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Detecting sub-clinical disease activity in patients with chronic rheumatic valvular heart disease

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ABSTRACT

Objective: Valve disease progression in rheumatic heart disease(RHD) is generally attributed to recurrent attacks of acute rheumatic fever(ARF). However, persistence of chronic sub-clinical inflammation remains a plausible but unproven cause. Non-invasive means to identify sub-clinical inflammation may facilitate research efforts towards understanding its contribution to disease progression.
Methods: Patients with chronic RHD, without clinical evidence of ARF, undergoing elective valve surgery were enrolled. Sub-clinical inflammation was ascertained by histological evaluation of left atrial appendage and valve tissue excised during surgery. We assessed the diagnostic utility of Gallium-67 scintigraphy imaging, and inflammatory biomarkers, hsCRP, IL-2, IL-6, Tumor Necrosis Factor-Alpha(TNF-α), Interferon-gamma(IFN-γ), and Serum Amyloid A(SAA), in identifying patients with sub-clinical inflammation.
Results: Of the 93 RHD patients enrolled(mean age 34 $\pm$ 11 years, 45% females), 86 were included in final analysis. Sub-clinical inflammation was present in 27 patients(31.4%). Patients with dominant regurgitant lesions were more likely to have sub-clinical inflammation compared to those with stenotic lesions, though this association was not statistically significant(dominant regurgitant lesions vs isolated mitral stenosis: OR 3.5, 95%CI 0.68–17.96, $p = 0.133$). Inflammatory biomarkers were elevated in the majority of patients: hsCRP, IL-2, IL-6, TNF-α, and IFN-γ in 44%, 89%, 90%, 79%, and 81% patients, respectively. However, there was no significant association between biomarker elevation and histologically ascertained sub-clinical inflammation. Ga-67 imaging was unable to identify inflammation in the 15 patients in whom it was performed.
Conclusion: Sub-clinical inflammation is common in RHD patients. Conventional inflammatory markers are elevated in the majority, but aren't discriminatory enough to identify the presence of histologic inflammation.

1. Introduction

Acute rheumatic fever (ARF) and rheumatic heart disease (RHD) remain important causes of morbidity and mortality in the developing world.[1,2] Acute rheumatic fever is an inflammatory disease which occurs as a sequela of Group A Streptococcal pharyngitis in susceptible individuals, usually children between the ages of 5 and 15 years.[2] Amongst the children affected by ARF, the majority develop carditis, of whom approximately half develop permanent valvular lesions resulting in clinically apparent RHD.[2] The acute damage to heart valves occurs by antigen mimicry. However, the pathogenesis of progression of valve lesions is debated. The most widely held belief is that progressive valve damage results from recurrent episodes of ARF. This is supported by

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the natural history studies and constitutes the basis on which secondary penicillin prophylaxis is advised.[3] However, there is evidence to suggest that chronic, sub-clinical inflammation may occur, and may potentially contribute to disease progression.[4] This hypothesis is supported by the Bland and Jones data which showed that a significant proportion of patients developed mitral stenosis on follow-up over 20 years without having developed ARF recurrences in the interim period.[5] Additionally, histopathological studies documenting the presence of active inflammation in excised left atrial appendages (LAA) in clinically and biochemically quiescent patients also point towards the presence of sub-clinical inflammation.[6] Ga-67 scintigraphy has been studied only in a small number of patients with acute carditis.[7] While several groups have studied peripheral blood lymphocytes and cytokine levels in patients with acute carditis and chronic RHD, [8–14] no attempt has been made to correlate these results with histopathological evidence of inflammation. The aim of this study was to assess the diagnostic utility of biochemical markers of inflammation and Ga-67 scintigraphic imaging for detection of sub-clinical cardiac inflammation in patients with established RHD, who have no clinical or conventional biochemical evidence of rheumatic activity.

2. Methods

This was a prospective observational study. Patients older than 18 years who were scheduled for elective surgery (closed mitral valvotomy, mitral valve repair or mitral valve replacement) for chronic RHD were enrolled. The study protocol was approved by the institutional ethics committee and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All patients gave written informed consent. Subjects were excluded if they were pregnant or if they had clinical or laboratory evidence of rheumatic activity in the 3 months prior to enrolment, suspicion of concomitant primary myocardial disease (inflammatory myocarditis, dilated cardiomyopathy, or infiltrative myocardial disease), or coexistent systemic inflammatory disorders (inflammatory arthritis, connective tissue disorders and vasculitis).

