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MINIREVIEWS

Amniocentesis: A contemporary review

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Abstract

Amniocentesis is an essential tool in obstetrics. Invasive testing remains the only modality for diagnostic genetic testing and the only way to provide comprehensive testing for chromosomal abnormalities. Despite increasing use of cell free fetal deoxyribonucleic acid (DNA) testing, amniocentesis should still be offered to all women who desire more complete and accurate genetic testing. Counseling patients on the limitations of screening tests is of the upmost importance and amniocentesis should continue to be recommended to confirm positive results from cell free fetal DNA testing or in the case of failed cell free fetal DNA test. As cell free fetal DNA screening has not adequately been studied in multiple gestations, its use is not recommended in this population and invasive testing should be offered. Amniocentesis is also very useful in providing additional information in settings other than genetic testing the second and third trimester. If intraamniotic infection is suspected, but the clinical findings are not enough to guide management, amniocentesis can provide testing that can both immediately clarify the picture (interleukin-6, gram stain, glucose levels) and finally confirm the presence of infection (culture). It can also be used to detect the presence of intrauterine viral infections. Additionally, amniocentesis may be used to test for markers of fetal lung maturity. The American Congress of Obstetricians and Gynecologists recommends that amniocentesis for this indication not be used in cases where late preterm delivery is indicated. It may be useful in guiding decision-making, however, when late preterm delivery is indicated, but when exact timing is unclear. Regardless of the indication, amniocentesis appears to be a relatively low risk procedure with minimal risk to the patient. Additional randomized controlled trials are not likely, as they are not feasible to due extremely high number of participants that would be needed to detect a difference in loss rates. Based on current literature, however, the risk of pregnancy loss from second trimester amniocentesis is low in both singleton and twin gestations. We counsel patients that technique has changed since the original studies in the 1970s and feel comfortable quoting a loss rate of 1/500-1/1000 based on contemporary data.

Key words: Amniocentesis; Prenatal diagnosis; Invasive genetic testing; Procedure related loss rates; Cell free fetal DNA testing; Fetal lung maturity; Intraamniotic

infection

Core tip: Invasive testing remains the only way to provide comprehensive testing for chromosomal abnormalities. Despite availability of cell free fetal DNA testing, amniocentesis should still be offered to all women who desire complete genetic testing. Amniocentesis is also useful if intraamniotic infection is suspected, but the clinical picture is unclear. Additionally, when late preterm delivery is indicated, amniocentesis need not be used. There are, however, some instances when delivery timing is unclear and amniocentesis for fetal lung maturity may provide information to guide delivery timing. Amniocentesis is a relatively safe procedure. We counsel patients that technique has changed since the original studies in the 1970s and feel comfortable quoting a loss rate of 1/500-1/1000 based on contemporary data.

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HISTORICAL CONSIDERATIONS

Amniocentesis has been widely used since it was first performed in 1956[1]. Originally reported as a method of determining fetal sex *in utero*, Fuchs and Riis then hypothesized that it could be possible to diagnose chromosomal abnormalities *in utero via* this technique. By 1963, it had been confirmed that the karyotype of fetal and amniotic cells are identical[2]. Steele and Brag then showed in 1966 that amniotic cells can be sampled and cultured in sufficient quantity to be karyotyped, showing that *in utero* chromosome analysis was possible[3]. Shortly thereafter, the first prenatal diagnosis of an abnormal karyotype was reported[4]. Since that time, its utility has only continued to expand in both the second and third trimesters.

In 1979, the National Institutes of Health (NIH) suggested that amniocentesis be routinely offered to women 35 years or older, based on the balance between the risk of procedure-related pregnancy loss and the incidence of aneuploidy based on age[5]. Its use for genetic testing has only continued to expand over the years and is now the most commonly used invasive genetic test in obstetrics[6]. With the recent advent of cell free fetal DNA screening, understanding the continued need for and risks of amniocentesis has become increasingly important.

