Vertebral Osteomyelitis Associated with *Granulicatella Adiacens*

Ryuki FUKUDA, Masayuki OKI, Akihiro UEDA, Hidetaka YANAGI, Masamichi KOMATSU, Masahito ITOH, Akiko OKA, Makoto NISHINA, Hideki OZAWA and Atsushi TAKAGI

Department of General Internal Medicine, Tokai University School of Medicine

(Received June 16, 2010; Accepted August 26, 2010)

Granulicatella adiacens **is a nutritionally variant streptococci. Only 3 cases of vertebral osteomyelitis due to these microorganisms have been reported. We experienced a 73-year-old male who consulted us due to fever and back pain of about 1-month duration. On examination, a presystolic murmur was heard in the apical region. Echocardiography showed prolapse of the mitral valve, but no vegetation was observed. MRI revealed osteomyelitis of lumbar vertebrae. As *G. adiacens* was detected in blood culture, it was determined as the cause of vertebral osteomyelitis, and combination antibiotics therapy was started. The condition improved, the patient underwent valvoplasty, and no trace of infective endocarditis was noted in the resected valve. All the previous cases had infection caused by *G. adiacens* and complicated with infective endocarditis. This is the first case without infective endocarditis. Vertebral osteomyelitis due to NVS is very rare. Since nutritionally variant streptococci do not grow in common culture media, and since the sensitivity of isolation by standard conventional biochemical methods is low, the condition may be misdiagnosed as blood-culture-negative vertebral osteomyelitis. Therefore, the possibility of nutritionally variant streptococci infection should be considered if a patient with vertebral osteomyelitis shows a positive Gram stain but negative blood cultures.**

Key words: Vertebral Osteomyelitis, Nutritionally Variant Streptococci, *Granulicatella adiacens*

INTRODUCTION

Vertebral osteomyelitis is frequently caused by hematogenic dissemination. The primary lesions of infection, which are identified in about half of patients, are often infective endocarditis (IE), urinary tract infection, and skin/soft tissue infection [1]. The causative microorganism is most frequently *Staphylococcus aureus*, followed by *Escherichia coli* [2]. We encountered a rare case of vertebral osteomyelitis caused by *Granulicatella adiacens* (*G. adiacens*), which belonged to a species of nutritionally variant streptococci (NVS).

CASE REPORT

A 73-year-old male was hospitalized due to fever and back pain in May 2009. The patient had been well until he experienced malaise and back pain about 1 month before admission and showed occasional episodes of fever at 37–38℃ from about 20 days before admission. Since the symptoms persisted, he consulted a local physician 15 days before admission, when a heart murmur was heard on physical examination, no abnormality was noted on chest radiography, but blood tests showed an abnormal elevation of the CRP to 7.18 mg/dL. The patient did not respond to treatment with loxoprofen sodium from 15 days before admission and Cefcapene from 4 days before admission, and was referred to our hospital.

The patient did not recently have history of dental treatment, upper respiratory infection, urogenital infection, or gastrointestinal infection. His history included surgery for appendicitis at the age of 26 years and hypertension and hyperlipidemia, both of which had been controlled well for 1 year prior to the present episode. On examination, the temperature was 36.4℃, the blood pressure 96/60 mmHg, the pulse regular at 68 beats per minute. A Levine III presystolic murmur was heard in the apical region, but no skin abnormality such as a Janeway lesion and Osler node, no Roth's spot was noted. There was no evidence of gingivitis, CVA tenderness or spinal percussion tenderness. On urinalysis, no abnormality was noted such as the presence of protein, glucose, blood, or sediment. The white blood cell count was 7,800/μL, hemoglobin level 12.6 g/dL, platelet count 26.6 × 10^4/μL, ESR 87 mm/h, C-reactive protein 9.59 mg/dL, AST 21 U/L, ALT 34 U/L, LDH 150 U/L, ALP 341 U/L, and gamma-GTP 71 U/L. Serum rheumatoid factor was negative. Gram-positive cocci were detected in all 4 sets of blood culture performed during the first 2 days after admission. However, no bacterial growth was observed in sheep blood, chocolate, or Gifu anaerobic medium. When blood samples were cultured in Brucella agar with hemin and vitamin K (Brucella HK), in consideration of the possibility of NVS, colony formation was noted. The microorganism that formed colonies was identified as *G. adiacens* on examination of the biochemical methods using API 20 STREP (bio-Merieux Vitek, Inc., Hazelwood, Mo). The genes of the microorganism was identified as *G. adiacens* (GenBank accession number D50540.1) by 16S rRNA sequencing using 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGCTACCTTGTTACGACTT-3') as polymerase chain reaction primers. This bacterial strain showed sensitiv-

