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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Keywords: *Ureaplasma urealyticum*, polyarthritis, spectrometry, antibiotics.

Acknowledgments: The authors would like to thank François Simon and Severine Mercier Delarue for their helpful contribution.
ABSTRACT

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.
A 18-year-old woman with no previous medical history was referred in October 2013 for fatigue and weight loss, without any bone pain nor arthralgia. She was diagnosed with common acute lymphoblastic leukemia (ALL) without central nervous system involvement. The initial white blood cells count was 2 X 10^9/L. The karyotype showed an isochromosome i(9)(q10) as sole abnormality. Initial gammaglobuline was 6.5 g/L. After a steroid prophase, she underwent a BFM-like induction course according to pediatric FRALLE-2000B trial including vincristine, oral prednisolone, daunorubicin, L-asparaginase and triple intrathecal chemotherapy (methotrexate, cytosine arabinoside and depomedrol).

On day 29, while the patient was recovering from neutropenia without granulocyte colony stimulating factor, joint pains (ankles and knees) with odynophagia and mild fever occurred. At that time, the patient was still receiving prednisolone 40 mg/sqm/d 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 axial involvement with lumbar spine pain with stiffness. Temperature was 38°C. C-reactive protein was 101 mg/L, ferritine 2652 μg/L, fibrinogen 7.31g/L; WBC count was 8 X 10^9/L, with lymphocyte 0.88 X 10^9/L, hemoglobin 97 g/L and gammaglobuline 3.1 g/L. Joint radiography and whole spine MRI were normal.

Hip fluid aspiration performed on day 45 revealed 51900 leukocytes/mm³ (70% polymorphonuclear cells), no crystals, no bacteria in culture and no leukemic blasts. Urine testing for Chlamydia trachomatis and Neisseria gonorrhoeae were negative. Broad viral genome detection including CMV, EBV, Parvovirus B19, Enterovirus, Adenovirus, HHV-6, HSV, VZV from blood and joint fluid was negative. Antistreptolysin, circulating anti-cyclic citrullinated peptide, rheumatoid factor and antinuclear antibodies were not detected. HLA B27 and B51 were absent. Lyme and brucellosis serologies were negative. Glycosylated ferritin was normal.

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

In this context of therapy escalation, we decided to test the hip fluid onto the PLEX-ID platform, which combines a broad-range PCR for bacterial DNA detection with electrospray ionization mass spectrometry. Nucleic acids were extracted, from 1mL of the hip fluid, by a magnetic beads based method with the nSP instrument, using the DNA Preparation kit (all from Abbott Ibis Biosciences). PLEX-ID analysis was performed with the PLEX-ID BAC Assay, devoted to bacterial broad Spectrum Amplification (AbbottIbis Biosciences, Carlsbad, CA, USA). After mass spectrometry of the amplicons, the PLEX-ID software converted the mass information into base compositions and determined the bacteria DNA present in the samples by comparing the base composition signature to a database. The system usually takes 6 hours from DNA extraction to provide final semi-quantitative results. In the present case, at day 71, the signal detected in the hip fluid sample suggested a strong positivity for *U. urealyticum*. On the PLEX-ID system, *Ureaplasma* are expected to be detected by 3 primer pairs. As previously reported, *Ureaplasma parvum* and *Ureaplasma urealyticum* may be differentiated by 2 of the 3 primer pairs (5).

In order to confirm presence of the bacteria and to test antibiotic susceptibility, a knee joint puncture was performed and grown on specific Shepard media containing urea. The Mycofast® RevolutionN kit (ELITech Microbiology Reagents) was used to determine susceptibility to a panel of antibiotics in agreement with the Clinical Laboratories Standards Institute breakpoints. Meanwhile, levofloxacin 500 mg twice daily and doxycycline 100 mg twice daily were started. *Ureaplasma* grew in synovial fluid (10^2 Color Changing Unit/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* (16). The strain was surprisingly identified as resistant to doxycycline that was withdrawn from patient therapy. Patient dramatic improvement upon antibiotic therapy (figure 1) allowed administrating acute lymphoblastic leukemia consolidation by high-dose methotrexate on day 82.

*U. urealyticum* is a commensal bacteria of the lower genitourinary tract found in 40-80% of sexually active women (14) and 25-40% of men (12) and rarely penetrates the submucosa or disseminates through the blood stream, except in immunocompromised patients. This
microorganism is primarily transmitted either by sexual contact, or vertically from mother to offspring, either in utero or perinatally (11).

*U. urealyticum* is causally linked to disseminated infections in newborns but also in immunocompromised patients suffering from hypogammaglobulinaemia (common variable immunodeficiency or other B-cell deficiencies) (6, 7), or after renal transplant (3), or lymphoma (1) or even after rituximab therapy for systemic lupus erythematosus without hypogammaglobulinaemia (8).

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) (4, 10) and confirmed by positive culture isolation (15).

The present case is remarkable for several reasons. First, in the literature, delay observed in identifying the causative agent *U. urealyticum* ranging from 4 weeks to 4 months (2), as ureaplasma are often not considered early in the clinical course. Here, time to diagnosis was four weeks, shortened by the use of the ESI TOF mass spectrometry, an innovating molecular biology technique overpassing the wide bacterial diversity. Indeed, the promising PLEX-ID system provides a rapid method for the detection of a broad array of bacteria directly in several specimens (articular fluid, blood, respiratory samples,...) and has the potential of impacting 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 common variable immunodeficiency (CVID) and hypogammaglobulinemia (2, 13), but to date, never described during ALL therapy. Patient medical history was not suggestive of CVID. Third, this case should raise clinician awareness of such infections with negative standard cultures in immunocompromised patients, since specific bacteriological techniques (specialized culture for detection) are required for early diagnosis and therapy. Fourth, the promptly response to antibiotics allowed *ad integram* recovery without any joint destruction. Fifth, ureaplasma resistance to tetracyclines is very low, approximately 5% (9). The culture isolation enabled to determine the antibiotic sensitivity profile, very useful in this case, and cannot be replaced by molecular techniques to date, although PCR-ESI-MS 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 the
detection of microorganisms, especially fastidious to grow bacteria. The present case illustrates how this technique could be used in conjunction with routine microbiological investigations to identify unusual infections in immunocompromised hosts.

REFERENCES


Figure 1: Progression of joint pain and inflammation according to treatment time.

Pain intensity was assessed using a verbal grading scale every day.
AraC: cytarabine; VP16: vepeside; IT: intrathecal; MTX: methotrexate.