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Molecular Imaging and Diagnostic Advances in *Brucella* Endocarditis: A Narrative Review

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ABSTRACT

Background:
Brucella endocarditis is a rare but fatal manifestation of brucellosis. Delayed diagnosis often leads to serious complications such as extensive valvular destruction. Traditional diagnostic methods have limited accuracy; therefore, the use of advanced diagnostic techniques is recommended.

Methods:
This review evaluates traditional, molecular, and advanced diagnostic techniques for the diagnosis of Brucella endocarditis, including blood culture, tissue and valve cultures, serology, Shell Vial assay, FDG PET/CT, cardiac CT, and MRI. While traditional tools like blood culture and echocardiography offer limited sensitivity for early diagnosis of Brucella endocarditis, advanced modalities—including PCR, NGS, FDG PET/CT, cardiac CT, and MRI—provide high accuracy in detecting occult lesions, paravalvular involvement, and culture-negative infections, especially in difficult cases.

Results:
The review includes studies related to blood culture, tissue and valve cultures, serology, Shell Vial assay, FDG PET/CT, cardiac CT, and MRI. The combination of advanced techniques with traditional approaches can help form a more complete diagnostic pathway and improve clinical outcomes.

Conclusion:
In conclusion, early and accurate diagnosis is important for clinical interventions, prevention of complications and decrease mortality. Wider access to modern molecular and imaging tools in endemic regions and high-quality studies on advanced diagnosis are needed for improvement of Brucella endocarditis survival outcomes. A structured, multimodality diagnostic strategy can have a key role in reducing mortality and improving long-term outcomes in patients with Brucella endocarditis.

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Introduction

Brucella endocarditis is a rare but fatal manifestation of brucellosis. Coccobacilli are Gram-negative intracellular bacteria of the genus Brucella. Coccobacillus causes brucellosis in endemic regions such as the Persian Gulf, Middle East, and Africa. Although the incidence of endocarditis in brucellosis patients is low, endocarditis is the most common cause of mortality in affected patients (1). Evidence suggests that Brucella endocarditis frequently results in irreversible and lethal cardiac and systemic complications, such as destruction of heart valves, intracardiac abscesses and fistulas into pericardial space, myocarditis and severe complications like systemic embolization with multiorgan damage. Some examples of systemic embolization are Myocardial infarction, acute renal failure, Roth spots, spleen and hepatic segmental infarctions (2).

Previous studies have shown that early diagnosis of this serious complication, infective endocarditis, and effective medical and surgical treatment reduce mortality and morbidities (3). This illustrates the importance of early detection of Brucellosis, however, there are no specific signs and symptoms that reliably indicate early diagnosis (4). Blood culture is the "gold standard" for diagnosing Brucella infection by confirming the organism, while its sensitivity varies from 15% to 70% based on the phase of the illness and laboratory techniques (5-7). Moreover, the other intervening factors are the prior antibiotics therapy and fastidious nature of Brucella needs relative clues that reduce the sensitivity of blood culture. This necessitates frequent samples and sometimes other sample sources, such as bone marrow (5, 7, 8). Although automated blood culture systems (e.g., BACTEC, BacT/Alert) have reduced diagnostic time to 4 days, diagnostic accuracy is affected by the bacterial load or biosafety conditions (5,9). Despite this, there are limiting factors for the widespread use of the automated blood culture system, including the labor-intensive process, costs and biosafety conditions.

Regarding traditional imaging modalities, echocardiography plays a crucial role in the evaluation of suspected heart involvement. Echocardiography is a fundamental traditional diagnostic tool for detecting vegetations and intracardiac complications of Brucella endocarditis (7, 10). However, the low sensitivity of TTE in diagnosis of small vegetations (<5mm) reported about 25–46% necessitates Transesophageal echocardiography which boosts both sensitivity and specificity of diagnosis (93–96%) as well as confirming the serious intra-cardiac complications (7,10-11). Vegetations, the hallmark of endocarditis, predominantly affect the mitral and aortic valves, often causing acute regurgitation. Serious complications such as fistulas are common, and a wide range of structural abnormalities may occur. TEE is strongly recommended in patients with non-diagnostic TTE and high clinical suspicion for infective endocarditis. Furthermore, TEE is recommended in all patients with a positive TTE for detailed evaluation of cardiac complications caused by vegetation. However, there are a number of diagnostic limitations of TEE, including the early stage of disease and multiple prosthetic valves transthoracic and leaflet perforation which surgical exploration is required for accurate definition (10, 12-16). Diagnosing prosthetic valve endocarditis remains a significant challenge, and TEE is recommended over TTE due to the latter's insufficient accuracy in detecting perivalvular complications, including abscess, pseudoaneurysm, partial

prosthesis dehiscence, and fistula. A systematic review reported the most common type of prosthesis mentioned the mechanical prosthesis (84.3%) and aortic valve and mitral valve prosthesis were affected in most patients (52.9% and 35.3%, respectively). In this systematic review, echocardiography confirmed diagnosis in 93% of patients. This review shows that the echocardiography is not a powerful diagnostic tool in all patients (17). In conclusion, Blood culture and echocardiography as the traditional diagnostic tools have limited sensitivity for early diagnosis of Brucella endocarditis. These limitations increase the risk of life-threatening cardiac complications and death. Therefore, this review focuses on the limitations and benefits of traditional diagnostic techniques and advanced molecular and imaging techniques. Finally, we review each diagnostic modality and compare their utility in the early and accurate diagnosis of Brucella endocarditis. We hope that this review will enhance awareness of advanced diagnostic techniques and contribute to improved patient outcomes in Brucella endocarditis.

Methods

This review used a narrative approach. Articles from PubMed, Scopus, Web of Science, and Google Scholar were searched. The keywords included Brucella endocarditis, diagnosis, echocardiography, PET/CT, PCR, and molecular imaging. English papers with clinical or diagnostic focus were included. Non-English papers and studies without full text were excluded. Data were collected on diagnostic methods, accuracy, and main findings. The results were arranged in sections about traditional tools, molecular imaging, and advanced diagnostic methods.

Diagnostic Evidence for Brucella Endocarditis

Epidemiology of Brucella Endocarditis (BE)

Brucella endocarditis (BE) is a rare but serious complication of brucellosis. An overall global prevalence of Brucella endocarditis (BE) is 1–1.5% among infected patients [18]. BE is a major cause of mortality in brucellosis with reported mortality rates between 20% and 40% in recent studies [3, 18] .Brucella endocarditis predominantly affects middle-aged men as a result of occupational exposure patterns and comorbidities [3]. BE is most common in endemic regions, such as the Mediterranean Basin, Middle East, Latin America, and parts of Asia, where exposure to livestock and unpasteurized dairy products is frequent (18-19). Main risk factors include pre-existing valvular heart disease, prosthetic valves, and close contact with infected animals or dairy products (3). Brucella melitensis is the most commonly implicated pathogen, attributed to its high virulence and endovascular tropism[6, 18]. The aortic valve is most frequently affected, followed by the mitral valve[3].Diagnosis is often delayed because of insidious onset, nonspecific symptoms, and potential negative blood cultures, necessitating reliance on serology, molecular testing, and echocardiography (18-19). Delayed diagnosis significantly increases mortality and the need for surgical valve replacement (3, 6).

