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REVIEW

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# Galectin-3 and fibrosis intensity in Chronic Chagas Cardiomyopathy: a systematic review

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## ABSTRACT

Chronic Chagas Cardiomyopathy (CCC) is the most prevalent type of myocarditis and the main clinical form of the Chagas disease, which has peculiarities such as focal inflammation, structural derangement, hypertrophy, dilation, and intense reparative fibrosis. Many cellular compounds contribute to CCC development. Galectin-3 is a partaker in inflammation and contributes to myocardial fibrosis formation. Some studies showed the connection between Galectin-3 and fibrosis in Chagas disease but are still inconclusive on the guidance for the early implementation of pharmacological therapy. This systematic review evaluated Galectin-3 as a biomarker for fibrosis intensity in CCC. Two independent reviewers have searched five databases (PubMed, EMBASE, Cochrane Library, Scopus, and Lilacs), using the following search terms: galectin-3, biomarkers, fibrosis, Chagas cardiomyopathy, and Chagas disease. Overall, seven studies met the inclusion criteria and made up this review. There were four trials conducted through animal model experiments and three trials with humans. Experimental data in mice indicate an association between Galectin-3 expression and fibrosis in CCC (75% of studies). Data from human studies showed no direct connection between myocardial fibrosis and Galectin-3 expression (80% of studies). Thus, human findings do not provide significant evidence indicating that Galectin-3 is related to fibrosis formation in Chagas disease. Based on the analyzed studies, it is suggested that Galectin-3 might not be a good fibrosis marker in CCC.

**KEYWORDS:** Galectin-3. Chronic Chagas Cardiomyopathy. Fibrosis.

## INTRODUCTION

Chagas disease is caused by the protozoa *Trypanosoma cruzi,* which leads to inflammatory cardiomyopathy and myocardial fibrosis[1] and affects millions of people worldwide. The infection is endemic in Mexico, Central, and South America and represents a severe public health problem[2]. The clinical course of Chagas disease comprises acute and chronic phases[3]. Chronic Chagas Cardiomyopathy (CCC) is the most relevant clinical manifestation of Chagas disease due to its frequency, severity, morbidity, and mortality[4].

Most Chagas disease patients present diffuse myocarditis with fibrosis and hypertrophy[5]. In CCC, fibrosis occurs due to the extensive and constant infiltration of inflammatory cells into the myocardium, mediated by cytokines and growth factors that regulate cell migration, proliferation, differentiation, production, and degradation of different extracellular matrix components, which are particularities that distinguish CCC from other diseases[6-8].

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Galectin-3 is a β-galactoside-binding lectin[9] found in several physiological and pathological cellular processes, including proliferation, migration, and cardiac fibrosis[10,11]. In the context of CCC, Galectin-3 participates in the migration of cells recruited into the heart and contributes to the fibrogenesis process; thus, Galectin-3 could also be a factor in maintaining inflammation and contributing to cardiac fibrosis formation[8]. Galectin-3 may be a potential therapeutic target and the control of its expression could benefit patients with CCC.

The biomarkers predicting the progression of CCC may guide the early implementation of pharmacological therapy in Chagas disease[12]. Furthermore, it is challenging to identify predicting factors associated with disease progression, morbidity, and mortality, to assist with the decision-making in the follow-up and treatment of this complex disease. The present study examines previous publications to establish whether Galectin-3 is an adequate biomarker for fibrosis intensity evaluation in CCC patients.

## MATERIALS AND METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)[13] was applied in the writing of this systematic review and registered in the PROSPERO database (CRD42019119309). This review aimed to answer the question "What is the real role of Galectin-3 as a biomarker in fibrosis intensity in CCC?"

### Search strategy

The literature search has been done in five electronic databases (PubMed, Embase, Cochrane Library, Scopus, and Lilacs) to recognize clinical trials that examined the role of Galectin-3 as a biomarker in fibrosis intensity in CCC, from database inception to December 2020. Furthermore, we searched all reference lists of qualified studies and related reviews to avoid any irrelevant publications. No search limitations concerning languages, time of publication, and article types were applied.

