Bartonella Species as a Cause of Culture-negative Endocarditis in South Africa

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Abstract

Background:

Previous reports have highlighted the high prevalence of blood culture negative endocarditis (BCNE) in South Africa.

Methods:

The Tygerberg Endocarditis cohort (TEC) study is a prospective cohort study of patients with confirmed or suspected IE presenting to Tygerberg Academic Hospital, Cape Town, South Africa.

Results:

To date, 44 patients have been included in this ongoing study. Fourteen of the 44 patients (31.8%) had BCNE. Further analysis of the patients with BCNE identified *Bartonella* species as the most common causative organism (n=6; 43%). Other causes included *Mycoplasma* species (n=2), *C. burnetii* (n=1), and non-bacterial thrombotic endocarditis due to anti-phospholipid syndrome (n=1). No cause could be identified in 4 of the 44 patients (9%). *Bartonella quintana* was identified with PCR of valvular tissue as the causative organism in 4 of the 5 patients that underwent urgent surgery.

The patients with Bartonella IE (n=6) had an average age of 39 years with equal gender distribution The common clinical features were clubbing (n=5; 83%), anemia (n=4; 66.6%), haematuria (n=3; 50%), acute on chronic severe valvular lesion (n=3; 50%) and acute severe valvular lesion (n=2; 33.3%).

The aortic valve was involved in 5 of 6 patients. During a mean follow-up period of 251 days after diagnosis, no major adverse events occurred.

Conclusion:

Bartonella IE is an important cause of BCNE in the Western Cape of South Africa. Imaging findings of significant valvular destruction with large vegetations on the aortic valve not affected by pre-existing congenital or rheumatic valve disease, should raise the suspicion of Bartonella IE.

Summary

Bartonella species is an important cause of blood culture negative endocarditis in the Western Cape of South Africa. Imaging findings of significant valvular destruction with large vegetations on the aortic valve should raise the suspicion of *Bartonella* associated infective endocarditis.

Background

Infective endocarditis (IE) is defined as an infection involving the endocardial surface of the heart. This can primarily affect native heart valves (native valve endocarditis or NVE), prosthetic heart valves

(prosthetic valve endocarditis – PVE), non-valvular endocardial surfaces (such as IE affecting ventricular septal defects) or any cardiac prosthetic devices.(1,2) The characteristics of IE have evolved in developed nations with a doubling of patient age, an increase in the prevalence of patients with IE due to *Staphylococcus aureus* in the setting of normal or non-rheumatic valves and a decrease in the prevalence of blood culture negative endocarditis (BCNE).(2,3) Data regarding the causes and epidemiology of IE in the developing world and specifically South Africa are limited. Current reports from South Africa suggest IE is still a disease of young patients with underlying rheumatic heart disease, predominantly caused by the viridans group streptococci and with a high rate of BCNE.(1,4) The postulated reason for the high rate of BCNE in the published South African literature has been the high rate of antibiotic use prior to blood culture sampling.(4) Data from the developed world would suggest that organisms that are difficult to culture and/or identify with standard laboratory methods are the commonest cause of BCNE. These organisms vary according to region, with developed countries reporting mostly *Coxiella burnetii* as a causative organism in cases of IE previously considered as BCNE.(1) Limited data is available for South Africa, but reports from Algeria (a developing country) suggest *Bartonella* species is a more common cause of BCNE than *C. burnetii*.(7) BCNE is associated with a higher rate of in-hospital adverse events, although recent publications have suggested similar long-term outcomes when compared to blood culture positive patients. (8,9)

Bartonella species are small Gram-negative bacilli that are generally transmitted by arthropod vectors. These fastidious intracellular bacteria cause various clinical syndromes and diagnosis is challenging due to difficulties isolating the organism using traditional culture methods.(10) *Bartonella quintana* is mostly associated with trench fever, IE and bacillary angiomatosis *whereas Bartonella henselae* is associated with cat scratch disease, bacillary angiomatosis and less commonly, with IE. *B. quintana* infection is often reported in homeless persons infested with body lice as compared to *B. henselae* infection which is usually associated with contact with cats. Single cases of other *Bartonella* species causing IE have been reported.(10,11)

The Tygerberg Endocarditis Cohort (TEC) study is a prospective cohort study of patients with definite or suspected IE according to the European Society of Cardiology (ESC) criteria.(2) All patients included are managed by an Endocarditis Heart Team with a set protocol to detect the causative organism as per the current ESC guidelines.(2) Tygerberg Academic Hospital is a public sector tertiary referral centre for a network of 17 hospitals and serves a population of approximately 2.4 million people.(12)

