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Five-year study assessing the feasibility and safety of autologous blood transfusion in pregnant Japanese women

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Abstract

Aim: To assess the feasibility and safety of autologous blood donation during pregnancy in Japanese women.

Material and Methods: We enrolled patients who were either at high risk for massive blood loss during delivery or had blood that was difficult to match for transfusion between March 2005 and February 2010. After delivery, we reviewed hospital records of these patients to collect data on blood donation procedures, obstetric outcome and blood transfusions received.

Results: We enrolled 314 patients during the study period and performed 809 blood donations. The median volume of donated blood was 1200 mL (range, 400–2000 mL). Vasovagal reflex as an adverse donor reaction occurred in 10 of the 314 patients (3.2%) during 11 of the 809 donations (1.4%). There were no cases of non-reassuring fetal heart rate patterns during blood donations. Twenty-five (7.8%) of the 322 neonates were admitted to the neonatal intensive care unit. All 322 infants were healthy 1 month after delivery. Among 314 patients, autologous blood re-transfusion was performed for 56 (17.8%) and homologous blood transfusion was performed concurrently for 5 (1.6%). Placenta previa was the indication with the highest re-transfusion rate (42.4%). All re-transfusions were performed without side-effects.

Conclusion: Autologous blood donation is feasible and safe for pregnant women and their infants. Although indications of autologous blood donation are controversial, it should be considered for cases of placenta previa.

Key words: adverse effect, autologous, blood, pregnancy, transfusion.

Introduction

Obstetric hemorrhage is a major cause of maternal death,[1] and its incidence has been increasing.[2–4] Transfusion therapy is one of the most important procedures for improving maternal outcome. Autologous blood donation (donation, storage and re-transfusion) may be effective for patients at risk for massive bleeding during delivery or are difficult to transfuse because of a rare blood type. The Royal College of Obstetricians and Gynecologists (RCOG) in the UK does not recommend autologous blood donation during pregnancy because of concerns regarding placental insufficiency, whether the woman can replace her hemoglobin before delivery, and whether the collected units will be sufficient in the event of major obstetric hemorrhage.[5] The American College of Obstetricians and Gynecologists (ACOG) also does not recommend this procedure.[6] However, autologous blood transfusion has two merits: the immediate availability of blood without cross-matched testing and reducing the need for homologous blood transfusion. For this reason, we suggest that autologous blood donation should be included as an option for pregnant women, especially

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for high-risk patients and those with rare blood types. A series of studies have demonstrated that autologous transfusion is safe in pregnancy.[7–12] Among these studies, two had a large sample size. McVay *et al.* reported 272 patients who donated in the third trimester of pregnancy. However, they were only able to analyze outcomes in 199 given the retrospective study design. Recently, Obed *et al.* reported the feasibility of autologous blood donation during pregnancy with 625 obstetric patients using a prospective study design. However, they did not evaluate the incidence of adverse effects (e.g. maternal vasovagal reflex), incidence of autologous blood use, quantity of blood loss at delivery, or outcomes of deliveries and neonates. Accordingly, the study by Obed *et al.* does not sufficiently address the feasibility and safety of autologous blood donation during pregnancy. The aim of this study is to prospectively assess the feasibility and safety of autologous blood donation and transfusion during pregnancy in Japanese women.

Material and Methods

This study was approved by the ethics committee of our institution. Candidates for enrollment were pregnant women scheduled to deliver at our center during the study period (between March 2005 and February 2010). The inclusion criterion was to be a patient at high risk for massive blood loss at delivery or a patient for which securing cross-matched blood for transfusion would be difficult.

At approximately 32 weeks of gestation, we explained the study to these patients and determined whether they met eligibility criteria. Patients with any maternal medical complication, fetal growth restriction or fetal structural anomalies were excluded from this study. We obtained written informed consent and initiated iron supplementation (100 mg/day) at the time of enrollment.

Patients who were scheduled to deliver by planned cesarean section began donating their blood approximately 5 weeks before the day of the operation and patients who were scheduled to deliver by spontaneous onset of labor began donating at approximately 36 weeks of gestation, because donated blood could be stored for up to 35 days. We scheduled patients at low risk for obstetric hemorrhage to donate 400 or 800 mL of autologous blood, and aimed for high-risk patients to donate 1200 mL or more.

The schedule on the day of blood donation was as follows:

1 Blood was sampled to examine whether the patient was anemic. Blood donation was postponed if the hemoglobin concentration was below 10 g/dL.
2 Lidocaine tape (Penles tape; Nitto Denko Corporation, Tokyo, Japan) was applied to lessen pain during donation.
3 Patients were hydrated orally (about 500 mL of isotonic water).
4 Patients donated approximately 400 mL of blood by gravity with fetal heart rate (FHR) monitoring.

All blood donations were performed by obstetricians. Donation was stopped and proper medical care was given if patients had an abnormal donor reaction or abnormal FHR patterns.

The policy of autologous blood transfusion was the same as for homologous blood transfusion. We did not determine the criteria for transfusion using patient hematocrit or hemoglobin levels because a blood test of an obstetric patient suffering from massive bleeding might not accurately reflect her condition. We performed autologous blood transfusion when a patient had unstable vital signs or low urinary output. If vital signs were stable, autologous transfusion was not performed. After delivery, we reviewed hospital records of these patients to collect data on blood donation procedures, obstetric outcome and blood transfusions received.

Results

A total of 314 patients (mean age ± SD, 34.8 ± 4.7 years) were enrolled during the study period; a total of 809 blood donations were performed. Gravidity and parity were 0, 0–5 and 0, 0–5 (median, range), respectively. Mean height and body weight before pregnancy were 159.0 ± 9.0 cm and 52.9 ± 7.1 kg, respectively.

Among the 314 patients, it would have been difficult to prepare matched blood for transfusion for 86 (56 with Rh negative blood type, 30 with positive irregular antibodies), and 228 patients were at high risk for massive bleeding during delivery (66 with total placenta previa, 62 with leiomyoma, 61 with low-lying placenta, 12 with previous cesarean section or past history of uterine surgery, 8 with twin pregnancy, and 21 with a history of massive bleeding during a previous delivery).

Data for autologous blood donations from pregnant women are shown in Table 1. Median donated blood volume and number of blood donations were 1200 mL

Table 1 Data of autologous blood donation during pregnancy

Volume of donated blood (mL)	1200, 400–2000	(median, range)
No. blood donations (times)	3, 1–5	(median, range)
Duration of blood donation (min)	7.3 ± 3.7	(mean ± SD)
Hemoglobin concentration at first donation (g/dL)	11.4 ± 0.8	(mean ± SD)
Hemoglobin concentration at delivery (g/dL)	10.8 ± 1.1	(mean ± SD)
Vasovagal reflex (per patient)	10/314 (3.2%)	
Vasovagal reflex (per donation)	11/809 (1.4%)	
Non-reassuring FHR during blood donation	0/809 donations	

FHR, fetal heart rate.

