Microscopy of Stained Urethral Smear in Male Urethritis; Which Cutoff Should be Used?

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Background: The microscopical diagnosis of male urethritis was recently questioned by Rietmeijer and Mettenbrink, lowering the diagnostic criteria of the diagnosis to ≥2 polymorphonuclear leucocytes (PMNL) per high power field (HPF), and adopted by Centers for Disease Control and Prevention in their 2015 STD Treatment Guidelines. The European Non-Gonococcal Urethritis Guideline advocates a limit of ≥5 PMNL/HPF.
Objective: To determine if syndromic treatment of urethritis should be considered with a cutoff value of ≥2 PMNL/HPF in urethral smear.
Methods: The design was a cross-sectional study investigating the presence and degree of urethritis relative to specific infections in men attending an STI clinic as drop-in patients.
Results: The material included 2 cohorts: a retrospective study of 13,295 men and a prospective controlled study including 356 men. We observed a mean chlamydia prevalence of 2.3% in the 0–9 stratum, and a 12-fold higher prevalence (27.3%) in the strata above 9. Of the chlamydia cases, 89.8% were diagnosed in strata above 9. For *Mycoplasma genitalium*, the prevalence was 1.4% in the 0–9 stratum and 11.2% in the stratum ≥10, and 83.6% were diagnosed in strata above 9. For gonorrhea, a significant increase in the prevalence occurred between the 0–30 strata and >30 strata from 0.2% to 20.7%. The results of the prospective study were similar.
Conclusions: Our data do not support lowering the cutoff to ≥2 PMNL/HPF. However, a standardization of urethral smear microscopy seems to be impossible. The cutoff value should discriminate between low and high prevalence of chlamydia, mycoplasma, and gonorrhea to include as many as possible with a specific infection in syndromic treatment, without overtreating those with few PMNL/HPF and high possibility of having nonspecific or no urethritis.

Urethritis is inflammation of the urethra, with an excess of leucocytes in urethral exudate. Etiology varies depending on local prevalence of infectious agents. *Neisseria gonorrhoeae* may account for 5–20%. *Chlamydia trachomatis* typically accounts for 15–40% of cases, *Mycoplasma genitalium* for 15–25%,[1] and *Trichomonas vaginalis* for 2–13%.[1,2]

Symptoms of urethritis are discharge from the urethra, dysuria, or itching in and around the meatus. Urethritis is confirmed by evidence of local inflammation and/or the presence of a known pathogen.[1] If urethritis is confirmed in the absence of gonorrhea, non-gonococcal urethritis (NGU) is diagnosed.

The NGU diagnosis is typically made when ≥5 polymorphonuclear leucocytes (PMNL) are seen averaged over 5 high powered fields (HPF), in the absence of intra-cellular diplococci.[3,4] This cutoff value was recently questioned by Rietmeijer and Mettenbrink,[5] with a proposed cutoff by ≥2 PMNL/HPF, and adopted by Centers for Disease Control and Prevention in their 2015 STD Treatment Guidelines.[6] Adopting a lower cutoff value for diagnosing urethritis has consequences. If a urethritis is microscopically confirmed in a patient with urethral symptoms, treatment with antibiotics is recommended.[4,6,7] A lower cutoff value for the syndromic diagnosis will thus increase the prescription of these remedies, risking resistance problems.[8] In the United States, mainly single-dose azithromycin is used for syndromic treatment of urethritis. Azithromycin treatment induces macrolide resistance in *M. genitalium* and select resistant strains, which will be further spread as long as no test for *M. genitalium* is available.[4] An impact on interpersonal relationship may also be the consequence of a false urethritis diagnosis.

To our knowledge, the diagnostic criteria adopted by Rietmeijer and Mettenbrink[5] have not been replicated for applicability in different settings in relation to geography, variation in STI prevalence, general antibiotic use, and sexual habits. The aim of this study was to determine if epidemiological treatment of urethritis in our setting should be considered with a cutoff value of ≥2 PMNL/HPF in urethral smear.

