Aspergillus Endocarditis in a Leukemia Patient Diagnosed by a PCR Assay

Yoshinobu KANDA, Hideki AKIYAMA, Yasusuke ONOZAWA, Takashi MOTEGI*, Somay YAMAGATA-MURAYAMA** and Hideyo YAMAGUCHI***

Division of Hematology and *Pathology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan, **Department of Microbiology and Immunology, Teikyo University School of Medicine and ***Teikyo University Research Center for Medical Mycology, Tokyo, Japan

(Received: October 23, 1996)
(Accepted: January 13, 1997)

Key words: *Aspergillus*, endocarditis, PCR

Abstract

A patient in the blastic phase of chronic myelocytic leukemia developed multiple arterial emboli that originated from mitral valve vegetation. The diagnosis of infective endocarditis was not confirmed because blood cultures, serological assays and other examinations detected no pathogens. He died of intracranial hemorrhage after thrombolytic manipulation for embolization of the abdominal aorta and an autopsy was performed. Polymerase chain reaction analysis and Southern blot analysis of tissues from the mitral valve revealed *Aspergillus* species as the cause of the endocarditis, although none of the tissue specimens were culture-positive. These molecular analyses will be useful in the diagnosis of various types of *Aspergillus* infections.

Introduction

The diagnosis of invasive aspergillosis is difficult to make because blood cultures are usually negative, a biopsy is not feasible, and direct histopathological examination of the tissue may not correctly identify the pathogen[1,2]. Although a serological test for detecting *Aspergillus*-specific galactomannan is commercially available, its clinical usefulness is severely hampered by a low level of sensitivity[3]. Here we report a case of *Aspergillus* endocarditis, in whom, using two PCR-based assays, *Aspergillus*-specific DNA was successfuly detected in autopsy tissue.

Case

A 46-year-old male was diagnosed with Philadelphia chromosome positive CML and four years later, he was considered to be in the blastic phase. Treatment with vincristine and prednisolone and multi-drug chemotherapy were given without any significant therapeutic effect. Meanwhile, he was found to have pneumonia but no significant pathogen was detected by cultural examinations of sputum or bronchoalveolar lavage fluid, serological testing of serum or histopathological examination of transbronchial lung biopsy. Since the patient did not respond favorably to any antibacterial or fluconazole therapy, pulmonary *Aspergillus* infection was suspected and empiric therapy with intravenous doses of amphotericin B was started. Pneumonia improved, however, only after the neutrophil count had recovered.

Correspondence to: Hideki AKIYAMA, M.D.
Tokyo Metropolitan Komagome Hospital 3-18-22, Honkomagome, Bunkyo, Tokyo, 113, Japan

平成9年3月20日

Fig. 1 Grocott-Gomori staining of the heart with vegetation showing mycelia.

Two months following the diagnosis of pneumonia, he suffered from splenic infarction. Heparin administration was started but symptoms of multiple arterial emboli became apparent. Althogh echocardiogram revealed vegetation in the mitral valve, none of the blood cultures was fungus-positive. Two weeks later, he suddenly suffered from an embolization of the abdominal aorta at the bifurcation. Urokinase was injected but was ineffective, and five hours later the patient developed intracranial bleeding and died. Autopsy revealed thrombi totally occluding the abdominal aorta at the bifurcation. Vegetation with the destruction of the mitral valve and multiple abscesses and multiple hemorrhages were also observed. Histopathological examinations of Grocott-Gomori methenamine silver-stained tissue sections detected mycelia in vegetation and the abscesses which were suspected to be *Aspergillus* species (Fig. 1). However, all of the tissue specimens examined were culture-negative.

Molecular analysis

DNA was extracted from the paraffin embedded material of the mitral valve and the lung while negative control DNA was extracted from parts of the myocardium of the same patient which wre determined to be fungus-free by histopathological examination. PCR using primer pairs (B2F and B4 R), which amplify a fragment within the 18S ribosomal RNA genes (18S-rDNA) amplified 687-bp products in the mitral valve and the lung, but not in the control DNA (Fig. 2A)[4]. Southern analysis of the products with the *Aspergillus/Penicillium*-specific internal probe revealed hybridizing bands (Fig. 2B)[5].

PCR was also performed using a primer pair specific for *Aspergillus/Penicillium* (B2F and S3R) and 385-bp products within the 18S-rDNA were amplified (Fig. 2C) (Murayama SY, Perera PD, Makimura K, Yamaguchi H. Identification of fungal species with PCR products. In preparation).

