9 Biochemical Screening for Neural Tube Defect and Aneuploidy Detection

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The use of biochemical screening in the field of obstetrics began in the 1970s with the identification of elevated levels of alpha-fetoprotein (AFP) in the amniotic fluid and maternal serum of pregnancies with fetal open neural tube defects (NTDs). Since that time, the role of biochemical screening has expanded to include multiple other maternal serum markers in both the first and the second trimesters of pregnancy, in screening not only for structural defects, but also for fetal aneuploidy, metabolic, and genetic disorders and even adverse pregnancy outcomes. This chapter will review the history of maternal biochemical serum screening, the factors that affect interpretation of these serum markers, the proposed evaluation and management of abnormal screening results, and the limitations of biochemical screening strategies. In addition, the role of ultrasound in the evaluation of abnormal serum screening results will be highlighted.

PHYSIOLOGY OF ALPHA-FETOPROTEIN

AFP is a 70,000-Da glycoprotein with its locus on the long arm of chromosome 4. Acting as an oncofetal protein, AFP is the major serum protein produced during fetal life. Early in gestation, AFP is synthesized by the yolk sac and then later by the fetal liver. AFP enters the amniotic fluid by diffusion across fetal skin as well as by excretion into urine by the fetal kidneys.[1] The concentration of both fetal serum AFP and amniotic fluid AFP peaks toward the end of the first trimester, with fetal serum AFP peaking between 10 and 13 weeks and amniotic fluid AFP reaching peak concentration between 12 and 14 weeks. Small amounts of AFP enter maternal circulation during early gestation, likely through diffusion across the amnion and transport across the placenta. In contrast to fetal serum and amniotic fluid levels, maternal serum AFP levels continue to rise throughout gestation, reaching a peak concentration between 28 and 32 weeks and then becoming nearly undetectable at term. The concentration gradient between fetal serum and amniotic fluid is approximately 150:1 to 200:1, whereas the concentration gradient between fetal serum and maternal serum is much greater at 50,000:1.[2,3] This difference in gradient forms the basis for the use of maternal serum AFP in screening for fetal anomalies, such as open neural tube and abdominal wall defects, as only a small amount of fetal blood leakage into the amniotic fluid can lead to an exponential rise in maternal serum AFP levels.

BIOCHEMICAL SCREENING FOR OPEN NEURAL TUBE DEFECTS

The use of AFP screening in obstetrics began in the 1970s, when Brock and Sutcliffe[4] observed that amniotic fluid AFP levels were elevated in pregnancies complicated by fetal open NTDs. They hypothesized that this elevation was due to transcapillary transudation of both cerebrospinal fluid as well as small amounts of fetal blood across the open spinal lesion and into the amniotic fluid. In the landmark United Kingdom Collaborative Study, it was demonstrated that an amniotic fluid AFP threshold of 2.5 times the multiple of the median (MoM) between 13 and 15 weeks would detect 98% of cases of anencephaly and open NTDs.[5] These findings were confirmed by other authors, yielding sensitivities ranging between 96% and 100% and specificities of greater than 99% for amniotic fluid AFP in the detection of open NTDs.[6,7] By the mid-1980s, amniotic fluid AFP as a screening test for open NTDs had been adopted into practice in the United States.

Shortly after this discovery, maternal serum AFP measurement was introduced as a screening tool for open NTDs. In 1977, Wald and colleagues[8] demonstrated that maternal serum AFP was greater than 2.5 MoMs between 16 and 18 weeks' gestation in 88% of fetuses with anencephaly and 79% with spina bifida. In 2003, the American Congress of Obstetricians & Gynecologists (ACOG) recommended that all women be offered screening for open NTDs with serum AFP in the second trimester of pregnancy.[9]

AFP levels are measured using enzyme immunoassays, and each laboratory performing this test is required to have established normal AFP reference ranges as well as undergo proficiency testing to ensure quality control. AFP levels are typically reported as MoMs of an unaffected population in order to normalize the distribution of results, accounting for interlaboratory variability and adjusting for other influential maternal factors. Today, most laboratories use thresholds of either 2.0 or 2.5 MoMs for reporting high AFP levels. Using a threshold of 2.0 MoMs, the detection rate is 95% for anencephaly and 75% to 90% for spina bifida. This results in a false positive rate (FPR) of 2% to 5%. Alternatively, when using the more stringent threshold of 2.5 MoMs, the detection rate decreases to approximately 90% for anencephaly and approximately 65% to 80% for spina bifida. However, the FPR also decreases to 1% to 3%.[6,9–11] These findings exemplify the necessary trade-off between higher detection rates at the expense of higher FPRs when defining a threshold for a screening test. Despite these promising detection rates, it is important to note that the positive predictive value (PPV) of maternal serum AFP screening is only 2% to 6%. While this PPV will increase in populations with a higher *a priori* risk of having an affected fetus, approximately 90% to 95% of NTDs occur in patients with no identifiable risk factors or family history.[9] This low PPV highlights the rather significant overlap in elevated maternal serum AFP levels in affected and unaffected pregnancies; therefore, maternal serum AFP should be used only as a screening test. All patients with positive results should undergo further diagnostic testing or imaging.

Traditionally, the gold standard for diagnosis of an open NTD following a positive screen for maternal serum AFP was amniocentesis. If amniotic fluid AFP levels were confirmed to be high, then an assay to detect the presence or absence of acetylcholinesterase (AChE) was performed. AChE is neuronally derived and, therefore, its presence in amniotic fluid is more specific to nervous system lesions. In a study of 10,000 pregnancies, the combination of amniotic fluid AFP and AChE detected 100%

of anencephaly cases and 100% of open spina bifida cases with a FPR of 0.2%.[12] Again, despite these findings, it must be noted that the presence of AChE in amniotic fluid is not 100% specific to nervous system lesions and has been reported in cases of other fetal anomalies.[13] Additionally, contamination of the amniotic fluid with fetal blood can also cause false positive results in both AFP and AChE levels. Finally, 10% to 15% of dorsal NTDs are skin-covered or "closed" and, therefore, will not be detectable using this technology.

ULTRASOUND SCREENING FOR OPEN NEURAL TUBE DEFECTS

During the 1980s, the detection rate for fetal open NTDs was only 50% to 80% using ultrasonography.[14,15] Such detection rates led many to believe that amniocentesis for AFP and AChE was still warranted even in the setting of a normal ultrasound.[14,16] However, with improvements in ultrasound technology and in the hands of experienced operators, targeted ultrasound for NTDs in the setting of an elevated maternal serum AFP level yields a sensitivity of 97% to 100% and a specificity of 100%.[17,18] In fact, Nadel and colleagues[18] demonstrated that a maternal serum AFP-based risk for an open NTD could actually be reduced by 95% after a normal targeted ultrasound exam. Given these advances, many providers feel comfortable using ultrasound as a diagnostic tool for the detection of NTDs, thereby eliminating the risks associated with invasive testing such as amniocentesis. However, in areas with limited ultrasound expertise and lack of resources, amniocentesis for AFP and AChE can still play a role in the diagnosis of NTDs.

