results.

In the subgroup of fetuses with abnormalities, the diagnostic rate of CMA was 8.8% (100/1130). Only 33 fetuses with pathogenic CMA results were not associated with structural abnormalities. Furthermore, compared with fetuses with isolated abnormalities (7.4%, 76/1029), fetuses with multiple abnormalities achieved higher positive yield (23.8%, 24/101).

WES

WES was carried out in ten families (parents and fetuses). Chromosomal disorders were identified in one fetus (1/10, 10%). Two copy number variations were considered to be likely pathogenic (2/10, 20%), while another two cases were likely benign (2/10, 20%).

Karyotyping had performed in 4 of 10 cases simultaneously, but two of them (one likely benign and one likely pathogenic) showed different results from WES. (Table 3)

Table 3
WES results

Maternal age	Timing of amniocentesis	Indication of amniocentesis	ES results	Karyotyping (-/results)	Fetal outcome
37	24 + 0	Abnormal childbearing history	Likely Benign	Normal	LB
30	25 + 1	CNS malformations: encephalocele; hydrocephalus; cerebellar hypoplasia	Likely pathogenic: c.674delC (p. A225Dfs*21); c.1106T > G (p.V369G)	-	TOP
33	28 + 0	Multiple abnormalities: ascites; pelvic cysts	Normal	-	TOP
25	24 + 0	CNS malformations: hydrocephalus	Normal	Normal	LB
23	33 + 0	Abnormal NIPT	Likely Benign	-	TOP
34	25 + 1	Facial malformations: cheilopalatognathus	Normal	-	LB
35	24 + 0	Facial malformations: hypoplasia of auricle	Normal	-	LB
41	27 + 0	FGR	CNV: c.625 + 1G > A	-	IUD
26	28 + 1	FGR	Likely pathogenic: c.337C > T (p. Arg113Ter)	Normal	Lost
31	24 + 1	Multiple abnormalities: ascites; pleural effusion; dextrocardia	Normal	Normal	LB
Abbreviations: WES-Whole exome sequencing; CNV- Copy number variants; IUD-Intra uterine death; FGR-Fetal growth restriction; NIPT-Noninvasive prenatal testing; CNS malformations-Central nervous system malformations; LB-Live birth; TOP-Termination of the pregnancy.					

The ultrasound findings supported two WES reports. In the first case, the pregnant woman with pathogenic WES underwent amniocentesis at 27 weeks 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) (12). The couple decided to continue the pregnancy, but IUD took place two months after the amniocentesis. In the second case, the couple was referred for genetic counseling in their fourth pregnancy due to fetal encephalocele, hydrocephalus and cerebellum dysplasia detected at 25 weeks.

WES reported ISPD gene mutations, c.674delC(p.A225Dfs*21) and c.1106T > G(p.V369G). it was consistent with Walker-Warburg syndrome(13). They finally decided to terminate the pregnancy.

Pregnancy outcome

The median turnaround time for receipt of CMA and WES results were 10 and 20 workdays, respectively. Except for 9 patients suffering from complications within one week after amniocentesis, all other pregnant women (1268/1277, 99.3%) received their genetic report before delivery or termination of pregnancies. (Table S1)

A total of 751 (57.3%) pregnancies resulted in live births, 469 (35.8%) pregnancies were terminated, 16 (1.2%) fetuses died in utero and 75 (5.7%) cases were lost of follow up. (Table 4)

Table 4
Pregnancy outcome

Category	#fetuses (CMA+ WES)	LB	TOP	Comment
Aneuploidies	66	4	61	
Trisomy 21	36	2	33	One IUD and two chose to continue pregnancy.
Trisomy 18	9	0	9	
Trisomy 13	5	0	5	
Others	16	2	14	Two XXX chose to continue pregnancy.
CNV	68	9	56	One lost to follow up, two IUD and one PTB happened before receiving results, eight chose to continue pregnancy.
VUS	50	22	26	Two IUD happened after receiving results, 22 chose to continue pregnancy.
Likely pathogenic	9	4	4	One lost to follow up, four chose to continue pregnancy.
Likely benign	7	6	1	
Normal	1111	706	321	73 lost to follow up, 11 IUD, five PTB happened before receiving results.
Total	1311	751	469	75 lost to follow up, 16 IUD and six PTB happened before receiving results.
Abbreviations: CNV-Copy number variants; VUS-Variants of uncertain significance; PTB-Preterm birth; IUD-Intra uterine death; LB-Live birth; TOP-Termination of pregnancy.				

