Prenatal Diagnosis, Fetal Surgery, Recurrence Risk and Differential Diagnosis of Neural Tube Defects

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SUMMARY

Prenatal screening with α-fetoprotein (AFP) and ultrasonography have allowed the prenatal diagnosis of neural tube defects (NTDs) in current obstetric care, and open spina bifida has been considered a potential candidate for *in utero* treatment in modern pediatric surgery. This article provides an overview of maternal serum AFP screening, amniotic fluid AFP assays, amniotic fluid acetylcholinesterase immunoassays and level II ultrasound for NTDs, prenatal repair of fetal myelomeningocele, recurrence risk of NTDs, and differential diagnosis of NTDs on prenatal ultrasound. [*Taiwan J Obstet Gynecol* 2008;47(3):283–290]

Key Words: acetylcholinesterase, α-fetoprotein, differential diagnosis, neural tube defects, prenatal diagnosis, recurrence risk, ultrasound

Introduction

Prenatal screening with α-fetoprotein (AFP) and ultrasonography have allowed the prenatal diagnosis of neural tube defects (NTDs) in current obstetric care, and open spina bifida has been considered a potential candidate for *in utero* treatment in modern pediatric surgery. This article provides an overview of maternal serum AFP screening, amniotic fluid AFP assays, amniotic fluid acetylcholinesterase immunoassays and level II ultrasound for NTDs, prenatal repair of fetal myelomeningocele, recurrence risk of NTDs, and differential diagnosis of NTDs using prenatal ultrasound.

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Maternal Serum AFP Screening, Amniotic Fluid AFP Assays, Amniotic Fluid Acetylcholinesterase Immunoassays and Level II Ultrasound for NTDs

Maternal serum screening for open NTDs using AFP began in the 1970s. In 1977, Wald et al [1] found that maternal serum AFP ≥2.5 multiples of the median (MoM) occurred in 88% of cases of anencephaly, 79% of cases of open spina bifida and 3% of unaffected singleton pregnancies when tested at 16–18 gestational weeks. Maternal serum AFP test is performed between 15 and 20 or 22 gestational weeks using the threshold of 2.5 MoM, with an anticipated sensitivity of 85% for NTD detection, a screen-positive rate of 5% or less and a positive predictive value of about 2% (2% of all women with a positive test result are carrying a fetus with an NTD) [2–4]. The American College of Obstetricians and Gynecologists (ACOG) [4] suggested that maternal serum AFP elevation is an effective screening test for

NTDs and should be offered to all pregnant women unless they plan to have amniotic fluid AFP measurements as part of the prenatal diagnosis of chromosomal abnormalities or other genetic diseases. Reichler et al [5] found that in a total of 529 patients with an elevated maternal serum AFP level and amniocentesis, there was a progressive increase of anomalies as a direct function of the level of maternal AFP, varying from 3.38% at a level of 2.5–2.9 MoM, 7.79% at a level of 3.0–3.9 MoM, 13.11% at a level of 4.0–4.9 MoM, 18.18% at a level of 5.0–6.9 MoM to 40.28% at a level ≥ 7.0 MoM. In the study of Reichler et al [5], the risk of NTDs varied from 1.45% (2.5–2.9 MoM), 3.25% (3.0–3.9 MoM), 4.92% (4.0–4.9 MoM), 10.90% (5.0–6.9 MoM) to 13.46% (≥ 7.0 MoM). Women with maternal serum AFP levels higher than a predetermined cutoff level (usually 2–2.5 MoM) should be referred for genetic counseling and consideration of a diagnostic test [4,6]. A typical NTD screening protocol has been suggested as follows [6]:

1. If the maternal serum AFP is < 2.0 MoM, no further action is required.
2. If the maternal serum AFP is ≥ 2.0 MoM, ultrasound is required to check dates.
3. Following date checking by ultrasound, if there is a difference of < 10 days from the last menstrual period, the screening remains positive. If there is a difference of ≥ 10 days from the last menstrual period and the maternal serum AFP is ≥ 2.0 MoM after date adjustment, the screening is positive. Genetic counseling for risks and benefits of amniocentesis and/or level II ultrasound should be offered when the screening is positive.

In the case of an elevated amniotic fluid AFP level in the presence of acetylcholinesterase, doctors should consider a diagnosis of NTD. In a study of 9,964 women with singleton pregnancies and known outcome (including six with anencephaly and 18 with open spina bifida), Loft et al [7] found that the acetylcholinesterase immunoassays and the amniotic fluid AFP assays yielded a 100% detection rate for anencephaly and for open spina bifida, with a false-positive rate of 0.22% and 0.08% for the acetylcholinesterase immunoassays and amniotic fluid AFP assays, respectively. Watson et al [8] found a 0.61% risk of chromosomal anomalies in pregnancies complicated by an elevated maternal serum AFP level but without any sonographically detected fetal structural abnormalities. In contrast, Harmon et al [9] found a 16.3% risk of chromosomal anomalies in pregnancies with prenatally diagnosed isolated NTDs, compared with a 0.3% risk in the same population based solely on maternal age.

