these two studies, IVIg was injected repeatedly. The Cochrane review of randomized controlled trials mentioned that there was no sufficient evidence to recommend use of any particular strategy to prevent mother-to-fetus transmission of CMV [15]. Pass et al. investigated the prevention of maternal CMV infection by administering a vaccine consisting of recombinant CMV envelope gycoprotein B to non-pregnant women [16]. The results of this trial suggest that CMV glycoprotein B vaccine has the potential to decrease incident cases of maternal and congenital CMV infection, however further phase 3 and 4 clinical trials are required . Pending the availability of an active vaccine, IVIg is thought to be one of the most promising candidates as prophylactic agent, because IVIg has been used safely in the previous studies [17-21]. The effects of IVIg may be related to not only the ability to inhibit the replication of CMV, but also the ability to inhibit the activity of immune cells producing harmful cytokines such as tumor necrosis factor α [22,23].

Number of subjects in the present study is very small, but the preliminary result suggests that IVIg injections in the present protocol are not effective for the prevention of mother-to-fetus CMV transmission. However, in our study, conventional polyclonal-immunoglobulin with a high titer of anti-CMV antibody was substituted for hyper-immunoglobulin. The titers of anti-CMV antibody contained in immunoglobulin used in our study may be much lower than that of hyper-immunoglobulin used in western countries, so we may have to use much higher doses of immunoglobulin. Therefore, we have discontinued the IVIg study of the present form. A new prophylactic study with use of a high dose IVIg has started.

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Original article

Nationwide survey of mother-to-child infections in Japan

Hideto Yamada [a, *], Shinya Tairaku [a], Ichiro Morioka [b], Ayako Sonoyama [a], Kenji Tanimura [a], Masashi Deguchi [a], Satoshi Nagamata [a], Yasuhiko Ebina [a]

[a] Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan
[b] Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan

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ABSTRACT

Objectives: The aim of this survey study was to evaluate a state of mother-to-child infections in Japan.
Methods: A nationwide survey on 2714 obstetric facilities where regular maternity checkups were carried out was conducted. A primary questionnaire assessed numbers of pregnancies including induced abortion, spontaneous abortion, still-birth as well as live-birth, which were affected by congenital infections of 6 pathogens during a year of 2011. The secondary questionnaire assessed clinical information, diagnostic modality, and the outcome for each case. The clinical features and diagnostic problems were evaluated.
Results: The high reply rates for the primary (73.7%) and the secondary questionnaire (100%) were achieved. The presence of congenital infections for 34 cases with cytomegalovirus (CMV), 1 with *Toxoplasma gondii*, 4 with rubella virus, 5 with *Treponema pallidum*, 8 with herpes simplex virus, and 69 with parvovirus B19 was confirmed after questionnaire assessment. The incidence of fetal demise among pregnancies with congenital parvovirus B19 infection was up to 71.0%. Eleven mothers with hydrops fetalis received prenatal fetal therapies involving fetal blood transfusion and immunoglobulin administration, whereas only three pregnancies (27.3%) ended in live-births.
Conclusions: This survey study for the first time revealed the annual frequency of pregnancies with mother-to-child infections of 6 pathogens in Japan. The results involve important information and are helpful for clinical practitioners. The majority of neonates with congenital infection of CMV or *T. gondii* might be undiagnosed in obstetric facilities.

1. Introduction

Mother-to-child infections, which develop if pathogens are transmitted from a mother to a fetus/neonate during pregnancy or the perinatal period, may lead spontaneous abortion, fetal death, fetal growth restriction and severe congenital diseases including anomalies and sequelae. To prevent or reduce the incidence of mother-to-child infections, maternal blood screening for some pathogens capable of mother-to-child infections is performed at regular maternity checkups in obstetric facilities. Recently, we have conducted a nationwide survey on obstetric facilities where regular maternity checkups were carried out, in order to assess a state of maternal blood screening for mother-to-child infections in Japan, 2011. It has been reported elsewhere that the performance rates of blood screening are more than 99% for rubella virus, *Treponema pallidum*, human immunodeficiency virus, human T cell leukemia virus type 1, hepatitis B virus, and hepatitis C virus, while the rates are found to be only 4.5% for cytomegalovirus (CMV), and 48.5% for *Toxoplasma gondii* [1].

The present study depicts the annual frequencies of pregnancies with congenital infections caused by CMV, *T. gondii*, rubella virus, *T. pallidum*, herpes simplex virus (HSV), and parvovirus B19, together with these clinical features and diagnostic problems that were found by the survey study.

2. Materials and methods

We conducted a nationwide survey from February 2012, in order to assess a state of maternal blood screening for mother-to-child

* Corresponding author. Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Tel.: +81 78 382 6000; fax: +81 78 382 6019.
E-mail address: yhideto@med.kobe-u.ac.jp (H. Yamada).

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infections and to determine numbers of pregnancies with congenital infections between January and December in 2011, with the approval of the Ethical Committee of Kobe University Graduate School of Medicine (No. 1441). The primary questionnaire survey was performed for 2714 obstetric facilities in Japan where regular maternity checkups were carried out. The primary questionnaire assessment involved an annual number of deliveries in 2011, scale of facilities, presence or absence of neonatal intensive care unit in facilities, and a state of maternal blood screening. The primary questionnaire also assessed annual numbers of pregnancies including induced abortion, spontaneous abortion (less than 22 weeks of gestation), still-birth (22 or more weeks of gestation), and live-birth, which were possibly affected by congenital infections of CMV, *T. gondii*, rubella virus, *T. pallidum*, HSV, or parvovirus B19.

The secondary questionnaire assessment for facilities that had possible cases with congenital infections and replied to the primary questionnaire was performed between July 2012 and May 2013. The questionnaire included maternal information (symptom, ultrasound abnormality, complications and treatment), diagnostic modality of infection for mothers and fetuses/neonates [antibody, serological test, histopathology, polymerase chain reaction (PCR), or virus culture], pregnancy outcome (induced abortion, spontaneous abortion, still-birth, live-birth, and weeks of gestation), and fetal/neonatal information (weight, symptom, anomaly, treatment, and prognosis). Whether the index case had confirmed, equivocal, or no congenital infection was determined from information about symptoms and results of the abovementioned diagnostic tests. The clinical features and diagnostic problems were also assessed.

3. Results

A high rate (73.7%) of reply to the primary questionnaire was achieved from 1990 facilities, where had a total of 788,673 deliveries covering 75.1% of annual number (1,050,806) of delivery in Japan, 2011 [2]. Two hundreds fifty-three facilities responded that they experienced pregnancies possibly affected by congenital infections of CMV, *T. gondii*, rubella virus, *T. pallidum*, herpes simplex virus or parvovirus B19. The frequencies of pregnancies with suspected congenital infections were determined from the replies to the primary questionnaire. The numbers of cases (induced abortion/spontaneous abortion/still-birth/live-birth) with suspected congenital infections were the following: CMV (5/3/3/58), *T. gondii* (2/1/1/72), rubella virus (4/0/2/18), *T. pallidum* (1/0/0/21), herpes simplex virus (0/0/1/21) and parvovirus B19 (4/48/28/146).

All the 253 facilities replied to the secondary questionnaire for each case of suspected congenital infection, therefore the 100% reply rate of the secondary questionnaire was achieved. Table 1 shows the frequency of pregnancies with confirmed and equivocal congenital infections that were determined from medical information obtained from the secondary questionnaire. The clinical features and diagnostic problems for each congenital infection were the following.

3.1. Cytomegalovirus

The congenital CMV infection for 34 cases consisting of 3 induced abortions, 2 still-births and 29 live-births were confirmed by positive tests for PCR analysis or virus culture of neonatal urine/amniotic fluid, and/or positive tests for specific IgM in the neonatal blood. Of the 34 cases of congenital infection, 19 (55.9%) had ultrasound abnormalities and other 4 (11.8%) had signs of recent infection on maternal antibody screening prenatally. The congenital infection of the remaining 11 cases was detected only after birth due to neonatal abnormalities. The fetal abnormalities found by prenatal ultrasound examinations in the 19 cases included fetal growth restriction in 13 fetuses, ventriculomegaly in 10, microcephaly in 7, hydrops/ascites/pleural effusion in 6, hyperechoic bowel in 5, cardiomegaly in 2, and hepatosplenomegaly in 1.

Seventeen mothers had primary infection with positive tests for IgM, low IgG avidity index, or seroconversion of IgG, while 6 had reinfection/reactivation with negative IgM or high IgG avidity index, and the remaining 11 were unknown because of no IgM measurements. The reporting rate of cases with confirmed congenital infection among facilities carrying out maternal CMV screening (6/90) was significantly ($p < 0.001$, chi-square test) higher than that among facilities with no screening (22/1900).

