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Association between Chemiluminescent Microparticle Immunoassay Signal-to-cutoff Ratio and Active Stage of Syphilis in Thai Pregnant Women

Piyachat Sakunborrirak, M.D.*,
Chenchit Chayachinda, M.D.*,
Jaruda Kobkitjaroen, M.D.**

** Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
*** Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

ABSTRACT

Objectives: To demonstrate the association between chemiluminescent microparticle immunoassay (CMIA) signal-to-cutoff (S/CO) ratio and active stage of syphilis as well as adverse perinatal outcomes.

Materials and Methods: A retrospective chart review was conducted in pregnant women with reactive CMIA (CMIA S/CO ratio ≥ 1) as the primary test in the reverse algorithm of syphilis screening. The participants were categorized into three groups: Group 1 CMIA+ venereal disease research laboratory (VDRL)+; Group 2 CMIA+ VDRL- *Treponema pallidum* haemagglutination test (TPHA)+; and Group 3 CMIA+ VDRL- TPHA-. CMIA S/CO ratio and perinatal outcomes were compared. Active stage of syphilis refers to having venereal disease research laboratory (VDRL) titer ≥ 1:8.

Results: Eighty-three out of 8,987 (0.92%) pregnant women who came for antenatal care at Siriraj Hospital between January 2020 and February 2021 were reactive for CMIA. Two twin gestations were excluded. The CMIA S/CO ratio was highest in group 1 (n = 39) at 23.1 ± 5.5, followed by 16.1 ± 5.2 in group 2 (n = 25) and 2.1 ± 3.2 in group 3 (n = 17), $p < 0.001$. Perinatal outcomes were not different among the groups, except for congenital syphilis (CS). All six neonates with CS were born to the participants in group 1 who had CMIA S/CO ratio ≥ 19.9. Most of the participants who delivered neonates with CS were diagnosed with syphilis in third trimester and had VDRL titer ≥ 1:8.

Conclusion: Instances of adverse perinatal outcomes and active stage of maternal syphilis were more frequent in pregnant women with higher CMIA S/CO ratio. The use of CMIA S/CO ratio as an adjunct to clinical evaluation may provide additional benefits to the syphilis screening.

Keywords: chemiluminescent microparticle immunoassay, perinatal outcomes, syphilis, screening, signal-to-cutoff ratio.

Correspondence to: *Jaruda Kobkitjaroen, M.D., Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. E-mail: jaruda.kob@mahidol.ac.th*

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ความสัมพันธ์ระหว่างค่าสัดส่วน chemiluminescent microparticle immunoassay กับระยะแพร่กระจายเชื้อมากของโรคซิฟิลิสในหญิงตั้งครรภ์ไทย

ปิยฉัตร สกุลบริรักษ์, เจนจิต ฉายะจินดา, จารุดา กอบกิจเจริญ

บทคัดย่อ

วัตถุประสงค์: *เพื่อแสดงความสัมพันธ์ระหว่างสัดส่วนค่า chemiluminescent microparticle immunoassay (CMIA) signal-to-cutoff (S/CO) กับระยะแพร่กระจายเชื้อมากของโรคซิฟิลิสในหญิงตั้งครรภ์ไทย และผลต่อทารกแรกเกิด*

วัสดุและวิธีการ: *ทำการศึกษาแบบ retrospective chart review ในหญิงตั้งครรภ์ที่ได้รับการตรวจคัดกรองโรคซิฟิลิสโดยการตรวจเลือดด้วย CMIA เป็นวิธีแรก และได้ผล CMIA เป็นบวก (CMIA S/CO ratio ≥1) จากนั้นจะตรวจยืนยันด้วย Venereal Disease Research Laboratory (VDRL) หากผลไม่ตรงกัน จะทำการตรวจยืนยันอีกครั้งด้วยวิธี Treponemal pallidum hemagglutination (TPHA) มีแบ่งกลุ่มการศึกษาเป็น 3 กลุ่ม ดังนี้ กลุ่ม 1 CMIA+ VDRL+ กลุ่ม 2 CMIA+ VDRL- TPHA+ และกลุ่ม 3 CMIA+ VDRL- TPHA- จากนั้นหาความสัมพันธ์ระหว่างสัดส่วนค่า chemiluminescent microparticle immunoassay (CMIA) signal-to-cutoff (S/CO) กับระยะแพร่กระจายเชื้อมากของโรคซิฟิลิสในหญิงตั้งครรภ์ไทย และผลต่อทารกแรกเกิด โดยที่ระยะแพร่กระจายเชื้อมากของโรคซิฟิลิสหมายถึงกลุ่มที่มีระดับ VDRL titer ≥1:8*

