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Prenatal risk stratification for isolated congenital diaphragmatic hernia: results of a Japanese multicenter study

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Abstract

Background/Purpose: The aim of this study was to establish a prenatal prognostic classification system for risk-stratified management in fetuses with isolated congenital diaphragmatic hernia (CDH).

Methods: A multi-institutional retrospective cohort study of isolated CDH, diagnosed prenatally in fetuses delivered during the 2002 to 2007 period at 5 participating institutions in Japan, was conducted. The risk stratification system was formulated based on the odds ratios of prenatal parameters for mortality at 90 days. The clinical severity in CDH infants were compared among the stratified risk groups.

Results: Patients were classified into the 3 risk groups: group A (n = 48) consisted of infants showing liver-down with contralateral lung–to–thorax transverse area ratio (L/T) ratio ≥0.08; group B of infants showing liver-down with L/T ratio <0.08 or liver-up with L/T ratio ≥0.08 (n = 35), and group C of infants showing liver-up with L/T ratio <0.08 (n = 20). The mortality at 90 days in groups A, B, and C were 0.0%, 20.0%, and 65.0%, respectively. The intact discharge rates were 95.8%, 60.0%, and 5.0%, respectively. This system also accurately reflected the clinical severity in CDH infants.

Conclusions: Our prenatal risk stratification system, which demonstrated a significant difference in postnatal status and final outcome, would allow for accurate estimation of the severity of disease in fetuses with isolated CDH, although it needs prospective validation in a different population.

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Congenital diaphragmatic hernia (CDH) remains one of the most challenging anomalies facing pediatric surgeons and neonatologists, as it has a broad spectrum of severities dependent on components of pulmonary hypertension and hypoplasia of the lungs. An accurate prenatal severity assessment is essential for standardization of prenatal and postnatal care for individual cases because severity directly affects mortality and morbidity. Prenatal prognostic classification of CDH would provide the family with more precise information about the course of treatment and allow a management protocol based on risk to be established. This may minimize excessive treatment and medical expenses for low-risk patients while maximizing effective management in high-risk patients.

Several prenatal prognostic parameters for fetal CDH have previously been proposed by other investigators [1-14]. It has been validated by multiple centers that the presence of liver herniation is among the most reliable predictors of severity and mortality in CDH [1-6]. Stomach position was also studied as a prognostic indicator along with liver herniation [8-10]. Moreover, estimation of fetal lung size or evaluation of fetal lung characteristics has reportedly been used for severity prediction [11-18]. Among these fetal lung assessments, the lung area–to–head circumference ratio (LHR) is the most commonly used as a prenatal prognostic factor [8,11,18]. However, LHR is no longer considered to be independently predictive of survival by several investigators [2,19-21] because it has been shown to increase according to gestational age [15,16,22,23]. The observed to expected LHR has been proposed to provide a constant value throughout the gestational period [22], but in that study, this value was standardized by the normal lung size value of each population corresponding to gestational age without taking individual fetal growth into consideration.

In contrast, the contralateral lung–to–thorax transverse area ratio (L/T ratio) appears to be a reliable predictive parameter in fetal CDH, as it was originally reported to be constant throughout the gestational period in normal fetuses [12], and is reportedly not strongly influenced by gestational age even in fetuses with CDH [15,16]. Although combining several reliable prognostic parameters including the L/T ratio may contribute to the establishment of a prenatal risk stratification system for fetal CDH, such approaches have not been successful to date. The aim of this study was to establish a prenatal prognostic classification system for risk-stratified management of fetuses with isolated CDH based on a combination of fetal ultrasonographic findings including liver position and L/T ratio.

1. Materials and methods

1.1. Patient selection and data collection

We conducted a multicenter retrospective review of the medical records of 117 fetuses with isolated CDH, diagnosed prenatally, born at 5 participating centers during the period between January 2002 and December 2007 [10,24]. Patients with serious associated anomalies such as major cardiac malformations and chromosomal abnormalities were excluded. Two cases with bilateral diaphragmatic hernia and 12 without L/T ratio measurements were excluded from the analysis. All 103 eligible patients were managed by maternal transport, with immediate resuscitation followed by neonatal intensive care mostly with high-frequency oscillatory ventilation. In all institutions, the blood gas parameter goals were $Paco_2 < 60$ to 70 mm Hg and preductal $Spo_2 > 90\%$, under the concept of permissive hypercapnia [25] and permissive hypoxia. All institutions had extracorporeal membrane oxygenation (ECMO) and nitric oxide inhalation (iNO) capability, which were initiated according to the clinical decisions of each institution; indication criteria were not defined prospectively. Diaphragmatic repair was performed when respiratory and circulatory functions had stabilized. As the criteria of preoperative stabilization were not defined prospectively, operability of each patient was determined according to the clinical decisions of each institution. This study was approved by the institutional review boards of all 5 participating centers.

The primary outcome measure was mortality at 90 days. Prenatal ultrasonographic findings including polyhydramnios, fetal liver position, fetal stomach position, and the L/T ratio were collected at 3 times, according to gestational age at diagnosis: the earliest determination before 30 weeks of gestation, between 30 and 35 weeks of gestation, and after 35 weeks of gestation. Polyhydramnios was regarded as positive if the maximal vertical pocket was more than 8 cm. Only those patients with obvious liver herniation (ie, whose liver occupied more than one third of the thoracic space) were regarded as liver-up. Those with slight liver herniation or with liver herniation first recognized during surgery were regarded as liver-down. Fetal stomach position was categorized as contralateral stomach herniation, defined as more than half of the stomach having herniated into the contralateral thoracic cavity (equivalent to grade 3 in our previous report [10]) or others. The L/T ratio was measured at the transverse section containing the 4-chamber view of the heart by ultrasonography [12]. Briefly, the L/T ratio was defined as the area of the contralateral lung, which was determined by tracing around the contralateral lung, divided by the area of the thorax surrounded by the inner border of the bilateral ribs, the sternum, and the vertebra [15]. The cutoff value of the L/T ratio was set at 0.08 based on our previous studies [15,16,26]. Polyhydramnios, liver-up, and contralateral stomach herniation were categorized as positive if 1 of the 3 determinations was positive. The L/T ratio value was represented by the minimal value of 3 determinations, as in our previous report [16].

Postnatal factors, including sex, gestational age at birth, birth weight, mode of delivery, hernia side, Apgar scores at 1 and 5 minutes, preductal arterial blood gas data within

24 hours after birth, use of circulatory support (ECMO, prostaglandin I_2 administration, prostaglandin E_1 administration, iNO), ductus arteriosus (DA) shunt direction within 24 hours after birth, size of diaphragmatic defect judged intraoperatively, need for patch closure, duration of respiratory support (iNO, mechanical ventilation, oxygen administration), duration of hospitalization, 90-day survival, survival to discharge, intact discharge, and survival time, were collected as secondary measures. Intact discharge was defined as being discharged from the hospital with no need for home treatments such as ventilatory support, oxygen administration, tube feeding, and/or parenteral nutrition.

1.2. Analysis of prenatal factors and formulation of the prenatal risk stratification system

Odds ratios (OR) of prenatal parameters for mortality at 90 days were compared by univariate and multivariate analyses. According to the magnitude of the OR, patients were first stratified by the most powerful factor, and then each stratified group was subsequently divided into 2 subgroups by the second most powerful factor. The risk stratification system was formulated based on the results of the mortality at 90 days in each subgroup. Patient demographics and prenatal and postnatal profiles including parameters indicating the respiratory status, circulatory status, surgical findings, and outcome were compared among the groups classified using this approach.

1.3. Statistical analysis

Univariate analyses were performed to assess the magnitude of risks associated with prenatal variables for mortality at 90 days using the χ^2 test and Fisher exact test. Multiple logistic regression analysis was also performed to estimate the ORs for prenatal variables adjusted for confounding. The stepwise selection method (P value criteria <.20) was used to select variables correlating with the mortality at 90 days. Crude ORs and adjusted ORs with 95% confidence intervals (CI) were calculated. The mean and SD were used to describe continuous variables. The median and interquartile range were used to describe ordinal scales or durations of treatment in the cases with censoring. The frequency and percentages were used to describe categorical data. One-way ANOVA with Tukey post hoc honestly significant difference test was used for comparison of continuous variables. The Kruskal-Wallis test was used for comparison of Apgar scores. The χ^2 test and Fisher exact test were used for analysis of categorical data. The log-rank test and Kaplan-Meier method were used to compare the treatment durations and survival times. P values lower than .05 were considered statistically significant. Statistical analyses were performed with JMP (version 8.02; SAS Institute, Cary, NC).

