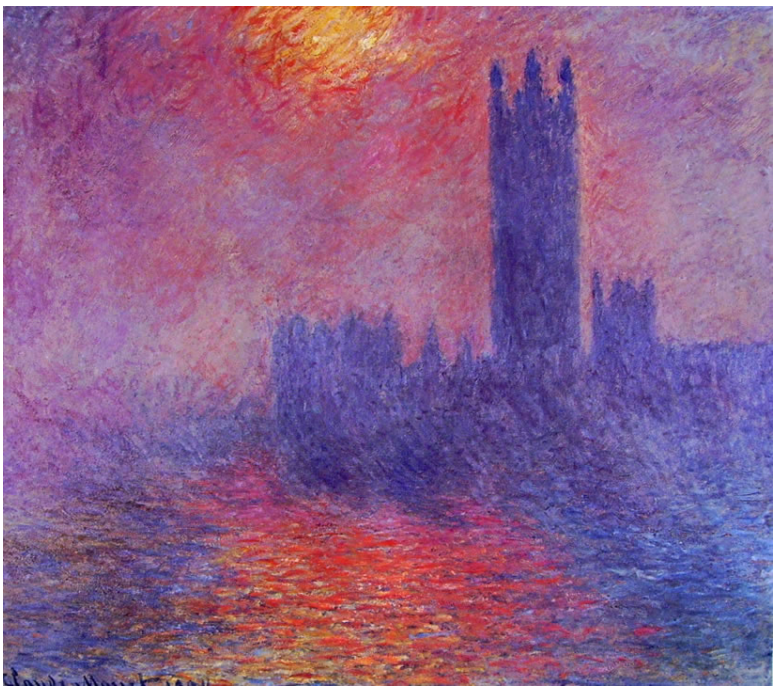

Heart Failure and Biomarkers. Unravelling new pathophysiological pathways, outcome predictors, and therapeutic targets.

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‘…Above all, always be capable of feeling deeply any injustice committed against anyone, anywhere in the world…’
(E.C.G.)

Cover image: Monet Claude, Houses of Parliament, 1900-1905

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   2.2. **Salzano A**§, Marra AM, Proietti M, Raparelli V, Heaney LM. Biomarkers in Heart Failure and Associated Diseases. **Dis Markers**, vol. 2019, Article ID 8768624, 2 pages, 2019. https://doi.org/10.1155/2019/8768624
   2.3. Suzuki T, Israr ZM, **Salzano A**. Biomarkers in cardiovascular disease: the dilemma of racial differences. **J Am Heart Assoc** 2019;8:e014295 doi: 10.1161/JAHA.119.014295

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      3.1.2. D'Assante R*, Napoli R*, **Salzano A**, Pozza C, Marra AM, Arcopinto M, Perruolo G, Milano S, Formisano P, Saldamarco L, Cirillo P, Cittadini A. Human heart shifts from IGF-1 production to utilisation with chronic heart failure. **Endocrine**. Endocrine. 2019 Sep;65(3):714-716. doi: 10.1007/s12020-019-01993-y. Epub 2019 Jul 2 PMID: 31267324
      3.1.3. Piccioli L, Arcopinto M, **Salzano A**, D'Assante R, Schiavo A, Stagnaro FM, Lombardi A, Panicara V, Valente P, Vitale G, Sarullo FM, Giallauria F, Marra AM. The impairment of the Growth Hormone/Insulin-like growth factor 1 (IGF-1) axis in heart failure: A possible target for future therapy. **Monaldi Arch Chest Dis**. 2018 Sep 5;88(3):975. doi: 10.4081/monaldi.2018.975. PubMed PMID: 30183158.
      3.1.4. D'Assante R*, Arcopinto M*, Rengo G, **Salzano A,** Walser M, Gambino G, Monti MG, Bencivenga L, Marra AM, Aberg DN De Vincentiis C, Ballotta A, Bossone E, Isgaard J, Cittadini A. Myocardial expression of somatotropic axis, adrenergic and calcium handling genes in HFpEF and HFrEF. **ESC Heart Failure**. 2020 Sep (in press).

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3.2.1. **Salzano A**§, Cittadini A, Bossone E, Suzuki T, Heaney LM. Multiple hormone deficiency syndrome: a novel topic in chronic heart failure. **Future Sci OA**. 2018 Apr 16;4(6):FSO311. doi: 10.4155/fsoa-2018-0041. eCollection 2018 Jul. PubMed. PMID: 30057788; PubMed Central PMCID: PMC6060397.

3.2.2. **Salzano A**, Marra AM, Bossone E, Suzuki T, Cittadini A. Letter to the Editor: "Cardiometabolic biomarkers and their temporal patterns predict poor outcome in chronic heart failure (Bio-SHiFT study)". **J Clin Endocrinol Metab**. J Clin Endocrinol Metab. 2019 Mar 1;104(3):734-735. doi: 10.1210/jc.2018-02043 PubMed PMID: 30339197.

3.2.3. Bossone E, Arcopinto M, Iacoviello M, Triggiani V, Cacciatore F, Maiello C, Limongelli G, Masarone D, Perticone F, Sciacqua A, Perrone-Filardi P, Mancini A, Volterrani M, Vriz O, Castello R, Passantino A, Campo M, Modesti PA, De Giorgi A, Monte I, Puzzo A, Ballotta A, Caliendo L, D'Assante R, Marra AM, **Salzano A**, Suzuki T, Cittadini A; TOSCA Investigators. Multiple hormonal and metabolic deficiency syndrome in chronic heart failure: rationale, design, and demographic characteristics of the T.O.S.CA. Registry. **Intern Emerg Med**. 2018 Aug;13(5):661-671. doi: 10.1007/s11739-018-1844-8. Epub 2018 Apr 4. PubMed PMID: 29619769.

3.2.4. Cittadini A*, **Salzano A***, Iacoviello M, Triggiani V, Rengo G, Cacciatore F, Maiello C, Limongelli G, Masarone D, Perticone F, Cimellaro A, Perrone Filardi P, Paolillo S, Mancini A, Volterrani M, Vriz O, Castello R, Passantino A, Campo M, Modesti PA, De Giorgi A, Monte IP, Puzzo A, Ballotta A, D'Assante R, Marra AM, Arcopinto M, Gargiulo P, Bruzzese D, Colao A, Napoli R, Suzuki T, Eagle KA, Ventura HO, Bossone E. On behalf of the T.O.S.CA. Investigators. 'Multiple Hormonal and Metabolic Deficiency Syndrome predicts outcomes in heart failure. The T.O.S.CA. Registry' (unpublished data, under review)

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3.3.2. **Salzano A**, Marra AM, D'Assante R, Arcopinto M, Suzuki T, Bossone E, Cittadini A. Growth Hormone Therapy in Heart Failure. **Heart Fail Clin**. 2018 Oct;14(4):501-515. doi: 10.1016/j.hfc.2018.05.002. Epub 2018 Aug 17. Review. PubMed PMID: 30266359.

3.3.3. **Salzano A**, D'Assante R, Lander M, Arcopinto M, Bossone E, Suzuki T, Cittadini A. Hormonal replacement therapy in heart failure: focus on growth hormone and testosterone. **Heart Fail Clin**. 2019 Jul;15(3):377-391. doi: 10.1016/j.hfc.2019.02.007. Epub 2019 Apr 6. Review. PMID: 31079696.

3.3.4. **Salzano A**, Marra AM, Arcopinto M, D'Assante R, Triggiani V, Coscioni E, Pasquali D, Rengo G, Suzuki T, Bossone E, Cittadini A. Combined effect of growth hormone and testosterone replacement treatment in heart failure. A hypothesis generating pilot study **ESC Heart Fail**. 2019 Nov 7. doi: 10.1002/ehf2.12520. PMID: 31696666

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4.2. Israr MZ, **Salzano A**, Suzuki T. Gut Feeling: the role of gut microbiota in immunomodulation of ischemia-reperfusion injury. **Arterioscler Thromb Vasc Biol** 2020 Sep;40(9):1967-1969. doi: 10.1161/ATVBAHA.120.314941 PMID: 32845773

4.3. Cassambai S, **Salzano A**, Yazaki Y, Bernieh D, Wong M, Israr MZ, Heaney LM, Suzuki T. Impact of acute choline loading on circulating trimethylamine N-oxide (TMAO) levels. **Eur J Prev Cardiol**. 2019 Feb 18:2047487319831372. doi: 10.1177/2047487319831372 PMID: 30776913

4.4. Yazaki Y*, **Salzano A***, Nelson CP, Voors A, Anker S, Cleland JG, Lang C, Metra M, Samani N, Leong LN, Suzuki T. Geographical location affects the levels and association of trimethylamine N-oxide with heart failure mortality: a post-hoc analysis of BIOSTAT-CHF. **Eur J Heart Failure** 2019 Jul 28. doi: 10.1002/ejhf.1550. PMID: 31353762

4.5. Yazaki Y*, Aizawa K*, Israr MZ*, Negishi K, **Salzano A**, Saitoh Y, Kimura N, Kono K, Heaney LM, Cassambai S, Bernieh D, Lai F, Imai Y, Kario K, Nagai R, Ng LL, Suzuki T. Ethnic differences in association of outcomes with trimethylamine N-oxide in acute heart failure patients. **ESC Heart Fail.** 2020 Jun 29. doi: 10.1002/ehf2.12777 PMID: 32598563

4.6. **Salzano A**, Israr ZM, Yazaki Y, Heaney LM, Kanagala P, Singh A, Arnold JR, Gulsin G, Squire IB, McCann G, Leong LN, Suzuki T. Combined use of trimethylamine N-oxide with BNP for risk stratification in heart failure with preserved ejection fraction: findings from the DIAMONDHFpEF study. **Eur J Prev Cardiol**. 2019 Aug 14:2047487319870355. doi: 10.1177/2047487319870355. PMID: 31412713

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5.1. **Salzano A**, Israr MZ, Fernandez Garcia D, Middleton L, D'Assante R, Marra AM, Arcopinto M, Yazaki Y, Bernieh D, Cassambai S, Page K, Rengo G, Bossone E, Cittadini A, Shaw JA, Suzuki T. Circulating cell-free DNA levels are associated with adverse outcomes in heart failure: testing liquid biopsy in heart failure. **Eur J Prev Cardiol**. 2020 Mar 25:2047487320912375. doi: 10.1177/2047487320912375. PMID: 32212838

5.2. Israr MZ*, **Salzano A***, Yazaki Y, Voors AA, Ouwerkerk W, Anker SD, Cleland JG, Dickstein K, Metra M, Samani NJ, Ng LL, Suzuki T; on behalf of BIOSTAT-CHF consortium. Implications of serial measurements of natriuretic peptides in heart failure: insights from BIOSTAT-CHF **Eur J Heart Fail.** 2020. 10.1002/ejhf.195

6. Chapter 6. Miscellaneous

6.1. **Salzano A**[§,] Suzuki T, Squire IB, Cittadini A. Are heart failure observational studies still useful? 'No need to argue'. **Eur J Prev Cardiol.** 2020 Jun 24:2047487320932258. doi: 10.1177/2047487320932258. PMID: 32580568

6.2. **Salzano A**, D'Assante R, Stagnaro FM, Valente V, Crisci G, Giardino F, Arcopinto M, Bossone E, Marra AM, Cittadini A. Heart failure management during COVID-19 outbreak in Italy. Telemedicine experience from a heart failure university tertiary referral centre. **Eur J Heart Fail.** 2020 May 28:10. doi: 10.1002/ejhf.1911. PMID: 32463534

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Chapter 1.

General Introduction

Heart failure (HF) is described as a clinical syndrome characterised by typical symptoms (e.g. ankle swelling, fatigue, dyspnoea) or signs (peripheral oedema, pulmonary crackles, or elevated jugular venous pressure), in which structural and/or functional cardiac abnormalities induce an impairment of cardiac output or an increase of intra-cardiac pressures at rest and/or during stress [1,2]. Importantly, due to different underlying aetiologies, demographics, co-morbidities, and response to therapies, the main terminology used to describe HF is based on measurement of left ventricle ejection fraction (EF). Classically, patients with normal EF (typically considered as ≥50%) are said to have HF with preserved EF (HFpEF), with those with reduced EF (typically considered as <40%) termed as HF with reduced EF (HFrEF). In the latest European Society of Cardiology (ESC) guidelines, cases where EF lies between 40–49%, previously considered as a 'grey area', are now defined as HF with mid-range EF (HFmEF) [1] .

Although in recent years overall mortality from cardiovascular disease has been reduced by about two-thirds, HF represents an exception to this rule, maintaining high levels of mortality that are known to be higher than those of many cancers [3]. This unacceptable high mortality rate associated with a slow but steady increase in prevalence, represent ominous HF peculiarities [4] leading to a huge economic and social burden [1,3]. Therefore, scientific community is trying to modify this trend, and one of the most active field is the research and development of novel biomarkers. Indeed, in the last years, the level of interest in discovery of new cardiovascular biomarkers has progressively grown[5]. Nowadays, biomarkers are routinely used in clinical care for diagnosis, monitoring (response to treatment), and risk stratification of patients with HF[6]. However, to date none of the novel biomarkers has demonstrated enough prognostic power when employed alone. Thus, it is commonly recognised that rather than a single biomarker, it is the integration of multiple biomarkers that yields the best strategy, although few studies have tested this hypothesis[7]. Circulating biomarkers are the most commonly employed in clinical scenarios, although emerging novel categories are actively tested in several trials.

Today, the word biomarker is commonly used, and in some cases abused, to describe a number of emerging tools, technologies, and strategies widely aimed to improve knowledge about several diseases[8]. In 2001, translating the necessity of reach a general agreement on a proper definition and classification of biomarkers, being the discover of the ideal biomarker a main goal of

the research community, the Biomarkers and Surrogate End Point Working Group proposed the following definition: "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*" [9]. In this context, biomarkers have also been categorized in three different types [10]:

- Type 0: biomarkers related to a specific disease correlated with clinical indices that can be monitored longitudinally;
- Type I: biomarkers used to evaluate in parallel the effects of a pharmacological intervention associated with the mechanism of action of a therapeutic drug;
- Type II: biomarkers considered surrogate end points that are able to predict clinical benefit

A surrogate biomarker is used in therapeutic trials and is representative of the patient clinical status/outcome. It is supposed to predict the effect of pharmacological therapy being an indicator of disease prognosis and progression benefit [10]. However, before employing a biomarker as a surrogate endpoint, several fundamental information is needed. First, it is necessary a strong correlation between the impact of an intervention on the biomarker and the impact of the intervention on a clinical meaningful endpoint; second, the outcome of interest should be modifiable through the therapeutic intervention; third, a biomarker should reflect both benefits and risks related to the intervention; finally, the sampling strategies and relative risk related should be well known as well as the time course between the change in biomarker and the outcome [11,12].

In particular, the hunt for the ideal biomarker is focused on the improvement in disease treatment and the reduction of healthcare costs [13]. Moreover, a biomarker should be specific for a particular disease, easy to detect, cost effective, and able to provide accurate results [14]. It should be taken into account that although many studies report the identification of new disease biomarkers, some of them do not satisfy the analytical validation based on well-established criteria of sensitivity and specificity that must be fulfilled to take a step towards for market applications [15]. For this reason, it is necessary to implement appropriate strategies starting from an accurate selection of patients, sample preparation, improvement of laboratory assays, to the final steps of statistical/analytical validation [14]. In addition, Morrow and de Lemos have recently defined three criteria a biomarker should have to be useful in clinic context [16]:

i) has to be accurate, repeated measurement must be possible and with a reasonable cost and in short time
ii) the biomarker must provide information that are not already available from a careful clinical assessment;

iii) the measurement of the biomarker should support clinician in medical decision making.

In conclusion, according to this definition, biomarkers may derive from the blood, the urine, genetic studies, imaging, physiological tests, and tissue-specimen biopsies [17].

Outline of the thesis

The present thesis, result of the international experience I had during the last 3 years in the context of the International Philosophiæ Doctor programme in Cardiovascular Pathophysiology and Therapeutics (CardioPaTh), disserts on the known and unknown role of biomarkers in HF.

The thesis is divided in several chapter, each of them dwelling upon specific aspects that have been investigated during the 3 years of my PhD curriculum.

After a generalist paragraph dwelling upon the use of biomarkers in HF, its associated diseases, and the argument around the dilemma of racial differences (chapter 2), the important and emerging role of hormone deficiencies (HD), alone ore combined in the multiple hormone deficiency syndrome (MHDS) is discussed (chapter 3), together with the presentation of the results of the main project of my PhD, the T.O.S.CA. project, an international effort aimed to investigate the role of MHDS in HF.

The following chapter (chapter 4) is based on the even strong relationship between the heart and the gut (the so-called Gut Axis), followed by a chapter showing the possible role of novel biomarkers in HF (chapter 5). In conclusion, a last chapter about my other HF research activity (not strictly related to biomarkers) is present.

Chapter 2. Biomarkers and Heart Failure

2.1. Biomarkers and Imaging in Heart Failure: Complementary or Subtractive?

This section is a review written with regard to the role of blood biomarkers and imaging in heart failure for the Heart failure clinic journal. As conclusion, the multi-biomarkers strategy, in particular if circulating and imaging biomarkers are combined and information from different pathophysiological pathways are provided, appears the most promising strategy that goes beyond the limits of the current management of HF.

Salzano A, Marra AM, D'Assante R, Arcopinto M, Bossone E, Suzuki T, Cittadini A. Biomarkers and imaging: complementary or subtractive? Heart Fail Clin. 2019 Apr;15(2):321-331. doi: 10.1016/j.hfc.2018.12.008. PMID: 30832821

Circulating Biomarkers.

Considering that an impressive number of circulating biomarkers has been studied [6,17,18], the purpose of this section is to provide a brief overview of the best characterized and promising of them. For this reason, we will discuss first the *classical HF biomarkers*, used in the every-day clinical practice worldwide and then *emerging* biomarkers separately.

Classical biomarkers

Circulating biomarkers are undeniable tools in HF management for several reasons: 1) they are essential for HF diagnosis 2) they are helpful in assessing prognosis of HF-patients 3) they are potentially useful in guiding therapy[19].

The most studied and useful biomarkers in HF are natriuretic peptides, namely B-type natriuretic peptide, N-terminal pro BNP (NT-proBNP) and the mid-regional precursor of the Atrial Natriuretic Peptide (MR-proANP). Natriuretic peptides mirror the volume/pressure overload, being secreted in response to end-diastolic wall stress[20,21]. According to the ESC guidelines, the presence of serum BNP < 35 pg/mL and NT-proBNP < 100 pg/mL is enough to rule out HF, even when signs symptoms highly suggestive of HF are present[22]. Furthermore, in the acute setting, higher cut-offs (BNP < 100 pg/mL or NT-proBNP < 300) yield a 99% negative predictive value of excluding HF-diagnosis[23]. MR-proANP has a lower diagnostic power when compared with BNP and NT-proBNP[24]. Besides their relevance

for HF diagnosis, natriuretic peptides are powerful biomarkers with regard to prognosis assessment of HF patients. According to the Acute Decompensated Heart Failure National Registry (ADHERE), BNP levels at the admission are strongly predictive of in-hospital mortality regardless of any other clinical and laboratory variables, both in HFrEF as well as HFpEF[25]. Both BNP[26] as well as NT-proBNP[27] levels are able to predict significantly outcomes in HF, whereas with regard to therapy guidance conflicting results were reported [28-31]. Although useless in HF-diagnosis, a rise in MR-proANP levels is strongly associated with outcomes in HF[32].

Serum Troponins (TnI/TnT) are often elevated in HF patients, but their specificity for HF diagnosis is questionable. Of note, since the clinical presentation of HF and acute coronary syndrome (ACS) might be quite similar, the assessment of TnI and TnT is useful in differentiating the two clinical syndromes. Specifically, during ACS troponins usually rise after 6 hours, whereas remains stable in HF in such timeframe[19]. On the other hand, elevated serum troponins are able to predict the decline of systolic function[33] and survival in HF patients[34].

Emerging biomarkers in HF

In the last decade, an enormous attention was paid to the role of micro ribonucleic acids (MiRNAs) in cardiovascular diseases. MiRNAs regulate protein translation and several evidence suggest their role in facilitating HF diagnosis[35]. However their prognostic role in HF is questionable[36,37].

Inflammation plays an active role in HF. Bowel hypoperfusion may lead to bacterial and endotoxin translocation into the bloodstream[38]. In these regards, C-reactive protein (CRP) is associated with HF severity[39] and mortality[40,41]. Other markers of inflammatory activation are able to predict survival in HF such as Interleukin-9 (IL-9)[42] or source of tumorigenicity 2 (ST2)[43,44] a member of the interleukin 1 receptor family. Interestingly ST2 showed also a correlation with right ventricular size and function[44]. Galectin-3 (G-3) is associated with macrophages activation and cardiac fibroblast proliferation which ultimately lead to LV stiffness [45] and predicts outcome in HFrEF as well as in HFpEF[46]. Likewise also growth differentiation factor 15 (GDF-1) may predict all-cause mortality in HFrEF[47]. Taken all together, inflammatory activation is an important feature of HF and is strictly associated with prognosis. However, since inflammation belongs to a broad spectrum of diseases, inflammatory biomarkers lack on specificity.

Strictly related to the inflammation burst, a robust body of evidence suggests the importance of hormonal deficiencies in HF [48-55]. This is not surprising considering the well-known relationship between cardiovascular diseases and hormonal pathways [56-60]. The most studied hormonal

abnormality in HF is the deficiency of the growth hormone (GH) /Insulin-like growth factor 1 (IGF-1) axis, which is independently correlated with all-cause mortality in HF [61-63]. Interestingly GH deficiency replacement has demonstrated in some preliminary studies to be a potential therapeutic target in HF patients[64-67].

Another intriguing features of HF pathophysiology is the interplay between the gut and the cardiovascular system. Recently, in the context of the *gut hypothesis* of HF [68], trimethylamine N-oxide (TMAO), a gut microbiome-mediated metabolite, has been shown to be a strong prognostic biomarker in both acute and chronic (stable) HF [69-74].

Taken together, natriuretic peptides represent the cornerstone of HF diagnosis, management and prognosis. However a multi-biomarkers approach might grant broader information, which in turn might be helpful for the daily clinical management of this clinical condition. In table 1 are summarized evidences regarding biomarkers in HF.

Imaging Biomarkers

Along with established circulating biomarkers, findings from different imaging techniques are extremely useful in prognosis estimation and in guiding therapy in HF. Three specific role have been identified for cardiac imaging in HF[75]: it identifies HF phenotype, assesses severity of heart dysfunction, and monitors responses to interventions. In the future, another important role of imaging will likely be the selection of proper patients for cardiac devices (see figure 1).

Transthoracic echocardiography

Transthoracic echocardiography (TTE) is the indispensable tool in the management of patients with HF for its safety and availability[1]. Main TTE parameters in HF include those strictly related to left ventricular (LV) dysfunction and remodeling (e.g. LV ejection fraction (EF), LV volumes and sphericity, LV deformation pattern) and those related to other cardiac abnormalities (e.g. left atrial (LA) volume and contractile function, right chambers dimensions and function, pulmonary artery pressures (PAP) and signs of congestion). All of them carry prognostic information.

LVEF is best measured with the biplane Simpson's method and it is one of the most important parameter in assessing HF[76]. Further, it has an important role in guiding device therapy (i.e. ICD/CRT implantation in patients with EF < 35%) [1]. LV mass (truncated ellipsoid method) and LV sphericity (LV diameter/ length ratio) are easy-available secondary prognostic measures[77].

Furthermore, recent technologies shed novel lights on LV systolic mechanics by imaging its

deformation pattern during ejection (longitudinal and circumferential shortening and radial thickening). Such deformation can be estimated by strain (change in the length of a myocardial segment relative to its resting length) and strain rate (strain divided by time) by speckle-tracking echocardiography (STE). These speckles are stable acoustic markers in the ultrasound images that can be tracked to generate myocardial deformation curves[78]. Among such measures, global longitudinal strain adds prognostic value to LVEF and is independent predictor of MACE in large trials[79].

LA enlargement (biplane area–length method) is common in HF patients and represents an adverse prognostic marker since it is associated with Atrial Fibrillation and MACE[80]. LA contractile function, possibly estimated with STE, is gaining importance to better elucidate LA physiology although less robust evidence is available as a prognostic marker in HF[81].

A glimpse of the right heart is of paramount importance to properly evaluate HF patients [82-85]. Elevated systolic PAP - estimated by peak velocity of the tricuspid regurgitation jet and right atrium estimated pressure- is widely prevalent in patients with HF and represents an established prognostic marker[86]. Independent of systolic PAP, the presence of right ventricular systolic dysfunction also has prognostic implications in HF[87]. Although TTE is not the gold standard technique for RV quantification, several parameters are available for this purpose, including tricuspid annular plane systolic excursion (TAPSE), fractional area change, and RV free wall strain (STE) [88]. Moreover, increased IVC diameter, reflecting congestion[89], identifies patients with an adverse outcome[90].

Finally, with regard to hospitalized patients, a recent study suggested that patients who performed an echocardiogram had a lower in-hospital mortality[91]

Cardiac magnetic resonance

Unlike echocardiography, cardiac magnetic resonance (CMR) can image any plane, eliminating one of the limit of TTE. Indeed, with CMR use geometrical assumptions are not needed, warranting more precision than TTE. The tradeoff is that the CMR has higher cost, lower availability and it is more time-consuming than TTE. To date, CMR should be proposed only to selected patients for which there is a specific clinical question to answer. Indeed, CMR has decisive relevance, for example, to strengthen the clinical suspicion of rare HF etiologies such as myocarditis, arrhythmogenic right ventricular cardiomyopathy, iron overload cardiomyopathy, amyloidosis, sarcoidosis, and Fabry disease[92].

Apart from rare etiologies, the differentiation between ischemic and non-ischemic causes of HF is a common challenge not always resolved by coronary angiography; in this regard, gadolinium-chelated

contrast agents are employed in CMR for infarct/fibrosis imaging. Of note, the physiological basis of *late gadolinium enhancement* (LGE) are the increase in its volume of distribution within areas of scarring or fibrosis and a prolonged washout in the irreversibly injured myocardium[93]. Patients who had LGE of any pattern showed an 8-fold higher risk of MACE and appropriate implantable defibrillator discharges compared with patients without LGE[94]. In addition, the presence of scarring on LGE-CMR in non-ischemic HF is predictive of inducible ventricular tachycardia even after adjustment for LVEF[95]. Besides, it has been demonstrated that the presence of LGE is the best independent predictor of CMR derived indexes in HF patients[96].

Moreover, dedicated protocols for quantification of RVEF and volume are available in CMR and are significantly more accurate than TTE estimation, thus allowing a more precise prognostication[97].

Further, the CMR has specific protocol to evaluate the epicardial fat. This has been associated with the presence of comorbidities and seems to be very useful in particular in the setting of HF with preserved and mid-range reduced EF [98,99].

Recently, Arvidsson et al. suggested a possible use of intracardiac hemodynamic forces analysis for risk stratification and CRT implantation guidance[100]. Indeed, the assessment of intracardiac hemodynamic forces computed from 4-dimensional flow CMR could provide independent biomarkers of cardiac function, in particular in patients with left ventricular dyssinchrony.

Taken all together, these findings support the idea that CMR makes a substantial contribution to both diagnosis and management in HF patients over-and-above standard echocardiography[101]. Further studies are needed to clarify the role of CMR in the HF management.

Nuclear imaging

Cardiac sympathetic nervous system is a key pathophysiological player and a primary therapeutic target in HF[102]. Metaiodobenzylguanidine (^{123}I-MIBG), a iodine radiolabeled norepinephrine analog used in nuclear imaging for scintigraphy and SPECT/PET, is retained within myocardial nerve endings and can be imaged to characterize myocardial uptake and thus obtain a noninvasive assessment of cardiac sympathetic innervation (cardiac adrenergic receptor density)[103].

In general, healthy individuals have high cardiac uptake of ^{123}I-MIBG, whereas those with HF have lower myocardial uptake, reflecting decreased receptor density. The heart-to-mediastinum ratio (HMR) is the main measures of ^{123}I-MIBG uptake. In the ADMIRE-HF trial, the risk of major cardiac

events was significantly lower for participants with higher HMR than for those with a lower HMR (using a cut off 1.6)[104]; other researchers found that the addition of the ^{123}I-MIBG HMR to validated HF score (e.g. Seattle Heart Failure Model) improved risk stratification with a net reclassification improvement more than 20%[105].

Moreover, it has been postulated that the MIBG pattern and its modification after therapy can predict therapeutic response and further improve the prognosis prediction and may have role in therapy tailoring in the future[106]. Further, recently cardiac 123I-MIBG scintigraphy demonstrated to be helpful in the selection of HF patients who might not benefit from ICD implantation[107] .

To date, although PET imaging has superior quantitative capabilities, ^{123}I-MIBG SPECT is the only widely available nuclear imaging method for assessing regional myocardial sympathetic innervation[108].

Recently, in the BETTER-HF trial, authors demonstrated that myocardial contractility as assessed by global longitudinal strain is correlated with autonomic denervation assessed by 123I-mIBG scintigraphy, and was able to discriminate the severe cardiac denervation[109].

Combining biomarkers

Despite the number of biomarkers studied in the past decades has increased dramatically[17,18], relatively few of them may change our decision making in the clinical arena.

To date, even if they are influenced by a number of cardiac and non-cardiac conditions, natriuretic peptides (BNP and NT-proBNP) represent the gold-standard biomarkers in HF [1,110-112]. An interesting matter of debate, considering the improvement in the field of biomarkers, is if circulating and imaging biomarkers could be considered subtractive or complementary.

On the one hand, it is important to highlight that circulating biomarkers have an important role in risk prediction models[113]. Indeed, the single biomarker or more often a combination of different circulating biomarkers are combined with clinical variables to predict mortality or hospitalization or both in HF. In addition, the most promising models are made by a combination of biomarkers related to different pathophysiological pathways[114]. Of note, recently has been reported that only few of the published models have been validated in independent cohorts and not all of them have been adequately validated in their original population [115].

On the other hand, in last year even more study have been published with regard to the role of novel imaging biomarkers in HF[75].

To date, most of the data available are based on 2 dimensional TTE[116]. Even if interesting information derived from 3-dimensional TTE, from CMR and from other imaging techniques, for its worldwide diffusion and for its availability and cost-effectiveness, TTE remains the most used technique in all the stage of HF. For this reason, almost all the studies in which circulating and imaging biomarkers have been combined are confined to TTE.

In this context, in the Cardiovascular Health Study, Authors demonstrated that the combination of echocardiography and NT-proBNP significantly reclassifies 5-year HF risk in older adults when added to a clinical model, in particular in intermediate-risk individuals[117]. Further, Lupon et al demonstrated that a multimarkers strategy combining circulating and imaging biomarkers was useful for risk stratifying in HF[118].

The combination strategy is endowed with a good performance not only in HF with reduced Ejection Fraction. Recently, in HF with preserved ejection fraction (HFpEF), it has been demonstrated that a 2-step algorithm combining imaging and circulating biomarkers (i.e. echocardiographic evaluation followed by the assessment of galectin-3) improves the diagnosis and prognostic assessment of patients with suspected HFpEF[119].

In conclusion, as recently reported[120], the management and the assessment of HF extend beyond basic clinical evaluations, and the currently available numerous tools need to be applied in a coherent and effective manner within each phase of the patient management cycle. In the modern medicine, these tools are a combination of clinical information, circulating and imaging biomarkers.

In the future, even more strings to clinicians' bows will be available.

Summary

Despite the number of biomarkers investigated in HF in the past decades has increased dramatically, to date relatively few of them modify our decision making in the clinical arena and may therefore be termed "clinically useful".

Natriuretic peptides (BNP and NT-proBNP) represent the gold-standard circulating biomarkers in HF, whereas TTE represents the indispensable tool in the management of patients with HF for its safety, low cost and availability.

The multi-biomarkers strategy, in particular if circulating and imaging biomarkers are combined and information from different pathophysiological pathways are provided, appears the most promising strategy that goes beyond the limits of the current management of HF.

In the near future we can expect the discovery of an ever-increasing number of novel blood-derived and imaging biomarkers capable of better predict HF prognosis, at the same time providing innovative pathophysiological mechanisms and guiding HF therapy. Major challenges remain particularly the definition of an optimal multi-biomarkers strategy, but patients with heart diseases are likely to benefit from these efforts.

Table 1. Circulating biomarkers and their role in the management of heart failure.

Biomarkers	Pathophysiological background	Diagnosis	Prognosis assessment	Therapy guidance
Natriuretic peptides (BNP, NT-proBNP, MR-proANP)	Reflect pressure/volume overload due to increased filling pressures	+++	+++	++ (MR-proANP useless)
Troponins	Myocardial damage	- (Lack of specificity)	++	--
CRP	Inflammation	-- (Lack of Specificity)	++	+
ST2, G-3 AND GDF-15	Cardiac fibrosis	--	++	-
Hormones (GH, IGF-1, testosterone)	Reflect the Anabolic imbalance	+	++	+
TMAO	Gut-microbiota involvement in HF	+	++	-
MiRNAs	Remodeling of Heart chambers	--	+	+

HF: Heart Failure. BNP: brain natriuretic peptide. NT-proBNP: amino terminal fragment of the pro-hormone brain type natriuretic peptide. MR-proANP: mid-regional precursor of the atrial natriuretic

peptide. CRP: C-reactive protein. ST2: source of tumorigenicity 2. G-3: Galectin-3. GDF-15: growth differentiation factor 15. GH: growth hormone. IGF-1: insulin like growth factor-1. TMAO: trimethylamine N-oxide. MiRNAs: microribonucleic acids.

+++ use recommended; ++very useful; + possibly useful (small or lack of evidences), -- not useful, - maybe not useful (small or lack of evidences).

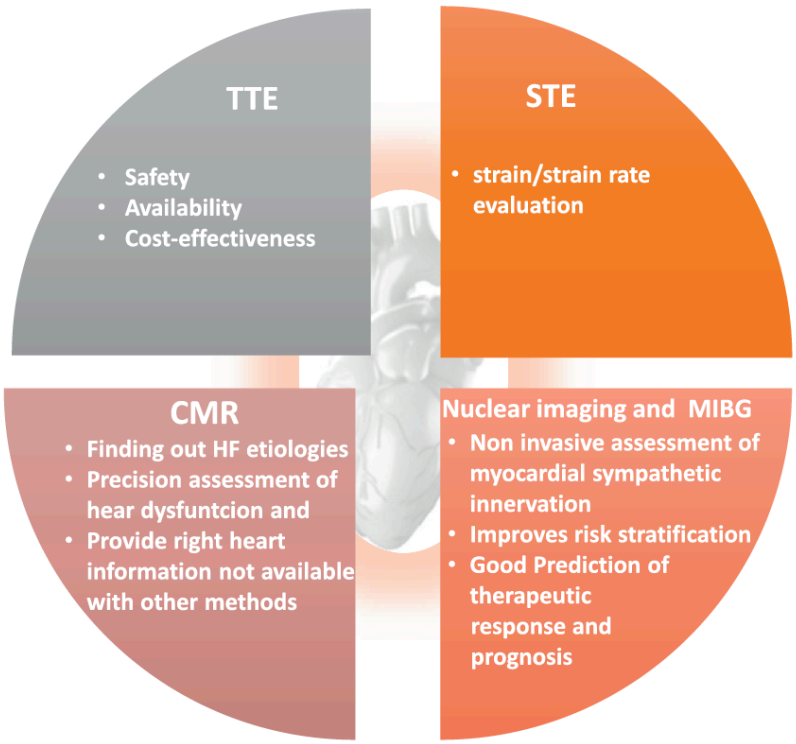


Fig. 1. Specific roles identified for cardiac imaging in heart failure: (1) heart failure phenotype identification; (2) severity assessment of heart dysfunction; and (3) monitoring of treatment responsiveness. Future roles include the selection of appropriate patients for cardiac devices. CMR, cardiac magnetic resonance; MIBG, metaiodobenzylguanidine; STE, speckle-tracking echocardiography; TTE, transthoracic echocardiography.

2.2 Biomarkers in Heart Failure and Associated Diseases

In December 2017 I have been invited to be Lead Guest Editor of a Special Issue dwelling upon the role of biomarkers in Heart Failure and Associated Diseases for the Journal *Disease Markers*.
The following is the accompanying Editorial, written by me and my international editorial team.

Salzano A§, Marra AM, Proietti M, Raparelli V, Heaney LM. Biomarkers in Heart Failure and Associated Diseases. Dis Markers, vol. 2019, Article ID 8768624, 2 pages, 2019. doi.org/10.1155/2019/8768624

Despite considerable improvement in the management of heart failure (HF), unsustainable levels of morbidity and mortality coupled with an increasing economic and social burden have been observed over the previous three decades [3]. A rational explanation of this is the fact that no single pathophysiologic paradigm of HF has been clarified, resulting in failure of our current models to completely explain disease progression [121].

Classically, seven categories of biomarkers in HF have been described, reflecting the different pathophysiological pathways involved in disease progression [17]. These include myocardial stretch, myocyte injury, matrix remodelling, inflammation, neurohumoral activation, oxidative stress, and indices of renal dysfunction [13]. Moreover, growing evidence supports the key role of alternative pathophysiological pathways (e.g., the gastrointestinal system, the anabolic/catabolic imbalance, and multiple hormonal deficiency syndrome), with ever-increasing identifications of novel biomarkers that demonstrate their importance in HF [7,42,55,63,122]. In this context, a growing interest in multimarker approaches to biomarker panels to assess multiple pathophysiologic pathways has been realised, including the combined use of proteins, lipids, metabolites, hormones, and genetic markers [16].

Owing to these recent advances in biomarker research, the aim of this special issue was to focus on the role of biomarkers in HF and associated diseases.

Ischemic heart disease is to date the most frequent cause of HF [121], with atherosclerosis the major pathophysiological mechanism. In this issue, Mocan-Hognogi et al. review the role of adipokines (in particular visfatin, apelin, leptin, and resistin) as a biomarker of ischemic cardiac disease and conclude that "*there is no doubt that inflammation is viewed as an important pathophysiological step in the development of atherosclerosis*".

Importantly, the identification of patients at high risk of poor prognosis is one of the principal aims of current clinical research [49]. With this regard, Alavi-Moghaddam et al. conducted a pilot study involving 21 patients diagnosed with acute myocardial infarction and demonstrated that plasma levels of microRNA-208b, of which levels of expression have been demonstrated to be increased in the blood of patients with acute myocardial infarction, were 2-fold higher in patients who died after 6 months than in those which survived.

Further, Banach et al. investigated plasma concentration of procalcitonin (PCT) in 130 patients with chronic HF with reduced ejection fraction, assessing its prognostic value during a 24-month follow-up period. Indeed, PCT levels were significantly higher in HF patients when compared to a control group. Further, Kaplan-Meier survival curves revealed that patients with PCT in the highest quartile had a significantly reduced probability of survival. This is additional evidence supporting the role of inflammation in HF [42].

Diabetic cardiomyopathy (DCM) is a common cardiac dysfunction, affecting approximately 12% of diabetic patients, and is featured by ventricular diastolic and (or) systolic dysfunction. Li et al. provided a comprehensive and novel illustration of gene expression profiles to identify differentially expressed genes in myocardial tissue, which may play critical roles in the occurrence and development in patients with DCM. This is of great interest considering that diabetes mellitus has been described in approximately 20-25% of HF patients [61,63].

HF is a progressive condition in which myocardial damage, caused by cardiovascular risk factors, leads to the development of myocardial dysfunction. Thus, an ever-worsening condition is present until the patient eventually develops end-stage heart failure. Heart transplantation is the only survival option for end-stage patients [121]. Cardiac allograft vasculopathy (CAV) is the leading cause of cardiovascular adverse events during follow-up of heart transplantation. Mirabet et al. demonstrated that high-sensitivity cardiac troponin T, measured during a long-term follow-up, appears as a helpful biomarker to identify patients at low risk of adverse CV outcomes. On the other hand, the soluble form of AXL (sAXL) and a biomarker of endothelial dysfunction was not able to predict outcome.

In conclusion, this issue collected novel findings and shed light upon the role of biomarkers in HF.

2.3 Biomarkers in cardiovascular disease: the dilemma of racial differences

In August 2019, being Prof Suzuki's team a group leader in the field of biomarkers, we have been invited to write for the Journal of the American Heart Association, an editorial focused on the dilemma of racial differences in the field of biomarkers.

