The clinical utility of tissue polymerase chain reaction, tissue culture and tissue histology in blood culture-negative infective endocarditis in South Africa – insights from the Groote Schuur Hospital Infective Endocarditis Registry

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Background. Infective endocarditis (IE) poses significant diagnostic and therapeutic challenges, especially in cases of blood culture-negative infective endocarditis (BCNIE). Among patients undergoing surgery for IE, valve tissue may be evaluated for additional microbiological information by performing a broad-range 16S rDNA polymerase chain reaction (PCR) test, tissue culture and tissue histopathology. In patients with BCNIE, these diagnostic tests may identify the causative agents, guide further clinical management and improve local IE-related epidemiological data. The clinical utility of additional analysis of explanted tissue in a local South African (SA) setting has yet to be described.

Objectives. To assess the clinical utility of performing PCR, culture and histopathology on the tissue of surgically explanted valves in BCNIE patients in an SA public sector hospital. We assess their diagnostic yield and treatment impact in a cohort of BCNIE patients treated with empirical antibiotic regimens.

Methods. We analysed data from the Groote Schuur Hospital (GSH) Infective Endocarditis Registry, a prospective observational study of adult patients with infective endocarditis. Participants for this analysis were selected based on clinical and pathological criteria for IE and negative blood cultures. All participants were treated between January 2017 and March 2021.

Results. During the study period, we identified 165 IE cases, 57 (34.5%) of which were blood-culture negative. BCNIE patients had a mean (standard deviation) age of 40.2 (13.4) years, and 41 (71.9%) were male. Twenty-seven of the 57 BCNIE patients underwent cardiac surgery and had tissue analysis performed. Tissue PCR identified an aetiological agent in 17/27 (63%) cases, with *Bartonella* spp. (12/27, 44%) being the most common organism. Tissue culture was positive in 3/27 (11%) cases, but the organisms identified were thought to reflect sample contamination. Tissue histopathology was performed in 22/27 (81.5%) cases and provided macroscopic confirmation of IE, but did not identify any specific organisms in any of the specimens. Only a small subset of the overall BCNIE cohort (11/57 (19.3%)) had serum serology for *Bartonella* spp. and *Coxiella* spp. performed, and 5/11 (45.5%) were positive for *Bartonella* spp. There was a 100% concordance rate between positive serum serology and tissue PCR. Tissue PCR impacted the antimicrobial regimen in 20/27 (74%) cases. Tissue culture and tissue histopathology did not influence antibiotic regimens in any patients.

Conclusion. In this single-centre study, perioperative serum serological testing was underutilised in BCNIE. Tissue PCR was valuable in determining the aetiology of BCNIE, and influenced management. Tissue culture and histopathology had a poor yield and added little value in identifying the microbiological cause of BCNIE. Finally, the most common BCNIE causative organism identified by additional non-culture testing in our setting is *Bartonella* spp.

Keywords: infective endocarditis; bartonella; tissue polymerase chain reaction; blood culture-negative infective endocarditis

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Blood culture-negative infective endocarditis (BCNIE) is defined as infective endocarditis (IE) that remains without an identifiable aetiology following a work-up that includes at least two independent blood culture samples.[1] Blood cultures are defined as negative following routine periods of incubation, usually 72 hours, depending on local laboratory practices.[2-5] BCNIE is often caused by fastidious organisms, but it may also result from diagnostic or therapeutic factors such as antibiotic administration before blood culture sampling.[3]

BCNIE is a significant clinical problem in South Africa (SA). Recent local studies have demonstrated that between 41% and 55% of IE cases are blood-culture negative.[6-8] This is important because BCNIE is associated with higher mortality when compared with blood culture-positive IE. For example, the ESC-EORP EURO-ENDO (European IE registry) showed higher 30-day mortality in patients with BCNIE than in patients with blood culture-positive endocarditis.[9] In addition, a lower 1-year survival rate was observed for BCNIE patients in the subgroup of patients who received medical treatment alone without surgical intervention.[9] In addition, there are important regional variations in the aetiology of BCNIE. For example, a recent SA study demonstrated that *Bartonella* spp. is the most common cause of BCNIE in SA.[10] This finding was consistent with a previous African study, which also showed *Bartonella* spp. as the most common aetiological agent identified in BCNIE patients.[5] This contrasts with European studies in which *Coxiella* spp. is the most common organism.[11]

