Original Article

Clue Cell as a Single Diagnostic Tool for Bacterial Vaginosis during Pregnancy

Chayachinda C, MD, MSc[1], Baukaew L, MD[1], Thamkhantho M, MD, FRCOG, MSc[1], Bangpichet A, BNs[1], Sodsee S, BNs[1], Pharkjaksu S, BSc, MSc, PhD[1]

[1] Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Objective: To demonstrate the diagnostic accuracy for bacterial vaginosis (BV) by using the sole detection of clue cells

Materials and Methods: All pregnant women who received antenatal care (ANC) at Siriraj Hospital between October 2017 and March 2018 were invited. Eligibility criteria included age of 18 years or older and gestational age (GA) of 12 weeks or more, absence of blood or amniotic fluid, and no antibiotics taken in prior two weeks. Vaginal discharge was obtained, air-dried, gram-stained, and sent to two microbiologists. Nugent's scoring system, which was done by one microbiologist, was used as the diagnostic gold standard. Percentage of clue cells was estimated by evaluating 100 consecutive squamous cells by another microbiologist and reported in the interval of ten. Diagnostic accuracy was derived by 2×2 tables using different cut-off percentage of clue cells.

Results: Of 748 participants, the average age was 29 years with GA of 28 weeks. Around 10% had leukorrhea or pruritus. The prevalence of BV was 18.7% (140/748). Clue cells were detected in 746 slides, which were just presence (n=204), 10% (n=151), 20% (n=142), 30% (n=99), 40% (n=62), 50% (n=30), 60% (n=31), 70% (n=15), and 80% (n=12). Using less than 10% of clue cells as the reference, the detection of clue cells at 20% or more appeared to be the most promising BV diagnostic tool with a sensitivity at 87.1%, specificity at 55.8%, positive predictive value at 31.2%, negative predictive value at 95.0%, diagnostic odd ratio at 8.57, accuracy at 61.6, and Youden index at 0.43.

Conclusion: Detection of clue cells at 20% or more can be an alternative diagnostic tool of BV, especially when other diagnostic methods are not applicable.

Keywords: Bacterial vaginosis, Clue cell, Pregnancy

Received 22 Feb 2020 | Revised 6 Mar 2020 | Accepted 10 Mar 2020

J Med Assoc Thai 2020;103(4):353-8
Website: http://www.jmatonline.com

Bacterial vaginosis (BV) is one of the most common reproductive tract infections (RTIs)[1]. It represents the dysbiosis of vaginal ecosystem resulting in homogeneous whitish or greyish vaginal discharge with fishy odor[2]. This impairs natural protective immune system allowing bacteria or other pathogenic organisms to gain access into the body via cervical os[3]. As being demonstrated in previous reports, BV associates with the occurrence of chorioamnionitis, pelvic inflammatory disease (PID), and endometritis[4-6]. At Siriraj Hospital, which is a University teaching hospital, BV accounts for 35.3% of women with RTIs[7] and 19.3% of asymptomatic pregnant women during early third trimester[8].

The high prevalence and considerable impact of BV urge health care providers to find simple diagnostic tool. As known, BV is caused by reduction of normally predominate vaginal bacteria like *Lactobacilli* spp. and overgrowth of anaerobic bacteria such as *Gardnerella vaginalis* and *Mobiluncus*. Thus, Nugent scoring system, which is the interpretation of gram stain, is currently the gold standard diagnostic test[9]. However, its impracticality is clearly demonstrated by great consumption of labor and time.

Many alternative diagnostic tools have been reported[10]. The most widely used one is the Amsel's

Correspondence to:

Thamkhantho M.

Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wang Lang Road, Bangkoknoi District, Bangkok 10700, Thailand.

Phone: +66-2-4194775, **Fax**: +66-2-4194997

Email: manopchai.tha@mahidol.ac.th

How to cite this article: Chayachinda C, Baukaew L, Thamkhantho M, Bangpichet A, Sodsee S, Pharkjaksu S. Clue Cell as a Single Diagnostic Tool for Bacterial Vaginosis during Pregnancy. J Med Assoc Thai 2020;103:353-8.