Suzuki T, Israr ZM, **Salzano A**. Biomarkers in cardiovascular disease: the dilemma of racial differences. **J Am Heart Assoc** 2019;8:e014295 doi: 10.1161/JAHA.119.014295

Racial differences in medicine is still a matter of debate, with the scientific community divided on the real meaning and relevance of the notion of race in medical research[123,124]. On the one hand, the view that classical racial categories identify subgroups of patients with a different disease epidemiology, pattern, and prognosis, posed the basis for so-called *racial medicine*. On the other hand, the concept that race is only a social construct, without biological roots, led to the statement that the use of race as a biological factor is '*problematic at best and harmful at worst'*[125]. Indeed, there is a tendency to assume that differences between subgroups are due to genetics - when they might be due to socio-economic or cultural factors that just happen to correlate with what is commonly defined as race - hiding the real pathophysiological background of a finding. This concept led to the use of the term ethnicity rather than race, with the aim of indicating a group of people who identify with each other on the basis of a supposed shared genealogy or ancestry or on cultural similarities (e.g. common language or dialect, history, society, culture or nation). Further, some scientists fear that the use of race as a variable in medicine can perpetuate historical discriminatory attitudes [124] (e.g. limiting the use of particular drugs or procedures to a particular racial subgroup).

However, genetic studies show that there seem to be more genetic variation (95%) within a group than between so-called racial groups (5%)[126]. To date, nearly all geneticists reject the concept that biological differences are due to racial differences[127], while epidemiological and clinical studies continue to find association between clinical findings and the social identities of research participants. In particular, in cardiovascular disease (CVD), the difference in drug response and its association with race has been well demonstrated[128]; the attenuated response to angiotensin-converting-enzyme inhibitor therapy in black as compared with white patients in heart failure and hypertension[129-131], prevalence of cardiovascular and metabolic diseases[132,133], and in prognosis[134], when subjects are grouped on the basis of racial categories.

In European populations, statistics on ethnicity or race present challenges with acquiring data. Challenges may include legal prohibitions, data protection provisions and political reluctance to emphasise ethnic diversity. Mapping Europe based on geographical location allow for collecting 'race or ethnic statistics' that adjust for the aforementioned challenges, amongst other proposed methods of collecting data for ethnic statistics[135]. In Europe, even when geographical location rather than race is considered, a recent report by the authors showed regional differences in levels of a gut microbiome-related biomarker, trimethylamine N-oxide (TMAO), in a European population with more than 99% of patients being Caucasian regardless of confounders[136]. These discrepancies are a complex interaction between factors that include socioeconomic status, structural differences and ethnic influences.

In this context, in the current issue of the Journal of the American Heart Association (JAHA), Hackler et al reported on the association between race and a panel of biomarkers - with known or possible informative cardiac and metabolic roles - in a multi-ethnic population cohort without known CVD[137] enrolled in the Dallas Heart Study (DHS)[138]. In a final cohort of 2635 subjects (1638 black and 997 white), with a 10 years follow-up, 32 biomarkers were investigated. These biomarkers were reflective of lipid state (e.g. cholesterol, triglycerides, lipoprotein a), adipokine levels (i.e. leptin and adiponectin), inflammation (e.g. high sensibility C-reactive protein, IL-18, ANA, D-dimer, soluble receptor for advanced glycation end products), endothelial function (e.g. sVCAM, SICAM), myocyte injury/stress (e.g. hsTNT, NT-proBNP, ST2), and kidney function (i.e. Cystatin C and microalbuminuria). The results showed the rate of CVD events in blacks was more than twice higher than in whites. In line with other studies[139], diseases such as arterial hypertension and diabetes were more frequent in blacks, and a difference in echocardiographic parameters (left ventricular mass, left ventricular end diastolic volume and coronary calcium) were observed between blacks and whites[140]. For differences in biomarker levels after multivariate adjustment, when compared to whites, blacks showed significant differences in Lp(a) concentration, adipokine levels (lower leptin and adiponectin), inflammatory biomarkers, endothelial biomarkers, and myocyte injury/stress (lower NT-pro BNP, higher ST2). Furthermore, black women had higher rates of microalbuminuria whereas black men had higher hsTnT levels. Notably, when these biomarkers where used in exploratory analyses for association with outcomes, differences in the rate of CVD were no longer significant, suggesting that these pathways can contribute or mediate the observed difference in CVD rate amongst the two groups.

The present study showed that multiple biomarkers reflective of different biological pathways differed by race. The observed association of CVD events with blacks is mediated by the described different biological patterns expressed by the subjects (resulting in the difference in biomarkers). However, some additional considerations are needed. Even if the DHS has been deeply phenotyped, with data on traditional risk factors and possible confounders available (e.g. socioeconomic status), the design of the study argues that these differences can be explained as genetic differences rather than as the presence of other factors (e.g. social state, educational state, dietary habits, nutritional state, physical activity) – hence ethnic differences more than racial differences. Accordingly, it has been demonstrated that the higher incidence rate of venous thromboembolism in blacks when compared with whites can be mostly explained by a difference in distribution of risk factors[141]. In line with this, in the present study blacks had more insulin resistance and diabetes, black men were more often smokers and black women had higher BMI when compared to white women. Consistent with well-documented socioeconomic differences between blacks and whites that impact on CVD rate and prognosis[142], black participants in the present study reported lower education and income compared with white participants.

Biomarkers related to different pathophysiological patterns were investigated in this study. It is a notable strength of the present report to investigate racial differences in CVD. Following a definition provided in the past by one of the authors of the present investigation[16], there are three criteria that define the clinical usefulness of a biomarker: i) it has to be accurate, repeated measurement must be possible, cost effective and time effective; ii) the biomarker must provide information that are not already available from a careful clinical assessment; and iii) the measurement of the biomarker should support the clinician in medical decision making. In line with these concepts, the present investigation focused on the strategy of investigating multiple biomarkers of several pathophysiological pathways that provided information that may be potentially useful in legitimate epidemiological observations[16]. To date, this appears the most promising strategy that can help to go beyond the limits of the current management of CVD [143].

In conclusion, the present report describes differences of multiple biomarkers -possibly or known to be - related to cardiometabolic diseases in healthy subjects grouped on the basis of the definition of race as black and white. Even if limited by the fact that there is a consensus that 'race', whether categorized or self-identified, is a weak surrogate for various genetic and non-genetic factors in correlations with health status[144], the finding of the present study can be considered as

hypothesis generating, providing additional information about the dilemma of racial differences in medicine, and allowing the pursuit of advancing tailored medicine.

3. Chapter 3. Hormonal and metabolic imbalance in heart failure

Chronic Heart failure (CHF) is a major healthcare issue with increasing prevalence, huge estimated cost, and poor prognosis, still approaching a 5-year mortality of 50%.[145,146] Given such disheartening statistics, an imperative need is to search for novel therapeutic approaches capable of slowing disease progression and improving survival.[147,148] In this regard, the neurohormonal model, conjecturing CHF pathophysiology as sustained by an excessive activation of numerous pathways, including the sympathetic, renin-angiotensin-aldosterone, and cytokine systems, provided a theoretical framework supporting the actual therapy.[147,148] Such an approach, although partially successful, has not fulfilled all the promises, and CHF prognosis remains modest.[145,146] However, to complement the paradigm of neurohormonal activation, a concomitant reduction of anabolic hormonal axes seems to potentially play an important role in CHF progression and prognosis.[149] Specifically, the so-called multiple hormonal deficiency syndrome (MHDS) encompasses several anabolic systems that are down-regulated or impaired in CHF: the somatotropic axis [including growth hormone (GH) and its tissue effector insulin-like growth factor-1 (IGF-1)], anabolic steroids (testosterone and DHEA-S), and thyroid hormones.[149-153] Notably, MHDS does not appear to be a mere marker of disease progression, insofar as each defect is associated with impaired clinical status, functional capacity, and increased mortality.[149-154] In addition, insulin resistance (IR), with or without coexisting overt hyperglycaemia and type 2 diabetes (T2D), appears to have an independent role in the pathogenesis and prognosis of CHF.[155] Indeed, it has been showed that T2D prevalence in HF is much higher than in the general population, with a strong impact on HF prognosis, suggesting that a negative role is played by hyperglycaemia and/or IR.[156] However, although IR and T2D should be therefore considered among the defects of the hormonal anabolic axes (i.e. expression of a down-regulation of insulin action), IR and T2D have never been analysed in the context of MHDS. Indeed, in previous preliminary report investigating the impact of HD in CHF, the role of IR has been ignored, and the effect of T2D disregarded. Furthermore, promising results observed in recent trials investigating the effect of antidiabetic drugs (i.e. gliflozins) support the idea that hormonal and metabolic players might be involved in the progression of CHF.[157] Despite these premises, to date no large study has focused on the relative role played by MHDS in the progression and survival of patients with CHF. In particular, no registry-based study has been specifically designed to evaluate

the overall impact of hormone deficiencies (HD, comprises insulin resistance and T2D) on CHF morbidity and mortality.

In the present chapter, the complex interplay between hormone deficiencies (alone or combined) and heart failure is deeply discussed.

3.1 Growth Hormone and IGF-1 axis

The impairment of Growth Hormone (GH)/Insulin Growth Factor-1(IGF-1) plays a crucial role in Chronic Heart Failure (CHF). Several studies have shown that patients affected by this condition display a more aggressive disease, with impaired functional capacity and poor outcomes. Interestingly, GH replacement therapy represents a possible future therapeutic option in CHF. In this section, we focused on the assessment of the main abnormalities in GH/IGF-1 axis in CHF, the underlying molecular background, and their impact on disease progression and outcomes.

3.1.2 Growth Hormone as biomarker in Heart Failure

Marra AM, Bobbio E, D'Assante R, **Salzano A,** Arcopinto M, Bossone E, Cittadini A. Growth Hormone as Biomarker in Heart Failure. **Heart Fail Clin**. 2018 Jan;14(1):65-74. doi: 10.1016/j.hfc.2017.08.008. Review. PubMed PMID: 29153202.

GH/IGF-1 axis physiology

Several biological processes require the pituitary secretion of GH. One of the main effects of the interaction between GH and its specific receptors (GHR),is the activation of a complex signaling cascade that leads to the hepatic production of its major biological mediator Insulin-like Growth Factor-I (IGF-I).

GH and IGF-1 are linked by a long-loop feedback since the IGF-1 produced in the liver in response to GH, inhibits GH release through the stimulation of somatostatin release[10].

The GH/IGF-I axis is regarded as the most powerful anabolic system in nature. Although this pivotal mechanism is still poorly understood, it is well known that GH/IGF-I axis is responsible for post-natal growth by increasing both bone length and density, and muscle mass during childhood and adolescence[11]. Moreover, it has important effects by regulating carbohydrates and lipids metabolism, the latter preferentially on visceral adipose tissue[12]. It has been documented that IGF-I is also

released by a number of other tissues, thus IGF-1 acts not only as classic endocrine hormone, but also in an autocrine and paracrine manner. IGF-1 circulates in blood either free or bound to specific binding proteins that prolong the its half-life[13]. To date, six IGF-1 binding proteins (IGFBPs) have been identified and represent an elaborate system for regulating IGF-1 activity. In particular, almost of 90% of circulating IGF-I is part of a ternary complex, also composed of IGF-specific binding protein 3 (IGFBP-III) and acid- labile subunit (ALS)[14]. This complex allows IGF-I to reach several tissues, where it binds to its own receptor (IGF1R), leading to the activation of the PI3K/Akt signaling pathway, which in turn promotes cell growth, enhances glucose transport, inhibits apoptosis and acts along with Interleukin 6 to protect cells from TNF-α cytotoxicity[9].

It should be noted that besides the regulation of the somatic growth, this anabolic axis has a significant impact on the cardiovascular system, by supporting cardiac growth and performance. The activation of IGF-I receptors expressed in cardiomyocytes, determines a direct effect on the reduction of the systemic vascular resistance by inducing the production of nitric oxide (NO), promotes the contractility of cardiomyocytes mainly by increasing intracellular calcium concentration and calcium sensitization of the myofilaments, and preserves capillary density[15]. Of note, both GH and IGF-1 are endowed with growth promoting properties within the myocardium, increase protein synthesis in the cardiomyocytes[16].

IGF-1 also induces the reuptake of calcium by the sarcoplasmic reticulum by regulating the sarco/endoplasmic reticulum Ca2+-ATPase (SERCA2), which is involved in diastolic function.

Moreover, several studies performed in experimental models of heart failure demonstrated that GH/IGF-1 activation augments SERCA2 myocardial content, attenuates left ventricular remodeling and enhances intracellular Akt signaling[17]. In addition, it plays a pivotal role by regulating cardiac growth, cardiomyocyte size and metabolism, by stimulating aminoacid uptake for protein synthesis, and promoting the transcription of genes specifically expressed in the cardiac muscle[9]. Of note, GH *per se* increases protein synthesis in the isolated perfused heart by augmenting amino acid trasport[18].

Pathophysiology of GH/IGF-1 impairment in heart failure

GH deficiency is a common finding in CHF with a prevalence ranging from 32% to 53% according to different reports [19–21]. IGF-1 has remarkable positive effect on the cardiovascular system, including anti-apoptotic and growth-promoting action, vasodilation and endothelial protection, and increase of cardiac contractility [10,16]. Most of the studies performed in CHF reported reduced IGF-1 serum levels when compared with healthy controls [19,22–24]. IGF-1 levels were remarkably reduced in patients

with advanced heart failure [25] or cachexia [26]. Many explanations were provided regarding the underlying mechanism of impaired GH/IGF-1 secretion in CHF. The first hypothesis is rooted in hypo-perfusion and reduced oxygen supply, which is the typical hallmark of CHF clinical syndrome. Indeed, 25 children with GH deficiency (GHD) were evaluated with brain MRI and compared to healthy controls. In these series pituitary stalk enhancement were significant lower in GHD than controls, probably related to a mismatch between arterial perfusion and venous drainage [27]. The hemodynamic impairment occurring in CHF is likely to alter the local perfusion of the pituitary gland, specifically by venous drainage stasis and/or deteriorated arterial blood supply leading to cell death and consequently GHD. This speculation is not sufficient to explain alone the high GHD prevalence observed in CHF. Indeed this theory was never proved by a neuroimaging study of the hypothalamic-pituitary axis of CHF patients. A primary hypothalamic damage was also hypothesized by Broglio et al., that found in their report in a CHF cohort a blunted response to different provocative tests such as Growth Hormone realizing hormone (GHRH), GHRH + Arginine, GH-related peptides [19]. The same authors also excluded the presence of an hyperactivity of somatostatine pulse, which physiologically counteracts GH secretion [19].

An additional central role might be also played by several other factors, albeit not completely understood. As matter of fact, abnormalities of GH/IGF-1 secretion are likely to be found in several chronic wasting condition characterized by inflammatory activation and cytokine overexpression[28–30]. In chronic illness reduced hepatic synthesis of peptides hormones produced by the liver is dramatically impaired and at the same stage a peripheral GH resistance was reported [26]. Many patients affected by CHF experience secondary pulmonary hypertension and consequent right heart failure and backward liver congestion, that may also impair IGF-1 secretion [31] . Another explication might be found in background therapy of CHF. Indeed, both ACE-inhibitors [32] as well as β-blockers [33] are likely to modify IGF-1 secretion through a direct inhibitions of IGF-1 signaling pathway.

Taking all together, it is not possible to put forward a single explanatory pathophysiological mechanism of the occurrence of GH/IGF-1 impairment in CHF. Most probably synergy and interaction of different molecular pathways and concomitant pharmacological issues might represent the underpinnings of this phenomena (Fig.1).

Growth hormone deficiency in Heart Failure

Adult GH deficiency (GHD) is a heterogeneous disorder characterized by unspecific clinical features. It may arise during childhood or adult life, resulting from several causes including pituitary adenoma,

trauma, genetic abnormalities, structural or iatrogenic lesions and infiltrative diseases[34].

Diagnosing GH deficiency in adults may be challenging because of the lack of a single biological endpoint such as growth failure and the pulsatile endogenous GH secretion, influenced by anthropometric factors, physical activity and sleep patterns[35].

International consensus guidelines have endorsed the insulin tolerance test (ITT) as the gold standard test for evaluation of adult GHD, but its safety concerns restrict its broader use in the United States[36–38].

Because of its high discriminatory power, convenience and reproducibility, the contemporary administration of Growth Hormone Releasing Hormone (GHRH) and arginine has gained wide acceptance for GHD diagnosis[39–41], in particular in CHF patients, in whom hypoglycemia could be unsafe, and should consequently be avoided.

Since the arginine potentiates the GHRH stimulatory effect by inhibiting somatostatin tone, the GHRH stimulus is significantly potentiated[42,43]. An intravenous bolus of GHRH (1 mcg/kg, maximum dose 100 mcg) is administered after an infusion of arginine (0.5 g/kg, maximum dose 30 g) and blood samples for GH measurement are obtained every 15-30 minutes during the next two hours[44]. Critical for the correct diagnosis of GH deficiency is an accurate measurement of GH levels since the results of the tests could be influenced by the analytical method. Other molecules GH-like (e.g. prolactin) may potentially affect the measurement cross-reacting with monoclonal antibodies usually used to limit detection to the 22 kDa GH isoform. Indeed, there are many different isomers and isoforms of circulating GH, but the 22 kDa GH variant is the most common one[45] . Several population studies reported Growth Hormone deficiency (GHD) being *per se* associated with impaired cardiac performance, increased peripheral vascular resistance and reduced exercise capacity[10] with a positive correlation between GHD severity and cardiac impairment[46]. Moreover, at the beginning of nineties a landmark paper demonstrated premature mortality due to cardiovascular disease in adults with hypopituitarism under routine replacement therapy except GH, thus suggesting a role for GH deficiency in cardiovascular diseases[47]. Interestingly, one third of patients affected by CHF have a concomitant GHD, when the latter is assessed with appropriate dynamic test[19,20,48,49]. Growing evidences support the concept of the relevance in several features of CHF. A recent prospective work published by our group, performed on 130 CHF patients prospectively recruited undergoing a GHRH+ Arginine provocative test, showed that coexistence of GHD and CHF identifies a subgroup of patients with worse clinical status and increased all-cause mortality, higher depression scores, impaired quality of life, presence of left ventricular (LV) remodeling, lower physical performance, and

increased NT-proBNP levels[49]. Specifically, when compared to GH sufficient patients, GHD patients displayed larger LV volumes with elevated wall stress, as well as higher filling pressures, and impairment of right ventricle function. Moreover, these patients also presented with worse cardiopulmonary performance as demonstrated by a significantly lower peak VO_2 and reduced ventilatory efficiency[49]. GH might play an influential role also in acute heart failure exacerbation. Bhandari et al.[50] recently evaluated serum GH concentrations in 537 patients admitted for acute heart failure (AHF), both with HFrEF phenotype (n=415) as well as with the Heart Failure with preserved ejection fraction (HFpEF) phenotype (n=122). The authors found increased GH levels in both HFrEF and HFpEF (p< 0.001 and p: 0.02 respectively) in patients who experienced one of prespecified outcome measures (either death or readmission within 1 year)[50]. GH concentrations were able to independently predict (HR 1.54, 95% CI 1.19–1.99, P = 0.001) outcomes in HFrEF but not in HFpEF. In the same study the author performed a further analysis aimed in comparing the usefulness of GH with NT-proBNP and the acute decompensated heart failure national registry (ADHERE) score[51], with regard to event prediction. Interestingly, the authors reported that the addition of GH to ADHERE multivariate logistic model (which in turn is composed by age, sex, urea, heart rate, and systolic blood pressure) , and to ADHERE model + NTproBNP lead to a net reclassification improvement of both the prognostic scores[50]. Despite the limitations due to the evaluation of a single GH measurement taken in unfasted patients, the paper for the first time demonstrated incremental prognostic utility of the assessment of a marker of GH activity also in HF acute settings.

IGF-1 in Heart Failure

Although the statements form international endocrinology societies suggests to consider the 2.5 percentile of an age-sex matched normal population as cut-off for IGF-1 deficiency[52,53], there is a lack of consensus regarding the reference values for CHF population, taking into account the inhomogeneity in the published reports. As shown in table 1, different criteria were used in the different studies in order to define the condition of IGF-1 deficiency. According to population studies, age should be considered as the main determinant of IGF-1 cut-off levels, whereas gender and ethnicity are likely to play both a negligible role[52]. Table 1 summarizes the studies evaluating GH/IGF-1 axis in cohorts of patients affected by CHF. Even if the most of the studies in CHF patients show reduced total serum IGF-1 levels, other showed normal and few even increased circulating levels. Several factors could explain the discrepancies observed among the studies: different therapeutic and anthropometric backgrounds, inhomogeneous disease severity, and also high assay

variabilities[58]. For instance, Jankowska and coworkers reported extraordinary high IGF-1 value in their study (median values above 250 ng/mL in all age categories of controls. Broglio et al.[19,40] reported low IGF-1 levels in patients with severe left ventricle (LV) dysfunction as well as dulled response to GHRH. Similar results were confirmed by Anwar et al.[59] in an elderly population of patients hospitalized for CHF and by Kontoleon[60] in 23 stable HF patients. Anker et al.[26] evaluated the GH/IGF-1 axis in cachectic and non-cachectic patients with severe heart failure. Increased GH levels were increased in the first group whereas IGF-1 levels were decreased compared with non-cachectic ones, who showed normal IGF-1 levels. Since the IGF-1/GH ratio was 12 fold higher in non-cachectic patients than in cachectic patients, these data have introduced the concept of GH resistance, defined as high GH levels and low IGF-1 levels[26]. The same year Al-Obaidi[61] and coworkers showed that patients with CHF in NYHA class I and II (mild-to-moderate symptoms) displayed elevated IGF-1 serum concentration ($p = 0.005$ vs control subjects), whereas patients with more severe disease (NYHA classes III and IV) had values comparable to healthy controls. According to these aforementioned results[61], one can speculate that IGF-1 rises at the initial stages of the disease in order to compensate and restore the heart function at a paraphysiological level, afterwards other factors may interfere and lead to a condition of reduced IGF-1 secretion up to a frank state of GH resistance[26].

As mentioned above the different (and increasing) use of CHF medications in the last two decades may have played a role for these discrepant results. Indeed, ACE-inhibitors and ARBs have been shown to increase IGF-1 levels in patients with CHF[32] and beta-blockers may exert a depressive action on the GH/IGF-1 axis[33]. A consistent body of evidences suggests that low IGF-1 levels in CHF are noteworthy for many reasons. Indeed, IGF-I deficiency is associated with greater activation in cytokines and neurohormonal system, endothelial dysfunction, impairment of skeletal muscle performance[23], and a worse outcome[9,24,50,62,63]. Interestingly, a landmark study performed by Jankowska and co-workers showed that the number of hormonal deficiencies (IGF-1, DHEA-S, testosterone) was an independent predictor of mortality in men with HF, leading to the speculation that anabolic deficiency has an intricate interaction and that the concomitance of more hormonal abnormalities has a detrimental synergistic action on prognosis[22]. This result was also confirmed by Arcopinto and coworkers, that demonstrated that low IGF-1 levels (< 122 ng/ml) independently predicted higher mortality rate[24]. In countertrend with these aforementioned findings, Andreassen et al.[64] were not able to find neither any relevant differences in left ventricular systolic function, NT-proBNP nor prognosis in patients with low IGF-1 levels.

Indeed, as shown by Petretta and coworkers, the presence of a low IGF-1/GH ratio independently predicts outcomes in a small cohort of CHF patients[63]. The condition of low IGF-1 and high GH depicts the state of GH resistance, which in turn was demonstrated to be associated with cachexia[26], a terminal stage of almost all chronic illness characterized by spread muscle wasting and poorer outcomes[65]. It is also worth to mention the relationship between IGF-1 and its blood transporters in the stream flow, the so-called IGF-1 binding proteins (IGF-BP), which in turn are able to determinate IGF-1 bioavailability. In these regards, Watanabe et al. demonstrated that a low IGF-1/IGFBP3 ratio is associated with increased rates of all-cause mortality, cardiac death, and a composite of cardiac death and re-hospitalization[62]. The evaluation of IGF-BP might be helpful not only in HFrEF but also in heart failure with preserved ejection fraction (HFpEF). Indeed Barroso and collaborates demonstrated a continuum in IGF-1/IGF-BP 7 ratio from subjects with normal diastolic function, asymptomatic left ventricular diastolic dysfunction and HFpEF[66]. Recently Faxen et al. demonstrated that patients with HFpEF displayed higher IGF-1/IGF-BP 1 when compared with HFrEF, specifically due to higher serum IGF-1 concentration[67]. However in the same report, low levels of IGF-1 were associated with higher mortality in HFrEF but not in HFpEF[68]. Salzano et al also found lower IGF-1 and higher GHD in HFrEF than HFpEF[69]. It is worth to mention that it is to date missing a prospective study dwelling upon IGF-1 levels in AHF. Taking all the evidences together, growing evidences lead to consider low IGF-1 levels in CHF not only an epiphenomenon, but also a key factor mechanistically linked to CHF and its severity. IGF-1 might be useful in clinical practice for risk stratification in order to identify a cluster of patients needing a more aggressive therapy.

Treating GH/IGF-1 abnormalities in Heart Failure

Apart the identification of a high risk population and prognostic value, the GH/IGF-1 axis might represent a possible therapeutic target in CHF.

Experimental studies in different animal models reported beneficial effects on cardiac function, peripheral vascular resistance, and survival[70–72]. Furthermore, early treatment of large myocardial infarction with GH reduces pathologic LV remodeling and improves LV function [73]. The translation of these results onto the clinical arena did not lead to unequivocal results. Although the encouraging data coming from a wide number of preliminary open-labeled pilot studies[74–80], two randomized controlled clinical trials[77,81] provided neutral results. However, a pooled meta-analysis of all study of GH in CHF showed an increase of LV Ejection Fraction (EF) and a reduction in systemic vascular

resistance[82]. Moreover, GH treatment reduced LV diastolic diameter and an increases LV wall thickness, determining a positive long-term modification in cardiac morphology[82]. In another meta-analysis treatment with GH resulted in an increase of exercise duration, maximum oxygen uptake, LVEF, cardiac output, and improvement in systemic vascular resistance and NYHA class level[83]. Several explication are likely to be possible in order to explain the inconsistent results of GH administration trials, such as different study duration, target dose, end-points and the assessment of GH status[84]. In this regard, it is worth to mention that also in placebo controlled studies, those patients who consistently increased IGF-1 due to GH administration experienced a significant improvement, whereas those without IGF-1 increase not[77]. This could lead to two conclusions. First, GH should be probably administered only in those patients with an impairment of GH/IGF-1 status. Second, GH therapy should not be administered in patients with advanced CHF, which are probably already in a GH resistance state[26]. On these premises, we performed a randomized, single-blind controlled trial, aimed in comparing the effect of GH replacement therapy in CHF with regard of GHD status[20]. In this study patients underwent a GHRH + Arginine provocative tests, in order to enroll only those with a real state of GH deficiency[20]. After 6 months, GH replacement therapy improved quality of life score, increased LVEF, peak oxygen uptake and exercise duration and flow-mediated vasodilation. On the other hand, it decreases circulating N-terminal pro-brain natriuretic peptide levels[20]. Considering these encouraging results, we extended this study with a 4-year follow up in order to assess whether this effects were sustained or tend to vanish over time[48]. After a 4-years follow-up, GH replacement therapy was still associated with LV reverse remodeling, as documented by the significant reductions of both LV end-diastolic and end-systolic volumes indexes and circumferential wall stress, with an increase in LVEF[48]. With regard of cardiopulmonary performance, despite the treatment effect did not reach statistical significance, in the GH group peak VO2 increased remarkably. Although the study was not designed for hard clinical endpoints, it was noteworthy that there was a marked difference in the aggregate of death and hospitalization for worsening CHF in replacement therapy arm[48]. However, the usefulness of GH replacement therapy must be still proved in a double-blind placebo controlled trial.

Future Prospective and Conclusions

The relationship between hormones of cardiovascular diseases is quite complex[15,85–88]. In this intricate scenario, the impairment of GH/IGF-1 plays a crucial role in CHF. The vast majority of studies showed that patients affected by this condition display a more aggressive disease, impaired functional and exercise capacity, higher neurohormonal activation, a more pronounced left ventricle

remodeling and poorer outcomes (mortality and hospitalization), regardless the assessment method employed (GH serum levels, GHD assessment, IGF-1 and its binding proteins measurement). Given this solid background, we sought to implement a prospective multicenter clinical registry aimed in investigate the impact of multiple and concomitant Anabolic Deficiencies (including therefore also GH and IGF-1 assessment, Testosterone, Insulin Resistance, Thyroid, etc.) on clinical status, exercise capacity, neurohormonal activation, left ventricle architecture and function, quality of life, hospitalization and mortality rate in patients affected by CHF: The T.O.S.C.A (Trattamento Ormonale nello Scompenso CArdiaco), whose preliminary results will be available within few months[89,90].

Interestingly, GH replacement therapy represents a fascinating future therapeutic option in CHF. Although the usefulness of GH replacement therapy in CHF must be still proven in a double-blind randomized controlled trial, this results shed new lights on the potential use of GH status assessment, not only in risk stratification and prognosis, but also as the target of innovative therapies, meeting all the needs of a biomarker as more close as possible to the “ideal” one[91].

3.1.3. D'Assante R*, Napoli R*, Salzano A, Pozza C, Marra AM, Arcopinto M, Perruolo G, Milano S, Formisano P, Saldamarco L, Cirillo P, Cittadini A. Human heart shifts from IGF-1 production to utilisation with chronic heart failure. Endocrine. Endocrine. 2019 Sep;65(3):714-716. doi: 10.1007/s12020-019-01993-y. Epub 2019 Jul 2 PMID: 31267324

Introduction

Chronic heart failure (CHF) is characterized by multiple hormonal and metabolic deficiencies (MHD) [151,152]. In this context, abnormalities of growth hormone (GH) and its effector insulin like growth factor-1 (IGF-1) play a distinct and relevant role [158,159]. Circulating levels of IGF-1 have been associated with cardiovascular events and mortality in CHF, but the benefit of the use of IGF-1 as prognostic marker in CHF is still unresolved [160]. Stimulation of IGF-1 receptors by the hormone can occur in response to the plasma circulating IGF-1 or to the local paracrine/autocrine production. Cardiomyocytes are able to produce IGF-1 mRNA and experimental conditions of heart hypertrophy are associated with an increase in such production, suggesting that modulation in local IGF-1 production might be an adaptive response to the need of local growth [161]. However, no data are available on the handling of IGF-1 by the human heart *in vivo* in healthy subjects or in patients with CHF. Thus, aim of the current study was to clarify whether human heart *in vivo* is an organ that generates or rather extracts IGF-1 and whether in patients with CHF there was a change in IGF-1 handling by the heart. For this purpose, we studied the difference in IGF-1 concentrations between aorta and coronary sinus in patients with or without CHF. In addition, we also evaluated inflammatory cytokine plasma glucose concentrations and their transcoronary concentration gradients (TCG).

Methods

Consecutive patients with or without clinical diagnosis of CHF and undergoing elective coronary angiography were recruited and enrolled in the study. CHF patients were affected by ischemic/non-ischemic CHF diagnosed according to the guidelines [147]. The patients without CHF (control group), were affected by factual or alleged stable coronary artery disease with no signs of CHF. Before any diagnostic procedure was performed, blood samples were simultaneously obtained from coronary sinus (CS) and ascending aorta (Ao) and collected in pre-chilled Vacutainer tubes containing sodium

citrate. After centrifugation, plasma samples were stored at -80°C until assayed. Plasma concentration of IGF-1 and cytokines (IL-1β, IL1-Rα, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-17, and TNFα) was measured by Bioplex-Technology. The relative TCG were calculated as the difference between CS and Ao concentrations. Therefore, a positive TCG would indicate net release of the hormone/cytokine from the heart, whereas a negative value would rather represent a net extraction by the heart. Categorical variables were expressed as counts and percentages. Normally distributed continuous variables were expressed as mean ± Standard Deviation whereas continuous data with skew distributions were expressed as the median and interquartile range [IQR]. Groups were compared using the Student *t*-test and Mann-Whitney U-Test as appropriate. A *p* value of <0.05 was considered statistically significant. Statistical analysis was performed using the R statistical programming environment, Version 3.5.

Results

Eleven CHF patients and ten non-HF subjects (CTR group) were enrolled in the study. Demographic and clinical characteristics of the study population are depicted in Table 1. No differences were observed between CHF and CTR group except for left ventricular ejection fraction (LVEF) (41.2±5.6 *vs* 56.5±4.7, p<0.001).

Compared to CTR patients, CHF patients showed higher, although non-statistically significant, IGF-1 Ao levels [91.6 (69.3-124.6) and 117.2 (84.8-151.1) ng/mL], whereas the CS concentrations were similar in the two groups [95.7 (68.8-130.7) and 94.3 (85.8-149.4) ng/mL, respectively). Therefore, whereas patients without CHF showed a positive TCG [12.9 (0-22) ng/mL], indicating net release of IGF-1 by the heart, patients with CHF were characterized by a negative TCG [-2.4 (-8.1-0.33) ng/mL] pointing to a net uptake of IGF-1 (p<0.05). In contrast with the heart of patients with CHF that showed a negative TCG and the need of extracting circulating IGF-1 for its functioning, CTR patients showed a TCG above zero, suggesting strongly that their heart is capable of producing much more IGF-1 than necessary locally.

Arterial and coronary concentration of all cytokines was within the normal range in the two groups. As a consequence, TCGs for all the above-mentioned cytokines were similar in CHF and CTR patients (data not shown).

Discussion

The present study investigated IGF-1 handling by the heart *in vivo* in humans. We show for the first time that there is a meaningful difference between patients with or without CHF in the way their heart uses IGF-1. Furthermore, we evaluated plasma concentration and TCG of several inflammatory biomarkers potentially involved in the progression of CHF.

Impaired tissue generation and a reduction in the circulating IGF-1 concentration have been involved in the loss of cardioprotective functions against ischemia and cellular aging. IGF-1 exerts a pro-survival effect by decreasing apoptosis and reducing ischemic damage, promotes an increase in the number of ventricular muscle cells, and induces the recruitment of cardiomyoblasts in the aging murine heart. In addition, IGF-1 enhances cardiac contractility by increasing the synthesis of proteins involved in the regulation of cardiac contractility and intracellular calcium concentration . Furthermore, IGF-1 acts by preserving endothelial function and nitric oxide-mediated vasodilation and promoting the re-endothelialization of the injured arteries through the mobilization of progenitor endothelial cells . IGF-1 effects on the heart might theoretically be due to the action of the circulating hormone on the IGF-1 receptors and/or to the stimulation of them by the locally produced IGF-1, the latter acting through paracrine/autocrine mechanisms.

We show for the first time in humans in vivo that the heart of subjects without CHF is a contributor to the circulating pool of IGF-1, exerting the role of a true endocrine organ. Since we did not measure coronary blood flow, we cannot exactly quantify the entity of such contribution or dissect the relative component of TCG, i.e. cardiac production from local consumption of IGF-1 (circulating or locally produced). However, since the concentration of IGF-1 in the CS exceeds the one in the arterial bed (positive TCG) the concept that the IGF-1 produced by the heart overcomes the amount of the hormone locally used appears undisputable. In patients with CHF, a substantial shift of the way the heart handles the IGF-1 is evident. The concentration of IGF-1 in the CS is lower than the concentration in the aorta, i.e. the TCG changes to negative, therefore, the heart subtracts IGF-1 from the circulating pool. For the reasons discussed above, we cannot establish whether the shift toward a negative TCG is due to an increase in the heart IGF-1 utilization or a decrease in its production or the combination of the two. What we can affirm is that the amount of IGF-1 locally used is higher than the amount produced. From experimental model and data *in vitro*, the presence of myocardial hypertrophy or frank HF associates with an increase in IGF-1 mRNA content, pointing from one side to an increased production of IGF-1, from the other side suggesting an increased need of the hormone by the derailing organ, in order to compensate the damage occurred after the cardiac

insult and/or promote the survival of unaffected cells [161]. We might speculate that CHF induces a local extreme need of IGF-1 so that the heart becomes a utilizer rather than a producer. In our own data, an increased cardiac production of IGF-1 is perfectly compatible with the shift from a positive to a negative TCG: the amount of IGF-1 taken-up by the heart largely exceeds the amount produced by the same organ, whatever it is.

Low circulating IGF-1 levels have been associated with systemic and local release of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) [162]. In addition, in experimental studies, IGF-1 gene transfection reduces the mRNA expression of IL-1β and TNF-α [163]. In our population, circulating inflammatory biomarkers and TCG were comparable in patients with or without CHF. This is not surprising since the patients studied were in stable clinical conditions.

In the context of CHF as MHD, hormonal replacement therapies are gaining increasing attention as possible adjuvant therapeutic approaches [164,165].

Our data show that the heart of patients with CHF requires much more IGF-1 than normal, suggesting that after the exogenous supply of GH, the increase of IGF-1 would fulfil a profound need of the failing organ. Therefore, a better understanding of cardiac handling of IGF-1 would substantiates such an approach and improve our knowledge of its pathophysiological relevance.

Table 1 Demographic, clinical characteristic, and plasma levels of IGF-1 of the patients studied

	CHF ($n = 11$)	CTR ($n = 10$)	p-value
Age (yrs)	66.5 ± 11-8	62.8 ± 6.9	0.38
Sex (M/F)	10/1	6/4	0.25
EF (%)	41.2 ± 5.6	56.5 ± 4.7	<0.001
BMI (kg/m^2)	27.6 ± 2.6	28.9 ± 2.3	0.30
SBP (mmHg)	140.5 ± 18.3	145.5 ± 18.7	0.56
DBP (mmHg)	83.0 ± 7.1	86.1 ± 7.0	0.35
HR (%)	62.7 ± 12.7	67.7 ± 10.8	0.39
eGFR (mL/min)	82.0 ± 48.2	104.4 ± 29.6	0.25
Hb (g/dL)	13.1 ± 1.8	13.7 ± 1.8	0.45
Ao IGF1 (ng/mL)	117.2 [84.8–151.1]	91.6 [69.3–124.6]	0.20
SC IGF1 (ng/mL)	94.3 [85.8–149.4]	95.7 [68.8–130.7]	0.47
TCG (ng/mL)	−2.4 [−8.1–0.33]	12.9 [0–22]	<0.05

CHF chronic heart failure, *CTR* control subjects, *EF* ejection fraction, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HR* heart rate, *EGFR* estimated glomerular filtration rate, *Hb* haemoglobin, *SC* coronary sinus, *Ao* aortic root

3.1.4. D'Assante R*, Arcopinto M*, Rengo G, Salzano A, Walser M, Gambino G, Monti MG, Bencivenga L, Marra AM, Aberg DN De Vincentiis C, Ballotta A, Bossone E, Isgaard J, Cittadini A. Myocardial expression of somatotropic axis, adrenergic and calcium handling genes in HFpEF and HFrEF. ESC Heart Failure. 2020 Sep (in press).

Introduction

In the last decades, a relevant piece of scientific literature has contributed to increase the knowledge of the molecular mechanisms underlying heart failure (HF) onset and progression enabled the identification of novel therapeutic targets. Mounting evidence supports the role of anabolic deficiencies in HF pathophysiology [166], a key player being the impairment of the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis [151,159], which molecular regulation still represents a huge gap in evidence. However, the vast majority of investigations reporting data on circulating levels of GH, IGF-1 or binding proteins have been conducted in HF with reduced ejection fraction (HFrEF), while limited evidence is available in the setting of HF with preserved ejection fraction (HFpEF). HF progression is also known to be influenced by various factors including the expression of proteins related to hyperactive adrenergic signaling and impaired myocardial contractility, which pharmacological modulation is part of HF therapeutic armamentarium. Increased G-protein-coupled receptor kinases (GRKs) levels/activity and reduced Sarco-Endoplasmic Reticulum Calcium ATPase (SERCA2) expression, widely implicated in HF-dependent beta-adrenergic receptor (ADRB) dysfunction and altered calcium homeostasis, play a pivotal role in HFrEF pathophysiology but their involvement in HFpEF remains undefined[167]. HFpEF appears a disease with multiple proposed pathophysiological mechanisms, though many remain speculative due to limited access to human heart tissue. Further, an additional limitation is that cardiac biopsies are commonly collected from end-stage HF patients, often treated with inotropic agents or placed on heart transplant waiting lists, and frequently without appropriate healthy controls.

Aims

Taking advantage of the availability of left ventricule (LV) cardiac biopsies obtained from stable chronic HF patients, the aim of the present study was to compare, for the first time to the best of our knowledge, cardiac expression levels of genes involved in the somatotropic axis regulation, adrenergic signaling and calcium handling, among HFrEF, HFpEF patients, and a control group, as reference population.