ผลการศึกษา: *จากจำนวนหญิงตั้งครรภ์ที่มาฝากครรภ์และได้รับการตรวจคัดกรองโรคซิฟิลิส ที่โรงพยาบาลศิริราชในช่วงเดือน มกราคม พ.ศ.2563 ถึง เดือนกุมภาพันธ์ พ.ศ.2564 ทั้งหมด 8,987 ราย พบว่ามีหญิงตั้งครรภ์จำนวน 83 ราย (ร้อยละ 0.92) ตรวจพบ CMIA เป็นบวก มีจำนวนหญิงตั้งครรภ์สองรายถูกนำออกจากการศึกษาเนื่องจากเป็นการตั้งครรภ์แฝด ผลการศึกษาพบว่าในกลุ่มที่ 1 (จำนวน 39 คน) มีค่าสัดส่วน CMIA S/CO ที่สูงที่สุด โดยค่าเฉลี่ยคือ 23.1 ± 5.5 รองลงมาคือในกลุ่มที่ 2 (จำนวน 25 คน) และกลุ่มที่ 3 (จำนวน 17 คน) ที่มีค่า 16.1 ± 5.2 และ 2.1 ± 3.2 ตามลำดับ, $p < 0.001$ ผลของทารกแรกเกิดไม่ได้มีความแตกต่างกันอย่างมีนัยสำคัญในทั้งสามกลุ่ม ยกเว้นพบทารกทั้งหมดจำนวน 6 รายที่เป็นโรคซิฟิลิสแต่กำเนิดโดยทั้งหมดเกิดจากมารดาในกลุ่มที่ 1 ที่มีค่าสัดส่วน CMIA S/CO ที่ ≥19.9 และหญิงตั้งครรภ์ส่วนใหญ่ที่คลอดทารกที่ได้รับการวินิจฉัยว่าเป็นโรคซิฟิลิสแต่กำเนิดพบว่าได้รับการวินิจฉัยว่าเป็นซิฟิซิสในไตรมาสที่สาม และตรวจพบว่ามีค่า VDRL titer ≥1:8*

สรุป: *ภาวะแทรกซ้อนของทารกแรกเกิดและระยะแพร่เชื้อมากของโรคซิฟิลิสพบได้มากกว่าในหญิงตั้งครรภ์ที่ตรวจพบว่ามีค่าสัดส่วน CMIA S/CO ที่สูง และการใช้ค่าสัดส่วน CMIA S/CO เสริมในการตรวจคัดกรองโรคซิฟิลิสอาจเป็นประโยชน์ในการดูแลรักษาทางคลินิก*

คำสำคัญ: *chemiluminescent microparticle immunoassay, ผลของทารกแรกเกิด, ซิฟิลิส, สัดส่วน*

Introduction

Syphilis is a destructive infectious disease caused by *Treponema pallidum* subspecies *pallidum*. It is a sexually transmitted infection, spreading mainly through lesion contact. In pregnant women, *T. pallidum* can infect in-utero fetus through placenta causing congenital syphilis (CS). Over the past 10 years, CS in Thailand is on the rise with an increase in the incidence rate from 7.48 per 100,000 live births in 2011 to 161.78 per 100,000 live births in 2021[1]. The risk factors include ineffective syphilis screening methods, failure to complete course of treatment, teenage pregnancy, commencing treatment for less than four weeks before delivery, and reinfection during pregnancy[2].

Diagnosis of syphilis can be done either by detection of the etiologic organism or serological diagnosis[3, 4]. The serological diagnosis of syphilis, which requires both treponemal and nontreponemal tests, is widely applied due to the quick resolution of the clinical presentations. Nontreponemal test detects antibodies to lipoidal antigens being released from damaged host tissues which occur most obviously during early stage of syphilis. Therefore, its titers indicate disease activity. In other words, higher titers relate to the more active stage of syphilis[5]. On the contrary, treponemal tests detect specific antibodies against *T. pallidum*, which remain detectable for life. A positive treponemal test may refer to current or past infection. At the moment, the clinical use of treponemal test is only reactive or non-reactive result, but their antibody titers have no clinical application[3, 4].

