OBSTETRICS

Amniocentesis in pregnancies at or beyond 24 weeks: an international multicenter study

Roni Zemet, MD; Mohamad Ali Maktabi, MD; Alexandra Tinfow, MS, CGC; Jessica L. Giordano, MS, CGC; Thomas M. Heisler, MS; Qi Yan, PhD; Roni Plaschkes, MD; Jenny Stokes, MD; Jennifer M. Walsh, MD; Siobhán Corcoran, MD; Erica Schindewolf, MS, LCGC; Kendra Miller, MS, CGC; Asha N. Talati, MD; Kristen A. Miller, MGC, LCGC; Karin Blakemore, MD; Kate Swanson, MD; Jana Ramm, MD; Ivonne Bedei, MD; Teresa N. Sparks, MD; Angie C. Jelin, MD; Neeta L. Vora, MD; Juliana S. Gebb, MD; David A. Crosby, MD; Michal Berkenstadt, PhD; Boaz Weisz, MD; Ronald J. Wapner, MD; Ignatia B. Van Den Veyver, MD

BACKGROUND: Amniocentesis for genetic diagnosis is most commonly done between 15 and 22 weeks of gestation but can be performed at later gestational ages. The safety and genetic diagnostic accuracy of amniocentesis have been well-established through numerous large-scale multicenter studies for procedures before 24 weeks, but comprehensive data on late amniocentesis remain sparse.

OBJECTIVE: To evaluate the indications, diagnostic yield, safety, and maternal and fetal outcomes associated with amniocentesis performed at or beyond 24 weeks of gestation.

STUDY DESIGN: We conducted an international multicenter retrospective cohort study examining pregnant individuals who underwent amniocentesis for prenatal diagnostic testing at gestational ages between 24w0d and 36w6d. The study, spanning from 2011 to 2022, involved 9 referral centers. We included singleton or twin pregnancies with documented outcomes, excluding cases where other invasive procedures were performed during pregnancy or if amniocentesis was conducted for obstetric indications. We analyzed indications for late amniocentesis, types of genetic tests performed, their results, and the diagnostic yield, along with pregnancy outcomes and postprocedure complications.

RESULTS: Of the 752 pregnant individuals included in our study, late amniocentesis was primarily performed for the prenatal diagnosis of structural anomalies (91.6%), followed by suspected fetal infection (2.3%) and high-risk findings from cell-free DNA screening (1.9%). The median gestational age at the time of the procedure was 28w5d, and 98.3% of pregnant individuals received results of genetic testing before birth or pregnancy termination. The diagnostic yield was 22.9%, and a diagnosis was made 2.4 times more often for fetuses with anomalies in multiple organ systems (36.4%) compared to those with anomalies in a single organ system (15.3%). Additionally, the diagnostic yield varied depending on the specific organ system involved, with the highest yield for musculoskeletal anomalies (36.7%) and hydrops fetalis (36.4%) when a single organ system or entity was affected. The most prevalent genetic diagnoses were aneuploidies (46.8%), followed by copy number variants (26.3%) and monogenic disorders (22.2%). The median gestational age at delivery was 38w3d, with an average of 59 days between the procedure and delivery date. The overall complication rate within 2 weeks postprocedure was 1.2%. We found no significant difference in the rate of preterm delivery between pregnant individuals undergoing amniocentesis between 24 and 28 weeks and those between 28 and 32 weeks, reinforcing the procedure's safety across these gestational periods.

CONCLUSION: Late amniocentesis, at or after 24 weeks of gestation, especially for pregnancies complicated by multiple congenital anomalies, has a high diagnostic yield and a low complication rate, underscoring its clinical utility. It provides pregnant individuals and their providers with a comprehensive diagnostic evaluation and results before delivery, enabling informed counseling and optimized perinatal and neonatal care planning.

Key words: chromosomal microarray analysis, exome sequencing, fetal anomalies, genetic testing, late amniocentesis, next-generation sequencing panel, prenatal diagnosis, preterm birth, third trimester amniocentesis

Introduction

Since the introduction of amniocentesis in 1970, it has become the most widely accepted method for obtaining fetal samples for genetic testing.[1–3] Amniocentesis for this indication is usually performed between 15 and 22 weeks but can be carried out later in gestation.[4] Amniocentesis is often offered to pregnant individuals to determine if there is a chromosomal or single-gene disorder in the fetus. Genetic indications for amniocentesis include fetal congenital anomalies detected by prenatal ultrasound, a family history of a genetic condition, or high-risk results on prenatal genetic screening tests.[5] Nongenetic indications include evaluation for fetal infection, work-up for fetal anemia and thrombocytopenia, and assessment of fetal lung maturity.[5,6]

Numerous genetic tests can be performed on amniotic fluid, including karyotyping, chromosomal microarray analysis (CMA), targeted tests for single-gene disease-causing variants, gene panel sequencing, exome sequencing (ES), and genome sequencing (GS). Currently, CMA is the recommended first-tier laboratory test for pregnant individuals undergoing prenatal diagnostic procedures for fetal structural anomalies with an overall incremental diagnostic rate of 6% to 7% after normal karyotype results.[7,8] A recent systematic review and

Cite this article as: Zemet R, Ali Maktabi M, Tinfow A, et al. Amniocentesis in pregnancies at or beyond 24 weeks: an international multicenter study. Am J Obstet Gynecol 2024;XXX:XX–XX.

0002-9378/$36.00

https://doi.org/10.1016/j.ajog.2024.06.025

Click Supplemental Materials and Video under article title in Contents at ajog.org

AJOG at a Glance

Why was this study conducted?

There is an important need for comprehensive data on the benefits and risks of late amniocentesis for diagnostic indications to support informed pretest counseling.

Key findings

Late amniocentesis has an overall genetic diagnostic yield of 22.9%. The diagnostic yield is 2.4 times higher for fetuses with anomalies involving multiple organ systems (36.4%) than for fetuses with anomalies in a single organ system (15.3%). Delivery occurred at a median gestational age of 38 weeks 3 days, with a mean 59-day interval from amniocentesis to birth. The postprocedure complication rate was 1.2%, with no significant difference in preterm delivery rates between the early (24−28 weeks) and later (28−32 weeks) gestation groups.

What does this add to what is known?

Late amniocentesis for genetic indications has a high diagnostic yield, particularly when using advanced genetic testing for fetuses with multiple congenital anomalies, and a low complication rate, confirming its clinical utility.

meta-analysis demonstrated that prenatal ES, which targets coding exons that making up 1% to 2% of the genome, can provide a diagnosis in an additional 31% of fetuses with structural anomalies when CMA results are nondiagnostic.[9] GS analyzes the entire genome, including noncoding regions; however, its use for prenatal diagnosis is currently primarily in the research setting.

Amniocentesis is an important diagnostic procedure for prenatal care, but it carries certain inherent risks and potential complications. Its procedure-related risks and complications have been primarily studied for procedures performed in the second trimester, before 24 weeks, and are low at this stage, with a procedure-related risk for pregnancy loss of 0.1% to 0.3%.[4,10] Other potential complications include preterm prelabor rupture of membranes (PPROMs) in 1% to 2%, bleeding in 2% to 3%, and postprocedural chorioamnionitis in <0.1%.[3] Needle injury to the fetus, although rare, is also a possibility. Another potential complication is Rhesus (Rh) factor sensitization, but this has been mitigated by the administration of Rh immune globulin to Rh-negative individuals after the procedure. It is important to note that with advancements in ultrasound guidance and technique, the overall risk of complications from amniocentesis has decreased over time.[3,5,10−13]

Late amniocentesis, performed from 24 weeks onward,[12,14] may be done for pregnant individuals presenting late to prenatal care, with suspected fetal infections, or when testing was deferred for other reasons. More commonly, it is offered for genetic testing following detection of congenital anomalies later in pregnancy.[14−21] The prenatal phenotype of genetic syndromes can evolve as pregnancy progresses, and previous studies have reported that structural anomalies manifest later in pregnancy in 5.6% to 16.7% of pregnancies following a normal anatomy scan performed before 24 weeks.[22,23] Bardin et al[24] highlighted that 5.3% of fetuses with late-appearing abnormal sonographic findings and a normal karyotype had abnormal CMA results. A more recent study shows that certain fetal anomalies, particularly those affecting the central nervous system (CNS), are more frequently first identified during third-trimester ultrasound examinations.[25] Furthermore, advances in high-resolution fetal imaging and the implementation of prenatal exome and GS have enhanced diagnostic capabilities throughout pregnancy, resulting in an increase in indications for prenatal diagnostic procedures offered at later gestational ages. Prenatal genetic diagnosis enhances informed counseling and optimized perinatal and neonatal care planning.