Twenty-five (86.2%) of 29 neonates with congenital CMV infection were symptomatic and 4 (13.8%) were asymptomatic. Of the 25 symptomatic neonates, 19 (76.0%) had abnormalities on the central nerves system; 2 died; 13 developed moderate or severe sequelae and 1 unilateral hearing difficulty at the time of the questionnaire. Twenty-one (72.4%) of 29 neonates with congenital infection underwent urine PCR or virus culture showing positive results, the remaining 8 (27.6%) had positive tests for IgM in the blood without urine CMV analyses. Seventeen (58.6%) neonates received medication of ganciclovir, valganciclovir and foscarnet; and 3 did immunoglobulin therapy only.

3.2. Toxoplasma gondii

One live-birth with asymptomatic congenital *T. gondii* infection was confirmed by a positive test for PCR analysis of the neonatal blood but specific IgM tested negative.

Thirty-eight mothers with positive tests for specific IgM were reported by the questionnaires. Nineteen (50%) of them underwent IgG avidity index measurements, and 5 (13.2%) did amniotic fluid PCR analyses. Twenty-nine (76.3%) mothers received medication of acetylspiramycin. Risk factors for *T. gondii* infection including "raw or undercooked meat" in 5 mothers, "gardening or intake of soil" in 2, and "a cat of pet or stray in the neighborhood" in 1 were reported.

3.3. Rubella virus

Asymptomatic congenital rubella virus infection for 2 live-births from 1 mother with primary infection and 1 reinfection mother was

Table 1
Results of secondary questionnaire: The frequency of pregnancies with confirmed and equivocal congenital infections in Japan, 2011.

Congenital infection	Total	Induced abortion	Spontaneous abortion	Still-birth	Live-birth	Adjusted live-birth/100,000 live-births[a]
Cytomegalovirus	34 (8)	3 (1)	0 (3)	2 (1)	29 (3)	3.7
Toxoplasma gondii	1 (16)	0 (2)	0 (0)	0 (1)	1 (13)	0.13
Rubella virus	4 (4)	1 (1)	0 (0)	1 (0)	2 (3)	0.25
Treponema pallidum	5 (5)	0 (0)	0 (0)	0 (0)	5 (5)	0.63
Herpes simplex virus	8 (1)	0 (0)	0 (0)	1 (0)	7 (1)	0.89
Parvovirus B19	69 (77)	3 (1)	35 (13)	14 (0)	17 (63)	2.2

Numbers in parentheses indicate equivocal cases in which diagnostic procedure for fetuses/neonates from mothers with definite infection was insufficient or inappropriate; therefore the presence of congenital infection was possible or not denied.

[a] Adjusted live-birth rates of confirmed congenital infections, calculated from questionnaire data covering 788, 673 live-births.

confirmed by positive tests for specific IgM in the neonatal blood. There was no report of congenital rubella syndrome (CRS). PCR analyses tested positive on the amniotic fluid in 1 induced abortion and 1 still-birth, in which primary infection was confirmed by IgG seroconversion in maternal blood.

3.4. *Treponema pallidum*

Five live-births with congenital syphilis were confirmed by positive tests for *T. pallidum* hemagglutination (TPHA) or fluorescence treponemal antibody absorption IgM in the blood of four neonates and hepatosplenomegaly with a positive test for rapid plasma reagin (RPR) in one neonate. Three of the five neonates had symptoms including hepatosplenomegaly in two and small for the gestational age in one. Four of the five neonates received medications of penicillin.

3.5. *Herpes simplex virus*

Seven live-births with neonatal HSV infection including four with disseminated disease and three with disease localized skin, eye or mouth (SEM) were confirmed by positive tests for specific IgM and/or positive tests for PCR analyses. The genital HSV infection was unrecognized prior to labor in 6 of the 7 mothers who had neonatal HSV infection. Five of the seven mothers did not undergo specific IgM measurement or antigen test prior to deliveries. Only 1 mother underwent selective cesarean section and the remaining 6 did vaginal deliveries. Two infants with disseminated disease died, and three received medications of aciclovir. One still-birth with HSV infection at 24 weeks of gestation was confirmed by positive tests for specific IgM in the blood of both mother and baby.

3.6. *Parvovirus B19*

The congenital parvovirus B19 infection for 69 cases consisting of 3 induced abortions, 35 spontaneous abortions, 14 still-births, and 17 live-births was confirmed by positive tests for PCR analysis of neonatal urine/amniotic fluid/placenta, positive tests for specific IgM in the neonatal blood, and/or characteristic symptoms (hydrops, ascites, pleural effusion, anemia, cardiomegaly, and hepatosplenomegaly) of the fetus/neonate. Forty-nine (71.0%) of the 69 pregnancies with congenital parvovirus B19 infection ended in spontaneous abortions and still-births. Additionally, 3 (4.3%) pregnancies ended in induced abortions. Three neonates (17.6%) of the 17 live-births had symptoms (hydrops, anemia, and cardiomegaly) and the remaining 14 neonates were asymptomatic. Of the 69 mothers, 58 (84.1%) were multiparous, and 37 (53.6%) had their family with symptoms of erythema infectiosum (34 children, and 3 others). Twenty-seven (39.1%) mothers experienced the erythema infectiosum, and 34 (49.3%) had no symptoms. Twenty of the 34 mothers without symptoms lived with their family who had the erythema infectiosum (18 children, and 2 others).

The prenatal ultrasound examinations detected fetal abnormalities including hydrops, ascites, pleural effusion, cardiomegaly, and hepatosplenomegaly at median 17 ranging from 10 to 26 weeks of gestation for 65 cases (Fig. 1). These fetal abnormalities disappeared spontaneously until delivery in 10 (15.4%) cases. The second questionnaire also inquired weeks of gestation of the erythema infectiosum appearing among 27 of the 69 mothers with congenital infection. The incidences of fetal/neonatal symptoms (hydrops, ascites, pleural effusion, cardiomegaly, and hepatosplenomegaly) were 100% (2/2) when the onset of maternal erythema infectiosum was at less than 10 weeks of gestation, 63.6% (14/22) at 10–19 weeks of gestation, 50.0% (1/2) at 20–24 weeks of gestation, and 0% (0/1) at 31 weeks of gestation. The interval between the erythema infectiosum appearing in mothers and detection of ultrasound fetal abnormalities was median 3.5 weeks ranging from 1 to 9 weeks.

Eleven mothers with hydrops fetalis received prenatal fetal therapies involving fetal blood transfusion ($n = 9$), and fetal blood transfusion plus immunoglobulin administration ($n = 2$). Three pregnancies with fetal blood transfusion ended in full term deliveries of asymptomatic live-births; and the remaining 8 (72.7%) did in abortions and still-births between 20 and 26 weeks of gestation.

4. Discussion

In this nationwide survey on obstetric facilities, we for the first time revealed the annual frequencies of pregnancies with congenital infections caused by 6 pathogens including induced abortion, spontaneous abortion, still-birth as well as live-birth in Japan.

The incidence of congenital CMV infection is known to be 0.2–2% of all births in developed countries [3]. Recently, a multicenter study of neonatal urine screening has demonstrated that

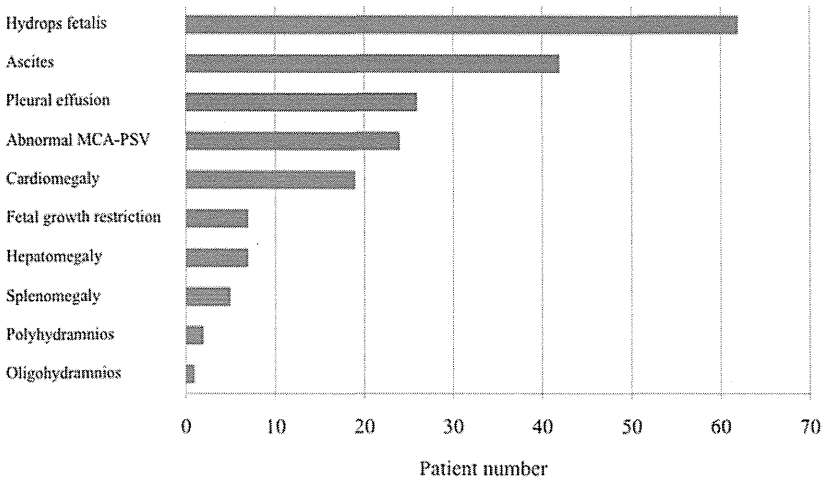

Fig. 1. Frequencies of ultrasonographic abnormalities in congenital parvovirus B19 infection MCA-PSV, middle cerebral artery-peak systolic velocity.

congenital CMV infection develops in 0.31% of 21,272 infants and 22.7% of the infected infants are symptomatic in Japan [4]. The annual number of live-birth has been reported to be 1,050,806 in 2011 [2]. Therefore, annual frequencies of symptomatic and asymptomatic neonates with congenital CMV infection in Japan are estimated to be 739 and 2518, respectively. However, in the present survey the congenital CMV infection were confirmed for only 29 cases consisting of 25 symptomatic and 4 asymptomatic neonates. Similarly, the incidence of congenital *T. gondii* infection is estimated to be 1.26 per 10,000 live-births in Japan [5], and 1–10 per 10,000 live-births in Europe [6] and the US [7]. However, only 1 neonate with congenital *T. gondii* infection was confirmed in the present study. Many neonates with congenital infection of CMV or *T. gondii* might be undiagnosed during pre- and postnatal period in obstetric facilities. The universal screening of CMV or *T. gondii* for all pregnant women is not recommended by Society of Obstetrics and Gynecology or the Ministry of Health, Labor and Welfare in Japan. IgG avidity or PCR assay for CMV and *T. gondii*, of which measurement costs are not yet covered by health insurance, has not been standardized in Japan. Pregnant women who have recent infection or high risks for mother-to-child infection would not be found efficiently prior to delivery, and so their neonates would not receive a workup for the congenital infection. These might be reasons why the majority of neonates infected with CMV or *T. gondii* were undiagnosed.

