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ORIGINAL PAPER

Diagnosis of blood culture-negative endocarditis and clinical comparison between blood culture-negative and blood culture-positive cases

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Abstract
Purpose To analyze the clinical characteristics of blood culture-negative endocarditis (BCNE) and how it compares to those of blood culture-positive endocarditis (BCPE) cases and show how molecular tools helped establish the etiology in BCNE.
Methods Adult patients with definite infective endocarditis (IE) and having valve surgery were included. Valves were studied by polymerase chain reaction (PCR). Statistical analysis compared BCNE and BCPE.
Results One hundred and thirty-one patients were included; 53 (40 %) had BCNE. The mean age was 45 ± 16 years; 33 (62 %) were male. BCNE was community-acquired in 41 (79 %). Most patients were referred from other hospitals (38, 73 %). Presentation was subacute in 34 (65 %), with fever in 47/53 (90 %) and a new regurgitant murmur in 34/42 (81 %). Native valves were affected in 74 %, mostly left-sided. All echocardiograms showed major criteria for IE. Antibiotics were used prior to BC collection in 31/42 (74 %). Definite histological diagnosis was established for 35/50 (70 %) valves. PCR showed *oralis* group streptococci in 21 (54 %), *S. aureus* in 3 (7.7 %), *gallolyticus* group streptococci in 2 (5.1 %), *Coxiella burnetii* in 1 (2.5 %) and *Rhizobium* sp. in 1 (2.5 %). In-hospital mortality was 9/53 (17 %). Fever ($p = 0.06$, OR 4.7, CI 0.91–24.38) and embolic complications ($p = 0.003$, OR 3.3, CI 1.55–6.82) were more frequent in BCPE cases, while new acute regurgitation ($p = 0.05$, OR 0.3, CI 0.098–0.996) and heart failure ($p = 0.02$, OR 0.3, CI 0.13–0.79) were less so.
Conclusions BCNE resulted mostly from prior antibiotics and was associated with severe hemodynamic compromise. Valve histopathology and PCR were useful in confirming the diagnosis and pointing to the etiology of BCNE.

Keywords Infective endocarditis · Negative blood cultures · Polymerase chain reaction · Valve pathology · Diagnostic criteria

Introduction

Despite progresses in blood culture media and automated growth detection systems, blood culture-negative endocarditis (BCNE) still represents 5–69.7 % of all cases of endocarditis. Its main causes are early antibiotic treatment started prior to blood sampling; endocarditis caused by fastidious microorganisms that require prolonged incubation and/or specific media, including *Brucella* sp., defective streptococci, HACEK bacteria, *Legionella* sp., mycobacteria, *Propionibacterium acnes* or other anaerobes, and fungi; endocarditis caused by strictly (*Coxiella burnetii*, *Tropheryma whipplei*) or facultative (*Bartonella* sp.) intracellular bacteria; and lastly, endocarditis that is not infectious in etiology, such as that related to neoplasia and autoimmune diseases (lupus, Behçet, rheumatoid arthritis) [1].

In Europe, the incidence of BCNE has been reported to be 13 % in the UK [2], 14 % in Spain [3], 9 % in France [4], 24 % in Sweden [5] and 25 % in Italy [6]. By comparison, BCNE accounted for 20 % in Japan [7], 31 % in India [8], 23 % in Brazil [9], 48 % in Pakistan [10], 50 % in Turkey [11], 54 % in Tunisia [12], 55 % in South Africa [13], 56 % in Algeria [14], 61 % in the Lao PDR [15], 69 % in

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Thailand [16] and 69.7 % in Egypt [17]. Therefore, BCNE occurs more frequently in developing countries and presents a major clinical challenge for diagnosis and treatment.

This study focuses on patients submitted to surgery in a cardiac referral center in Rio de Janeiro, Brazil, and its objectives are, firstly, to analyze clinical characteristics of BCNE and how it compares to blood culture-positive cases and, secondly, to show how often etiologic diagnosis in cases of BCNE can be established using molecular tools in valve tissue excised at surgery.

