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Cardiac sympathetic innervation assessed by I¹²³MIBG Imaging in different settings of patients affected by heart failure with reduced ejection fraction

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1. Introduction

Heart failure (HF) is a leading cause of morbidity and mortality worldwide and has a relevant impact on socio-economic health aspects (1). It can be defined as an abnormality of cardiac structure or function leading to failure of the heart to deliver blood, and so oxygen, at a rate adequate with tissues' necessities, despite normal filling pressures or only at the expense of increased filling pressures (2). In addition, it can be clinically defined as a syndrome in which patients present typical symptoms (e.g. breathlessness, orthopnea, fatigue) and signs (e.g. elevated jugular venous pressure, pulmonary crackles, third heart sound) resulting from an abnormality of cardiac structure or function (2).

In developed countries, 1-2% of the adult population is affected by HF and the prevalence follows an exponential pattern rising rates $\geq 10\%$ among persons aged 70 years or older (1). In the classification of the international cardiology societies (European Society of Cardiology, American Heart Association) two prevalent forms of HF have been identified: HF with reduced left ventricular (LV) ejection fraction (HF-REF) and HF with preserved LV ejection fraction (HF-PEF). The former is most common and can be characterized by different degrees of LV systolic dysfunction. Moreover, it is mainly related to the presence of coronary artery disease, however other aetiologies may be identified, as viral myocardial infections, alcohol or drugs abuse, chemotherapy, hereditary myocardial proteins' genes defects and idiopathic dilated cardiomyopathy (2).

An insufficient cardiac function leads to the activation of several compensatory mechanism that simultaneously act in order to compensate the effects of a failing heart. These mechanisms mainly consist in neuro-hormonal activation, enhanced sympathetic nervous system activity and natriuretic peptides synthesis and release (3). All these mechanisms, whereas initially positively contribute to compensate peripheral effects of an impaired cardiac

function, on long-term become dangerous and activate a vicious circle in which “HF begets HF”.

1.1 Sympathetic nervous system activity in heart failure: significance and prognostic implications of I¹²³-labelled meta-iodobenzylguanidine (I¹²³MIBG) Imaging

As previously stated, HF is characterized by an increased activity of sympathetic nervous system that acts as a compensatory mechanism in the initial phase of the disease, but contributes to deleterious adverse effects in the long-term due to increased oxygen demand and myocardial work. The ‘hyperadrenergic state’ results in decreased responsiveness to b-adrenergic receptor agonists, mainly caused by a reduced number (downregulation) and functional impairment (uncoupling) of receptors. In addition, prolonged sympathetic hyperactivity leads to direct detrimental cardiovascular effects, including excitation-contraction coupling abnormalities and promotion of cell apoptosis, which significantly contribute to HF progression (4).

Imaging with MIBG, an analogue of norepinephrine (NE), depicts the status of cardiac innervation in patients with HF (5) and has been shown to yield prognostic information, with the potential to stratify patients for fatal and non-fatal cardiac events more accurately than traditional risk markers. In patients with HF, human NE transporter 1 activity is downregulated with an increase in NE cardiac spillover in the synaptic space and reduced NE concentration in the presynaptic nerve endings, resulting in reduced cardiac MIBG uptake and accelerated washout rate (WR) in patients with HF (4). I¹²³MIBG is the result of an iodinated chemical modification of guanethidine with a molecular structure similar to NE, sharing the same uptake, storage and release processes. However, unlike NE, I¹²³MIBG is not metabolized by monoamine oxidase or catechol-O-methyltransferase and it has no interaction

with the postsynaptic receptors. As a result, scintigraphic images obtained by labelling MIBG with ^{123}I depict the status of catecholamine storage at the level of the myocardial sympathetic presynaptic fibers (6). To evaluate neuronal activity, ^{123}I MIBG uptake is semiquantified by calculating a heart to mediastinum ratio (H/M) after tracing regions of interest over the heart and the mediastinum. Neuronal integrity is also assessed by the ^{123}I MIBG WR, calculated by comparing early and late images (Figure 1). SPECT images are then analysed in conventional orthogonal planes (short axis, vertical and horizontal long axes). In patients with HF, reduced NE re-uptake in presynaptic neurons results in low MIBG cardiac uptake, while accelerated release kinetics leads to an increased WR (6). Cardiac neuronal abnormalities have been linked to the occurrence of several adverse events in patients affected by HF, as fatal ventricular arrhythmias, HF progression and overall mortality, included death for cardiovascular causes (7-9). Boogers et al. (10) reported data from a cohort of 116 patients with HF (mean EF 28%) who underwent MIBG imaging before ICD implantation and were followed for the occurrence of appropriate ICD therapy and cardiac death. In this study, the late MIBG SPECT defect score resulted in an independent predictor of appropriate ICD therapy and of cardiac death. Moreover, Tamaki et al. showed that, in 106 patients with LVEF <40%, those with sudden cardiac death had a significantly lower H/M and higher WR (8). At multivariate analysis only WR and EF were independent predictors of sudden cardiac death. Notably, WR showed higher specificity and predictive accuracy than EF and maintained its predictive capacity in patients with EF >35%, suggesting a role for ^{123}I MIBG to identify ICD candidates not currently included in guideline indications. The large multicenter AdreView Myocardial Imaging for Risk Evaluation in Heart Failure trial (ADMIRE-HF) (9) provided relevant confirmatory evidence for the independent prognostic role of ^{123}I MIBG in patients with HF. In this study, 961 patients with NYHA functional class I-III and EF \leq 35%, while on optimised medical therapy, underwent cardiac ^{123}I MIBG imaging and myocardial perfusion

imaging and were followed for a mean of 17 months. A significantly lower risk (15%) of the composite end-point was observed in patients with late H/M ≥ 1.60 compared with those with H/M < 1.60 (38%; HR 0.40, $p < 0.001$), with a highly significant HR for each individual component of the composite primary end-point. Notably, 2-year all-cause mortality was more than fivefold higher in patients with H/M < 1.60 than in those with H/M ≥ 1.60 (16.1% vs 3%). The multivariate and interaction analysis provided relevant information about the incremental prognostic value of I¹²³MIBG above and beyond traditional risk markers. In particular, in the ADMIRE-HF population, multivariate analysis identified four independent predictors of the primary end-point (NYHA class, plasma levels of B-type natriuretic peptide, EF and late H/M) and, interestingly, plasma NE levels measured in the trial did not significantly contribute to the prognostic model, suggesting that cardiac direct assessment of neuronal activity by I¹²³MIBG may be more useful than systemic evaluation reflected by NE plasma levels.

Thus, assessment of cardiac sympathetic activity by MIBG imaging represents a useful tool to depict the occurrence of increased sympathetic hyperactivity in HF and to follow its deleterious effects on disease progression. For clinical applications, MIBG imaging identifies, in addition to conventional functional and biochemical parameters, patients at low or high risk of cardiac events and is able to track innervation changes induced by treatments.

1.2 Aims and background of the research project

Aim of the present research path was to evaluate sympathetic nervous system at myocardial level in different settings of patients affected by HF-REF by the use of I¹²³MIBG cardiac scintigraphic study. In particular, in the current report we present the results of cardiac sympathetic activity evaluation in 3 specific groups of HF-REF patients, as diabetic HF

patients, non-diabetic HF patients affected by insulin resistance (IR) and HF-REF patients also affected by sleep-disordered breathing (SDB).

In the first study, we assessed myocardial adrenergic innervation in HF patients with severely reduced systolic function also affected by diabetes mellitus (DM), comparing this group to HF non-diabetic patients and to diabetic patients with normal cardiac structure and function. Aim of this analysis was to investigate if diabetes is associated with a further impairment of sympathetic activity and if parameters related to glycemic control may predict modifications of I¹²³MIBG parameters, so of myocardial adrenergic activity. A strict relationship exists between diabetes and HF. DM is considered either cause or consequence of reduced cardiac function and adversely affects disease progression and occurrence of serious adverse events. Nevertheless, at the moment, few clinical data are available on the impact of DM on cardiac sympathetic activity in patients affected by HF, despite this relationship could be one of the mechanisms responsible for the negative impact of diabetes on HF evolution. This assumption suggests a potential working hypothesis for future mechanistic studies aimed at assessing whether glycation directly affects the process of cardiac sympathetic impairment.

In the second phase of the research project we excluded diabetic patients and concentrated on non-diabetic HF patients also affected by IR. IR is quite common in HF also in absence of DM and can be considered as cause and/or consequence of HF and it has been associated with adverse prognosis. A close interaction exists between IR and sympathetic nervous system and this association may represent one of the pathophysiological mechanisms of adverse prognosis in HF patients also affected by IR. Thus, in this second study we tested the hypothesis that IR is associated with more impaired cardiac sympathetic innervation in non diabetic HF patients compared to those with HF but not IR, trying to explain a detrimental prognostic role of IR.

Finally, our last research aims to clarify the relationship between cardiac sympathetic innervation and SDB in HF patients. The interest for this field comes from the high prevalence and prognostic relevance of sleep apnea in systolic HF. SDB are characterized by sympathetic activation that mediate their adverse effects ultimately leading to high mortality rates. Hence, the interest to evaluate MIBG imaging in these patients, in order to clarify the prognostic implication of this disorder.

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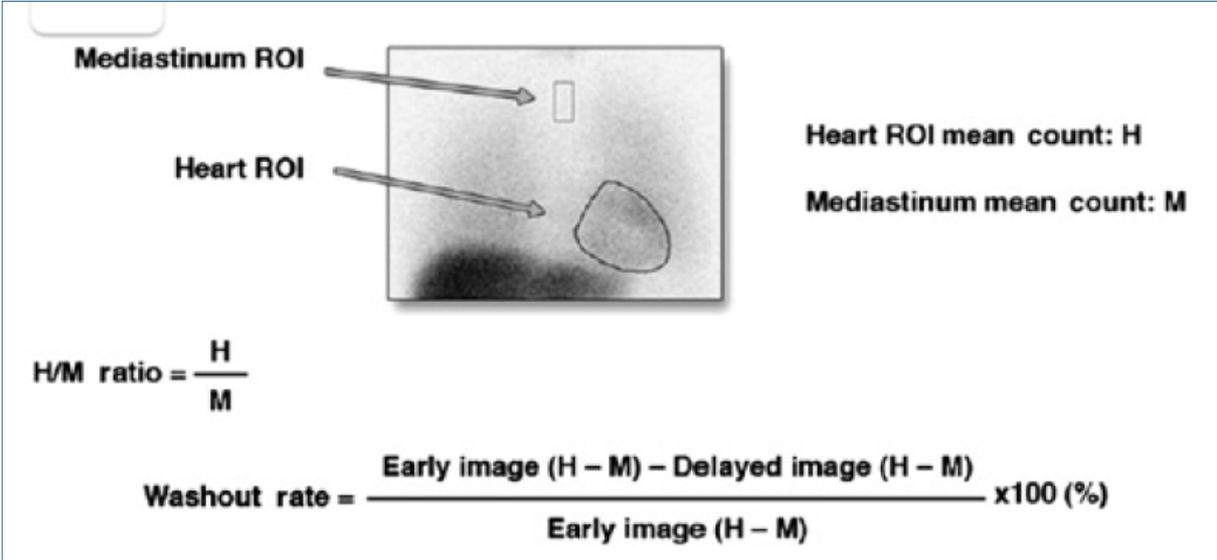
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1.4 Figure 1. Evaluation of meta-iodobenzylguanidine (I^{123} MIBG) activity in clinical studies.

Quantification of I^{123} MIBG heart to mediastinum ratio (H/M) and washout rate.

ROI, region of interest. *Modified from Carriò et al. (5).*



2. Impact of Diabetes Mellitus on Cardiac Sympathetic Innervation in Patients With Heart Failure. A Iodine-123 meta-iodobenzylguanidine (I^{123} MIBG) Scintigraphic Study

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HF is a leading cause of morbidity and mortality worldwide and is characterized by sympathetic nervous system hyperactivity that significantly worsens prognosis (1-8). Cardiac adrenergic nerve activity has been assessed I¹²³MIBG imaging (9) and, as demonstrated by the ADMIRE-HF study (10), the H/M ratio is an independent predictor of HF progression, arrhythmic cardiac events and cardiac death. Reduced I¹²³MIBG uptake, likely due to diabetic neuropathy, has been also demonstrated in patients with DM without HF and correlated with worse prognosis (11,12). DM is common in HF patients with a prevalence range from 10% to 30% (13), and adversely influences long-term morbidity and mortality of symptomatic and asymptomatic HF patients (14,15). In diabetic HF patients enrolled in the ADMIRE-HF trial, it has been recently demonstrated that the combination of DM and reduced I¹²³MIBG cardiac uptake is an independent predictor of HF progression (16). Yet, the distinct impact of DM on cardiac I¹²³MIBG uptake in patients with HF has not been largely investigated, and no previous studies have assessed cardiac innervation in matched HF patients with and without DM. Therefore, the aim of this study was to evaluate I¹²³MIBG uptake in matched DM and non DM patients with severe systolic HF.

Research Design and Methods

Population and study protocol

We enrolled 37 consecutive patients with systolic HF and type 2 DM and 38 HF patients without DM referring to the Outpatient Clinic for HF at the University of Naples Federico II, Italy. To be included in the study, patients needed to fulfill the following criteria: LVEF \leq 40% and dilated cardiomyopathy in at least two consecutive echocardiographic evaluations, diagnosis of HF since at least 6 months, stable clinical conditions (NYHA II-III), coronary angiography within 1 year from enrollment, no acute coronary syndrome or angina in the 6 months before inclusion in the study. Ischemic cardiomyopathy was defined as

ventricular dysfunction in myocardial regions subtended by significant (>70% diameter) coronary stenosis, with normal regional contractile function at echocardiography and/or invasive angiography in regions subtended by coronary arteries without significant stenosis. At the time of enrollment all patients were on optimized medical therapy for HF treatment including use of angiotensin-converting enzyme-inhibitors or AT1-antagonists when not tolerated, beta-blockers, loop diuretics, anti-aldosterone and digitalis when necessary in addition to conventional drugs used for the treatment of cardiovascular risk factors and for secondary prevention of coronary heart disease. Fourteen type 2 DM patients with normal cardiac function were also included in the study. The diagnosis of DM was confirmed by clinical history or through the assessment of at least 2 determinations of fasting plasma glucose ≥ 126 mg/dl or a random plasma glucose test ≥ 200 mg/dl or with blood glucose levels ≥ 200 mg/dl 120 minutes after an oral glucose tolerance test performed with 75 g of glucose dissolved in water and confirmed by repeating the test in another day (17). On the same day patients underwent clinical examination, venous blood sample collection, transthoracic echocardiography and I¹²³MIBG imaging. Demographic data including age, sex, height and body weight, body mass index, HF medications, NYHA class, tobacco use, hypertension, dyslipidemia, family history of coronary events, duration of DM, presence of comorbidities, and ischemic versus non ischemic HF etiology were also collected. A venous blood sample was collected in all patients to assess biochemical data, including hemoglobin A1c (HbA1c) and NT-proBNP; serum creatinine levels were obtained to estimate glomerular filtration rate and assess renal impairment, as previously described (18). Diabetic patients were also screened for the presence of diabetic neuropathy using the Michigan Neuropathy Screening Instrument (MNSI) examination (19-20). A standard transthoracic echocardiography was performed in all patients using a VIVID E9 ultrasound system (GE Healthcare) with second-harmonic capability and a 3.5 MHz probe. All measurements were performed according to the

European Society of Cardiology Recommendations for Chamber Quantification (21). LV diameters were obtained in the M-mode view. Global and regional LV function was evaluated and LV ejection fraction was calculated from apical four- and two-chamber views using the Simpson's biplane method (21). Wall motion score index was calculated to assess the extent of regional wall motion abnormalities. At the end of this initial evaluation, synaptic noradrenaline reuptake was assessed by I¹²³MIBG scintigraphy. All patients gave written informed consent and local ethic committee approved the protocol.

I¹²³MIBG Imaging Procedures

After blockage of the thyroid gland with 300 mg of perchlorate, an activity of 370 MBq (10 mCi ±10%) I¹²³MIBG (Covidien, Mallinckrodt) was administered over 1 to 2 minutes and a 10-minute planar anterior chest image was performed at 15 minutes ("early" image), and again at 3 hours and 50 minutes ("late" image). Imaging was performed with low-energy/high resolution collimators, and the camera peaked at 159 keV with a symmetrical 20% energy window. The images were acquired and stored in a 128x128 matrix (22). Two observers, blinded about patients' status (i.e. diabetic or non diabetic), analyzed I¹²³MIBG studies (10,23). H/M ratios were calculated from the early and late images after drawing regions of interest (7×7 pixels) over the entire heart and upper mediastinum Care was taken to exclude lung or liver from the myocardial and large vessels and lung from the mediastinum region of interest. I¹²³MIBG WR was calculated using the following formula: [(early heart counts/pixel – early mediastinum counts/pixel) – (late heart counts/pixel decay-corrected – late mediastinum counts/pixel decay-corrected)]/(early heart counts/pixel – early mediastinum counts/pixel).

Assessment of cardiac autonomic neuropathy

Evaluation of autonomic neuropathy was performed as previously described (11,24). In particular, five tests were used: 1) blood pressure change during standing up and 2) during

sustained handgrip; 3) heart rate response to Valsalva maneuver, 4) to standing up and 5) to deep breathing. Blood pressure response to standing up was evaluated through the difference of systolic blood pressure measured after 2 minutes of lying down and systolic blood pressure after standing up, while blood pressure response to 5 minutes of sustained handgrip at 30% of maximum voluntary contraction was evaluated through the difference of diastolic blood pressure assessed just before release of handgrip and diastolic blood pressure measured before starting the maneuver. For heart rate responses, Valsalva maneuver was continued for 15 minutes at 40 mmHg, then the ratio between the longest RR interval soon after the release and the shortest RR during the maneuver was evaluated; heart rate response to standing up was assessed as the ratio between the longest RR interval around the 30th beat and the shortest RR around the 15th beat (30:15 ratio) and finally heart rate changes to deep breathing was calculated through the mean of the differences of maximum and minimum heart rate of three consecutive deep breathings (6 breaths/minute). A mean autonomic score was then calculated referring to previously described normal, borderline or abnormal values (24) and presence of autonomic impairment was defined as an abnormal response to two or more of the five tests (11).

Statistical analysis

Data are expressed as mean \pm standard deviation. The Student *t* test was used for continuous variables. Correlation between variables was assessed by linear regression analysis and variables that revealed a statistical significance in univariate model were then included in a multivariate analysis. Categorical variables such as NYHA classification were analyzed by chi-square test. All data were collected in an Excel database and analyzed by SPSS 20.0. Statistical significance was accepted at $p \leq 0.05$.

Results

Patients characteristics

Mean age of the 75 patients with HF was 67.33 ± 9.6 years (84% male patients) with mean LV ejection fraction of $31.03 \pm 7.15\%$. In 52 subjects (69.3%) HF was of ischemic etiology and in 23 (30.7%) the etiology was an idiopathic dilated cardiomyopathy. All diabetic patients had a diagnosis of type 2 diabetes. No statistically significant differences between HF patients with and without DM were found for cardiovascular risk factors, demographic variables, comorbidities, LV systolic function, NYHA functional class, serum NT-proBNP levels and HF therapy, as shown in Table 1. Medical treatment of DM was as follows: 19 patients (51.4%) were on oral antidiabetic agents alone, 2 (5.4%) on insulin alone, 4 (10.8%) on oral drugs + insulin and 12 subjects (32.4%) were on diet only. Mean HbA1c was $6.61 \pm 0.69\%$ (49 mmol/mol), 22 patients (59.5%) had a HbA1c measurement $\geq 6.5\%$, whereas 11 subjects (29.7%) had a HbA1c value $>7\%$. Mean duration of DM was 62 ± 79 months.

In 14 patients with DM without HF mean age was 65.9 ± 8.8 years, mean DM duration was 58 ± 36 months and mean HbA1c was $7.5 \pm 1.5\%$ (58 mmol/mol).

I^{123} MIBG imaging

Early and late H/M ratios were significantly lower in patients with HF and DM compared to patients with HF without DM. In particular, DM patients showed a mean early H/M ratio of 1.65 ± 0.21 vs 1.75 ± 0.21 of non DM subjects ($p < 0.05$) and a mean late H/M ratio of 1.46 ± 0.22 vs 1.58 ± 0.24 in non DM subjects ($p < 0.03$). I^{123} MIBG washout rate did not significantly differ between the two groups (38 ± 22 vs $34 \pm 22\%$; $p = 0.44$). Both early and late H/M were significantly higher in DM patients without HF (2.22 ± 0.35 and 1.99 ± 0.24 , respectively) when compared to HF patients with ($p < 0.0001$) and without ($p < 0.0001$) DM.

Cardiac autonomic neuropathy

Mean autonomic score was 2.85 ± 0.80 in 20 patients with HF and DM and 3.06 ± 0.62 in 14 patients with DM without HF ($p=ns$). Autonomic impairment was found in all but 1 diabetic patients with HF and in 12 (86%) diabetic patients without HF. H/M ratios in 12 DM patients without HF with autonomic dysfunction were significantly higher compared to 19 DM patients with HF and with autonomic dysfunction (early H/M 2.24 ± 0.37 vs 1.62 ± 0.16 , respectively, $p < 0.0001$; late H/M 1.96 ± 0.24 vs 1.42 ± 0.16 , respectively, $p < 0.0001$). In the whole group of 34 DM patients evaluated for autonomic impairment, no correlation was found between autonomic score and both early and late H/M ratios ($r = -0.70$, $p = 0.723$ for early H/M; and $r = -0.787$, $p = 0.340$ for late H/M). In addition no correlation was found between mean autonomic score and HbA1c ($r = -0.006$, $p = 0.977$).

Determinants of I^{123} MIBG uptake in HF patients with and without DM

In the group of 75 patients with HF, in univariate analysis, early H/M ratio significantly correlated with age, LV ejection fraction, NYHA class, HF etiology, NT-proBNP, presence of diabetes and HbA1c (Table 2). In multivariate analysis, only HbA1c remained significant predictor of early H/M ratio (Table 2).

In univariate analysis, late H/M ratio significantly correlated with the same variables associated with early H/M, and, in addition, with glomerular filtration rate (Table 2). In multivariate analysis only etiology of HF remained significantly associated with late H/M. Interestingly, when presence of DM was eliminated from the multivariate analysis, HbA1c, in addition to HF etiology, resulted significantly correlated with H/M ratio, surely because HbA1c acted as a surrogate for DM.

Determinants of I^{123} MIBG uptake in HF patients with DM

In the group of HF patients with DM, in univariate analysis, early H/M ratio significantly correlated with LV ejection fraction, NT-proBNP and HbA1c (Table 2; Figure

1A). In multivariate analysis, LV ejection fraction and HbA1c remained significantly associated with H/M (Table 2). Late H/M ratio significantly correlated with age, LV ejection fraction, NT-proBNP and HbA1c (Table 2; Figure 1B). In multivariate analysis only HbA1c remained significantly associated with late H/M (Table 2).

Determinants of I¹²³MIBG uptake in HF patients without DM

In the group of HF patients without DM, in univariate analysis, early H/M ratio significantly correlated with age, NYHA class, HF etiology and NT-proBNP, (Table 2; Figure 1C). In multivariate analysis, none of these parameters remained significant predictor of early H/M ratio (Table 2). Late H/M ratio significantly correlated with the same variables associated with early H/M, and, in addition, with glomerular filtration rate (Table 2; Figure 1D). In multivariate analysis only NT-proBNP remained significantly associated with late H/M (Table 2).

Discussion

The present study demonstrates that in DM patients with severe systolic HF, I¹²³MIBG cardiac uptake is significantly impaired compared to matched HF patients without DM and to DM patients without HF. In addition, in HF patients I¹²³MIBG uptake significantly correlates with metabolic control of DM over the last 1 to 2 months, as indicated by the inverse association between H/M ratios and HbA1c levels.

Previous studies

Impaired I¹²³MIBG cardiac uptake was previously reported in DM patients without structural heart disease (12) and in DM patients with silent myocardial ischemia (11). In particular, Yufu et al. (12) recently demonstrated, in 108 subjects with type 2 DM but no cardiac diseases, that I¹²³MIBG washout rate predicts major adverse cardiac and cerebrovascular events. Moreover, Scholte et al. (25) reported that I¹²³MIBG imaging was able to

detect cardiac neuropathy in DM patients before the development of signs of cardiac autonomic imbalance, such as heart rate variability, and proposed that I^{123} MIBG imaging may provide early prognostic information in these patients. Mechanisms of reduced cardiac I^{123} MIBG uptake in DM patients without structural heart diseases are not completely understood and presumably different from mechanisms of reduced I^{123} MIBG uptake in HF patients with DM. Hyperinsulinemia exerts a sympathoexcitatory effect (26) that may lead to enhanced sympathetic tone and reduced I^{123} MIBG uptake in early stages of DM whereas cardiac sympathetic denervation, demonstrated at post-mortem studies, would be responsible for reduced I^{123} MIBG uptake in long-term diabetic patients with structural heart disease. However, few clinical data are available on the impact of DM on cardiac sympathetic activity in patients with HF. In fact, the only available data come from a recent retrospective analysis of the ADMIRE-HF trial (16). In this analysis, Gerson et al. (16) compared 343 DM patients to 618 non DM patients enrolled in the ADMIRE-HF study (10), and reported that HF patients with DM and I^{123} MIBG H/M ratio <1.68 had about 3-fold increased risk of HF progression compared to HF patients without DM and with H/M ratio <1.68 . It was also observed that DM patients in the ADMIRE-HF population showed significantly lower I^{123} MIBG H/M ratios (either early or late H/M) compared to non DM patients. At variance with our study I^{123} MIBG washout rate was also significantly higher in DM compared to non DM patients. However, due to the retrospective design of that analysis, DM and non DM patients were not matched for relevant characteristics that may have influenced differences observed in I^{123} MIBG parameters. In particular, DM patients had significantly worse clinical conditions, significantly less use of beta-blockers and were significantly older than non DM patients. Since it has been reported that beta-blocker therapy restores I^{123} MIBG uptake (27) and I^{123} MIBG uptake impairment correlates with the degree of clinical deterioration, it is

difficult to dissect from the data of the ADMIRE-HF trial the distinct influence of DM on cardiac I¹²³MIBG uptake in HF patients.

