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Infectious agents is a risk factor for myxomatous mitral valve degeneration: A case control study

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Abstract

Background: The etiology of myxomatous mitral valve degeneration (MVD) is not fully understood and may depend on time or environmental factors for which the interaction of infectious agents has not been documented. The purpose of the study is to analyze the effect of *Mycoplasma pneumoniae* (Mp), *Chlamydomphila pneumoniae* (Cp) and *Borrelia burgdorferi* (Bb) on myxomatous mitral valve degeneration pathogenesis and establish whether increased in inflammation and collagen degradation in myxomatous mitral valve degeneration etiopathogenesis.

Methods: An immunohistochemical test was performed to detect the inflammatory cells (CD20, CD45, CD68) and Mp, Bb and MMP9 antigens in two groups. The in situ hybridization was performed to detect *Chlamydomphila pneumoniae* and the bacteria study was performed using transmission electron microscopy. Group 1 ($n = 20$), surgical specimen composed by myxomatous mitral valve degeneration, and group 2 ($n = 20$), autopsy specimen composed by normal mitral valve. The data were analyzed using SigmaStat version 20 (SPSS Inc., Chicago, IL, USA). The groups were compared using Student's t test, Mann-Whitney test. A correlation analysis was performed using Spearman's correlation test. P values lower than 0.05 were considered statistically significant.

Results: By immunohistochemistry, there was a higher inflammatory cells/mm² for CD20 and CD45 in group 1, and CD68 in group 2. Higher number of Mp and Cp antigens was observed in group 1 and more Bb antigens was detected in group 2. The group 1 exhibited a positive correlation between the Bb and MVD percentage, between CD45 and Mp, and between MMP9 with Mp. These correlations were not observed in the group 2. Electron microscopy revealed the presence of structures compatible with microorganisms that feature *Borrelia* and *Mycoplasma* characteristics.

Conclusions: The presence of infectious agents, inflammatory cells and collagenases in mitral valves appear to contribute to the pathogenesis of MVD. *Mycoplasma pneumoniae* was strongly related with myxomatous mitral valve degeneration. Despite of low percentage of *Borrelia burgdorferi* in MD group, this agent was correlated with myxomatous degeneration and this may occur due synergistic actions between these infectious agents likely contribute to collagen degradation.

Keywords: Mitral valve, Myxomatous degeneration, *Borrelia burgdorferi*, *Mycoplasma pneumoniae*, *Chlamydomphila pneumoniae*, Metalloproteinases, Inflammatory markers

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Background

Myxomatous mitral valve degeneration (MVD) is the most common mitral valve disease in developed countries [1]. It occurs when the valve matrix is compromised due to an imbalance in the levels of acid mucopolysaccharides [2, 3]. Mitral valve prolapse is initiated by MVD, which serves as an anatomical substrate affecting the valve matrix. The prevalence of this condition is estimated at 2–3% and affects 150 million people around the world [4].

The etiology of this valvopathy is not fully understood. Familial forms show autosomal dominant transmission with variable penetrance, which may depend on age or environmental factors [5, 6]. In addition, inflammatory cells, autonomic imbalances of valve nerve endings and the action of metalloproteinases on collagen degradation can contribute to and enhance the degeneration of the valve matrix [7–9]. However, new contributing factors in the etiology of myxomatous degeneration should be sought to better understand this disease. Such knowledge could help to decrease postoperative recurrence, which occurs in approximately 69.2% of valve repair cases after 20 years of follow-up and is connected with degenerative progression [10]. Among potential contributing environmental factors, the participation of infectious agents in MVD pathogenesis has not been described in the literature as a causative factor; nevertheless, they may contribute to increasing the level of valve inflammation and collagen degradation. The presence of symbiotic bacteria is associated with tissue injury, as previously shown in studies analyzing atheromatous plaques in which proliferating *Mycoplasma pneumoniae* (Mp) and *Chlamydophila pneumoniae* (Cp) bacteria led to inflammation, collagen degradation and vulnerable plaque formation [11, 12]. *Borrelia burgdorferi* (Bb) is a bacteria that causes Lyme disease and leads to cardiac manifestations in 4% to 10% of cases; it may co-infect with various bacteria, including *Mycoplasma pneumoniae* [13, 14].

Based on this association between bacteria and tissue injury, the aim of this study was to analyze the presence and involvement of infectious agents in the increased inflammation and collagen degradation associated with the etio-pathogenesis of myxomatous mitral valve degeneration.

Methods

Valves studied

We analyzed 40 segments of mitral valve tissue, divided into 2 groups of 20 fragments each. Group 1 (myxomatous degeneration, MD) consisted of mitral valve fragments collected from patients undergoing replacement or mitral valve repair after mitral regurgitation with mitral valve prolapse (MVP). MVP is defined by the presence of a systolic murmur in the mitral focus by clinical

