Chapter 4

Blood Culture-Negative Endocarditis

Mio Ebato

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76767

Abstract

Blood culture-negative endocarditis is often severe and difficult to diagnose. Infective blood culture-negative endocarditis is classified into three main categories: (1) bacterial endocarditis with blood cultures sterilized by previous antibacterial treatment; (2) endocarditis related to fastidious microorganisms in which prolonged incubation is necessary; (3) true blood culture-negative endocarditis, due to intra-cellular bacteria that cannot be routinely cultured in blood with currently available. There are two major etiologies for noninfective endocarditis: (1) nonbacterial thrombotic endocarditis and (2) endocarditis related to systemic diseases (SLE and Behcet disease). Team approach including cardiologists, infection disease (ID) specialists, microbiologists, pathologist and immunologist is crucial for diagnosis and management of blood culture-negative endocarditis as it needs elegant and high-quality modern technics of histology, molecular analysis and essential epidemiological information.

Keywords: blood culture-negative endocarditis, fastidious microorganisms, intra-cellular bacteria, noninfective endocarditis

1. Introduction

Blood culture-negative IE (BCNIE) refers to infective endocarditis (IE) in which no causative microorganism can be grown using the usual blood culture methods. BCNIE accounts for 5–10% of all cases of endocarditis [1]. This variation is caused by differences in the diagnostic criteria and sampling strategies used. A European study included 820 cases indicated 20% of

patients with confirmed IE had negative blood cultures [2]. BCNIE often produces considerable diagnostic and therapeutic dilemmas, which result in poor prognosis.

2. Main etiologies of BCNIE

There are three main causes for BCNIE.

1. Administration to antimicrobial agents before blood culture.
2. Endocarditis related to fastidious microorganisms in which prolonged incubation is necessary.
3. True blood culture-negative endocarditis, due to intra-cellular bacteria that cannot be detected by currently available routine blood culture system.

If all microbiological assays are negative, noninfective endocarditis is considered, and systematically differential diagnosis should be performed. Nonbacterial thrombotic endocarditis (marantic endocarditis) in patients with malignant tumor and systemic diseases such as SLE and Behçet are two main causes of noninfective endocarditis.

3. Diagnostic approach

Definitions of the terms used in the European Society of Cardiology 2015 [4] modified criteria adapted from modified Duke Criteria [3] were shown in **Table 1**. Diagnosis of IE is drawn as follows:

3.1. Definition

Pathological criteria: Microorganisms demonstrated by culture or on histological examination of a vegetation, a vegetation that has embolized, or an intracardiac abscess specimen; or pathological lesions; vegetation or intracardiac abscess by histological examination showing active endocarditis.

Clinical criteria: two major criteria; or one major criterion and three minor criteria or five minor criteria.

Possible IE: One major criterion and one minor criterion or three minor criteria.

Rejected IE: Firm alternate diagnosis; or Resolution of symptoms suggesting IE with antibiotic therapy for ≤4 days; or No pathological evidence of IE at surgery or autopsy, with antibiotic therapy for ≤4 days; or Does not meet criteria for possible IE, as above.

When blood culture is negative, systematic diagnostic approach should be performed for rapid and correct management of BCNIE. Diagnostic work-up in blood culture-negative endocarditis is shown in **Figure 1** [1, 4].

Major criteria

1. Blood cultures positive for IE

a. Typical microorganisms consistent with IE from two separate blood cultures

**Streptococcus viridance*, *Streptococcus bovis*, HACEK group, *Staphylococcus aureus*

*Community-acquired enterococci, in the absence of a primary focus

b. Microorganisms consistent with IE from persistently positive blood cultures defined as follows

*>2 positive blood cultures of blood samples drawn >12 h part; or

*All of 3 or a majority of >4 separate cultures of blood (with first and last samples (drawn>1 h apart)

c. Single positive blood culture for *Coxiella burnetii* or phase I IgG antibody titer>1:800

2. Imaging positive for IE

a. Echocardiogram positive for IE: vegetation, abcess, pseudoaneurysm, intracardiac fistula, valvular perforation or aneurysma, new partial dehiscence of prosthetic valve

b. Abnormal activity around the site of prosthetic valve implantation detected by 18F-FDG PET/CT (only if the prosthesis was implanted for 3 months) or radiolabeled leukocytes SPECT/CT.

c. Definite paravalvular lesion by cardiac CT.

