



Preliminary study on the immunohistochemical expression of galectin-3 in hypertrophic hearts

Cecilia Salzillo,^{1,2} Lorenzo Giovannico,³ Giuseppe Fischetti,³ Gerardo Cazzato,¹ Mario Magistro,¹ Grazia Nucci,¹ Gabriella Serio,¹ Andrea Marzullo¹

¹Pathology Unit, Department of Precision and Regenerative Medicine and Ionian Area, "Aldo Moro" University of Bari; ²Department of Experimental Medicine, "Luigi Vanvitelli" University of Campania, Naples; ³Cardiac Surgery Unit, Department of Precision and Regenerative Medicine and Ionian Area, "Aldo Moro" University of Bari, Italy

Abstract

Background: Galectin-3 overexpression is associated with fibroblastic proliferation and production of collagen, resulting in increased cardiac fibrosis and remodeling. The aim of the study was to investigate the expression of galectin-3 in hypertrophic hearts. We examined 19 surgical specimens taken from interventricular septum of 8 patients with Tetralogy of Fallot, four patients with aortic valve stenosis, one cardiac explant affected from dilated cardiomyopathy, and six myocardial biopsies of patients submitted to heart transplantation.

Methods: All the samples were routinely processed, stained with hematoxylin-eosin, trichromic stain, and elastic fiber stain, and selected by having the morphological features of myocardial hypertrophy: myocytolysis, nuclear pleomorphism, interstitial fibrosis.

Results: At immunohistochemistry, myocardial fibers showed cytoplasmic expression of galectin-3 in four patients with aortic valve stenosis (diffuse in three and mild in one), in one patient with cardiac explant (mild) and the 4/6 transplanted hearts (mild and focal in three and diffuse in one). The eight patients affected by Tetralogy of Fallot and two patients with transplanted hearts resulted negative.

Conclusions: The results agreed with the hypothesis that galectin-3 may play a role in cardiac hypertrophy; its expression in myocardial fibers is not related to the morphological aspects as suggested by the absence in pediatric cases. The presence of myocardial biopsies taken from transplanted hearts would suggest a possible role in predicting the clinical outcome of such patients.

Key words: galectin-3; cardiac hypertrophy; immunohistochemical expression; heart transplantation; heart disease; cardiorenal syndrome.

Received: 13 November 2023; Accepted: 6 May 2024.

*Correspondence to: Cecilia Salzillo, Pathology Unit, Department of Precision and Regenerative Medicine and Ionian Area, "Aldo Moro" University of Bari, Piazza Giulio Cesare 11, 70121, Bari, Italy.
E-mail: c.salzillo@studenti.uniba.it

Introduction

Galectin-3 (Gal-3) is a member of the galectin family of carbohydrate-binding proteins expressed in the cytoplasm of different cell types (epithelial and endothelial cells), mainly by activated macrophages.^{1,2}

In the human genome, it is encoded by a single gene (LGALS3) located on chromosome 14, locus q21-22, composed of 6 exons and 5 introns covering about 17 kilobases.¹

Gal-3 regulates basic cellular functions, namely growth, proliferation, differentiation, and inflammation; the basic expres-

sion of Gal-3 is varied and unstable in different tissues and it is inducible.

Under physiological conditions in cardiac tissue, the basal expression of Gal-3 is very low but when cardiac damage occurs, it is readily induced. In fact, the overexpression of Gal-3 is associated with fibroblastic proliferation and collagen production, resulting in increased cardiac fibrosis and remodeling, with a probable predictive role in heart failure and heart transplantation.^{2,3}

Furthermore, Gal-3 is also present at the extracellular level and modulates the interaction between epithelial cells and the

extracellular matrix, playing a role in renal collecting tubule embryogenesis.⁴

In several experimental studies, the serum levels of Gal-3 appear to be related to the development of renal fibrosis, in an inversely proportional relationship to the estimated glomerular filtration rate in the adult population,⁵ with a probable predictive role in chronic renal disease and renal transplantation.

Materials and Methods

The aim of our study is to evaluate the immunohistochemical (IHC) expression of Gal-3 in the hypertrophic heart secondary to different morbid states in relation to the adaptive capacity of myocardial fibers and their distribution. Importantly, such an antibody has rarely been used for this application.