TECHNIQUE

Amniocentesis is generally performed after 15 wk gestation. The amniotic fluid volume at this gestational age is approximately 150 cc[5]. Prior to performing procedure, an ultrasound should be performed to evaluate the placental and fetal location and to confirm gestational age and fetal viability. After adequately prepping the maternal abdomen with antiseptic solution, the procedure should be performed under continuous ultrasound guidance to decrease the number of insertions and bloody taps[7]. If possible, transplacental passage of the needle should be avoided. If this is not possible due to an anterior placenta, care should be taken to avoid large vessels and echolucencies seen with ultrasound guidance. In the hands of experienced providers, there does not appear to be an increased loss rate with transplacental amniocentesis compared to procedures during which the needle does not pass through the placenta[8].

The effect of operator experience itself on outcomes of amniocentesis has independently been studied. In a retrospective review, Margioula-Siarkou *et al*[9], examined loss rates of a single operator over a 13 year period (n = 5913) and found the loss rates in the first 10% of amniocenteses performed to be significantly higher than the last 10%, suggesting there is benefit to experience. In a review of 6332 amniocentesis specimens that revealed male karyotype (46 XY), maternal cell contamination was seen more frequently in samples obtained by physicians who perform < 50 amniocenteses annually (0.67% *vs* 0.19%, P = 0.0021)[10]. There has never been a study defining an exact number of procedures after which a provider becomes "experienced," though reports of single institutions' experiences show that fetal loss rates are related to operator experience[11].

Needle size is another technical aspect of the procedure that has been postulated to have effect on loss rates; however studies on this issue are extremely limited. Athanasiadis *et al*[12], showed that while a larger caliber needle may facilitate a faster collection of fluid, it may also be associated with increased fluid leakage rates. From a review of the literature, it appears that 20 or 22 gauge needles are most commonly used today. When these two sizes were compared in a randomized trial with 200 participants, it was shown that procedure time was statistically significantly lower when a 20 gauge needle was used (9.6 s *vs* 26 s, P < 0.0001). There was no difference in intrauterine bleeding at the insertion site, patient discomfort 30 min post-procedure, or complication rates within 2 wk of the procedure between the two groups[12]. This decrease in procedure time is not clinically significant and we use a 22 gauge needle.

INDICATIONS

Amniocentesis allows for fetal DNA in the amniotic fluid to be analyzed for chromosomal abnormalities. This can be done in response to abnormal serum genetic screening, an abnormal ultrasound finding, or in order

to specifically test for a genetic condition for which a patient or partner is a carrier, including autosomal recessive, autosomal dominant, X-linked conditions or microdeletion/microduplication syndromes. This analysis of the fetal DNA is only possible with diagnostic testing via amniocentesis or chorionic villus sampling (CVS). One advantage of amniocentesis over CVS, however, is the ability to directly analyze fetal DNA. This avoids the potential issue of confined placental mosaicism that may be encountered in CVS samples. In 1%-3% of CVS samples, chromosomal mosaicism is seen[13]. This mosaicism is usually confined to the placenta, however is also present in the fetus in 10% of cases. In all cases of mosaicism on CVS, amniocentesis is recommended in order to determine whether it is confined to the placenta or is seen in the fetus as well.

Another important application for amniocentesis that deserves its own attention is in twin gestations. It has been previously shown that twin gestations are at an increased risk for chromosomal abnormalities. Further, the rate of multiple births is increasing. Between 1980 and 1999, the overall multiple birth ratio increased 59% and by 1999 multiples accounted for 3% of all live births[14]. Women of advanced age have experienced the greatest increase in rates of multiples[14]. As these women who are already at an increased risk for chromosomal abnormalities at baseline become increasingly pregnant with multiples, it is imperative that we have an accurate estimate of the risks of amniocentesis in this setting. As data is limited on cell free fetal DNA screening (see later) in the setting of multiple gestations, amniocentesis remains important for genetic diagnosis in these patients[15].