Typical ultrasound findings in the setting of an open NTD include a thin-walled cystic mass anterior to the lesion, splaying of the spinous processes in coronal views, and a wide separation of the lateral spinous processes in transverse views. Abnormal angulation of the spine may also be present. Depending on the size and location of the lesion, these findings may be subtle. In 1986, Nicolaides and colleagues revolutionized the concept of ultrasound screening for NTDs with the description of intracranial abnormalities that are invariably seen in a group of cases of open NTDs. He described frontal bone scalloping ("lemon sign") and obliteration of the cisterna magna with abnormal anterior curvature of the cerebellum ("banana sign") as the cerebellar vermis, fourth ventricle, and medulla are displaced through the foramen magnum[19] (Fig. 9.1). In addition, ventriculomegaly and microcephaly are frequently observed in cases of open NTDs. Of note, the "lemon" and "banana" signs are often not observed after the midtrimester; whereas, ventriculomegaly may develop as gestation progresses. Regardless, the presence of any of these intracranial findings increases the sensitivity of ultrasound for the detection of open NTDs to greater than 99%.[20]

The majority of studies evaluating the accuracy of ultrasound have been performed in high-risk populations (i.e., patients with an elevated maternal serum AFP). More recent studies have suggested that routine second trimester ultrasonography actually may be more likely to identify an open NTD compared with routine maternal serum AFP screening in the general population.[21] Finally, *first-trimester* screening for open NTDs is currently under active investigation. It has been proposed that in fetuses with open spina bifida, the downward displacement of the fetal brain will lead to nonvisualization of the fourth ventricle in early gestation. The fourth ventricle, described as the "intracranial translucency," is typically visualized in the midsagittal view of the fetal face used to obtain first-trimester nuchal translucency measurement in normal fetuses. While larger prospective studies are warranted, preliminary results demonstrate promise.[22,23] In addition, recent studies also have demonstrated that a small biparietal diameter in the first trimester of pregnancy may be a subtle marker for open spina bifida.[24,25] In a retrospective review, Bernard and colleagues[24] observed a 10-fold increased risk of open spina bifida when the biparietal diameter measured less than the 5th percentile between 11 and 14 weeks. Combining this potential for a first-trimester screening modality with the high detection rates observed in second-trimester ultrasonography, the future role of maternal serum AFP screening for open NTDs is uncertain.

FACTORS AFFECTING MATERNAL SERUM AFP INTERPRETATION

Accurate pregnancy dating is one of the key components to successful maternal AFP serum screening. Maternal serum AFP concentrations increase by 15% to 20% per week between 16 and 22 weeks; therefore, inaccurate dating may lead to erroneous results. The optimal timing to perform maternal serum AFP screening is between 16 and 18 weeks. Prior to this gestational age, there is considerable overlap between affected and unaffected pregnancies.[3] First-trimester crown-rump length measurement is the preferred method for accurate pregnancy dating; however, fetal biometry may be used after 14 weeks' gestation. Given that biparietal diameter measurements may lag in fetuses with open NTDs, attention to other biometric parameters is necessary.

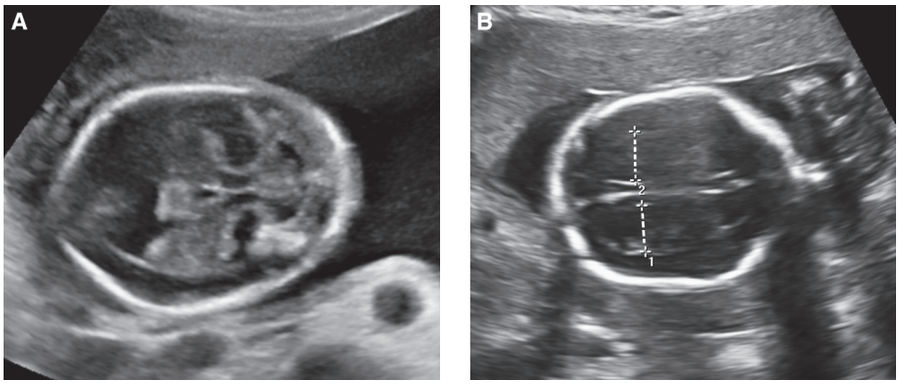

FIGURE 9.1: Intracranial ultrasound findings in fetuses with open NTDs: banana sign **(A)**, lemon sign **(B)**.

Other factors that must be accounted for when calculating maternal serum AFP concentration include maternal ethnicity, weight, and the diagnosis of diabetes mellitus. African Americans have a 10% to 15% higher serum AFP level compared with other races, despite the fact that African Americans have a lower incidence of NTDs overall.[26,27] While there may be small differences in serum AFP levels between other racial groups, these differences are not large enough to necessitate adjustment. Given that increasing maternal weight reflects an increased volume of distribution, higher body weights will result in a relative dilution of maternal serum AFP levels. Prior studies have shown that adjustment for maternal weight actually increases the detection rate of open NTDs and decreases the FPR.[28] The relationship between maternal serum AFP levels and diabetes mellitus remains controversial. It has been shown that serum AFP levels are approximately 20% lower in diabetic patients compared with nondiabetic patients; however, it remains uncertain whether the cause for this is the disease state itself or the state of glycemic control.[29] Notably, Baumgarten and Robinson[30] demonstrated a significant inverse relationship between maternal serum AFP and glycosylated hemoglobin, supporting the theory that uncontrolled glucose levels are the driving force behind this observed decrease. Additionally, it has been proposed that adjustment for maternal weight alone may be adequate. Although most laboratories continue to correct for diabetes in their AFP MoM calculation, the most recent literature suggests that this is likely unnecessary, regardless of insulin requirement.[31,32] Finally, maternal serum and amniotic fluid AFP levels are ineffective following multifetal or selective reduction procedures.[33] Ultrasound remains the optimal choice for open NTD screening in these patients.

ULTRASOUND EVALUATION FOR ELEVATED AFP LEVELS

False Positive Results

The first step in evaluating an elevated maternal serum AFP level is to perform an ultrasound to assess gestational age, if this has not previously been done. In approximately 50% of cases, underestimation of gestational age is identified, resulting in a false positive value.[3] In such cases, the initial serum AFP value can be adjusted, and often no further testing is needed. Fetal viability also should be assessed, as fetal demise can lead to an elevated maternal serum AFP level secondary to disruption in the maternal–fetal interface. Finally, it is imperative to evaluate for the presence of a multifetal gestation. Increased placental mass in multifetal gestations leads to increased AFP, approximately twofold higher in twin gestations compared with singletons. Typically, ultrasound evaluation for open NTDs is thought to be the gold standard evaluation for multifetal pregnancies; however, some laboratories do report an adjusted serum AFP value for twin gestations. Using this approach, either the maternal serum AFP MoM is divided by the median AFP level in unaffected twin pregnancies or a higher singleton threshold is used to define a positive screen. The issue with this approach is that a single serum value is being used to provide information on multiple fetuses. Cuckle and colleagues[34] demonstrated that by using a 2.5-MoM threshold, such as in a singleton pregnancy, the detection rate for open spina bifida in twin gestations was 89%; however, the FPR was as high as 30%. In order to maintain a FPR similar to that of singleton gestations, a 5.0-MoM threshold would need to be used, resulting in an open NTD detection rate of only 39% in twin pregnancies.