2. Results

2.1. Analysis of prenatal factors and formulation of the prenatal risk stratification system

Crude ORs for the factors significantly associated with mortality at 90 days were 18.6 for liver-up, 13.6 for L/T ratio <0.08, and 11.0 for contralateral stomach herniation. Adjusted ORs for liver-up and L/T ratio <0.08 were statistically significant, whereas that for contralateral stomach herniation did not reach statistical significance (Table 1). The mortality at 90 days of 4 subgroups, first stratified by the most powerful factor (liver herniation) and subsequently by the second most powerful factor (L/T ratio), are shown in Figure 1 (Fig. 1). The mortality at 90 days of all cases was 19.4%, whereas that of fetuses with liver-down and L/T ratio ≥0.08 was 0.0%. We thus defined the latter as group A (low-risk group). Fetuses with liver-down and L/T ratio <0.08 and those with liver-up and L/T ratio ≥0.08 were combined into group B (intermediate-risk group), since they had the same mortality at 90 days of 20.0%. The mortality at 90 days of fetuses with liver-up and L/T ratio <0.08 was 65.0%, and these constituted group C (high-risk group). The numbers of patients in groups A, B, and C were 48, 35, and 20, respectively (Fig. 1).

Table 1 Univariate and multivariate analyses for mortality at 90 days

Variable	n (%)	OR for mortality at 90 d (95% CI)	P
Univariate analysis		Crude OR	
Liver-up (ref: liver-down)	45 (43.7)	18.6 (4.04-86.3)	<.001
L/T ratio <0.08 (ref: ≥0.08)	30 (29.1)	13.6 (4.28-43.2)	<.001
Contralateral stomach herniation (ref: others)	25 (24.3)	11.0 (3.64-33.1)	<.001
Polyhydramnios (ref: no polyhydramnios)	31 (30.1)	2.27 (0.83-6.21)	.114
Multiple logistic regression analysis		Adjusted OR	
Liver-up (ref: liver-down)	45 (43.7)	9.34 (1.92-70.2)	.011
L/T ratio <0.08 (ref: ≥0.08)	30 (29.1)	8.28 (2.33-33.3)	.002
Contralateral stomach herniation (ref: others)	25 (24.3)	2.61 (0.64-10.5)	.173

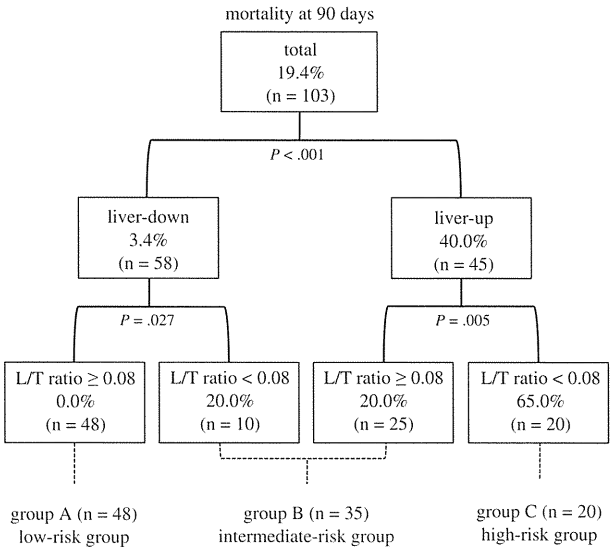

Fig. 1 Mortality at 90 days of subgroups stratified by liver position and L/T ratio. Group A includes patients with liver-down and L/T ratio ≥0.08; group B patients with liver-down and L/T ratio <0.08 or liver-up and L/T ratio ≥0.08; group C patients with liver-up and L/T ratio <0.08.

2.2. Comparison of CDH severity in each prenatal risk group

There were no significant differences in patient demographics, including sex, gestational age at diagnosis, gestational age at birth, and birth weight, among the 3 groups. The only exception was mode of delivery. Groups B and C had higher rate of cesarian sections, which were performed according to the clinical decisions of each institution, as the criteria of cesarian sections were not defined prospectively. Although the incidences of polyhydramnios did not differ significantly, not surprisingly, there were significant differences in liver-up and the L/T ratio as well as stomach herniation based on how the stratification system was formulated (Table 2). Apgar scores and preductal arterial blood gas data were significantly worse in group C. There were also significant differences in the duration of respiratory support such as iNO, artificial ventilation, and O_2 administration. Consequently, there were significant differences in the duration of hospitalization among the 3 groups (Table 3). DA shunt directions evaluated within 24 hours after birth, which suggest the severity of pulmonary hypertension, differed significantly among the 3 groups. The number of the patients who used circulatory support such as ECMO, prostaglandin I_2 administration, prostaglandin E_1 administration, and iNO were 14, 23, 35, and 86, respectively. There were significant differences in the use of such circulatory support to manage pulmonary hypertension among the 3 groups (Table 4). Although diaphragmatic repair could be performed in all group A patients, surgery was not possible in 4 group B patients (11.4%) and in 7 group C patients (35%) due to their unstable conditions. There were also significant differences in the proportions of patients with diaphragmatic defects exceeding 75%, as judged intraoperatively. Among the 13 group C patients undergoing surgery, 12 (92.3%) required patch closure, whereas only 10 (20.8%) required patch closure in group A (Table 5). There were significant differences in morbidity and mortality among the 3 groups. The rate of survival to discharge was 100.0% and the intact discharge rate was 95.8% in group A, whereas the corresponding rates were 74.3% and 60.0% in group B, and 20.0% and 5.0% in group C (Table 5). There were also statistically significant differences in survival curves among the 3 groups (Fig. 2).

3. Discussion

An accurate prenatal severity assessment for individual fetuses with CDH is essential for standardization of prenatal and postnatal treatments, since CDH has a broad spectrum of severities that directly affects the mortality and morbidity for the patients. We endeavored to establish such a risk stratification system by applying a combination of several reliable prognostic parameters previously proposed for use in fetuses with CDH [1-18]. These prognostic parameters are

Table 2 Demographics and prenatal findings of fetuses with CDH

	Group A (n = 48)	Group B (n = 35)	Group C (n = 20)	*P*
Sex (male/female)	23/25	23/12	12/8	.254
Side of hernia (right/left)	0/48	2/33	2/18	.119
Gestational age at diagnosis (wk), mean ± SD	29.6 ± 5.9	27.5 ± 5.2	28.3 ± 5.5	.231
Gestational age at birth (d), mean ± SD	266 ± 14.3	266 ± 12.4	266 ± 10.2	.995
Birth weight (kg), mean ± SD	2.82 ± 0.50	2.74 ± 0.51	2.61 ± 0.59	.302
Caesarian section (%)	23.8	60.0	75.0	.001
Polyhydramnios (%)	20.8	40.0	35.0	.148
Liver-up (%)	0.0	71.4	100.0	<.001
Stomach herniation (%)	4.2	25.7	70.0	<.001
L/T ratio, mean ± SD	0.121 ± 0.032*	0.099 ± 0.028†	0. 058 ± 0.016‡	<.001

* $P < .05$, A versus B; † $P < .05$ B, versus C; ‡ $P < .05$, C versus A.

Table 3 Respiratory status and respiratory support in CDH patients

	Group A (n = 48)	Group B (n = 35)	Group C (n = 20)	*P*
Apgar 1 min, median (interquartile range)	5 (3-7) (n = 48)	3.5 (2-6) (n = 34)	3 (2-4) (n = 19)	.002
Apgar 5 min, median (interquartile range)	6 (4.25-7.75) (n = 48)	5 (3.5-8) (n = 33)	3.5 (2-5.25) (n = 18)	.006
Highest pre-Pao_2 (mm Hg), mean ± SD**	284 ± 122* (n = 44)	211 ± 132 (n = 25)	129 ± 117‡ (n = 18)	<.001
Lowest pre-$Paco_2$ (mm Hg), mean ± SD**	33.4 ± 11.2 (n = 42)	36.8 ± 16.3† (n = 29)	49.8 ± 26.0‡ (n = 19)	.002
Duration of iNO (d), median (interquartile range)	8 (5-12)	11 (7-19)†	34 (22-40)‡	<.001
Duration of ventilation (d), median (interquartile range)	14 (9-28)*	30 (21-48)†	545 (30-747)‡	<.001
Duration of O_2 administration (d), median (interquartile range)	23 (15-38)*	43 (37-73)†	555 (529-748)‡	<.001
Duration of hospitalization (d), median (interquartile range)	48 (39-69)*	73 (56-108)	162 (95-545)‡	<.001

* $P < .05$, A versus B; †$P < .05$, B versus C; ‡$P < .05$, C versus A.

** Highest pre-Pao_2 and lowest pre-$Paco_2$ were measured within 24 hours after birth.

divided broadly into 2 categories. One is the indirect factor of pulmonary hypoplasia, which is an estimation of how much viscera (ie, liver herniation and stomach position) compresses the fetal lungs. The other is the direct parameter of fetal lung development itself. The magnitudes of ORs in univariate and multivariate analyses for mortality at 90 days were compared, and 2 powerful prenatal factors were eventually identified in each category.