The search strategy included Medical Subject Headings (MeSH), Descriptors in Health Sciences (DeCS), and Emtree, seeking terms such as "Galectin-3", "fibrosis", "Chagas disease", "Chagas cardiomyopathy", "biomarkers", using the operators of quotes, parentheses, "AND", "OR", "exp" and "mp". The study search strategy is shown in Figure 1.

### Eligibility and exclusion criteria

For eligibility and inclusion of the scientific studies, two

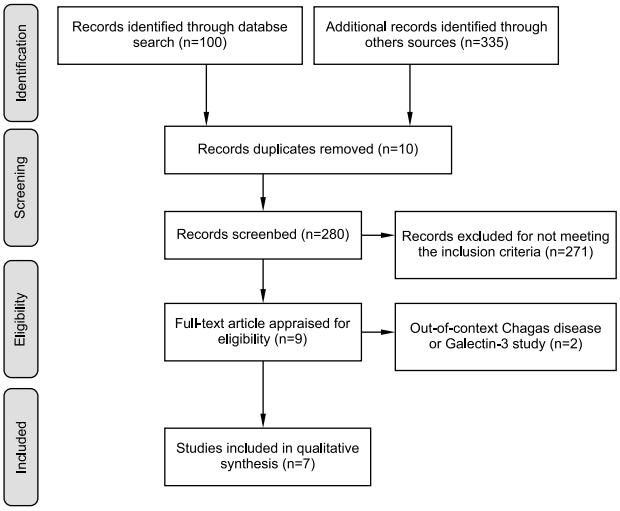


**Figure 1 -** PRISMA Flowchart describing the search strategy and selection of studies on the Galectin-3 assessment as a biomarker in fibrosis intensity in Chronic Chagas Cardiomyopathy.

reviewers read the titles and abstracts. In the second stage, the complete text of each study was evaluated by a third reviewer, and any disagreements were resolved by trying to answer the question that originated the study.

All original published papers regarding the topic of interest were eligible for inclusion, such as (1) cross-sectional, case-control, cohorts, clinical trials, or diagnostics studies; (2) studies with male and female humans or animals; (3) studies that evaluated Galectin-3 as a biomarker in CCC.

The exclusion criteria were: duplicates, narrative or systematic revisions and meta-analysis, case studies, book chapters, editorials, letters to the editor, studies on pregnant or breastfeeding women, children, and adolescents, studies involving themes not suitable for the review, and studies evaluating Gal-3 in relation to fibrosis and cardiomyopathy, but not Chagas disease.

### Data extraction

Two reviewers extracted all trial data independently; the third reviewer arbitrated the disagreements, when necessary. We withdrew data into separate spreadsheets. A specif dataset was created once the reviewers resolved all inconsistencies. For each eligible study, we extracted the author's first name, study design, year of publication, publishing means, impact factor, country, the population of studies, sample size, gender, age, tests used to detect fibrosis and Galectin-3, the statistical analysis used, and the main results of Galectin-3 and fibrosis. Seven articles met all inclusion criteria (Figure 1).

## RESULTS

Primarily, the database search generated 435 studies, of which, 280 published articles appeared to be relevant. Ten manuscripts, in duplicates, were removed. After that, 273 works were excluded for different reasons leaving seven eligible manuscript articles[14-20]. There were four trials conducted through animal model experiments and three trials with humans, as shown in Table 1. The selected articles were published in important academic journals that presented a range of impact factors from 1.35 to 4.73 and were conducted from 2014 to 2017 in four different countries: Brazil (57.1%), Colombia (14.3%), Argentina (14.3%), and Spain (14.3%). The population of the studies included in this systematic review comprised 216 humans, 103 males (47.7%) and 113 females (52.3%), with a mean age of 56 years old (Table 1). The total number of animals used in the experimental studies was 44. Of which, 29 (65.9%) were females and 15 (34.1%) were males, with a mean age of 6–8 weeks and 21 days, respectively.

Furthermore, in Table 2, we present the comparative events extracted from the seven reports that correlated Galectin-3 to the Chronic Chagas Cardiomyopathy in human or animal studies.

Cruz *et al.*[14] evaluated the myocardial fibrosis of patients with and without Galectin-3 polymorphism and compared myocardial fibrosis in individuals with AA, AC, and CC genotypes for the genetic variants at two single nucleotide polymorphic (SNP) sites of the Galectin-3 gene (rs4644 and rs4652). The results demonstrated that there was no statistical difference between the AA, AC and CC genotypes (p = 0.508) or between SNP rs4644 and SNP rs4652 (p = 0.903).

