A prospective cohort study of newborns born to mothers with serum *Toxoplasma gondii* immunoglobulin M positivity during pregnancy

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土方　みどり

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指導教員　森岡　一朗

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Original Article

A prospective cohort study of newborns born to mothers with serum *Toxoplasma gondii* immunoglobulin M positivity during pregnancy

Midori Hijikata [a,1], Ichiro Morioka [a,*,1], Aya Okahashi [a], Nobuhiko Nagano [a], Kaori Kawakami [b], Atsushi Komatsu [b], Kei Kawana [b], Shohei Ohyama [c,d], Kazumichi Fujioka [d], Kenji Tanimura [e], Masashi Deguchi [e], Miwa Sasai [g], Masahiro Yamamoto [g], Hideto Yamada [e,f]

[a] Department of Pediatrics and Child Health, Nihon University School of Medicine, 30-1 Oyaguchikamicho, Itabashi-ku, Tokyo, 173-8610, Japan
[b] Department of Obstetrics and Gynecology, Nihon University School of Medicine, 30-1 Oyaguchikamicho, Itabashi-ku, Tokyo, 173-8610, Japan
[c] Department of Neonatology, Hyogo Prefectural Kobe Children's Hospital, 1-6-7, Minatojima-Minamimachi, Chuo-ku, Kobe, 650-0047, Japan
[d] Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe, 650-0017, Japan
[e] Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe, 650-0017, Japan
[f] Center for Recurrent Pregnancy Loss, Teine Keijinkai Hospital, 1-40, Maeda 1-jho, 12-chome, Teine-ku, Sapporo, 006-8555, Japan
[g] Department of Immunoparasitology, Research Institute for Microbial Diseases, Osaka University, 3-1, Yamadaoka, Suita, Osaka, 565-0871, Japan

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ABSTRACT

Introduction: The aims were to investigate the clinical characteristics of *Toxoplasma gondii (T. gondii)* immunoglobulin (Ig) M-positive mothers and to clarify the incidences of serum *T. gondii* IgM or blood *T. gondii* DNA positivity in newborns born to the mothers and the actual congenital *T. gondii* infection.
Methods: Mothers with *T. gondii* IgM positivity and newborns born to the mothers from 2013 to 2020 were prospectively investigated. Serum *T. gondii* IgG and IgM were measured by enzyme-linked immunosorbent assay. Blood *T. gondii* DNA was detected by semi-nested polymerase chain reaction. Congenital *T. gondii* infection was diagnosed based on clinical characteristic manifestations with serum *T. gondii* IgG positivity at any age or *T. gondii* IgG positivity after 12 months of age.
Results: Among 71 *T. gondii* IgM-positive mothers, including one with triplets, 41% had low *T. gondii* IgG avidity index and 73% received maternal therapy. Among 73 newborns who were examined for serum *T. gondii* IgG and IgM at birth, none had clinical manifestations, and one (1.4%) had *T. gondii* IgM positivity. Among 32 newborns who were examined for blood *T. gondii* DNA at birth, two (6.3%) were positive. All patients with serum *T. gondii* IgM or blood *T. gondii* DNA positivity showed *T. gondii* IgG negativity within 12 months of age.
Conclusions: A few newborns born to *T. gondii* IgM-positive mothers were suspected of having congenital *T. gondii* infection based on serum *T. gondii* IgM or blood *T. gondii* DNA testing at birth. However, none developed congenital *T. gondii* infection.

1. Introduction

Primary infection with *Toxoplasma gondii* (*T. gondii*) during pregnancy causes congenital infection [1–4]. After birth, pediatric patients with congenital *T. gondii* infection may die or develop neurological and ophthalmic sequelae such as mental retardation, epilepsy, and impaired vision [1,4–7]. Indeed, our recently nationwide survey and literature review study revealed that in Japan, pediatric patients have died and developed neurological and ophthalmic sequelae due to this infectious disease [6]. Therefore, early diagnosis and treatment are vital to alleviate these sequelae due to therapeutic agents such as pyrimethamine, sulfadiazine, folic acid, and corticosteroids, although their use is off label in Japan. The treatment is recommended even for asymptomatic congenital infections [1,4,5].

In Japan, the incidence of congenital *T. gondii* infection, including abortion, is estimated to be 0.9–2.6 per 10,000 deliveries under the

Abbreviations: ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; OD, optical density; PCR, polymerase chain reaction; T. gondii, Toxoplasma gondii.

* Corresponding author.
E-mail address: morioka.ichiro@nihon-u.ac.jp (I. Morioka).
[1] These authors contributed equally.