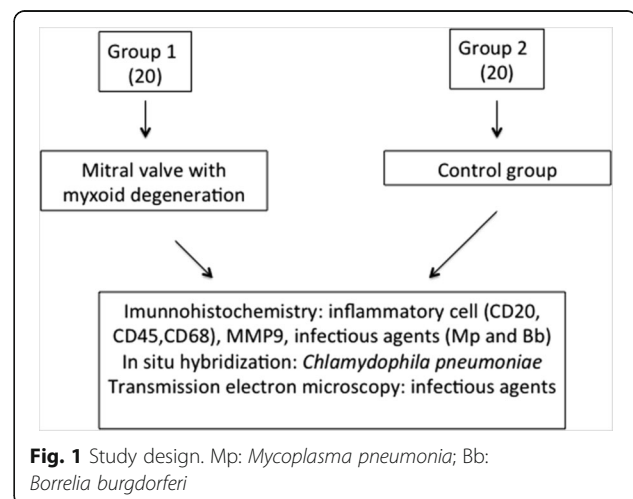
examination, supplemented by a transthoracic echocardiogram showing bulging of one or both leaflets at least 2 mm apart on the mitral valve ring plane, regardless of its thickness. MVD was further confirmed by histopathology analysis. Group 2 (control, CO) included segments of the mitral valve posterior cusp without signs of MVD collected in a macroscopic examination of the valve during necropsy of cadaver patients.

The exclusion criteria for the MD group were other cardiovascular diseases with a surgical indication and valve reoperation; for the CO group, samples from patients with known congenital or acquired heart valve disease or with any macroscopic signs of MVP were excluded. Additional exclusion criteria for both groups were age of less than eighteen years old and the non-agreement of the patient or legal representative for participation in the study.

The 40 fragments were analyzed using the following techniques: immunohistochemistry to detect *Mycoplasma pneumoniae*, *Borrelia burgdorferi*, inflammatory mediators and MMP9 antigens; in situ hybridization to detect *Chlamydophila pneumoniae*; and transmission electron microscopy to observe the infectious agents.

Study design (Fig. 1)

This was an observational, analytical, case-control study. For each specimen, measuring approximately $2 \times 2 \text{ cm}^2$, we prepared slices of 5- μm sections for histological analysis, immunohistochemistry and in situ hybridization (fixation in 10% formalin), and transmission electron microscopy (3% glutaraldehyde fixation). All the evaluations were performed at the Cardiac Pathology Laboratory in the Heart Institute of the Clinical Hospital of the Medical School of the University of Sao Paulo. The Bioethics Committee of the Clinical Hospital of Medical School of the University of Sao Paulo approved the study under the number 0029/04.



Histology and immunohistochemistry (IHC)

Five-micron serial sections of formalin-fixed, paraffin-embedded tissue were cut and Movat-stained for immunohistochemical detection of antigens from microorganisms (*Mycoplasma pneumoniae* and *Borrelia burgdorferi*), inflammatory cells (CD20, CD45 and CD68) and metalloproteinase. The tissue sections were deparaffinized in xylene, and antigen retrieval was performed in 0.01 mol-g/L citrate buffer, pH 6.0, for 10 min in a microwave. The slides were allowed to stand for 20 min at room temperature. To block endogenous peroxidase, the tissue sections were saturated with 6% hydrogen peroxide for 8 min, followed by avidin-biotin blocking for 20 min, incubation with a protein block for 30 min (Dako, Carpinteria, CA, USA), and then with primary antibodies against *Mycoplasma pneumoniae* (1:100; rabbit clone 10MR54; Fitzgerald International Inc., Concord, MA, USA), *Borrelia burgdorferi* (1:300; rabbit clone ab34970, ABCAM), MMP-9 (1:3200; rabbit clone RB1539P; Neo Markers Inc., Fremont, CA, USA), CD20 (1:1000; clone L26 M0755; Dako, Carpinteria, CA, USA), and CD45 (1:125; clone VCHL M0742 Dako, CA, California, USA) overnight. The reaction was visualized using a 3,3'-diaminobenzidine tetrahydrochloride solution. The positive controls were aneurysm sections for *Mycoplasma pneumoniae* and MMP9, positive myocardium for *Borrelia burgdorferi* and amygdala for inflammatory cells.

In situ hybridization (ISH)

In situ hybridization was performed to detect Cp DNA (50 ng/ μ l) using the probe ACAACGGCTAGAAATCAATTATAAGACTGAAGTTGAGCATATTCGTGAGGGAGTGACAGATTTAGATCATGGTGTGTCATTGCCCAAGGTTAAAGTCTACGT. For cell permeabilization, we used Tris / 10 mM EDTA pH 9.0, endogenous peroxidase blocking with 6% H₂O₂ and reduction of non-specific proteins with protein blocker (CAS Block - Invitrogen, MA, USA). The double-stranded DNA was denatured in an oven at 95 \pm 5 °C, and in situ hybridization was performed at 60 °C for 19 h in an oven. The signal was amplified using the Genpoint kit (Dako, Carpinteria, CA, USA), and the reaction was visualized with 3,3'-diaminobenzidine chromogen (Dako, Carpinteria, CA, USA). The probe was omitted for the negative control. Histological sections previously diagnosed as positive for *Chlamydia pneumoniae* were used as positive controls for the reactions.

Transmission electron microscopy (TEM)

Mitral valve specimens were fixed in 3% glutaraldehyde and postfixed in 1% osmium tetroxide solution. They were then washed in saline and kept until the next day in 0.5% uranyl acetate at 4 °C. The fragments were dehydrated in an ascending series of ethanol and propylene

oxide, followed by infiltration with a mixture of propylene oxide and araldite added to pure resin. The fragments were embedded in flexible molds containing silicone resin and polymerized for 48 h at 60 °C. Semithin samples (0.5 μ m) were collected and stained with methylene blue with 1% borax and 1% azure II. After selecting the best blocks, 70 nm-thick sections were cut with an ultramicrotome and placed on 200 mesh copper screens. The screens were contrasted with uranyl acetate and 5% lead citrate for examination under a transmission electron microscope (JEOL JEM-1010; JEOL Ltd., Akishima, Tokyo, Japan).

