Analytical and clinical evaluation of the chemiluminescent microparticle immunoassay for galectin-3 determination

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ABSTRACT

This study tested if the chemiluminescent microparticle immunoassay (CMIA) method on the Architect platform meets the analytical and clinical quality goals required for the galectin-3 (GAL-3) use in clinical practice. We evaluated results obtained from 121 apparently healthy adults and 382 patients with heart failure (HF). All healthy subjects and patients showed GAL-3 concentrations in plasma above the limit of detection (1.9 μg/L) and the limit of quantitation (2.4 μg/L). GAL-3 in healthy subjects ranged between 6.4 and 40.6 μg/L (median, 13.0 μg/L, interquartile range, 11.2-15.2 μg/L, 97.5th percentile, 33.7 μg/L). GAL-3 values were found significantly increased in patients with chronic HF (median, 15.1 μg/L, interquartile range, 11.7-19.4 μg/L) compared to healthy subjects (P <0.0001). HF patients with cardiac fibrosis, confirmed by magnetic resonance, showed significantly higher GAL-3 values (median, 15.3 μg/L, interquartile range, 11.2-21.9 μg/L) than those without cardiac fibrosis (median, 12.9 μg/L, interquartile range, 11.2-15.0 μg/L) (P=0.03). ROC analysis showed that GAL-3 discriminates the presence of cardiac fibrosis with an area under the curve of 0.635 (0.526-0.744), with a specificity of 76.7% and a sensitivity of 54.1% at the cut-off of 14.6 μg/L. Using multivariable models cardiac fibrosis was significantly associated with the logGAL-3.

INTRODUCTION

Galectins include a family of animal lectins, which have the ability to recognize β-galactoside-containing glycoconjugates through a conserved carbohydrate-recognition domain (CRD) (1, 2). They are involved in several physiological and pathological events, such as cell proliferation and differentiation, apoptosis, immune response, cell differentiation and tumor progression (1-4). In particular, galectin-3 (GAL-3) is a protein with a MW of ~30 kDa, implicated in several biological processes including cell adhesion, cell activation and chemoattraction, cell growth and differentiation, cell cycle and apoptosis (1-4). Recent studies suggested that GAL-3 is involved in pathophysiological mechanisms related to cardiac remodeling and fibrosis (3-6). Diffuse cardiac interstitial fibrosis impairs ventricular function and contributes to both systolic and diastolic dysfunction (7-10). According to international guidelines (9, 10), patients with heart failure (HF) are classified by ejection fraction (EF) into two groups: HF with preserved EF (HFpEF) and HF with reduced EF (HFrEF).

The 2013 American College of Cardiology Foundation/American Heart Association (ACCF/AHA) guideline for the management of HF recommends the use of biomarkers of myocardial fibrosis for additive risk stratification, although with a lower degree of evidence compared to natriuretic peptides and cardiac troponin I and T, in both ambulatory (class IIb, level B) and acute (class IIb, level A) HF patients (10). Compared to other suggested biomarkers for cardiac interstitial fibrosis, such as soluble ST2 protein and matrix metalloproteinase enzymes, GAL-3 has the advantage to be measured with fully automated platforms (11-19). On the other hand, from the analytical point of view, compared to others recommended cardiac biomarkers, such as natriuretic peptides and cardiac troponins, GAL-3 has the disadvantage of a narrower clinical range with a large superimposition between biomarker values measured in healthy subjects and HF patients (11-16). Previous studies reported that GAL-3 showed values ranging from 5 to ~70 μg/L in HF patients, while healthy subjects usually show GAL-3 values ranging from 5 to ~45 μg/L (11-16). As a result, it is conceivable that the analytical performance of GAL-3 methods should play an important role in discriminating between GAL-3 values found in

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healthy subjects and HF patients. Accordingly, a good reproducibility (i.e., CV ≤10%) at the cut-off level should be required for a GAL-3 method that should be used in clinical practice. Two studies recently evaluated the analytical performance of the chemiluminescent microparticle immunoassay (CMIA) for GAL-3 on the Architect platform (Abbott Diagnostics) (11, 12). Although these studies claimed to follow the same standardized protocols (20, 21) in order to evaluate the analytical sensitivity and the limits of detection (LOD), of blank (LOB) and of quantitation (LOQ), results reported in these articles were quite different. The performance values suggested by the manufacturer are also different (22).