Maternal–fetal hemorrhage can also result in a falsely positive elevated maternal serum AFP level. While this can often be elicited through a maternal history of vaginal bleeding, not all cases of maternal–fetal hemorrhage will lead to recognizable clinical signs. Ultrasound evaluation may demonstrate subchorionic hematoma, retroplacental hematoma, or placental sonolucencies in such cases.[35,36] Placental implantation site abnormalities such as placenta accreta have also been reported to be associated with elevated maternal serum AFP levels; therefore, ultrasound of the placental site is warranted, especially in cases of placenta previa and a prior uterine scar.[37,38] Other placental findings associated with elevated maternal serum AFP levels include chorioangiomas, angiomyxomas, and placental hypertrophy.[39,40] Rarely diagnosed during pregnancy, ovarian germ cell tumors and hepatic tumors also can cause elevated serum AFP levels; however, the magnitude of AFP increase is typically much higher than is typically observed in obstetric screening programs.

Other Fetal Anomalies

Apart from open NTDs, elevated maternal serum AFP levels also have been associated with other fetal malformations. The second most common group of anomalies associated with elevated AFP levels are fetal abdominal wall defects, including omphalocele, gastroschisis, and bladder extrophy. As in the case of NTDs, these open defects allow for leakage of fetal serum into the amniotic fluid, resulting in high amniotic fluid and maternal serum AFP concentrations. Saller and colleagues[41] observed that pregnancies with gastroschisis and omphalocele had elevated AFP levels compared with the unaffected population, 9.42 and 4.18 MoMs, respectively. Other studies have demonstrated a greater screening sensitivity for gastroschisis compared with omphalocele at any given AFP threshold.[42]

Congenital skin disorders such as epidermolysis bullosa and aplasia cutis have also been associated with elevated AFP levels. This is hypothesized to be caused by increased diffusion of fetal AFP through the weeping skin lesions and into the amniotic fluid. Reflux of intestinal contents in cases of duodenal atresia, annular pancreas, and intestinal atresia as well as reflux of lung fluid in cases of congenital lung lesions can also cause AFP elevations. A full listing of fetal anomalies reported to be associated with elevated AFP levels is shown in Table 9.1.[1,3]

Although extremely rare in the general population, congenital nephrosis should be considered to be a potential diagnosis in cases of an extremely elevated AFP level and normal ultrasound findings.[43] This autosomal recessive disorder is most common in Finland, where the incidence ranges from 1 in 2,600 to 1 in 8,000 pregnancies.[44] This lethal disorder causes early renal failure, and death typically occurs in infancy or early childhood. Abnormal filtering capacity of the fetal glomeruli is thought to cause extreme fetal proteinuria. Consequently, elevated amniotic fluid AFP levels are present. Maternal serum levels are usually elevated on the order of 5 to 6 MoMs, whereas amniotic fluid AFP levels are typically elevated to >10.0 MoMs.[45] While the fetal kidneys may appear slightly enlarged and echogenic, ultrasound is typically normal in these fetuses. Placentomegaly may occur; however, this finding usually is not observed until the third trimester. Definitive diagnosis is made by electron microscopy of a renal biopsy. In utero kidney biopsy has been

Table 9.1 Fetal Anomalies Associated with Maternal Serum AFP Elevations

Open NTDs
Gastroschisis
Omphalocele
Bladder extrophy
Esophageal atresia
Duodenal atresia
Annular pancreas
Pilonidal cysts
Autosomal recessive polycystic kidney disease
Epidermolysis bullosa
Aplasia cutis
Bilateral renal agenesis
Sacrococcygeal hematoma
Congenital cystic adenomatoid malformation
Cystic hygroma
Acardiac twin
Obstructive uropathy
Osteogenesis imperfecta
Triploidy

AFP, alpha-fetoprotein.

suggested and successfully performed in order to make an antenatal diagnosis in cases with high suspicion for this disorder.[46]

Unexplained Elevated AFP

In approximately 1% of patients with an elevated AFP level, no apparent structural cause can be identified. However, these patients still remain at increased risk for adverse pregnancy outcomes, including fetal loss, preterm delivery, fetal growth restriction, oligohydramnios, placental abruption, and intrauterine fetal death.[47–49] Milunsky and colleagues[47] demonstrated that patients with an elevated maternal serum AFP level were at a twofold increased risk for preeclampsia, a threefold increased risk for placental abruption, a fourfold increased risk for low birth weight, and an eightfold increased risk for fetal death. Subsequently, Waller and colleagues conducted a case-control study and again demonstrated that elevated maternal serum AFP levels were associated with intrauterine fetal demise. The odds ratio for fetal death in that study was 10.4 (95% CI, 4.9 to 22.0) in the setting of AFP levels exceeding 3.0 MoMs.[50] Most interestingly, that study also showed that the risk of intrauterine fetal death persisted through the third trimester, suggesting that there is potential for these high-risk patients to be identified and offered increased antenatal surveillance. Most recently, elevated maternal serum AFP levels have been linked to an increased risk of sudden infant death syndrome (SIDS); however, this association may be biased by an increased incidence of fetal growth restriction and preterm delivery in those patients.[51] Despite these associations, the majority of women with an unexplained elevated AFP level do have a normal pregnancy outcome.

The optimal management strategy for patients with an unexplained elevated AFP level remains unclear. A suggested algorithm for the evaluation and management of these patients is shown in Figure 9.2. It is hypothesized that these unexplained elevations may be due to abnormal placentation; therefore, it might be possible to predict which patients are at highest risk for adverse outcome by indirectly evaluating the placental circulation through uterine artery Doppler studies. Initial studies evaluating the utility of uterine artery Doppler studies in predicting preeclampsia and fetal growth restriction produced a wide range of sensitivities and predictive values, with significant variation depending on the parameter studied (i.e., pulsatility index [PI], resistance index [RI], or diastolic notching).[52–54] A recent systematic review and meta-analysis demonstrated that an increased uterine artery PI with notching was the best predictor of preeclampsia with a positive likelihood ratio (LR) of 21.0 in high-risk populations and 7.5 in low-risk populations.[55]

Relatively few studies specifically have addressed the role of uterine artery Doppler studies in populations with unexplained elevated maternal serum AFP levels. Aristidou and colleagues[56] reported that the presence of diastolic notching was a good

