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Brief report

Performance of a rapid molecular test to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women with pelvic inflammatory disease

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ABSTRACT

Objective: The aim of this study was to investigate the prevalence of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) in women with pelvic inflammatory disease (PID) and the usefulness and cost-effectiveness of a rapid molecular test for the diagnosis and clinical management of PID.
Methods: This observational study included 75 patients with mild-to-moderate PID ($n = 33$), severe PID ($n = 29$) and non-specific lower abdominal pain (NSAP) ($n = 13$). CT/NG infections were analyzed using a standard and a rapid test. A cost analysis was carried out.
Results: Samples of 19 patients (25.3%) were CT/NG positive. Concordance between rapid and standard tests was 100%. No significant differences were observed in the incidence of CT/NG in mild-to-moderate compared to severe PID. Costs differed according only to disease severity.
Conclusions: Rapid molecular tests could help with the diagnosis of PID in sexually active women in clinical settings in which a standard technique is not available.

Realización de una prueba molecular rápida para detectar *Chlamydia trachomatis* y *Neisseria gonorrhoeae* en mujeres con enfermedad inflamatoria pélvica

Palabras clave:
Chlamydia trachomatis
Neisseria gonorrhoeae
Técnicas moleculares
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RESUMEN

Objetivo: El objetivo de este estudio fue investigar la prevalencia de *Chlamydia trachomatis* (CT) y *Neisseria gonorrhoeae* (NG) en mujeres con enfermedad inflamatoria pélvica (EIP) y la utilidad y costo-efectividad de una prueba molecular rápida para el diagnóstico y manejo clínico de la EIP.
Métodos: Este estudio observacional incluyó a 75 pacientes con EIP leve a moderada ($n = 33$), EIP grave ($n = 29$) y dolor abdominal bajo inespecífico ($n = 13$). Las infecciones por CT/NG se detectaron mediante una prueba estándar y una prueba rápida. Se realizó un análisis de costes.
Resultados: Las muestras de 19 pacientes (25,3%) fueron positivas para CT/NG. La concordancia entre las pruebas rápida y estándar fue del 100%. No se observaron diferencias significativas en la incidencia de CT/NG en la EIP leve a moderada en comparación con la grave. Los costes difirieron solo según la gravedad de la enfermedad.
Conclusiones: Las pruebas moleculares rápidas podrían ayudar en el diagnóstico de la EIP en mujeres sexualmente activas en entornos clínicos en los que no se dispone de una técnica estándar.

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Introduction

Pelvic inflammatory disease (PID) is a clinical syndrome of the upper female genital tract which is mainly due to polymicrobial infection ascending from the endocervix.[1,2] PID may be also a sexually transmitted disease (STD), with *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) being the most common causal agents.[1,3]

The aim of this study was to investigate the prevalence of CT/NG in women suspected of having PID and the usefulness and cost-effectiveness of a rapid molecular test for the diagnosis and clinical management of PID.

Methods

Seventy-five women with suspected PID or non-specific lower abdominal pain (NSAP) were prospectively recruited in our hospital from April 2016 to April 2017. The study was approved by the Ethics Committee and all women provided written informed consent. Women meeting clinical criteria for PID diagnosis[2,4,5,6,7] or who referred NSAP in the emergency department were asked to participate. Blood analysis, microbiological tests and transvaginal ultrasonography were performed. Severe PID was defined as the presence of severe symptoms or signs and/or tubo-ovarian abscess.[8] Patients who did not meet these criteria were classified as mild-to-moderate PID.[1,6,8,9] All patients with PID were treated with broad-spectrum antibiotic regimens to cover likely pathogens, including CT/NG. Surgery was considered in cases of diagnostic uncertainty or severe cases presenting treatment failure. Women referring NSAP, in whom other causes for these symptoms were excluded but did not meet all PID criteria,[2,6,7] were also invited to participate in the study. Analgesic treatment was provided, if necessary. Follow-up was performed six weeks after diagnosis to evaluate clinical symptoms and patient improvement. CT/NG tests were repeated in patients previously positive for CT/NG.

Endocervical swabs and first void urine were collected for the detection of CT/NG. Vaginal smears were analyzed to evaluate bacterial vaginosis. Intraabdominal fluid samples from patients undergoing surgery were also tested. DNA from samples was extracted with the Biorobot EZ1® (Qiagen) and CT/NG were tested with real-time polymerase chain reaction (Anyplex® CT/NG, Seegene). The same samples were used to directly detect CT/NG with the GeneXpert® CT/NG assay (Cepheid). Unless the results from the rapid and standard tests were reported at the same time, the time from sample reception at the Microbiology Laboratory to result obtainment was registered for both methods. DNA was stored at −20 °C for retrospective test for *Mycoplasma genitalium* and *Trichomonas vaginalis* with the RealCycler® Monotest MGTVUS (Progenie).

