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Integrated approach to cardiovascular diseases: the role of imaging, biomarkers, aging and comorbidities

PhD Thesis

Leonardo Bencivenga, MD

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Leonardo Bencivenga, MD

29/11/1990 Napoli (Italy)

Promotor: Prof. Emanuele Barbato

Department of Advanced Biomedical Sciences,

Federico II University of Naples, Italy

Co-promotor: Prof. Bruno Vellas

Gérontopôle de Toulouse, Institut du Vieillissement, CHU de Toulouse, France

Naples, 03/02/2023

Federico II University of Naples, Faculty of Medicine,

Via Pansini n. 5, 80131 Naples, Italy

Contents

General introduction and outline of the thesis

Cardiovascular diseases (CVDs) constitute the leading cause of death, disability and loss of physical function in people aged over 50 years[1], resulting in the most relevant health, social and economic burden worldwide. Over 60 million people in the European Union suffer from CVDs (comprising ischemic heart disease, hypertension, stroke), accounting for an estimated economic cost of 210 billion € annually[2]; similarly, in the United States, the prevalence of CVDs is 48% in adults ≥ 20 years old and increases with age in both males and females[3]. Besides, several CVDs risk factors such as obesity, diabetes, and chronic kidney disease spread at a very rapid pace, and, since these conditions are also age-related, this trend is destined to continue, considering the epidemiological transition towards the aging of the population in developed countries[4].

Although it has greatly evolved in recent years, the management of CVDs is still generally conducted with a "one size fits all" approach, whereby all patients are treated with a gold standard medical regimen based on the best evidence[5]. This system certainly makes it possible to standardize disease management, however, especially in some cases, the specific characteristics of the patients would require a more personalized diagnostic and therapeutic evaluation, and also a more person-centred risk assessment.

Biomarker and imaging testing have become a crucial part of CVDs management, from diagnosis to prognostic estimation and surveillance, providing independent and complementary information. Nevertheless, these tools have often been considered separately, due to the lack of accurate and cost-effective methods to integrate the specific information into comprehensive algorithms. In addition, the ranges of “normal” for both biomarkers and imaging testing can be broader or borderline in specific populations, due to concomitant conditions, causing reduced specificity[6]. Indeed, comorbidities can significantly affect the results of tests and dosages, complicating clinical decisions and influencing the choice of more or less conservative therapeutic options.

As matter of the fact, the biology of the organism is complex, and, despite the great progress in diagnostic techniques in recent decades, many pathophysiological mechanisms underlying CVDs still remain unclear, as well as the very role of diagnostic tools in the management of the different phenotypes of the pathologies, and proper monitoring of therapies[7]. Adequate diagnosis of CVDs,

together with better understanding of the pathways involved, would lead to the development of more cost-effective management algorithm, and the identification of new care strategies.

This scenario is even particularly complex in the management of older adults, who are characterized by extreme heterogeneity and higher degree of homeostatic patterns variability, which is reflected in a wide range of phenotypic manifestations of late life, from successful aging to disability[8]. In particular, frailty and intrinsic capacity are two cornerstones of geriatric medicine, essential to consider in the assessment of older patients, especially when affected by systemic impact conditions such as CVDs[9]. This is especially important because the enhanced variability of physiological variables, together higher prevalence of comorbidities and geriatric syndromes may mislead about interpretation of typical CVDs evaluations and affect the outcome of the proposed therapies.

In the era of personalized medicine and patient-tailored care, it is crucial to integrate interdisciplinary knowledge in the management of CVDs, in order to achieve an integrated and multi-comprehensive approach for each patient. In the present thesis, the need and potential benefits to supplement specific imaging techniques in the management of patients with heart failure are discussed in section 1. Emerging circulating biomarkers that can support in the diagnosis, even differential, of this complex syndrome, as well as in the prognostic estimation, are proposed in section 2. The role of comorbidities and specific characteristics of aging of the cardiovascular system are also discussed in sections 3 and 4, with a focus on the specific resources of geriatric medicine to be implemented in the care of patient with CVDs.

Section 1

^{123}I-meta-IodineBenzylGuanidine imaging for the assessment of cardiac adrenergic innervation in heart failure

Heart failure (HF) constitutes a complex clinical syndrome, the end stage of several CVDs. It is associated with high morbidity and mortality, exerting a great impact on quality of life and constituting a major cost for healthcare systems[10]. The global prevalence of HF is 1–2% and increases with age; although the great progresses in diagnosis and therapies over the last decades, its mid-long-term prognosis is still poor[11].

The activation of neurohormonal systems, such as the sympathetic nervous system (SNS) and the renin–angiotensin–aldosterone system (RAAS), initially acts as compensatory mechanism to support cardiac function, but turns detrimental in the long term, promoting maladaptive ventricular remodelling (increased myocardial fibrosis and apoptosis), impaired tolerance to exercise, alterations in β-adrenergic receptor (β-AR) signalling, thus resulting in increased risk of fatal arrhythmias[12]. Indeed, higher levels of norepinephrine (NE) are detected in patients with HF, as epiphenomenon of sign of altered neuronal release and reuptake[13]. Accordingly, the current guidelines recommend Implantable Cardiac Device (ICD) implantation for the prevention of sudden cardiac death (SCD) in HF patients with left ventricle ejection fraction (LVEF) <35%[10]. Nevertheless, many ICD recipients never receive benefit from device therapy, which exposes them to not negligible device-related complications and/or adverse events, including inappropriate shocks, lead failure and infections[14]. Moreover, current indication does not adequately identify patients with HF and LVEF >35% at risk for SCD.

Accordingly, considerable efforts have been made in order to identify novel factors concurring to the risk of SCD in HF patients, and the assessment of cardiac adrenergic innervation has been proven to provide relevant diagnostic and prognostic information in this context[15–17]. In particular, ^{123}I-meta-IodineBenzylGuanidine (^{123}I-MIBG) imaging constitutes a useful and non-invasive tool to assess heart SNS, evaluate disease progression, and stratify prognosis in patients with HF[18], emerging

as a useful tool to screen patients with HF eligible for ICD implantation and identify those who might not benefit from this therapy[19].

Comorbidities and concomitant chronic conditions may affect SNS in HF, with an impact on disease progression and outcome, and also the efficacy of therapeutic approaches as ICD, therefore cardiac imaging with ^{123}I-MIBG represents a useful tool to guide the management of these patients, especially in particularly challenging clinical cases[20].

In this section, the role of ^{123}I-MIBG imaging in the management of patients with HF is explored in relation to particular clinical scenarios, very frequent in daily healthcare practice, such as overweight/obesity (Chapter 1)[21] and polymorbidity (Chapter 2)[22]. Furthermore, the association between eight risk scores currently employed for predicting overall mortality risk, and cardiac sympathetic innervation assessed through myocardial ^{123}I-MIBG imaging, is analysed to test the clinical benefit of integrating complementary prognostic information in the discrimination of HF patients eligible for ICD therapy (Chapter 3)(*).

(: this manuscript has been accepted for publication in European Journal of Clinical Investigation, as it is not yet available online on the publisher's website at the time of submission of the thesis, it is reported in the form of abstract in the Chapter 3)*

Chapter 1

Impact of body mass index on cardiac adrenergic derangement in heart failure patients: a ^{123}I-MIBG imaging study

The prevalence of obesity has increased during the last decades, and the World Health Organization (WHO) has identified it as one of the main public health concerns[23] [14]. It is widely recognized that overweight and obesity predispose to the development of pathological conditions, such as metabolic disorders and cardiovascular diseases; they are also associated with reduced quality of life[24].

Investigation of SNS activity through microneurography, baro- and chemo-reflex sensitivity, and norepinephrine spillover has demonstrated an increase in sympathetic tone in obese individuals[25,26]. These results suggest an association between obesity and SNS hyperactivity, nevertheless the implication of overweight and obesity on cardiac sympathetic drive is far to be completely clarified, in particular in the clinical setting of chronic HF.

Therefore, the aim of the research was to study the impact of Body Mass Index (BMI) on cardiac adrenergic derangement measured by MIBG imaging in a population of HF patients.

Methods

The study enrolled 249 consecutive HF patients referred to the Departments of Translational Medical Sciences and Advanced Biomedical Sciences of the University of Naples Federico II. Inclusion criteria were as follows: (a) ischemic or non-ischemic HF diagnosed at least 6 months earlier; (b) LVEF ≤ 45%; (c) stable clinical conditions during the month prior to inclusion; (d) optimal pharmacotherapy including beta-blockers, angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), anti-aldosterone, diuretics, and digitalis, if necessary and not contraindicated. Exclusion criteria were as follows: (a) acute coronary syndrome in the last 6 months prior to enrollment; (b) cardiac revascularization in the last 6 months prior to enrollment; (c) chronic kidney disease (CKD) with a glomerular filtration rate (GFR) < 30 ml/min.

Demographic and clinical data including age, gender, BMI, cardiovascular risk factors, and comorbidities were also collected. At the time of the enrolment, all patients underwent clinical examination, and a 2-dimensional echocardiography.

Heart adrenergic innervation was assessed through a ^{123}I-mIBG cardiac scintigraphy, performed following previously described standards[27] and the recommendation provided by the European Association of Nuclear Medicine Cardiovascular Committee and the European Council of Nuclear Cardiology recommendations[28].

Results

Characteristics of the overall study population and stratified by BMI values in obese (BMI ≥ 30 kg/m^2) and non-obese (BMI < 30 kg/m^2) patients are reported in Table 1. Obese patients showed a significant reduction in early H/M ratio (1.66±0.19 vs. 1.75±0.26; p=0.008), and a trend to reduction, although not significant, in washout rate (38.1±20.1 vs. 33.6±18.3; p=0.092 33.6±18.3 vs. 38.1±20.1; p=0.092).

	All (n = 249)	BM < 30 (n = 171)	BMI ≥ 30 (n = 78)	p value
Age, mean ± SD	66.4 ± 10.6	67.5 ± 10.5	64.1 ± 10.6	0.021
Gender, male % (n)	84.7 (211)	85.4 (146)	83.3 (65)	0.706
LVEF, mean ± SD	30.7 ± 6.4	30.5 ± 6.6	31.1 ± 6.0	0.521
BMI	28.3 ± 4.3	26.1 ± 2.5	33.2 ± 2.9	≤ 0.0001
NYHA class III, % (n)	31.3% (78)	29.2% (50)	35.9 (28)	0.306
Ischemic etiology, % (n)	69.5% (173)	70.8%(121)	64.7% (52)	0.554
Diabetes, % (n)	38.9% (97)	38.0% (85)	41.0% (32)	0.896
Dyslipidemia, % (n)	62.3% (155)	59.7% (102)	67.9% (53)	0.260
Hypertension, % (n)	75.1% (187)	71.4% (122)	83.3% (65)	0.057
GFR, mean ± SD	70.5 ± 22.4	70.0 ± 22.5	71.9 ± 22.3	0.521
CKD, % (n)	20.1% (50)	21.6% (37)	16.7% (13)	0.399
COPD, % (n)	29.3% (73)	30.4% (52)	26.9% (21)	0.653
ACE-I/ARBs, % (n)	77.5% (193)	73.1% (125)	87.2% (68)	0.014
Beta-blockers, % (n)	73.5% (183)	71.9% (123)	76.9% (60)	0.442
Aldos. antag., % (n)	42.2% (105)	41.5% (71)	43.6% (34)	0.783
Early H/M ratio	1.72 ± 0.24	1.75 ± 0.26	1.66 ± 0.19	0.008
Late H/M ratio	1.53 ± 0.24	1.54 ± 0.26	1.51 ± 0.19	0.380
Washout rate	36.7 ± 19.6	38.1 ± 20.1	33.6 ± 18.3	0.092

Table 1. Characteristics of the overall study population and of groups stratified by BMI

LVEF= left ventricular ejection fraction, BMI= body mass index, NYHA= New York Heart Association, GFR= glomerular filtration rate, COPD= chronic obstructive pulmonary disease, ACE-I= angiotensin converting enzyme inhibitors, ARB= angiotensin AT 1 receptor blockers, Aldos. antag.= aldosterone antagonists; H/M ratio= heart to mediastinum ratio.

The p value correspond to Student T test for continues variables, and chi square test for categorical data.

As reported in figure 1, a significant reduction in early and late H/M ratios has been observed among patients stratified in three BMI groups (≤24.9; 25-29.9 and ≥ 30 kg/m^2), while no significant difference has been detected in washout rate.

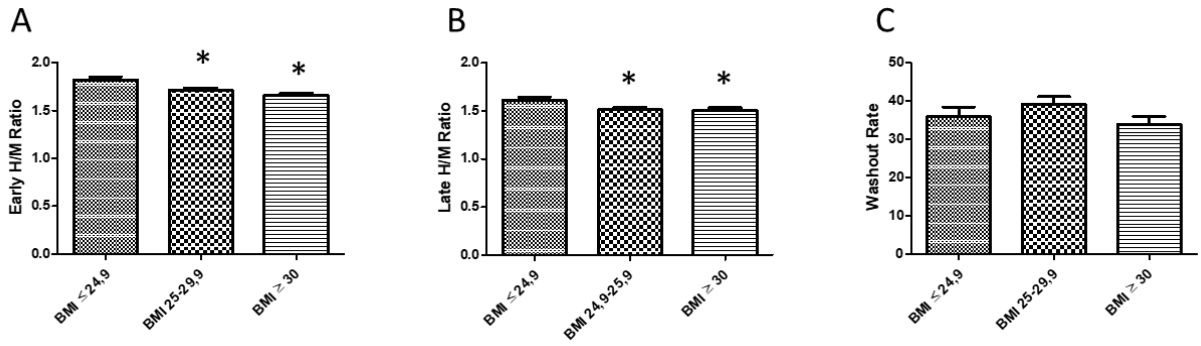

Figure 1. ^{123}I-mIBG imaging parameters in subgroups stratified by BMI.

*Early H/M ratio (panel A), Late H/M ratio (panel B) and Washout rate (panel C) in patients with BMI ≤24.9 (n=59), in the range 25-29.9 (n=108) and ≥ 30 kg/m2 (n=80). Data are presented as mean ±SD. One Way ANOVA with Bonferroni correction has been employed. * p< 0.05 vs. patients with BMI ≤ 24.9.*

At multivariable regression analysis, age, LVEF, HF ischaemic etiology and BMI resulted to be significantly and independently correlated with late H/M ratio (Table 2). The global strength of these associations was 0.23, as defined by R2, which measures the portion of the late H/M ratio variation explained by the regression analysis. Moreover, age, LVEF and BMI came out as significantly correlated with early H/M ratio (R2= 0.12). Washout rate was associated with age and LVEF, presenting a weaker global association (R2= 0.04).

	Global $R^2 = 0.12$					Global $R^2 = 0.23$					Global $R^2 = 0.04$				
	Ref.Coeff	p	R2(f)	BIF (%)	LS	Ref.Coeff	p	R2(f)	BIF (%)	LS	Ref.Coeff	p	R2(f)	BIF (%)	LS
Age	− 0.031	= 0.02	11.9	60.7	100	− 0.066	< 0.0001	31.9	99.3	91.3	4.14	= 0.02	51.5	77.7	100
LVEF	0.039	< 0.0001	34	84.5	100	− 0.065	< 0.0001	45.5	99.7	94	− 2.63	= 0.024	48.5	55	100
Gender	− 0.021	0.588		7.6	NA	− 0.059	0.125		33	NA	3.1	0.366		13.1	NA
NYHA	0.0029	0.368		13.3	NA	0.0004	0.99		3.8	NA	1.2	0.43		11.8	NA
Ischem.Etiology	0.055	0.07		42.4	NA	0.064	0.036	6.6	49.6	NA	4.2	0.12		31.7	NA
BMI	− 0.15	< 0.0001	54.1	99.9	100	− 0.013	< 0.0001	16	96.5	48.8	− 0.26	0.367		12.3	NA
Diabetes	0.021	0.402		12.8	NA	0.01	0.7		4.24	NA	− 1.9	0.393		7.1	NA
Hypertension	0.06	0.102		36.5	NA	0.04	0.25		24.6	NA	− 5	0.08		37.7	NA
Dyslipidemia	− 0.019	0.501		9.4	NA	− 0.022	0.469		15.9	NA	1.4	0.582		18.4	NA
COPD	− 0.041	0.202		25.5	NA	− 0.04	0.264		20.1	NA	− 1.29	0.646		7.8	NA
CKD	0.03	0.41		11.4	NA	0.06	0.214		23.2	NA	− 6.35	0.053		41.3	NA
ACE-I/ARBs	0.06	0.074		40.5	NA	0.042	0.232		22.6	NA	1.02	0.738		6.5	NA
Aldos.antag	− 0.004	0.988		5.6	NA	− 0.03	0.32		20.5	NA	2.9	0.247		21.8	NA
Beta-blockers	0.017	0.618		8.5	NA	− 0.003	0.925		3.9	NA	0.23	0.938		5.1	NA

Table 2. Multivariate Regression of Late, Early H/M and Washout rate.

R^2(f): fraction of the global R^2, BIF: bootstrap inclusion frequency, LS: linear stability, LVEF: left ventricular ejection fraction, Ischaem.Etilogy: Ischaemic Etiology; BMI: body mass index, NYHA: New York Heart Association, CKD: chronic kidney disease, COPD: chronic obstructive pulmonary disease, ACE-I: angiotensin converting enzyme inhibitors, ARB: angiotensin AT 1 receptor blockers, Aldos.antag.: aldosterone antagonists, H/M: heart to mediastinum.

The relationship between age, LVEF and BMI with all MIBG uptake parameters, after adjustment for all the remaining statistically significant variables, are reported in Figure 2. All the relationships analyzed were linear. With increasing age, a progressive decline in early and late H/M ratios and increase in washout rate have been observed (Figure 2, panels A-C), while a progressive increase in early and late H/M ratios and decrease in washout rate have been detected with increasing LVEF (Figure 2, panels D-F). Importantly, linear relationships between BMI and early and late H/M ratios have been noticed, with increasing BMI paralleled with a progressive reduction in early and late H/M ratios (Figure 2, panels G, H). Any linear correlation between washout rate and BMI was not found (Figure 2, panel I).

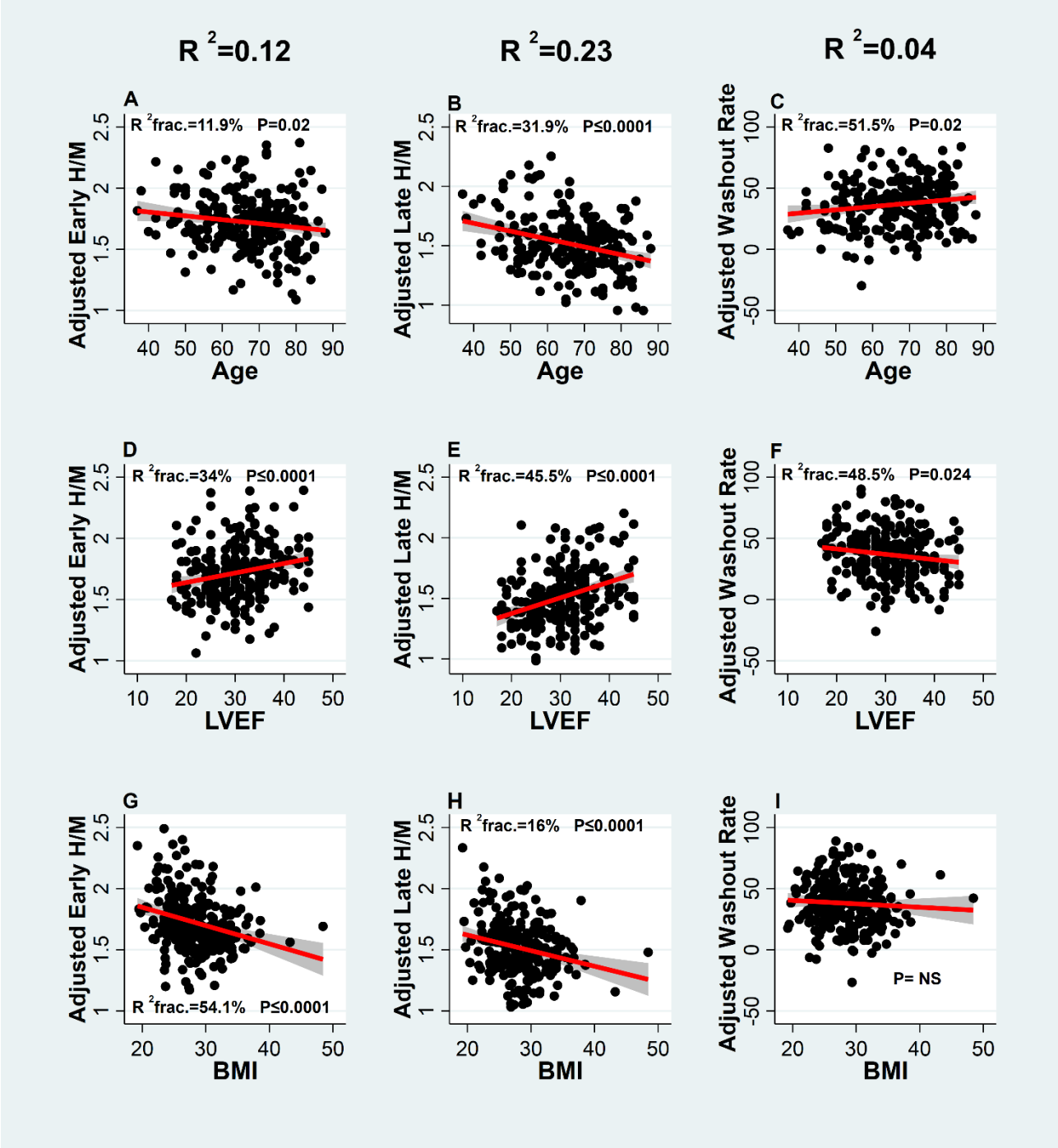

Figure 2. Relationships among age, LVEF, BMI and ^{123}I-mIBG imaging parameters.

Adjusted Early H/M (panels a, d and g), adjusted Late H/M (panels b, h and e) and adjusted Washout rate (panels c, f and g) plotted versus age panels a, b and c), LVEF (panels d, e and f) and BMI (panels g, h and i). In each plot, H/M ratios, washout rate and BMI adjusting was carried out at the mean value of the other significant variables. The shaded area indicates the 95 % confidence interval of the linear fit (continuous line). H/M: heart to mediastinum, LVEF: left ventricular ejection fraction, BMI: body mass index, R^2frac: R^2 fraction.

Conclusive Remarks

BMI is an independent predictor of cardiac mIBG uptake in a population of HF patients, with overweigh and obesity associated with a reduced early and late H/M ratios. If further studies in larger population will confirm these data, BMI should be considered as a factor influencing cardiac sympathetic innervation and the risk of adverse cardiac events in HF patients.

Chapter 2

Impact of the number of comorbidities on cardiac sympathetic derangement in patients with reduced ejection fraction heart failure

HF is frequently associated with comorbidities and chronic conditions, which negatively impact disease progression and outcome[29]. It has been widely recognized that concomitant conditions are often responsible for worsening of HF symptoms and limit the use of guidelines-guided therapies[30]. Of interest, comorbidities, including diabetes mellitus (DM)[31], chronic kidney disease (CKD)[32] and sleep-disordered breathing[27], are known to aggravate HF-related abnormalities in cardiac adrenergic innervation.

Although SNS derangement has been associated with increased incidence of arrhythmic events, several comorbidity-based risk scores indicate that high-risk HF patients present an elevated probability of all-cause mortality but a low risk of arrhythmias or appropriate ICD shocks, an equivalent of SCD in the population undergoing device implantation. Consistently, a meta-analysis has evaluated the impact of comorbidities on the efficacy of primary prevention ICD therapy in patients with systolic HF, highlighting that the presence of more concomitant pathologies/conditions attenuates the benefit on survival of this therapeutic approach[33]. Accordingly, the stratification of systolic HF population, through a simple risk score based on 5 clinical items, revealed that ICD efficacy is limited in the high-risk groups in terms of survival[34].

Even though the negative impact of concomitant cardiovascular and non-cardiovascular diseases on the prognosis of HF patients is widely recognized, whether the number of associated comorbidities may influence cardiac adrenergic innervation in HF has not been adequately investigated. Following these premises, the aim of the present study was to evaluate the impact of the number of comorbidities on cardiac adrenergic innervation, assessed through ^{123}I-mIBG imaging, in patients with systolic HF.

Methods

Participants have been enrolled at the Departments of Translational Medical Sciences and Advanced Biomedical Sciences of the University of Naples "Federico II". Inclusion criteria were: patients aged ≥18 years; diagnosis of HF with altered LVEF <50% from at least 6 months from study enrollment; stable clinical conditions during the month prior to inclusion; optimal pharmacotherapy. Acute coronary syndromes and/or cardiac revascularization in the previous 6 months represented exclusion criteria, together with dialysis-dependent kidney failure and inability to understand and/or consent to study participation.

Enrolled patients underwent medical history collection, accurate clinical examination and evaluation of the main demographic/clinical factors, including body mass index (BMI) and cardiovascular risk factors. Furthermore, transthoracic echocardiography and assessment of the cardiac adrenergic innervation through myocardial scintigraphy with ^{123}I-MIBG were performed in all patients.

In order to test the impact of comorbidities on cardiac adrenergic innervation, the presence of 7 comorbidities/risk factors, considered in the metanalysis by Steinberg and collaborators[33], has been documented in the study population (smoking, COPD, DM, peripheral artery disease, AF, ischemic heart disease [IHD], CKD). Kidney disfunction was defined by a glomerular filtration rate <60 mL/min, according to CKD-EPI equation. Based on the median number of associated comorbidities, the study population was subsequently divided into two groups: 3 or more comorbidities identified the high-risk group while 2 or less comorbidities the low-risk group.

Results

Characteristics of the overall study population, consisting of 269 HF patients. are reported in Table 1. Regarding the distribution of the number of comorbidities in the study population, 11 patients (4%) showed no comorbidity, 47 patients (18%) presented a single comorbidity, 58 (22%) two comorbidities, 87 (32%) three comorbidities, 46 (17%) four comorbidities, 17 (6%) five comorbidities and 3 (1%) six comorbidities. Of note, no patient presented all the seven comorbidities considered.

Characteristics	Overall Population (n=269)
Age mean, ± SD	66.2 ± 10.8
Gender (male), n (%)	227 (84.4)
BMI (Kg/m^2), ± SD	28.4 ± 4.2
LVEF mean, ± SD	31.1 ± 7.0
Early H/M mean, ± SD	1.71 ± 0.24
Late H/M mean, ± SD	1.53 ± 0.25
Washout rate mean, ± SD	35.83 ± 19.85
NYHA II, n (%)	186 (69.1)
NYHA III, n (%)	80 (29.7)
NYHA IV, n (%)	3 (1.1)
β-blockers, n (%)	198 (73.6)
RAAS, n (%)	209 (70.7)
MRA, n (%)	110 (40.9)
Smoking, n (%)	156 (58)
COPD, n (%)	81 (30.1)
PAD, n (%)	41 (15.2)
AF, n (%)	56 (20.8)
IHD, n (%)	187 (69.5)
CKD, n (%)	80 (29.7)
Diabetes, n (%)	110 (40.9)
Arterial Hypertension, n (%)	197 (73.2)
Dyslipidemia, n (%)	168 (62.5)
Pulmonary Hypertension, n (%)	198 (73.6)
Polipharmacotherapy, n (%)	181 (67.3)
Anemia, n (%)	50 (18.6)

Table 1. Characteristics of the overall population

AF, Atrial Fibrillation; BMI, Body Mass Index; COPD, Chronic Obstructive Pulmonary Disease; CKD, Chronic Kidney Disease; H/M, heart to mediastinum ratio; IHD, Ischemic Heart Disease; LVEF, Left Ventricular Ejection Fraction; MRA, Mineralocorticoid Receptor Antagonist; NYHA, New York Heart Association; PAD, Peripheral Artery Disease; RAAS, Renin-Angiotensin-Aldosterone-System; SD, Standard Deviation.

Data on the population stratified by the median value of 3 comorbidities are reported in Table 2. At univariate analysis, the high comorbidity group presented lower late H/M ratio values compared to low comorbidity group (p=0.04), while no differences were evident in early H/M ratio (1.70± 0.24 vs. 1,73 ± 0.24; p=0.40) and washout rate (35.60 ± 18.53 vs. 36.14 ± 21.54) between the two groups.

Characteristics	≥ 3 Comorbidities *(n=153)*	< 3 Comorbidites *(n=116)*	p-value
Age, mean ± SD	68.3 ± 9.4	63.4 ± 11.7	≤ 0.001
Gender (male), n (%)	130 (85)	97 (83.6)	0.446
BMI (Kg/m^2), mean ± SD	28.5 (4.7)	28.2 (3.6)	0.544
LVEF (%), mean ± SD	30.7 (6.7)	31.6 (7.3)	0.311
Early H/M, mean ± SD	1.70 ± 0.24	1.73 ± 0.24	0.402
Late H/M, mean ± SD	1.51 ± 0.21	1.57 ± 0.29	0.047
Washout rate (%)	35.60 ± 18.53	36.14 ± 21.54	0.829
NYHA II, n (%)	93 (60.8)	93 (80.2)	0.020
NYHA III, n (%)	57 (37.2)	23 (19.9)	
NYHA IV, n (%)	3 (2)	0	
β-blockers, n (%)	104 (68)	94 (81)	0.011
RAAS, n (%)	114 (74.5)	95 (81.9)	0.097
MRA, n (%)	91 (59.5)	48 (41.4)	0.493

Table 2. Characteristics of the study population stratified by number of comorbidities.

BMI, Body Mass Index; H/M, Heart to Mediastinum ratio; IHD, Ischemic Heart Disease; LVEF, Left Ventricular Ejection Fraction; MRA, Mineralocorticoid Receptor Antagonist; NYHA, New York Heart Association; RAAS, Renin-Angiotensin-Aldosterone-System; SD, Standard Deviation.

The p value derives from Student's t test for continues variables, and chi square test for categorical data.

At multivariable regression analysis, age, BMI and LVEF came out to be significantly and independently associated with both early and late H/M ratios, while age and LVEF were also corelated to washout rate (Table 3). Importantly, the number of comorbidities did not influence cardiac sympathetic innervation, as it did not show any significant correlation with all the mIBG parameters.

	Late H/M R^{2a}: 0.235			Early H/M R^{2a}: 0.125			Washout Rate R^{2a}: 0.047		
Variables	*B*	*SE*	*Sig.*	*B*	*SE*	*Sig.*	*B*	*SE*	*Sig.*
Gender	-0.054	0.037	0.147	-0.026	0.038	0.496	1.970	3.265	0.547
Age	-0.006	0.001	≤ 0.0001	-0.003	0.001	0.013	0.244	0.117	0.038
BMI	-0.013	0.003	≤ 0.0001	-0.016	0.003	≤ 0.0001	-0.188	0.281	0.505
LVEF	0.013	0.002	≤ 0.0001	0.008	0.002	≤ 0.0001	-0.602	0.173	≤ 0.001
Comorbidities*	-0.010	0.011	0.373	0.004	0.011	0.691	-0.317	0.973	0.745

Table 3. Regression analysis for ^{123}I-mIBG cardiac scintigraphy parameters.

*BMI, Body Mass Index; LVEF, Left Ventricle Ejection Fraction; R2a, Adjusted R2; SE, Standard Error; *Comorbidities included: Smoking, Chronic Obstructive Pulmonary Disease, Diabetes Mellitus, Peripheral Artery Disease, Atrial Fibrillation, Ischemic Heart Disease and Chronic Kidney Disease*

Conclusive Remarks

Since myocardial denervation is known to increase the arrhythmic risk in HF patients, these results may be consistent with the observation that an elevated number of comorbidities attenuates the benefit of ICD therapy in HF patients. Indeed, very comorbid HF patients display a high risk of all-cause death but may not present an elevated risk of SCD, comorbidities do not confer an additional contribute to the denervation of the failing heart. Accordingly, the study of adrenergic innervation may be essential in clinical decision making in this setting.

The present study shows that the number of comorbidities does not influence cardiac adrenergic innervation assessed through ^{123}I-mIBG scintigraphy, which is in turn affected by age, BMI and LVEF. These data may explain, at least in part, the previous observation that very comorbid HF patients present a lower arrhythmic risk and receive less benefit from ICD therapy compared to HF patients with a low number of concomitant associated pathologies.

Chapter 3

Cardiac sympathetic innervation and mortality risk scores in patients with heart failure

Background

Cardiac dysfunction represents a key feature of chronic Heart Failure with reduced Ejection Fraction (HFrEF) and exerts a crucial role in the onset, progression and prognosis of this syndrome. Critical clinical implications derive from these pathophysiological mechanisms, as chronic systolic dysfunction is burdened with high arrhythmic mortality risk[35]. Accordingly, international societies guidelines recommend therapy with Implantable Cardioverter Defibrillator (ICD) for primary and secondary prevention of Sudden Cardiac Death (SCD) in well-selected patients[10,36]. Nevertheless, the proportion of those who actually benefit from this treatment, which is not free from complications such as infections and inappropriate shocks, is low, and, importantly, several reports indicate the rate of non-arrhythmic deaths to significantly negatively impact on devices utility, especially in multimorbid patients[34].

Scientific community has focused on the development of tools to ameliorate risk stratification and increase the accuracy of the selection of candidates for ICD therapy. ^{123}I-meta-IodineBenzylGuanidine (^{123}I-mIBG) scintigraphy has emerged as effective non-invasive imaging method to assess cardiac adrenergic innervation, with independent role in predicting HF decompensation, major arrhythmic events, cardiac mortality and even appropriate ICD intervention[37]. Similarly, several clinical risk scores and models have been proposed to identify patients with HF at the highest risk of all-cause mortality, for whom the net clinical benefit of device positioning would presumably be lower[38].

Nevertheless, the association between the two classes of tools, one suggestive of significantly increased arrhythmic risk, the other of all-causes mortality, has not yet been adequately investigated.

Objective

Therefore, the aim of the present study was to test the relationship between the main risk scores for predicting mortality and cardiac sympathetic innervation, assessed through myocardial ^{123}I-MIBG imaging, in a population of HF patients with LVEF <50%.

Methods

Participants were recruited from patients referred to the Departments of Translational Medical Sciences and Advanced Biomedical Sciences of the University of Naples "Federico II". Inclusion criteria listed: adult patients able to understand study protocol and consent to participation; diagnosis of HF with LVEF <50%, at least 6 months before enrollment; stable clinical conditions during the month prior to inclusion; optimal pharmacotherapy according to European Society of Cardiology (ESC) Guidelines[10]. Exclusion criteria were acute coronary syndromes and/or cardiac revascularization in the previous 6 months, congenital heart diseases and dialysis-dependent kidney failure.

Eight risk stratification models were identified, through literature research, as applicable to the study population: AAACC[39], FADES[40], MADIT[34], MADIT-ICD non-arrhythmic mortality score[41], PACE[42], Parkash[43], SHOCKED[44] and Sjoblom[45]. Overview of the employed models, with list of variables and scores, is reported in Table 1.

Risk models	**Variables and scores**
AAACC[39]	➢ Age > 75 years (3 points) ➢ CKD (3) ➢ Anaemia (2) ➢ AF (1) ➢ COPD (1)
FADES[40]	➢ 65 < Age < 75 (0.5) or Age ≥ 75 years (2) ➢ NYHA ≥ III (1) ➢ Diabetes mellitus (1) ➢ LVEF ≤ 25% (1) ➢ Smoking (1)
MADIT[34]	➢ NYHA ≥ III (1) ➢ Age > 75 years (1) ➢ BUN > 26 mg/dL (1) ➢ QRS duration > 0.12 sec (1) ➢ AF (1)
MADIT-ICD non-arrhythmic[41]	➢ Age ≥ 75 years (2) ➢ BMI < 23 Kg/m^2 (2) ➢ LVEF ≤ 25% (2) ➢ AF (2) ➢ NYHA ≥ II (1) ➢ Diabetes mellitus (1)

	➢ CRT-D (-1)
PACE[42]	➢ Age ≥ 70 years (1) ➢ Serum creatinine ≥ 2.0 mg/dL (1) ➢ LVEF ≤ 20% (1) ➢ PAD (1)
Parkash[43]	➢ NYHA ≥ II (1) ➢ Age ≥ 80 years (1) ➢ Serum creatinine ≥ 1.8 mg/dL (1) ➢ AF (1)
SHOCKED[44]	➢ CKD (100) ➢ Age ≥ 75 (62) ➢ COPD (62) ➢ Diabetes mellitus (41) ➢ NYHA ≥ II (36) ➢ LVEF ≤ 20% (28) ➢ AF (27)
Sjoblom[45]	➢ NYHA ≥ II (1) ➢ Age > 70 years (1) ➢ Serum creatinine > 106 μmol (1) ➢ QRS duration > 0.12 msec (1) ➢ Diabetes mellitus (1) ➢ AF (1)

Table 1. Overview of mortality risk models with individual variables and scores

AF, Atrial Fibrillation; BMI, Body Mass Index; BUN, Blood Urea Nitrogen; COPD, Chronic Obstructive Pulmonary Disease; CRT-D, Cardiac Resynchronization Therapy; CKD, Chronic Kidney Disease; LVEF, Left Ventricular Ejection Fraction; NYHA, New York Heart Association functional class; PAD, peripheral artery disease.

Heart adrenergic innervation was assessed through a ^{123}I-MIBG cardiac scintigraphy, as previously described. Abnormal cardiac innervation was defined as late H/M <1.6 based on previous literature[46].

Results

Overall population consisted of 269 patients suffering from HF. As reported in table 2, very weak negative correlation for early H/M only emerged with FADES (r = -0.12, p = 0.047) and SHOCKED (r = -0.15, p = 0.018) scores. Late H/M showed significant negative correlation with all the predicting models, although generally weak, ranging from -0.15 (p = 0.013) for PACE to -0.32 (p < 0.001) for FADES. Similar results were obtained for the washout rate, whose stronger positive relationship emerged with FADES score (r = 0.17, p = 0.004), and other significance tresholds

reached with lower coefficients for MADIT, MADIT-ICD non-arrhythmic mortality score and Sjoblom.

Mortality Risk Scores	*Early H/M*	*Late H/M*	*Washout Rate*
AAACC	-0.075	-0.170	0.027
sig.	0.244	0.008	0.676
FADES	-0.120	-0.319	0.174
sig.	0.047	<0.001	0.004
MADIT	-0.011	-0.176	0.141
sig.	0.852	0.003	0.020
MADIT-ICD-NA	-0.024	-0.194	0.169
sig.	0.695	0.001	0.005
PACE	-0.099	-0.151	0.033
sig.	0.105	0.012	0.580
Parkash	-0.073	-0.152	0.078
sig.	0.231	0.012	0.199
SHOCKED	-0.155	-0.286	0.101
sig.	0.010	<0.001	0.097
Sjoblom	-0.082	-0.219	0.124
sig.	0.177	<0.001	0.041

Table 2. The relationship between cardiac ^{123}I-mIBG imaging parameters and all-cause mortality scores.

p value corresponds to Pearson's r correlation coefficient

All the scores showed poor discrimination for cardiac denervation, defined as late H/M <1.6, with areas under the curve (AUC) ranging from 0.546 for Parkash to a maximum of 0.621 for FADES (Figure 1).

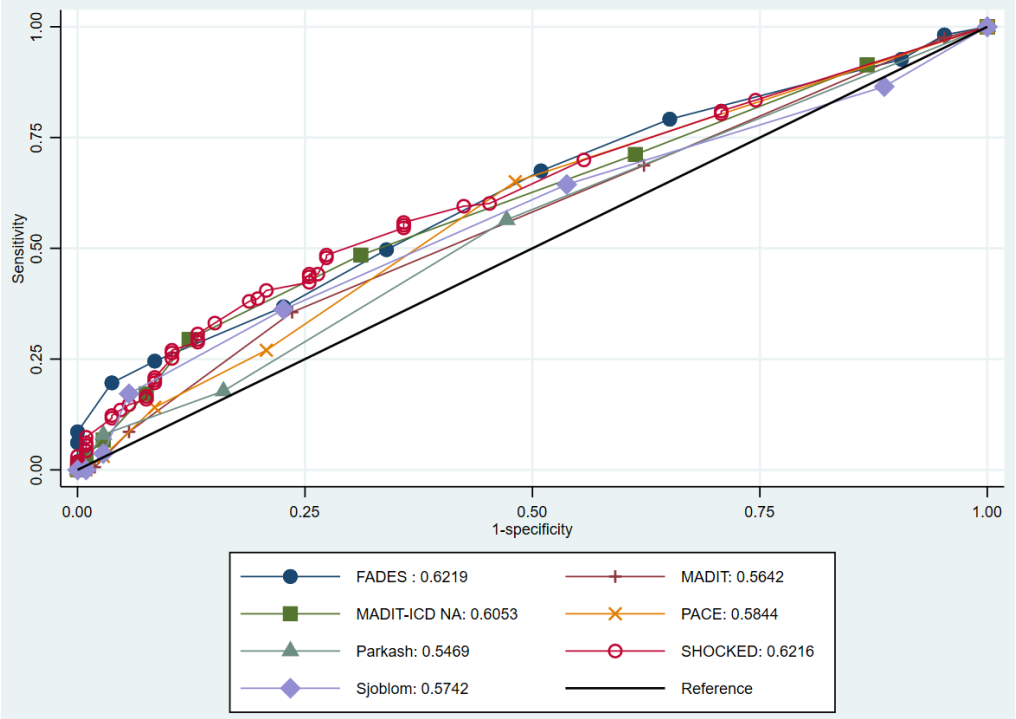

Figure 1. ROC curves of mortality risk scores for impaired cardiac innervation

Conclusive Remarks

In a population of HF patients, a weak association has been observed between cardiac innervation, assessed through ^{123}I-MIBG parameters, and eight all-cause mortality risk scores. This study suggests a poor discriminatory power of the stratification models, validated for overall mortality risk assessment, in the evaluation of altered cardiac adrenergic innervation. Thus, these results unveil the opportunity to integrate in the clinical practice tools assessing both arrhythmic- and overall-mortality risks, especially in the management of HF patients with unclear eligibility for ICD implantation (figure 2).

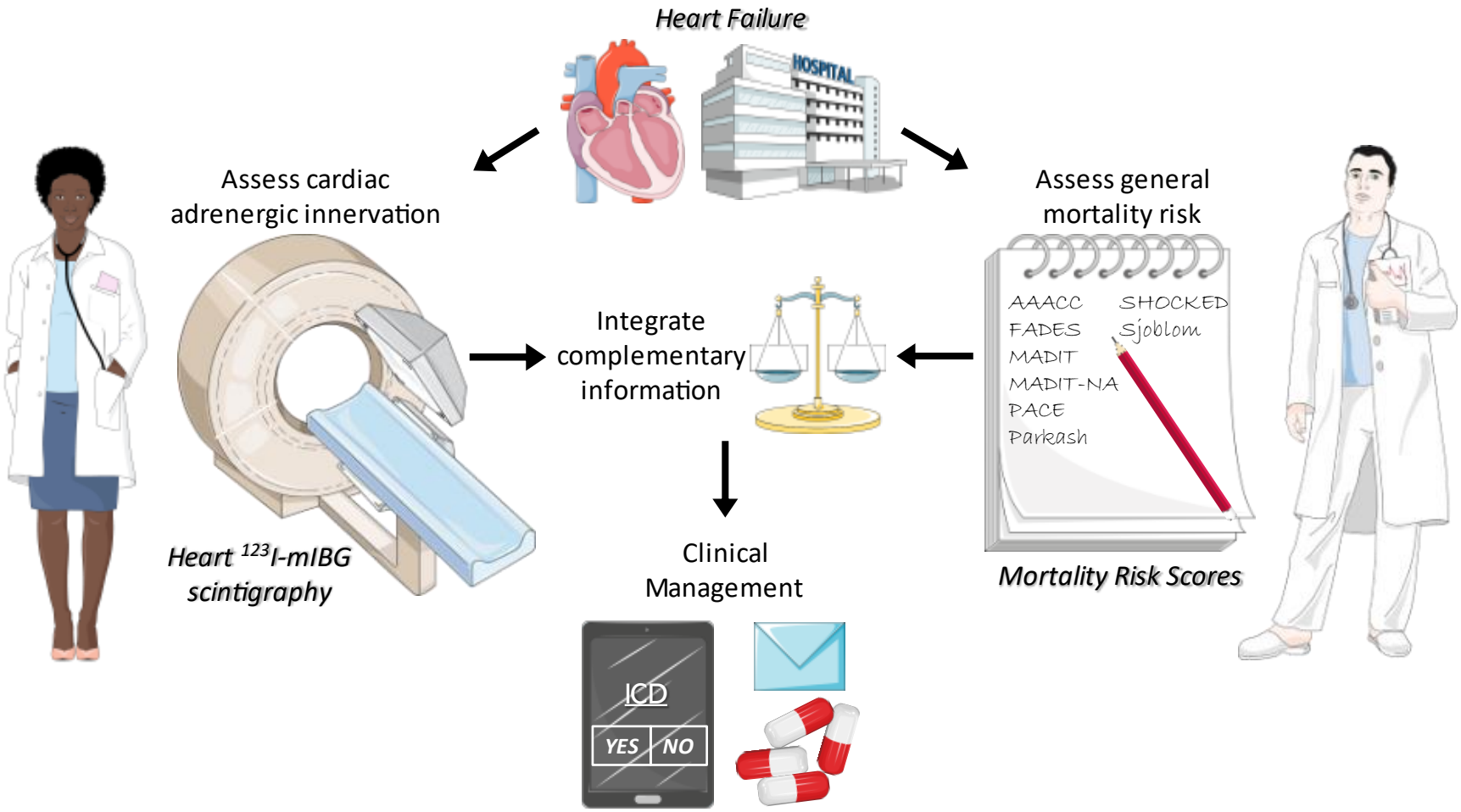

Figure 2. Integrated approach to HF patients with unclear eligibility for ICD implantation.

Section 2

Circulating biomarkers in heart failure: emerging pathophysiological mechanisms and phenotyping

The development of HF determines micro and macrostructural modifications, each involving the activation of different signalling pathways, including the inflammatory and neurohormonal systems, which in turn determine the release of different biomolecules with the aim of compensating the failing heart failure. As a consequence, large amounts of cytokines and regulatory molecules are released into blood vessels, constituting potential circulating biomarkers[47]. Indeed, a biomarker is defined as an easily accessible and measurable biological compound with the following characteristics: the accuracy of the method employed to measure; the tests used to measure the new biomarker should be robust; the compound should reflect a relevant pathophysiological pathway concurring to HF; the biomarker should provide additional information to those collectable through routine physical examination and laboratory evaluation; the biomarker should provide additional clinical judgment for deepen the diagnosis, prognosis, or management of this syndrome[48].

Beyond the natriuretic peptides, widely used in the diagnosis and follow-up of patients with HF, both HFrEF and preserved ejection fraction (HFpEF), in the past decades several biomarkers have been proposed to improve understanding of the complex syndrome process, but their clinical value is still uncertain. As matter of the fact, ESC guidelines on the management of HF list among gaps in evidence: the need for studies "on the role of biomarkers, focusing on their additive value in the diagnosis of HF" and "on biomarkers showing the impact on outcome of their measurements for the identification of subjects at risk of developing HF as well as to guide treatment in patients with HF"[10]. Therefore, it is essential to identify novel circulating biological markers with the aim to improve risk stratification in specific populations, support HF phenotyping and provide care personalization.

In this section, some evidence is reported to support the potential use of new circulating biomarkers of HF, starting from a literature review on the possible use of lymphocyte G protein-coupled receptor kinase 2 (GRK2) levels as an indicator of the activation of SNS in HF (Chapter 4)[49],

complementing the topics addressed in the previous part. Afterwards, the impact of circulating galectin-3 levels in older adults with HFrEF is tested in relation to their frailty status (Chapter 5)[50], anticipating a theme of geriatric medicine that will be treated in the following sections. Moreover, due to the ascertained role of inflammatory and immunomodulatory patterns in HF onset and progression, several regulators of vascular permeability and inflammation have been measured in HF patients, in order to analyse the potential of these molecules to discriminate between the different aetiologies (Chapter 6)[51] and phenotypes (Chapter 7)[52] of this complex syndrome.

Chapter 4

Why Do We Not Assess Sympathetic Nervous System Activity in Heart Failure Management: Might GRK2 Serve as a New Biomarker?

The hyperactivation of the SNS observed in HF is related to increased risk of arrhythmias and left ventricular dysfunction, and it represents a pathophysiological prerequisite for therapy with β-blockers[53]. Indeed, alteration of the complex β adrenergic receptor (β-AR) signalling exerts a pivotal role in HFrEF onset and progression, in particular the crucial interplay of GRK2 with other involved molecules has been extensively documented[17] (Figure 1).

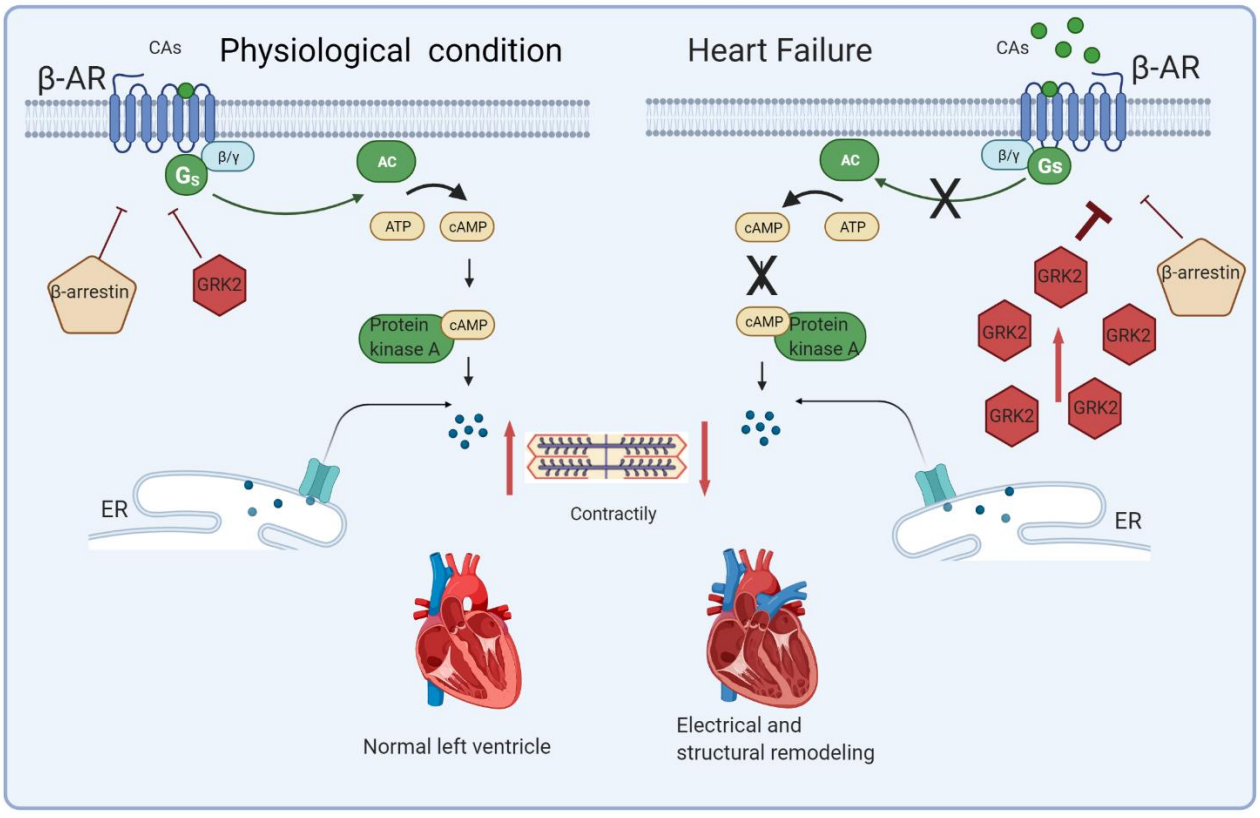

Figure 1. Graphical representation of β-AR signaling in physiological condition (left) and HF (right).

In physiological condition (left panel) β-AR stimulation by CA results in the dissociation of the stimulatory G-protein α-subunit (Gαs) from Gβγ. Gαs stimulates adenylate cyclase (AC) to produce

cAMP, which leads to increased contractility by activating protein kinase A (PKA). The G-protein–coupled receptor kinase (GRK2) translocates to the membrane and phosphorylate agonist-bound β-AR, leading to the decoupling of G protein. Finally, β-arrestins binds the complex triggering receptor internalization and downregulation. In heart failure (right panel) the increase in CA levels determines β-AR hyperactivation and consequent GRK2 up-regulation, which in long term results in βAR dysfunction. (AC, Adenylate Cyclase; ATP, Adenosine Triphosphate; CAs, Catecholamines; β-Ars, β-adrenergic receptors; cAMP, cyclic adenosine monophosphate; ER, Endoplasmic Re-ticulum; GRK2, G-protein–coupled Receptor Kinase 2; Gs, stimulatory G protein). Created with BioRender.com

Several tools are available to provide an estimation of SNS activity in HF, and these include cardiovascular reflex tests, resting heart rate, heart rate variability (HRV) measures, NE spillover, clinical microneurography, cardiac ^{123}I-MIBG and ^{11}C-hydroxyephedrine (^{11}C-HED) imaging, pupillometry and blood biomarkers.

Nevertheless, the techniques currently employed in the evaluation of adrenergic derangement of patients with HF are burdened by poor performance or intrinsic limits of the method that hinder their diffusion and large-scale use, confining them to research protocols or few specific clinical conditions. The main criticalities are the poor ability to discriminate against the sinoatrial response of sympathetic/vagal stimulation, the non-specificity for cardiac sympathetic modulation, the costs of machinery and tracers and the exposure of patients to radionuclides. Accordingly, on top of the above-mentioned tools to non-invasively assess SNS function in HF patients, an expanding field in this area is the one related to the evaluation of blood biomarkers (Table 1). However, they are slow to be implemented in the routine practice for several limitations due to their assessment and clinical impact.

In this context, lymphocyte GRK2 levels reflect myocardial β-adrenergic receptor function in HF and have been shown to add independent prognostic information related to ANS overdrive.