Traditional Diagnostic Approaches of Brucella Endocarditis

History & Lab data in diagnosis of Brucella Endocarditis

Brucella endocarditis (BE) is a major cause of mortality in brucellosis patients (1, 20). Brucellosis has three phases of presentation: acute (<2 months), subacute (2–12 months), and chronic. As a multisystem disease, brucellosis presents with a wide spectrum of clinical severity that varies from mild to fulminant forms, with an overall morbidity rate of 30–40%. The most common symptoms include fever, arthralgia, weakness, hepatosplenomegaly, and other constitutional signs. The incubation period is approximately 2–6 weeks, however, some patients may experience a longer course of disease (Fig. 1).

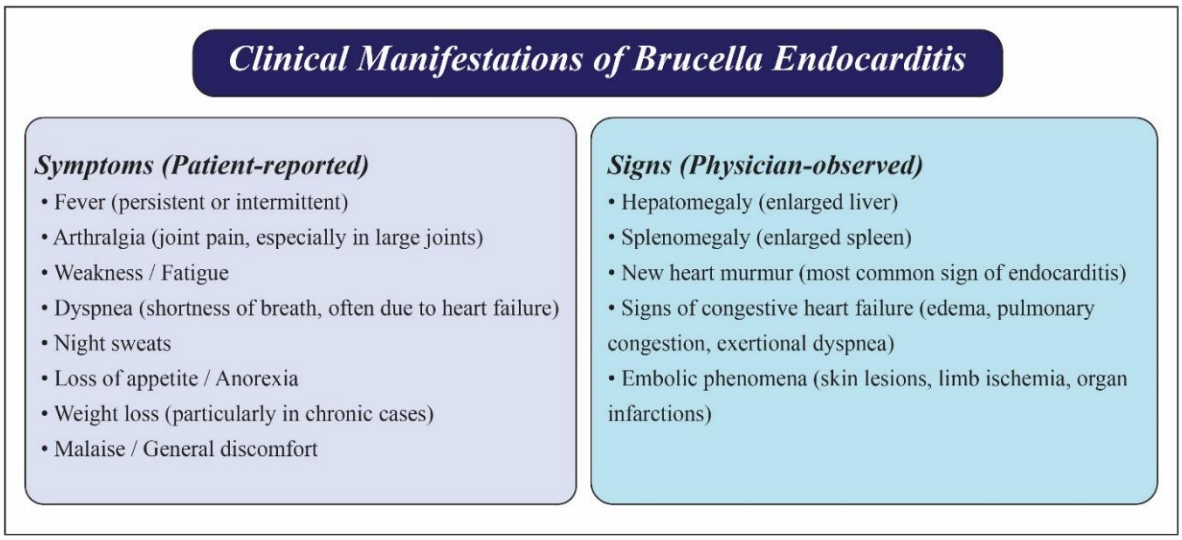

Figure 1. Clinical Manifestations of Brucella Endocarditis

Extracardiac complications such as sepsis, septic shock, renal failure, pneumonia, disseminated pulmonary abscess, liver abscess, sacroiliitis, and encephalopathy have been reported (7, 21). Cardiac involvement may present as pericarditis, myocarditis and infective endocarditis (Fig. 2).

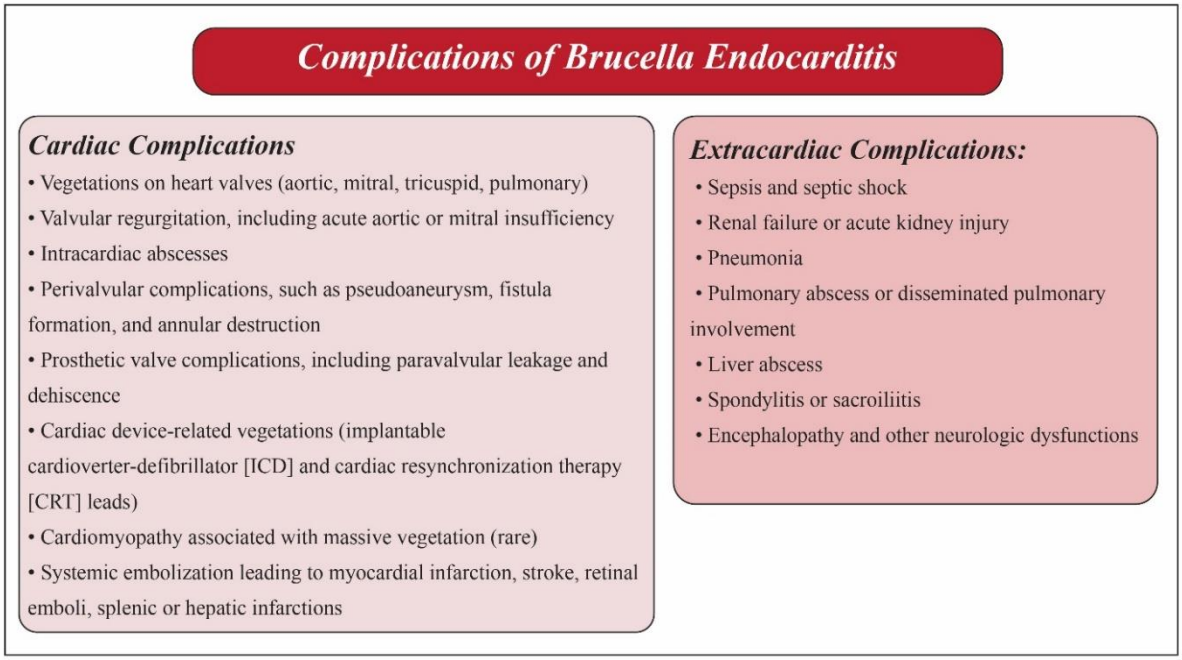

Figure 2. Complications of Brucella Endocarditis

Dyspnea as the most common cardiac symptom is due to congestive heart failure (CHF), occurring in 75–90% of patients, while a new murmur is the most common clinical sign in endocarditis (22). The incidence of embolic events is not higher than that observed with other causative pathogens (23-24). Accurate diagnosis requires a combination of history, clinical examination, and imaging evaluation. Echocardiography, especially TTE and TEE when needed, plays a key role in detecting vegetations and intracardiac complications (7, 10,23-24). Diagnosis of infective

endocarditis in cases with causative organisms from fastidious or slow-growth bacteria remains a major clinical challenge, as these organisms require special media and specific conditions (25). For years, the Beth Israel criteria served as the main diagnostic reference (26), but since 1994, the Duke Endocarditis Service expanded the criteria to include echocardiographic findings as well as clinical and laboratory data (37). Newer criteria proposed the inclusion of Coxiella burnetii serology or culture as major criteria (27-28). According to the Duke criteria, definite infective endocarditis is diagnosed when: (1) a microorganism is identified from a vegetation, embolus, or intracardiac abscess by culture or histology; (2) histopathology confirms active endocarditis in a vegetation or intracardiac abscess; or (3) two major criteria, one major plus three minor criteria, or five minor criteria are met.