Noya-Rabelo *et al.*[15] studied the percentage of myocardial fibrosis by magnetic resonance imaging and the plasma concentration of Galectin-3 by enzyme-linked immunosorbent assay (ELISA) in different groups of patients (indeterminate cardiac form and cardiac form with or without ventricular dysfunction)[15]. The results showed that the proportion of myocardial fibrosis was 9.4% (IQI: 2.4–18.4), with a progressive increase as the disease worsened. Myocardial fibrosis was detected in 6 of 17 individuals with the indeterminate form (median, 4.1%, IQI: 2.1–10.7), in 7 of 16 individuals with the cardiac form without ventricular dysfunction (median 2.3%, IQI: 1.0–5.0), and in 22 of 28 individuals with the cardiac form with ventricular dysfunction (median 15.2%, IQI: 7.8–25.0, p=0.001). The median Galectin-3 concentration was 12.1 ng/mL (IQI: 9.4–14.4). There were no statistically significant differences in Galectin-3 concentration between the groups of patients, with a median concentration of 12.1 ng/mL (IQI: 8.8–18.3). The median Galectin-3 concentration in the indeterminate group was 12.1 ng/mL (IQI: 10.1–13.9). In the cardiac group with or without ventricular dysfunction, the median Galectin-3 concentration was 12.0 ng/mL (IQI: 11.0–14.8). No correlation was found between myocardial fibrosis and Galectin-3 concentration (r = 0.098; p = 0.47).

Echeverria *et al.*[16] determined the concentration of Galectin-3 by fluorescence enzyme-linked immunosorbent assay (ELFA) and correlated the data with left ventricular ejection fraction (LVEF). The study applied three different models: (1) gross probability ratio; (2) crude probability ratio adjusted for body mass index, age, gender, and estimated glomerular filtration rate; and (3) further adjustment for angiotensin-converting enzyme inhibitor

**Table 1** - General characteristics of the studies included in the systematic review.

| Article, Country | Journal/Impact factor | Population | Total n | n (male/female) | Age |
|---|---|---|---|---|---|
| Cruz *et al.*[14], Brazil | Arquivo Brasileiro de Cardiologia/1.514 | Human | 55 | 23/32 | 58 years |
| Noya-Rabelo *et al*.[15], Brazil | Cardiology/1.350 | Human | 61 | 25/36 | 58 years |
| Echeverría *et al.*[16], Colombia | International Journal of Cardiology/3.229 | Human | 100 | 55/45 | 54 years |
| Ferrer *et al.*[17], Argentina | Parasitology/2.783 | Animal (mice) | 15 | 15/0 | 21 days |
| Souza *et al.*[18], Brazil | The American Journal of Pathology/3.491 | Animal (mice) | 29 | 0/29 | 6–8 weeks |
| Souza *et al.*[19], Brazil | Stem Cells International/3.869 | Animal (mice) | NI | NI | 6–8 weeks |
| Pineda *et al*.[20], Spain | The Journal of Infectious Diseases/4.730 | Animal (mice) | 10 | NI | 6–8 weeks |

NI = not informed.

or angiotensin receptor blocker, beta-blocker, aldosterone antagonist, and diuretic use. In the first model, there was a positive correlation between LVEF and Galectin-3 concentration (β = 0.421; IQI: -0.618 to -0.224; p<0.001). In the second and third models, no correlation was found between LVEF and Galectin-3 concentration (model 2: β = 0.154, IQI: -0.378 to -0.699, p = 0.175; model 3: β = 0.123, IQI: 0.320–0.073, p = 0.217).

Regarding the four studies involving animal models 17–20, three of them used mice aged 6 to 8 weeks, while in one of the studies, the authors included 15 males with a mean age of 21 days.