The main aim of this study was to demonstrate, using a standardized and robust protocol, that CMIA method has the analytical performance required to discriminate between healthy subjects and HF patients with or without cardiac fibrosis. The second study aim was to evaluate the clinical relevance of CMIA method for GAL-3 assay in HF patients and the association of increased concentrations of this biomarker. In particular, in a subgroup of HF patients, we evaluated the discriminatory power of GAL-3 assay for presence of cardiac fibrosis assessed by magnetic resonance imaging (MRI) using the late gadolinium enhancement technique (23).

MATERIALS AND METHODS

Assays

GAL-3 was assayed with the STAT GAL-3 method in EDTA samples using the Architect *i*1000SR platform, according to the manufacturer's instructions. This method is a two-step sandwich CMIA, which uses two monoclonal antibodies (B7/B5 and M3/38) specific for the protein. In step 1, 25 μL of sample and M3/M38 anti-GAL-3-coated paramagnetic microparticles are incubated. GAL-3 molecules present in the sample bind the M3/M38 antibody. After a wash step, the second antibody B7/B5, labeled with an acridinium-conjugate, is added to the reaction vessel in order to create an antibody-antigen sandwich. After a further washing cycle, a pre-trigger and trigger solutions are added to the reaction vessel in order to start the chemiluminescent reaction. The light emission is then measured as relative light units (RLU) by the photomultiplier of the Architect platform. There is a linear relationship between the logarithmic transformed values of plasma GAL-3 concentrations and the relative RLU values measured by the photomultiplier (Figure 1). The assay is completed within 18 min. The working range suggested by the manufacturer is from 4 to 114 μg/L (22).

Plasma amino-terminal propeptide of B-type natriuretic peptide (NT-proBNP) was measured with the monoclonal version of the electrochemiluminescence assay (ECLIA) (Roche Diagnostics), as previously described (24), using the Cobas e411 platform. The values of serum creatinine, measured with an enzymatic standardized assay (CR-E Creatinine for Synchron system, Beckman Coulter), were used for calculation of the estimated glomerular filtration rate ($eGFR_{creat}$) as recommend by Kidney Disease: Improving Global Outcomes (KDIGO) international guidelines using the 2009 Chronic Kidney Disease Epidemiology Collaboration (CDK-EPI) creatinine equation (25).

Blood samples

For GAL-3 and NT-proBNP assays, blood sampling was performed at 8am after an overnight fasting and a 20-min rest in a supine position. After withdrawal, blood samples (8-10 mL) were placed in disposable polypropylene tubes containing EDTA (1 g/L of plasma). Plasma samples were obtained shortly after venipuncture by centrifugation for 15 min at 4 °C. The collected plasma was divided in several aliquots of 0-5 mL and stored at -20 °C and -80 °C. The aliquots stored at -20 °C were assayed within two months, while the aliquots stored at -80 °C were used when assays were performed after several months of storage.

Subjects

We enrolled 121 apparently healthy subjects [63 females and 58 males, mean age 42.4 (±11.3) years] from January 2014 to September 2015 as reference population study group. No statistically significant difference in age between genders was detected. These subjects were screened by means of an accurate clinical examination, laboratory tests (creatinine, glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, bilirubin, aminotransferases, γ-glutamyltransferase, alkaline phosphatase, C-reactive protein, ferritin, iron, transferrin, homocysteine, thyroid-stimulating hormone, NT-proBNP, complete blood count) and an electrocardiogram (ECG) in order to exclude acute or chronic cardiac or extra-cardiac diseases (26). In subjects with age ≥60 years, a complete