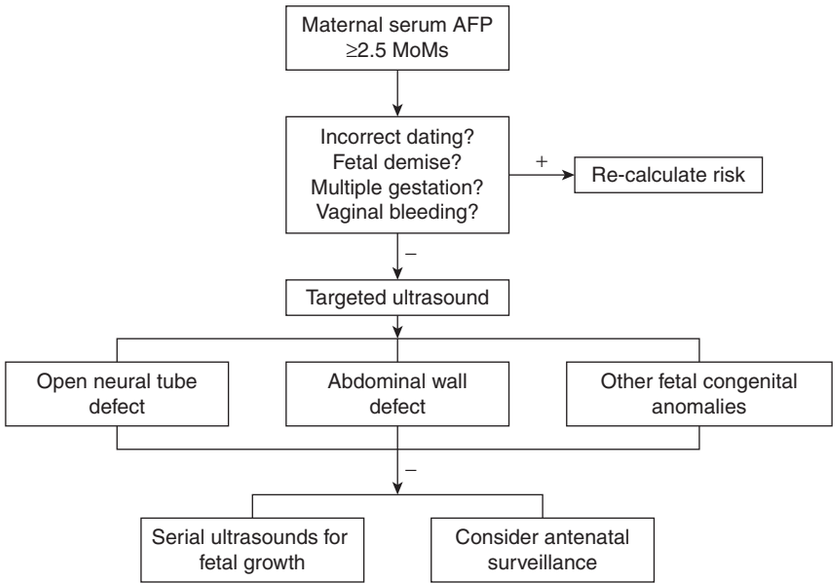

FIGURE 9.2: Suggested algorithm for the evaluation and management of elevated maternal serum AFP.

predictor of poor perinatal outcome in patients with an elevated maternal serum AFP level. Bromley and colleagues[57] demonstrated similar results; however, they observed that only severe grade II notching was associated with a significant increase in adverse outcomes (RR 3.4, 95% CI, 1.9 to 6.0). Finally, Konchak and colleagues demonstrated that both uterine artery notching and a RI > 95th percentile were predictive of adverse outcomes in this population. The best test characteristics were observed for the prediction of preeclampsia. Uterine artery notching demonstrated an 83.3% sensitivity and 95.6% specificity for preeclampsia, whereas an elevated RI demonstrated an 83.3% sensitivity and a 93.8% specificity for preeclampsia. Lower sensitivities were noted for the outcomes of fetal growth restriction and preterm delivery. Despite these results, PPVs ranged only between 44% and 55%.[58] While second-trimester uterine artery Doppler studies can be used in the evaluation of patients with an unexplained elevated maternal serum AFP level, there remain no specific guidelines for management and surveillance in these patients.

Umbilical artery Doppler studies as well as ultrasound evaluation of placental morphology have also been proposed as tools to evaluate patients with an unexplained elevated AFP level. Abnormal umbilical artery Doppler studies alone have not shown to be predictive of adverse outcomes in this population; however, in fetuses with identified fetal growth restriction, umbilical artery Doppler interrogation is useful in evaluating for worsening placental dysfunction.[57] Abnormal placental thickness, texture, and shape on ultrasound, especially in the setting of abnormal uterine artery Doppler studies may also be a predictor of adverse perinatal outcome in patients with elevated AFP levels.[59]

Given the increased risk for fetal growth restriction in these patients, serial ultrasound assessments for fetal growth are warranted in the third trimester of pregnancy. If abnormal growth is detected, then umbilical artery studies and antenatal testing with biophysical profiles or nonstress tests should follow. It is unclear whether the risk for intrauterine fetal demise in these patients is independent of fetal growth restriction; therefore, many institutions propose antenatal testing even in the absence of fetal growth abnormalities. In a 2001 retrospective cohort study of 136 patients with unexplained elevated maternal serum AFP levels, Huerta-Enochian and colleagues[49] demonstrated that intensive antenatal monitoring consisting of twice weekly nonstress tests and amniotic fluid volume assessment did not achieve earlier or improved detection of adverse outcome compared with routine prenatal care. The decision to perform antenatal testing in these patients remains an individualized decision between provider and patient.

USE OF BIOCHEMICAL MARKERS IN THE FIRST TRIMESTER

Aneuploidy Screening

In the mid-1990s, it was discovered that first-trimester alterations in pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) were associated with an increased risk for fetal aneuploidy.[60–62] These biochemical markers are placentally-derived, pregnancy-specific glycoproteins which are detectable in maternal serum. In pregnancies affected by trisomy 21, PAPP-A is reduced to 0.4 MoMs, and free β-hCG is increased to 1.98 MoMs between 9 and 11 weeks' gestation, on average. When combined with maternal age, low PAPP-A alone will identify approximately 50% of pregnancies affected by trisomy 21, whereas elevated free β-hCG alone will detect only 40% of pregnancies affected by trisomy 21.[63] When combining both PAPP-A and free β-hCG with maternal age, that detection rate increases from 60% to 65% at an FPR of 5%.[62]

Prior studies have evaluated the impact of measuring the intact hCG molecule rather than the free β-hCG subunit, as discussed above. Although both serum analytes are increased in fetuses affected by Down syndrome, levels of intact hCG do not begin to increase in affected fetuses until approximately 11 weeks compared with levels of free β-hCG, which begin to increase at 9 weeks. In a meta-analysis by Evans and colleagues,[64] the use of the free β-hCG subunit achieved a higher detection rate of trisomy 21 with a lower FPR compared with the intact hCG molecule. Today, most first-trimester aneuploidy screening protocols use the free β-hCG subunit; however, its impact on screening efficiency is likely greater during the earlier gestational age window of first-trimester serum screening.

In addition to these serum analytes, the discovery that an increased size of the fluid collection on the back of the neck (i.e., the "nuchal translucency") was also associated with an increased risk for trisomy 21 further revolutionized first-trimester aneuploidy screening.[65] First-trimester nuchal translucency measurement alone has a detection rate of 64% to 70% for trisomy 21; however, combining nuchal translucency measurement with PAPP-A and free β-hCG measurement increases that detection rate from 82% to 87% at a 5% fixed FPR.[66] This combined approach to first-trimester screening is now routinely offered to all patients, regardless of maternal age, between 10 and 13 6/7 weeks' gestation.

Combined first-trimester screening can also be used in the detection of patients at high risk for other aneuploidies, including trisomy 18 and trisomy 13. In contrast to trisomy 21, both PAPP-A and free β-hCG levels are *decreased* in these particular chromosomal abnormalities, while nuchal translucency is increased.[67,68] Tul and colleagues[67] demonstrated that free β-hCG MoM levels were decreased to <5th percentile of normal in 64% of trisomy 18 cases. Similarly, PAPP-A MoM levels were decreased to <5th percentile of normal in 78% of trisomy 18 cases. Results from the BUN study by Wapner and colleagues[69] showed a 90.9% detection rate for trisomy 18 at 2% FPR. Additional studies have demonstrated similar results for combined first-trimester screening with detection rates of 92.3% for trisomy 18 and 88.9% for trisomy 13.[70]

Adverse Pregnancy Outcomes

In the setting of a normal karyotype, low PAPP-A levels have been found to be associated with multiple adverse pregnancy outcomes. In a secondary analysis of the FASTER trial, Dugoff and colleagues demonstrated that low PAPP-A levels <5th percentile were significantly associated with fetal loss, preterm birth, gestational hypertension, preeclampsia, and low birth weight. Additionally, there was a pattern of an increased magnitude of risk as the PAPP-A level became more extreme. For example, the adjusted odds ratio (aOR) for spontaneous fetal loss <24 weeks was 1.95 (95% CI, 1.44 to 2.62) for PAPP-A levels <10th percentile; however, this risk increased to an aOR of 5.22 (95% CI, 3.09 to 8.80) for PAPP-A levels <1st percentile.[71] PAPP-A is derived from the placenta and serves as a protease for insulin-like growth factor (IGF) binding protein-4.[72] Decreased levels of PAPP-A are associated with high levels of bound IGF and, subsequently, lower levels of free IGF in the circulation.