Table 2 Delivery and neonatal outcomes of patients who donated blood during pregnancy

Gestational age at delivery (weeks)	37.8 ± 1.6	(mean ± SD)
Mode of delivery		
Normal vaginal delivery	106 (33.8%)	
Cesarean section	181 (57.6%)	
Instrumental vaginal delivery	27 (8.6%)	
Blood loss during delivery ($n = 314$)	1039 ± 942	(mean ± SD)
Transfused patients ($n = 56$)	2243 ± 1090	(mean ± SD)]*
Non-transfused patients ($n = 258$)	777 ± 664	(mean ± SD)]*
Birth weight (g)	2845 ± 430	(mean ± SD)
Apgar scores at 1 min	8	(median)
Apgar scores at 5 min	9	(median)
Cord blood pH of umbilical artery	7.28 ± 0.41	(mean ± SD)
NICU admission	25/322 infants (7.8%)	

*Student's t-test, $P < 0.05$. NICU, neonatal intensive care unit.

(range, 400–2000 mL) and three times (range, 1–5 times), respectively. Twenty-nine patients donated once, 109 donated twice, 150 donated three times, 18 donated four times, and 8 donated five times. Mean hemoglobin concentrations at the first donation and at delivery were 11.4 ± 0.8 g/dL and 10.8 ± 1.1 g/dL, respectively. Vasovagal reflex (VVR) as an adverse donor reaction was observed in 10 of the 314 patients (3.2%) during 11 of 809 donations (1.4%). No cases of non-reassuring fetal heart rate pattern occurred during the 809 blood donations.

The delivery and neonatal outcomes of patients who donated blood during pregnancy are shown in Table 2. Mean gestational age at delivery was 37.8 ± 1.6 weeks. One hundred and six patients (33.8%) delivered vaginally, 181 (57.6%) underwent cesarean section, and 27 (8.6%) delivered by forceps or vacuum extractor. Mean blood loss during delivery was 1039 ± 942 mL. There was a significant difference in the volume of blood loss between transfused (2243 ± 1090 mL) and non-transfused patients (777 ± 664 mL) using Student's t-test ($P < 0.05$). The mean birth weight of 322 infants was 2845 ± 430 g. Median Apgar scores at 1 and 5 min were 8 and 9, respectively. Mean blood pH of the umbilical artery at delivery was 7.28 ± 0.41. Twenty-five infants (7.8%) were admitted to the NICU. Of these, 18 infants had low birth weight, 4 had transient tachypnea, 1 had a Group B streptococcal infection, and 1 was affected by maternal general anesthesia. None of the infants had abnormal findings 1 month after delivery.

The numbers of transfusions performed grouped by primary indication for study enrollment are shown in Table 3. Autologous blood transfusion was performed for 56 of the 314 patients (17.8%), and homologous blood transfusion was performed concurrently for 5 patients (1.6%). Three of 56 patients with Rh negative blood type (5.4%) and none of the 30 patients with positive irregular antibodies received an autologous transfusion. Of 66 patients with total placenta previa, 28 (42.4%) received autologous blood transfusions and 4 also received transfusions of homologous blood. Nine patients (14.5%) with leiomyoma, 12 (19.7%) with low-lying placenta, 1 (8.3%) with previous cesarean section, and 3 (14.3%) with a past history of massive bleeding during a previous delivery received autologous blood transfusions. One patient with a low-lying

Table 3 Transfusions grouped by primary indication for study enrollment

Indication	*n*	Autologous blood transfusion	Homologous blood transfusion
Patients with blood difficult to match for transfusion			
Rh-negative blood type	56	3	0
Positive irregular antibodies	30	0	0
Patients at high risk for massive bleeding at delivery			
Total placenta previa	66	28	4
Complicated with leiomyoma	62	9	0
Low-lying placenta	61	12	1
Previous cesarean section or past history of uterine surgery	12	1	0
Twin pregnancy	8	0	0
History of massive bleeding during previous delivery	21	3	0
	314	56 (17.8%)	5 (1.6%)

Table 4 Five cases that required transfusion of homologous and autologous blood

Diagnosis	Blood loss (mL)	Autologous blood transfusion (mL)	Homologous blood transfusion (red cells) (units†)	Homologous blood transfusion (fresh frozen plasma) (units†)
Placenta previa	5100	400	8	0
Placenta previa	2732	400	2	0
Placenta previa	4500	1200	9	6
Placenta previa	4340	1200	5	0
Low-lying placenta	3800	1200	5	5

†One unit of blood product originates from 200 mL of whole blood.

placenta also received homologous blood. None of the transfused patients experienced side-effects from bacterial contamination in the donated blood.

Table 4 shows data of 5 patients who were transfused with homologous and autologous blood. Four patients had placenta previa and 1 had a low-lying placenta. Blood loss for each patient was 5100, 2732, 4500, 3800 and 4340 (mL), respectively. The 2 patients with 4500 and 3800 mL blood loss and placenta previa were transfused fresh frozen plasma (FFP) with red blood cells.

Discussion

Some reports have pointed out that the volume of blood donated during pregnancy is insufficient for transfusion during obstetric hemorrhage because most pregnant women are anemic. However, in this study, 285 of the 314 patients could donate at least twice (800 mL). Patients at low risk for obstetric hemorrhage were scheduled to donate 400 or 800 mL, and high-risk patients were scheduled to donate at least 1200 mL. Almost all patients were able to donate as scheduled.

The incidence of vasovagal reflex as an adverse donor reaction is reported to be about 1%.[13,14] The incidence of VVR for donations from pregnant women in this study (1.4%) was consistent with these reports. In addition, the 10 patients who suffered from VVR all had mild symptoms and easily recovered by stopping donation and undergoing bed rest. In other words, pregnant patients were not at increased risk for VVR, and if it happened, the symptoms could be managed.

In the present study, FHR monitoring was performed for all patients and no abnormal findings were observed during donations. Previous reports have shown similar findings.[7–11] In the case of maternal VVR, FHR monitoring showed a reassuring fetal status. The 400 mL of blood donated by the mother scarcely influenced feto-placental circulation. The infants were all in good condition when examined 1 month after birth. The long-term cohort studies seem to increase the reassurance of autologous blood donation during pregnancy.

There was a significant difference between transfused and non-transfused patients because we decided that the indication for re-transfusion of autologous blood was the same as for homologous blood. Given the risk of side-effects, such as bacterial contamination in the autologous blood, we believed that

re-transfusion of autologous blood should not be performed when vital signs were stable.

Re-transfusion of autologous blood was performed for 42.4% of patients with placenta previa, 19.7% with low-lying placenta, 14.5% with leiomyoma, 14.3% with a past history of massive bleeding during a previous delivery, 5.4% with an Rh negative blood type, and 8.3% with previous cesarean section. In the present study, we did not assess the cost-benefit analysis of autologous blood donation during pregnancy. However, as reported previously,[15] patients with placenta previa seem to be the best candidates for autologous blood donation during pregnancy because of the high frequency of re-transfusion. Other indications are accompanied by the problem of wasted blood. If autologous blood had not been prepared, banked blood suitable for high-risk patients would generally be stocked for deliveries. In these situations, homologous blood products as a social resource would have been wasted. Reducing the demand on homologous blood supply is very important because the number of blood donors in Japan has been decreasing (Ministry of Health, Labor and Welfare, Japan 2009). It is also important to note that there was the benefit of being prepared for emergent massive bleeding.