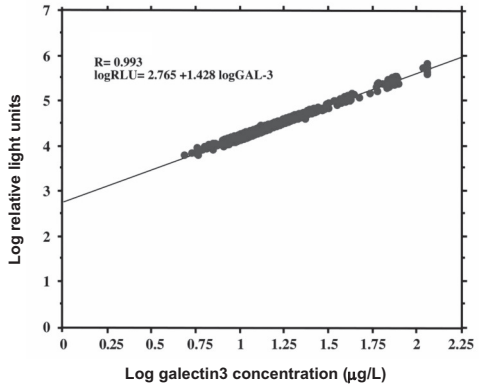

Figure 1
Linear regression between the log-transformed galectin-3 (GAL-3) values and the relative light unit (RLU) values obtained with the Architect system.
The linear regression was obtained using 618 couples of GAL-3 and RLU values, including samples from healthy subjects, patients and calibrators. These measurements were performed in 4 different runs using 3 different lots of reagents and calibrators throughout 12 months.

Table 1
Analytical performance of the Abbott galectin-3 assay

LOB (μg/L)	LOD (μg/L)	LOQ (μg/L)	Reference
0.8	1.0	4.0	Manufacturer
0.1-0.3	1.4-2.1	3.0-3.3	11
0.2-1.2	0.5-1.7	0.4-2.1	12
1.2-1.6	1.7-1.9	2.0-2.8	Present study

LOB, limit of blank; LOD, limit of detection; LOQ, limit of quantitation.

echocardiography examination was also performed. This reference group was recruited from the clinic and laboratory staff personnel and from subjects participant to screening and preventive medicine programs organized by our institution (26). All subjects belonged to the Caucasian ethnic group.

We also enrolled 382 adult patients with chronic HF [mean age 61.4 (±13.7) years; 21% women] referred from January 2012 to May 2015 to the Division of Cardiovascular Medicine of the Fondazione CNR-Regione Toscana G. Monasterio for HF management. The diagnosis of HF was made according the European Society of Cardiology (ESC) and ACCF/AHA guidelines (9, 10). Acute coronary syndrome within 6 months before the enrollment, acute HF at time of enrollment and chronic kidney failure requiring dialysis were the only exclusion criteria. Left ventricular EF was assessed in all HF patients by means of the Simpson biplane method at transthoracic two-dimensional echocardiography. Further to left ventricular systolic function, a number of other echocardiographic variables were also assessed, including left ventricular end-diastolic (reference value: mean 61 mm, SD 8 mm) and end-systolic diameter (50±9 mm), septal (10.5±1.9 mm) and posterior wall (9.6±1.5 mm) thickness, estimated pulmonary artery systolic pressure (40±11 mmHg) and longitudinal right ventricular systolic function (tricuspid annular plane systolic excursion, TAPSE, 23±11 mm).

Among HF patients who underwent cardiac MRI for clinical decision (n=104), the presence of cardiac fibrosis was assessed by the late gadolinium enhancement technique (23). A subset of these patients (n=47) participated to a previous study by our group (23). For the MRI study, all patients were examined with 1.5-T unit (CVi, GE-Healthcare) using the same protocol.

Statistical analysis

Standard statistical analyses were performed by means of the Stat-View 5.0.1 and JPM 12 programs (1992-98, SAS Institute Inc.). Parameter tests and linear/logistic regressions were performed using log-transformed data, because GAL-3 and NT-proBNP values were not normally distributed. In linear or logistic regression analyses the dichotomous variables were expressed as follows: gender: females 2, males 1; diabetes mellitus, hypertension, co-morbidity or polytherapy (chronic use of more than one drug): presence 1, absence 0. The parameters concerning the analytical sensitivity and reproducibility were evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP17-A (20) and EP5-A2 protocols (21). The ROC analysis was performed using the bootstrap method with 1000 iterations (27). P-values <0.05 were considered as statistically significant.