The diagnosis is rejected if an alternative diagnosis fully explains the symptoms, fever subsides within four days of antibiotics, or no pathological evidence is found during surgery or autopsy after ≤4 days of antibiotic treatment. Therefore, to explain this issue, it is emphasized that the traditional diagnostic tools for detection of Brucellosis, including blood culture and echocardiographic imaging modalities, have limited sensitivity and specificity for diagnosis. This leads to under-diagnosis of these patients resulting in the high probability of drastic complications as mentioned before (Table 1).

Table 1. Modified Duke Criteria Proposed by 2023 Duke-International Society for Cardiovascular Infectious Diseases Infective Endocarditis (IE) Criteria

Category	Criteria	Definition / Notes
Major Criteria	Positive blood culture	Isolation of Brucella spp. from ≥2 separate blood cultures, or a single positive culture with compatible clinical features.
	Evidence of endocardial involvement	Echocardiography: vegetations, abscess, prosthetic valve dehiscence, or new valvular regurgitation. FDG PET/CT, cardiac CT, or MRI may support diagnosis.
Minor Criteria	Predisposing heart condition or injection drug use	Pre-existing valvular disease, prosthetic valves, or other risk factors.
	Fever	≥38°C (≥100.4°F).
	Vascular phenomena	Embolism, infarction, or mycotic aneurysm.
	Immunologic phenomena	Positive serology (e.g., Wright agglutination, Coombs), glomerulonephritis, or Roth spots.
	Microbiological evidence	Positive serology or PCR/NGS for Brucella spp. not fulfilling major criterion.
	Echocardiographic minor findings	Suspicious mass or small vegetation not meeting major criterion.

Brucella spp. is classified as ACDP category 3 pathogens and therefore require handling in a containment level 3 laboratory (20). These bacteria grow slowly in artificial culture and therefore need prolonged incubation periods. Blood and bone marrow cultures must be incubated for at least three weeks and require broth-based selective media such as trypticase soy agar, Brucella agar, or serum dextrose liver infusion agar (21). Growth occurs in commercial medium in the BACTEC system, and the Casteñeda technique facilitates prolonged incubation with a vertical slope of agar and a liquid phase (30). In cases of Brucella endocarditis, bacterial isolation presents a major

challenge due to the organism's slow growth and fastidious nature. Standard blood culture methods often yield negative results and necessitate manual monophasic techniques with extended incubation periods up to 30 days and repeated subcultures to maximize recovery (31-33). Limitations include large incubation space, labor-intensive procedures, and delayed confirmation of diagnosis (31-33). This limitation explains the shortcomings of traditional diagnostic tools for Brucellosis. Blood culture and echocardiographic imaging modalities have limited sensitivity and specificity for early diagnosis of this pathogen. This leads to under-diagnosis of these patients resulting in the high probability of severe complications as mentioned before.

Blood culture is the diagnostic gold standard for providing direct bacterial isolation. The sensitivity of blood culture varies from 15% to 70% based on the phase of the illness and laboratory techniques. Furthermore, the diagnostic sensitivity of blood culture is even lower (5–20%) due to the fastidious nature of Brucella species, slow growth (2–6 weeks), and prolonged incubation requirement often more than 7 days, which frequently results in false-negative blood culture results in routine laboratory workflows (5,7,19,34-35). Moreover, prior antibiotic therapy and the intracellular nature of Brucella spp. are major factors for the limited sensitivity of blood culture. This necessitates frequent samples and sometimes other sample sources, such as bone marrow (5,7-8). Persisting the low sensitivity of blood culture as a conventional diagnostic tool in Brucella endocarditis is the major limitation of traditional diagnostic tools. Although automated blood culture systems (e.g., BACTEC, BacT/Alert) have reduced diagnostic time to 4 days, diagnostic accuracy is affected by bacterial load, which frequently results in false-negative blood culture results (5,9). However, there are limiting factors for the worldwide usage of the automated blood culture system, including the labor-intensive process, costs, and biosafety conditions. Serologic tests are major diagnostic tools that offer more sensitivity but lower specificity compared with blood culture (36). Common serological diagnostic methods include ELISA, indirect immunofluorescence, Rose Bengal, and Wright agglutination tests, and ELISA provides the highest sensitivity and specificity. A Wright agglutination titer >1:160, a Coombs anti-Brucella test >1:320, or an indirect immunofluorescence titer >1:512 are considered diagnostic. The 2-mercaptoethanol Wright test is applied in the detection of relapse. However, due to lower specificity of serologic tests in endemic regions, serologic results should be interpreted cautiously (37-39).

Echocardiography plays a crucial role in the evaluation of suspected heart involvement. Echocardiography is a fundamental traditional diagnostic tool for detecting vegetations and intracardiac complications of Brucella endocarditis, and the aortic valve is the most frequently involved, ranging from 29% to 75%, followed by the mitral valve (40). Secondary infection of a pre-damaged mitral valve is more common than primary infection of the aortic valve. Prosthetic valve endocarditis contributes about 8.3% of cases (7,41-42). Transthoracic echocardiogram as a first-line imaging provides a wide range of information regarding the structure of heart valves, cardiac performance, and intracardiac complications (7). Vegetation on the valves, abscess formation, and destruction of valves are the major cardiac complications in Brucella endocarditis (11). However, the low sensitivity of TTE in the diagnosis of small vegetation (<5 mm) is reported about 25–46%, which necessitates Transesophageal echocardiography that boosts both sensitivity and specificity of diagnosis (93–96%) as well as confirming the serious intra-cardiac complications (10-11). Vegetation in endocarditis typically affects the aortic and mitral valves. They commonly cause acute regurgitation. Serious intracardiac complications such as fistula may also occur. TEE is strongly recommended in patients with non-diagnostic TTE and high clinical suspicion for infective endocarditis. Furthermore, TEE is recommended in all patients with a positive TTE for detailed evaluation of cardiac complications caused by vegetation. However, there are a number of diagnostic limitations of TEE, including early stage of the disease, multiple

prosthetic valves, inconclusive TTE, and drastic intracardiac complications like leaflet perforation for which surgical exploration is required for accurate definition (10,12-16).