Apart from the ADMIRE-HF data, no previous studies evaluated the impact of DM on cardiac I¹²³MIBG uptake in patients with overt HF, whereas an influence of DM on I¹²³MIBG uptake and an association with subclinical HF was previously observed (28,29). In fact, it was reported that DM patients with normal LV function at rest who developed contractile dysfunction during stress show more impaired I¹²³MIBG uptake compared to patients with normal response to stress (28,29).

A provocative observation of the current study is the inverse correlation between HbA1c and either early or late H/M, observed in the whole population and in the subgroup of DM patients but not in non DM patients. The strength of this association was supported by multivariate analysis that identified HbA1c as the only significant predictor of late H/M ratio and as an independent predictor of early H/M ratio in the subgroup of DM patients. To our knowledge this observation is novel, and, indeed, no such correlation was found in the ADMIRE-HF population (10). However, consistent with our findings, in a previous study Ziegler et al. (30) observed in a small series of 12 type-1 DM subjects followed up for 4 years, that poor glycaemic control, assessed by HbA1c, represents a determinant of cardiac I¹²³MIBG uptake impairment that might be prevented by normoglycaemia. In our study HbA1c assessment and I¹²³MIBG imaging were obtained in the same day, which may explain the lack of correlation observed in the ADMIRE-HF populations. Notably, MIBG uptake in diabetic and non diabetic HF patients was significantly lower than that observed in diabetic patients with autonomic dysfunction and normal LV function, suggesting that autonomic dysfunction does not explain the impairment of MIBG uptake in HF diabetic patients. These previous observations and the findings of the current study suggest a potential working

hypothesis for future mechanistic studies aimed at assessing whether glycation directly affects the process of noradrenaline reuptake at synaptic level.

Limitations

There are limitations of the study that need to be acknowledged. The first is the relatively small number of patients that makes our findings preliminary and warranting further confirmation. However, the dispersion of data observed in the present study was of the same magnitude of that observed in the larger ADMIRE-HF population (16), as indicated by the similar coefficient of variations of H/M ratios in the two studies (data not shown). The small number of patients may have prevented differences in the use of beta-blockers and ACE inhibitors between DM and non DM patients with HF to reach statistical significance. However, both classes of drug demonstrated to improve I¹²³MIBG uptake (31). Thus, the higher percent of patients taking beta-blockers and ACE-inhibitor observed in DM patients with HF may only have undermined the differences in H/M ratios observed in the current study. In addition, no influence of type of drugs on MIBG uptake was found in univariate analysis. In the present study, I¹²³MIBG uptake was evaluated from planar images and, therefore, the value of SPECT I¹²³MIBG imaging, reported in previous studies (32), remains to be investigated. Likewise, although our findings were not influenced by wall motion score, lack of SPECT perfusion rest/stress data does not enable us to exclude an impact of myocardial necrosis or myocardial inducible ischemia on our observations.

Conclusion

In patients affected by chronic severe systolic HF, DM is associated with reduced cardiac I¹²³MIBG uptake compared to non DM patients and to DM patients without HF, and I¹²³MIBG uptake independently correlates with glycemic control over the last 1 to 2 months.

Additional pathophysiological studies are warranted to assess the biological relevance of these findings and their potential clinical implications for management of diabetic HF patients.

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Table 1. Baseline characteristics of HF patients with and without DM.

Characteristic	All	Patients with DM	Patients without DM	<i>p</i> value
Male sex % (n)	84 (63)	78.4 (29)	89.5 (34)	0.222
Age (y)	67.33±9.6	68.46±9.89	66.24±9.301	0.319
LVEF* (%)	31.03±7.15	29.78±6.63	32.24±7.52	0.139
NYHA† class II-III % (n)	38.7 (29) 58.8 (40)	32.4 (12) 67.6 (25)	44.7 (17) 55.3 (21)	0.154
Ischemic vs. non-ischemic % (n)	69.3 (52)	73 (27)	65.8 (25)	0.618
NT-proBNP‡ (ng/L)	1475.08±1169.73	1453.92±1131.36	1495.68±1220	0.878
HbA1c§ (%)	6.17±0.74	6.61±0.69	5.75±0.49	0.0006
GFR¶ (ml/min/1.73m ²)	78.4±32	77.3±34.3	79.5±30.1	0.788
DM§ duration (months)		62±79		
Cardiovascular Risk Factors				
Familiar history of CAD# % (n)	32 (24)	37.8 (14)	26.3 (10)	0.329
Hypertension % (n)	76 (57)	81.8 (30)	71.1 (27)	0.419
Smokers % (n)	69.3 (52)	64.9 (24)	73.7 (28)	0.460
Dyslipidemia % (n)	66.7 (50)	73 (27)	60.5 (23)	0.329
Comorbidities				
COPD** % (n)	42.7 (32)	43.2 (16)	42.1 (16)	1.000
Chronic kidney disease % (n)	18.7 (14)	21.6 (8)	15.8 (6)	0.565
Peripheral artery disease % (n)	10.7 (8)	16.2 (6)	5.3 (2)	0.147
Atrial fibrillation % (n)	18.7 (14)	10.8 (4)	26.3 (10)	0.137
Therapy				
ACE inhibitors % (n)	53.3 (40)	59.5 (22)	47.4 (18)	0.357
AT1 blockers % (n)	24 (18)	18.9 (7)	28.9 (11)	0.419
β-blockers % (n)	73.3 (55)	81.1 (30)	65.8 (25)	0.192
- Carvedilol % (n)	53.3 (40)	59.4 (22)	47.3 (18)	1.000
- Bisoprolol % (n)	20 (15)	21.6 (8)	18.4 (7)	1.000
Ca ⁺⁺ -channel antagonists % (n)	6.7 (5)	8.1 (3)	5.3 (2)	0.674

Aldosterone antagonists % (n)	38.7 (29)	35.1 (13)	42.1 (16)	0.637
Loop diuretics % (n)	69.3 (52)	73 (27)	65.8 (25)	0.618
Antidiabetic therapy				
Diet % (n)		32.4% (12)		
Oral antidiabetic agents % (n)		51.4% (19)		
Insulin % (n)		5.4% (2)		
Oral agents+Insulin % (n)		10.8% (4)		

Data are expressed as mean±1 standard deviation. Mean follow-up times.

*LVEF= left ventricular ejection fraction; †NYHA= New York Heart Association; ‡NT-proBNP= N-terminal pro-brain natriuretic peptide;

§HbA1c= Hemoglobin A1c; ¶GFR= glomerular filtration rate; ¶DM= diabetes mellitus; #CAD= coronary artery disease; **COPD= chronic obstructive pulmonary disease

Table 2. Determinants of I¹²³MIBG in all patients and in DM and non DM patients.

	Early H/M*				Late H/M*			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	β	p	β	p	β	p	β	p
<i>All patients</i>								
Age	-0.337	0.003	-0.184	0.079	-0.424	0.000	-0.218	0.138
LVEF[†]	0.360	0.001	0.092	0.506	0.398	0.000	0.070	0.619
NYHA[‡] class	-0.291	0.011	-0.088	0.399	-0.362	0.001	-0.184	0.083
Ischemic vs non-Ischemic	0.281	0.015	0.094	0.365	0.377	0.001	0.233	0.040
NT-proBNP[§]	-0.404	0.000	-0.193	0.158	-0.462	0.000	-0.235	0.098
WMSI	-0.229	0.179	-	-	-0.194	0.256	-	-
GFR[¶]	0.198	0.106	-	-	0.331	0.006	0.057	0.699
Diabetes	-0.227	0.050	0.086	0.475	-0.258	0.025	-0.067	0.580
HbA1C[#]	-0.473	0.001	-0.444	0.000	-0.382	0.001	-0.215	0.074
ACE-Is[©]	0.128	0.274	-	-	0.124	0.288	-	-
ARBs⁺	-0.044	0.706	-	-	-0.012	0.915	-	-
BBs[^]	0.067	0.568	-	-	0.135	0.247	-	-
<i>DM[°] patients</i>								
	β	p	β	p	β	p	β	p
Age	-0.284	0.088	-	-	-0.369	0.025	-0.247	0.072

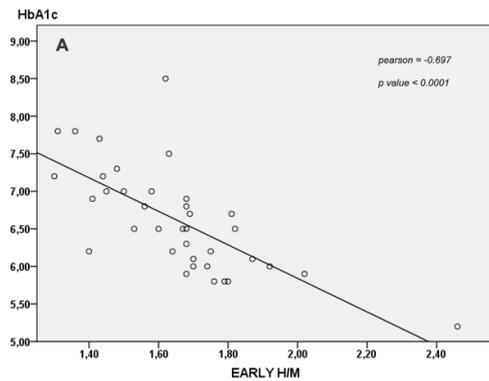
LVEF[†]	0.578	0.000	0.396	0.010	0.446	0.006	0.237	0.195
NYHA[‡] class	-0.196	0.245	-	-	-0.266	0.112	-	-
Ischemic vs Non-Ischemic	0.149	0.379	-	-	0.267	0.110	-	-
NT-proBNP[§]	-0.488	0.002	-0.034	0.818	-0.405	0.013	-0.025	0.890
WMSI	-0.334	0.206	-	-	-0.336	0.203	-	-
DM[°] duration	-0.054	0.752	-	-	0.001	0.995	-	-
MNSI^{**}	0.109	0.521	-	-	0.170	0.316	-	-
GFR[¶]	0.218	0.224	-	-	0.297	0.093	-	-
HbA1C[#]	-0.697	0.000	-0.579	0.000	-0.579	0.000	-0.478	0.001
ACE-Is[§]	0.153	0.367	-	-	0.093	0.586	-	-
ARBs⁺	0.011	0.950	-	-	0.162	0.337	-	-
BBs[^]	0.211	0.210	-	-	0.271	0.104	-	-
Antidiabetic therapy	-0.107	0.528	-	-	0.028	0.868	-	-
<i>non DM[°] patients</i>								
	β	p	β	p	β	p	β	p
Age	-0.359	0.027	-0.180	0.277	-0.452	0.004	-0.252	0.212
LVEF[†]	0.125	0.456	-	-	0.315	0.054	-	-
NYHA[‡] class	-0.337	0.038	-0.202	0.217	-0.403	0.012	-0.210	0.161
Ischemic vs Non-Ischemic	0.385	0.017	0.240	0.152	0.454	0.004	0.254	0.104

NT-proBNP[§]	-0.357	0.028	-0.158	0.350	-0.546	0.000	-0.329	0.039
WMSI	-0.334	0.206	-	-	-0.336	0.203	-	-
GFR[†]	0.173	0.320	-	-	0.375	0.026	-0.022	0.916
HbA1C[#]	-0.075	0.653	-	-	0.034	0.841	-	-
ACE-Is[°]	0.169	0.311	-	-	0.222	0.180	-	-
ARBs⁺	-0.146	0.383	-	-	-0.200	0.230	-	-
BBs[^]	0.030	0.858	-	-	0.130	0.435	-	-

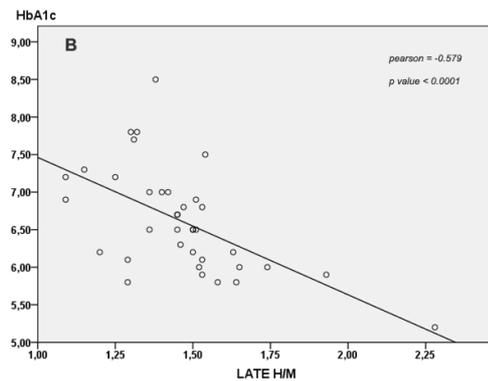
*H/M= Heart to Mediastinum; †LVEF = Left Ventricular Ejection Fraction; ‡NYHA= New York Heart Association; §NT-proBNP= N-terminal pro-brain natriuretic peptide; ||WMSI= Wall Motion Score Index; †GFR= Glomerular Filtration Rate; #HbA1c= Hemoglobin A1c; °DM= diabetes mellitus; **MNSI= Michigan Neuropathy Screening Instrument; °ACE-Is= Angiotensin Converting Enzyme Inhibitors; †ARBs= Angiotensin II Receptor Blockers; ^BBs= Beta-Blockers.

Figure 1. Correlation between HbA1c and early and late H/M ratio in HF patients with and without DM.