According to the European Society of Cardiology (ESC) IE guidelines, the initial evaluation for BCNIE should consist of blood serological testing for *Coxiella burnetii*, *Bartonella* spp., *Aspergillus* spp., *Legionella pneumophila*, *Brucella* spp. and *Mycoplasma* spp. Following this step, blood PCR testing and testing for rheumatoid factor, anticardiolipin-, anti-b2-glycoprotein-, antinuclear- and anti-pork antibodies should be performed. The latter antibodies are performed to exclude non-infective mimics of BCNIE. The ESC recommends performing tissue cultures, histological and PCR evaluations of any excised valvular tissue.[1]

Our study aims to assess the clinical utility of performing valve tissue PCR, tissue culture and tissue histopathology in BCNIE patients in an SA public-sector hospital. We describe these tests' diagnostic yield and impact on treatment in a cohort of patients diagnosed with BCNIE.

Methods

Patient selection and data collection

The Groote Schuur Hospital (GSH) Infective Endocarditis Registry is a prospective observational study of patients presenting or referred to GSH with definite or possible IE, based on the 2015 ESC IE diagnostic criteria.[1]

Adult patients (>18 years of age) were recruited to the GSH Infective Endocarditis Registry. The registry was approved by the University of Cape Town (UCT) Faculty of Health Sciences Human Research Ethics Committee (HREC) (ref. no. R037/2017), and the present sub-study is an analysis of BCNIE patients enrolled between January 2017 and March 2021 (ref. no. 538/2021).

GSH is a quaternary care hospital affiliated with UCT in Cape Town, SA. After obtaining informed consent, demographic data, clinical presentation and past medical and surgical history, data on recent hospitalisation, dental procedures and endoscopies were recorded. We also collected data on clinical findings, electrocardiograms, microbiological findings, echocardiographic findings, use of other imaging techniques (computed tomography and magnetic resonance imaging), medical therapy, complications and indications for surgery. Surgically removed valvular specimens were sent for tissue PCR and culture. For tissue PCR testing, valvular samples underwent 16S rDNA amplification using conventional PCR with primers spanning the internal fragment of the 16S rDNA gene. Sequencing results were manually analysed and followed by a basic local alignment search tool (BLAST) to identify the organisms.

The histopathological examination consisted of microscopy and special staining methods, including the Verhoeff-van Gieson elastic, Alcian blue, Brown-Brenn and periodic-acid Schiff stains. Immunohistochemistry for specific organisms was performed in only select cases.

During our study period, there was no set protocol for the workup of BCNIE cases at our institution. Serum serological testing was performed only if deemed necessary by the treating clinician; no blood PCR testing was performed, and the treating clinicians determined empiric antibiotic regimens. All surgery participants had tissue samples sent for PCR testing and culture.

Participant data were extracted from source documents and captured onto standardised electronic case report forms on Research Electronic Data Capture (REDCap), a secure online database hosted by UCT. The data evaluated included demographic information, medical and surgical therapy and microbiological and laboratory results of all identified cases.

Definitions

Patients were defined as having IE based on the 2015 ESC IE diagnostic criteria.[1] Patients without bacterial growth after 5 days, using standard blood culture techniques, were defined as having BCNIE.[1] If serological testing was done for both *Coxiella* spp. and *Bartonella* spp., it was considered performed. Serology was considered positive for *Coxiella* spp. if IgG phase I was positive at a titre >1:800, and positive for *Bartonella* spp. IgG was positive at a titre >1:128.[1,12]

Statistical analysis

Descriptive statistics were used to summarise the results. Categorical variables are presented as numbers and percentages, and continuous variables as means (standard deviation (SD)) when normally distributed and medians (interquartile range) when skewed.