The study was performed on 19 myocardial fragments from the hearts of patients undergoing myocardial band resection for Tetralogy of Fallot and aortic stenosis, heart explant, and biopsies taken from follow-up transplanted patients to monitor rejection reactions.

The following parameters of each patient were considered: age, sex, clinical diagnosis, evaluation of histological characteristics (cardiomyocyte hypertrophy and interstitial and subendocardial fibrosis), and immunohistochemical expression of Gal-3.

We used hematoxylin-eosin, trichromic stain, and elastic fiber

stain to select cases that have the features of hypertrophic myocardium, namely myocytolysis, nuclear pleomorphism, and interstitial fibrosis.

Results

In eight patients (Table 1 and Figure 1) with a clinical diagnosis of Tetralogy of Fallot, aged between 9-15 months, five males and three females, the following were highlighted: in two patients only cardiomyocyte hypertrophy; in one patient only interstitial fibrosis; in five patients cardiomyocyte hypertrophy and endocardial or subendocardial fibrosis.

In all cases the immunohistochemical expression of Gal-3 is negative.

In the other five patients (Table 2 and Figure 2), of which four with aortic stenosis and one with cardiac explant from dilated cardiomyopathy (CMD), aged between 48-76 years, one male and four female, we highlight: all five patients with cardiomyocyte hypertrophy, associated with subendocardial fibrosis in three patients, one patient with endocardial fibroelastosis, one patient with endocardial fibroelastosis and interstitial fibrosis. Gal-3 is positive in all cases, two with weak band positivity, two diffuse subendocardial positivity, and one focal subendocardial positivity.

Of the 6 patients (Table 3 and Figure 3) undergoing heart transplantation for various pathological conditions (CMD,

Table 1. Patients with a tetralogy of Fallot.

Patients	Age (months)	Gender	Clinical diagnosis	Histology	IHC Gal-3
1TF-A1	9	M	TF	Cardiomyocyte hypertrophy	Negative
2TF-A1	11	M	TF	Mild interstitial fibrosis	Negative
3TF-A1	14	F	TF	Interstitial fibrosis and cardiomyocyte hypertrophy	Negative
4TF-A1 and B1	13	M	TF	Cardiomyocyte hypertrophy	Negative
5TF-A1	15	M	TF	Hypertrophic-regressive cardiomyocytes and endocardial fibrosis	Negative
6TF-A1	12	F	TF	Hypertrophic-regressive cardiomyocytes and subendocardial fibrosis	Negative
7TF-A1	13	F	TF	Hypertrophic-regressive cardiomyocytes and endocardial fibrosis	Negative
8TF-A1	11	M	TF	Hypertrophic-regressive cardiomyocytes and endocardial fibrosis	Negative

IHC, immunohistochemical; Gal-3, galectin-3; TF, Tetralogy of Fallot.

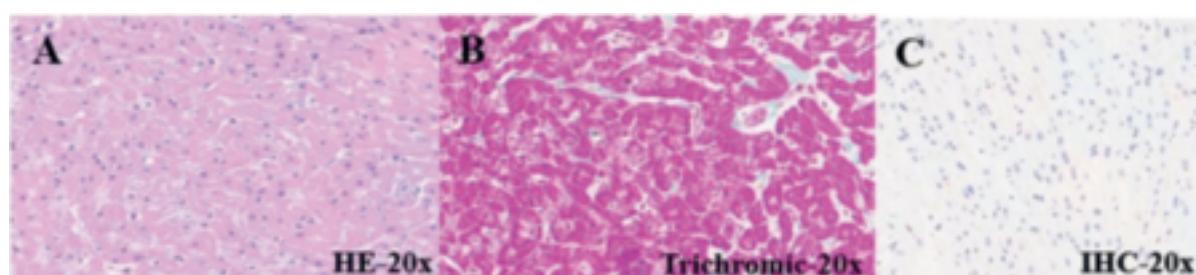


Figure 1. Patient with Tetralogy of Fallot. A) Hematoxilin and Eosin staining which highlights cardiomyocyte hypertrophy; B) minimal interstitial fibrosis is highlighted on trichrome staining; C) on immunohistochemistry, galectin-3 is negative. HE, Hematoxilin and Eosin; IHC, immunohistochemistry.

acute heart failure, and chronic ischemic heart disease) and in follow-up by heart biopsy for rejection, aged between 25-77 years, four males and two females, we went on to measure the peri-graft and post-graft serum Gal-3 values (normal values of serum Gal-3 17 ng/mL) buying them with the histological features and IHC expression of Gal-3.