In addition to its utility in genetic testing, amniocentesis has been used in the third trimester to test the amniotic fluid for biochemical markers suggestive of fetal lung maturity. This indication for amniocentesis has recently come under closer scrutiny. The issue of timing when delivery is indicated in the late-preterm or early-term time period is based mostly on expert opinion. To analyze the obstetric, fetal and maternal conditions that often lead to late-preterm or early-term birth, the National Institute of Child Health and Human Development (NICHD) and the Society for Maternal-Fetal Medicine held a workshop in February 2011. In this meeting, the issue of using amniocentesis for fetal lung maturity to guide decision-making was directly addressed. The consensus was that if there is an indication for delivery, amniocentesis to assess fetal lung maturity should not be used to assist in delivery timing[16]. There are several rationales for this recommendation. The first is that if significant fetal or maternal risk exists, delivery should occur regardless of fetal lung maturity. The second issue is that confirmation of fetal lung maturity with amniocentesis does not translate into maturity of organ systems other than the lungs[17]. A committee opinion from the American Congress of Obstetricians and Gynecologists (ACOG) supports this recommendation, based on the same two issues[18]. In an editorial statement arguing the dissenting opinion, Towers *et al*[19] asserts that fetal lung maturity testing provides more information than just lung maturity. He argues that many of the serious morbidities for preterm neonates born after 34 wk (intraventricular hemorrhage, necrotizing enterocolitis) are highest in those infants who are intubated, and that the risk of intubation in the neonatal period after an amniocentesis showing fetal lung maturity is < 1%[20-22]. Further, he argues that there is a role for amniocentesis when the clinical scenario is not clear. For example, in the setting of uncertain dates and a quasi-urgent fetal indication for delivery, such as suspected fetal growth restriction with less-than-optimal interval growth at < 34 wk. We believe that it is reasonable to use amniocentesis to assess fetal lung maturity in this setting, when there is an indication for an early delivery but no imminent danger to mother or fetus would likely occur while awaiting results.

Another use for amniocentesis is in the diagnosis of intraamniotic infection. This diagnosis can usually be made clinically, based on maternal fever often with associated maternal or fetal tachycardia, uterine tenderness, or foul smelling amniotic fluid[23]. There are situations, however, where infection is suspected or likely, but the clinical picture is not this clear. It is extremely important in these situations to clarify the diagnosis and obtain further information to guide management, as undiagnosed infection would put the patient at risk. One example of this is in the case of a candidate for a physical exam indicated cerclage, as subclinical intraamniotic infection is seen in 13%-28% of these women[24]. Amniocentesis can be utilized in these cases, as an amniotic fluid culture remains the most specific test for documentation of intraamniotic infection. There are several other tests that have been used to aid in the diagnosis, as it is not always practical to wait several days for final culture results in the setting of a possible infection. Romero et al. studied the diagnostic value of each of these tests and found that a high interleukin-6 (IL-6) level was 82% sensitive and a negative gram stain was 99% specific for the detection of amniotic fluid containing bacteria[25]. The correlation between high amniotic fluid IL-6 levels and chorioamniotic infection has been supported by other authors as well[26]. Analysis of amniotic fluid for these parameters remains an invaluable tool in detecting infection when the clinical picture is not straightforward.

Amniocentesis should also be used to diagnose intrauterine viral infections, such as Cytomegalovirus or Parvovirus. Whether there are ultrasonographic signs that a fetus has been affected by one of these viruses or maternal serum indicates infection, amniocentesis can be performed. Polymerase chain reaction studies for these viruses should then be performed on the amniotic fluid obtained[27]. This information is essential to guide further fetal assessment and possible intrauterine treatment, depending on the clinical scenario.

An exciting new application for amniotic fluid and,

thus, amniocentesis is its potential use for the ascertainment of stem cells. There has been much attention and research aimed at the potential clinical uses of stem cells from bone marrow, blood, embryonic tissue and umbilical cord blood. Their widespread use has been limited by small cell number, potential tumorigenesis, and some ethical concerns with the use of embryonic tissue. The use of amniotic fluid cells obtained from discarded fluid after second trimester amniocentesis has shown promise as a way to circumvent some of these limitations. The ability to expand these multipotent cells in culture and to cryopreserve them for delayed differentiation and use has already been documented[28]. They have been shown to differentiate along adipogenic, osteogenic, myogenic, endothelial, neurogenic and hepatic pathways without giving rise to tumors[29]. These initial studies make these cells available to be used and studied for potential clinically significant therapeutic purposes.