With experienced sonologists, sonography is sensitive and specific for the diagnosis of open NTDs [10–17]. In view of the increasing resolution of sonographic equipments and the 0.5% risk of post-procedure pregnancy loss in second-trimester amniocentesis, the need for amniocentesis in pregnant women with elevated levels of maternal serum AFP but normal ultrasonographic examination has been questioned [10,11,14,17]. Hogge et al [10] suggested that targeted ultrasonography alone may be a reasonable alternative for amniocentesis in the evaluation of pregnancies at risk for NTDs. In a study of the relative efficacy of amniocentesis versus targeted (detailed) ultrasonography in 225 patients referred because of an elevated maternal serum AFP level (79.6%) or a family history of NTDs (20.4%), Hogge et al [10] found that sonographic examination alone detected all 26 fetal anomalies, including 11 cases of anencephaly, 10 of open spina bifida and five of other anomalies. Nadel et al [11] reviewed the ultrasound findings in 51 consecutive fetuses with spina bifida, encephalocele, gastroschisis or omphalocele and found that all 51 cases were correctly diagnosed. The authors suggested that women with elevated maternal serum AFP levels should be referred to institutions that can perform level II ultrasound; if the level II ultrasound is normal, the patients can be told that the risk of an anomaly is low and can make an informed decision about whether or not to proceed with amniocentesis. Katz et al [12] detected all nine open NTDs among patients with elevated maternal serum AFP using ultrasound. The authors suggested that informed consent prior to amniocentesis should include a discussion of the ultrasound evaluation for evaluation of elevated maternal serum AFP. Lennon and Gray [13] found that ultrasound alone was 97% sensitive and 100% specific in diagnosing open NTDs. In a study of 4,430 cases of mid-trimester amniocentesis, Mandruzzato et al [14] found that all 13 cases presenting with structural malformations were correctly diagnosed by ultrasound. The authors suggested that if there is optional ultrasound scanning, routine assessment of amniotic AFP at the time of mid-trimester genetic amniocentesis is no longer justified. Norem et al [15] retrospectively identified 189 NTD cases among 219,000 consecutive pregnancies and found that among the 102 NTD cases who had received maternal serum AFP screening, 25 cases (25%) had negative maternal serum screening results, including 15 (38%) of the 40 spina bifida cases screened, six (67%) of the nine encephalocele cases screened and four (8%) of the 53 anencephaly cases screened. They additionally found that of the 186 NTD cases diagnosed prenatally, 115 (62%) were initially detected by routine ultrasound without knowledge of maternal serum AFP screening, 69 (37%) were diagnosed by targeted ultrasound with knowledge of elevated maternal serum AFP values and two (1%) were

diagnosed by pathologic examination after miscarriage. Norem et al [15] concluded that second-trimester ultrasound was more likely to detect NTDs, with 93% (50/54) sensitivity for the detection of spina bifida, 100% (59/59) sensitivity for the detection of anencephaly, 94% (16/17) sensitivity for the detection of encephalocele and 96% (125/130) sensitivity for the detection of NTDs using standard ultrasound. They suggested that maternal serum AFP screening should not be the sole screening method for detecting NTDs, and that routine second-trimester ultrasound should be included as part of standard prenatal care. Dashe et al [16] found that standard ultrasound improved NTD detection over maternal serum AFP screening alone, by improving maternal serum AFP test sensitivity from 65% to 86% if the gestational age used for AFP calculation was confirmed with ultrasound, and by identifying NTDs in low-risk pregnancies with 100% sensitivity using standard ultrasound. In a study of 6,501 women who underwent amniocentesis and had amniotic fluid measurements taken, Kooper et al [17] found that 27 out of 6,188 pregnancies (0.4%) without any increased NTD risk had amniotic fluid AFP levels >2.5 MoM, and two of which were associated with NTDs; two out of 258 pregnancies (0.8%) with an increased NTD risk had an increased amniotic fluid AFP level and were associated with NTDs; and 44 out of 55 pregnancies (80%) with clinically diagnosed fetal NTDs had an increased amniotic fluid AFP level. The authors suggested that high-quality ultrasound imaging will accurately detect NTDs and that fetal anomaly scan will potentially replace routine amniotic fluid AFP testing, although NTDs may be difficult to be detected by standard ultrasound in cases of maternal obesity and fetal persistent spine posterior position.

