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Original Article

A cross-sectional study on the relationship between endocervical polymorphonuclear cell counts and chlamydial cervicitis in female patients in Hong Kong

香港女性患者的宮頸內膜多形核白血球細胞計數與衣原體宮頸炎之間關係的橫斷面研究

NM Lau 劉顏銘 and HF Ho 何慶豐

Background: The reliability of polymorphonuclear cell (PMN) counts on endocervical smear and non-specific genital infection (NSGI) as a predictor of chlamydial cervicitis remains uncertain. ***Objectives:*** To investigate the relationship between endocervical PMN counts, NSGI and chlamydial cervicitis and to identify risk factors and cervical signs associated with chlamydial infection. ***Methods:*** The study was conducted in five female Social Hygiene Clinics in Hong Kong from August 2018 to May 2019. The attending doctor collected clinical information and performed speculum examination. Endocervical smears were examined for PMN counts under high-power field. *Chlamydia trachomatis* infection was diagnosed by nucleic acid amplification test. ***Results:*** A total of 556 participants were recruited. After exclusion, statistical analysis was performed on 517 participants. Among them, 16.6% was diagnosed chlamydial cervicitis and 198 (38.3%) fulfilled the criteria of NSGI by endocervical swab. NSGI (OR 4.115; 95% CI 2.244-7.546; p<0.001), age ≤24 (OR 2.000; 95% CI 1.108-3.611; p=0.022), *C. trachomatis* contact (OR 66.417; 95% CI 18.541-237.912; p<0.001), cervical erythema and oedema (OR 3.016; 95% CI 1.369-6.642; p=0.006) were independently associated with chlamydial cervicitis. The use of NSGI for the detection of chlamydial cervicitis had a positive predictive value (PPV) of 30.3%. ***Conclusion:*** With a low PPV, NSGI had limited utility for the prediction of *C. trachomatis* infection in the clinical setting.

背景：宮頸內膜塗片多形核白血球細胞計數和非特異性生殖器感染作為衣原體宮頸炎的預測因子的可靠性仍不確定。**目的**：研究宮頸內膜多形核白血球細胞計數、非特異性生殖器感染和衣原體宮頸炎之間的關係，並確定與衣原體感染相關的風險因素和宮頸體徵。**方法**：本研究於 2018 年 8 月至 2019 年 5 月在五間女性社會衛生服務診所進行，主治醫生收集臨床資料並進行陰道窺器檢查。在高倍視野下檢查宮頸內膜塗片的多形核白血球細胞計數。通過核酸增幅檢驗確診沙眼衣原體感染。**結果**：共招募了 556 名參與者。篩選後，對 517 名參與者進

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行了統計分析。其中，16.6% 的人被診斷為衣原體宮頸炎，另 198 人（38.3%）通過宮頸內膜拭子符合非特異性生殖器感染的診斷標準。非特異性生殖器感染（OR 4.115;95% CI 2.244-7.546; p<0.001）、24 歲或以下（OR 2.000; 95% CI 1.108-3.611; p=0.022）、沙眼衣原體接觸史（OR 66.417；95% CI 18.541-237.912；p<0.001）、宮頸紅斑及水腫（OR 3.016；95% CI 1.369-6.642；p=0.006）皆與衣原體宮頸炎獨立相關。使用非特異性生殖器感染查找衣原體宮頸炎的陽性預測值為 30.3%。**結論**：由於陽性預測值較低，非特異性生殖器感染在臨床環境中預測沙眼衣原體感染的效用有限。

Keywords: Chlamydial cervicitis, chlamydial infection, non-specific genital infection, polymorphonuclear cell counts

關鍵詞：衣原體宮頸炎、衣原體感染、非特異性生殖器感染、多形核白血球細胞計數

Introduction

Chlamydia trachomatis (*CT*) infection is one of the most prevalent sexually transmitted infections (STI) worldwide.[1] Previous studies showed that it is asymptomatic in approximately 70% of women.[2] Studies reported that chlamydial infection was associated with increased risk of pelvic inflammatory disease,[3,4] which can result in sequelae in the reproductive system, including infertility and ectopic pregnancy.[5] Early identification and treatment of *C. trachomatis* infections is important to prevent these complications.