Aortic insufficiency is the dominant lesion in the patients, but a wide range of cardiac involvements have been reported (20,43). Echocardiography often shows large, mobile vegetations—often exceeding 10 mm—on the aortic or mitral valves, resulting in severe regurgitation. Perivalvular complications—including abscesses, pseudoaneurysms, and fistulous tracts—are relatively common, especially in prosthetic valve infections. Paravalvular leakage and annular destruction have been observed, underscoring the aggressive, tissue-destructive nature of Brucella spp. infection. Intracardiac abscesses are frequently visualized as echo-free spaces adjacent to the annulus, sometimes extending toward the aortic root or left ventricular outflow tract. Compared to other invasive species such as staphylococci and streptococci, BE with extensive calcification and fibrosis often results in delayed diagnosis (44). It should be mentioned that valvular complications are not necessarily acute and may also occur in a chronic setting, which can lead to valve leaflet perforation and cardiac dilation (45). In a systematic review of 207 Brucella endocarditis cases, the aortic and mitral valves were the most frequent complications (58% and 19.8%, respectively), and 70% of patients had underlying heart lesions, like aortic valve insufficiency and rheumatic valvular disease (21.7% and 17.4%, respectively). Intra-cardiac abscess and embolism were the most common complications. In 2021, the new presentation of Brucella endocarditis as a pseudoaneurysm of the ascending aorta was reported in patients with bicuspid aorta diagnosed by echocardiography (46). Brucella endocarditis may result in post-surgical complications. It was reported in a patient with pseudoaneurysm after Bentall surgery, which resulted in acute neurological symptoms. TTE revealed an aortic pseudoaneurysm at the anterior and right side of the aorta extending from 9 O'clock to 1 O'clock in the short axis view, communicating with LVOT anteriorly. TEE finding showed a circumferential mass measuring 18 mm in diameter with left ventricular outflow tract communication (47). Brucella endocarditis may appear in patients with congenital heart disease. A case with Brucella endocarditis in an atrial septal defect diagnosed by echocardiography showed a secundum ASD with large vegetations of 0.5×1 cm arising from the rim of the ASD (30). In a five-year, single-center retrospective evaluation of infective endocarditis in children from 2016 to 2022, congenital heart disease was found as the dominant underlying disease like Tetralogy of Fallot, atrioventricular septal defect, truncus arteriosus, ventricular septal defect, and bicuspid aortic valve, following acquired heart disease (46% and 15%, respectively). The other predisposing factors were immunosuppressive therapy in children suffering from acute leukemia and rhabdomyosarcoma (48). In 2021, a rare case of endocarditis and spondylitis was reported due to Brucella melitensis biovar 3. Mitral valve vegetation and insufficiency led to heart failure and cold abscesses on both sides of the psoas major muscles were the predominant complications of the patient (49). Pulmonary valve endocarditis is another rare complication of brucellosis diagnosed by echocardiography which revealed 15×6 mm vegetation on the structurally normal pulmonary valve (50).

Moreover, the presence of cardiomyopathy and IE is a very rare presentation of brucellosis. In 2023, a case of massive cardiac vegetation associated with cardiomyopathy was diagnosed by trans-thoracic echocardiography. The patient had an ejection fraction =45%, and large vegetation on the aortic valve. TEE revealed a bicuspid aortic valve with severe aortic insufficiency due to a large, highly mobile mass (21×15 mm) on the ventricular side of right coronary cusp, protruding into the aorta during systole (51). The systemic embolization remains one of the most serious consequences of IE. Myocardial infarction, acute renal failure, retinal embolization, spleen and hepatic segmental

infarctions, and stroke are well-known complications of IE. Cerebrovascular accident is reported as a rare consequence of large mobile aortic valve vegetation with moderate regurgitation (49, 52). Intra-cardiac devices endocarditis is a rare manifestation of Brucellosis and oscillating vegetation on intra-cardiac leads could be diagnosed by TTE (47). Right-sided endocarditis, an extremely rare complication of brucellosis, may have a better prognosis. In a patient with multiple pulmonary infiltrations, transthoracic echocardiography illustrated a motile mass of 20×17 mm on the atrial side of the tricuspid ring, resulting in tricuspid regurgitation and pulmonary hypertension. The favorable result was achieved after surgery with excision of tricuspid and pulmonary vegetation and tricuspid repair under cardiopulmonary bypass. Early diagnosis of the extremely harmful Brucella endocarditis, and efficient medical as well as surgical treatment provide patients with the reduction of mortality and morbidities. This illustrates the importance of early detection of Brucellosis; however, there is no specific sign and symptom for a high clinical suspicion towards early diagnosis of Brucellosis. Making decisions for surgical treatment of IE depends on vegetation size and location, valvular dysfunction severity, the presence of heart failure, intracardiac abscess, and fistula to pericardial space. The other challenge is the diagnosis of prosthetic valves endocarditis which is extremely rare and it remains a diagnostic and therapeutic challenge. Despite advancements, traditional tools such as blood culture and echocardiography often fail to diagnose Brucella endocarditis promptly. Their low sensitivity results in delayed detection, increasing the risk of severe cardiac complications and mortality. Fluorine-18 fluorodeoxyglucose (^{18}F-FDG) PET/CT has emerged as a promising imaging modality for culture-negative endocarditis, particularly in patients with prior antibiotic use. However, its accessibility remains limited, especially in low-resource settings (53). Therefore, combining serology, PCR, and metagenomic sequencing with imaging modalities is recommended for early and accurate detection, improving prognosis and reducing mortality (35,53-54).

A study compiled from 21 reports (18 articles and 3 congress abstracts) included 1270 patients with a diagnosis of infective endocarditis (38.3% female, mean age 46.2, 28% prosthetic valve endocarditis). An underlying heart disease was present in 51.9% of patients, and a history of dental procedure was reported in 6.7%. Fever (94%), heart murmur (71.4%), and fatigue (69%) were the most common clinical manifestations. Mitral valve was the most commonly involved site (43.3%), followed by aortic valve (33.8%) and tricuspid valve (6.4%). Staphylococcus aureus (22.8%), coagulase-negative staphylococci (9.7%), and enterococci (7.5%) were the most commonly isolated organisms, while 31.1% of cases were culture-negative. The overall mortality rate was 23.4% (55). In a systematic review, the average duration between prosthesis implantation and the episode of Brucella PVE was 8±7 years (extremes: 4 months–28 years). Early Brucella PVE was noted in 15% of cases. A number of intra-cardiac complications were reported including abscess, paravalvular leakage, an aorta-left ventricle fistula, a massive aortic root infected pseudoaneurysm, mitral prosthetic valve obstruction, and severe leak in the aortic prosthetic valve (17). The other complex case reported a patient with total aortic valve prosthesis dehiscence with severe paravalvular regurgitation, abscess in aortic valve that reduced LV systolic function and severe pulmonary hypertension, leading to death (13). Pulmonary valve prosthesis involvement has also been reported as a rare complication of brucellosis. In one case, TTE showed severe right ventricular dilation and two pulmonary valve vegetations, the larger measuring 12 mm, which were later confirmed by TEE (56).