Minor criteria

1. Predisposition such as predisposing heart condition, or injection drug use.

2. Fever defined as temperature > 38°C

3. Valvular phenomena (including those detected by imaging only) major arterial emboli, septic pulmonary infarcts, infectious (mycotic) aneurysm, intracranial hemorrhages, conjunctival hemorrhages and Janeway's lesions.

4. Microbiological evidence: positive blood culture but does not meet a major criterion as noted above or serological evidence of active infection with organism consistent with IE

CT, computed tomography; FDG, fluorodeoxyglucose; HACEK, Haemophilus parainfluenzae, H. aphrophilus, H. paraphrophilus, H. influenzae, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae, and K. denitrificans; IE, infective endocarditis; Ig, immunoglobulin; PET, positron emission tomography; SPECT, single photon emission computerized tomography.

Table 1. Definitions of the terms used in the European Society of Cardiology 2015 modified criteria adapted from modified Duke criteria.

3.2. Past history and clinical examination

A precise interview about epidemiological factors, history of prior infections, exposure to antimicrobials, should be made in all patients with suspected BCNIE [1, 4].

Previous exposure to antibiotics is the most common cause of BCNE, and even a short course of antibiotics can cause long-lasting suppression of bacterial activity. A history of animal exposures may predispose to certain microbiologic etiologies. Immunosuppression or prolonged antibiotic therapy suggests endocarditis due to fungi. The epidemiological clues for defining the etiology of BCNIE are shown in **Table 2** [1].

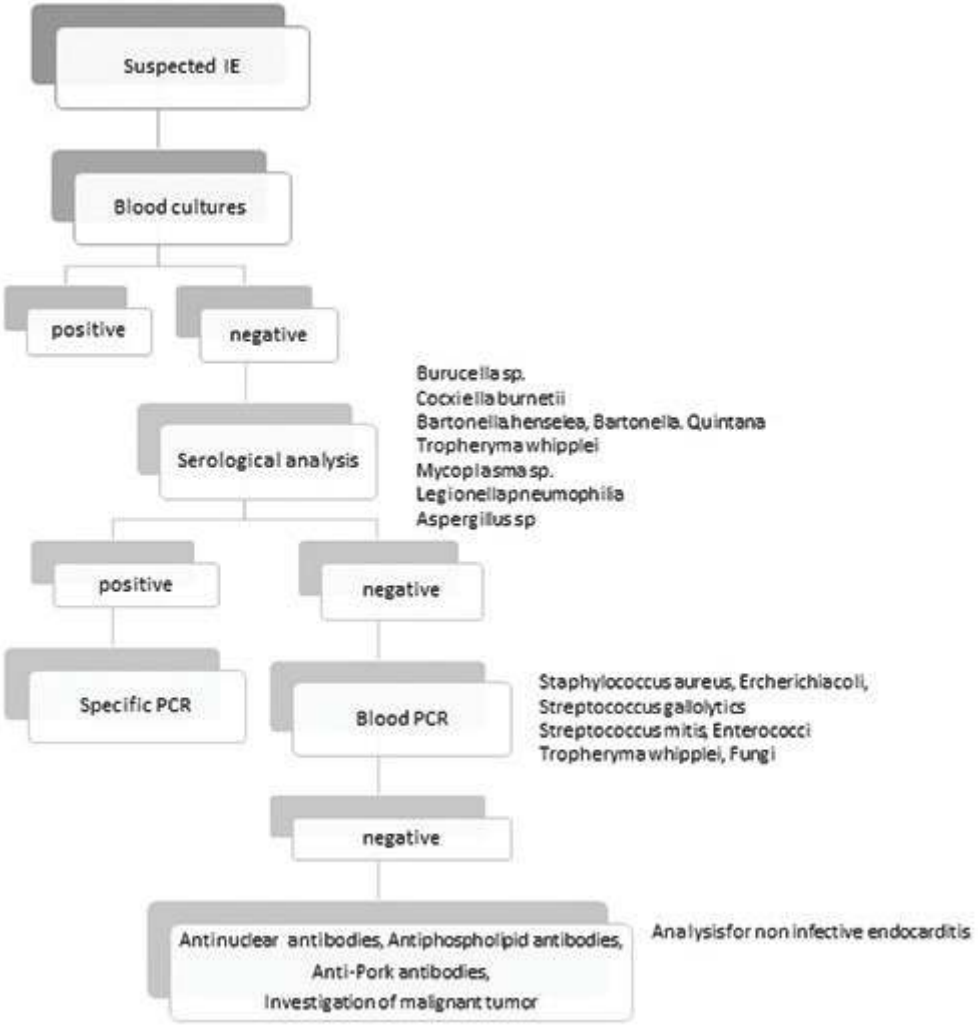

Figure 1. Diagnostic workup in blood culture-negative endocarditis.

