Ureaplasma parvum as a Cause of Sternal Wound Infection[▽]

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Few reports in the literature have documented the isolation of *Ureaplasma* species from sternal wounds. A case of sternal wound infection likely due to *Ureaplasma parvum* is described. When routine bacterial cultures from a sternal wound infection fail to yield a pathogen, diagnostic testing for mycoplasmas and ureaplasmas should be considered.

CASE REPORT

A 66-year-old Aboriginal male presented to a tertiary care hospital in Winnipeg, Manitoba, Canada, with an episode of chest pain that was unrelieved by nitroglycerin. His past medical history was significant for known ischemic heart disease, hyperlipidemia, hypertension, type 2 diabetes mellitus, remote tuberculosis, and bilateral knee arthroplasty. Laboratory investigations performed at the time of arrival (and repeated 18 h later) demonstrated a troponin T level of <0.01 µg/liter. The patient was admitted under the cardiology service with a diagnosis of unstable angina. Therapy with heparin was initiated.

A coronary angiogram performed 2 days postadmission demonstrated 70% stenosis of the left anterior descending coronary artery, complete occlusion of the right coronary artery, and an ejection fraction of 44%. Following consultation with a cardiac surgeon, the patient underwent three-vessel coronary artery bypass grafting. Cefazolin was administered before the procedure, and the treatment was continued for 5 days postoperatively. The patient was transferred out of the surgical intensive care unit to the general cardiac surgery ward on postoperative day 1. His postoperative course was complicated by ileus that spontaneously resolved over several days. On postoperative day 5, he developed a new onset fever of 38.6°C and increasing shortness of breath. He was also having runs of sustained ventricular tachycardia. On postoperative day 6, he was readmitted to the surgical intensive care unit with a diagnosis of possible pneumonia. Empirical antimicrobial therapy with piperacillin-tazobactam was initiated. His condition stabilized over the next few days. Blood cultures were negative.

On postoperative day 10, purulent drainage from the sternal wound was observed. The sternum was also found to be unstable on clinical examination. A chest computed tomography scan demonstrated soft tissue stranding and fluid around the sternum, consistent with expected postoperative changes. No definite evidence of osteomyelitis was noted. A wound swab of the sternal drainage was sent to the microbiology laboratory on postoperative day 10. A Gram stain demonstrated at most one polymorphonuclear leukocyte per oil immersion field. No bac-

teria were isolated on culture. A second wound swab, submitted on postoperative day 11, also did not yield bacterial growth.

On postoperative day 15, due to sternal instability and ongoing purulent drainage from the sternal wound, the patient was taken back to the operating room. The sternal incision was opened, and a substantial amount of pus was drained. The edges of the sternum were debrided, and the sternal wires were removed. The wound was closed again with stainless steel wires. Given the lack of positive cultures to date, a wound swab for mycoplasma/ureaplasma was obtained.

For the isolation of mycoplasma and ureaplasma, wound swabs were inoculated into bromothymol blue broth (B broth), prepared as described by Robertson (14) but without lincomycin. In a further modification to the broth described by Robertson, arginine was added to allow for the detection of *Mycoplasma hominis* in addition to *Ureaplasma* species. Current laboratory protocol at our institution is for B broth to be incubated for 5 days at 37°C. Broth that undergoes a color change to green is submitted for further evaluation by PCR assays.

