Case Report

Campylobacter fetus subspecies *fetus* spondylodiscitis

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Campylobacter spp. are common causes of gastrointestinal infections. *Campylobacter fetus* is a much rarer pathogen in humans, and usually causes bacteraemia and systemic complications in patients with predisposing conditions. We report a case of spondylodiscitis caused by *C. fetus* subsp. *fetus* as revealed by vertebral biopsy culture. This identification was confirmed by sequencing the 16S rRNA gene and by phylogenetic analysis. Treatment consisted of 6 weeks antimicrobial therapy combined with a strict initial immobilization, followed by a re-education program. The patient's recovery was uneventful.

Case report

A 91-year-old woman, suffering from pain in the left hip and in the lumbar region, had a fever 2 weeks before hospitalization. Radiographs of the lumbar spine revealed only arthritic lesions. She was given a monodose of 400 mg ofloxacin because of a possible urinary tract infection. She was admitted to the University Hospital Centre (2 Boulevard Tonnellé, 37044 Tours Cedex 9, France) because of a persistent fever (38.2 °C) and lower back pain. Her blood pressure was 140/70 mmHg, her absolute polymorphonuclear count was 10 900 cells mm⁻³, she had a C-reactive protein level of 111.2 mg l⁻¹ and a creatinine clearance rate of 35 ml min⁻¹.

An initial lumbar computed tomography (CT) scan showed an abnormal enhancement at the L2–L3 and L3–L4 disc space level, and in the left psoas muscle after contrast injection (Fig. 1), suggestive of a spondylodiscitis. Magnetic resonance imaging was contra-indicated due to the presence of a pacemaker.

A CT-guided discostebral biopsy with a posterolateral approach was performed. Three discitis samples were sent to the microbiology department of the University Hospital Centre. Samples were also sent to the mycology department and to the cytology department of the University Hospital Centre in accordance with French guidelines (SPILF, 2007).

The patient was placed in strict dorsal decubitus, and an empiric broad-spectrum antibiotic therapy using ofloxacin

(400 mg twice daily) plus rifampicin [100 mg (kg body weight)⁻¹ twice daily] was initiated (for the first week). The patient was afebrile 24 h later. Blood cultures (aerobic and anaerobic vials) remained negative (Bactec 9240; BD).

Culture of two of the three disc samples and fluid aspirations produced grey-white non-haemolytic colonies after 48 h incubation on 5 % blood Columbia agar (bioMérieux) at 37 °C under aerobic conditions. The growth on trypticase soy agar supplemented with 5 % horse blood and on chocolate agar plus PolyViteX (bioMérieux) was very weak. Microscopic examination of these colonies after Gram staining revealed curved Gram-negative rods, and both catalase and oxidase reactions were positive. A *Campylobacter* species was suspected and colonies were subcultured under microaerophilic conditions (10 % CO₂, 5 % O₂, 85 % N₂) at 37 and 42 °C. Bacteria grew only at 37 °C. Hydrolysis of sodium hippurate was negative. The pattern of susceptibility to antibiotics was resistance to nalidixic acid (30 mg disc) and susceptibility to cefalotin (30 mg disk). These phenotypic findings suggested *Campylobacter fetus* (Penner, 1988; Pigrau *et al.*, 1997). Identification was confirmed by PCR of the 16S rRNA gene followed by sequencing.

DNA was extracted with an Invisorb spin tissue kit (Invitek). PCR was performed with a 10 µl DNA sample in a mixture containing 0.5 µM primers U1IS 5'-CCAG-CAGCCGCGGTAATACG-3' and U2IS 5'-ATCGGYTAC-CTTGTTACGACTTC-3', 200 µM dNTPs (Boehringer), 2.5 mM MgCl₂ (Applied Biosystems) and 2.5 U AmpliTaq DNA (Applied Biosystems) in a final volume of 50 µl (Lu

Abbreviations: CT, computed tomography; SPILF, Société de Pathologie Infectieuse de Langue Française.

