Viruses identified in endomyocardial biopsy samples in idiopathic dilated cardiomyopathy patients in central South Africa

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ABSTRACT

Background: Heart failure and cardiomyopathy are problematic in South Africa. Viruses are important causes of myocarditis and cardiomyopathy. This study aimed to determine the distribution of viruses in endomyocardial heart biopsy samples in patients with dilated cardiomyopathy.

Methods: Endomyocardial biopsies (EMB) were analysed using histology, immunohistochemical staining, and polymerase chain reaction. The level of fibrosis, presence and type of cellular infiltration, and the prevalence of viral genomes with their replication activity were determined.

Results: Viral genomes were found in 73.7% of patients, with parvovirus B19 (B19V) present in 96.4%. No Coxsackievirus was identified, and 2 patients presented with transcriptional intermediates, which indicated active B19V viral replication. Most patients (71.4%) presented with single infections, but some (28.6%) with co-infections. Three patients presented with acute myocardial inflammation and moderate / severe increased lymphocytic infiltration.

Conclusions: This study found B19V predominant and present in almost all virus-positive EMB samples. Our results support the possible virus etiological shift towards B19V. These findings underscore the need to further investigate the pathophysiological role of B19V in the development and progression of DCM.

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INTRODUCTION

Cardiovascular disease remains a leading cause of death. In 2021, it was estimated that 20.5 million (31.8%) of worldwide deaths were due to circulatory and cardiovascular diseases.[1] Cardiomyopathies are more frequently observed in poorer societies where pestilence and famine predominate.[2] This is exacerbated by nutritional deficiencies and chronic persistent viral infections that render individuals more susceptible.[3] Dilated cardiomyopathy (DCM) is defined as contractile dysfunction associated with left ventricular dilation in the absence of coronary artery disease and abnormal loading conditions.[4,5] Familial and idiopathic cardiomyopathies and inflammatory myocarditis[6] are the most prevalent causes of DCM and are a major contributor to heart failure globally.[7,8]

Africa is home to some of the poorest countries and communities in the world,[9] and non-communicable diseases are becoming more prominent in Africa compared to the 1990s.[10] Cardiovascular disease is a significant health burden in Africa and accounts for 38.3% of non-communicable disease deaths.[10] The "Hearts of Soweto" study showed that dilated cardiomyopathy was the most common cause of heart failure in South Africa, responsible for 35% of heart failure cases.[11] In the Sub-Saharan Africa Survey of Heart Failure (THESUS-HF), dilated cardiomyopathy was the second most common cause of heart failure in almost a third of patients.[12] More recently, the African Cardiomyopathy and Myocarditis Registry Programme (IMHOTEP) reported the largest cardiomyopathy series in South Africa. Dilated cardiomyopathies were the most common cardiomyopathy observed, accounting for 72% of all cases.[13]

Determining the etiology of cardiomyopathy is critical as it allows focused and specific treatment.[14,15] Infectious causes such as viruses can lead to myocarditis, which may progress to inflammatory dilated cardiomyopathy.[8,16] The infection may cause chronic or autoinflammatory reactions in the body.[17,18] Patient outcomes depend on the virulence of the virus and the response of the host immune system, which could result in diseases ranging from subclinical to severe heart failure.[19] Performing endomyocardial biopsies (EMB) for suspected inflammatory cardiomyopathies remains the gold standard according to the European Society of Cardiology (ESC) 2023 guidelines.[20]

Enteroviruses have traditionally been regarded as the most common cause of myocarditis, especially until the 1990s.[21-23] In recent years, however, human parvovirus B19 (B19V) has become more prevalent in high-income countries.[24-26] The most frequently detected viruses in biopsies using polymerase chain reaction (PCR) are B19V,[27-31] human herpes virus 6 (HHV-6),[28,30] enterovirus species,[28,30] Epstein-Barr virus (EBV) [28, 30] and adenovirus (ADV).[28,30,32]

The number of studies conducted in sub-Saharan Africa on cardiomyopathies and myocarditis using EMB is limited. The first study that analysed endomyocardial biopsies with molecular testing was conducted in Cape Town in 2013 in patients with and without human immunodeficiency virus (HIV). In the HIV-positive cohort, they reported mostly EBV (64%), herpes simplex virus (HSV), B19V, and cytomegalovirus. In patients without HIV but with idiopathic dilated cardiomyopathy, the majority of patients had CVB3 (56%), followed by EBV, HSV, ADV, and B19V.[33] Almost a decade later, another local study was conducted on 102 myocarditis patients, where viral genomic material was detected in 60 (59%) study subjects. The most frequently detected virus was B19V, which was detected in 76.67% of the positive patients, followed by EBV, HHV-6, and human bocavirus. Co-infections were also identified with 3 double co-infections of B19V / EBV and 1 triple co-infection of B19V / EBV / HHV6.[34] The detection of B19V transcriptional activity in the heart muscle is important in establishing the clinical significance of infection.[35] To distinguish between latent and active parvovirus B19V infection, the detection of novel biomarkers such as ribonucleic acid (RNA) replication intermediates such as nonstructural protein 1 (NS1) and capsid protein (VP1) are used.[36] Active viral replication has been shown to lead to altered cardiac gene expression, cardiac damage, dysfunction, and increased inflammation compared to a control group with latent B19V infection.[37] In patients with acute disease, interferon beta (IFN-ß) can be administered, which has been shown to be very effective in patients with acute dilated cardiomyopathy due to B19V.[15,38,39]

Differences in South African demographics and socioeconomics could also indicate that the viruses frequently associated with cardiomyopathy in South Africa differ from those in high-income countries. South African cardiotropic infections have to be better understood and characterised to determine the true underlying causes of idiopathic dilated cardiomyopathy in sub–Saharan Africa.