Methods

Thirteen HF patients undergoing elective cardiac surgery for valvular replacement, mitral valvuloplasty, aortic surgery or coronary artery bypass, assigned to HFrEF (LVEF≤40%; n=6) and HFpEF (LVEF≥50%; n=7) groups according to 2016 European Society of Cardiology Guidelines [147], were enrolled at the Department of Cardiac Surgery, IRCCS Policlinico San Donato, between 2014 and 2017. All HFrEF patients were suffering from ischemic heart disease compared with only one in the HFpEF group, in line with the well-known different etiologies of HFpEF and HFrEF, with ischemic etiology more prevalent in HFrEF than HFpEF. Moreover, all HFpEF patients presented diabetes (n=3) and hypertension (n=6) as major comorbidities. For the purpose of the study, patients with EF between 40-50% were intentionally excluded. Nine subjects with normal cardiac structure and function, without symptoms or sign of HF, undergoing thoracic surgery were recruited as healthy controls. At the time of enrollment, all participants underwent a complete clinical examination, including NYHA functional class assessment and trans-thoracic echocardiography, and peripheral blood samples were collected. Cardiac biopsies were obtained during surgical procedures by highly qualified and expert surgeons, following the maximum safety standards. Samples with a minimum size of 200 mm^3 were collected from the apical portion of the LV or the junctional portion between the free wall of the left ventricle and the interventricular septum (IVS). Each sample was taken by direct vision, through the aortic valve, and with the scalpel. No full tickness samples were taken and no biopsy needle was used. The techniques and the modalities were exactly the same adopted to remove the muscle spur in the context of obstructive hypertrophic cardiomyopathy. No histopathologic evaluation was performed.

Afterwards, each sample was transferred in a sterile tube containing RNA later (Qiagen) and stored at -80°C until assayed. Total RNA was extracted from cardiac biopsies through TRIREAGENT kit (Sigma). Real-Time PCR (TaqMan Gene Expression Assays) was performed using predesigned, TaqMan® Gene Expression Assays to evaluate the mRNA expression levels of genes involved in somatotropic axis regulation [IGF-1, IGF-1 receptor (IGF-1R), GH receptor (GHR)], adrenergic signaling (GRK2, GRK5, ADRB1 and ADRB2), myocardial calcium handling (SERCA2) and Tumor necrosis factor-alpha (TNF-α). Each sample was analysed in duplicate. The relative comparative C_T method was used to analyse the qPCR data (Sequence Detector User Bulletin #2, Applied Biosystems). In the C_T method, the amount of target normalized to an endogenous reference and relative to a calibrator sample is given by $2^{-\Delta\Delta CT}$. The amount of each transcript was normalized to the amount of the reference gene

PPIA (Cyclopheline A) expressed in the same sample. The calibrator is the same defined stock sample analysed in triplicates in each of the quantitative qPCRs. Thus, the values represent arbitrary but linear relative amounts of each transcript.

The distribution of the parameters was tested with the Kolmogorov-Smirnov test. The intergroup differences were tested with the one-way ANOVA, with Holm correction as appropriate. Correlations between IGF-1 circulating levels and IGF-1R mRNA were examined using Spearman rank-order correlation. A *p-value*<0.05 was considered statistically significant. Statistical analysis was performed using the R-statistical programming environment, Version 3.5.

The study was approved by the Ethical Committee (Protocol Number 2535) and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All participants gave written informed consent.

Results

No differences were observed in terms of age, sex, kidney function, and hemoglobin levels among the three study groups. Notably, HFrEF and HFpEF patients displayed comparable stable clinical status, as suggested by the similar NYHA functional class and serum BNP levels, with average values <100 pg/mL (Table 1). Further, both HFpEF and HFrEF patients showed reduced IGF-1 circulating levels, when compared to controls, with lowest values observed in HFrEF (160±13.2 ng/mL, 138±11.5 ng/mL, 102±35.6 ng/mL, p<0.001, respectively, *Figure 1A*). Interestingly, no differences in the local expression of IGF-1 mRNA were detected among the groups (*Figure 1B*). When compared to controls, a significant increased myocardial expression of IGF-1R was found in HFrEF (0.72±0.12, 1.52±0.9, p<0.05, *Figure 1C*), while a trend to increase, although not significant, was observed in HFpEF (*Figure 1C*). A negative and significant correlation between IGF-1 circulating levels and cardiac IGF-1R expression was found in controls, HFpEF and HFrEF patients (R=-0.79, p=0.01; R=-0.92, p>0.001; R=-0.85, p=0.03, respectively).

With regard to GHR, a significantly reduced expression was observed in HFrEF when compared to HFpEF (0.54±0.27, 0.94±0.25, p<0.05, *Figure 1D*). Further, HFrEF revealed a marked decrease in cardiac SERCA2 expression compared to controls and HFpEF (0.19±0.39, 0.87±0.32, 0.82±0.15, p<0.05, respectively, *Figure 1E*). Of note, no differences were observed between controls and HFpEF. With regard to adrenergic signaling, cardiac mRNA levels of GRK5 (1.32±0.70, 0.71±0.14, 0.77±0.15, p<0.05) and GRK2 (3.45±2.94, 0.93±0.12, 0.80±0.14, p<0.01) were significantly increased in HFrEF when compared to HFpEF and controls, respectively (*Figure 1F-G*). ADRB1 expression showed a trend

to decrease (~24%), although not significant, in HFrEF (0.66±0.4) when compared with controls (0.86±0.4) while ADRB2 expression levels were comparable between HFrEF (0.65±0.3) and controls (0.68±0.1). In HFpEF, the expression levels of ADRB1 (0.83±0.3) and ADRB2 (0.66±0.2) are comparable to that measured in healthy subjects (*Figure 1H-I*).

Finally, no changes in the local expression of TNF-α were detected among groups (*Figure 1L*).

Discussion

This is the first investigation dwelling upon the comparison of myocardial expression of the somatotropic axis, adrenergic signaling and calcium handling genes among HFrEF, HFpEF patients and healthy controls. HFrEF display significantly increased levels of GRK2 and GRK5, consistent with the well-known HF-dependent sympathetic nervous system overdrive, which becomes detrimental in the long-term and facilitates HF progression. Cardiomyocyte beta-adrenergic receptor hyperactivation represents a trigger for GRK upregulation which, in turn, activates the processes of receptor desensitization/downregulation, thus resulting in dysfunctional adrenergic signaling [168-170]. Moreover, the results obtained on beta-adrenergic receptors 1 and 2 expression in HFrEF are consistent with a previous report indicating that, in human biopsies from HFrEF patients, ADRB2 levels do not show any HF-related change, while a decrease in ADRB1 mRNA expression are evident only in patients with advanced NYHA class[171]. Indeed, our study population consisted of mildly symptomatic HF patients, mostly NYHA class II, under beta-blocker therapy, that may interfere with beta-adrenergic receptor expression and signaling.

Of relevance, similar molecular abnormalities were not detected in HFpEF, whose pathophysiology is more related to the presence of comorbidities and systemic inflammation, allowing to better understand the results of randomized clinical trials that failed to demonstrate a beneficial effect on survival exerted by beta-blockers in HFpEF[172].

While a vast magnitude of studies, conducted in both animal models and in human cardiac tissue of HFrEF patients, indicates that SERCA2a expression/activity is impaired in HF, leading to an altered cardiac calcium homeostasis and consequent cardiomyocyte reduced contractility, only one study had evaluated expression patterns of genes related to myocardial contractile function in HFpEF patients, selected with different criteria, and considering other genes[173]. With regard to SERCA2, our findings could contribute to better understand the differences in the contractile capacity between patients with HFrEF and HFpEF. Indeed, HFrEF displays a significant decrease in cardiac SERCA2 levels

compared with HFpEF, suggesting that calcium transients might be faster in HFpEF resulting in an increased calcium reuptake and preserved intracellular calcium sensitivity.

With regard to the GH/IGF-1 axis, our results indicate that HFrEF and HFpEF exhibit a different pattern of gene expression while circulating IGF-1 levels are equally reduced, regardless of EF. Indeed, when compared to HFpEF and healthy controls, HFrEF showed a significant decreased GHR and an increased IGF-1R expression. Such divergent behaviour of GHR and IGF-1 receptor mRNA in HF has been described in other pathological conditions, including diabetes and malnutrition, in which the two mRNAs are not coordinately regulated in all tissues studied[174]. Moreover, it has been also documented that HFrEF and HFpEF may show a different behaviour regarding the anabolic drive, altered in HFrEF and unmodified in HFpEF [152,175]. On the other hand, the increased cardiac expression of IGF-1R in HFrEF may reflect a compensatory mechanism in response to low circulating IGF-1 levels, in order to augment myocardial hormone uptake and the activation of downstream molecular pathways. These observations, as well as the local increase in IGF-1 mRNA and the negative correlation between IGF-1 circulating levels and IGF-1R mRNA, are in line with our recent evidence of myocardial increased utilization of IGF-1 in HFrEF [176]. HFpEF showed a different pattern with a mild reduction of IGF-1 circulating levels and non-significant changes in the myocardial expression of IGF-1, IGF-1R, and GHR mRNAs when compared to healthy controls. The similar cardiac expression of TNF-α found across the study groups confirms a mild-to-moderate HF status where the myocardial catabolic drive has not yet taken over the anabolic one [177].

Conclusions

This study further supports the hypothesis that distinct molecular patterns underlie HFrEF and HFpEF pathophysiology, extending previous knowledge to genes involved in somatotropic axis regulation, calcium handling, and adrenergic derangement in HF patients with stable clinical status.

Table 1. Clinical characteristics of subjects enrolled in the study.

Characteristics	**Values**			**ANOVA**		**Chisq**	
	CTRL (n=9)	**HFpEF (n=7)**	**HFrEF (n=6)**	**F**	***p***	**χ^2**	***p***
Age (Yrs)	66.8 ± 14.4	74.3 ± 10.6	64.2 ± 13.3	1.10	0.35		
Sex (M/F)	4/5	5/2	5/1			2.62	0.3
Weight (kg)	75.7 ± 15.9	74.6 ± 12.7	72.2 ± 6.6	0.1	0.9		
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.05	0.1	0.9		
BMI (kg/m^2)	27.1 ± 5.6	26.1 ± 3.7	25.4 ± 2.5	0.2	0.8		
Creatinine (mg/dl)	0.80 ± 0.1	1.1 ± 0.5	1.19 ± 0.8	1.16	0.33		
eGFR (ml/min)	82.2 ± 16.9	76.4 ± 32.8	78.0 ± 33.6	0.09	0.91		
Hemoglobin (g/dl)	13.1 ± 1.3	12.1 ± 1.5	12.8 ± 1.5	0.91	0.42		
BNP (pg/mL)	14.1 ± 5.3*;**	55.6 ± 27.4	77.5 ± 22.6	20.4	<0.001		
Ejection Fraction (%)	60.0 ± 6.6**	61.0 ± 11.4***	32.0 ± 7.8	23.41	<0.001		
NYHA Class (I/II/III)	-	2/4/1	1/4/1			0.3	0.9
Time from diagnosis	-	4 ± 2	5 ± 3				0.6
Diastolic Dysfunction§	5	7	6			7.1	0.03
Ischemic etiology	-	1	6			5.5	0.02
Beta-Blockers	4	5	5			1.1	0.5
Type/average dosage of Beta-Blockers§§	Bisoprolol (4) / 5.625 mg	Bisoprolol (4) / 6.25 mg	Bisoprolol (5) / 5.5 mg Carvedilol (1) / 12.5 mg bid				

		Carvedilol (1) / 12.5 mg bid					
Diuretics	0	1	4			6.1	0.048
ACE/ARBS	3	1	5			4.4	0.1
Calcium channel blockers	0	4	0			10.48	0.01
Antidiabetics	0	3	2			3.88	0.14
MRA	0	0	1			2.79	0.25
Statins	1	2	4			3.3	0.19
OAC/Aspirin	1	2	1			0.64	0.72
Type of cardiac surgery	Correction of ascending aorta aneurysm (n=9)	Correction of ascending aorta aneurysm (n=6); CABG (n=1)	Mitral valvuloplasty + CABG (n=2); Correction of ascending aorta aneurysm + aortic valve replacement (n=4)				

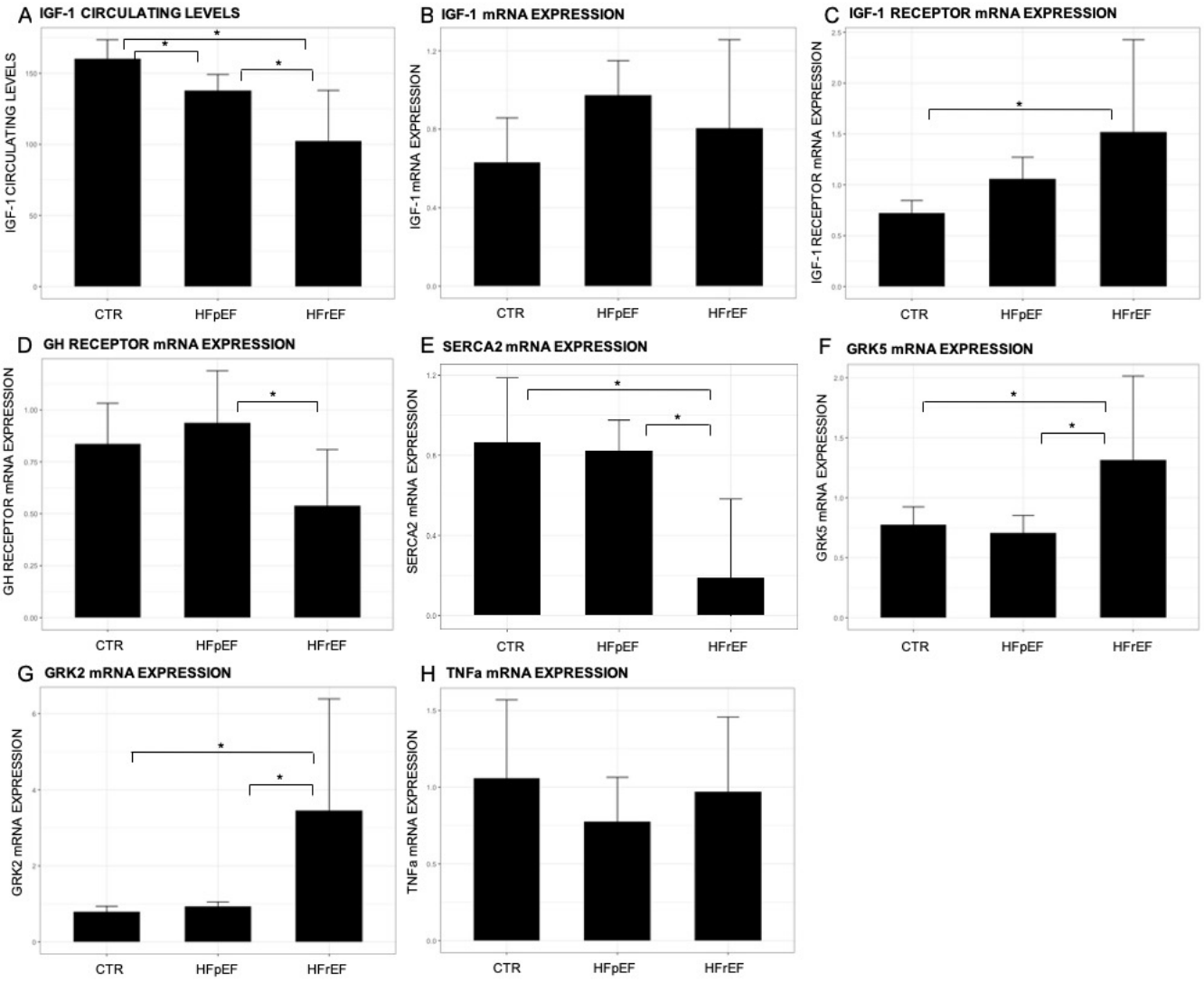
CTRL: non-HF-patients; HFrEF: Heart failure with reduced ejection fraction; HFpEF: heart failure with preserved ejection fraction; BMI: body mass index; EGFR: estimated glomerular filtration rate; BNP: brain natriuretic peptides; NYHA: New York Heart Association; MRA: Mineralocorticoid Receptor Antagonists; OAC: Oral Anticoagulant; CABG: Coronary Artery Bypass Graft.

§ Diastolic Dysfunction was established according to the ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC (Eur J Heart Fail. 2012 Aug;14(8):803-69. doi: 10.1093/eurjhf/hfs105).

§§Beta-blockers up-titrated to target dose.

*p value comparing CTRL *vs* HFpEF. ** p value comparing CTRL *vs* HFrEF. *** p value comparing HFpEF *vs* HFrEF.

Figure 1. mRNA expression of genes evaluated in cardiac biopsies in controls (CTR), CHF patients with preserved ejection fraction (HFpEF) or reduced ejection fraction (HFrEF). **1**A: IGF-I circulating levels. 1B: IGF-1 mRNA. 1C: IGF-IR mRNA. 1D: GHR. 1E: SERCA2. 1F: GRK5. 1G: GRK2. 1H: TNFa. *$p<0.05$.



3.2 Multiple Hormone and Metabolic Deficiency Syndrome

3.2.1 Salzano A§, Cittadini A, Bossone E, Suzuki T, Heaney LM. Multiple hormone deficiency syndrome: a novel topic in chronic heart failure. Future Sci OA. 2018 Apr 16;4(6):FSO311. doi: 10.4155/fsoa-2018-0041. eCollection 2018 Jul. PubMed. PMID: 30057788; PubMed Central PMCID: PMC6060397.

Heart failure (HF) is described as a clinical syndrome characterized by typical symptoms (e.g. ankle swelling, fatigue, dyspnoea) or signs (peripheral oedema, pulmonary crackles, or elevated jugular venous pressure), in which structural and/or functional cardiac abnormalities induce an impairment of cardiac output or an increase of intra-cardiac pressures at rest and/or during stress [1,2]. Importantly, due to different underlying aetiologies, demographics, co-morbidities, and response to therapies, the main terminology used to describe HF is based on measurement of left ventricle ejection fraction (EF). Classically, patients with normal EF (typically considered as ≥50%) are said to have HF with preserved EF (HFpEF), with those with reduced EF (typically considered as <40%) termed as HF with reduced EF (HFrEF). In the latest European Society of Cardiology (ESC) guidelines, cases where EF lies between 40–49%, previously considered as a 'grey area', are now defined as HF with mid-range EF (HFmEF) [1] .

Although in recent years overall mortality from cardiovascular disease has been reduced by about two-thirds, HF represents an exception to this rule, maintaining high levels of mortality that are known to be higher than those of many cancers [3].

This trend is not surprising if we consider the nature of the classical neurohormonal model that provides the pathophysiological basis for the natural progression of HF, resulting in the main target for drugs currently used in HF management (beta-blockers, ACE-I/ARBs/ARNI, and aldosterone receptor blockers). This model involves hyperactivity of neurohormonal pathways (the renin–angiotensin–aldosterone or the adrenergic system) that, although offering a compensatory role in early stages of disease, becomes a factor responsible for the worsening and progression of HF by triggering a cascade of further and deleterious increases in neurohormonal action. Moreover, it is well known that this model is present despite pharmacological interventions, via activation of alternative pathways (e.g. myocardial chimases) [178] .

For these reasons, researchers are looking for alternative models that could improve the understanding of the mechanisms underpinning HF progression. In particular, a growing body of evidence suggests that in addition to the increase of the pathways regulated by neurohormonal hyperactivity, the loss of equilibrium between the activation of these catabolic pathways and the impairment of anabolic hormonal axes depict the progression of the disease [48].

In this context, in 2009 Sacca' published an elegant review that first described the concept that HF could be considered as a multiple hormone deficiency syndrome (MHDS) [48]. Each component of MHDS (e.g. GH/IGF-1 axis, thyroid hormones, androgens, insulin resistance) is associated with impaired functional capacity and poor clinical outcome. Furthermore, prognosis has been shown to relate to the number of coexistent deficiencies [53]. This is not surprising considering the important relationship between hormones and the cardiovascular system [56,57,59,179].

Recently, initial investigations conducted by our group[180,181], demonstrated that not only the prevalence of hormonal deficiency is elevated in HF [63,182] and consequently related to poor cardiovascular performance and prognosis [63,182,183], but also that targeted hormone replacement therapy [51,65] leads to an improvement in cardiovascular performance and outcomes. These data provide strong evidence to suggest that the reversal of MHDS should be considered as an exciting and novel strategy in HF management [67,184].

Each deficiency is associated with reduced functional capacity and is a powerful and independent predictor of poor clinical outcome [48]. In particular, Arcopinto et al demonstrated that in a population of around 200 stable chronic HF patients, less than one fifth presented with no signs of hormonal deficiency [63]. Moreover, in patients with two or more deficiencies cardiovascular performance was impaired, as demonstrated by a decreased ability for maximal oxygen uptake (measures by a cardiopulmonary exercise test) and elevated levels of circulating NT-pro-BNP (a well-known marker of severity and prognosis in HF). Furthermore, the presence and number of deficiencies were related with a poor prognosis for all-cause mortality.

These data were in line with those presented by Jankowska et al [53], who demonstrated that multiple anabolic deficiencies occur frequently in HF (only 10% of the diseased population presented with no hormonal deficiency) and can predict long-term outcome. In particular, they found that reduced levels of multiple serum anabolic hormones (testosterone, DHEAS, and IGF-1) are strong markers of poor prognosis that are independent of conventional risk predictors. This reinforced the relationship

between the number of hormonal deficiencies and the subsequently profound impact on the progression of HF and associated outcomes.

Interestingly, Arcopinto et al [63] demonstrated that HF heavily impacted the age-related decline of anabolic hormones. Specifically, this decline was attenuated for DHEAS and IGF-1, with a paradoxical inversion observed for testosterone. This could suggest that the anabolic decline is age-independent and that the impact of MHDS could be more severe in young over elderly patients. This phenomenon could clearly have an impact on quality of life of patients diagnosed at an earlier age.

In 2016, a similar finding was demonstrated for the first time in HFpEF patients [182]. Despite in this group fewer patients presenting with reduced levels of MHDS compared to a HFrEF population, a remarkable prevalence of hormonal deficiency was noted. In particular, approximately 45% of the study population displayed two or more signs of hormone deficiency. However, the fact that there is a higher level of anabolic drive in HFpEF could further support the notion that HFpEF should be considered as distinct from HFrEF. Also for HFpEF, anabolic deficiencies could provide additional utility for prognostic biomarker investigations, as well as identifying hormonal systems for targeted and personalized treatment strategies.

Considering the importance of the findings on MHDS and HF to date, the T.O.S.CA registry is an important trial to further understand the implications of hormonal treatments on HF severity and progression[185]. Results from this prospective multicentre observational outcome-oriented study designed to evaluate the prevalence of MHDS and its impact on clinical outcomes in patients with chronic HF, are expected to provide important milestones surrounding this topic.

In conclusion, we recommend that screening for MHDS should be routinely performed in patients diagnosed with chronic HF, with a view to better characterize its impact on disease. This stance is taken due to the elevated prevalence of MHDS in HF and its impact on disease progression, coupled with preliminary data derived from small studies that show an improvement in cardiovascular performance and patient outcome following hormonal replacement therapy.

3.2.2. Bossone E, Arcopinto M, Iacoviello M, Triggiani V, Cacciatore F, Maiello C, Limongelli G, Masarone D, Perticone F, Sciacqua A, Perrone-Filardi P, Mancini A, Volterrani M, Vriz O, Castello R, Passantino A, Campo M, Modesti PA, De Giorgi A, Monte I, Puzzo A, Ballotta A, Caliendo L, D'Assante R, Marra AM, **Salzano A**, Suzuki T, Cittadini A; TOSCA Investigators. Multiple hormonal and metabolic deficiency syndrome in chronic heart failure: rationale, design, and demographic characteristics of the T.O.S.CA. Registry. **Intern Emerg Med**. 2018 Aug;13(5):661-671. doi: 10.1007/s11739-018-1844-8. Epub 2018 Apr 4. PubMed PMID: 29619769.

Introduction

Chronic Heart failure (CHF) is recognized worldwide as a major healthcare issue for its increasing prevalence and related extensive direct and indirect costs, and the ominous prognosis that is worse than that of many cancers [186]. HF pathophysiological underpinnings are represented by an excessive activation of ubiquitous pathways such as the sympathetic, renin-angiotensin-aldosterone, and cytokine systems, as the neuro-hormonal model dictates [3,187]. Besides such molecular pathways, an emerging body of evidence suggests the pivotal role played by the reduction of the anabolic drive, a common phenomenon in chronic diseases. Specifically, multiple hormonal deficiency syndrome (MHDS) encompasses several anabolic axes that are down-regulated in CHF: growth hormone (GH) and its tissue effector insulin-like growth factor-1 (IGF-1), thyroid hormones, anabolic steroids and insulin signaling [48,52,53,55,63,181,188]. MHDS does not appear to be a mere marker of disease progression, insofar as it is associated with impaired clinical status, functional capacity, and increased mortality [48,53,55,63,181,188].

A wide array of abnormalities of the GH/IGF-1 axis has been described in CHF ranging from decreased or normal activity, to overt GH resistance (i.e. high GH and low IGF-1 circulating levels) that may be present in different combinations according to the severity of heart disease [48,62,189]. GH Deficiency (GHD) affects about one-third of CHF patients and is equally associated with worse cardiac function, physical performance, and outcome [50,61]. Notably, preliminary studies have shown that correction of GHD leads to a remarkable and sustained improvement in cardiac and exercise performance both after 6 months of therapy [64] as well as at 4 years [65]. Reduced IGF-1 levels, the principal mediator of GH actions, are associated with increased cytokines and neurohormonal

activation, reduced skeletal muscle performance, endothelial dysfunction, and poor outcome [42,190,191].

The function of the thyroid gland is commonly impaired in CHF. Indeed, the "low triiodothyronine syndrome" (LT3S) is one of the most common thyroid abnormalities observed in CHF, characterized by reduced T3 levels and normal or near-normal thyroid-stimulating hormone (TSH) levels. This deficiency is due to reduced enzyme activity of 5'monodeiodinase responsible for converting thyroxine (T4) into T3 in peripheral tissues [192,193]. In CHF, LT3S prevalence is approximately 20-30% and strongly predicts patients mortality [194].

Interestingly, therapeutic correction of LT3S reduces sympathetic activation and circulating levels of N-terminal pro Brain Natriuretic Peptide (NT-proBNP) which commonly associate with improved prognosis [194].

Serum levels of free testosterone (FT) and dehydropianoandrosterone sulfate (DHEA-S) are equally decreased in male and female CHF patients [53], with their reduction associated to both CHF severity and prognosis [53]. Preliminary data from randomized trials of testosterone replacement therapy in men and women support its use as a promising therapeutic approach in CHF patients [195,196].

Insulin resistance (IR) – whether or not associated to type 2 diabetes mellitus (T2DM) – is another frequent finding among CHF patients, with a prevalence ranging from 33 to 70% [48,67]. The degree of IR is significantly related with worse clinical status and functional capacity, and poor outcome [197]. Experimental data, as well as preliminary clinical studies in insulin-resistant CHF patients, have shown that metformin prevents CHF progression and improves exercise capacity [188,198,199].

The prevalence, clinical implications and therapeutic options of anabolic deficiencies in CHF are summarized in Table 1.

Taken together, these abnormalities depict a scenario of hormonal/metabolic imbalance between anabolic drive and catabolic forces in CHF patients, with the latter prevailing over the former. Importantly, such reduction of the anabolic drive does not appear to be a mere epiphenomenon. Moreover, any component of this deranged anabolic pattern may negatively interact with the deficiency of other hormones (see Figure 1), as suggested by the additive effect on mortality of combined anabolic deficiencies [4]. However, the prevalence and diagnostic criteria of anabolic deficiencies in CHF have still not been established. Furthermore, the natural history of MHD and its impact on progression and outcomes in CHF patients need further investigation. For this reason, we hereby present the T.O.S.CA. (Trattamento Ormonale nello Scompenso CArdiaco; Hormone Therapy

in Heart Failure) Registry, a national multicenter initiative, designed to investigate the prognostic impact of MHD in CHF.

Methods

Study hypothesis

The T.O.S.CA. Registry seeks to test the hypothesis that the presence of pre-specified anabolic/metabolic deficiencies bears prognostic implications in a large population of mild-to-moderate CHF patients (Table 2).

Study design

The T.O.S.CA. Registry is a prospective multicenter observational outcome-oriented study designed to evaluate the prevalence of MHD and its impact on clinical outcomes in patients with CHF. The T.O.S.CA. Registry was initiated in April 2013 and includes participating centers (Endocrinology, Cardiology, and Internal Medicine Units with special interest in CHF management) situated throughout Italy (Figure 2).

The T.O.S.CA. Registry is designed to collect hormonal and clinical data of CHF patients who fulfil the following criteria: no history of current hormonal treatment, recent acute decompensation, acute coronary syndrome (< 6 months) or any other condition potentially impacting on hormonal balance: any relevant endocrine disease, active malignancy, severe liver disease (Cirrhosis CHILD B-C), and serum creatinine level > 2.5 mg/dL. All CHF patients with reduced ejection fraction (≤45%), on stable medications for at least three months including beta-blocker that had to be started at least 6 months before entering the study, that meet the inclusion/exclusion criteria are considered eligible. After enrollment, all subjects are followed as outpatients, as specified below.

The evaluation of circulating hormones includes measurements of thyroid hormones (TSH, free T_3, and free T_4), GH/IGF-1 axis, total testosterone, DHEA-S and insulin.

Serum hormones are analyzed in a dedicated core-lab (IRCSS-SDN, Naples, Italy) collaborating with the Clinical Coordinating Center. BNP and TMAO levels are also centrally evaluated in a laboratory (T.S.) Routine clinical chemistry and cardiovascular functional assessments are handled in the peripheral centers. Each procedure and blood sample collection is repeated annually for the duration

of the study. Intermediate visits are scheduled for clinical follow-up, therapy monitoring and to collect relevant clinical event information (mortality, hospitalization, see Table 2 and Table 3).
Hormone deficiencies are defined as follows:

- IGF-1 deficiency: serum IGF-1 levels lower than the 33rd percentile of a healthy control population: 122 ng/ml (age < 55 y); 109 ng/ml (55 y < age < 64.9 y); 102 ng/dl (65 y < age < 74.9 y); 99 ng/dl (age > 75 y) [63];
- Testosterone deficiency: serum Testosterone levels lower than 300 ng/dL [23];
- DHEA-S deficiency: serum DHEA-S levels lower than 80 µg/dL [23];
- Low T3: serum free T3 lower than 2 pg/mL (3.1 mmol/L) [20];
- Insulin-resistance: presence of type 2 diabetes mellitus or HomeOstasis Model Assessment (HOMA-Index) greater than 2.5 (according to the formula: IR=insulin (µU/mL) x glucose (mmol/L)/22.5) [197].

For the purpose of the study, patients are categorized into two groups: 1) patients with 1 anabolic deficiency or with no deficiency and 2) patients with 2 or more anabolic deficiencies.
No additional therapeutic interventions are administered to enrolled patients other than optimal medical therapy at the discretion of the prescribing physician.

Data collection

Following enrolment of a patient that meets the inclusion/exclusion criteria for the T.O.S.CA. Registry, the front pages of an electronic case report form (eCRF) are completed and submitted online. The eCRF collects clinical data and index events from enrolled patients. Data is updated on a web-based platform (URL: www.registrotosca.com).

To ensure anonymity and avoiding duplicate data, a unique identifier number (ID) is assigned to all patients. This system also facilitates data query and enables revision by local clinical investigators through personal log in and password credentials.

The data elements collected in the T.O.S.CA. Registry were originally developed and determined collegiately by the study Director (A.C.), representatives of each involved center (Figure 2), the CRO implementing the eCRF, and a statistician.
At the study entry, all patients undergo the following procedures:

1) Clinical examination and medical history;

2) Collection of anthropometric data and history of drug therapy
3) Serum and plasma samples, for routine clinical chemistry and research purpose;
4) Electrocardiogram (EKG);
5) Doppler Echocardiography;
6) Cardiopulmonary exercise stress testing (CETP);
7) 24-hour Holter EKG;
8) Six-minute walking test (6MWT);
9) Minnesota Living with Heart Failure Questionnaire (MLWHFQ).

Details are reported in Table 3. Data are acquired and submitted via a secure web site by the local investigators at each participating site. Local investigators are required to collect all data during hospitalization and periodically update the eCRF until the end of the follow-up phase.
All events (including death, cardiovascular events, admittance to emergency department, hospitalizations) are reviewed and collected either by telephone or in person at scheduled visits. All deaths are confirmed from medical records, the CHF clinic database or telephone interview with relatives of the patients. An independent endpoint committee adjudicates the endpoint. Each *ad interim* and *post-hoc* analysis will be performed only after the approval of the steering committee.
The study is conducted in accordance with Good Clinical Practice, Declaration of Helsinki 2002. The trial has been registered on Clinicaltrials.gov (NCT02335801).

Follow-up

The study duration is 5 years with a patient-average follow-up of 36 months. A complete work-up is performed at the study enrollment (see Table 3).
Aiming at capturing all planned and unplanned visits and clinical events, follow-up exercises are performed at 6, 12, 18, 24, 24, 30 and 36 months (Figure 3). Data and samples are obtained from patients at the participating sites. Sample collection and all procedures performed at baseline are repeated annually (Table 3); intermediate visits serve as clinical assessment and clinical event collection.

Sample size calculation

Sample size calculations are based on the 95% confidential intervals for estimates of the registry

primary endpoint (composite all-cause mortality or cardiovascular hospitalization). Data from 31 pre-existing cohort studies of unselected heart failure patients (six randomized clinical trials and 24 observational registries) reviewed in the Meta-analysis Global Group in Chronic Heart Failure (MAGGIC) report a 24% all-cause mortality during a median follow-up of 2.5 years [200]. Taking into consideration that: a) a conservative incidence of the primary endpoint during the median follow-up of 3 years of approximately 35% in the whole CHF population; b) the increase of composite of all-cause mortality or hospitalization by one-third in the subgroup classified as MHD (group 2) compared with the non-MHD subgroup (group 1); c) the ratio group 1/group 2 of about 0.5. [55,63]; and d) the probability of Type I Error (α) at 0.05, the power of the study (1 - β) at 0.80; the original calculated sample size is 456. A subsequent planned *interim* analysis was conducted after 18 months of follow-up and revealed a lower all-cause mortality rate than originally planned, ≈ 5% yearly, while the combined endpoint all-cause mortality and cardiovascular hospitalization yielded a similar event rate than originally calculated for all-cause mortality. It was therefore decided by the Steering Committee to modify the primary endpoint from all-cause mortality to the combined all-cause mortality and cardiovascular hospitalization. Of note, the observed event rate was congruent with more recent data on CHF patients [73,201].

Endpoints:

1. Primary endpoint**:** composite of all-cause mortality and cardiovascular hospitalization;
2. Secondary endpoint: composite of cardiovascular mortality and hospitalization, composite of all-cause mortality and hospitalization, and changes in peak VO2;
3. Tertiary endpoint: changes in LV ejection fraction, changes in LV volumes, changes in NT-proBNP levels, and changes in MLWHFQ score.

Results

From April 2013 to July 2017, a total of 526 patients were enrolled in the 19 centers distributed across all major geographical Italian regions (see figure 2). Early preliminary data are provided with regard to demographics, anthropometrics and main clinical characteristics at study entry (Table 4). At baseline, patients were aged (mean ± SD) 62.5 ± 12.2 years, 19% were female, and 84% were in New

York Heart Association (NYHA) class II-III with a left ventricular ejection fraction (LVEF) of 31.7 ± 7.4%. The aetiology of HF was ischemic in 52% of patients. The median [IQR] years of disease of the population was 9 [5-15] years.

Discussion

The total population available for data analysis is n=526, thus exceeding the pre-specified sample size needed for effective statistical analysis. The sample characteristics reflect the general epidemiology of the disease with a vast majority of male patients (81.1%) and a mean age of 62.5 years with ischemic etiology accounting for 52% of patients. NYHA class distribution can be considered as expected in a cohort of CHF outpatients (Table 4). Main demographic variables of TOSCA patients are in line with the most recent studies on heart failure [202,203], in particular, the ESC Heart Failure Long-Term Registry [202] (in which 7401 CHF patients from 21 EU countries were enrolled) has a similar mean age (66 vs 65 years, in ESC HF and TOSCA Registry, respectively) and sex distribution (71 vs 81% of males). Patients in our registry present with a more advanced clinical condition as highlighted by NYHA class distribution (III/IV class 25% vs 35%), difference in etiology (43 vs 52% post-ischemic), and more compromised LVEF (35 vs 31%). CHF-specific drug and device therapy in patients enrolled in the TOSCA Registry can be considered in line with EU patients in most cases, with renin–angiotensin system blockers therapy in 92.2 vs 85% of cases, in ESC HF and TOSCA Registry, respectively; beta-blockers in 92.7 vs 88.3%. Moreover, while there is a difference in percentage of ICD (23.6% in the ESC Registry vs 45.6% in TOSCA) a similar rate is observed in regard to CRT implants (12.7% vs 14.8%).

Cross-sectional data analysis will be performed in 2018 and carried out as follows:

1. Assessment of the prevalence of individual anabolic axis deficiency in the registry population;
2. Verification of the assumption that about 50% of patients present >2 anabolic deficiencies;
3. Cross-sectional analysis (functional data / hormonal data);

Following cross-sectional investigations, longitudinal analyses (survival analysis, hormonal influence on CHF progression rate) will be performed.

Future perspectives and research agenda

The T.O.S.CA. Registry results are expected to shed light on several pathophysiological and clinical features of CHF including:

1. Implementation of the current neuro-hormonal model with the characterization of novel, emerging hormone-metabolic pathways potentially related to the disease progression (*hormonal remodeling* hypothesis in CHF) [48]. This will be determined by testing the association between single and/or multiple hormone deficiency with known clinical and functional markers of clinical status and determining specific patterns of hormonal defects clusters in CHF;
2. Assessment of the prevalence of MHD and proposal of cut-off values for diagnosis of hormonal deficiency in heart failure to serve as a reference for future studies;
3. Description of the "natural history" of hormonal abnormalities in a representative sample of a mild-to-moderate CHF population;
4. Creating a large bio-bank as a valuable resource for future analysis. On the basis of preliminary analysis, other biomarkers can be assayed and new research hypotheses tested.
5. Collection of longitudinal data regarding echocardiography, measures of physical performance such as CPET, and biochemistry will help elucidating a wide array of the multifaceted CHF syndrome, insofar as most intervention studies are designed with a relatively short follow-up.

In conclusion, the T.O.S.CA Registry represents the most relevant observational trial on multiple hormonal and metabolic deficiency syndromes in CHF. The study findings will form the basis for generating hypotheses for future randomized clinical trials and might pave the way for promising single and multiple hormonal replacement therapy in CHF. In this context, the Registry Director, Prof. Antonio Cittadini, has been recently awarded with the 2016 GGI International Award, proposing a double-blind study of GH replacement therapy in CHF patients with coexisting GH deficiency.

