Association Between the Level of Inflammation at Each Anatomical Sexual Activity from Gram Staining and *Neisseria gonorrhoeae* and *Chlamydia trachomatis* Infections

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ABSTRACT

Objective: This study aimed to determine the association between the severity of inflammation at each anatomical sexual activity from gram staining with *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) infections.

Materials and Methods: This study was conducted using laboratory test data from patients at the Bangrak Sexually Transmitted Infections Center. The data obtained consisted of gram staining, which was divided by the number of polymorphonuclear leukocytes (PMNL), NG culture, and Nucleic Acid Amplification Test (NAAT) for NG and CT results.

Results: For the diagnostic association between PMNL and NG infection, the results revealed that samples with urethral PMNL 3+ or 4+ carried a significant likelihood ratio (LR) for positive infection, LR 5.61 (P<0.001) and LR 59.66 (P<0.001), respectively. Cervical, rectal, and pharyngeal PMNL was not related to infection. For CT infection, urethral gram stains with PMNL levels were greater than or equal to 2+ and cervical specimens with PMNL 4+ were associated with CT infection. Rectal and pharyngeal PMNL showed no significant association with CT infection.

Conclusion: Determination of PMNL levels from gram staining contributes to the diagnosis of patients with NG and CT in the urethra, particularly for patients with a high degree of inflammation.

Keywords: Gram staining; culture; nucleic acid amplification test; *Neisseria gonorrhoeae*; *Chlamydia trachomatis*
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INTRODUCTION

Gonorrhea and nongonococcal urethritis (NGU) are sexually transmitted infections (STIs) that remain major public health problems in many countries around the world. According to an estimation from the World Health Organization in 2020, there will be 374 million new STIs in all regions around the world, with 82 million being gonorrhea, and 129 million being chlamydia.[1]

From epidemiological surveillance data in Thailand, it was found that STIs increased the morbidity rate from 28.8 per 100,000 people in 2017 to 33.6 per 100,000 people in 2020, which then slightly decreased to 29.2

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per 100,000 people in 2021. The most common disease was gonorrhea, followed by NGU. In 2021, the rate of gonorrhea was 9.2 per 100,000 people, while NGU was 2.7 per 100,000 people. It was found that the age group that experienced the most STIs comprised those aged 15-24 years.[2]

Gonorrhea and NGU are STIs that cause inflammation at the site of infection. Gonorrhea is caused by *Neisseria gonorrhoeae* (NG), while NGU is caused by several types of organisms such as *Chlamydia trachomatis* (CT), *Mycoplasma genitalium, Ureaplasma urealyticum*, and *Trichomonas vaginalis*.[3,4] The most common organism that causes NGU is CT.[3,5,6] In addition, gonorrhea can also be found in coinfection with other sexually transmitted diseases.[5,7] Screening for gonorrhea and NGU can be carried out by gram staining, which can detect pathogens that are gram-negative intracellular diplococci in polymorphonuclear leukocytes (PMNL) in gonorrhea[3,8] and PMNL can be detected in both gonorrhea and nongonococcal urethritis.[3]

Previous studies found that urethral gram staining had sensitivity to the diagnosis of gonorrhea in 95% of symptomatic male patients, with 97% specificity, and cervical gram staining had 40-60% sensitivity.[9] The sensitivity of gram staining is reduced in the rectum, pharynx, and in those who are asymptomatic.[10] The sensitivity of gram staining in diagnosing chlamydia in males is 23%.[11] In females, it was found that using only gram staining had low sensitivity.[12]

Studies that address the association between the markers of inflammation (i.e. PMNL levels) and NG and CT infections are limited. This study aimed to determine the association between the severity of inflammation from gram staining with NG and CT.

MATERIALS AND METHODS

This study was conducted using laboratory test data from patients at the Bangrak STIs Center during the period from January 1, 2019, to December 31, 2021. The data were obtained from routine services consisting of gram staining, NG culture, and Nucleic Acid Amplification Test (NAAT) for NG and CT results. The data were obtained with permission from the Division of AIDS and STIs, and the Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP).