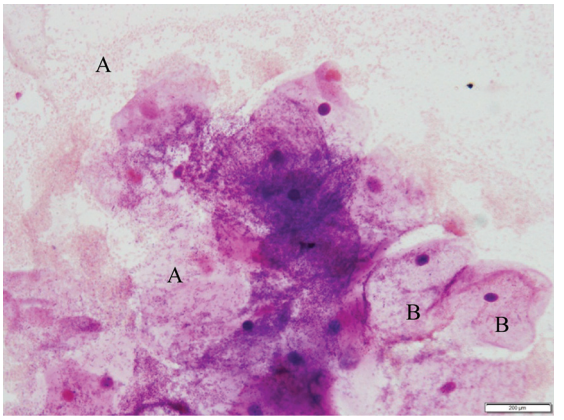

Figure 1. Gram-stained vaginal smear shows small cocco-bacilli (A) or cylindrical short rods kinking on epithelial squamous cell (B).

criteria because of its affordability and accessibility. Mohammadzadeh et al explored the BV diagnostic performance of each criterion of the Amsel's criteria, including detection of clue cells at 84.9%, amine test at 74.4%, grayish-whitish discharge at 68.1% and vaginal pH greater than 4.5 at 53.4%, respectively[11]. According to the highest diagnostic yield of clue cell and the availability of microscopes at all health-care settings, the present study aimed to demonstrate the potential of using 'presence of clue cells' as the sole diagnostic tool of BV.

Materials and Methods

The cross-sectional study was conducted at the Siriraj antenatal care (ANC) clinic after being ethically approved by the Siriraj Institutional Review Board. (COA Si488/2017).

Participants

All pregnant women who attended the Siriraj ANC clinic between October 2017 and February 2018 were invited into the study. The eligible criteria included being at least 18 years-old and having gestational age (GA) of at least 12 weeks. Those who took antibiotics within two weeks prior to the enrolment, or had vaginal bleeding, rupture, or leakage of amniotic fluid were excluded.

Procedure

After the study was explained in detail, the participants who signed the informed consent were asked about the demographic data. Then, they were sent to the pelvic examination room for vaginal swab. A sterile speculum without lubricant was inserted. Next, a sterile cotton swab was used to collect vaginal discharge from posterior fornix and immediately smeared on dry glass slide. Each slide was coded numerically and anonymously. The slide was left air-dried, gram-stained, and sent to two microbiologists who worked independently and blinded to the clinical features of the participants. Nugent's scoring was done by one microbiologist and used as the diagnostic gold standard. Percentage of clue cells was estimated by evaluating 100 consecutive squamous cells by another microbiologist and reported in the interval of ten.

Evaluation of gram-stained slides

Nugent's scoring system was used as the gold standard diagnostic method of BV. It took *Lactobacillus* morphotypes, *Gardnerella* and *Bacteriodes* spp. morphotypes, and curved gram-variable rods into consideration and reported in score 0 to 10. A score 0 to 3 was considered as normal flora, a score 4 to 6 as intermediate flora, and a score 7 to 10 as BV[9]. At least 100 consecutive squamous cells were examined for clue cells and recorded in percentage (interval of 10). Clue cell is a squamous epithelial cell being adhered by plenty of small anaerobic bacteria resulting in a unique morphological change (Figure 1). Adding to that, detection of *Neisseria gonorrhoeae*, *Candida* spp. and *Trichomonas vaginalis* was also demonstrated. *N. gonorrhoeae* could be seen as gram-negative diplococcic bacteria both intracellularly and extracellularly. *Candida* spp. was positive when pseudohyphae, a long-branching filamentous structure without septum, appeared. And, *T. vaginalis* was diagnosed when the microbiologist saw a leucocyte-sized organism with five flagella.

Outcome measures[12]

Accuracy of diagnostic tests using different percentage of clue cells as the cut-off value was derived from creating 2×2 table (Table 1). Sensitivity expressed the rate of participants with BV and positive test among those with BV. Specificity showed the rate of participants with no BV and negative test among those without BV. Positive predictive value (PPV) varied with prevalence in each population and represented a proportion of participants with BV in those who were tested positive. Negative predictive value (NPV) represented among participants with negative test, what the proportion of those without BV was. Positive likelihood ratio (LR+) directly estimated the possibility of having BV when being tested positive. Negative likelihood ratio

Table 1. The 2×2 table for testing diagnostic accuracy

	Bacterial vaginosis	No bacterial vaginosis
Positive test	a	b
Negative test	c	d

* Positive test=detection of clue cells at different percentage and above

Sensitivity=a/(a+c)