While numerous large multicenter studies have established the safety and genetic diagnostic accuracy of amniocentesis before 24 weeks,[3,10,11,26,27] comprehensive data on the safety of amniocentesis ≥24 weeks for genetic diagnostic indications remain sparse, resulting in knowledge gaps on its diagnostic yield, associated complications, pregnancy outcomes, and overall safety. This has contributed to hesitancy among healthcare providers and patients about performing late amniocentesis because of perceived associated risks. As a result, some practitioners choose to delay the procedure until the risk of prematurity is lower, typically around 30 to 32 weeks, although this timing can vary.[12,15,17,18,21] The few studies that investigated the safety of late amniocentesis have some shortcomings, including limited focus on the 24- to 28-week gestational window, variability in reported indications, study design, definitions of complications, and limited information on diagnostic yield and genetic results.[12,14,15,17−21,28] Thus, there is a need for more comprehensive data on the benefits and risks of late amniocentesis, which are essential to support informed pretest counseling that effectively balances potential advantages and drawbacks. Recognizing the ethical constraints associated with a prospective randomized controlled trial to address this knowledge gap, we conducted a retrospective international multicenter study to evaluate the indications, diagnostic yield, safety, and maternal and fetal outcomes of amniocentesis conducted at or after 24 weeks of gestation.

Materials and methods

Study design and population

We conducted an international multicenter retrospective cohort study of pregnant individuals who underwent amniocentesis for diagnostic testing at gestational ages between 24w0d and 36w6d from January 2011 to July 2022.

We established the collaborative 'Late-amnio' network that included 9 sites: Baylor College of Medicine (BCM), Columbia University Irving Medical Center, The Children's Hospital of Philadelphia, Johns Hopkins University, University of North Carolina at Chapel Hill, University of California San Francisco, Sheba Medical Center in Israel, The National Maternity Hospital in Ireland, and Justus-Liebig University Giessen in Germany. BCM served as the coordinating institution for the network and secured a Data Base Provider Agreement with each individual contributing site for sharing of limited, deidentified datasets for academic purposes. Patient medical records were abstracted using a standardized obstetric data dictionary developed at BCM with input from investigators from all contributing sites. The deidentified data from all sites were integrated into a comprehensive database housed at BCM. Data collection and management were conducted using Research Electronic Data Capture, a secure, web-based application designed to support data capture for research studies. Local institutional review board approval with a waiver of consent was secured individually by each participating site before initiating the study.

Inclusion and exclusion criteria

We included medical records from the 'Late-amnio' network's institutions of pregnant individuals with singleton or twin pregnancies who underwent amniocentesis between 24w0d and 36w6d for diagnostic testing. The majority underwent amniocentesis for prenatal genetic testing, and a small number of pregnant individuals were evaluated for fetal infection, particularly when this was suspected based on maternal serology results or sonographic findings. We excluded individuals who were pregnant with higher-order multiple gestations, cases where pregnancy outcomes were unavailable for review, or instances where amniocentesis was performed for obstetric indications, such as suspected chorioamnionitis, for amnioreduction, or to assess fetal lung maturity. Additionally, pregnancies involving other invasive procedures during the current term, such as amnioreduction, intrauterine transfusion, or twin-to-twin transfusion syndrome fetoscopic laser surgery, were also excluded.

Procedure and diagnostic testing

Amniocentesis was performed using standard approaches by or under the supervision of experienced providers at each institution. The performed genetic testing varied by year of inclusion and clinical indications. Until 2014, genetic testing was mostly limited to chromosome analysis (karyotyping), with a notable increase in the use of CMA from 2014 onward. For pregnancies where the fetal clinical features or family history suggested a single-gene disorder, targeted molecular testing for specific variant(s), a multigene panel, fetal exome or GS (for 2 cases) were done. Sequencing was done on fetal DNA directly extracted from amniotic fluid or from amniocyte cultures and, where applicable, from parental peripheral blood or buccal swabs. For a subset, quantitative fluorescence polymerase chain reaction (QF-PCR) or fluorescence in situ hybridization (FISH) for common aneuploidies involving chromosomes 21, 18, 13, X, or Y, were done to achieve faster results or according to local policy for first-line aneuploidy testing. When a fetal infection was suspected based on maternal serology results or sonographic findings, polymerase chain reaction (PCR) for specific viruses (cytomegalovirus, herpes simplex virus, parvovirus B19) or toxoplasma gondii was employed.

Definitions

The genetic tests were performed either within the institution or in accredited local laboratories, and the interpretation of the genetic findings was adherent to established regional protocols, such as the guidelines from the American College of Medical Genetics in North America,[29,30] which are also widely recognized and implemented in comparable laboratories in Europe. Pathogenic and likely pathogenic variants were considered 'diagnostic results,' which were categorized into 3 distinct groups for clarity and precision in interpretation. First, 'diagnostic results considered causative' referred to findings where the genetic abnormalities identified were directly linked to known genetic disorders, clearly explaining the observed fetal phenotype. Second, 'possible explanations for the fetal presentation' encompassed genetic results that were potentially associated with the observed phenotypes but lacked a definitive causal relationship. Lastly, 'incidental findings' were genetic anomalies uncovered during testing but not directly related to the fetal phenotype. This categorization of pathogenic and likely pathogenic genetic test results allowed for a nuanced understanding of the impact of various genetic findings on fetal development and aided in guiding appropriate clinical management and counseling. Variants of uncertain significance (VUS) are genetic changes (either single nucleotide variants or copy number variants [CNVs]) whose effect on individual health are not clearly understood based on the current knowledge and available data; these were categorized separately. Of note, regions of homozygosity (ROH) in CMA indicate stretches of DNA where the genetic material from both chromosomes in a pair is identical, which can be indicative of consanguinity, a population isolate, or uniparental disomy. ROH were not considered diagnostic results, unless an autosomal recessive or imprinting disorders were strongly suspected based on further evaluation.

Collected data and outcome measures

We extracted data from medical records for each pregnancy undergoing late amniocentesis and systematically documented the following parameters: indication for late amniocentesis, type of genetic testing and their results, and diagnostic yield. We further classified the diagnostic yield according to the presence and complexity of fetal anomalies by comparing 3 groups: 2 or more organ systems involved, a single system involved, and no anomalies

detected on imaging. For the purpose of our data analysis, we classified isolated fetal growth restriction (FGR) and isolated hydrops fetalis under a single entity, given that they represent a singular constellation of symptoms. We documented complications that occurred within the 2-week period following the procedure. The recorded complications included vaginal bleeding, chorioamnionitis, PPROM, preterm labor, placental abruption, and fetal demise. The recorded pregnancy outcomes encompass live birth, fetal demise, and pregnancy termination. Obstetrical outcomes data collected included mode of delivery, gestational age at delivery, preterm birth (PTB) before 32 weeks and before 37 weeks of gestation, and instances of PTB occurring within 1 week and 1 month following the amniocentesis, as well as cases of neonatal death within the first 28 days of life.

To further refine our understanding of these outcomes, 3 separate subgroup analyses were performed. The first evaluated the rate of PTB by gestational age at which the amniocentesis was performed (24w0d−27w6d, 28w0d−31w6d, and 32w0d−36w6d). The second compared pregnancy outcomes between pregnant individuals with diagnostic results to those with nondiagnostic results. The third focused on pregnancy outcomes associated with the specific indications for conducting late amniocentesis.

Statistical analysis

Continuous variables are presented as median and range, or interquartile range (IQR). Categorical variables are reported as numbers and percentages. Comparison between continuous variables was conducted using Student's *t* test or Mann-Whitney *U* test, and qualitative variables were compared using the chi-square test or Fisher's exact test, as appropriate. All tests were 2-tailed, and the significance threshold was set as a P value$<.05$. Statistical analyses were conducted using the IBM Statistical Package for the Social Sciences (IBM SPSS Statistics for Mac V29.0; IBM Corporation Inc, Armonk, NY).

Results

Demographic and clinical characteristics

We collected data on 777 pregnancies, of which 747 were retained for analysis after all exclusion criteria were applied. The analyzed cohort consisted of 723 singleton pregnancies and 24 twin pregnancies, of which 21 were dichorionic diamniotic and 3 were monochorionic diamniotic. Both fetuses were sampled in 14 twin pregnancies, the presenting fetus was sampled in 6 pregnancies, and the upper fetus was sampled in 4 pregnancies. Most excluded pregnancies had incomplete data, 5 had amnioreduction, and for 2 there was maternal cell contamination affecting the reliability of genetic test results. Of the 747 analyzed pregnancies, 652 resulted in a live birth, 41 ended in fetal demise, and 54 were electively terminated (Figure 1). The mean age of the pregnant individuals at the time of amniocentesis was 31.1 years, with a standard deviation of 5.8 years. A history of PTB in previous pregnancies was reported by 58 participants, representing 7.8% of the cohort. The median gestational age at the time of amniocentesis was 28w5d. In 338 cases amniocentesis was performed at 24 to 27w6d, 239 cases at 28 to 31w6d, and 170 cases at 32 to 36w6d. The demographic and clinical characteristics of the study group are presented in Table 1.