Prenatal Repair of Fetal Myelomeningocele

Management of Myelomeningocele Study (MOMS) trial

Prenatal repair of fetal myelomeningocele is an acceptable fetal surgery but with unproven benefits [4,18,19]. The role of the prenatal repair of fetal myelomeningocele is presently being investigated in the Management of Myelomeningocele Study (MOMS) trial, which began in February 2003, had approximately 112 patients in March 2007 and is estimated to be completed in 2009 [19]. The MOMS trial is an unblinded, randomized, controlled clinical trial of 200 patients in three institutions, i.e. The Children's Hospital of Philadelphia (CHOP), Vanderbilt University Medical Center (VUMC) and the University of California at San Francisco (UCSF), and is sponsored by the National Institute of Child Health and Human Development (NICHD) in the United States [19]. The primary end-point of the trial is the need for shunt at 1 year, and the secondary end-points are neurologic function, cognitive outcome and maternal morbidity following prenatal repair of fetal myelomeningocele [19]. The research agenda include reversal of hindbrain herniation, changes in fetal posterior fossa volume in myelomeningocele fetuses who undergo fetal surgery and those who do not, indications for shunting myelomeningocele patients who have ventriculomegaly but no evidence of overt increased intracranial pressure, and incidence of inclusion dermoid cysts in fetal surgery patients and in postnatally closed patients [19]. The inclusion criteria of the trial include myelomeningocele at level T1–S1 with hindbrain herniation, maternal age of 18 years or older, gestational age of 18–25 weeks at randomization, and normal karyotype [19]. Patients are centrally randomized to one of the following two different management protocols: (1) intrauterine repair of the myelomeningocele at 18–25 gestational weeks and cesarean delivery at 37 gestational weeks with lung maturity; or (2) cesarean delivery at 37 gestational weeks with lung maturity and neonatal repair of the myelomeningocele.

"Two-hit" hypothesis

Heffez et al [20] first proposed the "two-hit" hypothesis of which the first hit is the primary developmental abnormalities of NTDs, and the second hit is the secondary spinal cord injury caused by environmental exposures such as amniotic fluid, contact with the uterine wall, and/or pressure from the birth canal. Heffez et al [20] suggested that intrauterine protection of the exposed spinal cord may prevent some of the paralysis. In a study of eight stillborn human fetuses with myelomeningocele, Hutchins et al [21] found that the acquired spinal cord injury and the injury or destruction of the dorsal spinal cord were recent and consistent with occurrence during delivery. In a histologic study of the myelomeningocele lesions with surrounding tissues from 10 human fetuses between 19 and 23 gestational weeks, Meuli et al [22] found that the exposed neural tissue had undergone varying degrees of recent traumatic injury as a result of its exposed position, and the neural tissue remaining in the myelomeningocele contained hemorrhage and abrasions from recent injury, suggesting that injury occurred during passage through the birth canal. Drewek et al [23] found that amniotic fluid became toxic at approximately 34 gestational weeks. In a study of human myelomeningocele placodes using immunohistochemical caudal spinal cord

markers and structural markers, George and Cummings [24] found abnormal patterning along the dorsoventral and rostrocaudal axes, a paucity of maturing neurons, and significant inflammatory infiltrate, gliosis and fibrosis consistent with secondary injury.

Human experience with intrauterine myelomeningocele repair

Reversal of hindbrain herniation and need for ventriculoperitoneal shunting

In a study of 29 patients with intrauterine myelomeningocele repair performed at the VUMC and 23 controls, Bruner et al [25] found that intrauterine repair decreased the incidence of hindbrain herniation (38% *vs*. 95%; $p<0.01$) and the incidence of shunt-dependent hydrocephalus (59% *vs*. 91%; $p=0.01$). At VUMC, Tulipan et al [26] found that intrauterine myelomeningocele repair reversed preexisting hindbrain herniation. In a study of 10 patients undergoing fetal myelomeningocele closure at CHOP, Sutton et al [27] found that all nine surviving neonates showed improvement in the hindbrain hernia at the 3-week postoperative fetal scan, and only one neonate required placement of ventriculoperitoneal shunt. In a study of 104 patients with intrauterine myelomeningocele repair performed at either VUMC or CHOP and 189 controls, Tulipan et al [28] found that intrauterine myelomeningocele repair significantly reduced the incidence of shunt-dependent hydrocephalus (54.8% *vs*. 85.7%). However, patients with lesions above L3 did not share in this benefit, and patients with fetal surgery at >25 gestational weeks had a shunt rate of 75% in comparison with 44.1% of those with fetal surgery at ≤25 gestational weeks. In a study of 50 fetuses with intrauterine myelomeningocele repair performed at CHOP, Johnson et al [29] found that all fetuses had reversal of hindbrain herniation, and 20 of the 47 surviving fetuses (42.6%) had required ventriculoperitoneal shunting compared with 100% for thoracic, 88% for lumbar and 68% for sacral lesions (85% overall) in 297 historic controls who underwent postnatal neurosurgical closure. In a study of fetal head biometry by fetal magnetic resonance imaging in 22 patients with intrauterine myelomeningocele repair performed at CHOP, Danzer et al [30] found significant reversal of hindbrain herniation and normalization of the posterior fossa cerebrospinal fluid (CSF) spaces when compared with the controls.

