Universal Screening With Use of Immunoglobulin G Avidity for Congenital Cytomegalovirus Infection

Tanimura, Kenji ; Tairaku, Shinya ; Morioka, Ichiro ; Ozaki, Kana ; Nagamata, Satoshi ; Morizane, Mayumi ; Deguchi, Masashi ; Ebina,…

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Universal screening with use of IgG avidity for congenital cytomegalovirus infection

Kenji Tanimura,[1] Shinya Tairaku,[1] Ichiro Morioka,[2] Kana Ozaki,[1] Satoshi Nagamata,[1] Mayumi Morizane,[1] Masashi Deguchi,[1] Yasuhiko Ebina,[1] Toshio Minematsu,[3] and Hideto Yamada[1]

K. Tanimura and S. Tairaku contributed equally to this manuscript.

[1]Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan

[2]Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan

[3]Reserch Center for Disease Control, Aisenkai Nichinan Hospital, Miyazaki, Japan

Corresponding author: Hideto Yamada M.D., Ph.D., Professor and Chairman

Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine,

7-5-1, Kusunoki-Cho, Chuo-ku, Kobe 650-0017, Kobe, Japan

Tel: +81-78-382-6000; fax: +81-78-382-5756

E-mail: yhideto@med.kobe-u.ac.jp

Alternate corresponding author: Kenji Tanimura M.D., Ph.D.

Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho Chuo-ku, Kobe 650-0017, Japan

Phone: +81-78-382-6000; fax: +81-78-382-5756

E-mail: taniken@med.kobe-u.ac.jp

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Running title: Screening for congenital CMV infection

Summary of the article's main point:

The universal screening of CMV IgG avidity in low-risk women is inefficient for the prediction of congenital CMV infection.

Abstract

Background

The aim of this prospective cohort study was to evaluate the efficacy of maternal screening for congenital cytomegalovirus (CMV) infection (CCI) using CMV IgG and IgG avidity index (AI).

Methods

Pregnant women underwent screening of CMV IgG and AI measurements. IgG-negative women underwent re-measurement of IgG after educational intervention. Women with an AI≤45% received further examinations, including measurement of CMV IgM. All newborns received polymerase chain reaction analyses of the urine, and CCI was diagnosed by the detection of CMV-DNA in the urine. Primary infection was defined as an AI<35% and/or positive IgM (>1.20 index). Serum samples from women with an AI>45% were stored, and the IgM levels were measured after delivery. The efficacy of AI and IgM for CCI screening was compared.

Results

One thousand five hundred and sixty-two (71.2%) women tested positive for IgG. In this study, ten newborns with CCI were detected. The presence of infection in three newborns from mothers with primary infection was predicted by screening of IgG and AI<35%. However, infection in seven newborns from women with non-primary infection could not be

predicted by screening of CMV IgG, AI<35% or IgM. The application of an AI<35% for CCI screening yielded 22.2% sensitivity, 95.0% specificity, 2.5% positive predictive value, and 99.5% negative predictive value and was similar to that of IgM (11.1% sensitivity, 93.2% specificity, 0.9% positive predictive value, and 92.7% negative predictive value).

Conclusions

Maternal screening using CMV IgG and AI can identify pregnancies with CCI from primary infection, but overlooks a number of those from non-primary infection.

Human cytomegalovirus (CMV) is one of the most common causes of mother-to-child infection worldwide. The prevalence of congenital CMV infection is 0.2–2.4% in newborns in developed countries [1-3], and 10–15% of infected fetuses show symptomatic congenital CMV infection at birth. The clinical manifestations of CMV infection, including fetal growth restriction, low birth weight, and central nervous system and multiple organ involvement, can be so severe that they lead to a high perinatal mortality rate and major neurological sequelae in approximately 90% of surviving infants. In addition, 10–15% of infants with asymptomatic congenital infection develop long-term sequelae, including progressive sensorineural hearing difficulty, and mental retardation [4]. It was recently demonstrated that early diagnosis and early intervention with antiviral drugs can improve neurological outcomes in symptomatic congenital CMV-infected infants [5, 6]. Therefore, prenatal detection of pregnancies at high-risk of congenital CMV infection may be important for the accurate diagnosis of infected newborns and early commencement of antiviral therapy on symptomatic newborns.