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situation that all pregnant women underwent *T. gondii* infection screening [3]. Generally, the maternal *T. gondii* screening and treatment policy for primary infection in Japan is as follows. Serum *T. gondii* immunoglobulin (Ig) G is measured during the first trimester. When serum *T. gondii* IgG is positive, serum *T. gondii* IgM is measured. *T. gondii* IgG avidity index may also be measured, if possible. If serum *T. gondii* IgM is positive or *T. gondii* IgG avidity index is low, primary *T. gondii* infection is suspected and closed examinations, such as fetal ultrasounds, and maternal therapy are considered. Amniocentesis is performed with informed consent when the pregnant women desired it because of their anxiety. The amniotic fluid is examined by polymerase chain reaction (PCR). If fetal infection is diagnosed due to *T. gondii* DNA positivity, pyrimetamine, sulfadiazine and folinate are given during the 16–27 weeks of gestation as a maternal therapy. Pyrimethamine and spiramycin are given after 28 weeks of gestation, because of the risk of bilirubin encephalopathy in the newborn when sulfadiazine is used [1].

Fetal infection is prevented by acetylspiramycin or spiramycin therapy in pregnant women with primary *T. gondii* infection [1]. From August 2018, spiramycin therapy for pregnant women suspected with primary *T. gondii* infection has been covered under insurance in Japan [1]. As aforementioned, serum *T. gondii* IgM and *T. gondii* IgG avidity index can be used to identify pregnant women with primary *T. gondii* infection [1–3]. However, because the *T. gondii* IgG avidity assay is not standardized and cannot be generally used in clinical practice [1], the serum *T. gondii* IgM is mainly used in current clinical practice in Japan. Cohort studies on newborns born to *T. gondii* IgM-positive mothers in Japan are limited.

Pediatric patients are suspected to have congenital *T. gondii* infection after birth based on any of the following test results: 1) characteristic clinical findings with serum *T. gondii* IgG positivity; 2) serum *T. gondii* IgG and IgM positivity; 3) *T. gondii* DNA positivity on PCR analysis of the cerebrospinal fluid, blood, or urine (a non-approved *in vitro* diagnostic assay in Japan); 4) an increase in serum *T. gondii* IgG titer during the first year of life; and 5) serum *T. gondii* IgG positivity beyond 12 months of age; [1,4,5]. Among these criteria, criterion 2) or 3) is commonly used to suspect congenital *T. gondii* infection in newborns early after birth in current clinical practice in Japan. The exact incidence of serum *T. gondii* IgM or blood *T. gondii* DNA positivity in newborns born to *T. gondii* IgM-positive mothers is unknown.

This study aimed to investigate the clinical characteristics of *T. gondii* IgM-positive mothers and to clarify the incidence of serum *T. gondii* IgM or blood *T. gondii* DNA positivity in newborns born to *T. gondii* IgM-positive mothers early after birth in a prospective cohort study and show whether these newborns had actual congenital *T. gondii* infection based on their follow-ups.

2. Materials and methods

2.1. Study design and patients

In this prospective cohort study, newborns born to *T. gondii* IgM-positive mothers at Nihon University Itabashi Hospital, Tokyo and Kobe University Hospital, Kobe from January 2013 to December 2020, regardless of the results of serum *T. gondii* IgG avidity index, were enrolled. In our regular clinical practice, with informed consent, pregnant women with serum *T. gondii* IgG and IgM positive are screened during the first trimester, and *T. gondii* IgG avidity is tested at the discretion of the attending obstetricians according to the institutional policy [1,8]. After obtaining informed consent, pregnant women received acetylspiramycin (1200 mg/day) or spiramycin therapy (9 million international units/day) until delivery. Until spiramycin was approved in clinical practice in 2018, acetylspiramycin was used to prevent fetal *T. gondii* infection. If the amniotic fluid test by PCR was a positive result, in addition to the acetylspiramycin or spiramycin, the pregnant women received pyrimethamine and sulfadiazine until 27 weeks of gestations. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethics Committees of Nihon University Itabashi Hospital (approval number: RK-181009-8), Kobe University Graduate School of Medicine (approval number: 170071), and Osaka University Research Institute for Microbial Diseases (approval number: 29–6). Formal written consent was obtained from the parents of the newborns.

2.2. Study methods and protocols

From January 2013, all newborns born to *T. gondii* IgM-positive mothers underwent serum *T. gondii* IgG and IgM testing, physical examinations, ophthalmofundoscopy, and brain ultrasound or computed tomography within 5 days after birth. From July 2017, neonatal blood *T. gondii* DNA testing was also performed. According to serum *T. gondii* IgG and IgM results, the newborns were classified as follows—those with IgG and IgM negativity, those with IgG negativity and IgM positivity, those with IgG positivity and IgM negativity, and those with IgG and IgM positivity (serological test study). Similarly, according to the results of the *T. gondii* DNA test using neonatal blood, newborns were classified as positive or negative (genomic test study). The incidence of serum *T. gondii* IgM or blood *T. gondii* DNA positivity was investigated.