Molecular detection was performed initially using three separate PCRs to test for *M. hominis*, *Ureaplasma* species, and members of the *Mycoplasma* group with primers previously described by Abele-Horn et al. and van Kuppeveld et al. (1, 15). Briefly, a small aliquot of B broth (0.5 ml) was placed in a sterile microcentrifuge tube and spun at 5,000 rpm for 5 min at 4°C. A 2.5-µl aliquot of supernatant was then added to 47.5 µl of master mix (consisting of 0.5 µM of each primer, 2.5 units of *Taq* polymerase, 0.5 units of uracil DNA glycolase, 0.2 mM of dATP, dCTP, and dGTP, 0.6 mM of dUTP, 0.1 mM of dTTP, 2.5 mM MgCl₂, 5 µl of 1× PCR buffer, and sterile distilled water). Amplification was performed with an Eppendorf thermocycler (Eppendorf AG, Hamburg, Germany), involving 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s. Amplified products were visualized under UV light after being run on a 1.5% (wt/vol) agarose gel containing 25 µg of ethidium bromide. Control organisms run with each PCR include *Ureaplasma* strain ATCC 27618, *M. hominis* ATCC 23114, and *Escherichia coli* ATCC 25922 (negative control).

The patient's wound swab yielded *Ureaplasma* species, demonstrated by the presence of PCR product with both the *Mycoplasma* group primers and the *Ureaplasma* species primers. The B broth subculture was shipped frozen on dry ice to the

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University of Alabama at Birmingham Diagnostic Mycoplasma Laboratory for further genotypic characterization and antimicrobial susceptibility testing.

Genomic DNA from the *Ureaplasma* subculture was extracted by centrifuging 200 μ l of culture at $14,000 \times g$ for 20 min at 4°C; the pellet was digested with 200 μ l proteinase K (1 mg/ml) lysis buffer for 1 h at 60°C. Proteinase K was then inactivated by incubation at 95°C for 5 min. Identification of the isolate to the species level was performed by a multiplex real-time PCR based on the hypothetical genes OFR00127 and UU063 as described previously (8). The PCR assay demonstrated that the organism was *Ureaplasma parvum*. Following species determination, four real-time PCR assays were performed as described by Xiao et al. (18), which determined the organism to be serovar 6. MICs determined by broth microdilution as described by Waites et al. (16) were 0.5 μ g/ml doxycycline, 1.0 μ g/ml tetracycline, 1.0 μ g/ml levofloxacin, and 1.0 μ g/ml erythromycin.

On postoperative day 17, treatment with 500 mg ciprofloxacin given orally twice daily was initiated in response to the detection of *Ureaplasma*. Treatment with piperacillin-tazobactam was discontinued on postoperative day 21. At the same time, 100 mg doxycycline given orally twice daily was added to the ciprofloxacin to provide additional *Ureaplasma* coverage. By postoperative day 28, the patient's sternal wound was clinically improving. The patient was discharged from the hospital on a combination of ciprofloxacin and doxycycline. Given the possibility of sternal bone involvement based on clinical presentation, a decision was made to continue therapy for at least 3 months. The patient was subsequently evaluated in a follow-up 1 month after discharge. At this time, his sternal wound appeared to be healing well.

Ureaplasma parvum belongs to the class *Mollicutes* (17). *Ureaplasma* spp. appear as coccoid cells, ranging from 0.2 to 0.3 μ m in diameter (17). Like other members of the class *Mollicutes*, *Ureaplasma* spp. lack a cell wall and, consequently, cannot be visualized by Gram staining. Culture may be used to identify *Ureaplasma* spp. in the clinical microbiology laboratory. The presence of sterols is required for growth. Media that can be used for isolation include Shepard's 10B broth and A8 agar (17). The generation time for *Ureaplasma* spp. is approximately 1 h, and most cultures will be positive in 2 to 4 days. On agar plates, colonies of *Ureaplasma* spp. are small (15 to 60 μ m), and low-power microscopic magnification is required for visualization (17). *Ureaplasma* spp. may be distinguished from other human mycoplasmas by their ability to hydrolyze urea (17). Identification may also be achieved through molecular methods, as described in the case presented here. The most active antimicrobials in vitro against *Ureaplasma* spp. are macrolides, fluoroquinolones, and tetracyclines (17).

Ureaplasma spp. colonize the lower genitourinary tract in 40 to 80% of sexually active women (11). These organisms have been implicated as etiologic agents causing nongonococcal urethritis, stillbirth/premature delivery, postpartum bacteremia, and wound infection post-caesarean section (3, 7, 12, 13). They have also been associated with the development of neonatal pneumonia, meningitis, and chronic lung disease (8, 11, 17).