The data used for analysis consisted of gram staining results, which were divided by the number of PMNL/oil immersion field (OIF). They were categorized as no PMNL, rare (1-4 cells/OIF), 1+ (5-10 cells/OIF), 2+ (11-20 cells/OIF), 3+ (21-40 cells/OIF) and 4+ (≥41 cells/OIF).[13] Results of NG culture and NAAT for NG and CT, which are both standard tests for the diagnosis[8] of urethra, cervix, rectum, and pharynx, were collected. Gram staining data that yielded no culture or NAAT results were excluded.

Statistical methods

Data were analyzed using STATA version 17.0. Categorical data were reported as numbers and percentages. Diagnostic accuracy was measured by sensitivity, specificity, likelihood ratio (LR), and area under the receiving operating characteristics (ROC) curve. Confidence intervals for each diagnostic parameter were also estimated at a 95% level. A p-value < 0.05 was considered statistically significant.

RESULTS

Of the 1,474 gram-stained patients, 883 cases were tested for NG by cultures or NAAT and 225 cases were identified as NG infection: urethra (145 cases; 40.7%), cervix (54 cases; 13.8%), rectum (20 cases; 18.5%), pharynx (6 cases; 21.4%); 1,179 cases were tested for CT by NAAT and 431 cases were identified as CT infection: urethra (267 cases; 53.8%), cervix (102 cases; 22.5%), rectum (31 cases; 25.0%), pharynx (31 cases; 29.5%) (Table 1).

TABLE 1. Number of samples with NG and CT infections.

Gram stain samples	Total samples n	Total samples with culture/NAAT for NG n	Positive for NG n (%)	Total samples with NAAT for CT n	Positive for CT n (%)
Urethra	660	356	145 (40.7)	496	267 (53.8)
Cervix	553	391	54 (13.8)	454	102 (22.5)
Rectum	139	108	20 (18.5)	124	31 (25.0)
Pharynx	122	28	6 (21.4)	105	31 (29.5)

Abbreviations: CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; NAAT, Nucleic Acid Amplification Test

For the diagnostic association between PMNL and NG infection, our results revealed that samples with urethral PMNL 3+ or 4+ carried a significant likelihood ratio for positive infection, LR 5.61 (95% CI 2.57 to 12.24, P<0.001) and LR 59.66 (95% CI 25.37 to 140.27, P<0.001), respectively. At a PMNL cutoff point equal to or more than 3+, the sensitivity and specificity for NG infection were 75.2% (95% CI 67.4 to 82.0) and 95.7% (95% CI 92.1 to 98.0), respectively, whereas the sensitivity and specificity were 56.5% (95% CI 48.1 to 64.8) and 99.1% (95% CI 96.6 to 99.9), respectively, for a cutoff point at 4+. Cervical, rectal, and pharyngeal PMNL were not related to infection (Table 2).

For CT infection, urethral gram stain with PMNL levels 2+, 3+, and 4+, the LRs were significantly higher than 1 [LR 1.89 (95% CI 1.09 to 3.27, P 0.024) for PMNL 2+, LR 2.24 (95% CI 1.28 to 3.92, P 0.005) for PMNL 3+, and LR 6.00 (95% CI 3.02 to 11.91, P <0.001) for PMNL 4+]

TABLE 2. Diagnostic accuracy for the number of PMNL for NG infections.

