Objectives  After completing this article, readers should be able to:

1. List the common sequelae of twin-to-twin transfusion syndrome (TTTS).
2. Explain the pathophysiology of central nervous system and cardiac sequelae of TTTS.
3. Review the two staging systems for TTTS.
4. Compare and contrast treatment strategies for TTTS.
5. Describe appropriate placental examination in TTTS.

Abstract
Most anomalies seen in fetuses and neonates who have chronic twin-to-twin transfusion syndrome (TTTS) represent sequelae of cardiovascular dysfunction or vascular disruption. The placental examination can provide critical information to the neonatologist caring for infants who have a history of TTTS, especially in instances wherein the twins are not necessarily growth-discordant but have cardiovascular or renal dysfunction or neuropathologic findings. In this review, we present an updated discussion of the fetal and neonatal pathologies and adverse sequelae associated with TTTS and advances in antenatal diagnosis and clinical interventions for monochorionic gestations complicated by TTTS. We also present highlights of the placental examination so the neonatologist can inspect the placenta at the time of delivery and possibly gain insights that may affect patient care.

Introduction
Chronic twin-to-twin transfusion syndrome (TTTS) is a gestational condition in which a sustained net imbalance of blood volume transduces between twins of a diamniotic monochorionic (DiMo) placentation via placental anastomoses. It occurs in approximately 10% to 20% of all monochorionic twin gestations but may complicate as many as 35%. (1) One twin becomes the net donor and the co-twin becomes the effective recipient. In severe TTTS, cardiovascular, central nervous system (CNS), and other organ system sequelae or anomalies due to vascular disruption can occur in one or both twins. The neonatologist may be confronted with cardiovascular abnormalities or other organ dysfunction in liveborn infants from gestations complicated by TTTS that are difficult to explain. This article focuses on the fetal and neonatal pathologies due to TTTS and advances in antenatal diagnosis and treatment of TTTS that have been instituted in an effort to reduce the frequency and severity of such adverse sequelae. Clues to the pathologies may be found in the placenta, but due to logistical problems of specimen delivery to pathology and the detailed examination that may be required, several days may elapse before the report is available. However, the neonatologist may be able to identify important pathology, such as anomalous velamentous cord insertion and unequal sharing of the placenta disc, on inspection near the time of delivery. Cogent aspects of the placental examination may enable the neonatologist to make basic observations of the monochorionic placenta in TTTS and better interpret the subsequent pathology report.
Anomalies in Infants Affected by TTTS

Most anomalies seen in fetuses and neonates that have TTTS represent cardiovascular sequelae or vascular disruption. Some 45% to 50% of recipients have abnormal cardiac function in the neonatal period, reflecting their intrauterine compromise as fetuses, and 5% to 10% sustain long-term cardiac dysfunction. Donors are prone to impaired arterial distensibility. (2)

Injuries Related to Ischemia

Intrauterine cardiac dysfunction and hemodynamic derangements lead to ischemic brain lesions, including white matter infarction and leukoencephalopathy, intraventricular hemorrhage, hydranencephaly, and porencephaly, which can be detected by prenatal ultrasonography. (3)(4)(5)(6)(7) Up to 8% of TTTS cases have evidence of CNS injury on fetal magnetic resonance imaging (MRI) at the time of presentation prior to treatment. Such CNS findings range from ischemic or hemorrhagic changes in the brain to marked dilation of the cerebral venous sinuses due to central venous hypertension. The latter has been shown to correlate with worse survival when detected in TTTS. (6) When these lesions are seen in surviving twins of instances of co-twin death (66% of cases with intrauterine death involve demise of the donor twin (8)), they have been attributed to sudden acute TTTS (5)(9) through arterioarterial (A-A) anastomoses. (5) Due to the shared placental circulation, if one co-twin dies, there is an acute fall in blood pressure that causes placental resistance to decrease. This decrease in resistance across the placental vascular connections can result in reduced cerebral perfusion pressure and ischemic injury in the brain of the surviving twin. Quintero and associates (10) reported endoscopic evidence of feto-fetal hemorrhage from a recipient to donor twin within 3 hours of the spontaneous demise of the donor, noting endoscopic and middle cerebral artery Doppler evidence of paradoxic anemia in the recipient and erythrocythemia in the donor. TTTS can result in significant neurologic damage, with 5% to 27% of surviving twins having evidence of CNS sequelae on postnatal MRI or ultrasonography. (9)(11)(12)(13)(14) Brain injury, however, can occur in TTTS even when both twins survive. When both twins survive, neurologic damage in the recipient may be related to secondary polycythemia and venous stasis. In the donor, neurologic injury may be due to anemia and hypotension.