IGF plays an important role in both fetal growth and trophoblast invasion into the maternal decidua.[73,74] Given that low PAPP-A levels are a reflection of low free circulating IGF levels, these observed associations with placenta-mediated adverse pregnancy outcomes appear to be biologically plausible.

Despite these associations, the test performance characteristics of low PAPP-A limit its use as a primary screening tool for adverse pregnancy outcomes. In a population-based cohort study, Krantz and colleagues[75] demonstrated that PAPP-A <1st percentile had a sensitivity of 3.3%, specificity of 99.3%, and PPV of 24.1% for fetal growth restriction. Pihl and colleagues[76] observed similar test characteristics for PAPP-A <5th percentile for fetal growth restriction, citing a detection rate of only 13% and a PPV of 14%. For preeclampsia, the PPV for PAPP-A <5th percentile is much lower, ranging from 3.5% to 7.6% in prior studies.[71,77,78] Incorporating a maternal risk factor-based scoring system to improve the screening efficiency of low PAPP-A for preeclampsia resulted in a sensitivity of 36.4%, specificity of 86.8%, and a positive LR of 2.8. Combining low PAPP-A, African American race, overweight maternal BMI, and maternal history of chronic hypertension and pregestational diabetes resulted in an area under the curve of 0.70, indicating only a modest predictive capability, at best, for the outcome of preeclampsia.[79]

Consideration of combining low first-trimester PAPP-A levels with elevated second-trimester maternal serum AFP levels has also been given, considering the association between elevated AFP levels and adverse outcomes, as discussed earlier in this chapter. Smith and colleagues[80] demonstrated a synergistic effect between low PAPP-A and high AFP in the identification of women at risk to develop fetal growth restriction and preterm birth. Subsequently, Dugoff and colleagues[81] demonstrated that the detection rate of patients at risk for early fetal loss <24 weeks was as high as 46% when combining low PAPP-A, high AFP, and low unconjugated estriol, another serum analyte used in second-trimester aneuploidy screening. Unfortunately, the exceedingly small number of patients who undergo sequential screening and actually have this combination of abnormal serum analytes precludes their use as clinical screening tools.

Alternatively, high levels of PAPP-A have not been consistently linked to adverse outcomes. While several studies have suggested a trend toward an increasing risk of macrosomia with high PAPP-A levels, this finding has not been confirmed in subsequent reports.[71,82]

The association between abnormal levels of first-trimester free β-hCG and adverse pregnancy outcomes has been less consistent. The most commonly cited adverse outcome associated with low free β-hCG in the first trimester has been early pregnancy loss.[71,83] Dugoff and colleagues[71] demonstrated that free β-hCG levels <1st percentile had a sensitivity of 3.7% at a 1% FPR and a PPV of 2.8% for spontaneous pregnancy loss <24 weeks. Similar to high levels of PAPP-A, high levels of free β-hCG in the first trimester have not been reliably linked to an increased risk for adverse pregnancy outcomes.

On the basis of their overall poor screening efficiency, first-trimester PAPP-A and free β-hCG cannot be recommended as primary screening tools for adverse pregnancy outcome. Additionally, subjecting a population to universal screening for outcomes for which there are currently no proven prevention strategies may lead to increases in fetal surveillance that are neither cost-effective nor clinically indicated. Nevertheless, serial ultrasounds to evaluate fetal growth may be considered in the small number of patients at the highest risk for adverse outcome such as those with PAPP-A levels <1st percentile. Conversely, patients with *normal* first-trimester PAPP-A and free β-hCG levels can be counseled so that their risk of adverse pregnancy outcome may be reduced.

Ultrasound Evaluation

Patients with an abnormal first-trimester aneuploidy screen who decline invasive prenatal diagnostic testing should undergo a detailed genetic ultrasound during the second trimester to evaluate for major fetal anomalies as well as minor markers of aneuploidy. In patients with an abnormal first-trimester serum screen and a normal karyotype, the protocol for subsequent evaluation is less clear. As opposed to abnormal maternal serum AFP levels, abnormal first-trimester PAPP-A and free β-hCG levels have not been linked to many structural fetal defects. In a 2008 report from a secondary analysis of the FASTER trial, free β-hCG levels >2.0 MoMs were significantly associated with both unilateral and bilateral multicystic dysplastic kidney disease and hydrocele. PAPP-A levels >2.0 MoMs were also associated with hydrocele.[84] Targeted fetal renal ultrasound may be considered in this subset of patients.

In an attempt to improve the screening performance of low PAPP-A levels for the prediction of preeclampsia and fetal growth restriction, many authors have proposed the addition of uterine artery Doppler studies.[85,86] Poon and colleagues[85] demonstrated that the detection rate of early preeclampsia (requiring delivery <34 weeks) was increased when combining a model of maternal risk factors, low PAPP-A, maternal mean arterial pressure, and uterine artery Doppler PI between 11 and 13 weeks. Other authors have demonstrated that the addition of second-trimester uterine artery Doppler studies in patients with low first-trimester PAPP-A levels improves the screening efficiency for preeclampsia and fetal growth restriction; however, the optimal timing to perform this study during the second trimester remains unclear.[87,88] Currently, uterine artery Doppler studies are not routinely performed during first-trimester aneuploidy screening at all centers; however, the addition of second-trimester uterine artery Doppler studies may be considered in patients with extremely low PAPP-A levels to provide further risk stratification. The addition of novel maternal serum analytes such as A-Disintegrin and Metalloprotease 12 (ADAM12), placental protein 13 (PP13), and placental growth factor (PlGF) to uterine artery Doppler studies is actively being investigated to improve the prediction of adverse pregnancy outcomes in the first trimester.

USE OF BIOCHEMICAL MARKERS IN THE SECOND TRIMESTER

Aneuploidy Screening

Prior to the 1980s, aneuploidy risk assessment was based solely on maternal age. During that time, only women aged 35 or older were offered diagnostic testing with chorionic villus sampling (CVS) or amniocentesis. The risk of having a child affected by trisomy 21 is 1 in 270 at age 35; therefore, these women were considered to be of the highest risk. This threshold was chosen for two reasons: (1) The risk of having an affected child rapidly increases after age 35 and (2) the risk of pregnancy loss following amniocentesis was thought to be equivocal or less than the

risk of having an affected child after this age. In 1984, Merkatz and colleagues[89] made the discovery that low levels of maternal serum AFP between 15 and 20 weeks' gestation were associated with trisomy 21. On average, maternal serum levels of AFP are 0.7 MoMs below the unaffected mean in pregnancies affected by trisomy 21.[90] This finding revolutionized the concept of aneuploidy screening, which could then be made available to all pregnant women, including those under the age of 35. Soon after this discovery, it was also noted that hCG levels were increased and unconjugated estriol levels were decreased in pregnancies with a trisomy 21 fetus. HCG levels are typically increased from 2.3 to 2.5 MoMs above the unaffected mean in trisomy 21 pregnancies, whereas unconjugated estriol levels are typically decreased below 0.7 MoMs.[91,92] Both hCG and unconjugated estriol are secreted from the syncytiotrophoblast, suggesting that aneuploid fetuses demonstrate placental immaturity, which results in both unregulated hypersecretion and undersecretion of placental products.[3]