There is one additional use for amniocentesis that is no longer widely used, though is worthy of discussion: spectral analysis of amniotic fluid (ΔOD450) to quantify the severity of fetal anemia. *In utero*, bilirubin from the fetal pulmonary and tracheal effluents is found in the amniotic fluid. The level of bilirubin in the fluid can be obtained *via* amniocentesis and then be used to estimate fetal hemolysis[30,31]. This technique was compared to middle cerebral artery (MCA) Doppler assessment in a prospective study by Oepkes *et al*[32]. MCA Doppler assessment was found to be 85% accurate, whereas ΔOD450 measurements were 76% accurate using the Liley curve and 81% accurate using the Queenan curve. Based on the findings of this study, MCA Doppler assessment has been widely accepted as the primary screening tool in the detection of fetal anemia[5]. We agree that amniocentesis should no longer be the first line surveillance tool in this situation, given that the noninvasive option has been shown to be superior.

COMPLICATIONS

Amniocentesis is a relatively safe procedure with minimal risk to the patient. With sterile technique, chorioamnionitis is seen in less than 0.1% of cases[11]. Other infrequent complications include transient vaginal spotting or leakage of amniotic fluid. Patients should be counseled that if leakage occurs, it usually occurs within 48 h and that fetal survival is greater than 90% in these cases[11].

Pregnancy loss is the most serious and feared risk to an amniocentesis. Generally quoted loss rates are primarily based on 3 main studies in the 1970s that were not randomized[33-35]. Based on these studies, the Centers for Disease Control and Prevention (CDC) promulgated a loss rate of 0.5% following amniocentesis. Despite the fact that these studies were not randomized and amniocenteses were not performed with the use of concurrent ultrasound guidance, this CDC estimation of loss after amniocentesis is still often quoted. There is only one randomized trial evaluating loss after amniocentesis published by Tabor *et al*[36] in 1986. This trial reports a 1% increased risk in the amniocentesis group when compared to the control group who did not undergo amniocentesis[36]. This study has been criticized for being carried out on young, low-risk women, which is generally not the group of women most commonly undergoing this procedure. The results from this trial, which was carried out 30 years ago, may not be applicable to our current practice, as their equipment was far inferior to what we have today. Nonetheless, this is the only randomized trial comparing amniocentesis to no amniocentesis, and it is likely to remain so due to societal pressures and ethical concerns.

Absent randomized controlled trials (RCT), researchers have sought to refine the reported risk of amniocentesis by utilizing non-randomized studies that, although not randomized, mitigate some of the criticisms of older studies. For example, Eddleman *et al*[37] used the large database of approximately 35000 patients who were enrolled in the FASTER trial. In this multi-center, prospective clinical trial, there was a 1% loss rate in the amniocentesis group and a 0.94% loss rate in the no amniocentesis group. This difference of 0.06% (1/1600) is the loss rate attributable to the amniocentesis. Another very large, contemporary study that included 51557 patients was done by Odibo *et al*[38], with loss rate of 0.13% (1/769) attributable to amniocentesis. A meta-analysis that included 21 studies performed after 2000 showed a 0.11% procedure-related loss rate[39]. This study only analyzed studies which included greater than 1000 procedures and only those who examined loss rates < 24 wk gestation in order to determine loss rates attributable to amniocentesis. Other recent trials have continued to demonstrate this trend in loss rates lower than previously seen by Tabor *et al*[38,40-43]: Typically in the range of 0%-0.5% loss rate attributable to amniocentesis. This more contemporary analysis of loss rates is reassuring and should be included in current patient counseling[38-42].

Overall, reported loss rates for amniocentesis in recent years are consistently low, but have been criticized for various reasons. Due to their nonrandomized nature, many of these studies do not have a control group that would provide a background loss rate. Even in the studies that do have a control group, they are often not appropriately matched in terms of baseline risk factors for the women in each group. Another issue with current literature is that there has not been a standard manner by which to report procedure-related loss rates. Studies to date have used varying definitions of pregnancy loss in terms of cutoffs for gestational age and length of time from procedure to loss. As mentioned earlier, there will likely not be any future RCT to assess contemporary loss rates. An RCT would require > 400000 patients in each arm to have adequate power to detect a difference

of 0.05% in loss rates between those who do and do not undergo amniocentesis[37]. Thus, using large scale, multicenter, prospective trials, such as the FASTER Trial, as a surrogate appears to be the best option. Given it was carried out in multiple centers and that there were no specifications as to the technique, the results are generalizable to the larger "national" community. Given that more recent literature suggests loss rates lower than seen in the 1970s, amniocentesis remains a safe option for genetic testing. We believe it is reasonable to counsel patients of an approximate 1/500-1/1000 risk of loss attributable to amniocentesis. All women should be offered genetic testing and we recommend a customized risk assessment for each individual patient, rather than using an arbitrary age cut-off to guide recommendation for amniocentesis. We also believe that patients today, with appropriate counseling, are able to understand the reasons that we cannot give them an exact number for the risk of loss and accept a "range of risk" as our best estimate.