In the present study, we were able to manage cases requiring transfusion using only autologous blood for almost all patients with placenta previa (24/28 patients). Although the UK guidelines[1] point out that autologous blood is insufficient in cases of massive blood loss during delivery, our study showed that donated blood could generally provide adequate transfusion in cases of placenta previa. We speculate that the observed differences between patients may be due to autologous blood containing various levels of clotting factors and fibrinogen.[16] In addition, a recent study[17] reported that the transfusion of whole blood might decrease adverse side-effects of transfusion for obstetric hemorrhage. As autologous blood transfusion uses whole blood, fewer side-effects are expected.

In conclusion, autologous blood donation is feasible and safe for pregnant Japanese women and their infants. Although indications of autologous blood donation are controversial, it should be considered in cases of placenta previa. For other indications, the circumstances of blood supply at the institution should be considered.

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Acquisition of anti-Diego b antibodies possibly resulting from feto-maternal hemorrhage during pregnancy

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Abstract

Anti-Diego b (Di^b) antibodies, rare antibodies against red blood cell antigens, can cause severe hemolysis. We report a patient who most likely acquired anti-Di^b antibodies during pregnancy. The patient was a 39-year-old Japanese woman who delivered by cesarean section at 38 weeks of gestation. She required a second operation to treat re-bleeding of the surgical scar, but it was difficult to schedule this surgery because we could not obtain enough blood for transfusion due to the presence of anti-Di^b antibodies. These antibodies were likely acquired during pregnancy; she did not have irregular antibodies at 11 weeks of gestation. We speculate that she became sensitized to fetal blood due to feto-maternal hemorrhage. The infant had no hemolytic conditions. Testing for the presence of irregular antibodies should be performed during late stages of pregnancy as well as early stages.

Key words: Diego-b, feto-maternal hemorrhage, irregular antibody, pregnancy.

Introduction

Irregular antibodies (anti-red blood cell antibodies) most often develop after a blood transfusion. Certain types of irregular antibodies can prevent safe blood transfusions by causing severe hemolysis. Anti-Diego b (Di^b) antibodies are rare and can cause severe hemolysis. It is difficult to find compatible red cells for patients with these antibodies. We present a case of a pregnant woman who may have acquired anti-Di^b antibodies as a result of feto-maternal hemorrhage during pregnancy.

Case Report

A 39-year-old Japanese woman, gravida 3, para 3, came to our hospital for prenatal care at 11 weeks of gestation. Her second pregnancy had resulted in intrauterine fetal death at 36 weeks of gestation. She did not have a history of blood transfusion. Her blood type was B and RhD positive, and she tested negative for irregular antibodies at 11 weeks of gestation. Irregular antibody screening for red cells was performed using the Ortho BioVue Screen J kit (Ortho Clinical Diagnostics, Raritan, NJ, USA) and the anti-globulin method with low-ionic-strength solution (LISS; Ortho BioVue O.A.E.S, Raritan, NJ, USA). This kit contained four sets of panel cells, including three sets of panel cells with the following antigens: C, c, D, E, e, f, K, k, Kpb, Jsb, Fya, Fyb, Jka, Jkb, Xga, S, s, M, N, P1, Lea, Leb, Lub, and one set of panel cells confirmed to have the Di^a antigen for the Japanese population. In addition, panel cells used for this patient had the Di^b antigen. The patient was not screened for irregular antibodies during the third trimester.

The patient delivered a male infant weighing 2712 g at 38 weeks of gestation by elective cesarean section due to prior cesarean section. Perioperative blood loss was 623 g and the operative course was unremarkable.

The patient was recovering well until she suddenly began experiencing strong abdominal pain on day 3 after the cesarean section. Ultrasound revealed a

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hematoma, measuring 14 cm in diameter, located on the anterior wall of the uterus. The patient gradually developed anemia (hemoglobin was 9.7 g/dL on postoperative day 1 and 8.3 g/dL on postoperative day 3). We diagnosed a hematoma resulting from re-bleeding of the surgical scar of the uterus. We determined that blood loss was slow and decided against immediate removal of the hematoma. Instead, we focused on treating the patient's pain. However, the abdominal pain could not be controlled, and we decided to drain the hematoma on postoperative day 5. Attempts were made to prepare for blood transfusion, but compatible red cells were difficult to find due to the presence of irregular antibodies. Furthermore, we could not determine the type of irregular antibodies that caused incompatibility. We therefore postponed drainage of the hematoma, given the risks of operating without sufficient blood products. The Red Cross Blood Center confirmed positivity for anti-Di^b by the LISS antiglobulin method. The anti-Di^b antibody titer was 1:16 and the dominant immunoglobulin class was IgG using red cells typed as Di(a+b+). The patient's Diego blood type was Di(a+b-). We were able to collect 5 units of Di(b-) red blood cells the day after we began preparing compatible red cells. Surgery was performed to remove the hematoma on postoperative day 6, with a total blood loss of 890 g. Blood transfusion was unnecessary, and postoperative anemia was resolved with iron medication. The patient and her infant were discharged from our hospital on day 22 after cesarean section and day 16 after hematoma removal. The Diego blood type of the infant was Di(a+b+), but the infant did not develop hemolytic disease. We were also able to ascertain the Diego blood group of the first child (Dia(+)Dib(+)) but not of the husband.

Discussion

There are four Diego blood group types: Di(a-b+), which is found in >99.9% of Caucasians and those of African descent and in >90% of Asians; Di(a+b+), which is found in <0.1% of Caucasians and those of African descent and in 10% of Asians; Di(a+b-), which is found in <0.01% of Caucasians, those of African descent; and Di(a-b-), of which there is only one reported case in the literature.[1] Di(a+b-) phenotype has been detected in 0.2% of the Japanese population.[2] In the present case, our patient was Di(a+b-) by postoperative blood type analysis. It was difficult to obtain sufficient Di(b-) blood for transfusion and, in fact, it took half a day to acquire 5 units of blood. Certain types of irregular antibodies are harmless, but anti-Di^b antibodies can cause severe hemolysis. As such, it is important to transfuse compatible red blood cells in patients with anti-Di^b.

Irregular antibodies are created through the sensitization of red blood cells with antigens to other blood types. However, in the present case, the patient did not present with any irregular antibodies early in pregnancy and had no transfusion history. We speculate that she became sensitized to fetal blood due to feto-maternal hemorrhage. Although fetal blood should be isolated from the maternal circulation, it often flows into the maternal circulation during pregnancy and delivery. This condition is called feto-maternal hemorrhage, and can cause maternal sensitization.[3] According to Bowman *et al.*,[4] feto-maternal hemorrhage occurs in 75% of pregnant women, of whom 60% experience, at most, 0.1 mL of fetal blood inflow (1% exceed 5 mL and 0.25% exceed 30 mL). The frequency and amount of bleeding increase as gestation progresses; 5–15% of pregnant women experience feto-maternal hemorrhage of up to 0.1 mL during the first two trimesters, while 45% experience it during the third trimester. The frequency of feto-maternal hemorrhage reportedly increases with gestational age, and 75% of cases experience feto-maternal hemorrhage of up to 0.19 mL prior to birth.

In the present study, the patient may have acquired anti-Di^b antibodies during pregnancy because she had no irregular antibodies early in her pregnancy. The most likely cause of sensitization to fetal blood is feto-maternal sensitization, as she had no other incidents during her pregnancy where contact with another person's blood was made. In addition, we speculate that the reason why the past three pregnancies did not produce anti-Di^b antibodies was that not enough fetal blood had flowed into the maternal circulation. Another possible explanation is that the patient was already sensitized prior to pregnancy, but that the titer was too low. Re-exposure during pregnancy may have boosted the titer, a mechanism referred to as sensitization.