If newborns showed characteristic clinical symptoms or findings for congenital toxoplasmosis with serum *T. gondii* IgG positivity, neonatal treatments using pyrimethamine, sulfadiazine, folic acid, or corticosteroids were considered [1,5]. If newborns were positive for serum *T. gondii* IgM or blood *T. gondii* DNA without characteristic clinical symptoms or findings for congenital toxoplasmosis, the introduction of neonatal treatments was entrusted to the attending neonatologists based on a comprehensive clinical assessment of the newborns. All newborns enrolled in this study were followed up until a negative result for serum *T. gondii* IgG was confirmed [1].

In our standard follow-up schedule, all newborns underwent a physical examination at 1 month of age and physical examination and serological tests for *T. gondii* IgG and IgM at 6 and 12 months of age. According to the infants' conditions or test results, physical and blood examinations, ophthalmofundoscopy, or brain imaging examinations at the other age were allowed at the discretion of the attending neonatologists [1]. The final diagnosis criteria for congenital *T. gondii* infection are based on characteristic clinical symptoms or findings for congenital toxoplasmosis with serum *T. gondii* IgG positivity at any age or serum *T. gondii* IgG positivity after 12 months of age [5]. Based on these criteria, newborns with serum *T. gondii* IgM or blood *T. gondii* DNA positivity were confirmed to have an actual congenital *T. gondii* infection. Based on the chronological age, psychomotor development was assessed as a developmental quotient using the Kyoto scale of psychological development or Tsumori-Inage developmental test.

2.3. Measurements of serum T. gondii IgG, T. gondii IgM, and T. gondii IgG avidity

Serum *T. gondii* IgG and IgM were measured using enzyme-linked immunosorbent assay (ELISA) with commercially available kits (PLATERIA™ TOXO IgG and PLATERIA™ TOXO IgM, Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Based on the assay manufacturer's criteria, the following threshold values were defined as positive: *T. gondii* IgG $\geq$9 IU/mL and IgM $\geq$1.0 cutoff index.

T. gondii IgG avidity index was measured using a commercially available kit (PLATERIA™ TOXO IgG AVIDITY, Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions or in the commercial laboratory (The Daiichi Kishimoto Clinical Laboratories, Sapporo, Japan) [3]. Briefly, this method relies on the measurement of the avidity of *T. gondii* IgG. The use of an agent, including urea, dissociating the link between antigen and antibody in parallel with the usual technique of IgG measurement allows comparison between the optical density (OD) obtained after dissociating agent action and that obtained without dissociating agent action (avidity index, % = urea-treated OD/untreated

OD × 100). The avidity index is "low" when the antigen–antibody link is readily dissociated. An avidity index of <35% is considered "low" [3]; however, this value has not been standardized [1,3].

2.4. Detection of *T. gondii* genomic DNA by semi-nested PCR

Blood samples from patients were centrifuged at 3000 rpm at 4 °C for 30 min, and the sera were discarded. Lysis buffer (300–1000 μL, 100 mM Tris-HCl; pH 8.0, 200 mM NaCl, 5 mM EDTA, 0.4% SDS) with protease K (50 μg/ml) was added to the clot. They were then incubated overnight at 55 °C. The samples were centrifuged at 10000 rpm at 4 °C for 20 min, and the supernatants were collected for isolation of DNA using phenol/chloroform. DNA samples (100–200 ng) were used as PCR templates. Semi-nested PCR for *T. gondii* B1 gene locus was performed using rTaq DNA polymerase (Toyobo, Osaka, Japan) with the following cycling protocol: 94 °C for 3 min, followed by 45 cycles at 94 °C for 30 s, 61 °C for 30 s, and 72 °C for 1 min, and 72 °C for 2 min. The primer sequences used for semi-nested PCR were as follows: First PCR forward 5′-GGGGAAGAATAGTTGTCGCA-3′; Second PCR forward 5′-GCTCTAGCGTGTTCGTCTCC-3′; and reverse 5′- GATCCTTTTG-CACGGTTGTT-3′. The product lengths of the first and second PCR sequences were approximately 450 bp and 200 bp, respectively. An example of this is shown in Fig. 1.

3. Results

During the study period, 4385 mothers were screened for serum *T. gondii* antibody and delivered at two university hospitals. Among these, 133 (3.0%) were positive for *T. gondii* IgG and 60 (1.4%) were also positive for *T. gondii* IgM (Fig. 2). Four mothers with *T. gondii* IgG negativity and IgM positivity were found. Furthermore, seven mothers with *T. gondii* IgM positivity who delivered at another hospital and referred the newborns to our hospitals were added (IgG positivity and IgM positivity: n = 6; IgG negativity and IgM positivity: n = 1). Therefore, 71 mothers, including one with triplets, with serum *T. gondii* IgM positivity were enrolled. Among these, 66 (93%) were positive for *T. gondii* IgG. The *T. gondii* IgG avidity index was measured in 59 (83%) patients, with a median value of 39%. The prevalence of low *T. gondii* IgG avidity index was 41%. Fifty-two (73%) mothers were treated with acetylspiramycin or spiramycin during pregnancy. The remaining 19 pregnant women did not take the treatment due to high *T. gondii* IgG avidity index or refusal of the treatment. Seventy-three newborns were born to the *T. gondii* IgM-positive mothers, with a median gestational age at birth and birth weight of 39 weeks and 2916 g, respectively. None of the newborns showed clinical manifestations at birth, such as generalized symptoms, brain image abnormalities, and chorioretinitis (Table 1).

