Infective Endocarditis Caused by Panton-Valentine Leukocidin-producing Methicillin-susceptible *Staphylococcus aureus* Identified by the Broad-range PCR Method

Hiroshi Nakajima¹, Kaoru Dohi¹, Masaki Tanabe¹, Akiko Nakamura³, Shinji Kanemitsu³, Hideo Wada¹, Norikazu Yamada¹, Tsutomu Nobori⁴, Hideto Shinpo³ and Masaaki Ito¹

**Abstract**

A 76-year-old man was admitted to a community hospital due to a persistent high fever. He became afebrile after the administration of broad-spectrum antibiotics, but developed heart failure due to progressive aortic and mitral valve insufficiency and was transferred to our hospital. Although sequential blood cultures were negative, a broad-range polymerase chain reaction targeting the bacterial 16S-rRNA gene followed by the direct sequencing of whole blood revealed *spa*(+), *mecA*(-) and Panton-Valentine leukocidin (PVL)(+). He was finally diagnosed with infective endocarditis (IE) caused by PVL-producing methicillin-susceptible *Staphylococcus aureus* (MSSA), and underwent cardiac surgery. This is the first reported case of IE due to MSSA producing PVL.

**Key words:** infective endocarditis, polymerase chain reaction, Panton-Valentine leukocidin-producing methicillin-susceptible *Staphylococcus aureus*, heart failure, valve surgery

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**Introduction**

Infective endocarditis (IE) is a severe infection which can seriously damage the heart valves and which may cause other complications. Blood cultures, which have been the gold standard for the detection of pathogens, are often negative, particularly in patients who have received antibiotics. A molecular technique which combines broad-range polymerase chain reaction (br-PCR) amplification and direct sequencing is useful for the detection and identification of pathogens in patients with culture-negative endocarditis (1). Using this method, we identified Panton-Valentine leukocidin (PVL)-producing methicillin-susceptible *Staphylococcus aureus* (MSSA), a very rare causative agent of IE, from the whole blood and resected tissue samples of the heart valves of a patient with negative blood culture results.

**Case Report**

A 76-year-old man was diagnosed with moderate aortic valve regurgitation two years previously. Two months prior to his admission to our hospital he had been admitted to a community hospital due to a high fever, which was over 38.0°C and which persisted for 9 days, despite the provision of oral antibiotics. The patient had been physically active, and had not received any dental treatment nor had he suffered from skin infections for at least 1 year before the onset of symptoms. He had a history of bladder cancer and had undergone follow-up cystoscopy without antimicrobial prophylaxis one month before the onset of symptoms. On admission, a laboratory examination revealed an increased peripheral leukocyte count of 19,800 cells/mm³ and an elevated C-reactive protein (CRP) level of 10.8 mg/dL. Although the patient’s blood cultures were negative, empirical

¹Department of Cardiology and Nephrology, Mie University Graduate School of Medicine, Japan, ²Central Clinical Laboratories, Mie University Hospital, Japan, ³Department of Thoracic and Cardiovascular Surgery, Mie University Graduate School of Medicine, Japan and ⁴Department of Molecular and Laboratory Medicine, Mie University Graduate School of Medicine, Japan

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Correspondence to Dr. Hiroshi Nakajima, hirosh@wc4.so-net.ne.jp
broad-spectrum antibiotic treatment (ceftriaxone, azithromycin, minocycline and levofloxacin) was initiated and he became afebrile within 14 days. The patient was discharged from the hospital with oral levofloxacin. However, he was urgently readmitted to the same hospital 8 days after discharge because of orthopnea, and was diagnosed with acute heart failure. He was intubated for 4 days and treated with furosemide, carperitide, dobutamine and oral antibiotics [clarithromycin (200 mg/day)] via a nasogastric tube. Two weeks later, his heart failure symptoms improved, and he was transferred to our cardiology department in Mie University Hospital to undergo further treatment for heart failure.

