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Longitudinal Quantitative Detection by Real-Time PCR of *Mycoplasma genitalium* in First-Pass Urine of Men with Recurrent Nongonococcal Urethritis

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By using a TaqMan assay we monitored longitudinal changes in *Mycoplasma genitalium* loads in five men with recurrent *M. genitalium*-positive nongonococcal urethritis. We observed regrowth of *M. genitalium* persisting in hosts after treatment and a possible association of the increase in the *M. genitalium* load with emergence of symptoms and signs of nongonococcal urethritis in four of these patients.

In 1981, when *Mycoplasma genitalium* was initially identified, two strains were isolated from two urethral specimens in culture (16). Despite repeated attempts to isolate *M. genitalium* from the urogenital tract, however, culture of *M. genitalium* is still immensely difficult. In the 1990s, the advent of PCR-based assays facilitated studies on the association of *M. genitalium* with acute nongonococcal urethritis (NGU) (9, 11). This mycoplasma has been detected significantly more often in patients with acute NGU, particularly in patients with nonchlamydial NGU, than in subjects without urethritis (6, 8). In 2002 we developed a TaqMan assay for quantification of *M. genitalium* DNA and quantified *M. genitalium* in first-pass urine of men with urethritis and of asymptomatic men (17). We reported that the *M. genitalium* load was significantly higher in men with acute nonchlamydial NGU than in asymptomatic men (17). The various results reported to date suggest that *M. genitalium* may be associated with the development of acute NGU independent of *Chlamydia trachomatis* (2, 13).

For chronic NGU, it has been suggested that persistence of *M. genitalium* in the urethra after antimicrobial chemotherapy might be associated with this condition (5, 7, 10, 15). However, quantitative analysis of *M. genitalium* has been not performed in cases of chronic NGU. In the present study, therefore, we used the TaqMan assay to monitor longitudinal changes in *M. genitalium* loads in patients with recurrent NGU and to examine those cases for association of the *M. genitalium* load with clinical findings and inflammatory responses.

Five men with recurrent NGU, who had been included in a previous study (10), were enrolled in the present study. They attended the Department of Urology, Toyota Memorial Hospital, Toyota, Japan, between July 1999 and December 2001. Each of these patients had symptoms and signs consistent with acute urethritis at the first visit. For each patient, Gram-stained urethral smears showed five or more polymorphonuclear leucocytes (PMNLs) per high-power (×1,000) microscopic field in at least three fields. Urine specimens were subjected to AMPLICOR STD-1 assay (Roche Diagnostics, Indianapolis, Ind.) for detecting *Neisseria gonorrhoeae* and *C. trachomatis* and to a PCR- and phylogeny-based assay for detecting mycoplasmas and ureaplasmas. The AMPLICOR STD-1 assay was done according to the manufacturer's instructions. The PCR- and phylogeny-based assay, which could detect at least 10 copies of the 16S rRNA gene of *M. genitalium*, was done as described in our previous report (18). Two patients were positive for both *C. trachomatis* and *M. genitalium*, and three were positive only for *M. genitalium*. We treated all five patients with levofloxacin at 100 mg three times daily for 14 days. We asked them to practice sexual abstinence during treatment and to return for reexamination 7 days after the start of treatment and at the end of treatment, irrespective of the presence or absence of symptoms. With successive visits, we reexamined urethral smears and retested urine for pathogens.

DNA samples, which had been prepared from the first-pass urine for the phylogeny-based assay and stored at −70°C, were subjected to the TaqMan assay for quantification of *M. genitalium* DNA (17, 18). Briefly, precipitate from 1 ml of the first-pass urine was obtained by centrifugation and treated with digestion solution containing proteinase K (18). The DNA was purified by a classic phenol-chloroform procedure followed by ethanol precipitation and was then dissolved in 50 μl of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). A portion of the DNA solution was used for the PCR- and phylogeny-based assay, and the remaining solution was frozen and stored.

Sequences of primers and a probe for the TaqMan assay were derived from the 16S rRNA gene of *M. genitalium* (17). In our previous study this assay was confirmed to be highly specific for *M. genitalium* (17). For first-pass urine specimens the working range of this assay was from 5×10^7 to 5×10^1 copies of the *M. genitalium* 16S rRNA gene per 1 ml of urine (17). In this study, after thawing the DNA solutions we used 10 μl of each sample as template DNA for the TaqMan assay. All standard dilutions of *M. genitalium* DNA, negative controls including no DNAs, and clinical samples were run simulta-

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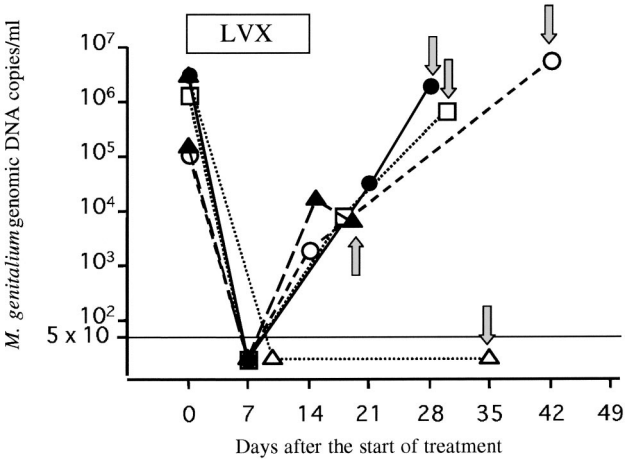