Indications for late amniocentesis

Late amniocentesis was performed for the prenatal diagnosis of fetal abnormalities, largely structural anomalies but inclusive of hydrops fetalis and FGR, either as the sole indication, or in combination with other indications, in 684 of 747 (91.6%). For 437 of those (58.5%), the detected anomalies were in a single organ system, and in 247 (33.1%) they affected multiple organ systems (Table 2). When single organ system anomalies were detected, the most commonly affected were the cardiovascular system (n=134; 30.6%) and the CNS (n=85; 19.4%), including microcephaly and macrocephaly.

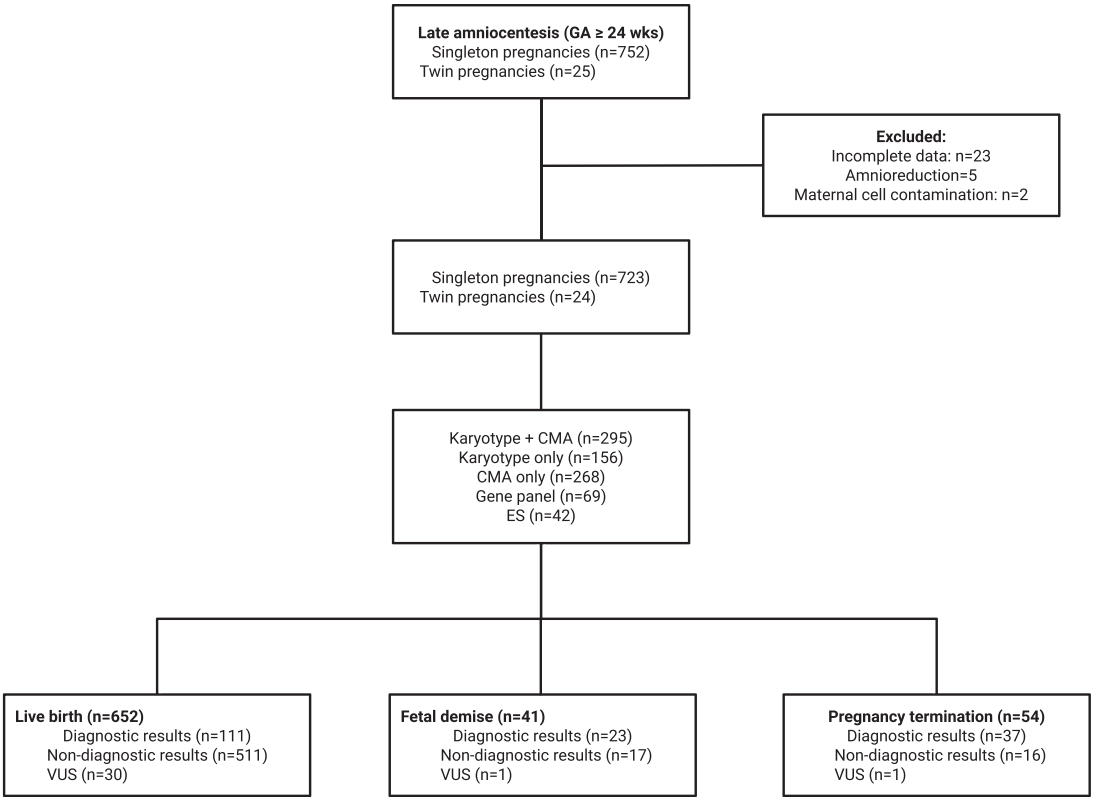
The indications for late amniocentesis for those pregnancies without detected structural anomalies included suspected fetal infection (17/747; 2.3%), high-risk or inconclusive results from cell-free DNA screening (14/747; 1.9%), potential inheritance of a monogenic disorder (9/747; 1.2%), abnormal second-trimester biochemical screening results (7/747; 0.94%), and advanced maternal age (4/747; 0.54%).

Genetic testing and diagnostic yield

In 156 pregnancies (20.9%), karyotype was the only performed test, while 268 (35.9%) had only CMA, and 295 (39.5%), had both karyotype and CMA for evaluation of chromosomal abnormalities. Testing for monogenic disorders was conducted in 110 (14.7%), of which 69 (9.2%) had gene panel testing, 42 (5.6%) had ES, and 2 (0.3%) had GS. Two ES and 1 GS were conducted following a nondiagnostic gene panel. Notably, the majority of those tested for monogenic disorders (92/110; 83.6%) also had preceding or concurrent CMA. For 402 (53.8%) of the pregnancies, rapid testing methods such as QF-PCR or FISH for common aneuploidies were used, with 392 (97.5%) of these fetuses also undergoing karyotype or CMA testing. PCR for 1 or more viruses (cytomegalovirus, herpes simplex virus, or parvovirus B19) and for toxoplasma gondii was performed for 59 (7.9%) pregnancies.

Diagnostic results were obtained in 171/747 (22.9%) tested pregnancies, of which 160 (93.6%) were interpreted as 'causative' of the ultrasound findings or other indications for testing, 7 (4.1%) as a 'possible explanation' for the fetal phenotype, and 4 (2.3%) as 'incidental' findings. The most common genetic diagnosis was aneuploidy found in 80/171 (46.8%) of diagnostic results. This included trisomy 21 (n=30), trisomy 18 (n=28), trisomy 13 (n=8), sex chromosome aneuploidies (n=6), ring chromosomes 13 and 18 (n=2), tetrasomy 9 (n=1), triploidy (n=1), mosaic tetrasomy 8 (n=1), mosaic trisomy 14 (n=1), mosaic trisomy 16 (n=1), and an unbalanced translocation (47,XY,+der(22)t(11; 22) (q23.2;

FIGURE 1
Study population of late amniocentesis



CMA, chromosomal microarray analysis; *ES*, whole exome sequencing; *GA*, gestational age; *VUS*, variant of uncertain significance.

911.21) (n=1)). Notably, in the earlier years from which data were collected, testing was limited to karyotyping in some of the centers. CNVs were present in 45/171 (26.3%) of pregnancies with diagnostic results. Of the 45 identified CNVs, 34 (75.6%) were microdeletions or microduplications that were too small to be detectable by karyotyping. Of all diagnostic results, 38/171 (22.2%) were single gene disorders, which were identified in 34.5% (38/110) of pregnancies in which sequencing was done, and included a yield of 28/69 (40.6%) with gene panel testing and 10/44 (22.7%) with exome/GS. Three fetuses were diagnosed with a single-gene disorder, attributed to known familial variants. Cytomegalovirus was detected in 8/171 (4.7%) of pregnancies with diagnostic results. VUS, either single nucleotide variants or CNVs, were reported in 32 (4.3%). Results were available before delivery, fetal demise, or pregnancy termination in 98.3% (734/747), with partial results observed in 6 of the 734 pregnancies (6/747; 0.8%). For 13/747 (1.7%), genetic test results were not available before delivery because there was spontaneous or induced early delivery or fetal demise. In these cases, genetic results enabled a diagnosis after delivery for 6 of these 13, including 2 trisomy 21, 1 trisomy 18, 1 CNV [46,XX,del(4) (q13.3q23)], and 2 monogenic disorders (Mitochondrial complex I deficiency, nuclear type 16 [MIM:618238], and Nephrotic syndrome, type 1 [MIM:256300]).

The diagnostic yield of late amniocentesis varied depending on the presence and complexity of fetal anomalies (Figure 2). For the total study population, not surprisingly, the highest diagnostic yield of late amniocentesis was for anomalies in 2 or more organ systems: 90/247 (36.4%), followed by testing for other indications in the absence of structural anomalies: 14/63 (22.2%), and testing for anomalies confined to a single organ system: 67/437 (15.3%).

The diagnostic yield, furthermore, varied by specific organ system with detected structural anomalies, including whether there was isolated or nonisolated hydrops fetalis, FGR, or polyhydramnios (Table 3). When only a single organ system was involved, including isolated FGR and hydrops fetalis as a single entity, the highest diagnostic rates were for musculoskeletal anomalies (11/30; 36.7%) and hydrops fetalis (4/11; 36.4%). The diagnostic yield was higher when multiple organ systems were affected, with the highest yield for CNS with other anomalies (44/95; 46.3%), closely followed by craniofacial anomalies (26/58; 44.8%), cardiovascular anomalies (60/137; 43.8%), and musculoskeletal anomalies (30/70; 42.9%) with other anomalies. Notably, testing for isolated

TABLE 1
Demographic and clinical characteristics of pregnant individuals who underwent late amniocentesis

Characteristics	Values
Maternal age (y)	31.1±5.8
Ethnic group	
White, not Hispanic	500 (66.9%)
White, Hispanic or Latino	105 (14.1%)
Black or African American	84 (11.2%)
Asian	29 (3.9%)
Unknown/not reported	29 (3.9%)
Gravidity	2 (IQR: 1–3)
Parity	1 (IQR: 0–2)
History of preterm birth	58 (7.8%)
History of miscarriages	206 (27.6%)
History of cesarean section	128 (17.1%)
Maternal body mass index at amniocentesis (kg/m^2)	29.1±5.7
Diabetes	
None	672 (90.0%)
Pregestational	22 (2.9%)
Gestational Diabetes Mellitus Class A1	36 (4.8%)
Gestational Diabetes Mellitus Class A2	13 (1.7%)
Unknown diabetes status	4 (0.5%)
Hypertensive disorders in pregnancy	
None	670 (89.7%)
Chronic hypertension	28 (3.7%)
Gestational hypertension	14 (1.9%)
Preeclampsia	26 (3.5%)
Unknown hypertensive status	9 (1.2%)
Median gestational age at amniocentesis	28w5d (range: 24w0d–36w6d)
Amniocentesis between 24w0d and 27w6d	338 (45.2%)
Amniocentesis between 28w0d and 31w6d	239 (32.0%)
Amniocentesis between 32w0d and 36w6d	170 (22.8%)

IQR, interquartile range.