Specificity=d/(b+d)

Positive predictive value=a/(a+b)

Negative predictive value=d/(c+d)

Positive likelihood ratio (LR+)=sensitivity/(1–specificity)

Negative likelihood ratio (LR–)=(1–sensitivity)/specificity

Diagnostic odd ratio=LR+/LR–

Youden index=sensitivity+specificity–1

(LR–) showed the likelihood of not having BV when being tested negative. Diagnostic odd ratio (DOR) demonstrated discriminative ability of test. Youden index represented overall measuring power of test ranging from 0 to 1 for poor to perfect performance.

Sample size calculation and statistical analysis

The sample size was calculated using the estimation of an infinite population proportion equation when the power was 90% and the allowed error was 5%. The required number of eligible participants was 139. As the prevalence of BV in pregnant women at Siriraj Hospital was 19.3%[8], at least 707 pregnant women were invited into the study until the required sample size was met.

The Stata, version 12.0 (StataCorp LP, College Station, TX, USA) was used for data analysis. Descriptive statistics were used as appropriate, including n (%) and mean ± standard deviation. Chi-square was used to compare categorical variables and student t-test was used to compare parametric continuous variable. A p-value less than 0.05 was considered as statistical significance. Accuracy of diagnostic tests comparing with Nugent scoring system, the gold standard of diagnosing BV, was done using manual calculation as described in Table 1.

Results

Of the 748 pregnant women, 140 were diagnosed with BV by Nugent scoring system (18.7%), 273 had intermediate flora (36.5%), and 335 was normal (44.8%). Around 10% reported either pruritus or leucorrhea.

Table 2 shows the comparable characteristics of those with and without BV. They were around 29 years of age and mostly in the 20 to 29 years age group. As

Table 2. Characteristics of the participants (n=748)

	Bacterial vaginosis (n=140) n (%)	No bacterial vaginosis (n=608) n (%)	p-value
Age (years); mean±SD	28.7±6.2	28.9±5.8	0.319
<20	9 (6.4)	27 (4.4)	0.633
20 to 29	70 (50.0)	289 (47.5)	
30 to 39	55 (39.3)	268 (44.1)	
≥40	6 (4.3)	23 (4.0)	
Body mass index (kg/m^2)			
Mean±SD	25.7±4.7	26.0±4.5	0.545
Education			
No/primary school	10 (7.1)	84 (13.8)	0.098
High school	85 (60.7)	338 (55.6)	
University	45 (32.1)	186 (30.6)	
Occupation			
Being unemployed	64 (45.7)	260 (42.8)	0.770
Temporary job	71 (50.7)	321 (52.8)	
Office job	5 (3.6)	27 (4.4)	
Primigravida	61 (43.6)	242 (39.8)	0.413
Previous caesarean section	57 (40.7)	230 (37.8)	0.527
Gestational age (weeks)			
Mean±SD	28.4±6.3	28.4±5.9	0.488
<14	5 (3.6)	16 (2.6)	0.829
14 to 28	45 (32.1)	195 (32.1)	
>28	90 (64.3)	397 (65.3)	
Presenting symptoms			
Leukorrhea	12 (8.6)	41 (6.7)	0.447
Pruritis	5 (3.6)	20 (3.3)	0.867
History of STDs	6 (4.3)	26 (4.3)	0.996

STD=sexually transmitted diseases; SD=standard deviation

the average GA was 28 weeks, their body mass index (BMI) was around 26 kg/m². Education level was mainly at high school and almost all were not working regularly. One third were primigravida, while another one third had previous Caesarean section. History of having sexually transmitted diseases (STDs) was reported in 32 participants. Among those with BV, four had herpes genitalis, one had genital warts, and one had both. Among those without BV, 19 had herpes genitalis, two had genital warts, one had gonorrhea, and four had PID.