The fetal liver position was the most powerful prognostic factor judging from OR magnitude. It has already been reported by many investigators that fetal liver herniation is the most reliable predictor of the severity and mortality in fetuses with CDH [1-6]. Although stomach position was also a good prognostic indicator, along with liver herniation [8-10], we selected fetal liver herniation based on the OR being larger than that for stomach herniation. Furthermore, these 2 predictors seemed to be mutually confounding factors. There is marked variation in the definition of liver herniation, and the "liver-up" concept differs markedly among authors [5,7]. In the present study, we defined liver-up as liver herniation in which the liver occupied more than one third of the thoracic cavity [10] based on previous studies [4,6], because there is a possibility of overestimating the affect of lung compression if situations such as slight liver herniation and liver herniation first recognized during surgery are included among the "liver-up" cases. Liver herniation occupying one third of the thoracic cavity turns out to be a good cutoff value for prediction, as liver-up defined by this means demonstrated a satisfactory OR.

The L/T ratio was the second most powerful prognostic parameter in our analysis. Many factors, such as lung area [11,12,16,18], volume [4,13,14], and signal intensity on magnetic resonance imaging [17], as well as pulmonary artery blood flow [27], have previously been proposed as means of estimating fetal lung development. Among these parameters, the simplest approach is to determine the fetal lung area using 2-dimensional ultrasonography, as is now widely done in multiple centers. LHR [8,11,18] has long been the most commonly used parameter for evaluating the fetal lung area. However, LHR was shown to increase according to gestational age [15,16,22,23]. Therefore, LHR is no longer considered to be independently predictive of survival by several investigators [2,19-21]. In contrast, we have previously demonstrated that the L/T ratio, which is not markedly influenced by gestational age even in fetuses with CDH [15,26]. We studied in detail to compare the reliability of L/T ratio and LHR in the same database and found that the L/T ratio is more reliable than the LHR [16]. Moreover, a manual tracing of the lung borders, which is conducted to obtain the L/T ratio, is reportedly a more reproducible measurement than the multiplication of the lung diameters, which is used for LHR determination [23,28]. The observed to expected LHR was proposed to provide a constant value throughout the gestational period, and it showed excellent receiver operating characteristic curve performance [22]. However, determining the observed to expected LHR requires the expected LHR in normal fetuses to be used for standardization of each relevant patient population, and the

Table 4 Circulatory status and circulatory support in CDH patients

	Group A (n = 48)	Group B (n = 35)	Group C (n = 20)	*P*
Left to right dominant shunt at DA (%)*	39.1 (n = 46)	36.4 (n = 33)	0.0 (n = 18)	.007
Right to left dominant shunt at DA (%)*	37.0 (n = 46)	51.5 (n = 33)	72.2 (n = 18)	.036
Use of ECMO (%)	2.1	14.3	40.0	<.001
Use of prostaglandin I_2 (%)	8.3	28.6	45.0	.002
Use of prostaglandin E_1 (%)	14.6	40.0	70.0	<.001
Use of iNO (%)	70.8	94.3	95.0	.005

* Shunt direction at the DA was evaluated within 24 hours after birth.

Table 5 Operative findings, morbidity, and mortality

	Group A (n = 48)	Group B (n = 35)	Group C (n = 20)	*P*
Inoperable cases (%)	0.0	11.4	35.0	<.001
Diaphragmatic defects exceeding 75% (%)	17.8 (n = 45)*	81.5 (n = 27)*	100.0 (n = 11)*	<.001
Need for patch closure (%)	20.8 (n = 48)	71.0 (n = 31)	92.3 (n = 13)	<.001
90-d Survival (%)	100.0	80.0	35.0	<.001
Survival to discharge (%)	100.0	74.3	20.0	<.001
Intact discharge (%)	95.8	60.0	5.0	<.001

* The size of diaphragmatic defect was not determined in several cases due to lack of intraoperative information.

availability is relatively low for some populations. Furthermore, there appears to be a problem in that individual fetal growth variation is not considered when determining the observed to expected LHR, relying instead on a standardized mean value from fetuses showing normal growth [22].

In the present study, ECMO and iNO were performed in 14 (13.6%) and 86 (83.5%) patients, respectively. As compared with previous reports [3,13,29-31], our results demonstrated less frequent use of ECMO and high use of iNO. In recent years, there has been an obvious trend in institutions in Japan to use ECMO less frequently with an associate increase in the use of iNO and prostaglandin E_1 administration for pulmonary hypertension [32-34], as was described by the CDH study group [35]. We usually use iNO without hesitation, if there is only a slight difference between the preductal and postductal oxygen saturation or blood gas data to reduce pulmonary artery resistance, with the ultimate goal of preventing right ventricular failure by removing the afterload on the right ventricle [32,33]. At present, in the 5 participating institutions, ECMO is being applied only for the most severe respiratory insufficiency cases. However, some infants with extremely severe pulmonary hypoplasia have been considered not to be indicated even for ECMO. This may account for the high mortality rate (64.3%) of our ECMO cases and the high inoperable rate (9.0%) of our non-ECMO cases.

One major limitation of this study is that the liver positions and L/T ratios were determined by various investigators at each participating institution, rather than by a small number of sonologists or other experienced judges, as would have been ideal to assure consistency. There may be some variation in the accuracy of the measurements in the present study and a prospective study in which the parameters determined by limited number of investigators may be needed to verify the accuracy of this risk stratification system. The other limitation of this study is that the risk group could not be confirmed until the end of gestation. In the present study, the presence of liver-up and an L/T ratio <0.08 were judged based on the worst value among the 3 representative measurements conducted before 30 weeks of gestation, between 30 and 35 weeks of gestation, and after 35 weeks of gestation. Because this process was applied for all of our determinations, the cases finally classified into the high-risk group were not always graded as being in this group from the beginning. Therefore, this system may not be useful for determining the need for maternal transport to a center offering fetal intervention [36]. Severity must be evaluated as early as possible, ideally before 28 weeks of gestation, to optimize the effects of early fetal intervention.

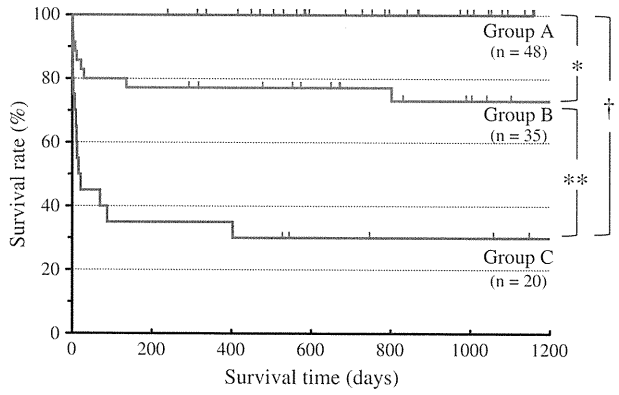

Fig. 2 Survival curves for patients with isolated CDH, compared using the prenatal risk stratification system. * $P < .001$; † $P < .001$; ‡ $P < .001$.

We have previously endeavored to devise a simple classification system based only on indirect factors reflecting how much the viscera compress the fetal lungs (ie, liver and stomach position) by applying a uniform multicenter survey [10]. This simple classification system has a clear advantage for screening candidates for fetal intervention, since the risk group can be determined by the findings of the earliest fetal evaluation. Compared with the previous simple classification system, the new risk stratification system was found to be more reliable in terms of accuracy and the ability to clearly separate the mortality and morbidity of the CDH patients, which suggests that it has an advantage for risk-stratified management after birth.

The incidence of right-sided CDH was relatively low in our cohort [24] compared with the previous reports [35,37]. The reason for this shift was unclear. However, the incidence did not seem to be markedly influenced by selective termination of pregnancy for fetuses with right-sided CDH, as only 13 (12.6%) cases had been diagnosed before 22 weeks of gestation, when the termination of pregnancy is legally accepted in our country [38]. We sought to treat right-sided CDH together with left-sided CDH in this study. Even though the incidence of liver-up and the original contralateral

lung area may differ between right- and left-sided CDH due to anatomical reasons, we considered it to be more practical to apply the same stratification to both sides in fetuses with CDH. All 4 cases with right-sided CDH were classified into the intermediate- or high-risk group according to their L/T ratios. Consequently, the results are therefore considered to be consistent with those of previous studies [8,39].

Our prenatal risk stratification system, which demonstrated a significant difference in postnatal status and final outcome, may allow for accurate estimation of the severity of disease in fetuses with isolated CDH such that management protocols could be established according to risk. This would minimize excessive treatment and medical expenses for low-risk patients and maximize effective management in high-risk patients. As the present study was a retrospective analysis, a prospective study in a different population will therefore be needed to verify the accuracy and the universal applicability of this risk stratification system.