MATERIALS AND METHODS

Study Design

The design was a cross-sectional study investigating the presence and degree of urethritis relative to specific infections in men attending an STI clinic as drop-in patients. The material included 2 cohorts: a prospective controlled study carried out October 2010 to March 2011 including 356 heterosexual men, and a retrospective study of 13,295 men attending the drop-in department during 66 months from Jan 2010 to June 2015.

Routine Clinical Patients, Retrospective Analysis

The Olafia STI clinic is a drop-in clinic situated in the center of Oslo, Norway, serving the whole city and surroundings, and the patients are registered in an electronic medical record. Men with genital symptoms are examined by a physician. Urethral smears are taken with a blunt metal spatula (www.pelomi.dk Bakkestrade 11, 2620 Albertslund, Denmark) gently inserted 0.5–1 cm into the urethra (Fig. 1).[9] Men with obvious discharge have a small sample of the discharge sampled with the spatula without inserting it into the urethra, and the fluid obtained is evenly smeared on a glass slide. The smear is heat-fixed and stained with a modified Löfflers methylene blue staining (LMBS) method (30 g saturated methylene blue ethanol solution mixed with water to 100 mL. Buffered with 1.5 mL 0.1 mol/L sodium hydroxide solution) in the examination room by the examining

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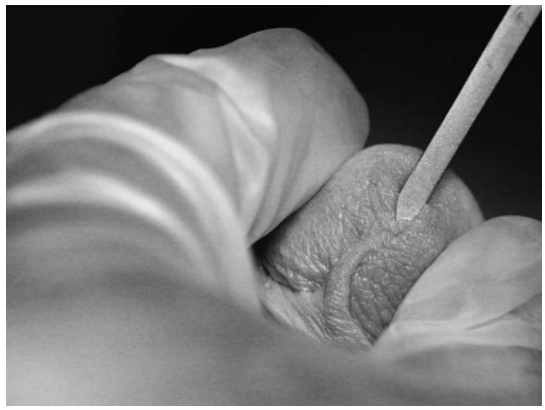

Figure 1. Urethral smear taken with a blunt metal spatula.

physician. In cases of intracellular diplococci with atypical morphology, sometimes an additional Gram stain was performed. All examination rooms have a microscope. Two different microscopes were used; a Nikon Eclipse E400 and an Olympus BX45.

The smears were examined under low power (×100) to identify areas with the highest concentrations of PMNLs. These areas were then examined under high power (×1000) oil immersion and the numbers of PMNLs in 5 microscopic fields with the highest leucocyte concentration were counted. The average results of PMNL count by microscopy in at least 5 HPF was recorded and categorized as follows: 0–4 PMNL/HPF, normal; 5–9 PMNL/HPF, mild urethritis; 10–30 PMNL/HPF, moderate urethritis; and >30 PMNL/HPF, severe urethritis. If non-gonococcal or gonococcal urethritis was diagnosed, the patient was treated with antibiotics according to the national guidelines.[10] During the study duration, 54 different physicians were on duty, most of them doctors in training for specialty in dermatovenereology. Each doctor would have been trained for 1 week by an experienced consultant before working independently. After the examination, the patient was asked to give 10-mL first void urine (FVU), which was sent to the laboratory for nucleid acid amplification tests (NAATs). NAAT for *C. trachomatis* and *M. genitalium* was performed as part of the routine in the clinic.[8] NAAT for *N. gonorrhoeae* on indication. All patients up to December 2012 were tested for *C. trachomatis* using Cobas TaqMan Ct test, v2.0 (Roche, Indianapolis, IN) as described[11] and thereafter all patients were tested using a kit from GeneProof a.s. (Brno, Czech Republic) as described.[12] Patients were tested for *M. genitalium* and *N. gonorrhoeae* as described.[11]

Prospective NGU Study

The protocol for the prospective NGU study was approved by the regional ethical committee, reference 2010/2229, and all participants gave written informed consent.[13] During a 6-month period from October 2010 to March 2011 men without symptoms of urethritis were randomly asked by the triage nurse and symptomatic men asked by the physician to participate in the prospective study and asked to sign a written consent. A total of 411 accepted.