Lower extremity function and urinary bladder function

Tubbs et al [31] found no improvement in the lower extremity function of 37 patients with intrauterine myelomeningocele repair performed at VUMC when compared with the controls. In a study of 50 patients with intrauterine myelomeningocele repair performed at CHOP, Johnson et al [29] found better-than-predicted leg function in 57% of thoracic and lumbar level lesion patients. However, the CHOP criteria demanded intact leg and foot motion to be presented prior to fetal surgery and only included early-gestation (≤26 gestational weeks) repair. Holzbeierlein et al [32] studied the urologic outcome in 16 patients with intrauterine myelomeningocele repair performed at VUMC and found no improvement in the urinary bladder function when compared with those myelomeningocele patients without prenatal repair.

Dermoid inclusion cysts and early spinal cord tethering after fetal surgery for myelomeningocele

Mazzola et al [33] reported that three girls who had undergone intrauterine myelomeningocele repair at CHOP suffered from spinal cord tethering and large dermoid inclusion cysts in infancy.

Neurodevelopmental outcomes

In a study of neurodevelopmental outcomes of 30 children at 2 years of age who had undergone intrauterine myelomeningocele repair at CHOP, Johnson et al [34] found that 67% of the children had normal cognitive language and personal–social skills, 20% had mild delays and 13% had significant delays. They also found that 13 children who had undergone ventriculoperitoneal shunting scored lower than those with nonshunted ventriculomegaly.

Perinatal survival and prematurity

In a study of 50 patients with intrauterine myelomeningocele repair performed at CHOP, Johnson et al [29] found perinatal survival in 47 patients (94%), and the mean age at delivery was 34^{+3} gestational weeks.

Recurrence Risk of NTDs

Only 5% of NTDs occur in families with a positive family history, and 95% of NTDs occur spontaneously in women with no family history [35]. The recurrence risk increases to 10-fold the population risk when a woman has had one previous affected pregnancy, doubles for two previous affected pregnancies and quadruples for three previous affected pregnancies [35]. Chromosomal abnormalities, risk factors, syndromes, disorders and other etiologies should be considered during counseling on the recurrence risk of NTDs. Main and Mennuti [36] reported the following estimated incidence of NTDs based on specific risk factors in the United States: general incidence, 1.4–1.6/1,000 live births; women undergoing

amniocentesis for advanced maternal age, 1.5–3.0/1,000 live births; women with diabetes mellitus, 20/1,000 live births; women on valproic acid in the first trimester, 10–20/1,000 live births; one sibling with NTD, 15–30/1,000 live births; two siblings with NTD, 57/1,000 live births; a parent with NTD, 11/1,000 live births; half sibling with NTD, 8/1,000 live births; first cousin with NTD, 10/1,000 live births; other first cousins with NTD, 3/1,000 live births; sibling with severe scoliosis secondary to multiple vertebral defects, 15–30/1,000 live births; sibling with occult spina dysraphism, 15–30/1,000 live births; and sibling with sacrococcygeal teratoma or hamartoma, ≤ 15–30/1,000 live births. The ACOG [4] suggested that folic acid supplementation of 400 μg/day is recommended for low-risk women, and folic acid supplementation of 4 mg/day is recommended for women at high risk for NTD or who have had a previous pregnancy with an NTD. Stevenson et al [37] found a decline in the prevalence of NTDs from 1.89/1,000 to 0.95/1,000 live births and fetal deaths in South Carolina during 1992–1998, and no NTD recurrences in 113 subsequent pregnancies to mothers of infants with isolated NTDs, who took periconceptional folic acid supplementation. They also reported that the rate of periconceptional folic acid use among women of childbearing years increased from 8% to 35% during the 6-year project period.

Differential Diagnosis of NTDs Using Prenatal Ultrasound

Differential diagnosis of NTDs using prenatal ultrasound should include sacrococcygeal teratoma, cystic hygroma, hemangioma, hemangiolymphangioma, scalp edema/cephalohematoma, epidermal scalp cyst, branchial cleft cyst, dermoid cyst of the anterior fontanelle, dacryocystocele, epignathus, and cervical teratoma.