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Efficacy of Double Vaccination With the 2009 Pandemic Influenza A (H1N1) Vaccine During Pregnancy

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OBJECTIVE: To evaluate the efficacy of double vaccination with the 2009 pandemic influenza A (H1N1) vaccine during pregnancy.

METHODS: A study of the 2009 H1N1 vaccine was conducted in 128 pregnant women, who were between 8 and 32 weeks of gestation in October 2009, to monitor the immune response to vaccination and the change in antibody positivity rate and to assess the immune response. Furthermore, the study aimed to assess the changes in these parameters after the first and second vaccination, monitor the maintenance of antibody titers in maternal blood, assess antibody transfer to umbilical cord blood, and evaluate the vaccine.

RESULTS: The antibody positivity rate increased from 7.2% before vaccination to 89.5% after the second vaccination. The vaccine was efficacious, producing a sufficient immune response in 90% of patients, regardless of the stage of gestation. The antibody titers were maintained until delivery, and were higher in umbilical cord blood at delivery than in maternal blood. Although the second vaccination increased the antibody titers in 27% of patients, and the antibody titers in maternal and umbilical cord blood at delivery tended to be higher in the double vaccination group than in the single, the differences were not statistically significant.

CONCLUSION: Single vaccination induces sufficient immune response and transfer of immunity to the fetus in pregnant women with no pre-existing antibodies.

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LEVEL OF EVIDENCE: III

Pregnant women are more vulnerable to infection than those who are not because the immune system is altered to tolerate the fetus, which is inherently antigenic. Moreover, early gestation is associated with an impaired maternal physical condition owing to nausea and vomiting, whereas middle and late gestation may be associated with impaired cardiopulmonary function owing to cardiac stress resulting from a decrease in lung capacity caused by the enlarging gravid uterus and increases of circulating plasma volume.[1] According to epidemiologic surveys of several previous influenza virus epidemics,[2–6] pregnant women are classified as at high risk[7–14]; influenza vaccination in all stages of gestation has been recommended by the American College of Obstetricians and Gynecologists and the Centers for Disease Control and Prevention since 2004. For the 2009 pandemic influenza A (H1N1) influenza strain, the percentage of patients hospitalized because of infection was more than four times higher in pregnant women than in those who were not, and pregnant women accounted for 13% of all the pandemic-related mortalities in the United States.[15] Despite the high risk of influenza-related complications reported in infants, vaccination is not indicated for those younger than 6 months, emphasizing the importance of vaccination of pregnant women. Accordingly, there are several reports on efficacy of vaccination from epidemi-

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ologic surveys of risk of infection or hospitalization or both in vaccinated pregnant women and their newborns.[16-23]

No immunologic studies have yet been conducted to support the epidemiologic surveys of the 2009 H1N1 vaccine and we expected that double vaccination might be better than single in women with no pre-existing antibodies during pregnancy. Because both maintenance of maternal antibody titers until delivery and antibody transfer to the fetus during pregnancy are important, the present study was designed to evaluate the efficacy of 2009 H1N1 vaccination during pregnancy by assessing the immunogenicity of the vaccine based on vaccination frequency-related change of antibody titers, maintenance of antibody titers during pregnancy, and antibody transfer to the fetus.

MATERIALS AND METHODS

This study was approved by the local ethics committee at the National Center for Child Health and Development and was conducted after informed consent was obtained from all study participants. A total of 128 pregnant women who were between 8 and 32 weeks of gestation in October 2009 were enrolled in this study. An additional 82 pregnant women who received a single vaccination during the same period were included in the study for comparison (Table 1). Women with complications involving immunologic abnormalities were excluded. Some of our procedures have been described previously.[24]

Before vaccination, the hemagglutination inhibition (HI) antibody titer against the HI antigen in the 2009 H1N1 virus was measured to determine the levels of pre-existing antibodies in maternal blood. In addition, white blood cell count, lymphocyte count, CD4, CD4/CD8, Th1/Th2 ratio, and natural killer (NK) cell activity were measured to investigate the maternal immune status. The 2009 H1N1 vaccine was subcutaneously injected into the upper arm twice at an interval of 3 weeks; antibody titers in maternal blood were measured 3 weeks after each vaccination and at the time of delivery. Antibody titers in umbilical cord blood were also measured at the time of delivery (Fig. 1).

Table 1. Characteristics of Patients

	Single (n=81)	Double (n=124)	*P*
Age (y)	35.7±3.6	34.8±4.1	.090
Gravidity	1.0±1.2	2.1±1.5	<.01
Parity	0.4±0.6	0.8±0.9	<.01
Interval between vaccination and birth (wk)	13.0±6.1	13.3±6.6	.844
Weeks of gestation at birth	38.6±1.9	38.5±1.7	.798

Data are mean±standard deviation unless otherwise specified.

The antibody titers in maternal blood and umbilical cord blood at time of delivery were measured according to the time from the first vaccination to delivery to monitor the immune response to vaccination and the resulting change in the antibody positivity rate, and to assess the immune response by Th1/Th2 ratio and stage of gestation, as well as assess changes of these parameters after the first and second vaccinations, to monitor the maintenance of antibody titers in maternal blood from the first and second vaccination to delivery, and to assess the differences in maternal–fetal antibody transfer.

The 2009 H1N1 vaccination during pregnancy was evaluated in mothers and fetuses by investigating the incidence of abortion or preterm delivery and the gestational age at delivery in mothers other than those with vaccination-related adverse reactions or multiple births, as well as the incidence of malformation, Apgar score, and birth weight in the newborns.

Specific staining of lymphocytes was performed by incubating whole blood with anti-CD4–PC5 or anti-CD8–PC5-conjugated monoclonal antibodies (Beckman Coulter). Red blood cells (RBCs) were removed by lysis (FACS Lysing solution; Becton Dickinson, BD Biosciences) and lymphocytes analyzed by flow cytometry (FACSCalibur; Becton Dickinson). After surface staining the activated whole blood samples with anti-CD4–PC5-conjugated monoclonal antibodies, RBCs were lysed and subsequently specific intracellular staining using FastImmune interferon γ fluorescein isothiocyanate/interleukin-4 phycoerythrin (Becton Dickinson) was performed according to the manufacturer's instructions. The stained cells were analyzed by flow cytometry; $CD4^+$ T lymphocytes that stained positive for interferon γ or interleukin-4 were used to assess numbers of Th1 and Th2 cells, respectively.

Serum samples from maternal or umbilical cord blood were treated with a receptor-destroying enzyme from *Vibrio cholerae* at 37°C for 18 hours to remove nonspecific inhibitors. After heat inactivation at 56°C for 30 minutes, the samples were diluted to ten times their volume with physiologic saline. To adsorb nonspecific agglutinins, receptor-destroying enzyme–treated serum samples were then incubated with 2.5% *volume/volume* chicken RBCs at 4°C for 1 hour. The serum samples used in subsequent hemagglutination inhibition tests were separated by centrifugation at 300×*g* for 5 minutes.

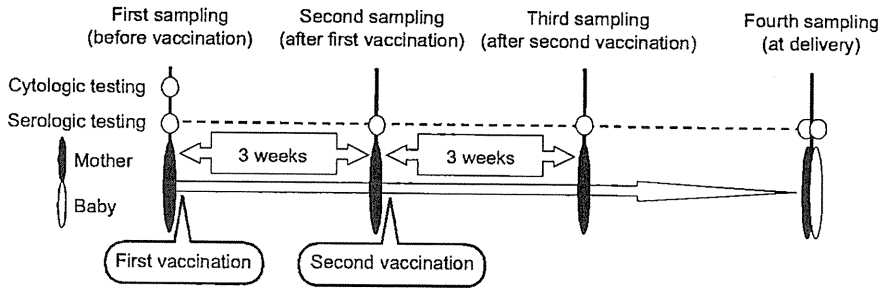

Fig. 1. Vaccination and protocol for blood sampling.
Horiya. H1N1 Vaccination During Pregnancy. Obstet Gynecol 2011.

The pretreated serum samples were serially double diluted in phosphate buffered saline using U-shaped 96-well microtiter plates and incubated with an equal volume of 4 U of various virus hemagglutinin antigens at room temperature for 10 minutes. An aliquot of 0.5% *volume/volume* chicken RBC suspension in phosphate buffered saline was added to each well and incubated at room temperature for 1 hour. The titers of the specific antibodies for the various strains were read by inverting the plates to produce a streak.[25,26]

The 2009 H1N1 vaccine (influenza hemagglutinin vaccine; Kitasato Institute Research Center for Biologicals) is a split vaccine made of the hemagglutinin fraction of viral proteins from concentrated and inactivated A/California/7/2009 virus, and contains neither an adjuvant nor preservative (thimerosal or 2-phenoxyethanol).