A smear for microscopy was taken as described above, and after performing microscopy, the slide was filed for follow-up standardization. Ten-milliliter FVU was sent to the laboratory for NAAT for *C. trachomatis* and *M. genitalium*. The *M. genitalium* assay was an in-house real-time polymerase chain reaction not approved by FDA.[9] The criterion for FVU was not having emptied the bladder for at least 1 hour.

The microscopy of smears was further standardized by blinding the smears and having 1 senior experienced clinician (H.M.) score all the samples for average PMNL/HPF again.

Patients were excluded from the study if they had used antibiotics in the prior 4 weeks, attending for test of cure, had recurrent urethritis, positive gonorrhea test, if they reported having sex with men, if they were younger than 16 years, or if sexual anamnesis form was missing. In addition, 3 cases without NAAT for chlamydia, and 3 coinfected *C. trachomatis* and *M. genitalium* were excluded. Based on these criteria, 55 patients were excluded and 356 were included in the analysis.

Epidemiological and Statistical Methods

Univariate analysis of the material was made using EpiInfo 7.0 and SPSS (version 19, IBM Corp, New York) and Excel 2013 (Microsoft) for binary distribution.

RESULTS

Retrospective Analysis of Men Visiting the Drop-In Clinic

A total of 44,651 urine NAATs for *C. trachomatis,* 44,180 for *M. genitalium* and 22,262 for *N. gonorrhoeae* from men who visited the clinic during the inclusion period of 66 months were analyzed. This includes asymptomatic males and test of cure. Of these men, 3363 (7.5%) were found to be infected with urethral chlamydia, 1674 (3.8%) with urethral *M. genitalium,* and 475 (2.1%) with urethral gonorrhea.

During the same period, 15,360 Löffler methylene blue stains were performed for evaluation of male urethritis, representing 34.4% of men who had a urine NAAT test for chlamydia. However, of these 15,360 men registered with urethral smear, 1942 were excluded because they were control visits, were missing NAAT for chlamydia and/or mycoplasma, or were included in the prospective NGU study. In addition, 54 were excluded because they had a double infection with chlamydia and gonorrhea, 57 had a double infection with chlamydia and mycoplasma,

TABLE 1. Ct Positivity by Löffler Methylene Blue Stain Strata

Löffler Stain Stratum	No	Ct Positive (NAAT)	Percent of All Ct Positivity	95% CI	Prevalence, %	95% CI
0–4	5495	80	4.9	3.9–6.0	1.5	1.1–1.8
5–9	1555	85	5.3	4.2–6.3	5.5	4.3–6.6
10–30	3389	692	42.7	40.3–45.1	20.4	19.6–21.8
>30	1943	763	47.1	44.7–49.5	39.3	37.1–41.4
0–9	7050	165	10.2	8.7–11.7	2.3	2.0–2.7
≥10	5332	1455	89.8	88.3–91.3	27.3	26.1–28.5
All	12382	1620			13.1	12.5–13.7

M. genitalium and *N. gonorrhoeae* excluded. Retrospective study 2010–2015.
Ct, *C. trachomatis.*

TABLE 2. Ng Positivity by Löffler Methylene Blue Stain Strata

Löffler Stain Stratum	No	Ng Positive (NAAT)	Percent of all Ng Positivity	95%, CI	Prevalence, %	95% CI
0–4	5422	7	2.1	0.6–3.7	0.1	0.03–0.2
5–9	1473	3	0.9	0–1.9	0.2	0–0.4
10–30	2709	12	3.7	1.6–5.7	0.4	0.2–0.7
>30	1486	307	93.3	90.6–96.0	20.7	18.6–22.7
0–30	9604	22	6.7	4.0–9.4	0.2	0.1–0.3
>30	1486	307	93.3	90.6–96.0	20.7	18.6–22,7
All	11090	329			3.0	2.7–3.3

Ct and Mg excluded. Retrospective study 2010–2015.
Ng, *Neisseria gonorrhoeae.*

9 had mycoplasma and gonorrhea, and 1 had triple infection. A total of 13,295 new consultations with LMBS were included (mean age, 32.6 (15–80) years). The same patients may have visited the clinic several times during the inclusion period because of new symptoms or risk.