Multiorgan ischemic sequelae also are seen. Renal failure occurs in 48% of survivors compared with 14% of aged-matched controls. (2) Renal cortical necrosis, intestinal atresia, and cutis aplasia may be seen in one or both twins. (3)(15)(16) Limb necrosis and other ischemic lesions and hemolytic jaundice in the surviving recipient twin may be related to hyperviscosity (erythrocythemia) (16) or thromboembolic phenomena due to placental chorangiopagus vessels. (17)

Cardiac Anomalies

DiMo twins are at increased risk for structural cardiac anomalies in at least one twin; 7% of DiMo twins have congenital heart defects (CHD) (18) compared with the overall prevalence of 0.5% seen in neonates. (19) Bahtiyar and associates, (19) in their multistudy review of the prevalence of CHD in DiMo twins, found an overall ninelfold increased risk for CHD over singletons, a 1.5- to 23-fold higher risk of CHD with TTTS over that of singletons, and a 2.78 times more frequent occurrence of CHD in the setting of TTTS compared with no TTTS. These overall and relatively increased risks are probably related to the greater frequency of abnormalities of placentation and the umbilical cord in DiMo gestations and their relative predominance in TTTS, respectively. However, the etiopathogenesis of cardiovascular malformations is poorly understood, even in singletons. Thus, the increased prevalence of cardiac malformations in DiMo twins, and especially in those pairs affected by TTTS, probably represents a complex interaction among many variables such as fetal flow fluctuations, vascular endothelial growth factors (VEGFs) and other mediators, the process of twinning itself, unequal sharing of the placenta, and inherent genetic factors.

The most common defects in DiMo twins are ventricular septal defect (VSD), pulmonary stenosis, and atrial septal defect. The prevalence of VSDs in TTTS, although higher, is not remarkably different from that in uncomplicated DiMo gestations (0.024 and 0.019, respectively). The detection of VSDs may represent sites of ischemic necrosis or excessive natriuretic proteins (see TTTS Part 1 in this issue) during early embryonic life. However, the membranous septum is a complex structure that has numerous embryologic components effecting its closure and VSDs, together with patent ductus arteriosus, are the most common cardiac defects in infancy. The pathogenesis of VSD is unclear, but its mildly increased incidence in TTTS infants may be related to a combination of factors, including its relatively greater general frequency and the increased risk of ischemia in TTTS due to abnormal placentation or ischemia due to hypovolemia. Bahtiyar and colleagues (19) postulated that because recent studies indicate that the cause
of CHD is partly due to aberrant angiogenic factors such as VEGF, the increased prevalence of CHD in TTTS may represent the deleterious effects of angiogenic factors. VEGF is upregulated in ischemia, and Dor and associates (20) found that VEGF is specifically upregulated during normal heart development in the atrioventricular field of the heart, soon after the onset of endocardial cushion formation. Premature induction of myocardial VEGF prevents formation of endocardial cushions in animal models.

The prevalence of pulmonary stenosis is fourfold greater in gestations involving TTTS compared with those not involving TTTS (0.028 versus 0.007), and atrial septal defects (0.024) are seen only in twins who have experienced TTTS. (19) However, these summations do not specify co-twin status (donor or recipient), although pulmonary stenosis presumably represents the recipient population. The original studies also do not separate donor or recipient twin status, so it is difficult to assess the potential relationship of atrial septal defects to fetal volume status or circulating mediators and angiogenic factors. Presumably, high-output failure in the donor twin or right ventricular outflow tract obstruction in the recipient both could result in increased shunting through the foramen ovale and acquired incompetence of the interatrial septum (passive enlargement such that the septum primum does not adequately cover the foramen ovale). It is unclear whether the clinically diagnosed “atrial septal defects” represent a true primary deficiency of tissue (due to ischemic necrosis, increased apoptosis, mesenchymal failure) or excessive secondary dilatation of the foramen due to shunt overload as the right ventricle fails. Recently, transposition of the great arteries has been identified in a recipient twin, but the donor additionally had a vein of Galen malformation. Although the coexistence of the lesions might reflect a common causal mechanism, their etiopathogenesis(es) is unclear. (21)

CHD in donor twins appears to be very rare, but left-sided obstructive lesions might be expected due to complications of hypovolemia. However, of the 830 DiMo gestations reviewed by Bahtiyar and associates, (19) the only instances of coarctation of the aorta or hypoplastic left ventricle (left-sided obstructive lesions) were isolated to two cases that did not have TTTS. Thus, although the prevalence theoretically would be expected to be higher in donor twins in TTTS and small twin size correlates with the presence of structural defects in DiMo twins, (22) the pathogenesis of these rarely encountered lesions appears to represent more than unusual sequelae of diminished blood flow.