Methods

All patients referred to the Division of Cardiology, Department of Medicine at Tygerberg Hospital in Cape Town, South Africa, with definite or suspected IE from November 2019 to August 2020 were included in this ongoing study. All patients underwent standard transthoracic echocardiography (TTE) with the majority also undergoing transoesophageal echocardiography (TEE) in the absence of identifiable contra-indications to TEE.(13,14) A stepwise protocol for organism detection was utilised to identify the common causative organisms of IE and to minimize the incidence of BCNE (Figure 1). Further

management and analysis of the samples were done according to current published guidelines.(2) Patients without an identified organism using standard culture techniques after 5 days, were defined as having BCNE.

All BCNE patients underwent further venous blood analysis for further testing, including:

- Serology for detection of IgM and IgG antibodies to *Bartonella* species*, Brucella* species*, C. burnetii, Legionella pneumophila* and *Mycoplasma pneumoniae*
- Antibody testing for antinuclear antibodies (ANF) and anti-cardiolipin antibodies (ACLA)
- Direct polymerase chain reaction (PCR) was performed on negative blood culture bottles for detection of the universal bacterial 16S rRNA and fungal 18S rRNA genes, followed by sequencing to identify the amplified DNA product.

A sample of heart valve tissue was collected from all patients who required surgery and this was submitted for:

All patients are treated by an Endocarditis team according to the current ESC guidelines.(2)

Results

To date, 44 patients have been included in this ongoing study. Fourteen of the 44 patients (31.8%) had BCNE. Further analysis of the patients with BCNE identified *Bartonella* species as the most common causative organism (n=6; 43%). Other causes included *Mycoplasma* species (n=2), *C. burnetii* (n=1), and non-bacterial thrombotic endocarditis due to anti-phospholipid syndrome (n=1). No cause could be identified in 4 of the 44 patients (9%). Two of the 4 patients had suspected *Mycobacterium tuberculosis* associated IE based on typical clinical and echocardiographic features, but without microbiological confirmation.(1)

The patients with Bartonella IE (n=6) had an average age of 39 years with equal gender distribution (Table 1). The common clinical features were clubbing (n=5; 83%), anemia (n=4; 66.6%), haematuria (n=3; 50%), acute on chronic severe valvular lesion (n=3; 50%) and acute severe valvular lesion (n=2; 33.3%).

Although echocardiography identified significant valvular destruction in all patients, no pre-existing underlying structural valve disease (rheumatic valve disease or congenital abnormalities) was identified. The aortic valve was involved in 5 of 6 patients. Acute severe aortic regurgitation was confirmed in 2 patients, acute on chronic severe aortic regurgitation in 2 patients, chronic severe aortic regurgitation in one patient and acute severe mitral regurgitation in one patient.

Vegetation size ranged from 10-15mm on linear measurement with an average circumference of 28mm. Only one patient had a raised white cell count (WCC). C-reactive protein (CRP) levels were mildly raised with only one value above 50. Two of our 6 patients were HIV-positive; one was on antiretroviral treatment

with undetectable viral load and one had a CD4 count of more than 1000 per microliter of blood. Four patients (66%) had low complement levels (Table 2).

B. quintana and *B. henselae* IgG and IgM were detected by an immunofluorescence assay (IFA). IgG was positive in all patients with titres ranging from 1:256 to 1:512, while IgM was positive in 5 of the 6 patients. The 16S and 18S PCRs performed on blood cultures were negative in all patients. Both patients with acute severe aortic regurgitation underwent emergency valve replacement, 3 of the other 4 patients underwent urgent/inpatient valve replacement. Valve repair was not possible in one patient. Sequencing of the 16S PCR product from valve tissue identified *B. quintana* in 4 of the 5 patients who had surgery. The patients who underwent surgery survived their hospital stay and were discharged home on oral doxycycline. One patient declined surgery but successfully completed 3 months of oral doxycycline. During a mean follow-up period of 251 days after diagnosis, no major adverse events (death, embolic events, renal failure requiring dialysis, or rehospitalization) occurred.