All 73 newborns underwent serological testing. Six (8.2%), zero (0.0%), 66 (90.4%), and one (1.4%) newborns had IgG and IgM negativity, IgG negativity and IgM positivity, IgG positivity and IgM negativity, and IgG and IgM positivity, respectively. Among 59 newborns with IgG positivity and IgM negativity, excluding seven who died during infancy or were lost to follow-up because of moving, all newborns were confirmed to have IgG negativity within 12 months of age (median age at confirmed IgG negativity: 6 months [minimum–maximum: 3–12 months]; Fig. 3). The performance characteristics of the serum *T. gondii* IgM test were as follows (n = 66): sensitivity, 0%; specificity, 98%; negative predictive value, 100%; and positive predictive value, 0%.

In the genomic test, 32 newborns were enrolled. Two (6.3%) were positive for blood *T. gondii* DNA (Fig. 3). The two newborns with positive blood *T. gondii* DNA showed IgG positivity and IgM negativity in their serums. The performance characteristics of the blood *T. gondii* DNA test were as follows (n = 32): sensitivity 0%, specificity 94%, negative predictive value 100%, and positive predictive value 0%.

Detailed clinical data of newborns suspected with congenital *T. gondii* infection are shown in Table 2. The mother of Cases #2 and #3 did not have a low *T. gondii* IgG avidity index during pregnancy (16 weeks of gestation). None of the mothers received acetylspiramycin or spiramycin therapy during their pregnancies. Case #1 was a newborn with serum *T. gondii* IgM positivity, and Cases #2 and #3 were newborns with blood *T. gondii* DNA positivity. Although none of the patients received neonatal therapies because of the absence of clinical

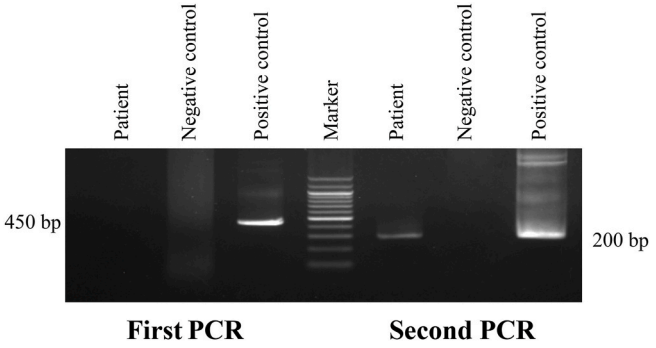

Fig. 1. Example results of *T. gondii* DNA detection by semi-nested PCR using B1 locus
When the second PCR showed a 200-bp band, blood *T. gondii* DNA was "positive".
bp, base pairs; PCR, polymerase chain reaction; *T. gondii*, *Toxoplasma gondii*.

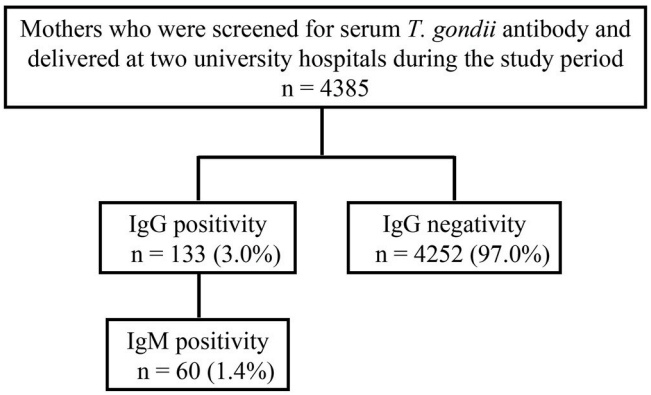

Fig. 2. Flowchart of mothers who were screened for serum *T. gondii* antibody and delivered at two university hospitals
Ig, immunoglobulin; *T. gondii*, *Toxoplasma gondii*.

Table 1
Clinical characteristics in enrolled mothers and newborns.

Mother	n = 71
T. gondii IgM positivity	71 (100)
T. gondii IgG positivity	66 (93)
T. gondii IgG avidity index, %, n = 59	39 (5–71)
Low *T. gondii* IgG avidity index	24/59 (41)
Therapy with acetylspiramycin or spiramycin during pregnancy	52 (73)
Newborns	n = 73
Gestational age at birth, weeks	39 (30–42)
Birth weight, g	2916 (1200–4115)
Male	36 (49)
Clinical manifestations at birth	
Generalized symptoms[a]	0 (0)
Brain image abnormalities[b]	0 (0)
Chorioretinitis	0 (0)

Data are shown as numbers (percent) or median values (minimum to maximum value).
Ig, immunoglobulin; *T. gondii, Toxoplasma gondii*.
[a] Anemia, jaundice, fever, petechiae due to thrombocytopenia, and hepatosplenomegaly.
[b] Intracranial calcification and hydrocephalus.