Among the 469 terminated pregnancies, pathogenic results were reported in nearly 1/4 cases (117/469, 24.9%). Except for 73 cases that were lost of follow up, five PTB happened before patients received the

results and 11 fetuses died in uterus, a total of 321 couples terminated the pregnancy despite the normal genomic results (321/1111, 28.9%) because of the fetal abnormalities detected by ultrasound, especially urinogenital and cardiovascular malformations.

Thirty-one of 66 (46.8%) fetuses diagnosed with a VUS, likely pathogenic or likely benign by CMA or WES in the report, they decided to terminate the pregnancy due 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 addition, 87.3% (117/134) of the fetuses with pathogenic results were terminated. In 66 fetuses with aneuploidy identified by CMA, there were 33 out of 36 pregnancies with trisomy 21 (one IUD), 9 out of 9 pregnancies with trisomy 18, 6 out of 6 pregnancies with trisomy 13 and 14 out of 16 with other aneuploidy were terminated after receiving the genetic reports. The remain four in the 66 women decided to give birth to the babies. Fifty-six out of 68 pregnancies with pathogenic CNVs identified by CMA or WES were terminated. Two fetuses with CNVs died and another one was associated with PTB before receiving the genetic result. The other eight women decided to continue the pregnancy and gave birth to the babies.

Discussion

The commonest indications of routine amniocentesis (16–24 gestational weeks) in China is advanced maternal age and the increased risk of serum screening(14). However, late detected fetal abnormalities (or a combination of fetal abnormalities with other indications) are the most common indication of late amniocentesis. It has accounted for 86.2% in our cohort, consistent with a recent study(9), the central nervous system abnormalities (16.3%, 184/1130) was the most common ones.

Our sample size is larger than the other studies. And the overall complication rate after the procedure is 7.8%, and is higher than that reported by Daum et al. (6.2%)(1), Liao et al. (1.9%)(15) and Geffen et al. (6.6%)(11), but it's in accordance with the 8% complication rate reported by Gabbay et al.(16) and consistent with a recent meta-analysis(17). Although our complication rate is higher than the second trimester amniocentesis(11), it is reasonable to speculate that at least some of the complications are unlikely to have a direct association with amniocentesis because 86.3% (88/102) complications took place in fetuses with abnormalities. In this study, complication rate within the first week was 0.7% and within one month was 1.5%, respectively.

We did not analyze all kinds of complications but focused on PTB and IUD which were the most common ones. PTB occurred in 33 women (2.6%) after amniocentesis, of which four had pathogenic chromosomal disorders, whereas four of sixteen women suffered from IUD (1.2%) had pathogenic chromosomal disorders. In our cohort, PTBs were associated with fetal malformations (24/25, 96%), mainly CNS anomalies (4/25, 16%) and FGR (4/25, 16%). PTB occurred in 0.7% (9/1243) in singleton pregnancies, while it occurred in 5.9% in twin pregnancies within one-month post-procedure. As for IUD, only two of the 16 IUD cases reported no fetal malformations. And IUD occurred in 0.2% in singleton pregnancies, while it occurred in 11.8% in twin pregnancies.

The presence of fetal malformations and pathogenic chromosomal disorders obviously increases the risk of both PTB and IUD, compared to others without these risk factors. In addition, twin pregnancies are more likely to be high-risk for PTB and IUD after late amniocentesis. This result may provide clinicians information to balance both the indication for late amniocentesis and the risks.