The performance rates of maternal blood screening in obstetric facilities were more than 99% for rubella virus and *T. pallidum* [1]. Two live-births with congenital rubella virus infection and 5 with congenital syphilis were confirmed, but some neonates from mothers with primary or active infection underwent no workup or serological tests, then classified as equivocal case in this study. The genital HSV infection was unrecognized prior to labor in 6 of 7 mothers whose neonates had HSV infection, and 1 mother underwent selective cesarean section. The majority of neonates with HSV infection are borne by mothers having unrecognized genital infection. Cesarean section alone does not completely remove the risk of transmission of HSV to neonates.

An epidemic of erythema infectiosum (Sticker disease) due to parvovirus B19 infection was present in Japan, 2011 succeeding 2007. The frequency of fetal demise (spontaneous abortion and still-birth) was up to 71.0% of cases with congenital parvovirus B19 infection. Thus, parvovirus B19 infection for pregnant women is etiologically associated with fetal demise, of which the incidence would increase during an epidemic period. Among approximately a half of mothers complicated by congenital parvovirus B19 infection, their family had symptoms; and the majority of them were their children. Women should be cautioned and educated about risks of parvovirus B19 transmission from their family prior to pregnancy, especially in the epidemic period. The fetal abnormalities appeared at median 17 ranging from 10 to 26 weeks of gestation, and 15.4% of those spontaneously disappeared until live-birth. The incidences of fetal/neonatal symptoms were 100% when their mothers experienced the erythema infectiosum at less than 10 weeks of gestation, 63.6% at 10–19 weeks of gestation, 50.0% at 20–24 weeks of gestation, and 0% at 31 weeks of gestation, suggesting that maternal infection earlier in gestation is causally associated with higher risks of symptomatic fetal/neonatal infection of parvovirus B19. This is concordant with results of a previous study [8]. Since 72.7% of hydropic fetuses receiving prenatal therapies demised in the present study, efficacy of the fetal therapies was limited and less effective than a previous survey [9].

This nationwide survey of mother-to-child infections has been performed for the first time on obstetric facilities. The results in the present study that demonstrates a state of mother-to-child infections in Japan, 2011 will involve important information in the field of epidemiology, public health, public administration and infectious disease, and are helpful for clinical practitioners as well.

Ethical approval

This study was conducted with informed consent from all of the subjects, and with the approval of the Ethical Committee of Kobe University Graduate School of Medicine.

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Conflict of interest

None of the authors have any conflicts of interest to declare.

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Short communication

Rapid increase in the serum *Cytomegalovirus* IgG avidity index in women with a congenitally infected fetus

Yasuhiko Ebina[a,*], Toshio Minematsu[b], Ichiro Morioka[c], Masashi Deguchi[a], Shinya Tairaku[a], Kenji Tanimura[a], Ayako Sonoyama[a], Satoshi Nagamata[a], Mayumi Morizane[a], Hideto Yamada[a]

[a] Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan
[b] Research Center for Disease Control, Aisenkai Nichinan Hospital, Miyazaki, Japan
[c] Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan

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ABSTRACT

Background: Human *Cytomegalovirus* (CMV) is the virus most frequently responsible for severe diseases of the fetus and newborn. The reported intrauterine transmission rate of CMV following primary maternal infection is approximately 40%. Invasive techniques are needed for the prenatal diagnosis of congenital CMV infection.
Objectives: The aim of this study was to evaluate whether the rapidity of change in the CMV IgG avidity index (AI) is associated with the presence of congenital CMV infection among mothers with suspected primary CMV infection.
Study design: The serum CMV IgG AI was repeatedly measured in 17 pregnant women with positive or borderline test results for CMV IgM together with an initial IgG AI value of <40%. Their neonates underwent polymerase chain reaction analyses for the presence of CMV DNA in the urine. The rapidity of change in the IgG AI per 4 weeks was defined as the ΔAI (%). The ΔAI of women with congenital CMV infection was compared with that of women with no infection.
Results: The ΔAI of nine mothers with congenital CMV infection (median,15.7%; range,7.8–42.8%) was significantly higher than that of eight mothers with no infection (median, 6.5%, range, 2.0–8.8%; $p < 0.001$). The incidences of congenital CMV infection were 100.0%, 16.7%, and 0.0% among mothers with a ΔAI of >10, 5–10, and <5%, respectively.
Conclusions: Measurement of the ΔAI in pregnant women might be useful for estimating the risk of mother-to-neonate CMV transmission.

1. Background

Human *Cytomegalovirus* (CMV) is the virus most frequently responsible for severe diseases of the fetus and newborn. The incidence of CMV IgG positivity among pregnant Japanese women decreased from 85% in 1988 to 68% in 2000 [1]. A recent multicenter screening of neonatal urine revealed that congenital CMV infection develops in 0.31% of newborns [2].

CMV IgM positivity is assumed to be a good indicator of acute or recent infection. However, it is not a specific marker of primary infection. Primary CMV infection is eventually diagnosed in only about 20–25% of IgM-positive women [3]. It has been demonstrated that the measurement of CMV-specific IgG avidity helps in diagnosing recent primary CMV infection [4–6]. IgG shows low avidity for the antigen during the early weeks following primary infection, but progressively matures, acquiring high avidity. We have demonstrated that ultrasound detection of fetal abnormalities and a low CMV IgG avidity index (AI) are independent risk factors for congenital CMV infection [7]. A CMV IgG AI with a cutoff value of <40% was found to be clinically useful for the prediction of congenital CMV infection, especially until 28 weeks of gestation [8].

The reported intrauterine transmission rate of CMV following primary maternal infection is approximately 40% [9]. Invasive techniques including amniocentesis [10–13] and cordocentesis [11,14] are generally used for prenatal diagnosis of congenital CMV infection.

* Corresponding author at: Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Tel.: +81 78 382 6005; fax: +81 78 382 6019.
E-mail address: ebiyas@med.kobe-u.ac.jp (Y. Ebina).

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Table 1
The characteristics of the women with and without congenitally infected fetuses.

	Women with congenitally infected fetus ($n=9$)		Women without congenitally infected fetus ($n=8$)		*P*-value
	Median	Range	Median	Range	
Age (years)	29	(19–38)	27	(22–36)	0.699
Number of previous gestations	1	(0–3)	1	(0–3)	0.828
Number of previous deliveries	1	(0–3)	1	(0–2)	0.915
Time of initial blood sampling (week of gestation)	25	(12–30)	21	(15–34)	0.963
Time between the measurements (days)	61.0	(22–86)	81.5	(38–98)	0.123

2. Objectives

The aim of this study was to evaluate whether the rate of change in the CMV IgG AI is associated with the presence of congenital CMV infection among mothers with positive or borderline CMV IgM test results.

3. Study design

This study was conducted after approval by the ethics committee of the Kobe University Graduate School of Medicine and after obtaining informed consent from the pregnant women involved. Serological and virological data were retrieved from pregnant women who had undergone maternity checkups from 2010 to 2013 or had been referred to Kobe University Hospital. During the study period, 232 women had positive or borderline tests for serum CMV IgM; 26 of these 232 women had an initial serum CMV IgG AI of <40% [8]. Of these 26 women, 17 who had given informed consent underwent reexamination of their CMV IgG AI until delivery.

