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Microscopy of Gram-stained urethral smear in the diagnosis of urethritis: Which threshold value should be selected?

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Abstract

The aim of this study was to investigate the sensitivity of GSS in the diagnosis of urethritis in patients who present to the outpatient clinic with symptoms of urethritis. Sixty-three male patients who presented to our outpatient clinic with symptoms of urethritis between January and March 2018 were evaluated. Urethral smear samples obtained from patients were evaluated both by GSS examination and by Real-time Multiplex Polymerase Chain Reaction (rt-MPCR) assay. The sensitivity and specificity of GSS in detecting gonococcal urethritis (GU) and nongonococcal urethritis (NGU) were calculated for threshold values of ≥5 and ≥2 PMNL/HPF. The mean age was 33.2 ± 7.1 years. According to the rt-MPCR results, 14 patients had GU and 27 patients had NGU. A threshold value of ≥5 PMNL/HPF in the GSS demonstrated 92.9% sensitivity in the diagnosis of GU and 55.6% sensitivity in the diagnosis of NGU. A threshold value of ≥2 PMNL/HPF reached 100% sensitivity for GU and 92.6% sensitivity for NGU. A cut-off value of ≥5 PMNL/HPF in the GSS has low sensitivity in the diagnosis of NGU. On the other hand, a threshold value of ≥2 PMNL/HPF seems to have higher sensitivity in the diagnosis of both GU and NGU.

KEYWORDS

gram-stained smear, nongonococcal urethritis, PCR, urethritis

1 | INTRODUCTION

Urethritis is a disease caused usually by sexually transmitted pathogens in males. Patients may be completely asymptomatic or present with symptoms such as dysuria, urethral itching and/or urethral discharge.(Moi, Blee, & Horner, 2015) Urethritis is defined as gonococcal when caused by *Neisseria gonorrhoeae* and as nongonococcal when caused by other types of pathogens.(Wang, Kong, Wang, Mckechnie, & Gilbert, 2010) Generally, the diagnosis of male urethritis is made by detecting ≥5 polymorphonuclear leucocytes (PMNL) averaged over five different high power fields (HPF) in the Gram-stained smear (GSS) of a urethral discharge sample.(Horner, Blee, Falk, van der Meijden, & Moi, 2016; Swartz et al., 1978) In order to establish the diagnosis of gonococcal urethritis (GU), the presence of intracellular Gram-negative diplococci should be confirmed. On the other hand, the presence of ≥5 PMNL/HPF coupled with a lack of intracellular diplococci indicate nongonococcal urethritis (NGU). However, especially in samples with mild inflammation, significant inter- and intra-observer differences occur in the evaluation of urethral samples.(Smith et al., 2003; Willcox, Adler, & Belsey, 1981) Therefore, the threshold value for GSS has become a matter of debate. Recently, Rietmeijer & Mettenbrink, 2012 proposed a cut-off value of ≥2 PMNL/HPF for the diagnosis of urethritis which was also approved by the 2015 Sexually Transmitted Disease (STD) Treatment Guidelines published by the Centers for Disease Control and Prevention (CDC).(Workowski & Bolan, 2015) Other methods for detecting urethral infections include cultures of urethral discharge and/or urine and molecular techniques such as polymerase chain reaction (PCR). PCR is superior to other methods because it is more

rapid and sensitive.(Stellrecht, Woron, Mishrik, & Venezia, 2004) A multiplex assay offers additional benefit by enabling concurrent detection of multiple pathogens in a single sample. In this study, we aimed to evaluate the GSS of urethral discharge samples in terms of PMNL/HPF in patients who presented to the outpatient clinic with urethritis and to compare the sensitivity of GSS to PCR assay.

2 | MATERIALS AND METHODS

Sixty-five male patients who presented to our outpatient clinic between January 2018 and March 2018 with dysuria, urethral itching and/or urethral discharge following sexual intercourse were included in this diagnostic study. The urethral swab samples obtained from patients were Gram-stained for microscopic evaluation. Simultaneous urethral smear and first-void urine samples were tested for pathogens using Real-Time Multiplex Polymerase Chain Reaction (rt-MPCR) assay. The PREP-NA PLUS and PREP-GS PLUS extraction kits manufactured by DNA-Technology®(Moscow, Russia) were used. The tests were analysed by Elite Prime Real-Time PCR, which is manufactured by the same company. Samples containing true pathogens (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*) were considered positive. In addition, samples containing opportunistic pathogens (*Ureaplasma urealyticum*, *Ureaplasma parvum*, *Candida albicans*, *Mycoplasma hominis*, *Gardnerella vaginalis*) were evaluated quantitatively in terms of microbial load. As recommended by the manufacturer, a microbial load of $>10^4$ was considered positive. The sensitivity and specificity of two different threshold values (≥5 PMNL/HPF and ≥2 PMNL/HPF) in the GSS examination of urethral discharge samples were evaluated. Two patients who had concurrent GU and NGU were excluded from the study.