Values are expressed as mean±standard deviation. Differences between groups were assessed by χ^2 test. Other data were analyzed by analysis of variance, with a post hoc test (Dunnett's test) when the F value was significant. Statistical significance was concluded with a 2-tailed $P<.05$. Analyses were performed using StatMate software.

RESULTS

The maternal mean age, white blood cell count, lymphocyte count, CD4, and CD4/CD8 ratio were not significantly different among the stages of gestation. On the other hand, Th1/Th2 ratio and NK cell activity tended to decrease as gestation progressed (Table 2). The change in Th1/Th2 ratio during pregnancy was consistent with the tendency that we reported previously.[24] Natural killer cell activity was measured because, on the assumption that NK cells are normally inhibited during pregnancy because the fetus is immunologically foreign to the mother, elevated NK cell activity might result in abortion or preterm delivery.[27]

HI antibody titers of 1:40 or higher usually are regarded as protective and are an objective for successful vaccination.[28] We applied the following criteria for classification of the immune responses. Participants with a prevaccination HI antibody titer of less than 1:10 and a postvaccination HI antibody titer of 1:40 or higher or a prevaccination HI antibody titer of 1:10 or higher and a fourfold or more rise in postvaccination HI antibody titer were classified as responsive. Those with a twofold or less increase or with no increase in postvaccination HI antibody titer were classified as poorly responsive and nonresponsive, respectively.

For the 2009 H1N1 virus, the antibody positivity rate increased from as low as 7.2% before vaccination to 89.5% after the second vaccination Whereas the first vaccination produced a good immune response in the presence of low titers of pre-existing antibodies

Table 2. Maternal Condition at the Time of Vaccination

	Trimester			
	First (n=17)	Second (n=79)	Third (n=29)	*P*
Age (y)	34.2±4.0	34.9±4.0	35.2±4.4	.699
WBC (/mL)	8,312±2,413	8,655±1,791	8,724±2,200	.174
Lymphocytes (%)	20.5±7.7	19.1±6.1	18.1±5.7	.458
CD4 (/mL)	45.6±6.5	44.7±7.5	44.6±7.0	.880
CD4/CD8 (ratio)	1.5±0.5	1.4±0.5	1.4±0.4	.802
Th1/Th2 (ratio)	15.5±12.5	14.4±8.2	10.0±4.0	<.01
NK cell activity (%)	37.6±13.1	32.4±12.0	30.6±9.5	.141

WBC, white blood cells; NK, natural killer.
Data are mean±standard deviation unless otherwise specified.

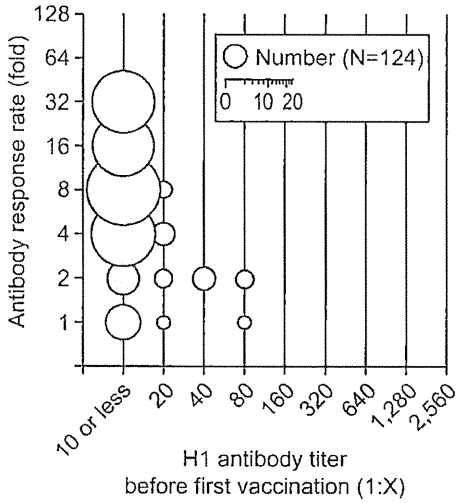

Fig. 2. Immune response after first vaccination. The fold-change in antibody titer is shown relative to the antibody titer before the first vaccination. Immune response to first vaccination was good because many participants had low titers of pre-existing antibodies.

Horiya. H1N1 Vaccination During Pregnancy. Obstet Gynecol 2011.

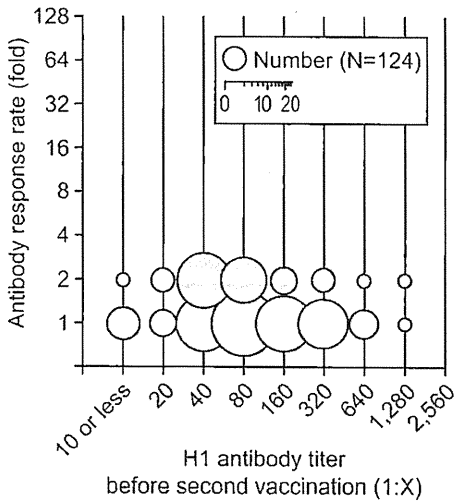

Fig. 3. Immune response after second vaccination. The fold-change in antibody titer is shown relative to the antibody titer before the second vaccination. The second vaccination was associated with a reduced postvaccination increase in antibody titers because many participants had high antibody titers before vaccination.

Horiya. H1N1 Vaccination During Pregnancy. Obstet Gynecol 2011.

(Fig. 2), the second vaccination was associated with a reduced postvaccination increase in antibody titers in participants with higher prevaccination antibody titers because of the presence of pre-existing antibodies (Fig. 3). Participants with an antibody titer of less than 1:4 after the first vaccination tended to demonstrate no increase after the second vaccination.

The vaccine produced a sufficient immune response in approximately 90% of participants regardless of Th1/Th2 ratio and stage of gestation (Table 3). No significant difference of responsive rate was observed among participants in each stratum of Th1/Th2 ratio (from 9.9 or less to 20 or more) both in the single-vaccination group (P=.352) and in those receiving double vaccination (P=.360) and among the participants in the first, second, and third trimester both in the single-vaccination group (P=.602) and in those receiving double vaccination (P=.685).

Participants with a poor response to the first vaccination had a poor response to the second vaccination as well, resulting in no significant increase in percentage of responsive participants after the second vaccination. However, 27% of participants had higher antibody titers after the second vaccination than after the first vaccination (Fig. 3).

The antibody titers tended to decrease over time after vaccination but were appropriately maintained until delivery, with a geometric mean antibody titer of 20 or more even 21 or more weeks after vaccination (Fig. 4). Antibody titers in umbilical cord blood at time of delivery showed a similar pattern to maternal antibody titers with regard to time from vaccination to delivery, and were higher than those in maternal serum. At the time of delivery, maternal antibody titers tended to be higher in the double vaccination group than in the single vaccination group regardless of gestational stage; those in umbilical cord blood tended to be higher in participants who received double vaccination within 10 weeks of delivery than in those who received a single vaccination during the same period (Fig. 5). However, statistical analysis revealed no significant difference in the antibody titers in maternal blood or umbilical cord blood between the two vaccination groups.

Redness at the vaccination site was the most common maternal adverse reaction after vaccination, followed by local symptoms such as pain and induration and systemic symptoms such as headache, malaise, fever, and nausea. However, no serious symptom requiring medical intervention was reported. These adverse reactions were not augmented or attenuated by the second vaccination, with no significant difference between the first and second vaccination detected (Fig. 6).

In the assessment of fetal adverse effects, there were no abortions, indicating that vaccination even at an early stage of gestation was unlikely to induce abortion. The incidence of preterm delivery was 7% in participants vaccinated in the first or third trimester

Table 3. Status of Immunity After Vaccination

	No. of Participants	Vaccination						
		Single			Double			
		R (n)	PR (n)	NR (n)	R (n)	PR (n)	NR (n)	*P*
All	106	87.7 (93)	5.7 (6)	6.6 (7)	88.7 (94)	4.7 (5)	6.6 (7)	.955
Th1/Th2 ratio								
9.9 or less	47	89.4 (42)	6.4 (3)	4.3 (2)	91.5 (43)	4.3 (2)	4.3 (2)	
10.0–14.9	22	91.0 (20)	0 (0)	9.1 (2)	91.0 (20)	0 (0)	9.1 (2)	
15.0–19.9	22	81.8 (18)	4.6 (1)	13.6 (3)	81.8 (18)	4.6 (1)	13.6 (3)	
20.0 or more	15	86.7 (13)	13.3 (2)	0 (0)	86.7 (13)	13.3 (2)	0 (0)	
Trimester								
First	15	86.7 (13)	6.7 (1)	6.7 (1)	86.7 (13)	6.7 (1)	6.7 (1)	
Second	69	85.5 (59)	7.2 (5)	7.2 (5)	87.0 (60)	5.8 (4)	7.2 (5)	
Third	22	95.5 (21)	0 (0)	4.5 (1)	95.5 (21)	0 (0)	4.5 (1)	

R, responsive; PR, poorly responsive; NR, nonresponsive.

and 2% in those vaccinated in the second trimester, with no significant difference detected. In addition, the incidence of preterm delivery in participants vaccinated at any time throughout gestation was similar to that from general statistics,[29] indicating that preterm delivery was unlikely to have been related to vaccination. In participants other than those with preterm delivery, the number of weeks of gestation at the time of delivery, Apgar score, and birth weight were within the standard ranges, showing no difference according to the gestational stage of vaccination (Table 4). The reasons for preterm delivery included maternal indication due to collagen disorder and spontaneous delivery, indicating no association with vaccination. Birth weight was similar to the standard body weight calculated based on the gestational age and sex and was not affected by vaccination. Five cases of malformation (three vaccinated in the second trimester and two in the third trimester) were observed, including one case each of ventricular septal defect, ear appendage, ankyloglossia, encephalocele and aplasia cutis congenita, and inguinal hernia. The incidence was not significantly different among

