Case Report

Recurrent infective endocarditis due to *Aggregatibacter actinomycetemcomitans*: reinfection or relapse?Anais Potron,^{1,2} Jean-Luc Mainardi,^{2,3,4} Isabelle Podglajen,^{2,3,4} Fabienne Meunier,¹ Marie-José Sanson-le Pors^{1,5} and Béatrice Berçot^{1,5}

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Aggregatibacter actinomycetemcomitans is commonly part of the normal microflora of the human upper respiratory tract. It has been implicated in periodontal disease and various infections, particularly endocarditis. We report here what we believe to be the first case of recurrent infective endocarditis due to *A. actinomycetemcomitans* in a 44-year-old woman occurring 5 years after the initial episode. Genomic analysis proved that the strains were closely related. Despite efficient antibiotic treatment, surgery was necessary for recovery.

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Case report

A 44-year-old woman (patient KHA) was admitted to our hospital for persistent fever in February 2005. Her medical history was remarkable for a reconstructive surgery of rheumatic mitral valvulopathy in 1982, followed by mitral valve replacement with a Sorin mechanical prosthesis and tricuspid valvuloplasty in 1993. In February 2000, she presented a first episode of infective endocarditis (IE). Even though no vegetations were evidenced by transparietal and repeated transoesophageal echocardiograms, the diagnosis of definite endocarditis was secured by the identification of *Aggregatibacter actinomycetemcomitans* from three blood cultures using the modified Duke's criteria (Li *et al.*, 2000). She was successfully treated with a combination of ciprofloxacin 750 mg twice daily and ceftriaxone 2 g once daily for a duration of 6 weeks. In 2003, aortic valve replacement with a mechanical prosthesis was required for calcific aortic stenosis. Microbiological culture of the aortic valve was negative.

In 2005, physical examination yielded a temperature of 38.2 °C, blood pressure of 99/57 mmHg and a pulse of 93 beats min⁻¹. Cardiovascular examination revealed a 3/6 mitral systolic murmur. Laboratory data showed anaemia

with a haemoglobin level of 8.2 g dl⁻¹, a white blood cell count of 6.4 × 10⁹ l⁻¹ and a C-reactive protein level of 147 mg l⁻¹ (normal level <10 mg l⁻¹). Transthoracic echocardiography evidenced a mobile vegetation on the mitral valve ring. Six out of 11 sets of blood cultures were positive and yielded a Gram-negative rod, identified as *A. actinomycetemcomitans* by phenotypic and genetic methods using *rrs* gene sequencing (Fihman *et al.*, 2007). The MICs of amoxicillin, ceftriaxone and ciprofloxacin determined by Etest (AB BIODISK) were 1, 0.032 and 0.023 mg l⁻¹, respectively. Antibiotic therapy was initiated by intravenous ceftriaxone 4 g once a day and gentamicin 90 mg twice daily. Aortic and mitral valve replacements were required 3 weeks later because transoesophageal echocardiography revealed persistence of the mitral vegetation and two aortic abscesses associated with disinsertion of the aortic valve. Under antibiotic treatment, the detection of bacteria in cardiac tissues using the 16S rRNA gene (Podglajen *et al.*, 2003) remained negative. After surgery, the aminoglycoside was switched to oral ciprofloxacin 750 mg twice daily associated with ceftriaxone for 1 month followed by ciprofloxacin alone for 1 month further. Clinical improvement under this regimen was evident within 10 days with apyrexia. C-reactive protein and haemoglobin levels were within normal limits after the 2 month course of antimicrobial therapy.

The genetic patterns of three isolates of *A. actinomycetemcomitans* from patient KHA [two isolates from 2000

Abbreviation: IE, infective endocarditis.

The GenBank/EMBL/DDBJ accession numbers for the sequences for *A. actinomycetemcomitans* isolate LRB2000-1 are HM630293–HM630301.

Fig. 1. PFGE patterns after *Xho*I restriction of chromosomal DNA from *A. actinomycetemcomitans* isolates. Lanes 1 and 2, strains isolated from blood cultures from patient KHA from the first IE episode in 2000 (lane 1, isolate LRB2000-1; lane 2, isolate LRB2000-2); lane 3, strain isolated from blood cultures from patient KHA from the second IE episode in 2005 (isolate LRB2005-1). Lanes 4–6, other non-linked clinical strains of *A. actinomycetemcomitans*. M, Molecular mass marker.

(LRB2000-1 and LRB2000-2) and one isolate from 2005 (LRB2005-1)] and three other non-linked clinical isolates of *A. actinomycetemcomitans* were compared by PFGE using the restriction enzyme *Xho*I, which has been shown to have excellent discriminatory power amongst *A. actinomycetemcomitans* isolates (Matsuda *et al.*, 2002). According to Tenover's criteria (Tenover *et al.*, 1995), the strains isolated in 2000 and 2005 were thus closely related since similar genetic patterns with only one band difference