Sacrococcygeal teratoma

When cystic myelomeningocele in the lumbosacral region is diagnosed, a differential diagnosis of sacrococcygeal teratoma should be considered. Lumbosacral myelomeningocele is usually a symmetric cystic, complex or solid mass in the lumbosacral region, and may be associated with ventriculomegaly and lemon or banana sign on prenatal ultrasound [38]. In contrast, sacrococcygeal teratoma is usually a solid mass arising from the buttocks, often has intrapelvic components, and is rarely a symmetric cystic mass [38]. Sacrococcygeal teratoma is a germ cell tumor arising from the presacral area. Sacrococcygeal teratoma has been classified by the American Academy of Pediatrics Surgical Section (AAPSS) on the basis of the amount of presacral and external tumor present, stage and timing of diagnosis, ease of resection, and malignant potential [39]. Sacrococcygeal teratoma is generally exophytic (AAPSS type I), but may retroperitoneally extend into the pelvis (AAPSS type II) or abdomen (AAPSS type III). In AAPSS type IV sacrococcygeal teratoma, the tumor is completely internal with no external component. The prognosis of fetal sacrococcygeal teratoma seems to be related to its content and extent rather than the size of the mass. Diffuse solid content, high vascularity, extensive intra-abdominal and intrapelvic hemorrhage may cause vascular steal from the umbilical artery blood flow to the placenta, resulting in fetal anemia, high-output cardiac failure, hydrops fetalis, placentomegaly, and an eclamptic-like disorder termed "maternal mirror syndrome" consisting of maternal hypertension, respiratory compromise and renal impairment. Large sacrococcygeal teratoma may cause dystocia, traumatic tumor rupture, and hemorrhage during vaginal delivery. Prenatal visualization of the vasculature of fetal sacrococcygeal teratoma can be made by three-dimensional color power angiography [40,41]. The three-dimensional color Doppler ultrasound helps the reconstruction of the vasculature, allowing visualization of the blood flow between the sacrococcygeal teratoma and fetal circulation [40,41]. Fetal magnetic resonance imaging is useful in providing a better contrast between the cystic and solid component of the tumor, and in delineating the intrapelvic extent of sacrococcygeal teratoma [41,42].

Cystic hygroma, hemangioma, hemangiolymphangioma, scalp edema/cephalohematoma, epidermal scalp cyst and branchial cleft cyst

When occipital encephalocele is diagnosed, a differential diagnosis of cystic hygroma, hemangioma, hemangiolymphangioma, scalp edema/cephalohematoma, epidermal scalp cyst and branchial cleft cyst should be considered. Cystic hygroma or lymphangioma is composed of large cystic spaces with endothelium-lined channels of varying dimensions. Congenital cystic hygroma is caused by anomalous embryologic development of the lymphatic system. The advent of ultrasonography has made possible the prenatal diagnosis of congenital cystic hygroma and observation of its progression *in utero* [43]. Occipital encephalocele always has a calvarial defect, usually contains herniated brain tissues in the sac, can be associated with microcephaly and hydrocephalus, and is located in the midline [44]. However, cystic hygroma seldom has a bone defect in the skull unless in association with coexistent encephalocele, contains only fluid in the sac, is associated with multiple septa, is rarely associated with microcephaly or hydrocephalus, and is located in the retrolateral

aspect of the neck [44]. Fetal scalp hemangioma may prenatally mimic encephalocele [45–49]. Color Doppler imaging is useful for differentiation of fetal cystic hygroma, hemangioma and hemangiolymphangioma, by demonstrating low-resistance flow and blood vessels within the hemangioma and hemangiolymphangioma. Hemangiolymphangioma often presents on prenatal ultrasound as a heterogeneous cystic and solid multiloculated mass with a propensity for rapid growth and invasion into adjacent tissues. Winter et al [47] reported the prenatal sonographic diagnosis of scalp edema/cephalohematoma mimicking an encephalocele at 31 gestational weeks, with no flow within the mass on color Doppler ultrasound. Shahabi and Busine [48] reported the prenatal sonographic diagnosis of an epidermal scalp cyst simulating an encephalocele at 17 gestational weeks with no associated ventriculomegaly. Tsai et al [50] reported the prenatal imaging findings of fetal branchial cleft cyst presenting as a nuchal cyst with homogeneous contents and no septa. Branchial cleft cyst is believed to be the result of incomplete involution of the branchial apparatus, and the lack of any solid elements or septa can be used to differentiate branchial cleft cyst from cervical teratoma [50–52].

Dermoid cyst of the anterior fontanelle and dacryocystocele

When frontal encephalocele is diagnosed, a differential diagnosis of dermoid cyst of the anterior fontanelle and dacryocystocele should be considered. Dermoid cyst of the anterior fontanelle may present with fluid in the mass and the appearance of a bony defect [38,53]. Dacryocystocele or lacrimal duct cyst is a small sonolucent cyst located inferomedially to the orbit and is caused by obstruction of the proximal and distal lacrimal ducts [54].

Epignathus and cervical teratoma

When basal encephalocele is diagnosed, a differential diagnosis of epignathus should be considered. Epignathus is a rare teratoma of the oropharynx and usually originates from the base of the skull in the posterior nasopharynx involving the hard palate or sphenoid bone [55]. Two- and three-dimensional ultrasound scans are useful in the prenatal investigation of fetal epignathus, by detailed assessments of the craniofacial structural abnormalities of the fetus and the vasculature of the tumor [56]. Cervical teratoma presents as a solid or mixed solid-cystic tumor that is located in the anterior or anterolateral neck [54]. Cervical teratoma can cause airway obstruction and may call for the need of tracheostomy or *ex utero* intrapartum treatment procedure during delivery [57–59].

References

1. Wald NJ, Cuckle H, Brock JH, Peto R, Polani PE, Woodford FP. Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural-tube defects. *Lancet* 1977; 1:1323–32.
2. Milunsky A, Jick SS, Bruell CL, et al. Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data. *Am J Obstet Gynecol* 1989;161:291–7.
3. ACOG educational bulletin. Maternal serum screening. Number 228, September 1996 (replaces no. 154, April 1991). Committee on Educational Bulletins of the American College of Obstetricians and Gynecologists. *Int J Gynaecol Obstet* 1996;55:299–308.
4. ACOG Committee on Practice Bulletins. ACOG practice bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 44, July 2003. (Replaces Committee Opinion Number 252, March 2001). *Obstet Gynecol* 2003; 102:203–13.
5. Reichler A, Hume RF Jr, Drugan A, Bardicef M, Isada NB, Johnson MP, Evans MI. Risk of anomalies as a function of level of elevated maternal serum α-fetoprotein. *Am J Obstet Gynecol* 1994;171:1052–5.
6. Canick JA, Kellner LH, Bombard AT. Prenatal screening for open neural tube defects. *Clin Lab Med* 2003;23: 385–94.
7. Loft AG, Hogdall E, Larsen SO, Norgaard-Pedersen B. A comparison of amniotic fluid alpha-fetoprotein and acetylcholinesterase in the prenatal diagnosis of open neural tube defects and anterior abdominal wall defects. *Prenat Diagn* 1993;13:93–109.
8. Watson WJ, Chescheir NC, Katz VL, Seeds JW. The role of ultrasound in evaluation of patients with elevated maternal serum alpha-fetoprotein: a review. *Obstet Gynecol* 1991;78: 123–8.
9. Harmon JP, Hiett AK, Palmer CG, Golichowski AM. Prenatal ultrasound detection of isolated neural tube defects: is cytogenetic evaluation warranted? *Obstet Gynecol* 1995;86: 595–9.
10. Hogge WA, Thiagarajah S, Ferguson JE 2nd, Schnatterly PT, Harbert GM Jr. The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects. *Am J Obstet Gynecol* 1989;161:520–3.
11. Nadel AS, Green JK, Holmes LB, Frigoletto FD Jr, Benacerraf BR. Absence of need for amniocentesis in patients with elevated levels of maternal serum alpha-fetoprotein and normal ultrasonographic examinations. *N Engl J Med* 1990;323: 557–61.
12. Katz VL, Seeds JW, Albright SG, Lingley LH, Lincoln-Boyea B. Role of ultrasound and informed consent in the evaluation of elevated maternal serum alpha-fetoprotein. *Am J Perinatol* 1991;8:73–6.
13. Lennon CA, Gray DL. Sensitivity and specificity of ultrasound for the detection of neural tube and ventral wall defects in a high-risk population. *Obstet Gynecol* 1999;94: 562–6.