Many irregular antibodies are IgG antibodies, and it is thought that these may cause fetal or neonatal hemolytic disease when they shift to the fetus transplacentally. Mochizuki *et al.* reported an association between maternal anti-Di(b) titer and severity of hemolytic disease in newborns.[5] Specifically, they reported that a high maternal titer (>1:64) was associated with a higher risk of severe hyperbilirubinemia for mismatched newborns. Given that the maternal

anti-Di^b titer was 1:16 in the present case, the titer for our patient could have been lower than the threshold level required for severe repercussions to occur in her baby. Another likely reason why the neonate did not develop a hemolytic condition was the short duration between antibody formation and birth.

Repeating antepartum antibody screening in Rh(D)-positive women in whom the initial screen was negative has been reported to be cost-ineffective because clinically significant alloimmunization is rare.[6] However, antibody screening only in early pregnancy is not sufficient to manage patients such as ours. The National Collaborating Centre for Women's and Children's Health recommends that all pregnant women be tested for red cell alloantibodies in early pregnancy and at 28 weeks of gestation.[7]

In conclusion, the present case suggests that the presence of irregular antibodies should be checked during the third trimester in addition to the first, given the possibility of maternal sensitization during later stages of pregnancy. This would provide the opportunity to prepare for an emergent transfusion, including an autologous blood donation, in women who have acquired rare antibodies against red cell antigens during pregnancy.

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Decrease in High Human Chorionic Gonadotropin in Twin-Twin Transfusion Syndrome following Fetoscopic Laser Surgery

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Key Words
Twin-twin transfusion syndrome · Human chorionic gonadotropin · Fetoscopic laser surgery · Monochorionic twins · Placental anastomoses

Abstract
Introduction: The purpose of this study was to investigate the concentration of maternal serum human chorionic gonadotropin (hCG) in twin-twin transfusion syndrome (TTTS) before and after fetoscopic laser surgery and to clarify the association between TTTS and hCG. ***Material and Methods:*** The concentration of maternal serum hCG was measured before fetoscopic laser surgery and 2 and 4 weeks after laser surgery in 120 patients diagnosed with TTTS. ***Results:*** The preoperative serum concentration of hCG was 6.34 multiples of the median (MoM; interquartile range 3.52–9.86). The concentration of hCG was higher in TTTS of Quintero stage III or IV (7.17 MoM, range 4.21–11.0) compared to stage I or II (3.37 MoM, range 2.35–7.74). When laser surgery for TTTS was effective, hCG gradually decreased to less than half the preoperative concentration 2 weeks after laser surgery, and the concentration was further reduced to within the normal range at 4 weeks. However, the concentration of hCG in 3 cases with TTTS recurrence did not decrease. ***Discussion:*** A close association was observed between the concentration of hCG and the condition of TTTS. A change in the concentration of hCG after laser surgery could be a useful marker to judge the effectiveness of laser surgery in TTTS.

Introduction

Twin-twin transfusion syndrome (TTTS) is a severe complication of monochorionic twin pregnancies, with high perinatal loss and significant morbidity. The pathogenesis of this syndrome remains unclear, but it is known that unbalanced placental intertwin vascular anastomoses play a key role in the pathophysiology of TTTS, wherein one twin becomes hypovolemic (the donor) and the other hypervolemic (the recipient). Laser ablation of placental anastomoses has gained widespread acceptance as the definitive treatment for severe TTTS between 16 and 26 weeks of gestation [1–3]. One randomized trial demonstrated that laser surgery is a more effective first-line treatment than serial amnioreduction for TTTS [4]. Recently, a metaanalysis of comparative studies found that compared with fetuses undergoing serial amnioreduction, fetuses undergoing laser ablation were twice as likely to survive and had an 80% reduction in neurologic morbidity [5].

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It has been reported that hCG in monochorionic twin pregnancies complicated by severe TTTS was significantly increased compared to uncomplicated twin pregnancies [6]. It has also been reported that the pathophysiology of TTTS is closely related to hCG [7, 8]. However, the changes in the concentration of hCG after laser surgery have not been studied sufficiently. Therefore, in the present study, the concentration of maternal serum hCG before and after fetoscopic laser surgery for TTTS was measured. The purpose of this study was to investigate the concentration of maternal serum hCG before and after fetoscopic laser surgery and to clarify the association between TTTS and the concentration of hCG.

Material and Methods

A total of 120 pregnant women diagnosed with TTTS who underwent fetoscopic laser surgery from March 2005 to October 2009 were studied. Inclusion criteria were that the pregnant woman was diagnosed with TTTS between 16 and 26 weeks of gestation and had undergone laser surgery at our institute. The diagnosis of TTTS was based upon ultrasonographic evidence of a single monochorionic placenta that was complicated by polyhydramnios with a maximum vertical pocket ≥8.0 cm (with a distended bladder) in the recipient twin and oligohydramnios with a maximum vertical pocket ≤2.0 cm (with a nondistended bladder) in the donor twin.

The exclusion criterion was the occurrence of fetal death after laser surgery, regardless of whether the fetal death was a single death or a double death. Further, recurrence was diagnosed when polyhydramnios/oligohydramnios reappeared after laser surgery.

The concentration of maternal serum human chorionic gonadotropin (hCG) was measured before laser surgery and 2 and 4 weeks after laser surgery. Maternal serum hCG was measured by a chemiluminescent enzyme immunoassay. Changes in the concentration of hCG in uneventful cases were compared with those in 3 recurrent cases. The concentration of hCG was shown as the multiple of the median (MoM). The median was derived from singleton pregnancies.

In our institute, laser surgery was performed for TTTS of Quintero stage [9] I–IV between 16 and 26 weeks of gestation. The laser surgery was performed as described in a previous report [10]. Prophylactic tocolytics and antibiotics were administered perioperatively. Antenatal care with weekly ultrasound surveillance was performed for 1 month at our institute and later at referral centers. All patients provided written consent to undergo fetoscopic laser surgery and to participate in this study, which was approved by the ethics committee of our institution.

Results

During this study of 120 cases, the twin survival rates at 1 month after laser surgery were as follows: 2 survivors in 97 cases (80.8%), 1 survivor in 21 cases (17.5%) and double death in 2 cases (1.7%). Among the cases with 2 survivors (n = 97), recurrence of TTTS with polyhydramnios/oligohydramnios occurred in 3 cases after laser surgery.

The median preoperative concentration of hCG was 6.34 MoM (interquartile range 3.52–9.86). There was no clear correlation between the concentration of hCG and gestational age (R = 0.039). The concentration of hCG was significantly higher in Quintero stage III or IV TTTS than in stage I or II disease (Wilcoxon test: p = 0.002; fig. 1). When laser surgery for TTTS was effective, the concentration of hCG gradually decreased to less than half the preoperative concentration 2 weeks after laser surgery (2.85 MoM, range 1.78–4.68; fig. 2). At 4 weeks after laser surgery, the concentration of hCG was further reduced to 1.67 MoM (range 1.12–2.32).

Polyhydramnios/oligohydramnios reappeared after laser surgery in 3 cases (cases 1, 2, 3; fig. 3). The concentration of hCG in these recurrent cases either remained elevated 2 weeks after laser surgery or increased to more than the preoperative concentration.