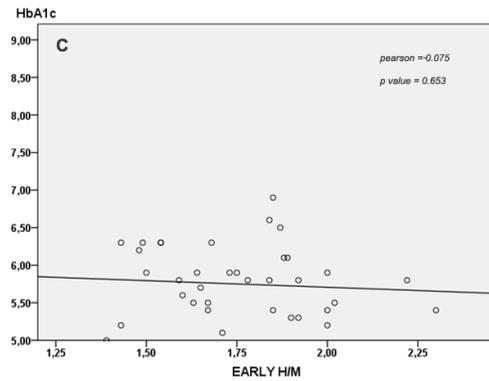
Figure 1 Correlation between HbA1c and early H/M ratio in DM patients



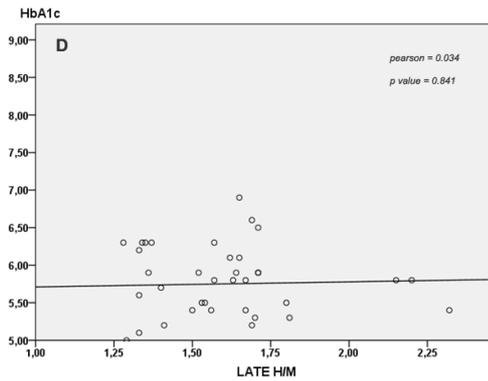
Correlation between HbA1c and late H/M ratio in DM patients



Correlation between HbA1c and early H/M ratio in non DM patients



Correlation between HbA1c and late H/M ratio in non DM patients



1A. Correlation between HbA1c and early H/M ratio in DM patients

1B. Correlation between HbA1c and late H/M ratio in DM patients

1C. Correlation between HbA1c and early H/M ratio in non DM patients

1D. Correlation between HbA1c and late H/M ratio in non DM patients

DM= diabetes mellitus; HbA1c= hemoglobin A1c; H/M= heart to mediastinum

3. Insulin Resistance is Associated With Impaired Cardiac Sympathetic Innervation in Patients with Heart Failure

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IR is common in non diabetic patients with HF and has been associated with adverse prognosis (1). However, pathogenetic mechanisms that link IR to unfavorable clinical outcome in non diabetic HF patients are not completely understood. Hyperinsulinemia, that characterizes IR, promotes sympathetic activation (2) and impaired cardiac sympathetic innervation has been showed in insulin resistant hypertensive patients with normal LV function (3). In addition, reduction of glycated hemoglobin in patients affected by DM with normal cardiac function has been correlated with preservation of cardiac sympathetic innervation (4). In fact, significantly more impaired cardiac sympathetic innervation has been recently reported in diabetic HF patients compared to non diabetic subjects (5) and associated with unfavorable clinical outcome (6). Yet, there are no studies that investigated the status of cardiac sympathetic innervation in non diabetic HF patients with IR.

Therefore, in the present study we tested the hypothesis that IR is associated with more impaired cardiac sympathetic innervation in non diabetic HF patients compared to patients with HF but not IR.

Methods

- Study Population

One-hundred fifteen consecutive patients (87% males; median age 65 ± 11.3 years) with severe-to-moderate systolic HF (mean LVEF $32.5\pm 9.1\%$) referring to the HF Unit at Federico II University of Naples, Italy, were enrolled in the study. To be included in the study, patients needed to fulfill the following criteria: LVEF $\leq 45\%$ in at least two consecutive transthoracic echocardiograms, diagnosis of HF since at least 6 months, stable clinical conditions (NYHA class I-III), coronary angiography within 1 year from enrollment, no acute coronary syndrome or angina in the 6 months before inclusion in the study. At the time of enrollment all patients were on optimized medical therapy for HF including angiotensin-

converting enzyme-inhibitors or AT1-antagonists, beta-blockers, loop diuretics, anti-aldosterone and digitalis when necessary, in addition to conventional drugs used for treatment of cardiovascular risk factors and for secondary prevention of ischemic heart disease. Based on IR index, patients were divided into two groups, with and without IR. All patients gave written informed consent and local ethic committee approved the protocol. Thirty-eight of 115 patients belong to the group of non-diabetic HF patients reported in previous study (5).

- Study Protocol and Procedures

On the first study day patients underwent clinical examination, venous blood sample collection and transthoracic echocardiography. The following day, I¹²³MIBG myocardial scintigraphy was performed. At clinical examination, demographic data and medical history were recorded. In particular, age, sex, height and body weight, HF medications, tobacco use, hypertension, dyslipidemia, family history of coronary events and presence of comorbidities were collected. NYHA class was estimated from patients' symptoms and ischemic versus non-ischemic HF etiology was assessed from clinical anamnesis and medical records.

Venous blood sample collection and assessment of IR

Venous blood sampling was obtained in all patients to assess biochemical data, including fasting glucose and insulin. IR was assessed through the evaluation of Homeostasis Model Assessment Insulin Resistance (HOMA-IR). In particular, HOMA-IR was calculated using the formula [fasting Glucose (mmol/L) x fasting Insulin (mIU/L)/22.5] and the presence of IR was defined as HOMA-IR value >2.5 (7).

Transthoracic echocardiography

A standard transthoracic echocardiography was performed in all patients using a VIVID E9 ultrasound system (GE Healthcare) with second-harmonic capability and a 3.5 MHz probe. All measurements were performed according to the European Society of Cardiology Recommendations for Chamber Quantification (8). LV diameters were obtained

in the M-mode view. Global and regional LV function was evaluated and LVEF was calculated from apical four- and two-chamber views using the Simpson's biplane method.

I¹²³ MIBG imaging procedures

After blockage of the thyroid gland with 300 mg of perchlorate, an activity of 111 MBq I¹²³MIBG (Covidien, Mallinckrodt) was intravenously administered over 1 to 2 minutes. A 10-minute planar image was acquired from an anterior thoracic view (256 × 256 matrix) 15 minutes ("early" image) and 3 hours and 50 minutes ("late" image) after tracer administration, as previously reported (9). Imaging was performed using a dual-head camera system (Skylight, Philips) equipped with low-energy, parallel-hole, high-resolution collimator, and peaked at 159 keV with a symmetrical 20% energy window. Two observers, blinded about patients' status, analyzed I¹²³MIBG studies (5,9). MIBG uptake was semi-quantified by calculating a H/M ratio after drawing regions of interest (ROI) over the heart and mediastinum. This approach provides a highly reproducible index of cardiac sympathetic activity (9). Briefly, H/M ratio was computed from the early and late images by dividing the mean counts per pixel within the myocardium by the mean counts per pixel within the mediastinum. Using dedicated post-processing software on a dedicated workstation (Philips), the cardiac ROI was assessed using a manually drawn polygonal ROI placed over the myocardium including the LV cavity on the MIBG images. Care was taken to exclude lung or liver from the myocardial ROI. The mediastinal ROI with a square shape was placed on the upper half of the mediastinum and had a size of 7 × 7 pixels. The location of mediastinal ROI was determined using as landmarks the lung apex, the upper cardiac border and the medial contours of the lungs. H/M ratio was computed for early and late imaging. By comparing early and late activities, the MIBG WR from the myocardium was derived, providing a parameter that reflects retention of norepinephrine by sympathetic neurons (9). MIBG WR was calculated using the following formula: [(early heart counts/pixel – early mediastinum

counts/pixel) – (late heart counts/pixel decay-corrected – late mediastinum counts/pixel decay-corrected)]/(early heart counts/pixel – early mediastinum counts/pixel). Reproducibility of I¹²³MIBG analysis in our laboratory has been recently reported (10). The absorbed dose per unit of activity of I¹²³MIBG was 0.018 mGy/MBq (9).

- Statistical analysis

Numerical variables that showed normal distribution were expressed as mean±SD; otherwise, variables non-normally distributed were expressed as medians and interquartile range. Unpaired t-test or non-parametric Mann-Whitney test were used when appropriate for between-group comparison. Categorical variables were analyzed by chi-square test. Correlation between variables was assessed by linear regression analysis. Multivariable regression analysis was performed in different steps to overcome collinearity between covariates included in the model as insulinemia and HOMA-IR. All data were collected in an Excel database and analyzed by SPSS 20.0. Statistical significance was accepted at $p \leq 0.05$.

Results

Of 115 patients, 15 (13%) were in NYHA class I, 66 (57%) in NYHA class II and 34 (30%) in NYHA class III. In 73 patients (63%) HF was of ischemic origin and in 42 (37%) patients etiology of HF was an idiopathic dilated cardiomyopathy. Seventy-six percent of patients were on treatment with inhibitors of renin-angiotensin system (ACE inhibitors or ARBs) and 72% took beta-blockers (77% on carvedilol, 14% on bisoprolol and 8% other beta-blockers), whereas 37% of patients were on mineralcorticoid receptor antagonists. Median early H/M ratio was 1.80 (IQR 1.60-1.88) and median value of late H/M ratio was 1.76 (IQR 1.36-1.70); mean washout rate was 10.08±9.50.

- Characteristics of IR and non-IR patients

Seventy-two (63%) patients showed IR (HOMA-IR >2.5) and 43 (37%) were non-IR (HOMA-IR ≤2.5). No significant differences between IR and non-IR patients were observed for age, LVEF, NYHA class, HF etiology and HF treatments (Table 1). IR patients showed significantly higher fasting insulinemia and fasting glucose levels compared to non-IR patients ($p<0.001$) (Table 1).

- MIBG uptake in IR and non-IR patients

IR patients, compared to non-IR patients, showed significantly reduced early H/M ratio (1.68 (IQR 1.53-1.85) vs 1.79 (IQR 1.66-1.95); $p=0.05$) and significantly reduced late H/M ratio (1.50 (IQR 1.35-1.69) vs 1.65 (IQR 1.40-1.85); $p=0.020$) (Figure 1A/B). Washout rate did not differ between IR and non-IR patients (10.46 ± 8.79 vs 9.45 ± 10.63 ; $p=0.578$).

- Determinants of I¹²³MIBG uptake

In the whole population early and late H/M ratio showed a significant inverse correlation with age, fasting insulinemia (Figure 2A/B), HOMA-IR (Figure 2C/D) and NYHA class and a significant direct correlation with LVEF and HF etiology. These variables were included in a multivariable model to assess the independent predictors of I¹²³MIBG uptake. At multivariate regression analysis, LVEF ($\beta=0.230$; $p=0.017$), fasting insulinemia ($\beta=-0.223$; $p=0.016$) and HOMA-IR ($\beta=-0.264$; $p=0.004$) remained independent predictors of early H/M ratio, whereas independent predictors of late H/M ratio were HF etiology ($\beta=0.203$; $p=0.039$) and LVEF ($\beta=0.243$; $p=0.014$).

Discussion

The findings of the present study demonstrate that MIBG uptake is significantly reduced in non diabetic HF patients with IR compared to matched non diabetic HF patients without IR. Since, reduced MIBG uptake reflects reduced pre-synaptic norepinephrine uptake

due to cardiac sympathetic nervous system overactivity, this observation might contribute to elucidate the interaction between IR and prognosis in patients with HF (1).

IR and cardiac sympathetic innervation in HF. The pathogenetic relationship between cardiac sympathetic innervation and IR is quite complex, since IR represents, at the same time, cause and consequence of HF. In fact, hyperinsulinemia, that characterizes IR, has been reported to increase neural sympathetic activation (2), which, in turn, may exert deleterious effects on cardiac structure and function, leading to impaired cardiac innervation (11). Very consistent with our observations, a previous study by Mongillo et al. (12) reported, in a small group of patients, a direct correlation between presynaptic noradrenaline re-uptake, evaluated by positron emission tomography using the noradrenaline analogue [¹¹C]meta-hydroxyephedrine, and insulin sensitivity in patients with LV dysfunction. In addition, impaired cardiac sympathetic innervation has been reported in type 2 diabetic patients with normal cardiac function compared to matched non diabetic patients (13,14), whereas Takahashi et al. (15) demonstrated a synergistic detrimental effect of hypertension and type 2 DM on cardiac innervation in patients with normal cardiac function, that did not correlate with glycated hemoglobin and fasting glucose plasma levels.

Conversely, IR may be consequence of HF. In fact, it has been demonstrated that overstimulation of beta-adrenergic receptors impairs insulin-sensitivity through an Akt-mediated effect (16). More recently, Ciccarelli et al., using a transgenic mice model, showed that ischemia-induced up regulation of G protein-coupled receptor kinase 2 promotes IR by interfering with insulin signaling (17). As it has been demonstrated that insulin signaling exerts protective effects in the heart through inhibition of apoptosis and oxidative stress (18) and enhances cardiomyocyte survival upon ischemic injury (19,20), development of IR may set a vicious pathogenetic circle along which IR begets IR through exacerbation of HF. Consistent with this hypothesis, it has been recently reported that improvement of loading

ventricular conditions, induced by mechanical ventricular assistance, restores insulin sensitivity in patients with advanced HF (21).

Current study provides further novel insights on the association between IR and HF. In our study, IR was 63% prevalent in HF patients, quite consistent with the 61% prevalence reported by AlZadjali et al. (22) in a population of 129 HF patients. In addition, impaired myocardial glucose uptake has been showed in DM patients with coronary artery disease and reduced EF (23) as well as in HF patients without DM (24) using positron emission tomography. In the current study, patients with HF and IR showed significantly reduced MIBG uptake, reflecting sympathetic nervous system overactivity, compared to insulin sensitive patients, despite no differences in clinical status, LVEF and HF treatments. The finding of impaired values of both early and late H/M ratios strengthens the conception that a complex and strict relationship exists between IR and cardiac sympathetic nervous system. Conversely, no differences in MIBG WR were found between patients with and without IR. This result might depend on the great variability of WR values observed in clinical practice, and the limited number of patients enrolled in the current analysis could have been not sufficient to demonstrate significant differences. In addition, to further support the interaction between IR and adrenergic system, a significant inverse correlation was found between HOMA-IR and both early and late H/M, and between fasting insulin plasma levels and early and late H/M. Since reduction of H/M ratios are independent prognostic predictors in HF patients (6), these data are consistent and further support the adverse prognostic impact of IR in patients with HF. The finding of independent role of HF etiology in the prediction of late H/M might depend on the higher prevalence of HF of ischemic etiology and related to a more clear and defined interaction between ischemia and sympathetic nervous system compared to adrenergic impairment observed in idiopathic dilated cardiomyopathy (25).