The study was approved by the Ethics Committee of Medical Park Hospital (Approval number: 003/2018). Written informed consent was obtained from all patients, and the study was performed in accordance with the Helsinki Declaration.

All statistical analyses were performed using the OpenEpi® version 3.01 (Atlanta, GA, USA). The sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy, positive likelihood ratio, and negative likelihood ratio were calculated within a 95% confidence interval.

3 | RESULTS

The mean age was 33.2 ± 7.1 (range 19-52) years. According to the rt-MPCR results, 14 patients tested positive for *Neisseria gonorrhoeae*, 27 patients tested positive for NGU pathogens, and no pathogens were detected in 22 patients.

A threshold value of ≥5 PMNL/HPF in GSS was 92.9% sensitive (95% Confidence Interval (CI) 68.5-98.7) and 86.4% specific (95%CI66.7-95.3) in the diagnosis of GU. When a threshold value of ≥2 PMNL/HPF was adopted, sensitivity increased to 100% (95%CI 78.5-100) while specificity stayed the same. A threshold value of ≥5 PMNL/HPF in GSS was 55.6% sensitive (95%CI37.3-72.4) and 86.4% specific (95%CI66.7-95.3) in the diagnosis of NGU. Sensitivity increased to 92.6% (95%CI76.6-97.9) and specificity stayed the same when a threshold value of ≥2 PMNL/HPF was adopted. (Table 1)

The distribution of pathogens in the 63 patients according to rt-MPCR results were as follows; *Chlamydia trachomatis* (25.3%), *Neisseria gonorrhoeae* (%22.4%), *Gardnerella vaginalis* (17.4%),

TABLE 1 Diagnostic utility of GSS in the diagnosis of urethritis

	GSS positivity for GU according to ≥5 PMNL/HPF threshold		GSS positivity for GU according to ≥2 PMNL/HPF threshold		GSS positivity for NGU according to ≥5 PMNL/HPF threshold		GSS positivity for NGU according to ≥2 PMNL/HPF threshold	
Parameter	Estimate	Lower-Upper 95% CI	Estimate	Lower-Upper 95% CI	Estimate	Lower-Upper 95% CI	Estimate	Lower-Upper 95% CI
Sensitivity	92.9%	68.5–98.7	100%	78.5–100.0	55.6%	37.3–72.4	92.6%	76.6–97.9
Specificity	86.4%	66.7–95.3	86.4%	66.7–95.3	86.4%	66.7–95.3	86.4%	66.7–95.3
Positive predictive value	81.3%	57.0–93.4	82.4%	59.0–93.8	83.3%	60.8–94.2	89.3%	72.8–96.3
Negative predictive value	95.0%	76.4–99.1	100.0%	83.2–100.0	61.3%	43.8–76.3	90.5%	71.1–97.4
Diagnostic accuracy	88.9%	74.7–95.6	91.7%	78.2–97.1	69.4%	55.5–80.5	89.8%	78.2–95.6
Positive likelihood ratio	6.8	3.5–13.2	7.3	3.8–14.1	4.07	1.9–8.7	6.8	3.5–13.1
Negative likelihood ratio	0.08	0.01–0.60	[a]	[a]	0.51	0.43–0.62	0.09	0.03–0.23

[a]The value was not calculated because one of the parameters was zero.

Ureaplasma urealyticum (9.5%), *Mycoplasma genitalium* (4.7%), *Mycoplasma hominis* (1.5%), and *Trichomonas vaginalis* (1.5%). None of the patients were tested positive for *Ureaplasma parvum* and *Candida albicans*.

4 | DISCUSSION

Urethritis is among the most commonly observed genitourinary diseases observed in males.(Bachmann et al., 2015) It is estimated that 2.8 million patients suffer from urethritis in the United States each year. Urethritis may lead to complications such as acute epididymitis, orchitis and prostatitis.(Brill, 2010) Traditionally, the GSS results of urethral samples were used to establish the diagnosis of urethritis. Currently, GSS is still important in differentiating between GU and NGU, and determining the appropriate treatment. The European NGU guideline has defined a threshold value of ≥5 PMNL/HPF in the GSS for the diagnosis of urethritis.(Horner et al., 2016) On the other hand, the CDC has lowered the threshold to ≥2 PMNL/HPF in the 2015 STD Treatment Guidelines. This reduction was based on the study by Rietmeijer et al. which demonstrated a significant rise from 6.6% to 16.2% in the prevalence of Chlamydia between the one and two PMNL/HPF strata.(Rietmeijer & Mettenbrink, 2012) In the study by (Geisler, Yu, & Hook, 2005), 82% of patients who tested positive for Chlamydia according to nucleic acid amplification tests had ≥5 PMNL/HPF in GSS and 6% had 1-4 PMNL/HPF, whereas 94% of patients with GU had ≥5 PMNL/HPF and 1% had 1-4 PMNL/HPF. Similar to the above-mentioned study, 92% of the patients with GU in our study had ≥5 PMNL/HPF. Regarding these results, we think that GSS has sufficient sensitivity in detecting GU. The main problem here is identifying NGU.