Biomarkers	Cut Off Values	Production	Increasing in	Hf Phenotype	Role
BNP	>35 pg/mL	released from myocytes under stress	HF, Aging, LVH, CKD, AS, MI, AF, Obesity	HFrEF > HFpEF	Diagnosis, Prognosis, Follow up
NT PRO BNP	>125 pg/mL	fragment of BNP precursor	HF, Aging, LVH, CKD, AS, MI, AF	HFrEF > HFpEF	Diagnosis, Prognosis, Follow up
MR PRO ANP	>127 pmol/L	atrial wall as result of stretch	HF, AS, Sepsis, MI, AF, Burns	HFrEF > HFpEF	Diagnosis, Prognosis, Follow up
HS-CIN TROPONIN	>34.2 pg/mL	cardiomyocytes injury	HF, MI, Myocarditis, CKD, Sepsis, Hypothyroidism, Trauma	HFrEF > HFpEF	Diagnosis, Prognosis
GALECTIN 3	<17.8 ng/mL	fibroblast proliferation and activation	HF, Aging, DM, CKD, IPF, Obesity, Cirrhosis, Cancer, Inflammatory states	HFpEF > HFrEF	Prognosis
MR-PROADM	0.10–0.64 nmol/L	released in several tissue as result of increased pressure and volume overload	HF, MI, CAD, Hypertension, CKD, Sepsis, Cancer	HFmrEF	Prognosis
ST2	>30 ng/mL	myocardial stretch	HF, CAD, IS	HFpEF > HFrEF	Prognosis
NGAL	50 ng/mL	neutrophils and endothelial cells, involved in response renal injury	HF, RI	HFrEF > HFpEf	Diagnosis, Prognosis
IRON DEFICIENCY	Ferritin <15 µg/L	multifactorial condition	HF, IDA, IM, Bleeding	HFrEF > HFpEF	Prognosis
NE	>480 pg/dl	Neuroendocrine cells as result of sympathetic overdrive	HF, MI, Hypertension, Pheochromocytoma, Cushing, Stress	HFrEF>HFpEF	Prognosis
NPY	>130 pg/mL	Neuroendocrine cells as result of sympathetic overdrive	HF, Obesity, Stress	HFrEF	Prognosis
GALANIN	To be determined yet	Neuroendocrine and gastrointestinal cells	HF, Hypertension, Pain, SL, Cancer	HFpEF	To be further elucidated
CHROMOGRANIN A /CST	>19.73 ng/mL	Neuroendocrine and myocardial cells	HF, CAD, Sepsis	Independent from LVEF	Prognosis

Table 1. Summary of the main HF biomarkers.

AF: Atrial Fibrillation; AS: Aortic Stenosis; BNP: Brain Natriuretic Peptide; CAD: Coronary Artery Disease; CKD: Chronic Kidney Disease; CST: Catestatin; DM: Diabetes Mellitus; HF: Heart Failure; HFmrEF: Heart Failure with Mid Range Ejection Fraction; HFpEF: Heart Failure With Preserved Ejection Fraction; HFrEF: Heart Failure With Reduced Ejection Fraction; HS-CIN: High Sensitivity Cardiac Troponin; IDA: Iron deficiency anaemia; IM: Intestinal Malabsorption; IPF: Idiopathic Pulmonary Fibrosis; IS: Inflammatory States; LVEF: Left Ventricular Ejection Fraction; LVH: Left Ventricular Hypertrophy; MI: Myocardial Infarction; NE: norepinephrine; NGAL: Neutrophil gelatinase associated lipocalin; NPY: Neuropeptide Y, PAH: Pulmonary Arterial Hypertension; RI: Renal Injury; SL: Sleep Regulation; ST2: Soluble Suppression of Tumorigenicity.

Lymphocyte GRK2 as biomarkers of HF

Sustained SNS hyperactivity determines enhanced cardiac GRK2 expression, which in turn results in β-AR downregulation/desensitization. Besides the relevant therapeutic implications of GRK2 inhibition in HF, cardiac GRK2 expression may provide relevant information regarding post-myocardial infarction (MI) cardiac remodelling and HF progression. Indeed, GRK2 protein levels, more closely reflecting sustained hyperactivation of β-AR by catecholamines (CAs), may represent a more stable surrogate of adrenergic nervous system hyperactivity than circulating norepinephrine (NE) levels, adding important information on cardiac βAR function[17]. Notably, it has been demonstrated that GRK2 levels, measured in HF patients' peripheral lymphocytes, mirror kinase expression in the myocardium, reflecting the loss of βAR responsiveness, the degree of cardiac dysfunction and the severity of the syndrome. Moreover, a direct correlation between lymphocyte GRK2 levels and peripheral NE circulating levels as been reported, and also an inverse correlation with cardiac βAR function in patients with HF. This study paved the way for a potential clinical application of lymphocyte GRK2 levels, allowing to speculate on white blood cells as a surrogate of cardiac GRK2 in HF patients[54].

Repeated evidence supports the role of white cell GRK2 to serve as a biomarker in patients with chronic HF, to both guide specific therapies and predict outcomes[55–58]. Moreover, GRK2 has the potentiality to add information, over the currently available biomarkers, on HF-related adrenergic nervous system hyperactivity and on its implication on cardiac βAR signalling function, which owns a crucial role in HF pathophysiology. White cell GRK2 levels have been reported to also increase in acute ST-segment elevation MI patients and to predict post-MI cardiac remodelling[59].

Conclusive remarks

One of the pillars of HF is represented by SNS hyperactivation, which importantly impacts patients' outcomes. To date, the assessment of adrenergic activity in HF is challenging due to the intrinsic features of both the proposed instrumental tools and tested biomarkers, which limit the exploration of this pathological mechanism in the affected patient. Given the ability to reflect the myocardial β-AR signalling pathway, the evaluation of lymphocyte GRK2 levels has been shown to provide prognostic in-formation related to sympathetic overdrive, additional and independent from other biomarkers. Further evidence will be needed to ascertain the effectiveness of adrenergic biomarkers in HF prognostic stratification and probably to develop other tools for implementation of ANS assessment in routine clinical practice.

Chapter 5

Impact of Galectin-3 Circulating Levels on Frailty in Elderly Patients with Systolic Heart Failure

Frailty is a geriatric syndrome characterized by a multidimensional and cumulative decline in many organs and systems, thus contributing to increased vulnerability to stressors and negative outcomes[8]. Within the elderly patient population, HF represents a relevant trigger for vulnerability and frailty development. Furthermore, frailty is particularly common in HF patients, with a prevalence ranging from 30 to 52%[60,61]. The burden of frailty in hospitalized patients with HF is described as accounting for 56 to 76%, higher than the frailty prevalence in community or non-community elderly without HF[62].

Inflammation has been reported to be a key regulator of HF onset and progression, and the identification of cardio-inflammatory phenotypes among HF patients may have relevant future implications, even regarding the therapeutic approaches[63]. Hypoperfusion and chronic congestion can contribute to tissue hypoxia, cellular apoptosis or necrosis, and the upregulation of inflammatory pathways. In turn, inflammation has also been associated with the pathophysiology of frailty, mainly through the activation of metabolic pathways and a direct influence on protein degradation[64].

Galectin-3 (Gal-3), a β-galactoside binding lectin, plays a significant role in systemic inflammation, cardiac fibrosis and HF progression[65,66]. Elevated Gal-3 levels are associated with negative outcomes, while the inhibition of Gal-3 has been described as improving cardiac remodeling in experimental models of systolic HF[67]. Gal-3 levels increase with age and have been associated with comorbidities[68].

Therefore, considering the role of Gal-3 in the systemic inflammation and cardiac fibrosis process, the objective of the present study was to investigate the association between Gal-3 and frailty in a population of elderly HF patients.

Methods

The population consisted of 128 consecutive elderly HF patients with systolic dysfunction, admitted at the Cardiac Rehabilitation Unit of the Salvatore Maugeri Foundation IRCCS, Institute of Veruno, Italy. The inclusion criteria were: (a) age ≥ 65 years; (b) diagnosis of HF from at least six months, due to ischemic or non-ischemic aetiology; (c) LVEF ≤ 40%; (d) stable clinical conditions for at least one month before enrolment; and (e) guidelines-based optimal medical therapy. The exclusion criteria listed: (a) chronic inflammatory diseases or ongoing infectious diseases; (b) severe renal or hepatic function impairment; (c) malignancies; (d) psychiatric disorders; (e) terminally ill patients; and (f) patients not able to understand the scope and methods of the study and/or to sign the informed consent.

At the time of enrolment, all patients underwent a complete clinical examination, including an assessment of the New York Heart Association (NYHA) functional class and an echocardiography to evaluate LVEF, a structured interview to collect data regarding demographic features, cardiovascular risk factors, comorbidities and HF medications, and a blood draw to laboratory tests, including a full blood count, creatinine, electrolytes, CRP, Gal-3 and N-terminal -pro-Brain Natriuretic Peptide (NT-proBNP).

Frailty was assessed through the Clinical Frailty Scale (CFS)[69]. This visuo-analogic scale includes the evaluation of several domains, such as cognition, mobility, function and co-morbidities, through a direct examination of history and medical records. According to their functional capacity, level of dependence and comorbidities, patients are scored from 1 to 9 points: scoring 1–4 classifies non-frail individuals, whereas 5–8 classifies frail patients, and a score of 9 identifies terminally ill patients (not included in this study). The Cumulative Illness Rating Scale (CIRS) was also administered to all patients, and the CIRS-Comorbidity Index (CIRS-CI) was calculated based on the count of the organ system with moderate to greater impairment[70].

Results

The characteristics of the overall study population, stratified by the presence or the absence of frailty, are reported in Table 1. Compared to non-frail patients, frail patients were older ($p = 0.008$), with a worse kidney function ($p = 0.001$), and they had lower haemoglobin levels ($p = 0.001$). Importantly, frail patients showed worse LVEF ($p = 0.02$), a worse NYHA functional class ($p < 0.0001$), and higher NT-proBNP levels ($p = 0.002$). Of note, higher CRP ($p < 0.0001$) and Gal-3 levels ($p < 0.0001$) characterized frail patients. The linear correlation analysis of Gal-3 vs. CFS

revealed a significant ($p < 0.0001$) progressive increment of the biomarker levels associated with a CFS score increase, with an estimated 5.7 ng/mL increase of Gal-3 for one unit increase of the score.

Characteristics	All Population (N = 128)	Frail (N = 54)	Non- Frail (N = 74)	*p*-Value
Age, years	69.2 ± 4.8	70.5 ± 5.4	68.2 ± 4.2	0.008
Gender M, N (%)	112 (87.5)	45/54 (83.4)	66/73 (90.4)	0.28
BMI, kg/m^2	25.4 ± 4.3	24.5 ± 4.6	26.0 ± 4.2	0.07
WBC/µL,	7833 ± 2623.5	8104.7 ± 4145.9	7430.6 ± 2099.6	0.78
Hbg g/dL	12.8 ± 1.5	12.2 ± 1.6	13.6 ± 1.7	0.001
Fibrinogen	400.2 ± 65.8	405.2 ± 112.4	394.8 ± 108.3	0.75
Na mmol/L	138.4 ± 3.2	138.3 ± 4.5	138.7 ± 3.8	0.68
GFR mL/kg	65.2 ± 18.4	59.1 ± 20.4	70.2 ± 14.8	0.001
CRP mg/L	7.9 ± 10.7	13.4 ± 13.8	3.7 ± 4.2	<0.0001
LVEF %	28.7 ± 8.5	26.7 ± 6.1	30.2 ± 10.2	0.02
NT proBNP pg/mL	5922.4 ± 15,099.9	11,427.9 ± 21,803.4	1856.4 ± 3570.1	0.002
Galectin-3 ng/mL	22.8 ± 16.9	34.4 ± 19.3	14.3 ± 7.6	<0.0001
Hypertension, N (%)	80 (62.5)	37/54 (68.5)	43/74 (58.1)	0.23
Dyslipidemia, N (%)	108 (84.3)	43/54 (79.6)	65/74 (87.8)	0.21
NYHA class III,IV N(%)	58 (54.7)	35/54 (64.8)	23/74 (31.1)	<0.0001
CIRS-CI	3.73 ± 2.2	4.6 ± 2.2	3.1 ± 1.9	<0.0001
CFS	4.27 ±1.7	5.9 ± 0.8	3.1 ± 0.8	<0.0001
ACEInhib/ARBs	104 (81.2)	41/54 (75.9)	63/74 (85.1)	0.19
Beta-blockers	85 (66.4)	40/54 (74.1)	45/74 (60.8)	0.12
Diuretics *	112 (87.5)	48/54 (88.9)	64/74 (86.4)	1.0
Drugs Number	4.84 ± 1.4	4.9 ± 1.2	4.8 ± 1.4	0.34

Table 1. Characteristics of patients in the overall study population, stratified as frail and non-frail.

*BMI: Body Mass Index; WBC: White Blood Cells; Hbg: Haemoglobin; GFR: Glomerular Filtration Rate; CRP: C Reactive Phase Protein; LVEF: Left Ventricular Ejection Fraction; NT proBNP: N-terminal -pro-Brain Natriuretic Peptide NYHA: New York Heart Association; CIRS-CI: Cumulative Illness Rating Scale Comorbidity Index; CFS: Clinical Frailty Scale; ACEinhb.: ACE inhibitors; ARBs: Angiotensin Receptor Blockers. * Diuretics and or Aldosterone Antagonists.*

The multivariable logistic regression analysis (Table 2) indicated that age, CIRS-CI, CRP, NT-proBNP and Gal-3 were significantly and independently associated with frailty and that the functional form of their relationship was linear. The global strength of these associations was 50%, as defined by the global pseudo R^2, thus indicating a robust global association. As documented by the percent contribution to the global pseudo R^2, Gal-3 together with NT-proBNP, CRP and CIRS were the factors that correlated most with frailty. Of note, Gal-3 explained a relevant portion of the global association (R^2 = 39.4%).

	Global Pseudo R^2 * = 0.5			
Variables	Odd's Ratio	95% CI	*p*-Value	Partial Contribution to Global R^2
Age (decades)	3.29	1.03–10.55	0.045	6.3%
Gender	0.86	0.19–4.03	0.854	NA
BMI	0.95	0.83–1.10	0.415	NA
CKD	1.47	0.34–6.36	0.605	NA
CIRS-CI (SD units)	1.85	1.03–3.32	0.039	10.3%
NYHA Class III, IV	1.53	0.46–5.13	0.456	NA
LVEF	0.93	0.84–1-03	0.172	NA
NT-proBNP (SD units)	2.39	1.22–4.73	0.012	24.5%
Hgb	0.95	0.64–1.40	0.828	NA
CRP (SD units)	3.73	1.24–11.22	0.019	19.5%
Gal-3 (SD units)	5.65	1.97–16.22	0.019	39.4%

Table 2. Multivariable logistic regression models.

*BMI: Body Mass Index; CKD: Chronic Kidney Disease defined as GFR ≤ 50 mL/kg/m2; CIRS: Standardized Cumulative Illness Rating Scale-Comorbidity Index; NYHA: New York Heart Association Class; LVEF: Left Ventricular Ejection Fraction; NT-proBNP: N-terminal -pro-Brain Natriuretic Peptide lnNT-proBNP: Standardized logarithmic transformation of NT-proBNP; Hgb: Haemoglobin; CRP: C-Reactive Protein; Gal-3: Standardized Galectin three, NA: Not Applicable. *Global pseudo R2 model including: age, CIRS, lnNT-proBNP, CRP and Gal-3.*

The linear correlation analysis of Gal-3 vs. CFS revealed a significant ($p < 0.0001$) progressive increment of the biomarker levels associated with a CFS score increase, with an estimated 5.7 ng/mL increase of Gal-3 for one unit increase of the score. A uniform increment is assumed given the lack of significance of the non-linear relationship.

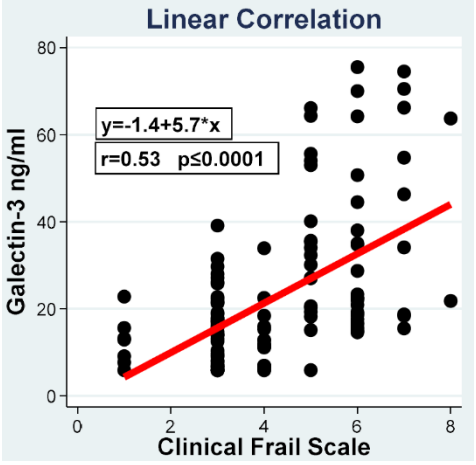

Figure 1. Linear correlation between the Galectin-3 and Clinical Frail values.

Conclusive remarks

This study identified Gal-3 serum levels as a circulating biomarker independently associated with frailty, in a population of elderly systolic HF patients. These findings may open the way for future investigations to better define the role of Gal-3 in the pathophysiology of frailty in HF and its utility as a biomarker in the prognosis of frail populations.

Chapter 6

Angiopoietins, Vascular Endothelial Growth Factors and Secretory Phospholipase A2 in Ischemic and Non-Ischemic Heart Failure

Within HFrEF, ischemic heart disease represents the most common cause of myocardial injury and ventricular dysfunction, leading in a significant percentage of cases to post-ischemic heart failure (IHF). Non-ischemic HF (NIHF), which accounts for less than 50% of HFrEF cases, comprises all the remaining heterogeneous HF aetiologies ranging from valvular diseases to toxic damage, up to metabolic conditions and genetic cardiomyopathies[71]. In a significant percentage (30%) of HF patients, the aetiology remains undetermined, and the syndrome is referred to as "idiopathic HF"[72]. Identification of these diverse aetiologies may be obtained through a complex diagnostic workup, frequently without a relevant therapeutic implication.

Neurohormonal and inflammatory activation are widely recognized as playing a pivotal role in HF onset and progression, irrespective of aetiology[73]. The angiopoietin (ANGPT) family is an important group of factors, specific for vascular endothelium, with roles in the modulation of angiogenesis[74], lymphangiogenesis, and also inflammation in several disorders, including cardiovascular diseases[75,76]. ANGPT1 is an anti-inflammatory molecule[77] contrarywise ANGPT2 is considered a pro-inflammatory molecule, responsible for vascular instability and leakage[78].

VEGFs and their receptors on blood and lymphatic endothelial cells play intricate roles in initiating and promoting inflammatory and cancer angiogenesis. VEGF-A is a key regulator of systemic and cardiac angiogenesis[79], VEGF-C and VEGF-D are the most important modulators of inflammatory and cancer lymphangiogenesis[80]. By contrast, the roles of VEGF-A, VEGF-C and VEGF-D in HF remain unclear or totally unexplored.

Phospholipases A2 (PLA2) modulates vascular permeability and activates inflammatory cells[81]. Circulating levels of secreted PLA2 (sPLA2) predict coronary events in patients with coronary artery disease[82] and serum sPLA2 levels also predict long-term mortality for HF after MI[83].

While some studies are available on ANGPTs, VEGF isoforms, and sPLA2 involvement in ischemic heart disease, very little is known in the clinical setting of IHF and especially in NIHF. Thus, the aim of the present study is to evaluate the circulating levels of ANGPTs, VEGFs, and sPLA2 activity in HF patients, particularly comparing the ischemic and non-ischemic aetiologies.

Methods

Patients with systolic HF were enrolled at the Department of Translational Medical Sciences of the University of Naples Federico II. Inclusion criteria were: age ≥ 18 years, diagnosis of HF from at least six months, LVFE ≤ 45%, stable clinical condition during the month prior to inclusion, and an optimal guideline-based pharmacotherapy from at least three months, if not contraindicated. Exclusion criteria were represented by chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), immune disorders (rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, Sjögren syndrome, vasculitis, psoriatic arthritis, dermatomyositis, ankylosing spondylitis), malignancies (also past), severe obesity as assessed through a BMI more than 32 kg/m^2, dialysis-dependent kidney failure, acute coronary syndromes and/or coronary revascularization in the previous 6 months, and an inability to provide informed consent. The control group was represented by subjects without HF and in accordance with the exclusion criteria.

All patients underwent medical history evaluation and collection of demographic/clinical data, including age, gender, BMI, cardiovascular risk factors, and comorbidities. Clinical examination, transthoracic echocardiography, and serum BNP determination were performed at the time of the enrolment. Blood samples were collected in patients under stable clinical conditions. The HF population was subsequently divided into two groups based on the HF aetiology: IHF or NIHF. Ischemic aetiology was established based on either previous documented myocardial infarction and/or significant coronary artery disease with indication of cardiac revascularization.

Results

The overall study population comprised 44 patients suffering from HF (19 with IHF and 25 with NIHF) and 42 healthy donors, carefully selected according to inclusion/exclusion criteria. As expected, IHF and NIHF showed higher BNP levels and lower LVEFs compared to healthy controls (Table 1).

Characteristics	Healthy Controls (*N* = 42)	IHF (*N* = 19)	NIHF (*N* = 25)
Age-median years (range)	75.5 (46–98)	77 (54–87)	65 (45–87)
Gender male-no. (%)	16 (38.1)	12 (63.1)	16 (64)
BMI (kg/m^2)	25.2 ± 4.1	25.4 ± 3.0	25.5 ± 4.2
Caucasian (%)	100	100	100
BNP (pg/mL)	50.6 ± 32.0	1025.8 ± 733.3 *	968.6 ± 802.2 *
Leukocytes (×10^3/mm^3)	7.2 ± 2.5	8.6 ± 4.1	7.9 ± 3.0
GFR (mL/min)	71.2 ± 23.3	48.5 ± 24.3	69.6 ± 32.4
LVEF (%)	61.6 ± 5.8	34.3 ± 6.9 *	34.6 ± 7.4 *

Table 1. Demographic and clinical characteristics of patients with ischemic heart failure (IHF) or non-ischemic heart failure (NIHF) and healthy controls.

*Data are expressed as the mean and standard deviation (BMI, BNP, Leukocytes, GFR, LVEF) or median value (Age). IHF: ischemic heart failure; NIHF: non-ischemic heart failure; BNP: B-type natriuretic peptide; GFR: glomerular filtration rate (assessed through CKD-EPI equation); LVEF: left ventricular ejection fraction. * p < 0.01 when compared to healthy controls analysed by one-way ANOVA and Bonferroni's multiple comparison test.*

As shown in Figure 1, lower concentrations of ANGPT1 and higher levels of ANGPT2 and ANGPT2/ANGPT1 ratios were detected in subjects suffering from HF compared to healthy controls. No differences were observed in plasma concentrations of VEGF-A and VEGF-C in the two groups (Figure 2). Otherwise, HF patients presented higher concentrations of VEGF-D compared to controls. Moreover, HF was associated with higher PLA2 activity (Figure 3).

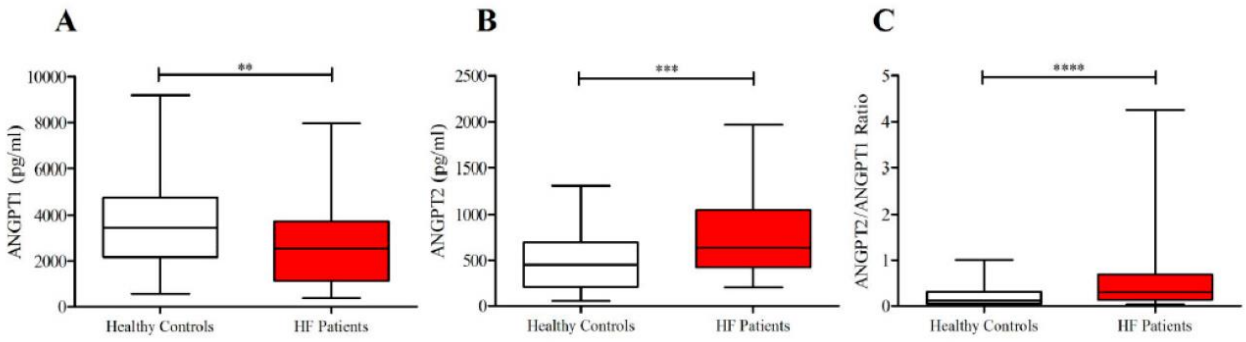

Figure 1. (A) Plasma concentrations of angiopoietin-1 (ANGPT1) in heart failure (HF) patients and in healthy controls; (B) Plasma concentrations of ANGPT2 in HF patients and in healthy controls; (C) ANGPT2/ANGPT1 ratio in HF patients and in healthy controls.

*Data are shown as the median (horizontal block line), the 25th and 75th percentiles (boxes), and the 5th and 95th percentiles (whiskers) (statistical analysis was performed by a Student's t-test). ** p < 0.01; *** p < 0.001; **** p < 0.0001*

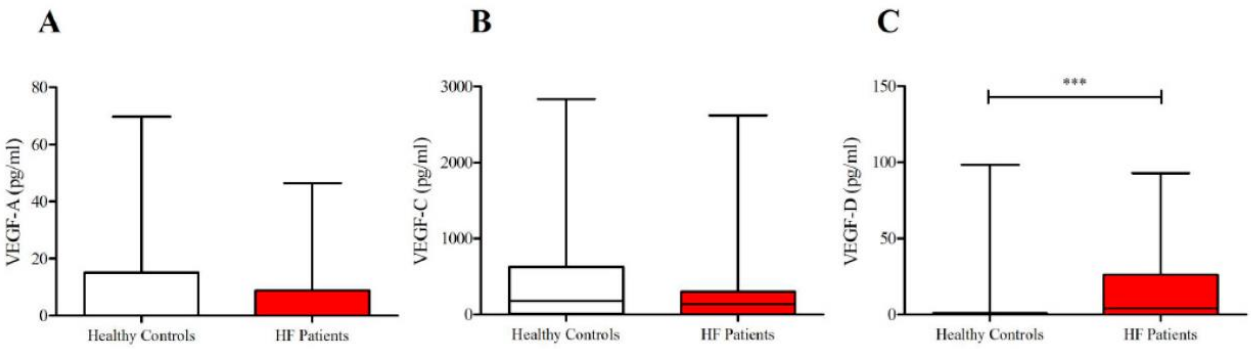

Figure 2. (A) Plasma concentrations ofvascular endothelial growth factor-A (VEGF-A) in heart failure (HF) patients and in healthy controls; (B) plasma concentrations of VEGF-C in HF patients and in healthy controls; (C) plasma concentrations of VEGF-D in HF patients and in healthy controls.

*Data are shown as the median (horizontal block line), the 25th and 75th percentiles (boxes), and the 5th and 95th percentiles (whiskers) (statistical analysis was performed by a Student's t-test). *** p < 0.001*

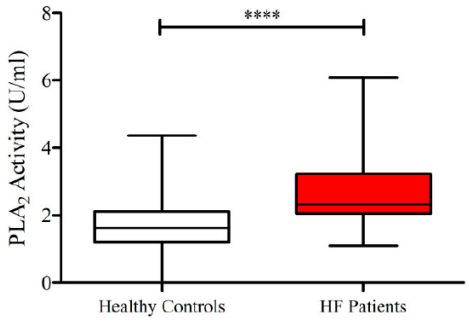

Figure 3. Plasma concentrations of sPLA2 activity in HF patients and in healthy controls.