### Staging of TTTS

#### Quintero Staging System

Quintero and coworkers (23) proposed a staging system for TTTS that considered a sequence of progressive ultrasonographic features:

- **Stage I:** Polyhydramnios in the recipient, severe oligohydramnios in the donor but urine visible within the bladder in the donor
- **Stage II:** Polyhydramnios in the recipient, a “stuck” donor, and urine not visible within the donor’s bladder
- **Stage III:** Polyhydramnios and oligohydramnios as well as critically abnormal Doppler waveforms (at least one of either absent or reverse end-diastolic flow in the umbilical artery, reverse flow in the ductus venosus, or pulsatile umbilical venous flow) with or without urine visible within the donor’s bladder
- **Stage IV:** Presence of ascites or frank hydrops (fluid collection in two or more cavities) in either donor or recipient
- **Stage V:** Demise of either fetus

Although this staging system has not been validated independently as prognostically important, it provides a useful shorthand description of the state of the twin gestation.

Taylor and associates (4) applied the Quintero staging system to a population treated with serial amnioreduction (AR), septostomy, and selective reduction alone or in combination and found no significant influence of staging at presentation on survival in their conservatively treated group. Survival was significantly poorer when stage increased rather than decreased. These authors concluded that the Quintero staging system should be used cautiously for determining prognosis at the time of diagnosis, suggesting that it may be better suited for monitoring disease progression. A subsequent larger study from the same institution, however, showed that Quintero stage at presentation, at first treatment, and at worst stage did, in fact, predict both perinatal (overall number of fetuses surviving of the total number of fetuses treated) and double survival (number of pregnancies with two survivors, but not survival of any twin (number of pregnancies with survival of one or both twins). (24) Duncombe and colleagues (25) also showed a correlation of Quintero stage at initial presentation and perinatal survival.

#### Cincinnati Staging System

The Quintero staging system, although useful in describing the progression of TTTS along the clinical spectrum of severity, has potential limitations in guiding therapy.
For patients who present at Stage I with only amniotic fluid discordance, it may be difficult to know with certainty if they actually have TTTS. Patients who have Stage II presentation usually are believed to be in the early stages of the disease. The use of echocardiography to identify findings of recipient TTTS cardiomyopathy can confirm the diagnosis in Stage I cases when it may be in doubt. In addition, echocardiographic findings can alert the clinician to more advanced disease than the Quintero Stage suggests. The largest group of patients tends to fall into Stage III, but this stage comprises a very broad spectrum of severity. At one end are patients whose only hemodynamic derangement is abnormal Doppler velocimetry, and at the other end of the spectrum are patients in whom the recipient twin has severe, end-stage, twin-twin cardiomyopathy. These latter patients may be premorbid but without the development of hydrops (Stage IV disease). The Quintero staging system is heavily weighted toward findings in the donor twin. The absence of a visible bladder in the donor upstages the case to Stage II. The critical Doppler abnormalities that are required for Stage III almost always are observed in the donor twin. Critical Doppler waveform abnormalities in the recipient twin are rare until end-stage TTTS has been reached. In addition, there is no assessment of the TTTS cardiomyopathy, which only occurs in the recipient and has a profound impact on survival of the recipient. (26)(27)(28)

The Fetal Care Center of Cincinnati has used fetal echocardiographic assessment of the recipient twin to stage patients (Table). This is in keeping with the view that TTTS is a fundamentally hemodynamic derangement. Fetal echocardiography can distinguish degrees of severity among the broad spectrum of severity in Stage III TTTS and identify sicker patients in Stages I and II. Echocardiographic features include the presence and severity of atrioventricular valvar incompetence, ventricular wall thickening, and ventricular function, as assessed by the Tei index. (29)(30) In a recent review of experience with the Cincinnati staging system, 20% of Quintero Stage I and II patients were upstaged to Stage III disease based on echocardiographic findings. (27)(28) The impact of TTTS cardiomyopathy on recipient twin survival had been demonstrated by Shah and associates (31) in patients treated by fetoscopic laser who were stratified by cardiovascular profile score and in the National Institutes of Health TTTS trial in which echocardiographic findings of TTTS was the single most important predictor of adverse recipient survival. (31) The upstaging of patients from Stage II to Stage III may influence counseling about treatment options. These echocardiographic features also are used to assess response to therapy. If a patient is treated initially with AR or microseptostomy, fetal echocardiography can be used to assess progression of TTTS despite therapy and as an indication for selective fetoscopic laser photocoagulation. (27)(32)(33)