Limitations. Lack of assessment of the effects of therapeutic interventions on IR and on MIBG uptake may represent a limitation of the study and deserves further investigation. Second, lack of follow up does not allow to establish the independent prognostic value of IR in non-DM patients with HF. Finally, the limited number of patients might have underestimated differences in WR between groups and studies on larger populations could be useful to clarify the value of this parameter in clinical practice.

Conclusions. Patients with HF and IR demonstrate significantly reduced MIBG uptake compared to matched non-IR patients. Thus, the findings contribute to shed light on the relationship among IR, HF and cardiac sympathetic nervous innervation. Additional studies are needed to elucidate the pathogenetic basis of this complex interaction.

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Table 1. Baseline characteristics, glycemic control and I¹²³MIBG Imaging in IR and non-IR patients.

	IR^a (n=72, 63%)	non-IR^a (n=43, 37%)	<i>p</i>
Age (y)	66±10	63±11	0.142
Male sex (%)	86	88	0.483
LVEF (%) ^b	30(25-37)	33(29-37.3)	0.299
NYHA ^c	2(2-3)	2(2-2.5)	0.192
HF ^d Ischemic etiology (%)	59.7	69.7	0.541
Ace-inhibitors (%)	50	44.1	0.574
AT II Receptor Antagonists (%)	25	32.5	0.388
Beta blockers (%)	73.6	69.7	0.570
Loop Diuretics (%)	61.1	58.1	0.658
Aldosterone Antagonists (%)	36.1	39.5	0.756
Glycemia (mg/dl)	100(91-116)	87(74-98)	<0.001
Insulinemia (mIU/l)	16.6(13-23.5)	5.4(3.5-8.8)	<0.001
HOMA-IR ^e	4.19(3.19-6.54)	1.24(0.65-1.94)	<0.001

^aIR insulin resistance

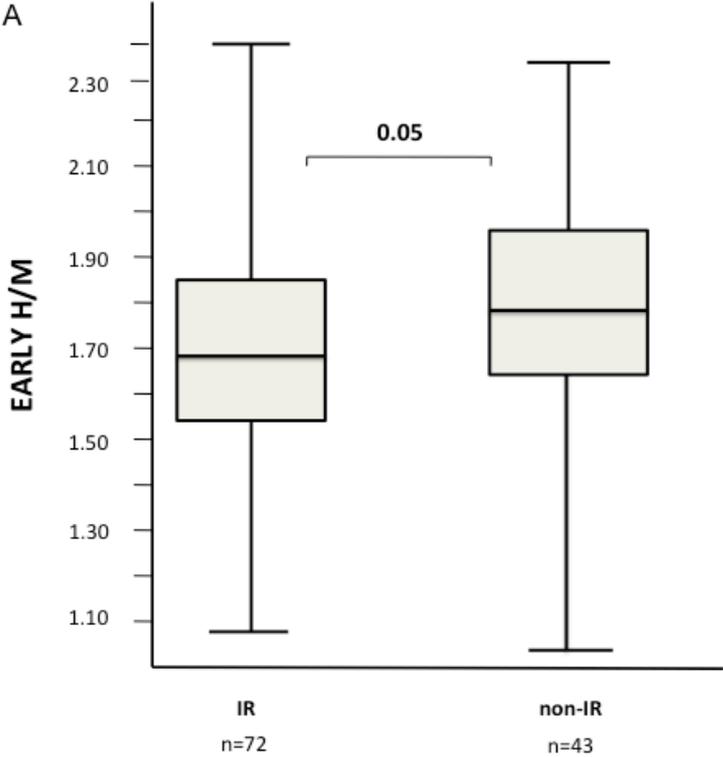
^bLVEF left ventricular ejection fraction

^cNYHA New York Heart Association

^dHF heart failure

^eHOMA-IR Homeostasis Model Assessment Insulin Resistance

Figure 1A. Early H/M ratio in IR vs non-IR patients

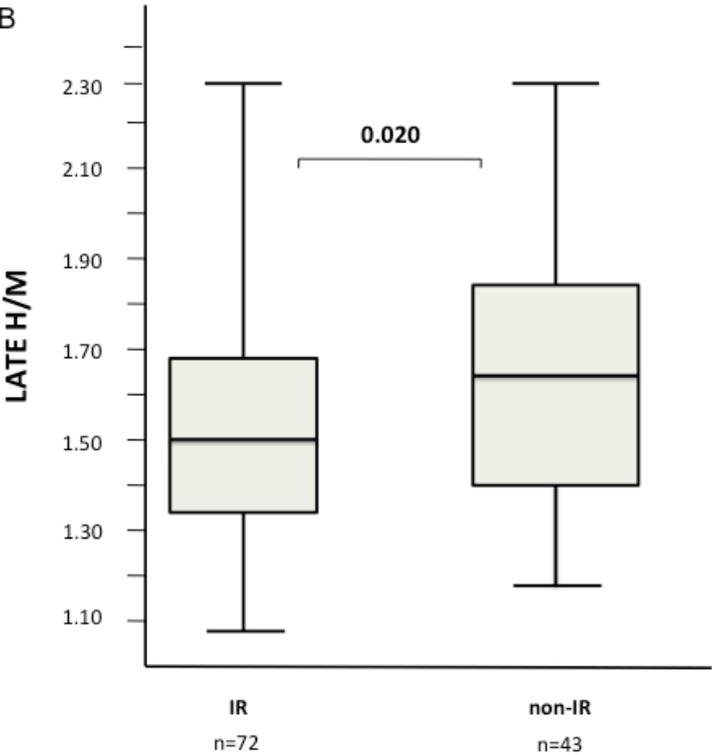


^{123}I MIBG: iodine-123 meta-iodobenzylguanidine

H/M: heart-to-mediastinum

IR: insulin resistance

Figure 1B. Late H/M ratio in IR vs non-IR patients

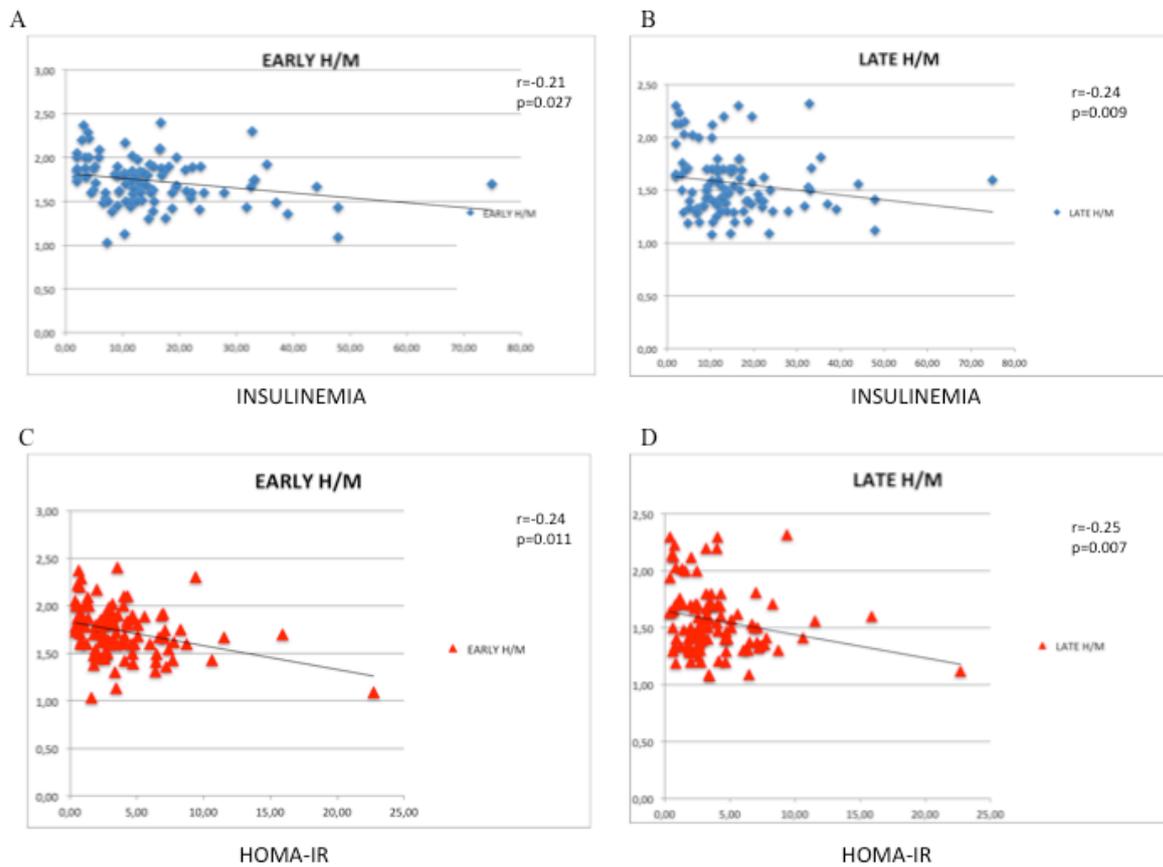


I^{123} MIBG: iodine-123 meta-iodobenzylguanidine

H/M: heart-to-mediastinum

IR: insulin resistance

Figure 2. Correlation between cardiac sympathetic innervation and fasting insulinemia and HOMA-IR.



Panel A: Inverse correlation between insulinemia and Early H/M ratio

Panel B: Inverse correlation between insulinemia and Late H/M ratio

Panel C: Inverse correlation between HOMA-IR and Early H/M ratio

Panel D: Inverse correlation between HOMA-IR and Late H/M ratio

H/M: heart-to-mediastinum

HOMA-IR: Homeostasis Model Assessment Insulin Resistance

3. Sympathetic cardiac innervation and sleep-disordered breathing incrementally predict prognosis in patients affected by heart failure with reduced ejection fraction

Submitted

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SDB are common in patients with HF, both in form of obstructive sleep apnea (OSA) and central sleep apnea (CSA), and have an impact on disease progression and prognosis (1,2). Adverse effects of SDB in HF are mainly mediated by increased sympathetic nervous system activity that increases myocardial oxygen demand, myocyte necrosis and apoptosis, beta-adrenoceptor desensitization and arrhythmogenesis, ultimately leading to high mortality rates (3,4). In fact, in patients with OSA, the combination of recurrent apneas, hypoxia and arousal is accompanied by an increase in chemoreflex-mediated adrenergic activity that also persists during daytime wakefulness (5-7). Similarly, in HF patients with CSA, a further increase in resting sympathetic drive has been demonstrated during apnea episodes (8,9) leading to adrenergic modulation of chemoreflex and altered ventilatory control (10). Increased urinary and blood levels of catecholamines, higher cardiac noradrenaline spillover (11) and impaired muscle sympathetic nerve traffic (4,9) have been demonstrated in HF patients affected by SDB and it has been reported that ventilatory therapy reduces sympathetic nervous system activity (12,13). However, few data are available on the relationship between SDB and impaired cardiac sympathetic innervation, assessed at myocardial level, in patients with HF. In the present study we sought to investigate the prognostic relationship between SDB and cardiac sympathetic innervation, assessed by I^{123} MIBG imaging, in patients affected by systolic HF.

Methods

Study Population

Ninety-four consecutive patients (93% male; mean age 66.1 ± 9.8 years) with moderate to severe systolic HF (median LVEF $32 \pm 7\%$) referring to the HF Unit at Federico II University of Naples, Italy, were included in the present analysis. Study inclusion criteria

were: diagnosis of systolic HF (LVEF $\leq 45\%$) evaluated by transthoracic echocardiograms in at least two consecutive determinations, stable HF since at least 6 months (NYHA I-III), no acute coronary syndrome or acute HF in the 6 months before inclusion in the study, ability to tolerate nocturnal cardiorespiratory monitoring and capability to sign informed consent and take part into the analysis. At enrollment all patients were on optimized medical therapy for HF including drugs for prevention of cardiac ischemic events when indicated. The study complies with the Declaration of Helsinki, all patients gave written informed consent and local ethic committee approved the protocol.

Study Procedures

On the first day patients' medical history and demographic data were collected. In particular, information on HF etiology, cardiovascular (CV) risk factors, ongoing therapy, and HF symptoms were recorded. A complete clinical examination and transthoracic echocardiography were performed and, in the same day, nocturnal cardiorespiratory monitoring equipment was applied, as subsequently reported. The day after patients returned to remove the device and underwent I¹²³MIBG myocardial scintigraphy.