Materials and methods

Study design: retrospective cohort study

Setting

Instituto Nacional de Cardiologia (INC) is a cardiac referral center in Rio de Janeiro, Brazil. Annually, approximately 270 valve replacement surgeries are performed at the institute, there are 8000 outpatient visits to the Heart Valve Disease Department, and 25 cases of definite infective endocarditis (IE) are admitted. Around 2/3 of patients referred for surgery are from other hospitals.

Patients

Adult patients with definite IE according to the modified Duke criteria [18] admitted for valve surgery were enrolled prospectively in the International Collaboration Study (ICE) after signing informed consent. The time period of the study was January 2006 to May 2014.

Variables

Demographic, microbiological, echocardiographic, surgical and outcome variables were collected prospectively using the International Collaboration on Endocarditis case report form. Laboratory parameters (ESR, CRP, creatinine levels) were also obtained. Data on histopathological examination of valves were sought retrospectively in the Pathology Department. Clinical criteria for endocarditis in the blood culture-negative cases were compared by the modified Duke criteria and the St. Thomas' (STH) modifications [19]. The STH modifications are shown in Box 1.

Blood cultures

Three sets of blood cultures were taken from peripheral veins in cases of IE with a subacute presentation, and two sets when the presentation was acute. Blood cultures were incubated in the BacT/Alert (Biomerieux, France) system, and pathogen identification was done by Vitek 1 (Biomerieux, France); from September 2009 onwards, Vitek 2 was used for this purpose.

Box 1 Proposed additional minor criteria for the Duke classification (STH additional minor criteria)

Newly diagnosed splenomegaly
Newly diagnosed clubbing
Splinter hemorrhages
Petechiae
High erythrocyte sedimentation rate (ESR)*
High C-reactive protein level, defined as >100 mg/L
Microscopic hematuria**
Central non-feeding lines
Peripheral venous lines

* High ESR is defined as more than one and a half times the upper limit of normal (ESR > 30 mm/h for patients <60 years of age; ESR > 50 mm/h for those >60 years of age; **hematuria was disregarded for patients with positive urine cultures, menstruating women, patients with end-stage renal disease and patients with urinary catheters. Adapted from Lamas and Eykyn [19]

Molecular techniques

These studies were performed in the Aix-Marseille Research Laboratory. Bacterial DNA was extracted from surgically excised valves that had been formalin-fixed and embedded in paraffin using the QIAmp Tissue kit (QIAGEN, Hilden, Germany) with the method as described by the manufacturer. Polymerase chain reaction (PCR) primers, targets and sequencing conditions are detailed elsewhere [20]. Formalin-fixed valves were tested by PCR for the detection of *C. burnetii*, *Bartonella* sp., *T. whipplei*, *Staphylococcus aureus*, *Streptococcus oralis* group, *S. gallolyticus* group, *Enterococcus* sp., *E. coli* and fungi.

Histological examination

This was done in all available valve samples using hematoxylin–eosin by the pathologist in INC (MZ) and in Marseille (HL), using the major and minor criteria for IE described in Lepidi et al. [21]. To detect microorganisms within tissues, the Brown–Brenn, periodic-acid Schiff and Grocott–Gomori stains were systematically used.

Statistical analysis

Data were stored in an Excel Microsoft Office spreadsheet; qualitative data were expressed as absolute and relative frequencies. Quantitative data were expressed as mean ± standard deviation (SD). Comparison between groups was made by using the Chi-square and Fisher's exact tests for

categorical variables and Student's *t* test for continuous variables. A *p* value <0.05 was considered significant. The R program, version 3.1.0, was used for statistical analysis.

Ethical issues

The ICE study has been approved by the Ethics Committee of INC under number 080/2005, and the permission for further data collection was obtained under number 875060/2014. Informed consent was obtained from each patient, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Results

One hundred and thirty-one patients had cardiac surgery for endocarditis between January 2006 and May 2014. Of these, 130 (99 %) had blood cultures taken, and 53 (40 %) of these were negative. Microbiological results of positive blood cultures were viridans group streptococci in 24/77 (31 %), *S. aureus* in 12 (16 %), coagulase negative staphylococci in 12 (16 %), *Enterococcus faecalis* in 8 (10 %), Candida spp. in 4 (5 %), other Gram-positive cocci in 12 (16 %) and Gram negatives in 5 (6 %).