Based on echocardiography, patients were divided into: combined stenotic lesions (isolated mitral stenosis + dominant stenotic lesions) and, combined regurgitant lesions (pure regurgitant lesions + dominant regurgitant lesions). Pre-operatively, Ga-67 scintigraphy scan was performed, and a blood sample was drawn for inflammatory markers. Both of these were done as close to the day of surgery as possible. Histopathological analysis of the LA appendages and other valve tissues was done within 48 h of excision. Fig. 1 outlines a schematic flow of patients through the study.

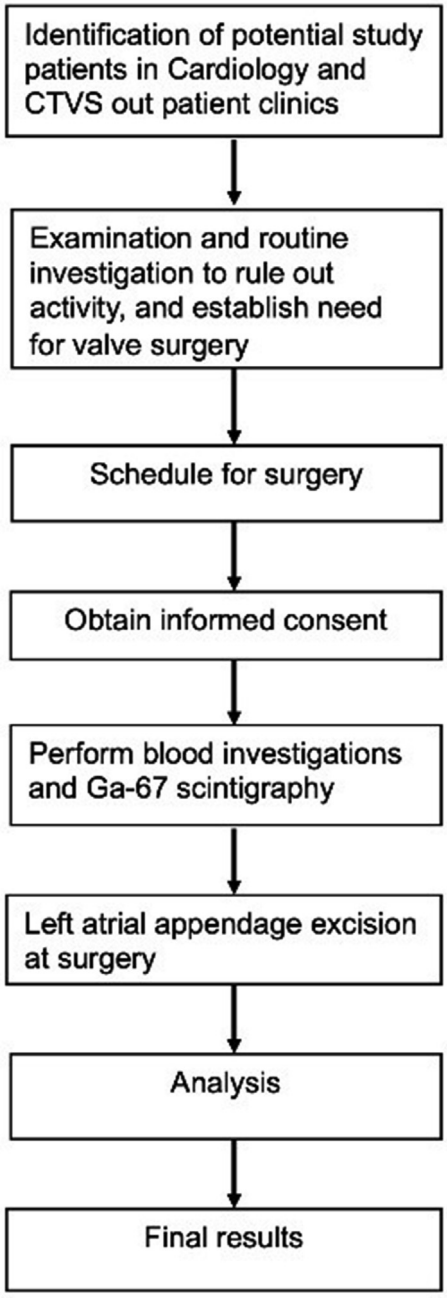

Fig. 1. Flowchart of patients through the study.

2.1. Ga-67 scintigraphy

Gallium-67 in a dose of 150–220 MBq (4–6 mCi) with a minimum dose of 9–18 MBq (0.25–0.5 mCi) was administered intravenously and images were acquired with a Millennium VG dual head gamma camera (GE Medical systems) equipped with a medium-energy parallel hole collimator. Planar images were obtained 24–72 h after injection of the radio-pharmaceutical. Once significant Ga-67 uptake was observed, SPECT images were acquired to localize the area of uptake, with a particular emphasis on imaging the LAA.

2.2. Inflammatory biomarkers

Blood samples were collected, and serum was separated and stored at −70 °C. Serum amyloid A (SAA), Interferon gamma (IFN-γ), Tumour Necrosis Factor alpha (TNF-α), Interleukin-2 (IL-2), Interleukin-6 (IL-6) and High Sensitivity C-Reactive Protein (hs-CRP) levels were determined by standard Enzyme-Linked Immunosorbent Assay (ELISA), using R&D Systems ELISA kits. Abnormal values were defined as per manufacturer instructions: hsCRP >3 mg/L, IL2 ≥ 10 pg/mL, IL6 ≥ 6.25 pg/mL, TNFα ≥25 pg/mL, IFNγ ≥12.5 pg/mL.