The aim of this study was to determine the distribution of viruses in endomyocardial heart biopsy samples in patients with dilated cardiomyopathy.

METHODS

This study was part of a main study that prospectively recruited patients that presented to Universitas Academic Hospital in Bloemfontein, South Africa, between January 2018 - December 2022. The main study recruited HIV-negative patients (≥18 years old) with idiopathic dilated cardiomyopathy, confirmed by the diagnostic criteria as per the ESC guidelines[20] for dilated cardiomyopathy. Only patients in whom endomyocardial biopsies were performed were included in this sub-study. The patients were divided into 2 groups according to whether viruses were detected or not, namely virus-positive and virus-negative.

DATA COLLECTION

Demographic data pertaining to age, ethnicity, and sex were collected. Clinical data (including LVEF%, NYHA classification) and laboratory data (viral PCR and histological and immunohistochemical analysis) were also recorded.

ENDOMYOCARDIAL BIOPSY

Jugular or femoral access was used to perform all the right ventricle septum biopsies using a Cordis (High Tech Medical, Johannesburg, South Africa) 5.5 or 5.4 French Maslanka (Maslanka Chirurgische Instrumente, Tuttlingen, Germany) bioptomes that were guided through 6 or 7 French flexor sheaths under real-time fluoroscopic and transthoracic echocardiogram (TTE) guidance.

A total of 8 specimens were taken from different areas on the septum, as inflammation could have been localised to one or more locations. Three of the specimens were fixed in a 4% buffered formalin solution for histological and immunohistochemical analysis. The remaining samples were fixed in

RNALater® solution (Thermo Fisher Scientific, Waltham, Massachusetts, United States) to ensure the preservation of deoxyribonucleic acid (DNA) and RNA for downstream analysis. The EMB samples were analysed by a specialised cardio-pathology laboratory in Germany, namely the Institut Kardiale Diagnostik und Therapie (IKDT).

VIROLOGICAL ANALYSIS

The total DNA was extracted from patient EMB samples using Gentra Puregene kits (Qiagen, Hilden, Germany) according to standard operating procedures. Total RNA was extracted using QIAzol reagent (Qiagen, Hilden, Germany) treatment with DNAse (Promega, Walldorf, Germany). cDNA synthesis was performed using random hexamer primers and the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States). Nested-PCR and quantitative reverse transcriptase (qRT)-PCR were used for the detection of the cardiotropic viruses, namely enteroviruses (Coxsackievirus B3 and echovirus) EBV, B19V, HHV6, and adenoviruses. To determine whether viruses such as B19V were actively replicating their transcriptional transcriptional activity was determined by testing for transcriptional intermediates such as messenger RNA (mRNA). Only viruses that were not latent would have these transcriptional intermediates present.[40]

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS

Histological examination was performed on formalin-fixed, paraffin-embedded specimens. Several stains were used, such as Azan, Elastika-van-Gieson (EvG), periodic acid-Schiff (PAS), Hematoxylin and Eosin (H&E), and Kryo H&E according to the standard procedures. Immunohistochemical staining was performed on RNAater-fixed cryo-embedded EMBs to allow for the detection and quantification of inflammatory cells. Myocardial inflammation was diagnosed using the European Society of Cardiology (ESC) statement[24] by the detection of ≥14 leukocytes per mm², with the presence of ≥7 clusters of differentiation (CD)3+ T-lymphocytes per mm². In addition, ≥14 lymphocyte function-associated antigen (LFA)-1+ lymphocytes per mm², ≥40 macrophage (MAC)-1+ macrophages per mm², ≥40 CD45R0+ memory T cells per mm², and ≥2.9 perforin+ cytotoxic cells per mm² were also considered pathologic. Endothelial activation was measured by the expression of intercellular adhesion molecule 1 [(ICAM)-1, threshold ≥2 area%)]. The immunohistochemical and histological slides were evaluated with a Leica DMR light transmission microscope and quantified by digital image analysis as described before.[41]

ETHICS AND STATISTICS

Continuous variables were described using the median and interquartile range, and counts and percentages were used to describe categories. The comparison between categories of virus positivity and participants' characteristics was performed using the Mann-Whitney U-test for continuous variables and the Fisher exact test for categories. All statistical tests were 2-tailed, and the type-I error rate was set to 5%.

The SAS software version 9.4 was used to perform the statistical analysis.

The main study and this sub-study were approved by the Health Science Research Ethics Committee of the University of the Free State and the Free State Department of Health with ethical approval numbers (UFS-HSD2017/0320-0008) and (UFS-HSD2017/1398-0002), respectively. Only patients with signed informed consent documents were included.

RESULTS

A total of 38 patients were included in the study. Details and demographics can be viewed in Table I. Patients were fairly young, with a median age of 42 years, and male and female patients were evenly distributed. Almost two-thirds of patients were African (58%), followed by Caucasian (24%) and mixed race (19%). Viral genomic material was detected in more than two-thirds of the study population (virus-positive group). Patients in the virus-negative group were 3 years younger than those in the virus-positive group. The virus-positive group of patients (n=28) presented with more patients who had moderate to severe heart failure (NYHA 3, 4) than those who tested negative for viral genomes. A lower LVEF% was observed in the virus-positive group of patients than in the virus-negative group (p=0.1352).