Overall, 1620 of 13,295 (12.2%; 95% confidence interval [CI], 11.6–12.7) had a positive NAAT for chlamydia (mean age, 29.1), 584 of 13,295 (4.4%; 95% CI, 4.0–4.7) were diagnosed with *M. genitalium* (mean age, 30.7 years), and 329 of 13,295 (2.5%; 95% CI, 2.2–2.7) with gonorrhea by NAAT (mean age, 33.8 years). However, only 6347 had a NAAT for gonorrhea, and the prevalence among those tested was 5.2% (95% CI, 4.6–5.7).

Chlamydia Urethritis

After exclusion of 329 men who were diagnosed with gonorrhea and 584 with mycoplasma based on urine NAAT, 12,382 LMBS were available for the chlamydia analysis, with an overall positivity rate of 13.1% (95% CI, 12.5–13.7). There was a progressive trend along the PMNL/HPF strata, increasing from 1.5% (95% CI, 1.1–1.8) at the 0–4 stratum to 39.3% (95% CI, 37.1–41.4) at the >30 stratum ($P = 0.001$). Examining the 95% CIs of the positivity rates, a statistically significant increase of the prevalence occurred between all LMBS strata, with prevalences in the strata below and above the 10 stratum of 2.3% (95% CI, 2.0–2.7) and 27.3% (95% CI, 26.1–28.5), respectively (Table 1).

M. genitalium Urethritis

The mycoplasma analysis was similar to that of *C. trachomatis*. A statistically significant increase occurred between all LMBS strata, with prevalences in the strata below and above the 10 stratum of 1.4% (95% CI, 1.1–1.6) and 11.2% (95% CI, 10.2–12.2), respectively (Table 8). Of all mycoplasma infections 488 of 584 (83.6%; 95% CI, 80.6–86.6) had moderate to severe urethritis with LMBS results above the 9 stratum.

Gonorrhea

Findings in the gonorrhea analysis were strikingly different; of men who had a LMBS and were diagnosed with gonorrhea by NAAT, a significant increase in the prevalence occurred between the 0–30 strata and >30 strata from 0.2% (95% CI, 0.1–0.3 to 20.7% (95% CI, 18.6–22.7), and 307 of 329 (93.3%; 95% CI, 90.6–96.0) had LMBS results above the 30 stratum (Table 2). Ninety-seven percent had a urethritis with ≥10 PMNL/HPF. Of the 329 patients, 316 had registered syndromic treatment in the data file. Two hundred ninety-nine (94.6%) of 316 received correct treatment with cephalosporin or cefixime at the first visit based on microscopy results before NAAT or culture results.

Distribution Between Less and Highly Experienced Physicians

A total of 54 physicians were on duty during the 66-month study period. Nine of them examined 6798 LMBS, about half of the smears, more than 500 each, with a mean of 758 (558–922). The other 45 physicians performed 6494 LMBS, with a mean of 144 (1–393) each. There was no significant difference between the 2 groups in the LMBS strata 0–9 and ≥10 for *C. trachomatis* and *N. gonorrhoeae* (Table 3 and 7). However, in those positive for chlamydia, the less experienced placed significantly more results in the >30 PMNL/HPF group than the 9 most experienced physicians. The same tendency was seen for gonorrhea and mycoplasma, but without significant differences between the groups.

Prospective Study

Three hundred fifty-six heterosexual men attending the Olafia drop-in clinic (mean age, 30.6 years; range, 16–66 years).