In this group, two cases show IHC of Gal-3 negative and the serum values are missing; four are positive to Gal-3; in three of them that date few weeks from the transplantation, post-graft serum Gal-3 is reduced as compared to peri-graft and in the remaining patient that received the heart in 2009 the serum Gal-3 is not available.

Discussion

The analysis of our preliminary study allows us to make 3 considerations: i) the hypertrophic-regressive morphological aspect does not necessarily correlate with the IHC expression of Gal-3; ii) the immunohistochemical expression of Gal-3 appears to be closely related to cardiac hypertrophy and remodeling; iii) in the studied cases, immunohistochemical expression and serum values of Gal-3 seem to be associated with cardiomyocyte hypertrophy more than with endocardial fibrosis.

In fact, in patients with Tetralogy of Fallot, the obstruction of the ventricular-pulmonary outflow is probably the expression of a malalignment of the infundibular portion of the septum, in which the hypertrophic component has not yet had the opportunity to manifest itself; in addition, the time interval between the onset of the disease and the resection is limited due to age of patients ranging from 9 months to 15 months. On the contrary, in patients with aortic stenosis and CMD, the phenomenon of adaptation-hypertrophy of the fibers correlates with Gal-3; positivity is commonly found in cardiac muscle subendocardial fibers with a progressively decreasing gradient as one moves away from the endocardial surface; moreover, it is found constantly below those areas affected by marked endocardial fibrosis, sometimes with the morphology of a real fibrous cushion and with fibroelastosis modifications of the endocardium.

In patients in follow-up for cardiac transplant rejection, it is observed that the immunohistochemical expression and the serum values of Gal-3 are correlated with the hypertrophic features of the cardiomyocytes.

Studies reported in the literature are conflicting on whether serum Gal-3 values are correlated or uncorrelated with myocardial fibrosis; in fact, in our preliminary study, they seem to be more correlated with cardiomyocyte hypertrophy.

In most studies, clinical data correlate serum Gal-3 levels with

Table 2. Patients with functional and anatomical stenosis.

Patients	Age (years)	Gender	Clinical diagnosis	Histology	IHC Gal-3
1S-A1	76	F	Aortic stenosis	Hypertrophic-regressive cardiomyocytes and endocardial fibroelastosis	Mild band positivity
2S-A1	48	F	Aortic stenosis	Hypertrophic-regressive cardiomyocytes and subendocardial fibrosis	Diffuse subendocardial positivity
3S-A1	48	F	Aortic stenosis	Hypertrophic-regressive cardiomyocytes and subendocardial fibrosis	Diffuse subendocardial positivity
4S-A2	55	M	Cardiac explant(CMD)	Hypertrophic-regressive cardiomyocytes and subendocardial fibrosis	Focal subendocardial positivity
5S-A1	75	F	Subaortic rim	Hypertrophic-regressive cardiomyocytes, endocardial fibroelastosis and interstitial fibrosis	Mild band positivity

IHC, immunohistochemical; Gal-3, galectin-3; S, stenosis; CMD, dilated cardiomyopathy.

