TITLE: First case of post-aneurysmal prosthetic vascular infection due to a non-superantigenic Yersinia pseudotuberculosis.

AUTHORS: Caroline Loïez¹,², Christophe Carnoy¹,³, Christophe Decoene¹,², Elizabeth Pradel¹,³, Caroline Fichel³, René Courcol¹,² and Frédéric Wallet¹,²

INSTITUTIONS: ¹Univ Lille Nord de France, F-59000 Lille France, ²CHU Lille F-59000 Lille France, ³INSERM U1019, CNRS UMR 8204, F-59019 Lille, France.

RUNNING HEAD: Yersinia pseudotuberculosis, ypm, aneurysm, prosthetic material, superantigen

REPRINT REQUEST: F. Wallet M. D.

Centre de Biologie-Pathologie
Pôle de Microbiologie
Bld du Pr Leclercq, 59037 Lille Cedex, France
frederic.wallet@chru-lille.fr
ABSTRACT

Among *Yersinia* spp., *Y. enterocolitica* is the most frequent species isolated from infected aneurysms. This report describes the first case of post-aneurysmal prosthetic vascular infection due to a superantigen-negative *Yersinia pseudotuberculosis* showing a potential affinity of this species for endovascular tissue.
CASE REPORT

A 58-year-old man was admitted to the cardio-vascular unit for abdominal pain with fever (38.5°C). One month before, this patient was treated by endovascular in situ graft for an aorto-iliac aneurysm rupture. On admission, the patient, known to be treated for a history of hypertension and presenting cirrhosis, showed an exacerbation of the spontaneous pain after abdominal palpation. The abdominal angioscanography revealed a retroperitoneal collection in contact with the vascular prosthesis due to a potential infection of the material. Blood tests showed a blood cell count of 16.5 X 10^3/µl (85% of polymorphonuclear leucocytes), the C-reactive protein concentration was 140 mg/l (normal range < 6 mg/l). The biochemical results were unremarkable except the gamma-glutamyl transferase at 220 UI/l. The patient did not present signs of severe sepsis. The retroperitoneal collection was rapidly evacuated by surgery without vascular prosthesis replacement, combined to a first antimicrobial treatment with piperacillin-tazobactam, ciprofloxacin and vancomycin.

Three blood culture sets (aerobic and anaerobic bottles) drawn on admission of the patient and a sample of the retroperitoneal collection were sent to the microbiological laboratory. A bipolar Gram-negative rod was isolated from the three blood culture sets by the BacT/Alert (bioMérieux, Marcy l’Etoile, France). The direct smear examination of the retroperitoneal collection, showing leucocytes without bacteria, yielded in pure culture with also a bipolar Gram-negative rod. This Gram-negative bacillus had the same aspect in the two pathological products. This non-motile, non-spore forming aerobic-facultative was able to grow on bromo-cresol purple agar, blood agar and chocolate agar plates within 18 hours. This rod was oxidase negative, did not produce indole. It was able to reduce nitrate to nitrite and hydrolysed urea. Phenotypical identification performed with the API 32 GN and the VITEK 2 GN system (bioMérieux, Marcy l’Etoile, France) gave Yersinia pseudotuberculosis (excellent identification, probability = 97%). To confirm this identification, a thin smear of this strain
was deposited on a MALDI-plate. Measurements were performed with a Microflex mass spectrometer (Bruker Daltonik, Wissembourg, France) using FlexControl software (version 3.0). The spectrum was imported into the BioTyper software (version 2.0; Bruker, Germany). The Biotype database contains approximately spectra of 3847 species and is regularly updated by the Bruker Company. The result of the pattern-matching process was expressed with a score of 2.27 giving a *Y. pseudotuberculosis* 29490 RKB as first choice (a score > 2.0 was considered as identification to species level). The routine biochemical phenotype identification and MALDI-TOF MS spectrum have exactly the same identification to the species level, the identification was considered final. This isolate belonged to *Y. pseudotuberculosis* serotype O:I. To evaluate the pathogenicity of this isolate, variants of *ypm* superantigens were search directly on the strain using PCR technique with the following primers: ypmA1:C1/ypmA2:C2 ACA-CTT-TCC-TCT-GGA-GTA-GCG / ACA-GGA-CAT-TTC-GTC-A and ypmB1/ypmB2 CTA-ATC-CCC-CGA-GGA-TAA-GTT / GCC-GAT-TCC-GAC-GAC-ATA-TAA-C (2). The strain did not harbour this superantigen gene (Figure 1). At last, the *in vitro* antimicrobial susceptibility tests of this strain was obtained by disk diffusion method on Mueller-Hinton agar plate as recommended by the Comité de l’Antibiogramme de la Société Française de Microbiologie (CA-SFM) criteria (http:www.sfm.asso.fr/) (4). The strain was susceptible to ampicillin, piperacillin, cefotaxime, imipenem, aminoglycosides, trimethoprim-sulfamethoxazole, fluoroquinolones and resistant to colistin.