2.3. Histopathological examination of the excised specimens

Left atrial appendages in all patients and valve tissue in those patients who underwent valve replacement were excised at the time of surgery. The excised specimens were fixed in neutral buffered formalin for histopathologic examination. Representative sections from the specimens were processed in paraffin and blocks were made. The slides were evaluated under light microscopy using

hematoxylin and eosin staining. Masson's trichome staining is done in all samples processed at our center to highlight the presence of fibrosis and staining with Verhoeff's Van Gieson when endocardial thickening is suspected. The presence of inflammation, characterized by lympho-mononuclear infiltrates and/or Aschoff bodies was used to define inflammation in our study. In the absence of well-defined criteria for inflammation in the left atrial appendage or valve tissue (unlike the Dallas criteria used to define inflammation in myocardium), the presence of inflammation was ascertained by an experienced cardiac pathologist (RR); presence of occasional lymphocytes was not considered positive for inflammation.

2.4. Statistical analysis

Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as frequency (percentage). The association of biomarkers and clinical variables with the presence of histopathological inflammation was determined using univariable and multivariable logistic regression. All analyses were performed using Stata 15 (StataCorp, College Station, Texas). A p-value of ≤ 0.05 was considered to be statistically significant.

3. Results

During the study period, 93 consecutive schronic RHD patients who were eligible for inclusion in the study were enrolled. Data of 7 patients was incomplete, and 86 patients were available for the final analysis. Baseline characteristics are summarized in Table 1. Mean age of the study population was 34 years; 45% were females. Stenotic lesions were more common than regurgitant lesions (combined stenotic lesions 61, 73%; combined regurgitant lesions 25, 27%). Majority of the patients were in NYHA class II (51%).

Histopathological examination of the excised valves and left atrial appendage detected lympho-mononuclear cell infiltration suggestive of chronic inflammation in 27 patients (31%) (Table 2). Aschoff bodies were not detected in any of the patients. Fibrosis was detected in 46 (53%) patients using Masson's trichome stain. None of the patients showed endocardial thickening. Representative histopathological specimens of left atrial appendage showing absence and presence of inflammation are shown in Fig. 2-A and 2-B. Inflammatory markers were elevated in a non-discriminatory fashion; proportion of patients with elevated biomarkers varied from 44% for hs-CRP to 99% for SAA (Table 2).

This was significantly higher than the number of patients who had inflammation on biopsy specimens. There was no association between histopathological and biochemical evidence of inflammation (Table 3, Graphical abstract). There was no significant association between the type of lesion on echocardiography and histological inflammation(Table 4). However, the presence of regurgitation compared to stenosis, was associated with a higher odds of histopathologically confirmed inflammation (dominant regurgitant lesions vs isolated mitral stenosis, OR 3.5, 95% CI 0.68−17.96, $p = 0.133$) (Fig. 3).

Fifteen patients underwent Ga-67 scanning but the quality of images obtained was poor. None of the scans identified the presence of inflammation, though 2 of the 15 patients who underwent scintigraphy had histopathologic evidence of inflammation. After careful consideration, Ga-67 scintigraphy was considered not promising, and was not performed in the remaining patients.

Table 1
Baseline characteristics.

Variable	n (%), or mean ± SD
N	93
Age (Y)	34.07 ± 11
Females	42 (45.1)
Valve involvement	
Isolated mitral stenosis	18 (19.4)
Dominant stenotic lesion[a]	48 (51.6)
Pure regurgitant lesion[b]	11 (11.8)
Dominant regurgitant lesion[c]	14 (15.1)
Organic tricuspid valve disease	2 (2.1)
NYHA class (n = 72)	
I	1 (1.4)
II	37 (51.4)
III	31 (43.1)
IV	3 (4.2)
Congestive heart failure	10[11]
Infective endocarditis	2 (2.2)

[a] Dominant stenotic lesion: severe mitral stenosis and/or aortic stenosis, with mild-moderate mitral regurgitation and/or aortic regurgitation.
[b] Pure regurgitant lesion: moderate-severe mitral regurgitation and/or aortic regurgitation with no mitral stenosis or aortic stenosis.
[c] Dominant regurgitant lesion: severe mitral regurgitation and/or aortic regurgitation, with mild-moderate mitral stenosis and/or aortic stenosis.

4. Discussion

In this study, we found that histopathologically confirmed subclinical cardiac inflammation was found in nearly a third of patients with chronic RHD, who did not have any clinical evidence of rheumatic activity. Serum markers of inflammation, including circulating cytokines, were elevated in the majority of patients, but did not correlate with the presence of histologic inflammation. Regurgitant lesions were more likely to have sub-clinical inflammation, though this association was not statistically significant.

4.1. Histopathological inflammation

Our results are consistent with Raizada et al who demonstrated the presence of inflammation in 25−33% of the RHD patients.[15] Another study found a substantially larger proportion of patients with sub-clinical cardiac inflammation. Chopra et al noted that 28 of 50 patients showed evidence of leucocyte infiltration in the excised LA appendage tissue.[6] It is plausible that younger patients may have a higher predilection to sub-clinical carditis. Patients in the study by Chopra et al were over a decade younger than those in our study (23 vs. 34 years). Moreover, among patients over the age of 30 years in that study, the proportion with sub-clinical inflammation was 29%, similar to our study. Likewise, average age in the Raizada study was above 30 years (32 years) with similar rate of histological inflammation (27%), which further supports this hypothesis.

Table 2
Myocardial inflammation and peripheral blood cytokine levels.

Variable (N = 86)	n (%)
Histopathology	
Inflammation	27 (31.4)
Blood biomarkers elevation	
hs-CRP	38 (44.2)
IL-2	77 (89.5)
IL-6	78 (90.7)
TNF α	68 (79.1)
IFN γ	70 (81.4)

hs-CRP, High Sensitivity C-Reactive Protein; IL-2, Interleukin 2; IL-6, Interleukin 6; TNF α, Tumour Necrosis Factor Alpha; IFN γ, Interferon Gamma.
Normal values: hsCRP ≤3 mg/L, IL2 <10 pg/mL, IL6 <6.25 pg/mL, TNFα <25 pg/mL, IFNγ <12.5 pg/mL.

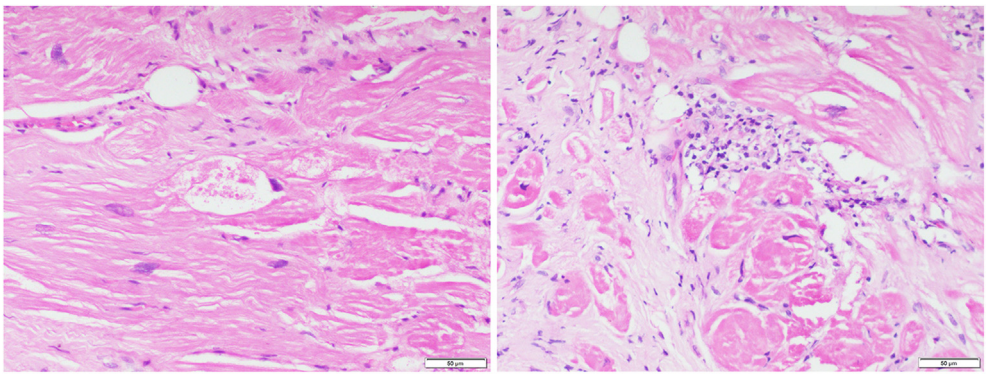

Fig. 2. A: Photomicrograph of left atrial appendage showing degenerative changes and absence of inflammation.B: Photomicrograph of left atrial appendage rich in lymphocytes depicting presence of inflammation.

Table 3
Correlation of inflammatory biomarkers with histological inflammation.

Biochemical markers		Inflammation		*p* value	Odds ratio – Uni variable analysis (95% CI)	Odds ratio – Multi variable (95% CI)
		Absent	Present			
hs-CRP	Normal	33	15	0.974		
	Elevated	26	12		1.01 (0.40–2.53)	0.75 (0.27–2.04)
IL-2	Normal	7	2	0.713		
	Elevated	52	25		1.68 (0.32–8.69)	1.26 (0.21–7.41)
IL-6	Normal	7	1	0.426		
	Elevated	52	26		3.49 (0.40–29.97)	3.44 (0.36–32.27)
TNF α	Normal	15	3	0.161		
	Elevated	44	24		2.72 (0.71–10.37)	2.49 (0.64–9.71)
IFN γ	Normal	13	3	0.371		
	Elevated	46	24		2.26 (0.58–8.71)	2.26 (0.54–9.39)

hs-CRP, High Sensitivity C-Reactive Protein; IL-2, Interleukin 2; IL-6, Interleukin 6; TNF α, Tumour Necrosis Factor Alpha; IFN γ, Interferon Gamma.
Normal values: hsCRP ≤3 mg/L, IL2 <10 pg/mL, IL6 <6.25 pg/mL, TNFα <25 pg/mL, IFNγ <12.5 pg/mL.

