Actinomyces neuii sp. neuii associated with periprosthetic infection in total hip arthroplasty as causative agent

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Actinomyces neuii has until now not been described as a pathogen associated with periprosthetic infection in total joint replacement. The case presented here suggests that A. neuii ssp. neuii is a causative pathogen. The discussion and review of the literature indicates the impact that detection of Actinomyces sp. could have.

Case report
A 78-year old woman suffering from serious coxarthrosis underwent total hip arthroplasty (THR) on her right hip in July 2006 without complications (cement-free acetabular and cemented femoral component). In June 2008 she consulted the orthopaedic outpatient department of St. Josef hospital in Wuppertal (Germany) with clinical signs of local pain in the replaced hip, radiating into the femur. No further relevant symptoms were elicited. A peripheral haematological white blood cell count revealed 11.8 x 10^9/liter leucocytes and C-reactive protein was measured at 3.2 mg/dl. Triple-phase bone scintigraphy with 99m-Tc showed an increased uptake of radioisotopes in all the phases, and in the radiographs a continuous radiolucent line at the bone-cement interface was visible. Joint aspiration revealed purulent synovial fluid. Microscopic analysis identified a mass of polymorphnuclear granulocytes, but no micro-organisms could be detected. No antimicrobial treatment had been administered four weeks prior to aspiration of synovial fluid. The day after admission to hospital the patient underwent revision surgery for removal of the prosthesis. Girdlestone arthroplasty was performed with implantation of a spacer made of antibiotic loaded bone cement. Intraoperatively, several biopsies of periprosthetic tissue were taken for
microbiological investigation. Subsequently, postoperative empirical intravenous antibiotic treatment was commenced with Cefazolin (2 gm tid) and Rifampicin (450 mg bid). Histopathological examination of the tissue samples showed a high degree of infiltration with inflammatory cells without any sign of malignancy.

The specimens, synovial fluid and intraoperative biopsies were subjected to culture and both the preoperative joint fluid as well as the intraoperative tissue showed growth of slow-growing gram-positive rods in pure culture. These were identified as *Actinomyces neuii* ssp. *neuii* by their biochemical properties and sequencing of bacterial 16S rRNA.

After seven days of empirical therapy the antibiotics were changed to penicillin G (5 million IU qid) according to the susceptibility pattern of the pathogen found.

The antimicrobial therapy was continued after procurement of biopsies for microbiological investigation taken at the time of re-implantation of a cemented total hip replacement.

Locally, antibiotic loaded bone cement with 2 g Vancomycin, 1 g clindamycin and 1 g gentamicin per 40 g PMMA bone cement was used for fixation of the prosthesis. The intravenous therapy was discontinued two weeks later and substituted by amoxicillin (1 gm tid) administered orally for another four weeks. Since the latest surgery cultures have revealed no evidence of micro-organisms after two weeks of incubation.

The postoperative radiological control showed a well-fixed implant, and the patient was discharged from hospital without any sign of local infection two weeks after re-implantation. The patient has not been seen for follow-up.

The genus *Actinomyces* contains several anaerobic and aero-tolerant gram-positive non-spore-forming organisms with variable morphology. Several species belong to the typical commensal of the oropharyngeal surface.

In 1994 the CDC group 1 and group 1-like coryneforme bacteria were reclassified as the genus of *Actinomyces* on the basis of 16S rRNA molecular and biochemical analysis. The new species was proposed by Funke et al. and named *Actinomyces neuii* sp. nov., containing *Actinomyces neuii* subsp. *neuii* for CDC group 1 and *Actinomyces neuii* subsp. *anitratus* for CDC group 1-like coryneforme bacteria (4, 5).