	Positive for NG		Negative for NG		Sensitivity % (95%CI)	Specificity % (95%CI)	LR (95%CI)	P-value	AuROC (95%CI)
	n	(%)	n	(%)					
Urethra	(n=145)		(n=211)						
No PMNL	4	(2.8)	18	(8.5)	NA	NA	0.32 (0.08, 1.01)	0.036	0.89 (0.85, 0.93)
Rare	13	(9.0)	133	(63.0)	97.2 (93.1, 99.2)	8.5 (5.1, 13.1)	0.14 (0.08, 0.25)	<0.001	
1+	12	(8.3)	28	(13.3)	88.3 (81.9, 93.0)	71.6 (65.0, 77.5)	0.62 (0.31, 1.26)	0.188	
2+	7	(4.8)	23	(10.9)	80.0 (72.6, 86.2)	84.8 (79.3, 89.4)	0.44 (0.19, 1.04)	0.061	
3+	27	(18.6)	7	(3.3)	75.2 (67.4, 82.0)	95.7 (92.1, 98.0)	5.61 (2.57, 12.24)	<0.001	
4+	82	(56.6)	2	(1.0)	56.5 (48.1, 64.8)	99.1 (96.6, 99.9)	59.66 (25.37, 140.27)	<0.001	
Cervix	(n=54)		(n=337)						
No PMNL	0	(0.0)	7	(2.1)	NA	NA	NE	NE	0.64 (0.56, 0.72)
Rare	7	(13.0)	80	(23.7)	100.0 (93.4, 100.0)	2.1 (0.8,4.2)	0.29 (0.13,0.62)	0.001	
1+	2	(3.7)	45	(13.4)	87.0 (75.1, 94.6)	25.8 (21.2, 30.8)	0.15 (0.42, 0.51)	0.003	
2+	12	(22.2)	61	(18.1)	83.3 (70.7, 92.1)	39.2 (33.9, 44.6)	0.65 (0.34, 1.25)	0.196	
3+	16	(29.6)	94	(27.9)	61.1 (46.9, 74.1)	57.3 (51.8, 62.6)	0.56 (0.32, 0.99)	0.047	
4+	17	(31.5)	50	(14.8)	31.5 (19.5, 45.6)	85.2 (80.9, 88.8)	1.12 (0.62, 2.03)	0.701	
Rectum	(n=20)		(n=88)						
No PMNL	5	(25.0)	44	(46.6)	NA	NA			0.65 (0.52, 0.78)
Rare	8	(40.0)	36	(40.9)	75.0 (50.9, 91.3)	46.6 (35.9, 57.5)	0.5 (0.18, 1.40)	0.187	
1+	3	(15.0)	4	(4.6)	35.0 (15.4, 59.2)	87.5 (78.7, 93.6)	0.98 (0.39, 2.43)	0.961	
2+	2	(10.0)	4	(4.6)	20.0 (5.7, 43.7)	92.0 (84.3, 96.7)	3.30 (0.73, 14.89)	0.119	
3+	1	(5.0)	3	(3.4)	10.0 (1.2, 31.7)	96.6 (90.4, 99.3)	2.20 (0.39, 12.46)	0.371	
4+	1	(5.0)	0	(0.0)	5.0 (0.1, 24.9)	100.0 (95.9, 100.0)	1.47 (0.15, 14.81)	0.744	
Pharynx	(n=6)		(n=22)						
No PMNL	1	(16.7)	4	(18.2)	NA	NA	0.92 (0.83, 10.17)	0.943	0.61 (0.34,0.89)
Rare	3	(50.0)	16	(72.7)	83.3 (35.9, 99.6)	18.2 (5.2, 40.3)	0.69 (0.15, 3.21)	0.630	
1+	1	(16.7)	2	(9.1)	33.3 (4.3, 77.7)	90.1 (70.8, 98.9)	1.83 (0.14, 24.11)	0.639	
2+	0	(0.0)	0	(0.0)	16.7 (0.4, 64.1)	100.0 (84.6, 100.0)	NE	NE	
3+	0	(0.0)	0	(0.0)	16.7 (0.4, 64.1)	100.0 (84.6, 100.0)	NE	NE	
4+	1	(16.7)	0	(0.0)	16.7 (0.4, 64.1)	100.0 (84.6, 100.0)	NE	NE	