*Data are shown as the median (horizontal block line), the 25th and 75th percentiles (boxes), and the 5th and 95th percentiles (whiskers) (statistical analysis was performed by a Student's t-test). **** p < 0.0001.*

The concentrations of ANGPT1 were significantly reduced in NIHF compared to controls (Figure 4A). By contrast, the plasma concentrations of ANGPT2 were selectively increased only in NIHF compared to healthy donors (Figure 4B). Similarly, the ANGPT2/ANGPT1 ratio, a parameter of vascular permeability, was also increased only in NIHF patients compared to controls (Figure 4C). Importantly, no difference emerged between IHF group and healthy controls in the ANGPT2/ANGPT1 ratio, whereas there was a significant difference between the ANGPT2/ANGPT1

ratio in NIHF vs. IHF (Figure 4C). There were no differences in ANGPT1 or ANGPT2 between male and female values in both controls and patients. Moreover, the age of patients and the concentrations of the different mediators examined did not correlate.

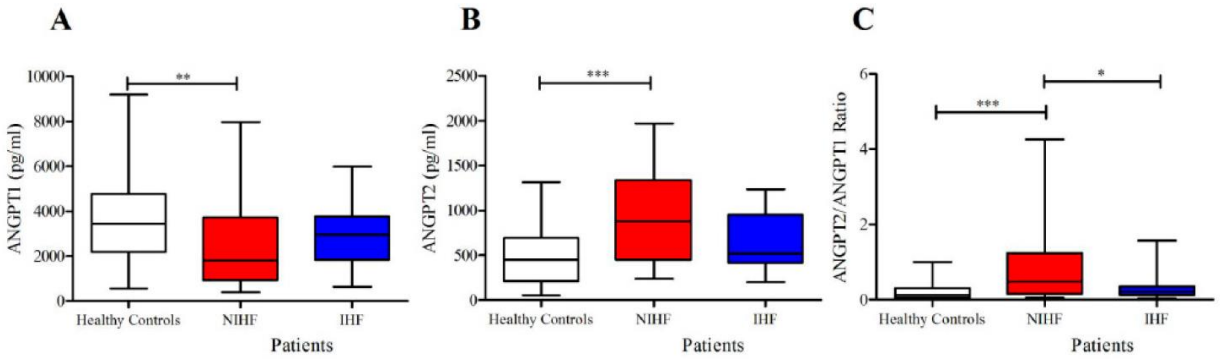

Figure 4. (A) Plasma concentrations of angiopoietin-1 (ANGPT1) in ischemic (IHF) and non-ischemic (NIHF) patients, and in healthy controls; (B) plasma concentrations of ANGPT2 in IHF and NIHF patients, and in healthy controls; (C) ANGPT2/ANGPT1 ratio in IHF and NIHF patients, and in healthy controls.

*Data are shown as the median (horizontal block line), the 25th and 75th (boxes), and the 5th and 95th percentiles (whiskers) (statistical analysis was performed by one-way ANOVA and Bonferroni's multiple comparison test). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$*

The mean plasma concentrations of VEGF-A were essentially similar in patients with different types of HF and controls (Figure 5A). The mean plasma concentrations of VEGF-C did not differ in patients with different HF types and controls (Figure 5B). In contrast, the plasma concentrations of VEGF-D were increased in IHF patients compared to healthy controls (Figure 5C). There were no differences in VEGF-A, VEGF-C, and VEGF-D concentrations between male and female values in either controls and patients. Moreover, the age of patients and the concentrations of VEGFs examined did not correlate.

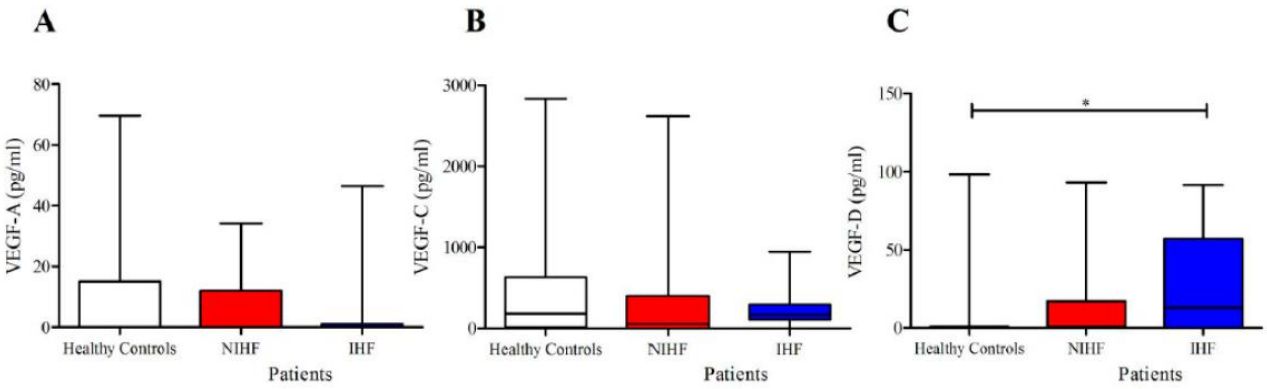

Figure 5. (A) Plasma concentrations of VEGF-A in IHF and NIHF patients and in healthy controls; (B) Plasma concentrations of VEGF-C in IHF and NIHF patients and in healthy controls; (C) Plasma concentrations of VEGF-D in IHF and NIHF patients and in healthy controls.

*Data are shown as the median (horizontal block line), the 25th and 75th percentile (boxes) and the 5th and 95th percentiles (whiskers) (Statistical analysis was performed by One-way ANOVA and Bonferroni's multiple comparison test). *p < 0.05*

Figure 6 shows that plasma activity of sPLA$_2$ activity was significantly increased in both groups of HF patients compared to healthy controls. There were no differences in sPLA$_2$ activity between male and female values in both controls and patients.

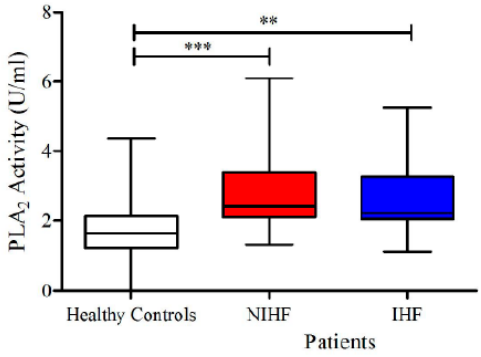

Figure 6. Plasma concentrations of sPLA$_2$ activity in IHF and NIHF patients and in healthy controls.

*Data are shown as the median (horizontal block line), the 25th and 75th percentile (boxes) and the 5th and 95th percentiles (whiskers) (Statistical analysis was performed by One-way ANOVA and Bonferroni's multiple comparison test). **p < 0.01; ***p< 0.001*

As shown in Figure 7, there was an inverse correlation between plasma concentrations of ANGPT2 and ANGPT1 (Figure 7A), and between sPLA$_2$ activity and ANGPT1 (Figure 7B) in NIHF patients. Furthermore, a positive correlation between PLA$_2$ activity and ANGPT2 was detected in NIHF (Figure 7C).

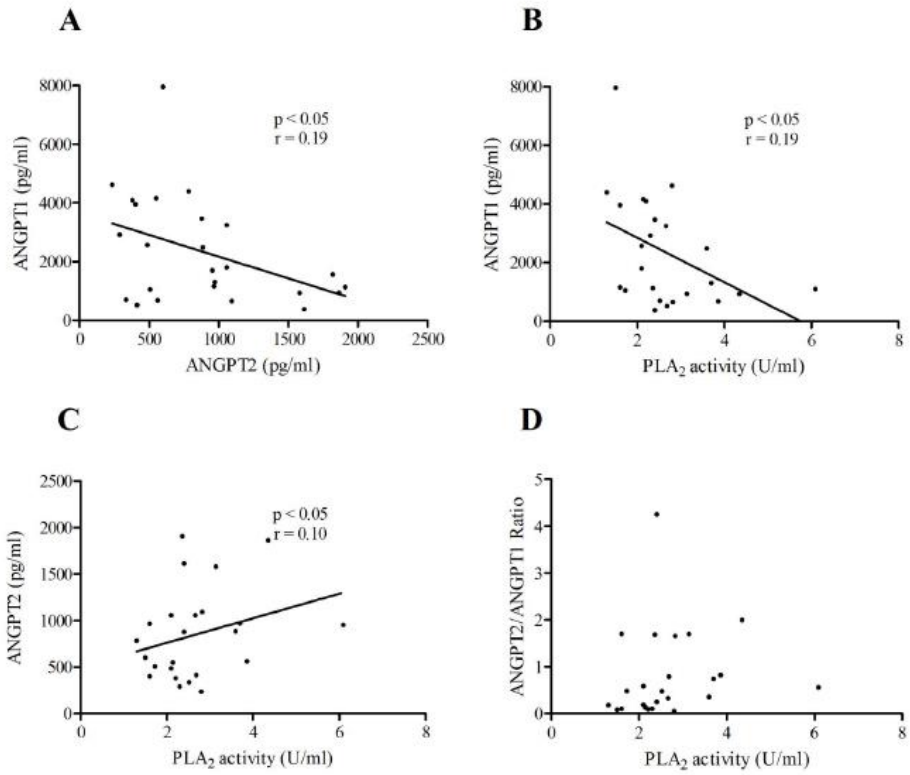

Figure 7. (A) Correlations between the plasma concentrations of ANGPT2 and ANGPT1 in NIHF patients; (B) Correlation between circulating $sPLA_2$ activity and the concentration of ANGPT1 in NIHF patients; (C) Correlation between the plasma concentration of $sPLA_2$ activity and ANGPT2 in NIHF patients; (D) Correlation between the plasma concentration of $sPLA_2$ activity and ANGPT2/ANGPT1 Ratio in NIHF patients.

Spearman's correlation coefficients (r) were calculated and are shown in the panels.

Contrariwise, no correlations were observed among the plasma concentrations of ANGPT1 and BNP, ANGPT2 and BNP, and sPLA2 activity and BNP in NIHF patients. Similarly, no correlations were found between plasma concentrations of ANGPT1, ANGPT2, and sPLA2 activity vs. LVEF in patients with IHF or NIHR.

Conclusive remarks

The findings of the present study suggest that the ANGPT system is selectively modulated in NIHF patients, with an increased ANGPT2/ANGPT1 ratio compared to IHF and controls, whereas

VEGF-D was exclusively augmented in IHF patients. In contrast, sPLA2 activity was increased in both IHF and NIHF patients compared to healthy controls. This represents the first evidence reporting that several regulators of vascular permeability and inflammation is specifically altered in patients with IHF and NIHF, paving the way for the identification of new molecular mechanisms underlying HF pathophysiology and novel therapeutic targets.

Chapter 7

Angiopoietins, vascular endothelial growth factors and secretory phospholipase A2 in heart failure patients with preserved ejection fraction

HFpEF is a leading cause of morbidity and mortality throughout the industrialized world, and currently represents approximately 50% of individuals with HF[84]. The complex and heterogeneous clinical phenotype that characterizes HFpEF stems from multiple comorbidities, including obesity, diabetes, hypertension, and atrial fibrillation[85]. An increasingly popular theory about HFpEF is that the syndrome reflects systemic and/or myocardial inflammation[86]. A deeper knowledge of the molecular and immunological mechanisms involved in this complex pathophysiology is needed for the identification of novel biomarkers and therapeutic targets to stratify prognosis and drive decision-making processes.

It is tempting to speculate that different patterns of inflammatory and vasoactive mediators reflect pathophysiological differences between HF phenotypes, therefore the aim of the present study was to evaluate the circulating levels of ANGPTs, VEGFs and $sPLA_2$ activity (the same molecules as Chapter 6) in patients with HFpEF or HFrEF, compared to healthy controls.

Methods

The study population consisted of Caucasian patients suffering from HF admitted to the Department of Translational Medical Sciences of the University of Naples Federico II. Inclusion criteria listed: age $\geq$ 18 years, diagnosis of HF from at least 6 months, stable clinical condition during the month prior to inclusion, optimal guideline-based pharmacotherapy from at least 3 months. Exclusion criteria were represented by COPD, DM, immune disorders (rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, Sjögren syndrome, vasculitis, psoriatic arthritis, dermatomyositis, ankylosing spondylitis), malignancies (also past), obesity as assessed through BMI more than 30 Kg/m^2, dialysis-dependent kidney failure, acute coronary syndromes and/or coronary

revascularization in the previous 6 months, and inability to provide informed consent. The control group was represented by Caucasian subjects without HF, in accordance with the exclusion criteria.

All patients underwent medical history evaluation and demographic/clinical data collection, including age, gender, BMI, cardiovascular risk factors and comorbidities. Clinical examination, transthoracic echocardiography and serum BNP determination were performed at the time of the enrolment. Blood samples were collected in patients under stable clinical conditions. In the present study, the population was divided into two groups based on LVEF: HFrEF (LVEF < 50%) or HFpEF (LVEF ≥ 50%). IHD was established based on either previous documented myocardial infarction and/or significant coronary artery disease with indication to cardiac revascularization.

Results

The study population comprises 47 patients with HFrEF, 31 patients with HFpEF and 47 healthy controls, carefully selected according to inclusion/exclusion criteria. As expected, HFpEF patients were older, more prevalently female, and showed higher LVEF and GFR, lower BNP levels and IHD etiology compared to HFrEF patients (Table 1).

Variable	Controls (n = 47)	HFrEF (n = 47)	HFpEF (n = 31)	*p*-value
Age (yrs)	71.17 ± 12.85	69.23 ± 11.56	75.80 ± 9.08	0.049
Gender, male (%)	18 (38.30)	29 (61.70)	12 (38.71)	0.042
BMI (kg/m^2)	25.89 ± 3.95	25.59 ± 3.70	26.21 ± 4.65	0.801
BNP (pg/mL)	50.58 ± 32.06	958.11 ± 763.94	550.5 ± 681.71	<0.001
Leukocytes (x 10^3/ mm^3)	7.43 ± 2.72	8.48 ± 3.52	8.26 ± 3.49	0.407
GFR (mL/min)	71.43 ± 22.55	51.93 ± 29.03	57.75 ± 30.06	0.032
EF (%)	61.55 ± 5.50	34.20 ± 7.1	61.82 ± 6.04	<0.001
Smoking (%)	7 (14.89)	19 (40.42)	5 (16.12)	0.007
Hypertension (%)	31 (65.95)	30 (63.8)	26 (83.87)	0.146
Coronary artery disease (%)	none	20 (42.55)	9 (29.03)	<0.001
Hyperlipidemia (%)	14 (29.78)	17 (36.17)	19 (61.29)	0.076
Atrial fibrillation (%)	6 (12.77)	17 (36.17)	12 (38.71)	0.013
Diuretics (%)	5 (10.63)	35 (74.47)	10 (32.26)	<0.001
ACEIs (%)	8 (17.02)	21 (44.68)	8 (28.81)	0.045
ARBs (%)	12 (25.53)	10 (21.28)	7 (22.58)	0.908
Beta-blockers (%)	15 (31.91)	37 (78.72)	18 (58.06)	<0.001

Table 1. Demographic and clinical characteristics of HFpEF, HFrEF and control groups.

BMI: Body Mass Index; BNP: B-type natriuretic peptide; GFR: glomerular filtration rate (assessed through CKD-EPI equation); LVEF: left ventricular ejection fraction.

Data are expressed as mean values for continuous variables and percentage (%) for categorical variables.

Figure 1A shows that lower concentrations of ANGPT1 were detected in HFrEF patients compared to healthy controls and HFpEF subjects; the plasma levels of ANGPT1 in HFpEF patients were similar to controls. Plasma concentrations of ANGPT2 were significantly higher in both HF groups than controls (panel B). ANGPT2/ANGPT1 ratio was higher in HFrEF but not in HFpEF patients, compared to controls (panel C). There were no differences in ANGPT1 or ANGPT2 between male and female values in both controls and groups of patients. Moreover, no correlations were observed between the age of patients and the concentrations of the different mediators examined.

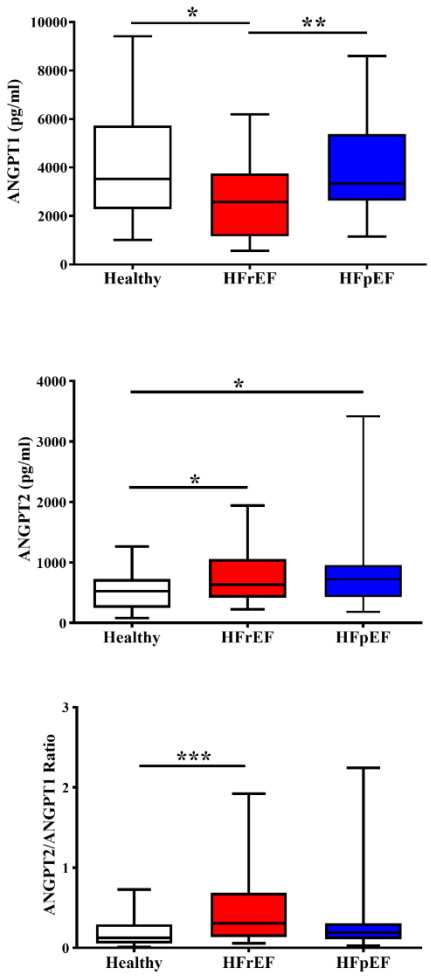

Figure 1. (A) Plasma concentrations of ANGPT1 in patients with HFrEF, HFpEF and healthy controls; (B) Plasma concentrations of ANGPT2 in patients with HFrEF, HFpEF and healthy controls; (C) ANGPT2/ANGPT1 ratio in patients with HFrEF, HFpEF and healthy controls.

**p <0.05; **p <0.01; ***p <0.001*

Within all the examined relationships (Table 2), the strongest significant correlation was observed between cardiac function assessed through EF and PLA2 (r= -0.435; p< 0.01). Weaker significant coefficients emerged with ANGPT1 (r= 0.307; p < 0.01) and ANGPT2/ANGPT1 (r= -0.244; p= 0.01), contrarywise no association was observed with the other examined molecules.

Mediator	EF	*p* value
ANGPT1	0.307	<0.01*
ANGPT2	−0.153	0.12
ANGPT2/ANGPT1	−0.244	0.01*
VEGF-A	−0.006	0.94
VEGF-C	0.017	0.86
VEGF-D	−0.095	0.349
PLA_2	−0.435	<0.01*

Table 2. Correlations between plasma concentrations of angiogenic, lymphangiogenic and proinflammatory mediators and ejection fraction (EF).

p value corresponds to Pearson's r correlation coefficient.

The mean plasma concentrations of VEGF-A were essentially similar in patients with HFrEF, HFpEF and controls (Figure 2A). VEGF-C and VEGF-D are the main lymphangiogenic factors [72,73]. The mean plasma concentrations of VEGF-C did not differ in all patients with different HF phenotypes and healthy donors (Figure 2B). By contrast, the plasma concentrations of VEGF-D were increased in both HFrEF and HFpEF patients compared to controls (Figure 2C). There was a trend in HFpEF to display a further increase in VEGF-D compared to HFrEF patients. There were no differences in VEGF-A, VEGF-C, and VEGF-D concentrations between male and female values in both controls and HF patients. Moreover, the age of patients and the examined VEGF concentrations did not correlate.

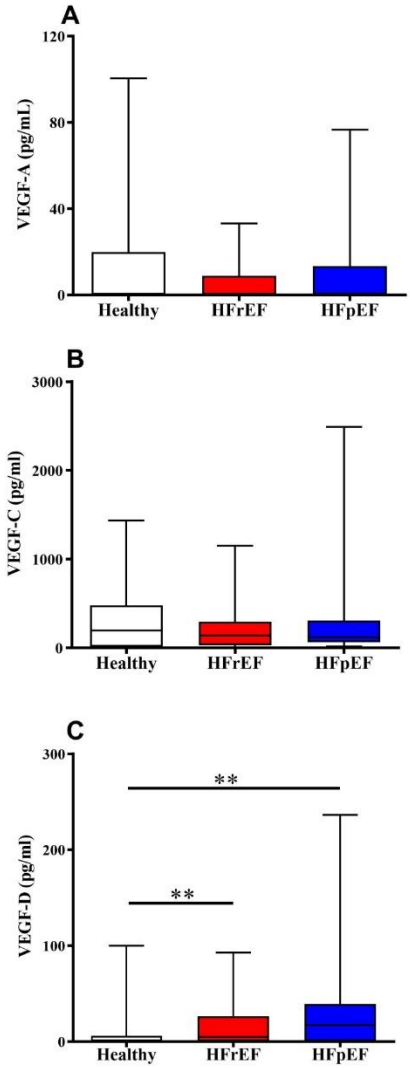

Figure 2. (A) Plasma concentrations of VEGF-A in HFrEF and HFpEF patients and healthy controls; (B) Plasma concentrations of VEGF-C in HFrEF and HFpEF patients and healthy controls; (C) Plasma concentrations of VEGF-D in HFrEF and HFpEF patients and healthy controls.

***p < 0.01*

Figure 3 shows that the plasma activity of sPLA2 was significantly increased in HFrEF patients compared to HFpEF and healthy donors. There were no differences in sPLA2 activity between male and female values in both controls and patients. Moreover, no correlations were observed between the age of patients and the concentration of sPLA2 activity.

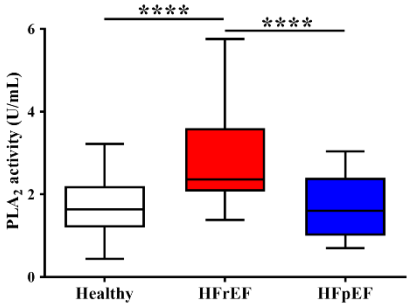

Figure 3. Plasma sPLA2 activity in HFrEF, HFpEF patients and healthy controls.

*****p < 0.0001*

The correlations among the altered mediators in patients with HFrEF or HFpEF were also analyzed. Figure 4A shows an inverse correlation between plasma concentrations of ANGPT2 and ANGPT1 in HFrEF patients. In addition, there was a negative correlation between $sPLA_2$ activity and ANGPT1 (Figure 4B) and between ANGPT2 and VEGF-D (Figure 4C) in HFrEF patients. Figure 4D shows a positive correlation between PLA_2 activity and ANGPT2 in HFrEF patients. No correlation was observed between ANGPT1 and VEGF-D (Figure 4E) and $sPLA_2$ activity and VEGF-D in HFrEF patients (Figure 4F). Moreover, no correlation was observed between ANGPT2 and VEGF-D plasma concentrations of HFpEF (Figure 4G).

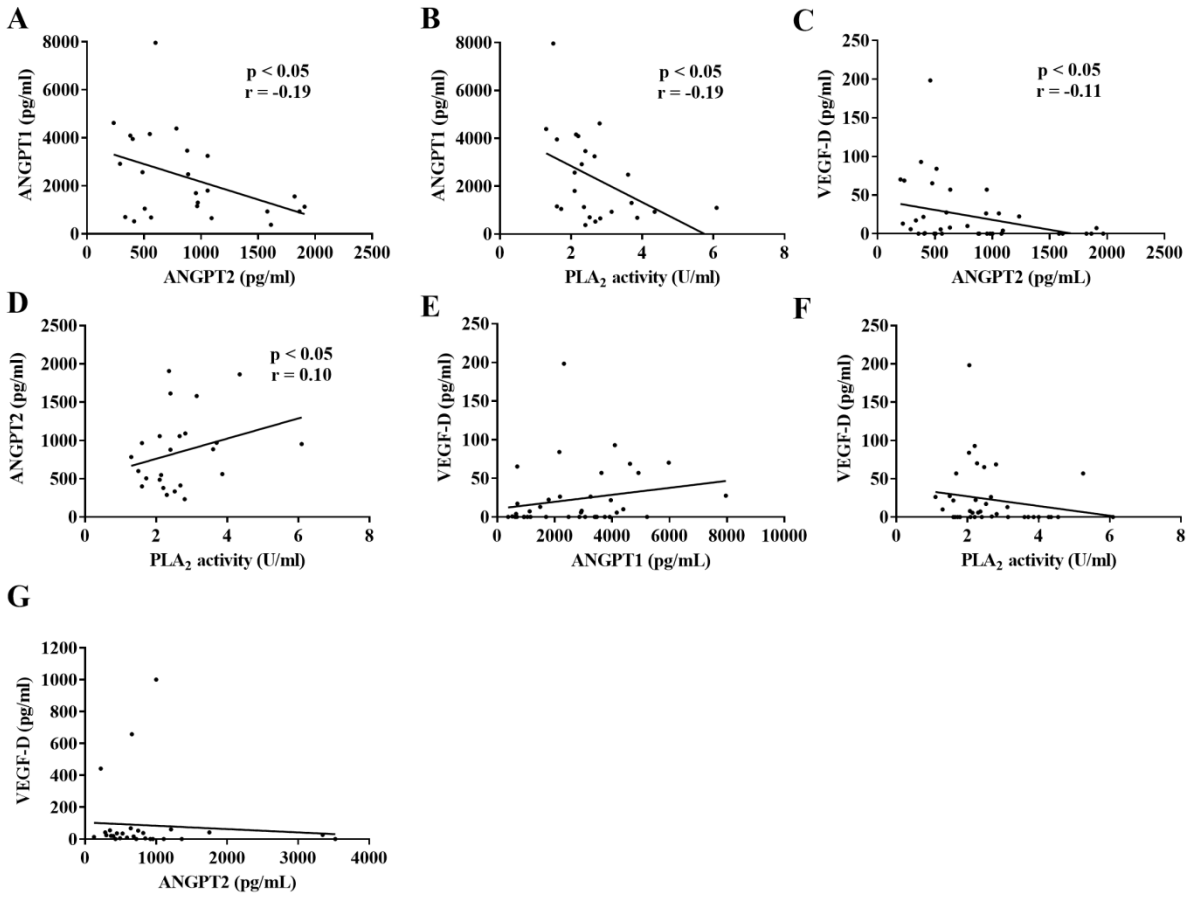

Figure 4. (A) Correlations between the plasma concentrations of ANGPT2 and ANGPT1 in HFrEF patients; (B) Correlation between circulating $sPLA_2$ activity and the concentration of ANGPT1 in HFrEF patients; (C) Correlation between the plasma concentrations of VEGF-D and ANGPT2 in HFrEF patients; (D) Correlation between the $sPLA_2$ activity and ANGPT2 in HFrEF patients. (E) Correlation between the plasma concentration of VEGF-D and ANGPT1 in HFrEF patients; (F) Correlation between circulating $sPLA_2$ activity and VEGF-D in HFrEF patients; (D) Correlation between the plasma concentration of VEGF-D and ANGPT2 in HFpEF patients.

Spearman's correlation coefficients (r) were calculated and are shown in the panels.

Conclusive remarks

The study reports significant and distinct alterations of plasma concentrations of three different classes of proinflammatory mediators essential for vascular development, integrity and remodeling (i.e., angiopoietins, VEGFs, and secretory phospholipase A_2) in patients with HFrEF or HFpEF compared to controls. These findings could pave the way for the identification of new inflammatory biomarkers underlying different forms of HF pathophysiology and novel therapeutic targets.

Section 3

Impact of aging and comorbidities on the management of cardiovascular diseases

Many of the biological pathways underlying CVDs, including and beyond those discussed in the previous sections, also concur to the development of typical chronic non-cardiovascular conditions of aging. Older adults are often burdened by multiple concomitant pathologies, determining reduced physical function, disability and polypharmacotherapy, which have been associated with an increased risk of worsening global health status[87]. The repeated effect of genetical and environmental factors over the decades contributes to the great heterogeneity of late life, indeed clinical phenotype of older people can widely vary, ranging from successful aging to frailty and disability.

In particular, irrespective of the several tools proposed to assess it, frailty can be defined as a geriatric syndrome, the most complex manifestation of aging process, a state of increased vulnerability to stress events determined by reduced homeostatic reserve and multiple comorbidities[8]. A strong bidirectional association has been documented between frailty and CVDs, in which both components contribute to the onset, development and outcome of the other, mutually complicating their course and influencing its diagnostic and therapeutic possibilities[88].

Interestingly, frailty prevalence is higher in HFpEF than HFrEF, probably because patients with maintained LVEF typically suffer a great burden of comorbidities, are older are more likely to undergo non-cardiac hospitalisations for non-cardiac causes[89]. In the wake of the previous chapters, in this section the first focus is dedicated to HFpEF, with a review on the role of comorbidities and specific heart abnormalities that determine this syndrome (Chapter 8)[85].

Moving to other highly prevalent CVDs in old age, the Chapters 9[90] and 10[91] (*) provide an overview of the complexity of older patients suffering from atrial fibrillation and aortic stenosis respectively, in which the role of comorbidities and complications can generate difficult clinical challenges, for which evidence-based support is still poor. Finally, the role of deficit accumulation frailty is analysed to determine its impact on the efficacy of cardiovascular rehabilitation program,

another crucial non-pharmacological therapeutic resource in the care of older patients affected by CVDs (Chapter 11)[92].

This section aims to bring further evidence in support of the need to integrate the multidimensional skills of geriatric medicine with the specialist knowledge of cardiovascular medicine, to allow a comprehensive assessment of the complex problems of older adults, also with the aim of personalizing the objectives and care pathways.

(: this manuscript was published before the update of the 2021 ESC/EACTS Guidelines for the management of valvular heart disease, therefore it is reported in a reduced form in Chapter 10, without indications on antithrombotic therapy, with the aim of highlighting the complexity of the management of the older patient with AS and comorbidities)*

Chapter 8

Heart failure with preserved ejection fraction: Squaring the circle between comorbidities and cardiovascular abnormalities

HFpEF is a complex clinical syndrome resulting from the interplay among several risk factors which in turn determine heart dysfunction. The prevalence of risk factors contributing to HFpEF development may vary between affected individuals, and they synergistically concur to increase the risk of HF onset and progression[93]. Although evidences on ethnic and racial differences are still limited, gender-based differences have been reported with black women suffering from higher HFpEF rates compared to other race- and sex-groups[94]. In almost all studies it has been shown that advanced age and female sex are more frequent in HFpEF than in HFrEF[84]. High prevalence of cardiovascular comorbidities and risk factors in HFpEF has been widely described, with arterial hypertension being the most prevalent condition[95]. Coronary artery disease (CAD), although more prevalent in HFrEF, is a common condition also in HFpEF, accounting for a percentage of patients ranging from 35 to 60% in epidemiological studies[96], and it is associated with increased risk of LVEF decline and higher mortality[97]. Furthermore, the burden of comorbidities in HFpEF also includes obesity, atrial fibrillation, diabetes mellitus, chronic kidney disease[96]. Of note, all the aforementioned conditions are highly prevalent in late life[9] and the growing percentage of elderly in the global population will be responsible for the predicted further increase in HFpEF incidence and prevalence in the next future.

Although no doubt remains on the pathophysiological correlation between HFpEF and these comorbidities, with a great impact on prognosis, it remains to be completely elucidated whether the burden of comorbidities is quantitatively higher than in HFrEF[98]. In this context, it has been described that specific conditions are more common in each HF subgroup (e.g. obesity is associated with higher incidence of HFpEF[99]) and that the mean number of comorbidities is higher in HFpEF patients than HFrEF ones[100] (Figure 1).

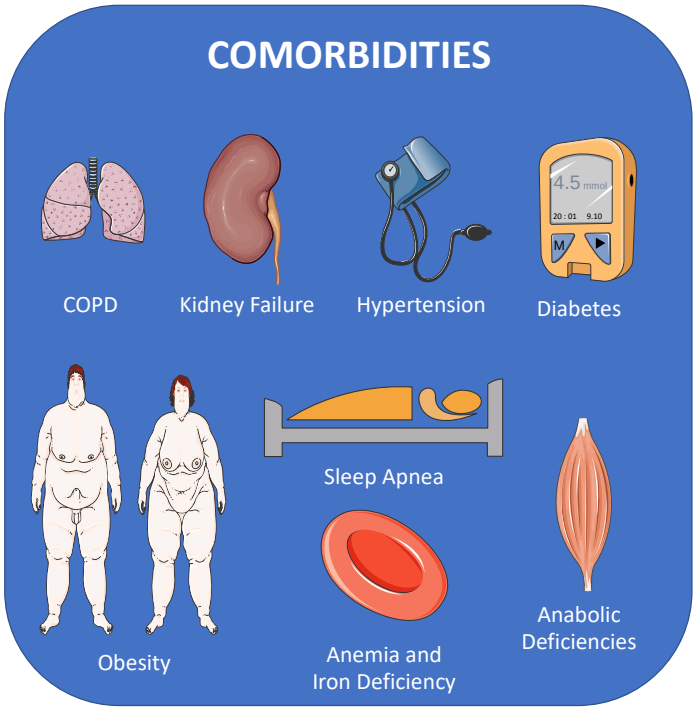

Figure 1. Comorbidities associated with HFpEF

As shown in Figure 2 low-grade chronic inflammation, endothelial dysfunction, cardiac fibrosis and increased ventricular stiffness, which constitute key pathological features of HFpEF[101].

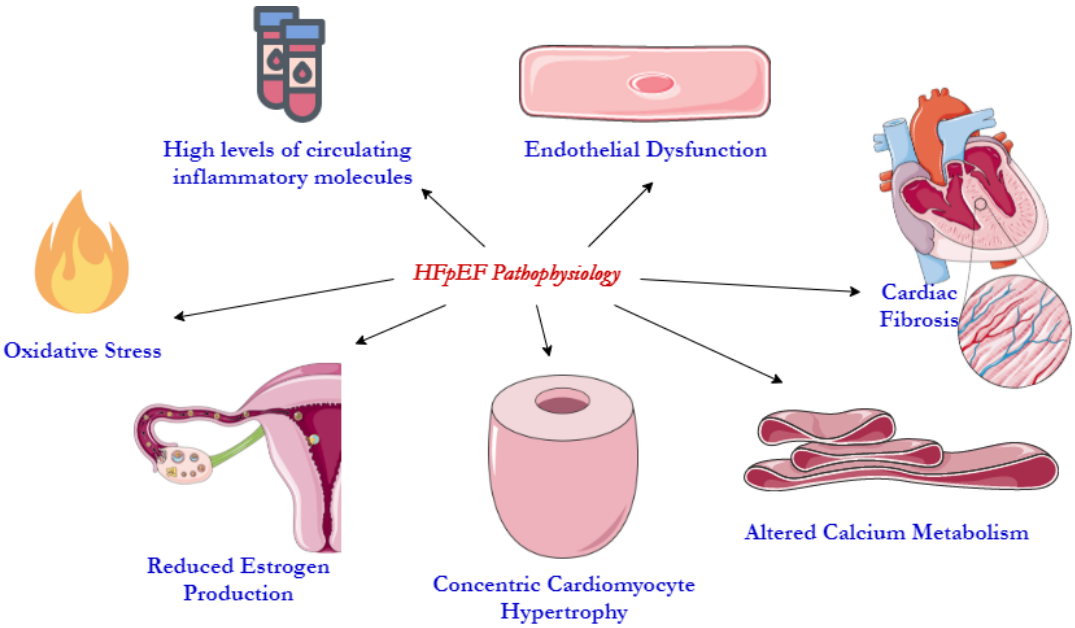

Figure 2. Key pathological features of HFpEF

A consistent body of evidence supports the possible existence of a specific pattern of cardiovascular abnormalities in HFpEF (Figure 3).

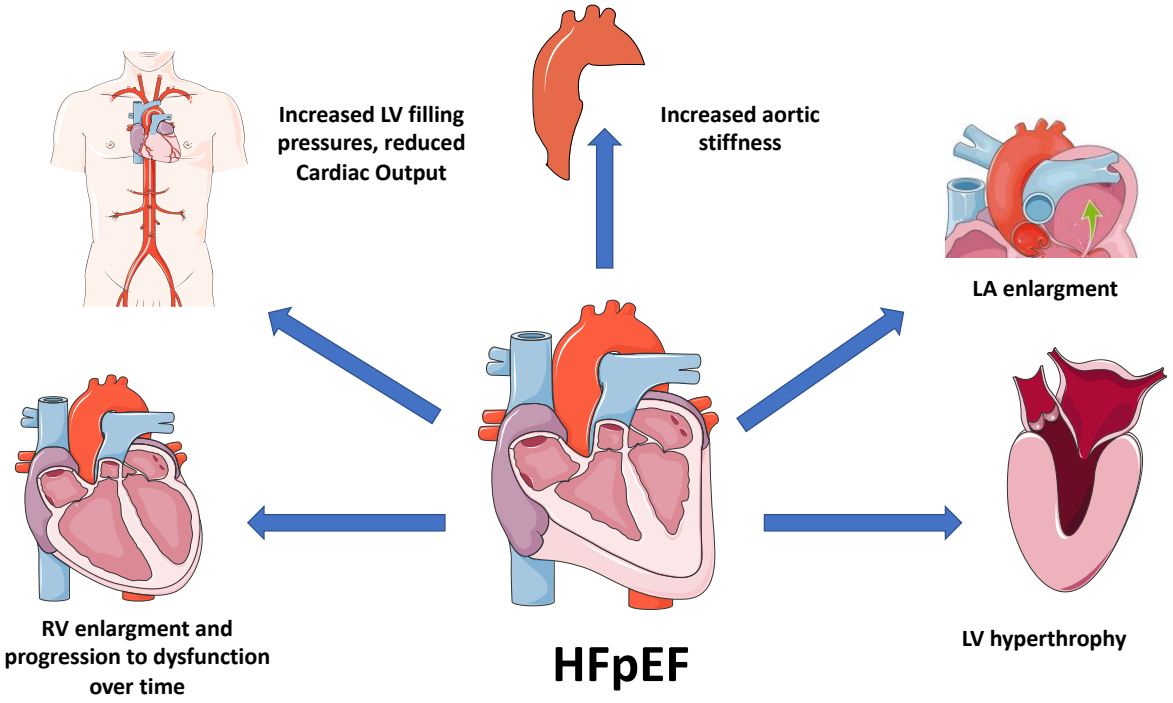

Figure 3. Specific cardiovascular abnormalities in HFpEF.

Table 1 summarizes the main results of the principal and larger randomized placebo-controlled multicenter trials in this condition. The most interesting finding is the non-negligible proportion of deaths due to non-cardiac causes in almost all study populations, ranging from 6 to 7%.

Acronym	Year of publication	Intervention	Sample size	Female (%)	HFpEF definition	Primary Outcome	Occurrence of the primary outcome	non-CV mortality
CHARM-Preserved [56]	2003	Candersartan (4 mg up to 32 mg daily) vs placebo	3023	40.1	LVEF ≧ 40% and Hospital admission for HF	Composite of cardiovascular death or hospital admission for HF	Candesartan: 22% Placebo: 24%	nr
PEP-CHF [57]	2006	Perindopril (4 mg daily) vs Placebo	850	54.9	LVEF ≧ 40% treated with diuretics for a diastolic HF	Composite of all-cause mortality and unplanned heart failure related hospi-talization	Perindopril: 23.6% Placebo: 25.1%	Perindopril: 1,65% Placebo:0,5%
DIG [58]	2006	Digoxin (0.125 mg up to 0.50 mg daily) vs Placebo	988	41.2	LVEF ≧ 45% plus Sinus Rhythm	Composite of HF mortality or HF hospitalization	Digoxin: 20.7% Placebo: 24%	Digoxin: 6,9% Placebo: 7,1
I-PRESERVE [59]	2008	Irbesartan (75 mg up to 300 mg) vs Placebo	4128	61.1	LVEF ≧ 45% plus history of HF in the previous 6-months	Composite of death from any cause or hospitalization for a protocol-specified cardiovascular cause	Irbesartan: 35.9% Placebo: 37.0%	Irbesartan: 6.5% Placebo: 6.5%
TOPCAT [60] (24,716,680)	2014	Spironolactone (15 mg up to 45 mg daily) vs placebo	3445	51.5	LVEF ≧ 45% plus history of HF in the previous 12-months and/or increased NP	Composite of death from cardiovascular causes, aborted cardiac arrest, or hospitalization for the management of heart failure.	spironolactone:18.6% placebo: 20.4%	Spirolonolactone: 92 (5.3%) Placebo: 98 (5.7%)
PARAGON—HF [61]	2019	sacubitril/valsartan (target dose, 97/ 103 mg twice daily) vs valsartan (target dose, 160 mg twice daily).	4822	51.4	LVEF ≧ 45% plus increased NP	Composite of total hospitalizations for heart fail- ure and death from cardiovascular causes.	Sacubitril/Valsartan: 37.2% Valsartan: 42.2%	Sacubitril/ Valsartan: 5.7% Valsartan: 5.7%
EMPEREOR-Preserved [4]	2021	Empagliflozin (10 mg once daily) vs placebo	5988	44,6	LVEF ≧ 40% + increased NP + evidence of cardiac structural abnormality (increased LV mass, increased LA volume)	Composite of cardiovascular death or hospital admission for HF	Empagliflozin: 13.8% Placebo: 17.1% (HR:0.79, 95% CI: 0.69–0.90, $p< 0.001$)	Empagliflozin: 6.7% Placebo: 6.1%

Table 1. The main randomized placebo-controlled multicenter trials in HFpEF.

LVEF: Left Ventricular Ejection Fraction; HF: Heart Failure, nr: not reported; NP: natriuretic peptides; LV: left ventricular; LA: left atrial

Conclusive remarks

HFpEF and HFrEF show different features in terms of cardiac abnormalities and concomitant comorbidities. For this reason, it is worth to consider them as two separate syndromes, characterized by increasing prevalence and high mortality. Both epidemiological and mechanistic studies support the concept that HFpEF represents true HF although aggravated by a collection of comorbidities. There is urgent need for improveing its phenotyping due to the high degree of disease heterogeneity within HFpEF, that lead to the failure of randomized clinical trials in demonstrating a remarkable impact of drugs in improving its morbidity and mortality. At this regard, the recently published data from the EMPEROR-Preserved trial demonstrated a remarkable impact of the Sodium-Glucose cotransporter 2 Empaglifozin in reducing a combined endpoint of cardiovascular death or hospitalization due to HFpEF decompensation. Interestingly, this trial showed that Empaglifozin was able to reduce the occurrence of the primary endpoint regardless of the presence of Type 2 Diabetes. This study represents the first evidence of beneficial treatment of a drug in HFpEF and may lead to the development of a new therapeutic approach in this disease.

Chapter 9

Atrial fibrillation in the elderly: a risk factor beyond stroke

Atrial Fibrillation (AF) represents the most common arrhythmia worldwide, affecting about 2% of the general population[102]. In the next decades, due to population aging, improvement in diagnosis, management, and therapies, AF prevalence will likely increase, outlining a global epidemic with even more significant impact on public health systems[103]. Similarly to the majority of cardiovascular pathologies, AF prevalence increases with age, accounting from less than 0.5% of the population younger than 50 years up to 10%–17% of people aged 80 years and older[9]. According to the "Expert consensus of the French Society of Geriatrics and Gerontology and the French Society of Cardiology", which has analysed the prevalence of AF stratified by age and sex in studies published from 1991 to 2011 in different countries, 70% of patients with AF are over 75 years old, thus suggesting this arrhythmia to be a pathology almost exclusively of the elderly[104].

AF can induce the formation of atrial thrombi and systemic embolism; therefore, it is often associated with a high number of complications. Among them, embolic stroke is undoubtedly the main one: the risk of stroke increases by almost fivefold in patients suffering this arrhythmia[105]. Aging is an independent risk factor for stroke in AF patients[106]; indeed the CHA_2DS_2-VASc score, recommended for thromboembolic risk stratification according to the most recent European Society of Cardiology (ESC) guidelines for the management of AF, attributes 1 point for age between 65 and 74 years and even 2 points for age ≥75 years, the same as history of previous stroke or thromboembolism[107]. Furthermore, the presence of other concurrent conditions substantially enhances the risk of stroke, and these are amplified in the characterization of the elderly patient: the high number of comorbidities, the increased risk of complications, the intrinsic vulnerability, the frequent lack of specific evidence-based indications, especially for the frail elderly. These features make the therapeutic management of this population particularly insidious, primarily if associated with specific acute or chronic disorders[108].

Beyond stroke, AF represents a risk factor for several other clinically relevant conditions, that can strongly impact the health status of elderly patients: HF, pulmonary embolism (PE), impairment

in physical performance, reduced quality of life (QoL) and development of disability, mood disorders and cognitive impairment up to dementia (Figure 1).

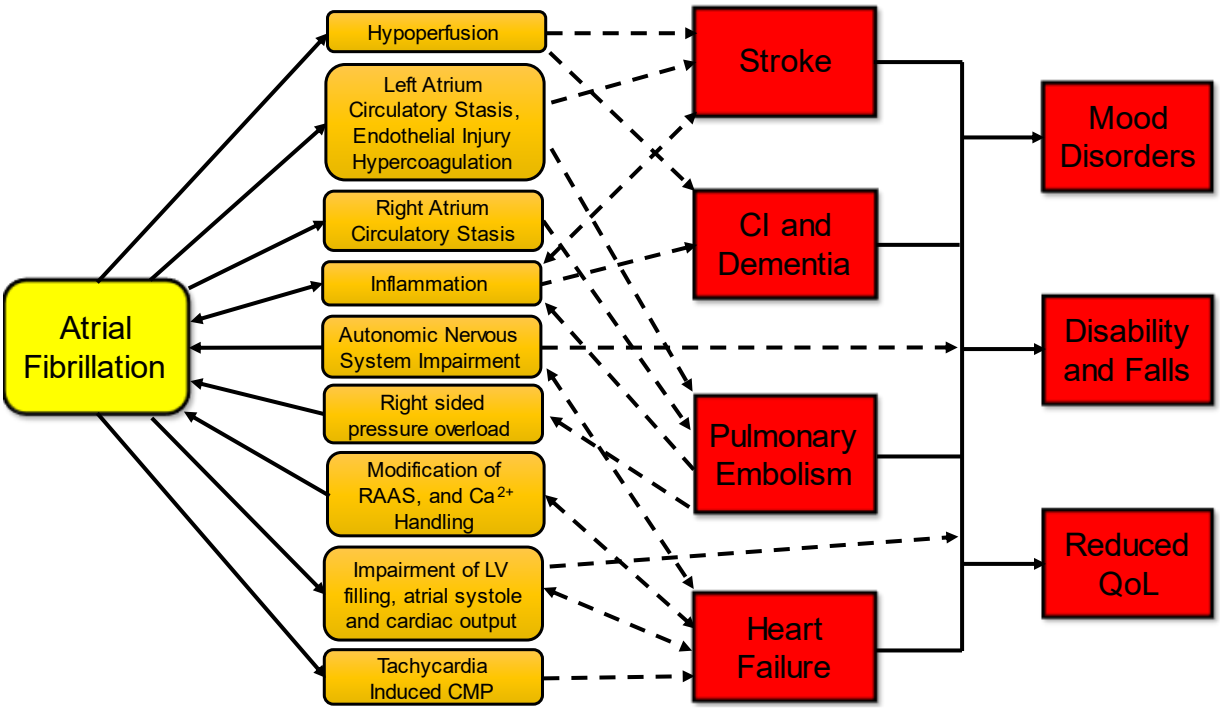

Figure 1. Atrial fibrillation as risk factor: an intricate relationship

AF constitutes a well-documented risk factor for stroke and other less investigated conditions. Although the lack of a specific cause-effect relationship, many widely accepted theories emerged on potential mechanistic linking AF with heart failure, pulmonary embolism, impairment in physical performance, reduced quality of life and development of disability, mood disorders, and cognitive impairment up to dementia.

AF: Atrial Fibrillation; CI: Cognitive impairment; CMP: Cardiomyopathy; LV: Left Ventricular; QoL: Quality of Life; RAAS: Renin Angiotensin Aldosterone System.

The association between AF and these conditions/pathologies in the elderly has been mainly highlighted by small observational, cross-sectional or longitudinal studies or data derived from the registry, whereas more scientifically rigorous studies, such as trials, are not available (Table 1). Further, AF is associated with a countless number of both cardiovascular-related (i.e., hypertension, diabetes, ischemic heart diseases, cardiomyopathies) and non-cardiovascular-related diseases (i.e., respiratory disorders and cancer) for which the boundary between epidemiological association and cause-effect relationship with AF is not entirely defined[109,110].

First Author, year	Study Design	Study population	Aims	Results
atrial Fibrillation and Heart Failure				
Stewart et al., 2002	Prospective	15406 patients, 7052 M, 8354 F, 45–64 yo; 100 patients with AF	AF effect on long term (20 years) outcomes	AF is an independent predictor of CV events [HR 3.0 in. F (2.1–4.2) and 1.8 in. M (1.3–2.5)]; stroke [HR 3.2 in. F (1.0–5.0), and 2.5 in. M (1.3–4.8)]; HF [HR 3.4 in. F (1.9–6.2), 3.4 in. M (1.7–6.8)].
Tsang et al., 2004	Retrospective	569 patients over 65 yo with abnormal LV relaxation. 60 % F.	AF and HF risk and outcome during a follow-up of 4.0 ± 2.7 years.	36 patients developed AF, 50 CHF, 19 AF and CHF; Age, myocardial infarction, diabetes mellitus, LV hypertrophy, and LA volume were predictors of AF and CHF in patients with abnormal diastolic relaxation.
Chiang et al., 2012	Large-scale, cross-sectional international survey	9816 AF patients	AF clinical characteristics in a real-life study	2606 (26.5 %) paroxysmal, 2341 (23.8 %) persistent, and 4869 (49.6 %) permanent AF. HF prevalence (32.9 %, paroxysmal, 44.3 % persistent, 55.6 % permanent AF)
Fummagalli S et al 2015	Prospective	3119 AF patients; 33.7 % over 75 yo; mean age, 815 years; 50.6 % F	Age-related differences in presentation, treatment, and outcome of AF.	Elderly had more frequently (47.5 % vs. 41.1 %; $p < 0.0001$) non-self- terminating forms of AF. CHF present in 55 % of elderly.
McManus et al., 2013	Retrospective Multiethnic Community-Based Cohort	23644 HFpEF and HFrEF patients, 47.7 % F.	AF impact on HFpEF and HFrEF outcome	AF was present in 43.2 % of HFpEF and 32.4 % of HFrEF; Preexisting and incident AF were significantly associated with hospitalization for HF (HR 1.22, 95 % CI 1.15–1.29; HR 2.00, 95% CI 1.83–2.18, respectively), with similar results in those with HF-PEF or HF-REF.
Startipy U et al 2017	Retrospective	41446 HF patients, HFrEF 55.4 % HFmEF 21.5 % HFp EF 23.1 %	AF effect on HFpEF, HFrEF and HFmEF outcome	AF prevalence: 53 % in HFrEF; 60 % in HFmEF; 65 % in HFpEF. Age is independently associated with HF, HFpEF of 2.23 (95 % confidence interval [CI]: 1.98–2.51), in HFmrEF of 2.28 (95% CI: 1.98 − 2.62), and in HFrEF of 2.34 (95% CI: 2.15 − 2.55). F sex and myocardial infarction are inversely correlated with AF.
Kotecha et al., 2016	Systematic Review and Meta-analysis	45100 patients, HFrEF 48.5 %; 32.3 % F HFpEF 51.5 %; 58.9 % F	Outcome in AF patients with HFrEF and HFpEF	All-cause mortality was significantly higher in AF-HFrEF; risk ratio (RR) 1.24, 95 % CI 1.12 − 1.36, $p < 0.001$. ($n = 45,100$), with absolute death rates of 24% compared to 18% in AF-HFpEF over 2 years $p < 0.001$. ($n = 45,100$), with absolute death rates of 24%
Roddux V. et al 2017	Systematic Review Meta-Analysis	220928 patients 17.5 % AF 49.1 % F	CV mortality, myocardial infarction and HF risk in AF patients.	AF was associated with an increased risk of myocardial infarction: (RR) 1.54, 95 % (CI) 1.26 − 1.85), all-cause mortality (RR 1.95, 95% CI 1.50 − 2.54) and HF (RR 4.62, 95% CI 3.13 − 6.83).
Odutayo et al., 2017	Systematic Review Meta-Analysis	43549 AF patients	Mortality and cardiovascular disease risk associated with AF in CHF, stratified our analyses by AF timing and pattern.	Mortality risk for AF varied between incident and prevalent AF (RR 2.21, 95 % CI 1.96 − 2.49 vs relative risk 1.19, 95% CI 1.03 − 1.38, respectively; $P < .001$). The RR of mortality associated with incident AF in HFpEF was 1.86 (95% CI: 1.37 − 2.53).
Chamberlain et al., 2011	Observational, Community based cohort	1664 HF patients, 54.6 % F; 553 AF prior to HF 384 AF after HF	Outcome in AF and CHF patients	AF prior to HF had a 29 % increased risk of death, while AF after HF more than a 2-fold increased risk of death.
Savarese G. et al 2017	Meta-Analysis	55011 patients with AF, 52 % with HF; 36 % F. 27,518 on DOACs.	Efficacy and safety of DOACs in AF and HF	HF patients had reduced rates of overall and intracranial bleeding (RR: 0.86; 95 % CI: 0.81 to 0.91; $p < 0.01$) (RR: 0.74 95 % CI: 0.63 to 0.88; $p < 0.01$) but increased rates of all-cause and CV death (RR: 1.70 95 % CI: 1.31–2.19; $p < 0.01$); (RR: 2.05 95% CI: 1.66–2.55; $p < 0.01$). DOACs, compared with warfarin significantly reduced overall bleeding, regardless of the presence or absence of HF.