3.3. Blood culture

BCNIE occurs frequently (45–60%) by common and easily grown staphylococci or streptococci in patients with preceding administration of antibiotics as it reduces the recovery rate of bacteria by 35–40% [5, 6]. In these cases, withdrawing antibiotics and repeating blood cultures are preferable methods to diagnose if the patient status allowed. The use of specific blood culture bottles for fastidious microorganisms is not recommended recently [1, 4, 5]. The extended incubation is applied only when cultures remain sterile after 48–72 h. Sophisticated automated systems allow isolating most pathogens that can grow slowly including Candida sp., deficient streptococci and HACEK group bacteria(Haemophilus, Aggregatibacter (previously

Epidemiological feature	Suspected microorganisms
Alcoholism, Cirrhosis	*Bartonella sp., Aeromnas sp., Listeria sp.*
Burn	*S. aureus*, Aerobic Gram-negative bacilli, Fungi
Chronic skin disorders	*S. aureus*, β-hemolytic streptococci
Genitourinary disorders	Enterococcus, GroupB streptococci, aerobic Gram-negative bacilli, *Neisseria gonorrhoeae*, Listeria monocytoogenes
Intravenous drug use, cardiovascular medical devices	*S. aureus*, CNS, Aerobic Gram-negative bacilli, β-Hemolytic streptococci, Fungi
Prosthetic valve replacement	Early(<1y): CNS, *S. aureus*, Aerobic Gram-negative bacilli, Fungi, *Corynebacterium sp., Legionella sp.*, Late(>1y): CNS, *S. aureus*, Viridance *Streptcoccus sp.*, Enterococcus sp., Fungi, Corynebacterium
Exposure to dog and/or cat	*Bartonella sp.,Pasteurella sp.*
Contact with contaminated milk or farm animal	*Brucella sp.*, Cociella bumetii
Homeless, body lice	*Bartonella sp.*
Gastrointestinal lesions	*S. gallolytics* (bovis), *Enterococcus sp.*, Clostridium spectrum
Dog or cat exposure	*Bartonella sp., Pasteurella sp., Capnocytophaga sp.*
Homeless, body lice	*Bartonella sp.*
Contact with contaminated milk or infected farm animals	*Brucella sp., Coxiella bumetii, Erysipelothrix sp.*
Diabetes mellitus	*S. aureus*, β-Hemolytic streptococci, *S. pneumoniae*
AIDS	*Salmonella sp., S. pneumoniae, S. aureus*
Organ transplantation	*S. aureus, Aspergillus fumigatus, Enterococcus sp., Candida sp.*

Table 2. Epidemiological clues for defining the etiology of blood culture-negative infective endocarditis *S. aureus*, *Staphylococcus aureus*; CNS, coagulase -negative staphylococci; *S. gallolytics*, *Streptococcus gallolyticus*; *Streptococcus pneumonie*.

Actinobacillus), Cardiobacterium, Eikenella, Kingella). Extending culture beyond 5 days is not contributive [1, 4–8]. The popular pathogens such as staphylococci, streptococci and enterococci are usually identified within 48 h. The European guidelines recommend that clinicians require prolonged incubation of vials only in the rare cases of cultures remaining negative at 48–72 h and if the diagnosis of IE remains plausible [4, 8].