A breakdown of the positive virological findings for the 28 patients can be viewed in Table II. B19V was found in almost all virus-positive biopsy samples (n=27, 96.4%), followed by HHV-6 and EBV. More than two-thirds of the virus-positive patients had only 1 virus present, whereas 28.3% had co-infections of several viruses. Co-infections consisted of B19V / HHV6 and B19V / EBV. Actively replicating, B19V mRNA was found in 2 patients.

Histological analysis of the EMB indicated that most patients (70%) did not show signs of fibrosis. Mild fibrosis was seen in 19%, severe fibrosis in 8%, and lipomatosis in 3% of patients (Figure 1).

TABLE I: Baseline characteristics.

Demographics	Total n=38	Virus-positive + n=28	Virus-negative – n=10	p-value
Age (year) (median)	42	42.5	39.5	0.3148
Q1;Q3	28, 58	34.5, 60.5	20, 55	
Sex n (%)				
Male	18 (47.4)	15 (53.6)	3 (30)	0.2778
Female	20 (52.6)	13 (46.4)	7 (70)	
Ethnicity n (%)				
Caucasian	9 (23.7)	6 (21.4)	3 (30)	0.4694
African	22 (57.9)	15 (53.6)	7 (70)	0.6731
Mixed	7 (18.4)	7 (25)	0	0.1564
Clinical				
LVEF % (median)	23	22	35.5	0.1352
Q1;Q3	18, 35	17.5, 32	20, 39	
NYHA n (%)				
Class 1	6 (15.8)	2 (7.1)	4 (40)	0.0314*
Class 2	12 (31.6)	10 (35.7)	2 (20)	0.4528
Class 3, 4	20 (52.6)	16 (57.1)	4 (40)	0.4681

**statistically significant, n: number, LVEF: left ventricular ejection fraction, NYHA: New York Heart Association, Q1: first quartile, Q3: third quartile.*

TABLE II: Description of the viral genomes detected in endomyocardial tissue.

Viral genomes detected in patients n=28	n (%)
Parvovirus B19 (B19V)	27 (96.4)
Human herpes virus 6 (HHV-6)	6 (21.4)
Epstein-Barr virus (EBV)	3 (10.7)
Adenovirus	0
Enterovirus	0
Replicating (B19V)	2 (7.1)
Single infection	20 (71.4)
Co-infection	8 (28.6)
B19V / HHV-6	5 (62.5)
B19V / EBV	3 (37.5)

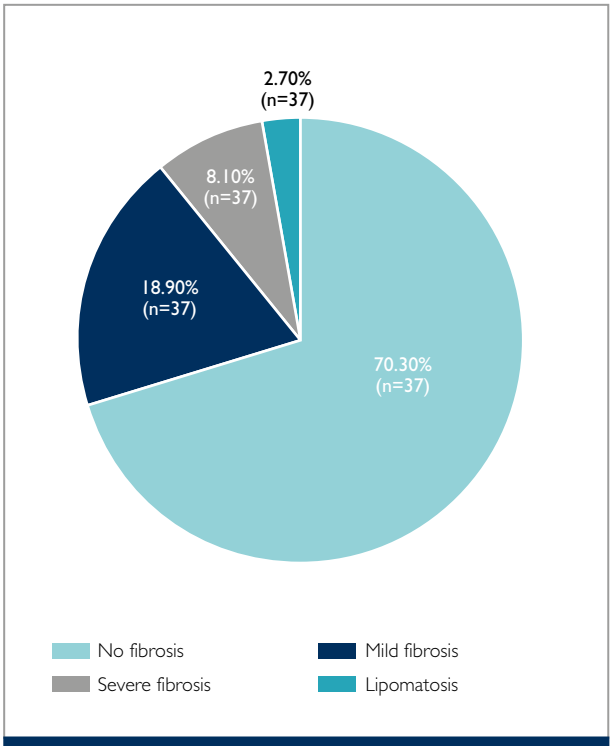

FIGURE 1: Histological analysis of endomyocardial biopsy sample showing the level of fibrosis.

The grade of fibrosis was confirmed with H&E and Kryo H&E histology and presented in Figure 2. No fibrosis was observed, as indicated by the normal endocardium and the normal size and arrangement of cardiomyocytes. Mild fibrosis showed partial fragmentation of myocytes in the interstitial space. Severe fibrosis was indicative of scars and missing cardiomyocytes. Lipomatosis showed fat cells of varying diameters near small vessels.

More than two-thirds of patients (n=25, 71%) had no signs of acute myocardial inflammation, as presented in Figure 3. Increased infiltration was not observed for CD3, CD11b, or CD45 positive lymphocytes. Minor CD11b / Mac-1 cell infiltration was seen but was below the pathological limit.