FGR had a low diagnostic yield of 4/69 (5.8%), but when combined with other anomalies, the yield increased to 31/83 (37.3%).

Pregnancy outcomes

As shown in Figure 1 and Table 4, 652 pregnancies resulted in live births, with diagnostic prenatal testing results in 111 (17.0%) of those. There were 41 fetal demises, 40 of which had congenital anomalies, with an overall high diagnostic rate of 23 (56.1%). Fifty-four pregnancies, of which 50 (92.6%) were found to have fetal structural anomalies, were terminated; the prenatal diagnostic yield for those was high at 37 (68.5%). There were 50 neonatal deaths, of which 24 (48%) had received diagnostic testing results.

The median gestational age at delivery was 38w3d (IQR: 36w6d–39w2d), and the average interval from the procedure to delivery was 59 days (IQR: 40–83 days). Of all live births, 163/652 (25.0%) were PTBs (either induced or spontaneous) before 37 weeks of gestation. For those for whom information on labor induction vs spontaneous labor was available, 61/619 (9.9%) were preterm due to spontaneous preterm labor. Notably, 93.3% (152/163) of PTBs before 37 weeks involved fetuses with structural anomalies. Finally, 10/652 (1.5%) of all deliveries occurred within a week and 59/652 (9.0%) within a month of the procedure and before 37 weeks of gestation.

Postprocedure complications

There were 9/747 (1.2%) pregnancies with complications occurring within 14 days after the late amniocentesis. Table 5 summarizes all identified complications, the interval from amniocentesis to complication, pregnancy outcomes, and whether pregnant individuals received the genetic testing results before delivery. There were 2 fetal demises among those 9 cases. The first was a demise 14 days after a procedure done at 27w0d for a fetus with multiple congenital anomalies, who was diagnosed with trisomy 18. The second was after an amniocentesis done at 24w1d for high risk for trisomy 21 on cell-free DNA screening. The pregnant patient developed fever and preterm contractions suggestive of chorioamnionitis the day after the procedure and had a fetal demise the next day. Trisomy 21 was confirmed after fetal demise. There were also 2 instances of placental abruption diagnosed 1 day after amniocentesis. For one, labor was induced 4 days after the procedure at 33w4d, and for the other, labor was induced at 33w1d after nearly 3 weeks of in-hospital observation following the procedure. There were 2 pregnancies with preterm labor and 2 with PPROM that resulted in deliveries within the first 2 weeks after the procedure. One pregnancy required labor induction due to nonreassuring fetal status within 3 days of the procedure, although fetal heart monitoring conducted postprocedure was reassuring.

TABLE 2
Indications for late amniocentesis

Indication	n (%)
Abnormal imaging	684 (91.6%)
Single organ system[a]	437 (58.5%)
Multiple organ system[b]	247 (33.1%)
Suspected fetal infection	17 (2.3%)
High-risk prenatal cell-free DNA screening	14 (1.9%)
Potential inheritance of a single-gene disorder	9 (1.2%)
Abnormal biochemical aneuploid screening	7 (0.9%)
Advanced maternal age (>35 y)	4 (0.5%)
Other indications[c]	12 (1.6%)

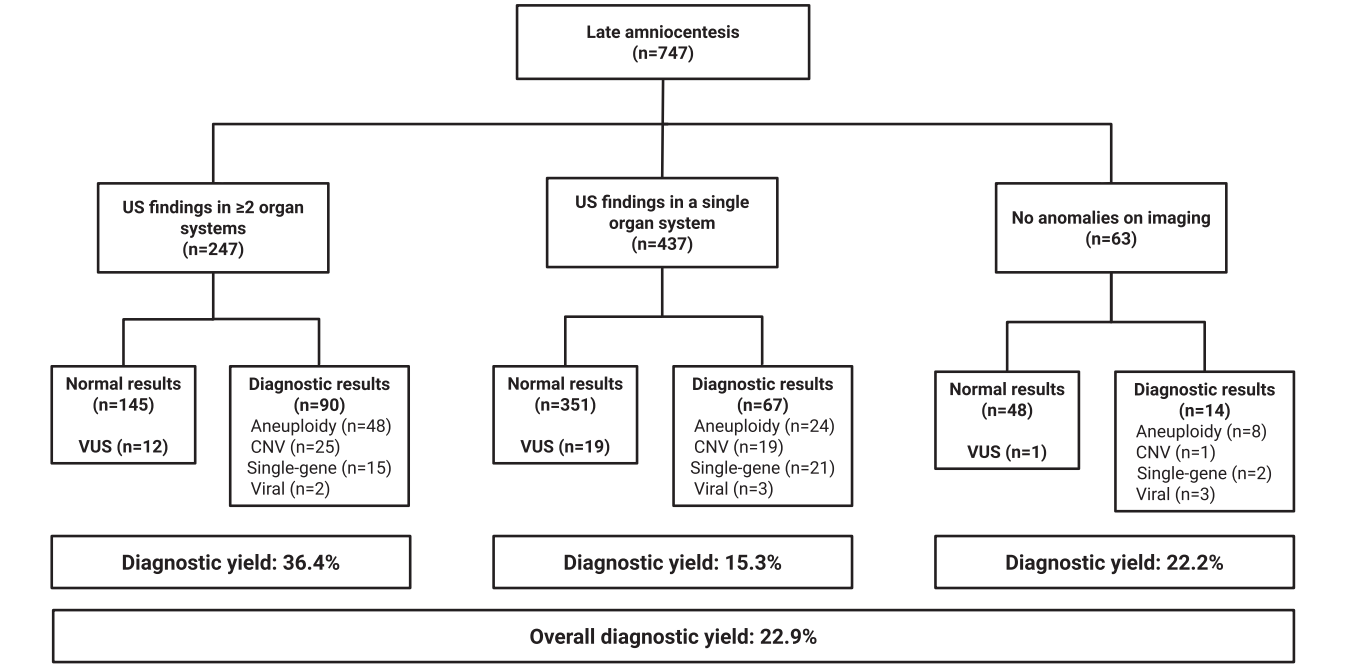
FGR, fetal growth restriction.

[a] Including isolated hydrops fetalis (n=11) and FGR (n=69); [b] Including nonisolated hydrops fetalis (n=16) and nonisolated FGR (n=85); [c] Other indications: soft markers on ultrasound (n=8), ABO blood group and Rhesus factor incompatibility (n=2), repeat failure of cell-free DNA screening (n=1), paternity testing (n=1).

Subgroup analyses

We subsequently conducted 3 subgroup analyses to address important clinically relevant questions concerning the risk and yield of late amniocentesis. The first subgroup analysis was performed to evaluate if the timing of amniocentesis ≥24 weeks impacts PTB outcomes. We stratified singleton pregnancies with livebirths into 3 groups by gestational age at the time of amniocentesis: 24w0d to 27w6d, 28w0d to 31w6d, and 32w0d to 36w6d (Table 6). The preterm delivery rates within 1 week or 1 month after the procedure were not significantly different between these groups overall, including for the 3 groups with respect to spontaneous deliveries. A trend was seen indicative of the lowest risk for spontaneous preterm delivery within a week or within 4 weeks postprocedure being in the earliest gestational age group (24w0d–27w6d) with slightly higher rates as the gestational age of the groups increased. In cases of spontaneous deliveries, when amniocentesis was performed between 24w0d and 27w6d, there were no deliveries within 1 week postprocedure (0/248), and there were 4 deliveries within the first month after the procedure (4/248; 1.6%). When amniocentesis was performed between 28w0d and 31w6d, there was 1 delivery within the first week after the procedure (1/195; 0.5%), and 7 deliveries within the first month (7/195; 3.6%). When amniocentesis was performed between 32w0d and 36w6d, there was 1 delivery within the first week after the procedure (1/155; 0.6%), and 6 deliveries within the first

FIGURE 2
Diagnostic yield of late amniocentesis, according to indication for the procedure



CNV, copy number variants; *US*, ultrasound; *VUS*, variant of uncertain significance.