At presentation, his temperature was 36.4°C, his blood pressure was 125/46 mmHg and heart rate was 89 beats per minute. A physical examination revealed a grade 4/6 systolic and diastolic murmur on the left sternal border. We did not observe skin lesions or pitting edema. A laboratory examination revealed a peripheral leukocyte count of 6,020 cells/mm³, a CRP level of 1.16 mg/dL and a brain natriuretic peptide (BNP) level of 816.2 pg/mL. A urinalysis revealed proteinuria and hematuria - findings which were suggestive of glomerulonephritis. Chest radiography revealed cardio-megaly, pulmonary congestion and bilateral pleural effusion (Fig. 1). A chest computed tomography scan showed no signs of pneumonia. Transthoracic echocardiography showed a small mobile mass on the anterior leaflet of the mitral valve, which was suggestive of vegetation (Fig. 2A) and transesophageal echocardiography showed the perforation of the non-coronary cusp of the aortic valve (Fig. 2E, F). Both valves had severe regurgitation (Fig. 2C, D). The patient fulfilled one major and three minor Duke criteria (vegetation with valvular destruction, high fever, a history of valvular disease and evidence of glomerulonephritis). He was therefore clinically diagnosed with IE. However, three consecutive sets of blood cultures were negative after 7 days of incubation and it was thus unclear whether the infection was active while the patient was afebrile with non-significant CRP results. The patient was hemodynamically stable and had no sign of active infection. Thus, the heart team planned an elective surgical operation following the provision of maximal medical heart treatment and after thorough risk stratification with multimodality imaging. Two weeks after his admission to hospital without antibiotic treatment, the mobile mass on the anterior leaflet of the mitral valve developed (Fig. 2B); the other clinical conditions remained stable. He was therefore assumed to have active IE, and ceftriaxone (2 g q. day) and gentamycin (60 mg q. 8h) were administered. We also performed a br-PCR targeting the bacterial 16S rRNA gene followed by a direct sequencing using whole blood, which revealed spa(+), mecA(-) and PVL(+) (Table). The patient was therefore clinically diagnosed with active IE caused by PVL-producing MSSA - despite the absence of skin lesions which could lead to MSSA bacteremia and/or endocarditis. He successfully underwent aortic valve replacement with a 23-mm Carpentier-Edwards PERIMOUNT Magna EASE aortic heart valve (Carpentier-Edwards, Irvine, USA), mitral valve plasty with a 30-mm Physio II annuloplasty ring (Carpentier-Edwards) and tricuspid valve plasty with a 30-mm Edwards MC3 tricuspid annuloplasty ring (Carpentier-Edwards). Perforation of the non-coronary aortic valve cusp (Fig. 3) and vegetation of the mitral anterior leaflet were visually confirmed. A pathological assessment showed that the only degenerative changes were those of the mitral valve and aortic valves; however, the br-PCR sequencing of the aortic valve and mitral valve revealed PVL(+)MSSA gene positivity in all tissues. Cefazolin sodium and cefaclor were administered for 2 weeks and 2 months, respectively. The patient was followed up, without complications, at our outpatient department.

**Discussion**

We herein presented the first case of IE caused by PVL-producing MSSA. IE is a serious disease that can damage the heart valves and cause other complications. The diagnosis of IE, which is usually based on the Duke criteria and involves positive results of multiple blood cultures. The identification of the causative pathogen is very important for successful treatment. However, the blood cultures of patients who receive antibiotic treatment can sometimes be negative. The br-PCR method, which overcomes this problem, has recently been reported to be helpful in the diagnosis of IE (1). The method is composed of universal PCR for the 16S rRNA gene, with the subsequent identification of bacteria from positive samples by a sequence analysis of the amplicons. In our case, the patient’s blood cultures were negative due to the administration of oral antibiotics, which were prescribed in the previous hospital despite echocardiographic evidence of the development of a mobile mass and valve destruction (which are strongly suggestive of active IE). We therefore decided to detect and identify the causative bacterium using the br-PCR method and successfully diagnosed the patient with IE due to PVL-producing MSSA. In suspected cases of culture-negative IE, br-PCR tests should be immediately performed because the correct identification of
the causative pathogen can help to determine an appropriate management plan for IE and can lead to better outcomes. In this case, however, the identification of the pathogen by the br-PCR test occurred immediately after the patient’s transfer from the community hospital, at which time it was not possible to avoid surgery because the patient had already developed severe aortic and mitral valve insufficiency secondary to IE. The patient should possibly have undergone immediate surgical treatment, irrespective of whether the infection was active or healed, in accordance with the guidelines for the prevention and treatment of IE (2). Eventually, however, the patient recovered and was discharged home despite being delayed surgical treatment.