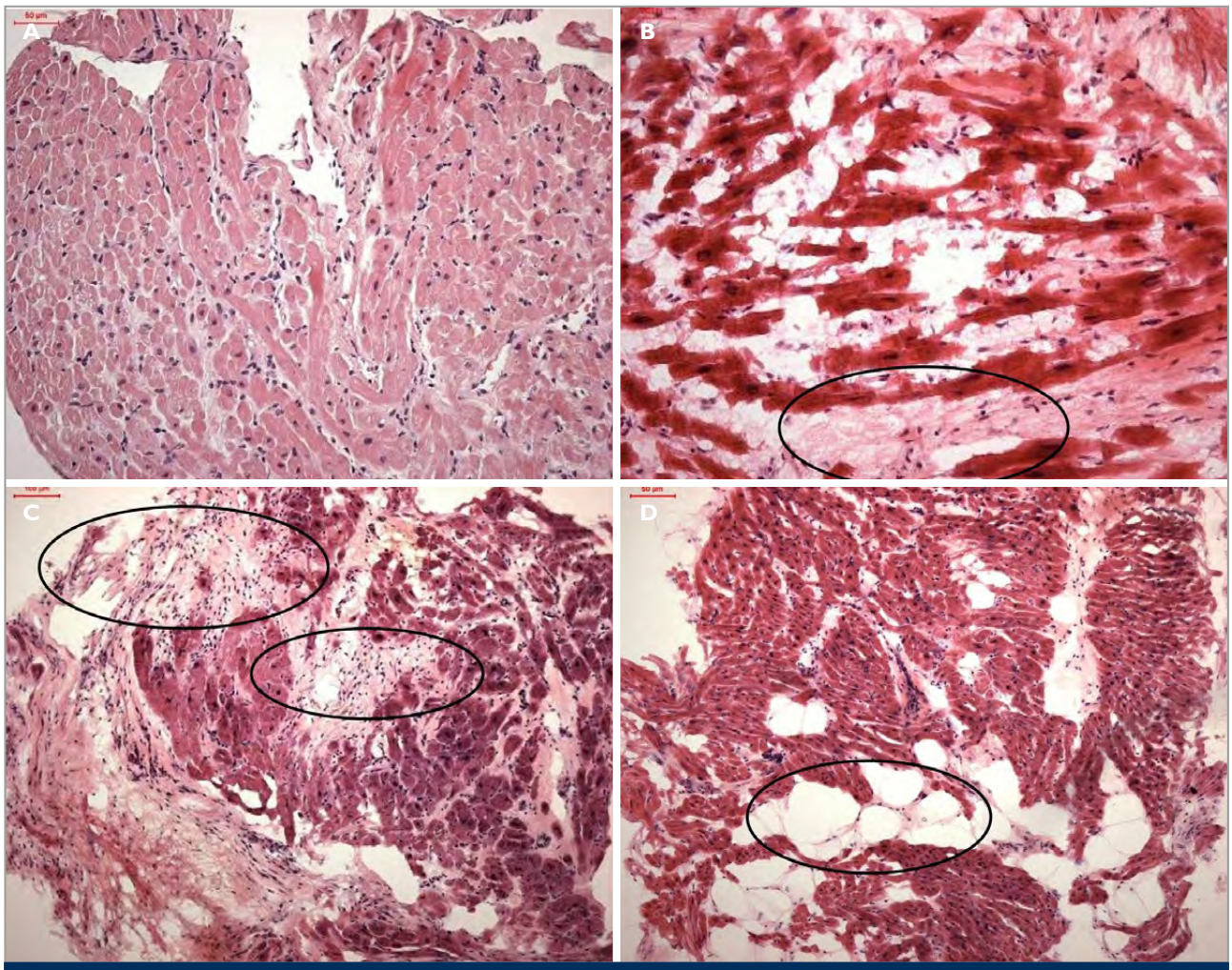

FIGURE 2: Histological presentation of fibrosis.
A. Normal endocardium with no fibrosis (H&E), B. Mild fibrosis (Kryo-H&E), C. Severe fibrosis with scarring (Kryo-H&E), D. Lipomatosis (L).

Mildly increased lymphocytic infiltration was observed in 20% (n=7) of patients and is presented in Figure 4. Mild increases of CD3, CD11a/LFA-1 lymphocytic infiltrates, and enhanced endothelial expression of ICAM-1 were observed.

Only 3 patients (9%) presented with acute myocardial inflammation and moderate / severe increased lymphocytic infiltration (Figure 5). This is indicated by the H&E stain in Figure 5A and the profound infiltration of CD3, CD11a/LFA-1, CD54/ICAM-1, and CD54 positive lymphocytes in Figure 5B - E.

DISCUSSION

Viral infection could be a contributing factor to cardiovascular disease and cardiomyopathy in central South Africa. Results of this study indicated the presence of viral genomes in the myocardium of the vast majority of patients (73.6%). The most prevalent virus was B19V, which was present in 96.4% of positive biopsy samples, with 2 being transcriptionally active, followed by HHV-6 and EBV; no enteroviruses were detected.

Almost two-thirds of the patients in our study population were African, corresponding to our region's demographic profile. The study population was relatively young at 42 years compared to the "Hearts of Soweto" study, which reported a 53-year-old mean age, and the Bloemfontein group, which reported a 51.8-year-old mean age.[11,42] Our results concur with the recent largest cardiomyopathy study (IMHOTEP) conducted in South Africa and published in 2024, which reported an overall median age of 35 years.[13] We found our LVEF% to be quite low, similar to the IMHOTEP study that reported an LVEF of 26%. Another local study in 2024 reported an LVEF of <30%.[13,43] There was a difference between the virus-positive group, which had an LVEF% of 13.5% lower than the virus-negative group, although not statistically significant. More patients in the virus-positive