FIG. 1. Longitudinal changes in *M. genitalium* loads in five patients with recurrent *M. genitalium*-positive NGU. The *M. genitalium* 16S rRNA gene in the first-pass urine samples of the patients was quantified by the TaqMan assay. The *M. genitalium* load was suppressed to below the detection level of 50 copies/ml in some urine samples during and after the levofloxacin (LVX) treatment, but all samples were positive for the mycoplasma according to the PCR- and phylogeny-based assay. Arrows indicate the *M. genitalium* load at the time when the patient returned to the clinic with recurrent urethritis symptoms. For four (○, ●, □, and ▲) of the five patients, the load at the time of recurrence of NGU was as high as it was at the time of the first visit when definite NGU was observed. For the remaining patient (△), no increase in the number of *M. genitalium* organisms was observed at the time of NGU recurrence.

neously (17). Amplification, data acquisition, and analyses were performed with the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, Calif.). The number of targets initially present was determined according to a standard curve (4).

Bacterial load in the five patients ranged from 1.1×10^5 to 3.3×10^6 copies of the *M. genitalium* 16S rRNA gene per 1 ml of urine (mean, 1.54×10^6 copies/ml) before treatment (Fig. 1). Four patients visited the clinic 7 days after the start of treatment. There were no urethritis symptoms, and the urethral smears were negative for PMNLs. The PCR- and phylogeny-based assay results were positive for the mycoplasma, but *M. genitalium* loads were suppressed to below the detection level of 50 copies/ml. The four patients returned again for follow-up examination within 7 days after completing the 14-day levofloxacin treatment. They complained of no urethritis symptoms, the smears were still negative for PMNLs, and two of them, who had been positive for *C. trachomatis* at the first visit, were negative for chlamydia; thus, their NGU was judged clinically cured. In these four patients, however, the *M. genitalium* load was higher than it was 7 days after the start of the treatment and ranged from 2.0×10^3 to 2.8×10^4 copies/ml (mean, 1.35×10^4 copies/ml) (Fig. 1). From 4 to 28 days after completion of the 14-day levofloxacin treatment, each of these four patients noticed urethral symptoms and returned to the clinic. Urethral smears were positive for PMNLs. We considered the recurrent NGU to have occurred by *M. genitalium* persisting in the urethra, since all four patients reported abstinence or protected sexual intercourse with the use of condoms after completing the treatment. The first-pass urine samples were positive for *M. genitalium* by the PCR- and phylogeny-based assay, and the *M. genitalium* load ranged from 6.0×10^3 to 5.5×10^6 copies/ml (mean, 2.01×10^6 copies/ml) according to the TaqMan assay (Fig. 1). The load at the time of recurrence of NGU was as high as it was at the first visit when definite NGU was observed.

The remaining patient, for whom the *M. genitalium* load was 3.0×10^6 copies/ml before treatment, visited the clinic 10 days after the start of treatment (Fig. 1). He had no symptoms of urethritis, and the urethral smear was negative. We judged the NGU to be clinically cured. The PCR- and phylogeny-based assay showed the first-pass urine to be positive for *M. genitalium*, but the bacterial load was <50 copies/ml according to the TaqMan assay. When he returned with symptoms 21 days after completing the 14-day levofloxacin treatment, his urethral smear was positive for PMNLs. Although the PCR- and phylogeny-based assay was positive for the mycoplasma, the *M. genitalium* load still remained <50 copies/ml. No increase in the number of *M. genitalium* organisms was observed at the time of NGU recurrence; thus, other pathogens might have been responsible for the presence of NGU in this case.

Presently, guidelines or recommendations are not available for the treatment of *M. genitalium*-positive urethritis. The in vitro antimicrobial susceptibility profile of *M. genitalium* is similar to that of *Mycoplasma pneumoniae* (14). Tetracyclines, macrolides, and some of the newer quinolones are active against *M. genitalium* (1, 14). However, clinical data on treating *M. genitalium*-positive NGU with such anitmicrobial agents are extremely limited. In managing *M. pneumoniae*-induced disease, the mycoplasma is not easily eliminated from the respiratory tract, even when individuals are given adequate doses of antibiotics to which the mycoplasma is known to be sensitive in vitro (3, 12). Antibiotic treatments decrease numbers of the mycoplasma to undetectable levels, but in some cases the mycoplasma reappears and persists in the respiratory tract without clinical signs (3, 12). In this study, *M. genitalium* loads from men with *M. genitalium*-positive NGU were suppressed to below the detection level of 50 copies/ml during levofloxacin therapy, though the PCR- and phylogeny-based assay results were positive for the mycoplasma and urethritis symptoms and signs disappeared. At the end of the treatment the mycoplasma loads became detectable by the TaqMan assay, but there were still no symptoms or signs. Such persistent *M. genitalium* in the urethra for NGU might be analogous to persistent *M. pneumoniae* in the respiratory tract. Although the number of cases was quite limited in our present study, we observed regrowth of *M. genitalium* persisting in hosts and a possible association of an increase in the *M. genitalium* load with the emergence of symptoms and signs of NGU. In our previous study we observed no recurrence of NGU in seven patients with *M. genitalium*-positive NGU who became negative for the mycoplasma by the PCR and phylogeny assay after antimicrobial chemotherapy. Further studies are required to establish a new treatment algorithm for NGU, including *M. genitalium*-positive NGU. However, our results suggest that the antimicrobial chemotherapy that is capable of eliminating the mycoplasma from the urethra may be recommended in the management of patients with *M. genitalium*-positive NGU to prevent the recurrence of NGU.

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