The rate of change in the CMV IgG AI per 4 weeks was defined as the ΔAI. The ΔAI (%/4 weeks) was calculated according to the following formula:

$$\frac{([AI_2 - AI_1] \times 28)}{(D_2 - D_1)}$$

where $(D_2 - D_1)$ represents the elapsed time in days between the initial measurement date D_1 and the second measurement date D_2, AI_1 represents the AI of the D_1 sample (%), and AI_2 represents the AI of the D_2 sample (%). The ΔAI of women with congenital CMV infection was compared with that of women with no infection. In the former group, the relationships among the ΔAI, neonatal symptoms, and CMV DNA amounts in the amniotic fluid, neonatal urine, or neonatal blood were examined. All neonates underwent PCR analyses for CMV DNA in the urine collected during the first week of life to determine the presence or absence of congenital infection [2].

Serological tests for CMV IgG (negative 0–230.99, borderline 231–239.99, and positive >240) and IgM (negative 0–0.89, borderline 0.90–1.99, and positive >2.0) were performed using an Enzygnost assay (Siemens Healthcare Diagnostics, Tokyo, Japan). CMV IgG avidity was measured at Aisenkai Nichinan Hospital as described previously [15]. Serological tests for CMV IgG were performed using the Enzygnost assay. In total, 220 μL of serum to be tested at a dilution of 1:231 was added to each of four antigen-coated wells, and the plates were incubated at 37 °C. The unbound IgG was aspirated, and the wells were washed twice with phosphate-buffered saline containing 0.05% Tween 20 (PBS-Tween). Next, two of the four wells were filled with PBS-Tween, and the other two were filled with PBS-Tween containing 8 M urea. After incubation at room temperature for 6 min, all wells were washed once with PBS-Tween. Subsequent steps were performed according to the manufacturer's instructions. The antibody AI (%) was calculated as the mean absorbance at 450 nm (OD450) of the urea-washed wells divided by the mean OD450 of the control wells untreated with urea. A cutoff IgG AI of <40% with 96.1% specificity and 64.3% sensitivity for congenital infection was determined in a previous study [8]. Real-time polymerase chain reaction analysis was performed at a commercial laboratory (SRL, Tokyo, Japan). All statistical analyses were conducted using the statistical package R (ver. 3.2.0, www.r-project.org). The Mann–Whitney *U* test was used

Table 2
The longitudinal serological data and outcomes.

Group and case number	ΔAI (%/4 weeks)	Time of initial blood sampling (week of gestation)	Initial measurement			Elapsed time (days)	Second measurement		CMV-DNA levels in the amniotic fluid (copies/mL)
			AI (%)	CMV IgG	CMV IgM		AI (%)	CMV IgG	
A: Women with congenitally infected fetuses ($n=9$)									
A1	**42.8**	25	38.3	13766	10.0	22	71.2	20812	1.3×10^4
A2	**21.8**	30	23.1	24291	7.3	48	60.4	12834	8.6×10^5
A3	**21.1**	12	2.3	1586	8.9	61	48.3	7177	Not done
A4	**19.6**	19	16.0	4712	2.5	29	36.3	6498	1.6×10^5
A5	**15.7**	22	10.9	3730	6.6	67	48.5	4172	6.4×10^7
A6	**11.1**	13	12.6	4144	13.2	79	44.0	12450	2.1×10^5
A7	**10.5**	26	38.5	6602	1.4	86	70.9	16998	Not done
A8	**10.5**	27	3.6	1726	6.6	37	17.5	2398	1.5×10^4
A9	**7.8**	26	31.9	5439	4.3	78	53.7	8057	Not done
B: Women without congenitally infected fetuses ($n=8$)									
B1	**8.8**	34	8.2	4744	6.5	38	20.1	13597	
B2	**8.6**	21	11.6	4320	1.4	55	28.4	4706	
B3	**8.3**	28	7.6	1731	9.1	85	32.7	7553	
B4	**6.8**	21	34.6	4830	1.4	98	58.3	2623	
B5	**6.1**	16	17.6	2796	1.1	87	36.7	3967	
B6	**4.0**	21	33.5	16599	0.9	97	47.5	15268	
B7	**3.9**	15	25.5	4921	3.9	54	33.1	7696	
B8	**2.0**	27	14.7	3331	10.0	78	20.3	4994	

Table 3
The serological results of the women with and without congenitally infected fetuses.

	Women with congenitally infected fetus ($n=9$)		Women without congenitally infected fetus ($n=8$)		*P*-value
	Median	Range	Median	Range	
CMV IgM at D_1	6.6	(1.4–13.2)	2.7	(0.9–10.0)	0.059
CMV IgM at D_1	4712	(1586–24291)	4532	(1731–16599)	0.815
CMV IgG at D_1	8057	(2398–20812)	6273	(2623–15268)	0.481
CMV IgG at D1	16.0	(2.3–38.5)	16.2	(7.6–34.6)	0.980
CMV IgG at D2	48.5	(17.5–71.9)	32.9	(20.1–58.3)	0.059

to analyze differences between two groups. The Spearman rank-order correlation coefficient was used for correlation analysis. A *P*-value of <0.05 was considered statistically significant.

4. Results

Of 17 neonates, nine were positive for urinary CMV DNA, while the remaining eight yielded negative results. Five of the nine positive neonates had symptomatic infections, including hepatosplenomegaly/hepatitis ($n=3$), thrombocytopenia ($n=3$), brain abnormalities ($n=3$), auditory brain stem response abnormalities ($n=3$), choroidoretinitis ($n=1$), and small for gestational age ($n=1$).

The characteristics of the women with and without congenitally infected fetuses are shown in Table 1. No statistically significant differences in maternal age, number of previous gestations/deliveries, gestational age in weeks at the initial blood sampling, or days between initial and second measurements were found between the two groups.

Table 2 illustrates the longitudinal serological data of individual serological data and outcomes. Table 3 shows the serological results of the women with and without congenitally infected fetuses. No statistically significant differences in the CMV IgM titers at D_1, CMV IgG titers at D_1/D_2, or the AI at D_1/D_2 were found between the two groups. The AI at D_2 in women with congenital CMV infection was higher than that in women without congenital infection, but without statistical significance. Fig. 1 shows the changes in CMV IgG AI according to weeks of gestation in the 17 women. Fig. 2 compares the ΔAI between the women with and without congenitally infected fetuses. The median ΔAI in women with congenital CMV infection was 15.7% per 4 weeks (range, 7.8–42.8%) and was significantly higher than that in women without congenital infection (6.5% per 4 weeks, 2.0–8.8%; $p<0.001$). The incidences of congenital infection were 100.0% (8/8), 16.7% (1/6), and 0.0% (0/3) in women with a ΔAI of >10%, 5–10%, and <5%, respectively. There was no relationship between the presence of neonatal symptoms and the ΔAI in women with congenital CMV infection. The median ΔAI in women with symptomatic congenital CMV infection ($n=5$) was 16.1% per 4 weeks, and that in women with asymptomatic congenital CMV infection ($n=4$) was 15.7% per 4 weeks. The median CMV-DNA levels in the amniotic fluid, neonatal urine, and neonatal blood were 1.9×10^5 copies/mL (range, 1.3×10^4 to 6.4×10^7 copies/mL), 1.6×10^7 copies/mL (range, 1.9×10^4 to 3.1×10^9 copies/mL), and 2.0×10^2 copies/10^6 WBC (range, 3.0×10 to 5.6×10^3 copies/10^6 WBC), respectively. There was no correlation between the ΔAI and amount of CMV DNA in the amniotic fluid ($r=-0.257$, $p=0.658$), neonatal urine ($r=0.524$, $p=0.197$), or neonatal blood ($r=0.333$, $p=0.428$).

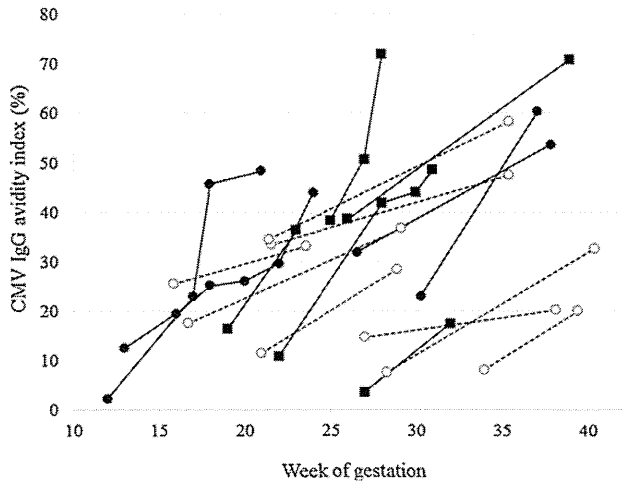

Fig. 1. Time-dependent changes in the CMV IgG AI in women with and without congenitally infected fetuses. Solid lines: CMV IgG AI in women with congenitally infected fetuses($n=9$); dashed lines: CMV IgG AI in women without congenital CMV infection ($n=8$). (■ symptomatic congenital CMV infection [$n=5$]; ● asymptomatic congenital CMV infection [$n=4$]; and ○no congenital CMV infection [$n=8$]).