Of the BCNE, 20 (38 %) patients were female and 33 (62 %) were male. The mean age was 45 ± 16 years. IE was community-acquired in 41 (79 %), hospital-acquired in 6 (11 %) and healthcare-related in 5 (10 %). Most patients were referred from other hospitals (38, 73 %).

Presentation was subacute in 34 (65 %). Fever was present in 47/53 (90 %) patients, new regurgitant murmur in 34/42 (81 %) and splenomegaly in 21 (39 %). Clinical features are shown in Fig. 1; denominators reflect the number of cases for which data were available.

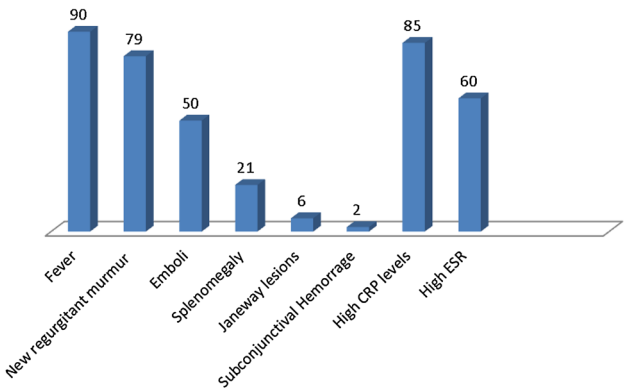

Fig. 1 Selected clinical features in 53 cases of patients with blood culture-negative IE submitted to surgery, INC, 2006–2014. *Numbers* represent %. *CRP* C-reactive protein level, *ESR* erythrocyte sedimentation rate

Affected valves were aortic 22 (42 %), mitral 15 (29 %) and both 15 (29 %); concomitant tricuspid involvement was seen in 5 (10 %). Native valve involvement only was present in 39/53 (74 %). Predisposing conditions for IE and comorbidities are given in Table 1.

All echocardiograms showed major criteria for IE. Transesophageal scans were done in 42/53 (79 %) of patients; TEE was not done when TTE was diagnostic except for prosthetic valves. Echocardiographic features were new valvular regurgitation in 48/52 (92 %), vegetations in 44/53 (83 %), valve perforation in 11 (21 %) and perivalvular abscess in 10 (19 %).

Antibiotic use prior to blood culture collection was recorded in 31/42 (74 %) cases, and the mean days of use were 14.6 ± 12. Only 2/34 (6 %) valve cultures were positive, with *S. gallolyticus* in one and *S. aureus* in the other. Definite histological diagnosis as per Lepidi et al. was established for 35/50 (70 %) available valves; major histological criteria present were vegetations in 22/50 (44 %), polymorphonuclear infiltrates in

Table 1 Predispositions to IE and comorbidities in 53 patients with blood culture-negative IE, INC 2006–2014

Cardiac predisposition	*n* (%)
Congenital heart disease[a]	4 (8)
IVDU	1 (2)
Native valve predisposition	8 (15)
Previous IE	3 (6)
Prosthesis only	3 (6)
RVD	8 (15)
RVD + prosthesis	7 (13)
RVD + IVDU	1 (2)
RVD + previous IE + prosthesis	1 (2)
None	17 (32)
Total	53 (100)
Comorbidities	
CHF	17 (32)
CRF[b]	11 (21)
Hypertension	12 (23)
Cerebrovascular disease	2 (4)
Diabetes mellitus	2 (4)
Cancer	2 (4)
HIV/AIDS	2 (4)
COPD	1 (2)
None	21 (40 %)