Results

Patients

At the time of analysis, 165 patients were enrolled in the GSH IE registry, and 57 (34.5%) were blood-culture negative. Of the 57 with BCNIE, 47 (82.5%) had definite IE, and 10 (17.5%) had possible IE. BCNIE patients had a mean (SD) age of 40.2 (13.4) years; 41 (71.9%) were male, 20 (35.1%) had rheumatic valvular heart disease and 43 (75.4%) had received antibiotics before having blood cultures taken. Further patient characteristics are displayed in Table 1.

Serum serology

Only a small subset of BCNIE patients (11/57 (19.3%)) had serum serology for *Bartonella* spp. and *Coxiella* spp. performed. Of these, 5/11 (45.5%) were positive for *Bartonella* spp. One patient who tested positive for *Bartonella* spp. on serum serology did not undergo cardiac surgery. Eight patients tested for serology underwent cardiac surgery and tissue PCR testing; 4 were positive on both serology and PCR; 2 were only positive on tissue PCR, and 2 were negative on both. There was a 100% concordance rate between positive serum serology and tissue PCR; all 4 cases revealed *Bartonella* spp.

Tissue PCR

Among the BCNIE patients, 27/57 (47.4%) underwent cardiac surgery and had tissue samples sent for further analysis. The indications for cardiac surgery included heart failure in 15/27 (55%), haemodynamic instability in 7/27 (26%), severe valvular regurgitation in 3/27 (12%), vegetations >1 cm in 1/27 (4%) and recurrent embolic complications in 1/27 (4%).

Tissue PCR was positive in 20/27 (74%) surgery cases. Genetic sequences were identified in three cases, but testing could not yield a specific organism, possibly reflecting multiple aetiological agents or inadequate genetic material for species identification. Tissue PCR identified an aetiological agent in 17/27 (63%) cases, with the most common organisms identified as *Bartonella* spp. (12/27, 44%), followed by *Streptococcus* spp. (2/27, 7.4%) and *Aggregatibacter* spp. (2/27, 7.4%). In patients in whom serum serology was performed and was negative, valvular PCR was positive in 2/4 (50%) cases; 1 case revealed *Streptococcus mitis* and the other *Aggregatibacter aphrophilus*. Fig. 1 displays testing from least invasive (serum serology) to most invasive (tissue PCR), and whether the test identified an aetiology, and Fig. 2 shows the organisms detected by tissue PCR testing.

Tissue culture

Tissue culture was positive in 3/27 (11%) cases. The tissue cultures in these 3 cases grew a mixed growth, *Staphylococcus warneri* and *Micrococcus* spp., respectively. In the cases that grew *Staphylococcus warneri* and *Micrococcus* spp., tissue PCR identified *Bartonella* spp. in both; as such, these tissue culture growths may reflect the result of sample contamination.

Tissue histopathology

Tissue histopathology was performed in 22 patients, in whom 19 (86%) had histological evidence of IE. In PCR-negative samples, 7/7 (100%) had histopathological evidence of IE. Tissue histopathology could not identify specific organisms.

Organisms detected

A definitive microbiological organism was identified in 18/57 (31.6%) BCNIE patients. Among the cases that underwent tissue PCR testing, an aetiology was identified in 17/27 (63%). In total, 7/27 (26%) surgery patients had no bacterial organisms identified. Table 2 provides the organisms identified among the 57 BCNIE patients.

Impact on treatment

Tissue PCR impacted antimicrobial treatment in 20/27 (74%) cases. Empiric antibiotic therapy was active against the organisms identified by PCR in most cases; however, it changed the antibiotic regimen in 4/27 (14.8%). Tissue PCR identified 12 cases of *Bartonella* spp., among which only 8 (66.6%) received preferred therapy in the form of doxycycline. Among the 8, 4 (50%) had doxycycline added after the results of preoperative serum serology, and the remaining 4 (50%) were given doxycycline empirically. Among the 4 *Bartonella* spp. cases not on preferred therapy before surgery, none had preoperative serum serology performed. Tissue PCR confirmed the appropriate empiric addition of doxycycline in 4/27 (14.8%) cases, and in 12/27 (44%) cases it was helpful to support the decision to withhold it. Tissue culture and tissue histopathology did not influence antibiotic regimens in any patients.