<table>
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<th>Stage</th>
<th>Donor</th>
<th>Recipient</th>
<th>Recipient Cardiomyopathy</th>
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<tr>
<td>I</td>
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<td>Polyhydramnios (DVP &gt;8 cm)</td>
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</tr>
<tr>
<td>II</td>
<td>Absent bladder</td>
<td>Bladder seen</td>
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<tr>
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<td>Abnormal Doppler finding</td>
<td>Abnormal Doppler finding</td>
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<tr>
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<td></td>
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<tr>
<td>IIIb</td>
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<td>IIIc</td>
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<td>&gt;3+ Z-score</td>
<td>&gt;4+Z-score</td>
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<tr>
<td>MPI</td>
<td>&gt; +2 Z-score</td>
<td>&gt;3+ Z-score</td>
<td>Severe biventricular dysfunction</td>
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</table>

*Definition of recipient cardiomyopathy in the Cincinnati TTTS staging system. AV=atrioventricular, DVP=deepest vertical pocket, LV=left ventricle, MPI=Myocardial Performance Index, RV=right ventricle
Treatment Options
Numerous treatments for TTTS have been proposed, including selective feticide, cord coagulation, sectio parva (removing one fetus), placental blood letting, maternal digitalis, maternal indomethacin, serial AR, microseptostomy of the inter-twin membrane, and nonelective or selective fetoscopic laser photocoagulation. For decades in the United States, serial AR has been the most prevalent therapy for TTTS, but in recent years, selective fetoscopic laser photocoagulation has become more widely accepted.

Amnioreduction
AR was employed initially for maternal comfort and as a means to control polyhydramnios in the hope of prolonging the pregnancy until the risks of extreme prematurity are lessened. In addition, AR improves uteroplacental blood flow, likely by reducing pressure from the amniotic fluid. In uncontrolled series, AR improved survival compared with the natural history of untreated TTTS. Moise, (34) in a review of 26 reports dating from the 1930s of 252 fetuses, found an overall survival of 49%. The survival in more recent series, with more consistently aggressive serial AR to reduce amniotic fluid volume to normal, have ranged from as low as 37% to as high as 83%. (35)(36)(37)(38)(39)(40) However, these retrospective series are comprised of small numbers of patients from a range of gestational ages as well as from a spectrum of severity of TTTS. Severity of TTTS and gestational age at diagnosis may have a profound impact on the observed mortality with any treatment strategy. The earlier in gestation that TTTS presents, the worse the prognosis. Mari and associates (41) found that patients presenting with advanced TTTS prior to 22 weeks’ gestation and absent end-diastolic flow in the recipient umbilical artery had a survival of both twins with aggressive AR of only 13%; with absent end-diastolic flow in the donor umbilical artery, survival was 33%.

Microseptostomy
The paradoxical resolution of oligohydramnios after a single AR was suggested initially by Saade and associates (42) to be due to inadvertent puncture of the inter-twin membrane. Inter-twin septostomy was proposed specifically as a treatment for TTTS to restore amniotic fluid dynamics without the need for repeated AR. One objection to this approach is the possible result in a large septostomy, creating an essentially monoamniotic sac with the attendant risk of cord entanglement. For this reason, a fetoscopic microseptostomy has been proposed to prevent this complication. In their small multicenter series of 12 patients, Saade and associates (42) reported an 81% survival with microseptostomy. However, not only was this series small and uncontrolled, but there was no report of neurologic or cardiac morbidity. In a direct comparison in a small retrospective single institution series of serial AR and microseptostomy, Johnson and associates (43) observed no survival advantage with either therapy. This was confirmed in a subsequent study by Moise and colleagues, (44) who reported the results of a multicenter prospective randomized clinical trial comparing AR to septostomy. The survival in each arm of the study was 65%, consistent with the concept that the effect of AR may be inadvertent septostomy. These studies, however, cannot prove that inter-twin microseptostomy is the mechanism by which amnioreduction works. The use of ultrasonographically guided microseptostomy has fallen out of favor largely because of the risk of creating a monoamniotic gestation and the attendant risk of cord entanglement and cord accident. (45)

Fetoscopic Laser Photocoagulation
The first treatment for TTTS that attempted to treat the anatomic basis for the syndrome was reported by De Lia and associates, (11)(46) who described fetoscopic laser photocoagulation of vessels crossing the inter-twin membrane. At least in theory, this treatment option should be superior because it not only arrests shunting of blood from the donor to recipient, but it also halts transfer of potentially vasoactive mediators. In their small series, these investigators reported a survival of 53% in 26 patients. (11) Although survival was not significantly better than previous reports with serial AR, the “neurologic outcome” in 96% of survivors was “normal,” as assessed by head ultrasonography. Other groups from Europe have reported similar survival with nonelective laser photocoagulation. Ville and associates (12) reported 53% survival with a fetoscopic laser technique, which was better than the survival observed with historical controls at the same center with serial AR (37%). They also observed a lower incidence of abnormalities dictated by neonatal head ultrasonography compared with historical controls.