Abbreviations: AuROC, area under the receiving operating characteristics curve; CI, confidence interval; LR, likelihood ratio; NA, not applicable; NE: not estimable; NG, *Neisseria gonorrhoeae*; PMNL: polymorphonuclear leukocytes

and, thus, were associated with the infection. At a PMNL cutoff point equal to or more than 2+, the sensitivity was 55.1% (95% CI 48.9 to 61.1), and specificity was 79.9% (95% CI 74.1 to 84.9). At PMNL 3+, the sensitivity was 38.6. % (95% CI 32.7 to 44.7), and specificity was 88.6% (95% CI 83.8 to 92.4). At PMNL 4+, sensitivity was 21.0% (95% CI 16.2 to 26.4), and specificity was 96.5% (95% CI 93.2 to 98.5). In cervical specimens, PMNL 4+ was modestly associated with CT infection, with LR 2.03 (95% CI 1.27 to 3.25, P 0.003). At a PMNL cutoff point equal to or more than 4+, sensitivity was 33.3% (95%CI 24.3 to 43.4) and specificity was 83.5% (95% CI 79.2 to 87.2) for diagnosis of CT infection. Rectal and pharyngeal PMNL showed no significant association with CT infection (Table 3).

In terms of discriminative ability, only urethral PMNL levels had an area under ROC higher than 0.70, the generally defined acceptable threshold, for both NG and CT infections (Figs 1 and 2).

TABLE 3. Diagnostic accuracy for the number of PMNL for CT infections.

	Positive for CT		Negative for CT		Sensitivity % (95%CI)	Specificity % (95%CI)	LR (95%CI)	P-value	AuROC (95%CI)
	n	(%)	n	(%)					
Urethra	(n=267)		(n=229)						
No PMNL	9	(3.4)	19	(8.3)	NA	NA	0.41 (0.18, 0.90)	0.025	0.72 (0.68, 0.76)
Rare	75	(28.1)	139	(60.7)	96.6 (93.7, 98.4)	8.3 (5.1, 12.7)	0.46 (0.33, 0.64)	<0.001	
1+	36	(13.5)	25	(10.9)	68.5 (62.6, 74.1)	69.0 (62.6, 74.9)	1.23 (0.72, 2.12)	0.443	
2+	44	(16.5)	20	(8.7)	55.1 (48.9, 61.1)	79.9 (74.1, 84.9)	1.89 (1.09, 3.27)	0.024	
3+	47	(17.6)	18	(7.9)	38.6 (32.7, 44.7)	88.6 (83.8, 92.4)	2.24 (1.28, 3.92)	0.005	
4+	56	(21.0)	8	(3.5)	21.0 (16.2, 26.4)	96.5 (93.2, 98.5)	6.00 (3.02, 11.91)	<0.001	
Cervix	(n=102)		(n=352)						
No PMNL	0	(0.0)	7	(2.0)	NA	NA	NE	NE	0.63 (0.57 ,0.69)
Rare	10	(9.8)	83	(23.6)	100.0 (96.4, 100.0)	2.0 (0.8, 4.1)	0.42 (0.21, 0.82)	0.011	
1+	12	(11.8)	45	(12.8)	90.2 (82.7, 95.2)	25.6 (21.1, 30.5)	0.92 (0.47, 1.81)	0.815	
2+	18	(17.7)	64	(18.2)	78.4 (69.2, 86.0)	38.4 (33.2, 43.7)	0.97 (0.55, 1.71)	0.926	
3+	28	(27.5)	95	(27.0)	60.8 (50.6, 70.3)	56.5 (51.2, 61.8)	1.02 (0.63, 1.64)	0.935	
4+	34	(33.3)	58	(16.5)	33.3 (24.3, 43.4)	83.5 (79.2, 87.2)	2.03 (1.27, 3.25)	0.003	
Rectum	(n=31)		(n=93)						
No PMNL	7	(22.6)	44	(48.8)	NA	NA	0.48 (0.20, 1.16)	0.100	0.63 (0.53, 0.73)
Rare	17	(54.8)	36	(38.4)	77.4 (58.9, 90.4)	47.3 (36.9, 57.9)	1.42 (0.70, 2.87)	0.332	
1+	3	(9.7)	4	(4.7)	22.6 (9.6, 41.1)	86.0 (77.3, 92.3)	2.25 (0.49, 10.30)	0.294	
2+	2	(6.5)	5	(4.7)	12.9 (3.6, 29.8)	90.3 (82.4, 95.5)	1.20 (0.22, 6.53)	0.832	
3+	2	(6.5)	3	(2.3)	6.5 (0.8, 21.4)	95.7 (89.4, 98.8)	2.00 (0.33, 12.22)	0.451	
4+	0	(0.0)	1	(1.2)	0.0 (0.0, 11.2)	98.9 (94.2, 100.0)	NE	NE	
Pharynx	(n=31)		(n=74)						
No PMNL	6	(19.4)	25	(33.8)	NA	NA	0.57 (0.21, 1.53)	0.264	0.61 (0.50, 0.71)
Rare	18	(58.1)	41	(55.4)	80.6 (62.5, 92.5)	33.8 (23.2, 45.7)	1.05 (0.52, 2.10)	0.895	
1+	4	(12.9)	6	(8.1)	22.6 (9.6, 41.1)	89.2 (79.8, 95.2)	1.59 (0.42, 6.01)	0.492	
2+	2	(6.5)	2	(2.7)	9.7 (2.0, 25.8)	97.3 (90.6, 99.7)	2.39 (0.34, 16.92)	0.382	
3+	1	(3.2)	0	(0.0)	3.2 (0.1, 16.7)	100.0 (95.1, 100.0)	NE	NE	
4+	0	(0.0)	0	(0.0)			NE	NE	