Formerly, the screening protocol of syphilis starts with a non-treponemal test which was called the 'traditional sequence'. A treponemal test will be done if only the primary test is reactive. This results in a big missing portion of patients in very early and late stage of syphilis[6-8]. Later, the reverse sequence which switches the order of the tests has shown a greater screening performance. It has been recommended by the World Health Organization (WHO)[9] and the Thai national guideline[10] as a pivotal tool to eliminate CS.

Chemiluminescent microparticle immunoassay (CMIA) is the recommended primary treponemal test in Thailand due to its high sensitivity[11, 12]. If CMIA is positive, a nontreponemal test will be performed to confirm the diagnosis of syphilis, which is either rapid plasma reagin (RPR) or venereal disease research laboratory (VDRL). In case of discordant results, false positive CMIA should be excluded by performing the second treponemal test, such as *Treponema pallidum* hemagglutination (TPHA). A reactive second treponemal test indicates syphilis infection, while a non-reactive one indicates that syphilis is unlikely. CMIA is run by an automated system and reported in a quantitative result with a signal-to-cutoff (S/CO) ratio. This ratio varies with the amount of antibody in a sample. The manufacturer defines reactive CMIA as the CMIA S/CO ratio at ≥1. Some studies reported the optimal cutoff points set at higher S/CO ratio to decrease false positive rate[13, 14]. Moreover, the association of the higher CMIA S/CO ratio and poorer neonatal outcomes were reported[15]. Therefore, the present study aims to demonstrate the association between CMIA S/CO ratio and clinical stage of syphilis during pregnancy and perinatal outcomes of pregnancy with syphilis.

Materials and Methods

This retrospective chart-review study was conducted at Siriraj Hospital, Thailand between January 2020, and February 2021. It was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University. (COA no. Si 938/2021).

Participants

All pregnant women who came for antenatal care (ANC) at Siriraj Hospital between January 2020 and February 2021; and had reactive CMIA were included in the study. Those who delivered at Siriraj Hospital were included for the analysis of the secondary outcomes. Exclusion criterion was multifetal pregnancy.

Laboratory investigations for syphilis screening and management

All laboratory investigations were conducted at the ISO 15189-accredited Laboratory at Siriraj Hospital. They were performed by using commercial reagent and following manufacturer instruction. The CMIA, using ARCHITECT Syphilis TP (Abbott Laboratories, Abbott Park, Illinois, USA), was performed to initially screen for antibodies to *T. pallidum*. CMIA was an immunoassay run by an automated instrument and reported quantitatively using S/CO ratio and considered reactive when the ratio was ≥1 according to manufacturer recommendation. Sera with reactive CMIA were later tested using the Venereal Disease Research Laboratory (VDRL) (Becton, Dickinson and Company, Sparks, Maryland, USA), which is a non-treponemal test. VDRL was a flocculation test performed manually and reported in titers. Those with reactive VDRL (CMIA+, VDRL+) were diagnosed with syphilis (Group 1). Those with discordant results (CMIA+, VDRL-) were further tested by *Treponema pallidum* hemagglutination (TPHA) (Rapid Labs, Colchester, Essex, UK). TPHA was an indirect hemagglutination assay performed manually and reported qualitatively as reactive and non-reactive. Those with reactive TPHA (CMIA+, VDRL-, TPHA+) were diagnosed with late syphilis (Group 2). Those with non-reactive TPHA (CMIA+, VDRL-, TPHA-) were considered as having false positive CMIA (Group 3)[4].

All pregnant women diagnosed with syphilis were treated in accordance with the standard guideline provided by the Center for Disease Control and Prevention and the Thai guideline[3, 16]. In the labor room, their blood was drawn for VDRL testing regardless of previous treatment to compare with that in neonate's blood. All neonates born to pregnant women with syphilis were examined thoroughly for the evidence of congenital syphilis and placentas were sent for pathological examination and detection of *T. pallidum*.

Neonates were considered to have CS if any of the following criteria was met: abnormal physical examination compatible with CS; fourfold higher than maternal titer of VDRL at delivery; or pathological reports representing evidence of *T. pallidum* infection[3]. Those with proven CS received aqueous crystalline penicillin for a total of 10 days.

Outcome measures

The association between CMIA S/CO ratio and activities of disease, including syphilis (Group 1), late syphilis (Group 2) and false positive CMIA (Group 3), was the primary outcome. Also, the CMIA S/CO ratio was divided into three intervals according to previous studies[13] for the comparison, including <9.9, 9.9 to <19.9 and ≥19.9.