Ryuki FUKUDA, Department of General Internal Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1143, Japan
Tel: +81-463-93-1121 Fax: +81-463-91-9372 E-mail: f.ryuki@is.icc.u-tokai.ac.jp

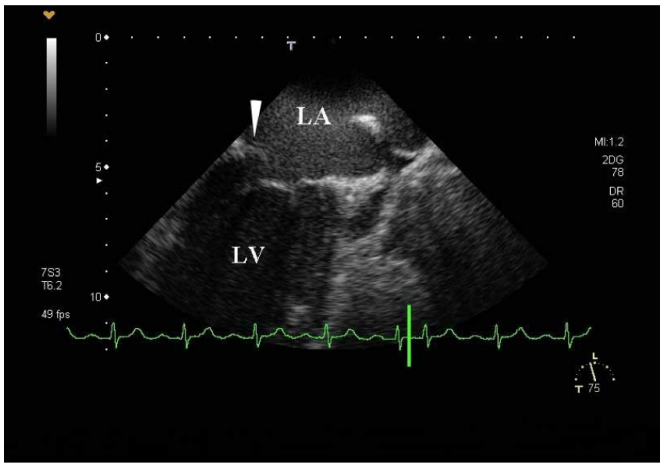


Fig. 1 Transesophageal echocardiography findings. LA: Left atrium. LV: Left ventricle. The triangle indicates the posterior cusp of the mitral valve prolapsed into the left atrium.

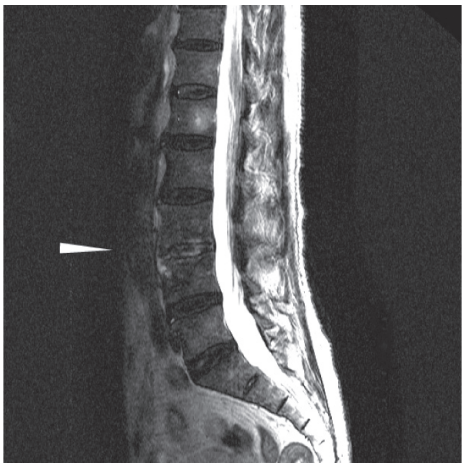
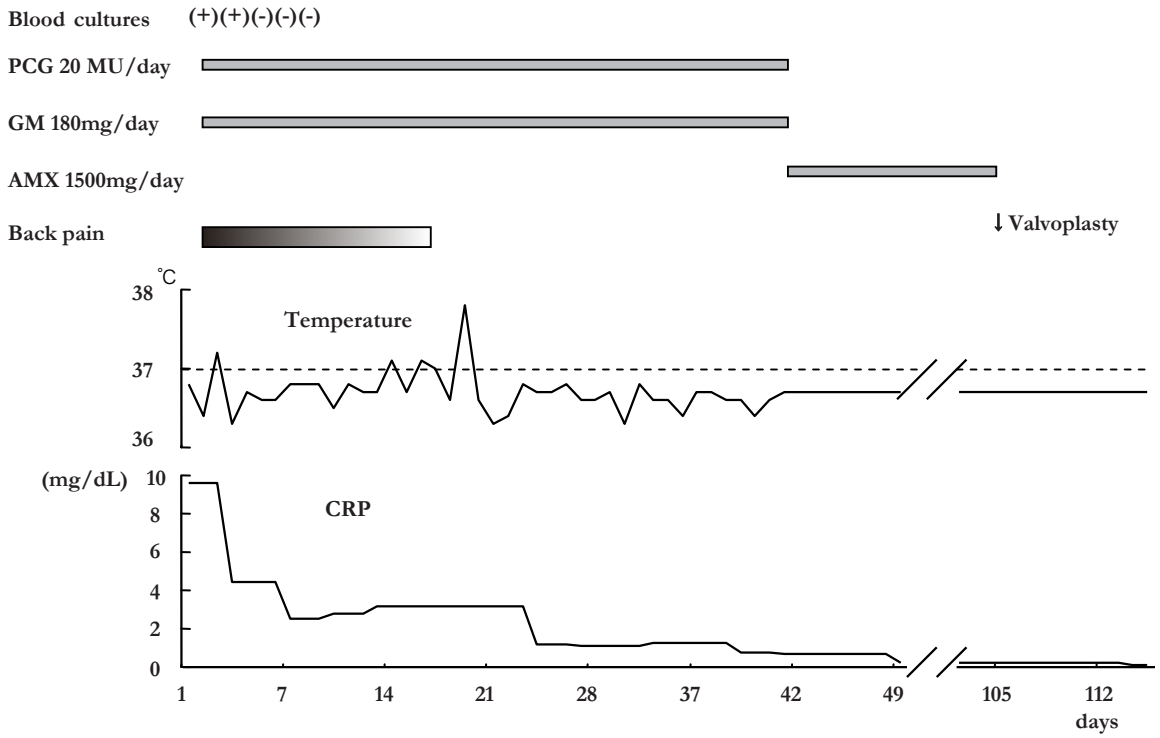