The risk of virus transmission to the fetus is highest during pregnancy for women with primary CMV infection [7, 8]. Maternal serological screening is considered effective for detecting pregnant women with primary CMV infection [9]. For this purpose, maternal blood tests for CMV-specific Immunoglobulin (Ig) G and CMV-specific IgM are widely employed [9]. Especially, testing for maternal serum CMV IgM is commonly used to identify primary

infection. Positive results of CMV IgM yield high sensitivity, low specificity, and low positive predictive value for the detection of primary infection [1, 10]. Pregnant women can produce CMV IgM during reinfection and reactivation of the virus [10]. In addition, CMV IgM may persist for months after primary infection and has been detected in some pregnant women 6–9 months after the primary infection [11]. However, serum CMV IgG avidity tests are highly specific and sensitive for the detection of primary infection [12], and CMV IgG avidity is considered the gold standard for the identification of primary infection during pregnancy [13-16]. Nonetheless, no studies have performed universal screening based on CMV IgG avidity during pregnancy, and the efficacy of this parameter for predicting congenital CMV infection is still undetermined. A prospective cohort study was conducted to evaluate the efficacy of maternal universal screening based on CMV IgG avidity for the prediction of congenital CMV infection, as compared with that based on CMV IgM.

Methods

Study design and participants

The institutional review board at Kobe University Hospital approved this prospective cohort study (reference no. 922), and written informed consent was obtained from all participants. All pregnant women who visited or were referred to Kobe University Hospital between February 2010 and April 2016 underwent maternal serological CMV screening.

These women underwent initial blood screening for CMV IgG before 22 gestational weeks (GW). CMV IgG-negative women received educational intervention to prevent primary infection during the rest of their pregnancies and CMV IgG levels were measured again at 34–36 GW. CMV IgG seroconversion in IgG-negative women was regarded as primary CMV infection during pregnancy. All CMV IgG-positive women were tested for serum IgG avidity 2–4 weeks after the initial CMV IgG measurements. Pregnant women who were referred after 22 GW received simultaneous CMV IgG and IgG avidity testing at the first visit.

All women who had a CMV IgG avidity index (AI) ≤ 45% underwent a series of examinations for CMV infection. These examinations included the measurement of serum CMV IgM levels, blood antigenemia (C7-HRP) testing (CMV antigen test “TEIJIN”, Teijin, Tokyo, Japan), and quantitative polymerase chain reaction (PCR) analyses for CMV-DNA in maternal serum, urine, and uterine cervical secretion. In the present study, women who had

an AI < 35% and/or a positive result for CMV IgM were considered to have primary CMV infection during pregnancy. Women with an AI > 45% were considered to have non-primary infection during pregnancy, and their serum were once stored at −80°C. Serum CMV IgM levels were later measured to evaluate their efficacy for the prediction of congenital CMV infection as compared with the efficacy of IgG avidity.

All newborns received polymerase chain reaction (PCR) analyses of the urine, and congenital infection was diagnosed with the detection of CMV-DNA in the urine. Women who were referred because of positive results for CMV IgM, those who were referred after 37 GW, and those who had deliveries in other hospitals were excluded from the present study.

Procedures

Serological tests for CMV-specific IgG (negative: ≤ 230, borderline: 230–240, and positive: ≥ 240) were performed using the Enzygnost assay (Siemens Healthcare Diagnostics, Tokyo, Japan). CMV-specific IgM (negative: < 0.8, borderline: 0.8–1.2, and positive: > 1.2 index) was measured using enzyme immunoassay kits produced by Denka Seiken (Tokyo, Japan). CMV-specific IgG avidity was measured as described previously (Aisenkai Nichinan Hospital, Miyazaki, Japan) [17]. The C7-HRP assay (CMV antigen test “TEIJIN”, Teijin, Tokyo, Japan) (positive: ≥ 1 positive cell/slide) was used for CMV antigenemia testing. Real-time PCR analyses for CMV-DNA in maternal serum, urine, and uterine cervical

secretion were performed (Special References Laboratories, Tokyo, Japan) (positive ≥ 1.0×10^2 copies/ml).