IVDU intravenous drug user, *IE* infective endocarditis, *RVD* rheumatic valvular diseases, *CHF* chronic heart failure, *CRF*[b] chronic renal failure, 2 on chronic hemodialysis, *HIV/AIDS* human immunodeficiency virus/acquired immunodeficiency syndrome, *COPD* chronic obstructive pulmonary disease

[a] Subaortic membrane 1, ventricular septal defect (VSD) + previous IE 1, VSD 1, aortic valvuloplasty

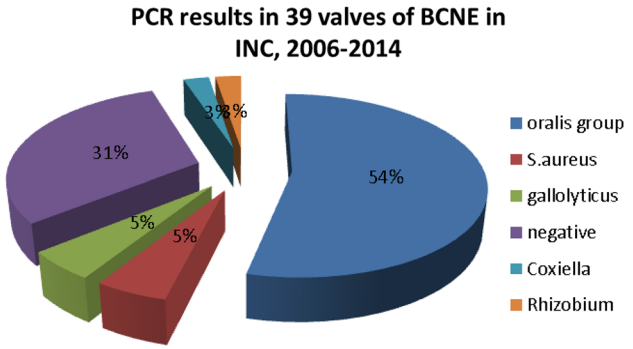

Fig. 2 Microorganisms identified by PCR in 39 valves from patients with BCNE submitted to surgery, INC, 2006–2014. The numbers in the graph are %

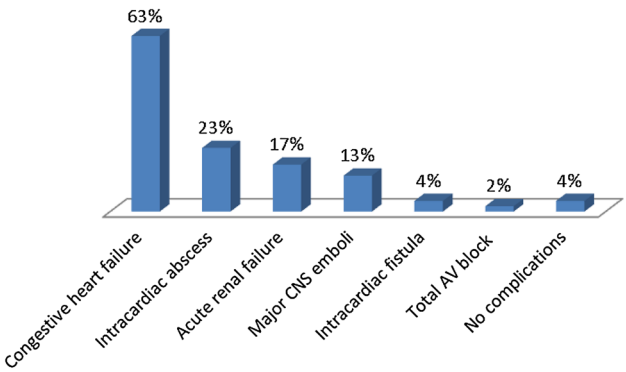

Fig. 3 Complications seen in 53 cases of BCNE, INC, 2006–2014. *CNS* central nervous system, *AV* atrioventricular

37 (74 %) and visible microorganisms in 15/48 (31.3 %) of valves.

Of the BCNE, 39/53 (74 %) had valves available for PCR study. PCR was negative in 11/39 (28 %) and showed *oralis* group streptococci in 21 (54 %), *S. aureus* in 3 (7.7 %), *gallolyticus* group streptococci in 2 (5.1 %), *C. burnetii* in 1 (2.5 %) and *Rhizobium* sp. in 1 (2.5 %). The distribution of these results is shown in Fig. 2.

Complications in the BCNE patients are presented in Fig. 3.

All patients in this study had cardiac surgery, as described in "Materials and Methods" section. In-hospital mortality was 9/53 (17 %) and refers to postoperative mortality. Six-month follow-up was known for 38 patients, and 8 (23 %) of them died.

A statistical analysis was performed, comparing variables in patients with blood culture-positive endocarditis (BCPE) and those with BCNE. Fever and embolic complications were more frequent in blood culture-positive cases, while new acute valvular regurgitation and heart failure were more frequent in those with BCNE. As expected, microorganisms were more often seen on histopathological examination of valve specimens from patients with BCPE. The statistical differences found are given in Table 2.

There was no difference regarding age (BCPE, mean age 41.1 ± 15.5 years, $p = 0.123$, Student's t test), sex or in-hospital postoperative mortality. No statistical difference was found in comorbidities such as previous chronic renal failure, hemodialysis, diabetes mellitus or heart failure nor in the predispositions to IE such as the presence of valve prosthesis or intracardiac devices, rheumatic valvular disease, previous IE or other types of predisposing valve lesions. There were no differences in frequency of splenomegaly or of high CRP levels. No statistical difference was found in the frequency of prosthetic and native valve involvement, or the presence of vegetations or abscesses on echocardiograms.