Chlamydia Urethritis

After exclusion of 16 with mycoplasma, *Chlamydia trachomatis* was diagnosed in 56 of 340 (16.4%; 95% CI, 12.5–20.4; mean age, 29.4 years). Of these 56 with chlamydia,

TABLE 3. *C. trachomatis* and *N. gonorrhoeae* Positivity by Löffler Methylene Blue Stain Strata

	Ct				Ng			
Löffler Stain Stratum	No	Ct Positivity (NAAT)	Percent of All Ct Positivity	95% CI	No	Ng Positivity (NAAT)	Percent of All Ng Positivity	95% CI
0–4	2956	45	5.4	3.8–6.9	2912	3	1.7	0–3.6
5–9	834	40	4.8	3.3–6.2	796	2	1.1	0–2.7
10–30	1729	394	47.1	43.7–50.5	1344	10	5.7	2.2–9.1
>30	816	357	42.7	39.4–46.1	620	161	91.5	87.4–95.6
0–9	3790	85	10.2	8.1–12.2	3708	5	2.8	0.4–5.3
≥10	2545	751	89.8	87.8–91.9	1964	172	97.2	94.7–99.6
All	6335	836			5672	176		

Nine physicians with >500 microscopic examinations.

TABLE 4. Ct Positivity by Löffler Methylene Blue Stain Strata

Löffler Stain Stratum	No	Ct pos (NAAT)	Percent of All Ct Positivity	95% CI	Prevalence, %	95% CI
0–4	138	2	3.6	0–8.4	1.4	0–3.4
5–9	43	3	5.4	0–11.3	7.0	0–14.6
10–30	65	11	19.6	9.2–30.0	16.9	7.8–26.0
>30	94	40	71.4	59.6–83.3	42.6	32.6–52.5
0–9	181	5	8.9	1.5–16.4	2.8	0.4–5.1
≥10	159	51	91.1	83.6–98.5	32.0	24.8–39.3
All	340	56			16.4	12.5–20.4

M. genitalium excluded. Prospective study.

51 (91%; 95% CI, 83.6–98.5) had a urethritis with ≥10 PMNL/HPF (Table 4). The prevalence was 5 of 181 (2.8%; 95% CI, 0.4–5.1) in those with less than 10 PMNL/HPF and 51 of 159 (32.0%; 95% CI, 24.8–39.3) in those with a moderate or severe urethritis (LMBS stratum ≥10) (Table 4).

M. genitalium Urethritis

After exclusion of 56 with chlamydia, *Mycoplasma genitalium* was diagnosed in 16 of 300 (5.3%; 95% CI, 2.8–7.9; mean age, 33.1 years). Of these 16 with mycoplasma, 13 (81.2%; 95% CI, 62.1–100) had a urethritis with ≥10 PMNL/HPF (Table 5). The prevalence was 3 of 179 (1.7%; 95% CI, 0–3.6) in those with less than 10 PMNL/HPF and 13 of 121 (10.7%; 95% CI, 5.2–16.3) in those with a moderate or severe urethritis (LMBS stratum ≥10) (Table 5).

We also conducted an analysis of the chlamydia strata including mycoplasma but excluding *N. gonorrhoeae* (Table 6).

DISCUSSION

Symptoms of urethritis, such as urethral discharge, dysuria, and urethral itching or discomfort, are the most common symptoms in male patients seeking care in STI outpatient departments. Urethritis should be confirmed with urethral smear microscopy in symptomatic patients.[4] The role of microscopy is 2-fold: To confirm the urethritis diagnosis to avoid unnecessary treatment, and to distinguish between gonorrhea and non-gonococcal urethritis,[14] to provide prompt correct treatment without waiting for laboratory results. One could imagine that the performance of less experienced physicians to evaluate the slides is less good than that of experienced consultants or laboratory technicians. However, comparing the results of the 9 most experienced physicians with the 45 less experienced, there was no statistical difference between the strata below and above 10 PMNL/HPF, indicating that judging the smear in the microscope is easy to learn (Table 3 and 7). The least experienced physicians tended to judge the number of PMNL/HPF significantly more often to be >30 PMNL/HPF instead of 10–30 PMNL/HPF in chlamydia-positive patients, without any clinical consequences, because the management of the patient is the same regardless of moderate or severe urethritis. The microscopy result may be biased by the clinicians' knowledge of the patient's history. However, in our study, there was no significant difference between the blinded microscopy in the prospective study and the microscopy results bedside in the retrospective study.