The nonselective fetoscopic laser technique photocoagulates all vessels crossing the inter-twin membrane. This approach may be problematic because the inter-twin membrane often bears no relationship to the vascular equator of the placenta. Thus, nonselective laser photocoagulation of all vessels crossing the inter-twin membrane may sacrifice vessels not responsible for the TTTS, resulting in a higher death rate of the donor twin from acute placental insufficiency. (47) More recently,
Quintero and associates (47) described a selective approach to fetoscopic laser photocoagulation in TTTS that does not photocoagulate every vessel crossing the inter-twin membrane. Only direct, A-A and venovenous connections are photocoagulated, along with any unpaired artery going to a cotyledon drained by a corresponding unpaired vein (and vice versa) going to the opposite (co-twin’s) umbilical cord. Vessels on the chorionic plate can be differentiated endoscopically because arteries usually cross over veins and are darker due to lower oxygen saturation (Figs. 1 and 2).

In a nonrandomized comparison of patients treated by serial AR at one center and selective laser photocoagulation at another, the overall survival was not statistically significantly different (61% for laser versus 51% for serial AR). (13) However, the survival of at least one twin with laser photocoagulation was 79% compared with 60% for serial AR ($P<0.05$).

Quintero and colleagues (48) retrospectively examined data from 78 patients treated by serial AR and 95 patients treated with selective laser photocoagulation and found no significant difference in patient distribution by stage. Perinatal survival was not significantly different in the laser versus AR group (64.2% versus 57.7%), although there was an inverse relationship between fetal survival and stage in the amniocentesis group but not in the laser group. For Stage IV disease, fetal survival in the AR group was significantly lower than in the laser group (20.6% versus 63.6%, $P = 0.001$). This information has important implications for evaluation of treatment options and the development of stage-based treatment protocols.

One potential limitation to the success of laser treatment is the presence of deep vascular arteriovenous (A-V) anastomoses that cannot be identified endoscopically. In one study, vascular casts of 8 of 15 placentas (53%) demonstrated potentially significant atypical A-V anastomoses such that two apparently normal cotyledons actually communicated below the chorionic surface. (49) A second type of atypical A-V anastomoses was noted in 11 of the 15 placentas (73%) in which shared cotyledons arose within larger apparently normal cotyledons. Such anastomoses would appear as shared cotyledons on endoscopy, and ablating these has the potential to destroy some surrounding normal cotyledon that, in the donor’s territory, could contribute to placental insufficiency. (49) In up to 20% of cases, communicating vessels on the chorionic plate are missed at the time of fetoscopic laser treatment, (50) but only 5% of these cases were associated with persistence of TTTS. These observations point to the necessity of a careful fetoscopic inspection of the chorionic plate to be certain that no vessel is missed.

**Fetoscopic Cord Coagulation**

Some centers have taken the view that the most definitive approach to treating TTTS is selective reduction using fetoscopic cord ligation or coagulation. The rationale is that cord occlusion and sacrifice of one twin arrests the syndrome, prolongs the gestation, and maximizes the outcome for one twin. Crombleholme and associates (32) have reserved this approach for instances in which advanced TTTS cardiomyopathy has irretrievably compromised the recipient twin and there is no hope for...
salvage. In such cases, due to unequal sharing between the donor and recipient, the selective fetoscopic laser procedure may result in death of the donor twin from acute placental insufficiency within hours of the procedure and the recipient twin from progressive TTTS cardiomyopathy. In this situation, fetoscopic cord coagulation may be the best option available. Cord coagulation preserves the vascular communications between the donor twin and the placenta in the recipient twin’s domain. In their series of 19 of 20 such cases, these investigators observed rebound fetal growth and restoration of amniotic fluid volume, and delivery of a neurologically intact donor twin at a mean gestational age of 34 weeks was achieved. (32) One survivor had grade I intraventricular hemorrhage but is otherwise doing well.