Table 4
Clinical correlation of individual valvular lesions with histological inflammation.

Valve lesions		Inflammation		*p* value	Odds ratio - Uni variable analysis (95% CI)
Type	n	Absent	Present		
Isolated Mitral Stenosis	17	14	3		1
Dominant stenotic lesion[a]	44	29	15	0.215	2.41 (0.59–9.73)
Pure regurgitant lesion[b]	11	8	3	0.547	1.75 (0.28–10.81)
Dominant regurgitant lesion[c]	14	8	6	0.133	3.5 (0.68–17.96)

[a] Dominant stenotic lesion: severe mitral stenosis and/or aortic stenosis, with mild-moderate mitral regurgitation and/or aortic regurgitation.
[b] Pure regurgitant lesion: moderate-severe mitral regurgitation and/or aortic regurgitation with no mitral stenosis or aortic stenosis.
[c] Dominant regurgitant lesion: severe mitral regurgitation and/or aortic regurgitation, with mild-moderate mitral stenosis and/or aortic stenosis.

4.2. Inflammatory biomarkers

Though serum markers of inflammation have been found to be elevated in patients with chronic RHD, the significance of this is not known. High-sensitivity CRP is the most commonly studied biomarker, and has been shown to correlate with the severity of valve disease. [16–21] Elevation of plasma homocysteine,[18] Pentraxin-3 (PTX3) levels(19), and raised Neutrophil-to-Lymphocyte Ratio (NLR)[20] have also been reported. However, some studies suggest that inflammatory biomarkers are elevated in ARF but not in RHD.[22] None of the studies so far have attempted to ascertain the prevalence of biomarker elevation in RHD patients.Various intermediate molecules of the inflammatory cascade such as IFN-γ, TNF-α and interleukins are elevated in patients with RHD.[8,9,14] In a Sudanese study, TNF-α was elevated while IFN-γ and IL-10 levels were normal in RHD patients.[23] Likewise, IL-17 and IL-23 levels were found to be significantly elevated in patients with RHD compared to age-matched controls.[24] Recently, Soares et al highlighted the increased expression of IL-6 and TNF-α in patients with more severe RHD.[25] However, since all our patients had severe RHD(requiring surgery), determination of the association between biomarkers and disease severity was not possible in our study. A new inflammatory molecule, Tenascin-C, has been shown to correlate with myocardial inflammation[26] and has been detected in the myocardium of RHD patients along with inflammatory cells.[27] Despite the large number of studies documenting the presence of inflammatory markers and cytokines in the serum of patients with chronic RHD, their relationship to sub-clinical carditis and disease progression has not hitherto been explored. In this study, evidence of biomarker elevation was seen in the majority of patients but was not discriminatory for the presence of histopathological evidence of carditis.

We do not understand the significance of elevated biomarkers in these patients. It could be that such nonspecific elevation is

