Journal of the American Heart Association

AHA SCIENTIFIC STATEMENT

Blood Culture–Negative Endocarditis: A Scientific Statement From the American Heart Association

Endorsed by the International Society for Cardiovascular Infectious Diseases

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ABSTRACT: Blood culture–negative endocarditis has been associated with worse outcomes when compared with blood culture–positive endocarditis, because pathogen-directed antimicrobial therapy and other management aspects have been difficult to achieve. Novel diagnostic tools, however, have changed the landscape of this syndrome and will likely improve patient outcomes. This American Heart Association scientific statement highlights these advances. The writing group, which represents a multidisciplinary team, provides an update on blood culture–negative endocarditis. Clinical scenarios representative of real-world experiences are included to assist frontline clinicians in the diagnosis and management of this syndrome.

Key Words: AHA Scientific Statements ■ blood culture–negative infective endocarditis ■ diagnosis ■ management ■ prevention

A positive blood culture is a major clinical criterion for confirming infective endocarditis (IE) and is often the first clue that IE may be considered in the differential diagnosis.[1] Moreover, identification of a pathogen and its susceptibility to antimicrobials is imperative for providing optimal therapy in IE cases. Unfortunately, in up to 30% of these cases, blood cultures are negative, primarily due to antibiotic exposure before blood culture collection or infection with either fastidious or nonculturable microorganisms. This so-called blood culture–negative endocarditis (BCNE) has been a bane of clinical practice for decades. The inability to provide pathogen-specific antibiotic treatment often leads to the use of broad-spectrum antibiotic coverage. For the individual patient, this could potentially increase the risk of selection of multidrug-resistant bacteria, *Candida* spp, and *Clostridioides difficile* infections.

Determining the cause of BCNE in the setting of recent antimicrobial exposure involves consideration of a variety of factors. These include epidemiological features, clinical course of illness, and type of antibiotic(s) that the patient has been exposed to before obtaining blood cultures. These features were outlined in the 2015 American Heart Association scientific statement "Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications,"[2] which addressed IE diagnosis, antimicrobial therapy, and management of complications. Recovery of pathogens in a culture from valve tissue specimens may also be limited due to prior antibiotic treatment.

For cases of BCNE not due to recent antimicrobial exposure, non–culture-based laboratory testing of clinical specimens has been available for detection of fastidious or nonculturable microorganisms. Of note, the 2015

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American Heart Association scientific statement included only a comment on the molecular technique polymerase chain reaction (PCR) and its limited availability in most clinical laboratories, with specimens being sent to reference laboratories. The 2023 European Society of Cardiology guidelines for the management of BCNE[3] provide a more contemporary approach to accommodate these laboratory advances. In addition, there have been advances in radiologic and nuclear medicine techniques that are critical in establishing an IE diagnosis and can also be helpful in defining a management strategy. For all patients with IE, including those with BCNE, prior antibiotic exposure could impact the sensitivity of tools used for the diagnosis of endocardial or device infection that includes, in particular, fluorine-18-fluorodeoxyglucose (^{18}F-FDG) positron-emission tomography (PET)/computed tomography (CT) imaging. Noninfectious causes of BCNE, termed nonbacterial thrombotic endocarditis (NBTE) are less common; the current diagnostic approach is to identify underlying conditions that include procoagulant and inflammatory states, including advanced malignancies, systemic lupus erythematosus, and antiphospholipid antibody syndrome.[4,5]

Several key advances achieved in imaging modalities as well as laboratory molecular techniques that enable detection of BCNE pathogens from blood and other specimens prompted, in large part, the development of this statement on BCNE (Figure 1). It is also important to acknowledge that, despite the ongoing advancements in medical technology, many health care centers worldwide lack access to such sophisticated molecular and imaging techniques. This limitation poses a notable challenge in formulating an optimal management strategy for BCNE. By acknowledging the diversity of available resources, the aim was to present a comprehensive perspective that accommodates the practical constraints faced by many health care facilities, thereby enhancing a more universally applicable understanding of BCNE diagnostics and management. To increase the clinical usefulness of this statement, 4 commonly encountered clinical scenarios are presented with questions proposed to provide the frontline clinician with a strategy to improve the diagnosis and management of patients with BCNE. The clinical variability and complexity in BCNE, however, dictate that this discussion be used to support and not supplant decisions in individual patient management.

CLINICAL SCENARIO

BCNE Due to Recent Antibiotic Exposure

A 74-year-old man with a past medical history of rheumatic heart disease presents to the hospital with a 1-week history of fever, chills, and general malaise. Notably, the patient had consulted his primary care physician 3 days prior, who prescribed a 7-day course of oral amoxicillin/clavulanic acid for possible pneumonia. Clinical examination revealed a new murmur, prompting further investigation. Blood cultures were obtained, yielding negative results. A transesophageal echocardiogram showed a large vegetation within the mitral valve with valve perforation. A diagnosis of BCNE was established. The patient subsequently underwent surgical valve repair. Tissue cultures were negative; however, 16S ribosomal RNA gene PCR/sequencing of the valve tissue confirmed the presence of *Streptococcus agalactiae*.

Definition of BCNE

BCNE is generally defined as IE without positive blood cultures. The Duke criteria were initially presented in 1994[6] to define cases of IE with incorporation of echocardiographic findings and have since been modified (2000)[7] and recently updated (2023).[1] These criteria have been extensively used both in clinical research and