Nocturnal cardiorespiratory monitoring

A Somté (Compumedics, Melbourne, Australia,) software polygraph was used to record nocturnal breathing patterns and identify the presence of SDB. Surface electrodes were applied to obtain continuous electrocardiogram recording. Airflow was monitored by use of thermistors placed at nose and arterial O₂ saturation was continuously monitored by a pulse oximeter. Thorax and abdomen movements were registered by pletismographic method. Time registration was computer-programmed, starting from patients sleep habits. A sleep diary was given to the patient to record the effective hours of sleep and possible awakenings. A registration of at least 7 hours was required to analyze the presence of SDB.

Nocturnal cardiorespiratory monitoring were analyzed and interpreted by a physician skilled in SDB (O.S.). According to the American Academy of Sleep Medicine Criteria (14), apnea was defined as a complete cessation of airflow for at least 10 seconds, while hypopnea was defined as a decrease in nasal airflow greater than 50% lasting for 10 seconds or more and associated to arterial O₂ desaturation. Presence and severity of SDB were defined by the assessment of Apnea/Hypopnea Index (AHI), defined as the number of respiratory events per hour of sleep. AHI value <5 allows to exclude respiratory sleep disorders, whereas AHI between 5 and 15 identifies mild disorder, between 16 and 30 a disorder of moderate intensity and >30 indicates a severe disturb (14). Based on AHI value patients were divided into two groups: SDB (AHI ≥5) and non-SDB (AHI <5). Furthermore, based on the pattern of abdominal and thoracic movements and from desaturation patterns, apneic events were divided into obstructive and central type. Patients groups' classification into OSA or CSA was determined by the presence of more than 50% of, respectively, obstructive or central apneic events.

Transthoracic echocardiography

A standard transthoracic echocardiography was performed in all patients using a VIVID E9 ultrasound system (GE Healthcare, Little Chalfont, United Kingdom) with second harmonic capability and a 3.5 MHz probe. All measurements were performed according to the European Society of Cardiology Recommendations for Chamber Quantification (15). LV diameters were obtained in the M-mode view. Global and regional LV function was evaluated and LVEF was calculated from apical four- and two- chamber views using the Simpson's biplane method.

I¹²³MIBG imaging procedures

After blockage of the thyroid gland with 300 mg of perchlorate, an activity of 111 MBq I¹²³MIBG (Covidien, Mallinckrodt) was intravenously administered over 1 to 2 minutes. A 10-minute planar image was acquired from an anterior thoracic view (256 × 256 matrix) 15 minutes (“early” image) and 3 hours and 50 minutes (“late” image) after tracer administration, as previously reported (16,17). Imaging was performed using a dual-head camera system (Skylight, Philips, Amsterdam, The Netherlands) equipped with low-energy, parallel-hole, high-resolution collimator, and peaked at 159 keV with a symmetrical 20% energy window. Two observers, blinded about patients’ status, analyzed I¹²³MIBG studies (16,18). ¹²³I-MIBG uptake was semi-quantified by calculating a heart-to-mediastinum (H/M) ratio after drawing regions of interest (ROI) over the heart and mediastinum. This approach provides a highly reproducible index of cardiac sympathetic activity (17). Briefly, H/M ratio was computed from the early and late images by dividing the mean counts per pixel within the myocardium by the mean counts per pixel within the mediastinum. Using dedicated post-processing software on a dedicated workstation (Philips), the cardiac ROI was assessed using a manually drawn polygonal ROI placed over the myocardium including the LV cavity on the I¹²³MIBG images. Care was taken to exclude lung or liver from the myocardial ROI. The mediastinal ROI with a square shape was placed on the upper half of the mediastinum and had a size of 7×7 pixels. The location of mediastinal ROI was determined using as landmarks the lung apex, the upper cardiac border and the medial contours of the lungs. H/M ratio was computed for early and late imaging. By comparing early and late activities, the I¹²³MIBG WR from the myocardium was derived, providing a parameter that reflects retention of norepinephrine by sympathetic neurons (16). I¹²³MIBG WR was calculated using the following formula: [(early heart counts/pixel – early mediastinum counts/pixel) – (late heart counts/pixel decay-corrected – late mediastinum counts/pixel decay-corrected)]/(early heart counts/pixel – early

mediastinum counts/pixel). Reproducibility of I¹²³MIBG analysis in our laboratory has been recently reported (19). The absorbed dose per unit of activity of I¹²³MIBG was 0.018 mGy/MBq (16).

Follow-up

Patients were followed up prospectively. Follow-up was carried out according to the local HF program and ended with the last clinical evaluation or with patients' death. If a patient died outside the hospital where he/she was on follow-up, medical records of the event and a report of the cause of death were considered. Follow-up analysis was carried out until November 2014. The study endpoint was the composite of CV death and hospitalization for worsening HF.

Statistical analysis

Numerical variables were recorded and analyzed as mean±SD or as medians and interquartile range when non-normally distributed. Categorical variables were expressed as percentage. Unpaired t-test or non-parametric Mann-Whitney test were used when appropriate for between-group comparison. Categorical variables were analyzed by chi-square test. Correlation between variables was assessed by linear regression analysis. Potential predictors of the study outcome were identified by multiple linear regression analysis, performed in different steps to overcome collinearity between covariates included in the model. Survival analysis was evaluated through Kaplan Meier curves and compared by Log-Rank test. All data were collected in an Excel database and analyzed by SPSS 20.0. Statistical significance was accepted at $p \leq 0.05$.

Results

Of 94 patients, 72 (77%) showed SDB and 22 (23%) did not. Median AHI value in the whole population was 14 ± 19 and 43 (46%) patients showed a moderate-severe disturb ($AHI > 15$). Among SDB patients, 49 (68%) showed prevalent OSA and 23 (32%) prevalent CSA. Mean early H/M ratio was 1.70 ± 0.21 and mean late H/M ratio was 1.53 ± 0.23 . In 62 patients (66%) HF was of ischemic origin and in 32 (34%) etiology of HF was an idiopathic dilated cardiomyopathy. Ten patients (11%) were in I NYHA class, 57 (60%) in II NYHA class and 27 (29%) patients were in NYHA class III.

I^{123} MIBG uptake in SDB and non-SDB patients

No significant differences between SDB and non-SDB patients were observed for age, gender, NYHA class, HF etiology, CV risk factors, NT-proBNP and LVEF (Table 1). SDB patients showed greater body mass index (BMI) compared to patients without SDB. As per protocol, SDB patients showed significantly higher AHI values compared to non-SDB patients ($p < 0.001$).

SDB patients, compared to non-SDB patients, showed significantly reduced early H/M (1.67 ± 0.22 vs 1.77 ± 0.13 ; $p = 0.019$) and late H/M ratio (1.50 ± 0.22 vs 1.61 ± 0.23 ; $p = 0.038$) (Table 1; Figure 1A-B). WR did not differ between groups (Table 1). No significant differences were found between OSA ($n = 49$, 53%) and CSA ($n = 23$, 24%) patients for age, gender, NYHA class, HF etiology, CV risk factors, NT-proBNP and LVEF (data not shown). AHI significantly differed between OSA and CSA patients (18 ± 11 vs 29 ± 15 ; $p = 0.001$), whereas early and late H/M ratios did not significantly differ between groups (early H/M 1.69 ± 0.24 vs 1.64 ± 0.19 , in OSA and CSA respectively, $p = ns$; late H/M 1.52 ± 0.24 vs 1.45 ± 0.19 , in OSA and CSA respectively, $p = ns$).

At multiple linear regression analysis, in which I^{123} MIBG parameters, age, BMI, LVEF and NT-proBNP were included, early and late H/M ratio remained independent

predictors of AHI ($\beta=-0.749$; $p<0.001$; $\beta=-0.830$; $p=0.001$, respectively). Similarly, AHI was the only predictor of early H/M ($\beta=-0.643$; $p<0.001$) and of late H/M ($\beta=-0.453$; $p<0.002$).

Prognostic impact of SDB and I¹²³MIBG parameters

Patients were followed up for a mean of 29 ± 18 months (range 6-43 months), and all of them completed at least 6 months of follow-up. In the whole population, 4 (4.2%) patients experienced CV death, while 17 (18%) were hospitalized for worsening HF. Analyzing event rate distribution and survival rates, median AHI value, SDB severity and late H/M showed the best discriminatory power identifying worse prognosis.

Dividing patients into two groups above and below the median AHI (median AHI=14), patients with AHI above the median ($n=49$) showed significantly higher event rates compared to patients with AHI below the median ($n=45$) (35% vs 9%, respectively; $p=0.003$). Kaplan-Meier analysis showed significantly worse prognosis in patients with AHI above the median for the composite outcome of CV death and HF hospitalization (Log-Rank= <0.001 ; Figure 2A).

When patients were divided into two groups according to SDB severity, patients with a moderate-severe disturb (AHI >15 ; $n=43$) showed significantly higher event rates compared to patients with mild or no disorder (AHI ≤ 15 ; $n=51$) (35% vs 11.7%; $p=0.007$). Kaplan-Meier analysis showed significantly worse survival in patients with moderate-severe disorder for the composite outcome of CV death and HF hospitalization (Log-Rank=0.001; Figure 2B).

In addition, event rates of the combined endpoint significantly differed in patients with normal (≥ 1.60) (20) or abnormal late H/M ratio (<1.60). Patients with late H/M ratio ≥ 1.60 ($n=36$) showed significantly lower event rates compared to patients with late H/M <1.60

(n=58) (11% vs 29%; p=0.039). Kaplan-Meier analysis showed significantly worse survival in patients with impaired cardiac sympathetic innervation (Log-Rank=0.042; Figure 2C).

Incremental prognostic impact of SDB and I¹²³MIBG parameters

An additional analysis was performed to assess the incremental prognostic value of H/M and SDB.

Addition of median AHI subgroups (<14 vs ≥14) to the binary late H/M classification (<1.60 vs ≥1.60) identified four new groups of patients (1:late H/M ≥1.60 and AHI <14; 2:late H/M ≥1.60 and AHI ≥14; 3:late H/M <1.60 and AHI <14; 4:late H/M <1.60 and AHI ≥14) and significant differences in event rates occurrence were observed among groups (5% vs 19% vs 12% vs 42%, respectively; p= 0.005). At Kaplan-Meier analysis the integration of these variables provided further stratification for the combined endpoint of CV death and HF hospitalizations (Log-Rank<0.001; Figure 3A). The worst prognosis was observed in patients with AHI above the median and late H/M ratio <1.60.

Similarly, highly significant differences in prognosis were found adding patients' stratification for SDB severity (mild or no SDB vs moderate-severe disorder) to the binary H/M result (<1.60 vs. ≥1.60). Event rates were significantly different among the new four groups (1:late H/M ≥1.60 and mild or no SDB; 2:late H/M ≥1.60 and moderate-severe SDB; 3:late H/M <1.60 and mild or no SDB; 4:late H/M <1.60 and moderate-severe SDB - event rates: 5% vs 19% vs 12% vs 44%, respectively; p= 0.008) and significant survival differences were found at Kaplan-Meier analysis (Log-Rank=0.001; Figure 3B), with the worst prognosis observed in patients with moderate-severe disorder and late H/M ratio <1.60.

Discussion

The findings of the present study demonstrate that SDB are highly prevalent (77%) in patients with chronic systolic HF and associated with more impaired cardiac sympathetic innervation. SDB and cardiac sympathetic innervation parameters incrementally predict the composite endpoint of CV survival and HF hospitalization, identifying a subgroup of patients with abnormal H/M ratio and moderate to severe SDB who show the worst prognosis.

In the present study significant lower values of early and late H/M ratios, indicating impaired cardiac sympathetic innervation due to chronically increased sympathetic drive, were observed in patients with HF and SDB, compared to patients without SDB, despite comparable LVEF and functional HF class. Notably, I¹²³MIBG parameters were the only independent predictors of SDB and, inversely, AHI was the best descriptor of cardiac adrenergic innervation status. Thus, these findings point to an independent contribution of SDB to sympathetic stimulation in patients with HF, leading to more pronounced beta-receptor desensitization and, hence, reduced H/M ratios measured at cardiac level.

In our study, early and late H/M ratio did not differ between patients with OSA compared to patients with CSA. This finding may appear not consistent with previous studies that investigated the impact of SDB in patients with HF on sympathetic activation using different approaches. In fact, Solin et al. (3) reported that overnight urinary norepinephrine levels were significantly increased in 90 HF patients compared to healthy subjects and to patients with OSA not affected by HF. Among HF patients, those with CSA revealed more increased norepinephrine levels compared to patients with HF and no SDB or with HF and OSA. However, patients with CSA had significantly reduced LVEF and significantly more compromised hemodynamic status compared to patients with HF and OSA. Similarly, in a study of 55 patients with severe systolic HF, Mansfield et al. (11) reported that total body and

cardiac norepinephrine spillover were more increased in patients with CSA compared to patients with OSA or without SDB, but also in this study these differences were attributed to the more impaired hemodynamic status of patients with CSA compared to patients with OSA. Yet, catecholamine levels do not strictly mirror the status of cardiac innervation as depicted from innervation imaging (20) and only few studies have used MIBG imaging to assess the impact of SDB on cardiac innervation in patients with HF. Nanjo et al. (21) reported in a study of 53 patients with stable chronic dilated cardiomyopathy, that late H/M ratio was significantly lower in HF patients with SDB compared to patients without SDB. However, in this study no nocturnal cardiorespiratory monitoring or complete polysomnography were performed and the presence of SDB was assessed using 24-h pulse oximetry. In a study of 59 HF patients, Tamura et al. (22) found that late H/M ratio was significantly lower and WR significantly higher in patients with HF and CSA compared to patients with HF and OSA. However, significantly higher BNP levels in patients with CSA indicated that hemodynamic status was more compromised in patients with CSA, potentially explaining their observation. Thus, to our knowledge, this is the first study that compared the status of cardiac sympathetic innervation of patients with HF and OSA or CSA, with similar degree of functional and hemodynamic LV impairment.