Methods of analysis

After immunohistochemical and in situ hybridization processing, the slides were scanned using a Scanscope CS System apparatus (Aperio Technologies, Inc., CA, USA) with a 20 \times Olympus UPlanSApo lens and 20 \times /0.75 specification. This device was coupled to a scanner that generated image files in .svs format. The scanned images were analyzed using the Aperio ImageScope View Software program (Aperio Technologies, Inc., CA, USA). The Image Scope Software is a new technology that eliminates the use of an optical microscope. The samples were analyzed using this tool, and an algorithm for nuclear and membrane marking was selected. For inflammatory cell analysis (CD20, CD45, CD68), the total number of positive cells was divided by the area in mm², and the rate is expressed as the total number of positive cells/mm². When the presence of a bacterial antigen and matrix metalloproteinase (MMP9) was detected, we counted the quantity of antigen signals in μ m² in an area 1 mm wide across the entire length of the valve.

Statistical analysis

The data were analyzed using SigmaStat version 20 (SPSS Inc., Chicago, IL, USA). The groups were compared using Student's t test for normally distributed variables; the results are expressed as the mean \pm standard deviation. The Mann-Whitney test was used for unpaired variables with asymmetrical distributions. Parametric data were expressed as the mean and standard deviation of the sample, and the independent groups were compared by Student's t test and analysis of variance. Nonparametric data were represented by the median, lower quartile (25th percentile) and upper quartile (75th percentile). A correlation analysis of the asymmetrically distributed ordinal variables was performed using Spearman's correlation test. Spearman's correlation test was also performed in different groups to test whether the infectious agents exerted their effects in association with another agent, inflammatory cells, or MMP9. *P* values lower than 0.05 were considered statistically significant.

Results

Studied population

Forty mitral valve samples with MVD or a normal appearance were included. In group 1, 14 (70%) patients were male, and 6 (30%) were female. The mean age was 67.4 ± 9.2 years. The intraoperative findings during surgery on the mitral valve were prolapse in 9 (45%) patients, ruptured chordae tendineae in 7 (35%) patients and prolapse associated with chordal rupture in 4 (20%) patients. According to the classification of Carpentier [15], the P2 segment was involved in 14 (70%) patients, the A2 segment in 1 (5%) patient, the A3 segment in 1 (5%) patient, the P1 and P2 segments in 1 (5%) patient, the P2 and P3 segments in 1 (5%) patient, the A2 and A3 segments in 1 (5%) patient, and the A2 and P2 segments in 1 (5%) patient (Table 1). In group 2, 11 (55%) patients were male, and 9 (45%) were female. The mean age was 67.6 ± 12.0 years. The cause of death was acute myocardial infarction in 5 (25%) cases, ischemic stroke in 4 (20%) cases, acute respiratory failure due to pneumonia in 3 (15%) cases, rupture of the ascending aorta during acute aortic dissection of the ascending aorta in 2 (10%) cases, pulmonary embolism in 1 (5%) case, hypertrophic cardiomyopathy in 1 (5%) case, ischemic cardiomyopathy in 1 (5%) case, acute pulmonary edema in 1

(5%) case, abdominal aortic aneurysm rupture in 1 (5%) case, and heart failure in 1 (5%) case (Table 2). The P2 segment was removed for analysis. None of the samples had signs of myxomatous degeneration in macroscopic analyses performed by two investigators, including the pathologist who performed the autopsies.

Percentage of degenerated tissue

The tissues were subjected to Movat staining to determine whether myxomatous degeneration was present. More degenerated tissue was observed in group 1, with a mean of $54.6\% \pm 23.7\%$ degenerated tissue, compared with group 2, with $35.5\% \pm 22.5\%$ degenerated tissue ($p = 0.013$; 95% CI 4.3 to 33.9) (Fig. 2).

Presence of inflammatory cells (Fig. 3)

An analysis of CD20⁺ (B lymphocyte marker) and CD45⁺ (T lymphocyte marker) cells revealed significantly more positive cells/mm² in valves with myxomatous degeneration (CD20⁺ cells: group 1 median = 17.8 (6.7–27.9), group 2 median = 4.6 (3.6–9.8), $p = 0.007$; CD45⁺ cells: group 1 median = 17.3 (3.4–92.5), group 2 median = 2.8 (1.4–10.1), $p = 0.008$). An analysis of CD68⁺ cells (macrophage marker) revealed more positive cells/mm² in the control group (group 1 median = 38.7 (26.6–81.8), group 2 median = 70 (42.7–120.4), $p = 0.098$) (Fig. 3a).