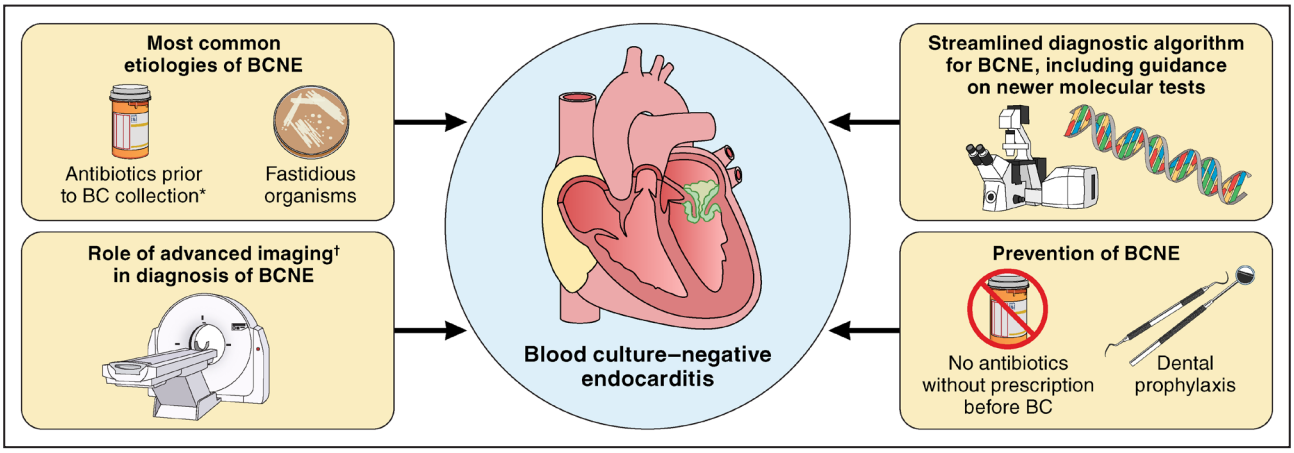

Figure 1. Blood culture–negative infective endocarditis. BC indicates blood culture; and BCNE, blood culture–negative endocarditis. *Antibiotics before BC collection remain the number 1 cause of BCNE. †Advanced imaging refers to mainly fluorine-18-fluorodeoxyglucose positron-emission tomography/computed tomography.

in the management of individual patients worldwide. All previous iterations of these criteria have included positive blood cultures as either a major (typical IE microorganisms) or minor criterion in the IE case definition. In the most recent version, the 2023 Duke–International Society for Cardiovascular Infectious Diseases (ISCVID) criteria,[1] the diagnostic test–based major criterion was expanded to include detection of nucleic acid of 3 microorganisms (*Coxiella burnetii*, *Tropheryma whipplei*, and *Bartonella* spp) and serologic evidence of infection of *Bartonella henselae* or *Bartonella quintana*; pathological diagnostic criteria for definite IE were extended to include nucleic-based tests using amplicon or metagenomic sequencing and in situ hybridization of tissue specimens. Additionally, the minor criteria were expanded to incorporate a positive nucleic acid–based test for an organism consistent with IE from a sterile body site other than cardiac tissue, prosthesis, or arterial embolus, or the presence of a single skin colonizer identified through PCR on a valve or wire without additional clinical or microbiological supporting evidence, while noting that test interpretation be in the context of clinical and histological evidence of IE.

Causes of BCNE

From a clinical standpoint, there are 2 scenarios where BCNE occurs:

1. Recent antibiotic administration before obtaining blood cultures. The most common pathogens in patients given empiric therapy are generally those that are seen in the bulk of IE cases and include methicillin-susceptible staphylococci, streptococci, and enterococci.
2. Pathogens that do not grow in routine blood cultures from patients without prior antibiotic exposure, which includes an array of bacteria, mycobacteria, and fungi.

Clinical presentation of BCNE may be different, depending on the scenario. For the minority of cases that are not related to recent antibiotic exposure as a cause of culture negativity, subacute or chronic presentation is likely. For prior antibiotic exposure cases, it would depend on the organism as to whether the presentation is acute or subacute. When the clinical presentation is not strongly suggestive of infection, it is essential to consider other noninfectious causes such as NBTE and rheumatological conditions in the differential diagnosis of BCNE.[4] Of note, activated partial thromboplastin time prolongation is a simple and useful laboratory marker of NBTE associated with antiphospholipid syndrome and systemic lupus erythematosus. Although noninfectious in origin, patients with NBTE can have a clinical presentation that satisfies the 2023 Duke-ISCVID criteria without positive blood cultures and is in the differential diagnosis of BCNE.

Patients with NBTE usually present with symptomatic embolic events with no fever or systemic signs of infection. Because euthermic endocarditis due to infection is seen in a minority of patients with IE, particularly older patients, an evaluation for infective versus noninfective endocarditis is required.[8] Two case series that have included contemporary patients with NBTE provide a clinical profile.[5,9] Women predominated in both series, with a mean age of 54 and 60 years, respectively. Malignancy and connective tissue diseases were most often associated with NBTE, with stroke as the most common (54.2% and 59.5%, respectively) presentation due to hypercoagulability. Transesophageal echocardiography (TEE) was superior to transthoracic echocardiography in identifying valvular abnormalities, most commonly vegetations. A recent multicenter case series that focused on marantic endocarditis associated with cancer demonstrated the importance of multimodality imaging (CT and ^{18}F-FDG PET/CT) in both cancer and NBTE diagnoses.[10] A systematic review and meta-analysis that covered almost 6 decades supported the findings of the above-cited case series that overall survival in NBTE cases associated with cancer was poor but improved in recent years.[11]

Suggested Diagnostic Approach for Patients With Suspected BCNE

Because most cases of BCNE are due to antibiotic administration before obtaining blood cultures, empiric antibiotic therapy may be initially administered after at least 2 (ideally 3) sets of blood cultures have been obtained. Blood cultures are to be obtained under a strict aseptic technique, with 1 aerobic and 1 anaerobic culture in each set. Separate venipunctures are no longer required to satisfy the updated 2023 Duke-ISCVID criteria[1]; however, this remains the strongly preferred technique to minimize risk of contamination and optimize sensitivity of pathogen detection. Although some guidelines still suggest specific timing,[2,3,12] evidence suggests that the yield of blood culture is directly related to the volume of blood (8–10 mL of blood per bottle), making a minimum of 2 sets (ie, 40 mL, essential to secure microbiological diagnosis).[13]

For patients already receiving antibiotics, it is crucial to conduct a comprehensive review of their recent antibiotic use, considering the type, timing, duration, route, and dosage. As mentioned earlier, methicillin-susceptible staphylococci, streptococci, and enterococci, are the most common pathogens when blood cultures yield negative results following recent antibiotic exposure. Nonetheless, clinicians need to consider

additional infectious causes for BCNE, such as fastidious or nonculturable pathogens, particularly in the presence of identifiable patient risk factors, exposure history, and epidemiological considerations (Table 1).