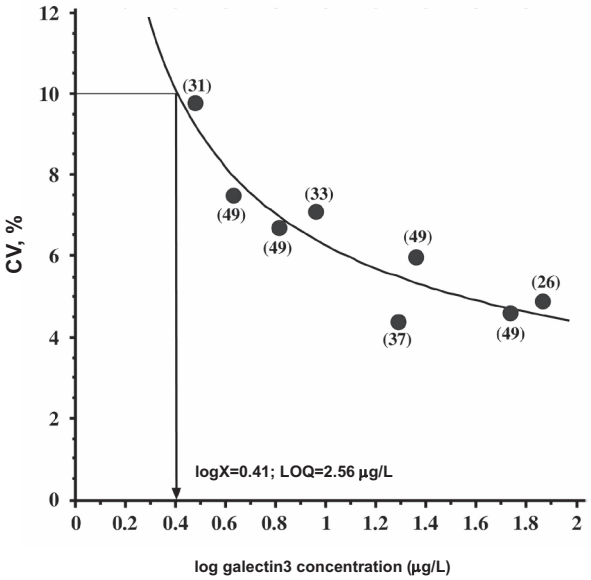

Figure 2
Imprecision profile of the chemiluminescent microparticle immunoassay for galectin-3 on Architect platform. The number of measurements of each sample, calibrator or control are indicated within brackets.
LOQ, limit of quantitation.

Results

Analytical aspects

LOB and LOD values are reported in Table 1. LOQ value at the 10% CV (i.e., 2.56 μg/L) was derived using the imprecision profile reported in Figure 2. We measured 3 plasma pools with mean GAL-3 concentrations of 6.6, 23.5 and 55.7 μg/L, respectively, and another plasma pool from healthy subjects diluted with saline in order to achieve a mean GAL-3 concentration of 4.3 μg/L. We measured also the 3 controls of the GAL-3 method with concentrations of 9.1, 19.3 and 72.8 μg/L, respectively. Finally, in order to achieve a GAL-3 concentration of ~3 μg/L, the calibrator with a 5.7 μg/L concentration (calibrator B) was dilute with the zero calibrator (calibrator A). All these samples were repeatedly measured

throughout 6 months using 3 different lots of calibrators (27225M500, 38142M500 and 41386M500).

The LOB, LOD and LOQ values in Table 1 are compared with the respective values observed in two previous studies (11, 12) as well as those suggested by the manufacturer for the GAL-3 assay on the Architect platform.

The reproducibility of the GAL-3 method was evaluated by measuring 3 plasma pools using two different lots of calibrators. The data are reported in Table 2.

Clinical aspects

The presence of GAL-3 outlier results in the reference population was assessed as previously reported (26). Only one value (59.9 μg/L) from a woman of 39 years was found to be outlier and was excluded from the study. Table 3 reports the values of GAL-3 concerning the reference population. The distribution of GAL-3 concentration value was not normally distributed, but it approximates a log-normal distribution. Females showed slightly, but significantly higher values than males (P=0.039). A weak positive correlation was found between GAL-3 values and age (Rho=0.222; P=0.015, Spearman's rank correlation test). Using a multiple regression analysis model, logGAL-3 values were significantly and independently associated to age (P=0.045) and gender (P=0.025).

Tables 4 and 5 report the characteristics of enrolled HF patients. When considering the 382 HF patients as

Table 2
Reproducibility of chemiluminescent microparticle immunoassay for galectin-3

Sample	n	Mean (μg/L)	Within-run CV	Total CV
A	20	6.57	3.88%	5.94%
B	20	23.1	3.02%	5.15%
C	20	55.1	1.70%	4.13%

Table 3
Reference intervals for plasma galectin-3

Subjects	n	Range, μg/L	Median, μg/L	25th P, μg/L	75th P, μg/L	97.5th P, μg/L
All	121	6.4-40.6	13.0	11.2	15.2	33.7
Females	63	8.4-40.6	13.5	11.6	16.2	36.7
Males	58	6.4-36.8	12.6	11.1	14.6	28.8

P, percentile.