Abbreviations: AuROC, area under the receiving operating characteristics curve; CI, confidence interval; LR, likelihood ratio; NA, not applicable; NE: not estimable; CT *Chlamydia trachomatis*; PMNL: polymorphonuclear leukocytes

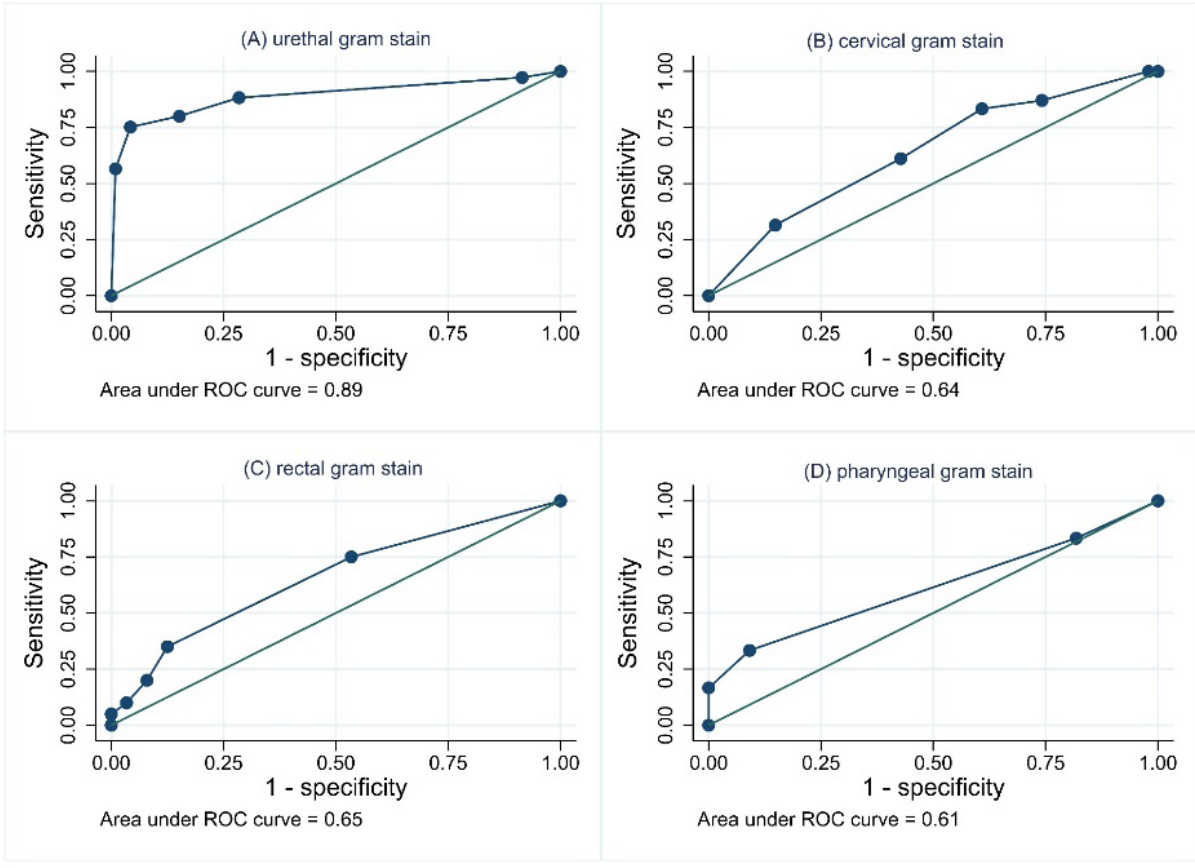


Fig 1. ROC curve for diagnosis of NG from each specimen.