Distribution of percentages of clue cells detected is shown in Figure 2. Clue cells were detected in 746 specimens and the proportion was the followings, just presence or less than 10% (204/748), 10% (151/748),

Table 3. Accuracy of diagnostic methods for bacterial vaginosis using different percentage of clue cells as the cut-off level (n=748)

Cut-off level of clue cell percentage	Sensitivity	Specificity	PPV	NPV	LR+	LR–	DOR	Youden index	Accuracy
10	90.7	31.7	23.4	93.7	1.33	0.29	4.59	0.22	42.8
20	87.1	55.8	31.2	95.0	1.97	0.23	8.57	0.43	61.6
30	72.1	75.7	40.6	92.2	2.97	0.37	8.03	0.48	75.0
40	55.7	88.2	52.0	89.6	4.72	0.50	9.44	0.44	82.1
50	37.9	94.2	60.2	86.8	6.53	0.66	9.89	0.32	83.7
60	29.3	97.2	70.7	85.7	10.46	0.73	14.33	0.27	84.5
70	14.3	99.5	74.1	83.4	28.60	0.86	35.26	0.14	83.0
80	6.4	100.0	69.2	82.2	NA	0.84	NA	0.06	82.0

DOR=diagnostic odd ratio; LR=likelihood ratio; NPV=negative predictive value; PPV=positive predictive value; NA=not applicable

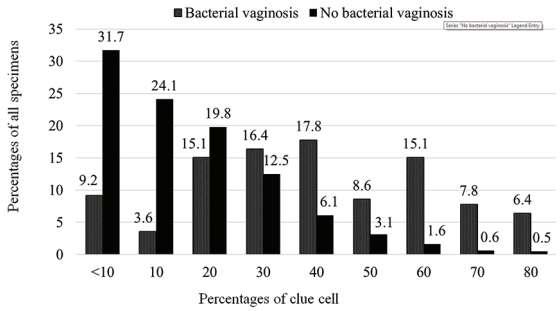

Figure 2. Distribution of percentages of clue cells detected in 'Bacterial vaginosis' group (n=140) and 'no bacterial vaginosis' group (n=608).

20% (142/748), 30% (99/748), 40% (62/748), 50% (30/748), 60% (31/748), 70% (15/748), and 80% (12/748). None of the participants had *N. gonorrhoeae* nor *T. vaginalis*. Around 15% of the participants had *Candida* spp., BV 15.7% versus non-BV 14.5%.

Figure 2 shows that the non-BV pregnant women had lower number of clue cells. Using the less than 10% as the reference, there was moderate diagnostic accuracy of detected clue cells for BV diagnosis when using 20%, 30%, or 40% as the cut-off values. Compared with the 20% or more group, the 30% or more group had higher Youden index (0.48 versus 0.43) but lower DOR (8.03 versus 8.57) (Table 3). Moving from the 20% or more group to the 30% of more group as the cut-off level, 21 participants with BV would be missed while 121 ones would receive unnecessary treatment. There was no adverse effect from the collection of vaginal secretion during pregnancy.

Discussion

Clue cell is the only objective evidence of the disturbed vaginal ecosystem that is not affected by exogenous factors like blood or amniotic fluid. Although, in the original Amsel's criteria, just presence of clue cells is counted, grading of the clue cells should relate to the level of the vaginal dysbiosis. The present study showed that the proportion of clue cells in all squamous epithelial cells at 20% or more can be a single diagnostic tool of BV with acceptable accuracy.

The sensitivity of using 20% or more clue cells in diagnosing BV (87.5%) appears comparable with that of Amsel's criteria (88.0%)[11] and that of BV Blue (88.0%)[13]. As known, presence of clue cells is the most important part of the Amsel's criteria[14]. Accordingly, concerning only one criterion out of four may dilute the diagnostic complexity without impairing the diagnostic competency. BV Blue is another bed-side diagnostic tool of BV that detects sialidase enzyme produced by *G. vaginalis*[13]. Despite the convenience, its high cost limits the use in low-resource settings.

Youden index represents overall measure of each cut-off level and shows that the sole detection of clue cells has up to moderate performance[12]. The best three cut-off levels were 20% or more, 30% or more, and 40% or more, with Youden index at 0.43, 0.48, and 0.44, respectively. Therefore, it may not fit the routine use as the diagnostic tool. However, during some situations such as pregnancy with rupture of membranes or vaginal bleeding, critical decision to treat or not to treat BV is urgently required. This tool may become extremely useful. As microscopes are now available in all levels of health-care settings and competency in detecting clue cells is included in the Medical education curriculum, only a small revision and awareness among healthcare providers is needed.