A comprehensive diagnostic algorithm about how to approach a patient with suspected BCNE is outlined in Figure 2. If after 72 hours, all blood cultures remain negative, laboratory testing can be expanded to include nonculturable and fastidious organisms, as outlined in the step-by-step approach for diagnosis of BCNE suggested in this statement. This may include prolonged incubation of blood cultures already sampled, serological testing for *C burnetii* and *Bartonella* spp (plus *Brucella* spp if the patient resides or resided in an endemic country), and metagenomics sequencing on plasma or whole blood.

Diagnostic Testing Is Typically Performed on Excised Cardiac Tissue or Prosthetic Material for Patients With Suspected BCNE

Evaluation by a cardiovascular surgeon is indicated when patients with suspected IE show signs of heart failure, severe valve dysfunction, paravalvular abscess or cardiac fistulas, recurrent pulmonary or systemic embolization, large mobile vegetations, or persistent sepsis despite adequate antibiotic therapy for >7 days.[2] Early consultation with the cardiovascular surgical team is critical to achieve optimal timing of surgical intervention. A discussion with the surgical team about what specific diagnostic tests to obtain on excised cardiac tissues is necessary to ensure optimal diagnostic testing is performed.

The inclusion of evidence of IE documented by direct inspection during cardiac surgery is important, particularly if further pathologic or microbiologic confirmation is not available. Therefore, "Surgical Evidence" was included as a new major criterion in the 2023 Duke-ISCVID definition.[1] Evaluation of excised cardiac valve tissue or prosthetic material offers an important opportunity for the definitive diagnosis of IE with detection of pathogens by culture or molecular diagnosis. At a minimum, excised specimens are sent for microbial stains and cultures (bacterial, fungal, mycobacterial) and pathologic evaluation. Pathologic evaluation may include gross anatomical review, immunohistochemical analysis for *T whipplei, Bartonella* spp, *C burnetii*, and fluorescence in situ hybridization where available[14]; it is also helpful to identify noninfectious causes of IE. When possible, PCR for fastidious organisms (Table 1) is also performed. In clinical settings where broad-range PCR/sequencing is available, tissue is sent for testing.[15] As for all diagnostic tests, false-positive results may occur, and findings need to be interpreted in the clinical context of the patient.

Approach to Empirical Management of BCNE

Consensus is currently lacking about the optimal empiric antibiotic regimen for cases of BCNE, highlighting the importance of consulting with an infectious diseases specialist. Within this writing group, there were divergent practices. Antibiotic regimens used in the United States and Europe vary due to discrepancies in antibiotic availability, organism prevalence, and resistant patterns, and valve type affected and timing of presentation (eg, acute versus subacute). A clear distinction must be made between BCNE in patients who received antibiotics before blood cultures were sampled, when empirical treatment should primarily target methicillin-susceptible staphylococci, streptococci, and enterococci, and BCNE in patients not previously treated with antibiotics, where empirical treatment active on fastidious organisms such as *Bartonella* spp, *C burnetii*, and *T whipplei* may be warranted. Figure 3 provides an overview of published[2,3,16] suggested empirical antibiotic treatments for BCNE.

After molecular, serologic, or pathologic test results identify a specific pathogen, empiric antimicrobial therapy can be subsequently tailored in accordance with pathogen-specific treatment,[2,3] always in consultation with an infectious diseases expert to assist with interpretation of test results and to guide treatment decisions.

In selected BCNE cases where the confirmed causative pathogen is either methicillin-susceptible staphylococci, streptococci, or enterococci, transitioning to highly bioavailable oral therapy may be considered in patients who are stable and meet the POET (Partial Oral Treatment of Endocarditis) trial criteria.[17]

CLINICAL SCENARIO

BCNE Due to Fastidious Microorganisms (*C burnetii*, *Bartonella* spp, *Brucella* spp, Whipple, Mycobacterial, and Fungal)

A 22-year-old man with a history of corrective surgery for D-transposition of the great vessels, ventricular septal defect, and pulmonary stenosis as a child, and a Contegra graft in place was evaluated for fever. He reported recurring fever, night sweats, and a 4-kg weight loss over the past 4 months after completing a course of azithromycin for suspected atypical pneumonia. TEE showed a torn medial pouch and small mobile vegetations on the distal end of the right ventricle to pulmonary artery Contegra graft. Three sets of blood cultures collected at admission did not identify a causative pathogen. Serology returned positive for *C burnetii* (phase I titer 1:2096; phase II titer 1:800).

Fastidious organisms remain a primary challenge in the diagnosis of BCNE. Incorporating epidemiologic factors, including travel, occupational, and social

Table 1. Most Common Fastidious Causes of Blood Culture–Negative Endocarditis With Epidemiological Clues, Diagnostic Methods, and Management Strategies