TABLE 3
Diagnostic yield for late amniocentesis by organ system with anomalies

	Single organ system			Organ system plus other anomalies[a]		
System involved	Total	Pathogenic	Diagnostic yield	Total	Pathogenic	Diagnostic yield
Cardiovascular	134	25	18.7%	137	60	43.8%
Central nervous system	85	11	12.9%	95	44	46.3%
Urogenital	34	5	14.7%	74	25	33.8%
Digestive	30	4	13.3%	44	17	38.6%
Musculoskeletal	30	11	36.7%	70	30	42.9%
Thoracic/pulmonary	20	2	10.0%	35	9	25.7%
Craniofacial	9	1	11.1%	58	26	44.8%
Polyhydramnios	12	0	0%	47	18	38.3%
Hydrops fetalis	11[b]	4	36.4%	16	2	12.5%
Fetal growth restriction	69[c]	4	5.8%	83	31	37.3%
Other anomalies	3	0	0%	9	2	22.2%

FGR, fetal growth restriction.

[a] Fetuses with multiple anomalies were counted several times; [b] Isolated nonimmune hydrops fetalis with no detected structural anomalies; [c] Isolated FGR with no detected structural anomalies.

month (6/155; 3.9%). The preterm delivery rate before 37 weeks was lower in the group that underwent amniocentesis between 32w0d and 36w6d compared to the other groups (P=.001). However, there was no difference in the overall preterm delivery rates for amniocentesis performed between 24w0d and 27w6d compared to amniocentesis between 28w0d and 31w6d. We note that the spontaneous preterm delivery rates before 37 weeks are comparable for all 3 groups (24w0d–27w6d: 9.3%; 28w0d–31w6d: 10.8%; 32w0d–36w6d: 5.8%; P=.26). Importantly, we observed a lower yield of amniocentesis at later gestational ages, with yields of 16.0% between 32w0d and 36w6d, 23.5% between 28w0d and 31w6d, and 26.0% between 24w0d and 27w6d (P=.04).

In a second subgroup analysis, we aimed to understand how the results of prenatal testing impact pregnancy outcomes after late amniocentesis by comparing outcomes for diagnostic results vs nondiagnostic test results (Table 7). There were significantly more fetal demises, pregnancy terminations, low Apgar scores at 5 minutes, and neonatal deaths for pregnancies with prenatal diagnostic results compared to those with nondiagnostic results. However, the median gestational age at delivery and the PTB rate were not different between these groups.

The third subgroup analysis examined the pregnancy outcomes by indications for late amniocentesis. This analysis focused on the presence and complexity of structural anomalies, comparing outcomes for pregnancies with fetal anomalies in multiple organ systems, a single organ system, and without fetal anomalies on imaging. We found overall less favorable outcomes for pregnancies with congenital anomalies in multiple organ systems compared to the other groups (Table 8).

Comment

Principal findings

To our knowledge, this study represents the first international multicenter effort to evaluate the indications, diagnostic yield, and pregnancy outcomes of late amniocentesis, at or after 24 weeks of gestation. In this cohort, late amniocenteses were performed at a median gestational age of 28w5d. The most common indication was genetic testing for fetal structural anomalies (91.6%), followed by suspected fetal infection (2.3%), and high-risk findings from cell-free DNA screening (1.9%). The overall genetic and viral diagnostic yield was 22.9%, but the yield was 2.4 times higher (36.4%) for fetuses with anomalies involving multiple organ systems vs involvement of a single organ system (15.3%). Furthermore, diagnostic yield was also influenced by the specific organ system involved and by the presence of hydrops fetalis. Not unexpectedly, the diagnostic rate was 0% for isolated polyhydramnios rising to 38.3% in the presence of additional anomalies, whereas for isolated FGR, it was low but not insignificant at 5.8%, rising to 37.3% for FGR in the presence of additional anomalies. A key finding is that 98.3% of pregnant individuals received their genetic test results before birth or pregnancy termination. The most common genetic diagnoses were aneuploidies (46.8%), followed by CNVs (26.3%) and monogenic disorders (22.2%).

The safety of late amniocentesis was underscored by our findings on delivery outcomes. The median gestational age at delivery after late amniocentesis was 38w3d, with an average interval of 59 days from the procedure to delivery. The overall complication rate within

TABLE 4
Pregnancy outcome

Parameter	Outcome
Live birth	652 (87.3%)
Fetal demise	41 (5.5%)
Pregnancy termination	54 (7.2%)
Gestational age at delivery (wk)	38w3d (IQR 36w6d–39w2d)
Birth weight (g)	2950 (IQR 2400–3465)
Procedure-to-delivery interval (d)	59 (IQR 40–83)
Rate of preterm delivery <37 wk of gestation	163/652 (25.0%)
Spontaneous preterm delivery <37 wk	61/619[a] (9.9%)
Rate of preterm delivery <34 wk of gestation	66/652 (10.1%)
Spontaneous preterm delivery <34 wk	24/619[a] (3.9%)
Rate of preterm delivery <32 wk of gestation	35/652 (5.4%)
Spontaneous preterm delivery <32 wk	10/619[a] (1.6%)
Delivery within 1 wk of amniocentesis	10/652 (1.5%)
Delivery within 4 wk of amniocentesis	59/652 (9.0%)
Mode of delivery	
Unassisted vaginal delivery	314/643 (48.8%)
Assisted vaginal delivery	17/643 (2.6%)
Cesarean section	312/643 (48.5%)
Apgar score at 5 min <7	64/652 (9.8%)
Neonatal death	50 (7.7%)
Diagnostic genetic testing	24 (48%)

IQR, interquartile range.

[a] Information on whether the delivery was spontaneous or induced was available for 619 out of the 652 deliveries.

2 weeks after the procedure was 1.2%, though some complications may have been unrelated to the procedure, instead stemming from fetal findings. Importantly, we found no difference in preterm delivery rates for amniocentesis done between 24w0d and 27w6d and those done between 28w0d and 31w6d, supporting the procedure's safety across these gestational periods. Together, these multicenter data show that late amniocentesis performed for pregnancies with fetal structural anomalies or other findings suggestive of genetic conditions or fetal infection has a high diagnostic yield with an acceptable safety profile, and that adverse outcomes are primarily driven by the complexity of the fetal anomalies rather than by procedure-related complications.

Results in the context of what is known

International guidelines offer broad directives concerning late amniocentesis. The American College of Obstetricians and Gynecologists practice bulletin on prenatal diagnostic testing for genetic disorders notes that while amniocentesis for the purpose of genetic diagnosis is typically conducted between 15 and 20 weeks of gestation, it may be carried out at later gestational ages. However, it does not provide specific information about diagnostic yield or risk of complications associated with the procedure when performed later in gestation.[4] Similarly, the International Society of Ultrasound in Obstetrics and Gynecology's practice guidelines for invasive prenatal diagnostics suggest amniocentesis from 15 weeks of gestation onward. They identify advanced gestational age at the time of the procedure as a factor that may increase the likelihood of culture failure but do not elaborate on other potential risks.[3]

A recent systematic review and meta-analysis that included 5 retrospective studies from 3 countries (Israel, India, and France), involving 911 singleton pregnancies, evaluated the obstetrical outcomes in pregnant individuals undergoing late amniocentesis.[12,14,15,17,20,21] The indications for late amniocenteses in this meta-analysis were comparable to those for pregnancies in our retrospective review, and included abnormal ultrasound findings, FGR, advanced maternal age, suspected fetal infection, abnormal biochemical screening, maternal desire, and family history of genetic disease. The authors also examined outcomes that were comparable to those in our study, including gestational age at delivery, spontaneous PTB before 37 weeks, PTB within 1 week of amniocentesis, PPROM, chorioamnionitis, placental abruption, fetal demise, and pregnancy termination. They found a 4.85% incidence of spontaneous PTB before 37 weeks and a 1.42% incidence within 1 week following amniocentesis. PPROM occurred in 2.85% of the cases, placental abruption in 0.91%, fetal demise in 3.66%, and 6.37% of pregnancies were terminated. We found comparable rates of fetal demise and pregnancy termination, at 5.5% and 7.2%, respectively. Although we found a higher rate of spontaneous PTB before 37 weeks (9.4%) than in the meta-analysis, it is similar to the US's PTB rate of 10.38% (2022 data),[31] and we note that the PTB rate within 1-week postprocedure was 1.5%, close to the 1.42% found in the meta-analysis, suggesting procedure-related PTB rates to be overall similar. We identified PPROM and placental abruption within the first 2 weeks postprocedure as potential procedure-related complications, with only 2 cases for each. It is also noteworthy that 2 other studies, focused on third-trimester amniocentesis primarily for assessing fetal lung maturity or elective reasons such as maternal age, but not