Since AFP, hCG, and unconjugated estriol are only weakly correlated and independent of maternal age, these analytes can be combined with maternal age to assess aneuploidy risk. In 1992, Haddow and colleagues[93] demonstrated that the combination of these three serum analytes was more effective in screening for fetal trisomy 21 than screening with AFP alone. This formed the basis for the first multiple marker aneuploidy screening tool, the "triple screen." For patients under the age of 35, the detection rate for trisomy 21 is approximately 60% at a 5% screen positive rate. However, given that the *a priori* age-related risk of trisomy 21 increases with maternal age, both the detection rate and the screen positive rate of the triple screen also increase with maternal age. For women aged 35 and older, the trisomy 21 detection rate increases to >85%, at the expense of a 25% screen positive rate.[93,94] The triple screen can also be used to assess trisomy 18 risk; however, in cases of trisomy 18, levels of all three serum markers are decreased.[95]

Since the adoption of the triple screen, it has been found that pregnancies affected by trisomy 21 also have increased levels of dimeric inhibin-A in maternal serum. Levels of this protein, produced by the placenta, are elevated approximately 1.8 MoMs above the unaffected mean in trisomy 21 pregnancies.[96,97] The addition of inhibin-A to AFP, hCG, and unconjugated estriol creates what is now known as the "quadruple screen." This screening protocol results in a trisomy 21 detection rate of 81% at a 5% screen positive rate and has become the standard of care for second-trimester aneuploidy screening.[66] Using this multimarker test, a composite LR is determined on the basis of the level of the above-mentioned four serum analytes. The *a priori* maternal age-related risk is then multiplied by this LR to calculate an adjusted *posttest* aneuploidy risk. Of note, inhibin-A is not used in the risk calculation for trisomy 18. The pattern of serum analyte changes associated with trisomy 21 and 18 in both first- and second-trimester serum screening is shown in Table 9.2. The threshold at which a test is considered "screen positive" varies by individual laboratories. Given that a "screen positive" test is often a trigger for further invasive diagnostic testing, the chosen threshold must represent a balance between detection rate, FPR, and pregnancy loss risk from amniocentesis. Traditionally, a cutoff of 1 in 270 has been used, consistent with the age-related aneuploidy risk at age 35.[98]

The "quad screen" can be performed between 15 and 20 weeks' gestation; however, the screen is most accurate if performed between 16 and 18 weeks. As discussed earlier in the chapter, levels of maternal serum AFP increase with gestational age. Unconjugated estriol levels also increase with gestational age, while hCG levels decrease; therefore, accurate pregnancy dating is essential in interpreting serum screening results. In contrast, inhibin-A levels remain relatively constant throughout gestation. Maternal weight also must be accounted for when interpreting second-trimester serum screening. The increase in maternal plasma volume observed with increasing maternal weight causes a relative dilution effect on the serum analyte concentrations. The impact of race and ethnicity on serum analyte levels has been extensively studied. While significant differences in serum analyte levels have been identified, the majority of these differences are too small to have a significant effect on screening efficiency.[26,27] The largest differences are observed when comparing African Americans to Caucasians. Watt and colleagues[99] demonstrated that African American women had serum AFP levels that were 22% higher than those of Caucasian women and hCG levels that were 19% higher. Although the overall effect of adjusting for African American race only increased the aneuploidy detection rate by approximately 0.5%, this practice is still recommended on account of its proven influence on AFP detection of open NTDs. Finally, both AFP and unconjugated estriol levels have been found to be significantly lower in pregnant women with insulin-dependent diabetes.[100,101] Controversy still exists as to whether these findings are secondary to the disease state itself or to poor glucose control.

As with open NTD screening with AFP, serum screening tests for aneuploidy are less sensitive in multiple gestations. It is possible to calculate a "pseudo-risk" for aneuploidy using maternal serum analytes in twin pregnancies; however, this approach incorporates assumptions regarding zygosity and chorionicity.[102] First-trimester screening using individual nuchal translucency measurements for each fetus is the preferred approach for aneuploidy screening at most centers.

Other serum analytes that could improve the screening efficiency for trisomy 21 have been investigated. Hyperglycosylated hCG (h-hCG), also known as invasive trophoblast antigen, initially demonstrated promise, given that both maternal serum and urinary concentrations of h-hCG were demonstrated to be elevated in pregnancies affected by trisomy 21.[103,104] Many laboratories began to incorporate h-hCG into aneuploidy screening as a fifth serum analyte, thus creating the "penta screen."

Table 9.2 Pattern of Serum Analyte Alterations in Fetal Chromosomal Abnormalities

First-Trimester Biochemical Screening

	PAPP-A	Free β-hCG
Trisomy 21	↓	↑
Trisomy 13/18	↓	↓

Second Trimester Biochemical Screening

	AFP	hCG	Estriol	Inhibin-A
Trisomy 21	↓	↑	↓	↑
Trisomy 18	↓	↓	↓	N/A

PAPP-A, pregnancy-associated plasma protein-A; hCG, human chorionic gonadotropin; AFP, alpha-fetoprotein.

Subsequent studies demonstrated that the improvement in the trisomy 21 detection rate with the penta screen over the quadruple screen was only modest, increasing from 79% to 83% at a 5% fixed FPR, a nonsignificant increase.[105] This is likely due to the strong correlation observed between h-hCG and both intact hCG and free β-hCG. While the "penta screen" is still offered by some laboratories, it has not been adopted as routine standard of care for second-trimester serum aneuploidy screening.

ACOG currently recommends that all patients, regardless of age, be offered options for aneuploidy screening.[98] Combinations of both first- and second-trimester screening are also available that can increase the detection rate of trisomy 21 to approximately 95%.[66] While first-trimester serum screening can provide risk estimates for trisomy 13, second-trimester serum screening does not provide risk estimates for trisomy 13 or other lethal chromosomal abnormalities. This should be taken into consideration in conjunction with any potential ultrasound findings in determining whether to pursue serum screening for aneuploidy.