Ferrer *et al.*[17] measured the expression of Galectin-3 associated with cardiac extracellular matrix (ECM) by analyzing the levels of procollagen I mRNA. Cardiomyopathy was induced by discrete typing unit strains I (DTU Tc I), named Ac and Hc, of *T. cruzi*. In samples from mice infected with Hc and Ac, the

**Table 2** - Characteristics of studies that evaluated Galectin-3 and myocardial fibrosis in Chagas chronic cardiopathy humans and mice.

| Article | Population description | Comparative events | Fibrosis detection/ Galectin-3 detection | Main results of Galectin-3 and fibrosis | Correlation between Galectin-3 and fibrosis? |
|---|---|---|---|---|---|
| Cruz *et al*.[14] | n = 55<br>IF: 16<br>CF: 39 | Myocardial fibrosis in patients with and without Galectin-3 polymorphism, and myocardial fibrosis in subjects with AA, AC, and CC genotypes for gene variants at two Galectin-3 single nucleotide polymorphism (SNP) sites (rs4644 and rs4652) | Magnetic r esonance imaging/RT-PCR | **No significant difference** in myocardial fibrosis between individuals with and without any of the rs4644 and rs4652 SNPs genotypes<br>The mean percentage of **myocardial fibrosis was not statistically different** between AA, AC, and CC genotypes (p = 0.508), or between SNP rs4644 and SNP rs4652 (p = 0.903) | No correlation |
| Noya-Rabelo *et al*.[15] | n = 61<br>IF = 17<br>CF without ventricular dysfunction: 16<br>CF with ventricular dysfunction: 28 | Myocardial fibrosis and Galectin-3 plasma levels | Magnetic r esonance imaging/ELISA | ***Myocardial fibrotic area***<br>All subjects: 9.4% (2.4%–18.4%)<br>IF: 4,1% (2.1%–10.7%)<br>CF without ventricular dysfunction: 2.3% ( 1.0%–5.0%)<br>CF with ventricular dysfunction: 15.2% (7.8%–25%)<br>**p = 0.004**<br>***Plasmatic concentration of Galectin-3***<br>All subjects: 12.1 ng/mL (9.4–14.4 ng/mL)<br>IF: 12.1 ng/mL (8.8–18.3)<br>CF without ventricular dysfunction: 12.0 ng/mL (11.0–14.8 ng/mL)<br>CF with ventricular dysfunction: 12.0 ng/mL (11.0–14.8)<br>**p = 0.900**<br>***Correlation between Galectin-3 and myocardial fibrotic area*** (r = 0.098; p = 0.47) | No correlation |
| Echeverría *et al*.[16] | n = 100 (patients with Chagas cardiomyopathy; CCM) Stage B (ECG abnormalities consistent with CCM and LVEF > 55%): 26 Stage C (ECG abnormalities consistent with CCM and LVEF 40%–55%): 29 Stage D (ECG abnormalities consistent with CCM and LVEF < 40%): n = 45 | Galectin-3 concentration with ejection fraction left ventricular (LVEF) in three models: **Model 1** (crude odds ratio), **model 2** (odds ratio adjusted for body mass index, age, sex, and estimated glomerular filtration rate), and **model 3** (model 1 + further adjustment for angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, beta-blocker, aldosterone antagonist, and diuretic) | LVEF/ELFA | ***Model 1***<br>β = -0.421 (-0.618 to -0.224)<br>**p < 0.001** | Positive correlation |
| | | | LVEF/ELFA | ***Model 2***<br>β = -0.154 (-0.378 to 0.699)<br>p = 0.175 | No correlation |
| | | | LVEF/ELFA | ***Model 3***<br>β = -0.123 (-0.320 to 0.073)<br>p = 0.217 | No correlation |

**Table 2** - Characteristics of studies that evaluated Galectin-3 and myocardial fibrosis in Chagas chronic cardiopathy humans and mice. (cont,)