Fig. 1. Lumbar CT scans after contrast injection. (a) Abnormal enhancement in the disc space and in the left psoas muscle after contrast injection. (b) Abnormal enhancement in the disc space and anterior extension in the prevertebral fat. (c, d) Perivertebral and psoas muscle enhancement after contrast injection; a low density lesion surrounded by abnormal enhancement within the right psoas muscle representing an abscess is visible. The arrows in (d) indicate the abscess. i.v., intravenous.

et al., 2000). Sequencing was performed using a BigDye terminator v 3.1 cycle sequencing kit (Applied Biosystems). Sequencing analysis 5.1.1 software (Applied Biosystems) was used to analyse the DNA sequence. BLAST software was used to compare the DNA sequence with sequences published in the National Center for Biotechnology Information database. The best identification, according to BLAST analysis, was *C. fetus* (two strains AB301966 and AB301967) with 97% identity for the 434 bases tested. Phylogenetic analysis of the partial nucleotide sequence performed with BIBI software (<http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi>) confirmed the identification.

In vitro susceptibility tests using the Etest method (AB Biodisk) on Mueller–Hinton agar (bioMérieux) supplemented with 5% horse blood, showed susceptibility to ampicillin and

levofloxacin, and resistance to erythromycin and ciprofloxacin (Table 1). Previous patterns of susceptibility to antibiotics determined with *C. fetus* isolates showed that resistance to erythromycin was rare (0 to 2.2% of 114 isolates in France in 2007, 0 of 105 isolates in France in 2008) (King *et al.*, 2008).

Pathological examination of disc samples showed no specific inflammatory reaction and no tumour cells were found. No organisms were seen on Gram staining. Ziehl–Nielsen staining and mycobacterial culture on Löwenstein Jensen medium (Bio-Rad) and in mycobacterial vials (BacT/ALERT; bioMérieux) were performed on disc samples. No acid-fast bacilli were observed and cultures remain negative after 3 months of incubation.

Following bacterial identification and antibiogram results, the antibiotic therapy was changed to oral amoxicillin, 6 g

Table 1. MIC determined by Etest method (AB Biodisk)

Antimicrobial agent	MIC against strain ($\mu\text{g ml}^{-1}$)	Breakpoint ($\mu\text{g ml}^{-1}$)*	
		S	R
Ampicillin	4	≤ 4	> 16
Ampicillin/clavulanate	3	≤ 4	> 16
Cefalotin	3	≤ 8	> 32
Imipenem	0.047	≤ 2	> 8
Ertapenem	0.75	≤ 2	> 4
Gentamicin	0.125	≤ 2	> 4
Erythromycin	6	≤ 1	> 4
Levofloxacin	1	≤ 1	> 2
Ciprofloxacin	2	≤ 0.5	> 1
Tetracycline	0.01	≤ 4	> 8

*Breakpoints for Gram-negative bacteria were used for imipenem, ertapenem and levofloxacin, and data for *Campylobacter* spp. were used for other antibiotics [French Society for Microbiology recommendations, 2009 (<http://www.sfm.asso.fr>)].

per day, for 5 weeks; the total antibiotic therapy being for 6 weeks. Strict dorsal decubitus was maintained for 3 weeks followed by progressive 'reverticalization' with a lumbosacral corset. At the 3 and 6 month follow-up stages, the patient remained well, with no fever or back pain, and no inflammatory biological syndrome.

Although the patient had no intestinal symptoms, the portal of entry of *C. fetus* subsp. *fetus* was probably the gastrointestinal tract. The patient had no history of gastrointestinal pathology. Stool cultures before specific antibiotic therapy remained negative for *Campylobacter* species, and colonoscopy was not performed because of underlying cardiac diseases such as atrial fibrillation, ischaemic cardiopathy and hypertension in this elderly patient. Despite negative blood cultures, transthoracic echocardiography was performed and no pacemaker-related infectious endocarditis was detected. A transoesophageal echocardiography was not performed, due to the age of the patient.

Discussion

C. fetus spondylodiscitis is very rare (Bachmeyer *et al.*, 1992; Mathieu *et al.*, 1991; Yamashita *et al.*, 1999). Therapy in the reported cases included a combination of doxycycline and erythromycin for 3 months or alternate oral administration of each antibiotic for 5 months (Mathieu *et al.*, 1991; Yamashita *et al.*, 1999). There are no international recommendations for the management of lumbar spondylodiscitis. A 10 year retrospective study by Roblot *et al.* (2007) suggested that antibiotic therapy of vertebral osteomyelitis could be safely shortened to 6 weeks without enhancing the risk of relapse. In the case presented here,

the patient had 6 weeks of treatment, with 5 weeks of amoxicillin oral monotherapy, combined with an initial strict dorsal decubitus for 3 weeks, followed by re-education. This treatment led to an uneventful recovery. Although 4–6 weeks is the minimum acceptable time for antimicrobial therapy, a much longer period of treatment may be required to cure individual patients (SPILF, 2007). Reviews of pyogenic vertebral osteomyelitis found that parenteral antibiotic therapy of less than 4 weeks resulted in therapeutic failure more often than therapy for more than 4 weeks did (McHenry *et al.*, 2002).