Urine samples were collected from newborns onto filter paper within 1 week after birth and the presence of CMV-DNA was assessed as described previously[18]. Liquid urine samples were obtained from CMV-positive newborns, and the CMV-DNA copy number was determined using real-time quantitative PCR. The presence of congenital CMV infection was confirmed by positive PCR results for CMV-DNA in the liquid urine samples of the newborns [19]. All newborns with positive PCR results for CMV-DNA in the urine received a workup to identify the symptoms of congenital CMV infection. Ophthalmoscopy, cerebral ultrasound, physical and neurological examinations, head computed tomography, head magnetic resonance imaging, and an auditory brain-stem response (ABR) test were performed. Symptomatic congenital CMV infection was diagnosed when newborns with positive PCR results had microcephaly, hepatosplenomegaly/hepatitis, thrombocytopenia, abnormality of brain images, CMV-associated retinopathy, or abnormal ABR [19, 20].

The efficacy of maternal serological screening based on IgG AI (cutoff values: ≤ 45% or < 35%) and positive results for CMV IgM (> 1.2 index) for the prediction of congenital CMV infection was compared.

Statistical analysis

Clinical characteristics and laboratory findings were compared between pregnancies with congenital CMV infection and those without congenital infection. Differences between the two groups were analyzed using the Mann–Whitney U test, Fisher's exact test, and the chi-square test. Statistical significance was considered present at p values less than 0.05. All statistical analyses were performed using SPSS software, version 19 (SPSS Inc., Chicago, IL, USA).

Results

A flowchart of the subjects in this prospective cohort study is shown in Figure 1. Four newborns with symptomatic congenital CMV infections (0.18%) and six with asymptomatic congenital infections (0.27%) were identified in the study.

Table 1 shows the clinical characteristics of the 2,193 women evaluated. Seven hundred and forty-six (34.0%) were referrals. These referred women had obstetric complications including multiple pregnancies, low-lying placenta, placenta previa, threatened premature labor and fetal growth restriction (n=339, 45.4%), maternal complications including hypertensive disorders, diabetes mellitus, autoimmune diseases and thyroid diseases (n=218, 29.2%), and their move to parent's home (n=189, 25.3%).

The proportion of the presence of maternal fever or flu-like symptoms in ten women who had congenital CMV infection was higher than that in 2,183 women with no congenital infection ($p < 0.05$). Maternal age, gravidity or parity, body mass index prior to pregnancy, the proportion of referrals, GW at the initial CMV IgG measurement, GW at delivery, and the birth weight of newborns were not significantly different between the two groups.

The results of maternal serological screening are shown in Figure 1. Six hundred and thirty-one pregnant women (28.8%) had negative results for CMV IgG during the initial tests, and five (0.79%) of the 631 women experienced IgG seroconversion during pregnancy. Among the five women with IgG seroconversion, one delivered a newborn with

asymptomatic congenital CMV infection. Five (1.1%) of 455 women with negative results for CMV IgG before 25 GW experienced IgG seroconversion between 25 GW and 36 GW.

One thousand five hundred and sixty-two pregnant women (71.2%) of the 2,193 had positive results for CMV IgG, and underwent measurements for CMV IgG AI. One hundred and eighty-three pregnant women with an AI ≤ 45% underwent CMV IgM measurements, C7-HRP tests, and PCR analyses for CMV-DNA in maternal serum, urine, and uterine cervical secretion.

Among 183 women with an AI ≤ 45%, 79 had an AI < 35%, 15 had positive results for CMV IgM, three had positive PCR results for CMV-DNA in uterine cervical secretion, two had positive PCR results in maternal serum, and none had a positive PCR result in maternal urine or a positive C7-HRP result. Eighty-eight (48.1%) of the 183 pregnant women with an AI ≤ 45% had an AI < 35% and/or positive results for CMV IgM, and were classified as having primary CMV infection during pregnancy. Two women had newborns with congenital CMV infection. One mother with symptomatic congenital infection had an AI < 35%, positive CMV IgM, and a positive PCR result in uterine cervical secretion. The other woman with asymptomatic congenital infection had an AI < 35% and a borderline result for CMV IgM.