| Article | Population description | Comparative events | Fibrosis detection/ Galectin-3 detection | Main results of Galectin-3 and fibrosis | Correlation between Galectin-3 and fibrosis? |
|---|---|---|---|---|---|
| Ferrer *et al*.[17] | n = 15<br>Control mice infected by simulation (mock): 5<br>Mice infected with *Trypanosoma. cruzi* Hc strains (Hc): 5<br>Mice infected with *T. cruzi* Ac strains (Ac): 5 | Galectin-3 expression associated with cardiac extracellular matrix (ECM) remodeling of chronic murine cardiomyopathy through analysis of pro-collagen I mRNA levels | RT-PCR and histopathology/ immunohisto-chemistry | ***Analysis of procollagen I mRNA levels in samples from mice infected with Hc and Ac***<br>Expression of procollagen I mRNA was higher in samples from animals inoculated with Ac ($p < 0.05$)<br>***Histology of the heart inoculated with Hc and Ac***<br>**An increase** in collagen matrix was observed in samples from animals infected with Hc and Ac. MEC remodeling was **less intense** in samples from mice inoculated with Hc compared to mice inoculated with Ac.<br>***Expression of the Galectin-3 antigen in the hearts of animals infected with mock, Hc, or Ac***<br>**Significantly increased Galectin-3** levels were observed in samples from mice infected with Ac compared to the simulated controls ($p < 0.001$).<br>***Immunohistochemistry staining of the Galectin-3 antigen in the hearts of animals infected with mock, Hc, or Ac***<br>**Galectin-3 antigen was mainly detected** in the cells located in the interstitium, and also at a higher level in the fibrotic areas. | Positive correlation |
| Souza *et al*.[18] | n = 29<br>Mice not infected by *T. cruzi* ***(Naive):*** 8<br>Mice with infection times of 30 dpi ***(30 dpi):*** 5<br>Mice with infection times of 90 dpi ***(90 dpi):*** 5<br>Mice with infection times of 180 dpi ***(180 dpi):*** 11 | Galectin-3 expression and areas of fibrosis in cardiac sections of mice at different times of infection (30 dpi, 90 dpi, and 180 dpi) by *T. cruzi* Colombian strain | Morphometric Analysis/ Immuno-fluorescence | **Increased myocardial Galectin-3** cell expression compared to naïve controls by confocal microscopy. Cardiac expression of Galectin-3 peaked at 30dpi but remained elevated during the chronic phase of infection compared to naive mice. Regarding the 30 dpi time for the naive mice, the $p< 0.01$, and 30 dpi for 180 dpi, the $p< 0.001$. The percentage of fibrosis increased with time.<br>Compared to the time of 180 dpi to 30 dpi $p- < 0.001$, and the time of 180 dpi to 90 dpi the $p < 0.05$. | Positive correlation |
| Souza *et al*.[19] | **Mouse model of CCC**<br>Mice not infected by *T. cruzi* ***(naive)***<br>Mice infected with *T. cruzi* with saline solution ***(saline)***<br>Mice infected with *T. cruzi* with mesenchymal stromal cells controls ***(MSC-WT)***.<br>Mice infected with *T. cruzi* with mesenchymal stromal cells Galectin-3 knockdown ***(MSC-Gal3KD)*** | Area of fibrosis analyzed in cardiac sections and gene expression of collagen type I (Col1a1) in cardiac tissue of mice infected with saline *T. cruzi,* animals infected with *T. cruzi* control mesenchymal stromal cells (MSC-WT), and animals infected with *T. cruzi* knockdown mesenchymal stromal cells from Galectin-3 (MSC-Gal3KD). | Morphometric Analysis/RT-PCR | Analysis of cardiac sections in mice infected with *T. cruzi* showed **extensive areas of fibrosis.** While the fibrosis content in the heart was not altered between groups, **collagen synthesis**, as measured by gene expression of type I collagen (Col1a1), **was reduced** with wild-type MSC but not with Galectin-3 knockdown MSC. | No correlations |
| Pineda *et al*.[20] | n = 10<br>Control mice: 5<br>Mice deficient in Galectin-3 : 5 | Expression of collagen I, III, and IV and laminin in myocardial samples from C57BL/6 and Galectin-3 -/- mice, 28 and 60 days after T. cruzi infection | Histopathology/ RT-PCR | **Reduced expression** of collagen I, III, IV, and laminin in the hearts of Galectin-3 -/- infected animals compared to the control group | Positive correlation |

IF = Indeterminate form; CF = Cardiac form; ECG = electrocardiogram and echocardiogram; ECM = cardiac extracellular matrix; ELISA = enzyme-linked immunosorbent assay; ELFA = enzyme-linked fluorescence assay; Galectin-3 = galectin-3; LVEF = left ejection fraction left ventricular; dpi = days post-infection; RT-PCR = Real time-polymerase chain reaction.

expression of procollagen I mRNA was higher in the animals inoculated with Ac ($p<0.05$). As for the cardiac histology of infected mice, an increase in the collagen matrix was observed in samples from animals infected with both strains. MEC remodeling was less intense in mice inoculated with Hc than Ac. At the same time, Galectin-3 levels were significantly higher in samples from mice infected with Ac than in the simulated controls ($p\leq0.001$). The Galectin-3 antigen was mainly detected in the interstitial cells and found in myocardial samples.