Sequential Treatment
The approach at the Fetal Care Center of Cincinnati has been to offer sequential therapy tailored to the needs of a given set of twins based on gestational age at presentation and evidence of progression of hemodynamic compromise according to Doppler velocimetry and echocardiographic changes. (26)(33) In this approach, only those cases in which less invasive approaches have failed are offered the more invasive fetoscopic treatments. For patients who present later than 24 weeks of gestation, we have favored AR, based on the much more favorable prognosis in these patients. For patients presenting prior to 24 weeks’ gestation in which advanced cardiac changes are not seen in the recipient (Stages I, II, III, IIIA), we have tended toward AR as an initial therapy. For patients who have echocardiographic evidence of moderate or severe TTTS (Stages IIIb, IIIC, IV), we favor selective fetoscopic laser treatment because there is insufficient time to see if the pregnancy will respond to AR before TTTS cardiomyopathy worsens. The group of patients presenting earlier in gestation tends to develop signs of more rapid hemodynamic progression of TTTS despite normal amniotic fluid dynamics. For this reason, all pregnancies undergo close serial ultrasonographic and echocardiographic surveillance for progressive cardiac and hemodynamic changes, which are indications for selective fetoscopic laser surgery. We reserve fetoscopic cord coagulation in TTTS for instances in which co-twin demise is imminent and fetoscopic laser surgery might adversely affect the available placental mass in the donor fetus, predisposing to acute placental insufficiency and risking demise of the donor as well. The one downside to an initial trial of AR is chorioamnionic separation, which occurred in one patient in this series (3%); chorioamnionic separation precludes a fetoscopic procedure. Fortunately, this patient had responded to AR.

Pathologic Evaluation of Placentas From Deliveries Complicated by TTTS
Placental examination is critical in all deliveries of twins and multiples, but it is especially important in cases of TTTS. There are numerous descriptions of the specifics of detailed pathologic examination of singleton placentas. Virtually all of the steps are applied to the placentas from multiple gestations, and several are added. (9)(51)(52)(53)(54)(55)(56) For example, the cord is examined to exclude excessive coiling and knots, the chorionic surface for greenish (meconium) or yellow-green (intrauterine infection) discoloration, maternal surface for the presence of retroplacental clot and infarction, and the parenchyma for infarction. The following description highlights the salient features of the examination of the DiMo placenta in TTTS that have bearing on the confirmation or amplification of clinical diagnoses or the detection of unsuspected pathology that has clinical implications for the neonate. It also focuses on gross pathology because neonatologists, if willing and reasonably informed, may be able to detect pathology that may have immediate bearing on infant care. Generally, due to logistical limitations, many hours and sometimes days elapse before the placental pathology examination and report are completed.

In DiMo placentations, the site of coaptation of the two amniotic membranous sacs is referred to as the “dividing membrane,” “interamniotic septum,” or “membranous equator.” The dividing membrane is a transparent structure; because of the absence of intervening chorion, the dividing membrane of a DiMo placenta consists of two oppositely facing monolayers of amnion separated by microscopic amounts of basement membrane material. As such, the individual layers are separated easily from one another, a physical feature that is useful to pathologists (Fig. 3). Unless the gestation was complicated by intrauterine infection, meconium release, or intra-amniotic or chorionic hemorrhage (ie, related to vascular rupture of velamentous vessels, procedure-associated bloody extravasation from intrauterine intervention(s) such as laser ablation or amnioreduction, marginal hematoma), the dividing membrane is colorless to faintly pink.

The most common misconception regarding the position of the dividing membrane in DiMo placentas (with or without TTTS) is the assumption that the clinical/gross position of the membranous equator correlates with that of vascular equator. The membranous equator is the
line of apposition of the two amniotic sacs and is seen most easily where it overlies the chorionic plate to form an inverted “T” as the amnions reflect upward from the chorionic surface. (Of note, the line of coaptation in TTTS also does not evenly divide the chorionic plate, due to the discrepant volumes of amniotic fluid, so the term “equator” is a misnomer.) The vascular equator is an irregular zone lying between the two cord insertion sites that demarcates the peripheral limits of each twin’s chorionic plate vascular ramifications. In TTTS, it may not be midline because the parenchymal territories of each twin are generally unequal. It is important for the clinician and the pathologist to remember that all anastomoses run under the dividing membrane, but not all vessels passing under the membrane are anastomotic. It is also important to remember that the superficial demarcation of the equatorial plate does not correlate with the parenchymal demarcations of the chorionic villous tree; the floating chorionic villous tree supplied and drained by one twin may extend into what would be perceived as the co-twin’s “territory” if only the chorionic surface is considered. The chorionic villous tree is a three-dimensional structure and, as such, may not “obey” expected lines of demarcation within the maternal space. Histologic sections of the dividing membrane and free membranes of each twin’s sac are obtained carefully so as to preserve the integrity of the dividing membrane and the relationship of the chorioamnion of each twin. The amniotic sac of each twin is applied loosely over, but not structurally attached to, the chorion and, like the dividing membrane’s location, can be avulsed easily from the chorionic surface. The absence of desmosomal connections between the amnion and chorion has implications for delivery and handling of the placenta.

Ideally, the cord of each twin is designated clinically by clamps, enabling correlation with the examination of the chorionic surface of each twin’s side. The amniotic surface of each twin’s side of the chorionic plate should be assessed grossly for its relative percentage of occupancy of the total placental dimension, color, and the existence of amnion nodosum. Amnion nodosum is diffusely deposited, grossly detectable but tiny (1 to 2 mm), white nodules on the chorionic surface. They are microscopically composed of aggregates of desquamated fetal skin epithelium and sebaceous material or hair and are attached to the chorionic surface of the donor twin, if the oligohydramnios and fetal skin maturation are of sufficient duration (Fig. 4). They are uncommon before late in the third trimester because of fetal skin immaturity. Cord insertion, both as it relates to distance from the
placental margin and from the dividing membrane, should be assessed. Cord vascular number and patency also should be evaluated. Cord color, coiling, length, and particularly diameter should be noted. Donor twins characteristically have thin cords; recipients’ cords are thick due to edema of Wharton jelly. Cord color should be white-gray. Reddish-brown discoloration due to hemolysis is seen in instances wherein intrauterine fetal death of one twin precedes delivery of the viable twin by 6 or more hours. In sudden demise just prior to delivery, no color change is generally detectable. Cords of stillborn infants should be examined carefully for thrombi, single umbilical artery, and vascular disruption. Velamentous vascular segments may be present in the free membranes or in the dividing membrane and should be examined for integrity and patency. Bright red, brownish-red, or yellow-green discoloration in the membranous tissue may represent acute, recent, or chronic hemorrhage, respectively. Marginal clots may be present in cases of marginal or velamentous insertion. Usually this type of margin-associated hematoma represents maternal venous extravasation, but its presence may have contributed to preterm delivery or it may be due to rupture of a cord vessel. The placental weight should be determined, but in the clinical setting, the weight is less reliable due to the presence of attached membranes and cords. Placental pathology reference ranges for twin placental weight are available for each week of gestation, but reflect trimmed specimen weight. (57)

Inspection of the maternal surface may reveal distinct differences in parenchymal color. The donor’s side may be pale and bulky or shrunken and the recipient’s congested and firm. However, often there is little difference. Evaluation of the chorionic plate for vascular anastomoses is best carried out by the pathologist, but clinical inspection may be of particular benefit in cases of suspected acute transfusion; larger venovenous (V-V) or A-A anastomoses may be detectable through the amnion at the time of delivery. A-A, V-V, and particularly A-V anastomoses can be seen, documented, and enumerated by careful inspection after removal of the amnion and should be followed by detailed injection studies. The examination involves specimen alteration and significant commitment of time and, therefore, is best left to the pathologist or specifically trained personnel. The amnion should be peeled back, except in the line of dividing septum, to expose the chorion. Arteries, which overlie veins, should be traced first from their origin at the cord insertion site to their termination. If they are normally distributed, the artery will be paired with a vein from the corresponding cord of analysis, and both will pierce/arise from the chorionic plate in close approximation. However, in A-V anastomoses, the afferent artery is unpaired and typically enters vertically into the placental parenchyma within millimeters of an unpaired vein that is a tributary of the co-twin’s venous system. The number of A-A, afferent arterial calibers, and direction of the anastomoses should be recorded. A-A and V-V anastomoses do not terminate in the parenchyma but demonstrate direct connection on the chorionic surface. All anastomoses should be documented photographically and preferably mapped, even if injection studies are not performed. (53) The vascular pattern (magistral, diffuse, or mixed) should be assessed, as should which twin has which pattern predominating. (57)

We strongly recommend use of injection studies. Although a variety of fluids has been used, (53) we find it simplest to use milk with added standard food coloring. Milk is nontoxic and easily available, and “backwash” can be wiped easily from the chorionic surface if overzealous injection pressure is applied. Prior to injecting a vessel, it should be emptied of blood by application of pressure such that blood is pushed toward the corresponding umbilical cord. The arteries should be injected gently and slowly, using a small-caliber needle (#19 to #20 gauge) attached to 10-mL syringe (Fig. 5). Injection of the vessels more distal from the cord insertion site, followed by application of gentle “anterograde” pressure (in the direction toward the vascular equator) on the injected vessel, enables demonstration of both superficial and deep anastomoses. A-V connections may be revealed by filling of the efferent vein. Serial photographs should be taken to document various phases of the injection study because progressive injections can result in vascular rupture, with leakage of fluid and obscuration of anatomy.