A cost study was carried out[10] based on information obtained from the clinical study, considering only direct costs. The economic variables included all resources used by each patient: pharmacologic treatments, need for hospitalization, visits, diagnostic imaging tests and laboratory tests. Unit costs in Euros 2018 for each resource used were obtained from the hospital database. The mean cost per patient was computed using individual patient data. Cost by disease severity (NSAP, mild-to-moderate PID, severe PID), and the presence of CT/NG (at least one positive *vs.* both negative) was also calculated.

Statistical analysis was performed with the Statistical Package for the Social Sciences software, v20.0 for Windows (SPSS, Chicago, Illinois). Continuous variables were compared using the parametric one-way ANOVA and presented as mean and standard deviations. Categorical variables were compared using the Chi-squared test or Fisher's exact test and presented as total count and relative percentages (%). Statistical significance was defined as a p-value < 0.05.

Results

Seventy-five patients were included in the study and classified into three groups: severe PID (n = 29), mild-to-moderate PID (n = 33) and NSAP with no clinical suspicion of PID (n = 13). All patients with severe PID were hospitalized except one, who refused to be admitted, and six required surgery; the median hospital stay in these patients was 6 days. Of patients with mild-to-moderate PID, 15 were hospitalized and three required surgery; the median hospital stay in these patients was 4.5 days. Table 1 shows clinical characteristics and the results of the microbiological tests performed in each group of patients.

For statistical purposes, CT/NG test results were also classified as at least one positive (CT and/or NG) or all negative (both CT and NG). Endocervical samples were positive in 19 patients (25.3%) by either CT (14/75, 18.7%) and/or NG (7/75, 9.3%) (two co-infections). In one case endocervical swab was NG-positive and urine NG-negative. Concordance between Anyplex® and GeneXpert® assays was 100% (75/75 endocervical swabs and 75/75 urines). The mean time to results was significantly shorter for GeneXpert® than for Anyplex®: 2.22 h *vs.* 24.37 h, respectively (p < 0.001). Intraabdominal fluid was cultured in nine patients requiring surgery and in four patients being positive: two *Escherichia coli*, one *Bacteroides fragilis* and one *Mycoplasma hominis*. Fluid was also tested for CT/NG: two patients were positive for NG (one was also positive for *E. coli*) and one for CT. No *T. vaginalis* was detected and only one case of *M. genitalium* was detected in a patient with CT. Only one among patients with NSAP was positive for CT, and appropriate treatment was administered.

All the patients were followed at six weeks after treatment. Endocervical samples and urine were only obtained from patients with a previously positive CT/NG result. All samples were negative except one from a asymptomatic woman, who was positive for CT: she was a sex worker and reinfection was more likely than persistent infection.

Table 2 provides the total cost and the mean cost per patient classified by severity of the condition and the presence or absence of CT/NG.

There were significant differences in costs across severity levels but not between the presence or absence of CT/NG. Patients with severe PID presented the highest mean cost per patient, with the NSAP group showing the lowest mean cost per patient. It should be noted that surgery was more frequent among patients diagnosed with severe PID (20.7%) compared to those with mild-to-moderate PID (9%). This must have increased the mean cost per patient, especially among patients with severe PID who were negative for CT/NG, five of whom required surgery (23.8%) compared with patients with severe PID with positive CT/NG test (12.5%).

Discussion

Despite the difficulty of case definition and diagnostic accuracy is a limitation for PID surveillance,[4] the present study followed the recommended diagnostic criteria for PID.[2,6,7] Furthermore, we also included a group of patients with NSAP who did not meet the minimum criteria for suspicion of PID[6] in order to establish if they could be misdiagnosed cases of subclinical PID, since it may be twice as common.[1,11]

According to the prevailing guidelines, all patients with suspected PID should undergo endocervical or vaginal tests for NG/CT, since a positive result supports its diagnosis.[1,6,7,11] In several studies, no etiological agent was detected in approximately 65-75% of women with clinically diagnosed PID[4,5] and non-identification

Table 1
Clinical characteristics and the results of the microbiological tests performed in each group of patients with severe pelvic inflammatory disease (PID), mild-to-moderate PID and non-specific abdominal pain (NSAP).