Fig. 2 T2-weighted MRI of the lumbar region. The triangle indicates discitis at L3/L4 and bilateral vertebral osteomyelitis of L3 and L4.



Pen G: Penicillin G. GEN : Gentamicin. AMX: Amoxicillin.

Fig. 3 Clinical course and changes in the body temperature and major examination findings. CRP: C-reactive protein. GM: Gentamicin. PCG: Penicillin G. AMX: Amoxicillin. MU: Million units.

ity to penicillin G. Transthoracic and transesophageal echocardiography revealed prolapse of the posterior cusp of the mitral valve and grade III mitral regurgitation, but no vegetation (Fig. 1). Lumbar MRI showed vertebral osteomyelitis at L3 and L4 and discitis at L3/L4 on T2-weighted imaging (Fig. 2). Head MRI showed no intracranial hemorrhagic lesion. A diagnosis of vertebral osteomyelitis and discitis due to *G. adiacens* was made, and the administration of penicillin G at 20 million U/day and gentamicin at 150 mg/day was started. The temperature had dropped below 37.0 ˚C, except for period of intravascular catheter-related infection during 15 to 20 days after admission (Fig. 3). No bacteria were detected on blood culture 2 or more days after the beginning of treatment, CRP tended to decrease, and back pain disappeared 13 days after the start of treatment. The antibiotics administered over 4 weeks. Thereafter, the treatment was changed to administration of amoxicillin at 1,500 mg/day. Mitral valvoplasty was performed 107 days after treatment initiation to resolve mitral valve prolapse. Gross examination showed no vegetation or inflammation in the

Table 1

Sex	Age	Symptom	Past history	Vegetation	Treatment	Outcome	References **No**
mal	45	back pain and fever	ND	Mitral valve	PCG, GM, CLDM	Cured	4)
mal	50	back pain and fever	ND	Aortic valve	PCG, GM, CTRX	Cured	4)
mal	68	back pain and fever	DM, AV block	Pacemaker lead	PCG, GM, RFP	Cured	5)
mal*	73	back pain and fever	HT, HL	not detected	PCG, GM,AMX	Cured	Our case

*Patient in this report, ND: not described, DM: diabetes mellitus, AV block: atrioventricular conduction block, HT: hypertension, HL: hyperlipidemia, PCG: Penicillin G, GM: Gentamicin, CLDM: Clindamycin, CTRX: Ceftriaxone, RFP: Rifampicin, AMX: Amoxicillin

mitral valve, prolapse of about 15 mm of the posterior cusp, and rupture of one tendinous cord. On histological examination, no bacterial plaque, fibrosis, nor acute or chronic inflammatory change was observed in the mitral valve, but myxomatous degeneration was noted. No bacteria grew in cultures of the resected mitral valve. There had been no evidence of a recurring sign such as fever, back pain, or increase in CRP 9 months after finished the antibiotics.

DISCUSSION

NVS are Gram-positive cocci first identified in 1961 from a patient with IE. They characteristically exhibit the satellite phenomenon around other bacteria. In 2000, NVS were classified into *Abiotrophia defectiva*, *G. adiacens*, *G. elegans*, and *G. balaenopterae* by 16S rRNA sequencing [3].