All cases except one were definite by histopathology or surgical findings; the one case had a positive valve PCR for an *oralis* group streptococcus. If pathological criteria were disregarded, nearly one-third of BCNE cases would be possible IE (16/53, 30 %). The modified Duke criteria would be able to make a definite clinical diagnosis for IE in 30/53 (57 %) of cases and the additional STH minor criteria, by adding one or more minor criteria, in 37 (70 %) of cases.

Discussion

One of the objectives of this study was to evaluate the molecular and histopathological diagnosis of BCNE on valve tissue obtained at surgery. Cases of left-sided endocarditis predominated, as the main indication for valve replacement therapy was severe valvular dysfunction leading to acute heart failure, or acute on chronic heart failure [22–24]. The most frequently isolated pathogens in blood were *viridans* group streptococci and staphylococci, but over 1/3 of cases were culture negative. The frequency of BCNE was higher than that of two recent Brazilian series, where it represented 23 % of 221 episodes of community-acquired IE [9] and 15.5 % of 71 cases of all IE [25]; however, the incidence of 40 % of BCNE in our institute is within the range of 15.5–69.7 % found in other developing countries [8, 11–17].

When valves excised at surgery were analyzed using the well established criteria for histological diagnosis [21], definite histological diagnosis was made in 70 % of patients with BCNE, which is in accord with Greub et al. [26] and Morris et al. [27], who studied all cases of endocarditis, and not only BCNE. In the first, the sensitivity and specificity of valvular histology were 63 and 100 %, respectively, using the same diagnostic criteria as we did; in the latter, the sensitivity was 79 %, but less stringent criteria were

Table 2 Statistical results comparing blood culture-negative and blood culture-positive cases of IE submitted to valve replacement surgery, INC 2006–2014

Variables	BCNE *n*/*N* (%)	BCPE *n*/*N* (%)	*p*	Test	OR	LCI	UCI
Age (years ± SD)	45 ± 16	41.1 ± 15.5	0.123	Student *t*			
Male gender	33 (62.3 %)	50 (65 %)	0.899	Chi-square			
Diabetes mellitus	2 (3.8 %)	9 (11.6 %)	0.198	Fisher's exact			
CHF	17 (32 %)	18 (23.3 %)	0.37	Chi-square			
CRF	10 (18.8 %)	19 (24.6 %)	0.57	Chi-square			
Hemodialysis	2 (3.8 %) 8	8 (10.4 %)	0.198	Fisher's exact			
Predisposition	21 (39.6 %)	37 (48.1 %)	0.44	Chi-square			
RVD	16 (30.2 %)	30 (38.9 %)	0.40	Chi-square			
Previous IE	5 (9.4 %)	9 (11.7 %)	0.90	Fisher's exact			
Native valve	38 (71.7 %)	56 (72.7 %)	1.00	Chi-square			
Prosthetic valve	15 (28.3 %)	21 (27.2 %)	1.00	Chi-square			
ICD	2 (3.8 %)	5 (6.5 %)	0.70	Fisher's exact			
Fever > 38 °C	47/52 (89 %)	74/76 (97 %)	0.06	Fisher's exact	4.7	0.91	24.38
Splenomegaly	11 (21.6 %)	27 (36 %)	0.125	Chi-square			
CRP levels	33 (84.6 %)	52 (86.7 %)	1.00	Chi-square			
Heart failure[a]	45/52 (85 %)	50/77 (65 %)	**0.02**	Chi-square	0.3	0.13	0.79
Acute renal failure	14 (26.4 %)	14 (18.2 %)	0.365	Chi-square			
Vegetations	44 (83.0 %)	69 (89.6 %)	0.406	Chi-square			
Abscess	10 (18.9 %)	14 (18.2 %)	1.00	Chi-square			
New moderate to severe valvular regurgitation	48/52 (92 %)	60/76 (79 %)	**0.05**	Fisher's exact	0.3	0.098	0.996
Embolic complications	16/52 (31 %)	45/77 (58 %)	**0.003**	Chi-square	3.3	1.55	6.82
Organisms seen on valve histology	14/47 (30 %)	35/68 (51.5 %)	**0.03**	Chi-square	2.5	1.147	5.48
In-hospital mortality	9 (17 %) 19	19 (24.7 %)	0.406	Chi-square			