The term non-specific genital infection (NSGI) was described as a distinct clinical entity in the British Medical Journal (BMJ) in 1974.[6] NSGI is diagnosed when there are non-specific cervical and vaginal infections but routine microbiological techniques do not identify any pathogens.[7] However, the diagnostic criteria may vary between different centres.[8,9]

In our service, NSGI is a diagnosis in females and comprises of inflammation of the endocervix or anterior urethra that is not caused by *Neisseria gonorrhoeae* The potential infective causes include *Chlamydia trachomatis* (Group D to K), *Ureaplasma urealyticum, Trichomonas vaginalis* and rarely herpes simplex virus.[10] Specimens from endocervix are Gram-stained and visually examined through a light microscope. polymorphonuclear cell (PMN) is counted with a high power field (magnification of 1000X) in five non-adjacent high power field (HPF) and averaged. NSGI is defined as:

1) 30 or more PMN/HPF by Gram stain in the endocervical smear and the average count in five HPF (with absent gram-negative intracellular diplococci); or
2) 15-29 PMN/HPF in the endocervical smear and the patient has symptom of abnormal discharge and risk factors such as practising high risk sexual behaviour, history of contact with a proven case of non-gonococcal urethritis (NGU), chlamydial infection or gonorrhoea; or
3) 15 or more PMN/HPF by Gram stain in the urethral smear with the presence of symptoms of dysuria and frequency.[10]

There is inconsistent evidence on utilisation of increased PMN counts on Gram stained endocervical smear as a predictor of chlamydial cervicitis from previous studies. There are also inconsistent results on clinical signs of cervicitis in predicting *C. trachomatis* infection.[11-14]

Objectives

Primary objective: To study the association between endocervical PMN counts, NSGI and chlamydial cervicitis in female patients at risk of sexually transmitted infections.

Secondary objective: To identify risk factors and clinical signs of cervicitis associated with chlamydial cervicitis.

Methodology

Study design

This is a cross-sectional study on female patients attending Social Hygiene Clinics of the Department of Health of Hong Kong for screening of sexually transmitted infections from August 2018 to May 2019.

Study population

Participants were recruited from five Social Hygiene Clinics in Hong Kong. Inclusion criteria were Chinese, aged 18-64 years, and sexually active within the past year. The exclusion criteria were pregnancy, menstruating, history of hysterectomy, intrauterine contraceptive device user and antibiotics use within the previous 21 days.

Case definition and diagnosis

A case of chlamydial cervicitis was defined as nucleic acid amplification test (NAAT) positive for C. *trachomatis* specific DNA or RNA by endocervical swab.

Data collection, clinical examination and specimen collection

Relevant data was collected by the medical officer in the consultation room. Speculum examination was performed by attending doctor to examine the cervix for the presence of mucopurulent endocervical discharge, endocervical contact bleeding, erythema and oedema, and ectropion. Gram stained urethral and endocervical smears would be examined for the number of PMN per HPF, presence of intracellular Gram-negative diplococci and yeast by RN. The number of PMNs was counted, averaged, and categorised as follows:

No PMN found	-	
Less than 5 PMNs	+	(1+)
5 - 14 PMNs	++	(2+)
15 - 29 PMNs	+++	(3+)
30 or more PMNs	++++	(4+)

For study purpose, those specimens with 5-14 PMNs (2+) were further classified as ≥10 or <10 by trained nursing staff. The specimens for detection of C. *trachomatis* were sampled by Cobas® PCR Media Dual Swab Sample Packet by properly trained nursing staff.

Primary and secondary outcomes

The primary outcomes were the association between endocervical PMN counts and chlamydial cervicitis, and the performance of NSGI for the detection of chlamydial cervicitis. The secondary outcome was to identify factors that were independently associated with chlamydial cervicitis.

Statistical analysis

Statistical analysis was performed using the Statistical Program for Social Sciences 21.0 for Windows (SPSS Inc., Chicago, Illinois, USA computer software).

Results

Recruitment of participants

A total of 556 participants were recruited from August 2018 to May 2019. After exclusion, 517 were included for statistical analysis (Figure 1).

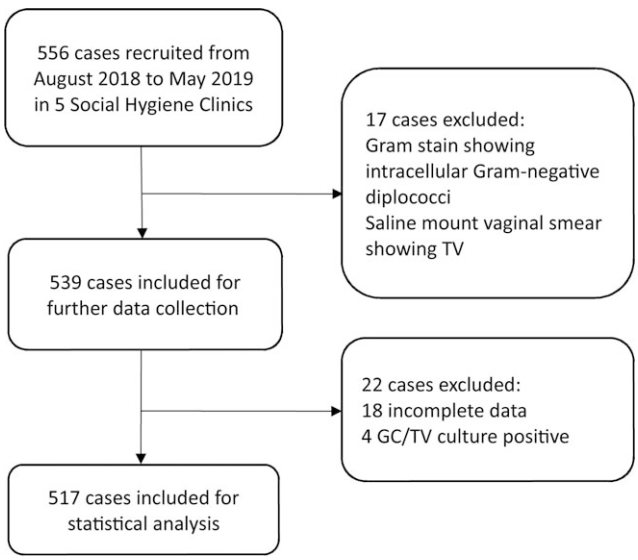

Figure 1. Study enrollment and analysis populations.