Table 2 shows the clinical characteristics and laboratory findings for three women who had newborns with congenital CMV infection due to primary CMV infection during

pregnancy.

Among 1,379 women with an AI > 45%, 92 had positive results for CMV IgM. The remaining 1,287 women with an AI > 45% and a CMV IgM ≤ 1.2 index were classified as having non-primary CMV infection (Figure 1). Seven (0.54%) of the 1,287 women with an AI > 45% together with a CMV IgM ≤ 1.2 index had newborns with congenital CMV infection, including three with symptomatic and four with asymptomatic infections.

Table 3 shows a comparison of the efficacy of maternal blood screening for the prediction of congenital CMV infection. Screening modalities included an AI ≤ 45%, an AI < 35%, and positive results for CMV IgM (> 1.2 index). The efficacy of maternal screening based on AI <35% was similar to that based on CMV IgM.

Figure 2 shows the serological status of 2,193 pregnant women and ten newborns with symptomatic/asymptomatic congenital CMV infection.

Discussion

This study evaluated 2,193 pregnant women. One (20.0%) of the five women who experienced IgG seroconversion had a newborn with congenital CMV infection, which was caused by primary CMV infection during pregnancy. Two (2.3%) of the 88 women who had an AI < 35% and/or positive results for CMV IgM had newborns with congenital infection, likely caused by primary CMV infection. In addition, seven (0.5%) of the 1,287 women who had an AI > 45% and a CMV IgM ≤ 1.2 index had newborns with congenital infection, probably caused by non-primary CMV infection (Figure 1). It has been reported that 30–80% of women who experience IgG seroconversion during pregnancy have newborns with congenital CMV infection [21-23], and 0.5–1.0% of women with non-primary CMV infection have newborns with congenital CMV infection [24, 25]. These values for the transmission rates of CMV to the fetus are compatible with the results of the present cohort study.

Many investigators have reported that the measurement of CMV IgM and CMV IgG avidity is useful for the detection of maternal primary CMV infection during pregnancy [9, 15, 26-28]. The level of CMV IgM and/or CMV IgG avidity for the prediction of congenital CMV infection has also been assessed in high-risk pregnant women who experience IgG seroconversion, women who have positive results for CMV IgM, and women who have prenatal ultrasound abnormalities suggestive of congenital CMV infection [16, 29-31]. The

investigators have concluded that positive CMV IgM and low AI in maternal blood yield positive predictive values of 9.8% [30] and 16.7–50% [16, 28, 30], respectively, for congenital CMV infection.

In this prospective cohort study, we determined for the first time the efficacy of maternal universal screening based on CMV IgG avidity for the prediction of congenital CMV infection in low-risk women. A cutoff value of CMV IgG AI < 35% yielded 22.2% sensitivity, 95.0% specificity, 2.5% positive predictive value, 99.5% negative predictive value, and 94.6% accuracy for the occurrence of congenital CMV infection. The efficacy of maternal screening based on an AI < 35% was similar to that based on the CMV IgM (> 1.2 index). The positive predictive values of low AI for predicting congenital CMV infection in the present study are much lower than those in previous studies [16, 27-30, 32-35]. These studies enrolled less than 200 women of which 30–80% had primary CMV infection during their pregnancies. CMV IgG avidity measurements may not be useful as a universal test for the prediction of congenital CMV infection in low-risk pregnant women.

Nearly all symptomatic congenital CMV infections are believed to be caused by a primary infection either during or just before pregnancy [8]. In the present study, seven (0.5%) of the 1,287 pregnant women with an AI > 45% coupled with either negative or equivocal results for CMV IgM (≤ 1.2 index), defined as non-primary CMV infection, had newborns with congenital infection (Figure 2). Three (42.9 %) of the seven newborns from

mothers with non-primary CMV infection had symptomatic CMV infection. Mothers with non-primary CMV infection were also at risk of giving birth to newborns with symptomatic congenital infection.