Perinatal outcomes including preterm birth, low birth weight (LBW), birth asphyxia and CS were the secondary outcomes. Preterm birth was defined as delivery prior to 37 weeks of gestational age (GA). Low birth weight (LBW) was defined as a neonate birth weight lower than 2,500 grams. Birth asphyxia was defined as having an Apgar score of less than 7 at one or five minutes.

Sample size calculation and statistical analysis

According to a study by Zofkie AC et al[15], which used chemiluminescence immunoassays (CIA), *Treponemal pallidum* particle agglutination (TPPA) and rapid plasma regain (RPR) for screening syphilis in pregnant women, the means and standard deviations of CIA S/CO titers were 18.3 ± 5.4 (CIA+/RPR+/TPPA+), 12.1 ± 5.3 (CIA+/RPR-/TPPA+) and 1.9 ± 0.8 (CIA+/RPR-/TPPA-), respectively. The sample size was calculated by nQuery Advisor. The alpha and power were set at 0.05 and 80%, respectively. Consequently, the minimum sample size required for our study was 75.

Stata program version 12.1 (Statacorp LP, College Station, Texas USA) was used for statistical analysis. Presentation of descriptive data was done with n (%), mean ± standard deviation (SD), and median with interquartile range (IQR). The comparisons between 3 groups were performed using one-way analysis of variance (ANOVA)

pairwise comparison by the Bonferroni for normal continuous data. Non-normal distribution data was analyzed using pairwise comparison Dunn's test's and Kruskal-Wallis test. Comparison of categorical data was done using chi-square test or Fisher's exact test. The t test and Wilcoxon Rank Sum test were used to compare parametric data and non-parametric data, respectively. A p value of < 0.05 was statistically significant.

Results

Between January 2020 and February 2021, there were 8,987 pregnant women who came for ANC at Siriraj Hospital. The total number of reactive CMIA samples was 83 (0.92%). Two participants had multifetal pregnancies and were excluded. The VDRL and TPHA results of 81 participants with reactive CMIA were shown in Fig. 1. Participants with reactive VDRL (CMIA+, VDRL+) were found most in CMIA S/CO ratio ≥19.9 (33/38 or 86.8%). On the contrary, participants with false positive CMIA (CMIA+, VDRL-TPHA-) were found most in CMIA S/CO ratio < 9.9 (19/23 or 82.6%) and not found in CMIA S/CO ratio ≥19.9.