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First Author, year	Study Design	Study population	Aims	Results
Kotecha et al., 2017	Meta-Analysis of RCT	3043 AF and HF 14,166 Sinus Rythm; median age 65 years; 24 % F.	Prognostic importance of heart rate in patients with HFrEF	Beta-blockers reduce mortality in patients with HFrEF in sinus rhythm, HR: 0.73 vs. placebo; 95 % CI: 0.67 to 0.79; p < 0.001), regardless of baseline heart rate (interaction p = 0.35). Beta-blockers had no effect on mortality in patients with AF (HR: 0.96, 95 % CI: 0.81–1.12; p = 0.58) at any heart rate (interaction p = 0.48).
Caldeira et al., 2011	Systematic Review and Meta-Analysis of RCT	2486 AF and HF patients.	Effects of rate and rhythm control in patients with AF and HF.	Hospitalisations were less frequent in rate control than in rhythm control patients (RR 0.92; 95 % CI: 0.86 − 0.98; p = 0.008). Mortality and stroke/thromboembolic events were not significantly different in rate and rhythm control arms (RR 1.03; 95% CI: 0.90 − 1.17) and (RR 1.09; 95% CI: 0.61 − 1.96).
Zhu et al., 2016	Systematic Review and Meta-Analysis of RCT	143 AF and HF patients.	Effectiveness of restoring the sinus rhythm by catheter ablation relative to medical rate control for persistent AF patients with HF.	Catheter ablation improved LVEF, exercise capacity, and quality of life for persistent AF patients with HF compared with the medical rate control strategy :MD: 6.22 %; 95 % CI: 0.7 − 11.74, P = 0.03) and peak VO2 (MD: 2.81 mL/kg/min; 95% CI: 0.78 − 4.85, P = 0.007; Minnesota Living with Heart Failure Questionnaires scores (MD: -11.05; 95% CI: −19.45 to −2.66, P = 0.01).
Kelly et al., 2019	Retrospective analysis from Get With The Guidelines-Heart Failure (GWTG-HF) registry data	15682 AF and HF patients; median age 83; 65.8 % F; 1857 rhythm control; 13,825 rate control.	Outcomes in patients with HFpEF complicated by AF.	Rhythm control in patients aged 65 and older with heart failure with preserved ejection fraction and AF was associated with a lower risk of 1 year all-cause mortality. Adjusted HR, 0.86; 95 % CI, 0.75 − 0.98; *P* = 0.02.
Atrial Fibrillation and Pulmonary Embolism				
Kukla et al., 2015	Retrospective	975 patients (59 % F) with AF; mean age 66 ± 15 yo (range 17–98)	RHT and AF prevalence and impact on survival and complications	AF and RHT in 16 patients (7%), mortality in patients with both AF and RHT 50 % vs 20 % in only AF, complications 40 % vs 22 %
Bikdeli et al., 2017	Systematic review	87063 patients with AF by 89 articles	Pathophysiological considerations, epidemiology, prognostic power, and potential impact of the association	Higher risk of PE in AF patients. Pooled analysis of 2 studies showed in AF patients and odds ratio of PE = 2.86 (95 % CI: 2.26–3.60)
Ng et al., 2016	Retrospective	1142 patients (55 % F) with PE; mean age 67.2 ± 16.6 yo	Prevalence and incidence of AF in patients with acute PE	Age-adjusted prevalence of AF 4672/100,000, (F 5358/100,000, M 4218/100,000). Age-adjusted incidence rate was 984/100,000 person-years (F 840/100,000 person-years, M 1167/100,000 person-years)
Tang et al., 2017	Retrospective	305 patients (≈53 % F) with PE and AF; age over 65 yo	CHADS2 score predicts prognosis of PE in patients with AF	CHADS2 and CHA2DS2-VASc scores were associated with adverse in-hospital outcome in patients with PE and AF. The combination of CHADS2 score ≥2 and sPESI ≥1 might help stratification of high-risk patients.
Guler et al., 2016	Cross-sectional, retrospective, observational	71 patients (F with No PE 38.5 %; F with PE 66.7 %) with persistent paroxysmal AF; No PE mean age 56.0 (26–83), PE mean age 66.0 yo (50–75)	Incidence and predictors of asymptomatic PE in patients undergoing AF ablation.	CHA2DS2-VASc scores were independent predictors of asymptomatic PE in patients undergoing AF ablation.
Hald et al., 2018	Prospective	29842 participants of the fourth and sixth surveys of the Tromsø study	Impact of AF on the cause-specific risks of PE and Ischemic Stroke	AF was related to higher risk of PE (HR, 10.88; 95 % CI, 6.23 − 18.89) and Ischemic Stroke (HR, 6.16; 95% CI, 4.47 − 8.48). When examining PE risk at different time points after AF diagnosis, the risk decreased over time and disappeared 3 years after onset of AF
AF, physical performance and falls.				
Rienstra et al., 2013	Cross-sectional prospective	3609 participants (59 % F) of the Framingham Heart Study; mean age 73 ± 8 yo	Relation among physical disability (Rosow-Breslau Functional Health Scale), subjective health (self-reported) and incident AF	24 % of patients suffered prevalent physical disability at baseline. 10-year age- and sex-adjusted incidence rate for AF was 13 %. Prevalent physical disability was related to incident AF (multivariable-adjusted HR 1.25; 95 %CI, 1.02–1.54)

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First Author, year	Study Design	Study population	Aims	Results
Magnani et al., 2016	Prospective	2753 Health ABC participants (F 52 %); mean age 73.6 ± 2.9 yo	Association of incident AF and 4-year interval decline in physical performance	AF patients experienced a significantly greater 4-year physical performance battery decline than the ones without AF at age 70, 74, 78, and 82, with mean estimated decline ranging from -0.08 to -0.10 U (95 % CI -0.18 to -0.01)
Donoghue et al., 2014	Prospective	4525 Irish Longitudinal Study on Ageing (TILDA) participants (F 52.1 %); mean age 63.5 ± 8.9 yo	Association between AF and objectively measured mobility	AF was independently associated with lower usual gait speed in community-dwelling adults
Sanders et al., 2012	Retrospective	442 patients; median age of 81 yo	Association between AF and non-accidental falls.	The prevalence of history of AF was significantly higher in patients with non accidental fall when compared to patients with accidental fall (26 % vs 15 %). AF was an independent risk factor for non accidental fall in patients ≤81 years old (2.5 times greater)
Wong et al., 2017	Retrospective	113600 individuals (F 46.6 %); mean age 55.8 ± 19.7 yo	AF association with fracture risk	After adjusting for covariates, the association between a history of AF and incident hip fracture was statistically significant in both men (adjusted HR 1.97 [95 % CI 1.61–2.42]) and women (adjusted HR 2.08 [95 % CI 1.80–2.39])
Hung et al., 2013	Cross-sectional prospective	401 geriatric subjects (F 24 %); mean age 82.2 ± 0.2 yo	Cardiovascular co-morbidities and medication associated with falls in older adults with AF.	Benzodiazepine use (OR 18.22, 95 % CI 2.71 − 122.38), a history of paroxysmal AF (OR 12.18, 95% CI 1.37 − 108.70) and hypertension (OR 9.49, 95% CI 1.19 − 75.57) were independent factors for falls in older adults with AF. Prevalence of falls or recurrent falls in AF subjects showed a linear relationship with the number of independent factors for falls.
Banerjee et al., 2014	Retrospective	7156 patients with non valvular AF; with prior history of falls (F 56.6 %) mean age 82.9 ± 8.9, with no prior history of falls (F 37.6 %) mean age 69.9 ± 15.1	Risk of cardiovascular outcomes associated with prior history of falls in patients with AF.	Prior history of falls was 1.1 %. By Cox regression analyses for all AF patients with heart failure, the association for ischemic stroke/ thromboembolism (HR 1.71; 95 % CI, 1.04 − 2.83; P 1/4 .04) and all-cause mortality (HR 1.68; 95% CI, 1.07 − 2.62; P 1/4 .02) only remained significant.
Leibowitz et al., 2017	Retrospective	410 > 65 yo subjects underwent surgical repair of hip fracture (F 66.1 %); mean age 80 ± 7.8 yo	Newly diagnosed AF in subjects underwent hip fracture repair surgery was predictive for one-year mortality	Mortality in patients with new onset AF was higher than in the group without AF (60 % vs 19.5 %; p = 0.001)
AF, disability and quality of life.				
Wallace et al., 2016	Longitudinal prospective	4046 subjects from the Cardiovascular Health Study (F 58.2 %); mean age 73 ± 5 yo	Associations between incident AF and disability-free survival / risk of disability	AF was associated with shorter disability-free survival and higher risk of ADL disability (HR 1.71, 95 % CI = 1.55 − 1.90 HR = 1.36, 95% CI = 1.18 − 1.58).
Ekerstad et al., 2018	Clinical observational study	408 elderly of the TREEE study; patients with AF (F 55.8 %) mean age 86.1 ± 5.1, patients without AF (F 56.9 %) mean age 85.3 ± 5.6 yo.	Prevalence of AF among hospitalized frail elderly patients	The prevalence of AF in hospitalized frail elderly population was 47 %.
Strømnes et al., 2019	Systematic Review	25 studies assessing sex differences in QoL in patients with AF (F 26 − 50%)	Sex differences in the QoL of patients with AF	Female AF patients report poorer QoL and were more symptomatic than male AF patients.
Freeman et al., 2015	Observational, community-based prospective	10087 adults who had AF (F 42.4 %); median age 75 (67 − 82)	Correlation between degree of AF symptoms severity and quality of life; the association between symptoms or quality of life with clinical outcomes (death, hospitalization, stroke, and major bleeding).	Inverse correlation between the EHRA AF symptom severity class and quality of life measured by the AFEQT. Patients with AF-related symptoms at baseline (EHRA ≥ 2) had a higher risk of hospitalization (adjusted hazard ratio 1.23 (95 % CI 1.15–1.31)
Thrall et al., 2006	Systematic Review	49 studies (528 patients)	Impact of AF on patients' QoL	Patients with AF showed significantly poorer QoL compared with healthy controls, the general population, and other patients with coronary heart disease
Wynn et al., 2015	Prospective, multicenter randomized controlled trial	146 patients with persistent AF of the SARA study (F 33 %); mean age 55 ± 9 yo	Quality of Life in AF patients undergoing catheter ablation	Catheter Ablation significantly improved QoL in persistent AF patients; medical therapy had no appreciable effect.
AF, anxiety and mood disorders.				
Thrall et al., 2007	Prospective	101 AF patients (F 38.6 %); mean age 66.3 ± 11.0 yo	Prevalence and persistence of depression and anxiety in patients with AF, and their effect on future quality of life	AF patients presented depression (30 %), state anxiety (23 %), and trait anxiety (22 %). Depression was the strongest independent predictor of future QoL

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First Author, year	Study Design	Study population	Aims	Results
Rewiuk et al., 2018	Clinical multicenter observational study	4049 AF subject enrolled in the PolSenior project (F 48 %); mean age 78.1 ± 8.3 yo	Prevalence of depression in Polish community-dwelling older patients with a history of AF.	After adjustment for age, gender and marital status AF was the second strongest cardiovascular risk factor of depression occurrence (OR = 1.69; 95 %CI: 1.43–2.00). After full adjustment for comorbidities, number of medications, and significant geriatric dysfunctions, AF remained the only cardiovascular comorbidity correlating with depression occurrence (OR = 1.35, 95 %CI: 1.10–1.67), beside stroke
Wändell et al., 2016	Retrospective	12283 AF subjects (F 46.2 %); mean age 74.4 ± 10	Effects of depression and anxiety on all-cause mortality in patients with AF.	30 % increased mortality risk in men with AF and concomitant depression compared to their counterparts without depression. Among women, the association between depression or anxiety and all-cause mortality was weaker and not statistically significant.
von Eisenhart Rothe et al., 2014	Controlled retrospective	702 AF patients of the ANTIPAF and Flec-SL trials (F 34.5 %); mean age 64.16 ± 13	Assessment of depressed mood frequency in persistent or paroxysmal AF and identification of cases of major depressive disorder in AF	Depressed mood was more severe in patients with persistent AF than in patients with paroxysmal AF (OR confounder controlled model = 1.44; 95 %CI: 1.13 − 1.75).
Hu and Lin, 2019	Retrospective	88259 patients with AF (F 43.5 %) and 88,259 without AF (F 44.5 %); with AF mean age 72.7 ± 13.4, without AF 72.4 ± 14.2	Incidence of suicide attempt in a Taiwanese population following admission for AF, adjusted for competing risks and comorbidities.	Incidence of suicide attempt after AF significantly increased in the following 10 years.
AF and cognitive impairment.				
Bunch et al., 2010	Retrospective	37025 patients by the Intermountain Heart Collaborative Study database (F 39.9 %); mean age 60.6 ± 17.9 yo	Verification of the independent risk of AF and AD. The impact of AF in those subsequently diagnosed with AD and other dementia types on risk of mortality.	27 % developed AF and 4.1 % developed dementia during the 5-year follow-up. AF was independently associated with risk of all forms of dementia. The highest risk of AD was in the younger AF group. The presence of AF in all dementia subtypes identified patients at higher risk of mortality, most prominent in the youngest population.
Singh-Manoux et al., 2017	Prospective	414 AF patients of the Whitehall II study (15.7 %); mean age 58.8 ± 5.9	Potential dose-response association of duration of exposure to AF on cognitive decline, and whether stroke and coronary heart disease subsequent to AF mediated this association.	AF was associated with a 6.22 times increased risk of stroke (95 % CI: 4.74, 8.16) and its association with dementia was not fully explained by stroke. AF increased risk of coronary artery disease (HR = 5.29; 95 % CI: 4.50, 6.22) and CVD (HR = 5.74; 95 % CI: 4.95, 6.65). The association between AF and dementia was present in those with CVD (HR = 1.79; 95 % CI: 1.04, 3.08) but not in those free of CVD (HR = 1.29; 95 % CI: 0.74, 2.24).
Chen et al., 2014	cross-sectional study	1906 participants of the U.S. community-based ARIC study (F 52.9 %); mean age 76.9 ± 5.2 yo	AF association with lower cognitive function	Persistent but not paroxysmal AF is associated with lower cognitive function in community-dwelling elderly individuals

Table 1. Main articles reporting the relationship between AF and non-stroke complications.

AD: Alzheimer's disease; ADL: activities of daily living; AF: atrial fibrillation; AFEQT: Atrial Fibrillation Effect on QualiTy-of-Life; CHF: Chronic Heart Failure; CV: Cardiovascular; CVD: Cardiovascular Disease; CI: confidence interval; DOACs: Direct Oral Anticoagulants; EHRA: European Heart Rhythm Association; F: female; Health ABC: Health, Aging, and Body Composition; HF: Heart Failure; HFmEF: Heart Failure with midrange Ejection Fraction; HFrEF: Heart Failure with Reduced Ejection Fraction; HFpEF: Heart Failure with Preserved Ejection Fraction; HR: hazard

ratio; OR: odds ratio; LA: left atria; LVEF: Left Ventricular Ejection Fraction; PE: pulmonary embolism; QoL: quality of life; RHT: right heart thrombi; RR: Relative Risk; MD: Mean Difference; sPESI: simplified pulmonary embolism severity index; yo: years old

Conclusive remarks

AF represents a tremendous public health problem, with a high impact in terms of mortality and morbidity, due to complications, such as stroke, and other less investigated conditions equally influencing the clinical status and survival of these patients. As typical in scientific literature, the geriatric population, whose management intrinsically results complex due to high heterogeneity in comorbidity, disability, and socioeconomic conditions, is underrepresented in clinical trials. Thus, more robust studies would be desirable to better define the impact of AF on heart failure, pulmonary embolism, mood disorders, QoL, disability, and cognitive impairment in elderly patients Moving toward the perspective of personalized medicine, biomedical research should mandatorily take greater account of the heterogeneity of the elderly, to construct evidence that allows physicians to no longer interpret the provided indications only based on their own experience.

Chapter 10

Antithrombotic therapy in patients undergoing transcatheter aortic valve replacement: the complexity of the elderly

Aortic stenosis (AS) represents the most common valvular heart disease in Europe and North America, with a steadily growing prevalence due to the ageing population[111]. Indeed, although the predictable variability among data derived from epidemiological studies and the slight contribution of bicuspid aortic valve and congenital forms, this condition particularly affects elderly patients. It is estimated that about 5% of the population at age 65 suffers from AS and it is becoming increasingly frequent in clinical practice[112].

Calcific degeneration of valve structure constitutes the most common cause of AS in the Western world, whereas rheumatic AS still remains the main aetiology in developing countries[113]. The pathophysiological mechanisms leading to valve stenosis are considered to be similar to those involved in the development of atherosclerotic plaques, with emerging therapeutic implications[114]. Actually, advanced age, male gender, dyslipidemia, systemic inflammatory status represents shared risk factors between coronary artery disease and AS[115]. Initially, aortic degeneration insidiously progresses, then symptoms' onset is paralleled by a fast worsening of valvular stenosis and calcifications. The symptomatologic manifestations, including breathlessness, angina, palpitations and syncope, are crucial for the assessment of aortic valve replacement therapy, though the heterogeneity of clinical presentation also poses controversies in approaching asymptomatic patients with instrumental evidence of severe AS[116].

In this context, transcatheter aortic valve replacement (TAVR) has quickly spread, indeed, it is nowadays also employed in treating patients with AS at intermediate operative risk. Nonetheless, the less invasive interventional strategy still carries relevant issues concerning post-procedural optimal antithrombotic strategy, given the current indications provided by guidelines are not completely supported by evidence-based data. Geriatric patients suffer from high bleeding and thromboembolic risks, whose balance is particularly subtle due to the presence of concomitant conditions, such as atrial fibrillation and chronic kidney disease, that make the post-TAVR antithrombotic management particularly insidious. This scenario is further complicated by the lack

of specific evidence regarding the "real-life" complex conditions typical of the geriatric syndromes (Figure 1), thus, the management of such a heterogeneous population, ranging from healthy aging to frailty, is far from being defined.

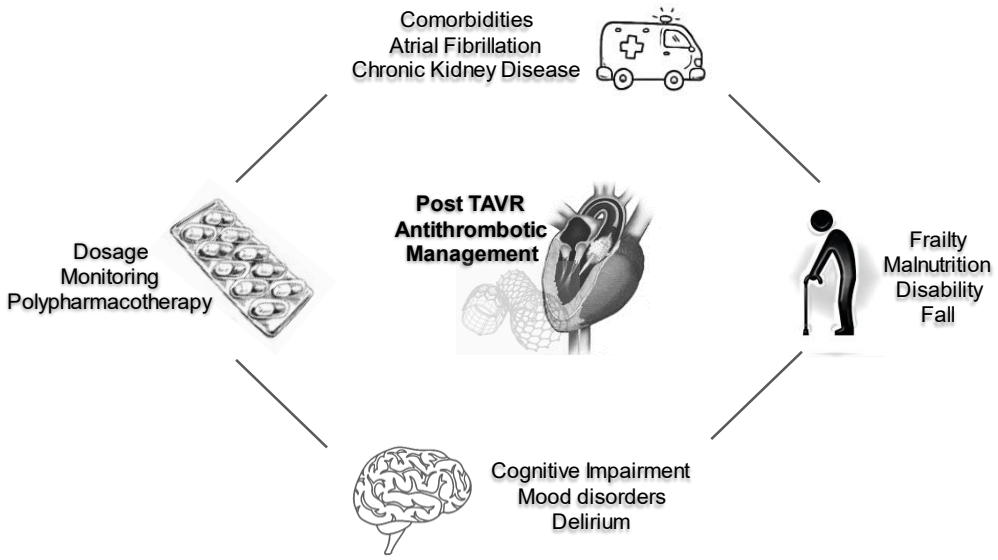

Figure 1. The complexity of antithrombotic strategy after TAVR in elderly.

Real-life geriatric patients suffer comorbidities and various degree of disability, which carry many issues concerning the correct choice of post-procedural optimal antithrombotic strategy. Cognitive impairment and polypharmacotherapy concur to make the balance between concomitant thromboembolic and bleeding risks even more unstable.

Conclusive remarks

Elderly patients, who constitute the vast majority of people undergoing TAVR, represent a heterogeneous population, with highly variable characteristics and great vulnerability, suffering from several comorbidities and various degrees of disabilities. Since multimorbidity is often listed as an exclusion criterion in most of the research protocols, consensus documents and guidelines rarely provide ad hoc recommendations for managing complex cases, as demonstrated by the difficulties in choosing optimal antithrombotic therapy in TAVR patients when concomitant geriatric syndromes, as AF and/or CKD, occur. In this scenario, a thorough assessment of possible ischaemic and bleeding complications and an attempt at attenuating these risks, also through behavioural measures, still remain the main challenges the physician has to face, waiting for further evidence aiming to provide suitable indications for the complex real-life clinical practice.

Chapter 11

Role of frailty on cardiac rehabilitation in hospitalized older patients

Cardiac rehabilitation (CR) is a comprehensive, multidisciplinary intervention, tailored on each single patient with CVD, which includes exercise training programs, lifestyle modification, and psychological support. It has been shown to improve exercise tolerance, patients' well-being and quality of life, and to reduce the risk of new cardiac events[117]. Interestingly, patients older than 75 years represent about one third of those referred to CR[117], therefore, frailty might be present in a large proportion of patients undergoing CR, but this aspect has not been thoroughly tested so far.

This research aimed to investigate whether multidimensional frailty might complicate the management of older patients undergoing CR. The main objective of the study was therefore to determine the relationship between frailty and CR outcomes in hospitalized older adults.

Methods

The participants have been recruited among patients referring to Istituti Clinici Scientifici Maugeri IRCCS, Telese Terme Institute, Italy for CR after HF exacerbation, IHD, valvular heart diseases (VHD), cardio-aortic surgery, and other cardiovascular conditions.

At admission, all patients underwent medical history collection, clinical examination and evaluation of the main demographic/clinical factors. At the time of enrollment, all stable patients underwent Comprehensive Geriatric Assessment (CGA), with the evaluation of the domains of health and functional status, psycho-cognition, socio-environmental condition. A CGA-based Frailty Index (FI) was adapted from the standard procedure proposed by Rockwood's research group, taking into account a total of 40 multidimensional health deficits including comorbidities, laboratory and diagnostic data, symptoms and sign of diseases[118]. Each deficit was awarded 1 point if present, or 0 in its absence. FI for single participant resulted by the ratio between her/his cumulative points and the total number of evaluated items, thus this ranged between 0 and 1. A cut-off of 0.25 was applied to define participants as frail ($FI \geq 0.25$) and non-frail ($FI < 0.25$).

At the end of CR, all patients underwent the 6-min walking test (6MWT) following indications provided by American Thoracic Society guidelines[119]. It was performed by trained physicians who

monitored the procedure and invited subjects to walk at their own maximal pace on a 20-m long hospital corridor, after 30 minutes of absolute rest from physical activity. Predicted 6MWT was determined for each participant through reference equations for both genders based on patients age, height and weight, while the 6MWT ratio was measured as the ratio between 6MWT distance and normal predicted values[120–123].

Results

The study population consisted of 559 older adults, mostly males (387, 69.2%). The patients mainly accessed the CR programs offered by the clinic for recovery from IHD (231, 41.5%), HF exacerbation (138, 24.7%), and correction of VHD (166, 29.7%). Data on the global sample and on groups stratified according to frailty status are reported in Table 1.

Characteristics	All patients ($n=559$)	Non Frail ($n=266$)	Frail ($n=293$)	sig p value
Age (years)	72 (69–76)	71 (68–75)	73 (69–77)	<0.001
Gender (male)	387 (69.2)	181 (68)	206 (70.3)	0.563
BMI (Kg/m^2)	28 (25–30.9)	27.05 (24.8–29.8)	28.8 (25.6–32)	<0.001
MMSE	24 (20–28)	25 (21–29)	23 (18–27)	<0.001
GDS	2 (1–4)	1 (1–3)	3 (1–5)	<0.001
BADL	6 (6–6)	6 (6–6)	6 (6–6)	<0.001
IADL	5 (4–7)	6 (4–7)	4 (3–5)	<0.001
MNA	25 (23.5–26)	25.5 (24.5–26)	25 (23–26)	<0.001
CIRS-C	5 (4–7)	5 (3–7)	6 (5–7)	<0.001
CIRS-S	2.2 (1.9–2.5)	2 (1.8–2.3)	2.2 (2–2.5)	<0.001
Number of drugs	9 (8–11)	8 (7–10)	10 (8–12)	<0.001
PASE	81 (41–121)	95 (70–126)	61 (20–106)	<0.001
Social support score	6 (4–7)	5 (4–7)	6 (5–8)	<0.001
Exton-smith	18 (17–19)	18 (18–19)	18 (16–19)	<0.001
SPPB	11 (8–12)	11 (9–12)	10 (7–12)	<0.001
POMA	28 (25–28)	28 (26–28)	28 (24–28)	0.003
Frailty Index	0.25 (0.19–0.3)	0.19 (0.16–0.22)	0.3 (0.26–0.33)	<0.001
Diagnosis at admission				
Heart failure	138 (24.7)	46 (33.3)	92 (66.7)	<0.001
Ischaemic heart disease	232 (41.5)	105 (45.3)	127 (54.7)	
Valvular heart disease	166 (29.7)	97 (58.4)	69 (41.6)	
Other CV conditions	23 (4.1)	18 (78.2)	5 (21.8)	
6MWT (m)	275 (203–348)	300 (236–360)	260 (180–330)	<0.001
Predicted 6MWT (m)	440.54 ± 57.42	451.5 ± 54.05	430.59 ± 58.65	<0.001
6MWT ratio	0.62 ± 0.21	0.66 ± 0.21	0.58 ± 0.21	<0.001

Table 1. Characteristics of the overall population and of subgroup according to frailty status

6MWT, 6-minutes walking test; GDS, Geriatric Depression Scale; BADL, Basic Activity of Daily Living; BMI: Body Mass Index; CIRS, Cumulative Illness Rating Scale; CV, cardiovascular; IADL, Instrumental Activity of Daily Living; MMSE, Mini Mental State Examination; MNA, Mini Nutritional Assessment; PASE, Physical Activity Scale for the Elderly; POMA, Tinetti's Performance Oriented Mobility Assessment; SD, Standard Deviation; SPPB, Short Performance Physical Battery.

The p value corresponds to Student's t test or Mann–Whitney U-test for continues variables, and χ^2 test for categorical data.

At enrollment, participants presented a mean FI of 0.25 (0.19-0.3) (Figure 1), whereas the predicted 6MWT and the 6MWT distance performed at the end of CR were 440.54±57.42 and 275 (203-348) m, respectively, accounting for an overall 6MWT ratio of 0.62±0.21. The relationship between FI and 6MWT ratio is shown in Figure 2.

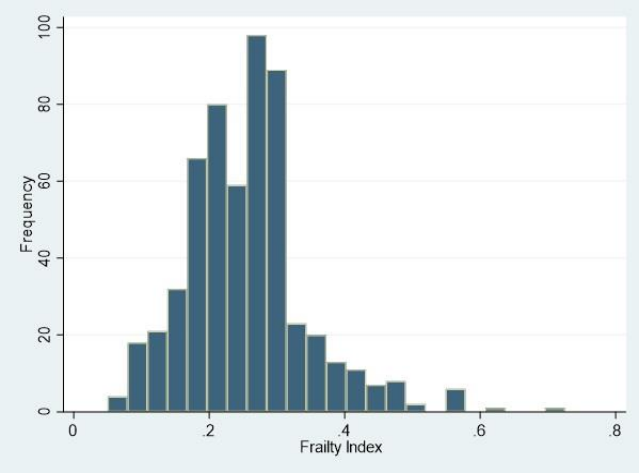

Figure 1. Frailty Index Distribution in the study population.