Case 2 underwent a second laser surgery (stage III TTTS before surgery). The concentration of hCG decreased and the condition improved in the 2 weeks following the second laser surgery. In contrast, in case 1, who had stage IV TTTS before surgery, polyhydramnios reappeared and an abnormal Doppler was observed after laser surgery. In this case, a cesarean section was performed 40 days after laser surgery (at 31 + 3 weeks of gestation). In case 3, who had stage I TTTS before surgery, polyhydramnios associated with maternal discomfort and an abnormal Doppler was observed after laser surgery. Because amniotic membrane detachment was seen after laser surgery, a second laser surgery was not performed. Placental examination of cases 1 and 3, but not case 2, showed residual anastomoses caused by incomplete coagulation of communicating vessels.

Discussion

In the present study, the concentration of maternal serum hCG before and after fetoscopic laser surgery for TTTS was measured. The preoperative concentration of hCG was significantly high. Two weeks after successful laser surgery, the concentration of hCG decreased to less than half the preoperative concentration, and 4 weeks after laser surgery, the concentration was further reduced. However, in recurrent cases of TTTS with polyhydramnios/oligohydramnios, hCG remained elevated or even

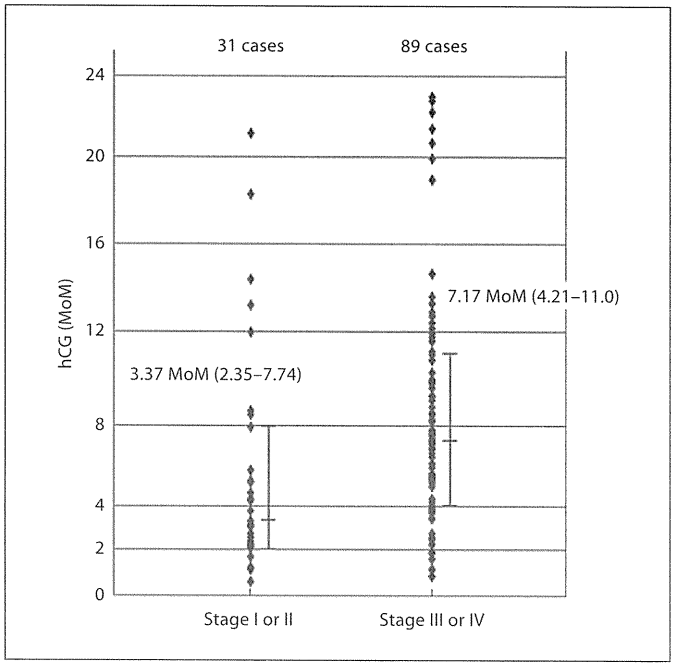

Fig. 1. Preoperative hCG concentration in TTTS according to Quintero stage (120 cases). Medians and interquartile ranges are shown. Preoperative hCG in stage III or IV TTTS (89 cases) was significantly higher than in stage I or II TTTS (31 cases) ($p = 0.0020$, Wilcoxon test).

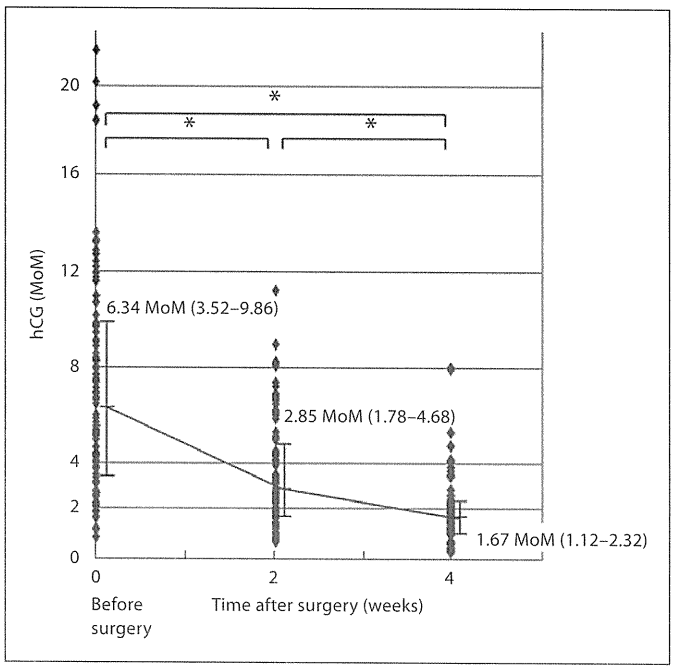

Fig. 2. Changes in hCG concentration after laser surgery (94 cases). Medians and interquartile ranges of the hCG concentrations before surgery and 2 and 4 weeks after surgery are shown. The concentration of hCG decreased to less than half the preoperative concentration 2 weeks after laser surgery, and the concentration of hCG was further reduced to within the normal range 4 weeks after laser surgery. * $p < 0.0001$ (Wilcoxon test).

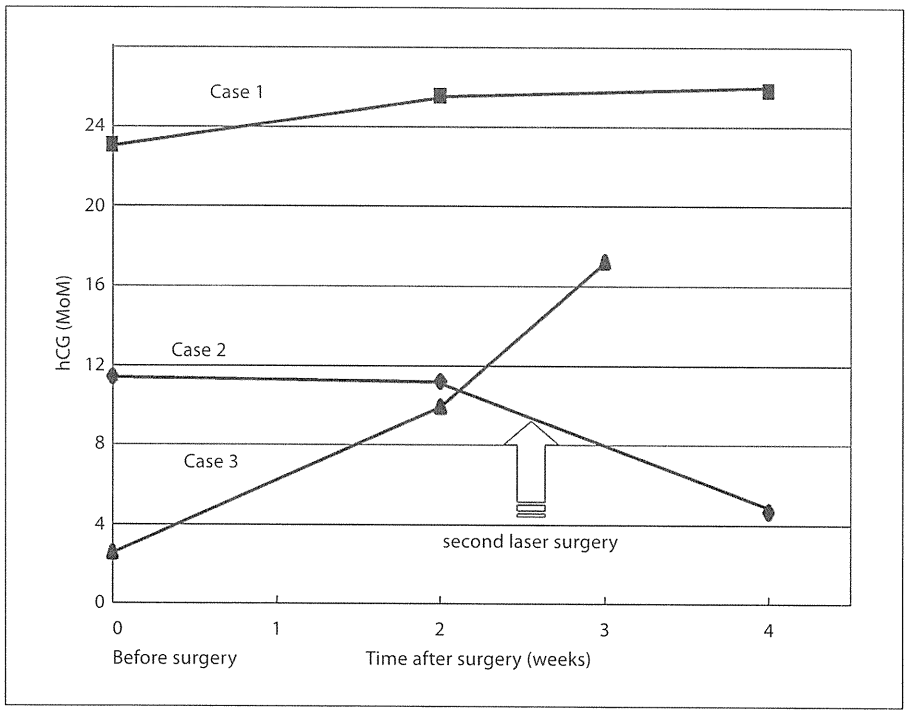

Fig. 3. Changes in hCG concentration after laser surgery in recurrent cases. TTTS recurred in 3 cases after laser surgery (cases 1, 2, 3). The concentration of hCG in recurrent cases either remained elevated 2 weeks after laser surgery or increased to more than the preoperative concentration. The hCG concentration decreased after the second laser surgery in 1 case (case 2).

increased 2 weeks after laser surgery. In the present study, a high concentration of maternal serum hCG was seen in TTTS; when laser surgery for TTTS was effective, the concentration of hCG decreased, but when the surgery was ineffective, hCG remained high. Thus, a close association was observed between the concentration of hCG and the condition of TTTS.