TEE is recommended due to the lack of sufficient accuracy of TTE in the diagnosis of prosthetic valves complications, like perivalvular abscess, pseudoaneurysm, new partial dehiscence of prosthesis, and fistula (14). In a systematic review from 1974 to 2021 with 51 patients with prosthetic heart valve endocarditis, the most common type of

prosthesis mentioned was mechanical prosthesis (84.3%), and aortic valve and mitral valve prosthesis were affected in most patients (52.9% and 35.3%, respectively). In this systematic review, echocardiography confirmed the diagnosis in 93% of patients and illustrated that echocardiography is not a powerful diagnostic tool in all patients. As a result, there are a number of limitations of diagnostic accuracy of echocardiography for timely detection and definition of Brucella endocarditis, like the early stage of disease, failure to detect small vegetations, complexity of diagnosis in multiple prosthetic valves, operator dependency, semi-invasive nature, and the lack of access to TEE in all centers (7, 10).

Resected heart valves or tissue biopsy specimens are inoculated into appropriate media to increase detection. A high density of organisms in the tissue enhances the efficiency of isolation, and positive serologic results assist in selecting proper culture medium, supporting early diagnosis and reducing false-negative cases of Brucella endocarditis (33). Brucella spp. are facultative intracellular bacteria with low environmental survival, which complicates their isolation from clinical specimens (57). Infection requires interaction with various host cell types (58-60). B. abortus shows tropism for the pregnant uterus of cows with high bacterial loads in placental cotyledons. Infection of trophoblast cells can result in necrosis and abundant extracellular bacterial aggregates (61). Three culture methods were compared for isolating B. abortus from bovine supramammary lymph nodes: Method I (halving and mincing the lymph node), Method II (placing small tissue pieces in phosphate-buffered saline), and Method III (inoculation into biphasic media). Out of 626 lymph nodes, B. abortus was isolated from 149 cows (52.3%) with the highest recovery obtained using Methods I and II. Three selective culture media—Brucella agar, Farrell medium, and CITA medium—are commonly used for isolation. Farrell medium inhibits most contaminants but can suppress growth of certain Brucella species. CITA medium, based on modified Thayer-Martin, improves recovery with balanced antimicrobial concentrations and Amphotericin B addition. Brucella agar is widely used for routine diagnosis and outbreak investigations (55-56).

The shell vial technique provides an effective method for isolating fastidious intracellular bacteria in Brucella endocarditis. Clinical samples are inoculated into and incubated in shell vials, and then bacterial presence is assessed with acridine orange, Gimenez, Giemsa, or immunofluorescence staining with the patient's serum. The bacteria are detected with PCR amplification and sequencing of the 16S rRNA gene. The use of this technique in routine clinical settings is limited because it needs specialized equipment and trained personnel. This technique can isolate the intracellular pathogens even from minimal sample material. This system has proven effective for Brucella spp. and other fastidious organisms, showing its value in cases where conventional culture methods fail (58). The shell-vial culture technique provides a valuable method for isolation of fastidious microorganisms when standard axenic cultures remain negative. In these cases, only cell cultures allow bacterial recovery. This technique successfully isolates Mycobacterium spp. from lymph node biopsies, pericardial fluid, cerebrospinal fluid, and bronchoalveolar lavage samples, as well as unusually fastidious bacteria such as Staphylococcus aureus small-colony variants with intracellular localization (60). The cell environment likely provides growth factors absent in axenic media, and the rinsing step of the shell vial procedure may remove inhibitory substances. Despite a low overall success rate, this method yields important diagnostic results in challenging cases of infection. The shell-vial culture assay demonstrates high utility for isolating fastidious microorganisms from clinical samples. Over a 13-year period, 580 bacterial strains (5%) were recovered from 11,083 clinical specimens. Additionally, 285 isolates of Rickettsia, Bartonella, or Coxiella burnetii were obtained from 7,102 samples, including 55 Rickettsia sp., 95 Coxiella burnetii, and 135 Bartonella sp. isolates. A centrifugation shell-vial technique, termed

JNSP ("je ne sais pas"), enabled recovery of 173 isolates from 3,861 clinical samples, 40 of which had not previously grown on standard axenic media. These included Staphylococcus aureus, Streptococcus sp., Mycobacterium sp., Nocardia asteroides, and Actinomyces sp., highlighting the value of shell-vial culture for detecting fastidious or previously uncultivable pathogens in clinical microbiology (31).

Fluorine-^{18}Fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) is an advanced hybrid imaging technique that uses a combination of metabolic and anatomical data that increases the detection of infectious and inflammatory cardiac diseases. Activated leukocytes at sites of infection show increased glucose metabolism that leads to elevated FDG uptake. Therefore, PET/CT scan detects and measures FDG uptake in activated leukocytes at sites of infection (42). This technique enables identification of both intracardiac and extracardiac infectious foci, supporting comprehensive clinical evaluation (73). The diagnostic accuracy of ^{18}F-FDG PET/CT is notably high in both prosthetic valve endocarditis and cardiac device pocket infections, with reported sensitivity and specificity exceeding 80–90% (73 , 74). Furthermore, this modality offers supplementary diagnostic value over the modified Duke criteria, as it can reclassify cases from "possible" to either "definite" or "rejected" infective endocarditis, thereby strengthening diagnostic confidence. Integration with cardiac CT angiography further improves the diagnostic precision for PVE. In Brucella endocarditis, FDG PET/CT has demonstrated significant value in detecting focal and multifocal Brucella infections, which are often difficult to localize using conventional imaging. A study conducted at Rambam Health Care Campus, where FDG PET/CT became the recommended imaging modality for suspected focal brucellosis, reported focal disease in 60% of cases—including previously undetected spinal infections—and revealed multifocal involvement in more than half of the affected patients (75). FDG PET/CT findings led to changes in clinical management in more than 50% of episodes, either initiating antibiotic therapy in spinal infections or discontinuation of treatment when no active disease is present. Radionuclide imaging includes ^{18}F-fluorodeoxyglucose PET/CT and single-photon emission computed tomography (SPECT/CT) with radiolabeled white blood cells (WBC) and is associated with improved diagnostic accuracy in suspected cardiovascular infections. These modalities could detect extracardiac involvement and allow precise evaluation of cardiac devices, such as leads and ventricular assist devices (76-77). SPECT/CT imaging with ^99mTc-HMPAO-labeled WBC has superior diagnostic performance compared to planar or stand-alone SPECT. It can show the presence of infection, extent of device involvement, and associated complications in patients with cardiac implantable devices. Currently, there is no published study that evaluates the radionuclide imaging techniques in Brucella endocarditis. Therefore, the direct scientific evidence in Brucella endocarditis is not available, but based on the results of these techniques in other cardiovascular infections, they may be a complementary diagnostic tool in the future (52).