Table 1 Prevalence and clinical associations of hormonal deficiencies and effects of hormonal therapies

Hormonal status	Observational studies			Interventional studies			Survival benefit
	Prevalence (%)	Prognostic information	Type of study	Acute administration	Chronic administration	Type of study	
GH deficiency	30–40	Low IGF-1 is associated with reduced muscle strength and increased neurohormonal activation Low IGF-1 predicts all-cause mortality GH deficiency is associated with poor functional status and increased mortality	PR, CS OB, PR PR, CS	Reversal of endothelial dysfunction Enhanced LV contractility	Improved exercise tolerance Reverse LV modeling	SB, RCT	Unknown
Testosterone deficiency	20–25	Associated with reduced exercise tolerance	PR, CS	Increased Cardiac Output	Improved exercise tolerance, insulin sensitivity and muscle strength	DB, PC, RCT	Unknown
Low T_3 syndrome	13–30	Associated with increased all-cause mortality	OB, PR	Neurohormonal deactivation	Increased cardiac output	PC, RCT	Unknown
Insulin resistance	30–35	Associated with severity of Heart failure symptoms, reduced functional capacity, and poor survival	PR, CS		Improved exercise tolerance (metformin) Improved exercise tolerance and LV ejection fraction (GLP-1 agonist)	DB, PC, RCT	Unknown

GH growth hormone, *IGF-1* insulin-like growth factor-1, *LV* left ventricle, *HF* heart failure, *GLP-1* glucagon-like peptide-1, *CS* cross-sectional study, *PR* prospective study, *OB* observational study, *SB* single blind, *DB* double blind, *PC* placebo-controlled, *RCT* randomized controlled trial

(Adapted from: Arcopinto et al.'s hormone replacement therapy in heart failure Current Opinion in Cardiology 2014 [15])

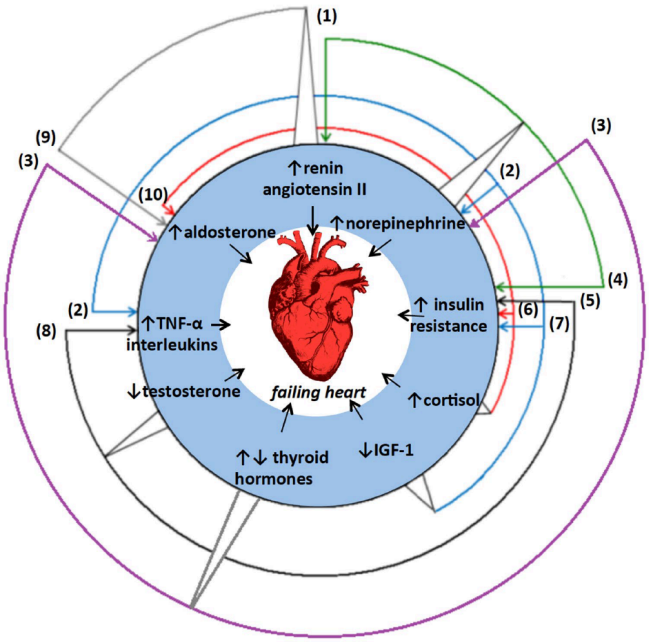


Fig. 1 Cross-talk among hormonal and metabolic pathways in CHF. (1) The insulin antagonistic effect of catecholamines. Epinephrine contributes to muscle insulin resistance [27]. (2) Lower IGF-1 levels are associated with higher levels of tumor necrosis factor-alpha, noradrenaline, and adrenaline [12]. (3) Infusion of T_3 decreases the circulating levels of key neurohormones such as norepinephrine and aldosterone [18]. (4) Reduced norepinephrine reuptake is associated with regional insulin resistance at cardiac level [28]. (5) Testosterone treatment in patients with moderate-to-severe heart failure improved insulin resistance [21]. (6) The net effect of the GH/IGF-1 status on insulin resistance depends on GH to IGF-1 balance, nutritional conditions, and, in case of GH therapy, acute or chronic administration [29]. (7) Testosterone may suppress the expression of the pro-inflammatory cytokines TNFα, IL-1β, and IL-6, IL-9 and potentiate the expression of the anti-inflammatory cytokine IL-10 [30]. (8) High cortisol levels exert a mineralocorticoid-like activity for the dysfunction of 11-hydroxysteroid dehydrogenase type 2 enzyme in cardiomyocytes of the failing heart [31]. (Adapted from: Arcopinto et al. [32])

Table 2 Main outcome measures and endpoints in the T.O.S.CA. Registry

Outcome	Details
Clinical events	All-cause mortality
	Cardiovascular mortality
	Hospitalization
	Cardiovascular Hospitalization
Disease progression	Changes in peak VO_2
	Changes in LV ejection fraction
	Changes in LV volumes
	Changes in NT-proBNP levels
	Changes in MLWHFQ score
Primary outcome	Composite of all-cause mortality and cardiovascular hospitalization
Secondary outcomes	Composite of cardiovascular mortality and hospitalization; composite of all-cause mortality and hospitalization; changes in peak VO_2
Tertiary outcomes	Changes in LV ejection fraction; changes in LV volumes; changes in NT-proBNP levels; changes in MLWHFQ score

LV left ventricle, *MLWHFQ* Minnesota Living with Heart Failure Questionnaire, *NT-proBNP* N-terminal prohormone brain natriuretic peptide, *peak* VO_2 peak oxygen consumption

Fig. 2 T.O.S.CA. Network

Table 3 Assessment of patients enrolled in the T.O.S.CA. Registry

Planned work-up for enrolled patients at baseline and annually follow-up	
Clinical data	Anthropometrics Body surface area CHF etiology* NYHA class Year of diagnosis* Medical/device therapy Comorbidity
Electrocardiography	Atrial fibrillation (yes/no) Left bundle branch block (yes/no) Pacemaker (yes/no)
Biochemistry	Electrolytes: K^+, Na^+, Ca^{++}, P Coagulation parameters Creatinine, BUN Bilirubin CPK, LDH, AST, ALT, ALP, uric acid Glycaemia, LDL, HDL and total cholesterol, triglycerides Serum proteins Complete blood cell count
Doppler Echocardiography	Interventricular septum (end-systole/end-diastole) Posterior wall (end-systole/end-diastole) Left ventricular diameter (end-systole/end-diastole) Left ventricular outflow tract Right ventricular outflow tract velocity time integral Pulmonary artery acceleration time Left ventricular volume (end-systole/end-diastole) Left atrial volume Right ventricular diastolic diameter Right atrial volume Tricuspid annular plane systolic excursion (TAPSE) Mitral E velocity Mitral A velocity Mitral deceleration time Tissue Doppler imaging: E′, A′, S′ Tricuspid regurgitation velocity peak Mitral regurgitation grading Aortic regurgitation grading Left ventricular outflow tract velocity time integral Aortic valve max and mean gradient Inferior vena cava diameter and collapse
24-h Holter EKG	Heart rhythm (sinus rhythm/atrial fibrillation/PMK) Mean, min, and max HR Supraventricular ectopic beats (*n*) Ventricular ectopic beats (*n*, Lown class)
Cardiopulmonary exercise stress testing	VO_2 at anaerobic threshold Workload at anaerobic threshold HR at anaerobic threshold VO_2 at peak exercise Workload at peak exercise SBP, DBP, HR at peak exercise VE/VCO_2 slope Respiratory exchange ratio (RER) Heart rate reserve Heart rate at 2 min recovery Exercise duration
Quality of life	Minnesota Living with Heart Failure Questionnaire (21 items)
6-min walking test	Total distance Baseline and end-test Borg Dyspnea Scale score Baseline and end-test SBP, DBP, HR, SpO_2
Hormonal evaluations (centralized)	Serum IGF-1 Serum total testosterone Serum DHEA-S Serum insulin

BUN blood urea nitrogen, *CHF* chronic heart failure, *CPK* creatinine phosphokinase, *DBP* diastolic blood pressure, *DHEA-S* dehydroepiandrosterone sulfate, *SBP* systolic blood pressure, *SpO_2* peripheral capillary oxygen saturation, *IGF-1* insulin growth factor-1, *NYHA* New York Heart Association, *PMK* pacemaker, *VE/VCO_2slope* The minute ventilation–carbon dioxide production relationship

*At baseline only

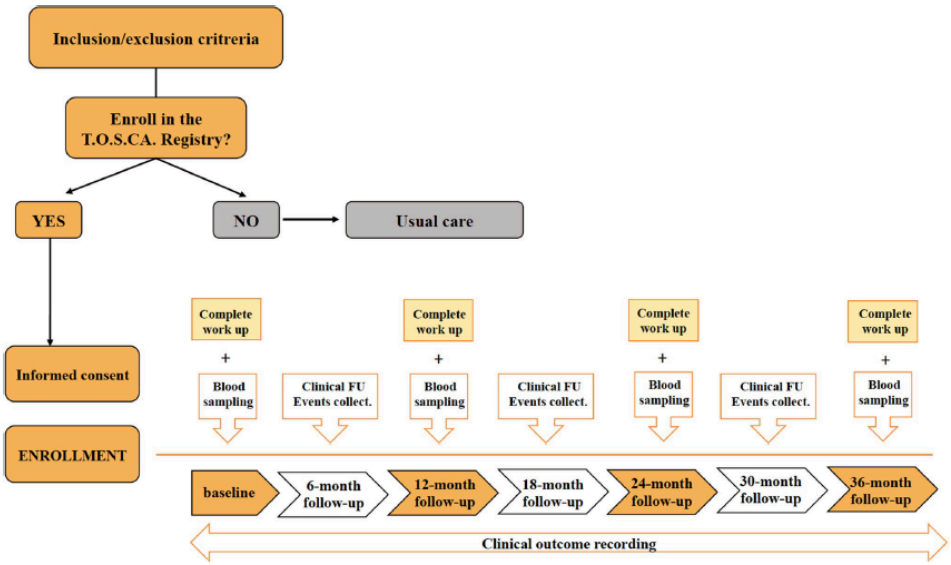


Fig. 3 Timetable of the T.O.S.CA. Registry

Table 4 Preliminary clinical characteristics of the population at baseline

Variables	Cohort ($n=526$)
Age (year)	62.5 ± 12.2
Sex (% male)	81.1%
NYHA (% I/II/III/IV)	13/54/30/3
Aetiology (% ischemic)	52
Year of disease*	9 (5–15)
Systolic blood pressure (mm hg)	120 ± 16.6
Diastolic blood pressure (mm hg)	75 ± 10.2
BMI (kg/m^2)	28.5 ± 5.3
eGFR (CKD-EPI formula, ml/min)	86.8 ± 40.1
NT pro BNP (pg/ml)*	950.5 (328.5–2758)
Left ventricular EF (%)	31.7 ± 7.4
Atrial fibrillation (%)	20
ICD (%)	45.8
CRT (%)	14.6
Medication (%)	
B-blocker	88.3
ACE-I/ARBs	85.0
MRA	49
Diuretics	74.7
Amiodarone	20.6
Digoxin	10.6
Antiplatelets	59.9
Antithrombotics	28.4
Lipid-lowering medications	60.5
Ivabradin	15.1
Antidiabetics	18
Insulin	14

Data expressed as mean ± SD

NYHA New York Heart Association, *BMI* body mass index, *eGFR* estimated glomerular filtration rate. *NT-proBNP* N-terminal prohormone brain natriuretic peptide, *EF* left ventricular ejection fraction, *ICD* implantable cardioverter defibrillator, *CRT* cardiac resynchronization therapy

*Data expressed as median (interquartile range)

3.2.3 Cittadini A*, **Salzano A***, Iacoviello M, Triggiani V, Rengo G, Cacciatore F, Maiello C, Limongelli G, Masarone D, Perticone F, Cimellaro A, Perrone Filardi P, Paolillo S, Mancini A, Volterrani M, Vriz O, Castello R, Passantino A, Campo M, Modesti PA, De Giorgi A, Monte IP, Puzzo A, Ballotta A, D'Assante R, Marra AM, Arcopinto M, Gargiulo P, Bruzzese D, Colao A, Napoli R, Suzuki T, Eagle KA, Ventura HO, Bossone E. On behalf of the T.O.S.CA. Investigators. 'Multiple Hormonal and Metabolic Deficiency Syndrome predicts outcomes in heart failure. The T.O.S.CA. Registry' (unpublished data, under review)

Introduction

Chronic Heart failure (CHF) is a major healthcare issue with increasing prevalence, huge estimated cost, and poor prognosis, still approaching a 5-year mortality of 50%.[145,146] Given such disheartening statistics, an imperative need is to search for novel therapeutic approaches capable of slowing disease progression and improving survival.[147,148] In this regard, the neurohormonal model, conjecturing CHF pathophysiology as sustained by an excessive activation of numerous pathways, including the sympathetic, renin-angiotensin-aldosterone, and cytokine systems, provided a theoretical framework supporting the actual therapy.[147,148] Such an approach, although partially successful, has not fulfilled all the promises, and CHF prognosis remains modest.[145,146] However, to complement the paradigm of neurohormonal activation, a concomitant reduction of anabolic hormonal axes seems to potentially play an important role in CHF progression and prognosis.[149] Specifically, the so-called multiple hormonal deficiency syndrome (MHDS) encompasses several anabolic systems that are down-regulated or impaired in CHF: the somatotropic axis [including growth hormone (GH) and its tissue effector insulin-like growth factor-1 (IGF-1)], anabolic steroids (testosterone and DHEA-S), and thyroid hormones.[149-153] Notably, MHDS does not appear to be a mere marker of disease progression, insofar as each defect is associated with impaired clinical status, functional capacity, and increased mortality.[149-154] In addition, insulin resistance (IR), with or without coexisting overt hyperglycaemia and type 2 diabetes (T2D), appears to have an independent role in the pathogenesis and prognosis of CHF.[155] Indeed, it has been showed that T2D prevalence in HF is much higher than in the general population, with a strong impact on HF prognosis, suggesting that a negative role is played by hyperglycaemia and/or IR.[156] However, although IR and T2D should be therefore considered among the defects of the hormonal anabolic axes (i.e. expression of a down-regulation of insulin action), IR and T2D have never been analysed in the context of MHDS. Indeed,

in previous preliminary report investigating the impact of HD in CHF, the role of IR has been ignored, and the effect of T2D disregarded. Furthermore, promising results observed in recent trials investigating the effect of antidiabetic drugs (i.e. gliflozins) support the idea that hormonal and metabolic players might be involved in the progression of CHF.[157] Despite these premises, to date no large study has focused on the relative role played by MHDS in the progression and survival of patients with CHF. In particular, no registry-based study has been specifically designed to evaluate the overall impact of hormone deficiencies (HD, comprises insulin resistance and T2D) on CHF morbidity and mortality.

Aims of the T.O.S.CA. (Trattamento Ormonale nello Scompenso CArdiaco; Hormone Treatment in Heart Failure, NCT02335801) Registry were to investigate, for the first time in a prospective multicentre observational registry, the prevalence, the clinical significance, and the prognostic impact of HD and diabetes, alone or combined (i.e. MHDS) in CHF.[165]

Methods

Study population

Study design and population baseline characteristics have been previously described.[165] In brief, the T.O.S.CA. Registry is a prospective multicentre observational study recruiting consecutive stable CHF patients with left ventricular ejection fraction (LVEF) ≤45%, without history of severe liver and/or kidney disease or active malignancy, on stable medications for at least 3 months, including any beta-blocker (started at least 6 months before entering the study). The study protocol was approved by the Ethics Committees of all participating centres and all patients gave written informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Study outcomes

The primary endpoint was a composite of all-cause mortality or cardiovascular hospitalization. Information regarding clinical outcome was obtained directly from patients or their relatives and an independent endpoint committee adjudicated the outcome. Secondary endpoint was the change in maximal oxygen consumption (peak VO_2).

Study procedures and statistical plan

Study procedures, previously described,[165] are available as supplementary material. In brief, blood samples were collected by venepuncture after overnight fast. To obtain serum and plasma, samples were centrifuged within 30 minutes, frozen, and stored at -80° C until assayed. Brain natriuretic peptide levels were assessed using a point-of-care device (RapidPIA™, Sekisui Medical Co, Tokyo, Japan) in a dedicated core-lab (John and Lucille van Geest Biomarker Facility, University of Leicester, UK). Single HD were defined as described in Table 1. Patients were divided in two groups: (1) patients with one or no hormone deficiency (NO-MHDS) and (2) patients with two or more hormone deficiencies (MHDS). All analyses were performed in the entire population and in the subgroup of patients without T2D. The effect of T2D per se on the primary endpoint was also evaluated.

Statistical analysis

The sample size calculation determined that 456 patients would provide the study with 80% power to detect a difference of the composite of all-cause mortality or CV hospitalization between the subgroup MHDS compared with the NO-MHDS, considering a conservative incidence of the primary endpoint during the median follow-up of 3 years of approximately 35% in the overall cohort. Notably, the observed event rate in our patient population was congruent with data from literature.

Normally distributed continuous variables were expressed as mean±standard deviation (SD), whereas continuous data with skewed distributions were expressed as median [interquartile range (IQR)]. Categorical variables were expressed as counts and percentages. The distribution of the variables was tested with Kolmogorov-Smirnov test. Patients for whom data regarding were not available, were excluded from the analysis.

The association between analysed variables and survival were established by using Cox proportional hazard regression analyses. Both univariate and multivariable linear models were used to assess potential predictors of survival.

Cox proportional-hazards model was also carried out to estimate the Akaike Information Criterion (AIC) for each HD and set up a model useful to find relevant interaction between HD and covariates.

With regard to anabolic status, we considered single HD, the sum of HD detected (from 0= no deficiencies to ≥3 deficiencies) or the presence of a MHDS.

For the multivariable analyses, established predictors of poor outcome in HF were employed as variables (i.e. age, sex, aetiology, NYHA class, LVEF, and BNP). Two different models were used to

test the primary endpoint: (i) model 1: diagnosis of MHDS (ii) model 2: patients stratified for number of HD. Results were as HR with 95% confidence intervals for a unit increase in the predictor value.

Kaplan-Meier curves for cumulative survival were constructed to assess the impact of severity of anabolic deficiency on endpoints. Differences in event rates between the groups were compared with the Cox-Mantel log-rank test. Schoenfeld residual test was performed to test the proportional hazards assumption for each covariate included in the Cox regression model.

No patient was lost to follow up.

Statistical analysis was performed using R version 3.0 (http: //www.r-project.org) and SPSS version 25.0 (SPSS Inc, Chicago, Illinois, USA). A value of $p<0.05$ was considered statistically significant.

Results

Study population

From the original cohort of 526 patients enrolled in 19 participating centres from April 2013 to July 2017, [14] complete hormonal data were available for 480 patients, who were used for the present analysis. Demographic characteristics at baseline of the final cohort are described in Table 2. There were no significant differences between patients included and not included in the present analysis (data not shown). Overall, MHDS (considering T2D or Homeostasic model assessment of insulin resistance - HOMA-IR- as one combined deficiency) was diagnosed in 358 patients (74·6%). Specifically, 5·8% displayed no HD, 19·6% only 1 HD, 32·9% 2 HD, 27·9% 3 HD, and 13·8% 4 or more HD. When patients with T2D were excluded from the analysis, among the remaining 360 patients, 8% displayed no HD, 22% only 1 HD, 34% 2 HD, 27% 3 HD, and 10% 4 or more HD.

Primary endpoint.

At the end of the follow-up (patient-average follow-up of 36 months), in the entire population, 271 patients (56%) experienced the primary endpoint (97 deaths and 174 cardiovascular hospitalisations). As shown in Fig. 1, panel A, 62% of patients in the MHDS group (221 events: 82 deaths and 139 cardiovascular hospitalisations), compared with only 41% of patients in the NO-MHDS group (50 events: 15 deaths and 35 cardiovascular hospitalisations), reached the primary endpoint, resulting in a 50% increase of patients with an event in the MHDS group ($p<0·001$). With regard to T2D patients, primary endpoint was reached in 70% of the patients with diabetes and 52% in the

patients without diabetes (p=0·001). When the 120 patients with diabetes were excluded from the analysis, 34% of patients in the NO-MHDS group (36 events: 11 deaths and 25 cardiovascular hospitalisations) and 60% of patients in the MHDS group (151 events: 52 deaths and 99 cardiovascular hospitalisations) experienced the primary endpoint (p<0·001), with an increased risk of outcome of more than 75% in the MHDS.

Prognosis

Single predictors

In the univariate Cox proportional hazard regression analyses (Figure 2), the following variables were associated with mortality and cardiovascular hospitalisation: age, advanced NYHA classes, LVEF, BNP, and anaemia. With regard to specific HD, Testosterone deficiency (TD) [1·59 (1·25-2·01), p<0·001], DHEAS deficiency (DHEAS-D) [1·40 (1·07-1·81), p=0·011], IGF-1 deficit [1·43 (1·13-1·82), p=0·003], and IR impairment [i.e. patients with abnormal HOMA index or T2D - 1·34 (1·03-1·73), p=0·03] were associated with the primary outcome. Further, when the analysis was performed separately for the presence of HOMA-IR or diabetes, only T2D was associated with the primary endpoint [1·55 (1·20-2·01), p<0·001], whereas HOMA-IR alone, did not show an increased risk of mortality or cardiovascular hospitalization [0·94 (0·74-1·2), p=0·064]. The number of HD [1·35 (1·21-1·52), p<0·001] as well as the presence of MHDS [2·09 (1·52-2·88), p<0·001] were associated with primary outcome. In particular, MHDS identified a group of patients with both higher mortality [2·2 (1·28-3·83), p=0·01] and cardiovascular hospitalisation [1·81 (1·29-2·54), p=0·001] (Figure 1, B-C). When the analysis was performed without the diabetic patients, MHDS was still associated with an increased risk of primary endpoint [HR 2·15 (1·49-3·09), p<0·01)].

Kaplan-Meier analysis performed across HD numbers showed that patients without HD had the best survival rate when compared with those with 1, 2, and ≥3 HD, with a graded relation between event rate and number of HD (Figure 3). Similar results were obtained when diabetic patients were excluded from the analysis: the presence of increasing number of HD was associated with an increased risk of primary outcome [1 HD: HR 1·43 (0·65-3·13), p=0·37); 2 HD: HR 2·53 (1·22-5·27), p<0·01; HD ≥3: HR 3·08 (1·49-6·38), p<0·002)].

Multivariate Analysis

MHDS

In the multivariable Cox proportional hazard regression analyses for model 1-full model (Figure 4A), the presence of MHDS was significantly associated with the primary endpoint when

adjusted for age, sex, NYHA class, aetiology, LVEF, BNP, and the presence of obesity, impaired eGFR, atrial fibrillation, and anaemia [1·74 (1·21-2·5), p=0·003]. In this model, also sex [1·47 (1·01-2·15), p=0·045], age [1·02 (1·00-1·03), p=0·023], and BNP [1·17 (1·04-1·31), p=0·007] were significantly associated with the outcome. In the multivariable Cox proportional hazard regression analyses for model 2-simplified (Figure 4B), the presence of MHDS was significantly associated with the primary endpoint when adjusted for age, sex, NYHA class, aetiology, LVEF, and BNP [1·93 (1·37-2·73), p<0·001]. When T2D patients were excluded, MHDS remained strongly and significantly associated with the primary endpoint [1·95 (1·31-2·90), p<0·001]. The presence of T2D was also significantly associated with the primary endpoint when adjusted for age, sex, NYHA class, aetiology, LVEF, and BNP [1·4 (1·05-1·85), p=0·02].

Schoenfeld residual test confirmed the proportional hazards assumption for each covariate included in the Cox regression model (χ^2=17·54, p=0·1).

Number of HD

With regard to model 3 (Figure 4C), the number of HD detected was significantly associated with outcome even when adjusted for age, sex, NYHA class, aetiology, LVEF, and BNP [1·28 (1·13-1·43), p<0·001]. When diabetic patients were excluded, the presence of increasing number of HD remained associated with an increased risk of primary outcome [1 HD: HR 1·72 (0·71-4·22), p=0·23); 2 HD: HR 2·85 (1·23-6·62), p<0·01; HD ≥3: HR 3·1 (1·33-7·23), p<0·01)].

Prognosis related to specific HD

Association between the presence of a specific single HD at baseline and outcomes was investigated. Kaplan-Meier analyses were performed across TD, DHEA-S D, low IGF-1, HOMA-IR, T2D, and low T3 for composite endpoint from enrolment (Supplementary Figure 1 and Supplementary figure 2).

The presence of TD, DHEAS-D, low IGF-1, or T2D, were independently associated with outcome (p<0·001, p=0·005, p<0·001, and p<0·001 respectively). Patients without HD had the best survival rate when compared with those with HD. Low T3 syndrome was not significantly associated with the primary endpoint. However, despite the small number of patients with this hormone defect and the consequent relatively low event rate, in a sub-analysis performed up to 30 months there was a significant association with the composite of all-cause mortality and cardiovascular hospitalisation

(p=0·02). No interactions were found between the five HD considered, suggesting a similar role of each HD in determining the primary endpoint.

Cardiopulmonary performance and echocardiographic findings.

At baseline, patients with MHDS displayed an impaired cardiopulmonary performance compared with NO-MHDS patients, as shown by significantly lower peak VO_2 (15·5±3·8 and 17·7±4·5 mL/Kg/min, respectively, p<0·01) and VO_2 at threshold (10·2±2·8 and 12·1±3·2 mL/Kg/min, respectively, p<0·01). With regard to the echocardiographic findings, no differences were found with regards to LV morphology, neither systolic nor diastolic function (LVEF 32·3±6·9 and 32·1±7·3%; LV End Diastolic diameter 63·5±8·5 and 62·3±8·5 mm; LV End Diastolic Volume indexed 97·2±33·2 and 96·3±34·3 ml/m^2, in MHDS and NO-MHDS, respectively).

With regard to changes in peak VO_2 during follow up, data available showed that MHDS patients displayed a more severe impairment when compared to NO-MHDS patients (delta changes: -4·5±0·3 vs -2·1±0·2 respectively, p=0·001).

Discussion

In the present study, the first prospective registry specifically designed to investigate the role played by HD and diabetes on prognosis and survival of HF patient with EF≤ 45%, we document several novel findings: 1) the prevalence of HD in CHF is very high, exceeding 90%. Specifically, more than two thirds of patients are characterized by two or more concomitant HD (i.e. multiple hormone and metabolic deficiency syndrome (MHDS; 2) the presence of MHDS identifies a group of patients at increased risk of mortality or cardiovascular hospitalization 3) there is a graded relation between the number of HD and total events, suggesting a possible causal role of MHDS in CHF; 4) roughly two thirds of the patients with CHF are characterized by abnormal insulin action, either insulin resistance (IR) or overt type 2 diabetes (T2D); 5) T2D, but not IR, exerts a negative role on CHF survival and prognosis.

Hormone deficiencies and heart failure

Recently, the new concept that CHF progression is associated with both the overactivity of adrenergic/renin-angiotensin-aldosterone/cytokine systems and the downregulation/impairment of

hormone activities has emerged.[149] Such a pathophysiological model stems from several independent studies documenting that isolated HD (i.e. adrenal, somatotropic, gonadal, thyroid, and insulin axes) were associated with the impairment of clinical status and physical performance, and with poor survival.[150,154,155,166,204,205] However, data available in the literature are scattered, lacking an overall view of the phenomenon. In addition, even when the effects of the combination of hormone deficiencies have been studied in smaller cohorts, IR or diabetes have been disregarded.[150] The T.O.S.CA. registry overcomes this limitation, adding valuable information in the topic, in view of the even more emerging role of IR or diabetes as relevant players in the pathogenesis of CHF.[157]

The T.O.S.CA. registry shows that more than 90% of the patients with CHF have at least 1 HD. Consistently with previous results, the presence of TD, DHEAS-D, or IGF-1 deficiency were independently associated with poor outcome. Specifically, the role played by each component of the somatotropic axis (IGF-1 and GH) has been extensively studied during the last 20 years, with several independent groups reporting lower circulating IGF-1 levels associated with worst NYHA class, impaired exercise capacity, sarcopenia, and increased inflammatory activation.[162,176,206] GH deficiency is also associated with impaired clinical status, LV remodelling, RV dysfunction, and increased mortality.[166] Likewise, TD is associated with significant impairment of skeletal muscle function and exercise capacity,[207-209] as well as a dysregulation of metabolic profiles, with increased risk of metabolic syndrome and new onset of T2D.[210] Similarly, low T3 syndrome has been associated with worse cardiovascular performance and increased mortality in CHF,[204] and restoring circulating thyroid hormone levels in low T3 syndrome CHF patients has been shown to improve cardiac output and induce neurohormonal deactivation.[211] IR and T2D have been associated with the severity of CHF, as testified by impaired six minute walking test and reduced peak VO_2.[154,211,212]

In the current study, we initially combined in a unique group the patients with T2D and the patients without T2D but with IR. This group, about two thirds of the entire population, was characterized by an increased occurrence of the primary outcome. T2D is a complex syndrome in which IR and hyperglycaemia coexist and weighting the relative role played by each of these two components is complex. However, when T2D alone was investigated, it exerts a tremendous impact on outcome, even when adjusted in the multivariate analysis, confirming observations from literature.[156] On the other hand, when patients with IR without T2D were investigated, we did not find any effect of IR on the prognosis of HF. In a small study on 105 CHF patients, mortality was linked to impaired insulin sensitivity, measured by intravenous glucose tolerance test associated with the minimal model technique.[155] However, it must be considered that in this previous report, mortality was very high

(40% vs 20% of the current report, in three years) and treatment was suboptimal (only 20% of patients were on beta-blockers). Furthermore, only men were included, whereas in the current study 20% of patients were women. Our finding suggests that, at least on the background of the treatment currently in use, IR does not appear to play a relevant role in the progression of CHF. Intriguingly, it has been demonstrated that drugs acting on insulin resistance, (i.e. glitazones), were non-effective or deleterious in clinical and experimental studies on CHF, whereas drugs having no direct effect on insulin sensitivity (i.e. sodium-glucose co-transporter-2 (SGLT2) inhibitors) have proved to be effective in reducing mortality and hospitalization in patients with CHF regardless diabetes. Considering that almost 40% of our patients are IR but non-diabetics, to clarify the role played by IR *per se* is very important, and further analyses are needed.

Taken together, the T.O.S.CA. registry confirmed that each of these HD in CHF is associated not only with a worse clinical status and impaired performance, but, more importantly, with reduced survival in CHF, further suggesting their causal role in CHF progression.[154-156,162,165,166,204-206] Current data do not display that a single hormonal defect has a predominant effect on morbidity and mortality compared with the others: except low T3, the survival curves related to the individual hormones display a similar divergence over time, pointing to the concept that each HD bears similar effect on outcome, while their aggregation portends to worse prognosis. Indeed, according to the interaction analysis performed in our work, none of the HD was able to drive the primary outcome.

Multiple hormone and metabolic deficiency syndrome in HF

Our report showed that the coexistence of at last 2 HD (i.e. MHDS) is very common, involving about 75% of subjects; notably, MDHS is strongly associated with impaired cardiovascular performance and increased risk of hospitalization and death.

Previous preliminary findings suggested that the coexistence of HD have a high prevalence among the patients with CHF and might have an impact on its prognosis.[150-152] Specifically, in a cohort of 208 male CHF patients, it has been demonstrated that the coexistence of at least 2 HD, among low levels of IGF-1, total testosterone, or DEHA-S (about 50% of the population) was associated with reduced survival.[150] More recently, in 107 male patients, a MHDS has been described in about 30% of the population, with an association between the number of HD and the outcome.[151] Finally, in a small cohort of 72 male patients, a prevalence of MHDS was found in about 60% of population, with a lower prevalence (about 40%) in HF with preserved ejection fraction.[152]

Going further these findings, we investigated a broader and more complete panel of hormones, showing also the impact of thyroid hormone deficiency and IR/T2D. In the current report, we demonstrate, for the first time, that MHDS has an independent and heavy effect of mortality or CV hospitalization. Further, The T.O.S.CA. registry describes the relationship between T2D and HD for the first time. Indeed, we analyse the entire phenotype of insulin action abnormalities, describing normal patients and insulin resistant one, with or without T2D. By excluding, as additional analysis, the diabetic patients from the study on the impact of hormone perturbations on CHF, we demonstrated that T2D could be considered as part of the MHDS.

A further strength of the present report is that female patients represent about 20% of the investigated cohort, whereas in all previous studies on the topic they have always been excluded. Therefore, we provide the first demonstration of the importance of MHDS in a mixed-sex population. This is of utmost importance, given the low representations of women in clinical studies engaging HF patients and the unmet need to better characterise female patients affected by this clinical condition.

Finally, we demonstrated for the first time that MHDS is associated with a more dramatic progression of the disease, as testified by the marked change in VO_2 between MHDS and NO-MHDS from baseline to follow-up. Indeed, even if no differences were observed with regard to echocardiographic findings, MHDS was associated with a more prominent decline of peak oxygen consumption. This might be due to the loss of many protective effects of hormones on myocardial mechanics, left ventricular remodelling, but also to the recognized action of anabolic hormones on skeletal muscle, endothelial function, and ventilator exchanges.[149]

Translational Perspective

The demonstration that hormones play a pivotal role in CHF progression and predict survival opens novel therapeutic horizons. While most circulating biomarkers are accepted as molecular signatures capable of predicting relevant disease states or clinical outcomes or guiding therapy, hormone deficiencies (HD) are endowed with the potential advantage of being correctable with appropriate replacement therapy, with preliminary positive results available in literature.[164,209,213-215]. Findings from the T.O.S.CA. Registry suggest to searching for HD in patients with HF as promising therapeutic targets and support the need for clinical trials aimed to demonstrate the potential benefits of hormonal replacement therapy.

Limitations

The observational character of our study is acknowledged.[165] Therefore, the study was not designed to elucidate the putative biological mechanism of HD in CHF. However, observational studies are more appropriate to describe the natural history of a disease and to generate or confirm new pathophysiological hypothesis.[216] Further, even if the cut-off values for defining the presence of HD may be regarded as somewhat arbitrary, they were chosen according with extant literature on the topic and, when available, with current guidelines.

Table 1. Definition of hormone deficiencies and their prevalence

HORMONE DEFICIENCY	VALUE FOR DIAGNOSIS	PREVALENCE OF HORMONE DEFICIENCIES		
		Total Population N=480	Men N=386	Women N=94
IGF-1 DEFICIENCY	Serum IGF-1 levels below the 33rd percentile of an age-matched healthy control population: • age < 55 years: 122 ng/ml • 55 y < age < 64.9 y) 109 ng/ml; • 65 y < age < 74.9 y:102 ng/dl • age > 75 y: 99 ng/dl	221 (46%)	171 (44·3%)	50 (53·2%)
TESTOSTERONE DEFICIENCY	Serum testosterone levels lower than 300 ng/dl in male or 25 ng/dl in female	204 (42·5%)	174 (45·1%)	30 (31·9%)
DHEAS-DEFICIENCY	Serum DHEA-S levels lower than 80 µg/dL	314 (65·4%)	241 (62·4%)	73 (77·7%)
INSULIN-RESISTANCE	Type 2 diabetes mellitus (T2D) or HomeOstasis Model Assessment (HOMA) greater than 2·5 (HOMA= insulin (mcU/mL) x glucose (mmol/L)/22.5	308 (64·2%)	244 (63·2%)	64 (68·1%)
LOW T3 SYNDROME	Serum free T3 lower than 3·1 mmol/L with TSH levels within normal range	33 (6·9%)	24 (6·2%)	9 (9·6%)

Serum hormones were analysed in a centralized core-lab (IRCCS SDN, Naples, Italy). Insulin and insulin growth factor-1 (IGF-1) were assayed by an enzyme-labelled chemiluminescent immunometric assay (IMMULITE 2000; IGF-1, interassay CV= 5·7%, Siemens Medical Solutions Diagnostics). Total testosterone was measured with a DPC Coat-A-Count RIA kit. DHEA-S was measured by a solid-phase, competitive chemiluminescent enzyme immunoassay.

Table 2. Demographic characteristics at baseline of final cohort

Variables	*Cohort (n = 480)*
Age (yr.)	63·7 ± 11·5
Sex (% male)	80.4
NYHA (% I/II/III/IV)	11/54/33/2
Aetiology (% ischemic)	52·7
Yr. of disease	7 [2-12]
Systolic blood pressure (mm/Hg)	121 ± 17
Diastolic blood pressure (mm/Hg)	74 ± 10
Type 2 Diabetes mellitus, n (%)	120 (25)
BMI (kg/m^2)	28·6 ±5·4
eGFR (, ml/min per 1.73 m^2)	86 ±41
NT pro BNP (pg/ml)	909 [284-2521]
Left Ventricular EF (%)	32·3 ± 7·2
Atrial fibrillation (%)	11·2
ICD (%)	36
CRT (%)	11·7
Medication (%)	
• *B-blocker*	87·5
• *ACE-I/ARBs*	86
• *MRA*	39
• *Diuretics*	69·4
• *Amiodarone*	15·8
• *Digoxin*	9·2
• *Antiplatelets*	49
• *Antithrombotic*	27·9
• *Lipid-lowering medications*	51·9
• *Ivabradine*	11
• *Antidiabetics*	15·2
• *Insulin*	10·6

Abbreviations: ACE-I: Angiotensin-converting-enzyme inhibitors; ARBs: angiotensin-receptor blockers; BMI: Body Mass Index; CRT: Cardiac resynchronization therapy; EF: Ejection Fraction; eGFR: estimated Glomerular Filtration Rate (CKD-EPI); ICD: implantable cardioverter-defibrillator; NYHA: New York Heart Association; NT pro BNP: N-terminal proB-type Natriuretic Peptide.

Figure 1. Occurrence of the primary endpoint in patients with MHDS.

Kaplan-Meier analysis of composite of all-cause mortality and cardiovascular hospitalizations [1·93 (1·37-2·73, p<0·001] (Primary endpoint, Panel A), all-cause mortality [1·02 (1·01-1·03, p=0·001] (Panel B), and cardiovascular hospitalization [1·02 (1·01-1·03, p=0·001] (Panel C), in patients with one or no hormonal deficiencies (NO-MHDS, n=122) *vs* patients with 2 or more hormonal deficiencies (MHDS, n=358). Log-rank testing was applied for calculation of p values.

(MHDS: multiple hormonal and metabolic deficiencies syndrome).

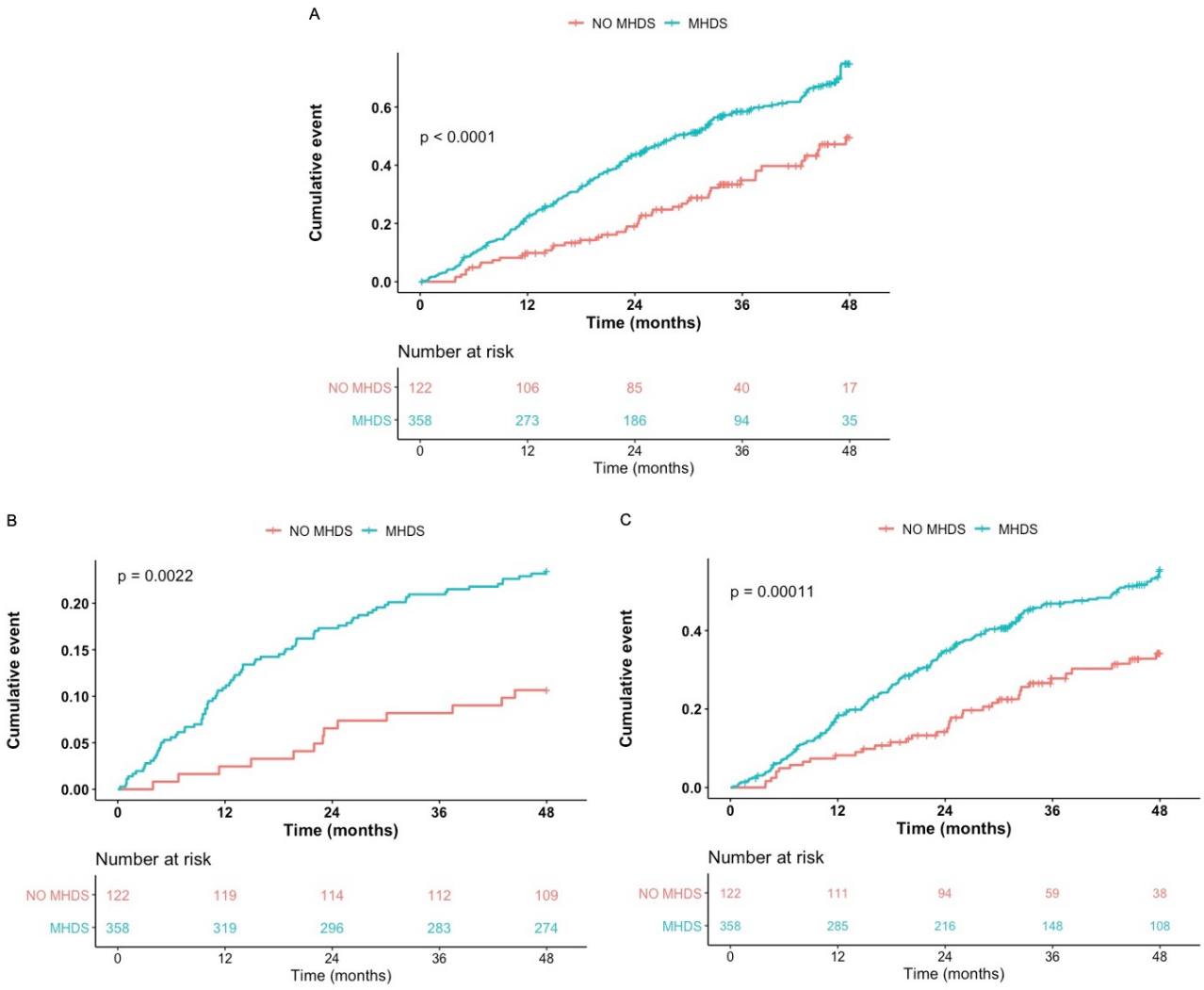


Figure 2. Single predictor models of Cox proportional Hazard Analysis

Forest plot of univariate Cox proportional hazard regression analyses of the effect several variables on the primary endpoint (composite of all-cause mortality or Cardiovascular hospitalization evaluated in the entire population n=480).

