Diagnosis of *Ureaplasma urealyticum* Septic Polyarthritis by PCR Assay and Electrospray Ionization Mass Spectrometry in a Patient with Acute Lymphoblastic Leukemia

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We report a case of polyarthritis with axial involvement in a young female patient treated for acute lymphoblastic leukemia. Detection in the hip fluid of *Ureaplasma urealyticum* by broad-range PCR followed by electrospray ionization mass spectrometry allowed the diagnosis of septic arthritis and ad integrum recovery upon adapted antibiotic therapy.

CASE REPORT

A 18-year-old woman with no previous medical history was referred in October 2013 for fatigue and weight loss without any bone pain or arthralgia. She was diagnosed with common acute lymphoblastic leukemia (ALL) without central nervous system involvement. The initial white blood cell (WBC) count was 2 × 10⁹ cells/liter. The karyotype showed an i(9)(q10) isochromosome as the sole abnormality. The initial gamma globulin level was 6.5 g/liter. After a steroid prophase, she underwent a Berlin-Frankfurt-Münster (BFM)-like induction course according to the pediatric FRALLE 2000B trial, including vincristine, oral prednisolone, daunorubicin, idarubicin, and cytarabine with lumbar spine pain with stiffness. Her body temperature was 38°C. Her levels were as follows: C-reactive protein, 101 mg/liter; ferritin, 2,652 μg/liter; fibrinogen, 7.31 g/liter; WBC count, 8 × 10⁹ cells/liter, with 0.88 × 10⁹ lymphocytes/liter; hemoglobin, 97 g/liter; and gamma globulin, 3.1 g/liter. Results for joint radiography showed involvement of the right hip, and the left temporomandibular joint, with limited mouth opening. Moreover, she had developed axial involvement without granulocyte colony-stimulating factor, joint pains (in the ankles and knees) with odynophagia and mild fever occurred. At that time, the patient was still receiving prednisolone at 40 mg/m²/day and prophylactic piperacillin-tazobactam.

On day 29, while the patient was recovering from neutropenia, she developed axillary lymphadenopathy and joint pains (in the ankles and knees) with odynophagia and mild fever occurred. At that time, the patient was still receiving prednisolone at 40 mg/m²/day and prophylactic piperacillin-tazobactam.

The pain worsened after corticosteroid withdrawal. On day 36, she was admitted with painful tender swelling and redness over the index proximal and distal interphalangeal joint, the right elbow, the right hip, and the left temporomandibular joint, with limited mouth opening. Moreover, she had developed axillary lymphadenopathy with lumbar spine pain with stiffness. Her body temperature was 38°C. Her levels were as follows: C-reactive protein, 101 mg/liter; ferritin, 2,652 μg/liter; fibrinogen, 7.31 g/liter; WBC count, 8 × 10⁹ cells/liter, with 0.88 × 10⁹ lymphocytes/liter; hemoglobin, 97 g/liter; and gamma globulin, 3.1 g/liter. Results for joint radiography and whole-spine magnetic resonance imaging (MRI) were normal.

A hip fluid aspiration performed on day 45 revealed 51,900 leukocytes/mm³ (70% polymorphonuclear cells), no crystals, no bacteria in culture, and no leukemic blasts. Results of urine testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were negative. Results for broad testing of blood and joint fluid for detection of viral genomes, including those of cytomegalovirus (CMV), Epstein-Barr virus (EBV), parvovirus B19, enterovirus, adenovirus, human herpesvirus 6 (HHV-6), herpes simplex virus (HSV), and varicella-zoster virus (VZV), were negative. Antistreptolysin, anticardiolipin, anticyclic citrullinated peptide, rheumatoid factor, and antinuclear antibodies were not detected. HLA B27 and B51 were absent. Results of serological tests for Lyme disease and brucellosis were negative. The glycosylated ferritin level was normal.

The patient had no personal history of psoriasis. Her mother had previously been diagnosed with elbow and knee rashes suggestive of psoriasis. Due to this first-degree-relative history, the presence of dactylitis, axial involvement, and the absence of rheumatoid factor, the diagnosis of psoriatic arthritis was made first. Prednisolone at 20 mg and ketoprofen at 100 mg twice daily were reintroduced on days 37 and 45, respectively. On day 61, due to pain exacerbation, tocilizumab at 400 mg was administered, with poor clinical efficacy.