Over the past decades, advanced imaging techniques such as cardiac computed tomography and magnetic resonance imaging have revolutionized the diagnostic approach to IE. These modalities improve accuracy and guide therapeutic decisions. Cardiac computed tomography can be a major complementary imaging technique, particularly in patients with indeterminate echocardiographic findings. Continuous developments in multiphase, ECG-synchronized, thin-section imaging protocols have led to marked improvement in diagnostic accuracy and spatial resolution. These advancements facilitated detailed visualization of valvular and perivalvular pathology such as abscesses, pseudoaneurysms, vegetations, fistulas, and prosthetic valve dehiscence. Apart from its diagnostic ability, CT provides noninvasive assessment of coronary anatomy, which is important for surgical planning and risk assessment in patients requiring valve replacement. The updated 2023 ESC and Duke-ISCVID criteria now recognize CT

findings—such as vegetations, perforations, abscesses, and pseudoaneurysms—as major imaging criteria in the diagnosis of IE. Studies have shown that although transesophageal echocardiography is more sensitive for detection of small vegetations due to its higher temporal resolution, CT shows comparable or superior performance in diagnosis of paravalvular abscesses and pseudoaneurysms, with reported sensitivities near 81–100%. Furthermore, integration of CT with TEE improves diagnostic sensitivity by approximately 15%, demonstrating the advantage of multimodality imaging in IE evaluation. Recent technological innovations, such as photon-counting CT, have increased image clarity and spatial resolution. Its use in both diagnosis and preoperative assessment has increased. Cardiac magnetic resonance imaging has also been introduced as a valuable noninvasive modality for the assessment of infective endocarditis. This technique provides superior soft-tissue contrast and detailed tissue characterization. Cardiac MRI can detect valvular vegetations, myocardial inflammation, and perivalvular infection, and can be visualized using contrast-enhanced sequences. In clinical studies, MRI has detected valvular vegetations and delayed enhancement patterns along the aortic root, cardiac chambers, and vascular walls, consistent with endothelial inflammation and infectious involvement—even when vegetations were not visible on echocardiography or CT. These features make MRI more useful in assessment of soft-tissue inflammatory extension, tissue health, and treatment planning. However, MRI has main limitations, such as longer acquisition time, the need for patient cooperation, and contraindications in patients with metallic or electronic implants. Overall, cardiac CT and MRI could provide anatomical and tissue-level information that helps in diagnosis of endocarditis. CT is effective in determination of structural complications such as abscesses, pseudoaneurysms, and prosthetic valve dehiscence. MRI could provide superior detection of soft-tissue inflammation and myocardial involvement. In conclusion, these modalities could increase diagnostic accuracy, earlier intervention, and improve patient outcomes. Their combination with current diagnostic criteria and imaging protocols could be useful in management of infective endocarditis, including Brucella endocarditis (47,52).

Polymerase chain reaction is a molecular-based test for detection of Brucella infections. PCR has higher sensitivity and specificity compared to traditional blood culture. Classical blood culture is time-consuming, needs a trained technician, and has great biohazard risks. PCR assays target conserved genetic regions of Brucella, such as bcsp31, omp2, and 16S rRNA genes, for rapid and accurate detection from clinical samples, such as blood, serum, and even excised tissue. These assays need robust DNA extraction protocols to minimize inhibitors that could affect amplification efficiency; commercial extraction kits are generally capable of detecting low amounts of Brucella DNA, sometimes down to 100 fg per sample. Multiplex and real-time PCR methods have further improved diagnostics, allowing for detection at genus and species levels, and even facilitating epidemiological investigations through strain typing. Real-time PCR utilizing fluorescent dyes or probes provides rapid results without the need for post-amplification processing, and its quantitative capabilities enable monitoring of bacterial load over the disease course. Moreover, multiplex PCR assays can concurrently detect Brucella alongside other causative pathogens in endocarditis, which is crucial for differential diagnosis, especially in regions where other fastidious organisms, such as Coxiella burnetii and Bartonella species, are prevalent causes of culture-negative endocarditis. The integration of PCR-based methods with established clinical criteria (such as Duke's criteria) enhances diagnostic certainty, reduces diagnostic delay, and informs timely management decisions. Nevertheless, widespread clinical implementation requires further standardization. Inhibitors present in cardiac tissue, variable DNA extraction efficiency, and occasional cross-

contamination remain technical concerns. Positive PCR from tissue or blood does not always indicate active infection. Sometimes, persistent DNA fragments can be detected long after microbiological treatment or in the setting of low-level colonization. This technique has limitations in application and inter-laboratory comparability due to the lack of current standardization in PCR target selection, primer design, and reporting conventions. Despite these barriers, PCR-based tools continue to be refined and validated for broader clinical use. This test is considered a complementary diagnostic algorithm for Brucella endocarditis (35, 49).

Next-generation sequencing has improved pathogen detection in infective endocarditis, such as Brucella endocarditis, especially in culture-negative cases due to prior use of antibiotics or slow-growing organisms. NGS-based metagenomic approaches can detect pathogen DNA directly from blood, valve tissue, or prosthetics. This technique could diagnose infective endocarditis even in the absence of viable organisms. This technology can detect not only Brucella species but also co-infecting or unexpected organisms. By sequencing the entire microbial spectrum, clinicians are prepared with data to direct pathogen-targeted therapies, decrease unnecessary broad-spectrum antibiotic exposure, and improve patient outcomes. NGS is also recommended for prosthetic valve endocarditis, fastidious or atypical presentations, and in immunocompromised hosts. NGS needs specialized laboratory capabilities and higher costs. NGS sensitivity in detecting rare or fastidious pathogens shows a significant advantage in culture-negative endocarditis. Increasing evidence supports the integration of NGS into diagnostic protocol, especially in referral or tertiary centers managing challenging infective endocarditis cases. Future directions include wider standardization, reduction in sequencing turnaround time, and reduction in cost, which may soon render NGS part of routine practice in difficult-to-diagnose infective endocarditis (3,9,25).