3.4. Serology

The list of serological tests to be performed in case of blood culture-negative endocarditis used to include: *Legionella pneumophila*, *Mycoplasma hominis*, *Chlamydophila pneumoniae*, Brucella sp., *Coxiella burnetii* (*C. burnetii*), and Bartonella sp. Two major series showed that only Bartonella sp. and *C. burnetii* serological tests are contributive: 348 cases of suspected BCNIE were investigated between 1983 and 2001, the diagnosis was documented by serological tests in 268 cases (77%),

including 266 cases of *C. burnetii* (n = 167) or Bartonella sp. (n = 99) [5]. The same team reported a second series of 745 patients presenting with suspected BCNIE having received a panel of serological tests between 2001 and 2009. They documented the predominance of Q fever and Bartonellosis. A total of 354 of the 356 cases documented by serological tests were positive for *C. burnetii* (n = 274) or Bartonella sp. (n = 80) [6]. In other words, if only Bartonella sp. and *C. burnetii* serological tests had been used, only 4 out of 624 diagnoses obtained by serological tests would have been missed. A review of endocarditis caused by fastidious pathogens shows that Mycoplasma sp. endocarditis is very rare (<10 reliable observations published to date, mostly due to M. hominis), as well as Legionella sp. endocarditis [7]. Moreover, most cases of endocarditis supposedly due to Chlamydophila sp. are probably cross-reactions with a Bartonella sp. In 2015, the only routinely recommended serological tests in case of negative blood cultures are tests for Q fever and Bartonellosis [4]. Brucellosis serological tests can be added in case of risk factors (living in endemic areas, occupational exposure, consumption of nonpasteurized dairy products). Serological tests for Mycoplasma sp. and Legionella sp. are still recommended in the 2015 ESC guidelines [4].

3.5. Evaluation of valve tissue

The more frequent use of valve replacement in the acute phase of infective endocarditis and the advent of molecular biology techniques have revolutionized the diagnosis of blood culture-negative endocarditis:

PCR systems based on universal bacterial 16S ribosomal RNA have demonstrated excellent sensitivity and specificity [8, 9], as well as PCR targeting bacteria specifically responsible for endocarditis with negative blood culture: Bartonella sp., *C. burnetii* [10] and *Tropheryma whipplei* (*T. whipplei*) [11].

Moreover, the microscopic examination of valves after Gram staining, and cultures on appropriate media provide important information not only for the identification of the pathogen involved when the data were not available preoperatively [12], but also information on its viability at the time of valve replacement, which will impact the duration of post-replacement treatment [11, 13]. The histological analysis of valves is not contributive to diagnose except some rare diagnoses such as porcine bioprosthesis endocarditis mediated by allergy to porcine proteins [22, 23]. Summary of diagnostic procedure of rare pathogens of BCNIE is shown in **Table 3**.

Pathogen	Diagnostic procedures
Brucella sp.	blood cultures, serology, immunohistology, PCR of surgical materials
Coxiella burnetii	serology (IgG phaseI >1:800, tissue culture, immunohistology, PCR of surgical materials
Bartonella sp.	blood cultures, serology, culture, immunohistology, PCR of surgical materials
Tropheryma whippplei	hystology and PCR of surgical materials
Mycoplasma sp.	serology, culture, immunohistology, PCR of surgical materials
Legionella sp.	blood cultures, serology, culture, immunohistology, PCR of surgical materials
Fungi	blood cultures, serology, immunohistology, PCR of surgical materials

Table 3. Summary of diagnostic procedure of rare pathogens of blood culture-negative infective endocarditis.

4. Treatment

4.1. Empirical therapy

Selection of medical therapy for patients with BCNIE is difficult. Some of the laboratory-based diagnostic techniques to define fastidious or rare pathogens are not available in most clinical laboratories. It consumed considerable time for completion of testing if specimens are sent to a referral laboratory. Patients with BCNIE are often treated empirically for the more common bacterial causes of IE during the waiting time. There is a need to provide empirical antimicrobials for all likely pathogens, though certain therapeutic agents, including aminoglycosides, have potentially toxic effects. Consultation with an ID specialist to define the most appropriate choice of therapy is recommended. Once additional clinical and laboratory data were brought, initial empirical therapy should be changed to more specific treatment. For patients with acute (days) clinical presentations of native valve infection, coverage for S aureus, β-hemolytic streptococci, and aerobic Gram-negative bacilli is reasonable. Empirical coverage could include vancomycin and cefepime as an initial regimen [1, 4, 14]. For patients with a subacute (weeks) presentation of native valve IE, empirical coverage of *S. aureus*, Viridance group streptococci (VGS), HACEK, and enterococci is reasonable. One treatment option could include vancomycin and ampicillin-sulbactam to provide some coverage for these organisms [1, 4, 14]. For patients with culture-negative prosthetic valve IE, coverage for staphylococci, enterococci, and aerobic Gram-negative bacilli is reasonable if the onset of symptoms is within 1 year of prosthetic valve placement. A regimen could include vancomycin, rifampin, gentamicin [1, 4, 14]. If symptom onset is >1 year after valve placement, then IE is more likely to be caused by staphylococci, VGS, and enterococci, and antibiotic therapy for these potential pathogens is reasonable [1, 4, 14]. One initial treatment option could include vancomycin and ceftriaxone. If subsequent blood culture results or other laboratory methodologies define a pathogen, then empirical therapy should be changed to focused therapy that is recommended for the specific pathogen identified.