The worldwide prevalence of NGU is higher compared to GU. About 5-20% of patients with urethritis have *Neisseria gonorrhoeae*.(Martin, 2008) In a recent study conducted in our country, 16% of patients with urethritis had GU, whereas 84% had NGU. (Sarier, Duman, Göktaş, Demir, & Kukul, 2017) This emphasises the importance of Gram staining in diagnosis. A threshold value of ≥5 PMNL/HPF may result in false-negative results, especially in NGU cases with low inflammation. It is noteworthy that, in our study, a threshold of ≥5 PMNL/HPF demonstrated only 55% sensitivity in the diagnosis of NGU. In the light of these data, the fact that the CDC has adopted a threshold value of ≥2 PMNL/HPF in GSS for the diagnosis of urethritis seems to have raised expectations regarding international urology guidelines. The 2015 European Association of Urology (EAU) guidelines(Grabe et al., 2015) state that "A Gram stain of a urethral discharge or a urethral smear that shows more than five leucocytes per high power field (×1,000) and eventually, gonococci located intracellularly as Gram-negative diplococci, indicate pyogenic urethritis". On the other hand, the 2017 EAU guidelines (Bonkat et al., 2017) only recommend to "use a Gram stain of urethral discharge or smear for the preliminary diagnosis of pyogenic urethritis". In other words, no threshold value has been provided in the latest EAU guideline. Moi et al. (Moi et al., 2017) stated that adopting a threshold value of ≥2 PMNL/HPF instead of ≥5 PMNL/HPF did not make a significant contribution to diagnosis. They explained this result by the fact that the prevalence of NGU pathogens may vary in different geographic regions and by the differences in the sexual habits of individuals. They concluded that the cut-off value for the diagnosis of clinical urethritis should be adjusted according to geographical regions with different prevalences of causative agents. Different from the study by Moi et al., we observed an increase in sensitivity when a threshold of ≥2 PMNL/HPF was chosen for the diagnosis of NGU. This is noteworthy.

It should be kept in mind that most cases of NGU are asymptomatic and that there may be only a few leucocytes in the Gram stain. Given the fact that most cases of NGU are positive for Chlamydia, we believe that adopting a threshold of ≥2 PMNL/HPF will help prevent false-negative results. In these types of patients, PCR assays are helpful for defining the causative agent within short notice, decreasing the risk of transmission, and directing treatment. Given the fact that NGU pathogens are time-consuming and difficult to culture, the CDC and EAU guidelines recommend nucleic acid amplification tests for the diagnosis of NGU. Among these, the rt-MPCR assay is especially useful in that it enables simultaneous recognition of multiple pathogens and their microbial loads in a single sample. In addition, it can also detect rare causes of NGU such as *Gardnerella vaginalis*, *Ureaplasma spp.*, *Mycoplasma spp.*, *Trichomonas vaginalis and Candida albicans*. By enabling timely diagnosis, PCR assays play an important role in the prompt treatment of infections caused by these rare pathogens. In addition, the quantitative assessment of these opportunistic pathogens (*Gardnerella vaginalis*, *Ureaplasma spp.*, *Mycoplasma hominis*, *Candida albicans*) by PCR is helpful in avoiding false-positive results, and thus, preventing unnecessary medical treatment. However, rt-MPCR has the disadvantages of being expensive and requiring experienced staff. On the other hand, the cost effectivity, easy applicability and rapidity of GSS still renders it an important tool in the diagnosis of urethritis.

This study has some limitations. Due to the single-centre design and the small sample size of the study, the sensitivity of GSS for each NGU pathogen individually could not be calculated. Nevertheless, we believe that this study will serve as a baseline for future studies with larger series which will investigate the sensitivity of GSS in detecting different NGU pathogens.

5 | CONCLUSION

Even though the presence of ≥5 PMNL/HPF in GSS is still considered to have high sensitivity in the diagnosis of GU, its sensitivity in the diagnosis of NGU is considerably low. It should be kept in mind that this might lead to problems concerning appropriate treatment. On the other hand, a threshold value of ≥2 PMNL/HPF provides high sensitivity in the diagnosis of both GU and NGU. Therefore, especially in high-risk patients and in countries with young population, adopting a threshold value of ≥2 PMNL/HPF

in GSS is important in terms of limiting disease transmission and complications by ensuring the initiation of prompt and correct treatment.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

ETHICAL APPROVAL

The study protocol was approved by the local ethics committees.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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