TABLE 5
Complications within 2 weeks after late amniocentesis

Indication for amniocentesis	History of preterm delivery	GA at amniocentesis (wk)	Complications	Amniocentesis-to-complication interval (d)	Genetic testing results	Genetic results available before delivery	Pregnancy outcomes
Multiple congenital anomalies: severe exencephaly, severe kyphosis with missing spinous processes, small chest, suspected ruptured omphalocele, clenched hands, abnormal feet placement	No	27w0d	Fetal demise accompanied by vaginal bleeding and PTL	14 d	47,XY,+18	No	Fetal demise at 29w0d, vaginal delivery
Increased risk for trisomy 21 by cell-free DNA screening	No	24w1d	Chorioamnionitis (temp 38°C and PTL) and fetal demise	1 d	47,XX,+21	No	Fetal demise at 24w3d, vaginal delivery
Suspected CMV infection and FGR	G1	32w6d	Placental abruption	1 d	CMV PCR negative	Yes	Induced vaginal delivery at 33w4d, birth weight 1640 g, Apgar score 8/9
FGR with short long bones	G1	30w2d	Vaginal bleeding and dynamic cervix, suspected for chronic abruption. Three-week admission for follow-up and betamethasone treatment, until delivery	1 d	Normal FISH and CMA	Yes	Spontaneous vaginal delivery at 33w1d, birth weight 1356 g, Apgar score 9/10
Increased risk for trisomy 18 in quad screen	No	24w2d	PTL	8 d	Normal karyotype, FISH, and CMA	FISH available before delivery for common aneuploidy	Spontaneous vaginal delivery at 25w3d, birth weight 750 g, Apgar score 1/5/9
Dilated and echogenic bowel, dilated stomach, ascites	G1	31w4d	PPROM and PTL	1 d	Normal FISH and CMA	FISH available before delivery for common aneuploidy	Spontaneous vaginal delivery at 31w6d, birth weight 1509 g, Apgar score 9/10. The neonate went through 2 surgeries for intestinal obstruction, and is healthy
Increased risk for trisomy 21 in second-trimester serum test and dangling choroid in ultrasound scan at 24w6d	G1	24w6d	PPROM	10 d	Normal karyotype, FISH, and negative CMV PCR	Yes	Spontaneous vaginal delivery at 27w2d, birth weight 740 g, Apgar score 4/6. Stayed in the NICU for 50 d, with small bilateral IVH, currently 10-y-old
FGR with short long bones	No	33w2d	PPROM	13 d	Normal CMA	No	Spontaneous vaginal delivery at 35w3d, birth weight 1825 g, Apgar score 9/10

(continued)

TABLE 5
Complications within 2 weeks after late amniocentesis *(continued)*

Indication for amniocentesis	History of preterm delivery	GA at amniocentesis (wk)	Complications	Amniocentesis-to-complication interval (d)	Genetic testing results	Genetic results available before delivery	Pregnancy outcomes
Microcephaly, ascites, echogenic bowels, absent ductus venosus	No	31w0d	NRFHR	3 d	Normal FISH, Karyotype, and CMA	FISH available before delivery for common aneuploidy	Delivered by CS due to biophysical profile 6/8 at 31w3d, birth weight 2295 g, Apgar score 6/6

CMA, chromosomal microarray analysis; *CS*, cesarean section; *FGR*, fetal growth restriction; *FISH*, fluorescent in situ hybridization; *GA*, gestational age; *IVH*, intraventricular hemorrhage; *NICU*, neonatal intensive care unit; *NRFHR*, nonreassuring fetal heart rate; *PCR*, polymerase chain reaction; *PPROM*, preterm premature rupture of membranes; *PTL*, preterm labor.

fetal structural anomalies, reported no higher risk for obstetric complications.[28,32]

While the meta-analysis referenced above allowed us to compare pregnancy outcomes and complications, it did not include data on the diagnostic yield of late amniocentesis, but those have been reported in a few other studies. The overall diagnostic rate in our study was 22.9%, which is higher than that reported in previous studies. For comparison, a retrospective study from a tertiary fetal center in China that included 1287 pregnancies found a 10.7% yield of abnormal genetic results from late amniocentesis.[19] Similarly, a single referral center study in India on 187 pregnancies reported a 13.33% yield of genetically abnormal results. The highest yields in this study were found in cases with multiple congenital anomalies (22%), CNS anomalies (22%), and skeletal anomalies (18%). In contrast, genetic or infectious testing did not identify any abnormality in cases of isolated FGR.[20] These findings are higher than the yields reported by Daum et al,[14] who found a 7.6% yield for 291 pregnancies, and Geffen et al,[17] who reported a 3% diagnostic yield for 167 pregnancies. The relatively high diagnostic yield in our study may be attributed to the data being primarily collected from referral centers, where pregnancies complicated by multiple fetal anomalies are more commonly managed, and from the increased availability of advanced diagnostic tools over the decade. The high-risk nature of our cohort likely accounts for the relatively high rates of fetal demise, pregnancy termination, and PTB observed. Fetal structural anomalies or FGR often independently increase the likelihood of PTB and fetal loss, irrespective of any procedure. Our observed diagnostic rate for isolated FGR was 5.8%, which was comparable to the incremental diagnostic yield of 4.8% of CMA after a normal karyotype for isolated early FGR fetuses with an estimated fetal weight <third percentile before 32 weeks of pregnancy tested at an average gestational age of 25.4 weeks.[33]

The median gestational age at the time of amniocentesis in our study was 28w5d

TABLE 6
Singleton pregnancies, live birth: gestational age at amniocentesis and preterm birth/spontaneous preterm birth

GA at amniocentesis (wk)	Number of cases after excluding IUFD and TOP	Preterm birth					
		<1 wk postprocedure		1–4 wk postprocedure		PTB <37 wk	
		All deliveries[a]	Spontaneous deliveries[a]	All deliveries[a]	Spontaneous deliveries[a]	All deliveries[b]	Spontaneous deliveries[a]
24w0d–27w6d (n=329)	267 Excluded: 62 (IUFD: 27, TOP: 35)	2/267 (0.7%)	0/248 (0%)	14/267 (5.2%)	4/248 (1.6%)	76/267 (28.5%)[c]	23/248 (9.3%)
28w0d–31w6d (n=229)	205 Excluded: 24 (IUFD: 8, TOP: 16)	4/205 (2.0%)	1/195 (0.5%)	16/205 (7.8%)	7/195 (3.6%)	51/205 (24.9%)	21/195 (10.8%)
32w0d–36w6d (n=165)	158 Excluded: 7 (IUFD: 4, TOP: 3)	4/158 (2.5%)	1/155 (0.6%)	13/158 (8.2%)	6/155 (3.9%)	20/158 (12.7%)	9/155 (5.8%)

In the spontaneous delivery columns, we included only deliveries for which explicit information confirmed whether the delivery was spontaneous or had been induced.

GA, gestational age; *IUFD*, intrauterine fetal demise; *PTB*, preterm birth; *TOP*, termination of pregnancy.

[a] No statistically significant difference between the 3 groups; [b] The preterm delivery rate before 37 weeks of gestation was lower in the group with amniocentesis between weeks 32w0d and 36w6d (P value=.001); [c] But similar in the groups with amniocentesis between weeks 24w0d and 27w6d, and amniocentesis between weeks 28w0d and 31w6d (P=.404).

(range: 24w0d–36w6d), with 338 amniocenteses done between 24w0d and 27w6d, and 239 between 28w0d and 31w6d, which is to our knowledge the largest reported number of amniocenteses performed at these gestational ages.[12,19] This provided a unique opportunity to compare the risks, outcomes, and diagnostic yields of amniocenteses performed earlier within the included gestational age ranges, when complications that cause unplanned preterm delivery could result in more significant prematurity compared to those performed later in gestation. This information is of immediate relevance for clinical care because there is a tendency among providers to delay amniocentesis until after 32 weeks to mitigate the risks of severe prematurity. This practice delays the completion of genetic testing, with a higher chance that results are not available until after delivery, precluding their use to inform prenatal, perinatal and immediate neonatal management. We found that amniocentesis performed at an earlier gestational age did not increase the risk of prematurity compared to amniocentesis performed at a later gestational age. In fact, the trend would indicate that the procedure-related risk for spontaneous PTB was the least in the earliest gestational age group, at 24w0d to 27w6d, and became progressively higher as the timing of amniocentesis progressed to the late preterm period (32w0d–36w6d). There may be a physiological basis for such a trend, given the greater uterine reactivity and contractility as pregnancy approaches term; however, this trend in our data did not reach statistical significance. Previous data on this topic are limited and contradictory. Our findings on risk are consistent with the work of Geffen et al, who also found no differences in total complication rates or specific complications between procedures performed before or after 30 weeks and those before or after 32 weeks. In a study from a single referral center in India, the risk of PTB within 4 weeks of amniocentesis performed after 29 weeks (7.7%) was higher than when it was done between 24 and 28 weeks (1.9%). This difference did not

TABLE 7
Pregnancy outcome associated with genetic results: diagnostic vs nondiagnostic results