Table 1 Baseline characteristics of patients with myxomatous mitral valves

Age, y	Gender	Clinical diagnosis / surgery	Cusp analyzed*
72	M	MD + chordal rupture	P2
51	F	MD + chordal rupture	A3
73	F	MD + chordal rupture	P2
72	F	MD + MVP + chordal rupture	P2
60	F	MD + MVP + chordal rupture	P2
59	M	MD + MVP	P1 + P2
72	M	MD + MVP + chordal rupture	P2
73	M	MD + chordal rupture	A2
72	M	MD + chordal rupture	P2 + P3
68	M	MD + chordal rupture	P2
68	M	MD + MVP	P2
73	M	MD + MVP	P2
79	M	MD + MVP	P2 + A2
56	M	MD + MVP	P2
63	F	MD + MVP	A2 + A3
69	F	MD + chordal rupture	P2
44	M	MD + MVP	P2
78	M	MD + MVP	P2
69	M	MD + MVP	P2
76	M	MD + MVP + chordal rupture	P2

MD myxomatous degeneration, MVP mitral valve prolapse.

*According to the Carpentier classification

Identification of bacteria by IHC, ISH and TEM (Fig. 4)

The numbers of *Mycoplasma pneumoniae*, *Chlamydo-philum pneumoniae* and *Borrelia burgdorferi* bacterial antigens are expressed as μm^2 antigens per 1 mm of analyzed valve segment length ($\mu\text{m}^2\text{Ag}/1\text{ mm}$). A significantly greater *Mycoplasma pneumoniae* antigen area was detected in the MD group. The median positive area in $\mu\text{m}^2\text{Ag}/1\text{ mm}$ for *Mycoplasma pneumoniae* was 180,993 (24,856–387,477) in group 1 (valves with myxomatous degeneration) versus 7970 (2736–15,992) in group 2 (normal valves) ($p < 0.001$). Similarly, for *Chlamydo-philum pneumoniae*, group 1 presented a greater antigen area (median = 9905 (4716–16,912) versus 5864 (2382–8692) in group 2; $p = 0.2$). By contrast, more *Borrelia burgdorferi* antigens were detected in the control group (group 1 median = 7596 (3203–13,519), group 2 median = 10,584 (7223–15,974); $p = 0.14$) (Fig. 4a). Electron microscopy revealed the presence of structures compatible with microorganisms with *Borrelia* and *Mycoplasma* characteristics. The microorganisms identified in the MD group were smaller and thinner than the bacteria found in the control group.

MMP9 analysis and correlation test (Fig. 5)

The presence of MMP9 is expressed in μm^2 of antigens per 1 mm valve segment length. We observed a significantly greater antigen area in the myxomatous

Table 2 Baseline characteristics of the control patients

Age, y	Gender	Cause of death	Post mortem interval, h
42	M	Acute myocardial infarction	6
59	M	Pulmonary thromboembolism	7
76	M	Ischemic stroke	3
66	M	Ischemic cardiomyopathy	6
82	M	Ischemic stroke	5
63	F	Dissection of the ascending aorta	9
79	F	Dissection of the ascending aorta	9
73	F	Acute respiratory failure - Pneumonia	10
75	M	Left Ventricular free wall rupture by acute myocardial infarction	5
54	F	Hypertrophic cardiomyopathy	9
74	F	Acute pulmonary edema	9
51	M	Acute myocardial infarction	10
52	M	Abdominal aortic aneurysm ruptured	12
80	M	Acute respiratory failure - Pneumonia	5
60	M	Acute myocardial infarction	6
61	F	Ischemic stroke	6
72	F	Ischemic stroke	8
84	F	Acute myocardial infarction	5
67	M	Acute respiratory failure - Pneumonia	14
82	F	Heart failure	9