The first therapy was modified by cefotaxime associated to levofloxacine after the results of the susceptibility tests. After 6 weeks, the treatment was relayed by levofloxacine alone for 6 months.
Discussion

*Yersinia pseudotuberculosis* is an enteric pathogen. In the human, this Gram-negative bacterium causes digestive illness (diarrhea, abdominal pain), but also systemic symptoms as fever, scarlatiniform rash, erythema nodosum and lymphadenopathy. *Y. pseudotuberculosis* is the only Gram-negative bacterium producing a superantigen. The 14.5 kDa exotoxin (designated YPM for *Yersinia pseudotuberculosis*-derived mitogen) might be involved in inflammatory post-infection complications (reactive arthritis, erythema nodosum) and in Kawasaki syndrome (11).

Hypertension is considered as a common underlying etiologic factor for atherosclerosis and is associated with increased risk for aortic aneurysm. Using conventional bacteriological techniques, the most common responsible organisms in primary infected aneurysm are *Salmonella* sp (50%) and *Staphylococcus aureus* (30%) followed by other *Enterobacteriaceae* such as *Escherichia coli*, *Streptococcaceae* and some Gram negative bacilli. In prosthetic vascular graft infections, the most frequent involved bacteria are *Staphylococcus aureus* in the early infections and coagulase negative staphylococci in the late infections (9, 10). A search in MEDLINE from 1975 up to now using the key words aneurysm and *Yersinia* reported a total of 16 cases. Among *Yersinia* spp., the most frequent species found in mycotic aneurysm is *Y. enterocolitica* (5, 8, 12, 14-18). *Y. pseudotuberculosis* was reported once in 2006 by Hadou *et al.* in an elderly patient with atherosclerosis cardiovascular disease (7). In the prosthetic vascular graft infections, only 2 cases were reported due to *Y. enterocolitica* (1, 19). Our case is the first describing a prosthetic vascular infection due to *Y. pseudotuberculosis*. In our case, predisposing factors such as cirrhosis may explain the susceptibility of the patient to *Yersinia* although no gastrointestinal disorder was found: the stool culture remained negative for *Y. pseudotuberculosis*. The tropism of *Y.
pseudotuberculosis to the vascular system may be explained by the possibility of the bacteria to harbour the *ypm* gene involved in microvascular disease like in Kawasaki syndrome (11).

Consequently, it seemed interesting to find this superantigen marker in this case. Unfortunately, the test was negative for our strain: the serotype O:1 is rarely positive for the *ypm* marker (6). The analysis of the *Y. pseudotuberculosis* genomes (4 completed, 3 in progress) did not revealed the presence of a gene encoding a superantigen-related toxin other than *ypmA*. Then, it is highly unlikely that the strain isolated in this study carry another version of the *Y. pseudotuberculosis* superantigenic toxin. Physicians and microbiologists should have a particular attention to *Y. pseudotuberculosis* having a predilection for vascular tissue. As our patient did not support the resection of the vascular materiel, a long antimicrobial treatment by fluoroquinolone was followed and seemed to be efficient since a good permeability of the prosthesis has been noted without inflammatory signs for 8 months.
REFERENCES


FIGURE 1

<table>
<thead>
<tr>
<th>ypmA/C</th>
<th>ypmB</th>
<th>yptb3405</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

bp

1000 800 600 400

196 197 198
Legend Figure 1: PCR-detection of the *ypm* superantigen genes in *Y. pseudotuberculosis* strains. 1. Strain AH (*ypmA*+); 2. Strain 487/90 (*ypmB*+); 3. Strain 2777 (non superantigenic); 4. Strain isolated in this study. Yptb3405 is an open reading frame conserved in *Y. pseudotuberculosis* species. PCR conditions have been described previously (3).