Discussion

This is the first study that identifies *Bartonella* species as an important cause of BCNE in the Western Cape province of South Africa. Previous cohort studies of patients with IE in the Western Cape and South Africa overall have not reported *Bartonella* species as a cause of IE.(4–6,15) The first case was described in 1993 and another case was reported recently.(16,17) Our findings are in keeping with data from other developing countries where *Bartonella* species are the most common cause of BCNE contrasting with developed countries that report *C. burnetii* as the commonest cause of BCNE.(7,11,18) The reasons for the lack of reporting of *Bartonella* species as a cause of BCNE in South Africa are probably multifactorial. We postulate that our systematic approach to organism detection, including serology and newer diagnostic modalities such as PCR performed on valves, explains this new finding, rather than the emergence of *Bartonella* species as a new cause of BCNE in South Africa. Should this be the case, it follows that a large group of patients previously labelled as BCNE were not adequately treated for Bartonella IE and this may have contributed to the adverse outcome of these patients.(8,9) Our data would suggest that Bartonella IE, if adequately treated, has a favourable in-hospital and short term outcome in keeping with other case series.(18,19)

PCR performed on blood and/or heart valves remains the gold standard for detection and identification of Bartonella to species level in patients with BCNE. Different techniques are available for analysis of heart valves; in this study, 16S PCR and sequencing of amplified bacterial DNA were used, although some reports suggest that real time PCR (RT-PCR) is more sensitive.(11) In our series, we identified *B. quintana* as the causative species in 4 of the 5 patients who underwent surgery. Of the 30 patients with culture positive endocarditis, 18 underwent valve surgery. None of these cases were PCR positive for Bartonella species on their valve tissue.

Two patients were diagnosed with Bartonella-associated IE without PCR confirmation on blood or heart valve. The decision was made by the Endocarditis team on the basis of the typical clinical features,

elevated serology titres and the absence of another causes in spite of a set protocol for organism detection. Currently criteria is lacking for the diagnosis of Bartonella IE if PCR on both the blood and heart valves is negative or unavailable in the setting of typical clinical and imaging findings and suggestive serology. Different serological cut-offs for the diagnosis of Bartonella IE has been suggested(11), with higher titers of IgG increasing the positive predictive value of serology assays. Serum samples of healthy volunteers typically have IgG titres by IFA of less than 1:128 and IgM titres less than 1:20, with no IgG titres above 1:256.(20) An IgM titre of more than 1:20 with an IgG titre of more than 1:128 suggest active Bartonella infection. We suggest that elevated Bartonella antibody titres in the setting of a typical clinical and imaging profile in the absence of another cause (after a set protocol for organism detection was followed), be utilised for the diagnosis (and thus initiation of therapy) of Bartonella IE.

Serology for *B. henselae* and *B. quintana* is not very useful in distinguishing between species due to the high rate of cross-reactivity between the assays.(11) Although it is reported that the likely causative organism will have higher titers by serology(20), in our series of 4 patients with PCR-confirmed *B. quintana* IE, the IgG titres for *B. quintana* was either equal or lower than the IgG titres for *B. henselae*, while the *B. quintana* IgM titres were either similar, higher or lower than *B. henselae* IgM titres, suggesting that it is less helpful in distinguishing between *Bartonella* species.

Previous case reports from South Africa and a case series from the United States also identified *B. quintana* as the causative species in BCNE.(16,17,21) A case series from Japan identified *B. henselae* as the causative species in five cases of BCNE.(19) *B. henselae* has been detected on blood PCR in up to 10% of HIV-positive patients in South Africa whereas no cases were detected in the non-HIV infected control cohort. No cases of IE due to *B. henselae* from South Africa has been published. The treatment for the different species of Bartonella is similar and currently no evidence is available to suggest the outcome of Bartonella IE is influenced by the specific causative species.

The cross-reactivity of the serological tests for the different *Bartonella* species extend to patients with other causes of BCNE, including *C. burnetii, Brucella* and *Mycoplasma* species.(22) One of our patients with confirmed *Mycoplasma hominis* IE also had positive serology for *B. henselae*, although the IgG titre was less than 1:256. This finding puts into perspective the importance of performing PCR on both blood and valve tissue to confirm the causative organism in patients with BCNE, even if serology is positive for Bartonella, Brucella, Coxiella or Mycoplasma. Although the 16S PCR on blood was negative in all our patients, it remains important to detect organisms associated with BCNE (e.g. Mycoplasma) that might cause false-positive serology.