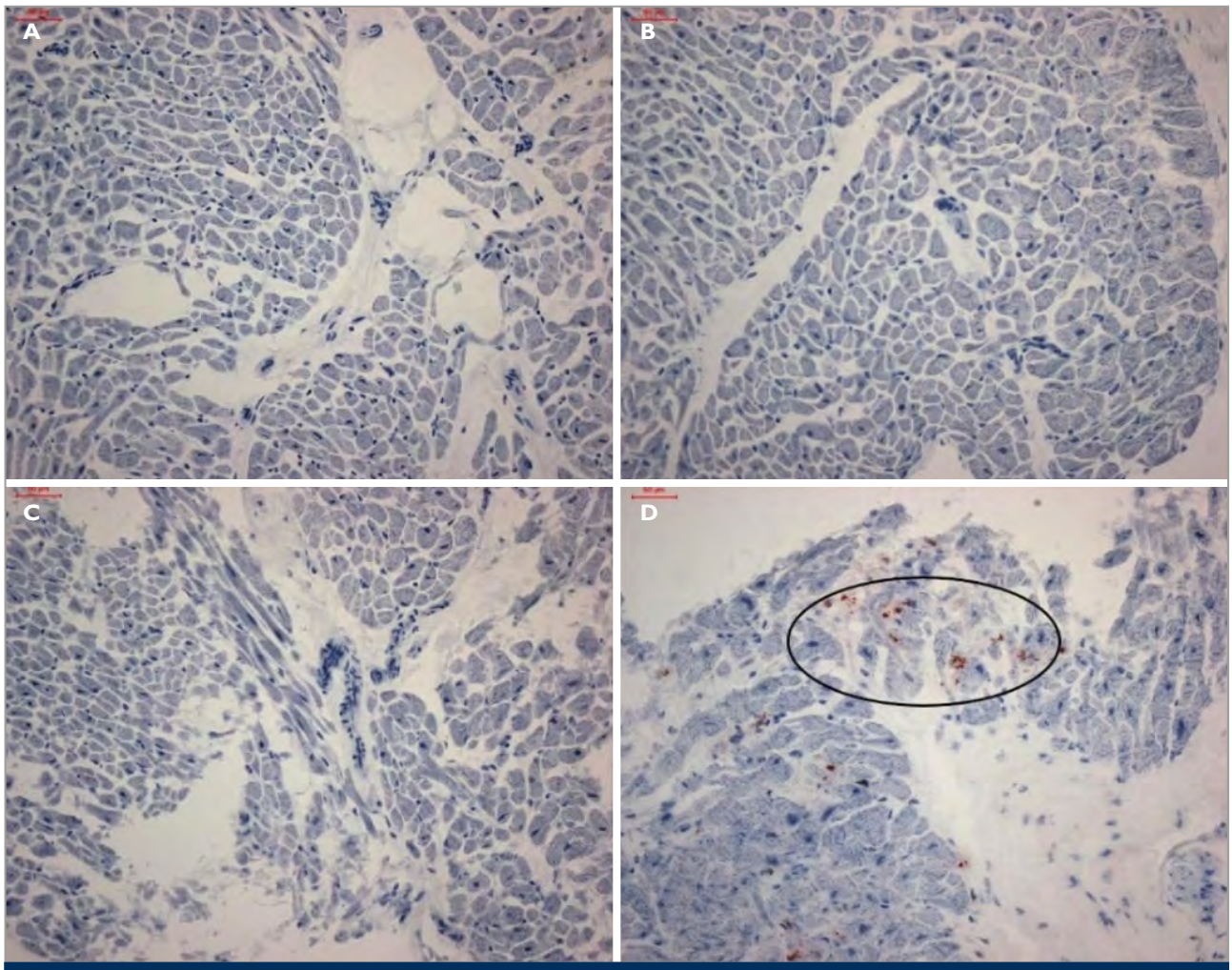

FIGURE 3: No myocardial inflammation.
A. No CD3 positive lymphocytes, B. CD11b positive cells, C. CD45RO, D. CD11b/Mac-1 cells.

group presented with moderate to severe heart failure (NYHA 3/4), although this difference was also not significant. Overall, patients in our study presented in a late stage of the disease, which is not uncommon in our public sector.[13,43]

Although we found a high positivity of viral genomes in our study population, with BV19 being the most prevalent, its significance still needs to be determined. Even though B19V viral genomes are commonly detected in endomyocardial biopsies of patients with heart failure, the clinical relevance is difficult to determine since viral genomes are detected in both symptomatic and asymptomatic patients.[30,44,45] Only 2 of the viral-positive patients had actively replicating B19V, confirmed by the detection of B19V mRNA. A German study in 2021 evaluated the clinical outcomes in a large cohort of 871 patients with positive parvovirus B19 DNA in their EMB samples. Patients were compared with and without parvovirus mRNA replicative intermediates, and those with replicating viruses had worse clinical outcomes. It demonstrated for the first time that transcriptionally active parvovirus B19 was pathologically and clinically relevant and not something that should just be dismissed.[40] It should be noted that there is still a debate on the significance of the contribution of B19V to DCM or whether it is only an innocent bystander and it has to be further investigated. Several authors consider B19V to be an innocent bystander.[35,45] Further studies would be required to determine the significance of active viral infection and its contribution to cardiovascular disease in our local populations.

Limited studies have been performed in sub-Saharan Africa that analysed EMB samples. In 2013, Shaboodien conducted the first study in South Africa on EMB samples that were tested for viral genomes. In contrast to our results, they showed that enteroviruses were important and present in 56% of patients with idiopathic dilated cardiomyopathy, and limited B19V was detected.[33] Several global studies have found that enteroviruses