Discussion

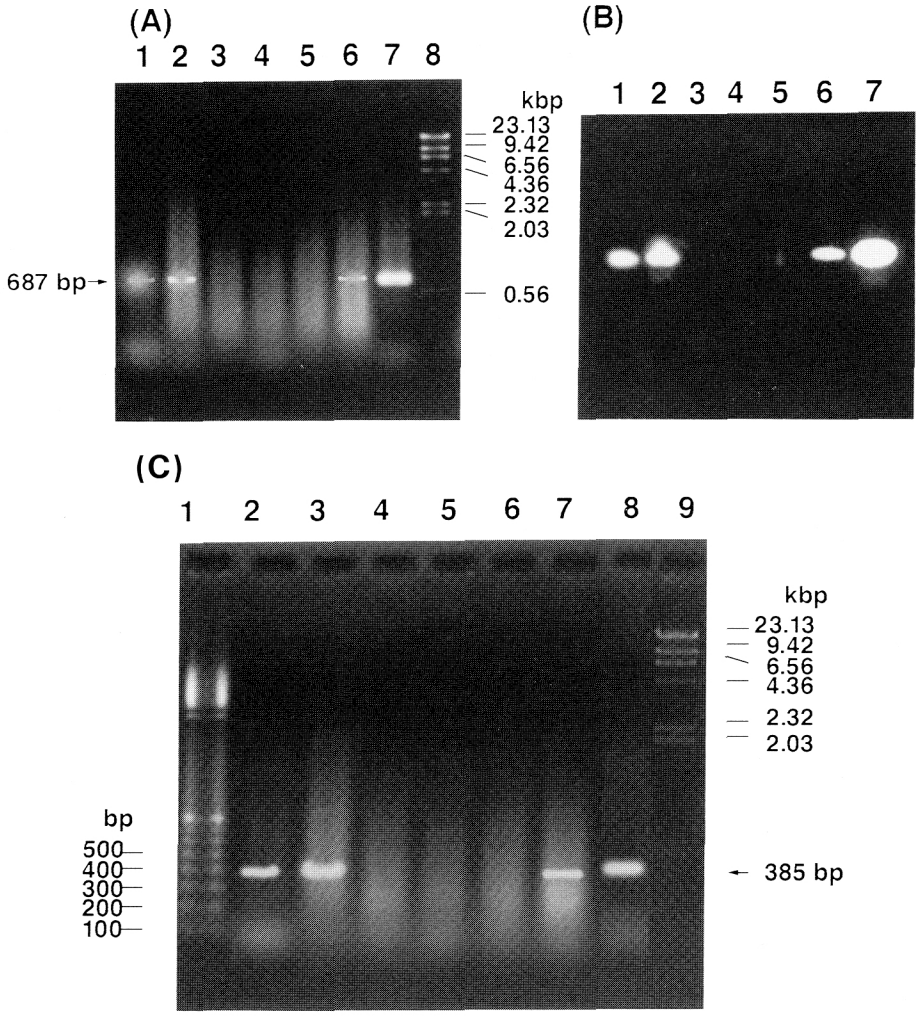
Invasive aspergillosis is now a growing clinical problem in the management of immunocompromised patients and it is important to establish a reliable and rapid method for diagnosis. Blood cultures are rarely positive for *Aspergillus*, however, and only few cases with true aspergillemia have been reported[6]. Although a serological test for detecting *Aspergillus*-specific galactomannan have been developed recently with a reasonable specificity, its clinical usefulness is severely hampered by its low level of sensitivity[3,7]. *Aspergillus* endocarditis is a severe complication after open heart surgery with a high incidence of systemic embolization and mortality of 93%, while the diagnosis of

Fig. 2 PCR amplification

A: Electrophoresis of the products of PCR amplification using the B2F/B4R primer pair. A 1.2% agarose gel was stained by ethidium bromide and visualized by UV irradiation.

B: Southern analysis with *Aspergillus/Penicillium* specific probe, detected by chemiluminescence with Polaroid 612, ISO 20,000 film. Lanes 1, 2. DNA extracted from the mitral valve. Lanes 3, 4. Negative control DNA extracted from the heart. Lanes 5, 6. DNA extracted from the lung. Lane 7. Ten ng of purified *Aspergillus fumigatus* genomic DNA. Lane 8. *Hind* III digested λ DNA marker.