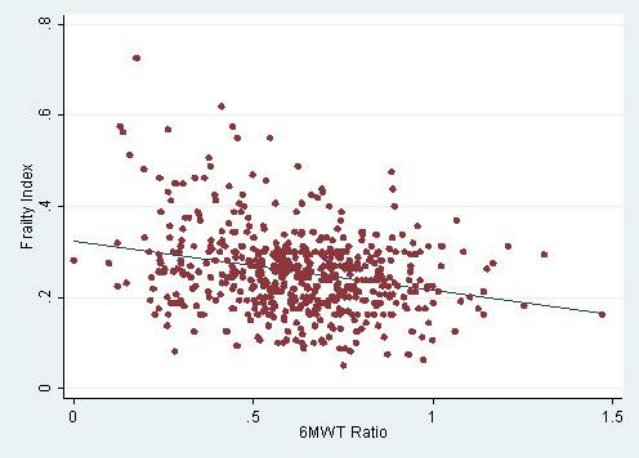

Figure 2. Scatter plot for 6MWT ratio and Frailty Index in the study population.

At univariate analyses, frail patients presented higher BMI, while no differences emerged in terms of gender. The frailer group presented worse score in all the CGA domains, as demonstrated by results in tests and scales exploring dependency [Activity of Daily Living (ADL), Instrumental Activity of Daily Living (IADL)], phyco-cognition [Mini Mental State Examination (MMSE), Geriatric Depression Scale (GDS)], comorbidities [Cumulative Illness Rating Scale (CIRS)], nutrition [Mini Nutritional Assessment (MNA)], polypharmacotherapy, global status (Exton-Smith, Social Support Scale), and physical performance [Physical Activity Scale for the Elderly (PASE), Short Performance Physical Battery (SPPB), Tinetti's Performance Oriented Mobility Assessment (POMA)].

At multivariable regression analysis for 6MWT ratio, the model included as independent variables age, gender, FI and diagnosis at admission and explained 23% of dependent variable variance in the study population (Table 2).

6MWT ratio R^{2a}: 0.226			
Variables	B	SE	Sig
Age	− 0.024	0.001	0.118
Gender (male)	0.166	0.017	< 0.0001
Diagnosis	− 0.036	0.01	< 0.001
Frailty Index	− 0.666	0.099	< 0.0001

Table 2. Regression analysis for 6MWT Ratio

The analysis revealed the 6MWT ratio to be significantly and independently associated with male gender, diagnosis and FI ($p < 0.0001$, $p < 0.001$ and $p < 0.0001$ respectively), while no relevant association emerged with chronological age.

Conclusive Remarks

FI resulted to be significantly and independently correlated to 6MWT ratio in a population of elderly patients undergoing CR. Frailty is a multifactorial geriatric syndrome whose assessment requires CGA and is essential for prognostic evaluation of older patients, also in CR clinical setting. Further studies are needed to establish the best strategies to assess frailty and physical function in CR setting, in order to incorporate them within the workflows of rehabilitation clinics and target specific geriatric deficits that would interfere with the success of training programs.

Section 4

Blood pressure variability as a new marker of aging

Accumulating evidence indicates that the cardiovascular system undergoes several phenotypic changes over time, from pathological cardiac remodelling to systolic and diastolic dysfunction, and microcirculatory impairment[124]. These biological modifications determine decline in cardiac function and higher vulnerability to stress, which in turn enhances the risk of CVDs. Accordingly, several alterations in neurocardiovascular mechanisms occurring with aging lead to impairment in physiological variability patterns, such as those implicated in the regulation of blood pressure (BP). BP values are well known to fluctuate over time as a result of interactions among external stressors, cardiovascular homeostasis and neurohormonal modulation, whose balance becomes less efficient with aging[125,126].

Beyond BP mean values, accumulating evidence has reported independent associations between blood pressure variability (BPV), and several health outcomes typical of late life[127]. Higher BPV has been found to increase with chronological age, and to be associated with greater risk of cardiovascular events, structural brain changes, altered cognition and increased dementia risk[128–133]. BPV has also demonstrated an independent predictive value for several non-cardiovascular health outcomes associated with aging and typical geriatric conditions[134–139].

Over the decades, many mechanisms of cardiovascular aging have been proposed, especially oxidative stress and mitochondrial dysfunction, nevertheless, clinical indicators of early alterations in cardiac homeostasis have not yet been identified. Following these premises, this section focuses on the role of BPV as clinical manifestation of the dysregulation in homeostatic patterns occurring during aging. The findings of a literature review on the pathophysiological mechanisms underlying altered BPV are reported in Chapter 12[140], together with its association with the hallmarks of aging. The latter topic has been further deepened through the assessment of circulating levels of molecules implicated in "inflammaging" and “mitochondrial dysfunction” in a population of community-dwelling older adults (Chapter 13)(*). Finally, the role of the BPV on the trajectories of the functional capacities of the individual is preliminarily shown in Chapter 14(**), evaluated through the Intrinsic

Capacity, an innovative approach of geriatric medicine, intended to reflect the dynamic biological reserve of each person and aimed at preserving successful aging.

(: this manuscript has been accepted for publication in GeroScience, as it is not yet available online on the publisher's website at the time of submission of the thesis, it is reported in the form of abstract in the chapter Chapter 13)*

*(**: this manuscript is under peer review in an international journal, therefore the results are not reported and the research project is presented schematically in Chapter 14)*

Chapter 12

Blood pressure variability: A potential marker of aging

BP is well known to fluctuate over time around average values, possibly as the result of complex interactions among external environmental stress, intrinsic cardiovascular regulatory mechanisms, humoral influences, and rheological factors[125]. It has been ascertained for several years that aging is associated with alterations in neurocardiovascular regulatory mechanisms that result in impaired physiological variability patterns[141]. Accordingly, dysregulated BP may constitute an epiphenomenon of the alterations in homeostatic mechanisms, typical of late life.

Far from being a background noise, a measurement artifact, or a random phenomenon, BPV has received growing attention in recent years[127], particularly due to its association with various health outcomes, independently of absolute BP values. Very interestingly, BPV has been found to increase with advancing age (≥ 75 years) in the Second Australian National Blood Pressure Study[142] and in several other findings[131,143,144].

The potential mechanisms involved in BPV in older adults may differ from those operating in younger adults[145]. Increased arterial stiffness and impaired baroreflex function are one of the mechanisms that may contribute to greater BPV with aging whereas in young people, BPV may merely occur in response to the activities of everyday life[146]. The growing interest in BPV is related to the repeated demonstration of its independent predictive value for target-organ damage and several health outcomes associated with aging (Table 1).

Health Outcome	*References*	*Study population*	*BPV assessment*	*Main findings*
Neurological and cardiovascular events	Mehlum et al. (2018)	13803 patients with hypertension and at least one additional risk factor for cardiovascular events, from the VALUE trial	Systolic visit-to-visit BP variability (SD) from all visits from 6 to 60 months	Patients in the highest quintile of SD had an increased risk of cardiovascular events (HR 2.1, 95 % CI 1.7–2.4)
	Proietti et al. (2017)	3843 patients with atrial fibrillation from AFFIRM trial	Systolic visit-to-visit BP variability (SD), median follow-up of 3.6 years	Patients in the 4th quartile of systolic BP variability (SD) had the highest risk for stroke (HR 2.33, 95 % CI 1.30–4.16,), major bleeding (HR 2.88, 95 % CI 1.79–4.61) and all-cause death (HR 1.38, 95 % CI 1.00–1.91)
	Tai et al. (2015b)	A meta-analysis of 13 prospective studies with total of 77299 patients	Systolic visit-to-visit BP variability (SD and CV), mean follow-up of 6.3 years	Adjusted HR for all-cause mortality and cardiovascular mortality: 1.03 (95 % CI 1.02–1.04) and 1.10 (1.02–1.17) per 1-mmHg increase in systolic BP variability (SD), respectively
Structural brain changes	Tully et al. (2020)	A meta-analysis of 12309 brain scans from 27 studies	Systolic and diastolic visit-to-visit BP variability (SD, CV, VIM, ARV, SV)	Higher systolic (OR, 1.27; 95 % CI, 1.14–1.42) and diastolic BPV (OR, 1.30; 95 % CI, 1.14–1.48) were associated with CSVD, independent of mean BP
	van Middelaar et al. (2019)	Post-hoc analysis in 122 community-dwelling people aged 70–78 years with hypertension from the prevention of dementia by intensive vascular care (preDIVA) magnetic resonance imaging substudy	Systolic visit-to-visit BP variability (CV) (baseline and after 2, 4, 6, and 7–8 years)	One point increase in CV of systolic BPV was significantly associated with 0.043 mL/y (95 % CI 0.015–0.072, unadjusted model) White Matter Hyperintensities progression
Cognitive Impairment and Dementia	Alpérovitch et al. (2014)	6506 non-institutionalized subjects aged 65 years, without dementia from the Three-City Study	Systolic visit-to-visit BPV from three measures (baseline and 2- and 4-year visits)	The risk of dementia (HR) for patients in the highest decile of the systolic BP variability (CV) was 1.77 (95 % CI 1.17–2.69)
	De Heus et al. (2021)	A meta-analysis on 7899,697 observations from 20 studies	Systolic and diastolic 24-hour, day-to-day, and visit-to-visit BPV, through all parameters	Higher systolic (OR 1.25, 95 % CI 1.16–1.35) and diastolic BPV (OR 1.20, 95 % CI, 1.12–1.29) were associated with a combined end point of dementia or cognitive impairment
	Ma et al. (2021)	13284 participants aged ≥ 50 years without dementia	Visit-to-visit SBP variability was quantified from repeated annual SBP measurements, over a median follow-up of 5.0 years	Higher visit-to-visit SBP variability was associated with cognitive deterioration (conversion from normal cognition to mild cognitive impairment MCI or dementia, or from MCI to dementia) (adjusted odds ratio in the highest quintile 2.64, 95 % CI 2.29–3.04)
	Rouch et al. (2020a)	3319 non institutionalized subjects from the S.AGES cohort aged ≥ 65 years	Systolic and diastolic visit-to-visit BP variability (SD, CV, VIM, ARV, SV), based on every 6 months BP measurements during 3 years	Higher BPV was associated with greater dementia risk (adjusted 1-SD increase of CV: HR=1.23, 95 % CI 1.01–1.50 for systolic BPV; 1.28, 1.05–1.56 for diastolic BPV)
Metabolic disorders	Joshipura et al. (2018)	950 Hispanics with no diabetes mellitus, from the San Juan Overweight Adults Longitudinal Study	Within-visit systolic BPV, the maximum difference among three measures	Systolic BPV accelerates development of pre-diabetes/diabetes in overweight or obese adults (RR = 1.77, 95 % CI: 1.30–2.42)
Kidney disease	Li et al. (2020)	A meta-analysis of 14 studies with a total of 11407535 participants	Visit-to-visit systolic and diastolic BPV using SD, CV and VIM	CKD onset is significantly higher in patients with greater baseline systolic BPV consistent through several indicators: RR of 1.69 (95 % CI, 1.38–2.08) for SD, 1.23 (95 % CI, 1.12–1.36) for CV, 1.40 (95 % CI, 1.15–1.71) for VIM. Similar results obtained for diastolic BPV
Sarcopenia	Hashimoto et al. (2018)	146 patients aged ≥ 65 years with diabetes from the KAMOGAWA-DM cohort study, without physical inactivity	Systolic visit-to-visit BP variability (CV) from at least four BP readings in 1 year	CV of systolic BP, rather than average systolic BP, is associated with sarcopenia in older patients with diabetes ($\beta = 0.20$, $p = 0.024$)
Hip fracture	Li et al. (2019)	21160 patients aged ≥ 50 years with diabetes retrospectively enrolled from the National Diabetes Care Management Program	Systolic and diastolic visit-to-visit BP variability (CV), with average follow-up period of 8.16 years	CV of systolic and diastolic BP constitute a predictor of hip fracture (HR 1.18, CI 95 % 1.00–1.40; and 1.21, 95 % CI 1.03, 1.43, in the third tertile, respectively)
	Yoo et al. (2021)	3256070 aged ≥ 50 years participants from the Korean National Health Insurance System database	Systolic and diastolic visit-to-visit BP variability (VIM), with median follow-up of 7.0 years	Systolic and diastolic BP variability (VIM) are independently associated with fracture incidence (respectively, HR 1.07, 95 % CI 1.06–1.08; HR 1.06, 95 % CI 1.05–1.07, in the higher quartile)
Frailty	Rouch et al. (2021)	1394 non frail community-dwelling participants aged ≥ 70 years from the MAPT study	Visit-to-visit systolic and diastolic BPV using SD, CV, VIM, ARV, SV. Systolic and diastolic BP were assessed regularly over a 5-year follow-up period	Higher systolic visit-to-visit BPV is associated with risk of incident frailty (1-SD increase of CV: HR = 1.17, 95 % CI: 1.01–1.35)

Table 1 Associations between BPV and age-related health outcomes.

ARV, Average Real Variability; BPV, Blood Pressure Variability; CI, Confidence Interval; CKD, Chronic Kidney Disease; CV, Coefficient of Variation; HR, Hazard Ratio; OR, Odds Ratio; RSD, Residual Standard Deviation; RR, Risk Ratio; SD, Standard Deviation; SV, Successive Variation; VIM, Variability Independent of the Mean

Several explanations have been proposed regarding the associations between BPV and different health outcomes. Most of the potential underlying mechanisms such as hemodynamic

instability, advanced artery remodeling and atherosclerosis, arterial stiffness, baroreflex impairment, endothelial dysfunction and subclinical inflammation, are related to aging[147]. Furthermore, the physiological process of aging is accompanied by a generalized loss of complexity (LOC) in the biological function of organs and systems (Figure 1).

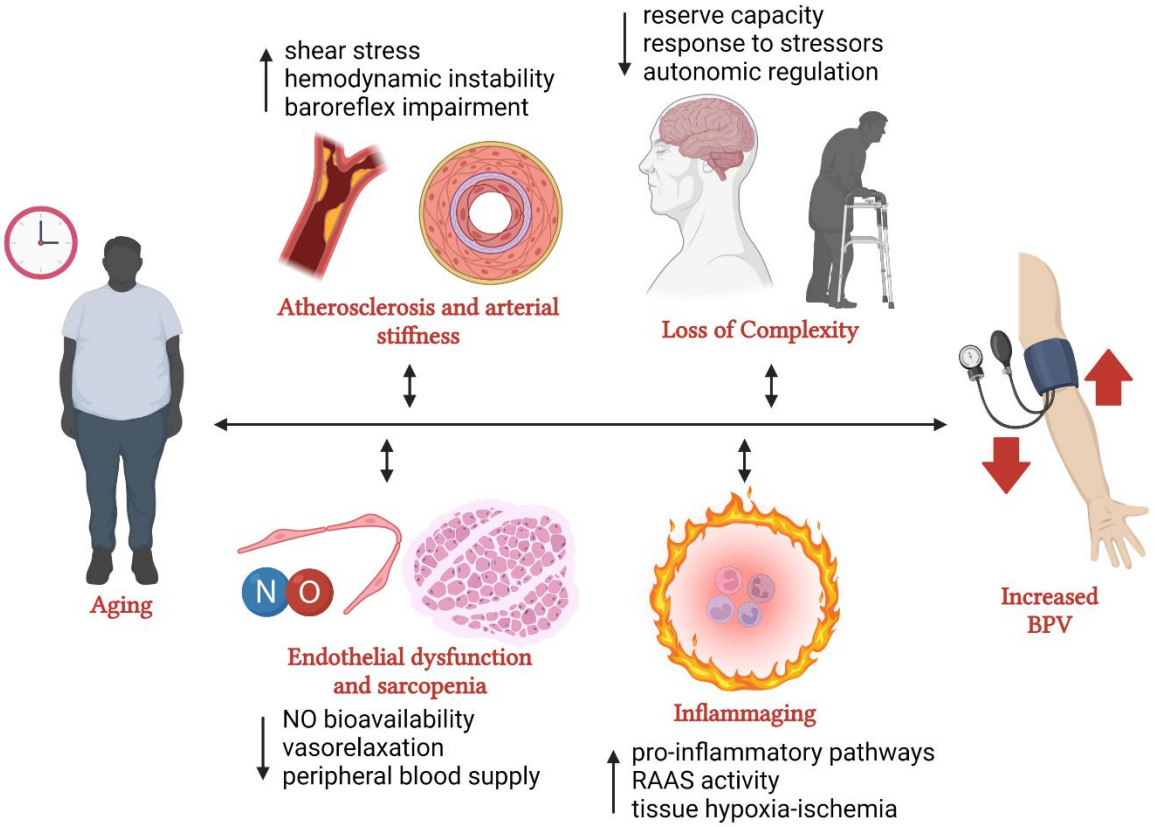

Figure 1. Mechanisms linking BPV and aging.

The pathophysiological mechanisms implicated in aging and BPV.

LOC contributes to the reduced capacity of homeostatic adaptation to stress, as evidenced by the alterations in the physiological dynamics of multiple physiological processes, especially cardiovascular and neurological stability or the pulsatile release of hormones[148]. BPV might help predicting the onset of several age-related conditions and diseases, independently of chronological age. In other words, BPV could be a potential clinical marker of biological aging.

Nowadays, the progress made in the field of Gerosciences has led to the identification of 9 cellular and molecular hallmarks of aging: a) genomic instability, b) telomere attrition, c) epigenetic alterations, d) loss of proteostasis, e) deregulated nutrient-sensing, f) mitochondrial dysfunction, g) cellular senescence, h) stem cell exhaustion, i) altered intercellular communication[149]. They can be

also grouped into three categories: 1) the primary hallmarks (a-d) determining alteration to cellular functions; 2) the antagonistic hallmarks (e-g), providing response to primary ones to counteract molecular damage, but becoming detrimental over time; 3) the integrative hallmarks (h-i) resulting from the previous two groups and ultimately concurring to the typical features of clinical ageing, as reduced resilience and impaired function[150]. Notably the majority of them has been shown to relate with pathophysiological modification found in cardiovascular aging and BPV (Figure 2).

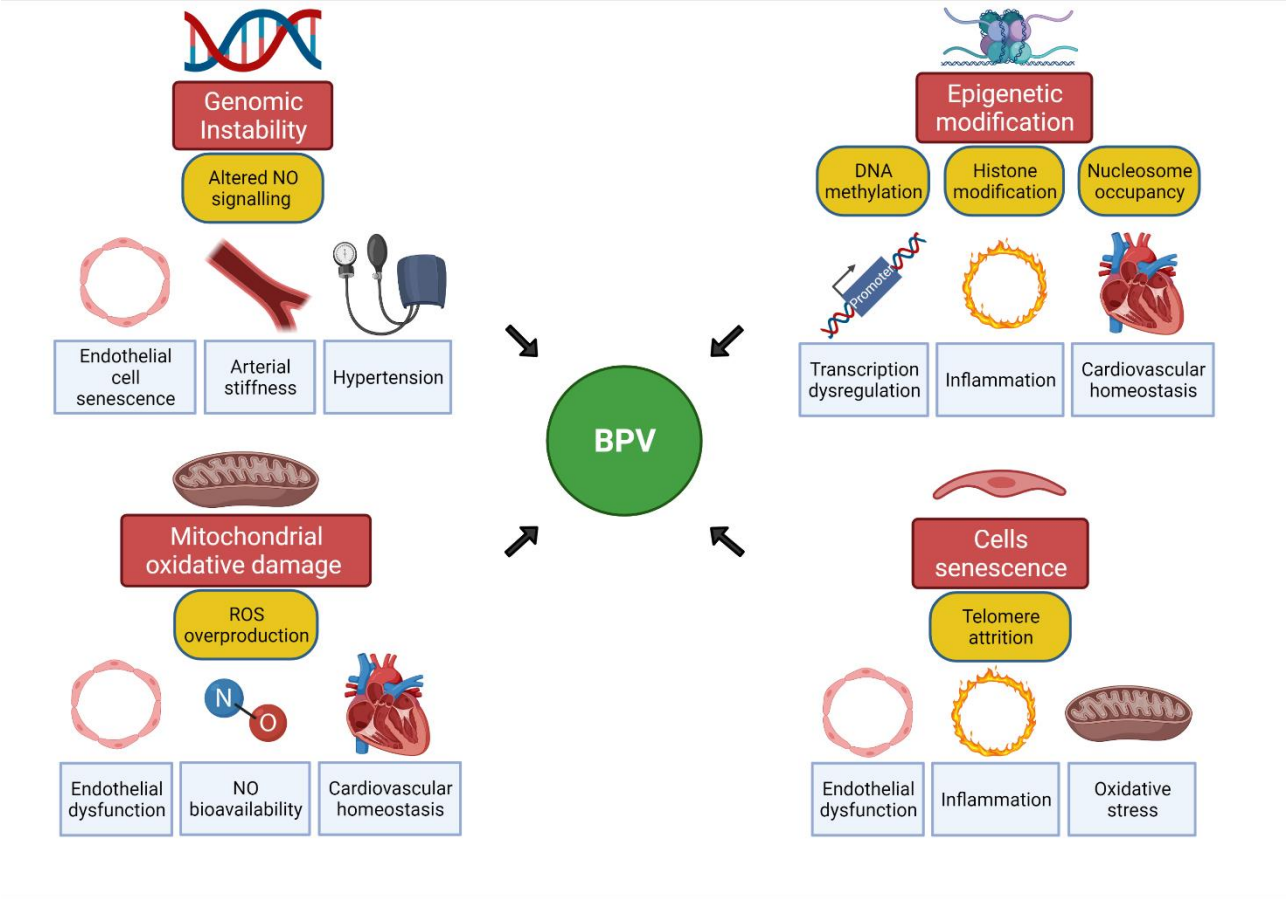

Figure 2. The relationship between BPV and the hallmarks of aging

The interplay between BPV and molecular mechanisms that underlie the hallmarks of aging.

Conclusive remarks

Accumulating evidence from basic and clinical sciences suggest that BPV could be a potential candidate marker of aging. Further work is certainly required to assess whether controlling BP instability could also be a promising interventional target to promote healthy aging. Converting multidisciplinary knowledge and discoveries of basic research into results that directly benefit

humans could help researchers test new hypotheses and ascertain the potential role of BPV as a clinical marker of aging.

Chapter 13

Biomarkers of mitochondrial dysfunction and inflammaging in older adults and blood pressure variability

Background and Aim

Increased Blood Pressure (BP) Variability (BPV) may represent an alteration in BP physiological homeostatic patterns. Most physiopathological mechanisms underlying BPV are implicated in aging. Vascular aging is associated with chronic low-grade inflammation occurring in late life, known as "inflammaging", and the hallmark “mitochondrial dysfunction” associated to stress due to age-related disorders, which in turn might contribute to higher BPV and risk of cardiovascular disease. We aimed to determine whether plasma levels of the pleiotropic stress-related mitokine Growth/Differentiation Factor 15 (GDF-15) and two inflammatory biomarkers, Interleukin 6 (IL-6) and Tumor necrosis factor receptor 1 (TNFR-1), are associated with visit-to-visit BPV in a population of community-dwelling older adults.

Methods

The study population consisted of 1,096 participants participants [median age 75 (72-78) years; 699 females, 63.7%] selected among community-dwelling participants aged ≥70 years from the MAPT study (table 1).

Variables *n(%) or median(IQR)*	**Whole population** *(1096 participants)*
Age (years)	75 (72-78)
Gender, male	397 (36.2)
BMI (Kg/m^2)	25.6 (23.2-28.3)
Asthma/COPD	77 (7.0)
Stroke	23 (2.1)
Active cancer	38 (3.4)
IHD	67 (6.1)
Diabetes	106 (9.6)
Heart Failure	17 (1.5)
Atrial Fibrillation	39 (3.5)
Antihypertensives	553 (50.4)
CKD	194 (17.7)

Table 1. *Baseline characteristics of the population.*

BMI, Body Mass Index; CKD, Chronic Kidney Disease; COPD, Chronic Obstructive Pulmonary Disease; IHD, Ischemic Heart Disease

Plasma blood sample was collected 12 months after enrolment and BP was assessed up to seven times over a subsequent 4-year period (figure 1).

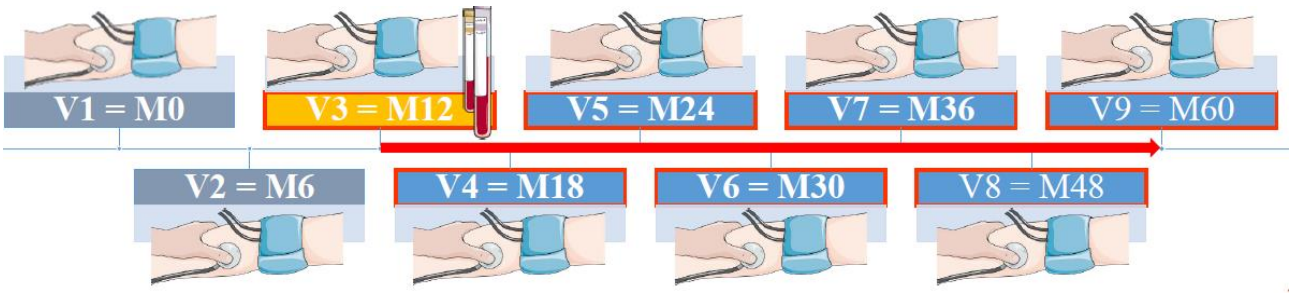

Figure 1. Timeline of blood sample collection and BP assessments.

The baseline assessment of BP was considered at 12 months after enrollment, corresponding to the time of blood biomarkers assessment. Visit-to-visit BPV was thus evaluated over a 4-year period using 7 BP measurements.

Systolic BPV (SBPV) and diastolic BPV (DBPV) were determined through several indicators including the coefficient of variation (CV%) and taking into account BP change over time, the order of measurements and formulas independent of mean BP levels.

Results

Higher values of GDF-15 were significantly associated with increased SBPV (all indicators, 3 shown in table 2) after adjustment for demographics, body mass index, MAPT randomization group, baseline systolic BP, antihypertensive drugs, diabetes mellitus, cardiovascular and non-cardiovascular comorbidities [adjusted 1-SD increase in GDF-15: β (SE)= 0.07 (0.04), p< 0.044, for CV%]. GDF-15 levels were not associated with DBPV. No significant associations were found between IL-6 and BPV, whereas TNFR1 was only partially related to DBPV.

4-year visit-to-visit BPV	*SBPV*				*DBPV*			
	Unadjusted		*Adjusted**		*Unadjusted*		*Adjusted**	
	β (SE)	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**
SD	0.11 (0.03)	<0.001	0.07 (0.03)	0.03	0.04 (0.03)	0.12	-0.00 (0.03)	0.88
CV%	0.09 (0.03)	<0.01	0.07 (0.04)	0.04	0.05 (0.03)	0.06	-0.00 (0.03)	0.98
VIM	0.08 (0.03)	<0.01	0.07 (0.03)	0.04	0.05 (0.03)	0.08	-0.00 (0.03)	0.93

Table 2. Association between GDF-15 levels and BPV.

Multivariable regression models adjusted on Age (years), Gender, BMI, randomization group, Antihypertensive agents, Baseline BP, Cardiovascular disease, Non-cardiovascular Disease, Diabetes.

CV, Coefficient of Variation; SD, Standard Deviation; VIM, Variation Independent of Mean.

Conclusive remarks

Unlike inflammation biomarkers, higher GDF-15 levels were associated with greater SBPV. Our findings support the age-related process of mitochondrial dysfunction underlying BP instability, suggesting that BPV might be a potential marker of aging.