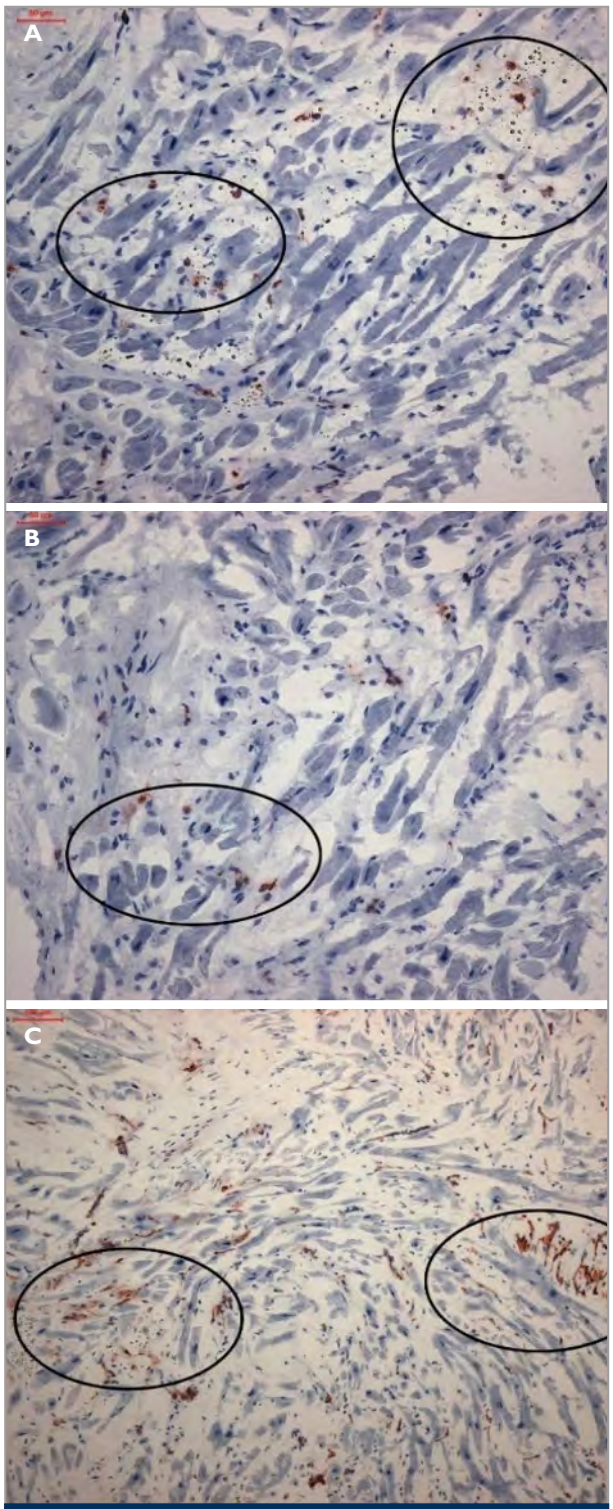

FIGURE 4 : Mild increased infiltration.
A. CD3 positive cells marked brown, B. CD11a/LFA-1 marked brown, C. Enhanced endothelial expression of ICAM-1.

were the most common virus associated with cardiomyopathy / myocarditis until the 1990s.[21-23] However, this seems to have started to shift, with B19V becoming the most frequently associated cardiotropic virus.[18,24-26,28-31] In 2022, a group in Stellenbosch, South Africa, evaluated myocarditis patients who were tested for cardiotropic viruses. Their findings support our results, as no enteroviruses were found. Most of their patients also had B19V, followed by HHV-6 and EBV.[34] Our findings and the findings of Hassan, et al. concur with the global trend of a declining involvement of enteroviruses and the overwhelming presence of B19V.[25,28-30,34]

Immunohistochemical analysis, which allows the differentiation and quantification of infiltrative inflammatory cells, remains an invaluable tool for identifying cardiac inflammation.[20,27] Our study found that only a third of our patients had mild to severely increased infiltrates and inflammation. This, combined with the limited number of patients with actively replicating viruses, meant that few patients in our cohort had acute cardiac inflammation and disease. This supports the concept of late presentation, which is seen in our clinical units and other similar ones.

Understanding and finding the cause of heart disease is important for improved scientific management. In South Africa, the proportion of dilated cardiomyopathy that remains idiopathic is very large.[11,12] Endomyocardial biopsy plays a role in allowing further investigation and testing. In South Africa, EMB is not routinely performed due to cost implications, and the role of EMB in advanced disease needs to be defined. This leads to a high percentage of dilated cardiomyopathy patients who keep the diagnosis of "idiopathic cardiomyopathy". Patients are only being managed and treated for their symptoms and not the underlying cause of the disease. It is important to keep investigating the use of non-invasive testing to filter through patients. Newer, less invasive diagnostic techniques have to be considered to guide which patients would require an EMB. This includes cardiac magnetic resonance imaging (CMR), taking blood samples, and testing for elevated levels of microribonucleic acid (miRNA) such as miR-133a and miR-155 in blood, which have been shown to have a positive correlation with viral myocarditis and inflammatory cell counts.[27,46] These could be explored further in the future, but currently, limited hospitals in our setting offer CMR services. The viability and usefulness of miRNA testing are promising, but the assays for the analysis of miRNAs would have to be developed, optimised, and standardised before they can be used routinely for patient screening.