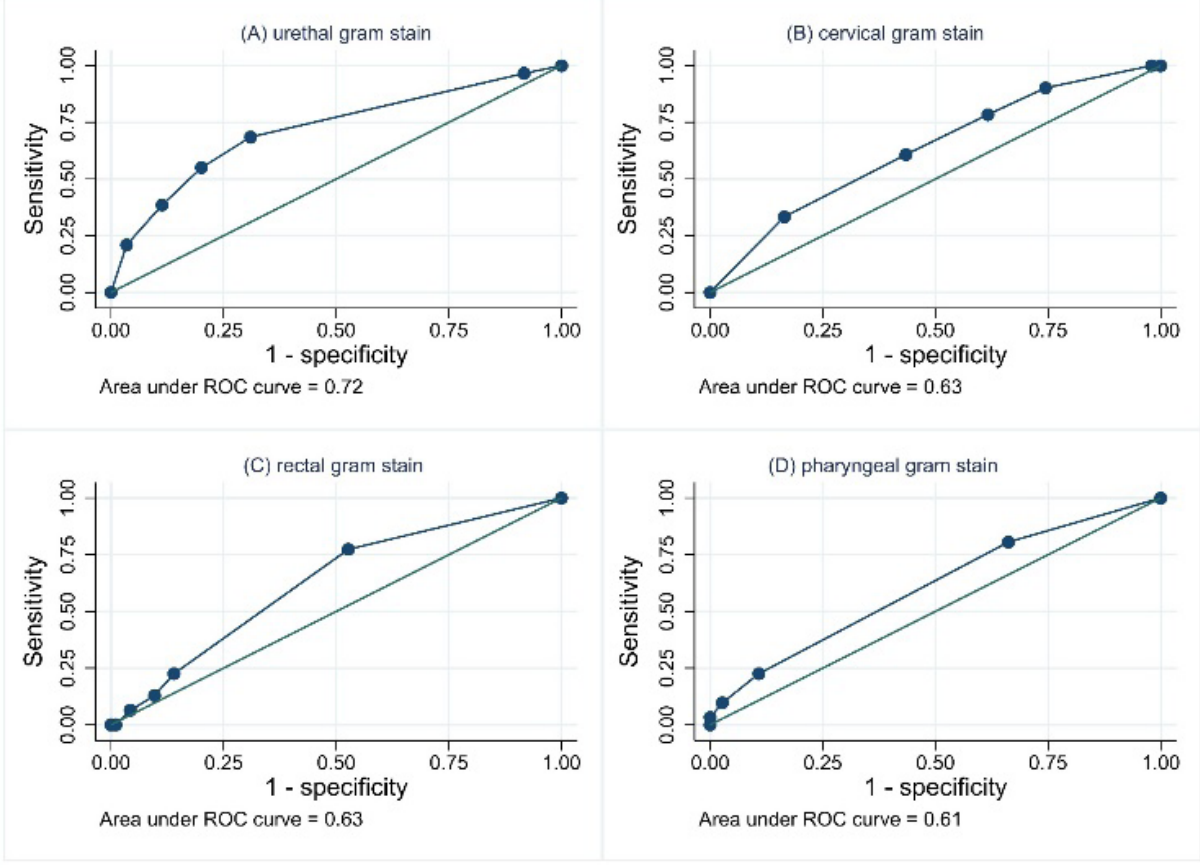


Fig 2. ROC curve for diagnosis of CT from each specimen.

DISCUSSION

Gonorrhea and chlamydia can cause inflammation at the site of the infection. The patient may or may not have symptoms. Screening for inflammation from gram staining can aid in diagnosis and treatment in cases where a culture test or NAAT is not available. The US Center for Disease Control defined PMNL ≥2 WBC/OIF as urethritis and PMNL >10 WBC/high-power field as cervicitis.[3] However, some studies used PMNL values >30 WBC per high-power field for cervicitis.[14,15]

From this study, the inflammation defined by PMNL from gram staining at the urethra was significantly associated with NG and CT infections. Those infected with NG were associated with higher PMNL levels than those with CT (3+ or above in NG and 2+ or above in CT). Inflammation of the cervix was statistically associated with CT infection at the PMNL level 4+. The severity of anal and pharyngeal inflammation was not associated with NG and CT infection.

Therefore, the detection of inflammation from PMNL levels may help in the diagnosis of gonorrhea and chlamydia in the urethra. The high levels of PMNL will have a relatively high specificity found in urethral and cervical infections, so it can help in the diagnosis of infection at the urethra and cervix. However, it may not be used to aid in the diagnosis of gonorrhea or chlamydia in the rectal and pharyngeal region. However, higher PMNL levels have been found to have low sensitivity, while lower PMNL levels have high sensitivity but low specificity, so other factors such as the patient's symptoms, and history of sexual behavior should be used to support the diagnosis.