Discussion

Local epidemiological data are crucial in selecting empiric antibiotic regimens when faced with BCNIE cases. In this prospective observational study, we investigated the clinical utility of performing tissue PCR, tissue culture and tissue histopathology in BCNIE patients. The significant findings of our study were: (*i*) among the 165 patients in our IE registry, 57 (34.5%) cases were blood-culture negative; (*ii*) a large number, 43/57 (75.4%), received antibiotics before blood culture sampling; (*iii*) there is underutilisation of serum serological testing in the diagnoses of BCNIE our setting; (*iv*) tissue PCR is a valuable diagnostic tool in BCNIE cases; (*v*) tissue PCR revealed patients not receiving appropriate antimicrobial therapy before surgery, and influenced antibiotic treatment in several cases; and (*vi*) tissue culture and tissue histopathology did not add diagnostic and antimicrobial therapeutic value.

Previous SA studies have demonstrated that between 41% and 55% of IE cases are blood-culture negative.[6-8] This is higher than in developed countries, where the incidence rates are between 7% and 31%.[1,5,13,14] This may reflect local antibiotic practices. For example, in one SA study by Koegelenberg *et al*.,[7] 23/26 (88%) BCNIE cases had received oral or intravenous antibiotics 48 hours prior to blood culture collection. Similarly, in the present study, 43/57 (75.4%) BCNIE patients received antibiotics before blood culture collection.

BCNIE may also be the result of fastidious organisms.[11] Previous studies have shown that using a diagnostic strategy that includes serum serology, blood and tissue PCR and tissue culture yields an organism in up to 63% of BCNIE cases.[11,15] The ESC recommends performing serum serology in all BCNIE cases.[1] Serum serological assays have been developed against *Coxiella burnetii*, *Bartonella* spp., *Mycoplasma* spp., *Chlamydia* spp., *Legionella pneumophila* and *Brucella melitensis*.[4,16] In our study, serum serology was performed in only 11/57 (19.3%) BCNIE patients, and in only 8/27 (29.6%) surgical cases preoperatively. Considering the high prevalence of *Bartonella* spp. in BCNIE patients requiring cardiac surgery (12/27, 44.4%), preoperative serum *Bartonella* spp. serology is a valuable diagnostic tool in our setting.

Tissue or blood PCR testing may be especially useful in cases of prior antibiotic exposure, since bacterial DNA may remain detectable even if routine methods cannot culture the organism.[17] Universal primers against 16S rDNA (for bacteria) or 18S rDNA (for fungi) are used to amplify microbial genomic material, which is then sequenced for pathogen identification. However, Pecoraro *et al*.[10] found an exceptionally low yield for blood PCR in BCNIE cases, with only a single positive result in 22 patients (4.5%). Similarly, Fournier *et al*.[11] found that blood PCR assays detected pathogens in only 3/177 (1.7%)

Table 1. Patient characteristics, *N*=57

Characteristic	*n* (%)*
Age (years), mean (SD)	40.2 (13.4)
Male	41 (71.9)
Female	16 (28.1)
Received antibiotic before blood culture	43 (75.4)
Rheumatic valvular heart disease	20 (35.1)
Previous infective endocarditis	3 (5.3)
Congenital heart disease	9 (15.8)
Current IV drug use	8 (14)
HIV	13 (22.8)
DM	2 (3.51)
Current smoker	26 (45.6)
Alcohol use	26 (45.6)
Chronic kidney disease	1 (1.75)
Hypertension	14 (58.3)

*Unless otherwise indicated.
SD = standard deviation; IV intravenous; DM = diabetes mellitus.