14. Mandruzzato GP, Fischer-Tamaro L, De Seta F, et al. Does amniotic fluid alpha-fetoprotein have diagnostic or prognostic value at the time of second midtrimester genetic amniocentesis? *Fetal Diagn Ther* 2002;17:147–52.
15. Norem CT, Schoen EJ, Walton DL, Krieger RC, O'Keefe J, To TT, Ray GT. Routine ultrasonography compared with maternal serum alpha-fetoprotein for neural tube defect screening. *Obstet Gynecol* 2005;106:747–52.
16. Dashe JS, Twickler DM, Santos-Ramos R, McIntire DD, Ramus RM. Alpha-fetoprotein detection of neural tube defects and the impact of standard ultrasound. *Am J Obstet Gynecol* 2006;195:1623–8.
17. Kooper AJA, de Bruijn D, van Ravenwaaij-Arts CMA, Faas BH, Creemers JW, Thomas CM, Smits AP. Fetal anomaly scan potentially will replace routine AFAFP assays for the detection of neural tube defects. *Prenat Diagn* 2007;27: 29–33.
18. Hirose S, Farmer DL, Albanese CT. Fetal surgery for myelomeningocele. *Curr Opin Obstet Gynecol* 2001;13:215–522.
19. Sutton LN. Fetal surgery for neural tube defects. *Best Pract Res Clin Obstet Gynaecol* 2008;22:175–88.
20. Heffez DS, Aryanpur J, Hutchins GM, Freeman JM. The paralysis associated with myelomeningocele: clinical and experimental data implicating a preventable spinal cord injury. *Neurosurgery* 1990;26:987–92.
21. Hutchins GM, Meuli M, Meuli-Simmen C, Jordan MA, Heffez DS, Blakemore KJ. Acquired spinal cord injury in human fetuses with myelomeningocele. *Pediatr Pathol Lab Med* 1996;16:701–12.
22. Meuli M, Meuli-Simmen C, Hutchins GM, Seller MJ, Harrison MR, Adzick NS. The spinal cord lesion in human fetuses with myelomeningocele: implications for fetal surgery. *J Pediatr Surg* 1997;32:448–52.
23. Drewek MJ, Bruner JP, Whetsell WO, Tulipan N. Quantitative analysis of the toxicity of human amniotic fluid to cultured rat spinal cord. *Pediatr Neurosurg* 1997;27:190–93.
24. George TM, Cummings TJ. The immunohistochemical profile of the myelomeningocele placode: is the placode normal? *Pediatr Neurosurg* 2003;39:234–9.
25. Bruner JP, Tulipan N, Paschall RL, et al. Fetal surgery for myelomeningocele and the incidence of shunt-dependent hydrocephalus. *JAMA* 1999;282:1819–25.
26. Tulipan N, Bruner JB, Hernanz-Schulman M, et al. Effect of intrauterine myelomeningocele repair on central nervous system structure and function. *Pediatr Neurosurg* 1999;31: 183–8.
27. Sutton LN, Adzick NS, Bilaniuk LT, Johnson MP, Crombleholme TM, Flake AW. Improvement in hindbrain herniation demonstrated by serial fetal magnetic resonance imaging following fetal surgery for myelomeningocele. *JAMA* 1999;282:1826–31.
28. Tulipan N, Sutton LN, Bruner JP, Cohen BM, Johnson M, Adzick NS. The effect of intrauterine myelomeningocele repair on the incidence of shunt-dependent hydrocephalus. *Pediatr Neurosurg* 2003;38:27–33.
29. Johnson MP, Sutton LN, Rintoul N, et al. Fetal myelomeningocele repair: short-term clinical outcomes. *Am J Obstet Gynecol* 2003;189:482–7.
30. Danzer E, Johnson MP, Bebbington M, et al. Fetal head biometry assessed by fetal magnetic resonance imaging following *in utero* myelomeningocele repair. *Fetal Diagn Ther* 2007;22:1–6.
31. Tubbs RS, Chambers MR, Smyth MD, Bartolucci AA, Bruner JP, Tulipan N, Oakes WJ. Late gestational intrauterine myelomeningocele repair does not improve lower extremity function. *Pediatr Neurosurg* 2003;38:128–32.
32. Holzbeierlein J, Pope JC 4th, Adams MC, Bruner J, Tulipan N, Brock JW 3rd. The urodynamic profile of myelodysplasia in childhood with spinal closure during gestation. *J Urol* 2000;164:1336–9.
33. Mazzola CA, Albright AL, Sutton LN, Tuite GF, Hamilton RL, Pollack IF. Dermoid inclusion cysts and early spinal cord tethering after fetal surgery for myelomeningocele. *N Engl J Med* 2002;347:256–9.
34. Johnson MP, Gerdes M, Rintoul N, Pasquariello P, Melchionni J, Sutton LN, Adzick NS. Maternal-fetal surgery for myelomeningocele: neurodevelopmental outcomes at 2 years of age. *Am J Obstet Gynecol* 2006;194:1145–50.
35. Aitken DA, Crossley JA, Spencer K. Prenatal screening for neural tube defects and aneuploidy. In: Rimoin DL, Connor JM, Pyeritz RE, Korf BR, eds. *Emery and Rimoin's Principles and Practice of Medical Genetics*, 5th edition. Philadelphia: Elsevier Churchill Livingstone, 2007:636–78.
36. Main DM, Mennuti MT. Neural tube defects: issues in prenatal diagnosis and counselling. *Obstet Gynecol* 1986;67: 1–16.
37. Stevenson RE, Allen WP, Pai GS, Best R, Seaver LH, Dean J, Thompson S. Decline in prevalence of neural tube defects in a high-risk region of the United States. *Pediatrics* 2000; 106:677–83.
38. McGahan JP, Pilu G, Nyberg DA. Neural tube defects and the spine. In: Nyberg DA, McGahan JP, Pretorius DH, Pilu G, eds. *Diagnostic Imaging of Fetal Anomalies*. Philadelphia: Lippincott Williams & Wilkins, 2003:291–334.
39. Altman RP, Randolph JG, Lilly JR. Sacrococcygeal teratoma. The American Academy of Pediatrics Surgical Section Survey – 1973. *J Pediatr Surg* 1974;9:389–98.
40. Chen CP, Shih JC. Prenatal visualization of the vasculature of fetal sacrococcygeal teratoma by three-dimensional color power angiography. *Ultrasound Obstet Gynecol* 2002;20:636–7.
41. Chen CP, Shih JC, Huang JK, Wang W, Tzen CY. Second-trimester evaluation of fetal sacrococcygeal teratoma using three-dimensional color Doppler ultrasound and magnetic resonance imaging. *Prenat Daign* 2003;23:602–3.
42. Chen CP, Sheu JC, Huang JK, Lin YH, Tzen CY, Wang W. Second-trimester magnetic resonance imaging of fetal sacrococcygeal teratoma with intrapelvic extension in a co-twin. *Prenat Diagn* 2004;24:1015–7.
43. Chen CP, Wang W, Lin SP, Sheu JC, Tzen CY. Favorable outcome in a fetus with an early-onset extensive cystic hygroma colli and intralesional hemorrhage. *Am J Perinatol* 1998;15: 601–5.
44. Pearce JM, Griffin D, Campbell S. The differential prenatal diagnosis of cystic hygromata and encephalocele by ultrasound examination. *J Clin Ultrasound* 1985;13:317–20.
45. Bronshtein M, Bar-Hava I, Blumenfeld Z. Early second-trimester sonographic appearance of occipital haemangioma simulating encephalocele. *Prenat Diagn* 1992;12:695–8.
46. Sherer DM, Perillo AM, Abramowicz JS. Fetal hemangioma overlying the temporal occipital suture, initially diagnosed