5. Discussion

The present study demonstrated that the serum CMV IgG AI increased more rapidly in pregnant women who were subsequently found to have congenital CMV infection than in women without

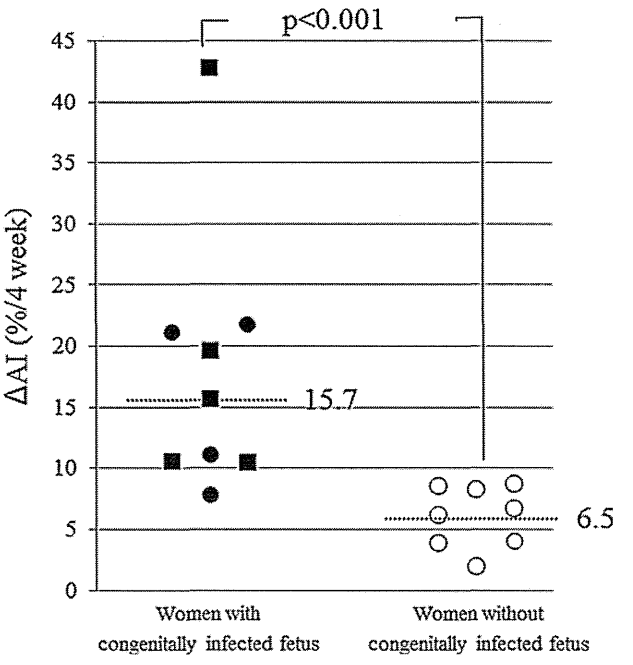

Fig. 2. ΔAI in women with and without congenitally infected fetuses (■ symptomatic congenital CMV infection [$n=5$]; ● asymptomatic congenital CMV infection [$n=4$]; and ○no congenital CMV infection [$n=8$]).

congenital infection. The incidences of congenital CMV infection were 100.0%, 16.7%, and 0.0% in women with a ΔAI of >10, 5–10, and <5%, respectively. Patients with clinically apparent primary infection reportedly have a more intense and prolonged IgG antibody response to active CMV replication than do patients with subclinical infection [16]. CMV IgG avidity is correlated with IgG neutralizing activity [17]. Therefore, vigorous viral replication might lead to a corresponding rapid IgG maturation for the antigen, leading to high ΔAI values in pregnant women with congenital CMV infection. Similarly, a recent study demonstrated that an incidence of congenital CMV infection in women with a rapid increase in the serum IgG AI was higher than that in women with a slow increase in the IgG AI (62.5% vs. 24.1%, respectively) [18]. Meanwhile, the dynamics of reduction of IgM levels can also help to understand the course of avidity maturation. Sera with the highest IgM levels had the lowest IgG avidity index values [19].

Measurement of the ΔAI in pregnant women with positive or borderline test results for serum CMV IgM and a low IgG AI might be useful to estimate the risk of mother-to-neonate CMV transmission during pregnancy. Pregnant women with ΔAI values of >10% per 4 weeks seem to have a high risk of congenital CMV infection. The present study thus contains important information that could be helpful for clinical practitioners.

However, this study included a relatively small number of patients, resulting in several uncertainties. Limitation of this study include the overlapping ΔAI values (ΔAI 5–10%) between maternal transmitters versus non-transmitters. The correct interpretation of avidity results is crucial because serological tests vary among different laboratories; thus, the method used and its reference values must be carefully assessed. In addition, the gestational ages of IgG avidity measurement and the elapsed time varied broadly. Further studies are necessary to confirm the conclusions of the present study.

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Competing of interests

None.

Ethical approval

This study was conducted with the informed consent of all patients. The study was approved by the institutional ethics boards of Kobe University Hospital (No. 922).

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Accurate Prediction of the Stage of Histological Chorioamnionitis before Delivery by Amniotic Fluid IL-8 Level

Satoshi Yoneda[1], Arihiro Shiozaki[1], Mika Ito[1], Noriko Yoneda[1], Kumiko Inada[1], Rika Yonezawa[1], Mika Kigawa[2], Shigeru Saito[1]

[1]Department of Obstetrics and Gynecology, University of Toyama, Toyama, Japan;
[2]Department of Public Health Faculty of Medicine, University of Toyama, Toyama, Japan

Keywords
Amniotic fluid, body temperature, histological chorioamnionitis, interleukin-8, preterm labor

Correspondence
Shigeru Saito, Department of Obstetrics and Gynecology, University of Toyama, 2630 Sugitani, Toyama, 930-0194, Japan.
E-mail: s30saito@med.u-toyama.ac.jp

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Objective
To estimate the stage of histological chorioamnionitis (h-CAM) antenatally using clinical data.

Materials and methods
Four hundred and twenty-eight singleton mothers were recruited. Clinical data including the levels of white blood cell count (WBC), C-reactive protein (CRP), amniotic fluid interleukin-8 (AF-IL-8) at Cesarean section, and maternal body temperature (MBT) were collected.

Results
Histological chorioamnionitis was present in 45.3% of the cases. Poor neonatal prognosis was highest (59.1%) in cases with h-CAM stage III. AF-IL-8 (odds ratio: 8.5, 95% CI: 5.1-14.8, $P < 0.0001$) and MBT (odds ratio: 2.3, 95% CI: 1.13–4.1, $P = 0.0192$) were independent risk factors for h-CAM. The cutoff value of AF-IL-8 for predicting each stage of h-CAM (stage I or higher, stage II or higher, and stage III) were ≥9.9 ng/mL, ≥17.3 ng/mL, and ≥55.9 ng/mL, respectively.

Conclusion
The stage of h-CAM was able to be predicted accurately by the level of AF-IL-8 before delivery.

Introduction

Histological chorioamnionitis (h-CAM) is an antenatal inflammatory state of the intrauterine environment strongly associated with preterm delivery. Around 33–83% of infants born before 30–32 weeks of gestation have been exposed to h-CAM,[1–5] which often is a clinically silent process. Exposure to h-CAM is known to induce several organ failures in the fetus.[6] Its presence in placentas from preterm infants increases the incidence of bronchopulmonary dysplasia (BPD),[7–9] necrotizing enterocolitis (NEC),[2,10] periventricular leukomalacia (PVL),[11,12] and cerebral palsy (CP).[12–17] These are recognized as symptoms of fetal response inflammatory syndrome (FIRS).[18–20] These effects have been generally shown to be more pronounced when additional signs of fetal inflammation, such as funisitis, are present.[21–24]

Histological chorioamnionitis is classified into three stages according to Blanc's classification: stage I (deciduitis), stage II (chorionitis), and stage III (amnionitis).[25] van Hoeven et al.[26] reported that the risk of FIRS becomes higher with an increase in the severity of h-CAM. However, the associations between h-CAM and inflammatory markers in maternal circulation have not been fully clarified. On the other hand, clinical CAM was defined by Lenki et al.[27] as maternal fever (≥38.0°C), elevated white blood cell count (≥15,000/μL), uterine tenderness, maternal or fetal tachycardia, and malodorous

vaginal fluid. Although clinical CAM may be clearly diagnosed before delivery, neonatal outcomes may already be deteriorated by that point in time,[14,28,29] suggesting that clinical CAM is a final stage of CAM. Park et al.[30] reported that the involvement of the amnion in the inflammatory process of the extraplacental membranes is associated with a more intense fetal inflammatory response than chorionitis alone. Therefore, an accurate prediction of h-CAM and its degree are needed to manage mothers with pre-clinical symptoms of clinical CAM before the appearance of clinical symptoms.

To overcome this problem, the ability of biological markers to detect h-CAM before birth has been investigated. There have been some reports evaluating h-CAM during pregnancy by biological markers, such as maternal body temperature (MBT),[27,31] maternal white blood cell count (WBC),[27,31] maternal C-reactive protein (CRP),[30,32,33] maternal or amniotic interleukin (IL)-6,[34–37] and amniotic IL-8.[38] However, there have been no reports predicting antenatally the severity of h-CAM by biological and clinical markers.

Information about the severity of h-CAM during the antenatal preterm period may be clinically useful for the management of cases at risk for preterm labor or with cervical incompetency. Additionally, with detection of strong intrauterine inflammation before clinical CAM, early termination of the pregnancy may be considered. On the other hand, no detection of inflammation or weak inflammation in the uterus would allow an extended period of pregnancy by maintenance tocolysis. Therefore, the degree of uterine inflammation should be evaluated before delivery, and individual strategies to improve neonatal prognosis for each case should be developed even if there is no sign of clinical CAM.

This study is the first report to show that amniotic fluid IL-8 levels are a good marker for estimating the stage of h-CAM in the antenatal period.