Participant characteristics

Characteristics of participants

Characteristics of participants are shown in Table 1. The mean age of the 517 participants was 33.6 years (standard deviation 11.2). Among them, 27.7% were ≤24 years old.

Personal STI history and contact history

A previous diagnosis of *C. trachomatis* infection was reported in 18.8% of the participants. Among all the subjects, 25.9% had current or history of other STI. For contact history, 6.4% reported sexual contact within 60 days before partner's diagnosis of *C. trachomatis* infection or onset of symptoms.

Genitourinary symptoms and cervical signs

42.9% of the participants reported one or more genitourinary symptoms. The most commonly reported symptom was abnormal vaginal discharge (39.2%), followed by dysuria (4.4%), urinary frequency (2.9%), lower abdominal pain (2.1%) and dyspareunia (0.4%). The cervical signs on vaginal speculum examination were distributed as follows: endocervical purulent discharge (28.6%), contact bleeding (1.0%), cervical ectopy (10.3%) and cervical erythema and oedema (8.5%).

Clinical outcomes

Results of univariate analysis

The results of univariate analysis of relationship between PMN/HPF and chlamydial cervicitis are shown in Table 2.

Findings from the logistic regression analysis

The results of logistic regression analysis are summarised in Table 3.

To summarise, young age (≤24 years old), CT contact, NSGI by endocervical smear, cervical erythema and oedema were significantly associated with chlamydial cervicitis.

The performance of NSGI for the detection of chlamydial cervicitis

Among all the participants, 86 (16.6%) was diagnosed with chlamydial cervicitis as tested by NAAT on endocervical swab. Sixty of them were diagnosed NSGI by endocervical smear before NAAT result was available (Table 4).

Of the 198 patients diagnosed NSGI by endocervical smear, 138 of them had negative *C. trachomatis* NAAT result (Figure 2).

Table 1. Characteristics of patients recruited

Patient characteristics	
Age (year)	**Mean (+/-SD)/Number (%)**
	33.6 (+/- 11.2)
≤24	143 (27.7%)
>24	374 (72.3%)
Education level	**Number (%)**
Primary and below	43 (8.3%)
Secondary	277 (53.6%)
Tertiary	197 (38.1%)
Smoking status	**Number (%)**
Smoker	122 (23.6%)
Non-smoker	395 (76.4%)
Marital status	**Number (%)**
Single	260 (50.3%)
Married	188 (36.4%)
Divorced	69 (13.3%)
Use of oral contraceptives	**Number (%)**
Yes	54 (10.4%)
No	463 (89.6%)
Condom use	**Number (%)**
Never	237 (45.8%)
Sometimes	223 (43.1%)
Always	57 (11.0%)
Sexual behaviour	**Number (%)/Median (IQR)**
≥2 sexual partners within 12 months	191 (36.9%)
≥1 new sexual partners within 3 months	126 (24.4%)
Number of lifetime sexual partners	3 (2-6)

Table 5 summarises the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio of NSGI for detection of chlamydial cervicitis. The use of NSGI for detection of chlamydial cervicitis had a sensitivity of 69.8% and a specificity of 68.0%. It had a positive predictive value of 30.3% and positive likelihood ratio 2.18. The negative predictive value is 91.8%.

Table 2. Univariate analysis of correlation of PMN/HPF with chlamydial cervicitis

	***C. trachomatis* NAAT (endocervical)**		
	Positive (n=86) Number (%)	**Negative (n=431) Number (%)**	**P value**
Endocervical PMN/HPF			
None	4 (4.7%)	37 (8.6%)	<0.001
1+	8 (9.3%)	97 (22.5%)	
2+	8 (9.3%)	106 (24.6%)	
3+	25 (29.1%)	93 (21.6%)	
4+	41 (47.7%)	98 (22.7%)	
Endocervical PMNs ≥10	69 (80.2%)	255 (59.2%)	<0.001
Urethral PMN/HPF			
None	15 (17.4%)	169 (39.2%)	<0.001
1+	33 (38.4%)	159 (36.9%)	
2+	13 (15.1%)	63 (14.6%)	
3+	25 (29.1%)	40 (9.3%)	
NSGI (Endocervical)	60 (69.8%)	138 (32.0%)	<0.001
NSGI (Urethral)	5 (5.8%)	6 (1.4%)	0.023