Abnormal Serum Analytes in Pregnancies with Normal Karyotype

Similar to first-trimester serum screening, extreme abnormal values of the second-trimester serum analytes have also been associated with other structural defects and adverse pregnancy outcomes in the presence of a normal fetal karyotype. The association between unexplained elevations in maternal serum AFP and adverse pregnancy outcome has been discussed earlier in this chapter. Low AFP levels <0.25 MoMs also have been linked to both pregnancy loss and low birth weight; however, these associations are less robust compared with those observed with high AFP levels.[48,106–108]

Extremely elevated levels of hCG in the second trimester have also been linked to adverse pregnancy outcomes such as preeclampsia, preterm delivery, intrauterine growth restriction, pregnancy loss, and intrauterine fetal demise.[109–111] The strongest association appears to be with intrauterine fetal demise, with an increased risk as high as four fold quoted for patients with hCG levels ≥2.0 MoMs.[111] The proposed mechanism for these associations stems from the hypothesis that the placenta produces an increased amount of hCG in response to the decreased oxygen supply that occurs in the setting of early placental vascular damage.[112] This supports the theory that placental dysfunction is reflected in abnormal levels of maternal serum analytes. As with other serum analytes, the magnitude of risk appears to increase as serum hCG levels become more extreme. In a case-control study by Lepage and colleagues,[110] only 2 of 15 women with extremely high hCG levels (≥10 MoMs) gave birth to a live-born neonate without complication. The risk of these adverse pregnancy outcomes is significantly greater in patients with the rare combination of both elevated AFP and elevated hCG levels.[111]

Low estriol levels below the range of 0.5 to 0.75 MoMs have also been linked to adverse pregnancy outcome; however, this finding in a fetus with a normal karyotype should also prompt investigation into other potential genetic and metabolic disorders of the fetal–placental unit.[109,113,114] The two most common disorders associated with extremely low (<0.15 MoMs) or absent maternal serum unconjugated estriol levels are Smith–Lemli–Opitz (SLO) syndrome and placental sulfatase deficiency. To understand these associations, it is first necessary to understand the biosynthesis pathway of unconjugated estriol. Dehydroepiandrosterone sulfate (DHEAS) is produced in the fetal adrenal gland and then converted to 16α-OH-DHEAS in the fetal liver. In the placenta, 16α-OH-DHEAS is then deconjugated by placental sulfatase, yielding molecules that will later be aromatized into unconjugated estriol.[3] Any disorder that impacts any step of this biosynthesis pathway will result in low- to absent-unconjugated estriol levels in maternal serum that may be detected at the time of second-trimester serum aneuploidy screening.

First described in 1964, SLO is an autosomal recessive disorder characterized by multiple fetal anomalies, moderate to severe mental retardation, growth failure, and characteristic-appearing facies.[115] The primary defect for this disorder involves cholesterol biosynthesis, leading to an increased amount of the cholesterol precursor 7-dehydrocholesterol and, subsequently, a decreased or absent amount of unconjugated estriol in maternal serum. In a case series of 33 women with SLO, 24 of 26 women who had second trimester unconjugated estriol levels measured had values <0.5 MoMs.[116] While universal risk assessment for SLO is possible using second-trimester screening, the yield of positive cases is low.[117,118] Consideration of the diagnosis of SLO is typically entertained in fetuses with a normal karyotype and an extremely low unconjugated estriol level, typically <0.3 MoMs. Prenatal diagnosis is currently based on the detection of increased levels of the cholesterol precursor 7-dehydrocholesterol in amniotic fluid.[119]

Placental sulfatase deficiency is another disorder associated with extremely low unconjugated estriol levels.[120] Lack of the placental sulfatase enzyme leads to decreased estrogen biosynthesis in the placenta. Placental sulfatase deficiency is X-linked and manifested clinically as icthyosis. Although usually mild and treatable, a small proportion of patients can have a more severe phenotype, including mental retardation. Prenatal diagnosis is available through molecular cytogenetic testing. Other disorders that have been associated with low unconjugated estriol levels include congenital adrenal hyperplasia, adrenocorticotropin deficiency, Kallman syndrome, hypothalamic corticotropin deficiency, and anencephaly.

Elevated levels of inhibin-A have been most extensively studied for their association with preeclampsia.[121,122] Aquilina and colleagues[123] demonstrated that inhibin-A levels ≥2.0 MoMs had a sensitivity of 48.6% and a specificity of 90% for preeclampsia, a predictive efficiency that was significantly greater than that of elevated hCG. A subsequent study demonstrated elevated inhibin-A levels to have a sensitivity of 71.4%, a specificity of 96.3%, and a PPV of 62.5% for preeclampsia.[124] In summary, abnormal levels of second-trimester serum analytes have a significant association with many adverse pregnancy outcomes in the absence of fetal aneuploidy; however, their relatively low predictive capability limits their use as primary screening tools for this purpose. Although it has been demonstrated that having two or more abnormal serum markers does increase sensitivity and PPVs, even when used in combination with each other and with first-trimester markers, serum analytes display only a modest accuracy for predicting adverse outcome overall.[125,126]

Ultrasound Evaluation

After a positive second trimester serum screen, patients are routinely offered invasive testing for definitive genetic diagnosis. All patients with a positive screen should undergo a targeted

genetic sonogram evaluating for major fetal anomalies as well as minor markers of aneuploidy. Common major anomalies seen in trisomy 21, 13, and 18 are listed in Table 9.3. Less-specific sonographic findings have been described in association with fetal aneuploidy, especially trisomy 21. These findings have become known as sonographic markers as the entities are nonspecific, often found in normal fetuses. The sonographic markers are listed in Table 9.4. Using these markers, an LR for fetal aneuploidy has been reported by several authors (Table 9.5).

In patients with a normal karyotype, consideration to the above-mentioned abnormalities and pregnancy complications should be given. Elevated inhibin-A levels ≥2.0 MoMs have been associated with multicystic dysplastic kidney disease and two-vessel umbilical cord.[84] These structural abnormalities should be readily apparent on routine fetal anatomic survey. As discussed earlier, patients with an extremely low unconjugated estriol level should be evaluated for SLO. The following ultrasound findings in conjunction with a low maternal serum estriol level are highly suggestive of SLO: syndactyly of two or three toes, growth restriction, microcephaly, and cleft palate. Cardiac defects, central nervous system malformations, and ambiguous genitalia can also be observed in cases of SLO.[127]

As in the case of first-trimester serum screening, the addition of uterine artery Doppler studies has been proposed to increase the detection rate of adverse outcome in patients with abnormal levels of second-trimester serum analytes. The combination of

Table 9.3 Common Features of Major Trisomies

	Trisomy 21	Trisomy 18	Trisomy 13
Major features	Cardiac defects, duodenal atresia, cystic hygroma, hydrops	Cardiac defects, spina bifida, cerebellar dysgenesis, micrognathia, diaphragmatic hernia, omphalocele, clenched hands/wrists, radial aplasia, clubfeet, cystic hygroma	Cardiac defects, central nervous system abnormalities, facial anomalies, cleft lip/palate, urogenital anomalies/echogenic kidneys, omphalocele, polydactyly, rocker-bottom feet, cystic hygroma
Markers or subtle findings	Nuchal thickening, hyperechoic bowel, EIF, shortened limbs, pyelectasis, mild ventriculomegaly, widened pelvic angle, shortened frontal lobe, clinodactyly, widened sandal gap, hypoplastic or absent nasal bone	Choroid cysts, brachycephaly, shortened limbs, IUGR, single umbilical artery	EIF, mild ventriculomegaly, pyelectasis, IUGR, single umbilical artery

EIF, echogenic intracardiac focus; IUGR, intrauterine growth restriction.