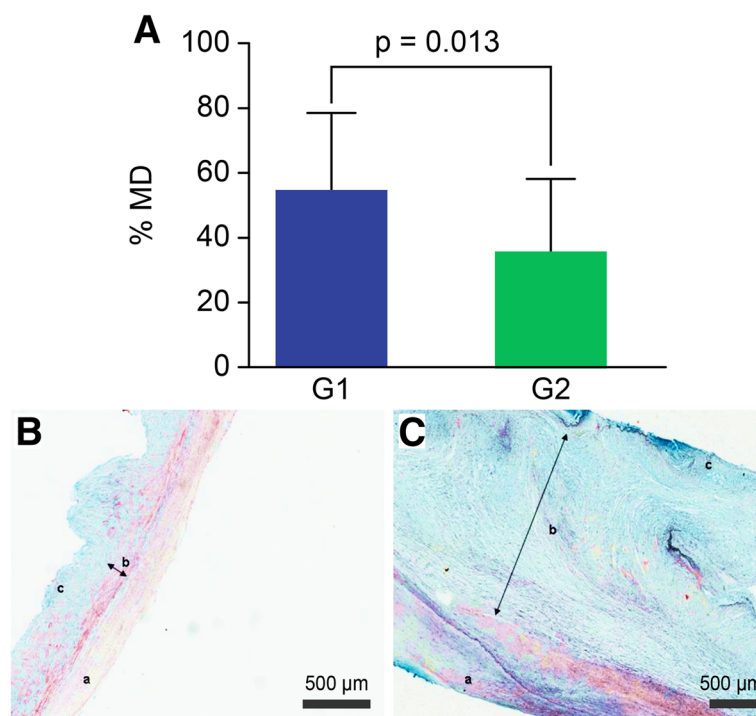
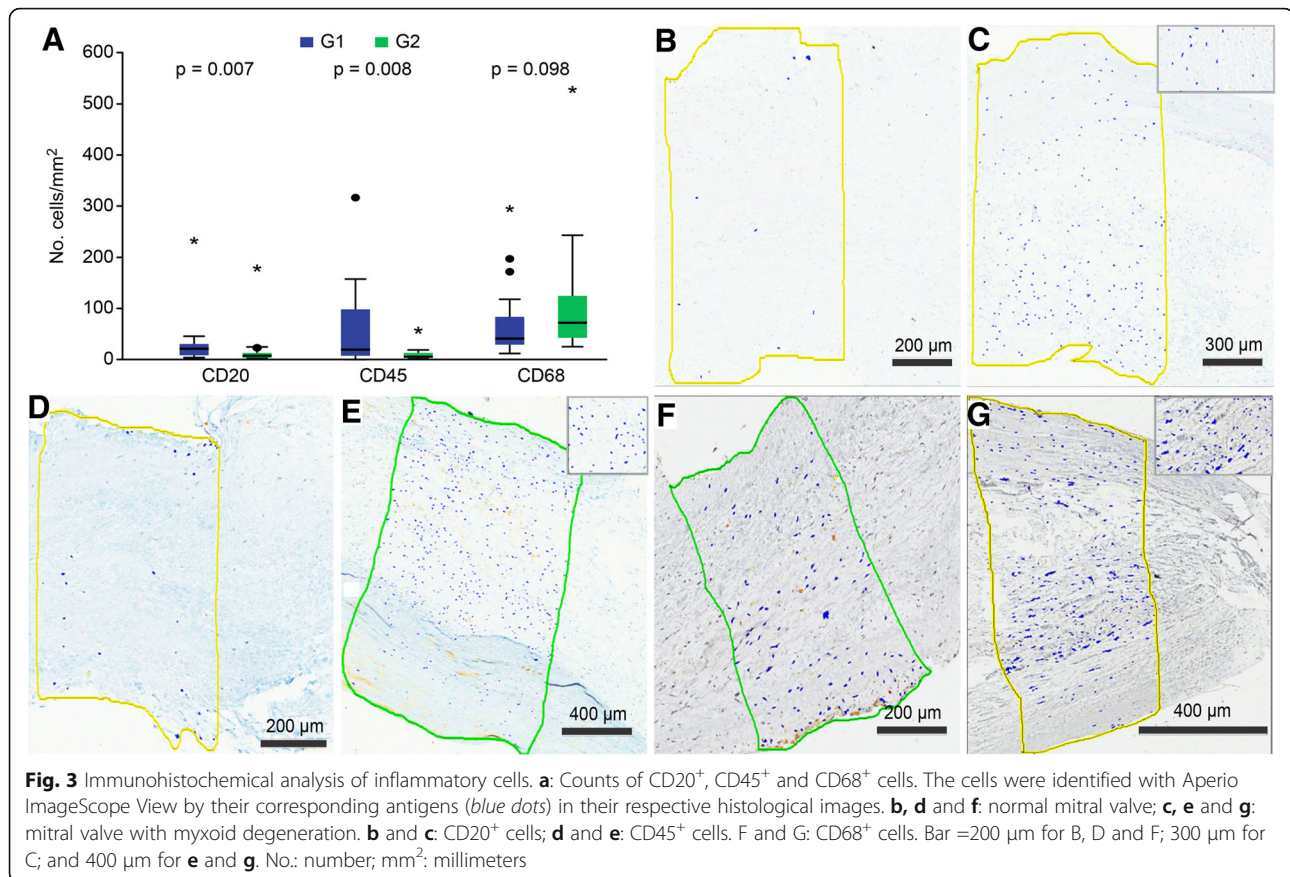


Fig. 2 Histological analysis of myxoid degeneration. **a:** analysis of myxomatous degeneration percentage (% MD) in groups 1 and 2. **b:** Normal valve. **c:** Mitral valve with MVD. The valve with myxomatous degeneration shows diffuse and homogeneous thickening, distorted architecture, spongy layer expansion and proteoglycan accumulation, indicated by the bluish Movat staining (a: atrial layer, b: spongy layer, and c: fibrous layer). Bar =500 µm. G1: group 1; G2: group 2; p: p value



degeneration group (median = 389,844 (214,459–679,711) versus 144,397 (29,894–247,453) in group 2; $p < 0.001$) (Fig. 5a).

The valves with myxomatous degeneration exhibited significant correlations between the quantity of bacterial antigens, inflammatory cells and MMP9: *Borrelia burgdorferi* was positively correlated with the MD percentage ($r = 0.52$ and $p = 0.018$), and *Mycoplasma pneumoniae* was positively correlated with CD45 ($r = 0.51$ and $p = 0.02$) and MMP9 ($r = 0.45$ and $p = 0.04$). These correlations were absent in the control group (Table 3).

Discussion

To the best of our knowledge, this is the first report describing the presence of bacteria in the pathogenesis of myxomatous degeneration. Our results also show increased expression of CD20, CD45 and MMP9 in MVD.