Microorganism	Epidemiological clues	Diagnostic methods	Management strategy	Comments/follow-up
Coxiella burnetii	Contact with contaminated milk or infected farm animals, including living near farms with infected animals Abattoir worker or other occupational exposure	Serology (IgG phase 1>1:800), tissue culture, tissue IHC, PCR from tissue specimens, PCR (including cell-free) from blood/serum specimens In rare cases, patients will have a phase I IgG of <1:800	Doxycycline* plus hydroxychloroquine† or doxycycline* plus quinolone‡ Treatment duration is for least 18 mo, with serial monitoring of serology Treatment duration may need to be extended based on clinical response Surgical management may be necessary Exclusion of G6PD deficiency	Other manifestations of Q fever include vascular infection (predominantly aortic) and abscesses Regular retinal exams while taking hydroxychloroquine to assess for possible retinal toxicity Treatment can be discontinued after at least 18 mo. A 4-fold decline in phase I IgG titers suggests a good response to treatment. However, titers will remain elevated to >1:800 in some patients despite clinical cure and absence of active disease
Bartonella spp	Exposure to body lice, homelessness or housing insecurity, rural residence without running water (*B quintana*) Exposure to cats (particularly kittens) and fleas (*B henselae*)	Serology, targeted PCR of whole blood, broad range PCR and metagenomics, tissue culture, IHC Blood culture (requires special conditions and prolonged intubation of at least 2 wk)	Doxycycline* (preferred) or azithromycin (12 wk) plus rifampin (6 wk)	Gentamicin§ is also effective against *Bartonella* spp but is not preferred due to elevated risk of immune complex-mediated glomerulonephritis in *Bartonella* spp BCNE
Tropheryma whipplei	Living in a rural area and occupational exposure to soil or animals Constitutional symptoms like fever, fatigue, weight loss, night sweats, joint pain, pleural effusion, cognitive impairment, and diarrhea	IHC of tissue, targeted PCR, broad-range PCR, metagenomics sequencing	Initial phase: 4-wk course of IV penicillin G‖ or ceftriaxone¶ Maintenance phase, at least 11 mo: oral trimethoprim/sulfamethoxazole If sulfa allergy: doxycycline plus hydroxychloroquine Surgical management may be necessary	Hepatitis, cytopenias Be aware of the development of Jarish-Herxheimer reaction (especially with penicillin G therapy) and increased rates of clinical failure/relapse
Slowly growing mycobacteria (*Mycobacterium chimaera*)	Previous cardiopulmonary bypass surgery with valve replacement	Mycobacterial blood or valve cultures, mycobacterial species–specific PCR, metagenomics, pathology	Treatment based on susceptibilities in conjunction with a local health care professional At least 24 mo Surgery	Monitoring ophthalmology Hepatitis Drug interactions
Rapidly growing atypical mycobacteria	Use of bioprosthetic material at index surgery	Mycobacterial blood or valve cultures, mycobacterial species–specific PCR, metagenomics, pathology	Treatment based on susceptibilities in conjunction with a local health care professional At least 24 mo Surgery	Monitoring ophthalmology Hepatitis Drug interactions
Fungi	Injection drug use Intracardiovascular medical devices Immunocompromised Prosthetic valve placement	Blood cultures, serology (aspergillus antigen, β-D glucan), pathology, broad-range PCR, metagenomics sequencing	Treatment regimen varies, depending on the organism isolated	Consult infectious diseases specialist

For less common causes of blood culture–negative endocarditis and their epidemiological features, see Table 6 in the 2015 American Heart Association scientific statement "Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications."[2] BCNE indicates blood culture–negative endocarditis; G6PD, glucose-6-phosphate dehydrogenase; IgG, immunoglobulin G; IHC, immunohistochemistry; and PCR, polymerase chain reaction.

*Doxycycline dose is 100 mg q 12 h oral.

†Hydroxychloroquine dose is 200 mg q 8 h oral.

‡Quinolones include ciprofloxacin 500 mg q 12 h oral, levofloxacin 500 mg q 12 h oral, or moxifloxacin 400 mg q d oral.

§Gentamicin dose is 3 mg/kg q 24 h intravenous.

‖Penicillin G dose is 2 million units q 4 h.

¶Ceftriaxone dose is 2 g q 24 h.