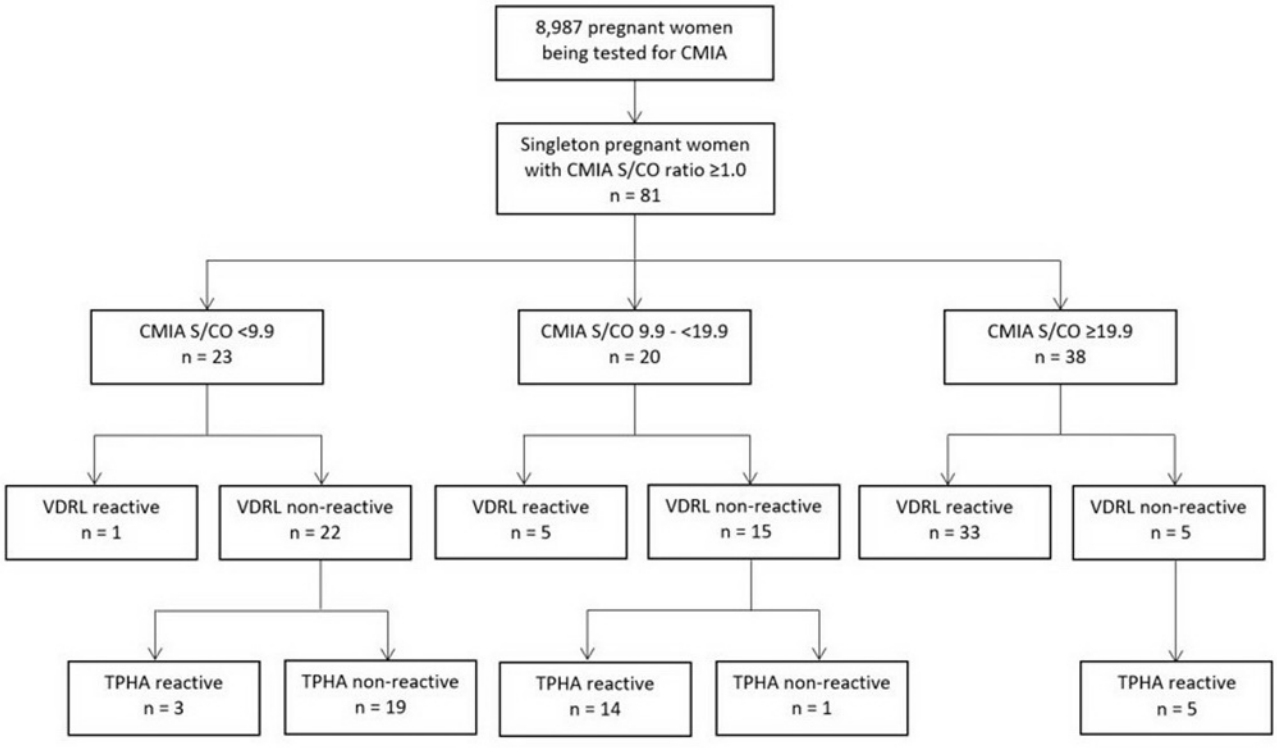

Fig. 1. Diagram of the participants.
CMIA: chemiluminescent microparticle immunoassay, S/CO: signal-to-cutoff ratio, VDRL: venereal disease research laboratory, TPHA: *Treponema pallidum* hemagglutination.

Participants' characteristics were compared among Group 1 (CMIA+ VDRL+), Group 2 (CMIA+ VDRL- TPHA+) and Group 3 (CMIA+ VDRL- TPHA-) as shown in Table 1. The participants in Group 1 were younger and more likely to be primiparous. Three of them had HIV infection. The body mass index (BMI), parity, previous abortion, and GA at diagnosis were comparable. CMIA S/CO ratio was highest in Group 1 at 23.1 ± 5.5, followed by 16.1 ± 5.2 in Group 2 and 2.1 ± 3.2 in Group 3, p < 0.001. (Fig. 2)

Table 1. Characteristics of pregnant women with reactive CMIA (n = 81).

	CMIA+, VDRL+ (n = 39)	CMIA+, VDRL-, TPHA+ (n = 22)	CMIA+, VDRL-, TPHA- (n = 20)	p value
Age (years)	22.7 ± 5.4	26.7 ± 5.5	30.2 ± 5.7	< 0.001
Age (years)				0.013
< 20	9 (23.1)	1 (4.5)	1 (5.0)	
20-30	26 (66.7)	15 (68.2)	10 (50.0)	
> 30	4 (10.3)	6 (27.3)	9 (45.0)	
BMI (kg/m^2)	23.4 ± 3.7	25.5 ± 5.7	24.5 ± 3.9	0.189
Primiparity	18 (46.2)	2 (9.1)	7 (35.0)	0.013
Previous abortion	11 (28.2)	9 (40.9)	4 (20.0)	0.321
GA at diagnosis (weeks)	20.0 ± 10.9	21.3 ± 13.3	16.0 ± 11.3	0.300
GA at diagnosis (weeks)				0.532
< 14	16 (41.0)	10 (45.5)	12 (60.0)	
14-28	12 (30.8)	4 (18.2)	4 (20.0)	
> 28	11 (28.2)	8 (36.4)	4 (20.0)	
HIV infection	3 (7.7)	0 (0)	0 (0)	0.187
CMIA S/CO ratio	23.1 ± 5.5	16.1 ± 5.2	2.1 ± 3.2	< 0.001

Data presented in n (%), mean ± standard deviation (SD) and median ± SD

CMIA: chemiluminescent microparticle immunoassay, VDRL: venereal disease research laboratory, TPHA: *Treponema Pallidum* hemagglutination assay, Rx: treatment, BMI: body mass index, GA: gestational age, HIV: human immunodeficiency virus, S/CO: signal-to-cutoff ratio

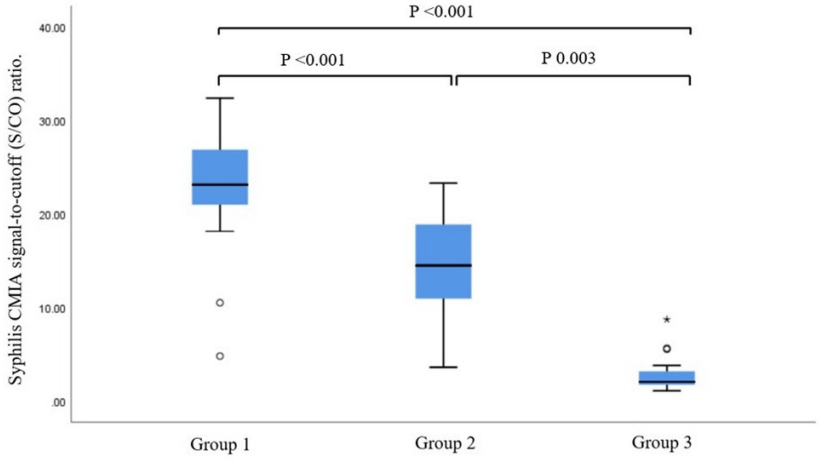

Fig. 2. Chemiluminescent microparticle immunoassay signal to-cutoff ratio by groups. Group 1 CMIA+/VDRL+; Group 2 CMIA+/VDRL-/TPHA+; Group 3 CMIA+/VDRL-/TPHA-.