Nearly all pregnant women with primary infection seem to have both positive results for CMV IgM and low IgG avidity indices [28, 35]. Our previous study targeted high risk pregnancies, and found that forty-five (15%) of the 300 women with positive results for CMV IgM showed low AI (< 35%) [38]. The present study enrolling low risk pregnancies excluded referrals with positive results for CMV IgM, and showed only six (3.3%) of one hundred eighty women with positive CMV IgM (> 1.2 index) had low AI (< 35%). Therefore, the number of pregnant women who truly experienced primary infection during pregnancy in the present study might be much fewer than that in previous studies.

Prophylactic education for women who are not immune to CMV during pregnancy reduces the incidence of IgG seroconversion [36]. In the present study, although prophylactic education was provided to pregnant women with negative results for CMV IgG, 1.1 % of these women experienced IgG seroconversion during pregnancy. The incidence of CMV IgG seroconversion was similar to that (1–2%) reported in previous studies [37].

Recently, we reported that the presence of CMV-DNA in uterine cervical secretion was predictive of the occurrence of congenital CMV infection in high-risk pregnant women who were positive for CMV IgM [38]. In this study, three of the 183 pregnant women with an

AI ≤ 45% had positive PCR results for CMV-DNA in uterine cervical secretion, and one (33.3%) had congenital CMV infection. The maternal screening of congenital CMV infection based on PCR assays for CMV-DNA in the uterine cervical secretion may be inefficient in the low-risk population.

The present study demonstrates, for the first time, that the efficacy of maternal universal screening based on CMV IgG avidity for congenital CMV infection was similar to that based on CMV IgM. However, newborns with congenital infection from mothers with non-primary infection and those from mothers who had negative results but experienced CMV IgG seroconversion during pregnancy were overlooked. The universal screening of CMV IgG avidity in low-risk women is inefficient for the prediction of congenital CMV infection, since the majority of newborns with congenital infection cannot be anticipated by measuring a high AI, which is not considered a source of reassurance. Universal screening of PCR tests for CMV-DNA in newborn urine can identify all newborns with congenital CMV infection. If universal CMV screening of newborn urine is not possible, PCR tests for CMV-DNA in the urine should be performed for newborns delivered by women who carry a high risk of congenital CMV infection. A high risk of congenital CMV infection is indicated by CMV IgG seroconversion, an AI < 35%, positive CMV IgM, and/or ultrasound abnormality. However, it is still difficult to detect newborns with congenital infection born to mother with non-primary infection.

There are some potential limitations associated with the present study. Kobe University Hospital has a maternal-fetal center where many pregnant women with a variety of complications are referred from clinics and hospitals. Therefore, the gestational age at the first CMV IgG measurement varied widely. This might have partially influenced the results of the present study.

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Potential conflicts of interest

All authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the Editors consider relevant to the content of the manuscript have been disclosed.

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Figure legends

Figure 1: Flow diagram for the study participants, results of maternal serological screening, and occurrence of congenital CMV infection among all subjects.

During the study period, 2,634 pregnant women underwent blood screening for CMV IgG and CMV IgG avidity. After applying the exclusion criteria, 2,193 pregnant women were enrolled in this study. A total of 631 (28.8%) pregnant women had negative results for CMV IgG, and five (0.8%) of these women had IgG seroconversion. One of the five women with IgG seroconversion had asymptomatic congenital CMV infection. A total of 1,562 (71.2%) of the 2,193 pregnant women had positive results for CMV IgG. A total of 183 pregnant women with an AI $\leq$ 45% underwent measurements of CMV IgM and C7-HRP, and PCR analyses for CMV-DNA in maternal serum, urine, and uterine cervical secretion. Eighty-eight (48.1%) of the 183 pregnant women with an AI $\leq$ 45% had an AI < 35% and/or positive results for CMV IgM, and two had congenital infection. A total of 1,379 women had an AI > 45%, and seven of these women had congenital CMV infection.

CMV, cytomegalovirus; IgG, immunoglobulin G; IgM, immunoglobulin M; GW, gestational weeks; AI, avidity index; PCR, polymerase chain reaction.

Figure 2: Serological status of 2,193 pregnant women and ten newborns with

symptomatic/asymptomatic congenital CMV infection.

Numbers in Venn diagram represent the number of pregnant women who had unique or non-unique results in serological tests.