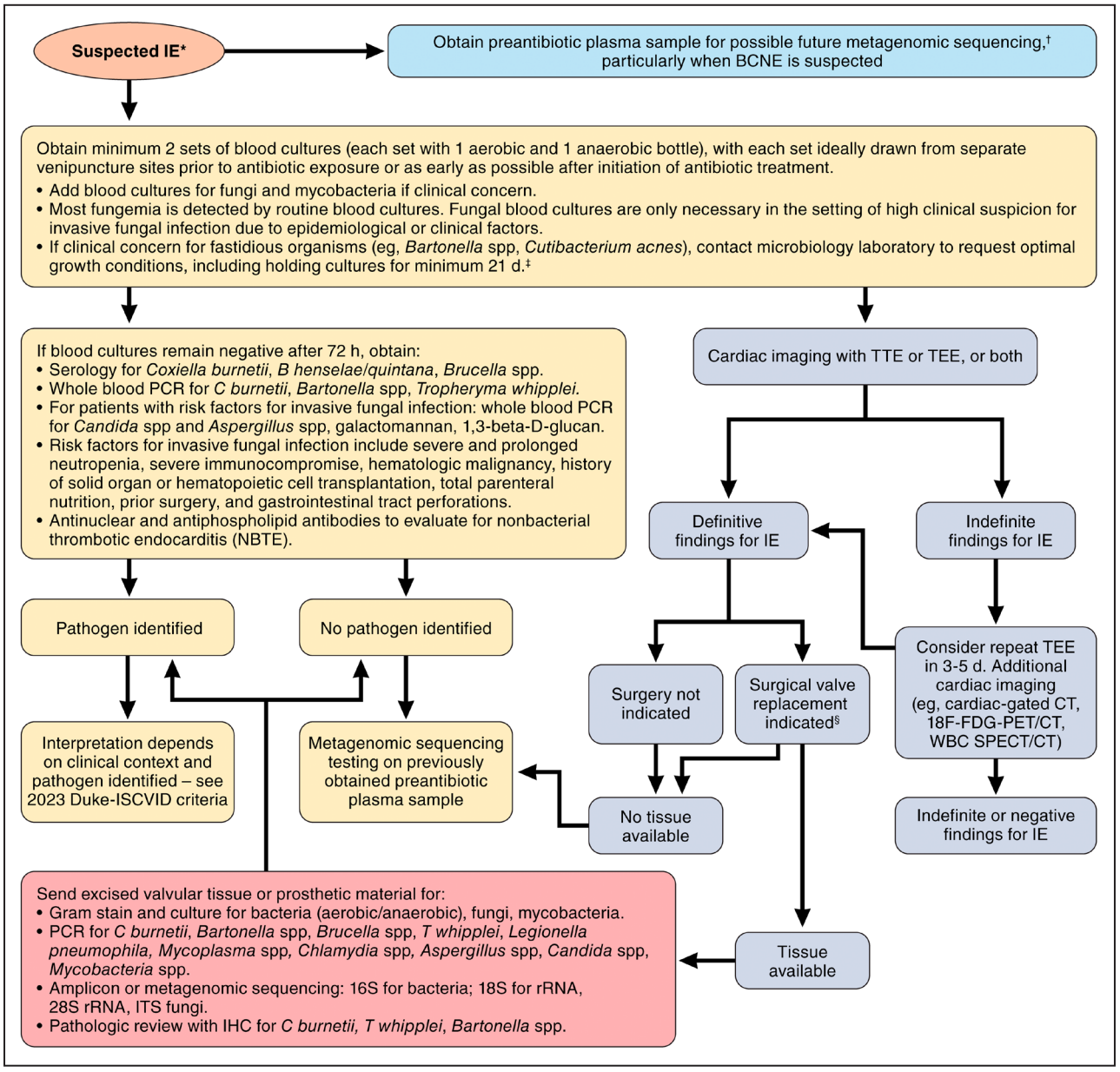

Figure 2. Suggested diagnostic algorithm for blood culture–negative infective endocarditis.
18F-FDG-PET/CT indicates fluorine-18-fluorodeoxyglucose positron-emission tomography/computed tomography; BCNE, blood culture–negative endocarditis; CT, computed tomography; IE, infective endocarditis; IHC, immunohistochemistry; ITS, internal transcribed spacer; NBTE, nonbacterial thrombotic endocarditis; PCR, polymerase chain reaction; TEE, transesophageal echocardiogram; TTE, transthoracic echocardiogram; and WBC SPECT/CT, white blood cell single-photon emission tomography/computed tomography. *IE should be considered in patients with symptoms of endocarditis (eg, new glomerulonephritis, cerebrovascular event, Janeway lesions/Osler nodes, prolonged unexplained fevers, new heart failure, new valvular dysfunction, new cardiac murmur), especially in patients with identifiable risks, including patient exposures (eg, recent surgery, history of intravenous drug use, homelessness, body lice infestation, animal exposure), anatomic risks (eg, congenital heart disease, prosthetic heart valves, implantable cardiac devices, valvular disease) or comorbid conditions (eg, immunosuppression, hemodialysis), and indwelling catheters. †Contact the microbiology laboratory for instructions on sample collection and specimen submission. ‡Communication with the laboratory is key in cases of BCNE. §Communication with the surgical team before an operative procedure is highly encouraged to ensure appropriate specimen collection and that diagnostic tests are submitted.

		USA-based regimens†		European-based regimens‡	
		Regimen	Dose and Route	Regimen	Dose and Route
NVE	Acute and Subacute	Vancomycin Plus Ceftriaxone	30-60 mg/kg per 24 h IV in 2 or 3 equally divided doses to achieve an AUC_{24}/MIC of 400/600 mg x h/L 2 g IV every 12 h	Community-acquired NVE or late PVE: Ampicillin Plus Ceftriaxone Or Flucloxacillin Plus Gentamicin‖	12 g per 24 h IV in 4-6 equally divided doses 2 g IV every 12 h 12 g per 24 h IV in 4-6 doses 3 mg/kg per 24 h IV or IM in 1 dose
PVE	Early	Vancomycin Plus Rifampin§ Plus Gentamicin‖ Plus Cefepime	30-60 mg/kg per 24 h IV in 2 or 3 equally divided doses to achieve an AUC_{24}/MIC of 400/600 mg x h/L 900 mg per 24 h IV or orally in 3 equally divided doses 3 mg/kg per 24 h IV or IM in 2 or 3 equally divided doses 2 g IV every 8 h	Nosocomial and non-nosocomial HCA-IE or early PVE: Vancomycin Or Daptomycin Plus Gentamicin‖ Plus Rifampin§	30-60 mg/kg per 24 h IV in 2 or 3 equally divided doses to achieve an AUC_{24}/MIC of 400/600 mg x h/L 10 mg/kg per day IV in 1 dose 3 mg/kg per 24 h IV or IM in 1 dose 900-1200 mg per 24 h IV or orally in 2 or 3 doses
	Late	Vancomycin Plus Ceftriaxone	30-60 mg/kg per 24 h IV in 2 or 3 equally divided doses to achieve an AUC_{24}/MIC of 400/600 mg x h/L 2 g IV every 12 h		

Figure 3. Empirical antibiotic treatment options for blood culture–negative endocarditis: literature-based recommendations.*[2,3]

AUC_{24}/MIC indicates area under the serum concentration versus time curve for 0 to 24 hours/minimum inhibitory concentration; BCNE, blood culture–negative endocarditis; HCA-IE, health care–associated infective endocarditis; NVE, native valve endocarditis; and PVE, prosthetic valve endocarditis. *A clear distinction must be made between BCNE in patients who received antibiotics before blood cultures were sampled, when empirical treatment should primarily target methicillin-susceptible staphylococci, streptococci, and enterococci, and BCNE in patients not previously treated with antibiotics, where empirical treatment active on fastidious organisms such as *Bartonella* spp, *Coxiella burnetii*, and *Tropheryma whipplei* may be warranted. Current recommendations are only based on expert opinion and clinical practice. †USA-based regimens per the 2015 AHA scientific statement "Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications."[2] Some members of the writing group use a combination of vancomycin and ceftriaxone only to treat NVE. ‡European-based regimens per the 2023 European Society of Cardiology guidelines for the management of endocarditis.[3] In France, amoxicillin plus cefazolin is the preferred empirical treatment for acutely ill patients with community-acquired native valve or late prosthetic valve IE, because it allows optimal coverage of the 3 major pathogens in this setting: cefazolin for methicillin-susceptible staphylococci, amoxicillin for penicillin-susceptible streptococci, and the synergistic effect of both for *Enterococcus faecalis*. §The writing group favors adding rifampin once source control is attained. Some writing group members include rifampin in late PVE. ‖Gentamicin is often avoided by writing group members due to risk of toxicity and side effects.

histories, can offer important clinical clues to support tailored diagnostic testing (see Table 1).