The cutoff for diagnosis of urethritis in European NGU guideline is ≥5 PMNL/HPF.[4] However, Center for Disease Control and Prevention in their 2015 STD treatment guidelines has decreased the cutoff limit to ≥2 PMNL/HPF.[6] This decision was made mainly on the background of one study, which found a significant increase of chlamydia prevalence from 6.6% (5.2–8.1) in the 1 PMNL/HPF strata to 16.2% (95% CI, 12.2–20.8) in the 2 PMNL/HPF strata.[5] The consequence of this decision is that a symptomatic male with 2 PMNL/HPF and above should be given epidemiologic treatment.

Our study indicate a higher cutoff than ≥2 PMNL/HPF for urethritis because we find a very low prevalence (1.5%; 95% CI, 1.1–1.8) of *C. trachomatis* in the 0–4 PMNL/HPF stratum and low also in the 5–9 PMNL/HPF stratum (5.5%; 95% CI, 4.3–6.6), with a mean of 2.3% (95% CI, 2.0–2.7) in the 0–9 stratum, and a 10-fold higher prevalence in the strata above 9 (27.3%; 95% CI, 26.1–28.5). 89.8% of the chlamydia cases were diagnosed in strata above 9, and 95.1% in strata above 4 (Table 1).

For *M. genitalium*, 83.6% (95% CI, 80.6–86.6) was diagnosed in strata above 9, and 92% in strata above 4. The prevalence was 1.4% (95% CI, 1.1–1.6) in the 0–9 stratum and 11.2% (95% CI, 10.2–12.1) in the stratum ≥10 (Table 8). Thus, the distribution between the strata was similar for *M. genitalium* and *C. trachomatis*, but with slightly less inflammatory cells in *M. genitalium*. The results were similar to those published in 2009.[9]

Findings in the gonorrhea analysis were strikingly different and similar to the results of Rietmeijer and Mettenbrink; of men who had a LMBS and were diagnosed with gonorrhea by NAAT, a significant increase of the prevalence occurred between the <30 strata and >30 strata from 0.2% to 20.7%, and 93% was diagnosed in the stratum above 30 PMNL/HPF (Table 2).

The prospective, controlled study (Tables 4 and 5) showed similar results as the retrospective study, with low prevalences of

TABLE 5. Mg Positivity by Löffler Methylene Blue Stain Strata

Löffler Stain Stratum	No	Mg pos (NAAT)	Percent of All Mg Positivity	95% CI	Prevalence, %	95% CI
0–4	136	0	0		0	
5–9	43	3	18.75	0–37.9	7.0	0–14.6
10–30	57	3	18.75	0–37.9	5.3	0–11.1
>30	64	10	62.5	38.8–86.2	15.6	6.7–24.5
0–9	179	3	18.8	0–37.9	1.7	0–3.6
≥10	121	13	81.2	62.1–100	10.7	5.2–16.3
All	300	16			5.3	2.8–7.9

C. trachomatis excluded. Prospective study.

TABLE 6. Ct Including Mg without Ng by Löffler Methylene Blue Stain Strata

Löffler Stain Stratum	No	Ct and Mg Positivity (NAAT)	Percent of All Ct and Mg Positivity	95% CI	Prevalence, %	95% CI
0–4	5547	83	5.0	3.9–6.0	1.5	1.1–1.8
5–9	1604	87	5.2	4.1–6.2	5.4	4.3–6.5
10–30	3646	718	42.8	40.4–45.2	19.7	18.4–21.0
>30	2226	789	47.0	44.7–49.4	35.4	33.4–37.4
0–9	7151	170	10.1	8.7–11.6	2.3	2.0–2.7
≥10	5872	1507	89.9	88.4–91.3	25.7	24.5–26.8
All	13,023	1677			12.9	12.3–13.5