The morphology of the cultivated strain provided small whitish colonies with the best growth on blood agar supplemented with 5% sheep blood at 37 °C in a 5 % CO₂ atmosphere but could also be isolated from anaerobically incubated plates after 72 hours of incubation. Gram staining revealed short Gram-positive rods without branching filaments. In initial screening reactions the presence of katalase, and a positive CAMP-test was observed. A set of
biochemical tests produced the following results: reduction of nitrate, (key reaction for
differentiation from *Actinomyces neuii subsp. anitratus*), no hydrolysis of urea, esculin and
gelatine, activity of α-glucosidase and β-galactosidase, no activity of N-acetyl-β-
glucosaminidase, acid production from glucose, ribose, xylose, and mannose, but acid was not
produced from maltose, lactose, sucrose and glycogen. The reactions were generated by the
commercial identification kit API Coryne (Version 3.0) (BioMérieux, Marcy l’Etoile, France)
that had a correspondence of 99% with *Actinomyces neuii subsp. neuii* (Profil-Code-number
3410714). The molecular 16S rRNA partial gene sequence analysis showed a similarity of
100% (879/879 nucleotides) compared to the reference sequence of the type strain
*Actinomyces neuii subsp. neuii* (accession number Z 33613/GI 1729442 NCBI).

Due to the slow growth, the MIC were determined by the E test (Oxoid, Basingstoke,
Hampshire, United Kingdom) and carried out on Mueller-Hinton agar supplemented with 5%
sheep blood with an inoculum corresponding to McFarland standard 0.5. The following
antimicrobial agents were chosen and revealed low MIC to penicillin G, 0.008 µg/ml,
ampicillin, < 0.015 µg/ml; clindamycin, < 0.016 µg/ml; levofloxacin, 0.5µg/ml; vancomycin,
0.25µg/ml; Rifampicin, 0.003 µg/ml and the MIC of gentamicin was 1.0µg/ml

**Discussion**

The literature contains several cases of isolates with *Actinomyces neuii subsp. neuii*. After the
species was renamed in 1994 the majority were isolated from abscesses and associated with
mixed anaerobic flora (6). During recent years the species was cultured from various clinical
specimens, and was reported to be the causative organism in infected mammary prosthesis
(1), chronic osteomyelitis (13), endophthalmitis (7), infective endocarditis (2), and
ventriculoperitoneal shunt infection (15).

In periprosthetic joint infections coryneform bacteria and *Actinomyces spp.* do not belong to
the commonly cultured micro-organisms. Many species are regarded as low-virulent bacteria
that are part of normal mucocutaneous flora. Therefore, mucocutaneous lesions are probably
the main cause for local (dental-oral) and remote infections.

Since implant infections can occur during the entire lifetime of the joint replacement,
clinicians are eager to characterize the origin of infection. Depending on the onset of
symptoms after primary implantation, prosthetic joint infections are divided into early,
delayed and late infections. Early and delayed infections are mainly regarded as having been
acquired during replacement, whereas late infections are predominantly caused by
haematogenous seeding (11). As the period of late infection varies up to one year depending
on the classification scheme used, it is limited in its significance. In our case two years had
passed between primary implantation and revision of the total hip prosthesis and in any
scheme this would be considered as a late infection. According to the physiological oral colonization of coryneforme bacteria, oral-pharyngeal lesions are an important risk factor for infections. The patient has had a complete dental prosthesis for many years, so that a dental infection could be excluded as possible source of bacteraemia. Other frequent sources like skin, respiratory tract or urinary tract infection could not be found in the patient’s history. Therefore, due to the natural habitat of Actinomyces spp., it is difficult to estimate the clinical significance of isolates recovered from human specimens, but in this case it is strengthened by the findings of the same micro-organism in multiple specimens from preoperative synovial fluid and intraoperative periprosthetic tissue in pure culture, through the presence of inflammation on histopathological examination, and the good clinical response to the high dose therapy with penicillin G.

A review of the literature indicates that coryneforme bacteria (14) and different recognized strains of Actinomyces spp. (9) such as A. naeslundii (10, 16), A. israelii (8, 12, 17) and A. viscosus (3) have to be considered as causative agents of periprosthetic joint infections. In one case it was associated with an apparent focus after dental extraction, and in another the micro-organism was isolated from an intravenous drug user. In all other cases the origin of infection remained unclear. Nevertheless, these reports underline that coryneforme bacteria cultured from synovial fluid or periprosthetic tissue should not simply be regarded as contamination of the specimen, but have to be identified to the species level, because they could be of clinical significance. In five of the six publications mentioned above reference was made to antibiotic therapy, and the patients were successfully treated either with penicillin, as in our case, or with a cephalosporine. We are reporting on a new case and to the authors’ knowledge, after its renaming in 1994, Actinomyces neuii subsp. neuii has so far not been described as an etiologic pathogen of prosthetic hip infection.

References:


