International Journal of Infectious Diseases 161 (2025) 108151

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Systematic serological testing in infective endocarditis: Limited clinical impact despite high usage—a 14-year cohort study

Noelia Ruiz-Alonso[1], Claudia González-Rico[1,2], Javier Queipo Menéndez[3], Concepción Fariñas-Álvarez[2,4], Jesús Rodríguez-Lozano[2,5], Manuel Gutiérrez-Cuadra[1,2], Manuel Cobo-Belaustegui[6], José Francisco Gutiérrez-Díez[7], Carlos Armiñanzas[1,2], Francisco Arnaiz de las Revillas[1,2,8], Itxasne Cabezón[1], Marta Fernández-Sampedro[1,2,8], Raúl Parra-Fariñas[1], Paula Runza[1], Inés de Benito-Población[5], María Carmen Fariñas[1,2,8,*]

[1] *Infectious Diseases Service, Hospital Universitario Marqués de Valdecilla-IDIVAL, Santander, Spain*
[2] *CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III (CIBERINFEC, ISCIII), Madrid, Spain*
[3] *Internal Medicine Service, Hospital Universitario Marqués de Valdecilla-IDIVAL, Santander, Spain*
[4] *Division of Health Care Quality, Hospital Universitario Marqués de Valdecilla-IDIVAL, Santander, Spain*
[5] *Microbiology Service, Hospital Universitario Marqués de Valdecilla-IDIVAL, Santander, Spain*
[6] *Cardiology Service, Hospital Universitario Marqués de Valdecilla-IDIVAL, Santander, Spain*
[7] *Cardiovascular Surgery Service, Hospital Universitario Marqués de Valdecilla-IDIVAL, Santander, Spain*
[8] *Departamento de Medicina y Psiquiatría, University of Cantabria, Santander, Spain*

ARTICLE INFO

Article history:
Received 30 September 2025
Revised 19 October 2025
Accepted 22 October 2025

Keywords:
Infective endocarditis
Serology
Blood culture
Diagnostic utility

ABSTRACT

Objectives: To evaluate the clinical utility of systematic serological testing in infective endocarditis (IE), determine the prevalence of blood-culture negative IE (BCNIE), and characterize its clinical presentation in our cohort.

Methods: Retrospective analysis of 296 consecutive IE episodes (2008-2021) at a tertiary hospital. We compared clinical characteristics, serological testing patterns, and outcomes between BCNIE and blood-culture-positive IE (BCPIE) cases.

Results: BCNIE accounted for 22.3% (66/296) of cases. Prior antibiotic use was significantly higher in BCNIE (27.3% vs 2.2%, $P < 0.001$). Serological testing was performed in 81.8% of BCNIE and 71.3% of BCPIE cases. Despite positive serological results for *Coxiella burnetii* phase I IgG (24.2% of tested cases), *Bartonella henselae* IgG (14.9%), *Mycoplasma pneumoniae* IgM (6.9%), and *Brucella* spp. (1.5%), only one patient (1.9% of all positive results) received targeted antimicrobial therapy. In multivariate analysis, no serological marker was associated with improved clinical outcomes.

Conclusions: Systematic serological testing in IE provides limited diagnostic and therapeutic value. A selective approach targeting BCNIE cases with specific epidemiological risk factors appears more appropriate and cost-effective.

Introduction

Infective endocarditis (IE) is a severe, life-threatening disease in which successful treatment depends on accurate identification of the causative pathogen. Blood culture remains the gold standard for determining the responsible microorganism in IE cases. The major Duke criteria for IE diagnosis include either multiple positive blood cultures for typical IE-causing organisms or more than two positive blood cultures for less common agents [1].

However, blood-culture-negative infective endocarditis (BCNIE) still accounts for up to 31% of IE cases and is associated with increased long-term mortality [2]. BCNIE may result from prior empirical antibiotic administration, infection by intracellular pathogens not detectable by conventional blood culture, or difficulties in cultivating fastidious microorganisms using standard media [3]. These limitations underscore the need for alternative laboratory diagnostic approaches.