Table 4
Characteristics of the entire group of patients with heart failure (n=382)

Variable	
Age, years	62±14[a]
Male, n (%)	229 (83)
Body mass index, kg/m^2	27.1±14[a]
Ischemic/non-ischemic, %	34/66
NYHA class I-II/III-IV, n	296/86
Left ventricular ejection fraction, %	33.9±10.0[a]
Atrial fibrillation, n (%)	84 (22)
Diabetes, n (%)	75 (20)
Hypertension, n (%)	185 (48)
Serum creatinine, mg/dL	1.08±0.35[a]
CDK-EPI eGFR$_{creat}$, mL/min/1.73 m^2	74.9±23.3[a]
NT-proBNP, ng/L	793 (264.5-1736.0)[b]
Galectin-3, μg/L	15.1 (11.7-19.4)[b]
Furosemide, %	74
β-blockers, %	90
ACE inhibitors/ARBs, %	92
Spironolactone, %	61

NYHA, New York Heart Association; ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers.
[a] *Mean±SD.*
[b] *Median (25th-75th percentile).*

Table 5
Characteristics of the enrolled patients affected by non-ischemic cardiomyopathy who underwent the magnetic resonance study

Variable[a]	All patients (n=104)	No LGE (n=30, 29%)	LGE (n=74, 71%)	P
Age, years	57.8±14.1	53.0±16.1	59.8±12.8	0.033
LVEF, %	35.9±13.4	38.9±12.1	34.7±13.8	NS
Body mass index, kg/m^2	27.0±4.5	27.2±4.3	27.0±4.6	NS
eGRF, mL/min/1.73 m^2	81.8±20.4	86.9±25.3	79.9±18.0	NS
Galectin-3, μg/L	14.3 (11.2-20.5)	12.9 (11.2-15.0)	15.3 (11.2-21.9)	0.031
NT-proBNP, ng/L	500.0 (131.5-1702.3)	437.5 (93.25-1577.5)	582.5 (168.3-1857.5)	NS

LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; NS, not significant.
[a]*Values are expressed as mean±SD for continuous normally distributed variables and as median (25th-75th percentile) for continuous non-normally distributed variables.*

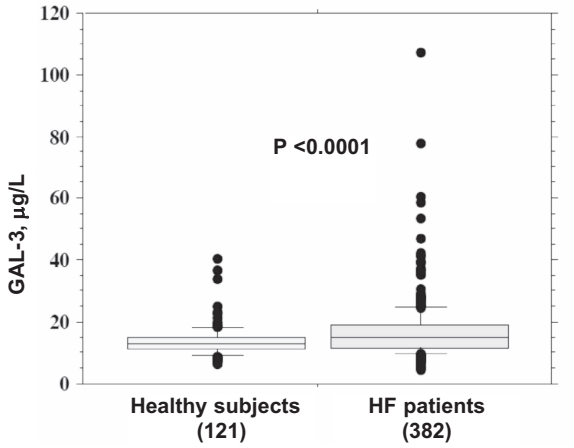

Figure 3
Comparison of galectin-3 (GAL-3) values measured in control subjects and patients wih heart failure (HF).
Data are reported as boxes indicating 10th, 25th, 50th (median), 75th and 90th percentiles; values >90th percentile or <10th percentile are indicated as separated black circles.

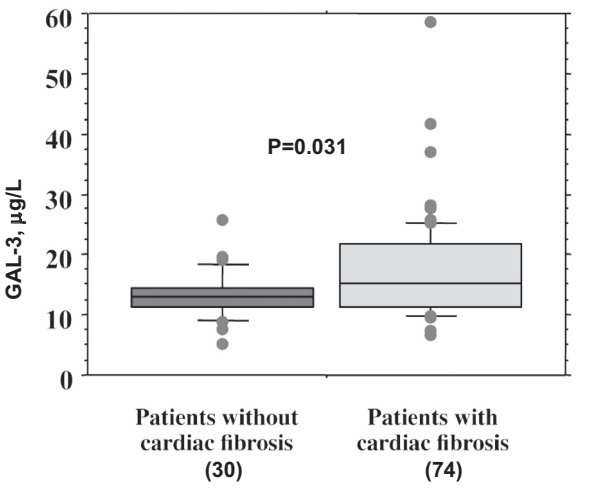

Figure 4
Box plots of galectin-3 (GAL-3) values measured in patients with heart failure evaluated by magnetic resonance imaging for cardiac fibrosis.
Data are reported as boxes indicating 10th, 25th, 50th (median), 75th and 90th percentiles; values >90th percentile or <10th percentile are indicated as separated black circles.

whole group, GAL-3 values (median, 15.1 μg/L; interquartile range, 11.7-19.4 μg/L) were found significantly increased compared to group of healthy subjects (P <0.0001) (Figure 3). GAL-3 values were positively correlated with age (Rho=0.332, P <0.0001), NT-proBNP (Rho= 0.375, P <0.0001) and functional New York Heart Association (NYHA) class (Rho=0.261, P <0.001), and negatively associated with eGFR (Rho=-0.510, P <0.0001), but not with gender, body mass index (BMI), serum creatinine, left ventricular EF, presence of comorbidity (including hypertension, dyslipidemia and diabetes mellitus) and polytherapy [one or more drugs, including diuretics, β-blockers, angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers and spironolactone]. Table 6 summarized the stepwise multiple regression analysis model between logGAL-3 values and logNT-proBNP values (P <0.0001), eGFR values (P <0.0001, negatively correlated) and severity of HF disease, as expressed by functional NYHA class score (P <0.0001). Using a multiple logistic model, we was evaluated the relation between the clinical severity of HF disease (patients in I or II functional NYHA class compared to those in III or IV class) with log-tranformated GAL-3, eGFR, BMI, left ventricular EF, NT-proBNP, age, gender, comorbidity and polytherapy (Table 7).

The clinical characteristics of 104 patients, in whom the presence of cardiac fibrosis was assessed by MRI, are reported in Table 5. Among these patients, 88 were in NYHA class I or II (n=45 and n=43, respectively), while 16 were in NYHA class III or IV (n=15 and n=1, respectively). Moreover, 29% of patients had preserved systolic function (defined as left ventricular EF ≥45%). HFpEF was more commonly found in patients without (13/30) than in those with (17/74) myocardial fibrosis, but this difference was not significant. HF patients with cardiac fibrosis, confirmed by MRI, showed significantly higher GAL-3 values than those without cardiac fibrosis (Table 5 and Figure 4). Patients with cardiac fibrosis were on

Table 6
Stepwise multiple analysis model between logGalectin-3 values (dependent variable) and logNT-proBNP values, eGFR values and severity of heart failure disease, as expressed by functional New York Heart Association (NYHA) class score (predictive variables)

	Coefficient	SE	Standard coefficient	F-to-remove
Intercept	1.30	0.071	1.30	332.709
eGFR	-0.003	4.301 E^{-4}	0.044	64.971
logNTproBNP	0.035	0.016	0.117	4.727
NYHA	0.024	0.011	0.098	4.356

Table 7
Multiple logistic model between the clinical severity of heart failure patients in functional New York Heart Association (NYHA) class I or II compared to those in III and IV class (dependent variable) and logGalectin-3, logNT-proBNP, eGFR, age, gender, ejection fraction (EF), polytherapy and comorbidity (predictive variables)

