Pitfalls in the interpretation of results returned by multiplex real-time PCR panels in the diagnosis of non-gonococcal male urethritis: The case of *Ureaplasma urealyticum*

Dificultades en la interpretación de los resultados obtenidos por paneles de PCR multiplex en tiempo real en el diagnóstico de la uretritis no gonocócica masculina: el caso de Ureaplasma urealyticum

Ureaplasma urealyticum (UU) has been linked to non-gonococcal urethritis (NGU) only when present at high loads and other pathogens have been excluded.[1] Despite the extensive use of molecular syndromic panels targeting UU in the diagnosis of NGU, the qualitative results obtained complicate the straightforward attribution of UU as the cause of NGU. The aim of the present study was to evaluate the potential utility of the PCR cycle threshold (C_t) value in "real life" for discriminating between colonization and the potential pathogenetic involvement of this microorganism. We retrospectively analyzed all data obtained from men with suspicion of urethritis attended at the Department of Health Clínico-Malvarrosa (Valencia, Spain) between October 2018 and December 2019. According to Centers for Disease Control and Prevention (CDC) guidelines, patients were categorized as having urethritis in the presence of urethral discharge and/or ≥2 polymorphonuclear leukocytes (PMNL) per high-power field (1000×) in Gram staining.[2] NGU was defined by the absence of Gram-negative intracellular diplococci on urethral smears and negative PCR and culture for *Neisseria gonorrhoeae* (NG).[2,3] The control group included asymptomatic subjects attending for sexually transmitted disease (STD) screening with no evidence of urethral inflammation.

All individuals provided one first-void and middle-stream urine for mycoplasma culture (Mycoplasma IST2, BioMérieux)[4] and quantitative culture on BBL™ CHROMagar™ plates (Becton-Dickinson), and three urethral exudates (Dry Swabs 162C, COPAN®) for Gram staining, conventional culture on GC II agar with IsoVitaleX (Becton-Dickinson) and BBL™ Thayer-Martin selective agar (Becton-Dickinson), and a multiplex real-time PCR (RQ-SevenSTI, AB Analitica) detecting NG, UU, *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), *Trichomonas vaginalis* (TV), *Mycoplasma hominis* (MH) and *Ureaplasma parvum* (UP). DNA extraction was carried out using the automated QIAsymphony SP extractor and the Virus/Pathogen Mini extraction kit (QIAGEN GmbH). Since it is not possible to distinguish between UU and UP by mycoplasma culture, all samples positive for UP were excluded. The Fisher's exact test and the Mann–Whitney *U*-test were used to compare frequencies and PCR C_t values across groups, using SPSS version 20.0 (Chicago, USA). *P*-values < 0.05 were considered statistically significant.

A total of 175 subjects were included, 101 patients with NGU and 74 asymptomatic controls. Demographics, clinical data and microorganisms detected in both groups are shown in Table 1. As expected, CT and MG were the most frequently pathogens found in NGU group.[5] However, the frequency of detecting UU by PCR was comparable in both groups, whether they presented coinfections or if we considered those patients in whom UU was the only detected microorganism. This differs from the findings in a recent meta-analysis where the PCR detection rate of UU in suspected NGU patients was 18.31%, significantly higher than that in controls (13.7%).[6] However, a direct comparison with our study is limited because some studies in the meta-analysis lacked information on co-infections with other pathogens. Notably, most studies in the meta-analysis used a cut-off of ≥5 PMNL/1000×, in contrast to our study's ≥2, as recommended by the CDC in high-prevalence settings.[2] In our study, among the 9 of the 10 NGU patients in whom UU was the only detected microorganism and clinical data was available, all but one patient received empirical or targeted antimicrobial therapy (macrolides, tetracyclines, or quinolones) and resolved their clinical symptoms. These findings may suggest the casual involvement of UU in our study. However, a notable limitation of our series is the inability to rule out other non-cultivable pathogens contributing to NGU such as herpesvirus and adenovirus.[5] In 2018, the Editorial Board of the European Guidelines for STD recommended antimicrobial treatment for men diagnosed of NGU attributable to UU only when the bacterial load is ≥10^3 genome equivalents/ml of urine. In our study, the median C_t value for UU was similar (*P* = 0.41) in cases (29; IQR, 28–33) and controls (31; IQR, 28–35), irrespective of whether other microorganisms were co-detected (28; IQR, 26–32) or not (32; IQR, 28–35). In conclusion, these findings suggest that C_t values do not correlate with clinical presentation and do not endorse the clinical utility of PCR C_t values in inferring the potential causal involvement of UU in NGU in a real-life setting. The limitations in ruling out other non-cultivable pathogens contributing to NGU emphasizes the need for further research in understanding the role of UU in urethritis.

Table 1
Demographics and microorganisms detected in NGU and control group.