* Corresponding author: María Carmen Fariñas, Infectious Diseases Service. Hospital Universitario Marqués de Valdecilla-IDIVAL, Av. Valdecilla, s/n., Santander, 39008, Spain.
E-mail address: mcarmen.farinas@scsalud.es (M.C. Fariñas).

https://doi.org/10.1016/j.ijid.2025.108151

The modified Duke criteria now consider antibody titers against specific pathogens as major diagnostic criteria: an anti-phase I IgG titer ≥1:800 for *Coxiella burnetii,* and both IgG and IgM detection for *Bartonella quintana* or *Bartonella henselae* with IgG titers ≥1:800 [1]. Serological testing is also commonly employed for other fastidious, slow-growing, or non-culturable microorganisms, including *Brucella* spp., *Legionella pneumophila*, and *Chlamydia* spp. [4,5].

Although serology provides a rapid and culture-independent diagnostic tool, it has several limitations. These include cross-reactivity between different genera and prolonged persistence of IgM antibodies, which can lead to false-positive results [4]. Moreover, serological testing is frequently performed in patients in whom causative microorganisms have already been identified by blood culture, limiting its added diagnostic value while consuming time and resources [6].

The primary objective of this study is to assess the clinical impact of serological findings and to evaluate the usefulness of systematic serological testing in an IE cohort. The secondary objectives are to determine the prevalence of culture-negative cases and to characterize their clinical presentation.

Methods

Study design and population

We conducted a retrospective analysis of a prospectively maintained cohort that included all consecutive cases with a final diagnosis of possible or definite IE, as defined by modified Duke criteria, recorded between January 2008 and December 2021 in the IE cohort at University Hospital Marqués de Valdecilla. Patients transferred from other hospitals were excluded to avoid including already diagnosed episodes with incomplete data. Only the first episode was included for patients who experienced recurrences during the study period.

Data collection and quality

We collected variables related to sociodemographic data, comorbidities, IE clinical presentation, microbiological data, clinical and surgical interventions, and outcomes of each registered case. Data were collected from patients' medical records included in the Spanish Infective Endocarditis Group (GAMES) database, a group of the Spanish Society of Cardiovascular Infections (SEICAV), following predefined definitions and criteria that remained unchanged throughout the study period. The database is maintained by personnel with clinical and microbiological expertise and is regularly monitored by a dedicated external data entry professional to ensure accuracy and consistency. All IE cases included in the study had been previously quality-checked by the GAMES coordination team. This study followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations [7].

Definitions

Hospital-acquired IE was defined as episodes with IE-related symptoms or signs that developed during or after hospitalization and were not present or incubating at admission time. Healthcare-associated IE was defined as episodes occurring in patients who had undergone any invasive procedure within the preceding 3 months. In-hospital mortality was defined as all-cause mortality occurring during hospital stay for the episode.

Serological tests were categorized into diagnostic serology, conducted during the acute episode, and follow-up serology, performed up to 8 weeks after hospital discharge. Serology results were interpreted as positive according to thresholds defined by respective commercial kits (1:80 for *B. henselae* IgG and *C. burnetii* IgG total and phase I, 1:20 for *Brucella* spp., and 1.1 index for *M. pneumoniae* IgM). For patients with available phase-specific *C. burnetii* serologies, IgG phase I positivity was considered the main serological marker associated with chronic Q fever and included in analysis. For patients before 2009, only total IgG was available, and these cases were analyzed separately due to lack of phase differentiation. Equivocal serology results were carefully managed to avoid misclassification. If no repeat testing was available, the equivocal result was excluded from positivity. This approach ensured that uncertain results were translated into a definite result whenever possible, without overestimating positivity and avoiding false negatives.

Targeted therapy for these pathogens was determined based on established guidelines and clinical practice, considering combinations with doxycycline for *B. henselae, Brucella* spp. and *C. burnetii*, and combinations with macrolides, doxycycline, or fluoroquinolones against *M. pneumoniae*, initiated after positive serology results [8–13].

Full recovery was defined as patients who completed the episode cured, excluding those who died, relapsed, or developed reinfection within the first 3 months post-discharge.

Statistical analysis

Categorical variables are expressed as frequencies and percentages. Quantitative variables are expressed as mean values and standard deviations for symmetrically distributed data or as median values and interquartile ranges for asymmetrically distributed data. Chi-squared or Fisher's exact tests were performed for comparisons of categorical variables between groups. Student's t-test and Mann-Whitney U test were used for continuous variables after distribution normality verification using the Shapiro-Wilk test.

Multivariable logistic regression was performed to examine independent associations between predictor variables and binary outcomes, adjusting for potential confounders. Separate logistic regression models were constructed for each microorganism (serology result coded as 0 = negative, 1 = positive) with full recovery as the dependent variable (coded as 0 = no, 1 = yes). To ensure model stability, subgroups with very small number of positive results were excluded. Each model included the serological result as the main predictor and was adjusted for age, sex, and the Charlson comorbidity index. Adjusted odds ratios (aOR) with 95% confidence intervals (CI) were reported.

Differences were considered significant when the *P*-value was <0.05. Owing to the thorough quality control of the database, the majority of variables were fully available for all patients. In instances where data were not available, we excluded the specific missing values from the corresponding descriptive statistical analyses. No data imputation or replacement was performed, ensuring that all reported results are based solely on observed data. Missing values are reported when applicable. Statistical analyses were performed using IBM SPSS Statistics (version 19).

Ethics

This study was conducted in accordance with principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the collection of any clinical data. Participant confidentiality and data anonymity were maintained throughout the study.

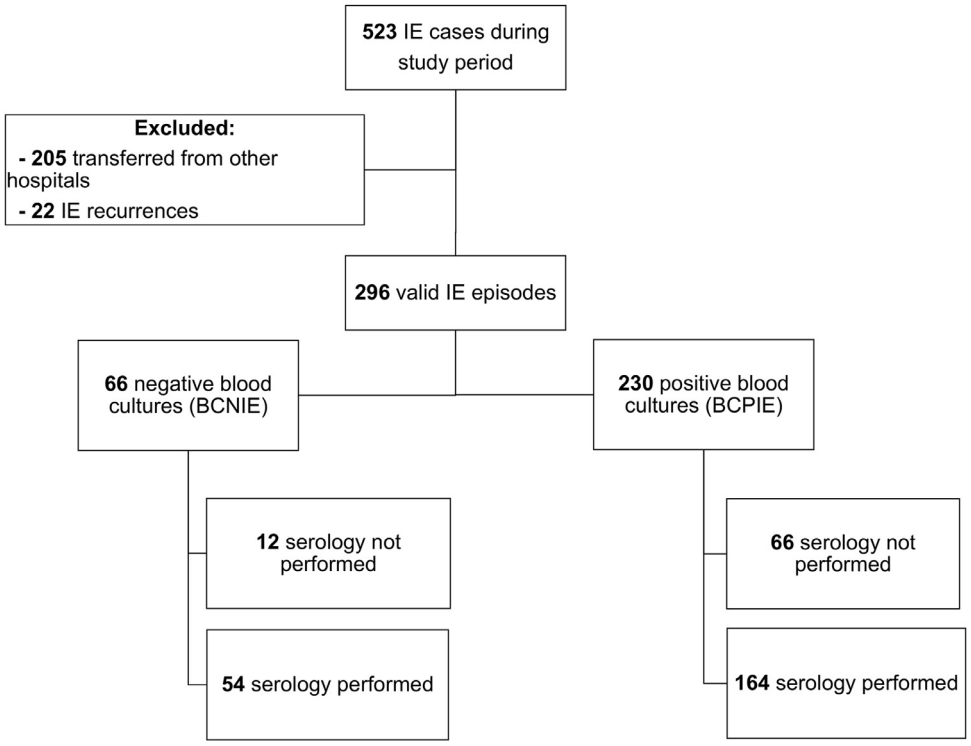

Figure 1. Study flow chart showing patient selection and classification. Valid IE episodes were classified in BCNIE (blood-culture negative infective endocarditis) and BCPIE (blood-culture positive infective endocarditis).

Results

Patient characteristics

A total of 296 IE episodes were included during the study period (Figure 1), with 95 (32.1%) patients being women, and a median age of 71.0 (interquartile range, IQR: 60.5-77.6) years. Sixty-six (22.3%) cases were classified as BCNIE, of which 18 (27.3%) had received antibiotics before blood culture collection, compared to only 5 (2.2%) among blood-culture positive infective endocarditis (BCPIE) cases ($P < 0.001$) (Table 1).

Patients with BCNIE and BCPIE showed similar baseline characteristics, with some notable exceptions. BCNIE patients had slightly lower median age (67.9 vs 71.9 years, $P = 0.066$) and body mass index (25.5, IQR: 23.5-29.7 vs 26.0, IQR: 23.6-29.3 kg/m^2, $P = 0.035$). Hypertension was more prevalent in BCNIE patients (74.2% vs 56.5%, $P = 0.032$), while myocardial infarction and chronic renal disease were less frequent in this group (1.5% vs 12.2%, $P = 0.011$ and 7.6% vs 16.5%, $P = 0.036$, respectively).

Clinical presentation

Clinical characteristics of IE episodes according to blood culture results are summarized in Table 2. Distribution of affected valves and acquisition setting was similar across groups, although surgery tended to be more frequently required among BCNIE patients (48.5% vs 35.2%, $P = 0.050$). Notably, a higher proportion of BCNIE cases were classified as "possible" rather than "definite" IE according to Duke criteria (43.9% vs 10.9%, $P < 0.001$). In-hospital mortality and length of hospital stay did not differ significantly between BCNIE and BCPIE groups (27.3% vs 24.3%, $P = 0.629$ and 38.4 ± 21.9 vs 41.3 ± 38.6 days, $P = 0.555$, correspondingly).

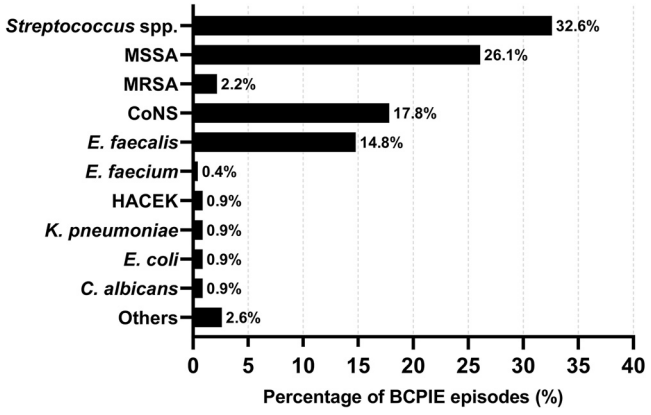

Figure 2. Distribution of microorganisms isolated in blood-culture positive IE cases (BCPIE). The pathogens identified were *Streptococcus* spp., methicillin-sensitive *Staphylococcus aureus* (MMSA), methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-negative staphylococci (CoNS), *Enterococcus faecalis* (*E. faecalis*), *Enterococcus faecium* (*E. faecium*), HACEK bacteria (including *Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella* and *Kingella), Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E. coli)* and *Candida albicans (C. albicans)*. The "Others" category comprised one isolate each of *Enterobacter cloacae, Campylobacter fetus, Abiotrophia defectiva, Providencia rettgeri, Serratia marcescens* and *Corynebacterium atriatum*.

Microbiological findings

Among the 230 BCPIE episodes, the spectrum of microorganisms isolated showed that *Streptococcus* spp., *Staphylococcus aureus* (both methicillin-susceptible and resistant) and coagulase-negative staphylococci were the most common pathogens, followed by *Enterococcus faecalis*. Less frequent isolates included HACEK group bacteria, gram-negative bacilli, and *Candida albicans* (Figure 2).

Table 1
Baseline demographic characteristics and comorbidities of IE patients.

	Group (no. of cases)			*P*-value[a]
	Total (296)	BCNIE (66)	BCPIE (230)	
Sex				
Female	95 (32.1)	24 (36.4)	71 (30.9)	0.399
Male	201 (67.9)	42 (63.6)	159 (69.1)	
Median age (IQR), years	71.0 (60.5-77.6)	67.9 (58.6-74.5)	71.9 (61.2-78.7)	0.066
Median BMI (IQR), kg/m² [b]	25.9 (23.6-29.2)	25.5 (23.5-29.7)	26.0 (23.6-29.3)	0.035
Median Charlson comorbidity index (IQR)	5 (3-7)	4 (3-6)	5 (3-7)	0.731
Diabetes mellitus	79 (26.7)	17 (25.8)	62 (27)	0.846
Hypertension	179 (60.5)	49 (74.2)	130 (56.5)	0.032
Hyperlipidemia	141 (47.6)	30 (45.5)	111 (48.3)	0.166
Solid malignancy	63 (21.3)	11 (16.7)	52 (22.6)	0.227
Hematological malignancy	6 (2)	2 (3.0)	4 (1.7)	0.760
COPD	59 (19.9)	18 (27.3)	41 (17.8)	0.232
Congestive heart failure	99 (33.4)	22 (33.3)	77 (33.5)	0.899
Myocardial infarction	29 (9.8)	1 (1.5)	28 (12.2)	0.011
Coronary disease	93 (31.4)	19 (28.8)	74 (32.2)	0.849
Chronic renal disease	43 (14.5)	5 (7.6)	38 (16.5)	0.036
Liver disease	37 (12.5)	5 (7.6)	32 (14)	0.167
Prior antibiotic use[c]	23 (7.8)	18 (27.3)	5 (2.2)	<0.001