SD standard deviation, *BCNE* blood culture-negative endocarditis, *BCPE* blood culture-positive endocarditis, *CHF* chronic heart failure, *CRF* chronic renal failure, *RVD* rheumatic valve disease, *ICD* intracardiac device, *CRP* C-reactive protein, *p p* value, *OR* odds ratio, *LCI* lower confidence interval, *UCI* upper confidence interval

Significant *p* values are highlighted in bold

[a] Heart failure as a consequence of valvular dysfunction resulting from IE

used. However, only when microorganisms are seen is it possible to guess more accurately what the etiology was; this was the case in one-third of the valves studied, which compares to 60 % in 31/52 cases of valves from patients with BCNE [27] and 9 % in 11 patients with BCNE [28].

Diagnosis of IE, by the time a patient is sent to operating room, has usually been made based on clinical and imaging findings. Therefore, the pathologist can confirm the diagnosis of endocarditis, but, more importantly, there is the chance to identify the causative pathogen by tissue staining properties, what was the case for one-third of the BCNE in this series. Although the yield of valve culture was low (6 %), it provided the opportunity to identify two typical pathogens for IE and determined the full-length course of treatment, an important clinical decision. If valve culture is negative, a shorter treatment course might suffice, depending on the preoperative treatment duration and also on surgical findings, such as abscess or fistula formation. Total duration of treatment also may rely on histopathological findings that show evidence of active endocarditis, such as the presence of microorganisms and polymorphonuclear infiltrate, and these may indicate the need for further antimicrobial treatment. Therefore, we suggest resected valve tissue is sent to both the microbiology laboratory and the pathology department in patients with BCNE.

Regarding molecular diagnosis of IE, PCR was recognized as a useful tool nearly 20 years ago [29, 30] and has been proposed as a Duke major diagnostic criterion [31], since, in active endocarditis, it is able to detect the causative pathogen. A recent British and Irish study reinforces the importance of PCR in cases of BCNE, where it provided an etiological agent in 43/69 (62 %) of examined valves [32]. PCR should not be performed in valves of patients who are not suspected of IE, as it may show positivity resulting from past infections. Previous studies have demonstrated that bacterial and fungal genetic material may be present months or years following previous episodes of IE [33–35]. One must also carefully consider possible PCR contamination, and

appropriate controls are crucial, as well as a careful interpretation of results [26]. All valves analyzed by PCR in this study were from patients operated emergently or urgently for IE, and most showed pathogens that are frequently found in IE, notably oral streptococci, *S. gallolyticus* group streptococci and *S. aureus*. Only one patient presented a zoonotic infection, *C. burnetii*. In another surgical referral center in São Paulo, Brazil, *Bartonellae* were responsible for 10/51 (19.6 %) cases of community-acquired BCNE and *C. burnetii* for 4/51 (7.8 %), and these diagnosis relied mostly on serological analysis, although all 6 patients with *Bartonella* IE who had valves removed at surgery had diagnosis confirmed by valve PCR [36]. We have reported 2 cases of *Bartonella* in 51 cases of BCNE which had valves available for study from 1998 till 2009 in our center [37]. Also, seroepidemiological studies have found a high antibody prevalence for *Bartonella* spp. in blood donors and in asymptomatic HIV-positive individuals in the city of Rio de Janeiro, as well as in their pets [38]. Serological testing for zoonotic agents is not routinely done in our center, and it may be that some diagnoses were missed. However, there was a good correlation between valve PCR and serology in the mentioned recent study [36], which suggests we did not miss cases. Several references point to the zoonotic bacteria *C. burnetii* and *Bartonella* spp. as the main agents of BCNE, in both developed [20] and developing countries [14, 15, 17], and therefore, our current findings are different in this respect, pointing to antibiotic use as the main cause of blood culture negativity.