C: Electrophoresis of the products of PCR amplification using the B2F/S3R primer pair. Lane 1. 100 bp DNA ladder. Lanes 2, 3. DNA extracted from the mitral valve. Lanes 4, 5. Negative control DNA extracted from the heart. Lanes 6, 7. DNA extracted from the lung. Lane 8. Ten ng of purified *Aspergillus fumigatus* genomic DNA. Lane 9. *Hind* III digested λ DNA marker.



this infection is difficult because of the low incidence of the disease and the low sensitivity of microbiological tests[1]. Only 8% of the cases with *Aspergillus*-induced endocarditis were reported to yield a positive blood culture[1].

平成9年3月20日

Accordingly, the use of molecular biological techniques to diagnose *Aspergillus* infections have recently been extensively studied and sensitive methods to screen the majority of clinically important fungi using the PCR technique have been investigated[4]. Moreover, a PCR assay to specifically detect aspergilli and penicillia has became available[5,8)~10]. In the present case, although *Aspergillus* endocarditis was suspected from the microscopic findings, the invaded tissues were culture-negative. On the other hand, two different PCR assays revealed the existence of *Aspergillus* or *Penicillium* in the tissue lesion. All of the clinical, histopathological and molecular findings suggested the occurrence of *Aspergillus* infection.

In this context, the application of adequate PCR assays in clinical specimens, such as blood and tissue biopsy materials, will be useful in the diagnosis of various types of *Aspergillus* infection and may become a powerful diagnostic method in clinical medicine in the near future.

References

1) Rubinstein E, Lang R: Fungal endocarditis. Eur Heart J, 1995; 16 (Supple B): 84-89.
2) Mehta G: *Aspergillus* endocarditis after open heart surgery; an epidemiological investigation. J Hosp Infect. 1990; 15: 245-253.
3) De Repetigny L: Serodiagnosis of candidiasis, aspergillosis, and cryptococcosis. Clin Infect Dis. 1992; 14 (Supple 1): S11-22.
4) Makimura K, Murayama SY, Yamaguchi H: Detection of a wide range of medically important fungi by polymerase chain reaction. J Med Microbiol. 1994; 40: 358-364.
5) Makimura K, Murayama SY, Yamaguchi H: Specific detection of *Aspergillus* and *Penicillium* species from respiratory specimens by polymerase chain reaction (PCR). Jpn J Med Sci Biol. 1994; 47:141-156.
6) Duthie R, Denning DW: *Aspergilus* fungemia: report of two cases and review. Clin Infect Dis. 1995; 20: 598-605.
7) Rogers TR, Haynes KA, Barnes RA: Value of antigen detection in predicting invasive pulmonary aspergillosis. Lancet. 1990; 326: 1210-1213.
8) Tand CM, Holden DW, Aufauver-Brown A, Cohen J: The detection of *Aspergillus* spp. by the polymerase chain reaction and its evaluation in bronchoalveolar lavage fluid. Am Rev Resp Dis. 1993; 148: 1313-1317.
9) Spreadbury C, Holden D, Aufauver-Brown A, Bainbridge B, Cohen J: Detection of *Aspergillus fumigatus* by polymerase chain reaction. J Clin Microbiol. 1993; 31: 615-621.
10) Melchers WJ, Verweij Pe, van den Hurk P *et al*.: General primer-mediated PCR for detection of *Aspergillus* species. J Clin Microbiol. 1994; 32: 1710-1717.

Polymerase Chain Reaction 法により診断された *Aspergillus* 性心内膜炎を合併した慢性骨髄性白血病の1例

[1)]東京都立駒込病院血液内科，[2)]同病理
[3)]帝京大学医学部細菌講座
[4)]帝京大学医真菌研究センター

神田 善伸[1)] 秋山 秀樹[1)] 小野澤康輔[1)] 茂木 孝[2)]
村山 琮明[3)] 山口 英世[4)]

要 旨

Aspergillus による心内膜炎を合併した慢性骨髄性白血病の一例を提示する．症例は46歳．急性転化後の化学療法に伴う顆粒球減少時に肺炎を合併，次いで多発性の動脈血栓症を合併し，心内膜炎と診断された．各種培養は陰性であり，血清学的検査も陰性であったため，細菌性心内膜炎の診断は困難であった．腹部大動脈の血栓症に対し血栓溶解療法を施行したが，頭蓋内出血により死亡した．解剖により真菌性心内膜炎が証明され，polymerase chain reaction (PCR) 法と Southern 法により原因菌として *Aspergillus* が同定された．*Aspergillus* 感染症の診断において，PCR 法は有力な手段となると考えられた．