Variables	HR	95% CI	p
Age (1yr)	1.02	1.01-1.03	0.001*
Sex (Female)	0.84	0.61-1.14	0.25
NYHA class			
II	1.40	0.90-2.20	0.14
III-IV	1.95	1.23-3.09	0.004*
Aetiology (Ischemic)	1.14	0.90-1.45	0.29
EF (%)	0.97	0.96-0.99	0.004*
lnBNP	1.23	1.12-1.35	0.000*
Obesity	0.99	0.77-1.28	0.98
Impaired Hgb	0.53	0.34-0.81	0.003*
AF	1.03	0.77-1.39	0.83
Impaired eGFR	1.12	0.84	0.45
Testosterone Deficiency	1.59	1.25-2.01	0.001*
DHEAS Deficiency	1.40	1.07-1.81	0.011*
IGF-1 Deficiency	1.43	1.13-1.82	0.003*
IR / DM	1.34	1.03-1.73	0.03*
Low-T3	1.21	0.76-1.92	0.42
DM	1.55	1.20-2.01	0.000*
IR	0.94	0.74-1.20	0.6
MHDS	2.09	1.52-2.88	0.000*
Number of HD			
1	1.63	0.76-3.51	0.21
2	2.73	1.32-5.61	0.006*
>=3	3.39	1.66-6.90	0.000*

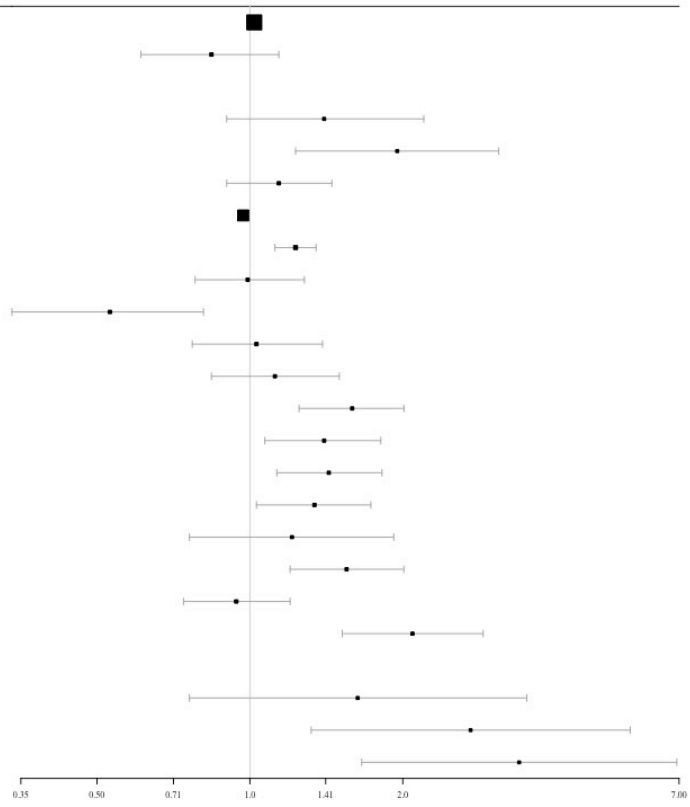


Figure 3. Occurrence of the primary endpoint according to the number of hormonal deficiencies. Kaplan-Meier analysis of survival for all-cause mortality or cardiovascular hospitalization in patients with 0 (n=28), 1 (n=94), 2 (n=158), 3, or more than 4 (n=200) hormonal deficiencies [1·35 (1·21-1·52), p< 0·001].

(MHDS: multiple hormonal and metabolic deficiencies syndrome). Log-rank testing was applied for calculation of p values.

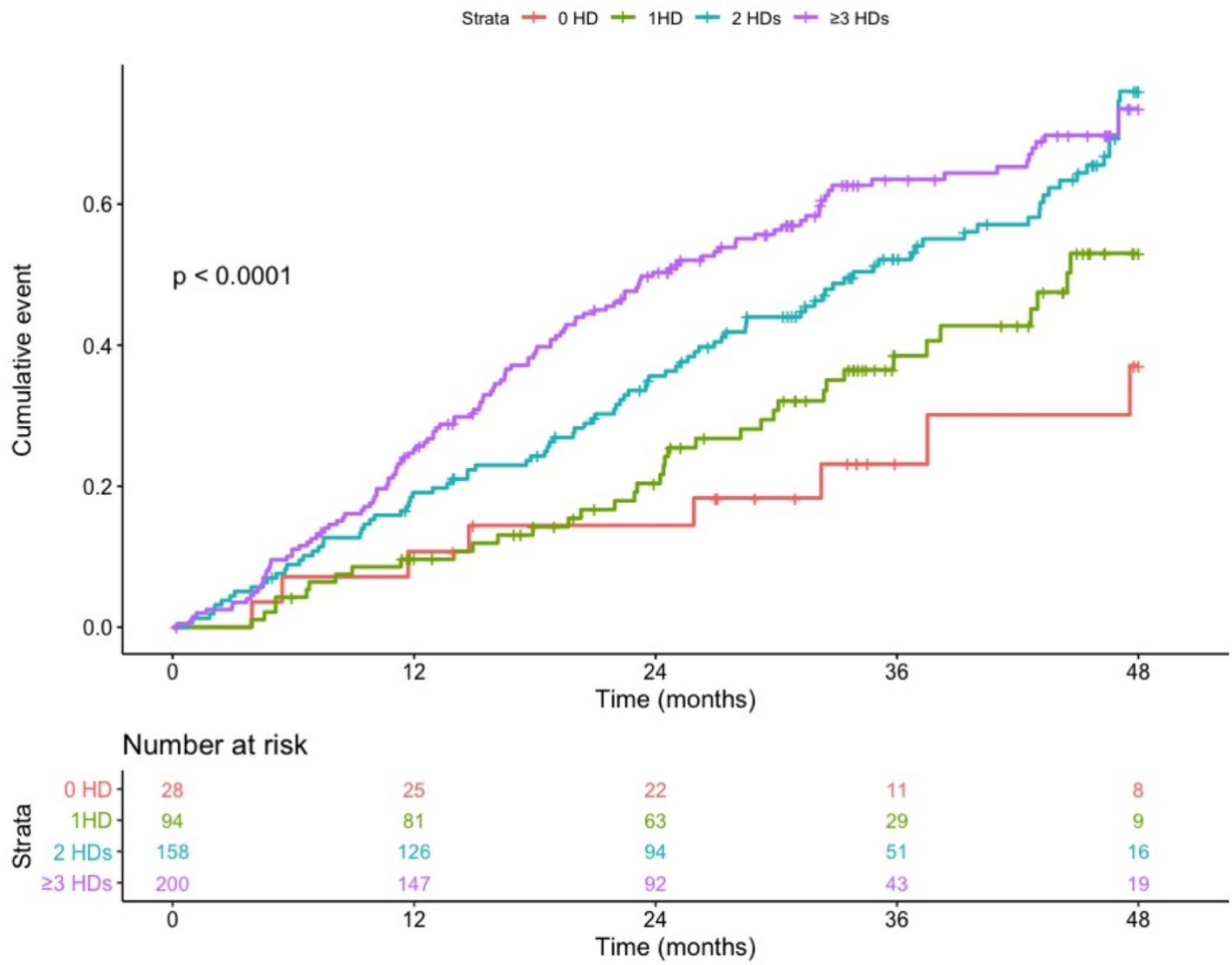


Figure 4. Multivariable Models of Cox Proportional Hazard Analysis

Adjusted Hazard Ratios for outcome (composite of all-cause mortality or Cardiovascular hospitalization evaluated in the entire population n=480) according to the presence of MHDS (A) full model, simplified model (B) or the number of hormonal deficiencies with simplified model (C). (BNP: B-type natriuretic peptide; EF: Ejection Fraction; NYHA: New York Heart Association; MHDS: multiple hormonal and metabolic deficiency syndrome; Anaemia: defined as Hgb<10 mg/dl; Obesity: defined as BMI>30g/m^2; impaired eGFR defined as creatinine clearance <60ml/min; AF: atrial fibrillation).

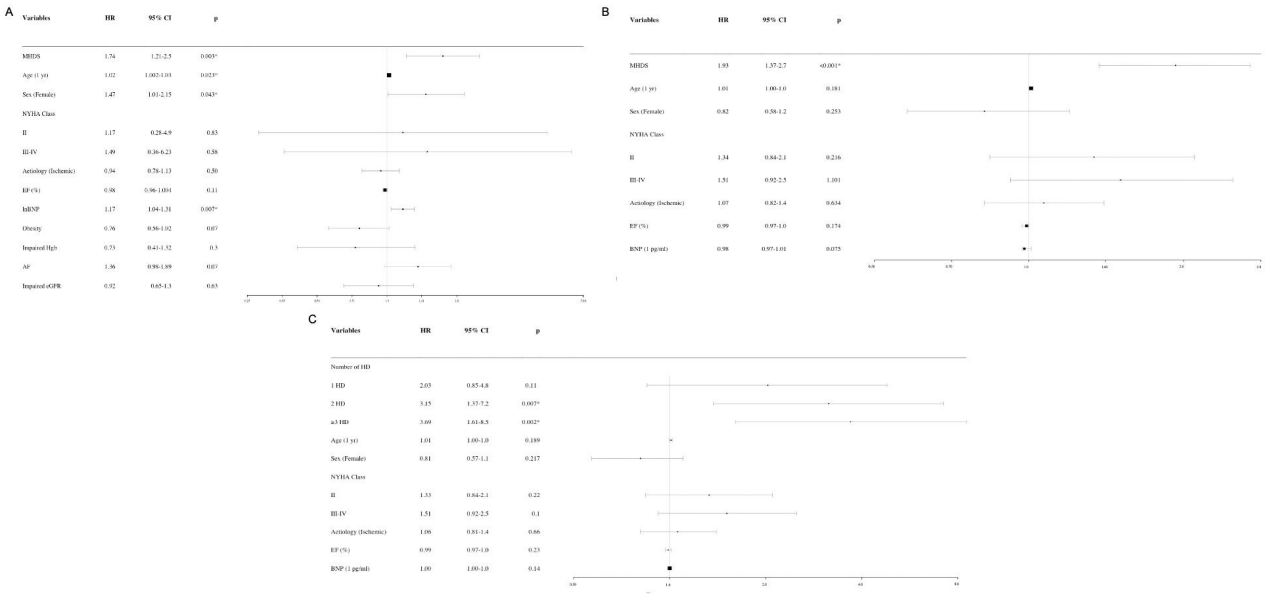


Figure 5. Occurrence of the primary endpoint according to a specific hormonal deficiency.

Kaplan-Meier analysis of composite of all-cause mortality or Cardiovascular hospitalization in patients with or without (A) Testosterone [1·57 (1·24-1·99), p<0·001] (B) DEHA-S [1·4 (1·1-1·8), p=0·014], (C) IGF-1 [1·42 (1·11-1·8), p=0·004], (D) or T3 [1·21 (0·76-1·94), p=0·42] (D) deficiency.

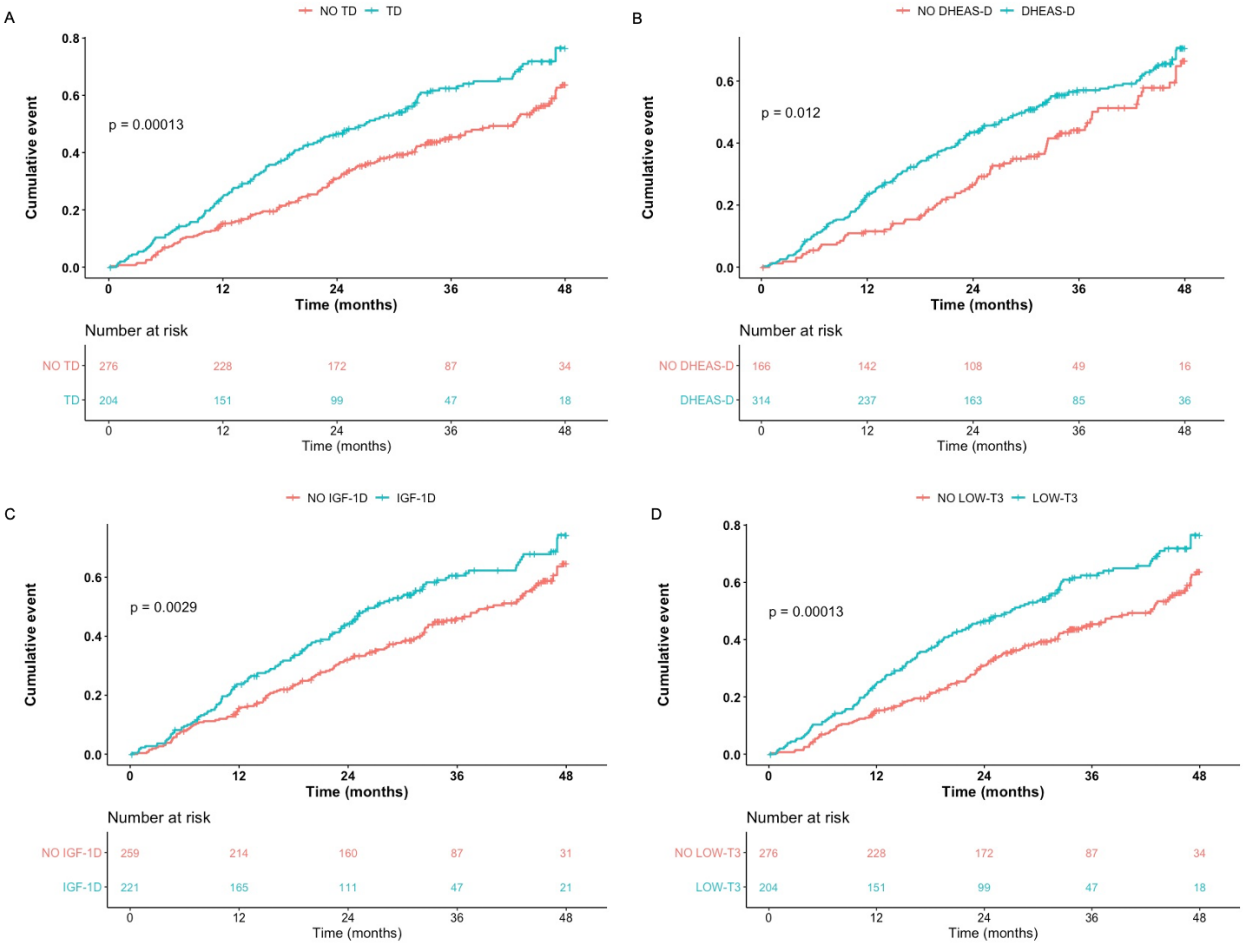
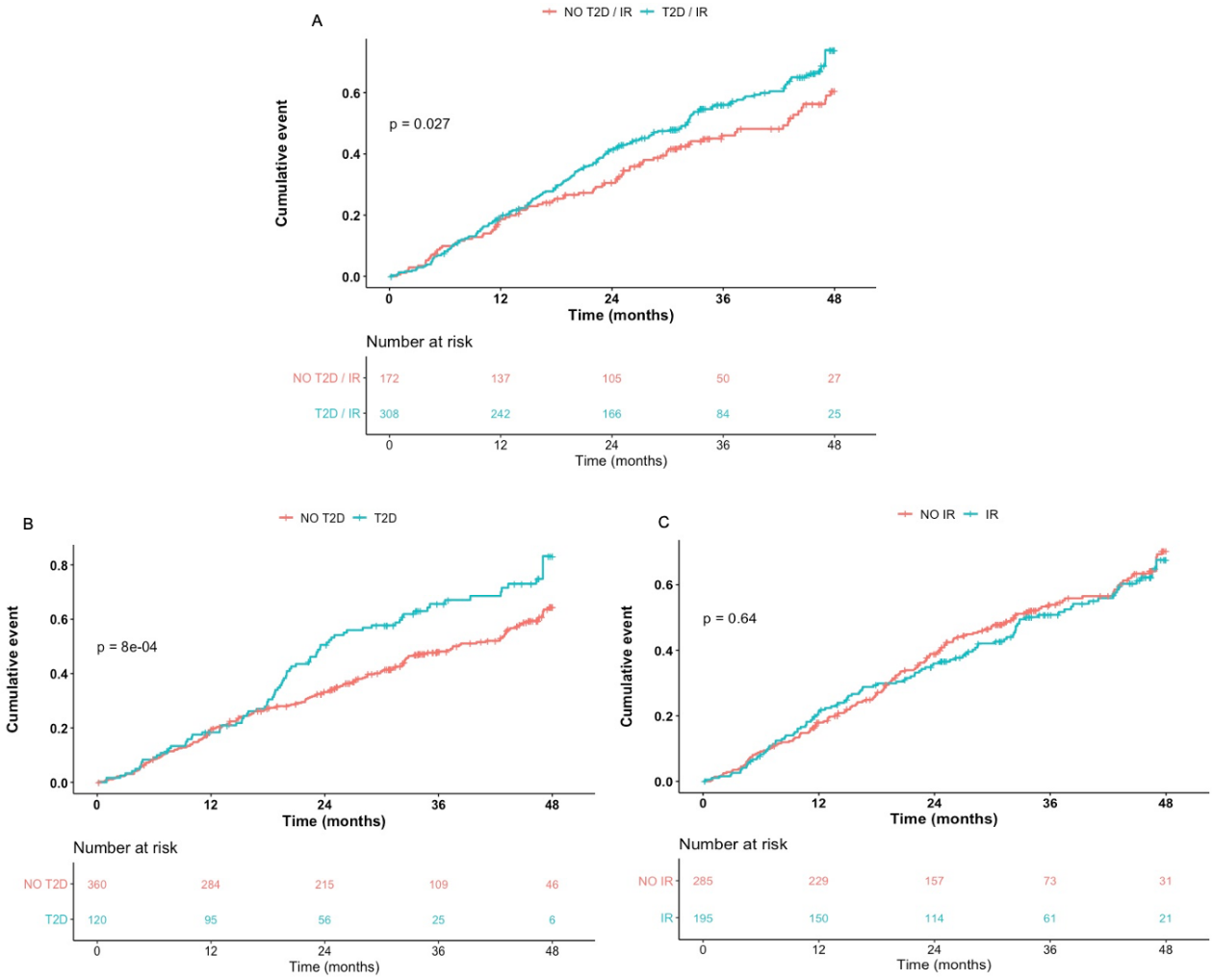


Figure 6. Occurrence of the primary endpoint according to insulin impairment.

Kaplan-Meier analysis of composite of all-cause mortality or Cardiovascular hospitalization in patients with or without (A) insulin signaling impairment [1·34 (1·03-1·73), p=0·03], (B) Type 2 Diabetes (T2D) [1·55 (1·20-2·01), p=0·001], or Homeostatic model assessment for insulin resistance (HOMA-IR) impairment [0·94 (0·74-1·20), p=0·64].



3.3 Treatment of hormone deficiencies in heart failure

3.3.1 D'Assante R, Piccioli L, Valente P, Stagnaro FM, Schiavo A, Lombardi A, Panicara V, Arcopinto M, Vitale G, Sarullo FM, Giallauria F, Marra AM, **Salzano A**§. Testosterone treatment in chronic heart failure. Review of literature and future perspectives. **Monaldi Arch Chest Dis**. 2018 Sep 5;88(3):976. doi: 10.4081/monaldi.2018.976. PubMed PMID: 30183157.

Introduction

Since the previous view of chronic heart failure (CHF) as a syndrome merely based on disordered hemodynamic and fluid balance has switched into a view of the disease involving different molecular pathways in disarray, our understanding of CHF grew very fast [3,18]. CHF is now considered not only as a "cardiac disease" caused by alterations in the structure and function of the heart but as a "systemic disease" in which the interplay between myocardial factors, systemic inflammation, comorbidities, and neurohormonal activation plays a pivotal role[17]. With the aim of improve the assessment and the treatment of patients with CHF, the actual vision has progressed from a focus on recover and support only hemodynamic aspects of the patients to a more targeted view aimed at modifying the maladaptive molecular processes that contribute to progression of disease[217-219].

In this context, mounting evidence showed that hormonal deficiencies (HD) seem to have an important role in CHF [50,55,63,183,220] . In particular, androgen depletion is common in men with CHF and is associated with increased morbidity and mortality [53,54]. In this review, we summarize clinical data and potential applications of testosterone treatment in CHF.

Testosterone and HF. Pathophysiological side

The interaction between hormones and cardiac structure and function is something of well-known[57,59,89,221,222]. In this context, there are several studies sought to investigate the pathophysiological mechanisms underpinning this relationship.

The fact that androgen receptors (AR) are expressed in atrial and ventricular myocardial cells, suggests that testosterone may act directly at the cellular level[223]. Testosterone and its active counterpart Dihydrotestosterone exhibit biological effects by interacting with AR expressed on cell surface as well as in the cytosol. Androgens can mediate different response depending on the type of interaction with the receptor. AR expressed on the surface seem to be more responsible for the activation of ion channels and signaling pathways, while intracellular AR are involved in the

modulation of androgen target genes that regulate myocardial and vascular cell activity[224]. In addition, also polymorphisms of AR seem to play a role, in particular on metabolic profile[225]. Independently from its genomic effect, however, testosterone interacts with myocardial cells by regulating intracellular $Ca2^{+}$ homeostasis. Experiments performed on cardiomyocytes of rats, showed that testosterone inhibits voltage-dependent $Ca2^{+}$ channels, resulting in a rapid vaso-relaxation in both large arteries and smaller resistance vessels[224]. Moreover, it has been evaluated whether testosterone may have effects on the action potential duration (APD). Some observations suggest that low testosterone levels prolong APD, increase intracellular $Ca2^{+}$ release and, consequently, the duration of contraction[226]. On the other hand, results obtained in gonadectomized female mice showed an increased neutrophil infiltration in the border zone of injured tissue after induction of myocardial infarction when compared with the same group supplemented with testosterone. These data suggest that testosterone is able to increase inflammation through neutrophil local infiltration after acute Myocardial infarction. Moreover, this inflammatory status results also in a higher rupture rate during left ventricular remodeling and higher mortality rate secondary to cardiac rupture[227]. There are no clear data about the role and the importance of this inflammation.

Of note, evidences emerging form studies on testosterone deficiency, suggest that testosterone is linked to the regulation of metabolic profile. In particular, endogenous testosterone levels improve lipid profile and reduce insulin resistance. Currently, there is no clear consensus on the association between endogenous testosterone levels and the effect of lipid profile, even though several evidences suggest that testosterone inhibits the maturation of preadipocytes into mature adipocytes, reduces total fat mass and increases net lean mass[228,229]. In addition, recent evidence suggests that testosterone is a metabolic hormone that differentially regulates the expression of key targets of lipid and glucose metabolism in a tissue-specific manner to potentially reduce fat deposition in pathologically relevant locations such as liver and the arterial tree[230]. Moreover, testosterone improves fasting glucose levels and glucose tolerance. Indeed, it has been reported that men with low testosterone levels have double the risk of developing new onset type 2 diabetes and metabolic syndrome[231].

For this reason, it has been hypothesized that testosterone plays a pivotal role in the prevention of the metabolic syndrome[232].

Testosterone and HF. Clinical side.

Several studies have investigated the role of testosterone in the treatment of CHF (table 1).

The first of these was conducted in 12 stable male patients by Pugh et al [233]. After administration of a single dose of testosterone (60 mg orally), the authors monitored central haemodynamic over 6h, using a pulmonary flotation catheter. Subjects received the second treatment on day 2 and haemodynamic monitoring was repeated. They observed an increase in cardiac output in the treated arm, mainly mediated by a reduction in peripheral resistance and left ventricular afterload. It is interesting to note that no side effects were reported and that results were more evident in patients with lower baseline levels of testosterone.

A few years later, the same group performed a double-blind, placebo-controlled study, enrolling 20 male CHF patients [234]. The treatment group (100mg every 2 weeks for 12 weeks) showed a significant improvement in the 6 minute walking distance and in the Minnesota living with heart failure questionnaire score. Following this promising results, they performed a larger randomized, double-blind, placebo controlled trial in 76 CHF patients[235]. In the treated group (5 mg/day administered by an adhesive skin patch), authors demonstrated an improvement of clinical severity disease as demonstrated by the shift of at least one class of NYHA class (30% of patients vs 8 % in treated and untreated respectively). In addition, even if testosterone did not change left ventricular morphology or function, a significant improvement in shuttle walk distance was demonstrated. On the other hand, treatment had no significant effect on measurements of skeletal muscle bulk and strength, heart rate, blood pressure, and weight. With regard to plasma concentrations of cytokines (e.g. TNFα, IL-1β, and IL-6), which role in CHF is emerging in last years[42], no differences where described. This was in line with previous report [236], where the administration of physiologic concentrations of testosterone to male patients with CHF had no effect on the serum concentrations of TNF-α, whether administered acutely over 6 hours or in the longer term over a 12-week period, either via intramuscular injection or transdermal patch.

Of note, even if the same group demonstrated, in a previous report, that testosterone therapy was able to reduce QT duration in men with CHF[237], data confirmed by an independent group a few years later[238], in this larger cohort they cannot confirm this finding.

Finally, testosterone treatment was well tolerated, even though some local reactions caused by the patch preparation was described.

Considering that elderly patients displays a deterioration of skeletal muscle strength, affecting early fatigue and limiting exercise tolerance [239,240], in a double-blind, randomized, placebo-controlled study focused on these patients, Caminiti et al [195] demonstrated that testosterone therapy (12 weeks with very long acting intramuscular 1000 mg testosterone undecanoate) improves functional exercise

capacity and muscle strength. In 70 elderly patients with stable CHF and median age of 70years, peak VO_2 and index of muscular strength (e.g. quadriceps maximal voluntary contraction and peak torque) improved in testosterone group but not in placebo group. Further, also glucose metabolism (HOMA-IR) and baroreflex sensitivity improved.

In 2014, Miramadi et al[241] performed a double-blind, placebo-controlled trial, in which 25 male patients received an intramuscular (gluteal) long-acting androgen injection (1mL of testosterone enanthate 250mg/mL) once every 4 weeks for 12 weeks. When compared with the placebo arm, the treatment group displayed a significant increasing trend in 6 minute walking distance and quality of life.

Further, Stout et al [242] demonstrated that testosterone supplementation added to a program of exercise rehabilitation was feasible and can positively impact on a range of key health outcomes in elderly male patients with CHF who have a low testosterone status. Indeed, testosterone treatment (an intramuscular (gluteal) injection of 100 mg testosterone/mL once a fortnight for 12 weeks) induced positive changes in aerobic fitness, leg strength, depression, and androgen deficiency symptoms that were not observed in placebo group. In this context, a very interesting study was performed by dos Santos and colleagues[243]. In this study, 39 male patients with advanced CHF (NYHA class III) and testosterone deficiency were randomized to training (4-month cycloergometer training), testosterone (intramuscular injection of testosterone undecylate for 4 months), and training + testosterone. Authors not only confirmed that testosterone therapy was able to potentiate the beneficial effects of exercise training in patients with CHF, but provide interesting information about the effects of the combined therapies on total body composition, functional capacity, hormonal status, and quality of life, supporting the concept that is the peripheral effect of testosterone the cause the improvement in cardiovascular performance.

Considering the important topic of sex and gender differences [82,179,244-246] , the effect of testosterone treatment has been investigated also in female population. In 36 female patients treated with testosterone (300 mg, patch transdermal, twice per week), an improvement in 6 minute walking distance and in peak oxygen consumption was observed. In this group, a positive effect was observed on metabolic pattern too (insulin resistance)[196]. In addition, also in female patients, a direct effect of testosterone to shorten ventricular repolarization in vivo was demonstrated[238].

Clinical implication

About 5 years ago, a meta-analysis performed by Toma and collegues[247] demonstrated that testosterone supplementation in patients with CHF is associated with a clinically significant improvement in the exercise capacity, expressed as 6 minute walk test distance. Of note, this improvement was greater than that seen with other CHF treatment (e.g. beta blockers and ACE inhibitors etc). Also VO_2 peak and NYHA class improved in patients receiving testosterone treatment. Because this progress are not associated with an improvement in cardiac structure or function, the cause of these findings is most likely due to peripheral mechanisms, as showed by dos Santos et al[243].
It is important to underline that in current literature only small trials are available, with different kind of patients and using different routes and dosage of testosterone. Further, all studies are focused in the setting of CHF with reduced ejection fraction and no data are available about CHF with mildly reduced or preserved EF. Finally, all these studies, because of the short term of their design, are focused only on the study of change in clinical parameters and not on strong clinical outcome, as mortality and/or hospitalization.
Of note, none of the trials showed a significant change in prostate specific antigen. In the studies using topical testosterone, skin reactions were described. Thus, there are no real safety concerns reported in any of the trials.
Another intriguing issue to argue is that the basal testosterone status was not used in all the studies as a parameter of inclusion or exclusion. Recently, in the context of HD in CHF [180,181,185,248,249], the idea that not all the patients need a hormone therapy, but only patients with a deficit of the axis of interest, has improved the outcome and represents the most promising option in this field [64,65,67,184]
.
Finally, considering the high frequency of multiple HD in CHF, no data are available about combined hormone treatment.

Conclusion

Taken all together, findings from the most recent literature demonstrated that testosterone treatment in CHF is a promising issue that needs further investigation.

Table 1. Summary of studies on testosterone treatment in chronic heart failure.

First author [cit] (year)	Sample size	Sex	Mean age (years)	NYHA class (mean ± SEM)	Testosterone deficiency	Testosterone supplementation	Type of trial	Trial duration
Pugh PJ [29] (2003)	12	Male	NR	NR	NR	60 mg orally day one and day 2	R, DB, PC, cross-over	2 days
Malkin CJ [34] (2003)	20	Male	61.5	2.5±0.5	NR	Sustanon 100 mg IM every 2 weeks	R, DB, PC	12 weeks
Pugh PJ [30] (2004)	20	Male	62	NR	NR	Sustanon 100 mg IM every 2 weeks	R, DB, PC	12 weeks
Malkin CJ [31] (2006)	76	Male	64	2.5±0.6	NR	Androderm 5 mg every 24 hours	R, DB, PC	12 months
Caminiti G [38] (2009)	70	Male	70	2.5±0.5	NR	Long-acting testosterone undecanoate (Nebido) IM at 0, 6, 12 weeks	R, DB, PC	12 weeks
Iellamo F [48] (2010)	32	Female	68.7	3±0	NR	Transdermal testosterone	R, DB, PC	6 months
Schwartz JB [35] (2011)	84	Male (69%) and Female (31%)	70.4	NR	NR	Long-acting testosterone undecanoate 1,000 mg IM at 0, 6, 12 weeks	R, DB, PC	12 weeks
Stout M [40] (2012)	41	Male	67.2	2.5±0.5	low testosterone status	Sustanon 100 mg IM every 2 weeks	R, DB, PC	12 weeks
Mirdamadi A [39] (2014)	50	Male	60	2.4±0.6	NR	Long-acting testosterone enanthate 250 mg IM every 4 weeks	R, DB, PC	12 weeks
Dos Santos MR [41] (2016)	39	Male	51	3	Testosterone deficiency	intramuscular injection of long-acting depot testosterone undecylate	R	48 weeks

R, randomized; DB, double blind; PC, placebo-controlled; NR, not reported; SEM, standard error of the mean

3.3.2 **Salzano A**, Marra AM, D'Assante R, Arcopinto M, Suzuki T, Bossone E, Cittadini A. Growth Hormone Therapy in Heart Failure. **Heart Fail Clin**. 2018 Oct;14(4):501-515. doi: 10.1016/j.hfc.2018.05.002. Epub 2018 Aug 17. Review. PubMed PMID: 30266359.

Introduction

Despite considerable improvement in the management of Heart failure (HF), unsustainable levels of morbidity and mortality coupled with an increasing economic and social burden have been observed over the previous decades [3]. One possible explanation might be that no single pathophysiologic paradigm of HF may completely explain disease progression.

The classical neuro-hormonal model, rooted on over-expression of different molecular pathways such as the sympathetic nervous, the renin-angiotensin-aldosterone, and the cytokines system, represented the theoretical background for the implementation of milestone clinical trials, which in turn have dramatically changed the natural history of this disease [3,250]. Nowadays, drugs that contrast the sympathetic nervous system (β-blockers) and the renin-angiotensin-aldosterone pathway (Angiotensin Converting Enzyme inhibitors/Angiotensin Receptor Blockers, angiotensin receptor neprilysin inhibitor, and Mineralocorticoid Receptor Antagonists) are considered the first line pharmacological therapy of heart failure [1,2,217,218].

However, CHF is still burdened by increased mortality, worse than that of many cancers, frequent comorbidities and, consequently, remarkable associated health care costs [3]. For this reason, other pathophysiological models to complement the paradigm of neuro-hormonal hyperactivity were proposed. In this regard, several studies have showed that hormonal deficiencies are common in CHF [48,53,55,63] and, more importantly, are significantly related with several indexes of physical performance and survival. The importance of these data led some authors to consider CHF as a Multiple Hormone Deficiency Syndrome [48].

In this context, the reduced activity of Growth Hormone (GH) and its tissue effector Insulin-like Growth Factor 1 (IGF-1) [62] plays a pivotal role. This review is focused on the involvement of GH/IGF-1 axis in CHF, on the role and prevalence of GH Deficiency (GHD) in HF, and on the effects of GH therapy both as add—on as well as replacement the correction of GHD in HF.

The effects of GH-IGF1 on the cardiovascular system

The pituitary secretion of GH exerts several biological effects through the interaction between GH and its specific receptors (GHR) that leads to the hepatic production of Insulin-like Growth Factor-I (IGF-I), its major biological mediator [251,252]. Through a long-loop feedback, IGF-1 produced in the liver in response to GH, prevents GH release (see figure 1).

Among all the anabolic systems existing in nature, the GH/IGF-1 pathway is considered the most powerful. It regulates post-natal growth by increasing muscle mass, bone length, and density during childhood and adolescence. Furthermore, carbohydrates and lipids metabolism are regulated by its effects, mainly on visceral adipose tissue [253]. IGF-1 circulates in blood either free or bound to particular binding proteins (called IGFBPs) that prolong the its half-life [254]. Nevertheless almost of 90% of circulating IGF-1 is part of a ternary complex composed of IGF-specific binding protein 3 (IGFBP-3) and acid- labile subunit (ALS) [255]. Through this complex, IGF-1 is able to reach its target-organs, where the interaction with its own receptor (IGF1R) activates the PI3K/Akt pathway [256]. This complex pathway promotes the main effect of GH: cell growth, enhances glucose transport, inhibits apoptosis and acts along with Interleukin- 6 (IL-6) to protect cells from TNF-α cytotoxicity [254].

The cardiovascular system is an important target of this anabolic axis [251,257]. It is well established that GH plays a pivotal role by regulating cardiac growth, cardiomyocyte size and metabolism, by stimulating amino acid uptake for protein synthesis [258], and promoting the transcription of genes specifically expressed in the cardiac muscle [258]. Moreover, systemic vascular resistance are likely to be reduced by the activation of IGF-1 receptors through the production of nitric oxide (NO). IGF-1 leads to augmented contractility of cardiomyocytes mainly by increasing intracellular calcium concentration and calcium sensitization of the myofilaments, and preserves capillary density [259]. Through regulation of the sarco/endoplasmic reticulum Ca2+-ATPase (SERCA2), IGF-1 also induces the reuptake of calcium by the sarcoplasmic reticulum. Moreover, enhancement of akt signaling, which lies downstream of IGF-1 receptor, augments myocardial contractility and diastolic function and such effects are associated with increased SERCA2 myocardial content, a pivotal regulator of calcium handling [260].

GH/IGF-1 deficiency in heart failure

According with literature, GH deficiency (GHD) is a common finding in CHF with a prevalence ranging from 32% to 53% [55,63,64,261]. Less congruent are data regarding IGF-1 in CHF. Most of the studies

reported reduced IGF-1 serum levels when compared with healthy controls [53,61,191,261,262], in particular in patients with more advanced heart failure [190,263] or cachexia [264,265].

Since GH levels and daily response are influenced by anthropometric factors, physical activity and sleep patterns [266] the diagnosis of GHD in adults may be challenging. The most recent guidelines considered the insulin tolerance test (ITT) as the gold standard test for evaluation of adult GHD [267-269]. Considering that in CHF patients hypoglycemia could be unsafe, alternative tests are used. In particular, because of its high discriminatory power, convenience and reproducibility, the contemporary administration of Growth Hormone Releasing Hormone (GHRH) and arginine has gained wide acceptance for GHD diagnosis [261,270]. Novel alternative tests to the ITT for the diagnosis of GHD are under evaluation [271].

Blood samples for GH measurement are taken every 15-30 minutes during the next two hour after an intravenous infusion of arginine (0.5 g/kg, maximum dose 30 g) followed by an intravenous bolus of GHRH (1 mcg/kg, maximum dose 100 mcg) [272]. To avoid that molecules GH-like (e.g. prolactin) may potentially affect the measurement cross-reacting with monoclonal antibodies, is usually used to limit detection to the 22 kDa GH isoform, the most common of the different isomers and isoforms of circulating GH [273].

It was already ascertained by several independent groups that GHD is *per se* associated with impaired cardiovascular performance and increased peripheral vascular resistance. A positive correlation between GHD severity and cardiac impairment was described [274]. Furthermore, population studies reported an increased cardiovascular mortality associated with GHD [275]. With the aim of systematically evaluate GH/IGF-1 activity in a large cohorts of CHF patients, combining measures of the IGF-1 system with stimulated pituitary responses, our group recently performed a GHRH+ Arginine provocative test [61] on 130 CHF patients, demonstrating a GHD prevalence of ≈30%. Of note, GHD patients displayed larger LV volumes with elevated wall stress, as well as higher filling pressures, and impairment of right ventricle systolic function compared with GH sufficient patients. Moreover, GHD patients also displayed impaired peak VO_2 and reduced ventilator efficiency, resulting in a worse cardiopulmonary performance. The coexistence of GHD and CHF identifies a subgroup of patients with increased mortality, as showed by the Cox-regression analysis showed that GHD cohort has higher risk of mortality compared to GH sufficient cohort (HR 2.11, p = 0.021) independent of age, sex, NT-proBNP, peak VO2 and LVEF. With regard to GHD in HFpEF, recently Salzano et al. demonstrated that such endocrine defect is also common in HFpEF, although to a lesser extent than in HFrEF [55].

Few data are available dwelling upon GH and acute HF. Recently, Bhandari et al. [276] evaluated serum GH concentrations in 537 patients admitted for AHF. GH levels were increased in all patients who experienced one of outcome measures (either death or readmission within 1 year), without regard of EF. In this context, GH levels were independently predictors of outcomes in HF with reduced EF but not in HF with preserved EF. Interestingly, GH improved risk classification as measured by continuous net reclassification improvement (NRI) when added to the ADHERE multivariate logistic model (which in turn is composed by age, sex, urea, heart rate, and systolic blood pressure) [277], and to ADHERE model + NT-proBNP. The authors concluded that GH could be used as incremental prognostic biomarker over the ADHERE score clinical predictors and NT-proBNP for risk stratification of acute HF patients. Even with the strong limitations due to the evaluation of a single GH measurement taken in unfasted patients, this work, for the first time, demonstrated incremental prognostic utility of the assessment of a marker of GH activity also in HF acute settings. No data are available as to the prevalence of GHD in AHF.