In our work, we identified enhanced *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae* antigens in mitral valves with MD compared with controls, with a median of 180,993 (24,856 to 387,477) versus 7970 (2736 to 15,992) μm^2 Ag/1 mm valve segment extension ($p < 0.001$) and 9905 (4716–16,912) \times 5864 (2382–8692) μm^2 Ag/1 mm valve segment extension ($p = 0.2$), respectively. We also observed positive correlations

between *Mycoplasma pneumoniae* and CD45⁺ cells ($r = 0.51$ and $p = 0.02$) and MMP9 ($r = 0.45$ and $p = 0.04$). The presence of *Borrelia burgdorferi* antigens was positively correlated with the percentage of MD ($r = 0.52$ and $p = 0.018$). In previous studies, the presence of various symbiotic bacteria has been associated with the formation of tissue injury. In the aortic valve, *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae* proliferation was associated with tissue calcification and aortic valve stenosis [16, 17]. Roggerio et al. studied the relationship between *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae* in aortic aneurysms and identified these bacteria in the adventitia of the aorta, concluding that infectious agents, acting symbiotically, can contribute to the development of aortic aneurysms, initiating inflammation and contributing to the disease process [18].

Both groups had an average age of approximately 67 years. The valve impairment in the control group can be explained by age-related progressive degeneration of the extracellular matrix through the action of collagenolytic enzymes secreted by interstitial cells that cause abnormalities in collagen [19].

Infectious agents are often present in cardiovascular disease, with roles for these pathogens in its genesis or

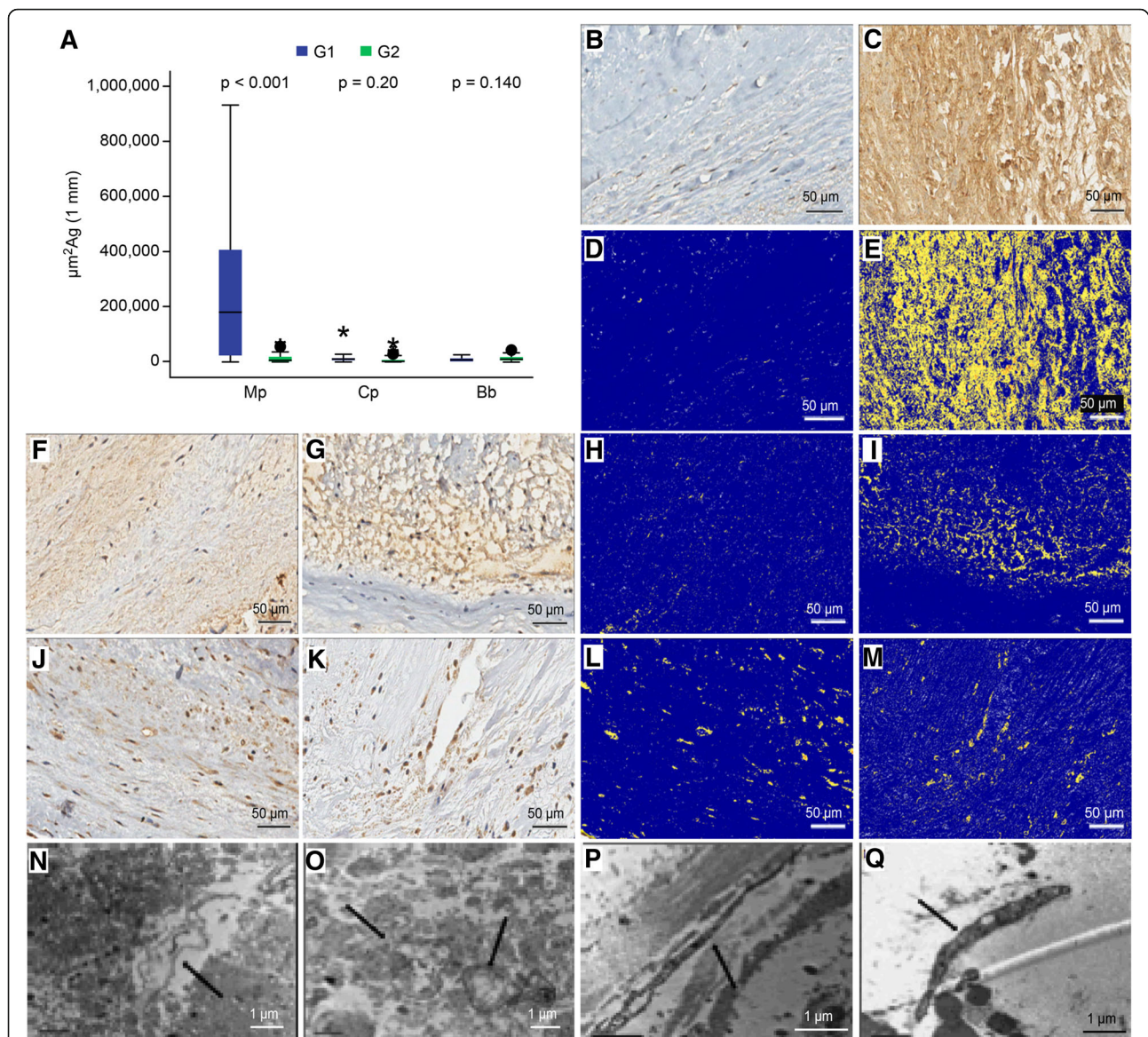
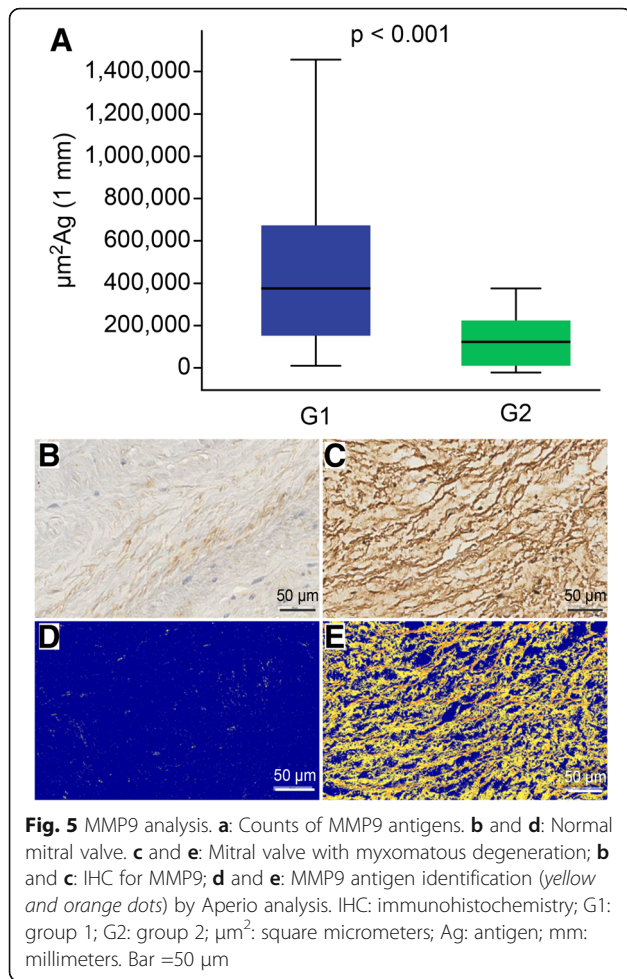