BCNIE, blood-culture negative infective endocarditis; BCPIE, blood-culture positive infective endocarditis; IQR, interquartile range; BMI, body mass index; COPD: chronic obstructive pulmonary disease.
[a] Two-tailed Chi-squared test or Fisher's exact test for categorical variables, as corresponding. Due to the non-normal distribution of the quantitative variables, two-tailed Mann-Whitney U test was used.
[b] Missing values: $n = 78$.
[c] In the 7 days preceding blood culture collection.

Table 2
Clinical characteristics of IE episodes according to blood culture results.

	Group (no. of cases)			*P*-value[a]
	Total (296)	BCNIE (66)	BCPIE (230)	
IE Duke classification				
Definite	242 (81.8)	37 (56.1)	205 (89.1)	<0.001
Possible	54 (18.2)	29 (43.9)	25 (10.9)	
IE location				
Aortic	149 (50.3)	36 (54.5)	113 (49.1)	0.438
Mitral	134 (45.3)	28 (42.4)	106 (46.1)	0.598
Tricuspid	18 (6.1)	3 (4.5)	15 (6.5)	0.554
Pulmonary	5 (1.7)	2 (3.0)	3 (1.3)	0.310
Device	18 (6.1)	6 (9.1)	12 (5.2)	0.246
Valve type				
Native	205 (69.3)	44 (66.7)	161 (70.0)	0.605
Prosthetic	76 (25.7)	18 (27.3)	58 (25.2)	0.986
Early	33 (11.1)	9 (13.6)	24 (10.4)	0.519
Late	43 (14.5)	9 (13.6)	34 (14.8)	
IE acquisition				
Community	228 (77.0)	51 (77.3)	177 (77.0)	0.223
Hospital-acquired	54 (18.2)	10 (15.2)	44 (19.1)	
Healthcare-associated	9 (3.0)	2 (3.0)	7 (3.0)	
Unknown	4 (1.4)	3 (4.5)	2 (0.9)	
Fever	222 (75.0)	45 (68.2)	177 (77.0)	0.147
Surgery	113 (38.2)	32 (48.5)	81 (35.2)	0.050
In-hospital mortality	74 (25.0)	18 (27.3)	56 (24.3)	0.629
Mean stay length ± SD, days	40.6 ± 35.6	38.4 ± 21.9	41.3 ± 38.6	0.555

BCNIE, blood-culture negative infective endocarditis; BCPIE, blood-culture positive infective endocarditis; IE, infective endocarditis; SD, standard deviation.
[a] Two-tailed Chi-squared test or Fisher's exact test for categorical variables, as appropriate. Due to the normal distribution of the quantitative variable, a two-tailed t-test was used.

Serological testing results

Serological testing was performed in 54 (81.8%) BCNIE cases and 164 (71.3%) BCPIE cases at diagnosis ($P = 0.087$), while follow-up serology was tested in 26 (39.4%) BCNIE cases and 79 (34.3%) BCPIE cases ($P = 0.450$). The most frequently detected pathogen during acute IE episodes was *C. burnetii*, followed by *B. henselae* and *M. pneumoniae*. Only 3 (1.5%) episodes were positive for *Brucella* spp. Differences in positivity between groups were significant for *C. burnetii* total IgG at diagnosis, with a higher frequency in BCNIE patients (Table 3). None of the serology-positive cases could be confirmed by molecular techniques.