Figure 5. Dye injection of the artery.
Following injection studies, the placenta should be sectioned serially and the parenchyma of each twin’s territory, as well as sites of A-V anastomoses, sampled and processed for histologic examination.

The use of injection studies is extremely important in examining placentas from gestations treated with laser fetoscopic occlusion procedures (Fig. 6). Laser surgeries are performed at select medical centers. However, as pointed out by De Paepe and associates, (58) treated women may return to their primary obstetricians for continued clinical care and delivery at their local hospitals. Thus, the practicing neonatologist (and pathologist) may encounter infants and placentas from these gestations even though the procedure was not performed in their institutions. The pathologist may be asked to verify the presence of vascular occlusion at the sites of photoocoagulation, determine the existence of any remaining anastomoses, and interpret the placental findings with reference to infant outcomes. Lasered sites are usually fibrotic and pale, with superficial coagulative necrosis. The proximal segments of the lasered afferent and distal efferent vessels may be thrombosed distant from the site. Variable thrombosis and involution of dependent branch villi also are common.

**Summary**

Although TTTS is a pathologic condition of net transfusion from a one twin to the other, the process is not simply a continuous, unidirectional siphon of blood from the donor to the recipient. As was presented in TTTS Part 1 and further amplified in TTTS Part 2, TTTS is a dynamic condition. Abundant clinicopathologic evidence and animal research indicate that although choriangiopagus is a necessary prerequisite for TTTS, the underlying cause remains unknown. Moreover, both the volume of blood passed from donor to recipient and its contents, as well as the degree of discrepancy in placental territory between the donor and recipient, act together to determine the time of onset, rapidity of progression and severity, and morbidity and mortality associated with TTTS. Reversed transfusion from recipient to donor may occur as hypervolemia and polyhydramnios ensue and may serve to correct the imbalance in blood volume and maladaptive responses in the donor. However, as the recipient twin and donor twin move toward euvoelmic and cardiac function in the recipient and donor improve, the net shunt from donor to recipient can recur. Clinical monitoring and appropriately timed interventions through serial AR or selective fetoscopic laser photoocoagulation of anastomoses have significantly improved the severe mortality associated with TTTS. The neonatologist is well served to remember that the morbidity and mortality of TTTS is related to multiple and changing intrauterine factors and that surviving infants may have or develop sequelae that are nonuniform. Monochorionic twins’ improvement and response to treatment may
reflect the discontinued shunting of blood, but unexplained reduction in expected clinical response or the persistence of organ dysfunction may reflect response of circulating mediators and cardiac, renal, and hepatic pathologies that have their roots in intrauterine life. Such sequelae may require greater durations of time and therapies to correct. Others, such as increased peripheral vascular resistance and nephrosclerosis and speech deficits, potentially may present later or progress and appear to be temporally unrelated to the TTTS. Some twins may sustain long-term cardiac or renal dysfunction. Further studies should continue to shed light on this complex condition and, with continued progress with intervention procedures, hopefully reduce the number and severity of pathologic sequelae and further improve infant outcomes.

References

3. Chronic twin-to-twin transfusion syndrome (TTTS) is associated with sequelae of cardiovascular dysfunction or vascular disruption, resulting in the occurrence of congenital anomalies in one or both twins. Of the following, the most common congenital anomaly associated with chronic TTTS is:

A. Cutis aplasia.
B. Hydranencephaly.
C. Intestinal atresia.
D. Limb necrosis.
E. Ventricular septal defect.

4. Quintero and associates have proposed a staging system for TTTS that considers a sequence of progressive ultrasonographic features. You observe the following findings in a pregnancy complicated by TTTS: polyhydramnios in the recipient twin, oligohydramnios in the donor twin, and reverse end-diastolic blood flow in the umbilical artery of the donor twin. Of the following, the stage of TTTS in this pregnancy is most likely to be:

A. Stage I.
B. Stage II.
C. Stage III.
D. Stage IV.
E. Stage V.

5. You are following a 28-year-old primigravida woman who has a twin pregnancy via serial ultrasonographic examinations for TTTS. At 24 weeks' gestation, you observe polyhydramnios in the recipient twin, oligohydramnios in the donor twin, and mild cardiomyopathy in the recipient twin. Of the following, the favored treatment in this pregnancy is:

A. Amnioreduction.
B. Fetoscopic cord ligation.
C. Fetoscopic laser photocoagulation.
D. Placental blood letting.
E. Septostomy of the inter-twin membrane.