LIMITATIONS

Our sample size was limited. It should be noted that the main study included patients with dilated cardiomyopathy based on the ESC criteria; the emphasis of this sub-study was on the histology and virology of EMB samples, and therefore, clinical correlations were not investigated. Cardiac MRIs were unavail-

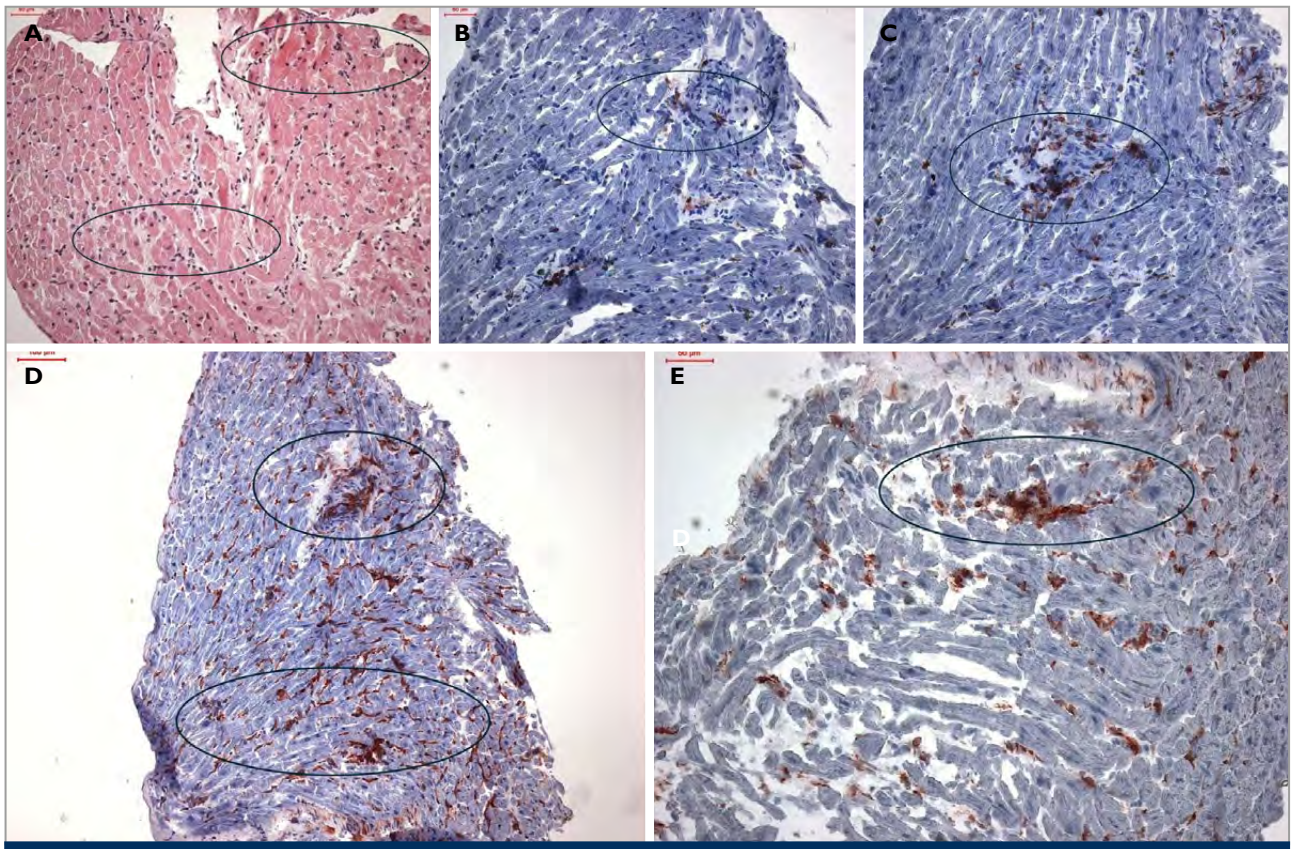

FIGURE 5: Moderate to severe increased infiltrates.
A. H&E stain, B. CD3, C. CD11a/LFA-1, D. CD54/ICAM-1, E. CD54.

able in our public sector facility at the time of the study. Minimal acute infections were detected - this can be ascribed to patients presenting late once advanced symptoms have already appeared. Only the viruses most commonly found were tested for. However, it still remains to be proven whether B19V is an innocent bystander or plays a role in the underlying pathophysiology. Further studies are warranted. It should be emphasised that the role of EMB in advanced disease should be investigated, especially in resource-limited environments.

RECOMMENDATIONS

Further studies should be undertaken to investigate a larger sample size. HIV-positive patients should also be considered to investigate the effect of HIV on myocardial disease and the viruses associated with the EMB in central South Africa. Techniques such as next-generation sequencing could be invaluable in enabling the non-targeted detection of viruses that might be important in the developing world.

CONCLUSION

Our findings indicate that B19V is the predominant virus detected in virus-positive endomyocardial biopsy (EMB) samples (96.4%), with no enteroviruses identified, suggesting a potential etiological shift toward B19V in the context of dilated cardiomyopathy (DCM). These findings underscore the need to further investigate the pathophysiological role of B19V in the development and progression of DCM. Of particular concern is the observation that severe cardiac inflammation was present in only a small subset of patients, indicating that many patients only presented at an advanced stage of the disease.

SUPPLEMTARY DATA

A supplementary table with the raw data endomyocardial biopsy analysis is provided in Supplementary Table 1 to provide an overview of the biopsy results per patient.

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Conflict of interest: none declared.

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SUPPLEMENTARY TABLE I: Raw data endomyocardial biopsy analysis.