NVS are normal flora of the upper respiratory, urogenital, and gastrointestinal tracts of humans and are isolated as causes of various infections including IE, otitis media, eye infections, pancreatic abscess, artificial joint infection, intramammary foreign body infection, and central nervous system infections. Vertebral osteomyelitis is a rare condition, with only 3 cases having been reported to our knowledge [4, 5]. In all 3 cases, vertebral osteomyelitis was caused by *G. adiacens* and complicated by IE, and vegetation was observed on the valve or the pacemaker leads (Table 1). Since vertebral osteomyelitis is frequently caused by hematogenous dissemination, it is related to IE, and 12% of the primary foci of vertebral osteomyelitis are IE [2]. Furthermore, 4.6–19% of the patients with IE have vertebral osteomyelitis [6, 7]. Therefore, the complication of vertebral osteomyelitis by IE in all 3 cases does not contradict previous reports.

On the other hand, NVS are isolated as the causative microorganisms of IE in 5–6% of patients [8]. Compared with the frequency of the complication of IE by vertebral osteomyelitis, reports of vertebral osteomyelitis due to NVS are extremely rare. One of the reasons may be the growth characteristics of NVS, which require L-cysteine and pyridoxal to grow. Therefore, they grow only in media containing L-cysteine or pyridoxal such as Brucella HK. Furthermore, responses to Gram staining vary with the nutritional environment. NVS are detected as Gram-positive cocci or coccobacilli when grown in an optimal environment, but otherwise exhibit various morphological features [9]. The second reason is the difficulty in identifying NVS. API 20 STREP system apply the criteria as follows: excellent and good identification rate are $\geqq$ 99.9% and $\geqq$ 90, respectively, T index which is close to 1 indicates that identical bacteria are less deviation from a typical profile number of a bacterial species in database. The identification rate and T index using the system are 99.8% and 0.92, respectively, for *G. adiacens*, indicating the practicality this examination [10]. However, the sensitivity for identification of *G. adiacens* of 86% has been reported to be insufficient in comparison with 16S rRNA sequenceing [11]. Using commercial automated systems such as API 20 STREP, the false-negative rate is high, and NVS may not be identified even if isolated. Because of mentioned the above characteristics, NVS have been regarded historically as a cause of culture-negative IE. Blood cultures are negative in 42% of patients with vertebral osteomyelitis [2], and such patients may be classified as culture-negative cases similarly to culture-negative IE. 16S rRNA sequenceing is the technique for identifying NVS when API 20 STREP data suggests false-negative.

The case presented here differs from the previous report in that vertebral osteomyelitis probably was not complicated by IE. The present patient did not fulfill the Duke criteria. Vegetation of IE due to NVS is large, being about 10 mm in diameter, and frequently result in embolization [12], but the present patient showed no vegetation or symptoms of embolization throughout the course. Moreover, while histological changes such as fibrosis, thickening, chronic inflammation, acute inflammation, and the presence of bacteria are noted in 90% of patients with IE even after the end of standard antibiotic treatment [13], no such change was demonstrated in our patient, who exhibited the myxedema-like changes observed in mitral prolapse syndrome. Detection of PCR products from *G. adiacens* in the resected valve may have been useful for more definite diagnosis or exclusion of IE [14]. Unfortunately, we could not conduct the method. However, European guideline [15] and textbook [16] have pointed out that molecular biology techniques such as PCR should be only performed in patients with negative blood cultures who undergo valve surgery. Therefore, this is the first case of vertebral osteomyelitis due to *G. adiacens*, who is not complicated with IE in ordinary clinical practice.

Concerning treatment, there is discrepancy between the *in vitro* sensitivity to antibiotics and clinical outcome in patients with IE due to NVS. It has been reported that bacteriological failure occurs in 41% of patients, that post-treatment recurrence is observed in 17%, and that the prognosis is poor [17]. For these reasons, long-term multiple drug combination therapy such as the administration of penicillin and gentamicin for 4–6 weeks has been recommended [18]. Treatments for vertebral osteomyelitis have varied: Penicillin and gentamicin for 4 weeks, followed by penicillin alone for 2

weeks and then clindamycin for 2 weeks, or penicillin and gentamicin for 2 weeks, followed by ceftriaxone [4]; and penicillin, gentamicin, and rifampine simultaneously [5] (Table 1). However, all reported cases including ours followed an uneventful course without bacteriological failure or recurrence.

In summary, vertebral osteomyelitis due to NVS may be misdiagnosed as blood-culture-negative vertebral osteomyelitis. If a patient with vertebral osteomyelitis shows positive Gram staining and negative blood cultures, NVS infection should be considered, and culturing in media containing L-cysteine and pyridoxal should be carried out. If bacteria have been isolated, they should be biochemically identified using the API 20 STREP system and confirmation by 16S rRNA sequencing might be considered.