●, Newborns with symptomatic congenital CMV infection (n = 4). ○, Newborns with asymptomatic congenital infection (n = 6).

Table 1. Clinical characteristics of 2,193 women

	All n = 2,193	Congenital infection n = 10	No congenital infection n = 2,183	*p* value
Age, years old	33.2 ± 5.5	32 (28–40)	34 (14–49)	0.5
Gravidity	1.3 ± 1.5	1 (0–3)	1 (0–13)	0.3
Parity	0.6 ± 0.8	0 (0–2)	0 (0–6)	0.4
BMI prior to pregnancy, kg/m^2	21.4 ± 3.7	22.9 (17.4–27.7)	20.5 (13.8–44.9)	0.1
Referral	34.0%	60.0%	33.9%	0.1
Maternal fever or flu-like symptoms	16.6%	50.0%	16.4%	0.02
Gestational weeks at the initial CMV IgG measurements	17.9 ± 8.5	17 (8–32)	16 (4–36)	0.4
Gestational weeks at delivery	37.4 ± 2.9	36 (26–40)	38 (22–42)	0.1
Birth weight, g	2,727.7 ± 634.2	2,362 (1,080–3,320)	2,820 (314–4,748)	0.06

BMI, body mass index.

Data are expressed as the mean ± standard deviation, median (range), or %.

Table 2. Three women who had newborns with congenital CMV infection due to primary CMV infection

Case	Age, years old	Gravidity / parity	Gestational weeks at flu-like symptoms	CMV IgG (Gestational weeks at measurements)	IgG avidity index (%)	CMV IgM (index)	CMV-DNA in maternal serum/ urine/ uterine cervical secretion	C7-HRP tests	Gestational weeks and days at birth	Birth weight, g	Symptoms of the newborn
1	35	1/0	28	24,290 (30)	23.1	1.05	Neg./ Neg./ Neg.	Neg.	39 w 6 d	2,784	None
2	28	1/1	16	1,726 (27)	3.6	6.6	Neg./ Neg./ +	Neg.	32 w 6 d	1,396	Abnormal ABR, small-for-gestational age
3	31	1/0	None	Neg.(17)/ 9,600 (35)	N.A.	N.A.	N.A.	N.A.	36 w3 d	3,238	None

Neg., negative; ABR, auditory brain stem response; N.A., not applicable.

Table 3. Comparison of efficacy of maternal blood screening for prediction of congenital CMV infection

Maternal blood screening	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
CMV IgG avidity index ≤45%	22.2	88.4	1.1	99.5	87.9
CMV IgG avidity index <35%	22.2	95.0	2.5	99.5	94.6
CMV IgM >1.2 index	11.1	93.2	0.9	99.5	92.7

PPV, positive predictive value; NPV, negative predictive value.

Accuracy (%) is calculated by the following formula: (number of true positive population + number of true negative population) / number of all populations.

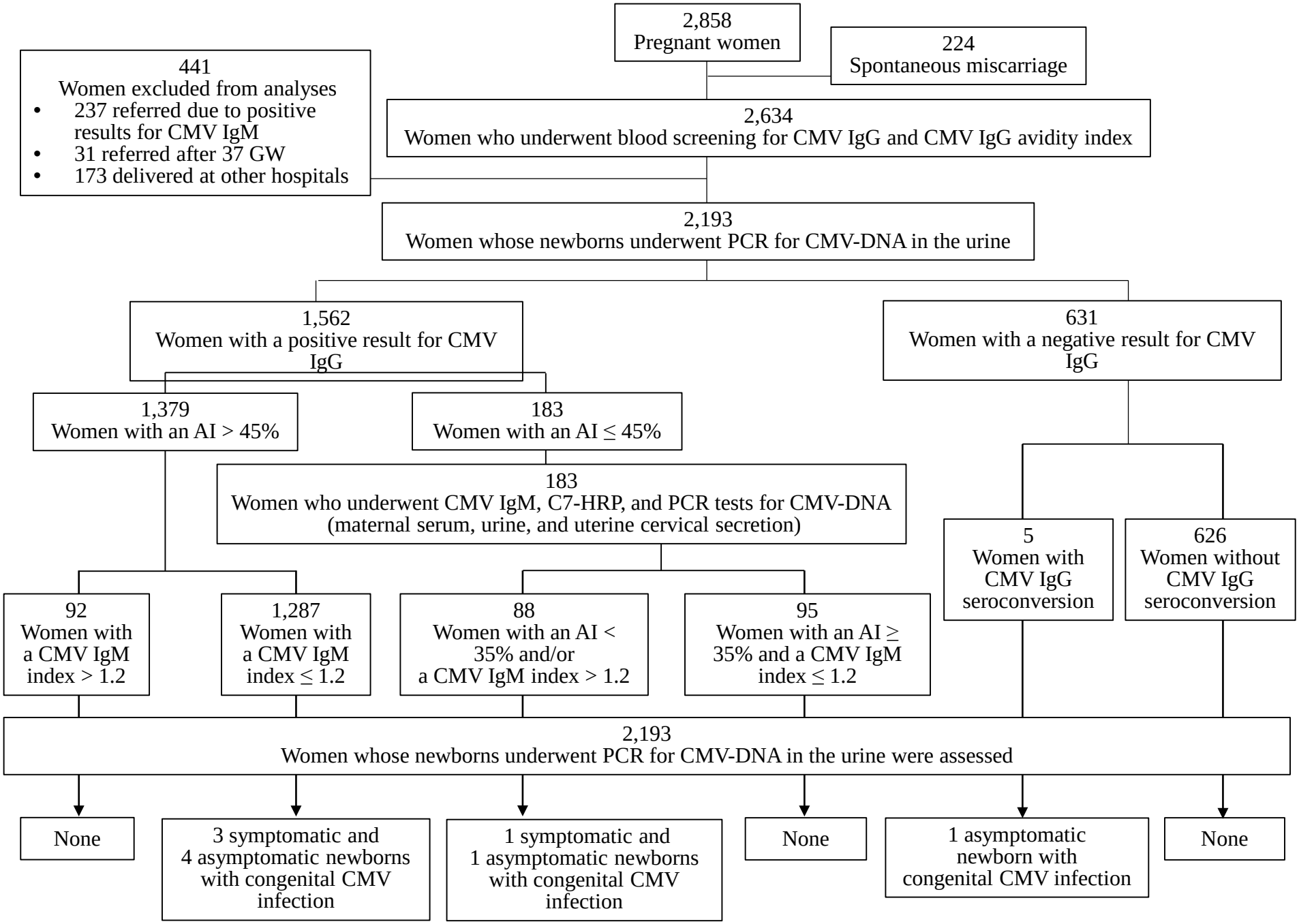
2,858
Pregnant women
224
Spontaneous miscarriage
441
Women excluded from analyses
• 237 referred due to positive results for CMV IgM
• 31 referred after 37 GW
• 173 delivered at other hospitals
2,634
Women who underwent blood screening for CMV IgG and CMV IgG avidity index
2,193
Women whose newborns underwent PCR for CMV-DNA in the urine
1,562
Women with a positive result for CMV IgG
631
Women with a negative result for CMV IgG
1,379
Women with an AI > 45%
183
Women with an AI ≤ 45%
183
Women who underwent CMV IgM, C7-HRP, and PCR tests for CMV-DNA (maternal serum, urine, and uterine cervical secretion)
5
Women with CMV IgG seroconversion
626
Women without CMV IgG seroconversion
92
Women with a CMV IgM index > 1.2
1,287
Women with a CMV IgM index ≤ 1.2
88
Women with an AI < 35% and/or a CMV IgM index > 1.2
95
Women with an AI ≥ 35% and a CMV IgM index ≤ 1.2
2,193
Women whose newborns underwent PCR for CMV-DNA in the urine were assessed
None
3 symptomatic and 4 asymptomatic newborns with congenital CMV infection
1 symptomatic and 1 asymptomatic newborns with congenital CMV infection
None
1 asymptomatic newborn with congenital CMV infection
None

Figure1.

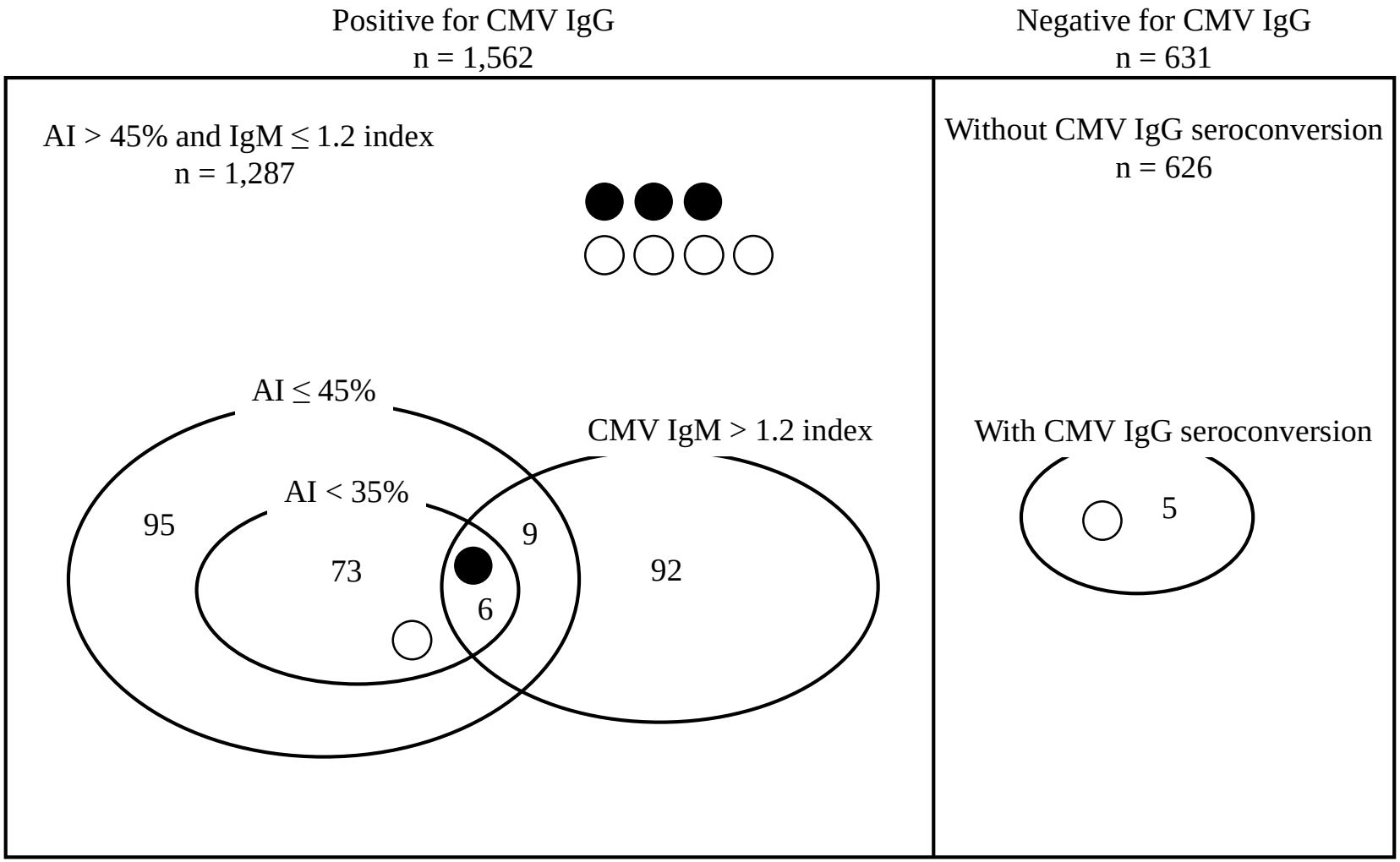
Positive for CMV IgG
n = 1,562
Negative for CMV IgG
n = 631
AI > 45% and IgM ≤ 1.2 index
n = 1,287
Without CMV IgG seroconversion
n = 626
AI ≤ 45%
CMV IgM > 1.2 index
With CMV IgG seroconversion
AI < 35%
95
73
9
6
92
5

Figure 2.