Mechanism of GHD in CHF

Despite it is not possible to put forward a single explanatory pathophysiological mechanism of the occurrence of GH/IGF-1 impairment in CHF, many explanations could be provided regarding the underlying mechanism of impaired GH/IGF-1 secretion in CHF: (I) local hypo-perfusion of the hypothalamic-pituitary axis; (II) alterations related to chronic disease, inflammation, and liver congestion; (III) effects of CHF therapy on GH/IGF-1 axis. The systemic hypo-perfusion and reduced oxygen supply, a typical hallmark of CHF clinical syndrome is likely to alter pituitary perfusion, likewise what happens in other in other splanchnic districts. This mismatch between arterial perfusion and venous drainage is likely to cause somatotropic cells death resulting ultimately in GHD. Although the association between perfusion delay and GHD has been proved in non CHF patients [278], to best of our knowledge no neuroimaging study of the hypothalamic-pituitary axis of CHF patients has never been conducted. Moreover Broglio et al., after exclusion of the presence of an hyperactivity of somatostatin pulse, which physiologically counteracts GH secretion, in a cohort of 38 DCM patients found a reduced response to different provocative tests such as GHRH, GHRH + Arginine, and GH-related peptides and hypothesized a primary hypothalamic damage [261]. Considering that defects of GH/IGF-1 secretion are founded also in other several chronic wasting conditions characterized by inflammatory activation, cytokine overexpression, and liver congestion [82], another hypothesis is rooted in a possible role of this conditions, that lead to a reduced hepatic synthesis of peptides

hormones produced by the liver and at the same stage a peripheral GH. Considering that both ACE-inhibitors [279] as well as β-blockers [280] are likely to modify IGF-1 secretion through a direct inhibitions of IGF-1 signaling pathway, another explication for GHD in CHF might be found in therapy of CHF *per se*.

In all likelihood, no single mechanism is sufficient to explain the impairment of the GH/IGF-1 axis in CHF, but probably the synergy and interaction of different pathophysiological processes and concomitant pharmacological issues might represent its underpinnings.

Growth Hormone therapy in HF

A dangerous liaison exists between hormones and cardiovascular diseases [56-59,179]. Nowadays, a growing body of evidence supports the role of hormonal therapy in CHF [67,281]. In this intricate scenario, the impairment of GH/IGF-1 axis plays a critical role in CHF [50]. The vast majority of studies showed that patients affected by this condition display a more aggressive disease. The impact of multiple and concomitant anabolic deficiencies (including therefore also GH and IGF-1 assessment, Testosterone, Insulin Resistance, Thyroid, etc.) on different end-points including hospitalization and mortality rate in CHF are being investigated by a prospective multicenter clinical registry, the T.O.S.C.A (Trattamento Ormonale nello Scompenso CArdiaco), [52,181]. Among hormonal therapy, GH replacement therapy represents a promising therapeutic opportunity in CHF. Data derived from animal models showed beneficial effects of GH on peripheral vascular resistance, cardiac function, and survival [282-286]. Among the pathophysiological mechanisms, is well recognized that early treatment of large myocardial infarction with GH reduces pathologic LV remodeling and improves LV function [287]. Despite this solid background, when translated onto clinical field, these results did not lead to unequivocal results [51,248,288-302] (see table 1). At the beginning of the nineties, several preliminary pilot studies tested the effect of GH therapy leading to encouraging results both in CHF [248,288,289,292-294,297,299] as well as other clinical setting [303]. However, more robust randomized controlled clinical trials [248,290,291,296-299] failed to confirm previous results. In particular, in a cohort of 50 CHF patients, Osterziel et al. [291] demonstrated a significant increase in left-ventricular mass, strongly related to changes in serum IGF-I concentrations but without significant improvement in clinical status. Of note, ejection fraction increased markedly in patients with larger increases of IGF-1 during GH treatment [296]. Isgaard et al. [290] and Acevedo et al. [299] showed no effects of GH therapy, despite the last demonstrated a positive correlation between the changes in oxygen consumption and

ejection fraction in GH treatment group. Adamopoulous et al., in a randomized crossover trial, demonstrated that GH administration modulates beneficially circulating cytokine network and soluble adhesion molecules in patients with idiopathic dilated cardiomyopathy, whilst enhancing contractile reserve and diminishing LV volumes [298]. Of note, the relationship between cytokine network, cardiac performance and survival in HF was demonstrated also by our group [42]. Fazio et al. [248] in a randomized, double blind placebo controlled trial, demonstrated that GH, but not placebo, increased IGF-I serum concentration, improved New York Heart Association functional class, improved cardiopulmonary performance (improved exercise duration, peak power output, peak minute ventilation , peak oxygen consumption, and anaerobic threshold) without affecting lung function parameters.

However, in an elegant pooled meta-analysis of effects of GH therapy in CHF, Le Corvoisier et al. demonstrated that sustained GH treatment improves several cardiovascular parameters in HF [304]. In particular, an increase in LV Ejection Fraction (EF) and a reduction in systemic vascular resistance were showed, both associated with beneficial long-term changes of cardiac architecture related to an increase in LV wall thickness and a reduction in LV diastolic diameter [304]. In another meta-analysis, Tritos et al. demonstrated that GH therapy resulted in an increase of LVEF, cardiac output, an improvement in systemic vascular resistance and NYHA class level, and an amelioration of exercise duration and of maximum oxygen uptake, with no adverse effects on diastolic function [305] . A possible explanation of these inhomogeneous data could be find in the different study duration, target dose, end-points, and the lack of assessment of GH status [67]. In particular, Le Coirvoisier et al., performing a subgroup analysis according to target dose of all clinical trials, demonstrated that while high-dose GH therapy was associated with significant overall effect for ventricular morphology, cardiopulmonary performance and clinical status, low GH dose was associated only with exercise duration and NYHA class. Moreover, when trials were separated in two groups according to the median of IGf-1 increase, studies with larger IGF-1 increase reported a significant overall effect size for LV morphology and cardiopulmonary performance, while no significant effects were showed by trials with smaller IGF-1 increases. The take home messages of this work are: i), a proven impairment of of GH/IGF-1 status, e.g. GHD should be the preliminary condition before starting to administer GH, ii) physicians should consider an individualized dose titration of GH in patients with HF, iii) GH therapy should not be administered in all patients with CHF, but only in mild to moderate CHF, considering the hypothesized GH resistance state of the more advanced disease [265]. On these premises, ten years ago our group implemented a novel approach, performing a randomized, single-blind

controlled proof-of-concept trial in which, for the first time, the effects of GH therapy were tested only in patients affected by both GHD and CHF [64]. In this study, to check GHD status, patients underwent a GHRH + Arginine provocative tests. After 6 months, GH replacement therapy decreased circulating NT-pro-BNP levels, augmented LVEF (from 33 ± 2 to 36±2 %, $p<0.01$), increased peak oxygen uptake (from 12.9 ± 0.9 to 14.5± 1.0 ml/kg/min, $p<0.005$), improved quality of life score and flow-mediated vasodilation. Considering these encouraging results, with the aim to evaluate for the first time in HF a long-term GH therapy, we extended this study with a 4-year follow up in order to assess whether these effects were sustained or tend to vanish over time [65] . At the end of the study, GH replacement therapy was still associated with a significant reduction of both LV end-diastolic and end-systolic volumes indexes and circumferential wall stress, accordingly with a LV reverse remodeling, as documented by an increase in LVEF. In the GH group peak VO_2 increased remarkably (with a treatment effect of 7.1 ± 0.7 ml/kg/min in the GH group while the control group experienced a change of -1.8 ± 0.5, $p < 0.001$). Moreover, a difference in the composite of death and hospitalization for HF was observed, even though the study was not designed for hard clinical endpoints.

However, the usefulness of GH replacement therapy must be still proved in a double-blind placebo controlled trial. In this context, our group has been recently awarded with the 2016 Grant for Growth Innovation (GGI) International Award, proposing a double-blind study of GH replacement therapy in CHF patients with coexisting GH deficiency.

Side effects

Taken all together, data from clinical studies showed that GH therapy, if used with correct dosage, is safe and without major collateral effects. The worsening of ventricular arrhythmias reported by Frustaci et al. [289] are not reported by other studies. A possible explanation for this apparent discrepancy is the very high dosage used by Frustaci et al. It is well known that supraphysiologic levels of GH, as in Acromegaly, could lead to an increase in ventricular arrhythmia. Moreover, Le Corvoisier et al [304] demonstrated that there were no differences in the frequency of major adverse effects between GH and placebo treatments: death, worsening of heart failure, increased salt retention and ventricular arrhythmias are not significant between treatment or placebo arms. Moreover, Cittadini et al., in the trial that lasted longer on GH therapy in CHF, reported no major adverse events in the patients who received GH therapy. In particular, in this population, only two cases of arthralgia are reported. This is a well-known side effect of GH replacement, and improved after GH therapy

withdrawal. The relatively low prevalence of adverse effects is congruent with the largest database available, which indicates that GH replacement in adults is well tolerated [306].

Conclusions

Growing evidence support the hypothesis that CHF could be viewed as a Multiple Hormone Deficiency Syndrome. Deficiencies of the main anabolic axes are very common in CHF and are associated with poor cardiovascular performance and prognosis appearing not to be only a mere cluster of biomarkers. Among these, GHD plays a pivotal role and its correction is likely to be considered as an innovative therapie that have to be considered in CHF.

In this regard, most recent statement of the American Heart Association on management of Dilated Cardiomyopathies [307] , recommends with strong level of consensus the testing for GH deficiency in patients with DCM who have other signs and symptoms of those clinical disorders (Level of Evidence C) and recommends that appropriate therapy of the primary disorder of GHD should be performed in all patients with coexisting DCM.

On the basis of the aforementioned evidence acquisition and authors 'own experience, a suggested flowchart addressing the management of GHD in CHF patients has been developed for clinical use (see figure 2). In particular, we suggest that (I) GH status should be investigated in all CHF patients, in particular in who displays signs and/or symptoms of GHD and/or have past medical history of condition commonly associated with GHD (e.g. structural hypothalamic/pituitary disease, surgery or irradiation in these areas, head trauma, or evidence of other pituitary hormone deficiencies); (II) GH should be administered only in those patients with an impairment of GH/IGF-1 status, e.g. GHD, as a replacement therapy; (III) an individualized dose titration of GH should be considered for each patient depending on neuro-humoral response; (IV) GH therapy should not be administered in patients with advanced CHF, which are probably already in a GH resistance state.

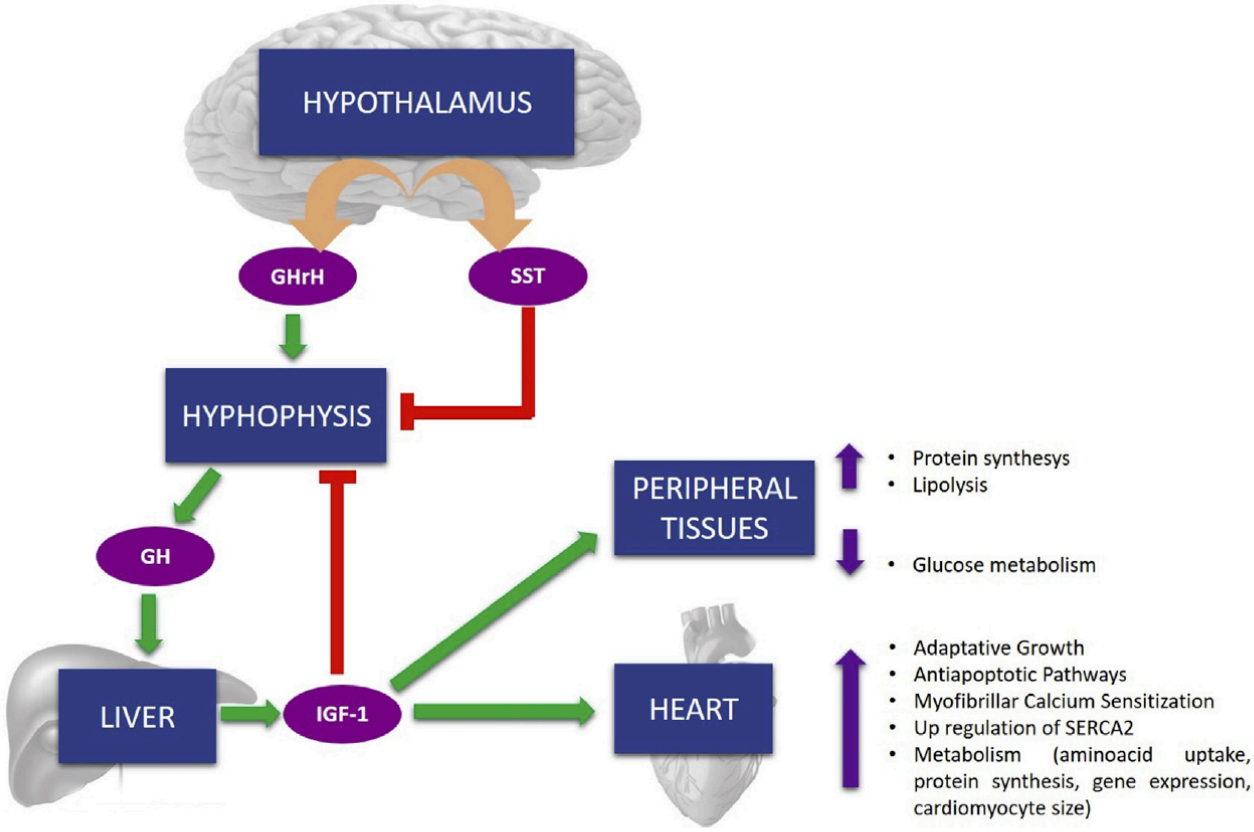


Fig. 1. Pathophysiologic background of hypothalamic-pituitary axis and GH/IGF-1 systemic effects. GHrH, growth hormone–releasing hormone; SERCA2, sarcoplasmic/endoplasmic reticulum Ca2+-ATPase; SST, somatostatin.

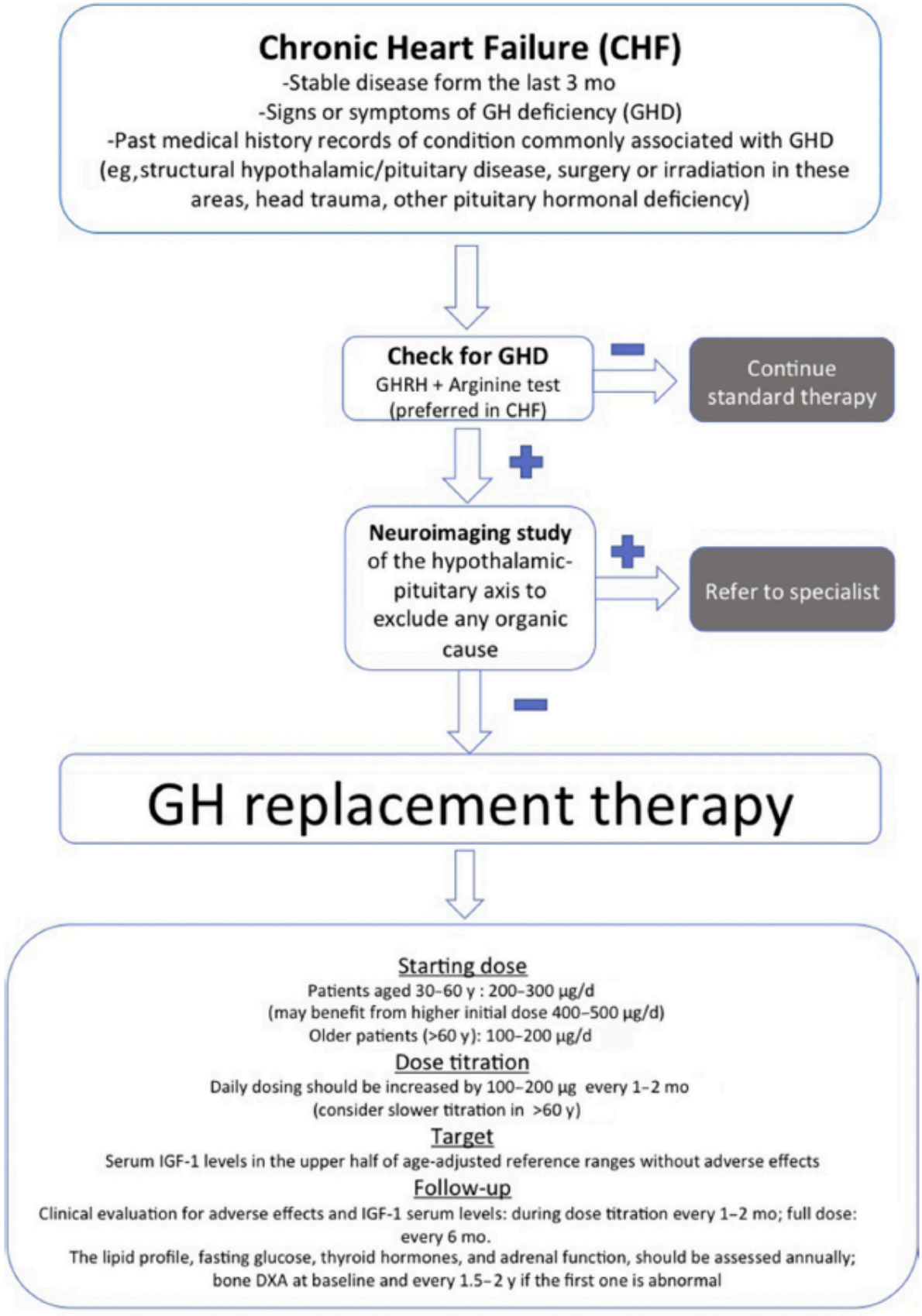


Fig. 2. Management of GHD in CHF. Based on the evidence acquisition, more recent guidelines, and authors' own experience, a suggested flowchart addressing the management of GHD in patients with CHF has been developed for clinical use.

Table 1
Summary of studies on GH therapy in heart failure

Author, Year of Publication (Ref.)	Study Design, Treatment Duration, Daily Dose, Target Dose (Calculated on a Mean Weight of 70 kg)	Patients (n) and GH Status	Age (Mean ± SD), Ischemic (%), Women (%)	Increase of IGF-1 (%)	Effects of GH Therapy
Fazio et al,[63] 1996	• Open, crossover trial • 3 mo of therapy and 3 mo after therapy • 4 IU every other day sc • 12–16 IU/wk	• 7 • NA	• 46 ± 9 • 0 • 29	105.1	• Increased LV mass and LVWT and reduced the size of LV chamber • Improvement in hemodynamic variables (decreased mean pulmonary arterial and PCWP, increased stroke volume) • Beneficial changes in myocardial energy metabolism (the increase in oxygen consumption in response to physical exercise was significantly reduced) • Improved clinical status • No side effects
Frustaci et al,[64] 1996	• Open trial • 3 mo • 4 IU intramuscularly daily • 32 IU/wk	• 5 • NA	• 28 ± 8.1 • 0 • 75	NA	• Mild reductions in LV end-diastolic diameter and mild improvement in the LVEF • Worsening of arrhythmias (Lown class increased) improved after GH withdrawal
Isgaard et al,[65] 1998	• Placebo-controlled trial • 3 mo • Initial dose of 0.1 IU/kg/wk for 1 weekend thereafter 0.25 IU/kg/wk • 7 IU first week, 18 IU per week	• 22 • NA	• 60 ± 11 • 36 • 36	137.1	• No significant effect on systolic or diastolic cardiac function, exercise capacity, or neuroendocrine activation • No overall improvement in functional class or dyspnea grade • The treatment was safe and without serious side effects
Osterziel et al,[66] 1998 and Perrot et al,[71] 2000	• Randomized, double-blind placebo-controlled trial • 3 mo • 0.5 IU or placebo daily sc The dose was increased every second day by 0.5 IU until a final dose of 2 IU/d was reached • 14 IU/wk	• 50 • NA	• 54 ± 10 • 0 • 14	78.8	• GH treatment increased LV mass (small increase in LVWT) a relation between the change in myocardial mass and change in serum IGF-I concentrations • The treatment was safe and without serious side effects

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Table 1
(*continued*)

Author, Year of Publication (Ref.)	Study Design, Treatment Duration, Daily Dose, Target Dose (Calculated on a Mean Weight of 70 kg)	Patients (n) and GH Status	Age (Mean ± SD), Ischemic (%), Women (%)	Increase of IGF-1 (%)	Effects of GH Therapy
Genth-Zotz et al,[67] 1999	• Open, crossover • 3 mo of therapy, 3 mo after therapy • 2 IU daily sc • 14 IU/wk	• 7 • NA	• 55 ± 9 • 100 • 0	110.1	• An attenuation of LV remodeling (a significant reduction in end-systolic and end-diastolic volume indexes and an increase in LVWT) and improvement of diastolic function (an increase of early diastolic filling velocity [E] and a decrease of the deceleration time of early diastolic filling) • Mean PCWP significantly decreased at rest and after exercise. After 3 mo of discontinuation, PCWP at rest was still lower than at baseline; increased cardiac output at rest and in response to physical exercise and significantly decreased systemic vascular resistance • Increased in exercise capacity: maximal oxygen uptake (Vo_{2max}) rose during therapy and fell 3 mo after GH was discontinued • Decreased NYHA functional class during treatment; this improvement had deteriorated 3 mo after discontinuation
Jose et al,[69] 1999	• Open, crossover • 6 mo of therapy and 6 mo after therapy • 2 IU on alternate days • 6–8 IU/wk	• 6 • NA	• NA • NA • NA	NA	• Improvement in the symptomatic class with treatment • Significant increase in the interventricular septal wall thickness and posterior wall thickness • These changes were partially reversed by the end of 6 mo of treatment but the symptomatic status of these patients was better than before therapy

Spallarossa et al,[68] 1999	• Open, controlled trial • 6 mo • 0.006 U/kg body weight daily sc: during the first month the dosage was gradually increased up to a full treatment regimen of 0.02 U/kg/d • 10 IU/wk	• 20 • NA	• 62.1 ± 8 • 100 • 0	89	• GH did not change LV diameters or LVWT. • Improved clinical status (reduction of Nottingham Health Profile score) in treatment group • Increased duration of the exercise test in GH group • A trend toward decreased serum triglyceride levels and adipose body tissue associated with an increase in high-density lipoproteins
Smit et al,[70] 2001 and Van Thiel et al,[75] 2004	• Open, randomized, controlled trial • 6 mo • Started with 0.5 IU sc around increased after 2 wk to 1.0 IU/d and 4 wk after entering the study, the final dose of 2.0 IU/d • 14 IU/wk	• 19 • NA	• 65.5 ± 8.5 • 100 • 15	36.7	• No beneficial effect on LV function
Napoli et al,[72] 2002	• Randomized double-blind placebo-controlled trial • 3 mo • 4 UI sc every other day • 12–16 wk	• 16 • NA	• 54.5 ± 11.3 • 31 • 25	85.5	• Corrected endothelial dysfunction (through greatly improved Ach-mediated endothelium-dependent vasodilation) and improved nonendothelium-dependent vasodilation (nonsodium nitroprusside dependent vasodilation) • Increased Vo_2 max
Acevedo et al,[74] 2003	• Randomized double-blind placebo-controlled trial • 2 mo • 0.035 U/kg/d subcutaneous • 16–20 IU/wk	• 19 • NA	• 57.7 ± 4.5 • 35 • 10	40.1	• No significant differences in body weight, lean body mass and dynamometry • No significant effect in left ventricular ejection fraction, left ventricular volumes, and peak oxygen consumption • A positive correlation between the changes in oxygen consumption and ejection fraction was found in GH treatment group • No adverse effects

(*continued on next page*)

Table 1
(*continued*)

Author, Year of Publication (Ref.)	Study Design, Treatment Duration, Daily Dose, Target Dose (Calculated on a Mean Weight of 70 kg)	Patients (n) and GH Status	Age (Mean ± SD), Ischemic (%), Women (%)	Increase of IGF-1 (%)	Effects of GH Therapy
Adamopoulos et al,[73] 2003	• Randomized crossover • 3 mo of therapy and 3 mo after therapy • 4 IU every other day • 12–16 IU/wk	• 12 • NA	• 50 ± 13.8 • 0 • 33	NA	• A significant decrease in the circulating proinflammatory cytokines (TNF-α, interleukin-6, GM-CSF and its soluble receptor GM-CSFR), chemotactic chemokines (MCP-1) and soluble adhesion molecules (sICAM-1 and sVCAM-1), and an increase in the serum anti-inflammatory/proinflammatory balance, • A reduction in end-systolic volume and wall stress associated with an increase in myocardial wall thickness and contractile reserve • Significant correlations were found between the GH-induced improvement in contractile reserve and the increase in Vo_2 max
Fazio et al,[76] 2007	• Randomized, double-blind, placebo-controlled trial • 3 mo • 4 IU sc every second day • 12–16 IU/wk	• 22 • NA	• 55 • 40 • 31	101	• Increased IGF-I serum concentration • Improved NYHA functional class • Improved cardiopulmonary performance (improved exercise duration, peak power output, peak minute ventilation, peak oxygen consumption, and anaerobic threshold) without affecting lung function parameters

Cittadini et al,[23] 2009	• Randomized, single-blind, controlled trial • 6 mo • 0.012 mg/kg every second day • 2.5–3.36 IU/wk	• 56 • GHD	• 62 ± 2 • 53 • 28	55	• GH therapy is associated with reverse remodeling, as shown by the reduction of cavity size, induction of mild hypertrophy, and a remarkable reduction of LV systolic stress • Resting systolic blood pressure decreased significantly in the GH group • Significantly improved exercise capacity and cardiopulmonary performance: exercise duration, peak workload, and peak Vo_2 consumption increased at the anaerobic threshold, Vo_2, and workload • Clinical status improved after GH therapy (MLHFQ score decreased in the active treatment group and anxiety and depression score) • NT-proBNP levels decreased and IGF-1 levels increased • Remarkable improvement of FMD • GH therapy was not associated with untoward effects during the treatment period
Cittadini et al,[84] 2013	• Randomized, single-blind, controlled trial • 48 mo (extension of previous study) • 0.012 mg/kg every second day • 2.5–3.36 IU/wk	• 31 of 56 • GHD	• 62 ± 2 • 77 • 16		• LV volumes and ejection fraction showed the highest response at 2 y and then tended to stabilize • Although the study was not designed for hard clinical end points, it was noteworthy that there was a marked difference in the aggregate of death and hospitalization for worsening CHF

Abbreviations: FMD, flow mediated dilation; GM-CSFR, granulocyte-macrophage colony–stimulating factor; LVWT, left ventricular wall thickness; MCP, membrane cofactor protein; MLHFQ, Minnesota living with heart failure questionnaire; NA, data not available; NYHA, New York Heart Association; PCWP, pulmonary capillary wedge pressure; sc, subcutaneous; SD, standard deviation; sICAM, Serum intercellular adhesion molecule; sVCAM, Serum vascular adhesion molecule; TNF-α, tumor necrosis factor-α.

3.3.3 Salzano A, D'Assante R, Lander M, Arcopinto M, Bossone E, Suzuki T, Cittadini A. Hormonal replacement therapy in heart failure: focus on growth hormone and testosterone. Heart Fail Clin. 2019 Jul;15(3):377-391. doi: 10.1016/j.hfc.2019.02.007. Epub 2019 Apr 6. Review. PMID: 31079696

Introduction

To date, the mainstay of Heart Failure (HF) treatment is considered the counteraction of neurohormonal over-activation [1]. Indeed, according to the namesake pathophysiological model, even if the renin–angiotensin–aldosterone (RAAS) together with the adrenergic system plays a compensatory role in the early phases of the disease, they soon themselves become responsible for the progression of HF, triggering a vicious cycle of ever more deleterious increasing neurohormonal action [3,178]. Thus, the main target of HF therapy is to block this pattern using drugs as angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARBs), beta-blockers, mineral receptor antagonist (MRA), and the novel angiotensin receptor-neprilysin inhibitor (ARNI). However, because of the activation of alternative escape pathways limiting the ability to completely prevent the natural progression of HF, the positive effects of these drugs are limited.

In this context, a dangerous link exists between hormones and cardiovascular system [56-58,179,181]. To date, growing evidence supports the concept that hormonal deficiencies (HD) in HF have a high prevalence and a strong impact on the disease, with a promising possible role of hormonal therapy [67,184]. In this intricate scenario, growth hormone deficiency (GHD) and testosterone deficiency (TD) seem to play a critical role [50] in particular. Most studies in the field demonstrated that patients affected by these disorders display a more aggressive phenotype of HF. However, the impact of multiple and concomitant HD (including GH and IGF-1, Testosterone (T), Insulin Resistance, Thyroid, etc.) on adverse cardiovascular outcomes (e.g. hospitalization and mortality rate) in CHF is not completely elucidated although a growing body of evidence suggests that treatment of these might have a role in HF [247,304,305,308].

This review gives an overview of the current evidence regarding GH and T deficiencies in HF, reviewing the impact of their treatment in HF.

Growth Hormone treatment in HF

GH deficiency in heart failure

The prevalence of GH deficiency (GHD) ranges from 32% to 53% [55,63,64,261]. The literature suggests IGF-1 serum levels are decreased in HF patients when compared with healthy subjects [53,61,191,261,262]. Of note, this finding is particularly evident in more advanced HF patients [190,263] or those with cachexia [264,265].

Despite current guidelines suggesting insulin tolerance testing (ITT) as the gold standard test for diagnosis of GHD in adults [266-269], because hypoglycemia could be unsafe in HF setting, alternative tests are often used instead. The Growth Hormone Releasing Hormone (GHRH) and arginine test could be considered the more convenient option [261,270-273].

Several independent groups have demonstrated that GHD is *per se* related to impaired cardiovascular performance and increased peripheral vascular resistance, with a clear association between GHD severity, cardiac impairment [274], and an increased cardiovascular mortality [275]. Recently, combining stimulated pituitary responses with measures of the IGF-1 system in a cohort of HF patients, our group demonstrated that GHD patients displayed higher filling pressures with larger left ventricular (LV) volumes, and a more severe impairment of right ventricle systolic function respect of non-GHD patients [61]. Further, a significantly lower peak oxygen consumption (peak VO_2) and reduced ventilator efficiency have been demonstrated in the GHD group, resulting in impaired cardiopulmonary performance. Of note, patients with coexistent GHD and CHF display a higher risk of mortality compared to the GH sufficient cohort (HR 2.11, p = 0.021). With regard to HF with preserved EF (HFpEF), it has been demonstrated that GHD is a common finding, although to a lower degree when compared to HF with reduced EF [55].

In the acute setting, a relationship between GH status and outcome has been demonstrated [276]. In 537 acute HF patients, GH levels were increased in all patients who died or were readmitted to hospital within one year, regardless of their EF. In this setting, GH levels were independently predictors of poor outcome in HFrEF but not in HFpEF. When added to the ADHERE multivariate model [277], GH was able to ameliorate the net reclassification improvement (NRI) as well as when added to the ADHERE model + NT-proBNP.. Despite some limitations (e.g. the single GH measurement taken in non-fasted patients) for the first time GH activity has shown promise as a prognostic marker in the acute settings. To date, no data is available around the prevalence and role

of GHD in acute HF.

Mechanisms of GHD in CHF

Local hypo-perfusion of the hypothalamic-pituitary axis (with somatotropic cell death from mismatched arterial and venous flow) [261,278], alterations related to chronic disease (as well as in other several chronic wasting conditions characterized by liver congestion, inflammatory activation, and cytokine overexpression) [82], and/or effects of CHF therapy on GH/IGF-1 axis [279,280]have been considered as possible explanatory pathophysiological mechanism of the occurrence of GH/IGF-1 impairment in CHF. Current understanding suggests a multiple model in which the synergy and the interaction of these different molecular pathways with the concomitant pharmacological action could explain the GH/IGF-1 axis alterations [309,310].

Growth Hormone treatment in HF

Data derived from animal models have shown several beneficial effects of GH on cardiac morphology and function, peripheral vascular resistance, and survival [282-286]. With regard to the pathophysiological mechanisms, it is well recognized that GH therapy in myocardial infarction in the early stage is able to improves LV function, mainly through a reduction of the pathologic LV remodeling [287]. However, results in the human CHF population remain inconsistent [51,288-302,311] when applied into clinic studies (see table 1). In the early nineties, preliminary pilot studies verified the effects of GH treatment leading to positive results both in CHF [288,289,292-294,297,299,311] as well as in other clinical settings [303]. However, when these results were tested in randomized controlled clinical trials [290,291,296-299,311] previous encouraging results were not confirmed. Specifically, in a population of 50 CHF patients, Osterziel et al. [291] demonstrated an increase in LV mass strongly related to serum IGF-I changes, but not a clinical improvement in patients with CHF. Of note, EF increased noticeably in patients with the larger increases of IGF-1 during GH treatment [296]. Isgaard et al. [290] as well as Acevedo et al. [299] showed neutral effects of GH therapy in CHF patients, although the latter demonstrated a positive relationship between the changes in oxygen consumption (peak VO_2) and EF in GH therapy group. In patients with idiopathic dilated cardiomyopathy, a beneficial modulation of circulating cytokines and soluble adhesion molecules has been demonstrated by Adamopoulous et al. in a randomized crossover trial, together with positive effects of GH in contractile reserve and LV volumes [298]. Our group confirmed the relationship between cytokine network, cardiac performance, and survival in HF [42]. Finally, in a double blind placebo randomized controlled trial,

Fazio et al. [311] demonstrated that GH increased IGF-I serum levels, improved New York Heart Association (NYHA) functional class, and cardiopulmonary performance without affecting lung function parameters. None of these effects were observed in the placebo group.

Considering these results, with the aim of clarifying the overall effect of GH treatment in HF, Le Corvoisier et al. performed a pooled meta-analysis of effects of GH therapy in CHF [304]. In brief, sustained GH treatment improves cardiovascular parameters in HF (in particular LVEF) with a reduction in general vascular resistance. These changes were associated with a positive long-term modification in cardiac morphology, due to an increase in LV wall thickness and a reduction in LV diastolic diameters [304]. Tritos et al, in different meta-analysis, demonstrated that GH treatment resulted in an improvement in systemic vascular resistance, an increase of cardiac output, LVEF, and NYHA class level together with a significant improvement in exercise duration and in peak VO_2 [305]. No effects where observed with regard to diastolic function. It is necessary to underline that there is a great inhomogeneity of data with regard to study duration, target dose, end-points, and the lack of assessment of GH status [67]. When trials were separated in two groups according to target dose of all clinical trials, Le Coirvoisier et al. demonstrated that a low GH dose affected only NYHA class and exercise duration, whereas a high GH dose positively affected ventricular morphology, cardiopulmonary performance and clinical status. Subgroup analysis according to the median of IGF-1 increase showed that larger IGF-1 increases corresponded with a more significant effect size for LV morphology and cardiopulmonary performance. However, a neutral effect was observed in trials with smaller IGF-1 increases [304]. Recently Brankovic et al, in a cohort of 263 CHF patients from the Bio-SHiFT study, demonstrated that the temporal patterns of insulin-like growth factor binding proteins (IGFBPs), in particular IGFBP-1, IGFBP-2, and IGFBP-7, predict adverse clinical outcomes and could in part explain the non-responsiveness of some patients to GH treatment [312-314],

Taken all together, several findings from these meta-analyses can be considered and translated into clinical practice. First, before starting GH administration a proven impairment of GH/IGF-1 status, (e.g. GHD) should be confirmed. Second, dose titration should be individualized. Third, GH therapy should not be administered in all patients with CHF, but rather the subgroup of patients in which GH treatment is more effective (e.g. mild to moderate CHF, considering the GH resistance state of the more advanced disease [265]).

In a randomized, single-blind controlled proof-of-concept trial, our group were the first to assess the effects of GH therapy were assessed only in those patients suffering from GHD [64]. In this study patients underwent a GHRH + Arginine provocative test with the aim of understanding GHD status;

thus, only when GHD was diagnosed GH treatment was started. After 6 months of GH replacement treatment, we observed a decrease in circulating NT-pro-BNP levels, an improvement of LVEF (from 33 ± 2 to 36±2 %, p<0.01), and of peak VO_2 (from 12.9 ± 0.9 to 14.5± 1.0 ml/kg/min, p<0.005), together with a better quality of life score and flow-mediated vasodilation. Because of these positive findings, we extended this study with a 4-year follow up [65] to establish the long-term effects of GH therapy in HF. At 48 months, GH replacement therapy was still associated with a significant reductions of LV end-diastolic, end-systolic volumes, and circumferential wall stress, with LV reverse remodeling accordingly, resulting in an increase in LVEF. Further, in the GH group the improvement of peak VO_2 was remarkable (final treatment effect of 7.1 ± 0.7 ml/kg/min in the GH group compared to of -1.8 ± 0.5, p < 0.001 in the control group). In addition, whilst the study was not designed for hard clinical endpoints, a significant difference in the composite of death and hospitalization for HF was observed. To date, this is the longest study that investigated the effect of GH in CHF.
Further research is needed to verify the efficacy of GH replacement therapy and our group is beginning a randomized double-blind placebo controlled trial of GH replacement therapy in CHF patients with coexisting GHD.

Side effects

General data derived from clinical studies have showed the safety of GH treatment [257,315]. If used with the correct dosage, GH treatment could be considered without major collateral effects. The initial warning of worsening ventricular arrhythmias, perhaps related to the very high dosage of GH used by Frustaci et al [289] has not been reported by subsequent studies.

It is well known that an excess of GH, such as in acromegaly, could lead to an increase in ventricular arrhythmia[316]. When all clinical studies were revised by Le Corvoisier et al [304], there were no differences in the incidence of major adverse effects (death, worsening of HF, increased salt retention and ventricular arrhythmias) between treatment and placebo arms. Finally, after 48 months of follow up, Cittadini et al. reported no major adverse events in the patients treated with GH at long-term [65]. In particular, in the final cohort of 17 patients, only two cases of arthralgia, a well-known transient side effect of GH replacement, were reported. The relatively low prevalence of adverse effects observe in this study is in line with data derived from the largest database on GH treatment available [306].

Testosterone treatment in HF

Testosterone deficiency

The interaction between hormones and cardiac morphology and function is something well recognized [50,57,89,181,222], with testosterone (T) showing a key role.

Some authors suggest the hypothesis of a direct action of T on the androgen receptors (AR) in myocardial cells [223]. T and its active counterpart dihydrotestosterone (DHEA) interact with AR expressed externally on the cell surface and internally in the cytosol. Extracellular AR seem to be more linked to the activation of ions channels and signaling pathways. Intracellular AR are more involved in the androgen target genes modulation, resulting in a fine regulation of myocardial and vascular cell activity [224]. Additionally, there is evidence that metabolic profile is affected by polymorphisms of AR [225].

In the general population, the decline in androgen levels with aging contributes to sarcopenia, visceral adiposity deposition, and osteopenia. From the forth decade of life, there is a decrease in serum T level of about 3.5 ng/dL per year (the so-called phenomenon of andropause) [317]. A similar decline has been described for circulating DHEA-S levels, from the second-third decade of life[318].

To date, it is important to underline that the criteria for TD are not widely accepted. Previous guidelines recommended to consider a TD when morning total T level drops below 300 mg/dL in two or more occasions in presence of consistent symptoms and signs [319]. Current guidelines suggest that if the total T has been measured with an assay that has been certified by an accuracy-based standardization or quality control program (e.g. Centres for Disease Control and Prevention (CDC)) clinicians can use as the lower reference range for total T in healthy, non-obese young men (aged 19 to 39 years) a value of 264 using the 2.5th percentile, or 303 using the 5th percentile (with a preference for the first one value) [320]. In patients whose serum TT concentrations are just above or below the lower limit of normal (e.g., 200 to 400 ng/dL), clinicians should also measure free T. It is important to underline that low T concentrations without symptoms or signs of TD do not establish a diagnosis of hypogonadism. Diagnosing TD in the elderly is challenging due to the significant overlap of signs and symptoms with those of normal aging. Epidemiological studies demonstrated that the incidence of hypogonadal levels of testosterone in men is 20% in people 60 or older and 30% in 70 or older [321].Independent groups have showed that TD in older men is associated with an increased risk of death [322,323] supporting the finding that low serum T levels might be used as a marker of poor survival [322].

It has been demonstrated that the prevalence of TD in patients with CHF syndrome is about 25-30% [55,63,262,324-326] associated with an impairment of skeletal muscle function and cardiovascular performance [196] and contributes to the negative outcome [234,327,328]. In addition, decreased circulating

levels of DHEA-S are able to predict mortality in elderly [329]. Recently, Enina and colleagues demonstrated that T levels could be useful as predictors of CRT response in male HF patients [330] .

TD has also been linked to the dysregulation of other metabolic profiles. The association between T and the lipid profile is still a matter of debate but there is some evidence that T inhibits the evolution of preadipocytes into mature adipocytes, thus reducing total fat mass and increasing net lean mass [228,229]. Current evidence suggest that metabolic effects of T on the regulation and on the expression of lipid metabolism are tissue-specific with the final result of reduced fat deposition in sites such as the arterial wall and the liver [230]. However, testosterone improves IR; there is evidence that those with low testosterone levels have an increased risk of metabolic syndrome and new onset type 2 diabetes [231,232].