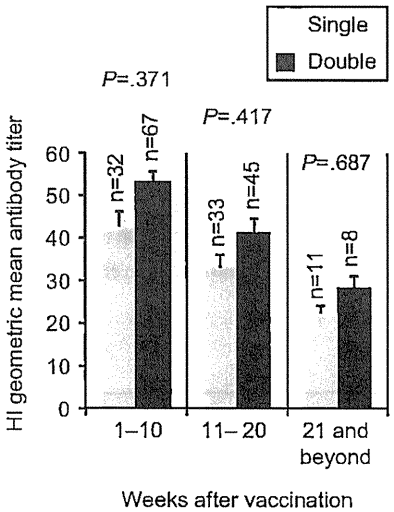

Fig. 4. Geometric mean antibody titer in maternal blood at time of delivery. The geometric mean antibody titer decreased as the time from vaccination to delivery increased, but high antibody titers of 20 or more were maintained until delivery. High antibody titers tended to be maintained more efficaciously after double vaccination than after single vaccination.

Horiya. H1N1 Vaccination During Pregnancy. Obstet Gynecol 2011.

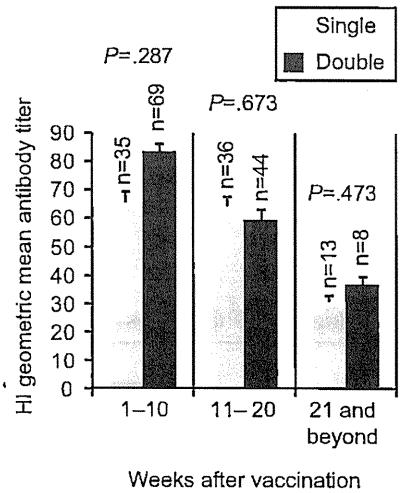

Fig. 5. Geometric mean antibody titer in umbilical cord blood. The geometric mean antibody titer in umbilical cord blood was higher than in maternal blood. Antibody titers tended to be higher in participants who received double vaccination within 10 weeks of delivery than in those who received a single vaccination during the same period.

Horiya. H1N1 Vaccination During Pregnancy. Obstet Gynecol 2011.

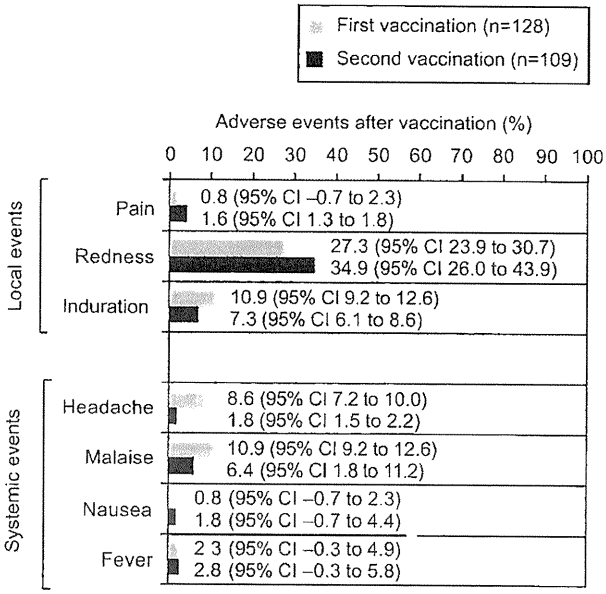

Fig. 6. Adverse reactions. The most common adverse reaction was redness at the vaccination site; the incidence of other individual adverse reactions was less than 10%. No severe adverse reaction requiring medical intervention was reported. There was no significant difference between the first vaccination and the second vaccination in terms of adverse events. CI, confidence interval.

Horiya. H1N1 Vaccination During Pregnancy. Obstet Gynecol 2011.

the gestational stages of vaccination (P=.488), and the number of occurrences was proportional to the number of participants vaccinated during each stage of gestation. The total incidence was 4.2%, which is within the range of spontaneous incidence.[29,30] In addition, because there have been no reports of specific malformation(s) related to seasonal influenza vaccines, it is unlikely that any of the cases of malformation was related to vaccination.

DISCUSSION

Because pregnant women have a high risk of being infected with and having morbidity from influenza, vaccination with an inactivated influenza vaccine during pregnancy is recommended. Although the efficacy of vaccination has been extensively evaluated, it is difficult clinically to assess the acquisition of immunity because of the presence of pre-existing antibodies, or in epidemiologic surveys because of differences between the vaccine strains and the post-vaccination epidemic viral strain. Vaccination against the 2009 H1N1 virus had the following characteristics: those vaccinated had no pre-existing antibodies because it was a new type of virus; because the viral strain that might cause a pandemic was predicted, the vaccine strain that could intercept infection was predicted accurately and the vaccine was manufactured before the epidemic; only one viral strain was used in the vaccine.

In the present study, the prevaccination antibody positivity rate (pre-existing antibodies) was as low as 7.2% because vaccination was initiated before the epidemic. After the second vaccination, the antibody positivity rate increased to 89.5%, which was consistent with approximately 90% in our previous study of seasonal influenza vaccination in pregnant women.[24] In addition, the vaccine produced a sufficient immune response regardless of the alterations in immunity as classified by Th1/Th2 ratio or stage of gestation. Vaccination was immunologically useful in all stages of gestation.

As for the vaccination frequency, 87.9% of participants (95% confidence interval 82.2–93.6) developed antibodies after the first vaccination, with a seroconversion rate of 81.5% (95% confidence interval 74.7–88.3) and change in antibody titers of 7.6-fold (data not shown). These numbers meet the

Table 4. Vaccination Timing and Outcomes

	Trimester			
	First (n=15)	Second (n=74)	Third (n=29)	All (n=118)
Maternal				
Abortion	0	—	—	0
Preterm	6.7	1.4	6.9	4.2
Gestational age (wk)	38.5±1.6	38.7±1.6	38.8±1.5	38.7±1.6
Neonatal				
Malformation (case)	0 (0%)	3 (4.1%)	2 (6.9%)	5 (4.2%)
Apgar score less than 7 at 5 min (case)	0 (0%)	1 (1.4%)	0 (0%)	1 (0.8%)
Birth weight (g)	3,025.1±407.5	3,034.0±453.2	3,026.3±394.8	3,035.3±430.6
Actual weight/median weight (%)	103.3±16.2	98.7±12.0	98.3±12.3	99.2±12.7

Data are % or mean±standard deviation.

criteria (more than 70%, more than 40%, and more than 2.5, respectively) specified by the European Medicines Evaluation Agency. Single vaccination is thus considered sufficiently potent, whereas subsequent maintenance of adequate antibody titers until delivery and antibody transfer to the newborn are also important.

In this study, second vaccination did not significantly increase the percentage of responsive participants, although it did increase antibody titers in 27% of participants. When the participants were classified by the time from vaccination to delivery, antibody titers in maternal blood at the time of delivery tended to be higher in those who received double vaccination. Among participants who received vaccination within 10 weeks of delivery, transfer to umbilical cord blood was higher in those who received double vaccination than in those who received single vaccination. However, differences were not statistically significant, suggesting that single vaccination is useful even in the absence of pre-existing antibodies and when considering maintenance of antibody titers and antibody transfer to the fetus. The optimal vaccination frequency may have to be determined based on factors such as specific epidemiologic data, the cost, labor, and adherence.

The overall incidence of adverse reactions was low, less than 10% for all adverse reactions except for redness, because the vaccine is a split vaccine containing no adjuvant. In participants who received double 2009 H1N1 vaccination during pregnancy, adverse reactions were not markedly augmented or attenuated by the second vaccination. Moreover, early delivery or abortion, malformation, and birth weight were not significantly affected. Nonetheless, the sample size was insufficient to fully evaluate the safety of the vaccine; additional information from larger studies is needed to determine this.

Historically, there have been few opportunities to evaluate an influenza vaccine under conditions where those vaccinated had no pre-existing antibodies, and the vaccine strain corresponded perfectly to the epidemic strain. Thus our results may provide valuable information. We hope that, complementary to epidemiologic assessments, this immunologic assessment will be helpful in discussions of countermeasures against a possible outbreak of highly pathogenic influenza viruses.

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Ultrasound prognostic factors after laser surgery for twin–twin transfusion syndrome to predict survival at 6 months

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Objective To evaluate the significance of ultrasound findings, detected one or two weeks after laser surgery for twin–twin transfusion syndrome, in predicting the mortality at 6 months of age.