In many regions of the world *Staphylococcus aureus* is the most common causative pathogen of bacteremia and/or IE. *Staphylococcus aureus* causes more acute and severe IE than the majority of other pathogens (1, 3). Žaloudíková et al. demonstrated that positive results in either blood or valve

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**Figure 2.** On admission, transthoracic echocardiography showed a mobile mass (yellow arrowhead) on the anterior leaflet of the mitral valve (A). Transthoracic echocardiography showing a mobile mass (yellow arrowhead), which developed two weeks later (B), severe mitral regurgitation (C) and severe aortic regurgitation (D). Transesophageal echocardiography showing perforation (green arrow) of the non-coronary aortic valve cusp (E, F). LA: left atrium, RA: right atrium, and RV: right ventricle.
tissue PCR tests showed high diagnostic accuracy, with a sensitivity of 89%, a specificity of 95%, a positive predictive value of 92% and a negative predictive value of 94% for the diagnosis of IE among 60 patients with suspected staphylococcal IE and 59 surgically-treated controls (1). *Staphylococcus aureus* produces two types of biocomponent toxin: gamma-hemolysin and PVL. The former is made by virtually every strain of *Staphylococcus aureus*, while the latter is made by only 2-3% of the strains (4). PVL-producing *Staphylococcus aureus* have been reported to be associated with skin and soft tissue infections. In this case, the patient had not received dental treatment nor had he suffered from skin infections for at least 1 year before the onset of symptoms. He had a past history of bladder cancer and had undergone follow-up cystoscopy without antimicrobial prophylaxis one month before the onset of symptoms. Since the incidences of IE due to MSSA following cystoscopy have never been reported, the source and route of IE remain undetected in this case.

The clinical sequelae of PVL-producing *Staphylococcus aureus* infections tend to be more severe than those of non-PVL-producing *Staphylococcus aureus*. For example, pneumonia associated with PVL-producing *Staphylococcus aureus* is more frequently associated with sepsis, high fever, leukopenia, hemoptysis, pleural effusion and death (5). PVL genes are frequently detected in community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) (6) and several cases of IE due to community-acquired MRSA with PVL have been reported (7). However, the prevalence of MSSA with PVL is very low and differs from one region to another. PVL was found to be significantly more common in North American MSSA isolates (22%) than in European isolates (0%) (8). In a Japanese study, Kono et al. reported that PVL was found in 11.1% of MRSA cultures and 5.1% of MSSA cultures of 86 *Staphylococcus aureus* (MRSA) (6) and 59 MSSA cultures of 86 *Staphylococcus aureus* skin and soft tissue pus isolates (9). To our knowledge, this is the first case of IE due to MSSA producing PVL. Interestingly, MSSA affected only the cardiac valves but not the skin or lung of the patient in the present case. Since the br-PCR method is not widely used in the clinical setting for detecting and identifying pathogens in Japan, the incidence of IE caused by PVL-producing MSSA may have been underestimated. This approach may help us to gain a precise understanding of the impact of specific bacterial genotypic and phenotypic characteristics on clinical outcome. The clinical and pathological role of PVL-producing MSSA in IE warrants further investigation.

The authors state that they have no Conflict of Interest (COI).
References


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