Testosterone treatment

Taking into account the relationship between T and cardiovascular system, several studies have investigated the role of T in the treatment of CHF [331] (see table 2).

Pugh et al conducted a study on 12 stable male patients [233] to assess the effects of T in HF. In particular, using a pulmonary flotation catheter, central haemodynamics were measured over 6h after administration of a single dose of T (60 mg orally) on two consecutive days. An increase in cardiac output in the active arm was observed. Of note, effects were more evident in patients with a lower baseline circulating T level and no side effects were reported.

This research continued with a double-blind, placebo-controlled trial, enrolling 20 male CHF patients few years later [234]. In this trial, the treatment group (100mg every 2 weeks for 12 weeks) displayed a significant improvement in the 6 minute walking test (6MWT), in particular in the maximum distance, and in quality of life, assessed by the Minnesota living with heart failure questionnaire (MLWHFQ).

The same group performed a further larger randomized, double-blind, placebo control study, involving 76 CHF patients [235]. In the active group (5 mg/day administered by an adhesive skin patch), authors demonstrated a clinical improvement as testified by the decrease of at least one class of NYHA class (30% of patients in treated arm vs 8 % in untreated). Even if T did not change LV morphology or function, a significant improvement in cardiovascular performance was demonstrated (i.e. shuttle walk distance). With regard to QT duration, despite in a previous report the same group showed that T therapy was able to reduce QT duration [237] and these data were confirmed by a

subsequent study by an independent group [238], in this larger cohort no effects of T on QT duration were observed.

Reassuringly, no significant effects were observed with regard to skeletal muscle bulk and strength, weight, and other cardiovascular parameters (e.g. heart rate and blood pressure). In line with a previous report [236] where different methods of T administration were tested (i.e. acute versus chronic and either via transdermal patch or intramuscular injection) no differences were detected with regard to circulating concentrations of cytokines (e.g. TNFα, IL-1β, and IL-6)[42].

To further explore this, Miramadi et al [241] performed a double-blind, placebo-controlled trial, involving 50 male patients. The treatment group received an intramuscular long-acting androgen injection (1mL of T enanthate 250mg/mL) once every 4 weeks for 1, 5 months. They displayed a significantly increased trend in the 6 minute walking distance and in quality of life scores.

However, recently Navarro-Penalver et al performed a prospective, randomized, double-blind, placebo-controlled, and parallel-group trial comparing T with placebo in males with HFrEF and TD on 25 CHF patients [332]. Their finding were not consistent with previous reports. Indeed, after 12 months of long-acting intramuscular T administration, neither clinical symptoms (e.g. NYHA class, MLWHFQ score), functional capacity (e.g. 6-minute walk test, LVEF) nor other secondary parameters (NT-pro BNP levels, lipid profile, BMI) were observed to improve. With regard to safety, the prostate specific antigen (PSA) levels did not change, and no other adverse side effects were observed.

In the elderly para-physiological decrease of skeletal muscle strength, resulting in early fatigue and reduced exercise tolerance is well known [239,240]. With this in mind, Caminiti et al [195] performed a double-blind, randomized, placebo-controlled study focused on elderly patients with stable CHF (median age 70 years). In this trial, the authors demonstrated that T treatment (12 weeks with very long acting intramuscular 1000 mg testosterone undecanoate) improved functional exercise capacity and muscle strength. Of note, peak VO_2 and index of muscular strength (e.g. peak torque and quadriceps maximal intentional contraction) improved in the active group whereas no effects were observed in the placebo group. Glucose tolerance (expressed as HOMA-IR) and baroreflex sensitivity also showed a significant improvement.

The role of exercise rehabilitation in HF is recognised worldwide [333]. Stout et al [242] investigated the effect of T supplementation when added to a program of exercise rehabilitation. In this report, 28 male patients with CHF and low T status (defined as <432 ng/dL), were randomly allocated to exercise with T or exercise with placebo. T treatment (an intramuscular (gluteal) injection of 100 mg testosterone/mL once a fortnight for 12 weeks) induced positive changes of TD symptoms, with

improvements in aerobic fitness, leg strength, and depression. These effects were not observed in the placebo group. Following this, an interesting study by dos Santos and colleagues [243] investigating male patients with advanced CHF (NYHA class III) and TD were randomized to 3 arms: cycloergometer training, T (intramuscular injection of T undecylate), or both. The authors not only confirmed beneficial effects of T when added to an exercise training in patients with CHF, but also supported the concept that is the peripheral action of T the main reason for the improvement in cardiovascular performance, providing novel information about the effects of the combined therapies on total body composition, cardiovascular performance, hormonal status, and quality of life.

Current cardiovascular research asks how and whether sex and gender differences are able to modify outcomes [82,179,244-246,334]. The effect of T treatment was investigated in 36 female patients treated with testosterone (300 mg, patch transdermal, twice per week). In this study, an improvement in 6MWT distance and in peak VO_2 consumption were observed, with a positive effect on metabolic parameters (e.g. insulin resistance) [196]. A separate study of female patients demonstrated a direct effect of T in the ventricular repolarization in vivo (a shortening effect) [238].

Meta-analysis performed by Toma and colleagues [247] demonstrated that T supplementation in patients with CHF is associated with a clinically significant improvement in the cardiovascular performance, expressed as 6MWT distance, VO_2 peak, and NYHA class. Of note, this improvement was superior when compared to the effect of other CHF treatment strategies (e.g. beta blockers and ACE inhibitors).

Few years later, Wang at al performed a meta-analysis including 8 trials [308]. Authors showed that T treatment improved significantly exercise capacity (6MWT and shuttle distance), muscle strength, and electrocardiogram indicators (i.e. decreased Q-T intervals) but did not significantly change EF, NT-proBNP, clinical parameter (i.e. blood pressure and heart rate), and Inflammatory markers (i.e. TNF-α, high-sensitivity C-reactive protein and IL-6).

Side effects

None of the trials for T replacement showed safety concerns. No major events were reported associated to the treatment [247,308] and there were no significant changes in prostate specific antigen assays. Skin reactions were described in the studies using topical T but were transient and not severe. Testosterone treatment could be considered safe in HF.

Clinical perspectives

A growing body of evidence led to the hypothesis that CHF could be considered as a Multiple Hormone Deficiency Syndrome (MHDS). It has been demonstrated that deficiencies in the main

anabolic axes cannot be considered as mere epiphenomena, and are very common in HF, with a clear association with poor cardiovascular performance and outcomes. Among these, GHD and TD play a pivotal role and the replacement treatment is an innovative therapy that should be considered in CHF. A prospective multicenter clinical registry: The T.O.S.C.A (Trattamento Ormonale nello Scompenso CArdiaco) will investigate this issue [181,185].

Several points need further discussion. First, it is important to underline that, to date, different patient populations and different routes and dosage of hormone replacement treatment have been tested. Second, all studies only involved patients with reduced EF and no data is available regarding patients with mildly reduced or preserved EF. Thirdly, due to the short-term nature of most trials, there is a paucity of data regarding strong end-points (e.g. mortality and/or hospitalization).

With regard to GHD, the latest American Heart Association (AHA) statement on management of dilated cardiomyopathies [307] recommends, with a strong level of consensus, that GHD should be investigated in patients with DCM when other signs and/or symptoms of this clinical disorders are present (Level of Evidence C). They recommend that treatment of the primary disorder be given to all patients with coexisting DCM.

Another consideration is that basal T status (i.e. TD) was used in only a few studies as a parameter of enrolment. Recently, in the context of HD in CHF [52,82,185,248,249], the idea that hormone treatment should be used only when a deficit of the axis of interest is diagnosed rather than in all patients, represents a paradigm shift and has improved the overall outcome. To date, this represents the most promising question in this field [64,65,67,184]. With this in mind, a flowchart to manage the GHD in CHF patients has been developed and suggested for clinical use [315].

Conclusions

The use of GH and T in the treatment of CHF is promising and in need of further review. Considering all the literature and the authors' own experiences, some suggestion can be considered:

(I) GH status and T status should be investigated in all CHF patients, in particular in who displays signs and/or symptoms of these conditions

(II) GH and T should be administered only in those patients with an impairment of GH/IGF-1 status, e.g. GHD, or androgens, i.e. TD, as a replacement therapy

(III) An individualized dose titration of GH and T should be considered for each patient depending on clinical and neuro-hormonal response.

Considering this high frequency of multiple HD in CHF, no data is available regarding combined hormone treatment. This is important, considering that these two treatments seem to exert different action on cardiovascular system, being GH more effective on central pathophysiological mechanisms and T more effective on the peripheral mechanisms (figure 1).

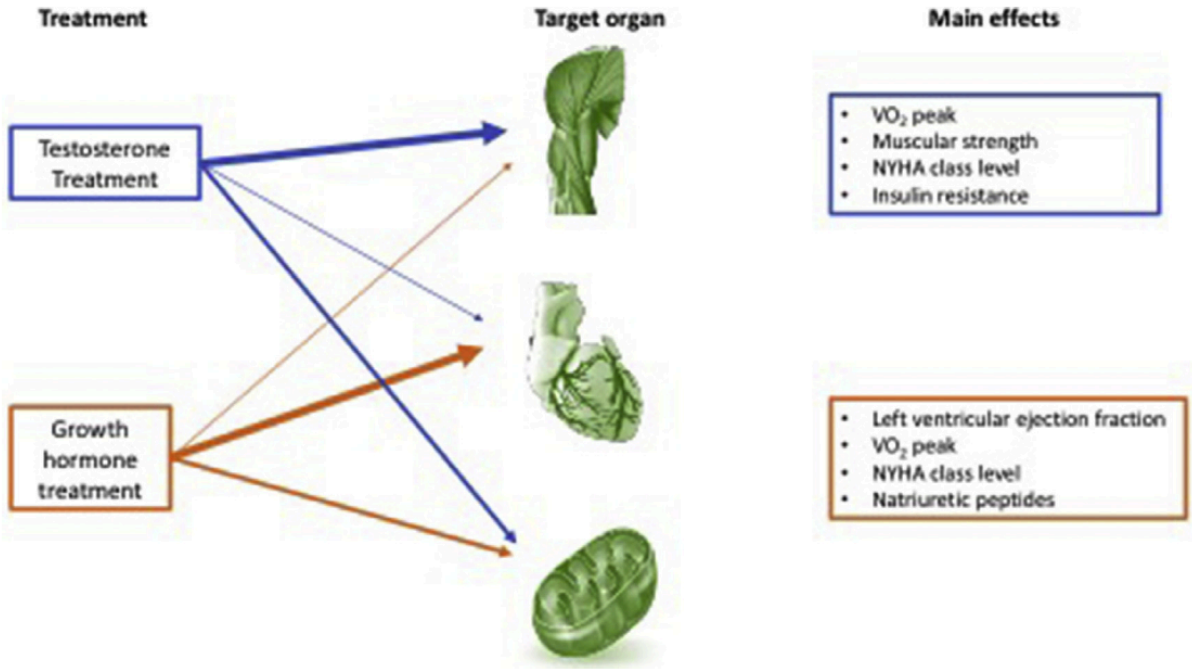


Fig. 1. Effects of testosterone treatment (*blue arrows*) and growth hormone treatment (*orange arrows*). The weight of the arrows indicates the impact of the treatment with regard to the target organ: muscle, heart, and metabolism.

Table 1. Summary of studies on growth hormone treatment in heart failure

First author (year)	Sample size	Gender	Mean age (years)	Baseline NYHA Class (mean)	Growth hormone status	Growth hormone supplementation	Type of trial	Trial duration
Fazio S, 1996 [288]	7	Male (71) Female (29)	46	2.7± 0.2	NA	4 IU every other day sc		3 months treatment and 3 months after
Frustaci A, 1996 [289]	5	Male (25) Female (75)	28	NR	NA	4 IU daily im		3 months
Isgaard J, 1998 [290]	22	Male (64) Female (36)	60	3 ± 0.5	NA	0.1 IU/kg/wk for 1 week, thereafter 0.25 IU/kg/week	R, DB, PC	3 months
Osterziel KJ, 1998 [291,296]	50	Male (86) Female (14	54	2.3 ±0.6	NA	0.5 IU or placebo daily, sc. Dosage increased every second day by 0.5 IU until a final dose of 2 IU daily was reached	R, DB, PC	3 months
Gent-Zotz S, 1999 [292]	7	Male	55	2.4 ± 0.5	NA	2 IU daily sc		3 months treatment and 3 months after
Jose VJ , 1999 [294]	6	NA	nr	3.4 ± 0.5	NA	2 IU every other day sc		6 months treatment and 6 months after
Spallarossa P, 1999 [293]	20	Male	62	2.7 ± 0.4	NA	0.0006 IU/kg daily sc. Gradual increase until 0.02 IU/kg/ daily	R, PC	6 months
Smit JW, 2001 [295,300]	19	Male (85) Female (15)	65	2.5 ± 0.7	NA	Start 0.5 IU daily sc Dosage increased to 1.0 IU/ day after 2 weeks and to 2.0 IU/day final dose	R, C	6 months
Napoli R, 2002 [297]	16	Male (75) Female (25)	54	2.25 ± 0.4	NA	4 IU every other day sc	R, DB, PC	3 months
Acevedo M, 2003 [299]	19	Male (90) Female (10)	57	3	NA	0.035 IU/Kg daily sc	R, DB, PC	2 months
Adamopoulos S, 2003 [298]	12	Male (67) Female (33)	50	3	NA	4 IU every other day	R	3 months treatment and 3 months after
Fazio S, 2007 [311]	22	Male (69) Female (31)	55	2.45 ± 0.5	NA	4 IU every other day	R, DB, PC	3 months
Cittadini A, 2009 [64]	56	Male (72) Female (28)	62	2.6 ± 0.7	GHD	0.012 mg/kg every other day	R, SB, C	6 months
Cittadini A, 2013 [65]	31	Male (84) Female (16)	62	2.7 ± 0.1	GHD	0.012 mg/kg every other day	R, SB, C	48 months

IU: International Unit; sc: subcutaneous; NYHA: New York Heart Association; NR: not reported; NA: not assessed; R: randomized; SB: single blind; DB: double blind; PC: placebo-controlled; C: controlled

Table 2. Summary of studies on testosterone treatment in heart failure

First author, year	Sample Size (n)	Gender (%)	Mean age (years)	Baseline NYHA Class (mean±ds)	Testosterone deficiency	Testosterone supplementation	Type of trial	Trial duration
Pugh PJ , 2003 [233]	12	Male	NR	NR	NR	60 mg orally day one and day 2	R, DB, PC	2 days
Malkin CJ, 2003 [237]	20	Male	61.5	2.5±0.5	NR	Sustanon 100mg IM every 2 weeks	R, DB, PC	12 weeks
Pugh PJ, 2004 [234]	20	Male	62	NR	NR	Sustanon 100 mg IM every 2 weeks	R, DB, PC	12 weeks
Malkin CJ, 2006 [235]	76	Male	64	2.46±0.6	NR	Androderm 5 mg every 24 hours	R, DB, PC	12 months
Caminiti G, 2009 [195]	70	Male	70	2.46±0.5	NR	Long-acting testosterone undecanoate (Nebido) IM at 0, 6, 12 week	R, DB, PC	12 weeks
Iellamo F, 2010 [196]	32	Female	68.7	3±0	NR	Transdermal testosterone	R, DB, PC	6 months
Schwartz JB, 2011 [238]	84	Male (69%) and Female (31%)	70.35	NR	NR	Male: long-acting testosterone undecanoate 1000 mg IM at 0, 6, 12 week. Female: transdermal testosterone 33 mcg (Intrinsa)	R, DB, PC	12 weeks
Stout M, 2012 [242]	41	Male	67.2	2.5±0.5	low testosterone status	Sustanon 100 mg IM every 2 weeks	R, DB, PC	12 weeks
Mirdamadi A, 2014 [241]	50	Male	60	2.38±0.57	NR	Long-acting testosterone enanthate 250 mg IM every 4 weeks	R, DB, PC	12 weeks
Dos Santos MR, 2016 [243]	39	Male	51	3	Testosterone deficiency	Long acting testosterone undecylate IM	R	48 weeks
Navarro-Penalver, 2018 [332]	29	Male	64.5	2.3±0.47	Testosterone deficiency	long-acting undecanoate testosterone 1000mg, IM at 0 and then every 3 months	R, DB, PC	12 months

R: randomized; DB: double blind; PC: placebo-controlled; NR: not reported; sc: subcutaneous; IM: intramuscular

3.3.4 Salzano A, Marra AM, Arcopinto M, D'Assante R, Triggiani V, Coscioni E, Pasquali D, Rengo G, Suzuki T, Bossone E, Cittadini A. Combined effect of growth hormone and testosterone replacement treatment in heart failure. A hypothesis generating pilot study ESC Heart Fail. 2019 Nov 7. doi: 10.1002/ehf2.12520. PMID: 31696666

Background

Growing evidence suggests that multiple hormone deficiencies (MHD) are common in heart failure (HF) patients and are related to impaired cardiovascular performance and poor outcome [48,53-55,61,63]. Preliminary clinical trials of single hormone replacement therapy to treat growth hormone deficiency (GHD) [64,65,335] or testosterone deficiency (TD) [195,234,235] have reported promising results, showing both safety and effectiveness in HF patients.

Notably, although MHD syndrome affects at least one third of the HF population [53,55,63], no data are available so far dwelling upon combined GH and T treatment in HF patients. This information is of great relevance since both GH and T are endowed with potential adverse effects.

Aims

The aim of the current hypothesis generating pilot study was to assess the effects of combined correction of MHD on cardiovascular performance and clinical status in HF patients with reduced ejection fraction (HFrEF). Thus, we have investigated for the first time the effectiveness and safety of combined GH and T replacement therapy in the clinical setting of stable chronic HF.

Methods

Five stable chronic HFrEF patients (New York Heart Association - NYHA classes II to III), a sub-group of the control cohort of a previous protocol (NCT01576861) [64,65], with the diagnosis of both GHD, using the growth hormone releasing hormone (GHRH) plus arginine stimulation test, and TD, according to published guidelines [266,320], underwent 1 year of GH replacement therapy to correct GHD by subcutaneous injections of somatotropin (recombinant DNA origin) (Saizen©, Merck-Serono International, Geneva, Switzerland) at a dose of 0.012 mg/kg every second day, on top of guideline based HF therapy [1]. After 12 months, because of the presence of signs and symptoms of TD, patients were evaluated eligible to add T replacement treatment (intramuscular testosterone undecanoate, Nebid©, Bayer, Germany) at a dosage of 1000 mg every 3 months. Due to the lack of data regarding safety of combined treatments, in order to minimize potential side effects (e.g. water retention,

peripheral oedema) GH and T administration was not started simultaneously. Of note, this strategy allowed us to better characterize additional or synergistic actions of both hormones.

Each patient underwent a complete M-mode, 2-dimensional and Doppler echocardiographic examination, and an incremental symptom-limited cardiopulmonary exercise test (CPET) on a bicycle ergometer at baseline (BL), after 1 year of GH treatment (V1), and after 1 year of combined GH + T treatments (V2). Samples collection and all procedures performed at baseline were repeated annually, while intermediate visits (each 6 months) included clinical assessment and record of clinical events. Patients were treated with evidence-based therapies (beta-blockers, ACE/ARBs, and MRA) at a targeted dose [1] from at least three months before the start of hormone deficiencies (HD) replacement therapy in order to minimise possible confounding effects.

Normally distributed continuous variables were expressed as mean ± Standard Deviation (SD), whereas continuous data with skew distributions were expressed as median and interquartile range [IQR]. Categorical variables were expressed as counts and percentages. The distribution of the parameters was tested with Kolmogorov-Smirnov test. The intergroup differences were tested with the one-way ANOVA, with Bonferroni correction as appropriate. Normally distributed parameters were compared between two groups using the t test paired sample test. Non-normally distributed parameters were compared between two groups using Mann-Whitney U-Test. *P* values <0.05 were deemed statistically significant. All data were analysed using IBM SPSS Statistics (v24, IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics of enrolled patients are depicted in Table 1. One-year of GH treatment resulted in a significant increase in left ventricular (LV) ejection fraction (EF) of 5.4% and in VO_2 peak of 19.3% (approximately 1.8% and 3.4 ml/min/kg respectively), paralleled by a non-significant trend in decreasing VE/VCO_2 slope (about 7.6% corresponding to 2.2 ml/min/kg) (Table 2). Consistently, a statistically significant reduction in NT-pro BNP levels of 35% and a significant improvement in NYHA functional class were observed. A small increase in HOMA-IR was detected (although not statistically significant). No relevant effects were observed with regard to body mass index and handgrip performance. With the addition of T replacement therapy for an additional year, cardiopulmonary and skeletal muscle performance improved, while no effect was detected on LV structure. Notably, EF significantly increased of an additional 12% (final delta change: +18%, $p<0.01$) while VO_2 peak significantly increased by an average of 5.84 ml/min/kg, (+ 27.7%, final delta change from baseline

52.4%, p<0.01). A 17.5% improvement in handgrip performance was equally observed (final delta change of 25.8%, p<0.01). All these results were paralleled by a striking improvement in NYHA class and reduction in NT-pro BNP levels (p<0.01) (Figure 1). As expected, total testosterone (TT) and sex hormone binding globulin (SHBG) levels, that remained substantially stable during the first phase of the study, changed following T treatment. In particular, TT increased whereas SHBG decreased (respectively +132.9% and – 40.1%), resulting in a significant increase in free T and bioavailable T (+261.5% and +308.3% respectively). HF treatment dosages, (all patients were treated with evidence-based therapies - beta-blockers, ACE/ARBs, and MRA- at a targeted dose from at least three months before baseline) remained stable (expressed as >75% of the targeted dose) during the study period, as well as non-pharmacological approaches (e.g. diet). No patients received anticoagulant drugs. Adverse effects were reported neither during GH alone (e.g. fluid retention, hypertension, paraesthesia, joint stiffness, peripheral oedema, arthralgia, myalgia, and carpal tunnel syndrome) nor during combined therapy (GH+T). Following T treatment, no significant changes were observed with regard to prostatic specific antigen and haematocrit. One patient during GH treatment and 1 patient during the GH+T treatment had a self-limiting flu-like syndrome, that did not require any medical intervention and were not related to drug administration. Finally, no major adverse cardiovascular events occurred during the study period.

Conclusions

The current hypothesis generating pilot study suggests that combined hormone replacement therapy yields overall beneficial effects on a wide array of cardiovascular parameters. Consistently with previous findings related to GH or T treatment [247,304,305,315,336], in our population GH appears to improve mostly LV architecture and function (as mainly demonstrated by the EF improvement) [65,304,305], while testosterone therapy results in ameliorated skeletal muscle performance, possibly due to peripheral vasodilation and improvement in oxygen delivery to skeletal muscle, and slightly reduction in insulin resistance [195,247,308]. Of note, after the 2 years follow-up, no drug-related adverse effects were observed suggesting that both GH and T treatment are safe, even if combined, as previously showed in healthy individuals [337-339]. Finally, no major adverse cardiovascular events occurred during the study.

The present finding is in line with the notion that HD can be considered as a novel, safe, and promising therapeutic target in HF [49,67]. In this regard, the American Heart Association recommends to test for GHD in patients with dilated cardiomyopathy who have signs and symptoms of GHD [340],

and the eventual presence of the primary deficit in GH levels should be appropriately treated (namely GH Cardiomyopathy) [340]. Most recent European guidelines considered T therapy as a possible treatment for cachexia and sarcopenia in combination with nutritional supplements [1].

There are several limitations of the current study. First, it involved only a small group of patients enrolled from a single centre. Second, the lack of a placebo arm. Further, it has not been designed to provide mechanistic explanation of the observed phenomena. Finally, it is not possible to clearly discriminate the effects of the two treatments on each different variable. All these limitations are related to the design of the protocol, and further investigations in more robust randomized clinical trial studies are needed. However, the strength of the present report is to be the first that investigated the effects of combined GHD and TD replacement therapy in HF. Thus, these promising preliminary results could be viewed as a background for the implementation of more robust clinical trials.

In conclusion, despite several limitations, data from this hypothesis generating pilot study support the idea that the combination of GH and T replacement therapy in the treatment of concomitant HD seems to have beneficial effect on the cardiovascular performance in HF patients. However, further studies on larger populations and with a more robust study design are needed.

Table 1 Detailed characteristics of each patient at time of enrolment

ID patients	1	2	3	4	5
Age (year)	62	55	70	67	48
BMI (kg/m^2)	30	28	31	25	36
NYHA class	2	3	3	2	3
EF (%)	40	26	27	39	35
Peak VO_2 (mL/min/kg)	20.9	13.8	18.6	15.8	19.2
VE/VCO_2 slope	25.8	34.6	22.5	28.1	32.4
NT-pro BNP (pg/mL)	211	497	1031	352	992
ESVi (mL/m^2)	174	179	178	140	187
EDVi (mL/m^2)	291	242	244	229	288
Handgrip (kg)	31	33	21	27	43
Glycaemia (mg/dL)	87	98	92	85	79
Insulin (microU/mL)	17.15	5.05	3.38	4.11	14.5
HOMA IR index	3.68	1.22	0.77	0.86	2.82
IGF-1 (ng/mL)	51	63	46	30	133
Total Testosterone (ng/dL)	202	190	187	142	144
SHBG (nmol/L)	36.2	57.9	24.2	43.4	36.5
Free testosterone (ng/dL)	3.60	2.34	3.40	1.52	2.39
Bioavailable testosterone (ng/dL)	84.5	58.8	97.8	52	60.4

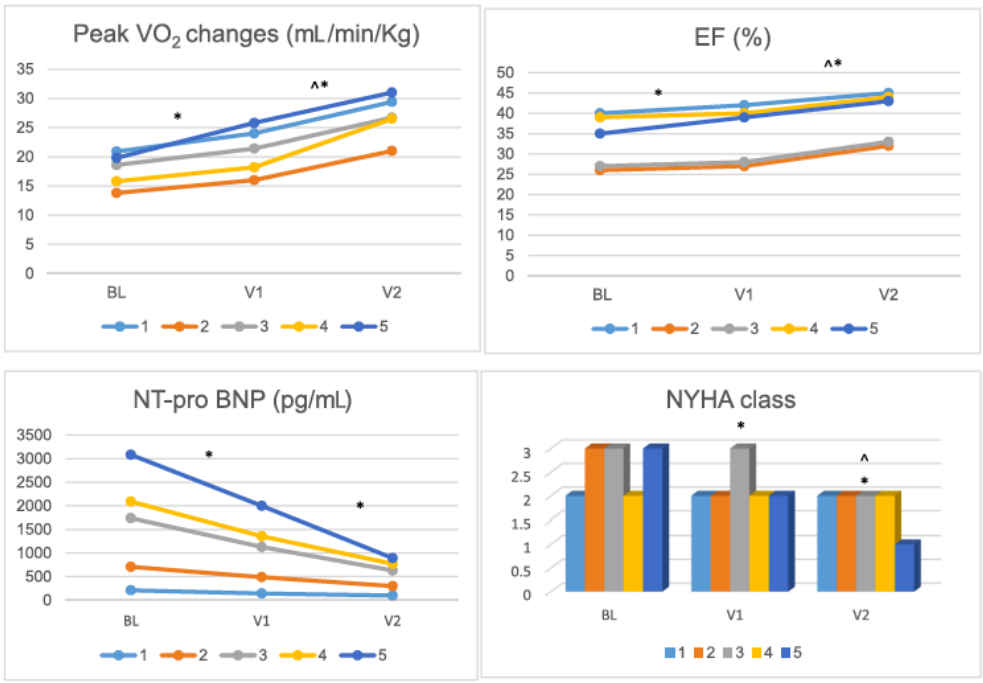
BMI, body mass index; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; HOMA-IR, homeostasis model assessment- insulin resistance; IGF-1, insulin growth factor-1; NT-pro BNP, serum amino terminal fragment of the pro-hormone brain type natriuretic peptide; NYHA, New York Heart Association; VCO_2, carbon dioxide production; VE, ventilation per minute; VO_2, oxygen consumption.

Table 2 Value at baseline (BL), after 1 year of growth hormone (GH) treatment (V1), and 1 year of GH treatment + testosterone treatment (V2); delta (Δ) change after 1 year of GH treatment (ΔV1), after 1 year of GH treatment + testosterone treatment compared from previous time point (ΔV2) and overall effect (ΔEP)

Characteristics	Values			ANOVA		Delta changes		
	Baseline	V1	V2	*f*	*P*	ΔV1	ΔV2	ΔEP
BMI (kg/m^2)	30 ± 4	29.8 ± 3.6	30.4 ± 3.8	—	—	−0.2 (−0.7%)	0.6 (2%)	0.4 (1.3%)
NYHA class (I/II/III)	0/2/3	0/4/1*	1/4/0*, §	3.7	0.05	−0.4 (15.4%)	−0.4 (18.2%)	−0.8 (30.8%)
EF (%)	33.4 ± 6.6	35.3 ± 7.12*	39.4 ± 6.34*, §	156	<0.001	1.8 (5.4%)	4.2 (11.9%)	6 (17.9%)
Peak VO_2 (mL/kg/min)	17.6 ± 2.8	21.1 ± 4.0*	26.9 ± 3.8*, §	24.5	<0.001	3.4 (19.3%)	5.84 (27.7%)	9.26 (52.44)
VE/VCO_2 slope	28.7 ± 4.9	26.5 ± 4.4	24.8 ± 4.2	—	—	−2.2 (−7.6%)	−1.7 (−6.4%)	−3.9 (−13.5%)
NT-pro BNP (pg/mL)	497 [352–1031]	347 [228–643]*	142 [120–330]*	8.5	0.01	−216 (−35.1%)	−221.6 (−55%)	−438.2 (−71.1%)
ESVi (mL/m^2)	171.6 ± 18.3	159.8 ± 13	145.4 ± 11.5	—	—	−11.8 (6.9%)	−14.4 (−9%)	−26.2 (−15.3%)
EDVi (mL/m^2)	258.8 ± 28.6	249.4 ± 26.7	242 ± 25.8	—	—	−9.4 (3.6%)	−7.4 (−2.9%)	−16.8 (−6.5%)
Handgrip	31 ± 8.1	33.2 ± 7.7	39 ± 8.2*, §	323	< 0.001	2.2 (7.1%)	5.8 (17.5%)	8 (25.8%)
Glycaemia (mg/dL)	88.2 ± 7.19	90.4 ± 2.97	87.2 ± 3.27	—	—	2.2 (2.5%)	−3.2 (−3.5%)	−1 (−1.14)
Insulin (microU/mL)	8.83 ± 6.47	9.96 ± 6.23	9.65 ± 6.07	—	—	1.12 (12.7%)	−0.3 (−3.1%)	0.82 (9.2%)
HOMA-IR index	1.9 ± 1.3	2.2 ± 1.3	2.1 ± 1.3	—	—	0.34 (18.8%)	−0.15 (−6.78)	0.19 (10.2%)
IGF-1 (ng/mL)	51 [46–133]	113 [100–158]*	146 [135–201]*	63	<0.001	45.5 (70.9%)	33.4 (30.3%)	79.2 (122.6%)
Testosterone (ng/dL)	187 [144–202]	179 [161–204]	389 [351–488]*, §	126	<0.001	−1 (−0.6%)	228.6 (132.9%)	227.6 (131.6%)
SHBG (nmol/L)	36.5 [36.2–57.9]	35.8 [35–59.3]	24.6 [17.5–33.1]*, §	3.98	0.05	0.7 (1.8%)	−16.2 (−40.1%)	−15.5 (−39.1%)
Free testosterone (ng/dL)	2.58 [2.51–4.17]	2.39 [2.34–3.63]	9.19 [7.8–12]*, §	23	<0.001	−0.36 (−11.9%)	6.9 (261.5%)	6.58 (218.3%)
Bioavailable testosterone (ng/dL)	60.4 [58.8–97.8]	54.9 [52.5–88.9]	230 [187–304]*, §	36.9	<0.001	−13.1 (−18.5%)	177.6 (308.3%)	164.5 (232.7%)

Data are expressed as mean ± standard deviation or median (interquartile range).
*$P < 0.05$ respect BL.
§$P < 0.05$ V2 respect V1.
BMI, body mass index; EDV: end diastolic volume; EF: Ejection fraction; ESV: End systolic volume; HOMA-IR: homeostasis model assessment-insulin resistance; IGF-1: insulin growth factor-1; NT-pro BNP: serum amino terminal fragment of the pro-hormone brain type natriuretic peptide; NYHA: New York Heart Association; VCO_2: carbon dioxide production; VE: ventilation per minute; VO_2: oxygen consumption.

Figure 1 Value at baseline (BL), after 1 year of growth hormone (GH) treatment (V1), and 1 year of GH treatment + testosterone treatment (V2) for selected parameters. EF, ejection fraction; NT-pro BNP, serum amino terminal fragment of the pro-hormone brain type natriuretic peptide; NYHA, New York Heart Association; VO_2,: oxygen consumption. $^{*}P < 0.01$ respect BL; $^{\wedge}P < 0.01$ V2 respect V1.



4. Chapter 4. The gut axis involvement in Heart Failure

A key model of interest and one of recent attention, is the association between HF and the gastrointestinal (GI) system [38], the so-called "gut hypothesis" [68]. In HF, it is well established that the presence of bowel hypo-perfusion, derived from reduced cardiac output, leads to mucosal ischemia that can increase bowel wall permeability and bacterial translocation. This bacterial translocation, through endotoxin production, is considered an important stimulus for elevated cytokine levels [341], which is associated with impaired cardiovascular performance and poor prognosis [42]. On the other hand, chronic bowel congestion causes oedema of the gastrointestinal tract, affecting the functions of the intestinal mucosa [342]; further, many other factors that may alter the gut flora (e.g. acute changes of fluid balance, acid/base disturbance, gastrointestinal dysmotility, and nutrient deprivation) are very common in HF, and might result in bacterial overgrowth or translocation. In this context, growing evidence suggests that one of the more prominent molecules associated with the link between HF and the gut is the choline and carnitine metabolic by-product, trimethylamine N-oxide (TMAO); produced by the gut microbiota from the precursor trimethylamine (TMA), with subsequent oxidation via the liver enzyme, flavin containing monooxygenase 3 (FMO3) [74,343,344].

In recent years, TMAO has been shown to be a strong prognostic biomarker in both acute [70] and stable chronic HF [69,72,73,345,346]. Furthermore, the magnitude of cardiovascular impairment and poor prognosis have been associated with the circulating levels of TMAO.

4.1 Salzano A, Cassambai S, Yazaky Y, Israr ZI, Wong M, Suzuki T. The gut axis involvement in heart failure. Focus on Trimethylamine N-oxide **Heart Fail Clin** 2020 Jan;16(1):23-31. doi: 10.1016/j.hfc.2019.08.001.PMID: 31735312

Role of TMAO in the pathophysiology of heart failure

TMAO metabolism

Trimethylamine N-oxide (TMAO), a gut microbiota mediated metabolite, is derived from the metabolism of choline and carnitine into trimethylamine (TMA). TMAO is thought to be the 'missing' link between the consumption of a 'Western diet' and cardiovascular disease risk [347]. Intestinal bacteria are implicated in the production of the precursor to TMAO, trimethylamine (TMA), through the anaerobic metabolism of choline and L-carnitine, derived largely from eggs and red meat as well as other sources (Figure 1). TMA lyase is the bacterial enzyme responsible for the generation of TMA, via *cutC/cutD* and *cntA/cntB* genes for choline and L-carnitine respectively [348]. The oxidation of TMA to TMAO occurs via the human liver enzyme, flavin containing mono-oxygenase 3 (FMO3) [349], which is involved in the detoxification of xenobiotics [350]. In healthy humans, TMAO is rapidly cleared from the body via urine [351] and is largely considered as a waste product of choline metabolism [352]. Therefore, renal function can significantly affect TMAO levels. However, individuals with FMO3 deficiency, have an accumulation of the toxic TMA, which is associated with a characteristic fishy body odour, known as trimethlyaminuria (TMAU). Consequently, TMAU sufferers are advised to limit the choline, lecithin, and carnitine consumption in their diet to ensure reduced levels of TMA production [353].

TMAO itself has a protective functional role in antagonising the destabilising effect of urea on proteins [354], particularly in saltwater fish and crustaceans [353]. Although TMAO is mainly excreted through urine in humans, a study investigating the direct effects of TMAO on pathological protein folding, found a stabilising chaperone effect whereby mutant protein folding such as with the cystic fibrosis transmembrane conductance regulatory (CFTR) protein, was rescued [355,356]. Whilst the protein stabilising effects associated with TMAO have been well known, TMAO has been shown to directly impact atherosclerotic plaque formation, reduce ventricular function in HF and induce renal fibrosis within *in vivo* and *in vitro* models [347,357]. These observations have been linked to an association with increased cardiovascular risk; population based studies within acute HF and acute myocardial infarction patients [70,358] have found elevated circulating levels of TMAO to be correlated with death and hospitalisation.

However, literature regarding the association of TMAO with cardiovascular disease is widely debated. Certain fish are known to have high levels of TMAO, yet the consumption of fish has been linked to cardioprotective effects due to omega-3 fatty acids [359,360], with no mention of the involvement of TMAO. Studies have also shown that the most significant increase in plasma TMAO levels is observed following the consumption of fish, compared to other sources of protein [361]. These studies highlight the discrepancies regarding the effect of TMAO *in situ*, yet studies focusing on mechanistic investigations have previously shown that TMAO is causal of atherosclerosis, platelet hyper-reactivity and cardiac fibrosis in a murine model of chronic HF [362], therefore associating a direct link between TMAO and the propagation of cardiovascular risk factors.

TMAO and diet

Plasma TMAO levels are determined by factors including diet. Excessive consumption of dietary sugars and saturated fats, known as the Western diet, has been linked with obesity, metabolic syndrome and impairment in cardiac function. The consumption of a Western diet has been shown to alter the gut microbiota composition, with decreased levels of Bacteroidetes and Bifidobacteria and increased levels of Firmicutes and Proteobacteria; the altered composition is thought to influence TMAO synthesis [363]. Studies have demonstrated the consumption of a Western diet leads to increased circulating TMAO levels in the blood [364] and cardiovascular disease risk [347]. A recent study showed Western diet-induced obese mice displayed impaired cardiac systolic and diastolic dysfunction, which following voluntary exercise resulted in slight decreases in weight gain and metabolic disorders, but completely prevented cardiac dysfunction. The results from this study suggest the cardioprotective effects of voluntary exercise in Western diet-induced obesity can be terminated via TMAO supplementation [365].

Protein-rich diets seem to have a positive correlation with TMAO excretion in the urine [366]. Large amounts of non-digestible carbohydrates can reduce TMAO formation by remodelling the gut microbiota, however studies have also shown that TMAO levels increase in the short term [367,368]. Nonetheless, the Western-diet, consisting mainly of red meat, is considered to be the major influence between TMAO levels and cardiovascular disease risk [369].

TMAO in preclinical models of HF

The recent interest in the intestinal microbiota has sparked the discussion of novel approaches to therapeutic medicine; dysbiosis of the gut microbiota has been associated with diseases such as obesity and atherosclerosis [370,371], therefore implicating the gut microbiota as a potential therapeutic

target. Studies in humans and animal models have shown the correlation of cardiovascular disease adverse events and increased levels of TMAO [70,347,357,358,372,373]. Despite the debated role of TMAO, it is clear from clinical and pre-clinical studies, that the gut microbiota is involved in facilitating the changes associated with diseases such as HF. Animal models of diet-induced obesity and pressure overload-induced heart failure have demonstrated a role for TMAO in accelerating cardiovascular risk such as renal dysfunction or atherosclerotic lesions [372,374]. Moreover, studies that have used animal models of myocardial infarction and a humanised microbiome have demonstrated that targeting the gut microbiota leads to attenuation of myocardial hypertrophy and HF [375], as well as reduced TMAO levels [376].

Several methods of targeting TMAO have been explored in a bid to identify the role of TMAO in cardiovascular disease risk, including inhibition of TMA lyases [348], and the use of faecal transplantation and probiotics to repopulate the intestinal flora [364,376]. However, these interventions have shown mixed results with animal models extrapolated into clinical settings or healthy volunteers. Additionally, there have been no studies to date, which show that lowering TMAO has any beneficial effect on 'heart health'. Whilst many studies indicate a correlative relationship, there are few studies that look at the mechanistic approach for interventions in the case of TMAO and cardiovascular risk. This leaves the mechanistic implications of pathogenesis largely unclear [377], which also limits successful intervention. Several studies have failed to show an intervention consistently capable of lowering TMAO [364,378]. In addition, whilst some methods to reduce TMAO, such as faecal transplantation show promise in animals and *in vitro* models, these have not been translated into human successfully, therefore highlighting the challenges that present when extrapolating experimental studies. However, it is clear that there is a role for the gut microbiota in heart health, which can be exploited for therapeutic purposes, and therefore on-going research is necessary to determine how the modulation of the gut microbiome may lead to improved cardiovascular health, with more research necessary in clinical settings.