It is known that the concentration of hCG in pregnancy reaches a peak between 60 and 80 days after the last menses. At 10–12 weeks, plasma levels begin to decline, and a nadir is reached by about 16 weeks. Plasma levels are maintained at this lower level for the remainder of pregnancy [11]. There are many reports regarding hCG in twin gestations, and most have found that the concentration of hCG is almost 2 MoM [12, 13]. In the present study, the preoperative concentration of hCG in TTTS was 6.34 MoM (interquartile range 3.52–9.86), which is significantly higher than that of normal twin gestations. The reason why high concentrations of maternal serum hCG are seen in TTTS is not known. One hypothesis is that polyhydramnios surrounding the recipient twin may be associated with relatively impaired uteroplacental blood flow that increases the risk of hypoxemia [14]. Unbalanced placental intertwin vascular anastomoses create hypervolemia in one twin (the recipient). This can then lead to polyhydramnios, recipient heart failure and placental edema. The increased maternal serum hCG may reflect the large placental size in TTTS and a change in placental oxygen tension secondary to uteroplacental hypoperfusion [6]. Mirror syndrome refers to a condition of generalized maternal edema, often with pulmonary involvement, that mirrors the edema of the hydropic fetus and placenta [15]. TTTS is one of the diseases associated with mirror syndrome [7, 16], in which hCG is increased [17].

The present study showed that when patients diagnosed with TTTS underwent laser surgery, the concentration of hCG decreased to less than half the preoperative concentration after 2 weeks and further decreased to 1.67 MoM (interquartile range 1.12–2.32), which is considered within the normal range for a twin pregnancy, after 4 weeks. In the recurrent cases, the concentration of hCG either increased or remained unchanged 2 weeks after laser surgery. However, in the recurrent cases that underwent an effective second surgery, the concentration of hCG decreased. Thus, the change in the concentration of hCG after laser surgery could be a marker for the effectiveness of laser surgery. The concentration of hCG 2 weeks after laser surgery could be used as one criterion for determining recurrence of TTTS with polyhydramnios/oligohydramnios. In this study, we excluded the cases in which fetal death occurred after laser surgery, regardless of whether the fetal death was a single death or a double death, due to concerns about the impact on hCG levels. The dynamics of hCG in the single death cases were almost same as in the 2 uneventful surviving cases after laser surgery. The concentration of hCG decreased to less than half the preoperative level after 2 weeks and further reduced to within the normal range of a singleton pregnancy after 4 weeks (data not shown).

Our study has both strengths and limitations. Though there have been some reports that have followed the changes in the concentration of hCG after laser surgery for TTTS, the number of cases studied has been limited [7] and the changes have only been followed for 1 week [6]. The strength of this study is that a considerable number of cases were studied for 1 month after laser surgery. The limitation is that there were only 3 recurrent cases and 1 case that underwent a second surgery. Investigation of more recurrent cases and cases with second surgery should confirm our findings.

In conclusion, we propose the adoption of a control curve of maternal serum hCG after laser surgery. This curve would show the median concentration of hCG in cases with an uneventful course after laser surgery. An excessive concentration of hCG is associated with TTTS, and the concentration of hCG in cases with an uneventful course decreased to less than half the preoperative concentration 2 weeks after laser surgery and to within the normal range 4 weeks after surgery. A close association between the concentration of hCG and the condition of TTTS after laser surgery was observed. hCG could be a useful predictive parameter for the effectiveness of laser surgery in TTTS.

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RESEARCH LETTER

Low prevalence of genetic prenatal diagnosis in Japan

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KEY WORDS: prenatal diagnosis; maternal serum marker screening; amniocentesis; chorionic villus sampling

Advances in molecular genetics, cytogenetics, and ultrasound diagnosis have revealed many abnormalities of fetuses *in utero*. In addition to technical advances and economic aspects, ethical considerations influence how prenatal diagnosis is used in communities. The outcome of prenatal diagnoses may be associated with trends in babies born with congenital disorders. We surveyed the number of genetic prenatal diagnoses to examine the trends in prenatal diagnosis in Japan.

In 2002, we conducted a survey on genetic prenatal diagnoses performed in Japan during the previous 5-year period (Sago *et al.*, 2005). This report was the first to explore the trends in prenatal diagnosis in Japan. The study surveyed clinical laboratories nationwide and was assumed to cover most cases of genetic prenatal tests performed in Japan. Adding the results of a recent 6-year survey, we have now completed an 11-year survey for the period between 1998 and 2008. Based on the methods in the previous survey, we sent questionnaires to both commercial and academic clinical laboratories thought to be performing prenatal diagnostic tests and obtained answers regarding the numbers of genetic tests of maternal serum markers, amniocentesis, and chorionic villus sampling (CVS) conducted between January 2003 and December 2008. We combined the data from this new 6-year survey with that of the previous survey conducted between 1998 and 2002 and examined the trends over this 11-year period. Of the 59 responding laboratories, 25 laboratories had actually conducted some sort of prenatal genetic test. Yearly changes in the numbers of maternal serum marker screenings, amniocentesis procedures, and CVS procedures are shown in Table 1. The annual number of maternal serum marker screenings reached a maximum in 1998 and then decreased markedly until 2001, after which time it increased. The annual number of amniocentesis procedures was about 10 000 between 1998 and 2002, increasing by approximately 30% in 2008. On the other hand, the number of CVS procedures was very low.

According to a report by the Ministry of Health, Labor, and Welfare, the Japanese population in 2008 was 127.7 million and the total number of births in 2008 was 1.09 million. The total number of prenatal genetic tests was about 32 000 in 2008. Consequently, only 3% of all pregnant women received prenatal diagnoses based on maternal serum marker screening or chromosome analysis; however, some pregnant women received both maternal serum marker screening and chromosome analysis (i.e. some overlap existed). The rate of prenatal genetic diagnosis is extremely low in Japan, compared with the rates in other advanced countries where the maternal ages are also high, similar to the situation in Japan.

Japanese law permits abortions for maternal economic or health problems but not for fetal abnormalities. Actually, abortions because of fetal abnormalities are, in many cases, performed with maternal health problems given as the reason. Pregnant women may be deterred from receiving a prenatal diagnosis because abortions for fetal abnormalities are not permitted legally and many people believe that abortions are unethical even if a fetus has serious abnormalities.