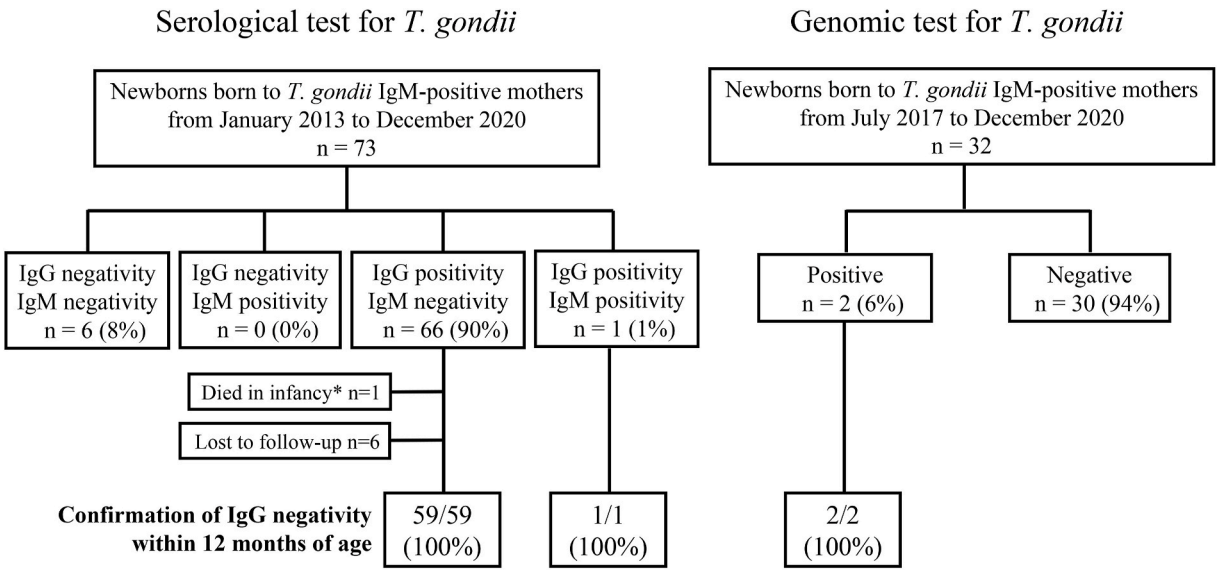

Fig. 3. Flowchart of newborns born to mothers with serum *T. gondii* IgM positivity during pregnancy for serological and genomic tests
* Sudden infant death syndrome at 4 months of age.
Ig, immunoglobulin; *T. gondii, Toxoplasma gondii.*

Table 2
Detailed clinical data of newborns who were suspected with congenital *T. gondii* infection.

Case	#1	#2	#3
Maternal *T. gondii* IgG avidity index (gestational weeks)	–	60% (16)	60% (16)
Maternal therapy	No	No	No
Reason of preterm delivery	pPROM	pPROM	pPROM
Gestational weeks at birth	35	30	30
Birth weight, g	2752	1200	1617
Sex	Male	Female	Female
Clinical manifestations at birth	No	No	No
T. gondii DNA in the blood at birth	Negative	Positive	Positive
T. gondii IgG (IU/mL)/IgM (COI)			
at birth	23/2.6	100/0.1	91/0.1
1 month of age	–	29/0.1	23/0.1
2 months of age	–	8/0.1	8/0.1
3 months of age	19/3.4	3/0.1	3/0.1
6 months of age	<7.5/4.5	–	–
12 months of age	<7.5/<0.8	–	–
18 months of age	<7.5/<0.8	–	–
Negative conversion of *T. gondii* IgG	Yes	Yes	Yes
Neonatal therapy	No	No	No
Growth and development (age)	Normal BW: 15.3 kg HT: 97.6 cm HC: 49.5 cm DQ: 97 (3 years of age)	Normal BW: 10.4 kg HT: 83.9 cm HC: 47.5 cm DQ: 96 (2 years of age)	Normal BW: 9.8 kg HT: 80.1 cm HC: 47.5 cm DQ: 100 (2 years of age)
Sequelae	No	No	No

Cases #2 and 3 were two triplets.
The following threshold values were defined as positive: T. gondii IgG ≥9 IU/mL and IgM ≥1.0 COI.
–, Not examined; BW, body weight; COI, cutoff index; DQ, developmental quotient; HC, head circumference; HT, height; pPROM, preterm premature rupture of the membrane; *T. gondii, Toxoplasma gondii.*

manifestations at birth and decreased *T. gondii* IgG titer levels with age, negative conversion of *T. gondii* IgG was confirmed within 12 months of age. None of the patients had growth and development disorders or sequelae of congenital toxoplasmosis.