Variable	Coefficient	SE	Coef/SE	Chi-square	P
Constant	-4.971	2.230	-2.229	4.969	0.0258
logGalectin-3	1.811	0.835	2.169	4.704	0.0301
eGFR	0.081	0.009	2.081	4.330	0.0375
Age	0.003	0.013	0.237	0.056	0.8124
Gender	0.154	0.323	0.477	0.228	0.6332
logNT-proBNP	-0.045	0.0255	-0.175	0.031	0.8608
Body mass index	0.080	0.030	2.649	7.259	0.0071
EF	-0.048	0.015	-3.164	10.010	0.0016
Polytherapy	-0.300	0.185	-1.623	2.653	0.1046
Comorbidity	-0.009	0.143	-0.061	0.004	0.9516

average older than those without fibrosis and presented a not significant trend for lower left ventricular EF (P=0.09). The presence of cardiac fibrosis was significantly associated with GAL-3 values (Rho=0.212, P=0.031) and age (Rho=0.210, P=0.033), while all the other variables were not significantly correlated. In these patients, the ROC analysis showed that GAL-3 discriminated the presence of cardiac fibrosis with an area under the curve of 0.635 (95% confidence interval: 0.526-0.744). The best threshold of 14.6 μg/L corresponded to a specificity of 76.7% (53.3-93.3%), a sensitivity of 54.1% (35.1-68.9%) and an accuracy of 60.6%.

DISCUSSION

Compared to natriuretic peptides, which are considered the first-line biomarkers for the diagnosis, prognosis and management of HF patients, GAL-3 has the limitations of being not cardiac specific and of showing a narrower clinical range (9, 10, 17, 19). Indeed, BNP and NT-proBNP circulating concentrations can increase up to 50 folds (BNP) and up to 500 folds (NT-proBNP) with respect to the upper limit of the reference population in HF patients with severe disease (28). This large clinical range of biomarker concentrations allows the identification of patients in the early stages of HF and/or with mild symptoms (28). BNP and NT-proBNP are also able to discriminate between patients in HF stage B and healthy subjects (28).

The distribution of GAL-3 concentrations in large cohorts of general population has been previously reported (12, 29, 30). Christenson et al. reported that the 90th, 95th and 97.5th percentiles of GAL-3 concentrations using an ELISA method were, respectively, 17.6 μg/L, 20.3 μg/L and 22.1 μg/L in a cohort including 1092 apparently healthy subjects (520 men and 572 women), with age ranging from 55 to 80 years (29). De Boer et al. (30) reported data regarding 7968 subjects (including also patients at high risk for cardiovascular events), enrolled in the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study. These authors observed a strong association between GAL-3 and increasing age and gender (30). Gaze et al. (12) enrolled 627 apparently healthy individuals (292 women and 335 males) with no evidence of cardiac disease; in this study, only a weak

trend was observed between increasing age and GAL-3 concentrations, with no differences between genders. In this cohort, the mean, median, 75th percentile, 95th percentile and 97.5th percentile values were 14.6 μg/L, 13.3 μg/L, 17.5 μg/L, 25.2 μg/L and 28.4 μg/L, respectively (12). Considering 274 subjects without clinical evidence of cardiac disease (133 women and 141 men, age ranging from 55 to 80 years), including different ethnic groups, the manufacturer of the CMIA Architect method indicates a 97.5th percentile of 28.7 μg/L in women and of 26.1 μg/L in men, respectively (22). The differences between the previously reported results and our data are probably attributable to the size of samples and study protocols of gender-specific populations. Systematic differences between the clinical results observed with ELISA and CMIA methods should be also taken into account (12, 22). However, the reference values found in the present study are similar to those reported by Gaze et al. (12) and suggested by the manufacturer's package insert (22).

