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 $\geq 10^3$ 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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Laboral cranio-facial cellulitis with perichondritis and external otitis, a case report

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

We present the case of a 62-year-old Spanish man, with a history of high blood pressure, dyslipidaemia, obesity and prediabetes mellitus. He was a former smoker of 45 pack-years. A farmer by profession.

He presented with general malaise, fever and an erythematous reaction on the left side of the face, radiating to the upper left thoracic area. He was prescribed doxycycline 100 mg/12 h and streptomycin 1 g/24 h, but with no improvement.

Given his worsening condition, he went to his health service to be re-assessed. He had an erythematous rash covering his body with extension towards the scalp, and left perichondral involvement with purulent content coming out of his ear canal. In addition to these regions, enlargement of his laterocervical, preauricular and retroauricular lymph node chains was detected. Notably, there were multiple erosions in his ear canal. His vital signs were as follows: temperature (T) 37.2°C; blood pressure (BP) 168/94 mmHg; heart rate (HR) 84 bpm; oxygen saturation (SatO2) 92%.

Blood tests showed a high C-reactive protein (289.80 mg/l, reference range 0–15 mg/l) with leucocytosis ($18.63 \times 1{,}000/\mu l$, reference range $4 \times 1{,}000$–$10 \times 1{,}000/\mu l$) and neutrophilia ($15.69 \times 1{,}000/\mu l$, reference range $2 \times 1{,}000$–$7.5 \times 1{,}000/\mu l$). The rest of the tests performed were within normal limits, including a chest x-ray.

The patient was admitted to the Infectious Diseases Unit. After an initial assessment, the symptoms were confirmed and it was decided to obtain two samples of the purulent content of the left ear canal, and initial treatment was proposed based on intravenous (IV) piperacillin – tazobactam 4 g–0.5 g/8 h plus linezolid 600 mg/12 h, replacing the previous regimen. After this, an Ear, Nose and Throat (ENT) assessment was requested and investigations were completed with a computed tomography (CT) scan of the facio-cervical structures to detect possible abscesses, collections or erosions of the surrounding bone material which could cause local fistulisation.

The ENT assessment and CT images ruled out the presence of local complications. It was also decided to expand the treatment with ciprofloxacin ear drops at 3 mg/6 drops/12 h.

During his hospital stay, the patient's condition improved, with a progressive reduction of the erythema on his body, and confirmed by an improvement in his analytical markers with C-reactive protein (150.10 mg/l), leucocytosis ($11.83 \times 1{,}000/\mu l$) and neutrophilia ($8.10 \times 1{,}000/\mu l$).

In addition to this, the results of the bacteriological cultures of the ear canal showed the presence of *Bacillus thuringiensis*, sensitive to the treatments given (Fig. 1).

Given his good clinical progress, with virtual resolution of his analytical parameters, with C-reactive protein (10.30 mg/l), leucocytosis ($11.03 \times 1{,}000/\mu l$) and neutrophilia ($6.88 \times 1{,}000/\mu l$), the patient was sent home on ciprofloxacin 500 mg/12 h and linezolid 600 mg/12 h, with subsequent follow-up at the Infectious Diseases Unit clinic.

The condition was classified as craniofacial cellulitis with perichondritis, external otitis and erythematous irradiation towards cervical/thoracic regions. The pathogen isolated was *B. thuringiensis*, an unusual germ as a skin isolate, but related to the patient's agricultural work.

B. thuringiensis is the most used biological insecticide worldwide. The pesticidal mechanism of this gram-positive bacteria[1] is based on the production of crystalline protein bodies, which are toxic to different invertebrates, especially their larvae.[2,3] These proteins are called Cry. The crystals of *B. thuringiensis* are ingested and solubilised in the insect's digestive system, after which the crystalline proteins are released in the form of protoxins, which are processed by intestinal proteases to form active larvicidal proteins.

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