Usually, gonorrhea is diagnosed using culture or NAAT, while chlamydia uses NAAT as the standard test. However, NAAT for chlamydia is not widely used in Thailand due to the high cost. The syndromic management is more common in gonorrhea and will lead to overtreatment which can cause antimicrobial-resistant microorganisms.[16] Therefore, it is necessary to step forward the diagnosis of gonorrhea and chlamydia from a syndromic approach to laboratory diagnosis by using at least gram staining which is feasible in all levels of hospitals.

From this study, the cutoff of PMNL from gram staining can be used for decision-making in the diagnosis and treatment of gonorrhea and chlamydia. This will reduce the rate of drug resistance from the overuse of antibiotics.

This study had several limitations. First, the number of rectal and pharyngeal samples was too low to draw rigorous conclusions on the diagnostic association between PMNL levels and infections. Second, there was no standardized protocol to collect the specimens from all sites from each included patient, and not all patients were verified for both infections. Therefore, we could not accurately estimate the proportion of patients with isolated infection of NG and CT or patients with co-infection from both organisms.

CONCLUSION

The determination of PMNL levels from gram staining contributes to the diagnosis of patients with NG and CT in the urethra, particularly patients with a high degree of inflammation. If the amount of inflammation is lower, however, other factors such as a history of sexual behavior or additional patient symptoms should be considered to support the diagnosis.

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Conflict of interest: none

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