Clinical impact of serological results

To evaluate the clinical relevance of positive serological results, we assessed use of targeted antimicrobial therapy. None of the cases with positive serology *for B. henselae, Brucella* spp., or *M. pneumoniae* were deemed clinically significant, as no directed treatment was prescribed. Among eight patients with positive anti-phase I IgG results for *C. burnetii,* only one (12.5%) received specific antimicrobial therapy with doxycycline and hydroxychloroquine. This patient, who had an antibody titer of 1:10,000, fulfilled a Duke major criterion for infective endocarditis but had previously yielded *S. aureus* in blood cultures. Another patient with an anti-

Table 3
Serological testing results during diagnosis and follow-up periods.

	Diagnosis				Follow-up			
	Total	BCNIE	BCPIE	*P*-value[a]	Total	BCNIE	BCPIE	*P*-value[a]
B. henselae								
No. of tested cases	194	48	146		97[b]	26	71	
Positivity	29 (14.9)	8 (16.7)	21 (14.4)	0.350	17 (17.5)	3 (11.5)	14 (19.7)	0.261
***Brucella* spp.**								
No. of tested cases	196	49	147		93	22	71	
Positivity	3 (1.5)	1 (2.0)	2 (1.4)	0.277	2 (2.2)	1 (4.5)	1 (1.4)	0.618
***C. burnetii* (total IgG)**								
No. of tested cases	26	5	21		17	4	13	
Positivity	5 (19.2)	3 (60.0)	2 (9.5)	0.048	2 (11.8)	1 (25.0)	1 (7.7)	0.627
***C. burnetii* (phase I IgG)**								
No. of tested cases	33	6	27		11	3	8	
Positivity	8 (24.2)	2 (33.3)	6 (22.2)	0.724	5 (45.5)	2 (66.6)	3 (37.5)	0.600
M. pneumoniae								
No. of tested cases	201	48	153		93	21	72	
Positivity	14 (6.9)	3 (6.3)	11 (7.2)	0.823	3 (3.2)	0 (0.0)	3 (4.2)	0.632

BCNIE, blood-culture negative infective endocarditis; BCPIE, blood-culture positive infective endocarditis; *B. henselae, Bartonella henselae; C. burnetii, Coxiella burnetii; M. pneumoniae, Mycoplasma pneumoniae*.

[a] Two-tailed Chi-squared test or Fisher exact test as corresponding.

[b] One *B. henselae* serology result during follow-up was equivocal and could not be reclassified as no retesting was performed. This result was excluded from the positivity rate.

body titer of 1:1,280 also met a major Duke criterion but did not receive targeted treatment. All *B. henselae* serologies were below Duke's threshold, with titers not exceeding 1:800.

Multivariable analysis

In multivariable logistic regression adjusting for age, sex, and Charlson comorbidity index, none of the microorganisms' serology results showed a statistically significant association with full recovery from the IE episode. The adjusted odds ratios were: *B. henselae* 0.76 (95% CI: 0.28-2.06; $P = 0.592$), *C. burnetii* total IgG 0.60 (95% CI: 0.04-10.34; $P = 0.726$), and *C. burnetii* phase I IgG 1.77 (95% CI: 0.31-10.19; $P = 0.522$). Due to the small number of positives for *Brucella spp.* and *M. pneumoniae*, these microorganisms were not analyzed.

Age (aOR=0.96; 95% CI: 0.94-0.98; $P = 0.001$) and Charlson index (aOR=0.89; 95% CI: 0.79-1.00; $P = 0.040$) were the only covariates significantly associated with recovery.

Discussion

This study characterized IE patients over 14 years at a tertiary care center. In our cohort, 22.3% of IE episodes were culture-negative, slightly higher than 14-17% reported in other European and Spanish series [14,15], although historical prevalence ranges from 2.5% to 31% [16]. More than one-fourth of BCNIE patients received antibiotics prior to culture collection, representing more than tenfold increase compared to BCPIE. This finding underscores the critical impact of pre-treatment on culture yield and highlights the need for optimized diagnostic strategies.

Baseline characteristics were largely similar between BCNIE and BCPIE patients. Surgery was more frequently required in BCNIE, which may be explained by the higher risk of complications associated with delayed or less effective antimicrobial therapy [17]; however, in-hospital mortality did not differ between groups, as previously reported in several cohorts [2,18].

Consistent with previous studies, *C. burnetii* and *Bartonella spp.* emerged as the most frequently detected pathogens in our cohort [19,20], while *Brucella* spp. and *M. pneumoniae* were rare. However, seropositivity seldom translated into clinical utility, as only a single *C. burnetii* case received targeted therapy.