Table 3. Logistic regression analysis of factors associated with chlamydial cervicitis

Variables	**Odds ratio (95% CI)**	**P value**
Age ≤24 years old	2.000 (1.108-3.611)	0.022
CT contact	66.417 (18.541-237.912)	<0.001
Cervical erythema & oedema	3.016 (1.369-6.642)	0.006
NSGI (endocervical)	4.115 (2.244-7.546)	<0.001

Table 4. The performance of NSGI for the detection of chlamydial cervicitis

	***C. trachomatis* NAAT (endocervical)**	
	Positive (n=86)	**Negative (n=431)**
NSGI (endocervical)	60	138
Not NSGI	26	293

Discussion

Association between PMN counts on Gram stained endocervical smear and chlamydial cervicitis

The Sexually Transmitted Diseases Treatment Guidelines published in 2015 by Centers for Disease Control and Prevention (CDC) stated that increased number of leukocytes (>10 PMNs) on Gram stained endocervical smear had a low positive predictive value in the diagnosis of chlamydial cervicitis.[15] From previous studies, there were conflicting results on clinical use of increased PMN counts on Gram stained endocervical smear as a predictor of chlamydial cervicitis. There was no standardised cut-off for significant PMN counts.[14,16-20]

In our practice, Gram-stained urethral and endocervical smears are examined for the number of PMN/HPF. Our service uses a higher cut-off for the significant number of leukocytes on Gram-stained endocervical smear than most previous studies. Our study found that the diagnosis of NSGI was significantly associated with chlamydial cervicitis. However, the use of NSGI for the detection of chlamydial cervicitis had a suboptimal sensitivity of 69.8% and specificity of 68.0%. It had a low positive predictive value of 30.3%. This showed that NSGI had limited utility for prediction of chlamydial cervicitis in the clinical setting.

Association between clinical signs of cervicitis and chlamydial cervicitis

Cervicitis is diagnosed by "a purulent or mucopurulent endocervical exudate visible in the endocervical canal or on an endocervical swab specimen and sustained endocervical bleeding easily induced by gentle passage of a cotton swab through the cervical os".[15] Earlier studies had inconsistent results on clinically significant signs of cervicitis in predicting *C. trachomatis* infection.[11-14,20,21]

Our study showed that after adjusting for confounding factors, cervical erythema and oedema was independently associated with chlamydial cervicitis. However, cervical erythema and oedema was not an objective finding and was observer-dependent. From earlier studies

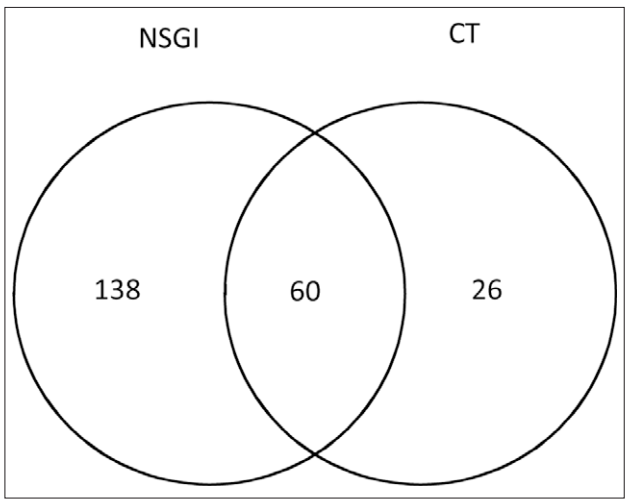

Figure 2. Participants diagnosed NSGI and/or chlamydial cervicitis (n=224). Circles represent fulfillment of criteria: NSGI: ≥30 PMN/HPF in endocervical smear or 15-29 PMN/HPF in cervical smear with symptom of abnormal discharge and risk factors; CT: Endocervical swab NAAT positive for *C. trachomatis*.

Table 5. Sensitivity, specificity, PPV, NPV and likelihood ratio of NSGI for detection of chlamydial cervicitis

	***C. trachomatis* NAAT**							
	Positive	**Negative**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**LR+**[†]	**LR-**[‡]
NSGI	60	138	69.8	68.0	30.3	91.8	2.18	0.44

[†] Positive likelihood ratio

[‡] Negative likelihood ratio

and our study, we concluded that clinical signs of cervicitis were non-specific findings with many different causes.