There are case reports of *Ureaplasma* spp. as a cause of meningitis, aortic graft infection, and intrarenal abscess in adults (4, 6, 9).

To date there have been few reports of *Ureaplasma* spp. as a cause of sternal wound infection/mediastinitis. A review of the English-language literature yielded three additional cases beyond the one presented here (2, 5, 10). Pigrau et al. described a 63-year-old male who developed a sternal wound infection 7 days after coronary bypass surgery (10). Both *M. hominis* and *Ureaplasma* spp. were isolated from cultures taken at the time of surgical wound drainage. The patient responded to combination therapy with clindamycin, gentamicin, and doxycycline (10). García-de-la-Fuente et al. published a case of a 77-year-old male who developed sternal wound dehiscence, mediastinitis, pleural effusion, and pericardial effusion following aortic valve replacement (5). Cultures demonstrated both *M. hominis* and *Ureaplasma* spp. This patient improved slightly in response to therapy with clindamycin and doxycycline. However, he subsequently developed gastrointestinal bleeding and died (5). Finally, Boyle et al. have reported the case of a 48-year-old male who developed an unstable sternum with associated fever and leukocytosis 30 days post-heart-lung transplantation (2). Mediastinitis was diagnosed at the time of sternal debridement. Wound cultures demonstrated heavy growth of *M. hominis* and moderate growth of *Ureaplasma* spp. The infection was successfully treated with a combination of doxycycline and erythromycin (2). In all three of these cases, *Ureaplasma* spp. were isolated in association with *M. hominis*. The case described in this report is unique in the respect that *U. parvum* was the sole organism detected.

All of the reports of *Ureaplasma* infections published prior to 2003 used the designation of *Ureaplasma urealyticum*, but since that time the genus has been subdivided into two separate species: *U. urealyticum*, which includes serovars 2, 4, 5, 7, 8, 9, 10, 11, 12, and 13, and *U. parvum*, which includes serovars 1, 3, 6, and 14 (17). There is debate as to whether there is differential pathogenicity between the two species or among the various serovars, but this has not been settled (17). *U. parvum* tends to be the more common of the two species in most clinical specimens (18). The only method to distinguish the two species from one another is through the use of the PCR assay. Due to the limited availability of PCR assays and the uncertainty of the significance of differentiating the two species and their respective serovars, species determination is not performed routinely for diagnostic purposes but may be valuable to characterize invasive isolates and for epidemiological purposes (17).

There are several limitations to the case presented here. First of all, the patient did not have a urethral swab submitted for *Ureaplasma* culture, so the source of the organism in his wound remains unclear. Second, antimicrobial therapy was initiated prior to sternal wound swabs being sent for routine bacterial culture. This may have interfered with our ability to identify bacteria more commonly associated with sternal wound infection. Finally, routine bacterial cultures were not sent at the time of sternal debridement, once again limiting our ability to attribute the current infection entirely to *Ureaplasma*. It is not known whether *Ureaplasma* spp. ever colonize sternal wounds. The frequency of ureaplasmas as pathogens in sternal wound infections alone or in association with other more fre-

quently recognized pathogens is also unknown since these organisms are seldom sought, except as in this case when infection persists without an apparent etiology and shows no response to β -lactam antibiotics. In our institution, we do not routinely perform diagnostic testing for either *M. hominis* or *Ureaplasma* spp. in the setting of sternal wound infection.

In summary, this report describes a case of sternal wound infection likely due to *U. parvum*. For patients with a sternal wound infection/mediastinitis for which routine bacterial cultures are negative, consideration should be given to pursuing diagnostic testing for *M. hominis* and *Ureaplasma* spp. Further research is required to determine whether *Ureaplasma* spp. ever occur as a colonizer of uninfected sternal wounds or wounds infected with other more common bacterial pathogens.

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