C. fetus subsp. *fetus* infections and especially bacteraemia usually occur in immunocompromised or debilitated elderly patients (Janssen *et al.*, 2008). In the 58 cases of bacteraemia due to *Campylobacter* species reported by Pigrau *et al.* (1997), almost all patients (93 %) had an underlying disease such as cirrhosis of the liver, immunosuppression or neoplasia. The exploratory tests for digestive neoplasia (such as colonoscopy) were not performed in this case but clinical findings did not support neoplasia or immune dysfunction. The case of vertebral osteomyelitis caused by *C. fetus* reported by Yamashita *et al.* (1999) also occurred in an elderly man with no predisposing conditions.

As with other Gram-negative organisms that colonize mucosal surfaces, the high serum resistance of *C. fetus* strains is related to the ability to cause bacteraemia (Blaser, 1998; Chuman *et al.*, 2003; Pigrau *et al.*, 1997). Various systemic complications may occur, such as meningitis and endocarditis. *C. fetus* endocarditis has been reported in less than 30 cases (Brouqui & Raoult, 2001; Farrugia *et al.*, 1994).

In this case, the patient received ofloxacin for 7 days, which was stopped 2 weeks before microbiological samples were taken. Moreover, blood cultures were negative even though the Bactec system is recognized to allow growth of *Campylobacter*. There was no evidence of infectious endocarditis with the self-limiting transthoracic echocardiography.

Identification of the pathogen was obtained by microbiological examination of disc samples and confirmed by molecular methods. The sensitivity of standard microbiological methods for identifying the cause of infectious spondylodiscitis microbiological cultures ranges from 50 to 80 % (Bontoux *et al.*, 1992; Grammatico *et al.*, 2008; Lecouvet *et al.*, 2004; Torda *et al.*, 1995). Isolation of *C. fetus* is rare in such samples (Bachmeyer *et al.*, 1992; Yamashita *et al.*, 1999). The French National Reference Centre for *Campylobacters* and *Helicobacters* (Centre National de Référence des *Campylobacters* et *Hélicobacters*) reported in 2008 that *C. fetus* represented 3.2 % of 3481 strains isolated, mainly from blood cultures (55.2 %) (King *et al.*, 2008).

In our case, the identification of a *C. fetus* subspecies was confirmed by amplification of the 16S rRNA gene by PCR followed by DNA sequencing. Furthermore, some data

highlight the sensitivity and specificity of DNA-based diagnostic methods and their contribution to the identification of pathogens in spondylodiscitis. In the 19 patients with spondylodiscitis reported by Lecouvet *et al.* (2004) the use of molecular methods in addition to microbiological techniques increased the identification rate of bacterial agents from 74 % (14 of 19 patients) to 100 % (19 of 19 patients). DNA-based methods are highly sensitive and can complement classical microbiological methods to optimize the identification of the causative agents of infectious spondylodiscitis.

This is, to the best of our knowledge, the fourth case of a *C. fetus* spondylodiscitis in a host with no apparent immunosuppression except for her advanced age. The diagnosis was based on discovertebral biopsy, and the bacterial identification was confirmed by PCR and phylogenetic analysis. Improvements in the detection of *C. fetus* and related microaerophiles by clinical microbiologists will help us appreciate the true scale of this problem. Amplification-based analysis of bacterial DNA should overcome some of the limitations of classical microbiological methods (Lecouvet *et al.*, 2004; Schulze *et al.*, 2006).