Materials and methods

Study Population

Four hundred and twenty-eight mothers who underwent Cesarean sections were recruited at Toyama University Hospital between January 2009 and December 2013. Two hundred and fifteen cases delivered preterm babies and 213 cases full-term babies. We excluded cases with premature rupture of membranes (PROM), preterm birth within 2 days of maternal steroid treatment leading to increased maternal WBC, severe fetal growth retardation (less than −2.0 S.D.), severe congenital abnormalities, polyhydramnios, gestational diabetes mellitus, preeclampsia, multiple pregnancies, and Cesarean sections due to failure of vaginal trial labor. The study was approved by the ethics committee of Toyama University Hospital. All the subjects included in this study provided informed written consent.

Definitions and Study Procedures

Gestational age was determined from the first day of the last menstrual period, or by fetal size by transvaginal ultrasound before 12 weeks of gestation.

The stages of h-CAM are defined by the degree of the neutrophil infiltration to the amnion–chorion–decidua. The stage I is defined that maternal neutrophils are between the decidua and chorionic plate. The stage II is defined that maternal neutrophils are in the connective tissues of the chorionic plate. Stage III is characterized by neutrophil infiltration of the amnion according to Blanc's diagnostic criteria.[25] And the funisitis was defined as the presence of any vasculitis in the umbilical cord. Section of tissue blocks were stained with hematoxylin–eosin and examined systematically for inflammation by some pathologists unaware of the proteomic results of the amniotic fluid in their laboratory rooms.

Demographic and clinical data (maternal data included age, parity, gestational age at delivery, intrapartum fever, and mode of delivery) were collected. Clinical CAM was defined as the combination of maternal fever during labor (more than 38°C) with any one of the following: maternal tachycardia (≥100 beats/min), uterine tenderness, malodorous amniotic fluid, or maternal leukocytosis (≥15,000 white blood cells/mL).

Poor neonatal outcome was defined as neonatal death or diagnosis of periventricular leukomalacia (PVL), intraventricular hemorrhage (IVH) ≥grade III, bronchopulmonary dysplasia (BPD), and necrotizing enterocolitis (NEC) during hospitalization in the NICU. PVL was defined by de Vries,[39] the classification of IVH was defined by Papile et al.[40] moderate/severe BPD was defined as an oxygen requirement at 36 weeks of gestational age according to the NICHD consensus conference paper,[41] and NEC was defined according to modified Bell's criteria[42] with ≥stage II considered to be significant.

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Sample Collection and Preparation

Maternal body temperature was measured just before Cesarean section. In preterm delivery, blood tests (maternal WBC and CRP) were performed within 48 hr before Cesarean section. In the case of elective Cesarean section, which was usually planned at about 39 weeks of gestation in full-term delivery, the blood test was performed within 2 weeks before the Cesarean section. However, in cases with high maternal fever before elective Cesarean section, levels of WBC and CRP were examined again just prior to Cesarean section. Amniotic fluid was extracted directly from the amniotic cavity just before delivery.

Management of Preterm Labor

When the patients are diagnosed as preterm labor, they are immediately hospitalized for bed rest and recommended the treatment of maintenance tocolysis (continuous intravenous infusion of ritodrine hydrochloride or magnesium sulfate) until 36 weeks of gestation to prevent the preterm birth.[43,44] During the long hospitalization, the clinical symptom would go on severer such as bag formation in the cervix, maternal WBC >15,000/μL, or maternal CRP >1.0 mg/dL; intravenous antibiotics (beta-lactam antibiotics 2–3 g/day or EM 800–1500 mg/day for 7 days) are empirically considered by obstetricians. Therefore, we could often use the antibiotics for preterm labor in long hospitalization.

Detection of AF-IL-8

The IL-8 is a chemokine produced by a variety of cell types, and various diseases related as a pro-inflammatory marker were reported.[45–47] We considered that it was a very good marker for the early stage of inflammation in the amnion, and routinely measured amniotic IL-8 level since 2001. In our previous study, amniotic IL-8 level was most reflected at each stage of h-CAM among the level of amniotic IL-8, TNFα, and IL-17.[48]

About 10 mL of amniotic fluid was obtained before delivery. AF-IL-8 was measured by an enzyme-linked immunosorbent assay (ELISA) as previously reported.[49] The detection limit of AF-IL-8 by ELISA was 32 pg/mL. On average, intra-assay and interassay coefficients of variation were 4.8% and 7.5%, respectively. The remaining sample of the amniotic fluid was stored at −80°C.

Statistical Analysis

To identify relevant clinical variables that varied between the h-CAM-positive and h-CAM-negative groups, univariate analysis was performed using the χ^2 test, Student's *t*-test, or Mann–Whitney *U*-test where appropriate. The association between the severity of h-CAM and lower gestational age at delivery was analyzed by ANOVA, and the risk factor of poor neonatal outcome was evaluated by logistic regression analysis. A cutoff value to predict h-CAM was proposed by receiver-operating characteristic (ROC) curves, and logistic regression analysis was performed to investigate the most reliable biochemical marker for the prediction of h-CAM. Odds ratios and 95% confidence intervals (95% CI) were also calculated. Diagnostic values of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were estimated to predict h-CAM. The cutoff values to predict the stage of h-CAM were determined using the most reliable biochemical marker. All analyses were performed using statistical analysis software (JMP 9.02; SAS Institute Inc, Tokyo, Japan). A P value of below 0.05 was regarded as significant.

Results

The patient characteristics of this study are shown in Table I. There were 215 (50.2%) cases of preterm delivery and 213 (49.8%) cases of full-term delivery. The frequency of stage I, stage II, and stage III h-CAM was 47.4, 35.0, and 17.6%, respectively. Clinical CAM was only detected in h-CAM(+) cases, with the frequency being very low (1.0%). Preterm birth was more frequent, and the gestational age was shorter in h-CAM-positive cases compared to h-CAM-negative cases. Funisitis was observed in 32.5% of h-CAM-positive cases, while it was observed in only 0.8% of h-CAM-negative cases.

There was a significant association between the severity of h-CAM and lower gestational age at delivery (ANOVA; $P < 0.0001$), and the incidence of poor neonatal outcome, such as neonatal death ($n = 4$), PVL ($n = 4$), IVH ≥grade III ($n = 2$), BPD ($n = 11$), and NEC ($n = 1$), was highest (59.1%) in stage III h-CAM (Fig. 1). The severity of h-CAM (odds ratio: 2.7, 95%CI: 1.6–5.0, $P = 0.0004$) and

Table I Characteristics of Patients ($N = 428$)

	h-CAM (–) ($N = 234$)	h-CAM (+) ($N = 194$)	P
Maternal age (year)	33 (18–43)	33 (20–44)	0.3036
Nulliparity (%)	35.9 (84/234)	40.2 (78/194)	0.3602
Antibiotic therapy (%)	6.4 (15/234)	29.9 (58/194)	<0.0001
Preterm birth (%)	41.4 (97/234)	60.8 (118/194)	0.0001
Delivery (w)	37 (24–41)	33 (24–41)	<0.0001
c-CAM (%)	0 (0/234)	1.0 (2/194)	–
Funisitis (%)	0.8 (2/234)	32.5 (63/194)	<0.0001
h-CAM stage I (%)		47.4 (92/194)	–
h-CAM stage II (%)		35.0 (68/194)	–
h-CAM stage III (%)		17.6 (34/194)	–

c-CAM, clinical chorioamnionitis; h-CAM, histological chorioamnionitis.

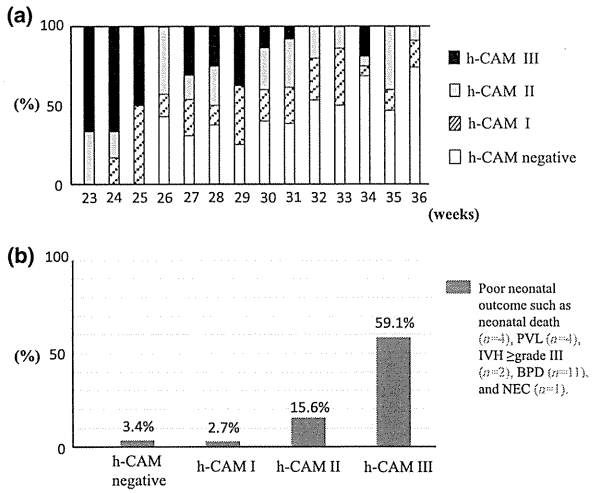

Fig. 1 The ratio of the stage of histological chorioamnionitis (h-CAM) to gestational age at delivery. There was a significant relationship between the severity of h-CAM and lower gestational age at delivery. (ANOVA; $P < 0.0001$) (a) Ratio of poor neonatal prognosis including neonatal death, PVL, IVH ≥stage III, BPD, and NEC. Cases of h-CAM stage III have the highest incidence of poor neonatal prognosis (59.1%) (b).

lower gestational age at delivery (odds ratio: 1.3/week, 95%CI: 1.1–1.6, $P = 0.0009$) were independent risk factors of poor neonatal outcome.