In our center, viridans group streptococci predominated in both BCPE and BCNE. It must be emphasized that rheumatic fever is still highly prevalent in Brazil, and it accounted for about 1/3 of predisposing heart lesions in patients with IE submitted to surgery. It must also be noted that oral health is poor in our patient population [39]. Therefore, damaged rheumatic valves and poor oral health are probably the main contributors to this scenario.

When clinical, echocardiographic and outcome variables were compared in BCNE and BCPE, complications such as new valvular regurgitation and new heart failure were more frequent in BCNE, and fever and embolic phenomena were less so. This is similar to a recent three-center study from Spain in which the profile of BCNE versus BCPE was investigated [3]. In this paper, patients with IE from 1996 to 2011 were included. There were 106 BCNE (although only 79 cases were definite) and 643 BCPE. In their analysis, heart failure was also more common in BCNE, and fever and embolic phenomena in BCPE. This may occur because pathogens of lower virulence predominated in BCNE, leading to a subacute course and also diagnostic delay, resulting in severe valve damage and heart failure with surgical indication when diagnosis was eventually made. More fever and embolic phenomena may be anticipated when more virulent pathogens, such as staphylococci, are involved, and also in cases where antibiotics have not yet been started. Mortality rates between BCPE and BCNE were similar in our study, which accords with data in the Spanish study.

Antibiotic use prior to blood culture sampling was higher in our sample (3/4 of BCNE), as compared to 47 % in Siciliano et al. ($n = 51$ cases of definite BCNE), 34 % in Ferrera et al. ($n = 79$ definite BCNE), 2/3 in Lamas and Eykyn ($n = 34$ cases of definite BCNE) and 44 % in Fournier et al. ($n = 549$ definite BCNE) [2, 3, 9, 20]. In the 13 patients with BCNE whose valves were studied by PCR by Greub et al. [26], 12 had received antibiotics. The high rate of antibiotic use in our sample may be due to the fact all our BCNE were surgical cases, mostly referred from other institutions, whereas in the mentioned papers, the surgical rate was about 50 %.

Finally, our results confirm that the modified Duke criteria perform poorly in the setting of BCNE [2, 19, 40], with 57 % of definite cases, versus 70 % when the STH additional minor criteria are added (new onset splenomegaly or clubbing, petechiae, splinter hemorrhages, hematuria, high ESR or high CRP levels, presence of any vascular line). These minor criteria were suggested based on the observation of several case series where Oslerian features of endocarditis were uncommon (less than 5 %), whereas petechiae and splinter hemorrhages were common (approximately one quarter of cases). They also recognize that rheumatoid factor is nowadays rarely requested in the scenario of infection, whereas ESR and CRP are routinely requested. Unfortunately, clubbing is not a feature included in the case report forms used in the study, and testing for hematuria is often not done in clinical practice (or the information was unavailable in the referred patients). Despite these shortcomings, the STH criteria performed better in BCNE, as has been previously shown in a British series of BCNE [2].

Study limitations are that data obtained refer to a single cardiac referral center, and therefore, there is the referral bias of patients who have a surgical indication for the treatment of IE. The setting is a developing country, and therefore, conclusions may not be extrapolated. Another limitation was that data were not available for all studied features, due to the partially retrospective nature of the study and to the referral pattern of the patients, where initial data were (not) obtained from other institutions.

Conclusions

BCNE has a high incidence in our hospital, and this resulted mainly from prior antibiotic use. Valve culture had a very low sensitivity for diagnosis, while histopathology confirmed IE in around 3/4 of cases. PCR of valves was the most helpful tool, *identifying* pathogens in over 2/3 of cases. *Viridans* group

streptococci predominated, as they are easily eliminated from blood with antibiotic use and are one of the most frequent agents of IE in developing countries, such as Brazil. Zoonotic agents such as *C. burnetii* and *Bartonella* sp. were infrequent, differently from other reported series of BCNE.

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Compliance with ethical standard

Conflict of interest The authors have no conflict of interest regarding this manuscript.

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