4. Discussion

This is the first prospective cohort study of newborns born to mothers with serum *T. gondii* IgM positivity during pregnancy over 8 years. Among 73 newborns included in the serological test study, one (1.4%) was positive for *T. gondii* IgM. Among 32 newborns included in the genomic test study, two (6.3%) were positive for blood *T. gondii* DNA. Although these three newborns were suspected of having congenital *T. gondii* infection at birth, none developed congenital *T. gondii* infection during the follow-up based on our definitions in this study.

In the present study, the prevalence of mothers with IgG positivity (3.0%) was consistent with a Japanese previous report (2–10%) [1]. However, out of 133 mothers with *T. gondii* IgG positivity, 60 (45%) were positive for *T. gondii* IgM (Fig. 2). This was a high percentage in comparison with a Japanese previous report (13–26%) [1], because this study consisted of two university hospitals where pregnant women with serum *T. gondii* IgM positivity are referred from the surrounding clinics or hospitals.

In adults, serum *T. gondii* IgM positivity is not always indicative of acute *T. gondii* infection because *T. gondii* IgM occasionally persists for several months or years after primary infection [1,9]. Therefore, the *T. gondii* IgG avidity test is often used to distinguish between recent and non-recent infections as a high *T. gondii* IgG avidity index can rule out infection in the last 3–4 months [9]. Yamada et al. reported that around 30% pregnant women with serum *T. gondii* IgM positivity during the first trimester had a low *T. gondii* IgG avidity index of <35% [3]. In our current study, because of inclusion of different subjects from the different institutions and during different study periods, 41% of 59 pregnant women serum *T. gondii* IgM positivity had a low *T. gondii* IgG avidity index, indicating that approximately 60% patients had non-recent infection. Findal et al. also have reported that a low *T. gondii* IgG avidity index may also persist for ≥3 months in some pregnant women [10]. Therefore, there may be fewer pregnant women with recent *T. gondii* infection in our cohort. Because only maternal *T. gondii* IgM screening may not be suitable for detecting fetal *T. gondii* infection, Yamada et al. have suggested a combination screening using *T. gondii* IgG avidity index and multiplex nested PCR test in maternal blood [2,3]. In our cohort, 73% of mothers were treated with acetylspiramycin or spiramycin during pregnancy. Therefore, the reason why the newborns did not develop congenital *T. gondii* infection might be that there were few mothers who were not truly primary infections with low *T. gondii*

IgG avidity index and that they had been appropriately treated with acetylspiramycin or spiramycin during pregnancy.

In newborns, serum *T. gondii* IgM positivity is used as a diagnostic tool for congenital *T. gondii* infection [4–6]. In our cohort study, one patient with *T. gondii* IgM positivity at birth did not develop congenital *T. gondii* infection. In a previous study, the sensitivity of *T. gondii* IgM measured by ELISA was approximately 75% (i.e., approximately 25% showed false-negative results) [4,11]. Moreover, in a Japanese case, umbilical cord blood was negative for *T. gondii* IgM and congenital toxoplasmosis was diagnosed based on intracranial calcification at birth and blood *T. gondii* IgG positivity at 18 months of age (i.e., a false-negative result) [12]. The most recent clinical manual by the Japan Agency for Medical Research and Development for mother-to-child infections in states that actual congenital *T. gondii* infection is approximately 25% of those with the *T. gondii* IgM positivity in newborn blood (i.e., a false-positive result) [1]. Therefore, the current *T. gondii* IgM test can show false-negative and false-positive results. Pediatricians or neonatologists should know that serum *T. gondii* IgM positivity in newborns may not be always indicative of a congenital *T. gondii* infection. A combination test of serum *T. gondii* IgM and IgA has been reported to increase the sensitivity to >90% [13], because IgM and IgA do not cross the placenta.

In newborns, blood *T. gondii* DNA positivity is also used as a diagnostic tool for congenital *T. gondii* infection [4–6]. In our cohort study, two patients with blood *T. gondii* DNA positivity at birth did not develop congenital *T. gondii* infection serologically. Multiplex nested PCRs using four loci in the *T. gondii* B1 gene, cyclin-dependent kinase, SAG5E, and bradyzoite surface antigen showed the similar results with blood *T. gondii* DNA positivity at birth, but without *T. gondii* IgG positivity at 12 months of age (4 of 4 cases) [3]. The reason for this is that the PCR analysis at birth could detect the genome even in non-active *T. gondii* or that the infant might have an immature immune response for *T. gondii* to develop congenital *T. gondii* infection. Further studies are needed to determine whether all cases with only blood *T. gondii* DNA positivity subsequently develop abnormal symptoms or findings. However, we believe that the newborn may not require medication if no clinical symptoms or findings of congenital toxoplasmosis are found at birth. In the study, neonatal blood-multiplex nested PCR revealed negative findings in two newborn patients, who later developed a congenital infection confirmed serologically, indicating false-negative results [3]. These data suggest that the blood *T. gondii* DNA test for detecting of congenital infection is limited as an *in vitro* diagnostic assay in newborns. *T. gondii* antigens, such as P35 surface antigen and dense granule antigens, have been identified as candidates for diagnosing congenital *T. gondii* infection [14–16]. Novel diagnostic assays using these antigens are urgently needed.