	NGU patients (*n* = 101)	Controls (*n* = 74)	*P*-value
Median age in years (range)	29 (14–81)	27 (17–61)	0.534
Clinical presentation (%)			
Urethral discharge	94/99 (94.9)	–	NA
Dysuria	47/99 (47.4)	–	NA
Itching	10/99 (10.1)	–	NA
Meatitis	6/99 (6.0)	–	NA
Microorganisms detected by PCR; n/total (%)			
Chlamydia trachomatis	23/101 (22.7)	6/74 (8.1)	0.003
Mycoplasma genitalium	11/101 (10.8)	2/74 (2.7)	0.049
Trichomonas vaginalis	8/101 (7.1)	1/74 (1.3)	0.035
Mycoplasma hominis	7/101 (6.9)	5/74 (6.7)	1.00
Ureaplasma urealyticum	19/101 (18.8)	13/74 (17.5)	0.713
Microorganisms detected by mycoplasma culture; n/total (%)			
Mycoplasma hominis	1/101 (0.009)	–	NA
Ureaplasma urealyticum[a]	9/101 (8.9)	6/74 (8.1)	0.621
Ureaplasma urealyticum coinfections; n/total (%)			
Chlamydia trachomatis	2/19 (10.5)	2/13 (15.3)	0.385
Mycoplasma genitalium	–	1/13 (7.6)	NA
Trichomonas vaginalis	4/19 (21)	1/13 (7.6)	0.010
Mycoplasma hominis	3/19 (15.7)	3/13 (23)	0.212

–: negative; NA: not applicable.
[a] No positive mycoplasma cultures with negative PCR results were observed. Both quantitative urine cultures and conventional cultures of urethral exudate were all negative.

Authors' contributions

María Jesús Castaño, María Jesús Alcaraz and Eliseo Albert: data curation and analysis. María Jesús Castaño and David Navarro: study design and manuscript drafting. All authors approved the final version of the manuscript.

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Conflict of interest

The authors have no relevant competing interest to disclose about this work.

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Celulitis craneo-facial con pericondritis y otitis externa profesional, a propósito de un caso

Laboral Cranio-Facial Cellulitis with Perichondritis and External Otitis, A Case Report

Se presenta el caso de un varón de 62 años, natural de España. Antecedentes de hipertensión arterial, dislipemia y obesidad; prediabetes mellitus y exfumador de 45 paquetes-año. Agricultor de profesión.

Acude por un cuadro de malestar generalizado, fiebre y una reacción eritematosa a nivel de la hemicara izquierda, con irradiación hacia el hemitórax superior. Fue a consulta y le prescribieron doxiciclina 100 mg/12 h y estreptomicina 1 g/24 h, sin objetivar mejoría alguna.

Dado el empeoramiento, acudió a los servicios sanitarios para ser reevaluado; presentaba una afectación corporal eritematosa con extensión hacia el cuero cabelludo y una afectación pericondral izquierda con salida de contenido purulento por el conducto auditivo. Además de dichas regiones, se detectaron adenopatías de las cadenas ganglionares laterocervicales, preauriculares y retroauriculares. A destacar, presentaba múltiples erosiones a nivel de dicho conducto auditivo. Sus constantes fueron: temperatura (T^{a}) 37,2 °C; tensión arterial (TA) 168/94 mmHg; frecuencia cardiaca (FC) 84 lpm; saturación de oxígeno ($SatO_2$) 92%.

En las pruebas complementarias se objetivó una proteína C reactiva elevada (289,80 mg/L, rango de referencia 0-15 mg/L) con leucocitosis (18,63 x 1.000/μL, rango de referencia 4 x 1.000–10 x 1.000/μL) y neutrofilia (15,69 x 1.000/μL, rango referencia 2 x 1.000–7,5 x 1.000/μL). El resto de las pruebas complementarias realizadas estaban dentro de la normalidad, incluyendo una radiografía de tórax.

El paciente fue ingresado en la Unidad de Enfermedades Infecciosas. Tras una evaluación inicial, se confirma el cuadro y se decide obtener dos muestras del contenido purulento del conducto auditivo izquierdo, además, se propone un tratamiento inicial con base en piperacilina – tazobactam 4 g – 0,5 g/8 h intravenoso (IV) y linezolid 600 mg/12 h, en sustitución de la pauta previa. Tras ello, se plantea una valoración por parte de otorrinolaringología y se completa el estudio con una tomografía computarizada de las estructuras facio-cervicales para detectar posibles abscesos, colecciones o una erosiones del material óseo circundante que puedan producir una fistulización local.

Tras una valoración por parte de otorrinolaringología y con el resultado de la tomografía computarizada, se descartó la presencia de complicaciones locales. Asimismo, se decidió ampliar el tratamiento con ciprofloxacino 3 mg/6 gotas/12 h ótico.

Durante su estancia hospitalaria, el paciente logró una adecuada evolución del cuadro, produciéndose una progresiva reducción del eritema corporal, lo cual se ve confirmado por una mejoría en sus marcadores analíticos con una proteína C reactiva (150,10 mg/L), una leucocitosis (11, 83 x 1.000/μL) y una neutrofilia (8,10 x 1.000/μL).

A ello se suma la obtención de los resultados de los cultivos bacteriológicos del conducto auditivo, los cuales muestran la presencia de un *Bacillus thuringiensis*, sensible a los tratamientos dispuestos (fig. 1).

Dada su buena evolución clínica, con una práctica resolución de los parámetros analíticos, con una proteína C reactiva (10,30 mg/L), una leucocitosis (11,03 x 1.000/μL) y una neutrofilia (6,88 x 1.000/μL), se procedió a derivar al paciente a su domicilio con ciprofloxacino 500 mg/12 h y linezolid 600 mg/12 h y su posterior revisión en consultas de la Unidad de Enfermedades Infecciosas.