	NSAP ($n = 13$)	Mild-to-moderate PID ($n = 33$)	Severe PID ($n = 29$)	p-value
Age (years)	28 (6)	32 (10)	35 (10)	NS
Cervical motion tenderness or uterine tenderness	4 (30.8%)	23 (69.7%)	21 (72.4%)	0.040
Adnexal tenderness	8 (61.5%)	16 (48.5%)	21 (72.4%)	NS
Body temperature >38 °C	3 (23.1%)	16 (48.5%)	18 (62.1%)	NS
Abnormal cervical mucopurulent discharge	2 (15.4%)	17 (51.5%)	13 (44.8%)	NS
Leukocytosis (>11 × 10^9/L)	1 (7.7%)	14 (42.4%)	22 (75.9%)	0.000
Prothrombin time < 70%	0 (0%)	9 (29%)	7 (25%)	NS
C-reactive protein > 5 mg/dL	1 (8.3%)	18 (54.5%)	21 (72.4%)	NS
Pyosalpinx	0	0	24 (82.8%)	0.000
Tubo-ovarian abscess	0	0	11 (37.9%)	0.000
CT positive	1 (7.7%)	7 (21.2%)	6 (20.7%)	NS
NG positive	0 (0%)	4 (12.1%)	3(10.3%)	NS
CT and/or NG positive	1 (7.7%)	10 (30.3%)	8 (27.6%)	NS
CT and NG negative	12 (92.3%)	23 (69.7%)	21 (72.4%)	NS
Bacterial vaginosis	0 (0.0%)	4 (12.1%)	9 (31.0%)	0.028

CT: *Chlamydia trachomatis*; NG: *Neisseria gonorrhoeae*; NSAP: non-specific abdominal pain; PID: pelvic inflammatory disease; NS: not significant. Continuous variables were compared using the parametric one-way ANOVA and presented as mean (standard deviation); categorical variables, using the Chi-squared test or Fisher's exact test and as total count (%). Statistical significance was defined as a p-value < 0.05.

Table 2
Total and mean costs per patient in each group of patients with severe pelvic inflammatory disease (PID), mild-to-moderate PID and non-specific abdominal pain (NSAP).

CT and NG tests	Study group	Number of patients	Total cost[a]	Mean cost[a] per patient
CT and/or NG positive	NSAP	1	243€	243€
	Mild-to-moderate PID	10	19,472€	1947€
	Severe PID	8	27,739€	3467€
	Total	19	47,454€	2497€
Both CT and NG negative	NSAP	12	3195€	266€
	Mild-to-moderate PID	23	37,010€	1609€
	Severe PID	21	95,788€	4561€
	Total	56	135,994€	2428€
Total	NSAP	13	3438€	264€
	Mild-to-moderate PID	33	56,482€	1711€
	Severe PID	29	123,528€	4259€
Total		75	183,448€	2445€

CT: *Chlamydia trachomatis*; NG: *Neisseria gonorrhoeae*; NSAP: non-specific abdominal pain; PID: pelvic inflammatory disease.
[a] Costs are expressed in Euros 2018.

of the causal pathogen does not necessarily exclude the presence of PID.[2,6,7] In our study, no etiological agent was found in 71% of women diagnosed with PID, similar to previous reports.[4,5,12,13] Concordance between rapid GeneXpert® and standard Anyplex® assays was 100%, being results more rapidly obtained with the rapid test.

No differences were observed in the presence or absence of CT/NG in mild-to-moderate compared to severe PID. Moreover, the presence of CT/NG was not found to be a risk factor for a complicated clinical course (33% of patients undergoing surgery were positive for CT/NG compared to 30% of those not requiring surgery). Previous studies have reported similar data and recommend that PID management should be based on clinical features.[5,14] Likewise, the economic analysis showed no cost differences between CT/NG-positive and negative patients. Patients diagnosed with severe PID presented the highest mean cost per patient due to the need for more complex treatments.

Our study had some limitations, such as the limited sample size and that the results from the rapid test were not available for decision-taking. Nevertheless, as described previously, patient management should be based on clinical criteria, irrespectively of the presence or absence of CT/NG.[1,2,4,6,7]

Our study also has several strengths: it was a prospective study and patients were classified into the three groups according to clinical criteria,[2,6,7] and two molecular methods were compared to detect CT/NG with total concordance between both of them. Finally, to our knowledge this is the first study to assess the diagnosis utility of a rapid molecular test for CT/NG in patients with clinical suspicion of PID, which could help in clinical settings where a standard technique is not available.

Conflict of interest

JM was contracted part time during six months in 2016 with funding obtained by Cepheid Inc.

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