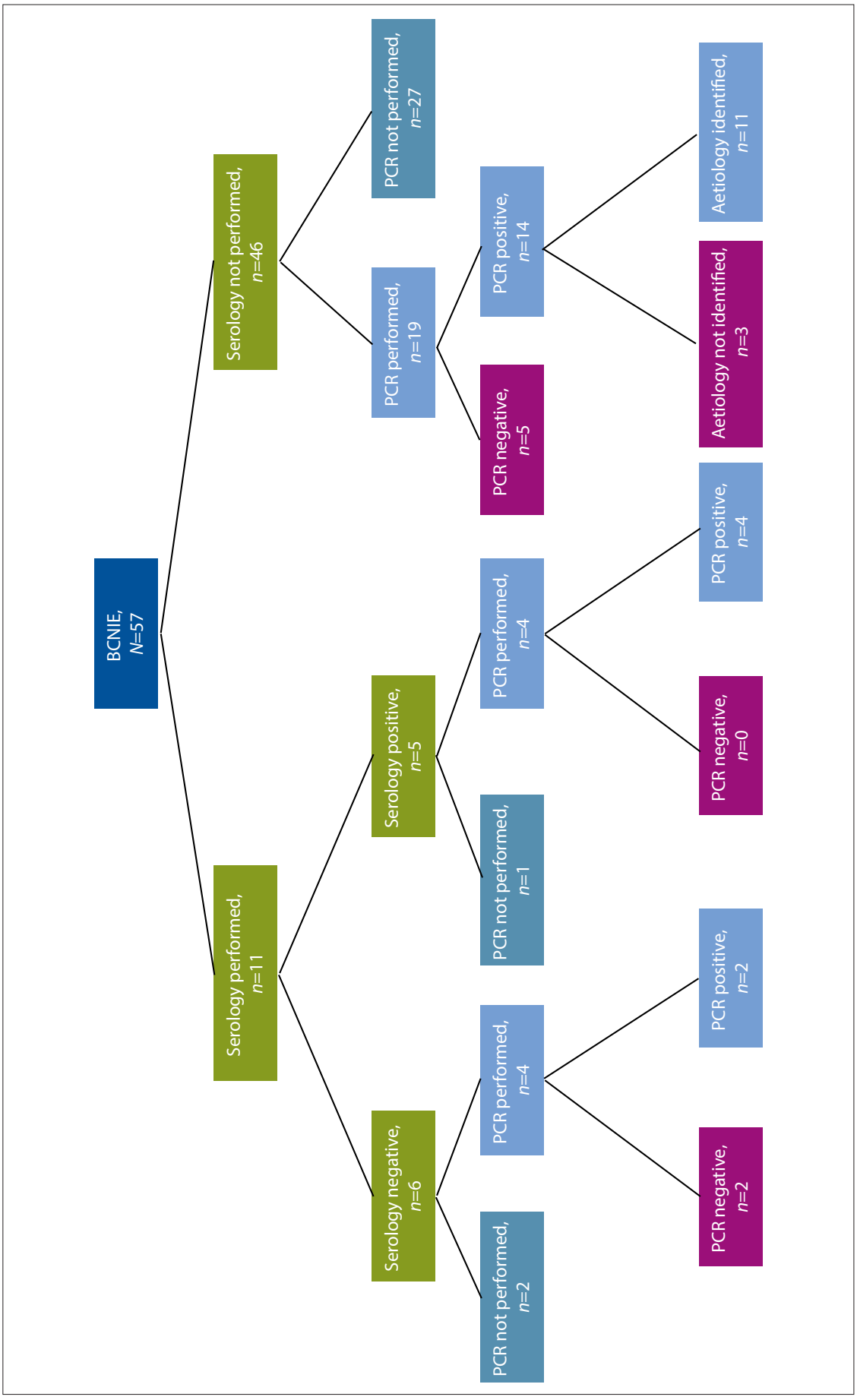

Fig. 1. Diagnostic tests performed. (BCNIE = blood culture-negative infective endocarditis; PCR = polymerase chain reaction.)