In 2015, Pineda *et al.*[20] evaluated the expression of collagen I, III, IV, and laminin in myocardial samples of C57BL/6 mice and those with the knockout of Galectin-3, 28, and 60 days after *T. cruzi* infection[20]. The reduced expression of collagen I, III, IV, and laminin in the heart of Galectin-3 knockout animals, as demonstrated, compared to the control group suggests that galectin-3 is strongly involved in Chagas disease, not only in the immune response against *T. cruzi* but also in mediating myocardial damage.

In 2017, Souza *et al.*[18] correlated the Galectin-3 expression area's percentage with the fibrosis area's percentage in cardiac sections of mice at different times of infection with trypomastigotes of the Colombian *T. cruzi* strain (30 dpi, 90 dpi, and 180 dpi). It was observed an increased myocardial Galectin-3 expression in infected animals compared to non-infected controls. When compared to the uninfected mice, the Galectin-3 cardiac expression peaked at one-month post-infection, remaining high during the chronic phase of the infection, but this expression decreased with time. Regarding infection points, Galectin-3 expression in 30 dpi mice was higher than that of 180 dpi mice ($p<0.001$). The percentage of fibrosis increased with infection time and fibrosis was more severe in 180 dpi mice than in 30 dpi ($p<0.001$) and 90 dpi ($p<0.001$) mice.

A second study[19] evaluated the fibrosis area percentage and the gene expression of type I collagen (Col1a1) in the mice's *T. cruzi*-infected heart. *T. cruzi*-infected mice received control mesenchymal stromal cells (MSC-WT) or mesenchymal Galectin-3 knockdown stromal cells (MSC-Gal3KD). The analysis of cardiac sections in *T. cruzi*-infected mice showed extensive areas of fibrosis while the fibrosis content in the heart was not significantly different between the groups. the Collagen type I (Col1a1) gene expression was reduced in infected mice that received wild-type MSC cells but not in those who received MSC knockdown Galectin-3. According to studies using animal models, three studies (75%) showed a positive correlation between Galectin-3 and myocardial fibrosis and one study (25%) presented no correlation between these variables.

## DISCUSSION

CCC is the most relevant clinical form of Chagas disease due to its high morbidity, mortality, and social/medical impact[21-24]. CCC has a characteristically slow and progressive course, although it can progress rapidly. Its clinical manifestations range from subclinical to severe presentations characterized by heart failure, arrhythmias of variable type and severity, conduction blocks of electrical stimulation, thromboembolism, ischemic stroke, and sudden death that may eventually constitute its first manifestation[21,23-26].

CCC is the most common and fibrous type of myocarditis worldwide[27,28]. CCC presents peculiarities such as variable intensities of focal inflammation, structural derangement, hypertrophy, dilation, and intense reactive and reparative fibrosis[8]. A recent study concerning this topic showed a correlation between Gal-3 and cardiac dysfunction, and severe human Chagas cardiomyopathy[29].

Inflammation is a commonly beneficial protective response to tissue injury, promoting healing and repair. Acute inflammation is the natural response of vascularized tissues to injury, irritation, and infection. In contrast, chronic inflammation is a detrimental process due to the inability to resolve acute inflammation or persistent inflammatory stimuli[10]. Chronic inflammation with fibrosis formation and functional impairment of organs is a significant cause of morbidity and mortality of many chronic diseases[30].

Tissue fibrosis is a progressive disorder characterized by the abundant accumulation of extracellular matrix, leading to organ lesions and dysfunction. Several cytokines, chemokines, growth factors, and angiogenic regulate cells' activation to produce extracellular matrix in fibrous processes. In most cases, it is relevant to identify the mechanisms of fibrosis formation by identifying irreversible lesions and, consequently, delineate possible antifibrotic therapeutic strategies[10,31].