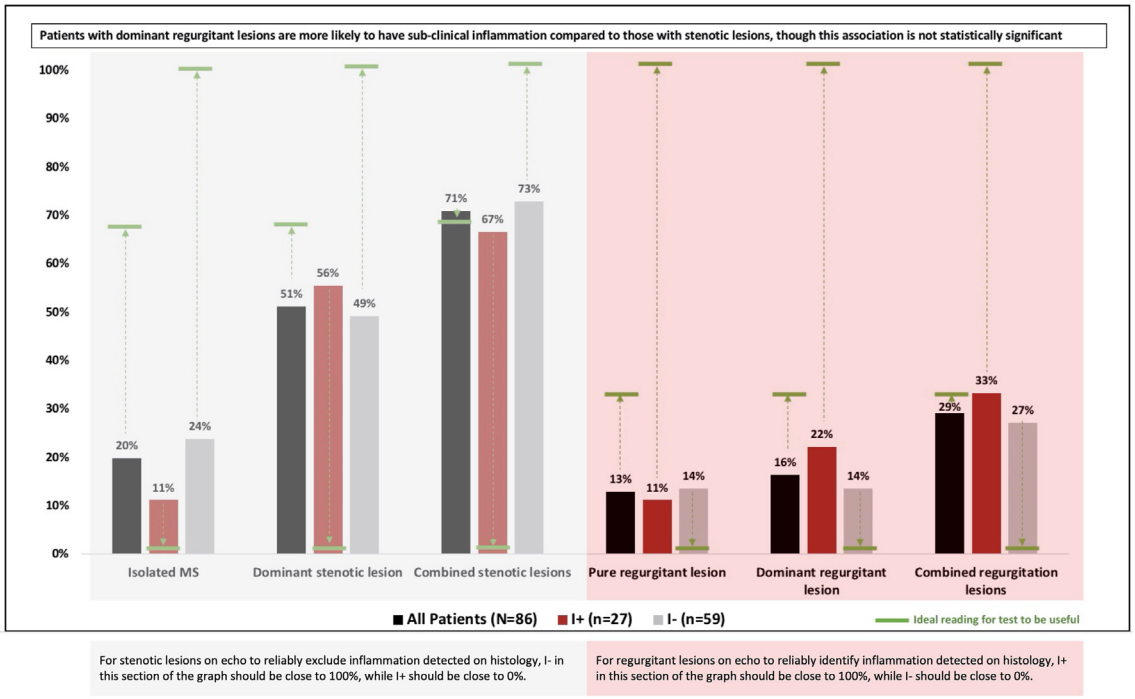

Fig. 3. Correlation of type of lesions on echocardiography with histological inflammation. (The small green horizontal lines represent the proportion of patients required to have the particular lesion's PPV to be 100%, which was 69% for stenotic lesions and 31% for regurgitant lesions. The length of broken green line represents the magnitude by which the lesion was not specific for inflammation. There was a statistically non-significant trend towards association of regurgitant lesions with histologically detected inflammation).

observed in patients with any longstanding disease states, akin to the elevations of hsCRP seen in patients with chronic coronary syndrome. Large prospective studies are needed to assess the importance of biomarker elevations on disease progression and prognosis in RHD.

4.3. Radionuclide imaging

Previous attempts at radionuclide-based inflammation imaging in RHD have all been in patients with ARF. Calegaro et al performed Ga-67 imaging in 30 patients with rheumatic fever carditis, where it was noted to have a 93.5% sensitivity for detecting carditis. There was a 39% greater uptake of Ga-67 in patients with carditis when compared to those without carditis.[7] Narula and colleagues used Indium labeled antimyosin antibodies and demonstrated a sensitivity of 80% for diagnosing acute rheumatic activity.[28] Gallium may be a better tracer than Indium because rheumatic carditis is only rarely associated with myocardial necrosis. We discontinued Ga-67 scanning after 15 patients because of poor image quality and inability to detect activity even among patients who had documented inflammation. Ga-67 scintigraphy may not be sensitive enough to detect the low level of inflammation present in chronic RHD.

4.4. Other imaging modalities

Using late gadolinium enhancement (LGE) as a surrogate for myocardial fibrosis, cardiac MRI (CMR) has been utilized in RHD patients with varying results. Significant myocardial fibrosis was shown in RHD patients with severe MS,[29] compared to another study of RHD patients with predominant MR where fibrosis on CMR was found to be relatively uncommon.[30] Similar studies looking at myocardial edema or inflammation have not been carried out till date. F-18 FDG PET/CT is an upcoming modality for inflammation detection in a myriad of systemic inflammatory disorders. However, the only study which looked at its role in rheumatic heart disease showed no difference in the imaging pattern between chronic RHD patients and controls.[31]

4.5. Study limitations

We used simple hematoxylin and eosin (H&E) staining for detection of inflammation. It is possible that immunohistochemical staining using CD3, CD4 and CD8 antibodies may have helped to better characterize the inflammation detected. Also, we did not include a control group in our study.

5. Conclusion

Histopathologic evidence of inflammation is commonly present in the heart valve and LA appendage specimens retrieved from patients with chronic RHD who do not have clinical rheumatic activity. Serum levels of inflammatory biomarkers are also commonly elevated in these patients but do not correlate with the presence of cardiac inflammation. Better ways to detect sub-clinical inflammation in chronic RHD may pave the way to understanding its role in disease progression.

6. One-line key messages

What is already known?

- Sub-clinical inflammation is often seen in patients with chronic RHD

What this study adds?

- Backspace Conventional inflammatory biomarkers are elevated in the majority of patients with chronic RHD, but do not correlate with the presence of histologic inflammation

Declaration of competing interest

None declared.

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