Patient Nr	Histology	Cardiomyocytes	Immunohistological	Diagnosis as per pathology report	Virus Detection	Replicating virus	Copies / µg DNA	Virus type
1	Not done - sample issue	Not done	Not done	No diagnosis given	+	N	1 669 copies	B19V
2	Mild fibrosis	Hypertrophied	No increased infiltrates	DCM	+	N	31 copies	B19V
3	Normal, without fibrosis	Normal	No increased infiltrates	DCM	+	N	4 copies B19V, 6 copies HHV6	HHV6, B19V
4	Normal, without fibrosis	Mild atrophied	Mild increased infiltrates	Inflammatory cardiomyopathy	+	N	54 copies	B19V
5	Endocardium thickened and fibrotic	Normal	No increased infiltrates	DCM	+	N	881 copies	B19V
6	Mild perivascular, interstitial fibrosis	Mild atrophied	Increased lymphocytic infiltrates, enhanced expression of ICAM- 1	Borderline myocarditis	+	N	304 copies	B19V
7	Normal, without fibrosis	Mild atrophied	No increased infiltrates	DCM	+	Y	2 copies mRNA B19V	B19V mRNA
8	Normal, without fibrosis	Normal	Mild increased infiltrates	iDCM, low grade inflammation	+	Y	942 copies / 2 mRNA	B19V mRNA
9	Normal, without fibrosis, pronounced myocyte atrophy	Moderate atrophied	No increased infiltrates	No diagnosis given	+	N	<LOD	B19V
10	Normal, without fibrosis	Normal	Moderate to severe increased infiltrates	Inflammatory cardiomyopathy	+	N	<LOD	B19V
11	Normal, without fibrosis	Mild atrophied	No increased infiltrates	No diagnosis given	+	N	<LOD	B19V, EBV
12	Normal, without fibrosis	Moderate atrophied	Moderate increased infiltrates	Borderline myocarditis / inflammatory cardiomyopathy	+	N	16 copies	HHV6, B19V
13	Normal, without fibrosis	Mild atrophied	No increased infiltrates	No diagnosis given	-	N	None	None detected
14	Normal, without fibrosis	Mild atrophied	No increased infiltrates	No diagnosis given	+	N	<LOD EBV and B19V	B19V, EBV
15	Normal, without fibrosis	Normal	No increased infiltrates	No diagnosis given	+	N	<LOD	B19V
16	Normal, without fibrosis	Mild atrophied	No increased infiltrates	No diagnosis given	+	N	694 copies	B19V
17	Normal, without fibrosis	Normal	No increased infiltrates	No diagnosis given	-	N	None	None detected
18	Normal, without fibrosis	Normal	No increased infiltrates	No diagnosis given	+	N	<LOD	B19V
19	Normal, without fibrosis	Normal	No increased infiltrates	No diagnosis given	-	N	None	None detected
20	Normal, without fibrosis	Moderate atrophied	No increased infiltrates	DCM	-	N	None	None detected
21	Normal, without fibrosis	Normal	No increased infiltrates	DCM	+	N	30 copies	B19V

SUPPLEMENTARY TABLE I: Raw data endomyocardial biopsy analysis - continued.

Patient Nr	Histology	Cardiomyocytes	Immunohistological	Diagnosis as per pathology report	Virus Detection	Replicating virus	Copies / µg DNA	Virus type
22	Normal, without fibrosis	Hypertrophied	No increased infiltrates	Due to moderate lipomatisis, biopsy results compatible with ARVC or toxic myocardial damage	+	N	<LOD	B19V
23	Moderate lipomatisis	Normal	No increased infiltrates	Mild inflammatory cardiomyopathy	-	N	None	None detected
24	Normal, without fibrosis	Normal	No myocardial tissue, only fatty tissue	No diagnosis given	+	N	<LOD	B19V
25	Normal, without fibrosis	Normal	No increased infiltrates	Cardio-myopathy	+	N	410 copies	B19V
26	Normal, without fibrosis	Normal	No increased infiltrates	No diagnosis given	-	N	None	None detected
27	Mild fibrosis, atrophied cardiomyocytes	Hypertrophied	No increased infiltrates	Low intramyocardial inflammation / DD postinfectious	+	N	<LOD	B19V
28	Fibrosis with amyloid	Normal	Mild increased infiltrates	Mild inflammatory cardiomyopathy	+	N	506 copies B19V, HHV6 77 copies	B19V, HHV6
29	Mild fibrosis	Mild atrophied	Mild increased infiltrates	No diagnosis given	-	N	None	None detected
30	Normal, without fibrosis. Mild lipomatosis	Normal	Mild increased infiltrates	Borderline myocarditis / inflammatory cardiomyopathy	-	N	None	None detected
31	Mild perivascular fibrosis, mild cardiomyocyte hypertrophy	Mild atrophied	No increased infiltrates	Cardio-myopathy	+	N	<LOD EBV and B19V	B19V, EBV
32	Mild fibrosis	Normal	Quality sample not good	Cardio-myopathy	+	N	1 493 copies	B19V
33	Normal, without fibrosis.	Normal	Mild increased infiltrates	Borderline myocarditis / inflammatory cardiomyopathy	+	N	132 copies	HHV6
34	Normal, without fibrosis.	Normal	No increased infiltrates	No signs of myocarditis or inflammatory cardiomyopathy	-	N	None	None detected
35	Mild fibrosis	Normal	Massive infiltration	Massive inflammatory cardiomyopathy	+	N	7 copies B19V, <LOD HHV6	B19V, HHV6
36	Normal, without fibrosis	Normal	No increased infiltrates	No diagnosis given	-	N	None	None detected
37	Normal, without fibrosis	Normal	No increased infiltrates	DCM	+	N	23 copies B19Vand 77 copies HHV6	B19V, HHV6
38	Fibrosis with amyloid	Normal	No increased infiltrates	DCM	+	N	57 copies	B19V

DCM: dilated cardiomyopathy, EBV: Epstein Barr virus, HHV6: human herpes virus 6, iDCM: inflammatory dilated cardiomyopathy, LOD: limit of detection, N: no, B19V: Parvovirus B19, Y: yes.