Biomarkers such as C-reactive protein and procalcitonin are usually measured in suspected infective endocarditis for diagnosis and monitoring of therapy response. In Brucella endocarditis, CRP is often elevated, around 22 mg/L, but procalcitonin stays low or slightly raised. This pattern reflects the intracellular nature of the organism and its limited capacity to trigger a strong acute-phase response. These findings show a major limitation. CRP and procalcitonin have limited sensitivity or specificity to differentiate Brucella endocarditis from other causes of endocarditis or systemic inflammation. The biomarkers can also be affected by comorbidities, co-infections, or other inflammatory conditions, which can make interpretation difficult. Current recommendations support using CRP and procalcitonin as adjuncts, not as standalone diagnostic tools, together with microbiological and molecular techniques for definite diagnosis.

Advanced molecular diagnostic techniques have important effects on Brucella endocarditis. Early and accurate diagnosis improves management choices and prognosis. Brucella infection of cardiac valves needs special management because of its destructive progression, large vegetations, paravalvular abscess, and high mortality without targeted therapy. Confirmation of the diagnosis with PCR and/or NGS could help improve management with an effective combination of suitable antibiotics such as doxycycline, rifampicin, and an aminoglycoside or fluoroquinolone in prolonged duration (usually 3–6 months), to eliminate intracellular bacteria. Surgical intervention is often needed when there is extensive tissue destruction, refractory heart failure, or uncontrolled infection. Early detection and multidisciplinary approach could improve outcomes. Delayed diagnosis due to atypical clinical features, low accuracy of culture, or limited access to molecular diagnostics is associated with increased morbidity, slow recovery, and mortality rates that can exceed 30% if the condition is not properly treated. Postoperative antimicrobial therapy, often maintained for several months, decreases the risk of relapse. Biomarkers such as CRP can help monitor inflammation and therapeutic response. But definitive decisions regarding

intervention are best guided by molecular diagnostic evidence, imaging findings, and clinical assessment. Multidisciplinary approaches, integrating molecular, serological, and imaging data, improve prognosis in Brucella endocarditis (Figure 3).

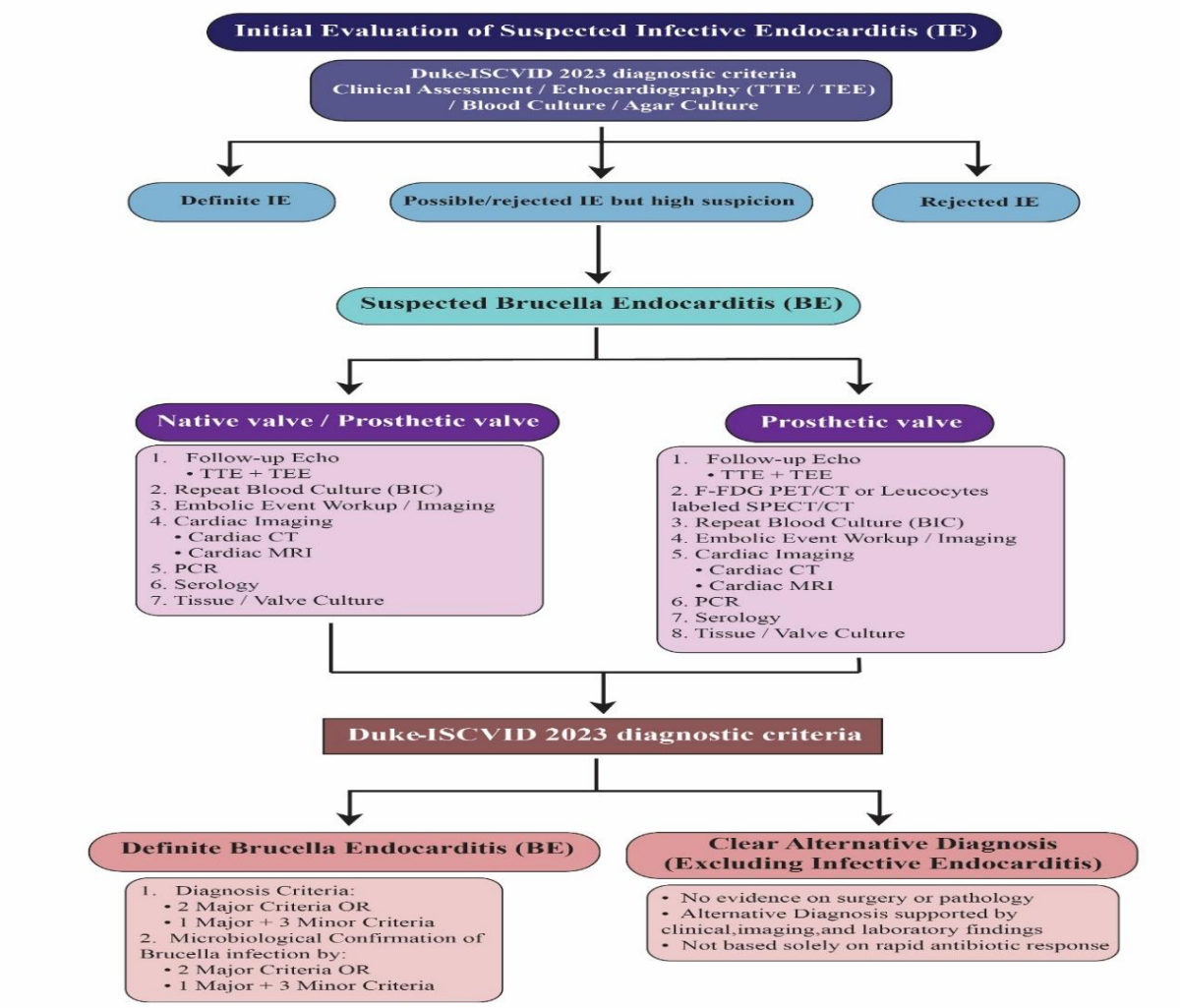

Figure 3. Diagnostic Algorithm for Brucella Endocarditis

In the differential diagnosis of Brucella endocarditis, some rare bacterial agents and non-infectious conditions must be considered. Some rare bacteria such as Coxiella burnetii, Bartonella spp., Tropheryma whipplei, and Mycobacterium spp. can cause blood culture-negative endocarditis and need molecular or serologic methods for diagnosis. Non-infectious endocarditis such as Libman-Sacks endocarditis and non-bacterial thrombotic endocarditis are important in patients with malignancy or autoimmune disorders. They usually present without systemic symptoms and with negative blood cultures. Antibiotic use prior to sampling is another common cause of BCNE, which shows the need for careful review of the patient's medication history. Fungal endocarditis should also be considered in the differential diagnosis. Although rare (1–3% of cases) and associated with high mortality (>70%), the most common causative agents include Candida spp., Aspergillus, and Histoplasma spp., with patients having prosthetic valves, prior cardiac surgery,

or injection drug use at increased risk. Prosthetic valve fungal endocarditis has particular importance; the most frequent agents are Candida spp. and Histoplasma capsulatum, and patients often present after aortic valve replacement with systemic symptoms. Finally, rheumatic heart disease should be considered in the differential diagnosis, especially in patients with valvular lesions and new heart murmurs, as valvular involvement can overlap with infectious endocarditis.