In this context of therapy escalation, we decided to test the hip fluid on the Plex-ID platform, which combines a broad-range PCR assay for bacterial DNA detection with electrospray ionization mass spectrometry (PCR–ESI-MS). Nucleic acids were extracted from 1 ml of the hip fluid by a magnetic bead-based method with an nSP instrument, using a DNA preparation kit (both from Abbott Ibis Biosciences). Plex-ID analysis was performed with the Plex-ID BAC assay, devoted to broad-spectrum bacterial amplification (Abbott Ibis Biosciences, Carlsbad, CA, USA). After mass spectrometry of the amplicons, the Plex-ID software converted the mass information into base compositions and determined the bacterial DNA present in the samples by comparing the base composition signature to sequences in a database. The system usually takes 6 h from DNA extraction to provide final semiquantitative results. In the present case, at day 71, the signal detected in the hip fluid sample suggested a strong positivity for *Ureaplasma urealyticum*. On the Plex-ID system, *Ureaplasma* species are expected to be detected on the basis of 3 primer pairs. As previously reported, *Ureaplasma parvum* and *Ureaplasma urea-

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lyticum may be differentiated on the basis of 2 of the 3 primer pairs (1).

In order to confirm the presence of the bacteria and to test for antibiotic susceptibility, a knee joint puncture was performed, and the sample was grown on specific Shepard medium containing urea. A Mycofast Revolution kit (ELITech Microbiology Reagents) was used to determine susceptibility to a panel of antibiotics in agreement with the Clinical and Laboratory Standards Institute breakpoints. Meanwhile, levofloxacin at 500 mg twice daily and doxycycline at 100 mg twice daily were started.

Urea-plasma organisms grew in synovial fluid (10^2 color-changing units/ml) and in urine, and biovar typing by real-time PCR (both in culture medium and in the hip sample) confirmed the presence of U. urealyticum (2). The strain was surprisingly identified as resistant to doxycycline, which was withdrawn from patient therapy. The patient’s dramatic improvement upon antibiotic therapy (Fig. 1) allowed for acute lymphoblastic leukemia consolidation by administration of high-dose methotrexate on day 82.

U. urealyticum is a commensal bacterium of the lower genito-urinary tract found in 40 to 80% of sexually active women (3) and 25 to 40% of men (4) and rarely penetrates the submucosa or disseminates through the bloodstream, except in immunocompromised patients. This microorganism is primarily transmitted either by sexual contact or vertically from mother to offspring, either in utero or perinatally (5).

U. urealyticum is causally linked to disseminated infections in newborns but also in immunocompromised patients suffering from hypogammaglobulinemia (common variable immunodeficiency [CVID] or other B-cell deficiencies) (6, 7) after renal transplant (8) or lymphoma (9) or even after rituximab therapy for systemic lupus erythematosus without hypogammaglobulinemia (10).

We report here the first patient who developed a rapidly progressive bilateral and symmetric septic polyarthritis with axial involvement due to U. urealyticum following induction course for acute lymphoblastic leukemia (ALL). The diagnosis was first oriented by a new assay based on broad-range PCR followed by electrospray ionization mass spectrometry (PCR–ESI-MS; Plex-ID system, Abbott Ibis Biosciences, Carlsbad, CA) (11, 12) and confirmed by positive-culture isolation (13).

The present case is remarkable for several reasons. First, in the literature, the delay observed in identifying U. urealyticum as the causative agent ranges from 4 weeks to 4 months (14), as ureaplasma is often not considered early in the clinical course. Here,
the time to diagnosis was 4 weeks, shortened by the use of the ESI-time of flight (TOF) mass spectrometry. Indeed, the promising Plex-ID system provides a rapid method for detection of a broad array of bacteria directly in several specimens (e.g., articular fluid, blood, and respiratory samples) and has the potential to affect patient outcome by reducing the need to wait for the culture. Second, septic arthritis with U. urealyticum is an infection typically seen in patients with CVID and hypogammaglobulinaemia (14, 15) but, to date, has never been described to occur during ALL therapy. The patient’s medical history was not suggestive of CVID. Third, this case should raise clinicians’ awareness of such infections occurring with negative standard cultures in immunocompromised patients, since specific bacteriological techniques (specialized culture for detection) are required for early diagnosis and therapy. Fourth, the prompt response to antibiotics allowed infections occurring with negative standard cultures in immunocompromised patients, since specific bacteriological techniques are required for early diagnosis and therapy. Fourth, the prompt response to antibiotics allowed ad integrum recovery without any joint destruction. Fifth, the resistance of ureaplasma to tetracyclines is very low, approximately 5% (16). The culture isolation enabled determination of the antibiotic sensitivity profile, which was very useful in this case, and this method cannot be replaced by molecular techniques to date, although PCR–ESI–MS-based detections allow for the initial antimicrobial therapy to be based on the organism present, resulting in more optimal care and outcome for this patient. In conclusion, the Plex-ID system provides a rapid method for detection of microorganisms, especially fastidious bacteria. The present case illustrates how this technique could be used in conjunction with routine microbiological investigations to identify unusual infections in immunocompromised hosts.

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