The clinical features of the patients with Bartonella IE had significant overlap with the known features of IE caused by the usual organisms, with clubbing, anaemia and haematuria being the most common.(4) In all but one of the patients, the aortic valve was involved, which is in keeping with previous reports.(16,21) All patients had hemodynamically severe incompetence of either the aortic (n=5) or mitral (n=1) valve with clinical features of acute (n=2), acute on chronic (n=3) or chronic (n=1) incompetence. Historically, acute IE and acute valvular incompetence is associated with *S. aureus* IE and these patients often do not

demonstrate the classical findings of clubbing and anemia as these features take time to develop.(23) In our series, patients with acute valve lesions had both clubbing and anemia; clubbing was also present in all of the patients with acute on chronic valve lesions. This would suggest a significant time from infection/bacteraemia to presentation, even though patients present with acute or acute on chronic valve incompetence. We postulate that patients with Bartonella IE has an early phase with minimal symptoms and low-grade underlying bacteraemia during which time the patient develops clubbing and anaemia of chronic disease. Patients only seek medical attention at the time of significant valvular destruction with the associated sequalae of dyspnea and hemodynamic compromise.

The majority of patients had severe destruction of the aortic valve with no evidence of underlying congenital heart/valve disease (e.g. bicuspid aortic valve, ventricular septal defect) or rheumatic valve disease (Figure 2). The propensity of *Bartonella* species to involve the aortic valve is well documented, although no clear explanation exists for this finding.(18)

Maximum vegetation length by two dimensional echocardiography of 10mm or more in patients with left-sided IE is associated with an increased risk of embolic events. Although large vegetations were observed in all our patients, no embolic events occurred.(24) In contrast to other causes of IE of the aortic valve, no peri-annular extension, e.g. peri-aortic abscess formation was noted. The fact that most patients underwent surgery early and were on appropriate antimicrobial therapy may have contributed to this finding. Due to the severe destruction of the aortic valve, 4 of 5 patients underwent aortic valve replacement with a mechanical valve. Mitral valve repair was attempted in the single patient with severe mitral regurgitation, but was converted intra-operatively to mitral valve replacement due to extensive tissue destruction. It would be difficult to draw meaningful conclusions from this small number of patients, but it seems that patients with Bartonella IE have a reasonably good short term outcome in spite of the significant valvular destruction and large vegetations observed with echocardiography.

Conclusion

Bartonella IE is an important cause of BCNE in the Western Cape of South Africa. A systematic approach to organism detection in patients with suspected or confirmed IE is essential for the diagnosis of the causative organism in patients with BCNE. Imaging findings of significant valvular destruction with large vegetations on the aortic valve not affected by pre-existing congenital or rheumatic valve disease, should raise the suspicion of Bartonella IE. Early initiation of appropriate antimicrobial therapy combined with early surgery seems to provide a good in-hospital and short term outcome.

Declarations

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Conflicts of interest/Competing interests: None

Ethics approval: Ethics approval was obtained from the committee for Human Research of the Faculty of Medicine, Stellenbosch University, Cape Town (ID 10660)

Consent to participate: All patients provided written, informed consent.

Consent for publication: The authors consent to publication of the data if accepted. Patients consented to the publication of the data and images.

Availability of data and material: All data is securely stored on a digital database that is password protected. Data is available for review.

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Tables

Table 1. Demographic information, clinical and imaging findings

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Demographic information						
Age (years)	39	26	36	41	57	37
Sex	Male	Female	Female	Male	Male	Female
Rural/Urban	Rural	Urban	Urban	Rural	Urban	Rural
Housing	Informal	Formal	Homeless	Formal	Formal	Informal
Clinical and imaging features						
Clubbing	Yes	Yes	Yes	No	Yes	Yes
Anemia	Yes	No	Yes	Yes	Yes	No
Hematuria	No	No	No	Yes	Yes	Yes
Valve involvement	Aortic	Aortic	Mitral	Aortic	Aortic	Aortic
Hemodynamic lesion	Acute	Acute on chronic	Acute on chronic	Chronic	Acute	Acute on chronic
Vegetation length (mm)	15	12	10	11	10	10
Vegetation circumference (mm)	47	29	22	25	25	22
Vegetation number	Multiple	Multiple	Single	Multiple	Multiple	Multiple
Pre-existing valvular structure	Normal	Normal	Normal	Normal	Normal	Normal