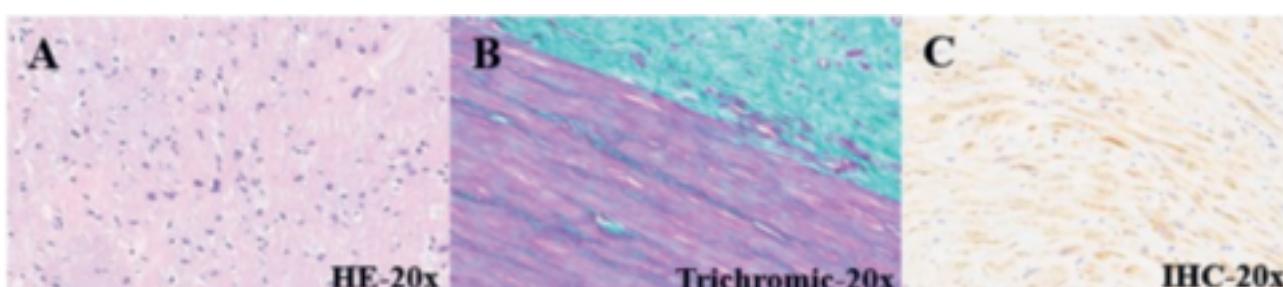


Figure 2. Patient with aortic stenosis. A) Hematoxilyn and Eosin staining highlights hypertrophic-regressive phenomena; B) trichrome staining reveals subendocardial fibrosis; C) on immunohistochemistry, galectin-3 has diffuse subendocardial positivity. HE, Hematoxilyn and Eosin; IHC, immunohistochemistry.

Table 3. Myocardial biopsies of patients submitted to heart transplantation.

Patients	Age (years)	Gender	Clinical diagnosis	Histology	IHC Gal-3	Peri-graft serum Gal-3 ng/mL	Post-graft serum Gal-3 ng/mL
1HT-A1	71	M	HT (deceased)	Hypertrophic-regressive cardiomyocytes and subendocardial fibrosis	Negative	NN	NN
2HT-A1	51	F	HT (2021)	Hypertrophic-regressive cardiomyocytes and subendocardial fibrosis	Mild and focal positivity	29	22
3HT-A1	77	M	HT (2009)	Hypertrophic-regressive cardiomyocytes and endocardial fibrosis	Mild and focal positivity	NN	33
4HT-A1	51	M	HT (2022)	Hypertrophic-regressive cardiomyocytes and discrete subendocardial fibrosis	Diffuse subendocardial positivity	45.6	26
5HT-A1	59	M	HT (2021)	Hypertrophic-regressive cardiomyocytes, discrete subendocardial and interstitial fibrosis	Mild and focal positivity	65	17
6HT-A1	25	F	HT (2019)	Modest interstitial lymphocytic infiltrate and marked interstitial and subendocardial fibrosis	Negative	NN	NN

IHC, immunohistochemical; Gal-3, galectin-3; HT, heart transplantation.

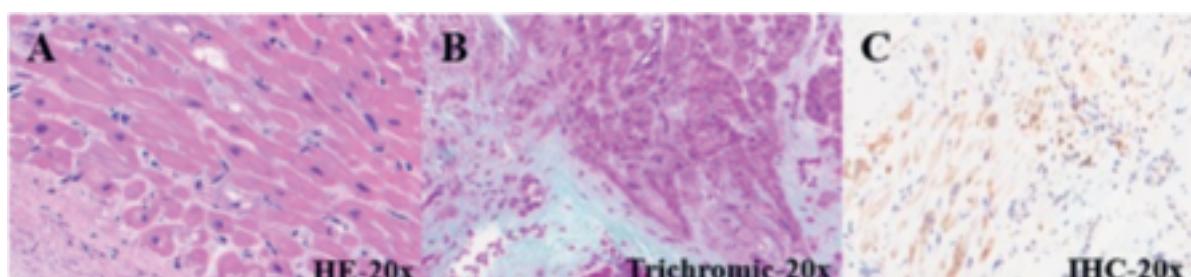


Figure 3. Myocardial biopsy of patients submitted to heart transplantation. A) Hematoxilin and Eosin staining highlights hypertrophic-regressive aspects; B) trichrome staining shows fibrosis; C) on immunohistochemistry, galectin-3 has diffuse positivity. HE, Hematoxilin and Eosin; IHC, immunohistochemistry.

myocardial fibrosis,⁶ as it is responsible for the regulation of pro-fibrotic pathways.^{7,8}

In other studies, no significant association was found between Gal-3 and myocardial fibrosis, suggesting that Gal-3 does not play a crucial role in the pathogenesis of fibrotic cardiomyopathy associated with pressure overload.^{2,9}