TABLE 7. *C. trachomatis* and *N. gonorrhoeae* Positivity by Löffler Methylene Blue Stain Strata

	Ct				Ng			
Löffler Stain Stratum	No	Ct Positivity (NAAT)	Percent of All Ct Positivity	95% CI	No	Ng Positivity (NAAT)	Percent of All Ng Positivity	95% CI
0–4	2539	35	4.5	3.0–5.9	2508	4	2.6	0.09–5.2
5–9	721	45	5.7	4.1–7.4	677	1	0.7	−0.6 to 1.9
10–30	1660	298	38.0	34.6–41.4	1364	2	1.3	−0.5 to 3.1
>30	1127	406	51.8	48.3–55.3	865	145	95.4	92.1–98.7
0–9	3260	80	10.2	8.0–12.3	3185	5	3.3	0.5–6.1
≥10	2787	704	89.8	87.7–91.9	2229	147	96.7	93.9–99.5
All	6047	784			5414	152		

Forty five physicians with <500 microscopic examinations.

C. trachomatis and *M. genitalium* in the PMNL strata below 10, and 91% and 81% of the positive NAATSs for *C. trachomatis* and *M. genitalium* in the strata above 9, respectively. This strengthens the credibility of the retrospective study.

Our study material differentiated from the study of Rietmeijer and Mettenbrink[5] in several ways. When examining men without discharge, they inserted a calcium alginate swab 1–3 cm into the urethra and then rolled onto the slide, whereas in men with evident discharge, a small sample of the discharge was directly collected on a glass slide. The slides were stained with Gram stain. In our clinic, we use a metal spatula in all patients, with or without discharge, as shown in Figure 1, followed by methylene blue staining. We are not aware of any comparison between methylene blue staining and Gram staining other than for gonorrhea,[14] but assuming equal sensitivity of the staining methods, we speculate that the sensitivity of the urethral smear microscopy is highly dependent on the sampling technique used, swab versus plastic loop versus spatula, with increasing yields in the same order.[1] A metal spatula seems to be a more sensitive sampling method and leads to recovery of more PMNL onto the slides, especially in men without evident discharge. However, studies which compare the sensitivity of various collection methods for urethritis diagnosis are lacking. The prevalence of chlamydia was double in the study by Rietmeijer and Mettenbrink[5] compared with our study, 25.3% versus 13.1%, and the gonorrhea prevalence 5 times higher, 14.8% versus 3.0%. As urine-based NAAT, Rietmeijer and Mettenbrink [5] used APTIMA COMBO 2 (Gen-Probe, San Diego, CA) during most of their study, we used NAATs with a somewhat lower sensitivity. However, the American study population has likely a higher STI prevalence to the Norwegian population. In addition, the studies are not direct comparable since we did not count the exact number of PMNL between zero and 10, but only grouped them in 2 strata below 10, <5 and ≥5–9, and two strata ≥10–30 and above 30. In the stratum ≥10, we had 18 times higher prevalence for chlamydia than in the stratum <5 (1.5% vs. 27.3%), compared with a 5 times higher prevalence (8.8% vs 43.5%) in the same strata in the study by Rietmeijer and Mettenbrink[5] (Table 1). They found 5359 cases in the strata 0–4 PMNL/HPF, of which 3695 were in the strata 0–1 PMNL/HPF, with a prevalence of chlamydia of 197 (5.3%) of 3695 compared with 275 (16.5%) of 1664 in the strata 2–4, 3 times higher. Given the same distribution in the strata 0–4 in our retrospective study, 3786 of 5495 would have been in the 0–1 strata, with a prevalence in 33 (0.9%) of 3786. One thousand seven hundred eight of 5495 would have been in the strata 2–4 in our study, with a prevalence in 47 (2.8%) of 1708, still too low for syndromic treatment indication.