Table 2. Special investigations and surgical outcome

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Special investigations						
B. quintana IgG	Positive	Positive	Positive	Positive	Positive	Positive
Titre	1:256	1:256	1:512	1:256	1:512	1:256
B. quintana IgM	Negative	Positive	Positive	Positive	Positive	Positive
Titre		1:256	1:64	1:128	1:512	1:64
B. henselae IgG	Positive	Positive	Positive	Positive	Positive	Positive
Titre	1:256	1:256	1:512	1:512	1:512	1:256
B. henselae IgM	Negative	Positive	Positive	Positive	Positive	Positive
Titre		1:64	1:64	1:64	1:512	1:128
16S PCR on valve tissue	*B. quintana*	Not done	Negative	*B. quintana*	*B. quintana*	*B. quintana*
16S PCR on blood cultures	Negative	Negative	Negative	Negative	Negative	Negative
HIV status	Positive	Positive	Negative	Negative	Negative	Negative
CRP	50	37	209	22	33	12
WCC (per microliter)	7700	10000	27600	7300	3150	8800
Complement level	Normal	Low	Normal	Low	Low	low
Valve replaced	Aortic	Refused	Mitral	Aortic	Aortic	Aortic
In-hospital mortality	No	No	No	No	No	No
Current follow-up period (days)	369	128	352	306	226	130