4.2. Antibiotic treatment for fastidious microorganisms

HACEK Gram-negative bacilli are fastidious organisms, and the laboratory should be made aware that infection with these agents needs consultation to specialist. Because of slow growth, standard MIC tests may be difficult to interpret. Some HACEK-group bacilli produce beta-lactamases, and ampicillin is therefore no longer the first-line option. They are susceptible to ceftriaxone, other third-generation cephalosporins and quinolones; the standard treatment is ceftriaxone 2 g/day for 4 weeks in native valve endocarditis and for 6 weeks in prosthetic valve endocarditis. If they do not produce beta-lactamase, ampicillin (12 g/day i.v. in four or six doses) plus gentamicin (3 mg/kg/day) divided into two or three doses for 4–6 weeks is an option [1, 4, 13]. Ciprofloxacin (400 mg/8–12 h i.v. or 750 mg/12 h orally) is a less well-validated alternative. Clinical outcome of HACEK endocarditis is favorable.

In cases with fungi, mortality is very high, and treatment necessitates combined antifungal administration and surgical valve replacement. Antifungal therapy for Candida sp. includes liposomal amphotericin B with or without flucytosine or an echinocandin at high doses; and for Aspergillus spp., voriconazole is the drug of choice and some experts recommend the addition

of an echinocandin or amphotericin B. Suppressive long-term treatment with oral azoles (fluconazole for Candida and voriconazole for Aspergillus) is recommended [1, 4, 14]. Consultation with an infectious doctor specialist in the Endocarditis Team is recommended.

4.3. Specific therapy for true culture-negative microorganisms

The recommended therapy for true culture-negative microorganisms in the European guidelines 2015 is shown in **Table 4** [4, 12]. Consultation with ID specialist is highly recommended for the treatment of these special organisms. This is an area with a very limited level of evidence. The treatment of *T. whipplei* endocarditis has not been standardized. Doxycycline + hydroxychloroquine for 12–18 months, with monitoring of plasma levels of these two agents (objective: achieving plasma concentrations of 0.8–1.2 mg/L for hydroxychloroquine, and < 5 mg/L for doxycycline), and of negativation of samples initially positive for *T. whipplei* was proposed. The treatment of Bartonella sp. endocarditis is a beta-lactam antibiotic (amoxicillin or ceftriaxone) or doxycycline for 4 weeks in combination with gentamicin for the first 2 weeks [1, 4, 14] the treatment of *C. burnetii* endocarditis, is doxycycline + hydroxychloroquine until a phase1 antibody rate <800 is reached for IgG, and <50 for IgM and IgA [1, 4, 14].

4.4. Surgical treatment of blood culture-negative IE

There is no specific recommendation for surgical treatment of BCNIE: cardiac surgery indications rely on the same criteria that apply for any type of endocarditis (heart failure, uncontrolled infection, risk of embolism [1, 4, 15]). However, an additional argument for the surgical treatment of BCNIE is the ability to harvest valve tissue, which often finally allows microbiological documentation.