Fig. 4 Bacterial analysis by IHC, ISH and TEM. **a**: Counts of *Mycoplasma pneumoniae* (Mp), *Chlamydomphila pneumoniae* (Cp) and *Borrelia burgdorferi* (Bb) antigens. **b, d, f, h, j** and **l**: Normal mitral valve. **c, e, g, i, k** and **m**: Mitral valve with myxomatous degeneration; **b** and **c**: IHC for Mp; **d** and **e**: Mp antigen identification (yellow dots) by Aperio analysis; **f** and **g**: IHC for Cp; **h** and **i**: Cp antigen identification (yellow dots) by Aperio analysis; **j** and **k**: IHC for Bb; **l** and **m**: Bp antigen identification (yellow dots) by Aperio analysis. **n, o, p** and **q**: Transmission electron microscopy of bacterial elements (arrows). **N**: A microorganism with *Mycoplasma* characteristics was identified in the control group, with a long, single envelope membrane and a structure similar to a spirochete in the cytoplasm amid fragmented collagen tissue. **O**: This structure is consistent with *Borrelia*, long and winding, and was identified in the control group. **P**: Structures compatible with *Mycoplasma* were also identified in the MD group. **Q**: A microorganism with a wavy structure compatible with spirochete-containing organelles or endosymbionts (consistent with *Borrelia*) was observed in the MD group. IHC: immunohistochemistry; ISH: in situ hybridization; TEM: transmission electron microscopy; Mp: *Mycoplasma pneumoniae*; Bb: *Borrelia burgdorferi*; G1: group 1; G2: group 2; μm^2 : square micrometers; Ag: antigen; mm: millimeters. Bar = 50 μm for B-M. Bar = 1 μm for N-Q

prior to its inception reported in several papers. Recently, Mangini et al. detected the presence of *Mycoplasma pneumoniae*, *Chlamydomphila pneumoniae* and *Borrelia burgdorferi* in hearts with dilated cardiomyopathy, including Chagas disease [20]. In the aortic valve, the proliferation of *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae* was related to tissue calcification and aortic valve

stenosis [21]. Inflammatory cells and metalloproteinases have also been associated with the MD process. Veinot et al. analyzed five valves with MD and observed an increase in markers of mast cells (CD117), suggesting the participation of an inflammatory process triggered by myxoid degeneration. CD117⁺ cells are young cells identified in mitral valves that represent a degree of heart regeneration



capacity against stress, trauma or ischemia [3]. Barth et al. analyzed the presence of CD34⁺ fibrocytes in 15 myxoid valves and 10 valves without degeneration and found that these cells appear to be morphologically altered [7]. Rabkin et al. identified a role for MMP9, as well as other collagenases like MMP1, MMP2 and MMP13, in the breakdown of collagen in myxomatous degeneration [19]. The involvement of bacteria in the etiology of MVD has not previously been described. It is possible that the

Table 3 Correlations between bacterial antigens versus inflammatory cells versus MMP-9

	MD		CO	
	r	p	r	p
Bb x % MD	0.52	0.018	0.15	0.5
CD45 x Mp	0.51	0.02	0.01	0.9
Mp x MMP9	0.45	0.04	0.17	0.6
Mp x % MD	-0.34	0.13	0.04	0.8
MMP9 x % MD	-0.02	0.91	0.4	0.07

MD myxomatous degeneration, CO control, Bb *Borrelia burgdorferi*, Mp *Mycoplasma pneumoniae*, MMP9 metalloproteinase 9.

presence of such agents could lead to collagen degradation and increase local inflammation, facilitating the process of myxoid degeneration. This scenario warrants further investigation.

