Discordant Blood Chimerism in Dizygotic Monochorionic Laser-Treated Twin–Twin Transfusion Syndrome

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BACKGROUND: Twin–twin transfusion syndrome occurs in 10% of monozygotic monochorionic twin gestations and results from an unbalanced exchange of blood from the donor to the recipient fetus through placental anastomoses.

CASE: We present a case of twin–twin transfusion syndrome with differing fetal sex treated with in utero laser surgery. Genetic analyses showed 46,XX/46,XY hematologic chimerism in both twins at birth and at 6 months, with the recipient twin being significantly more chimeric than the donor. Placental pathologic examination confirmed monochorionicity and laser ablation of all anastomoses.

CONCLUSION: Despite in utero separation of the fetal circulations remote from delivery, hematologic chimerism persisted after birth. We speculate that the greater degree of blood chimerism in the recipient compared with the donor was related to the pathophysiology of twin–twin transfusion syndrome before laser surgery.

M onochorionic twin gestations are associated with an increased risk of fetal and neonatal complications, mainly due to vascular communications that link the circulatory systems of the twins together. In the case of twin–twin transfusion syndrome, which occurs in 10% of monozygotic monochorionic twin gestations, there is a net transfer of blood from the donor twin to the recipient twin through these placental anastomoses. One treatment for twin–twin transfusion syndrome, selective laser photocoagulation of communicating vessels, involves fetoscopic identification and laser ablation of all the placental vascular anastomoses in utero, thereby separating the fetal circulations from one another.

Monochorionicity occurs in approximately two thirds of monozygotic (identical) twins, but it is extremely rare in dizygotic (fraternal) twins.1 However, the rate of dizygotic monochorionic twinning may be increasing owing to the use of assisted reproductive technology.2 Assisted reproductive technology has been hypothesized to increase the chance of monochorionicity in dizygotic twins by affecting cell fusion, adhesion, and embryo proximity.2 In cases of dizygotic monochorionic twins, blood chimerism, the presence of hematologic cells from more than one zygotic lineage, has been found.3–6 Monochorionic dizygotic twin gestations complicated by twin–twin transfusion syndrome are even less common.5,7

We present a case of twin–twin transfusion syndrome in a dizygotic monochorionic diamniotic twin gestation that was successfully treated using laser surgery. Despite in utero separation of the fetal circulations remote from delivery, hematologic chimerism persisted after birth. In addition, the recipient twin, the fetus receiving relatively increased blood volume before laser surgery, harbored significantly greater hematologic chimerism than the donor twin.

CASE

The patient was a 28-year-old gravida 1 para 0 who conceived through assisted reproductive technology. The patient’s oocytes were fertilized using intracytoplasmic sperm injection with two embryos transferred into the uterus. Although a monochorionic diamniotic twin gestation was suspected on early ultrasonography, discordant fetal sex was identified at 21 0/7 weeks of gestation, thereby putting into question the actual chorionicity. Subsequent serial ultrasonography showed an increasing amniotic fluid discordance on either side of the dividing membrane, and the patient was referred to our institution for evaluation of twin–twin transfusion syndrome.

Ultrasonas assessment at our center confirmed the presence of twin–twin transfusion syndrome. A thin dividing membrane with a T-shaped insertion into the placenta indicative of a monochorionic diamniotic twin gestation was identified. The female donor twin had grossly normal anatomy and an amniotic fluid maximum vertical pocket of
1.6 cm. The male recipient twin had significant cardiomegaly and a maximum vertical pocket of 16.1 cm.

After discussing all management options and obtaining signed informed consent, the patient underwent operative fetoscopy and sequential selective laser photocoagulation of communicating vessels at 26 0/7 weeks of gestation using surgical techniques described previously. The trocar was inserted into the amniotic sac of the polyhydramniotic male twin. On fetoscopic examination, the female donor fetus and the male recipient fetus had normal-appearing external female and male genitalia, respectively. Five arteriovenous vascular communications were identified and ablated without complication. Amniotic fluid from the male recipient sac was sent for chromosome analysis, which revealed a normal male karyotype (46,XY in 23 cells counted).

The postoperative course was uncomplicated, and the patient electively delivered at 37 4/7 weeks of gestation through caesarean delivery. Both twins had normal Apgar scores, were appropriately grown, and had uncomplicated neonatal courses. Placental pathologic evaluation using previously described techniques revealed a monochorionic placenta with complete ablation of all vascular communications. A subtle demarcation in the midline of the placenta was identified grossly. Pediatric genetic evaluation of the twins at 9 months of age revealed no dysmorphic features, including normal-appearing external genitalia. The children were alive and well, achieving normal developmental milestones, at 1 year of age.