TABLE 8. Mg Positivity by Löffler Methylene Blue Stain Strata

Löffler Stain Stratum	No	Mg Positive (NAAT)	Percent of All Mg Positivity	95% CI	Prevalence, %	95% CI
0–4	5464	49	8.4	6.1–10.6	0.9	0.6–1.1
5–9	1517	47	8.0	5.8–10.3	3.1	2.2–4.0
10–30	2928	231	39.6	35.6–43.5	7.9	6.9–8.9
>30	1437	257	44.0	40.0–48.0	17.9	15.9–19.9
0–9	6981	96	16.4	13.4–19.4	1.4	1.1–1.6
≥10	4365	488	83.6	80.6–86.6	11.2	10.2–12.1
All	11,346	584			5.1	4.7–5.6

C. trachomatis and *N. gonorrhoeae* excluded. Retrospective study 2010–2015.
Mg, *M. genitalium.*

TABLE 9. Demographic Characteristics and Prevalences

	Retrospective Study (n = 13295)	Prospective Study (n = 356)
Mean age	32.6 (15–80)	30.6 (16–66)
MSM or bisexual	2095 (16 %)	None
Prevalence *C. trachomatis*	5.8%	
Prevalence *M. genitalium*	2.9%	
Prevalence *N. gonorrhoeae*	8.7%	
Heterosexual	10,896 (82%)	356 (100%)
Prevalence *C. trachomatis*	13.6%	
Prevalence *M. genitalium*	4.7%	
Prevalence *N. gonorrhoeae*	1.3%	
Sexual preference not known	304 (2%)	
Urethral symptoms	7164 (54%)	218 (61%)
No urethral symptoms	6131 (45%)	138 (39%)
Overall prevalence *Chlamydia trachomatis*	1620 (12.2%) 95% CI, 11.6–12.7	56 (15.7%)
Overall prevalence *Mycoplasma genitalium*	584 (4.4%) 95% CI, 4.0–4.7	16 (4.5%)
Overall prevalence *Neisseria gonorrhoeae*	329 (2.5%) 95% CI, 2.2–2.7	None

All patients were new attendance drop-in patients.

The Rietmeijer and Mettenbrink study has no information about the proportion of men who have sex with men (MSM). In our retrospective study, 16% were MSM or bisexual. In MSM, the prevalence of chlamydia and mycoplasma was half compared with heterosexuals, and the prevalence of gonorrhea almost 7 times higher (Table 9). In the prospective study, MSM were excluded. It is not likely that this fact influence the results.

A "true" and "universal" cutoff cannot be made but is dependent on background prevalence and on sampling technique. A cutoff by ≥5 seems reasonable in Europe, with lower prevalence of chlamydia and mainly swab or plastic loop for urethral sampling. In our setting, using metal device for urethral sampling, a cutoff by ≥10 PMNL/HPF includes 90% of all chlamydia and 84% of *M. genitalium* cases, with a prevalence of chlamydia in the strata below 10 of 2.3% (95% CI, 2.0–2.7) compared with a 10-fold higher, 23.7% (22.6–24.8) in the strata 10 and above. However, with a prevalence of 5.5% chlamydia and 3.1% mycoplasma, the stratum 5–9 in our setting should be regarded as a "grey zone," and syndromic treatment considered if severe symptoms or other risk factors.

Rietmeijer and Mettenbrink[5] in their study did not include NAAT for *M. genitalium*. We therefore conducted an analysis of the chlamydia strata including mycoplasma but excluding *N. gonorrhoeae* (Table 6). This did not result in significant different prevalences in the LMBS strata compared with the strata without mycoplasma.

In conclusion, to confirm a diagnosis of NGU or gonococcal urethritis in symptomatic men, microscopy should be performed, and treatment should be given at the first visit. A standardization of urethral smear microscopy seems to be impossible. The cutoff value for the clinical urethritis diagnosis should discriminate between low and high prevalence of chlamydia and mycoplasma to include as many as possible with a specific infection in syndromic treatment, without overtreating those with few PMNL/HPF and high possibility of having nonspecific or no urethritis. The PMNL/HPF cutoff varies with different techniques for producing and staining of the smear, and different prevalence of specific infections.

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