The literature regarding loss rates after amniocentesis in twin gestation is even more limited[11]. There have been several published studies addressing this; however they are limited by small sample size[44-48]. Cahill published a retrospective review of a 16 year time period comparing women who underwent amniocentesis (n = 311) to a control group (n = 1623) who did not[49]. In this study, the attributable risk of pregnancy loss prior to 24 wk after an amniocentesis was 1.8%. The women who elected to have an amniocentesis were older, more likely to be ⩾ 35 years old and more likely to report alcohol exposure. It should be noted, however, that this increased risk of loss after amniocentesis remained after adjustment for maternal age, chorionicity, presence of anomaly on ultrasound, alcohol exposure, or race. Further, amniocentesis remained significantly associated with loss in patients who were younger than 35 years old and had normal ultrasound findings. In agreement with this finding, Yukobowich *et al*[47] found a statistically significant increase in fetal loss rate after amniocentesis (2.7% *vs* 0.6%). Conversely, other authors have found no difference in loss rates[45,46,50]. It is clear that additional studies are needed to further elucidate the true impact amniocentesis has on the loss rate in a twin gestation. The literature is extremely scant in regards to loss rates in the setting of higher order multiple gestations and further research is needed to guide counseling.

OTHER CONSIDERATIONS

The safety and accuracy of amniocentesis performed prior to 15 wk has been assessed in several randomized clinical trials. One study by the NICHD compared first trimester amniocentesis to first trimester transabdominal CVS[51]. They found an increase in spontaneous loss rate (RR-1.74) and a 4-fold increase in the rate of talipes equinovarus in the amniocentesis group. Those pregnancies which underwent CVS, however, are not an appropriate control group for those undergoing early amniocentesis. CVS is a different procedure that can be done at an earlier gestational age, which involves aspiration of the placental tissue and generally requires a larger gauge needle than used for amniocentesis. Nicolaides *et al*[52], found that the spontaneous loss rate after early amniocentesis (5.8%) was significantly higher than after CVS (1.8%). Other trials have been focused on comparing early amniocentesis to mid-trimester amniocentesis for a more direct comparison. Similarly, in a large study by the Canadian Early and Mid-Trimester Amniocentesis Trial Group[53] that included over 4000 amniocenteses, procedures done at 11-12 wk were compared to those done between 15-20 wk. The only difference between these two groups was the gestational age at the time of amniocentesis. There was a statistically significant increase in post-procedure spontaneous loss rate (2.6% *vs* 0.8%) and talipes equinovarus (1.3% *vs* 0.1%) in the early amniocentesis group. Given the established relative safety of amniocentesis in the mid-trimester, we recommend that these procedures be carried out after 15 wk gestation.

The safety of invasive procedures in the setting of maternal transmittable blood-borne illnesses is an additional concern, due to potential fetal contamination with maternal blood cells. Studies on amniocentesis in the setting of maternal human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are limited. There have been several small studies evaluating the risk of transmission in mothers with HBV. In the limited studies analyzing transmission rates after amniocentesis in women who were HBsAg-positive, there does not appear to be an increased risk of vertical transmission after amniocentesis[54,55]. In these studies, all infants did receive HBV vaccine and immunoprophylaxis at birth. The data on women with HCV is extremely limited, as there has been one prospective trial including 22 women. The amniotic fluid was positive for hepatitis C RNA in one woman at the time of amniocentesis. None of the infants born to these 22 mothers were positive for hepatitis C on postnatal testing[56]. Due to lack of evidence, there is insufficient data to estimate the risk of transmission in women with high hepatitis B or C viral loads. This preliminary data seems to suggest low risk of vertical transmission, however further studies with larger numbers are needed to adequately assess safety.