Table II shows the averages of four markers (MBT, WBC, CRP, and AF-IL-8) in h-CAM-positive and h-CAM-negative cases. MBT [36.8 (35.1–38.8)°C], WBC [8520 (4500–23,880)/μL], CRP [0.24 (0.02–9.9) mg/dL], and AF-IL-8 [19.7 (0.1–566.5) ng/mL] were significantly higher in h-CAM-positive cases than in h-CAM-negative cases [36.7 (35.0–37.6)°C, 7600 (2490–22,940)/μL, 0.20 (0.02–4.8) mg/dL, and 2.9 (0.1–162.6) ng/mL] ($P < 0.0001$, $P = 0.0002$, $P = 0.0006$, and $P < 0.0001$, respectively). The cutoff values to predict h-CAM were ≥9.9 ng/mL (AF-IL-8), ≥9800/μL (WBC), ≥0.44 mg/dL (CRP), and ≥37.1°C (MBT), respectively (Fig. 2). The area under curve (AUC) of AF-IL-8 (AUC = 0.7653) was significantly larger than those of WBC (AUC = 0.6070), CRP (AUC = 0.5970), and MBT (AUC = 0.6251), respectively ($P < 0.0001$, each) (Fig. 2).

AF-IL-8 (odds ratio: 8.5, 95% CI: 5.1–14.8, $P < 0.0001$) and MBT (odds ratio: 2.3, 95% CI:

Table II Clinical Data in Cases With or Without Histological Chorioamnionitis

	h-CAM (–) ($N = 234$)	h-CAM (+) ($N = 194$)	P
MBT (°C)	36.7 (35.0–37.6)	36.8 (35.1–38.8)	<0.0001
WBC (/μL)	7600 (2490–22,940)	8520 (4500–23,880)	0.0002
CRP (mg/dL)	0.20 (0.02–4.8)	0.24 (0.02–9.9)	0.0006
AF-IL-8 (ng/mL)	2.9 (0.1–162.6)	19.7 (0.1–566.5)	<0.0001

h-CAM, histological chorioamnionitis; MBT, maternal body temperature; WBC, white blood cell; CRP, C-reactive protein; AF-IL-8, amniotic fluid interleukin-8.

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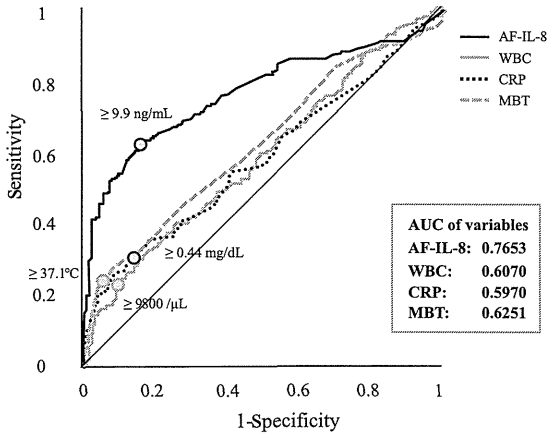

Fig. 2 Receiver-operating curves for amniotic fluid IL-8 (area under curve = 0.7653, $P < 0.0001$ for each AUC of maternal BT, CRP, WBC) for the prediction of histological chorioamnionitis.

1.13–4.1, $P = 0.0192$) were independent risk factors for h-CAM among the four markers (Table III). The level of AF-IL-8 predicted h-CAM with a sensitivity of 57.7%, a specificity of 88.9%, a PPV of 81.1%, and a NPV of 71.7%, while MBT predicted h-CAM with a sensitivity of 27.8%, a specificity of 91.4%, a PPV of 73.0%, and a NPV of 60.4% (Table IV).

Using the level of AF-IL-8, the cutoff value was evaluated to predict the stage of h-CAM before delivery. The cutoff values for h-CAM of stage I or higher, stage II or higher, and stage III were ≥9.9, ≥17.3, and ≥ 55.9 ng/mL, respectively (Fig. 3). The sensitivities for predicting h-CAM of stage I or higher, stage II or higher, and stage III were 57.7, 77.4, and 91.2%, with specificities of 88.9, 85.3, and 91.4%, respectively (Table V).

Although the frequency of funisitis was significantly increased with increase in the stage of h-CAM (Table VI), AF-IL-8 levels in each stage of h-CAM with or without funisitis were similar (Table VII).

Table III Logistic Regression Analysis for the Prediction of Histological Chorioamnionitis

	Odds ratio	95% CI	*P*
MBT (>37.1°C)	2.3	1.13–4.1	0.0192
WBC (>9800/μL)	1.1	0.62–1.9	0.7204
CRP (>0.44 mg/dL)	1.3	0.77–2.3	0.2986
AF-IL-8 (>9.9 ng/mL)	8.5	5.1–14.8	<0.0001

CI, confidence interval; MBT, maternal body temperature; WBG, white blood cell; CRP, C-reactive protein; AF-IL-8, amniotic fluid interleukin-8.

Discussion

Strengths and Weakness of the Study

The following are major strengths of this study. (i) This study was comprised of a large cohort of women with singleton babies ($N = 428$) and with histologic chorioamnionitis ($N = 194$). (ii) We evaluated several markers of infection and inflammation including AF-IL-8, MBT, WBC, and CRP. We found both AF-IL-8 and MBT were independent markers to predict h-CAM before delivery, and the predictive value for h-CAM was higher in AF-IL-8 compared to MBT. (iii) We have shown for the first time that the level of AF-IL-8 was able to estimate the stage of h-CAM before delivery. The cutoff value of AF-IL-8 to predict stage I or higher was ≥9.9 ng/mL with a sensitivity of 57.7% and a specificity of 88.9%. The cutoff value to predict stage II or higher was ≥17.3 ng/mL with a sensitivity of 77.4% and a specificity of 85.3%. The cutoff value to predict stage III of h-CAM was ≥55.9 ng/mL with a sensitivity of 91.2% and a specificity of 91.4%. (iv) In our study, poor neonatal prognosis, such as neonatal death, PVL, IVH ≥grade III, BPD, and NEC, was extremely high in cases of stage III h-CAM. When h-CAM stage III is estimated by AF-IL-8 level, termination of pregnancy may be considered. However, the gestational age at that point in time must also be considered for fetal prematurity.

Table IV Diagnostic Factors for the Prediction of Histological Chorioamnionitis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AF-IL-8 (>9.9 ng/mL)	57.7 (112/194)	88.9 (208/234)	81.1 (112/138)	71.7 (208/290)
MBT (>37.1°C)	27.8 (54/194)	91.4 (214/234)	73.0 (54/74)	60.4 (214/354)

AF-IL-8, amniotic fluid interleukin-8; MBT, maternal body temperature; PPV, positive predictive value; NPV, negative predictive value.

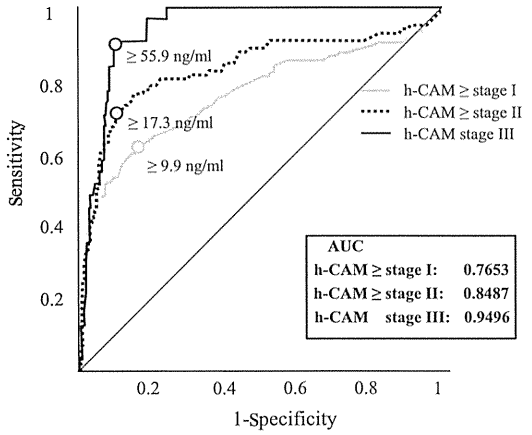

Fig. 3 Cutoff values of amniotic fluid IL-8 to predict the stages of histological chorioamnionitis. The area under curve for the prediction of histological chorioamnionitis stage I or higher, stage II or higher, and stage III is 0.7653, 0.8487, and 0.9496, respectively.

Table VI Frequency of Funisitis in Each Stage of Histological Chorioamnionitis

h-CAM	Frequency of funisitis		
Stage I	8.7 (8/92)	$P < 0.0001$	$P < 0.0001$
Stage II	43.9 (29/68)	$P = 0.0012$	
Stage III	76.5 (26/34)		

h-CAM, histological chorioamnionitis.

Table VII Association between Amniotic Fluid IL-8 and Funisitis in Each Stage of Histological Chorioamnionitis

	Funisitis (+)	Funisitis (−)	*P*
AF-IL-8 (ng/mL)			
Stage I	12.5 (0.1–40.2)	5.8 (0.1–240.6)	0.4142
Stage II	21.2 (0.1–379.5)	39.6 (0.1–376.7)	0.6944
Stage III	165.6 (17.5–566.5)	118.7 (26.5–237.5)	0.1679

h-CAM, histological chorioamnionitis; AF-IL-8, amniotic fluid interleukin-8.

The following are potential weaknesses of this study. (i) While in most cases WBC and CRP were examined just prior to Cesarean section, some full-term delivery cases without symptoms were examined within 2 weeks. (ii) We report no information about the levels of other cytokines in the amniotic fluid. Amniotic IL-8 level was measured because it has been reported that amniotic fluid IL-8 levels gradually increased with the h-CAM stage.[48] Amniotic TNF-α and IL-17 levels are only elevated in stage III of h-CAM.[48] Holst et al.[50] reported that among 27 proteins in the amnion, amniotic macrophage protein-1β (MIP-1β) is useful to predict delivery within 7 days. Keeler et al.[51] also reported that among 25 amniotic cytokines, monocyte chemotactic protein-1 (MCP-1) is the best marker for the prediction of early delivery. Using these suggested amniotic markers reflecting strong inflammation in the amnion, further detail of h-CAM may be able to be provided. Further studies are needed to clarify which cytokines are the best suited to evaluate the stage of h-CAM. (iii) When the stage III of h-CAM will be predicted by amniotic IL-8 levels, certainly, the best treatment was not leaded in this study. However, using this result of predicting the accurate stage of h-CAM antenatally, the new strategy might be effective to prevent the neonatal outcomes in the future studies.

Clinical Significance of this Study

Lenki et al.[27] reported that clinical CAM may be diagnosed by symptoms and blood examination, although the prognosis of the neonate is usually poor by the time clinical CAM is made evident.[10,28] This study reported a very low frequency of clinical CAM, suggesting that the majority of the h-CAM cases were terminated before the onset of clinical CAM. This evidence prompted us to establish a new

Table V Diagnostic Factors for the Prediction of Histological Chorioamnionitis Stages

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
h-CAM > stage I (>9.9 ng/mL)	57.7 (112/194)	88.9 (208/234)	81.1 (112/138)	71.7 (208/290)
h-CAM > stage II (>17.3 ng/mL)	77.4 (79/102)	85.3 (278/326)	62.2 (79/127)	92.3 (278/301)
h-CAM stage III (>55.9 ng/mL)	91.2 (31/34)	91.4 (360/394)	47.7 (31/65)	99.2 (360/363)

h-CAM, histological chorioamnionitis; PPV, positive predictive value; NPV, negative predictive value.

strategy for diagnosing h-CAM before the appearance of clinical signs. Therefore, recognition of h-CAM as a pre-stage of clinical chorioamnionitis may allow better management of women at risk for preterm labor or cervical incompetency and avoid poor neonatal prognosis such as neonatal death, PVL, IVH ≥grade III, BPD, and NEC.

An accurate prediction of the stage of h-CAM is important to provide proper treatment. Compared to cases with h-CAM, pregnancies without h-CAM should be extended as long as possible. Although there has been no evidence showing the efficacy of maintenance tocolysis for treatment, it may be effective for treatment of low-grade h-CAM predicted antenatally. Knowing the estimated grade of h-CAM, we may be able to determine an appropriate time for delivery in cases of severe intrauterine inflammation to avoid a poor outcome for the baby. We have reported that clinical symptoms and AF-IL-8 may be used to estimate the time of delivery in preterm labor cases.[52]

The aim of this study was only to lead the cutoff value of amniotic IL-8 levels to predict each stage of h-CAM by using large number of amniotic samples. Therefore, the sample included cases of the third trimester. The evaluation of amniocentesis to predict the stage of h-CAM was clinically for less than 30 weeks of gestation.

The Correlation between AF-IL-8 and Funisitis

It was reported that the risk of FIRS increases with the presence of h-CAM. The severity of h-CAM influences FIRS, although the degree of influence on the fetus is unknown. Funisitis can also increase the risk of FIRS.[21–24] In the present study, there was no correlation between the level of AF-IL-8 and funisitis in any of the stages of h-CAM (Table VI). The level of AF-IL-8 was strongly correlated with the stage of h-CAM, irrespective of the existence of funisitis. These data suggested that AF-IL-8 is a good marker for h-CAM, but not for predicting funisitis.

Questions in this Study

The antibiotic therapy has the effect just to microbes, not inflammation. And the preterm delivery was strongly correlated with amniotic inflammation (h-CAM) rather than the microbes in the amnion.[53,54] Therefore, antibiotic therapy was considered not to influence the amniotic fluid IL-8 levels.

Despite the high rate of preterm birth of 41% in h-CAM-negative group, the rate of delivery before 33 weeks of gestation was 14.9% (35/234) without h-CAM, while 38.6% (75/194) with h-CAM. The rate of preterm delivery of 41% was certainly high; however, most cases were delivered after 33 weeks of gestation without h-CAM.

The frequency of clinical CAM was only 1%: The reason was considered that (i) in this study, we excluded the premature rupture of the membranes (PROM) that often happened to clinical CAM. (ii) A large numbers of sample cases after 33 weeks of gestations were included in our study (73.8%).

Unanswered Questions and Proposals for Future Research

At present, the stage of h-CAM cannot predict the condition of the baby before delivery. Therefore, a prediction of the stage of h-CAM as part of the management of cases at risk for preterm labor or cervical incompetency may improve the prognosis of neonates. Maintenance tocolysis may be performed in cases without h-CAM or with h-CAM stage I, while a short-term tocolysis may be carried out in cases with h-CAM stage II or III. In cases with h-CAM stage III and with a positive microbubble test showing fetal lung maturation, delivery of the baby should be considered as an option, taking into account the gestational age. In conclusion, the stage of h-CAM may be predicted accurately by the level of AF-IL-8 before delivery.

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ARTICLE

Vaccination during the 2013–2014 influenza season in pregnant Japanese women

T. Yamada · K. Abe · Y. Baba · E. Inubashiri · K. Kawabata · T. Kubo · Y. Maegawa · N. Fuchi · M. Nomizo · M. Shimada · A. Shiozaki · H. Hamada · S. Matsubara · N. Akutagawa · S. Kataoka · M. Maeda · H. Masuzaki · N. Sagawa · A. Nakai · S. Saito · H. Minakami

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Abstract This questionnaire survey was conducted at 11 hospitals in Japan to determine vaccination coverage against seasonal influenza and the prevalence rate of influenza among pregnant Japanese women. Of 2,808 postpartum women who gave birth at the 11 hospitals during the study period from March 1, 2014, to July 31, 2014, 1,713 (61 %) participated in this study and 876 (51 %) reported having received vaccination against influenza in or after October 2013. Women aged <25 years had a significantly lower vaccination rate than those aged≥25 years (31 % vs. 53 %, respectively; p=0.0000). Eighty-seven (5.1 %) and 1,626 (94.9 %) women did and did not contract influenza, respectively. Although prior birth did not affect overall vaccination coverage (50 % for primiparous vs. 53 % for multiparous), multiparous women had a significantly higher rate of contracting influenza than primiparous women, irrespective of vaccination status (5.6 % vs. 2.2 % [p=0.0216] and 9.7 % vs. 3.5 % [p=0.0003] for women with and without vaccination, respectively). The 2013–2014 vaccination program significantly reduced the influenza infection rate by 35 % (3.9 % vs. 6.3 % for women with and without vaccination, respectively; p=0.0272). Seventy-two (83 %) of the 87 women took antiviral agents for the treatment of influenza and two (2.3 %) required hospitalization. These results suggested that pregnant Japanese women had a high level of concern regarding seasonal influenza. However, campaigns targeting young pregnant Japanese

T. Yamada (✉) · H. Minakami
Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, N15W7, Kita-ku, Sapporo 060-8638, Japan
e-mail: taka0197@med.hokudai.ac.jp

K. Abe · H. Hamada
Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

Y. Baba · S. Matsubara
Department of Obstetrics and Gynecology, Jichi Medical University, Tochigi, Japan

E. Inubashiri · N. Akutagawa
Department of Obstetrics and Gynecology, Sapporo Toho Hospital, Sapporo, Japan

K. Kawabata · S. Kataoka
Department of Obstetrics and Gynecology, Hakodate Central General Hospital, Hakodate, Japan

T. Kubo
Division of Obstetrics, National Center for Child Health and Development, Tokyo, Japan

Y. Maegawa · M. Maeda
Department of Obstetrics and Gynecology, Mie Chuo Medical Center, Tsu, Japan

N. Fuchi · H. Masuzaki
Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Medicine, Nagasaki, Japan

M. Nomizo · N. Sagawa
General Women's Medical and Health Science Center, Rakuwakai Otowa Hospital, Kyoto, Japan

M. Shimada · A. Nakai
Department of Obstetrics and Gynecology, Nippon Medical School Tama-Nagayama Hospital, Tokyo, Japan

A. Shiozaki · S. Saito
Department of Obstetrics and Gynecology, University of Toyama, Toyama, Japan