Prognostic impact of SDB in relation to I^{123} MIBG findings

Patients with AHI above the median showed significantly increased incidence of the combined endpoint of cardiac death or re-hospitalization for worsening HF compared to patients with AHI below the median. In addition, dividing patients according to SDB severity, significantly higher incidence of the combined endpoint was found in patients with moderate-severe disorder compared to patients with only mild or absent disorder, using an AHI threshold >15 to identify patients with moderate-severe SDB, as previously suggested (14).

Our findings appear very consistent with those reported by Wang et al. (23) in 164 patients with HF divided between patients without SDB or only mild OSA, identified by AHI ≤ 15 , and patients with untreated OSA identified by AHI > 15 . In that study, that excluded patients with CSA, an independent impact of OSA was found on mortality rate. However, no parameters of cardiac sympathetic status were reported. Taken together, these findings suggest that HF patients also affected by SDB develop more impaired cardiac sympathetic innervation, as a result of more increased chronic sympathetic drive, leading to more adverse prognosis. Consistent with our study, Lanfranchi et al. (24) reported that the presence of impaired respiration during sleep was associated with increased mortality rate in 62 patients with systolic HF, and that an AHI ≥ 30 was an independent predictor of prognosis. Bakker et al. (25) also reported, over a 10-year follow-up of 53 HF patients, a significant decreased survival in patients with CSA compared to those with OSA or those without SDB.

However, in these studies, at variance with the current analysis, SDB was analyzed as a single prognostic parameter and not integrated in a model that accounts for other prognostic parameters, including I¹²³MIBG findings, to assess its incremental prognostic value. In fact, the ADMIRE-HF trial (20) reported that a value of late H/M < 1.60 identifies patients at increased risk of cardiac death, arrhythmic events, HF progression and all-cause mortality with incremental prognostic value to natriuretic peptide levels and LVEF. Consistent with that study, our findings demonstrate an increased risk of CV death and HF hospitalizations in HF patients with late H/M value < 1.60 compared to patients with late H/M ≥ 1.60 . Inclusion in the model of both AHI and late H/M further stratified clinical outcome. In fact, when median AHI was combined with late H/M ratio, four groups of patients were identified with significant event rates differences, with the highest event rate observed in patients with late H/M < 1.60 and AHI above the median value (Figure 3A). Similarly, adding SDB severity classification to late H/M, significant event rates and survival differences were found among

the new four classes of patients with the worst event rate in subjects with late H/M <1.60 and SDB of moderate-severe grade (Figure 3B).

Limitations

This study did not assess the effects of intervention for SDB on cardiac sympathetic innervation. This prevents to verify whether changes of cardiac innervation status are associated with clinical outcome and can represent a meaningful surrogate prognostic endpoint in HF patients. In fact, a recent study reported that short-term continuous positive airway pressure was associated with a significant improvement of pre-synaptic catecholamine retention, indicating improved cardiac innervation status, in patients with HF and OSA (13). However, no prognostic data were reported. Collectively, these findings generate the hypothesis to be tested that therapeutic-induced changes of cardiac innervation parameters may represent a short-term marker of subsequent clinical outcome in HF patients with SDB. However, this study is the first investigating the association among SDB, cardiac innervation and prognosis, reporting the largest population of HF patients with SDB undergoing I¹²³MIBG imaging.

Conclusions

Patients with systolic HF and SDB show a greater impairment of cardiac adrenergic innervation and more adverse prognosis compared to HF patients without SDB. Further studies are warranted to assess whether SDB therapy, through reversal of sympathetic innervation abnormalities, improves prognosis in HF patients with SDB.

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Table 1. Characteristics and I¹²³MIBG uptake in the two groups of patients with SDB and no-SDB.

	*SDB (n=72)	no-SDB (n=22)	p
Age (y)	66.9±9.7	63.2±9.8	ns
Male sex (%)	92	95	ns
BMI [†]	29.0±4.1	26.4±3.7	0.01
LVEF [‡] (%; IQR)	32(8)	33(9)	ns
NYHA [§]	2.1±0.7	2.3±0.6	ns
NT-proBNP (pg/ml, IQR)	441(1720)	182(149)	ns
Ischemic Etiology (%)	62	77	ns
Hypertension (%)	82	73	ns
Diabetes (%)	36	32	ns
Dyslipidemia (%)	68	77	ns
Cigarette Smoking (%)	53	64	ns
Early H/M	1.67±0.22	1.77±0.13	0.019
Late H/M	1.50±0.22	1.61±0.23	0.038
Washout rate (%; IQR)	10.1(10)	8.4(9.4)	ns

*SDB sleep disordered breathing

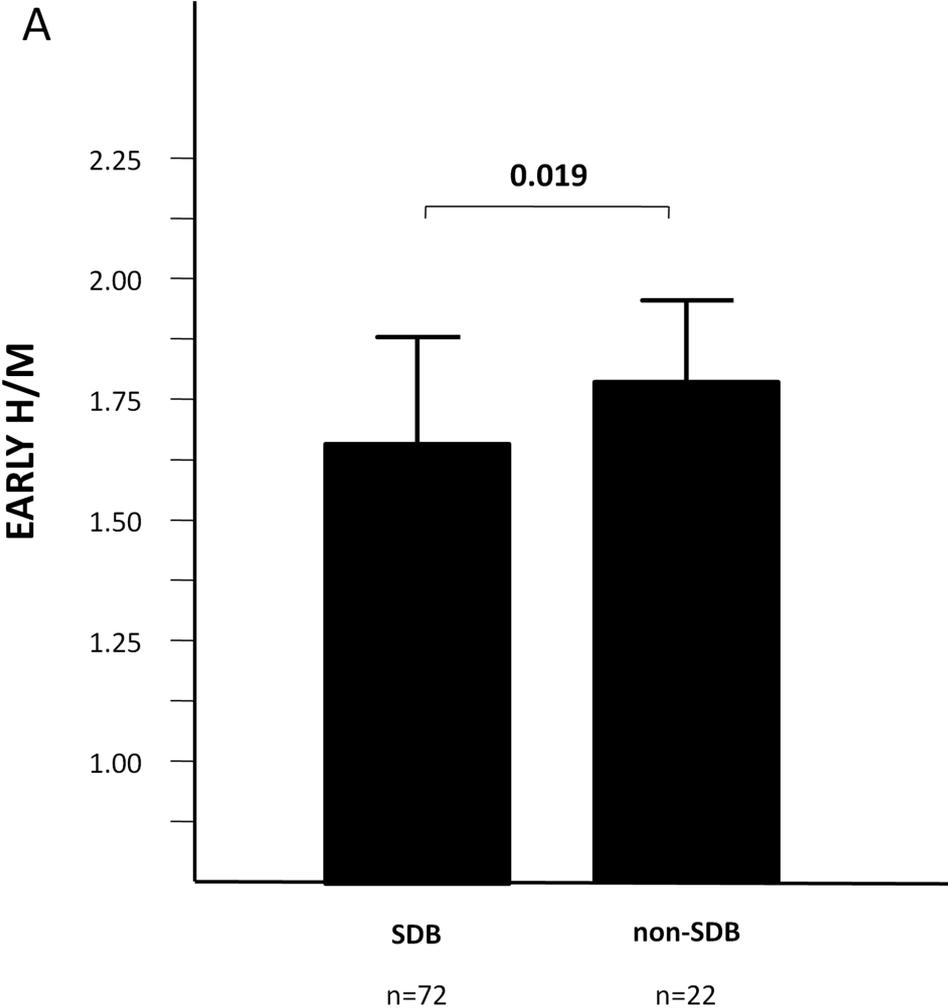
†BMI body mass index

‡LVEF left ventricular ejection fraction

§NYHA New York Heart Association

||H/M heart to mediastinum ratio

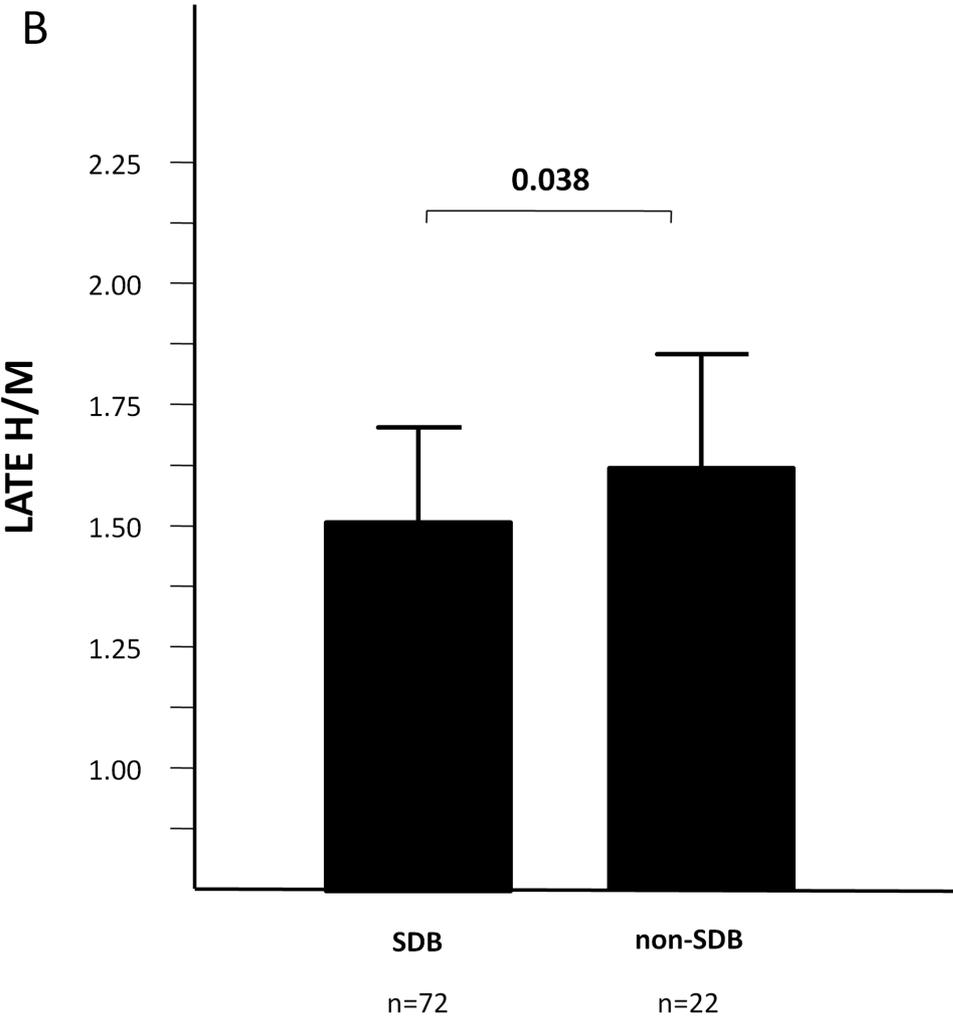
Figure 1A. Early H/M in the two groups of patients with and without SDB



SDB sleep disordered breathing

H/M Heart to Mediastinum ratio

Figure 1B. Late H/M in the two groups of patients with and without SDB



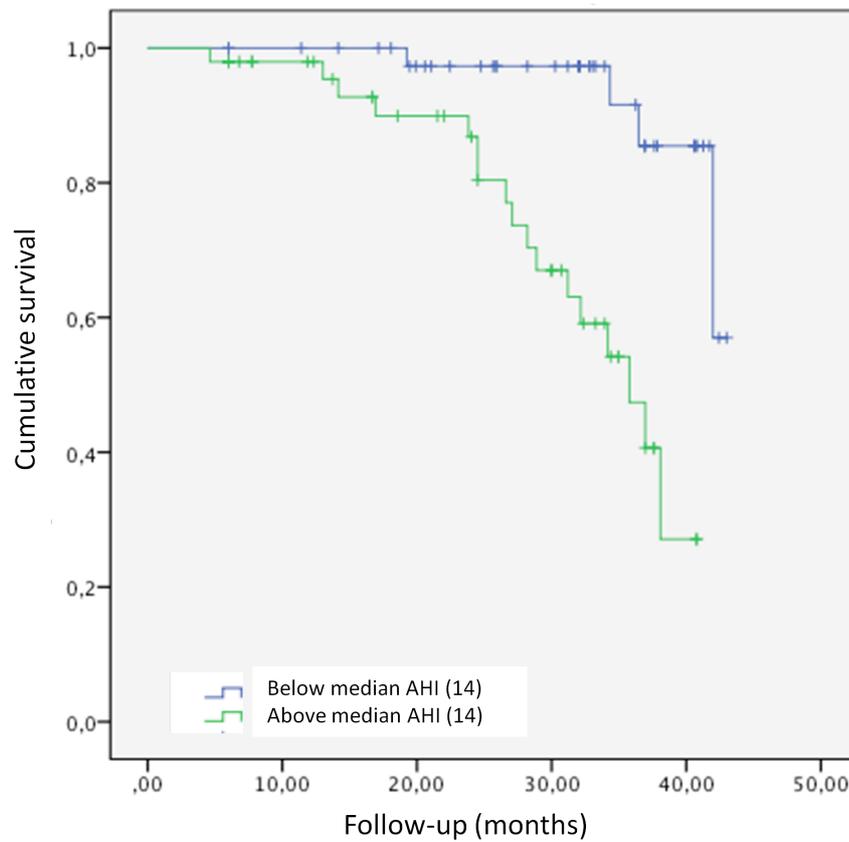
SDB sleep disordered breathing

H/M Heart to Mediastinum ratio

Figure 2. Kaplan-Meier analysis for the composite endpoint of cardiovascular death and hospitalization for worsening HF

Panel A. Kaplan-Meier analysis for the composite endpoint of cardiovascular death and hospitalization for worsening HF in patients above median AHI (green curve) and below median AHI (blue curve).

