Real-time PCR and serology for the diagnosis of primary syphilis

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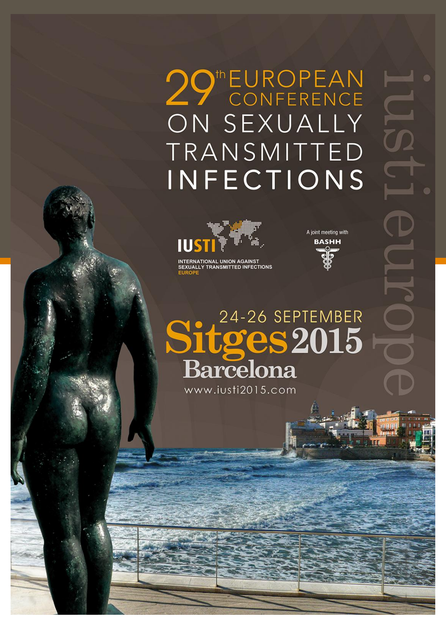

Abstract
Background: Syphilis is caused by *Treponema pallidum* (TP). Serological tests are still the standard for laboratory diagnosis, but they are relatively insensitive for detecting the primary stage of syphilis and positive serology can also be indicative of a past infection. PCR assays can be used to detect TP in samples from primary lesions.
Objective: To compare the results of a commercial real-time PCR (RealCycler THLV®, Progenie Molecular) for the detection of TP in the diagnosis of primary syphilis with the serology in these cases.
Methods: Results of a real-time PCR for the diagnostic of TP during a period of two years (January 2013 to December 2014) were analyzed. Swabs from genital, rectal and oral ulcers were processed from patients with suspected sexually transmitted infections. Results were compared with the serological tests (VDRL, IgG and IgM).
Results: 342 samples out of 287 patients were analyzed. 68 (19.9%) were positive for TP, corresponding to 65 episodes in 64 (22.3%) patients. The most common diagnosis was genital ulcer in 37 (56.9%) cases, followed by proctitis or rectal ulcer in 19 (29.2%) and oral ulcer in 8 (12.3%). Syphilis serology was performed in 53 (81.5%) of these patients. The PCR allowed the diagnosis in 14 patients (26.4%) with primary syphilis in which serology was negative or not suggestive of recent syphilis. When PCR was negative, syphilis serology was performed in 135 (49.3%) cases: 76 (54.3%) had negative serology and 44 (32.6%) had a serology compatible with a past infection. In 18 (13.3%) cases serology was suggestive of recent infection (VDRL > 1/32 and/or positive IgM) and medical charts were reviewed: 16 cases corresponded to treated, secondary or early latent syphilis and only two cases were probably primary syphilis.
Conclusions: The real-time PCR allows us to diagnose primary syphilis quickly and reliably. Only using serological techniques, some cases could remain undiagnosed.

Background:

Syphilis is caused by *Treponema pallidum* (TP). Serological tests are still the standard for laboratory diagnosis, but they are relatively insensitive for detecting the primary stage of syphilis and positive serology can also be indicative of a past infection. PCR assays can be used to detect TP in samples from primary lesions.

Methods

Results of a real-time PCR for the diagnostic of TP during a period of two years (January 2013 to December 2014) were analyzed. Swabs from genital, rectal and oral ulcers were processed from patients with suspected sexually transmitted infections. Results were compared with the serological tests (VDRL and ELISA IgG and IgM).

For the AANN extraction, we used the Biorobot EZ1® (Quiagen) and for the amplification, the SmartCycler® (Cepheid) with the RealCycler THLV® kit (Progenie Molecular). This real-time PCR allows the detection of TP, *Chlamydia trachomatis* (L1, L2 and L3) and *Haemophilus ducreyi* simultaneously. HIV co-infection and presence of reaginic and treponemic antibodies were also studied: an aglutination for VDRL (Spinreact) and an enzyme immunoassay for IgG and IgM (Trinity Biotech).

Image 1. Biorobot EZ1® (left) and SmartCycle® (right).

Results

342 samples out of 287 patients were analyzed. 68 (19.9%) were positive for TP, corresponding to 65 episodes in 64 (22.3%) patients. All patients were male, apart from two. The medium age was 37 years. 54 (83.1%) patients were HIV positive. The most common diagnosis was genital ulcer in 37 (56.9%) cases, followed by proctitis or rectal ulcer in 19 (29.2%) and oral ulcer in 8 (12.3%).

Syphilis serology was performed in 53 (81.5%) of these patients. The PCR allowed the diagnosis in 14 patients (26.4%) with primary syphilis in which serology was negative or not suggestive of recent syphilis.

When PCR was negative, syphilis serology was performed in 135 (49.3%) cases: 76 (54.3%) had negative serology and 44 (32.6%) had a serology compatible with a past infection. In 18 (13.3%) cases serology was suggestive of recent infection (VDRL > 1/32 and/or positive IgM) and medical charts were reviewed: 16 cases corresponded to treated, secondary or early latent syphilis and only two cases were probably primary syphilis.

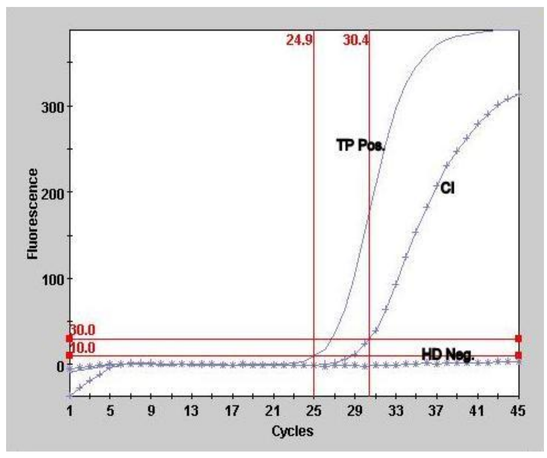

Image 2. Positive PCR for TP.

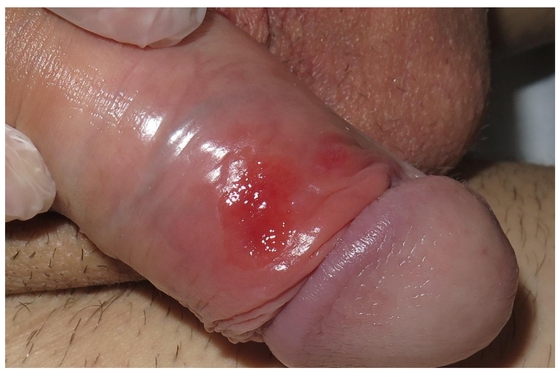

Image 3. Lesion in penis caused by TP.

Conclusions

The real-time PCR allows us to diagnose primary syphilis quickly and reliably. Only using serological techniques, some cases could remain undiagnosed.

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