CMIA: chemiluminescent microparticle immunoassay, VDRL: venereal disease research laboratory, TPHA: *Treponema pallidum* hemagglutination.

Miscarriages occurred in three participants at GA 10, 11, and 21 weeks. The first two cases were diagnosed with false positive CMIA and the last one was early syphilis. Eight participants delivered at other hospitals. Accordingly, 70 participants were included into the analysis of the perinatal outcomes.

The perinatal outcomes were similar among pregnant women with different ranges of CMIA except for the incidence of CS (Table 2). All six neonates diagnosed with CS were born to mothers with CMIA S/CO ratio ≥19.9 and reactive VDRL. The pregnant women who delivered neonates with CS tended to be younger; were diagnosed with syphilis in third trimester and had higher CMIA S/CO ratio and VDRL titer, compared to those who delivered neonates without CS (Table 3).

Table 2. Perinatal outcomes in pregnant women with reactive CMIA (n = 70).

	S/CO ratio ≥ 1 to < 9.9 (n = 17)	**S/CO ratio 9.9 to < 19.9 (n = 19)**	**S/CO ratio ≥ 19.9 (n = 34)**	**p value**
GA at delivery (weeks)	38.2 ± 1.1	38.5 ± 1.6	37.4 ± 2.8	0.251
GA at delivery (weeks)				0.131
< 34	0 (0)	0 (0)	3 (8.8)	
< 37	1 (5.9)	3 (15.8)	6 (17.6)	
≥ 37	16 (94.1)	16 (84.2)	25 (73.5)	0.424
Birth weight (gm)	3,055 ± 274	2,899 ± 296	2,832 ± 595	0.276
< 1500	0 (0)	0 (0)	2 (5.9)	
< 2500	0 (0)	1 (5.3)	5 (14.7)	0.263
Birth asphyxia	1 (5.9)	0 (0)	3 (8.8)	0.678
Congenital syphilis	0 (0)	0 (0)	6 (17.6)	0.040

Data are presented as n (%) or mean ± standard deviation (SD)
CMIA: Chemiluminescent microparticle immunoassay, S/CO: signal-to-cutoff ratio, GA: gestational age

Table 3. Characteristics of pregnant women delivering neonates with and without congenital syphilis (n = 70).

	Congenital syphilis (n = 6)	**No congenital syphilis (n = 64)**	**p value**
Age (years)	23.5 ± 7.6	25.7 ± 6.0	0.407
< 20	2 (33.3)	8 (12.5)	0.377
20-30	3 (50.0)	43 (67.2)	
> 30	1 (16.7)	13 (20.3)	
GA at diagnosis (weeks)	27.7 ± 9.0	19.5 ± 12.0	0.111
Trimester at diagnosis			
First	1 (16.7)	31 (48.5)	0.141
Second	1 (16.7)	15 (23.4)	
Third	4 (66.6)	18 (28.1)	
CMIA S/CO ratio	26.8 ± 4.4	16.0 ± 8.9	0.005
≥ 1 to < 9.9	0	17 (26.6)	0.025
9.9 to < 19.9	0	20 (31.2)	
≥ 19.9	6 (100)	27 (42.2)	
VDRL titer	1:16 [1:2,1:64]	0 [0,1:2]	< 0.001*
VDRL titer ≥ 1: 8	4 (66.6)	7 (10.9)	< 0.001

Data are presented as n(%), mean ± standard deviation (SD), median ± SD or median with interquartile range.
GA: gestational age, CMIA: chemiluminescent microparticle immunoassay, VDRL: venereal disease research laboratory, S/CO: signal-to-cutoff ratio.
*Wilcoxon rank sum test

Discussion

High CMIA S/CO ratio in pregnant women with syphilis relates to the occurrence of congenital syphilis. Both high CMIA S/CO ratio and high VDRL titer favor active stage of maternal syphilis. This supports the fact that early stage of syphilis in pregnant women associates with CS, at 70-100%[16], due to a high level of spirochetemia together with a greater tissue damage. Compatible with previous studies, in order to eliminate CS, the higher CMIA S/CO ratio may be applied as an adjunct to help identify the stage of syphilis and make a prompt decision for management[15, 17].