In this study, we observed that in patients with more severe HF, as well as those with cardiac fibrosis assessed by MRI, GAL-3 values were on average increased only by ~25% compared to values found in healthy subjects or in patients with less severe HF. As previously reported (19), the analytical performance of GAL-3 methods, especially the reproducibility, should play an important role in discriminating between healthy subjects and patients at high risk of cardiac fibrosis, who show an increase in concentrations of ~25%, roughly corresponding to an absolute amount of 5 μg/L GAL-3. To meet these clinical requirements, variability ≤20% CV at 5 μg/L should be expected. The present study confirms previous data (11, 12, 19), indicating that CMIA for GAL-3 on Architect platform has good reproducibility and analytical sensitivity. In particular, our results demonstrate that CMIA is able to measure a GAL-3 concentration of 5 μg/L with a between-run CV of 7.6%, assessed by the imprecision profile. Furthermore, all samples, including those from healthy subjects, showed GAL-3 values >LOQ: consequently, all samples are always measured with a CV ≤10%.

The data of the present study confirm previous clinical studies suggesting that GAL-3 is increased only in specific subgroups of HF patients, namely those with a more advanced disease or affected by comorbidities likely predisposing to a more extensive cardiac fibrosis (13-18, 23). In our group of 382 HF patients, log-transformed GAL-3 values were significantly and positively associated with age, serum creatinine, functional NYHA class and log-transformed NT-proBNP, and negatively associated with left ventricular EF values, all variables known to be associated with prognosis in HF. In addition, in the subgroup of patients who underwent MRI study, GAL-3 values were significantly increased in those patients exhibiting a more extensive cardiac fibrosis, confirming data from a previous report (23). Cardiac fibrosis is frequently observed also in patients with non ischemic HF and is associated with a more aggressive disease and a worse outcome (31). The relationship between circulating GAL-3 and myocardial fibrosis, in addition to the prognostic implications, may further support the implementation of the GAL-3 assay in the clinical setting as a tool for identification of high risk subsets, possibly needing a targeted therapeutic approach. From a clinical point of view, it is important to underline that recent studies have demonstrated that in patients with HFpEF, elevated plasma GAL-3 values are associated to an increase in mortality rate and major cardiovascular events (13-18). Patients with diastolic ventricular dysfunction are more frequently aged women with several comorbidities, including hypertension, diabetes mellitus, obesity, renal dysfunction, anemia and chronic pulmonary disease (10, 32, 33), conditions known to promote cardiac and systemic fibrosis. A recent study has provided the cost-effectiveness of the GAL-3 method using the Architect platform in HFpEF patients (34). The potential use of GAL-3 as a reliable, noninvasive marker of fibrosis may add value to the quality of the diagnostic and risk-stratification processes also in this subset of HF patients.

The present study suffers the limitation of a relative small number of enrolled patients. In particular, it is theoretically conceivable that no more than 6 up to 10 covariates should be considered in the multivariable logistic and linear regression analyses, considering only the 104 patients with MRI results (35). However, only variables of primary clinical interest and with low grade of correlations with other covariates should be taken into consideration in a multivariable model (35). Even if a smaller number of healthy individuals were enrolled in the present study as compared to others (12, 22, 29, 30), accurate clinical, instrumental and laboratory investigations were employed in our study in order to exclude the presence of both subclinical acute or chronic diseases. From the analytical point of view, the limitation of our study is that the analytical evaluation was performed in a single center compared to other recent multicenter studies (11, 12) (Table 1). Conversely, the study strength is that the analytical parameters were evaluated using different lots of reagent and materials throughout several working days. According to Spencer et al. (36), for an accurate evaluation of functional sensitivity (including both limit of quantitation and reproducibility), the inter-assay imprecision profile should be evaluated using more than 10 measurements for each sample, performed in different runs in a period of at least 6-8 weeks. In this study, from 26 to 49 measurements were performed for each plasma sample throughout a period of 6 months using 3 lots of calibrators and reagents in order to accurately estimate the imprecision profile.

In conclusion, our data indicate that the CMIA on the Architect platform meets the analytical quality goals required for GAL-3 determination in clinical practice. Further larger clinical studies are however needed to demonstrate the diagnostic and prognostic efficiency of GAL-3 assay in patients with chronic HF.

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CONFLICTS OF INTEREST

None.

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