The strengths of the present study are the homogeneity of participants and reliable diagnostic methods. As menstrual cycle affects vaginal microflora, conducting the BV-related study in pregnant women can better represent such vaginal ecosystem. Gram-stained slide can be interpreted for both Nugent scores and percentage of clue cells with high reproducibility. The present study used one slide for both microbiologists to minimize technical errors. Limitation of the study is that Amsel's criteria were not concurrently applied to compare the diagnostic performance. Generalizability to clinical practice in non-Thai women need to be validated.

Conclusion

The sole detection of clue cells at 20% or more can sufficiently support the BV diagnosis. Its usefulness stands out in some situations when other diagnostic methods are not applicable. From the present study the clue cell plays the crucial role of diagnostic tool of BV but the percentage of 20% and more in high power field of microscopic examination is also the key of specificity in diagnosis. Last, the microscopic examination by the well-trained personnel is highly recommended for the proper diagnosis of BV.

What is already known on this topic?

Amsel's criteria has been widely known as a diagnostic tool for BV, unfortunately the diagnostic criteria of Amsel's cannot be met properly due to the lack of appropriate tool such as vaginal pH paper, 10% potassium hydroxide, etc. The more complete combination tool we get, the more accuracy diagnosis of BV we have. The notion of sole diagnostic detection in the clinical application to diagnose will be fruitful to the healthcare professionals in poorly equipped health center.

What this study adds?

The sole detection of clue cells under microscopic examination at 20% and more can efficiently assist the diagnosis of BV when the diagnostic tools of Amsel's are not applicable. Surely, if the diagnosis was correct, the plan of treatment will be very good to the patients who have been suffering from vaginal dysbiosis, especially pregnant women who are prone to preterm birth.

Acknowledgement

The authors would like to convey our sincere gratitude with deep appreciation to all the stall member of Unit of gynecologic infectious diseases and female STD's who kindly participated in collecting data, statistically analysis in the present study.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. Am J Obstet Gynecol 2013;209:505-23.
2. Hillier SL, Krohn MA, Rabe LK, Klebanoff SJ, Eschenbach DA. The normal vaginal flora, H2O2-producing lactobacilli, and bacterial vaginosis in pregnant women. Clin Infect Dis 1993;16 Suppl 4: S273-81.
3. Amabebe E, Anumba DOC. The vaginal microenvironment: The physiologic role of lactobacilli. Front Med (Lausanne) 2018;5:181.
4. McGregor JA, French JI. Bacterial vaginosis in pregnancy. Obstet Gynecol Surv 2000;55:S1-19.
5. Nelson DB, Macones G. Bacterial vaginosis in pregnancy: current findings and future directions. Epidemiol Rev 2002;24:102-8.
6. Sharma H, Tal R, Clark NA, Segars JH. Microbiota and pelvic inflammatory disease. Semin Reprod Med 2014;32:43-9.
7. Chayachinda C, Thamkhantho M, Chalermchockcharoenkit A, Nuengton C, Thipmontree W. Characteristics of clients at the Siriraj Female STD Clinic during 2011-2015. Siriraj Med Bull 2018;11: 182-9.
8. Tachawatcharapunya S, Chayachinda C, Parkpinyo N. The prevalence of bacterial vaginosis in asymptomatic pregnant women during early third trimester and the pregnancy complications. Thai J Obstet Gynaecol 2017;25:96-103.
9. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991;29:297-301.
10. Sha BE, Chen HY, Wang QJ, Zariffard MR, Cohen MH, Spear GT. Utility of Amsel criteria, Nugent score, and quantitative PCR for Gardnerella vaginalis, Mycoplasma hominis, and Lactobacillus spp. for diagnosis of bacterial vaginosis in human immunodeficiency virus-infected women. J Clin Microbiol 2005;43:4607-12.
11. Mohammadzadeh F, Dolatian M, Jorjani M, Alavi MH. Diagnostic value of Amsel's clinical criteria for diagnosis of bacterial vaginosis. Glob J Health Sci 2014;7:8-14.
12. Šimundić AM. Measures of diagnostic accuracy: Basic definitions. EJIFCC 2009;19:203-11.
13. Kampan NC, Suffian SS, Ithnin NS, Muhammad M, Zakaria SZ, Jamil MA. Evaluation of BV((R)) Blue Test Kit for the diagnosis of bacterial vaginosis. Sex Reprod Healthc 2011;2:1-5.

14. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983;74:14-22.