The median CMIA S/CO ratio from participants with active syphilis in the present study is slightly higher than that from Thai pregnant women with untreated syphilis in the previous study (23.11 vs 21.27)[14]. The higher level of S/CO ratio can be partly explained by the fact that the participants in the present study were younger (26 vs 32 years). As widely known, a recent infection of syphilis leads to marked horizontal and vertical transmission as well as a greater tissue damage. Young women, particularly teenagers, who are not far from their sex debut, thus tended to be in the early stage of syphilis. This results in a higher VDRL titer and, as being shown in the present study, a higher CMIA S/CO ratio.

The false positive CMIA results using S/CO ratio ≥1 as a cutoff point was observed at 11.8-53.8% in previous studies[14, 15, 18]. In this study, the proportion of CMIA results which returned false positive was 24.7% (20 in 81). The false positive CMIA can be lessened by increasing S/CO ratio diagnostic point. In line with the previous study in non-pregnant women reporting a diagnostic specificity of 100% when using CMIA S/CO ratio ≥9.9 as a cutoff point[13], our findings showed that only 1.7% (1 in 58) of pregnant women with CMIA S/CO ratio ≥9.9 were false positive. Additionally, 82.6% of samples with reactive CMIA S/CO ratio < 9.9 were false positive. Because of the difference in population, each laboratory should determine its own optimal CMIA S/CO ratio cutoff point. However, as false positive tests appear more acceptable in terms of screening, CMIA S/CO ratio cutoff point at ≥1 remains for it has high screening performance.

The present study demonstrated that 27.2% (22 in 81) of CMIA-reactive participants had false negative VDRL (CMIA+, VDRL-, TPHA+). The diagnosis of syphilis would have been missed in those participants if the traditional sequence had been used, similar to the previous studies that found a missed diagnosis rate of 24.2-27.1%[19, 20]. The finding underlines the superiority of the reverse sequence in terms of syphilis screening.

Although perinatal outcomes were not different among the three groups of CMIA S/CO ratio in this study, all neonates with CS were born to mothers with high CMIA S/CO ratio and high VDRL titer. Additionally, the participants with younger age and greater GA at diagnosis tended to have neonates with CS. This is consistent with a previous report which showed that 50% of teenage mothers with syphilis gave birth to neonates with CS, and that the first ANC after 20 weeks of gestation was a predictive factor[5]. Late ANC may lead to delayed diagnosis and treatment of syphilis while intrauterine fetuses are increasingly harmed with GA[21]. Information regarding prevention of both sexually transmitted infections and pregnancy, together with proper management once either occurs should be more emphasized in Thai young population. The single study center is the strength in that all laboratory investigations were performed in one qualified center. Therefore, the whole process of the reverse sequence algorithm was completely adhered. Moreover, the good collaboration between the Department of Obstetrics and Gynaecology, that of Pediatrics and that of Clinical Pathology are well-established. The limitation was the lack of long-term follow-up of newborns as late-onset congenital syphilis may develop much later.

Conclusion

Instances of adverse perinatal outcomes, particularly congenital syphilis, and maternal syphilis with VDRL titers ≥1:8 are more frequent in pregnant