by ultrasonography as an encephalocele. *J Ultrasound Med* 1993;12:691–3.
47. Winter TC 3rd, Mack LA, Cyr DR. Prenatal sonographic diagnosis of scalp edema/cephalohematoma mimicking an encephalocele. *AJR Am J Roentgenol* 1993;161:1247–78.
48. Shahabi S, Busine A. Prenatal diagnosis of an epidermal scalp cyst simulating an encephalocoele. *Prenat Diagn* 1998; 18:373–7.
49. Mitchell CS. Vertex hemangioma mimicking an encephalocele. *J Am Osteopath Assoc* 1999;99:626–7.
50. Tsai PY, Chang CH, Chang FM. Prenatal imaging of the fetal branchial cleft cyst by three-dimensional ultrasound. *Prenat Diagn* 2003;23:605–6.
51. Choi SS, Zalzal GH. Branchial anomalies: a review of 52 cases. *Laryngoscope* 1995;105:909–13.
52. Suchet IB. Ultrasonography of the fetal neck in the second and third trimesters. Part 3. Anomalies of the anterior and anterolateral nuchal region. *Can Assoc Radiol J* 1995;46:426–33.
53. Kang AH, Bruner JP. Cephalocele, anterior fontanelle. *The Fetus* 1994;4:5.
54. Benacerraf BR, Nyberg DA. The face and neck. In: Nyberg DA, McGahan JP, Pretorius DH, Pilu G, eds. *Diagnostic Imaging of Fetal Anomalies*. Philadelphia: Lippincott Williams & Wilkins, 2003:335–79.
55. Gull I, Wolman I, Har-Toov J, Amster R, Schreiber L, Lessing JB, Jaffa A. Antenatal sonographic diagnosis of epignathus at 15 weeks of pregnancy. *Ultrasound Obstet Gynecol* 1999; 13:271–3.
56. Chen CP, Shih JC, Huang JK, Chin DT, Tzen CY, Lin YH, Wang W. Two- and three-dimensional ultrasound demonstration of a giant epignathus. *Ultrasound Obstet Gynecol* 2003;21:407–9.
57. Azizkhan RG, Haase GM, Applebaum H, et al. Diagnosis, management, and outcome of cervicofacial teratomas in neonates: a Childrens Cancer Group study. *J Pediatr Surg* 1995;30:312–6.
58. Liechty KW, Crombleholme TM, Weiner S, Bernick B, Flake AW, Adzick NS. The *ex utero* intrapartum treatment procedure for a large fetal neck mass in a twin gestation. *Obstet Gynecol* 1999;93:824–5.
59. Ward VM, Langford K, Morrison G. Prenatal diagnosis of airway compromise: EXIT (*ex utero* intra-partum treatment) and foetal airway surgery. *Int J Pediatr Otorhinolaryngol* 2000; 53:137–41.