Clinicians may disregard serological positives for several reasons. First, long-term serological follow-up in Q fever shows that *C. burnetii* phase I IgG can persist for years even without signs of chronic infection, complicating the interpretation of active disease [21]. Second, nonspecific reactivity can obscure interpretation: a recent study using chemiluminescent assays found that false-positive serologies for *B. henselae* and *C. burnetii* were significantly associated with the presence of extractable nuclear antigens and older age, suggesting that autoantibody-mediated interference can produce misleading results [22]. These limitations can lead to overestimating the infectious burden and reduce clinicians' confidence in serology alone, explaining why positive results often do not lead to treatment decisions.

Given limited clinical utility of serology, molecular techniques provide a more accurate alternative and can directly detect pathogen DNA in blood or valve tissue, thus providing higher diagnostic accuracy [23]. Independent evidence further supports this approach: in the Spanish GAMES cohort, approximately half of *Bartonella* spp. serology-positive cases were not concordant with valve polymerase chain reaction (PCR) results [24], and none of the seropositive cases in our study could be confirmed by molecular techniques. Similarly, Endres et al. [25] found that 50% of serology-negative cases were false negatives when evaluated by PCR, and tissue PCR results impacted antimicrobial treatment in 74% of cases—a significantly higher rate than observed for serology in our cohort.

These data argue against routine serology for all IE patients and favor a selective, epidemiology-guided approach. In this strategy, serology would be reserved for patients with negative blood cultures, high clinical suspicion for zoonotic or intracellular pathogens, or relevant epidemiological exposures, with molecular confirmation incorporated whenever possible. From a cost-effectiveness perspective, while no studies have explored serology-related economic impact, our data suggest that systematic serology for all IE patients imposes a substantial burden on healthcare resources relative to its limited actionable value. A targeted approach aligns with antimicrobial stewardship principles, reduces unnecessary testing, and focuses resources on patients most likely to benefit [26].

Our study has several limitations. First, its single-center design may have introduced geographic bias, while its retrospective nature may account for information bias, particularly regarding prior antibiotic exposure and animal contact history. Second, the use of commercial kit thresholds rather than Duke-recommended cut-offs may have led to classification of weakly positive results with un-

clear clinical meaning. However, this approach reflects real-world clinical practice and was necessary to accurately evaluate cases over a long period (2008-2021), during which the Duke criteria did not consistently account for less common pathogens such as *Bartonella* spp., *Brucella* spp., or *M. pneumoniae*. The thresholds provided by validated commercial kits corresponded to the serological evidence that guided clinical decisions at the time of diagnosis, ensuring consistency and appropriateness in our retrospective study. Third, the limited sample size of the BCNIE subgroup constitutes a potential limitation, as it may have decreased the statistical power of the analyses and hindered the detection of existing differences between groups. Finally, molecular diagnostics, including PCR, were not systematically performed in our cohort but may improve etiological diagnosis in future BCNIE cases.

While PCR is now widely available, metagenomics remains technically demanding and costly, limiting its routine use. As these technologies become faster and more affordable, integrating selective serology, PCR, and targeted metagenomics in a tiered diagnostic approach could optimize accuracy and resource utilization in BCNIE [27,28].

Conclusions

Routine serological testing in IE offers minimal diagnostic and therapeutic benefit. Despite positive serological results being relatively common, they rarely influence clinical management or improve patient outcomes. A selective approach restricted to BCNIE cases with epidemiological risk factors appears more appropriate and cost-effective. Future studies should prioritize molecular diagnostic methods to improve microbiological yield and guide targeted antimicrobial therapy in culture-negative infective endocarditis.

Declaration of competing interest

The authors have no competing interests to declare.

Author contributions

NRA and MCF conceived the study. NRA, CGR, JQM, CFA, JRL, MGC, MCB, JFGD, CA, FAR, IC, MFS, RPF, PR, IBP and MCF contributed to data collection. NRA and CFA analyzed the data. NRA wrote the original draft. All authors provided critical feedback, provided suggestions during the preparation of the manuscript, and approved its final version.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval

This study was approved by the Ethics Committee of Cantabria (reference number 2008.04; approval date: February 5, 2008) and conducted in accordance with the Declaration of Helsinki.

Acknowledgments

We thank the nursing staff and laboratory personnel for their contribution to data collection and patient care.

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