ACKNOWLEDGEMENT

This case report was supported by Department of Laboratory Medicine, Tokai University School of Medicine. We want to thank for their cooperation in identification of *Granulicatella adiacens*.

REFERENCES

1) Zimmerli. Clinical practice. Vertebral osteomyelitis. N Engl J Med. 2010; 362: 1022–9.
2) Mylona E, Samarkos M, Kakalou E, Fanourgiakis P, Skoutelis A. Pyogenic vertebral ostemomyelitis: a systematic review of clinical characteristics. Semin Arthritis Rheum 2009; 39: 10–7.
3) Collins MD, Lawson PA. The genus Abiotrophia (Kawamura *et al.*) is not monophyletic: proposal of Granulicatella gen. nov., Granulicatella adiacens comb. nov., Granulicatella elegans comb. nov. and Granulicatella balaenopterae comb. nov. Int J Syst Evol Microbiol. 2000; 50: 365–9.
4) Heath CH, Bowen SF, McCarthy, JS, Dwyer B. Vertebral osteomyelitis and discitis associated with Abiotrophia adiacens (nutritionally variant streptococcus) infection. Aust N Z J Med. 1998; 28: 663.
5) Rosenthal O, Woywodt A, Kirschner P, Haller H. Vertebral osteomyelitis and endocarditis of a pacemaker lead due to Granulicatella (Abiotrophia) adiacens. Infection. 2002; 30: 317–9.
6) Pigrau C, Almirante B, Flores X, Falco V, Rodríguez D, Gasser I, *et al*. A. Spontaneous pyogenic vertebral osteomyelitis and endocarditis: incidence, risk factors, and outcome. Am J Med. 2005; 118: 1287. e17–1287. e24.
7) Tamura K. Clinical characteristics of infective endocarditis with vertebral osteomyelitis. J Infect Chemother. 2010; 10: 1007/s10156–010–0046–8.
8) Roberts RB, Krieger AG, Schiller NL, Gross KC. Viridans streptococcal endocarditis: the role of various species, including pyridoxal-dependent streptococci. Rev Infect Dis. 1979; 1: 955–66.
9) Ruoff KL. Nutritionally variant streptococci. Clin Microbiol Rev. 1991; 4: 184–90.
10) Pompei R, Caredda E, Piras V, Serra C, Pintus L. Production of bacteriolytic activity in the oral cavity by nutritionally variant streptococci. J Clin Microbiol. 1990; 28: 1623–7.
11) Woo PC, Fung AM, Lau SK, Chan BY, Chiu SK, Teng JL, *et al*. Granulicatella adiacens and Abiotrophia defectiva bacteraemia characterized by 16S rRNA gene sequencing. J Med Microbiol. 2003; 52: 137–40.
12) Lin CH, Hsu RB. Infective endocarditis caused by nutritionally variant streptococci. Am J Med Sci. 2007; 334: 235–9.
13) Morris AJ, Drinkovic D, Pottumarthy S, Strickett MG, MacCulloch D, Lambie N, *et al*. Gram stain, culture, and histopathological examination findings for heart valves removed because of infective endocarditis. Clin Infect Dis. 2003; 36: 697–704.
14) Rovery C, Greub G, Lepidi H, Casalta JP, Habib G, Collart F, *et al*. PCR detection of bacteria on cardiac valves of patients with treated bacterial endocarditis. J. Clin. Microbiol. 2005; 43: 163–7.
15) Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology; European Society of Clinical Microbiology and Infectious Diseases; International Society of Chemotherapy for Infection and Cancer, Habib G, Hoen B, Tornos P, Thuny F, Prendergast B, Vilacosta I, *et al*;. Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009) 2009; 30: 2369–413.
16) Fowler VG jr, Scheld WM, Bayer AS. Endocarditis and intravascular infections. *In*: Mandell GL, Bennett JE, Dolin R, Eds. Churchill. Principles and Practice of Infectious Disease, 7th ed. Philadelphia: Churchill livingstone, Elsevier, 2010: 1067.
17) Stein DS, Nelson KE. Endocarditis due to nutritionally deficient streptococci: therapeutic dilemma. Rev Infect Dis. 1987; 9: 908–16.
18) Sinner SW, Tunkel AR. Viridans streptococci, groups C and G streptococci, and gemella species. *In*: Mandell GL, Bennett JE, Dolin R, Eds. Churchill. Principles and Practice of Infectious Disease, 7th ed. Philadelphia: Churchill livingstone, Elsevier, 2010: 2673.