The two patients with blood *T. gondii* DNA positivity at birth were born to the mothers with a high *T. gondii* IgG avidity index. They were not diagnosed with congenital *T. gondii* infection, because of a negative conversion of *T. gondii* IgG within 12 months of age. Therefore, the mother would be a non-primary infection with persistent *T. gondii* IgM (the high *T. gondii* IgG avidity index was correct). Because the nested PCR is too sensitive for diagnosing an infection [17], the genome of non-active *T. gondii* might be detected, as aforementioned. This is a limitation for the diagnosis using our current semi-nested PCR for *T. gondii* B1 gene locus. A quantitative PCR assay may have lower sensitivity in comparison with the nested PCR, but a quantitative PCR can provide the diagnostic threshold value [18]. Because our current semi-nested PCR is difficult to perfectly diagnose with congenital *T. gondii* infection at birth, we have to follow-up the patients for over 12 months to diagnose with congenital *T. gondii* infection.

A first limitation of this study is that it included a small cohort of newborns with congenital *T. gondii* infection. However, the incidence of congenital toxoplasmosis in newborns is extremely rare in Japan (0.1–1 case per 100,000 live births based on the nationwide survey) [6]. In a nationwide survey of maternity facilities in 2011, 49% of the facilities performed maternal serum *T. gondii* antibody screening [8]. The maternal management policies of the participating institutions in the present study, however, performed *T. gondii* antibody screening using blood samples. *T. gondii* IgG and IgM and maternal therapy can prevent fetal infection when primary *T. gondii* infection is suspected during pregnancy (maternal therapy rate: 73%). Therefore, our results represent the current Japanese situation when performing a *T. gondii* antibody screening during pregnancy and maternal therapy. Second, it was not possible to completely determine whether the three patients with serum *T. gondii* IgM positivity or blood *T. gondii* DNA positivity were uninfected or asymptomatically infected at birth, because there is a case of congenital *T. gondii* infection whose the antibodies (both IgG and IgM) was not produced for more than 12 months after birth [12]. It is difficult to diagnose as non-infection or asymptomatic infection in the patients without clinical characteristic manifestations of congenital toxoplasmosis in current clinical *in vitro* diagnostic assays during infancy. Therefore, newborns born to *T. gondii* IgM-positive mothers should be followed-up for at least one year with the serological tests, ophthalmofundoscopy, and brain imaging examinations in current clinical practice.

5. Conclusions

This is the first prospective cohort study of newborns born to mothers with serum *T. gondii* IgM positivity during pregnancy in Japan. Among 71 *T. gondii* IgM-positive mothers, including one with triplets, 41% had low *T. gondii* IgG avidity index and 73% received maternal therapy. Although a few newborns born to the mothers were suspected of having congenital *T. gondii* infection using the current serum *T. gondii* IgM or blood *T. gondii* DNA test at birth, none developed congenital *T. gondii* infection during their follow-ups. Because current clinical *in vitro* diagnostic assays are difficult to perfectly diagnose with congenital *T. gondii* infection at birth, the follow-up for over 12 months is needed to diagnose with congenital *T. gondii* infection. It is essential to develop novel *in vitro* diagnostic assays to introduce appropriate treatments early after birth.

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Authorship statement

All authors meet the ICMJE authorship criteria. IM and HY contributed to the conception of this study. MH, AO, NN, KaK, AK, KeK, SO, KF, KT, and MD were responsible for the clinical data analysis. MS and MY were responsible for the laboratory analysis. All have authors contributed to the writing of the final manuscript.

Declaration of competing interest

None of the authors have any conflicts of interest to declare in this study.

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主論文の要約

氏名：土方　みどり
博士の専攻分野の名称：博士（医学）
論文題名：A prospective cohort study of newborns born to mothers with serum *Toxoplasma gondii* immunoglobulin M positivity during pregnancy
（トキソプラズマ IgM 陽性のハイリスク妊婦から出生した新生児を対象にした前向きコホート研究）

【背景】我が国の妊婦のトキソプラズマ抗体保有率は 2～10%と諸外国に比べて低く、妊婦の感染により胎児に影響を与える恐れがある。ハイリスク母体に対して治療を行うことで胎児感染予防効果があるとする報告や、より早期から母体治療を行うことで児の重症化予防につながるとも報告されている。先天性トキソプラズマ症の発症予防薬であるスピラマイシンは、2018 年 8 月に保険収載され、ようやく整備されつつ。一方、新生児への治療の目的は、精神発達遅滞やてんかん、網脈絡膜炎などの神経学的・眼科的合併症の発症の抑制にあり、早期診断のうえ、症候性先天性トキソプラズマ症に対するピリメタミンやスルファジアジン、ロイコボリン、ステロイドによる早期治療介入は予後改善が望めるとの報告がある。無症候性感染の場合でも時間が経過してから治療を開始してもすでに症状が固定化することがあるため、治療を行うことが推奨されているがいずれにしても治療に関して確立されていないのが現状である。

【目的】トキソプラズマ（Tg）妊婦スクリーニングで Tg-IgM 陽性妊婦から出生した児の先天性 Tg 感染症の発症頻度を明らかにする。

【方法】2013 年 1 月から 2020 年 12 月に日本大学と神戸大学の 2 施設で、Tg-IgM 陽性妊婦から出生した児を対象とし、臨床所見、Tg-IgM、Tg-IgG、眼底検査、頭部超音波検査を行い、1か月健診以降は一般診察に加えて抗体検査のフォローを行った。2017 年以降に出生した児については Tg DNA semi nested PCR 検査を行った。先天性 Tg 感染は臨床症状を伴う Tg-IgG 陽性例または生後 12 か月以上まで持続する Tg-IgG 陽性例と定義し、Tg-IgG が陰性化を確認できた時点で、「感染なし」と判断しフォローアップを終了とした。

【結果】Tg-IgM 陽性母体は 71 人で、66 人(93%)はTg-IgG抗体陽性、妊娠中にアセチルスピラマイシンまたはスピラマイシンによる治療を行ったのは 52 人（73%）であった。出生児は品胎を含む 73 人で、臨床所見を認めた症例はいなかったが、Tg-IgG 陽性・Tg-IgM 陽性は 1 人（1%）であった。PCR 検査は 2 人（6%：2/32 人）で陽性であったが、いずれも生後 12 時か月以内に児の IgG は陰性化し、先天性感染の発生はなかった。

【考察】

今回の前向きコホート研究の中から本研究の定義上、感染児の発生がなかったことに対し、トキソプラズマ IgG、IgM を用いた妊婦スクリーニングでの測定方法に関して、本研究ではプラテリア®トキソーIgG・IgM のキットを使用した ELISA 法で行った。ELISA 法の原理として、種々の抗原抗体反応の組み合わせを利用し、最終的に酵素標識した抗原あるいは抗体を反応系に組込んで、酵素活性を検出しており、なかでも最も特異度が高いサンドイッチ法で行われているため、使用前に抗原を精製する必要がなく、非常に特異的であるため、検査自体は他の方法よりも高い検出精度で行うことができていると考えられた。しかし、トキソプラズマ IgM 陽性妊婦のうち、およそ 7 割はパーシスタント IgM ないしは偽陽性で本当の妊娠中初感染ではなかった[1)2)3)]とあり、また妊娠中は IgG avidity 低値も persistent になることがあるとの報告[4)]もあり、真の初感染が少なかった可能性が考えられた。また本研究では約 7 割で妊婦治療が行われており、適切に妊婦治療を行ったために母子感染が予防された可能性が示唆された。

【限界点】

本研究の limitation として、1 つ目に IgM 抗体陽性に関して母体が持っている測定系に影響する成分について母体の血液検査を全例調べてはいなかったが、プラテリア®トキソ IgG、IgM 法は抗核抗体やリウマチ因子などの妨害物質を含んだ血清について全くの非特異反応が認められなかったことが報告[5)]されており、特異性に優れたキットであると考えられる。

2 つ目に 2 施設のみ研究であり、コホート小さいという点だが、新生児の先天性トキソプラズマ症の発生率は 2011 年の産科施設の全国調査で 49%の施設が母体血清 Tg 抗体スクリーニングを実施した下で、出生 10 万人あたり 0.1-1 例[6)]とされており、非常に稀な疾患であり、。今回 2 施設のみの検討ではありますが、トキソプラズマ妊娠管理マニュアルに従い妥当に評価できる結果と考えられた。3 つめに臨床症状のない DNA-PCR 陽性や IgM 陽性例が、治療が必要なのかを新生児期に判断することは困難であり、現行の検査方法のみでは 1 歳までの抗体検査や眼底検査、頭部画像検査のフォローが必要とした。P35 表面抗原や高密度顆粒抗原などの Tg 抗原が、先天性トキソプラズマ感染を診断するための候補として同定されており、早期診断を可能とする新たな抗原を用いた検査手段の開発が課題である。

【結論】出生時に先天感染を疑われた症例はあったが、いずれも先天感染を発症しなかった。現行の臨床で行われている血清学的検査、PCR 検査では出生児の治療が必要な感染かを判定するには限界がある。出生後の早期に適切な治療法を導入するためには DNA に代わる新たな分子マーカーを用いた早期診断を可能とする体外診断技術開発が今後の課題である[7)8)9)]。

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