Brucella endocarditis often has a delayed diagnosis due to nonspecific and gradual clinical presentation such as fever, arthralgia, weakness, and hepatosplenomegaly. Blood culture often remains negative because Brucella species grow slowly and require special culture media, and prior antibiotic therapy reduces culture sensitivity. Transthoracic echocardiography shows limitations in detecting small vegetations or complex valve involvement, particularly in prosthetic valves, while transesophageal echocardiography allows definitive evaluation. These diagnostic limitations increase the risk of severe cardiac complications including valve destruction, abscess formation, fistula, and heart failure, resulting in higher morbidity and mortality. Advanced diagnostic methods such as PCR, serology, and metagenomic sequencing allow rapid pathogen detection, facilitate timely treatment, and improve patient outcomes.

Discussion

Brucella endocarditis is a rare but fatal form of brucellosis, with mortality rates reported from 20% to 40%. Early diagnosis is difficult due to nonspecific symptoms, slow growth of Brucella spp., prior use of antibiotics, and intracellular localization of the pathogen. Traditional diagnostic tools, such as blood culture and echocardiography, show limited sensitivity, particularly in culture-negative cases and in patients with prosthetic valves (16, 27-29). Blood cultures often result in false-negative results, even with automated systems, due to low bacterial load and slow proliferation. Echocardiography, while fundamental, fails to detect small vegetations, early disease, or complex prosthetic valve involvement. Prosthetic valve endocarditis presents additional diagnostic difficulties. In systematic reviews, mechanical prostheses accounted for the majority of Brucella PVE, and TEE confirmed diagnosis in 93% of patients; however, echocardiography did not provide definitive results in all cases. Some manifestations of Brucella endocarditis such as small vegetations, perivalvular abscesses, pseudoaneurysms, and fistulas remain underdiagnosed, and the patients face severe complications such as heart failure, embolic events, and death (3,6,17,23-28).

Molecular diagnostic tools, including PCR and next-generation sequencing, provide rapid and precise identification of Brucella DNA, even in culture-negative samples or after antibiotic therapy. PCR targeting bcsp31, omp2, and 16S rRNA genes shows high sensitivity and specificity. Metagenomic NGS can detect Brucella and co-infecting pathogens. Combination of molecular techniques and clinical protocols improves diagnostic rates and early initiation of prolonged therapy. Delayed diagnosis can lead to an increased need for surgical intervention and postoperative mortality. Advanced imaging modalities provide critical diagnostic advantages. FDG PET/CT increases detection of hidden infectious foci, paravalvular, and culture-negative infections, showing both metabolic and anatomical information. Radionuclide imaging, such as SPECT/CT with radiolabeled leukocytes, increases detection of device-related infections and

perivalvular complications (47). Cardiac CT and MRI could show abscess, pseudoaneurysm, fistula, and tissue-level involvement. These modalities have complemented echocardiography and improved preoperative assessment. The use of a multimodality strategy that includes echocardiography with CT, MRI, and PET/CT improves early diagnosis, interventions, and decreases mortality. Based on clinical evidence, biomarkers such as CRP and procalcitonin have a supportive role in the diagnosis of BE but are not diagnostic. Medical planning is guided by microbiology, molecular tests, and imaging results. Delayed diagnosis is a major problem due to subclinical symptom onset, culture-negative infections, and limited access to advanced diagnostic modalities in endemic regions. In conclusion, Brucella endocarditis needs fast and accurate diagnosis. Traditional tests have considerable limitations, especially in early disease and patients with prosthetic valve involvement. Molecular tests, such as PCR and NGS, FDG PET/CT, radionuclide imaging, cardiac CT, and MRI, improve diagnostic accuracy. Multimodality strategies and wider availability of advanced tools help improve outcomes, decrease mortality, and guide effective medical decisions (45,49,52-56).

There are multiple challenges in the diagnosis of Brucella endocarditis. Low incidence decreases clinician experience. Nonspecific clinical presentation such as fever, arthralgia, weakness, and hepatosplenomegaly delays suspicion. Blood cultures are usually negative as a result of slow growth, intracellular localization, and prior use of antibiotics. Standard imaging such as transthoracic echocardiography shows limited sensitivity for small vegetations, prosthetic valve involvement, and perivalvular infection (58). Transesophageal echocardiography improves diagnosis but is operator-dependent, semi-invasive, and unavailable in all centers. Serologic tests, such as ELISA, Wright agglutination, and Coombs anti-Brucella, show more sensitivity but lower specificity, especially in endemic regions. PCR and next-generation sequencing improve pathogen detection but need specialized laboratory infrastructure, trained personnel, and incur higher costs. Advanced imaging such as FDG PET/CT, cardiac CT, and MRI increase localization and assessment of valvular and extracardiac lesions. However, these techniques have limited access in low-resource areas. Delayed diagnosis results in severe complications such as valve destruction, abscess formation, fistula, heart failure, systemic embolization, and increased mortality. Future directions include a combination of traditional and advanced imaging modalities that will improve patient outcomes. Development of portable, rapid PCR and NGS platforms suitable for low-resource settings could decrease diagnostic delay. Standardization of imaging protocols for FDG PET/CT, CT, and MRI may increase reliability and comparability between centers. Large multicenter studies will be essential to improve diagnostic accuracy and guide clinical interventions. Brucella endocarditis is rare and some cases remain undiagnosed. Future use of artificial intelligence may support diagnosis and treatment. Artificial intelligence may help create diagnostic and therapeutic algorithms and support (36,47,52-53,60).

Conclusion

Brucella endocarditis is a rare but fatal manifestation of brucellosis. Early and accurate diagnosis is essential to guide medical interventions and reduce mortality. Traditional diagnostic tools such as blood culture and echocardiography have limited sensitivity, especially in early disease or prosthetic valve patients. Advanced techniques including FDG PET/CT, cardiac CT, cardiac MRI, PCR, and next-generation sequencing have improved detection of paravalvular complications, hidden infectious foci, and culture-negative disease.

The combination of advanced techniques with traditional approaches can help form a more complete diagnostic pathway and improve clinical outcomes. Wider access to modern molecular and imaging tools in endemic regions and high-quality studies on advanced diagnosis are needed for improvement of survival outcomes. A structured, multimodality diagnostic strategy can have a key role in reducing mortality and improving long-term outcomes in patients with Brucella endocarditis.

Acknowledgments

None

Conflict of Interest

None

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