Patients at Increased Risk of Infection With *C burnetii*

Most patients acquire *C burnetii* through inhalation of contaminated dust or other aerosols.[18] Although people who work with livestock or livestock processing are at increased risk, most patients who are diagnosed with Q fever do not report any recent contact with livestock.[19] *C burnetii* is a fastidious organism that can persist in the environment for years. Although residence within 5 km of infected farms has been associated with increased risk of developing Q fever due to the prolonged environmental persistence, risk of infection to humans persists after the epizootic has resolved.[20] Patients with congenital heart defects, heart valve defects, cardiac or vascular implants, and people who are immunocompromised are at increased risk of developing IE due to chronic Q fever.[18]

Discontinuing Treatment for *C burnetii*

The decision to discontinue therapy for a suspected cure is challenging and requires evaluation of both the

clinical and serologic response. Generally, at least a 4-fold decline in phase I IgG (immunoglobulin G) (with levels <1:200) would be expected in a patient with a clinical cure after an 18-month course of treatment (see Table 1).[21,22] However, some individuals may exhibit persistently elevated titers (including titers >1:1024) despite successful therapy. In such circumstances, consultation with an infectious diseases specialist is critical. The writing group suggests assessing the patient's symptoms and overall clinical condition. If the patient is asymptomatic at the time of follow-up, and symptoms have resolved despite elevated titers, it may not indicate treatment failure.[23] Ultimately, the decision to discontinue treatment and continue monitoring is individualized based on the patient's clinical status and the clinical judgment of the treating physician. For patients with prosthetic valves or nonbiological implants, surgical removal of the prosthetic material may be required; if this is not feasible, then prolonged or lifelong therapy is worth considering.[18,24]

Patients at Increased Risk for BCNE Due to *Bartonella* spp

Bartonellosis has emerged as a major cause of BCNE.[25] The 2 most common *Bartonella* spp to cause human disease are *B henselae* and *B quintana*. *B henselae* is transmitted by infected flea feces, most often spread to humans by cat scratches. Conversely, *B quintana* is transmitted by the human body louse. Patients with a history of inconsistent access to laundry and bathing (ie, people experiencing homelessness[26] or housing instability or those who reside in remote communities without running water[27]) are at increased risk of body lice infestation and *B quintana* infection. Asking all patients about animal exposures and about current and prior housing status is critical to inform assessment for bartonellosis risk.

Potential Challenges With Diagnosing *Brucella* spp Endocarditis Using Serological Tests

Agglutination serves as a confirmatory serological test for diagnosing brucellosis. However, early testing or the presence of blocking antibodies can result in failure to detect brucellosis.[28,29] Agglutination tests are to be avoided for complicated, chronic, or neurologic cases, and false-negative results can occur when there are no agglutinating antibodies. The principal immunodeterminant and virulence factor in *Brucella* spp is the smooth lipopolysaccharide located on the outer cell membrane, which shares antigenic similarities with lipopolysaccharides found in other gram-negative rods.[30,31] False-positive *Brucella* test results may arise from antibody cross-reactivity with *Escherichia coli* O157, *Francisella tularensis*, *Moraxella phenylpyruvica*, *Yersinia enterocolitica*, specific *Salmonella* serotypes, and in individuals vaccinated against *Vibrio cholerae*.[32,33] False-positive results may lead to unnecessary brucellosis treatment. Although serological tests lack specificity, they are pivotal in resource-poor settings.[34]

Relapsing Infection Due to *Brucella* spp

Challenges in managing brucellosis include the need for ongoing treatment to prevent recurrence, with relapse rates estimated at 5% to 15%. Relapses in brucellosis are primarily attributed to inadequate antibiotic selection, short treatment durations, and noncompliance. Studies suggest that *Brucella* strains remain susceptible to doxycycline and rifampin, which are commonly used antibiotics, although reports of potential resistance to rifampin have been noted globally. The risk of selecting for rifampin- and streptomycin-resistant *Mycobacterium tuberculosis* in regions where tuberculosis is prevalent is also of concern.[35] Whole-genome sequencing has facilitated the identification of virulence and resistance genes in *Brucella* spp, but additional research at proteomic and transcriptomic levels is needed to fully understand resistance mechanisms.

For patients experiencing persistent symptoms beyond 1 year posttherapy and diagnosed as chronic brucellosis, extended antibiotic therapy offers little benefit, suggesting symptomatic management; however, immunomodulation may provide some relief. Asymptomatic individuals with positive serological tests, indicating subclinical brucellosis, often include professionally exposed workers who require careful monitoring but may not necessitate immediate treatment.

New Molecular Diagnostics and Metagenomic Sequencing That Will Affect the Diagnosis of Whipple Disease

With the advancements of molecular techniques, *T whipplei* is increasingly recognized as a significant cause of BCNE. The lack of serological tests for identifying *T whipplei* complicates the diagnosis of IE, underscoring the importance of newer molecular tests when available. The 2023 Duke-ISCVID criteria[1] now include *T whipplei* identification using sequencing technology, PCR, or amplicon/metagenomic sequencing from blood as a major criterion for IE.[36] The same molecular tests, such as PCR or amplicon/metagenomic sequencing, can also be applied to sterile sites other than cardiac tissue as a minor criterion.[1] Whipple disease is often diagnosed by detecting *T whipplei* in intestinal biopsies using histological stains such as periodic acid–Schiff stain and immunohistochemistry along with these molecular tests. With the recognition

of the significance of molecular biology in the 2023 Duke-ISCVID criteria,[1] it is likely that more cases of IE due to *T whipplei* will be identified worldwide.

Suspected Mycobacterial BCNE

Mycobacterial endocarditis is rare. However, *Mycobacterium chimaera*, a nontuberculous mycobacterium from the *Mycobacterium avium* complex, needs to be suspected in patients with delayed-onset prosthetic valve BCNE.[37] A large multicontinental outbreak,[38] with an estimated incidence of 156 to 282 cases per 100 000 population annually before preventive measures were implemented, has been attributed to *M chimaera*–contaminated heater-cooler devices used during cardiopulmonary bypass. Diagnostic and management strategies are outlined in Table 1. Treatment duration may be monitored by yearly PET/CT until complete resolution of metabolic hyperactivity occurs at infected sites.

The importance of rapidly proliferating nontuberculous mycobacteria in cardiac infections has sparked debates. *Mycobacterium chelonae* has been associated with prosthetic valve endocarditis in the past (Table 1).[39–41] The precise origins and routes of transmission remain uncertain, with potential factors including colonization of donor animals, contamination during tissue sampling, or introduction during manufacturing procedures. Nontuberculous mycobacteria bioprosthetic IE might be significantly underdiagnosed, because it resembles mechanical prosthesis dehiscence, thereby often escaping consideration for histological and bacteriological analyses.