From Nyberg DA, Souter VL. Chromosomal abnormalities. In: Nyberg DA, McGahan JP, Pretorius DH, et al, eds. *Diagnostic Imaging of Fetal Anomalies*. Philadelphia, PA: Lippincott Williams & Wilkins; 2003:864.

Table 9.4 Sonographic Markers of Aneuploidy

Choroid plexus cysts
Strawberry-shaped head
Mild cerebral ventricular dilatation
Nuchal thickening
Hyperechoic bowel
Intraabdominal findings such as hepatic calcification
Shortened limbs or other skeletal anomalies
Echogenic intracardiac focus
Renal pyelectasis
Widened pelvic angle
Intrauterine growth restriction
Single umbilical artery/umbilical cord anomalies
Umbilical cord cysts/pseudocysts
Placental abnormalities, especially cystic changes
Abnormal amniotic fluid volume

Table 9.5 Comparison of Likelihood Ratios Reported for Sonographic Markers of Fetal Aneuploidy

	Likelihood ratio (95% confidence interval)		
Sonographic marker	Nyberg et al.	Bromley et al.	Smith-Bindman et al.
Nuchal thickening	11.0 (5.5–22.0)	12	17 (8–38)
Hyperechoic bowel	6.7 (2.7–16.8)	—	6.1 (3.0–12.6)
Short humerus	5.1 (1.6–16.5)	6	7.5 (4.7–12.0)
short femur	1.5 (0.8–2.8)	1	2.7 (1.2–6.0)
Echogenic intracardiac focus	1.8 (1.0–3.0)	1.2	2.8 (1.5–5.5)
Pyelectasis	1.5 (0.6–3.6)	1.3	1.9 (0.7–5.1)
Normal ultrasound	0.36	0.2	—

From Nyberg DA, Souter VL. Chromosomal abnormalities. In: Nyberg DA, McGahan JP, Pretorius DH, et al, eds. *Diagnostic Imaging of Fetal Anomalies*. Philadelphia, PA: Lippincott Williams & Wilkins; 2003:894; Bromley B, Liebermann E, Shipp T, Bernacerraf BF. The genetic sonogram: a method of risk assessment for Down syndrome: in the second trimester. *J ultrasound Med* 2002; 1087–1096.

second-trimester uterine artery Doppler with elevated levels of inhibin-A appears to provide the best prediction for preeclampsia, especially preeclampsia requiring early delivery.[128–130] Aquilina and colleagues[128] demonstrated that the sensitivity for preeclampsia improved from 27% to 60% when adding abnormal values of inhibin-A to bilateral uterine artery notching, which was a statistically significant increase. Alternatively, Ay and colleagues[124] demonstrated that although the sensitivity and specificity of uterine artery Doppler for the prediction of preeclampsia increases in the setting of abnormal inhibin-A levels, this increase was not clinically significant. Most recently, Filippi and colleagues demonstrated that abnormal second-trimester uterine artery Doppler studies conferred a high risk of adverse pregnancy outcome; however, *normal* uterine artery Doppler studies did not necessarily equate to *normal* pregnancy outcome. In that study, women with extreme levels of serum analytes but normal uterine artery Doppler studies still had a 26% risk of adverse pregnancy outcome.[131] The decision to perform second-trimester uterine artery Doppler studies in patients with unexplained abnormal serum analyte levels remains individualized.

NONINVASIVE PRENATAL TESTING AND CELL-FREE FETAL DNA

Noninvasive Prenatal Testing (NIPT)/Cell-Free Fetal DNA (cffDNA)

In late 2011, NIPT via analysis of cffDNA in maternal plasma was first offered to obstetricians and maternal fetal medicine specialists in the United States as a screening test for high-risk pregnancies.[132] Initially, NIPT was performed exclusively to screen for Down syndrome, trisomy 18, and trisomy 13. Since then its uses have expanded, and it is likely that other indications for this procedure will continue to grow. For example, some laboratories now offer NIPT to screen for sex chromosome abnormalities, triploidy, and even some microdeletion syndromes.[133]

This noninvasive screening method relies on the fact that cffDNA fragments from both the fetus and the pregnant woman are present in the mother's bloodstream during pregnancy. Cell-free fetal DNA fragments, mostly of trophoblast origin, cross the placental barrier and enter the maternal circulation. These cells clear from the maternal system within hours, so fetal DNA detected during a pregnancy represents DNA from the current gestation. The fetal fraction of cell-free DNA is approximately 11% of all cell-free DNA circulating in the maternal plasma.[134] The risk of aneuploidy for a particular chromosome can be assessed by comparing the amount of cffDNA of the chromosome of interest to the cell-free DNA counts of other chromosomes. The laboratory method and sensitivity of NIPT varies between testing sites and by the genetic abnormality that is being screened. Most laboratories report detection rates of 99% for Down syndrome, 97% to 99% for trisomy 18, 80% to 92% for trisomy 13, and over 90% for most sex chromosome abnormalities.[135]

There are several advantages to the NIPT screening method. First, it can be done as early as 10 weeks in pregnancy and throughout the remainder of pregnancy, unlike other maternal serum screens which require testing over specific time windows during the pregnancy. Second, it has a higher detection rate and lower FPR than any of the other first- or second-trimester screening modalities for Down syndrome, trisomy 18, and trisomy 13. This ultimately leads to fewer women choosing to pursue invasive testing through CVS or amniocentesis, both of which carry some negative risk to the pregnancy.[136] Last, it can screen for other genetic abnormalities for which historically there has been an absence of noninvasive maternal serum screening. These disorders include triploidy, sex chromosome abnormalities, and some microdeletion syndromes.

Despite many advantages, there are limitations to NIPT. First, NIPT is a screening test, not a diagnostic test. It is not as reliable as CVS or amniocentesis in detecting chromosome abnormalities. Its scope of use is also smaller. Many genetic anomalies such as mosaicism, partial trisomies, translocations, and single gene disorders, cannot yet be screened with NIPT. Second, NIPT is dependent on a sufficient fetal fraction of cell-free DNA. Approximately 0.5% to 1% of women will receive an inconclusive test result owing to insufficient fetal fraction.[135] Fetal fraction decreases with increasing maternal weight, for example, so women who are overweight (esp. >200 lb) are more likely to receive an uninterpretable result.[137] Lastly, although studies are currently being performed on low-risk populations, NIPT has to date been validated only in the high-risk (e.g., advanced maternal age, abnormal serum screen, personal or family history of aneuploidy, and abnormal ultrasound) patient population. In addition, it has not been validated in triplet or higher multiple pregnancies, or in pregnancies conceived using egg donation.[135] However, its use has dramatically changed the paradigm for prenatal screening and testing in the high-risk population, and should future studies validate similar results for other populations, it is likely to become the primary screening option for all pregnant patients.

In summary, NIPT is an effective first-line screening tool for high-risk pregnant women who are >10 weeks gestational age. NIPT should be offered in concert with proven diagnostic tests such as CVS and amniocentesis that have the ability to detect genetic conditions missed by NIPT. Genetic counselors should be consulted to explain the complexities of this screening modality to patients and their families.[138]

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