Maternal serum marker screening was introduced to Japan in 1994 and soon began to be used clinically; however, insufficient genetic counseling for this test facilitated anxiety in pregnant women, creating a social problem. Therefore, in 1999, the Expert Committee on Prenatal Diagnosis of the Sciences Council for Evaluating Advanced Medical Techniques of Japan published the 'View on Prenatal Serum Marker Screening'. This view stated that physicians were not required to give information on this test to pregnant women vigorously and that physicians should not recommend this test. Thereafter, the number of maternal serum screening tests decreased for a few years and remained at about 15 000 annually. However, the number began to increase in 2003, albeit slightly, and reached 18 000 in 2008. A nuchal translucency scan at 11–13 weeks of gestation was also rarely performed, although ultrasonography is performed during the first trimester to check the fetal heartbeat and growth in almost all pregnant women in Japan. The lack of information on prenatal diagnosis provided by doctors

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Table 1—The number of prenatal genetic tests and liveborns in Japan 1998–2008

Year	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Liveborn infant[a]	1 203 147	1 177 669	1 190 560	1 170 662	1 153 855	1 123 610	1 110 835	1 062 530	1 092 674	1 089 818	1 091 150
Pregnant women of ≥35 years[b]	127 442	130 497	141 653	142 784	147 636	157 369	169 516	173 754	192 905	211 711	228 444
Maternal serum screening	21 708	18 312	15 927	15 308	15 627	16 591	16 613	16 279	17 558	17 333	18 209
Amniocentesis	10 419	10 516	10 627	10 070	9 926	11 284	11 261	11 557	11 703	12 458	13 402
Chorionic villus sampling	76	58	96	120	129	65	80	43	36	36	46

[a] The number of liveborn infants has been significantly decreasing (number of infants = 1.200.079.13312 × (year-1998), ($R^2 = 0.85$).
[b] Advanced age pregnancies has been significantly increasing (proportion of over 35 years = 0.10 + 0.01 × (year-1998), $R^2 = 0.96$).

Table 2—Prenatal screening policies and screening rates in industrialized countries

Countries	Screening policies	Total fertility rates[a] (2000/2008)	Screening rates
Australia	W	1.76/1.97	98% (2007)[b]
Denmark	W	1.77/1.89	84.4% (2006)[c]
England and Wales	W	$1.64/1.90_{(2007)}$	88% (2009)[d]
France	W	1.88/2.00	—
Germany	W	1.38/1.38	—
Italy	W	1.26/1.41	—
Switzerland	W	1.50/1.48	—
USA	W	$2.06/2.12_{(2007)}$	—
Netherlands	S	1.72/1.78	—
Spain	S	1.23/1.46	—
Japan	S	1.36/1.37	3%(2008)

W, prenatal screening offered in whole country; S, no national policy but some form of screening in some area.
[a] From United Nations Statistics Division, 2008.
[b] From Genetics Education in Medicine Consortium, 2007.
[c] From Ekelund *et al.* (2008).
[d] From National Down Syndrome Cytogenetic Register, 2009.

is thought to be one of the reasons why relatively few pregnant women receive genetic prenatal diagnosis.

A comparison of the status of prenatal diagnosis between Japan and other advanced countries is difficult because nationwide data are scarce (Boyd *et al.*, 2008). Available data of screening policies and screening rates in industrialized countries are shown in Table 2. Many industrialized countries have a national policy to offer first or second trimester screening for all pregnant women. More than half of pregnant women are assumed to undergo genetic prenatal screening tests in advanced countries (Genetic Education in Medicine Consortium, 2007; Ekelund *et al.*, 2008; National Down Syndrome Cytogenetic Registry, 2009). On the other hand, only 3% of all pregnant women receive genetic prenatal screening or diagnostic tests in Japan and most chromosomal analyses were performed using amniocentesis in the 11-year survey.

Recently, the number of births in Japan has been decreasing yearly and the number of advanced-age pregnancies has been increasing markedly (Table 1). This situation raises the concern that the number of babies born with congenital disorders related to maternal age, such as Down syndrome, may increase. Kajii reported that if the current situation continues, the number of babies born with Down syndrome would reach 3000 per year in 2011, representing a 50% increase over the previous 5-year period (Kajii, 2008).

The present study clarified the genetic prenatal diagnosis trends in Japan over the past 11 years. Although the number of prenatal tests being performed has increased slightly, the rate of prenatal testing in pregnant women remains extremely low at about 3%, and amniocentesis was the main invasive procedure used to confirm a diagnosis. We should carefully observe how these results affect society in terms of the number of

babies born with congenital disorders. The data presented herein provides valuable information for assessing the effect of medical care on society.

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RESEARCH LETTER

A fetus diagnosed with Casamassima-Morton-Nance syndrome with *de novo* del(8)(p23.1)

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KEY WORDS: Casamassima-Morton-Nance syndrome (CMN syndrome); spondylo-costal dysostosis; del(8)(p23.1); prenatal diagnosis

Casamassima-Morton-Nance (CMN) syndrome is a condition in which spondylocostal dysostosis is accompanied by anal atresia and urogenital anomalies (Casamassima *et al.*, 1981). In the initial report on CMN syndrome, published in 1981, Casamassima *et al.* described Mennonite siblings from a consanguineous marriage, which classified this syndrome as an autosomal recessive disease in OMIM (#271 520). However, the causative genes are unknown. Additionally, the first reported case did not display either a congenital heart anomaly or abnormal karyotype. Here, we report a fetal case of CMN syndrome with a severe heart anomaly as well as an abnormal karyotype. This is the first report of CMN syndrome in Japan and only the fifth worldwide.

The affected fetus was the first pregnancy of a 35-year-old Japanese woman. There was no significant maternal or paternal family history of genetic disease and the parents were not consanguineous. Fetal ascites and obvious subcutaneous edema were found by ultrasound (US) at routine check-up at 26 weeks' gestation, the mother was referred to our center for further examination. The pregnancy had been uneventful until the fetal malformations were found by US examination. The detailed fetal echography suggested double outlet right ventricle (DORV), pulmonary stenosis (PS), ventricular septal defect (VSD), right hydronephrosis, vertebral irregularity and a single umbilical artery (right).

A fetal three-dimensional computerized tomography (3D-CT) (maximum intensity projection (MIP) image) revealed multiple skeletal abnormalities, such as bifid ribs (right No.7–8) and mid-thoracic (Th5–9) vertebral body multiple segmentation anomaly, which were indicative of spondylo-costal dysostosis (Figure 1). A calcified mass was also detected in the rectum. Furthermore, magnetic resonance imaging (MRI) demonstrated profuse peritoneal fluid, with an encapsulated intestinal mass, and marked subcutaneous edema, especially around the neck. T1WI images showed the meconium descending to the lower level of the colon, but not reaching rectum, suggesting an imperforate anus. T2WI images showed a narrow thorax and hydrocolpos with a vaginal septum and, on the upper side, a continuous uterus with two cavities, compatible with uterus bicornis.