Management and Prognosis for Suspected Fungal BCNE

Fungal IE is among the most difficult to treat and carries a dismal prognosis. Comprehensive investigations to ensure reliable microbiological documentation, through prolonged incubation of blood cultures, with or without specific media, and additional diagnostic testing including targeted nucleic acid amplification and the biomarkers (ie, 1,3-β-D-glucan and galactomannan) are important. Nucleic acid detection by PCR for both *Candida* spp and *Aspergillus* spp has proven to be both sensitive and specific for detection of invasive candidemia and aspergillosis, respectively.[42,43] 1,3-β-D-glucan and galactomannan may provide useful diagnostic and prognostic information in some cases,[44,45] but their usefulness in diagnosing and ruling out invasive fungal disease can be limited by suboptimal sensitivity and specificity.

Empirical treatment is usually not started when fungal IE is suspected, because optimal antifungal treatment tailored to the species involved and antimicrobial susceptibility testing is of utmost importance. However, given the high mortality without early effective treatment, antifungal medication must be started as soon as indirect clues for fungal IE are available (ie, elevated levels of 1,3-β-D-glucan or galactomannan). Early cardiac surgery with valvular replacement and excision of all infected tissues is critical. However, in selected cases of *Candida* endocarditis, when cardiac surgery would appear too risky, tailored antifungal treatment, including high-dose echinocandin for at least 6 weeks, followed by oral azoles for at least 2 years, has been associated with success rates of >50%. Fungal IE due to molds, primarily *Aspergillus* sp, are rare, with mortality rates of >80% in the literature (>95% in the absence of cardiac surgery).[46]

CLINICAL SCENARIO

BCNE With Negative Conventional Diagnostic Workup

A 40-year-old patient presented with a 1-week history of fever, fatigue, and arthralgias. The patient reported a recent history of gonorrhea treated with a single dose of ceftriaxone. Physical examination revealed a systolic murmur at the left lower sternal border. A transthoracic echocardiogram identified a 3-cm multilobular mass attached to the tricuspid valve with leaflet perforation. Despite the presence of vegetation, workup including bacterial, mycobacterial, and fungal blood cultures was negative. Additionally, serology for *Coxiella* and *Bartonella* spp was negative. No other risk factors were identified by history except for multiple sexual partners in the past year. Testing for sexually transmitted infections was negative, with the exception of a chlamydia/gonorrhea PCR in the urine, which was indeterminate. Metagenomics shotgun sequencing was detected on blood *Neisseria gonorrhoeae*.

Consideration of Shotgun Metagenomics Sequencing on Whole Blood in Patients With Suspected BCNE Due to Antibiotic Exposure Before Blood Culture Collection

In cases of suspected BCNE due to prior antibiotic therapy, the application of metagenomics sequencing may prove beneficial due to prolonged detectability of microbial DNA as compared with traditional culture methods. Identifying the organism early enables targeted therapy, which not only minimizes adverse drug events from broad-spectrum treatment but also effectively reduces the bacterial load if surgical procedures are necessary. However, caution is advised due to the risk of false-positive results or identification of contaminants. Therefore, interpretation of results requires the

involvement of infectious diseases and microbiology experts (see below). A notable constraint is the high and frequently unattainable costs of these technologies for many health care systems.

Optimal Timing for Obtaining Metagenomics Sequencing in Whole Blood in Patients With Suspected BCNE

Despite being less affected by recent antibiotic therapy compared with traditional cultures, the quantity of microbial DNA is reduced by effective antibiotics, and results can be negative after antibiotic exposure.[47,48] Additionally, the turnaround delays for available results due to sample processing, shipment to a sequencing facility, and analysis need to be carefully considered before ordering. The writing group favors a proactive approach by collecting an appropriate specimen for shotgun metagenomic sequencing as soon as BCNE is suspected. The sample can be stored under appropriate conditions in the local clinical laboratory, and submission for sequencing can be considered if conventional cultures and routine workup for BCNE yield negative results within the initial 72 hours.

Approach to Interpreting Results Obtained With Metagenomics Sequencing

Interpretation of sequencing results requires a multidisciplinary approach involving infectious disease and microbiology experts. According to the 2023 Duke-ISCVID criteria, only 3 pathogens (*C burnetii*, *Bartonella* spp, and *T whipplei*) identified by sequencing technology fulfill a major criterion for IE; nucleic acid–based testing that detects an organism not listed above from noncardiac tissue is now a minor criterion.[1] The writing group is of the opinion that if an organism that is unlikely to be a blood commensal or contaminant (eg, *Brucella* spp or *N gonorrhoeae*) is identified by metagenomic sequencing of whole blood/plasma in patients exhibiting symptoms compatible with BCNE, then the results are likely to be reliable and prompt consideration of a treatment targeted against the detected organism.

If multiple organisms are detected using these techniques, prioritizing pathogens known to cause IE is reasonable. It is important to emphasize that there is not a universally agreed-on microbial DNA quantification cutoff deemed relevant in clinical practice. Interpretation of microbial DNA abundance can be complex and contingent on several factors, including pathogen-specific factors, recent exposure to effective antibiotics, and the sensitivity of the sequencing technology. Therefore, relying solely on the reported microbial nucleic acid quantity is inadequate to determine the clinical relevance of an organism.

CLINICAL SCENARIO

BCNE With Equivocal Echocardiographic Findings

A 78-year-old male patient with aortic stenosis prompting transcatheter aortic valve replacement 1 year previously presented with fever and chills for the past 4 days. Blood cultures from admission remained negative for >48 hours. TEE revealed a severe paravalvular leak without vegetations. The patient underwent ^{18}F-FDG PET/CT that showed moderate fluorodeoxyglucose uptake involving the aortic valve.