Figures

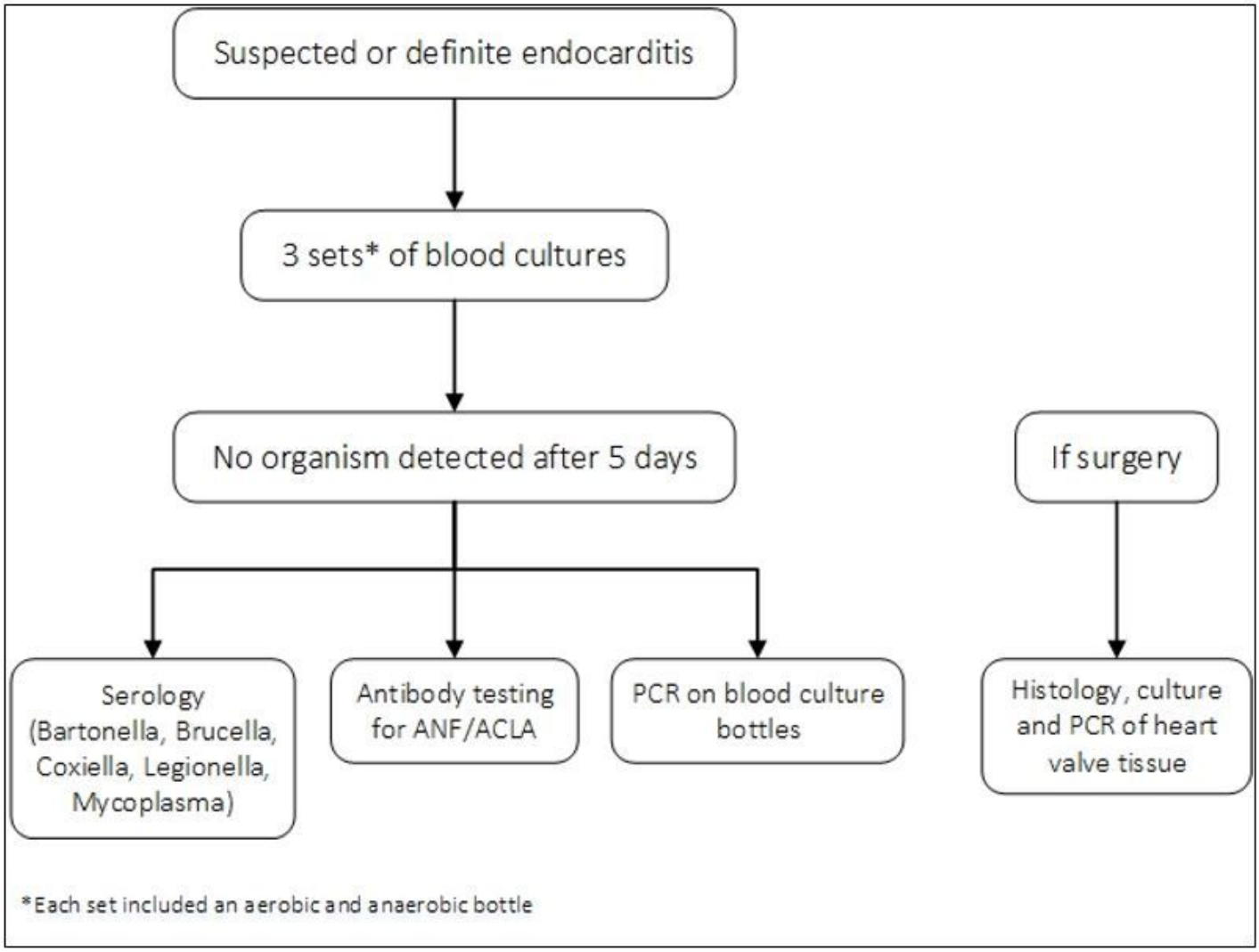

Figure 1

Protocol for organism detection

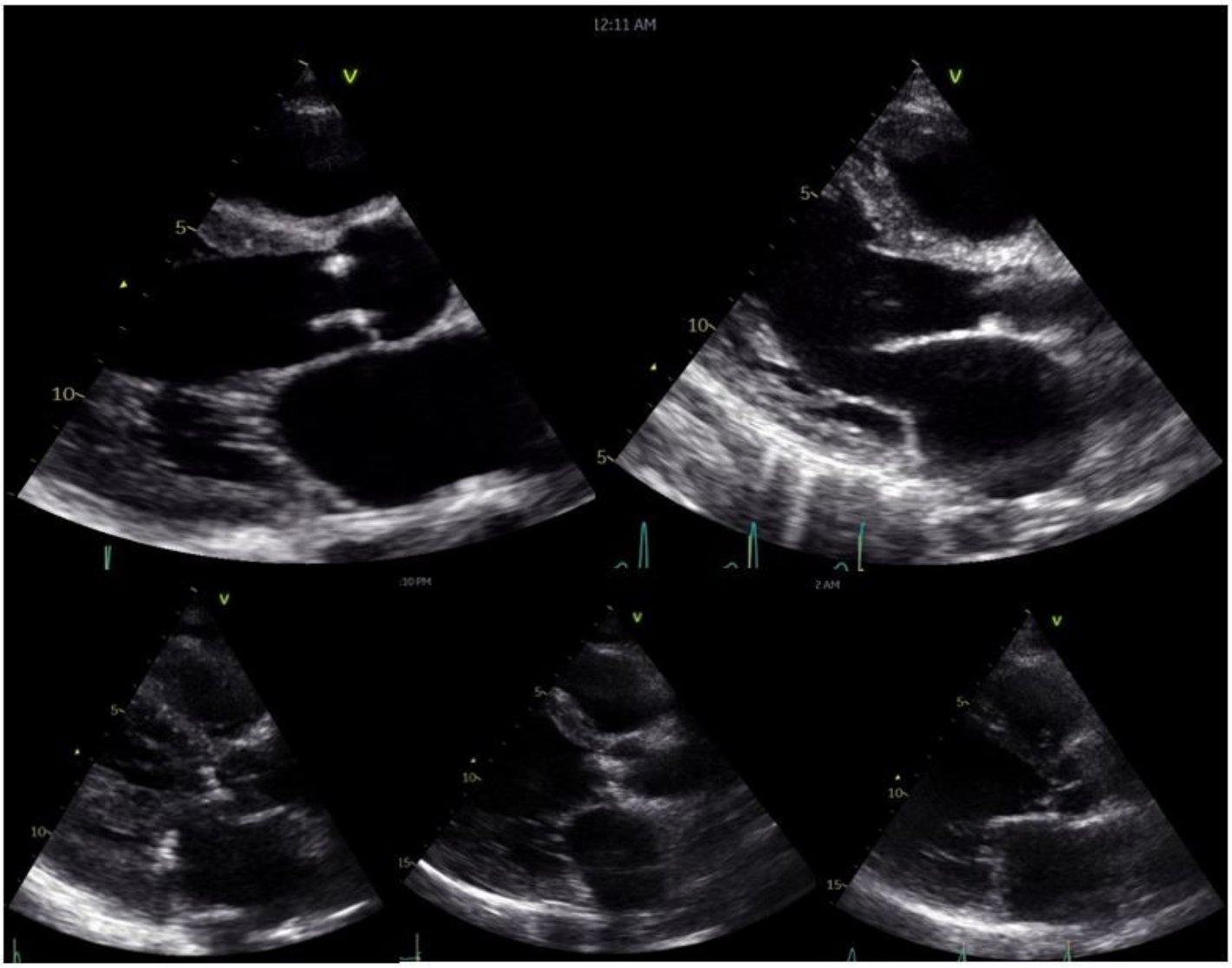

Figure 2

Parasternal long axis view of the aortic valve of patient 1, 2, 4-6 demonstrating severe valve destruction with large vegetations