At the time of delivery, cord blood chromosome analysis using standard cytogenetics and interphase fluorescence in situ hybridization using X and Y probes was completed to assess for hematologic chimerism. Chromosome analysis of the male recipient twin revealed two blood cell lines. Of the 20 cells examined, 14 were 46,XX and sex was 46,XY (30% male). These results were confirmed by fluorescence in situ hybridization, which revealed 46,XX[32]/46,XY[28] (47% male). Chromosome analysis of the female donor twin cord blood also revealed two blood cell lines. Of the 22 cells examined, 18 cells were 46,XX and 4 cells were 46,XY (82% female). These results were confirmed by fluorescence in situ hybridization, which revealed 46,XX[73]/46,XY[27] (73% female). Follow-up peripheral blood chromosomal analyses at 6 months of age revealed similar findings. The level of blood chimerism remained fairly consistent, with 84 of 300 cells in the recipient male twin 46,XY (28% male) and 218 of 300 cells in the donor female twin 46,XX (73% female). Buccal smears at 9 months of age revealed normal karyotypes for both fetuses in that cell line, with 46,XY[500] by fluorescence in situ hybridization noted in the male twin and 46,XX[500] by fluorescence in situ hybridization in the female twin, using the probes Yp11.3 SRY Red and CEP X Green.

**COMMENT**

We report a case of dizygotic monochorionic twins with discordant fetal sex complicated by twin–twin transfusion syndrome and treated in utero using laser surgery. Dizygotic monochorionic conceptions are rare, although it is believed that assisted reproductive technology has increased their occurrence. The event of dizygotic monochorionic twins complicated by twin–twin transfusion syndrome is even less common and to our knowledge has only been published twice previously. One remarkable observation that we discovered in this case was the significantly greater degree of blood chimerism in the recipient fetus compared with the donor fetus. We speculate that this finding is related to the pathophysiology of twin–twin transfusion syndrome that was present before the laser surgery, which severed the vascular communications between the twins, as subsequently confirmed with placental injection studies.

In this case, the male recipient twin, the fetus receiving relatively increased blood volume before laser surgery, had 53% to 70% 46,XX blood cells at birth; this level of chimerism was significantly different from the donor female twin, who had only 18% to 27% 46,XY blood cells at birth (P = .001, Fisher exact test). This discordance persisted at the 6-month re-evaluation, with the male recipient having 72% 46,XX and the female donor having 27% 46,XY blood cells (P = .001, Fisher exact test). The finding of a higher proportion of female donor blood cells in the male recipient twin is in keeping with the understood pathophysiology of twin–twin transfusion syndrome, which involves the net transfer of blood from the donor to the recipient. Because twin–twin transfusion syndrome did not become clinically apparent until the early third trimester, we offer one of two speculations for the discordant blood chimerism observed in this case: 1) the exchange of blood stem cells or lymphocytes or both resulting in chimerism may continue until the early third trimester, or 2) the pathophysiology of twin–twin transfusion syndrome may begin much earlier in the pregnancy than previously surmised but may not become clinically evident until later in the pregnancy.

Amniocytes taken from the male recipient sac at the time of operative fetoscopy and buccal smears obtained from both infants at 9 months of age did not show chimerism. In prior studies, it has been stressed that gonadal and other types of tissue are generally not chimeric in cases of blood chimerism. Thus, the peripheral blood chromosomal complements in dizygotic monochorionic twins may be considered similar to that occurring as a result of allogenic bone marrow transplants.

Chimerism occurs naturally in co-twins who share a common circulation during gestation. However, because this almost always occurs in monozygotic Monochorionic Gestation

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gous twins, the consequences are not significant from a clinical perspective. Complete hematopoietic chimerism occurs in bone marrow transplantation, in which all bone marrow-derived cells in the recipient are eliminated and replaced with donor cells. Partial or mixed chimerism in bone marrow transplantation occurs when milder forms of preconditioning are used, which at first do not entirely ablate the host hematopoietic system. Because the twins in our case have been engrafted with each other’s cells, including stem cells, without prior myeloablation and without evident clinical manifestations, they likely have full tolerance. This finding supports the concept of fetal tolerance to foreign antigens that has been cited by researchers who study in utero stem cell transplantation.

In conclusion, we present a case of dizygotic monochorionic twins with twin–twin transfusion syndrome, treated successfully using laser surgery, with discordant hematologic chimerism. We speculate that the greater degree of blood chimerism in the recipient fetus is related to the pathophysiology of twin–twin transfusion syndrome present before the dichorionization of the placenta by laser surgery.

REFERENCES