Galectin-3 is a multifunctional protein of the lectin family that binds to the cell surface and glycans of the extracellular matrix. It plays a crucial role in cell proliferation, adhesion, differentiation, apoptosis, and angiogenesis[10]. Galectin-3 exerts a pro-inflammatory effect by activating macrophages and mediating extracellular matrix-producing cells[32,33]. Furthermore, Galectin-3 regulates the inflammatory response through cell activation and migration by regulating apoptosis of immune cells. In acute inflammation, Galectin-3 seems to play a pro-inflammatory and protective role. After the transition to a chronic inflammatory state, it plays a different fibrogenic role in repairing the lesions; ultimately, this results in fibrosis and architectural

disruption of organs. A more detailed study of the role of Galectin-3 in cardiac remodeling revealed that it was localized in the fibrosis sites themselves, especially in fibroblasts and macrophages, but not in cardiomyocytes[33].

Most heart disease biomarkers, such as troponin, C-reactive protein, and BNP are released into circulation due to the pathogenic process, representing the result and not the cause of the damage. Extracellular Galectin-3 has a causal role in the remodeling process, the inhibition of which seems to block or reverse the process. These measurements and analysis may enable the development of disease-modifying therapies by inhibiting remodeling and stopping or delaying the progression of heart failure[10].

It is already known that Galectin-3 is an important factor in the progression of heart failure (HF). HF patients with low levels of Gal-3 present a better outcome than patients with high Gal-3 levels[34,35]. Gal-3 can be used to identify patients with HF at low risk for 30-day and 180-day mortality, and HF patients' hospital returns after an episode of acute HF[36].

The results of this systematic review show that 75% of animal model studies demonstrated a positive correlation between Galectin-3 and myocardial fibrosis. These findings provide evidence for a pro-inflammatory role of Galectin-3 in Chagas disease, which is indicative of its ability to promote tissue inflammation, cell infiltration, and cardiac damage.

Although experimental data in mice indicate an association between Gal-3 expression and fibrosis, the few studies in humans are controversial. They do not provide robust evidence for this association in Chagas heart disease. There were biases in these studies that may affect the interpretation of the results. Among them are the small sample size of the studies analyzed and the different methods used to measure the concentration of Gal-3 and the percentage of fibrosis in the myocardium. Moreover, in the clinical studies, Galectin-3 was measured in a single moment; thus, it was challenging to provide information about its importance over time. Furthermore, the circulating concentration of Galectin-3 may not accurately reflect its expression in the myocardium. There is experimental evidence that Gal-3 expression is associated with cardiac fibrosis in CCC. However, studies on humans are scarce, using different methods and presenting controversial results, indicating the need for studies on this subject.

### Study limitations

These results must be interpreted with caution and several limitations should be borne in mind. There are three major limitations in this study that could influence the conclusions. First, the small number of studies relating Galectin and cardiac fibrosis in human Chagas disease (3 articles) in association with the number of individuals evaluated in the 3 reviewed articles (216 individuals divided in control, indeterminate and cardiac groups). Second, the methodology used to evaluate the relation between Galectin and fibrosis was different in all three refereed articles (MRI, gene polymorphism, FEVE, plasmatic expression). Third, the evaluation of Gal-3 levels, when measured, was done only once and there was no follow-up to assess if any alteration in these biomarkers occurred over time. Based on this consideration, it would be necessary a multicentric study with a higher number of individuals evaluated and a pattern methodology to identify the real role of Galectin-3 in fibrosis production and maintenance in cardiac Chagas disease.

## CONCLUSION

Galectin-3 has been pointed out as an indicator of fibrosis in various tissues, but its specific role in detecting myocardial fibrosis is not well established. Through this systematic review, it was not possible to assume that Gal-3 can be useful to characterize fibrosis in CCC patients. Further research is needed to verify the association of Galectin-3 with cardiac connective tissue, remodeling and ascertaining the potential use of this molecule as a biomarker of fibrosis in CCC.

## AUTHORS' CONTRIBUTIONS

Study conception and design: ATC, ALGO, NSG, and ICM; data collection: ICM; analysis and interpretation of results: ATC, ALGO, NSG, and ICM; draft manuscript preparation: ATC, ALGO, NSG, CASM, and MOCR. All authors reviewed the results and approved the final version of the manuscript.

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