Pregnancy outcome	Diagnostic results (n=171)	Nondiagnostic results (n=576)	*P* value
Live birth	111 (64.9%)	541 (93.9%)	<.001
Fetal demise	23 (13.5%)	18 (3.1%)	<.001
Pregnancy termination	37 (21.6%)	17 (3.0%)	<.001
Gestational age at delivery (wk)	38w1d (IQR 36w6d–39w1d)	38w3d (IQR 36w6d–39w2d)	.61
Birth weight (g)	2906 (IQR 2370–3295)	2980 (IQR 2401–3485)	.29
Procedure-to-delivery interval (d)	60 (IQR 44–85)	59 (IQR 40–83)	.3
Rate of preterm delivery <37 wk of gestation	28 (25.2%)	135 (25.0%)	.95
Rate of preterm delivery <34 wk of gestation	11 (9.9%)	55 (10.2%)	.94
Rate of preterm delivery <32 wk of gestation	6 (5.4%)	29 (5.4%)	.99
Apgar score at 5 min <7	19/92 (20.7%)	45/496 (9.1%)	.001
Neonatal death	24/104 (23.1%)	24/530 (4.5%)	<.001

IQR, interquartile range.

reach statistical significance and was counterbalanced by better neonatal lung maturity, even if delivery occurred within 4 weeks of the procedure.[20]

In regard to testing failure, we observed 1 case with limited metaphases for analysis, and excluded 2 due to maternal cell contamination. Notably, there were no instances of cell culture failure. Prior research has shown that amniotic fluid cell culture fails more often (up to 10%) after 24 weeks of gestation,[12] thought to be partly due to the enhanced keratinization of fetal skin, resulting in fewer viable amniotic fluid cells for analysis.[34] Reid et al[35] also reported that the likelihood of culture failure correlates significantly with abnormal ultrasound findings. More recently, advanced diagnostic methods like CMA, QF-PCR, and ES, which in contrast to karyotyping do not necessarily require prior culture, have improved the diagnostic yield of late amniocentesis,[19] with higher diagnostic yield when there are multiple structural anomalies.[14,20]

Clinical implications

Our findings have important clinical implications for the use of late amniocentesis. We found that late amniocentesis has a high diagnostic yield, making it a valuable procedure, particularly for pregnancies complicated by multiple congenital anomalies, isolated musculoskeletal anomalies, or hydrops fetalis. Our findings, corroborated by a recent systematic review and meta-analysis[12] affirm that the procedure is relatively safe with a low risk of complications and PTB, unless there are multiple fetal anomalies, which implies that not the late amniocentesis procedure itself, but the indication for which it is done, is the main cause of PTB. The low complication rate at 24w0d to 31w6d should allay concerns about performing amniocentesis at these gestational ages. This, combined with the high diagnostic yield, especially when CMA and ES are done, supports the recommendation that healthcare providers and pregnant individuals consider timely amniocentesis after the initial diagnosis instead of delaying the procedure, a practice that increases the chance of delivery before the completion of genetic testing.

Some fetal abnormalities may not be detected during the routine second-trimester anatomy scan, even with advanced equipment and expertise. This can occur because the anomaly might be present earlier but not seen due to technical difficulties, or because some abnormalities, for example, of the genitourinary tract or CNS, may develop or become visible only after the second-trimester anatomy ultrasound.[36] Drukker et al conducted a systematic review of 13 studies involving over 140,000 women and found new fetal anomalies in 3.68/1000 pregnancies imaged during the third trimester. The most common anomalies were urogenital, CNS, and cardiac, accounting for 55%, 18%, and 14% of third-trimester diagnoses, respectively.[37] A Cochrane systematic review of 2 randomized controlled trials comparing universal screening with clinically indicated screening in the third trimester found a higher detection rate of anomalies in the universally screened group. However, there was no overall improvement in infant survival rates.[38] The International Society of Ultrasound in Obstetrics and Gynecology practice guidelines regarding the performance of third-trimester obstetric ultrasound recommend that depending on the objectives of the third-trimester scan, anatomical evaluation may be undertaken, and mention prenatal genetic analysis such as third-trimester amniocentesis as a potential benefit of late diagnosis of fetal anomalies.[36] However, the value of late pregnancy imaging leading to prenatal genetic analysis and diagnosis has not been directly studied, highlighting the need for future prospective research to explore this area comprehensively.

TABLE 8
Pregnancy outcome associated with the indication for late amniocentesis

Pregnancy outcome	No anomalies on imaging (n=63)	Single organ system (n=437)	Multiple organ systems (n=247)	*P* value[a]	*P* value[b]	*P* value[c]
Live birth	58 (92.1%)	402 (92.0%)	192 (77.7%)	<.001	.98	<.001
Fetal demise	1 (1.6%)	12 (2.7%)	28 (11.3%)	<.001	1.0	<.001
Pregnancy termination	4 (6.3%)	23 (5.3%)	27 (10.9%)	.02	.76	.006
Gestational age at delivery (wk)	39w0d (IQR 38w0d–40w0d)	38w3d (IQR 37w1d–39w2d)	37w6d (IQR 35w6d–39w0d)	<.001	.009	<.001
Birth weight (g)	3207.5 (IQR 2716.5–3447.5)	2998.5 (IQR 2469–3509)	2665 (IQR 2040–3377)	<.001	.25	<.001
Procedure-to-delivery interval (d)	76 (IQR 49–97)	59 (IQR 40–84)	57 (IQR 36–78)	.003	.004	.2
Rate of preterm delivery <37 wk of gestation	11 (19.0%)	84 (20.9%)	68 (35.4%)	<.001	.73	<.001
Rate of preterm delivery <34 wk of gestation	4 (6.9%)	34 (8.5%)	28 (14.6%)	.048	1.0	.02
Rate of preterm delivery <32 wk of gestation	3 (5.2%)	19 (4.7%)	13 (6.8%)	.6	.75	.30
Apgar score at 5 min <7	2/52 (3.8%)	25/364 (6.9%)	37/172 (21.5%)	<.001	.56	<.001
Neonatal death	1/57 (1.8%)	19/389 (4.9%)	28/188 (14.9%)	<.001	.49	<.001

IQR, interquartile range.

[a] *P* value - Comparison between the 3 groups of indication for the procedure; [b] *P* value - Comparison between no anomalies to a single organ system involved; [c] *P* value - Comparison between single organ system to multiple organ systems.

Research implications

Given the retrospective design of the study and the challenges in fully adjusting for confounding factors linked to high-risk pregnancies, including the presence of fetal structural anomalies, and maternal risk factors that can influence outcomes, there is a need for more controlled prospective research. Acknowledging the ethical constraints inherent to a prospective randomized controlled trial to overcome these limitations, a prospective international registry should be considered. By including cohorts both undergoing and not undergoing late amniocentesis, yet sharing similar risk factors, such a registry would enable a more precise identification of the risks attributable to late amniocentesis, as opposed to those inherently associated with high-risk pregnancies.

Strengths and limitations

Our study's key strength lies in the international and multicentric nature of our research, which involves 9 referral centers. This approach provided us with a large cohort, enhancing the generalizability and robustness of our findings. Another strength of our study is that we excluded patients having additional prenatal therapeutic or diagnostic procedures. Additionally, our comprehensive data collection, encompassing obstetric history, pregnancy follow-up and outcome, and genetic testing results, serves as a robust framework for assessing the safety and efficacy of late amniocentesis in the clinical setting. Yet, we acknowledge that its retrospective design restricts our ability to capture data on all late amniocenteses done at each site during the study period. It also limited our ability to determine the number of pregnant individuals who were offered late amniocentesis but declined, along with their reasons for doing so. Another limitation arises from the study's duration of 11 years (2011–2022), a time-span during which the number and types of genetic tests available for prenatal diagnosis have evolved, such that not all women received the same level of diagnostic examination, and advanced tests like

fetal ES were unavailable in the early stages. The differing legal frameworks for pregnancy termination across countries and states possibly introduced additional variability in our findings, since more individuals without this choice later in pregnancy may have declined diagnostic testing through late amniocentesis. Finally, the fact that our study was conducted in referral centers poses a risk of selection bias. Patients with suspected complex abnormalities are preferentially referred to these centers, which could have contributed to the higher diagnostic yield, as well as a higher rate of indicated preterm delivery.

Conclusions

Our study shows that late amniocentesis, at or after 24 weeks of gestation, has a low complication rate and high diagnostic value, particularly for pregnancies complicated by multiple congenital anomalies, supporting its clinical utility. The diagnostic yield is expected to further increase beyond that derived in our retrospective study with more advanced laboratory diagnostics that are now routinely utilized.