There have been more reports on amniocentesis in the setting of maternal HIV infection. There are studies that have shown 2-4 fold increased risk of vertical transmission of HIV after amniocentesis in the second or third trimester[57-59], though these studies were performed prior to the widespread use of combination antiretroviral therapy (ART) and many women had received no treatment at all prior to the procedure. There have been subsequent, promising small series reported after the use of combination ART has been shown to be effective. The previously shown increased risk of vertical transmission was not seen in these

women who were effectively treated[11]; in fact, the risk of transmission was not increased when compared to women who did not undergo amniocentesis[60-62]. The United States Department of Health and Human Services guidelines were updated to account for these new studies, stating that the risk of transmission does not appear to be increased in women treated with effective combination ART[63]. They do caution that although there have been no cases of vertical transmission in the 159 reported cases of amniocentesis in women who are on effective combination ART, a small increased risk of transmission may still exist. Due to this potential risk of vertical transmission, it is recommended that women in whom amniocentesis is indicated should be started on combined ART and ideally should have an undetectable viral load at the time of the procedure. Women should be counseled on the potential risk for transmission as well as the risks and benefits of noninvasive testing alternatives[11].

Recently, some obstetrical care providers have opined that with cell free fetal DNA technology, there will no longer be a need for amniocentesis. This technology in the maternal circulation has received much attention and press recently for screening, however it is limited in the information it can provide. Comprehensive pretest counseling is prudent, as this remains a screening modality. Patients must understand that this is not a diagnostic test, a negative test does not ensure a normal pregnancy, and that all positive results should be confirmed by invasive testing[64]. It most commonly only tests for trisomies 21, 18, and 13 and sex chromosome abnormalities, with varying degrees of accuracy[15]. In a study by Bianchi *et al*[65] of patients with abnormal cell free fetal DNA screening, aneuploidy was confirmed by invasive testing in only 93% with trisomy 21, 64% with trisomy 18, 44% with trisomy 13, and 38% with sex chromosomal abnormalities. Similarly, patients must be counseled on the genetic disorders that are not tested for by these blood tests. Women must be counseled that major trisomies screened for with cell free fetal DNA make up only about 50% of the cytogenetic abnormalities that would be found by karyotype following CVS or amniocentesis[5]. Further, there is a chance of test failure using this technique, due to a low fetal fraction of cell free fetal DNA recovered from maternal blood. In this circumstance, we believe that diagnostic testing should be offered due to an increased risk of aneuploidy in this setting (OR = 9.2)[66]. Testing with amniocentesis or CVS remain the only ways to definitively obtain genetic information from karyotype and microarray. Thus, ACOG still recommends offering invasive testing to all women[15].

CONCLUSION

Amniocentesis is an essential tool in obstetrics. Invasive testing remains the only modality for diagnostic genetic testing and the only way to provide comprehensive testing for aneuploidy and microdeletions. Despite increasing use of cell free fetal DNA testing, this test should still be offered to all women who desire more complete and accurate genetic testing. Counseling patients on the limitations of screening tests is of the upmost importance and amniocentesis should continue to be recommended to confirm positive results from cell free fetal DNA testing, in the case of failed cell free fetal DNA test, or with a positive first or second trimester screen. As cell free fetal DNA screening has not adequately been studied in multiple gestations, its use is not recommended in this population and invasive testing should be offered.

Amniocentesis is also very useful in providing additional information in settings other than genetic testing the second and third trimester. If intraamniotic infection is suspected, but the clinical findings are not enough to guide management, amniocentesis can provide testing that can both immediately clarify the picture (IL-6, gram stain, glucose levels) and confirm or exclude the presence of infection (culture). Additionally, in cases where late preterm delivery is indicated, but exact timing is unclear, amniocentesis to test for fetal lung maturity provides useful information to guide decision-making.

Regardless of the indication, amniocentesis appears to be a relatively low risk procedure with minimal risk to the patient. Though additional RCTs are not likely, based on current literature, the risk of pregnancy loss from second trimester amniocentesis is low in both singleton and twin gestations. We counsel patients that technology has changed since the original studies in the 1970s and feel comfortable quoting a loss rate of 1/500-1/1000 based on contemporary data.

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