Chapter 14

Visit-to-visit Blood Pressure Variability and Intrinsic Capacity in community-dwelling older adults

Background

Intrinsic Capacity (IC) represents an innovative approach of geriatric medicine, proposed by the World Health Organization as a marker of healthy aging[151], based on individual's functional abilities and intended at preserving successful aging[152]. The IC model reflects the trajectories of biological reserve of each person, through the assessment of key domains: locomotion, cognition, psychology, vitality and sensory[153].

Hypothesis

Higher BPV, as plausible epiphenomenon of less successful aging process, is associated with IC decline over time.

Study design

Secondary analyses from the Multidomain Alzheimer Preventive Trial (MAPT)[154].

Population

Non-frail community-dwelling volunteers aged ≥70 years.

Repeated clinical controls over a 5-year follow-up period (Figure 1).

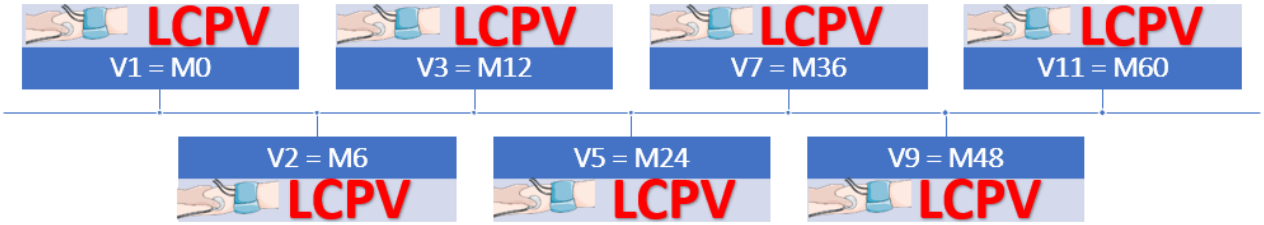

Figure 1. Timeline of IC and BP assessments.

Assessment of IC domains was performed at the time of enrollment and repeated over a 5-year period. Visit-to-visit BPV was evaluated in the same interval using 7 BP measurements.

L, Locomotion; C, Cognition; P, Psychology; V, Vitality; S, Sensory

Blood Pressure Variability

SBPV and diastolic DBPV were determined through six indicators, taking into account BP change over time, the order of measurements and formulas independent of mean BP levels.

Outcome

IC trajectory[155] over 5 years

Statistical Analysis

Unadjusted and multivariable-adjusted linear mixed models to explore the relation of IC with BPV over time

Findings

Both greater systolic and diastolic BPV are associated with IC decline over time. The relationship with SBPV is stronger.

Conclusions

CVDs affects millions of people worldwide, representing the leading cause of morbidity and mortality. The clinical manifestations of this group of pathologies are not limited to the cardiovascular system, with a systemic impact on individual's health status, poor quality of life and reduced life expectancy. Indeed, as documented for HF, some CVDs actually constitute complex syndromes, in which the pathophysiological component affecting the heart and vessels is closely connected to alterations in other body systems and apparatus, in an interplay that generates detrimental vicious circles. Moreover, the same disease can derive from multiple factors and aetiologies, whose discrimination can be crucial to select the appropriate therapeutic approach. For this reason, the study of new easily accessible and measurable circulating biomarkers, selectively expressed in different phenotypes of the pathologies, can stimulate the discovery of specific underlying pathophysiological mechanisms and pave the way for future innovative therapeutic resources.

Advances in this field of medical science have been dramatic in recent years, nonetheless the management of patients with CVDs still represents a complex challenge for physicians, particularly when there are specific conditions for which the support from evidence-based medicine evidence is poor. When the risk-benefit ratio of a clinical decision is blurred, as the selection of high-risk patients for ICD therapy, the integration of clinical information with that deriving from the available imaging tests can be crucial in guiding a therapeutic choice and go beyond the limits of available evidence for cases not envisaged by current indications and consensus.

This is also the case of the older adults, a large and growing population characterized by the lack of specific indications and by the concurrence of pathologies and conditions that make its management very complex. Notably, the prevalence of CVDs is particularly high in late life, and their association with the multiple age-related conditions often prevents the use of necessary therapeutic aids or increases the risk of failure and adverse events. Therefore, as already suggested by some guidelines, the approach to the geriatric patient with CVDs should always include a comprehensive multidimensional assessment, which can provide crucial additional information for correct management, address modifiable deficits and avoid the risk of both under- or over-treatment. In the last twenty years, the various models proposed for the study of the phenotypic heterogeneity of the aging process have led to the overcoming of the old fascinating concept of chronological age, in favour of biological age, whose impact on CVDs outcomes is much more relevant.

The next step in geriatric medicine would be to reverse the paradigm of care, moving from the management of accumulated deficits to the preservation of functionality, aiming at preventing disability before it manifests itself. In this scenario, geroscience will certainly bring new evidence to be integrated into clinical practice, starting from the translational study of the patterns of variability of measurable clinical features, as blood pressure and heart rate. The management of CVDs could particularly benefit from the integration of these findings, given the complex interactions that occur over time among the cardiovascular system and inflammaging, mitochondrial dysfunction and the other hallmarks of aging.

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121. Cañas, F., Giraldo, G. C., Murillo, A., Perafán, P. E. & Quintero, O. Benefits of Cardiac Rehabilitation on Functional Status and Mood Disorders in Elderly and Very Elderly Patients: A PROSPECTIVE COHORT STUDY. *J. Cardiopulm. Rehabil. Prev.* **41**, 113–115 (2021).

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123. Güngör, G. *et al.* The 6-minute walk test in chronic respiratory failure: does observed or predicted walk distance better reflect patient functional status? *Respir. Care* **58**, 850–857 (2013).

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130. Mehlum, M. H. *et al.* Blood pressure variability and risk of cardiovascular events and death in patients with hypertension and different baseline risks. *Eur. Heart J.* **39**, 2243–2251 (2018).

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133. Rouch, L. *et al.* Visit-to-Visit Blood Pressure Variability Is Associated With Cognitive Decline and Incident Dementia. *Hypertension* **76**, 1280–1288 (2020).

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135. Kawai, T. *et al.* The impact of visit-to-visit variability in blood pressure on renal function. *Hypertens. Res.* **35**, 239–243 (2012).

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138. Ogliari, G. *et al.* Visit-to-visit blood pressure variability and future functional decline in old age. *J. Hypertens.* **34**, 1544–1550 (2016).

139. Rouch, L. *et al.* Visit-to-Visit Blood Pressure Variability and Incident Frailty in Older Adults. *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.* **76**, 1369–1375 (2021).

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in elderly patients receiving antihypertensive therapy. *J. Hum. Hypertens.* **16**, 141–146 (2002).

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147. Donato, A. J., Machin, D. R. & Lesniewski, L. A. Mechanisms of dysfunction in the aging vasculature and role in age-related disease. *Circ. Res.* **123**, 825–848 (2018).

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154. Vellas, B. *et al.* MAPT STUDY: A MULTIDOMAIN APPROACH FOR PREVENTING ALZHEIMER'S DISEASE: DESIGN AND BASELINE DATA. *J. Prev. Alzheimer's Dis.* **1**, 13 (2014).

155. Giudici, K. V. *et al.* Effect of long-term omega-3 supplementation and a lifestyle multidomain intervention on intrinsic capacity among community-dwelling older adults: Secondary analysis of a randomized, placebo-controlled trial (MAPT study). *Maturitas* **141**, 39–45 (2020).

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Leonardo Bencivenga

Date of birth:29/11/1990 | **Nationality:**Italian | **Phone number:** (+39) 3381835996 (Mobile) | **Phone number:**(+39) 3346053365 (Work) | **Email address:** leonardobencivenga@gmail.com | **Email address:** leonardo.bencivenga@unina.it | **Website:** https://www.scopus.com/authid/detail.uri?authorId=57194680251 | **Website:** https://orcid.org/0000-0002-9575-8337 | **Website:** https://www.webofscience.com/wos/author/record/AAQ-3276-2020 | **LinkedIn:** https://www.linkedin.com/in/leonardobencivenga/ | **Twitter:** https://twitter.com/leonardobenciv |

Address: Via Sergio Pansini, 5, Building 2, IIIfloor, 80131, Napoli, Italy (Work)

About me:
Medical Doctor, Specialist in Geriatric Medicine, PhD in CardioPaTh

EDUCATION AND TRAINING

11/2019 – 10/2022 Napoli, Italy

INTERNATIONAL PHD STUDENT IN CARDIOVASCULAR PATHOPHYSIOLOGY AND THERAPEUTICS
Department of Advanced Biomedical Sciences, University of Naples "Federico II"

| **Website** http://www.cardiopath.eu/

09/2021 – 09/2022 Toulouse, France

VISITING RESEARCHER Gérontopôle de Toulouse, Institut du Vieillissement, CHU de Toulouse

Pathophysiological mechanisms of aging, cardiovascular risk factors, frailty and intrinsic capacity

10/2016 – 10/2020 Napoli, Italy

SPECIALIZATION IN GERIATRIC MEDICINE 50/50 CUM LAUDE Università degli Studi di Napoli "Federico II", Dipartimento di Scienze Mediche Traslazionali

06/10/2019 – 10/10/2019 Firenze, Italy

ADVANCED COURSE OF STATISTICS AND EPIDEMIOLOGY Società Italiana di Gerontologia e Geriatria

15/04/2018 – 18/10/2018 Firenze, Italy

COURSE OF STATISTICS AND EPIDEMIOLOGY Società Italiana di Gerontologia e Geriatria

08/04/2018 – 12/04/2018 Verona, Italy

2018 POSTGRADUATE COURSE IN SYSTEMATIC REVIEWS AND META-NALYSES FOR THE PRODUCTION OF EVIDENCE-BASED GUIDELINES IN HEALTHCARE Università degli Studi di Verona

03/09/2017 – 07/09/2017 Loiano, Italy

TRAINING COURSE "SUMMER SCHOOL ON... METODOLOGIA DEI TRIAL CLINICI" Fondazione GIMBE, within GIMBE4young program - Scholarship awarded on national selection

| **Website** https://www.gimbe.org/

07/2016 Napoli, Italy

MEDICAL LICENSE Ordine dei Medici-Chirurghi e degli Odontoiatri

2016 Napoli, Italy

MEDICAL DEGREE 110/110 CUM LAUDE Università degli Studi di Napoli "Federico II"

2009 Frattamaggiore, Italy

SCIENTIFIC HIGH SCHOOL DIPLOMA 100/100 CUM LAUDE Liceo Scientifico Statale "Carlo Miranda"

WORK EXPERIENCE

11/2022 – CURRENT Castellammare di Stabia, Italy

OUTPATIENT SPECIALIST DOCTOR ASL NAPOLI 3 SUD

UOSD Anziani Fragili e Demenze

06/2021 – 10/2022 Italy

OUTPATIENT SPECIALIST DOCTOR ASL REGIONE CAMPANIA

Geriatric Medicine Clinic
ASL Napoli 2 Nord, Distretto Sanitario 38
ASL Napoli 3 Sud, Distretto Sanitario 34
ASL Caserta, Distretto Sanitario 17 e 19
2017 – 2021

OUT-OF-HOURS MEDICAL SERVICE ASL NAPOLI 2 NORD

Medical Doctor in out-of-hours service
Distretto Sanitario 35 e 41

10/2016 – 10/2020 Napoli, Italy
SPECIALIST MEDICAL TRAINING AZIENDA OSPEDALIERA UNIVERSITARIA "FEDERICO II"

Integrated Activity Department of Cardiovascular Emergencies, Clinical and Aging Medicine

12/2019 – 02/2020 Milano, Italy
SPECIALIST MEDICAL TRAINING FONDAZIONE IRCCS CA'GRANDA OSPEDALE MAGGIORE POLICLINICO MILANO, GERIATRIC MEDICINE UNIT

Diagnostic-Therapeutic Day Hospital, Geriatric Medicine Clinic

07/2019 – 10/2019 Napoli, Italy
SPECIALIST MEDICAL TRAINING A.O.R.N. ANTONIO CARDARELLI, U.O.C. PRONTO SOCCORSO E OBI

First Aid Department

06/2019 Napoli, Italy
SPECIALIST MEDICAL TRAINING ASL NAPOLI 1 CENTRO, DISTRETTO SANITARIO 28

RSA Anziani Alto e Medio carico assistenziale, RSA Demenza, Centro Diurno Demenza e Poliambulatorio

01/2019 – 04/2019 Napoli, Italy
SPECIALIST MEDICAL TRAINING A.O.R.N. ANTONIO CARDARELLI, U.O.S.C. MEDICINA INTERNA 3

Internal Medicine Department

09/2017 – 12/2018 Napoli, Italy

INVESTIGATOR OF THE INTERNATIONAL MULTI-CENTER RANDOMIZED CONTROLLED TRIAL "PERSPECTIVE" UNIVERSITÀ DEGLI STUDI DI NAPOLI "FEDERICO II", DIPARTIMENTO DI SCIENZE MEDICHE TRASLAZIONALI

Patient enrollment and neurocognitive assessment - RCT promoted by NOVARTIS to evaluate the effects of LCZ969 compared to valsartan on cognitive function in patients with chronic heart failure with preserved ejection fraction

HONOURS AND AWARDS

10/2021

2021 Research Grant for "Polymorbidity and other common conditions in Internal Medicine" – Foundation for the Development of Internal Medicine in Europe (FDIME) Research Study Project: Role of frailty and polymorbidity on blood pressure variability in elderly

06/2021

Programma Star Plus 2020 - LINEA D'INTERVENTO 2 – MOBILITÀ GIOVANI RICERCATORI – Università degli Studi di Napoli Federico II Bando per l'Erogazione di Contributi per Soggiorni all'Estero – Annualità 2020

PROFESSIONAL SKILLS

2021 – CURRENT

Topical Advisory Panel Member for International Journal of Molecular Sciences

10/2021 – CURRENT

Special Issue Editor for Journal of Diabetes Research

Frailty and Type 2 Diabetes Mellitus in Older Patients https://www.hindawi.com/journals/jdr/si/152573/

09/2021 – CURRENT

Special Issue Editor for International Journal of Molecular Sciences

Section "Molecular Pathology, Diagnostics, and Therapeutics": Aging and Heart Disease https://www.mdpi.com/journal/ijms/special_issues/Aging_Heart

2021 – 2022

Research Topic Editor for Frontiers in Medicine, Section "Geriatric Medicine"

- Frailty: Risks and Managementhttps://www.frontiersin.org/research-topics/33513
- Post COVID-19 Physical Performance and Functional Capacityhttps://www.frontiersin.org/research-topics/22156/post-covid-19-physical-performance-and-functionalcapacity

02/2021 – CURRENT

Associate Editor for Journal of Basic and Clinical Physiology and Pharmacology

Sector Geriatrics

09/2020 – CURRENT

Review Editor for Frontiers in Physiology

Editorial Board of Clinical and Translational Physiology

2019 – CURRENT

Reviewer for International Journals

Frontiers in Medicine; Scientific Reports; PLOS ONE; Journal of Comparative Effectiveness Research; The Journal of Nutrition, Health & Aging; European Journal of Preventive Cardiology; Biomolecules; Current Oncology; Frontiers in Nutrition

30/11/2015 – CURRENT

Basic Life Support & Defibrillation (BLS-D) according to AHA Guidelines and I.L.C.O.R. 2015

Certification awarded by "Team Didattico del GIEC" (American Heart Association Authorized Training Center) on 01/12/2015, 27/09/2018, 10/10/2018, 23/01/2019

29/01/2019 – 30/01/2019

Specific training course on health and safety at work

Organized by the AOU "Federico II" of Naples, pursuant to Legislative Decree 81/08 and ss.ii.mm, of the State-Region Agreement of 21 december 2011 and of the State-Regions Agreement of 7 July 2016

03/2021

Introduction to Systematic Review and Meta-Analysis

Johns Hopkins University
https://coursera.org/share/856f875bdea1b2bdd753fe6cb43039eb

LANGUAGE SKILLS

01/2018

Certificate of Level B2 of the English Language, Common European Framework of Reference for Languages

Centro Linguistico di Ateneo dell'Università degli Studi di Napoli "Federico II"

06/2008

First Certificate In English - Council of Europe Level B2

University of Cambridge - Cambridge English for Speakers of Other Languages

06/2021 – CURRENT

Common European Framework of Reference for Languages (CEFR) Level C1

Academic IELTS: Overall Band Score 7.0
British Council - idp - Cambridge Assessment English

TEACHING

10/2020 – CURRENT

Academic Tutor

School of Specialization in Geriatric Medicine, Università degli Studi di Napoli "Federico II"
06/07/2019 – CURRENT

Concetto di Valutazione Multidimensionale, schede di valutazione e applicazione clinica: Multidimensional Prognostic Index (MPI)

Corso di Formazione Caregiver, Anno Accademico 2018-2019-2020-2021-2022
organized by the Department of Traslational Medical Sciences of Università degli Studi di Napoli "Federico II" with Comunità di Sant'Egidio, Napoli

NATIONAL ED INTERNATIONAL SCIENTIFIC SOCIETY

2015 – CURRENT

Società Italiana di Gerontologia e Geriatria

Founding member and deputy coordinator of the working group Young Epidemiologists SIGG - YES Editorial Board of SIGG Report

2018 – CURRENT

Società Italiana di Medicina Interna

Representative of the Campania Region for GIS (Young Internists SIMI, Italian Society of Internal Medicine)
2021-2024
Member of the Multimedia Information/Dissemination Committee

2018 – CURRENT
European Geriatric Medicine Society

2018
American Geriatrics Society

2018 – CURRENT
European Society for Medical Oncology

2017 – CURRENT
Associazione Italiana Psicogeriatria

2018 – CURRENT
Società Italiana Cardiologia Geriatrica

2020 – CURRENT
European Society of Cardiology

2020 – CURRENT
Associazione Italiana di Cardiologia Clinica, Preventiva e Riabilitativa

SPEAKER IN COURSES AND CONGRESSES

03/12/2022
Simposio Gruppo YES - Young Epidemiologist SIGG Un Caso Inter…Societario

67° Congresso Nazionale SIGG, Roma
Moderatore

01/12/2022
Associazione tra variabilità della pressione arteriosa e biomarcatori di invecchiamento in una popolazione di anziani residenti in comunità: evidenze dallo studio MAPT *67° Congresso Nazionale SIGG, Roma*

01/12/2022
Ricerca clinica e Biogerontologia: un legame infinito

67° Congresso Nazionale SIGG, Roma
Moderatore, sessione II

17/11/2022
Caso Clinico "Ultrà"

Corso SIMI: Approccio internistico al paziente cardiorenale: dai meccanismi fisiopatologici alle novità terapeutiche, virtuale

08/11/2022
Variabilité de la pression artérielle et Biomarqueurs plasmatiques de neuro-dégénérescence

42es Journées Annuelles de la Société Française de Gériatrie et Gérontologie, Paris

07/11/2022
Biomarqueurs du vieillissement et variabilité de la pression artérielle

42es Journées Annuelles de la Société Française de Gériatrie et Gérontologie, Paris

La Tana Dei Giovani Internisti SIMI: Ethical Life Support. Strumenti etici per decidere in medicina

123° Congresso Nazionale SIMI, Roma
Moderazione

01/10/2022
Le infezioni urinarie nel paziente anziano fragile

Le giornate itineranti del GIS: Le infezioni in Medicina Interna, Verona

13/05/2022
SIMPOSIO GIS: Vecchie e Nuove sfide per il giovane internista

Congresso SIMI Sezione Campania 2022, Napoli
Moderazione

02/12/2021
L'Ecografia Muscolare nello studio della Fragilità: un nuovo dominio strumentale di Valutazione Multidimensionale Geriatrica?

66° Congresso Nazionale SIGG "Geriatria e Rinascita", Roma

10/06/2021
Stroke ed endocardite aortica in paziente con epatocarcinoma sottoposto a TACE

Congresso SIMI Sezione Campania 2021, virtuale

2019
Biogerontologia e Medicina Traslazionale: i Giovani e La SIGG - Gruppo di Studio YES: Young Epidemiologists SIGG

65° Congresso Nazionale SIGG "Nessuno escluso. Dall'acuzie alla cronicità, alle malattie rare dell'invecchiamento", vituale

05/10/2018
Il Selfy-MPI

Corso Teorico Pratico Invecchiamento attivo e in salute in medicina generale: l'approccio multidimensionale, Padula (SA)

ABSTRACT AND POSTER IN CONGRESSES

2022
Cardiac sympathetic innervation and mortality risk scores in patients suffering from Heart Failure with reduced Ejection Fraction

Leonardo Bencivenga, Klara Komici, Giuseppina Gambino, Fabio Santillo, Laura Andreea Ceparano, Grazia Daniela Femminella, Nicola Ferrara, Alberto Cuocolo, Pasquale Perrone Filardi, Giuseppe Rengo *123° Congresso Nazionale della Società Italiana di Medicina Interna, Roma*

2022
Growth/Differentiation Factor 15 but not inflammation biomarkers are associated with higher blood pressure variability in older adults

Leonardo Bencivenga, Mathilde Strumia, YvesRolland, Laurent Martinez, Philippe Cestac, Sophie

Guyonnet, Sandrine Andrieu, Angelo Parini, Alexandre Lucas, Bruno Vellas, Philipe De Souto Barreto, Laure Rouch, for the MAPT/DSA group
18th European Geriatric Medicine Society, London and virtual

2022
Association between Plasma Growth Differentiation Factor 15 and Blood Pressure Variability in community-dwelling older adults: the MAPT Study

Leonardo Bencivenga, Mathilde Strumia, Bruno Vellas, Philipe de Souto Barreto, Laure Rouch *2nd Euro Geroscience Conference, Toulouse*

2021
Muscle Ultrasound as Imaging domain of Frailty

Leonardo Bencivenga, Francesco Picaro, Lorenzo Ferrante, Federico Ruggiero, Grazia Daniela Femminella, Dino Franco Vitale, Carlo Rengo, Nicola Ferrara, Giuseppe Rengo
17th European Geriatric Medicine Society, Athens and virtual

2021
Role of frailty on cardiac rehabilitation of hospitalized elderly patients

Bencivenga Leonardo, Cacciatore Francesco, Bosco Quirino, Formisano Roberto, Iannuzzi Gian Luca, Perrotta Giovanni, Tescione Girolamo, Gambino Giuseppina, Ferrara Nicola, Femminella Grazia Daniela, Vitale Dino Franco, Papa Antimo, Rengo Giuseppe
122° Congresso Nazionale della Società Italiana di Medicina Interna, virtuale

2021
Comprehensive Geriatric Assessment: Muscle Ultrasound as imaging domain of Frailty

Leonardo Bencivenga, Francesco Picaro, Lorenzo Ferrante, Dino Franco Vitale, Carlo Rengo, Giuseppe Rengo
19th European Congress of Internal Medicine, virtual

2020
Role of body mass index on cardiac adrenergic derangement in elderly patients with heart failure

Leonardo Bencivenga, Klara Komici, Dino Fraco Vitale, Roberto Formisano, Brunella Puzone, Antonio Cittadini, Alberto Cuocolo, Pasquale Perrone Filardi, Nicola Ferrara, Giuseppe Rengo
65° Congresso Nazionale SIGG "Nessuno escluso. Dall'acuzie alla cronicità, alle malattie rare dell'invecchiamento" vitual

2020
Impact of comorbidities on cardiac adrenergic derangement in heart failure patients assessed through 123I-mIBG imaging

Leonardo Bencivenga, Klara Komici, Dino Franco Vitale, Formisano Roberto, Giuseppina Gambino, Antonio Cittadini, Alberto Cuocolo, Pasquale Perrone Filardi,Nicola Ferrara, Giuseppe Rengo *121° Congresso Nazionale della Società Italiana di Medicina Interna, virtual*

2020
Role of body mass index on cardiac adrenergic derangement in elderly patients with heart failure

Bencivenga Leonardo, Komici Klara, Vitale D. Franco, Formisano Roberto, Puzone Brunella, Cittadini Antonio, Cuocolo Alberto, Perrone Filardi Pasquale, Ferrara Nicola, Rengo Giuseppe *16th European Geriatric Medicine Society, E-Congress*

2019

Impatto delle comorbidità sull'innervazione cardiaca simpatica valutata mediante scintigrafia miocardica con I131-MIBG in pazienti con insufficienza cardiaca a frazione d'eiezione ridotta

Bencivenga Leonardo, Nocella Pierangela, Grieco V. Fabrizio, Spezzano Angela, Puzone Brunella, Giuseppina Gambino, Komici Klara, Cannavo Alessandro, Vitale D. Franco, Perrone Filardi Pasquale, Cuocolo Alberto, Ferrara Nicola, Rengo Giuseppe
64° Congresso Nazionale SIGG "Continuità di affetti, continuità di cura", Roma

2019

Role of β3-adrenergic receptor on cardiac sympathetic innervation and on brain-derived neurotrophic factor levels in patients with chronic heart failure

Leonardo Bencivenga, Giuseppina Gambino, Daniela Liccardo, Claudia Perna, Klara Komici, Pierangela Nocella, Fabrizio Vincenzo Grieco, Angela Spezzano, Andrea Elia, Antonio Cittadini, Alberto Cuocolo, Pasquale Perrone Filardi, Dino Franco Vitale, Nicola Ferrara, Alessandro Cannavo, Giuseppe Rengo *120° Congresso Nazionale della Società Italiana di Medicina Interna, Roma*

2018

Impatto dello stato nutrizionale sulla mortalità a lungo termine in pazienti anziani con infarto miocardico acuto

Bencivenga Leonardo, Nocella Pierangela, Grieco V. Fabrizio, Conte Maddalena, Provenzano Sandra, Ronga Ilaria, Visaggi Lucia, Spezzano Angela, Ferrara Nicola, Rengo Giuseppe, Komici Klara *63° Congresso Nazionale SIGG "Gli anziani: le radici da preservare", Roma*

2017

La severità della malattia coronarica è associata al declino cognitivo negli anziani

Bencivenga Leonardo, Komici Klara, Mancini Angela, Conte Maddalena, Grieco V. Fabrizio, Provenzano Sandra, Ronga Ilaria, Morisco Carmine, Ferrara Nicola, Rengo Giuseppe *62° Congresso Nazionale SIGG "Invecchiamento Scenario 2.0", Napoli*

AUTHOR

2019

Scompenso Cardiaco

Chapter of the textbook *Paziente anziano, Paziente Geriatrico, Medicina della Complessità*, EdiSES

2018

La valutazione multidimensionale geriatrica

Chapter of the textbook *Manuale di Geriatria*, Edra

PUBLICATIONS

Management and Treatment of Cardiovascular Diseases in the Elderly – 2017

Leonardo Bencivenga, Fabrizio Vincenzo Grieco, Grazia Daniela Femminella, Claudio de Lucia, Klara Komici, Carlo Rengo, Nicola Ferrara, Giuseppe Rengo

Current Pharmacogenomics and Personalized Medicine

Antidiabetic Drugs in Alzheimer's Disease: Mechanisms of Action and Future Perspectives – 2017

Grazia Daniela Femminella, **Leonardo Bencivenga**, Laura Petraglia, Lucia Visaggi, Lucia Gioia, Fabrizio Vincenzo Grieco, Claudio de Lucia, Klara Komici, Graziamaria Corbi, Paul Edison, Giuseppe Rengo, Nicola Ferrara

Journal of Diabetes Research

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