The total yield of abnormal genetic results was 10.2% which was higher than a recent study by Geffen et al.(11) and Daum et al.(1) with similar indications. As a referral center, a significant number of our patients are referred due to guarded prognosis. However, 86.4% of pathogenic CNVs failed to be detected by karyotyping, demonstrated that both CMA and WES achieve better diagnostic yield than traditional karyotyping as that in the second trimester amniocentesis(3, 18). In our cohort, the diagnostic rate reaches the highest (35.7%) when abnormal NIPT results become the indication of amniocentesis, following by multiple abnormalities (23.8%). It is comparable with the diagnostic rate of second trimester amniocentesis for fetal structural abnormality(18). The vast majority of women (88.7%) decided to terminate the pregnancies after receiving pathogenic genetic results. Twenty-nine percentage of women with normal genetic results still opted for termination of pregnancies due to the severe ultrasound findings.

In China, termination of pregnancies after 24 gestational weeks is legal in cases with major fetal abnormalities. Therefore, late amniocentesis can provide more information for patients to decide whether to continue the pregnancy. Even in places where late termination is not allowed, performing late amniocentesis and exploring the etiology remains useful. It can provide patients opportunities to begin to anticipate lifestyle changes and apply for assistance from relevant supporting groups and resources.

A considerable disadvantage of late amniocentesis, especially late in pregnancy, is the identification of uncertain results like VUS, likely pathogenic and likely benign. Forty-seven percentage of fetuses with uncertain results were terminated. The reason for this is that 88.7% of them are with fetal abnormalities, while the other 12.3% are fetuses with abnormal NIPT results. Except one patient is lost and the other one died, half of the eight fetuses with pathogenic or likely pathogenic results are terminated regardless of the abnormal ultrasound finding.

Pregnant women paid close attention to their genetic test results, which made it reasonable to consider the importance and necessity of genetic information even if ultrasound findings are severely abnormal. Moreover, genetic information also plays an important role in future pregnancies. To increase the accuracy of diagnosis and save time, we also recommend offering CMA and WES simultaneously when sonographic abnormalities were identified. However, the cost-effectiveness should be assessed.

Strengths and limitations

Our study has some limitations. There were 75 women who were lost of follow-up. Our study started from 2014. However, WES was underdeveloped and unavailable at that time. So all our cases of WES 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. The assessment of complications and diagnostic yield could be inadequate.

This study also has several strengths. Although previous studies (1, 11, 15) have reported some data of late amniocentesis, a large clinical investigation is lacking. The sample size of our cohort is larger than the previous ones, so that our data could be more persuasive. Our study provided information of late amniocentesis which can improve the prenatal diagnosis and postnatal care for women.

Conclusion

Late amniocentesis is a reasonable procedure in modern genetic technologies, when late-onset sonographic abnormalities are identified. It is a quick and helpful tool for pregnant women after 24 gestational weeks. The majority of patients can get their genetic results before delivery, and enough time would be provided to make a decision about the pregnancy. The diagnostic yields of CMA and WES are much higher than that of traditional karyotype. In cases with normal karyotype results and sonographic abnormalities, CMA and WES should be considered.

The diagnostic yield achieved maximal when fetuses with suspected genetic disorders become the indication of late amniocentesis, following by sonographic abnormalities. The risk of PTB and IUD should be considered with the presence of sonographic abnormalities.

Before late amniocentesis, comprehensive genetic counseling is necessary to help women understand what their genetic test results mean, the potential risk and limitations, probable outcomes such as results that are uninterpretable or received post-delivery.

Abbreviations

CMA: Chromosomal microarray analysis

CNS: Central nervous system

CNV: Copy number variants

DCDA: Dichorionic diamniotic twin pregnancies

FGR: Fetal growth restriction

IUD: Intra uterine death

MCDA: Monochorionic diamniotic pregnancies

NIPT: Noninvasive prenatal testing

NT: Nuchal translucency

PTB: Preterm birth

TTTS: Twin to twin transfusion syndrome

VUS: Variants of uncertain significance

WES: Whole-exome sequencing

WGS: Whole-genome sequencing

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University. Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The dataset generated and/or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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 was responsible for modern genetic technologies. LY was responsible for data management and statistical analysis. CM and LY drafted the paper. CM and PC

contributed by revising the manuscript and providing important input. All authors read and approved the final manuscript.

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Figures

Figure 1

Study population. The selection of the study population from all women after 24 gestational weeks in our hospital, who underwent amniocentesis during January 2014 and December 2019.

Supplementary Files

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