Pathogens	Standard therapy	Treatment outcome
Brucella sp.	Doxycycline (200 mg/day) + contrimocazole (960 mg/12 h) + rifampicine (300~600 mg/day for ≥3~6 months orally	Treatment success defined as IgG < 1:60
Bartonella sp.	Doxycycline (100 mg/12 h) orally for 4 weeks + gentamicin (3 mg/day) iv for 2 weeks	Success rate > 90%
Coxiella.burnetii (Q fever)	Doxycycline (200 mg/day) + hydroxychloroquine (200–600 mg/day) orally for ≥18 months	Treatment success defined as phase I IgG < 1:200 IgM, IgA < 1:50
Legionella sp.	Levofloxacin (500 mg/12 h) iv or orally for ≥6 weeks or clarithromycin (500 mg/12 h) iv for 2 weeks, then orally for 4 weeks + rifampin (300–1200 mg/24 h)	Optimal treatment unknown
Mycoplasma sp.	Levofloxacin (500 mg/12 h) iv or orally for ≥6 weeks	Optimal treatment unknown
Treaponema whipplei (Whipple's disease)	Doxycycline (200 mg/day) + hydroxychloroquine (200–600 mg/day) orally for ≥18 months	Long-term treatment, optical duration unknown

Table 4. Recommended therapy for true culture-negative microorganisms in the European guidelines 2015.

5. Noninfective endocarditis

When all microbiological assays are negative, the diagnosis of noninfectious endocarditis should systematically be considered (**Figure 1**).

5.1. Nonbacterial thrombotic endocarditis

Nonbacterial thrombotic endocarditis (marantic endocarditis, Trousseau syndrome) is observed in 1.2% of patients with active cancer at autopsy [16]. Usually, the single or multiple small vegetation-like lesions are observed predominantly on the mitral and aortic valves with no underlying valve diseases. These are associated with an underlying hypercoagulable state that justifies routine anticoagulation. Control of pathologically altered coagulation mechanism is essential for the treatment and the prognosis is poor without resolving the problem. The differential diagnosis with an infectious cause of BCNIE is often difficult, and the prognosis is poor [17]. The initial lesion is usually breast, lung, prostate, ovarian or colon cancer. However, it should not be forgotten that undiagnosed infective endocarditis is also common in cancer patients with sterile blood cultures and/or fastidious organisms that are difficult to identify by conventional methods.

5.2. Systemic diseases

Inflammatory diseases can cause endocarditis and produce a syndrome similar to culture-negative IE. Perhaps the one most often encounter is antiphospholipid antibody (APA) syndrome [18], which has been described as both a primary and a secondary syndrome of systemic lupus erythematosus(SLE) and malignancies. Sterile valvular vegetations form and often embolize, clinically mimicking in many respects with IE. The mitral valve is most often affected, and valvular regurgitation is the frequent functional abnormality. To complicate matters, the APA syndrome may also develop secondary to IE [19].

In patients with SLE, valve abnormalities are common (15–75% of autopsy series, depending on the severity of the disease), but rarely progress to a clinical stage of Libman-Sacks endocarditis [20]. The patients are usually young individuals with a very severe lupus poorly controlled by treatments. Immunological manifestations (Osler nodes) and embolism (stroke, often in combination with an antiphospholipid syndrome) may be observed. Valve lesions are mainly found in the left heart. Endocardium involvement may occur in Behçet's disease [21]. It is a disease of young ± male patients with a predominantly aortic involvement. Endocardium involvement in Behçet's disease is a poor prognostic factor. The treatment is of course should be targeted on the systemic disease (immune-suppressants, immune-modulators) with lifelong curative anticoagulation. Checkup for antinuclear antibodies as well as antiphospholipid antibody {anticardiolipin antibodies [immunoglobulin (Ig) G and anti-b2-glycoprotein 1 antibodies [IgG and IgM]} should be performed for the patients who are suspected to have noninfective endocarditis.

5.3. Allergy for porcine valve

When the patient has a porcine bioprosthesis implanted during last 6 months, anti-pork antibodies should be sought [22, 23] to consider allergy for the valve.

6. Conclusion

Blood culture-negative endocarditis is still a clinical challenge with heterogeneous pathology. Remarkable progress has been made in methodologies to evaluate the main etiologies in past two decades. Team approach including cardiologists, infectious disease specialists, microbiologists and immunologist is crucial for the correct diagnosis that is able to reach rapidly the new diagnostic microbiological techniques, and high-quality epidemiological information.

Conflict of interest

There is no conflict of interest for the theme.

Author details

Mio Ebato

Address all correspondence to: ippeiaki@med.showa-u.ac.jp

Division of Cardiology, Showa University Fujigaoka Hospital, Yokohama, Kanagawa, Japan

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