Methods Ultrasound evaluation including fetal biometry, amniotic fluid volume estimation and Doppler examination was performed between 7 and 14 days after surgery for 181 cases. The presence of one or more effusions and single fetal death were also determined. Associations between ultrasound findings and mortality at 6 months of age were evaluated using multiple logistic regression analysis.

Results Of the total 181 pairs, 145 (80.1%) donor and 160 (88.1%) recipient twins survived *in utero* for more than 7 days after surgery, and hence were included in the analysis. The survival rate at 6 months was 66.9% for the donor and 80.7% for the recipient twins. Risk factors for death in the donor were the presence of severe intrauterine growth restriction and effusions. In recipients, elevation in the middle cerebral artery peak systolic velocity coincided with fetal death, but this occurred in only three cases.

Conclusion Ultrasound risk factors one week after surgery included severe intrauterine growth restrictions and effusions in the donor twins. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS: Doppler; fetal therapy; laser surgery; monochorionic twin; twin–twin transfusion syndrome; ultrasound

INTRODUCTION

Twin–twin transfusion syndrome (TTTS) develops in approximately 10% of cases of monochorionic twin pregnancies and is associated with a poor perinatal prognosis (Lewi *et al.*, 2008). The unbalanced blood flow from the donor to the recipient twin via the intertwin vascular anastomoses may result in profound hemodynamic disturbances in each twin (Diehl *et al.*, 2001; Bermudez *et al.*, 2002). Consequently, severe oligohydramnios occurs in the donor twin and polyhydramnios and cardiac failure occur in the recipient twin. Several recent studies, including a randomized controlled trial, have demonstrated that fetoscopic laser coagulation of placental vascular anastomoses results in a higher survival and lower neurological complication rate, when compared with serial amnioreduction (Ville *et al.*, 1998; Hecher *et al.*, 1999; Quintero *et al.*, 2003; Senat *et al.*, 2004).

The use of ultrasound, which reveals significant perioperative prognostic factors, facilitates the prediction of perinatal outcome of twins after surgery. Detection of absent or reversed end-diastolic flow in the umbilical artery of the donor twin may be the most significant prognostic factor for fetal demise, which frequently occurs within a few days after surgery (Martinez *et al.*, 2003; Cavicchioni *et al.*, 2006; Ishii *et al.*, 2007; Sago *et al.*, 2010). However, even fetuses that survive the acute period, that is, one week after surgery, sometimes die during the fetal or neonatal period (Sago *et al.*, 2010). The clinical features of fetuses that suffer *in utero* or neonatal death one week after surgery remain unknown. This study aimed to identify ultrasound parameters one or two weeks after laser surgery in predicting eventual mortality of fetuses.

METHODS

A total of 181 Japanese women were diagnosed with TTTS, for which they underwent fetoscopic laser surgery. The characteristics and perinatal outcomes of these cases have been previously reported (Sago *et al.*, 2010). TTTS was diagnosed on the basis of the following criteria: (1) the presence of polyhydramnios and a deepest vertical pocket (DVP) of >8 cm in the recipient twin and (2) oligohydramnios and a DVP of <2 cm in the donor twin. All patients were between 16 and 26 weeks of gestation and the Quintero stage of disease was between I and IV (Quintero *et al.*, 1999). Laser surgery was performed using previously described methods (Sago *et al.*, 2010) and

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vascular anastomoses were selectively coagulated (Quintero *et al.*, 1998.). Patients gave their written consent and the study protocol was approved by the institutional review board of each institution.

All mothers with at least one surviving fetus 7 days after laser surgery were examined between 7 and 14 days after the procedure. Ultrasound examination included fetal biometry and amniotic fluid volume estimation. According to the formula given by the Japanese Society of Ultrasound in Medicine (Shinozuka, 2002), an estimated fetal weight of < -2 SD was regarded as a severe intrauterine growth restriction (IUGR). Effusion in one body compartment was defined as the presence of at least one of the following signs: (1) ascites; (2) pleural effusion; (3) pericardial effusion; and (4) skin edema. Furthermore, the presence or absence of a single co-twin death was noted. This was followed by color and pulsed Doppler examination of the umbilical artery (UA), middle cerebral artery (MCA), and *ductus venosus* (DV) of the fetuses. Doppler sampling was performed using a 3.5 MHz or 5 MHz curved-array transducer with spatial peak temporal average intensities lower than 100 mW/cm^2. The high-pass filter was set at the lowest level. During Doppler studies for fetal vessels, the occurrence of absent or reversed end-diastolic flow (AREDF) in UA, elevated peak systolic velocity in the MCA (MCA-PSV), and reversed blood flow during atrial contraction in the DV (DVRF) were regarded as critically abnormal. Flow velocity waveforms were recorded during the absence of fetal breathing and/or movements. Umbilical artery waveforms were recorded at a free loop of the umbilical cord or at the placental cord insertion site. MCA-PSV was measured as described by Mari *et al.* (2000) and a value of >1.5 multiples of median (MoM) using their reference range was considered elevated. The insonation angle between the ultrasound beam and the direction of blood flow was kept as close as possible to 0 degrees. The sample volume for DV was determined from its inlet portion at the umbilical vein.

The study outcome was survival at 6 months of age in each twin and the odds ratio (OR) was used to estimate the relative risk of death for each fetus according to the ultrasound findings. Univariate analyses were used to estimate crude ORs and 95% confidence intervals (CIs) of the ultrasound risk factors. A multiple logistic regression model for death at 6 months of age in each twin was constructed using variables obtained by stepwise selection (significance level for entry into the model was <0.2). The reported p values were two-sided and analyses were performed using SAS software version 9.1.2 (SAS Institute Inc., Chicago, Illinois, USA).

RESULTS

Of the total 181 twin sets undergoing laser surgery (Table 1), 145 (80.6%) donor and 160 (88.9%) recipient twins survived for more than 7 days after surgery, and hence were included in the analysis. Ultrasound examination was performed at approximately 8.1 days (range: 7–14) for donor twins and 7.7 days (range: 7–14) for recipient twins. Pregnancy outcomes and perinatal survival rates are shown in Table 2. The median gestational age at delivery was 33 weeks in donor twins (interquartile range: 29.6–36.3 weeks) and 33.1 weeks in recipient twins (interquartile range: 29.6–36.1 weeks). The incidence of preterm delivery and the gestational ages of donors and recipients, respectively, were as follows: 2.8% and 3.1%, <24 weeks; 14.5% and 15.0%, <28 weeks; and 43.4% and 40.6%, ≥34 weeks. Intrauterine fetal death (IUFD) between 7 days and delivery occurred in 8.3% donor and 5.0% recipient twins. The survival rate at 6 months of age was 83.5% for donor and 91.3% for recipient twins.

Univariate logistic regression analysis showed that the significant risk factors for donor death at 6 months of age were the presence of AREDF in UA (OR: 3.03; 95% CI: 1.07–8.58; $p=0.031$), effusion in one body compartment (OR: 3.72; 95% CI: 1.09–12.6; $p=0.043$), and IUGR (OR: 5.33; 95% CI: 1.93–14.75; $p=0.001$) (Table 3). With regard to recipient death, MCA-PSV >1.5 MoM (OR: 4.95; 95% CI: 1.09–22.5; $p=0.058$) was defined as a single prognostic factor (Table 4). Variables such as DVP, DVRF, or single IUFD of the co-twins did not affect the outcomes (Table 3).

In the final multiple logistic model, prognostic factors for donor death were severe IUGR (OR: 6.17; 95% CI:

Table 1—Baseline characteristics ($n=181$)

Maternal age, mean ± SD	31.0 ± 4.5
Nulliparity - no. (%)	100 (55%)
Gestational age at surgery, mean ± SD	21.2 ± 2.5
Location of placenta - no. (%)	
Anterior	89 (49%)
Posterior	92 (51%)
Quintero stage - no. (%)	
Stage 1	14 (8%)
Stage 2	30 (17%)
Stage 3	113 (62%)
Stage 4	24 (13%)

SD, standard deviation.

Table 2—Pregnancy outcomes and survival rates of twins

	Donor ($N=145$)	Recipient ($N=160$)
Gestational age at delivery–weeks		
Median	33.1	33.0
Interquartile range	29.6–36.3	29.6–36.1
Gestational age at delivery, no. (%)		
< 24 weeks	4 (2.8%)	5 (3.1%)
24 to < 28 weeks	17 (11.7%)	19 (11.9%)
28 to < 32 weeks	30 (20.7%)	37 (23.1%)
32 to < 34 weeks	31 (21.4%)	34 (21.3%)
34 to < 36 weeks	16 (11.0%)	17 (10.6%)
≥ 36 weeks	47 (32.4%)	48 (30.0%)
IUFD, no. (%)	12 (8.3%)	8 (5.0%)
NND, no. (%)	6 (4.1%)	5 (3.1%)
Infantile death (<6 months), no. (%)	6 (4.1%)	1 (0.6%)
Survival at 6 months of age, no. (%)	121 (83.5%)	146 (91.3%)

Intrauterine fetal death (IUFD) after ultrasonographic assessment at least 7 days after laser surgery; NND, neonatal death.