women with higher CMIA S/CO ratio. All neonates with CS were born to mothers with CMIA S/CO ≥19.9. The use of CMIA S/CO ratio as an adjunct to clinical evaluation may provide additional benefits to the syphilis screening and raise clinicians' awareness of commencing immediate and proper management for pregnant patients.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Bureau of Epidemiology. Department of Disease Control. National Disease Surveillance (Report 506), Ministry of Public Health, Bangkok; 2012-2021. (Accessed 30 January 2022) Available from: http://doe.moph.go.th/surdata/disease.php?ds=37
2. Rungmaitree S, Mokkhamakkun C, Phongsamart W, Lolekha R, Wittawatmongkol O, Lapphra K, et al. Incidence of and factors associated with congenital syphilis at a tertiary hospital in Bangkok, Thailand. Southeast Asian J Trop Med Public Health 2019;50:1090-101.
3. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. Sexually transmitted diseases treatment guidelines, 2021. MMWR Recomm Rep 2021;70:1-187.
4. Centers for Disease Control and Prevention. Sexually transmitted infections treatment guidelines, 2021. United States; 2021. (Accessed 3 December 2022) Available from: https://www.cdc.gov/std/treatment-guidelines/syphilis.htm
5. Panchalee T, Chayachinda C, Panyakat W, Chen M, Quinones-Garcia V Z, Pernsoongnern P. Characteristics and neonatal outcomes of teenage pregnant women diagnosed with syphilis at Siriraj Hospital. Siriraj Med J 2018;70:298–301.
6. Centers for Disease Control and Prevention. Syphilis testing algorithms using treponemal tests for initial screening---four laboratories, New York City, 2005--2006. MMWR Morb Mortal Wkly Rep 2008;57:872-5.
7. Centers for Disease Control and Prevention. Discordant results from reverse sequence syphilis screening--five laboratories, United States, 2006-2010. MMWR Morb Mortal Wkly Rep 2011; 60:133–7.
8. Gratrix J, Plitt S, Lee BE, Ferron L, Anderson B, Verity B, et al. Impact of reverse sequence syphilis screening on new diagnoses of late latent syphilis in Edmonton, Canada. Sex Transm Dis 2012;39:528-30.
9. World Health Organization. WHO guideline on syphilis screening and treatment for pregnant women. Geneva: World Health Organization; 2017.
10. Division of AIDS and STIs. Prevention and management of mother-to-child transmission of syphilis guideline. Bangkok: Department of Disease Control, Ministry of Public Health, Bangkok;2021. (Accessed 27 September 2021) Available from: http://klb.ddc.moph.go.th/dataentry/handbook/form/128
11. Bangrak STI Center. National Guideline for Syphilis Testing: Laboratory Manual for Diagnosis and Monitoring. Bangkok, Bangrak STI Center; 2021. (Accessed 27 September 2021) Available from: http://klb.ddc.moph.go.th/dataentry/handbook/form/117
12. Seña AC, White BL, Sparling PF. Novel *Treponema pallidum* serologic tests: a paradigm shift in syphilis screening for the 21st century. Clin Infect Dis 2010;51:700-8.
13. Dai S, Chi P, Lin Y, Zheng X, Liu W, Zhang J, et al. Improved reverse screening algorithm for Treponema Pallidum antibody using signal-to-cutoff ratios from chemiluminescence microparticle immunoassay. Sex Transm Dis 2014;41:29–34.
14. Boonchaoy A, Wongchampa P, Hirankarn N, Chaithongwongwatthana S. Performance of chemiluminescent microparticle immunoassay in screening for syphilis in pregnant women from low-prevalence, resource-limited settings. J Med Assoc Thai 2016;99:119-24.
15. Zofkie AC, Seasely AR, Gaffney D, Rogers VL, Mcintire DD, Roberts SW, et al. Syphilis immunoassay signal strength correlates with active infection in pregnant women. Am J Perinatol 2020;37:671-8.
16. Chayachinda C, Chinhiran K, Chaithongwongwatthana S, Kittiyaowamarn R, Thamkhantho M. Elimination of congenital syphilis: what's new for obstetricians in the 2020 Thai National Guideline? Thai J Obstet Gynaecol 2021:29;2-9.
17. Adhikari EH, Frame IJ, Hill E, Fatabhoy R, Strickland AL, Cavuoti D, et al. Abbott ARCHITECT Syphilis TP chemiluminescent immunoassay accurately diagnoses past or current syphilis in pregnancy. Am J Perinatol 2020;37:112-8.
18. Li Z, Feng Z, Liu P, Yan C. Screening for antibodies against Treponema pallidum with chemiluminescent microparticle immunoassay: analysis of discordant serology results and clinical characterization. Ann Clin Biochem 2016;53:588-92.
19. Tong ML, Lin LR, Liu LL, Zhang HL, Huang SJ, Chen YY, et al. Analysis of 3 algorithms for syphilis serodiagnosis and implications for clinical

management. Clin Infect Dis 2014;58:1116-24.
20. Totten YR, Hardy BM, Bennett B, Rowlinson MC, Crowe S. Comparative performance of the reverse algorithm using Architect Syphilis TP Versus the traditional algorithm using rapid plasma reagin in Florida's Public Health Testing Population. Ann Lab Med 2019;39: 396-9
21. Thornton C, Chaisson LH, Bleasdale SC. Characteristics of pregnant women with syphilis and factors associated with congenital syphilis at a Chicago Hospital. Open Forum Infect Dis 2022;9:ofac169.