Because of oligohydramnios, fetal ascites was aspirated for cytogenetic analysis instead of amniotic fluid. At 28.6 weeks of gestation, 7 days after the mother's admission, intrauterine fetal death (IUFD) was confirmed. The baby weighed 1525 g and was 34 cm tall. According to autopsy findings, the prenatal diagnoses, including anal atresia, uterus bicornis, narrow throrax, heart anomalies (DORV, VSD and PA), enlarged kidney and a single umbilical artery, were confirmed. Additional morphological aberrations, such as a mucous-filled rectum–vagina connection, were also observed. The external examination showed microcephaly, hypertelorism, a flat nose with anteverted nares, low-set ears and a short neck. Other findings included bowel malrotation and abnormal pulmonary lobulation with the right lung composed of two lobes and the left lung composed of one lobe. Radiographic examination was concordant with prenatal CT findings, confirming spondylo-costal dysostosis. There was neither tracheoesophageal fistula with esophageal atresia nor radial dysplasia, both of which are specific for VATER association. The anatomy and histology of the other viscerae, including the brain, were normal. Placental examination showed no abnormalities. Cytogenetic evaluation (G-banding) of the fetal ascites identified the fetal expression of 46, XX,del(8)(p23.1). Parental cytogenetic examination indicated that both parents had a normal karyotype. For further examination, we tried to analyze array comparative genomic hybridization (CGH) (whole genome), but the fetal DNA derived from liver at autopsy was degenerated because of IUFD. Array CGH using whole genome amplification due to limited amount of DNA extracted from fetal ascites was

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Table 1—Reported cases of CMN syndrome

Year	1981	1981	1998	2007	2009	Present case
Sex	M	M	F	NA	F	F
Age	Neonate	Fetus	GA17w	GA14w	GA32w	GA26w
Growth	AFD	Short stature	AFD	FGR	AFD	AFD
Development	Normal	NA	NA	NA	NA	NA
Outcome	20m Healthy	IUFD	18 w Termination	14 w IUFD	7m Death	28 w IUFD
Vertebrae/rib anomaly	Scoliosis Hemivertebra	NA	Hemivertebra, supemumerary vertebra, absent vertebra and short and thin ribs	Scoliosis Hemivertebra	Hemivertebra Abnormal rib	Fused vertebra, hemivertebra and bifid rib
Urinary tract anomaly	Bilateral hydrocele	Urethral atresia, hydroureteronephrosis	Urethra invisible	Absence of kidney and bladder	Hydronephrosis	Hydronephrosis
Genital anomaly	–	Absent external genitalia	Absent external genitalia	Absent external and internal genitalia	Hydrometrocoipos	Hydrocoipos with septum and uterus bicomis
Anal atresia	+	+	+	+	+	+
Heart anomaly	–	–	–	–	PDA	DORV, VSD and PS
Hydrops	Protuberant abdomen	Protuberant abdomen	Distended abdomen	–	Ascites Pericardial fluid	Ascites and subcutaneous edema
Others	–	–	Oligohydroamnios and calcification mass in rectum	–	Intestinal volvuius	Abnormal pulmonary lobulation, bowel mairotation and calcification mass in rectum
Consanguity	+	+	–	–	–	–
Chromosome	46, XY	NA	t(6;9)	NA	46, XX	del (8)(p23.1)

NA, not assessed; AFD, appropriate for date; FGR, fetal growth restriction; DORV, double outlet right ventricle; F, female; IUFD, intrauterine fetal death; M, male; PS, pulmonary stenosis; VSD, ventricular septal defect.

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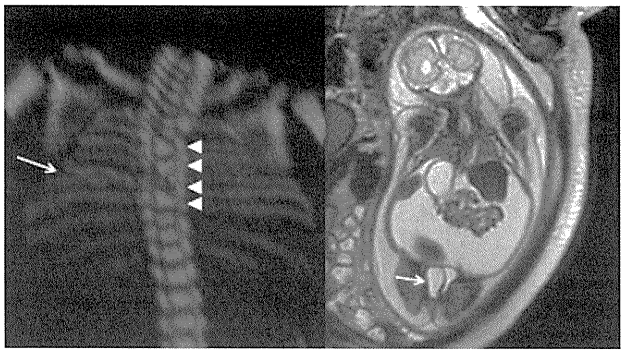

Figure 1—Fetal three-dimensional computerized tomography (3D-CT) revealed a mid-thoracic vertebral body multiple segmentation anomaly (arrow heads) and bifid ribs (arrow), indicating a diagnosis of spondylo-costal dysostosis. Fetal T2W1 magnetic resonance imaging (MRI) showed severe subcutaneous edema, small thoracic cage, and hydrocolpos with a vaginal septum (arrow)

performed but the results were insufficient to examine the detailed molecular-cytogenetic change of this case.

In the current report, we have identified a new fetal case of spondylocostal dysostosis, imperforate anus and genitourinary malformation. Collectively, these findings indicated a diagnosis of CMN syndrome. The combined fetal 3D-CT and MRI were helpful in confirming the diagnosis of this syndrome *in utero*.

Although the first case of CMN syndrome reported did not have any heart anomaly, the current case suffered from cardiac malformations, including DORV, PS and VSD. In 1995, Simpson *et al.* reported sibling cases of CMN-like syndrome, spondylo-costal dysostosis with heart anomaly (Simpson *et al.*, 1995). Considering that the current case displayed all of these findings, this case was quite rare. Among the total five cases reported (Table 1), karyotypes were analyzed in only three of them, and only one of those three cases was associated with a paternally inherited balanced translocation t(6;9)(p12;q12) (Daikha-Dahmane *et al.*, 1998). However, there is no data that demonstrates any relationship between the region of translocation of the breakpoints and deletion in this case.

In terms of the chromosomal abnormality, del(8)(p23.1) is known to be associated with a spectrum of anomalies including congenital heart malformations, congenital diaphragmatic hernia (CDH), developmental delay, and neuropsychiatric findings. And this region, 8p23.1, contains the transcriptional factor gene *GATA4* which is known to play a key role in heart development in humans (Wat *et al.*, 2009). In our case, the fetus did not have a CDH but cardiac malformations, including DORV, PS and VSD. Other fingings except congenital heart malformation, such as spondylocostal dysostosis, anal atresia, urogenital anomalies and fetal hydrops were not typical of del(8)(p23.1) but consistent with CMN syndrome.

With a certainty, it is important to consider VATER association as a differential diagnosis of current case, neither tracheoesophageal fistula with esophageal atresia nor radial dysplasia was seen in this case, and moreover VATER association was defined as sporadic, with no family history of malformation, no recognized teratogen and no chromosomal abnormality detected. Therefore, we excluded a VATER association.

Herein, we have described a fetus in which observed findings were consistent with CMN syndrome. Notably, it was complicated by congenital heart anomalies which may be related to the expression of *de novo* del(8)(p23.1). Further studies are needed to identify the candidate gene related to this syndrome.

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ORIGINAL ARTICLE

The Japanese experience with prenatally diagnosed congenital diaphragmatic hernia based on a multi-institutional review

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Abstract
Purpose To review the recent Japanese experience with prenatally diagnosed congenital diaphragmatic hernia (CDH) based on a multi-institutional survey.
Methods A multicenter, retrospective cohort study was conducted on 117 patients born between 2002 and 2007 with isolated prenatally diagnosed CDH. All patients were managed by maternal transport, planned delivery, immediate resuscitation and gentle ventilation. The primary outcome measurements were the 90-day survival and intact discharge. The examined prenatal factors included gestational age (GA) at diagnosis, lung-to-head ratio (LHR), lung-to-thorax transverse area ratio (L/T) and liver position. Physical growth and motor/speech development were evaluated at 1.5 and 3 years of age. Data were expressed as the median (range).
Results The mean GA at diagnosis was 29 (17–40) weeks. The LHR and L/T were 1.56 (0.37–4.23) and 0.11 (0.04–0.25), respectively. There were 48 patients with liver up. The mean GA at birth was 38 (28–42) weeks. The 90-day survival rate and intact discharge rate were 79 and 63%, respectively. Twelve patients had major morbidity at discharge, and 71% of these patients had physical growth or developmental retardation at 3 years of age.
Conclusion This multicenter study demonstrated that the 90-day survival rate of isolated prenatally diagnosed CDH was 79%, and that subsequent morbidity remained high.

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