References

- Bachmeyer, C., Grateau, G., Sereni, D. & Cremer, G. A. (1992). *Campylobacter fetus* spondylodiscitis. *Rev Rhum Mal Osteoartic* **59**, 77–79.
- Blaser, M. J. (1998). *Campylobacter fetus* – emerging infection and model system for bacterial pathogenesis at mucosal surfaces. *Clin Infect Dis* **27**, 256–258.
- Bontoux, D., Codello, L., Debais, F., Lambert de Cursay, G., Azais, I. & Alcalay, M. (1992). Infectious spondylodiscitis. Analysis of a series of 105 cases. *Rev Rhum Mal Osteoartic* **59**, 401–407 (in French).
- Brouqui, P. & Raoult, D. (2001). Endocarditis due to rare and fastidious bacteria. *Clin Microbiol Rev* **14**, 177–207.
- Chuman, Y., Takata, T., Sameshima, H., Takeuchi, S., Takatsuka, Y., Makino, T., Blaser, M. J. & Utsunomiya, A. (2003). *Campylobacter fetus* bacteremia in a patient with adult T cell leukemia. *Clin Infect Dis* **36**, 1497–1498.
- Farrugia, D. C., Eykyn, S. J. & Smyth, E. G. (1994). *Campylobacter fetus* endocarditis: two case reports and review. *Clin Infect Dis* **18**, 443–446.
- Grammatico, L., Baron, S., Rusch, E., Lepage, B., Surer, N., Desenclos, J. C. & Besnier, J. M. (2008). Epidemiology of vertebral osteomyelitis (VO) in France: analysis of hospital-discharge data 2002–2003. *Epidemiol Infect* **136**, 653–660.
- Janssen, R., Krogfelt, K. A., Cawthraw, S. A., van Pelt, W., Wagenaar, J. A. & Owen, R. J. (2008). Host-pathogen interactions in *Campylobacter* infections: the host perspective. *Clin Microbiol Rev* **21**, 505–518.
- King, L., Lehours, P. & Megraud, F. (2008). *Bilan de la Surveillance des Infections à Campylobacter Chez l'Homme en France en 2008*. Bordeaux: Centre National de Référence des Campylobacters et Hélicobacters. http://www.cnrch.u-bordeaux2.fr/Campylobacter_Bilan_surveillance_reseau_2008.pdf.
- Lecouvet, F., Irenge, L., Vandercam, B., Nzeusseu, A., Hamels, S. & Gala, J. (2004). The etiologic diagnosis of infectious discitis is improved by amplification-based DNA analysis. *Arthritis Rheum* **50**, 2985–2994.
- Lu, J. J., Perng, C. L., Lee, S. Y. & Wan, C. C. (2000). Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. *J Clin Microbiol* **38**, 2076–2080.
- Mathieu, E., Koeger, A. C., Rozenberg, S. & Bourgeois, P. (1991). *Campylobacter* spondylodiscitis and deficiency of cellular immunity. *J Rheumatol* **18**, 1929–1931.
- McHenry, M. C., Easley, K. A. & Locker, G. A. (2002). Vertebral osteomyelitis: long-term outcome for 253 patients from 7 Cleveland-area hospitals. *Clin Infect Dis* **34**, 1342–1350.
- Penner, J. L. (1988). The genus *Campylobacter*: a decade of progress. *Clin Microbiol Rev* **1**, 157–172.
- Pigrau, C., Bartolome, R., Almirante, B., Planes, A. M., Gavalda, J. & Pahissa, A. (1997). Bacteremia due to *Campylobacter* species: clinical findings and antimicrobial susceptibility patterns. *Clin Infect Dis* **25**, 1414–1420.
- Roblot, F., Besnier, J. M., Juhel, L., Vidal, C., Ragot, S., Bastides, F., Le Moal, G., Godet, C., Mulleman, D. & other authors (2007). Optimal duration of antibiotic therapy in vertebral osteomyelitis. *Semin Arthritis Rheum* **36**, 269–277.
- Schulze, F., Bagon, A., Müller, W. & Hotzel, H. (2006). Identification of *Campylobacter fetus* subspecies by phenotypic differentiation and PCR. *J Clin Microbiol* **44**, 2019–2024.
- SPILF (2007). Primary infectious spondylitis, and following intradiscal procedure, without prosthesis. Recommendations. *Med Mal Infect* **37**, 573–583.
- Torda, A. J., Gottlieb, T. & Bradbury, R. (1995). Pyogenic vertebral osteomyelitis: analysis of 20 cases and review. *Clin Infect Dis* **20**, 320–328.
- Yamashita, K., Aoki, Y. & Hiroshima, K. (1999). Pyogenic vertebral osteomyelitis caused by *Campylobacter fetus* subspecies *fetus*. A case report. *Spine* **24**, 582–584.