The presence of microorganisms as host symbionts is not well understood but is increasingly being studied. Scholars believe that microbial symbionts may fundamentally alter the host organism's physiology and sometimes impart new or optimized abilities that result in more adapted or healthier individuals [22]. When more than one microparasite species infects the same host, interactions between these parasites can exacerbate their symbiotic and immunological effects [23, 24]. In this study, the valves in the control group exhibited more *Borrelia burgdorferi* antigens than the valves in the MVD group. However, in the valves with MVD, the presence of *Borrelia burgdorferi* was associated with more myxomatous degeneration, and an interaction with *Mycoplasma pneumoniae* may have contributed to collagen degradation through a synergistic effect between these agents. Considering that we observed *Mycoplasma pneumoniae* and *Borrelia burgdorferi* in both groups but in different quantities and morphologies, these agents might also produce different responses with respect to collagen degradation. The presence of *Mycoplasma pneumoniae* can stimulate hyperactive macrophages, with subsequent cytotoxic T cell activation inducing fibroblast apoptosis [25]. In addition, the MVD group exhibited more *Mycoplasma pneumoniae*, which positively correlated with gelatinase MMP9, an enzyme that participates in collagen degradation, as reported by other authors [9, 19]. In our study, we also identified a greater number of CD68⁺ cells in the valve segments of the control group. Rabkin et al. associated the presence of macrophages in normal mitral valves with a minor amount of proteolytic enzymes [19]. Though valves with MD had fewer CD68⁺ cells, they still had higher quantities of B and T lymphocytes, including cytotoxic T lymphocytes that can release cytokines such as perforin and the granzina B leading to cell apoptosis and contributing to collagen degradation. It is possible that the CD68⁺ cells function as protective factors against collagen degradation.

Scholars have suggested that *Borrelia burgdorferi* strains interact competitively with the host [26]. In a recent study, Devevey et al. analyzed three different strains of *Borrelia burgdorferi sensu strictu* in 36 rats and detected a strong and competitive inhibitory interaction among the strains. In all cases, the strain that infected first exhibited a fitness advantage, mainly because colonization of the rat tissue by subsequent strains was inhibited. This inhibition is most likely due to the production of specific antibodies against *Borrelia* protein antigens [27]. The presence of bacteria and their relationships with collagen degradation as well as increased

local inflammation can facilitate myxomatous degeneration, and the synergistic actions of multiple symbionts seem to be a contributing factor in MVD pathogenesis.

We are aware of the limitations of our study, especially the restrictions of the methodological design, including the sample size. Another limitation of this work is that the control group included cases with cardiovascular diseases such as myocardial infarction and aortic aneurysm, though these conditions did not appear to influence the results (Additional file 1). Moreover, in the control patients, we identified several degeneration foci with histological features consistent with MD, though they were not enough to lead to a breakdown in the valve structure. The results of this study will form a strong foundation for future research into the hypothesis that symbiotic actions of the bacteria play a key role in the development of myxoid degeneration of the mitral valve. More studies will be needed to confirm this causal relationship.

Conclusion

The presence of infectious agents, inflammatory cells and collagenases in mitral valves appear to contribute to the pathogenesis of MVD. *Mycoplasma pneumoniae* was strongly related with myxomatous mitral valve degeneration. Despite of low percentage of *Borrelia burgdorferi* in MD group, this agent was correlated with myxomatous degeneration and this may occur due synergistic actions between this infectious agents likely contribute to collagen degradation.

Additional file

Additional file 1: Quantification of antigens of *Borrelia burgdorferi*, *Mycoplasma pneumoniae* and MMP 9 containing only the cases with cardiovascular disease associated. (DOCX 15 kb)

Abbreviations

Bb: *Borrelia burgdorferi*; CO: Control; Cp: *Chlamydomytila pneumoniae*; IHC: Immunohistochemistry; ISH: In situ hybridization; MD: Myxomatous degeneration; MMP9: Metalloproteinase 9; Mp: *Mycoplasma pneumoniae*; MVD: Mitral valve degeneration; MVP: Mitral valve prolapse; TEM: Transmission electron microscopy

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Availability of data and materials

All relevant data are available in the tables and figures. The raw data supporting our findings can be found by contact with autor across e-mail (mgtiveron@yahoo.com.br).

Authors' contribution

MGT are responsible conducted the experiment, the data collection and data analysis assistance of MMR. PMAP and MLH are responsible for the design and conception of the experiment and interpretation of final results.

Reviewed, revised, and edited the final manuscript. JK and RI conducted the immunohistochemistry and in situ hybridization experiment. JJP conducted the transmission electron microscopy experiment and analysis. MMR conducted the data analysis and interpretation. CMAB reviewed, revised, and edited the final manuscript. FBJ reviewed and revised the manuscript. All authors contributed to the final manuscript. All authors read and approved the final manuscript.

Authors' information

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The Bioethics Committee of the Clinical Hospital of Medical School of the University of Sao Paulo approved the study, approval no. 0029/04. A written informed consent was taken from all participants. Written consent for participating in the study and consent to publish was obtained from each participant prior to the tests.

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