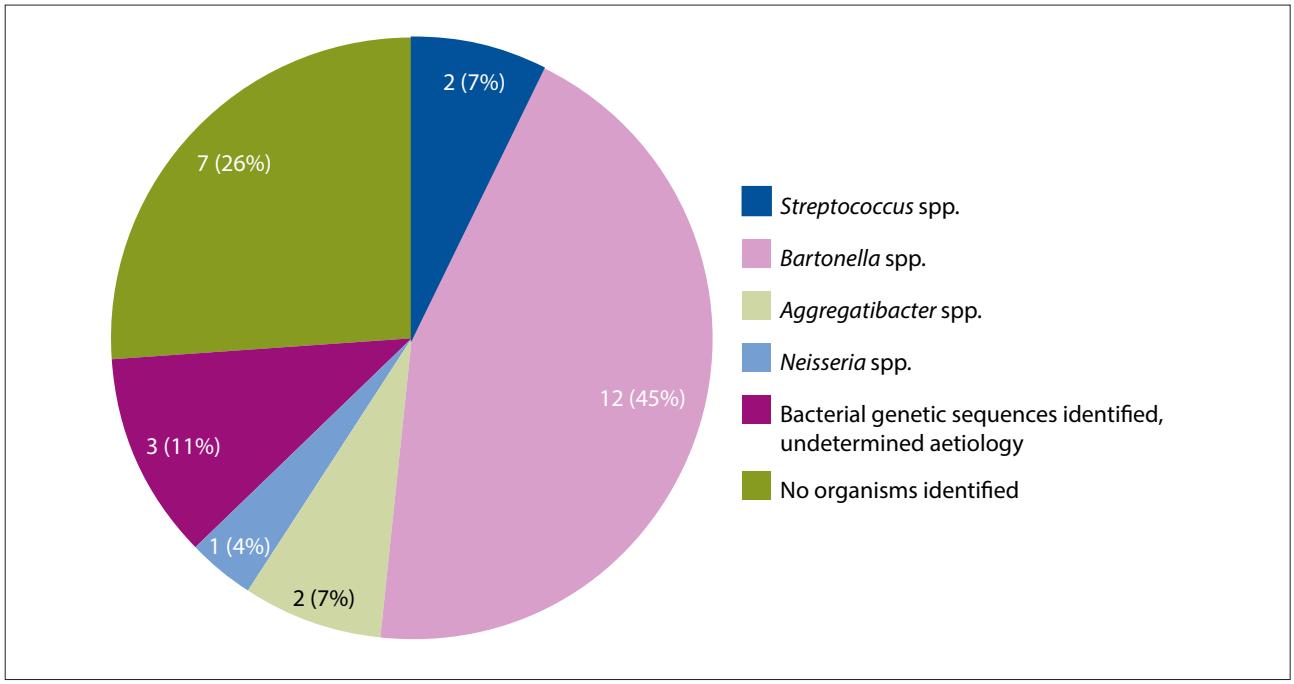

Fig. 2. Organisms identified by tissue polymerase chain reaction, N=*27.*

Table 2. Organisms identified, *N*=57

Organism	Serology, *n*=11	Valve PCR, *n*=27	Valve culture, *n*=27
Streptococcus spp.	-	2	-
Bartonella spp.	5	12	-
Aggregatibacter spp.	-	2	-
Neisseria spp.	-	1	-
Bacterial genetic sequences identified, undetermined aetiology	-	3	-
Other	-	-	3
No organisms identified	6	7	24

PCR = polymerase chain reaction.

BCNIE cases. Because of the low level of bacteraemia in IE, and the high frequency of antibiotic administration before the collection of blood cultures in BCNIE patients in our setting, the application of PCR-based methods to detect micro-organisms in blood samples has potential advantages. For example, in a study by Casalta *et al.*,[18] bacteria were identified in blood samples by PCR in 3/20 (15%) BCNIE patients who had received antibiotics before blood culture collection. The analytical specificity of the blood PCR in that study was 100%. Although the SA National Health Laboratory Service (NHLS) offers blood PCR at GSH, no blood PCRs were performed in this study. This could be due to a lack of physician awareness and/or a lack of perceived diagnostic benefit.