Late amniocentesis enables pregnant individuals to undergo a comprehensive diagnostic evaluation, receiving timely results before delivery. This is crucial for informed patient counseling, to allow decisions on pregnancy management and delivery options, and to optimize both the subsequent antepartum and intrapartum management, as well as neonatal care planning. The data presented herein enable more comprehensive genetic counseling before a late amniocentesis, focusing on the patient's expectations of the genetic test, clarifying its benefits and limitations, and discussing potential outcomes and risks associated with the procedure. ■

References

1. Sarto GE. Prenatal diagnosis of genetic disorders by amniocentesis. Wis Med J 1970;69:255–60.
2. Hunter AG, Thompson D, Speevak M. Midtrimester genetic amniocentesis in Eastern Ontario: a review from 1970 to 1985. J Med Genet 1987;24:335–43.
3. Ghi T, Sotiriadis A, Calda P, et al. ISUOG practice guidelines: invasive procedures for prenatal diagnosis. Ultrasound Obstet Gynecol 2016;48:256–68.
4. Practice Bulletin No. 162: prenatal diagnostic testing for genetic disorders. Obstet Gynecol 2016;127:e108–22.
5. Jindal A, Sharma M, Karena ZV, Chaudhary C. Continuing Education Activity. europepmc.org. Available at: https://europepmc.org/books/n/statpearls/article-17468/?extid=30247838&src=med. Accessed July 10, 2020.
6. Quinlan MP. Amniocentesis: indications and risks. Virtual Mentor 2008;10:304–6.
7. Committee on Genetics and the Society for Maternal-Fetal Medicine. Committee Opinion No. 682: microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools in obstetrics and gynecology. Obstet Gynecol 2016;128:e262–8.
8. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 2012;367:2175–84.
9. Mellis R, Oprych K, Scotchman E, Hill M, Chitty LS. Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: a systematic review and meta-analysis. Prenat Diagn 2022;42:662–85.
10. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015;45:16–26.
11. Salomon LJ, Sotiriadis A, Wulff CB, Odibo A, Akolekar R. Risk of miscarriage following amniocentesis or chorionic villus sampling: systematic review of literature and updated meta-analysis. Ultrasound Obstet Gynecol 2019;54:442–51.
12. Nassr AA, Hessami K, D'Alberti E, et al. Obstetrical outcomes following amniocentesis performed after 24 weeks of gestation: a systematic review and meta-analysis. Prenat Diagn 2023;43:1425–32.
13. Odibo AO, Gray DL, Dicke JM, Stamilio DM, Macones GA, Crane JP. Revisiting the fetal loss rate after second-trimester genetic amniocentesis: a single center's 16-year experience. Obstet Gynecol 2008;111:589–95.
14. Daum H, Ben David A, Nadjari M, et al. Role of late amniocentesis in the era of modern genomic technologies. Ultrasound Obstet Gynecol 2019;53:676–85.
15. Picone O, Senat MV, Rosenblatt J, Audibert F, Tachdjian G, Frydman R. Fear of pregnancy loss and fetal karyotyping: a place for third-trimester amniocentesis? Fetal Diagn Ther 2008;23:30–5.
16. O'Donoghue K, Giorgi L, Pontello V, Pasquini L, Kumar S. Amniocentesis in the third trimester of pregnancy. Prenat Diagn 2007;27:1000–4.
17. Geffen KT, Ben-Zvi O, Weitzner O, Peleg A, Biron-Shental T, Sukenik-Halevy R. The yield and complications of amniocentesis performed after 24 weeks of gestation. Arch Gynecol Obstet 2017;296:69–75.
18. Leytes S, Haratz KK, Grin L, et al. Procedure-to-delivery interval after late amniocentesis and the need for routine antenatal corticosteroids. J Matern Fetal Neonatal Med 2022;35:4338–45.
19. Li Y, Yan H, Chen J, et al. The application of late amniocentesis: a retrospective study in a tertiary fetal medicine center in China. BMC Pregnancy Childbirth 2021;21:266.
20. Sharma A, Kaul A. Late amniocentesis: better late than never? A single referral centre experience. Arch Gynecol Obstet 2023;308:463–70.
21. Gabbay-Benziv R, Yogev Y, Melamed N, Ben-Haroush A, Meizner I, Pardo J. Pregnancy outcome after third trimester amniocentesis: a single center experience. J Matern Fetal Neonatal Med 2012;25:666–8.
22. Yinon Y, Katorza E, Nassie DI, et al. Late diagnosis of fetal central nervous system anomalies following a normal second trimester anatomy scan. Prenat Diagn 2013;33:929–34.
23. Malinger G, Lerman-Sagie T, Watemberg N, Rotmensch S, Lev D, Glezerman M. A normal second-trimester ultrasound does not exclude intracranial structural pathology. Ultrasound Obstet Gynecol 2002;20:51–6.
24. Bardin R, Hadar E, Haizler-Cohen L, et al. Cytogenetic analysis in fetuses with late onset abnormal sonographic findings. J Perinat Med 2018;46:975–82.
25. Ficara A, Syngelaki A, Hammami A, Akolekar R, Nicolaides KH. Value of routine ultrasound examination at 35-37 weeks' gestation in diagnosis of fetal abnormalities. Ultrasound Obstet Gynecol 2020;55:75–80.
26. Giovannopoulou E, Tsakiridis I, Mamopoulos A, et al. Invasive prenatal diagnostic testing for aneuploidies in singleton pregnancies: a comparative review of major guidelines. Medicina (Kaunas) 2022;58:1472.
27. Alfirevic Z, Navaratnam K, Mujezinovic F. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst Rev 2017;9:CD003252.
28. Toutain J, Lemaire-Coustel MA, Begorre M, et al. Proportion of parents agreeing to delay fetal karyotyping until the third trimester of pregnancy in cases with an indication. Fetal Diagn Ther 2012;31:115–21.
29. Richards CS, Bale S, Bellissimo DB, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. Genet Med 2008;10:294–300.
30. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24.

31. Martin JA, Hamilton BE, Osterman MJK. Births in the United States, 2022. NCHS Data Brieff, no 477. Hyattsville, MD: National Center for Health Statistics; 2023.
32. Hodor JG, Poggi SH, Spong CY, et al. Risk of third-trimester amniocentesis: a case-control study. Am J Perinatol 2006;23:177–80.
33. Borrell A, Grande M, Meler E, et al. Genomic microarray in fetuses with early growth restriction: a multicenter study. Fetal Diagn Ther 2017;42:174–80.
34. Lam YH, Tang MH, Sin SY, Ghosh A. Clinical significance of amniotic-fluid-cell culture failure. Prenat Diagn 1998;18:343–7.
35. Reid R, Sepulveda W, Kyle PM, Davies G. Amniotic fluid culture failure: clinical significance and association with aneuploidy. Obstet Gynecol 1996;87:588–92.
36. Khalil A, Sotiriadis A, D'Antonio F, et al. ISUOG practice guidelines: performance of third-trimester obstetric ultrasound scan. Ultrasound Obstet Gynecol 2024;63:131–47.
37. Drukker L, Bradburn E, Rodriguez GB, Roberts NW, Impey L, Papageorghiou AT. How often do we identify fetal abnormalities during routine third-trimester ultrasound? A systematic review and meta-analysis. BJOG 2021;128:259–69.
38. Bricker L, Medley N, Pratt JJ. Routine ultrasound in late pregnancy (after 24 weeks' gestation). Cochrane Database Syst Rev 2015;2015:CD001451.

Author and article information

From the Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX (Zemet and Van Den Veyver); Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX (Ali Maktabi); Department of Obstetrics and Gynecology, Columbia University Irving Medical Center, New York, NY (Tinfow, Giordano, Heisler, Yan, and Wapner); Department of Obstetrics and Gynecology, Sheba Medical Center, Tel-Hashomer, Israel (Plaschkes and Weisz); Department of Obstetrics and Gynaecology, National Maternity Hospital, Dublin, Ireland (Stokes, Walsh, Corcoran, and Crosby); The Center for Fetal Diagnosis and Treatment, The Children's Hospital of Philadelphia, Philadelphia, PA (Schindewolf, K Miller, and Gebb); Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of North Carolina School of Medicine, Chapel Hill, NC (Talati and Vora); Division of Maternal-Fetal Medicine, Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD (KA Miller, Blakemore, and Jelin); Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, CA (Swanson and Sparks); Department of Prenatal Diagnosis and Fetal Therapy, Justus-Liebig University, Giessen, Germany (Ramm and Bedei); The Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel-Hashomer, Israel (Berkenstadt); and Division of Maternal-Fetal Medicine and Reproductive and Prenatal Genetics, Department of Obstetrics and Gynecology, Baylor College of Medicine and Texas Children's Fetal Center, Houston, TX (Van Den Veyver).

Received March 8, 2024; revised June 12, 2024; accepted June 19, 2024.

The authors report no conflict of interest.

I.B.V.D.V. receives research support from award P50HD103555 (for the use of the Administrative and Clinical Translational Core facilities) from the *Eunice Kennedy Shriver* National Institute of Child Health & Human Development of the National Institutes of Health. R.Z. is supported by T32 GM07526 from the National Institute for General Medical Sciences of the National Institute of Health. N.L.V. receives research support from award R01HD105868 from *Eunice Kennedy Shriver* National Institute of Child Health & Human Development of the National Institutes of Health. A.C.J. receives research support from award 5K23DK119949 from the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health. I.B.V.D.V., J.L.G., R.J.W., and N.L.V. receive research support from award R01HD055651 from the *Eunice Kennedy Shriver* National Institute of Child Health & Human Development of the National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The findings were presented at the ACMG Annual Clinical Genetics Meeting, March 14—18, 2023, in Salt Lake City, Utah.

Corresponding author: Ignatia B. Van Den Veyver, MD. iveyver@bcm.edu; Roni Zemet, MD. roni.zemetlazar@bcm.edu