Table 3—(a) Crude odds ratio of ultrasound factors for death at 6 months of age in donor twins. (b) Crude odds ratio of ultrasound factors for death at 6 months of age in recipient twins

a)

Variables	Mortality rate in donor twin	OR (95%CI)	*p*
DVP in donor		2.06 (0.77–5.51)	0.145
≤2 cm (*N* = 34)	23.5%		
2 cm < (*N* = 100)	13.0%		
AREDF in UA of donor		3.03 (1.07–8.58)	0.031
AREDF (*N* = 22)	31.8%		
Normal (*N* = 120)	13.3%		
DVRF in donor		2.29 (0.55–9.62)	0.371
DVRF (*N* = 10)	30.0%		
Normal (*N* = 127)	15.7%		
MCA-PSV (MoM)		—	1.000
<1.5 (*N* = 105)	16.2%		
1.5 ≤ (*N* = 3)	0.0%		
Own effusion		3.72 (1.09–12.6)	0.043
Effusion (*N* = 13)	38.5%		
Normal (*N* = 125)	14.4%		
IUFD of co-twin		0.61 (0.07–5.15)	1.000
Presence (*N* = 9)	11.1%		
Absence (*N* = 136)	16.9%		
Severe IUGR		5.33 (1.93–14.75)	0.001
≤ − 2SD (*N* = 53)	30.2%		
2SD < (*N* = 80)	7.5%		

b)

Variables	Mortality rate in recipient twin	OR (95%CI)	*p*
DVP in recipient		1.09 (0.34–3.43)	1.000
8 cm ≤ (*N* = 51)	9.8%		
<8 cm (*N* = 99)	9.1%		
AREDF in UA of recipient		—	1.000
AREDF (*N* = 1)	100.0%		
Normal (*N* = 154)	8.4%		
DVRF in recipient		1.36 (0.16–11.85)	0.563
DVRF (*N* = 9)	11.1%		
Normal (*N* = 143)	8.4%		
MCA-PSV (Mom)		4.95 (1.09–22.51)	0.058
<1.5 (*N* = 113)	8.0%		
1.5 ≤ (*N* = 10)	30.0%		
Own effusion		2.62 (0.50–13.79)	0.239
Effusion (*N* = 11)	18.2%		
Normal (*N* = 141)	7.8%		
IUFD of co-twin		2.52 (0.72–8.81)	0.230
Presence (*N* = 24)	16.7%		
Absence (*N* = 136)	7.4%		

P, *p*-value; OR, odds ratio; CI, confidence interval; DVP, deepest vertiacal pocket; AREDF in UA, absent or reversed end-diastolic flow in the umbilical artery; DVRF, reversed blood flow during atrial contraction in *ductus venosus*; MCA-PSV, peak systolic velocity of middle cerebral artery; MoM, multiples of median; Effusion, defined as when at least one sign, such as ascites, pleural effusion, pericardial effusion and skin edema, was noted; IUFD, intrauterine fetal death; IUGR, intra uterine growth restriction.

2.03–18.73; $p = 0.001$) and effusion in one body compartment (OR: 4.24; 95% CI: 1.09–16.53; $p = 0.037$) (Table 4 (a)). The statistically significant prognostic factor for recipient death was elevated MCA-PSV (OR: 1.21; 95% CI: 1.01–1.46; $p = 0.040$) (Table 4(b)). Interestingly, one of the three recipient twins with elevated MCA-PSV who died had an anemia–polycythemia sequence diagnosed postnatally.

Table 4—(a) Multivariate-adjusted odds ratio of ultrasound factors for death at 6 months of age in donor twins. (b) Multivariate-adjusted odds ratio of ultrasound factors for death at 6 months of age in recipient twins

a)

	Donor (*N* = 126)	
Variables	OR (95%CI)	*p*-value
Own effusion	4.24(1.09–16.53)	0.037
Severe IUGR	6.17(2.03–18.73)	0.001

b)

	Recipient (*N* = 119)	
Variables	OR (95%CI)	*p*-value
MCA-PSV (Mom)	1.21 (1.01–1.46)	0.040
Own effusion	1.22 (0.97–1.55)	0.090

OR, odds ratio; CI, confidence interval; Effusion, defined if at least one sign, such as ascites, pleural effusions pericardial effusion and skin edema was noted; IUGR, intra uterine growth restriction; MCA-PSV, peak systolic velocity of middle cerebral artery; MoM, multiples of median.

DISCUSSION

Ultrasound detectable prognostic features that predict 6-month survival in donor and recipient twins and which are obtained more than a week after laser surgery have not been elucidated earlier, although this information might be important when referring a patient back after laser surgery. In contrast, earlier studies have logically focused on perioperative sonographic indicators (Martinez *et al.*, 2003; Cavicchioni *et al.*, 2006; Ishii *et al.*, 2007; Sago *et al.*, 2010). According to the results of our previous study (Sago *et al.*, 2010), 13.3% donor twins and 6.1% recipient twins died within 7 days after surgery, while 69.1% donor twins and 82.9% recipient twins survived for more than 6 months.

Regarding the donor twins, a preoperative AREDF in UA is a negative predictor for fetal or neonatal survival (Martinez *et al.*, 2003; Cavicchioni *et al.*, 2006; Ishii *et al.*, 2007; Sago *et al.*, 2010), which may be associated with a small placental territory and/or vascular anastomoses (Chang *et al.*, 2006). On the other hand, AREDF in UA presented immediately after surgery correlate with perinatal outcome (Martinez *et al.*, 2003; Cavicchioni *et al.*, 2006). AREDF in the UA one week or more after laser surgery was neither predictive of donor death in previous studies (Martinez *et al.*, 2003; Cavicchioni *et al.*, 2006) nor in this one. Actually most donor deaths occurred within 7 days after laser surgery (Ishii *et al.*, 2007). Early donor death has often been explained by a drastic change in circulating blood volume. The presence of AREDF becomes less predictive later on, as the latter probably is more an indicator of the degree of growth restriction, which on itself may be an independent predictor of perinatal death. In the present study, the presence of severe IUGR rather than AREDF was indeed an independent predictor of survival. This has also been shown previously

in singletons (Kramer *et al.*, 1990; Spinillo *et al.*, 1995; McIntire *et al.*, 1999).

A second predictor of late donor death was the presence of one or more effusions. This has been earlier explained to be caused by a sudden increase in peripheral or placental vascular resistance causing an increased afterload. They were explained to be caused by an increase in umbilical venous blood flow volume after arrest of the intertwin transfusion process, leading to a relative increase in fetal volemia (Mahieu-Caputo *et al.*, 2000; Gratacos *et al.*, 2002a, 2002b; Ishii *et al.*, 2004). In a previous study by Gratacos *et al.*, in nine out of ten donor twins who developed hydropic signs, this disappeared within 14 days after surgery, whereas it worsened and the donor twin resulted in death in another case (Gratacos *et al.*, 2002b). In the present study, 13 donor twins had hydropic signs, of whom five (38.5%) actually died. The exact pathophysiologic mechanism of persisting hydrops remains unknown, hence the difference in results cannot be explained.

For recipients, postoperative MCA-PSV >1.5 MoM was predictive of the outcome, which may be indicative of fetal anemia. Preoperative-elevated MCA-PSV of the recipient twins was earlier reported as a risk factor for IUFD 24 h after laser surgery for TTTS (Kontopoulos and Quintero, 2009), whereas postoperative elevation of the MCA-PSV usually is benign and transient (Ishii *et al.*, 2008). In the present study, elevation of MCA-PSV more than 7 days after surgery was documented in seven patients, which normalized in six. The only one where it did not eventually died. Elevated MCA-PSV has also been earlier tied to subsequent fetal death in growth-restricted fetuses (Mari *et al.*, 2007). These authors speculated that an increased MCA-PSV indicated an increased blood flow to the brain through an elevated left cardiac output and increased placental vascular resistance. Because we did not have detailed assessment of the fetal hemodynamic status, including cardiac output, we cannot speculate on this particular feature. As only three of ten recipient twins with postoperative elevated MCA-PSV eventually died, this might not make it a significant prognostic factor.

In summary, factors that predict perinatal outcome between 7 and 14 days after laser surgery are different from those in the preoperative or immediate postoperative period. In this study, we have shown that the presence of severe IUGR and one or more effusions in the donor twin more than one week after laser surgery are predictive of the perinatal outcome of the donor. Such findings put the donor fetus at risk, which warrants a more close surveillance, and eventually in the viable period may lead to a more active management.

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