However, tissue PCR has been demonstrated in multiple clinical studies to be an important diagnostic tool in BCNIE.[5,10,15,17] For example, in a prospective study by Fournier *et al.*,[15] 16S rDNA PCR assays on excised valvular tissue samples were positive in 150/227 (66%) BCNIE cases. In the present study, tissue PCR was positive in 20/27 (74%) cases, and yielded a specific organism in 17/27 (63%) cases. In our study, the most common aetiological agent identified by tissue PCR was *Bartonella* spp., found in 12/27 (44%) patients. This is consistent with findings from a previous SA study.[10] The clinical utility of performing tissue PCR has not been previously studied in our setting. Previous studies have demonstrated that patients with *Bartonella* spp. IE have a higher mortality rate and undergo valvular surgery more frequently than patients with IE caused by usual pathogens.[19] As such, identifying *Bartonella* spp. is critical in guiding clinical decision-making. Our study highlights the high prevalence of *Bartonella* spp. in cases of BCNIE requiring valvular surgery in our setting. In addition, we identified 4/12 (33%) *Bartonella* spp. BCNIE patients not on appropriate antimicrobial therapy before surgery, so that performing tissue PCR altered the treatment strategy, as it is recommended that patients with *Bartonella* spp. IE receive an extended course of antibiotics, usually 6 weeks of doxycycline with or without an additional agent.[20, 21] In our study, empiric antibiotic regimens or antibiotics guided by serum serology would have been active against the organisms ultimately identified by tissue PCR in most cases; however, performing tissue PCR helped to confirm the appropriate empiric use of doxycycline in those with tissue PCR-positive for *Bartonella* spp., and provided confidence to withhold it in patients negative for *Bartonella* spp. The addition of empiric doxycycline to current guideline-directed therapy for BCNIE patients in countries with high rates of *Bartonella* spp. IE may be considered.[10]

Tissue cultures offer limited additional diagnostic value when performing serum serology and tissue PCR. In the study by Fournier *et al.*,[11] tissue cultures from valvular samples or implantable device leads were positive in only 41/119 (34%) BCNIE cases; tissue culture did not detect any micro-organisms that PCR had not identified. Other studies have similarly demonstrated a low diagnostic rate of tissue cultures in BCNIE cases. Armstrong *et al.*[17] obtained a positive

valvular culture in 8/46 (17%) BCNIE cases, and Pecoraro *et al.*[10] reported positive valvular tissue culture in 0/20 (0%) patients among the prospective BCNIE cohort. In our study, tissue cultures did not result in additional diagnostic value, and were positive in only 3/27 (11%) patients, 2/3 (66.6%) of which were likely due to contaminants. This demonstrates a poor diagnostic yield of performing tissue cultures in our setting.

Histopathology can help identify non-infective mimics of BCNIE, such as non-bacterial thrombotic endocarditis.[22] In our study, valvular histopathology did not provide any additional aetiological information. Still, it revealed evidence of IE in 7/7 (100%) cases where no pathogen could be identified using the combined diagnostic modalities.

Study limitations

This was a single-centre study; therefore the results are not necessarily generalisable, and the causative organisms associated with BCNIE vary according to geographical region.[10,11] Among our BCNIE patients, serology was not sent for *Mycoplasma* spp.; a previous SA study has identified this as an important aetiological agent.[10] In the present study, many BCNIE patients had neither serum serology nor tissue PCR performed. Part of this study was performed during the COVID-19 pandemic, which significantly influenced healthcare services in SA. The exact impact of the pandemic on our institutional practices during this time is difficult to quantify.[23]

Conclusion

We set out to determine the utility of non-invasive and invasive tests in identifying the bacterial aetiology of blood culture-negative IE. We found that serum serological testing was underutilised, while tissue PCR had a high diagnostic yield and an important impact on antimicrobial utilisation. This study identifies gaps in our approach to BCNIE. Further, it identifies tests that are not cost-effective and do not add value for money in the management of BCNIE.

Data availability. Data used for this study are available from the author on request.

Declaration. The research for this study was done in partial fulfilment of the requirements for WVE's MMed (Int Med) degree at the University of Cape Town.

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Author contributions. WVE designed the study, collected data and performed the data analysis. PM recruited GSH IE registry patients and supervised WVE in the study design and data analysis. MN supervised the study design and data analysis.

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Conflicts of interest. None.

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