Furthermore, in other studies, a specific association of Gal-3 with cardiac fibrosis has not been observed and Gal-3 appears instead to be elevated due to impaired renal clearance or renal dysfunction,¹⁰ due to the involvement of the cardiac-renal axis. Other authors believe that chronic kidney disease is a contributing cause of elevated concentrations of Gal-3 since in chronic renal failure there is renal fibrosis that can be associated with cardiac fibrosis.¹¹

All these hypotheses suggest that Gal-3 could be a potential biological marker for patients with cardiorenal syndrome and further studies are needed.¹²

Conclusions

The immunohistochemical expression of Gal-3 is associated with the cases studied with cardiomyocyte hypertrophy more than with endocardial fibrosis. Moreover, this was a preliminary and descriptive study, and Gal-3, to be considered as a novel biomarker for myocardial function, needs to be correlated with several clinical parameters.

There is probably a correlation with the Gal-3 values detected

in the serum which, however, requires studies on larger case series and for longer time intervals.

Contributions

AM, CS, conceptualization, methodology, writing/original draft preparation; GC, LG, GF, MM, investigation; GC, LG, GF, GN, data curation; AM, GS, writing/review and editing, supervision. All authors read and approved the published version to be published.

Conflict of interest

The authors declare no potential conflict of interest.

Funding

None.

References

1. Bi J, Garg V, Yates AR. Galectin-3 and sST2 as prognosticators for heart failure requiring extracorporeal life support: Jack n' Jill. *Bioolecules* 2021;11:166.
2. Frunza O, Russo I, Saxena A, Shinde AV, Humeres C, Hanif W, et al. Myocardial galectin-3 expression is associated with remodeling of the pressure-overloaded heart and may delay the hypertrophic response without affecting survival, dysfunction, and cardiac fibrosis. *Am J Pathol* 2016;186:1114-27.
3. Suthahar N, Meijers WC, Silljé HHW, Ho JE, Liu FT, de Boer RA. Galectin-3 activation and inhibition in heart failure and cardio-vascular disease: an update. *Theranostics* 2018;8:593-609.
4. Ochieng J, Furtak V, Lukyanov P. Extracellular functions of galectin-3. *Glycoconj J* 2002;19:527-35.
5. Tang WH, Shrestha K, Shao Z, Borowski AG, Troughton RW, Thomas JD, Klein AL. Usefulness of plasma galectin-3 levels in systolic heart failure to predict renal insufficient and survival. *Am J Cardiol* 2011;108:385-90.
6. Lin YH, Lin LY, Wu YW, Chien KL, Lee CM, Hsu RB, et al. The relationship between serum galectin-3 and serum markers of cardiac extracellular matrix turnover in heart failure patients. *Clin Chim Acta* 2009;409:96-9.
7. Grupper A, Nativi-Nicolau J, Maleszewski JJ, Geske JR, Kremers WW, Edwards BS, et al. Circulating galectin-3 levels are persistently elevated after heart transplantation and are associated with renal dysfunction. *JACC Heart Fail* 2016;4:847-56.
8. Bayes-Genis A, de Antonio M, Vila J, Peñafiel J, Galán A, Barallat J, et al. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. *J Am Coll Cardiol* 2014;63:158-166.
9. Abou Ezzeddine OF, Haines P, Stevens S, Nativi-Nicolau J, Felker GM, Borlaug BA, et al. Galectin-3 in heart failure with preserved ejection fraction. A RELAX trial substudy (Phosphodiesterase-5 inhibition to improve clinical status and exercise capacity in diastolic heart failure). *JACC Heart Fail* 2015;3:245-52.
10. Maisel A, Kim P, Steward W. Galectin-3 after heart transplantation: does it get better? *JACC Heart Fail* 2016;4:857-9.
11. O'Seaghda CM, Hwang SJ, Ho JE, Vasan RS, Levy D, Fox CS. Elevated galectin-3 precedes the development of CKD. *J Am Soc Nephrol* 2013;24:1470-7.
12. Iacoviello M, Aspromonte N, Leone M, Paradies V, Antoncechchi V, Valle R, et al. Galectin-3 serum levels are independently associated with microalbuminuria in chronic heart failure outpatients. *Res Cardiovasc Med* 2015;5:e28952.