2A

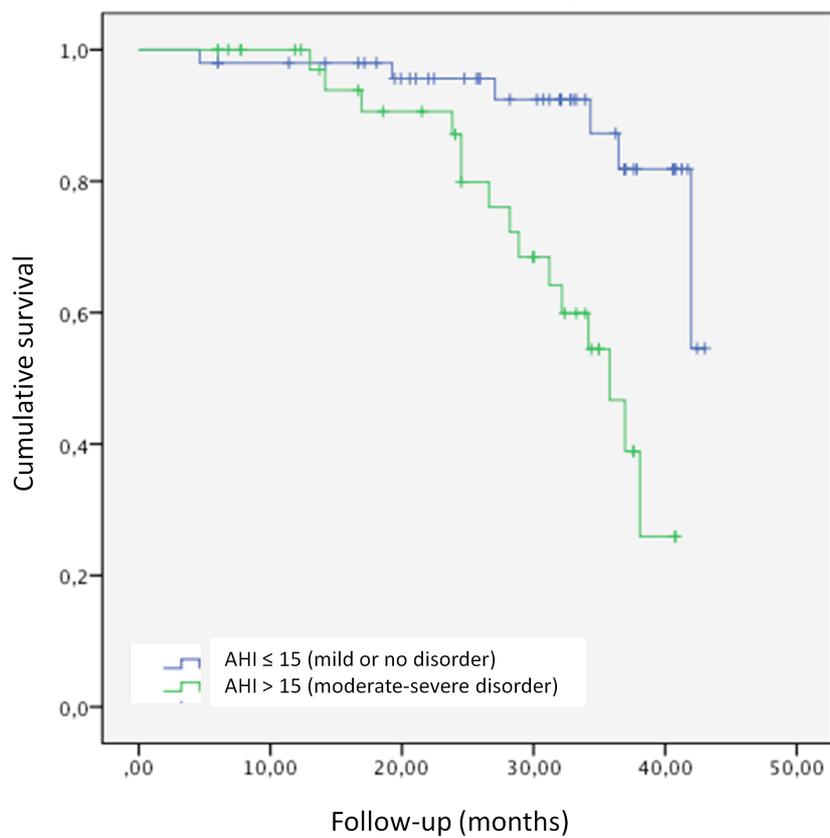


AHI Apnea/Hypopnea Index

Figure 2. Kaplan-Meier analysis for the composite endpoint of cardiovascular death and hospitalization for worsening HF

Panel B. Kaplan-Meier analysis for the composite endpoint of cardiovascular death and hospitalization for worsening HF in patients with mild or no SDB (blue curve) and with moderate-severe SDB (green curve).

2B

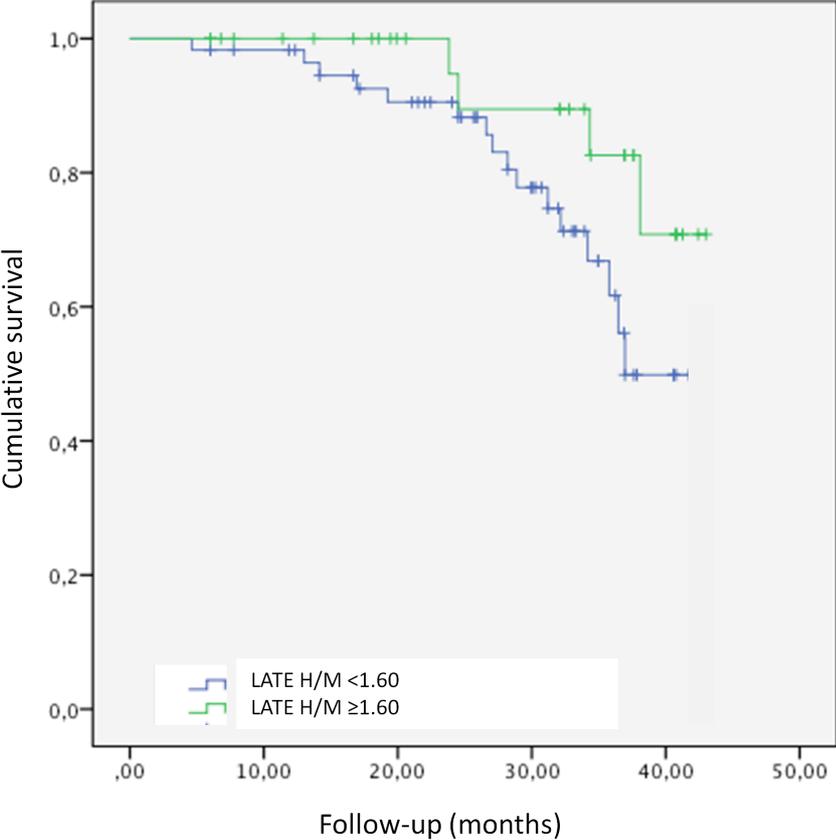


AHI Apnea/Hypopnea Index

Figure 2. Kaplan-Meier analysis for the composite endpoint of cardiovascular death and hospitalization for worsening HF

Panel C. Kaplan-Meier analysis for the composite endpoint of cardiovascular death and hospitalization for worsening HF in patients with late H/M <1.60 (blue curve) and late H/M \geq 1.60 (green curve).

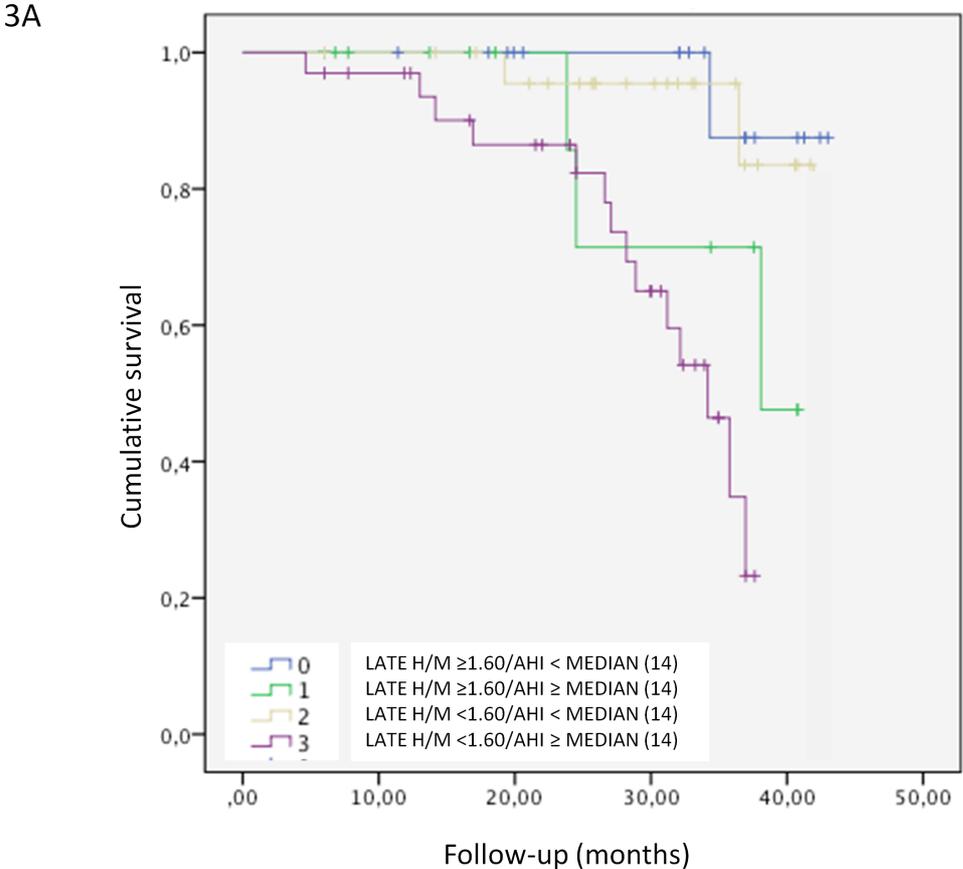
2C



H/M heart to mediastinum ratio

Figure 3. Kaplan-Meier analysis for the composite endpoint of cardiovascular death and hospitalization for worsening HF showing incremental value of late H/M to median AHI and SDB severity.

Panel A. Addition of binary late H/M division (<1.60 vs. ≥1.60) further stratifies patients already divided in the two groups above and below median AHI (14).



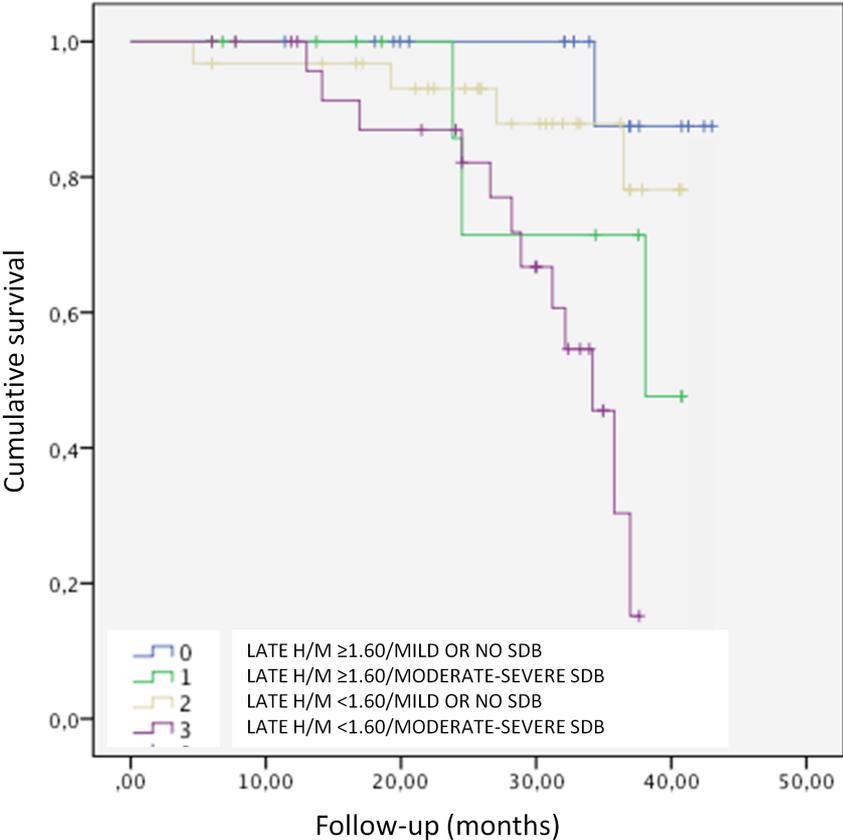
AHI Apnea/Hypopnea Index

H/M heart to mediastinum ratio

Figure 3. Kaplan-Meier analysis for the composite endpoint of cardiovascular death and hospitalization for worsening HF showing incremental value of late H/M to median AHI and SDB severity.

Panel B. Addition of binary late H/M division (<1.60 vs. ≥1.60) further stratifies patients already divided in the two groups of mild/no-SDB and moderate-severe SDB.

3B



SDB sleep disordered breathing

H/M heart to mediastinum ratio

5. Closing remarks

Morbidity and mortality related to HF-REF show an exponential growth due to population aging and prolonged life expectancy. Numerous factors have been already identified as prognostic indicators in patients affected by HF, however several pathogenetic mechanisms remain still unclear and continue to adversely affect patients' prognosis and disease progression.

Sympathetic nervous system hyperactivation play a detrimental role in the advancement of cardiac dysfunction and it is strictly related to several metabolic mechanisms that additionally influence prognosis. The identification of the pathophysiological pathway that relates adrenergic impairment with metabolic disorders might help in the identification of new therapeutic targets to improve HF survival and quality of life.

Assessment of cardiac sympathetic activity by I¹²³MIBG imaging represents an interesting and promising tool to assess adrenergic imbalance in HF and to follow its deleterious effects on disease progression. However, the lack of wide prospective outcome data currently remains a limitation for routine clinical use. Prospective clinical studies are needed, aimed at assessing whether I¹²³MIBG imaging will ultimately have an impact on the management of HF in clinical practice.