Risk factors associated with chlamydial cervicitis

Our study had identified several risk factors associated with chlamydial cervicitis. In accordance with previous studies,[22-24] this study demonstrated that age ≤24 years old was significantly associated with chlamydial cervicitis. Several international guidelines suggested all sexually active women younger than 25 years of age as well as older women at risk for chlamydia should be offered chlamydia screening annually.[15,25-27]

In keeping with previous studies,[28] a large proportion (57.1%) of our *C. trachomatis* positive cases were asymptomatic. This finding raised the awareness of launching a comprehensive screening programme in high risk group. With our finding that *C. trachomatis* infection is prevalent among young women attending STI clinics in Hong Kong, further studies are needed to evaluate whether a selective screening program that target asymptomatic young women is justified in local population.

This study revealed that CT contact was independently associated with chlamydial cervicitis. This was supported by previous study that nearly 70% of male partners of women with chlamydial infection were also infected.[15]

Clinical relevance

Practically, for timely treatment and prevention of further transmission, clinical and microscopic criteria are used to predict infection before diagnostic test results are available. Unlike urethritis in male patients, there is a lack of objective criteria for cervicitis for female patients. Previous studies have evaluated different clinical and microscopic criteria of cervicitis, but the overall agreement of these indicators has been inconsistent.

Our study showed that the use of NSGI for detection of chlamydial cervicitis had a low positive predictive value of 30.3% and positive likelihood ratio 2.18. This showed that NSGI was of limited clinical utility for the presumptive diagnosis of chlamydial cervicitis. This might be beneficial for patients who could not return for treatment when the final diagnostic test result is available. Moreover, though the sensitivity of the Cobas 4800 CT/NG test for endocervical swabs for detection of *C. trachomatis* is high (92.0-93.8%), a small number of patients may still have a false-negative result. Another advantage of using NSGI as a presumptive diagnosis is for earlier partner referral and epidemiological treatment. On the other hand, this may lead to anxiety and stress in patients who resent being labelled as having a sexually transmitted infection.

From our study result, absence of NSGI had a negative predictive value of 91.8% for chlamydial cervicitis. A case not fulfilling the criteria of NSGI indicated that cervicitis was much less likely. Patients not diagnosed NSGI should wait for NAAT result rather than have presumptive treatment.

The present study showed that although NSGI was independently associated with chlamydial cervicitis, it had a low positive predictive value. Of the 198 patients diagnosed NSGI (with *N. gonorrhoeae* and *T. vaginalis* excluded), 138 of them tested negative for *C. trachomatis*. This indicates that there were other causes of cervicitis apart from the routinely-tested pathogens (*C. trachomatis, N. gonorrhoeae* and *T. vaginalis*). These include *M. genitalium* (MG) and *U. urealyticum* (UU) as well as non-infective causes of NSGI.

There was increasing evidence showing MG as a cause of cervicitis. In women with clinical cervicitis, prevalence of MG varies from 6.3% to 28.6% in different studies.[15,29-31] Further prospective studies are necessary to determine its causal relationship. The pathological role of UU in cervicitis is still

controversial and routine screening is not recommended.[32,33] Based on result of the present study and previous studies, we need to consider other infective causes of cervicitis especially MG. It would be beneficial to patients if we need could liaise with our laboratory for testing of MG.

Our study has identified several risk factors associated with chlamydial cervicitis. Identification of risk factors can help future health education, which is an important part in prevention of sexually transmitted disease. Health education is an important in preventing re-infection. Proving that CT contact as a predictive factor for *C. trachomatis* infection is not only important for more timely treatment, but also for counselling on partner referral and practice of safe sex.

Limitations

This study had several limitations. First, we had no data on prevalence of MG and UU in our study population, which could be a cause of NSGI. The prevalence of MG varies from 4.5% to 28.6% in different studies.[29-31,34,35]

Secondly, subjects were recruited from those who attended Social Hygiene Clinics and agreed to participate in the study. They might not be representative of the general population.

Third, reporting bias might exist especially in questions about sensitive information such as number of sexual partners and history of STI. Furthermore, patient might not have correct information about contact history if no referral was provided by her partner.

Conclusion

To conclude, this study showed that NSGI, young age ≤24 years old, CT contact, cervical erythema and oedema were independently associated with chlamydial cervicitis. There was a lack of objective criteria for cervicitis and definitions varied in previous studies. With a low positive predictive value, the diagnosis of NSGI had limited utility for prediction of chlamydial cervicitis in the clinical setting.

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