Imaging Modalities for Diagnosis of IE

The importance of echocardiography in the diagnosis of IE has been well established. In recent years, the role of advanced imaging for the diagnosis of IE has received significant attention as an aid to increase both sensitivity and specificity, especially when echocardiographic findings are inconclusive. Revisions to IE diagnostic criteria and clinical guidelines have included the addition of advanced imaging, including cardiac-gated CT, ^{18}F-FDG PET/CT, and white blood cell single-photon emission tomography/CT findings in both major and minor criteria.[1–3]

Potential Advantages and Disadvantages of Advanced Imaging in the Diagnosis of IE

Each of these imaging modalities has advantages and disadvantages to echocardiography in the diagnosis of IE. Cardiac CT has similar sensitivity to TEE for detection of large vegetations, valve perforation, valve aneurysm, perivalvular abscess, fistula, pseudoaneurysm, and prosthetic valve dehiscence. A meta-analysis by Mahmood et al[49] on the use of ^{18}F-FDG PET/CT found a pooled sensitivity of 76.8% and specificity of 77.9% for native valve IE, and a sensitivity of 80.5% and specificity of 73.1% for prosthetic valve IE, and noted the potential to detect extracardiac sites of infections.[50] However, a more recent study[51] found that the sensitivity of ^{18}F-FDG PET/CT was only 22% in patients with native valve IE (compared with 93% for prosthetic valve IE). White blood cell single-photon emission tomography/CT also has high specificity for infection, but limited sensitivity, especially in native valve IE.[50] Both ^{18}F-FDG PET/CT and white blood cell single-photon emission tomography/CT provide a whole-body evaluation to detect extracardiac involvement, which may aid in the diagnosis of IE.

Advanced Imaging in Diagnosis of BCNE

Limited data are available on the use of advanced imaging in the diagnosis of BCNE. Moreover, these imaging

Table 2. Prevention of Blood Culture–Negative Endocarditis

How to avoid BCNE: messages to community and patients at higher risk
Directed to patients at higher risk
Directed education of patients with prosthetic valves or at risk for infective endocarditis: patient leaflet postcardiac surgery
Infective endocarditis prophylaxis for at-risk procedures
Schedule a biannual dental visit for oral hygiene treatment
Consult a dentist about a dental abscess or infection
Fever as a warning sign: obtain at least 2 sets of blood cultures before starting antibiotics
Maintain up-to-date recommended immunizations
To reduce risk of infection, consult a dermatologist to treat skin conditions that may lead to broken skin; consult a podiatrist for adequate foot care and early antiseptic for open skin cuts

BCNE indicates blood culture–negative endocarditis.

modalities are either not available or scarce at many centers. Our approach for patients with at least possible BCNE who have undergone TEE with inconclusive evidence is to proceed with advanced imaging. The presence of prosthetic material, recent surgery, perivalvular involvement, and extracardiac sites of infection will determine if cardiac CT, ^{18}F-FDG PET/CT, or white blood cell single-photon emission tomography/CT is needed. In some cases, >1 imaging modality may be required.

In the future, a synergistic approach combining diagnostic imaging modalities may not only expedite BCNE diagnosis but also refine treatment strategies, ensuring more favorable outcomes for patients. With improved diagnostics, the medical community anticipates a paradigm shift in managing BCNE, fostering a more nuanced and effective approach to this challenging condition.

FUTURE CONSIDERATIONS

The epidemiologic profile of IE is reflective of many factors, and this is equally true for BCNE. Because the predominant cause of BCNE is recent antibiotic exposure before blood culture collection, BCNE will likely continue to plague both patients and clinicians unless antibiotics are more thoughtfully prescribed. It is important to inform the community and high-risk patients both to consult their primary care physician and to get a minimum of 2 sets of blood cultures before commencing antibiotics (Table 2). Moreover, in some low- and middle-income countries, individuals obtain antibiotics without prescriptions. The risk of BCNE in low- and middle-income countries with easy antibiotic access is therefore compounded by diagnostic challenges due to limitations in advanced diagnostic techniques. In these limited-resource settings where laboratory testing and imaging are largely unavailable, it is critical to evaluate epidemiological factors that may be key in the selection of empiric antibiotic therapy.

It is conceivable that based on current and future advances in the clinical diagnostic laboratory coupled with an improvement in antimicrobial stewardship, the rates of BCNE will decline. This was speculated in a systematic review of IE epidemiology a decade ago, where the rate of BCNE based on findings that laboratory advances on the identification of pathogens may have accounted for a decrease in the BCNE rate over time (P<0.001).[52] More recent nationwide data from both Germany[53] and Denmark[54] also reported declines in BCNE prevalence among patients with IE. Thus, improvements in diagnosis and earlier effective treatment for patients with BCNE may become possible as laboratory molecular techniques (metagenomic testing, for example) become more available and affordable.

ARTICLE INFORMATION

The American Heart Association makes every effort to avoid any actual or potential conflicts of interest that may arise as a result of an outside relationship or a personal, professional, or business interest of a member of the writing panel. Specifically, all members of the writing group are required to complete and submit a Disclosure Questionnaire showing all such relationships that might be perceived as real or potential conflicts of interest.

This statement was approved by the American Heart Association Science Advisory and Coordinating Committee on October 9, 2024, and the American Heart Association Executive Committee on October 23, 2024. A copy of the document is available at https://professional.heart.org/statements by using either "Search for Guidelines & Statements" or the "Browse by Topic" area.

The American Heart Association requests that this document be cited as follows: DeSimone DC, Garrigos ZE, Marx GE, Tattevin P, Hasse B, McCormick DW, Hannan MM, Zuhlke LJ, Radke CS, Baddour LM; on behalf of the American Heart Association Council on Lifelong Congenital Heart Disease and Heart Health in the Young; Council on Clinical Cardiology; and Council on Quality of Care and Outcomes Research. Blood culture–negative endocarditis: a scientific statement from the American Heart Association. *J Am Heart Assoc*. 2025;14:e040218. doi: 10.1161/JAHA.124.040218

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Acknowledgments

Drs P Tattevin, B Hasse, and M Hannan were representatives of the International Society for Cardiovascular Infectious Diseases.

Disclosures

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This table represents the relationships of writing group members that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all members of the writing group are required to complete and submit. A relationship is considered to be "significant" if (1) the person receives $5000 or more during any 12-month period, or 5% or more of the person's gross income; or (2) the person owns 5% or more of the voting stock or share of the entity, or owns $5000 or more of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

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This table represents the relationships of reviewers that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all reviewers are required to complete and submit. A relationship is considered to be "significant" if (1) the person receives $5000 or more during any 12-month period, or 5% or more of the person's gross income; or (2) the person owns 5% or more of the voting stock or share of the entity, or owns $5000 or more of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

*Modest.

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