were observed (Fig. 1). In addition to the PFGE analysis, the sequence types of the *A. actinomycetemcomitans* isolates were determined by multilocus sequence typing (Haubek *et al.*, 2007). An internal fragment of six housekeeping genes, *pgi* (glucose-6-phosphate isomerase), *recA* (RecA protein), *adk* (adenylate kinase), *frdB* (fumarate reductase), *atpG* (γ -subunit of ATP synthase) and *mdh* (malate dehydrogenase), and two pseudogenes, *hbpA* (haemoglobin-binding protein gene) and *thpA* (transferring-binding protein), were amplified and sequenced using the multilocus sequence typing method previously described (Haubek *et al.*, 2007). The sequences were compiled and aligned using the BioEdit v5.0.9 software (Hall, 1999). Levels of similarity (1-distance) were calculated by maximum-likelihood distance using DNADIST (PHYLIP). The identity matrix for the eight studied gene nucleotide sequences from *A. actinomycetemcomitans* isolates is displayed in Table 1 and compared with those obtained from the complete genome sequence of the *A. actinomycetemcomitans* HK1651 isolate (Haubek *et al.*, 2007). The sequence types were exactly the same for the three isolates from patient KHA (no mutations were evidenced in the eight studied genes) whereas the similarity matrix identity between the other *A. actinomycetemcomitans* isolates varied from 0.975 to 0.999 (Table 1).

Discussion

Aggregatibacter (Actinobacillus) actinomycetemcomitans belongs to the HACEK group of bacteria, along with *Haemophilus* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species (Das *et al.*, 1997). These Gram-negative bacteria are frequently found as colonizers of the oral cavity and grow slowly (Zamboni, 1985). They are responsible for 3 % of all cases of IE (Das *et al.*, 1997), and, among them, *A. actinomycetemcomitans* is the most frequently involved. Recurrent IE due to the same micro-organism is rare (approx. 3 % of IE cases). A time-based

Table 1. Similarity matrix of the sequence types of *A. actinomycetemcomitans* isolates

Nucleotide sequences to a length of 4109 bp of the housekeeping genes *adk* (559 bp), *atpG* (494 bp), *frdB* (506 bp), *hbpA1* (439 bp), *hbpA2* (329 bp), *mdh* (434 bp), *pgi* (521 bp), *recA* (490 bp) and *thpA* (339 bp) were aligned by using the BioEdit program. The GenBank accession numbers for the *A. actinomycetemcomitans* isolate HK1651 genes are EF142164.1, EF142218.1, EF142336.1, EF142489.1, EF142408.1, EF142568.1, EF142653.1, EF142768.1 and EF142817.1, and those for the KHA isolate LRB2000-1 genes are HM630293, HM630294, HM630295, HM630296, HM630297, HM630298, HM630299, HM630300 and HM630301, respectively.

	KHA isolate LRB2000-1	KHA isolate LRB2000-1	KHA isolate LRB2005-1	BRE isolate	BOU isolate	MRA isolate	HK1651 isolate
KHA isolate LRB2000-1	1.000	—	—	—	—	—	—
KHA isolate LRB2000-1	1.000	—	—	—	—	—	—
KHA isolate LRB2005-1	1.000	1.000	1.000	—	—	—	—
BRE isolate	0.976	0.976	0.976	1.000	—	—	—
BOU isolate	0.976	0.976	0.976	0.999	1.000	—	—
MRA isolate	0.999	0.999	0.999	0.976	0.976	1.000	—
HK1651 isolate	0.975	0.975	0.975	0.998	0.998	0.975	1.000

clinical criterion, defined as the delay in recurrence of less or more than 6 months, is usually used to distinguish relapse from reinfection (Mansur *et al.*, 2001). To our knowledge, four cases of endocarditis clinically considered as reinfections but classified as relapses using molecular methods have been described. They involved *Staphylococcus aureus* (Chu *et al.*, 2005), *Propionibacterium acnes* (Chu *et al.*, 2005), *Pseudomonas stutzeri* (Grimaldi *et al.*, 2009) and *Streptococcus gallolyticus* (Mühlemann *et al.*, 1999) and occurred 9, 24, 48 and 96 months after the initial episode, respectively. Here, we report what we believe to be the first case of recurrent IE involving *A. actinomycetemcomitans*, which occurred after a 5 year period. Using the modified Duke's criteria of endocarditis (Li *et al.*, 2000), the patient was classified as having definite IE in 2000 (one major and three minor criteria) and in 2005 (two major and two minor criteria). Considering the fact that the genetic methods demonstrated the involvement of identical or closely related isolates in the two IE episodes, this raises the question of how this bacterium has been able to persist for 5 years. We cannot reliably determine whether the patient had a persistent cardiac focus or a persistent unknown abscess, which led to IE on two separate occasions. However, when cardiac surgery was performed in 2003, cultures of the aortic valve were negative after an incubation period of 30 days, and clinical examination of the mitral valve was normal during the surgery. It therefore appears more conceivable that these two episodes of endocarditis could have resulted from a chronic dental focus where the same *A. actinomycetemcomitans* isolate persisted. This hypothesis is supported by the fact that a periodontal disease was highlighted in the second episode and that *A. actinomycetemcomitans* is usually considered to be a major pathogen in the aetiology of chronic periodontitis. Unfortunately, we cannot confirm this hypothesis since detection of *A. actinomycetemcomitans* in the patient's oropharyngeal flora was not performed. In fact, oropharyngeal samples are never taken in clinical practice in the case of endocarditis due to streptococci or HACEK bacteria.

Molecular methods offer advantages for epidemiological investigation and are able to determine identity between clinical strains. Our case report highlights that the identification of two similar isolates can be related to either a relapse from a persistent cardiac focus or a reinfection from a persistent unknown abscess, which must be identified and removed.

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