TMAO in Heart Failure

Acute heart failure

To date, only one study has investigated the role of TMAO in acute HF patients [70]. In 972 acute HF patients, our group showed that individuals with higher TMAO levels were more likely to be older, have reduced renal function, decreased heart rate, blood pressure and haemoglobin levels, increased levels of NT-proBNP and potassium, and reduced left ventricular ejection fraction. With regard to

comorbidities, more patients in the higher TMAO group had diabetes or previous HF and evidence of atrial fibrillation. The clinical status of these patients was the poorest, with more patients in New York Heart Association (NYHA) class IV in the higher TMAO group. When clinical parameters associated with TMAO levels were investigated, TMAO was significantly and positively correlated to age, blood urea, serum creatinine and NT-proBNP, whereas a negative correlation was found with estimated glomerular filtration (eGFR), systolic blood pressure, and heart rate. Age, blood urea, and eGFR were the most significant independent predictors of TMAO levels, with a minor role for diabetes, smoking status, atrial fibrillation, and NYHA class.

With regard to prediction of outcomes, TMAO was a univariate predictor in a composite of death and hospitalisation due to HF at 1 year and of death at 1 year. Particularly, TMAO was predictive in a multivariate model with predictors such as age, NT-proBNP and NYHA class. However, for both endpoints, when renal function (eGFR and urea) were included, TMAO was no longer predictive of outcomes. With regard to in-hospital mortality, TMAO was able to improve the risk stratification in combination with clinical scores (i.e. ADHERE, OPTIMIZE-HF, and GWTG-HF), even when renal indices are included.

Notably, TMAO strengthened the prediction of the composite endpoint when combined with NT-proBNP. Indeed, patients with both markers elevated were at the highest risk of death/HF. This is not surprising, given these markers reflect two different pathways, and further support the hypothesis that a combination of biomarkers involved in different pathways could be the key point in the utilisation and interpretation of biomarkers within cardiovascular disease [8,379].

Chronic heart failure

Chronic heart failure (CHF) has been investigated more thoroughly in the context of TMAO. The first investigation of TMAO in chronic HF was performed a few years ago [73]; it demonstrated for the first time that TMAO levels were significantly higher in HF than in healthy subjects. In a cohort of 720 stable CHF patients, authors demonstrated an association between elevated TMAO levels and poor outcome in HF. In particular, patients with higher TMAO levels tended to be older, with a greater prevalence of diabetes mellitus (DM) and renal insufficiency. Furthermore, BNP values were higher in this category of patients and TMAO levels were significantly correlated with BNP levels and eGFR. With regard to mortality, TMAO stratified by quartiles showed a graded increase in mortality risk at 5 years. No differences were found when patients were stratified with regard to aetiology of HF, age, presence of DM, sex or smoking habits. Finally, even when corrected for eGFR, high sensitivity C-

reactive protein, and BNP levels, patients in the highest TMAO quartile remained at a significantly higher risk of mortality.

In a second study performed by the same group [345], high TMAO as well as choline and betaine (two substrates in the formation of TMAO, see figure 1) were associated with higher NT-proBNP levels and more advanced left ventricular diastolic dysfunction. Notably, levels of these metabolites were not related to systolic function, nor inflammatory or endothelial damage injury biomarkers. However, despite the association of all three metabolites with increased risk for 5-year adverse clinical events (death/transplantation), only TMAO remained independently associated when corrected for age, renal function, NT-proBNP levels, and echocardiographic parameters of diastolic dysfunction. Therefore, TMAO demonstrated a stronger prognostic value when compared to choline and betaine.

Another investigation conducted on TMAO, choline, and betaine in a cohort of 155 patients with HF, 100 patients with coronary artery disease (CAD), and 33 healthy matched individuals [69] showed HF patients with the highest levels of these metabolites when compared to the other groups. All three markers were associated with NYHA class, NT-proBNP, and inversely associated with eGFR and were significantly related to pulmonary pressure (estimated with right-sized catheterisation). Furthermore, TMAO was associated with previous diagnosis of ischemic disease and with age. Amongst the HF patients, TMAO levels tended to be higher in patients with a diagnosis of CAD, independent of several confounding factors (e.g. age, eGFR, CRP, NT-proBNP). Interestingly, the association of TMAO levels with echocardiographic markers of diastolic dysfunction were not confirmed in this study. For mortality, TMAO levels but not choline or betaine were associated with poor prognosis, with approximately half of the cases in the upper tertile resulting in death or heart transplantation. However, when adjusted for multiple confounders (e.g. eGFR, CRP, NT-proBNP), this association was no longer significant.

Recently, our group has demonstrated using the BIOSTAT-CHF cohort [203,380], that TMAO levels are associated with adverse outcome (mortality and mortality/HF) [346]. For the first time the response of circulating TMAO levels to treatment and its associations with outcome were investigated. We found that contrary to natriuretic peptides, TMAO levels did not respond to guideline treatment. In addition, patients with sustained elevated levels of TMAO had the worst outcome. In particular, low TMAO levels at either baseline and/or follow up were associated with better prognosis. TMAO levels

remained associated with outcomes even when adjusted for BIOSTAT risk models, which includes age, blood urea, BNP, and other biochemical biomarkers and comorbidities.

In the LURIC-study, TMAO levels and association with outcome was compared among HFrEF and HFpEF [72]. In particular, TMAO was significantly associated with outcome in HFrEF but not in HFpEF when adjusted for several factors, including age, sex, eGFR and DM. Notably, TMAO was more predictive in patients without DM and in patients with the lowest tertile of eGFR. In addition, TMAO showed a predictive value above and beyond NT-proBNP within this cohort, as NT-proBNP levels lose significance when adjusted for C-reactive protein.

Finally, in a small single-centre study, TMAO levels were assessed for the first time in a non-Caucasian population, confirming that TMAO levels were elevated in HF patients when compared to controls subjects [381]. Additionally, TMAO levels were related to the gut microbiome composition, in particular with the abundance of the Escherichia/Shigella genus.

Clinical context and future perspectives

Overall, the data from published studies show a positive association between TMAO levels and prognosis in HF (see Table 1), as explicated in a recent meta-analysis [382]. This observation is not surprising, given that high concentrations of TMAO are associated with increased risk of cardiovascular events and all-cause mortality, with a dose-dependent relationship [382]. In particular, the association of TMAO with outcomes are strongest in patients with coronary artery disease, and this could be linked with the proposed pro-atherogenic role of TMAO.

However, some points remain a matter of debate. Firstly, it is not possible to conclude whether high TMAO levels are causative of the increased risk of poor outcome in HF patients, or if the HF causes the increase in TMAO levels and this is associated with poor prognosis. Whilst these effects are correlative, several studies have suggested a mechanistic link between myocardial function and altered intestinal microbiota [38,68], which may underlie the association with poor outcome. The relationship between TMAO and left ventricular systolic function is unknown, however diastolic dysfunction has been observed [345], suggesting a stronger link with cardiac congestion rather than with impaired perfusion. This association may be explained by the intestinal congestion observed within patients with more severe HF, resulting in alterations of the microbiota composition, with subsequent impact on TMAO levels. Another issue not yet clarified is whether TMAO has the same role in HFrEF and in HFpEF. Indeed, only one study has tested the association of TMAO in HFrEF and HFpEF [72], and further research is needed to clarify the association of TMAO in these conditions.

The role of diet and kidney function are also highly debated regarding the association of TMAO with HF. Indeed, some reports showed attenuated association of TMAO levels with outcomes after adjustment for confounders [69,70]. In contrast, in other HF populations, TMAO levels were reported to be associated with mortality even after adjustment for renal function [73,345,346]. With this regard, a recent meta-analysis demonstrated significant differences in the relationship between TMAO and all-cause mortality in patients with and without chronic kidney disease [382], showing no significant difference between the two groups.

In addition, considering that TMAO levels were shown not to be affected by traditional guideline-HF treatment [346], this presents a novel field of research for investigation, whereby modification of the gut microbiota could be a novel therapeutic approach, and to date there are no large scale trials which have been conducted to test this hypothesis.

TMAO and HF: unmet needs

On the basis of data present in the literature, there are several limitations with regard to our knowledge about TMAO in HF. In brief:

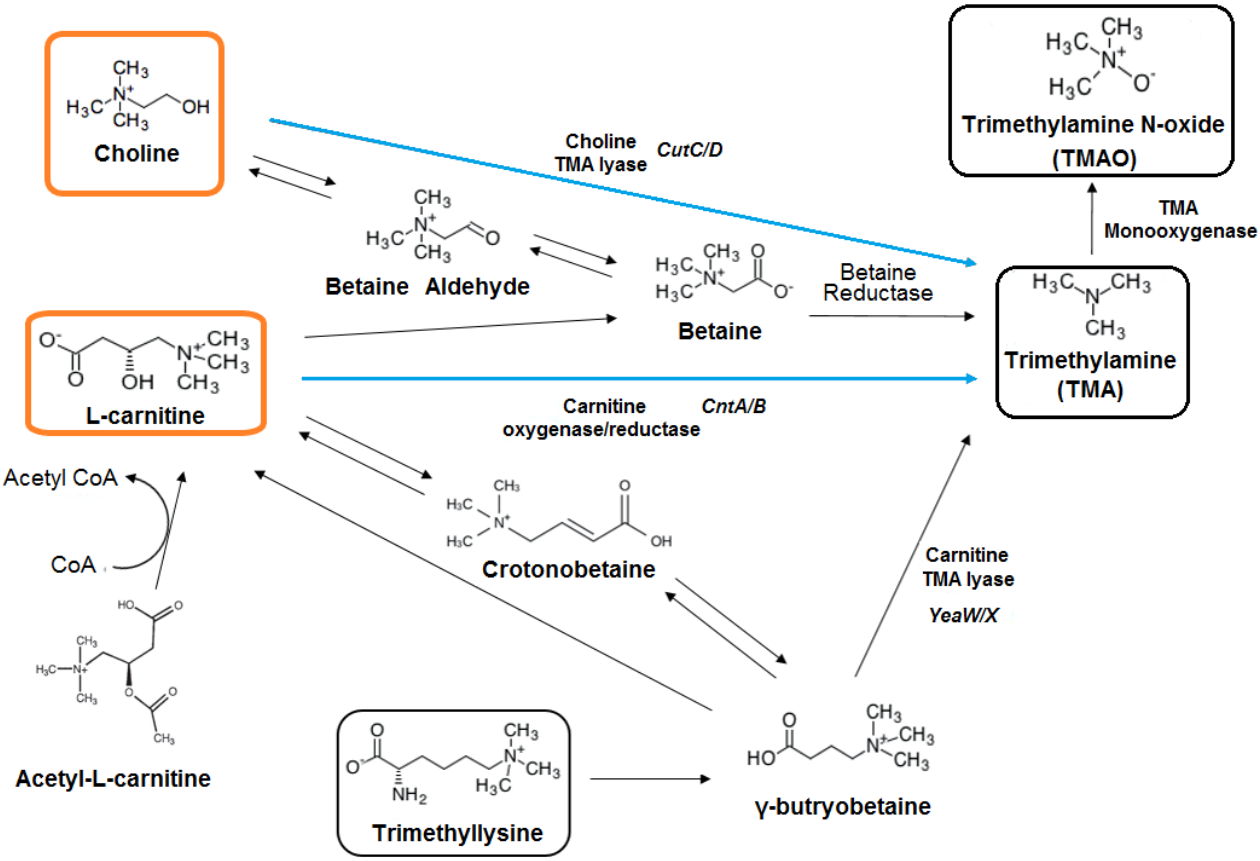
- Largely, the data obtained from these studies represent single ethnic groups, mainly Caucasian patients. Few studies are representative of other ethnic groups, and therefore drawing generalised conclusions based on a single ethnicity is misrepresentative.
- To date, it is not clearly understood whether HF affects TMAO levels or if TMAO is involved in the pathophysiology of HF.
- The role of confounding factors does not have a consensus amongst the literature and studies presented, therefore the interplay of several factors such as eGFR, age, and sex need to be clearly addressed concerning TMAO.
- All observations deriving from HF studies has limited or no data about the dietary condition of patients.
- There are no studies investigating pharmacological intervention on TMAO levels in HF.
- There is no clear evidence to suggest that lowering TMAO levels in patients shows better prognosis.
- It is not clear whether TMAO has an association with mortality in HFpEF.

In our opinion, these points need to be addressed in future research, in order to gain a full understanding of the role of TMAO in HF.

Conclusions

In conclusion, TMAO levels are increased in HF populations when compared to healthy subjects. Higher TMAO levels are associated with poor prognosis, whereas low TMAO levels either at baseline/follow up confer better prognosis. In order to draw appropriate conclusions regarding the role of TMAO in HF, additional studies are needed to address the current limitations highlighted. Interestingly, the finding that TMAO levels seem not be affected by guideline-HF treatment, may shed light upon TMAO as a novel and independent therapeutic target for HF, that requires specific treatment.

Figure 1. A schematic depicting the multiple pathways and metabolites associated with the production of TMAO in humans. Orange box – sources of TMAO, blue arrow – key pathways of TMAO metabolism, black box – metabolites with associated cardiovascular or noxious properties.



Authors	Year Journal	Location	Study population	Follow-up length	Outcomes	TMAO levels (µmol/L)
Tang et al. [15]	2014 *J Am Coll Cardiol*	USA	CHF n=720	5 years	All-cause mortality	5.0 [3.0-8.5]
Tang et al. [13]	2015 *J Cardiac Fail*	USA	CHF n=112	5 years	All-cause mortality and heart transplantation	5.8 [3.6-12.1]
Troseid et al. [12]	2015 *J Intern Med*	Norway	CHF n=155	5.2 years	All-cause mortality and heart transplantation	13.5 ± 18.5 (CAD) 7.1 ± 5.6 (DCM)
Suzuki et al. [11]	2016 *Heart*	UK	AHF n=972	1 year	All-cause mortality and a composite of mortality/rehospitalisation	5.6 [3.4-10.5]
Shuett et al. [14]	2017 *J Am Coll Cardiol*	Germany	CHF (HFpEF and HFrEF) n=823	9.7 years	All-cause mortality and cardiovascular mortality	4.7 [3.4-6.8] (HFrEF) 4.7 [3.2-6.9] (HFpEF)
Suzuki et al. [16]	2018 *Eur J Heart Fail*	11 European countries	Worsening or new-onset HF n=2234	3 years	All-cause mortality and a composite of mortality/rehospitalisation	5.9 [3.6-10.8]
Yazaki et al. [53]	2019 Eur J Heart Fail	11 European countries	Worsening or new-onset HF grouped by region n=2234	3 years	All-cause mortality and a composite of mortality/rehospitalisation	7.4 [4.1-13.6] NW 4.6 [2.9-7.5] CE 6.2 [3.6-11.5] S
Salzano et al. [54]	2019 Eur J Prev Cardiol	UK	CHF (HFpEF and HFrEF) n=156	5 years	Composite endpoint of all-cause mortality/hospitalisation for HF at 18 months (short-term) and at 60 months (long-term)	8.4 [3.7-13.8] HFrEF 6.6 [4.3-12.3] HFpEF
Hayashi et al [55]	2018 Circ J	Japan	Decompensated HF n=22	cross-sectional	TMAO levels (during de-compensation and during compensation phases) and gut microbioma composition were altered when compared to control subjects	17.3 ± 11.7 (Decomp) 17.7 ± 12.6 (Comp)

Table 1. Previous reports for associations between TMAO and outcome in heart failure patients.

4.2 Israr MZ, **Salzano A**, Suzuki T. Gut Feeling: the role of gut microbiota in immunomodulation of ischemia-reperfusion injury. **Arterioscler Thromb Vasc Biol** 2020 Sep;40(9):1967-1969. doi: 10.1161/ATVBAHA.120.314941 PMID: 32845773

The human gut microbiota encompasses the collection of bacteria, archaea, and eukarya colonising the gastrointestinal tract to form an intricate and life-long relationship [383]. By numerical standards, the number of microorganisms colonising the gastrointestinal tract exceeds 10^{14}, and is over 100 times the amount of genomic content (microbiome) of the human genome [384]. The development of the gut microbiota is believed to begin from birth with generally low diversity and develops along with the hosts growth through life, shaped by genetic and environmental stimuli [385]. The gut microbiota, dominated by *Firmicutes, Bacteroidetes, Actinobacteria,* and *Proteobacteria*, ensures beneficial physiological effects to the host including improving gut integrity, regulating host immunity and response, protecting against pathogens and harvesting energy [383]. In addition, the commensal microbiota and the host immune system is crucial for a thorough immune system with the microbiota providing support towards the immune response. The gut microbiota or flora is at regular competition against pathogenic bacteria for space and nutrients, protecting the host from harmful bacterial colonisation. This notion is well represented by the adverse effect of antibiotics whereby gut microbiota is reduced by antibiotics allowing pathogens to populate the area afterwards. On the other hand, alterations in the microbiota and gut dysbiosis, through the crosstalk with other organs, often lead to associated comorbidities including cardiovascular disease [386], with breakdown of the integrity of the gut mucosal lining and inflammation [387].

In the current issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Ascher and colleagues report on the role of the gut microbiota in acute mesenteric infarction [388]. This study, using gnotobiotic mouse models (i.e. in which all the forms of life present (microorganisms) within mice can be accounted for) showed that the gut microbiota influences the extension of ischemia/reperfusion (I/R) injury, and the interaction between leukocytes and I/R vessel walls. Specifically, leukocyte adhesion was increased by colonisation, with mono-organism or complex gut microbiota. Conversely, the use of antibiotics, decreasing the gut microbiota, reduced leukocyte adherence. From a mechanistic point of view, the present report showed that the presence of gut commensals restricts the formation of neutrophil extracellular trap (NET), with germ-free mice displaying more NET than colonised mice. The suppression of the NET by gut microbiota was

associated with attenuated neutrophil Toll-like receptor 4 signaling, and germ-free mice were more prone to NETing (Figure 1).

This study explored the dynamic changes that occur in the gut microbiota in response to stimuli (i.e. antibiotics) and the regulation of the immune response (NETosis) in I/R injury. Ascher et al suggest that the gut microbiota serves as an environmental factor that affects the immune response towards I/R injury. Similar to previous findings on the role of the microbiota in acute inflammatory responses, I/R injury was associated with local inflammatory responses of leukocyte activation, trafficking, endothelial barrier dysfunction and elevations in inflammatory mediators [389].

Whilst this study and many other studies have been conducted in animal models using germ-free/gnotobiotic models, the impact that the findings could have in humans represents an area that would benefit from further research. On this basis, an experimental human model of small intestine I/R injury has revealed that the gut barrier is resistant to short ischemic episodes and prevention of inflammation [390], in contrast to animal models which show an inflammatory response following intestinal I/R injury. However, prolonged ischemic episodes result in loss of intestinal barrier integrity, exposure to pathogens and activation of an inflammatory response, in line with animal models [391]. Although the precise role of I/R injury and the gut microbiota is a topic of debate, it has been suggested that the dramatic change in the environment after hypoxia upregulates pathogenic factors and the immune response. At the cellular level, molecular cross-talk involving Toll-like receptor signalling pathways, type II secretion mechanisms and quorum sensing support this hypothesis [392].

On the basis of effectors of the gut microbiota, one of the greatest influences is by dietary intake due to the different preferences of energy for the bacteria. This symbiotic relationship allows for modification of the host microbiota by altering the diet. For example, switching from a low fat, plant polysaccharide-rich diet to a high fat, high sugar diet results in a reduction of Bacteroidetes and increases in Firmicutes (Clostridia, Bacilli and Mollicutes class), changes in gene expression and metabolic pathways, and greater adiposity – all within 2 weeks [393]. Similarly, at the metabolic level, alterations to the gut microbiota have identified trimethylamine N-oxide (TMAO) amongst other metabolites (choline- and carnitine-related) to show perturbations linked with cardiometabolic disease [394,395]. Particularly representative of a Western diet (meat, fish and eggs), elevations in TMAO have shown associations with adverse outcomes in cardiovascular disease including heart failure and myocardial infarction [396-401]. With this in mind, therapeutic interventions and dietary supplements to improve the microbial community might offer a beneficial effect to host health. Use of probiotics in

regulation of the gut microbiota by ingesting beneficial bacteria has shown positive results. In children, a reduction in allergy rates has been associated with reduced antibiotic use, improved lifestyle, immune system, and daily intake of probiotic supplements [402]. Supporting this concept, preliminary reports have demonstrated the efficacy of probiotics (i.e. *Lactobacillus plantarum*) to show improvement in the immune response and to provide protection against I/R injury [403,404]. In myocardial I/R, probiotic supplements have demonstrated a reduction in infarct size and improved recovery of post-ischemic mechanical function [405].

In conclusion, the present report demonstrates the dynamic role of the gut microbiota in the immune response following I/R injury. Targeting the gut microbiota and its mediators might be a potential therapeutic intervention against I/R injury. Although an interesting hypothesis, whether I/R injury could be hypothetically monitored by altering commensal bacterial populations deserves further investigations.

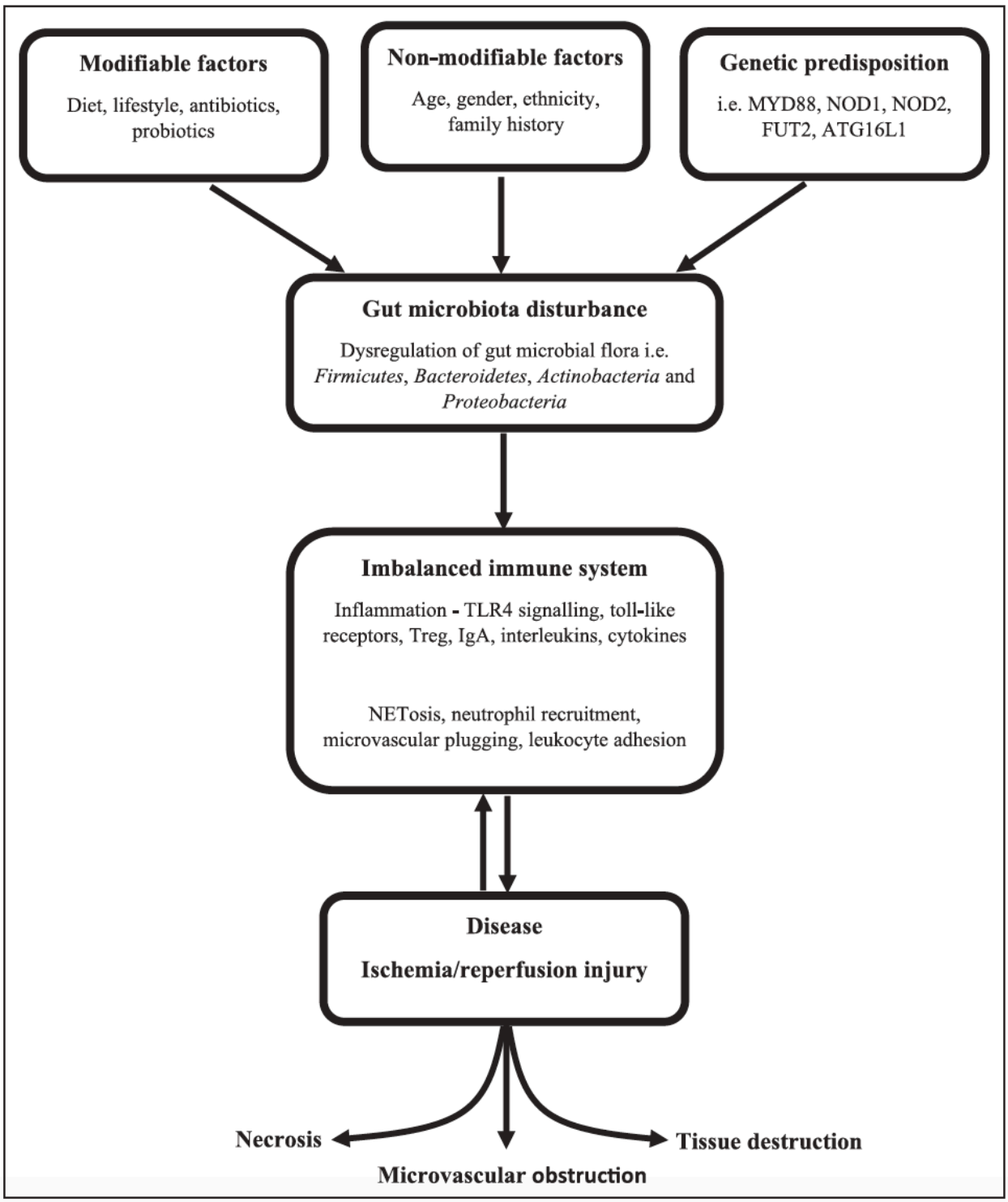


Figure. Gut microbiota dysregulation and its role in immunomodulation of ischemia-reperfusion injury.

4.3 Impact of acute choline loading on circulating trimethylamine N-oxide (TMAO) levels

Cassambai S, Salzano A, Yazaki Y, Bernieh D, Wong M, Israr MZ, Heaney LM, Suzuki T. Impact of acute choline loading on circulating trimethylamine N-oxide (TMAO) levels. Eur J Prev Cardiol. 2019 Feb 18:2047487319831372. doi: 10.1177/2047487319831372 PMID: 30776913

Despite recent efforts to reduce cardiovascular disease risk by dietary intervention (1), few markers are useful to assess the efficiency and progress of this. Circulating levels of trimethylamine N-oxide (TMAO) are associated with poor outcomes of cardiovascular disease (2-6). TMAO is generated via hepatic flavin monooxygenase 3 (FMO3) mediated oxidation of trimethylamine (TMA) (7), derived largely from carnitine and choline through gut microbial metabolism. These substrates are found in red meat and eggs which are representative of a Western diet. Therefore, TMAO levels could be used to monitor the effect of dietary intervention, particularly for the consumption of a Western diet. In this study, we examined the effect of acute choline loading on TMAO levels in healthy adult volunteers.

Eighteen healthy omnivorous adult volunteers, with no history of recent use of antibiotics/probiotics, completed this study. Baseline characteristics and blood samples were obtained from participants at baseline (BL). Participants were asked to complete a daily written food diary and returned after 2 weeks (visit 2, V2). The consumption of red meat, fish, and eggs was not permitted 24 hours before BL and V2. Prior to taking 700 mg of choline bitartrate capsules (Solgar, USA), a blood sample was collected to measure TMAO level at time zero (0), then at 2, 4, 6 and 8 hours post choline consumption. During V2, participants were given a standardised low-choline breakfast and lunch (total choline intake <50 mg) (8), between 0-2 and 4-6 hours respectively. This protocol was approved by the local ethics committee and adhered to the Declaration of Helsinki. All participants provided written informed consent. Plasma samples were quantified in duplicate for TMAO, using stable isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Shimadzu LC-30AD coupled with an 8050 triple quadruple mass spectrometer (TQ-MS), using an optimised version of a previously described method (9), with a run time of 1 minute and limit of quantification (LOQ) of 0.1 µmol/L. Individual responses of circulating TMAO levels in participants are shown in Figure 1a.

Participants showed a range in circulating TMAO levels between 0-20 µmol/L over the 8 hour period, with a few (n=4) showing higher TMAO levels following six hours post-choline consumption (>20 µmol/L). We observed that there were patients that showed raised TMAO levels in response to choline loading ('responders') and those that did not ('non-responders'). To further investigate the different responses to choline loading (response vs non-response), participants were split according to the median baseline TMAO level of 2.0 µmol/L (Figure 1b), into high TMAO (HT) and low TMAO (LT) groups. TMAO levels rapidly and significantly increased from 4 hours after choline loading in the HT group; in contrast, TMAO levels showed no change at any time point after choline loading in the LT group (Figure 1b) ($p=0.012$). TMAO levels in the HT group were higher than those in the LT group at all times during the acute oral choline challenge (3.1-16.3 µmol/L vs. 1.3-3.3 µmol/L, respectively). Participants in the HT group (n=9) tended to be older and had higher alcohol intake compared to those in the LT group (n=9); however this was not statistically significant ($p=0.094$ and $p=0.063$, respectively). Gender, ethnicity, BMI, and weekly physical activity were similar between groups ($p>0.05$). Dietary records from the two weeks prior to acute choline loading as well as baseline records did not show any differences in TMA rich food intake (red meat, eggs or fish) between groups (Table 1). No side effects (e.g. fishy body odour) were reported during the study.

The present study indicates that acute choline loading in healthy subjects produces a differential response in circulating TMAO levels (i.e. 'responders' and 'non-responders'). These two different response groups in TMAO production following choline challenge were demonstrated for the first time by the present study. To note, individuals with a 'responder' phenotype had higher baseline TMAO levels. Furthermore, food diaries from these individuals, assessed to determine protein intake in the two weeks before choline loading, showed no significant differences in TMA rich foods (e.g. red meat, eggs) compared to those with a 'non-responder' phenotype. This indicates that omnivorous individuals can have different responses to dietary sources of choline, independently of daily dietary conditions. TMAO levels were also analysed for association with participants' physical activity due to the beneficial effect of physical activity on cardiovascular risk, in particular when combined with dietary intervention (10). We did not observe differences in TMAO levels with respect to physical activity between the low and high TMAO groups. In previous studies, acute dietary supplementation of choline has been shown to increase TMAO levels in a general population (11). However, most studies have focused on carnitine, demonstrating the effect of L-carnitine supplementation with increased TMAO levels correlated to chronic carnitine intake (12). TMAO levels have been attributed more to carnitine rather than choline and from red meat rather than other

sources of protein (13). Whilst these studies showed that TMAO levels are significantly increased with L-carnitine and red meat intake, they did not account for baseline differences in the TMAO levels of participants and whether these could affect subsequent TMAO increase, underlying a responder or non-responder phenotype. Further, single TMAO time point cross-sectional studies are unable to ascertain whether there are temporal or individual differences in TMAO production, following dietary/loading challenge (e.g. response). The current study sheds light upon the hypothesis that individuals respond differently to dietary choline intake, which could be related to reduced FMO3 activity or gut bacterial conversion of choline to TMA, resulting in inefficient conversion of choline to TMAO. Further investigation of gut microbial composition and FMO3 activity to identify outstanding factors for response to choline loading is warranted.

In conclusion, our findings suggest the presence of different TMAO responses to acute choline loading. As a current goal of cardiovascular disease prevention is to target lifestyle factors (14), with dietary manipulation (e.g. to reduce red meat consumption) being one of the mainstays for intervention, the finding that not all subjects respond uniformly to dietary choline intake, and the use of a loading test to identify 'responders/non-responders' might help to further stratify patients into appropriate dietary interventions. Further studies that address whether the 'responder' phenotype affords a higher risk for cardiovascular risk and can be used to identify patients warranting dietary intervention are needed.

Table 1. Demographics data from the participants assessed for this study.

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	Total $N = 18$	Low TMAO ≤2.0 µmol/L	High TMAO >2.0 µmol/L	p value
Demographics				
Age	28 [23–36]	25 [21–29]	33 [27–49]	0.094
Male	5 (28%)	1 (11%)	4 (44%)	0.294
Caucasian	14 (78%)	7 (78%)	7 (78%)	1.000
BMI	21.7 [20.9–23.8]	20.9 [20.2–23.8]	22.6 [21.5–23.7]	0.258
Current smoker	1 (6%)	0 (0%)	1 (11%)	1.000
FH of CVDs	3 (17%)	2 (22%)	1 (11%)	1.000
Systolic blood pressure, mmHg	117 [110–129]	118 [110–124]	116 [115–133]	0.666
Diastolic blood pressure, mmHg	78 [74–86]	78 [70–86]	77 [76–82]	0.863
Heart rate, beats/min	68 [63–78]	65 [65–76]	70 [62–78]	0.605
Physical activity, days/week	3.5 [2.5–7]	4.0 [2.5–7]	3.0 [3–7]	0.876
Intake history				
Alcohol, units/week	1 [0–3]	0 [0–0.1]	2 [1–6]	0.063
Red meat, units/week	3 [1–4]	2 [1–3]	3 [3–4]	0.258
Fish, units/week	1 [1–3]	1 [0.1–3]	2 [1–2]	0.489
Eggs, units/week	1 [1–3]	1 [1–2]	2 [1–4]	0.297
Laboratory				
Cholesterol, g/dL	4.7 [4.0–5.2]	4.6 [4.4–5.2]	4.7 [3.9–5.2]	0.796
HDL, g/dL	1.6 [1.4–1.9]	1.5 [1.4–1.7]	1.9 [1.4–2.0]	0.546
TMAO, µmol/L				
Baseline	2.0 [1.3–4.0]	1.3 [0.7–1.6]	4.0 [3.3–5.9]	<0.001*
After choline loading				
0 h	2.1 [1.4–3.4]	1.5 [1.3–1.9]	3.4 [2.2–3.5]	0.024*
2 h	2.0 [1.3–3.1]	1.3 [1.2–2.0]	3.1 [2.0–4.0]	0.021*
4 h	1.9 [1.2–4.5]	1.3 [1.1–1.8]	4.5 [2.7–6.5]	0.001*
6 h	4.1 [1.0–10.3]	1.0 [0.8–1.9]	10.3 [7.3–34.0]	0.001*
8 h	6.6 [4.4–16.3]	3.3 [1.0–6.2]	16.3 [11.6–50.5]	0.001*

Analysed data were split into high and low TMAO groups, respectively, based on the median of baseline values. Mann–Whitney U test for continuous variables and chi-squared test for categorical variables were used to examine between-group differences. Subsequent analyses used a general linear model with repeated measures and Bonferroni correction to compare group TMAO means during the oral acute choline challenge. p values < 0.05 were deemed statistically significant. IBM SPSS Statistics (v25, IBM Corp., Armonk, NY, USA) and Prism (v.7, GraphPad) were used.
*$p < 0.05$.
BMI: body mass index; FH: familiar history; CVD: cardiovascular disease; TMAO: trimethylamine N-oxide; HDL: high density lipoprotein.

Analysed data were split into high and low TMAO groups (HT and LT groups respectively), based on the median of baseline values. Mann-Whitney U test for continuous variables and chi-squared test for categorical variables were used to examine between-group differences. Subsequent analyses used a general linear model with repeated measures and Bonferroni correction to compare group TMAO means during the oral acute choline challenge. P values <0.05 were deemed statistically significant. IBM SPSS Statistics (v25, IBM Corp., Armonk, NY, USA) and Prism (v.7, GraphPad) were used.

BMI: Body mass index; FH: familiar history; CVD: cardiovascular diseases; TMAO: trimethylamine N-oxide; HDL: high density lipoprotein. * P< 0.05

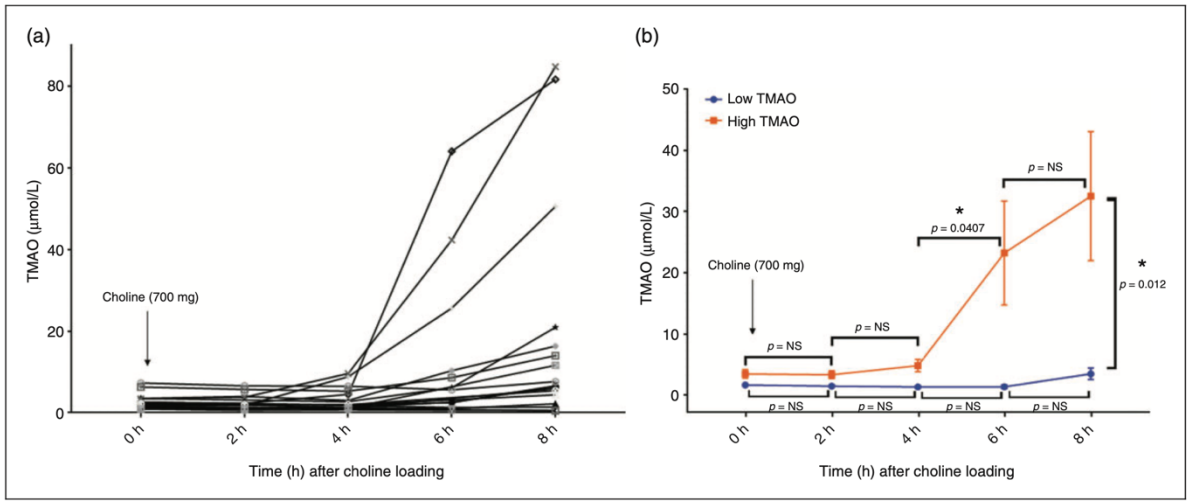


Figure 1. (a) Plasma trimethylamine N-oxide (TMAO) levels over an 8-h period following acute choline loading of 700 mg orally after 0 h (arrow). (b) Participants were split into two groups dependent on the median TMAO levels at baseline (high or low TMAO). Data are presented as the mean ± SEM.
*$p < 0.05$.
NS: non-significant.

4.4 Geographical location affects the levels and association of trimethylamine N-oxide with heart failure mortality

Yazaki Y*, **Salzano A***, Nelson CP, Voors A, Anker S, Cleland JG, Lang C, Metra M, Samani N, Leong LN, Suzuki T. Geographical location affects the levels and association of trimethylamine N-oxide with heart failure mortality: a post-hoc analysis of BIOSTAT-CHF. **Eur J Heart Failure** 2019 Jul 28. doi: 10.1002/ejhf.1550. PMID: 31353762

Elevated circulating levels of the gut microbiota-derived metabolite, trimethylamine N-oxide (TMAO), are associated with adverse outcomes in heart failure (HF) [72,122,346,406]. However, there are apparent discrepancies in the reported relationship between TMAO levels and outcomes. Independent reports from a British acute HF cohort [122] and from a Norwegian chronic HF cohort [406] showed attenuation of association of TMAO levels with outcomes after adjustment for main confounders, namely renal function. In contrast, in a German chronic HF population, TMAO levels were reported to be associated with mortality and had a better predictive value than N-terminal pro-B-type natriuretic peptide (NT-proBNP) even after adjustment for glomerular filtration rate (eGFR) [72]. We therefore wondered whether geographical location might account for this apparent difference [407] and investigated this hypothesis using the BIOSTAT-CHF cohort [346,408,409], a multinational study done across 11 countries in Europe in which we recently reported the association between TMAO levels and outcomes [346].

A diet-based categorization by country of enrolment was performed using the European Nutrition and Health Report classification [410] into the Northern/Western group (NW: France, Netherlands, Norway, Sweden, and United Kingdom), Central/Eastern group (CE: Germany, Poland, Serbia, and Slovenia), and Southern group (S: Greece and Italy) (Figure 1). The primary study endpoint was 2-year all-cause mortality. The association between TMAO and mortality was assessed by Cox proportional hazards analysis within each geographical group, adjusted for the modified BIOSTAT full risk model (Figure 1) [408] and for further specific TMAO confounders: eGFR, body mass index (BMI), and protein intake (as estimated by the Maroni formula). The effects of geographical location on addition of TMAO or NT-proBNP to the BIOSTAT risk model for mortality and interaction with TMAO were investigated. Further, genetic effects on TMAO by the enzyme critical for conversion of TMAO from its precursor TMA, the flavin-containing monooxygenase isoform 3 (FMO3) gene (i.e. four common gene polymorphisms of the A allele of rs2266782 and rs1736557, G allele of rs2266780, and

T allele of rs909530) [349], were investigated using linear regression under an additive mode of inheritance further adjusting for the first 10 genetic principle components. Genotyping was carried out on the Affymetrix Axiom UK Biobank array and called using Affymetrix Power Tools 1.16.1. A p-value of <0.05 was considered statistically significant.

Of 2234 patients in BIOSTAT-CHF, 952 (43%) were classified as NW, 714 (32%) as CE, and 568 (25%) as S. Geographical differences in demographics, comorbidities, and mortality were in line with previous reports on geographical characteristics of patients with HF (i.e. CE patients showing a younger age, a higher percentage of ischemic disease, and different outcomes) [407]. CE patients were younger, had higher eGFR, higher estimated protein intake, and higher percentage of ischemic aetiology compared to NW but were similar to S patients (Figure 1 and Table 1). When adjusted for age, eGFR, protein intake, and BMI, TMAO levels in CE remained significantly lowest amongst regions (median [IQR]: 6.2 µM [4.8-6.8], 7.2 µM [5.4-8.9], and 6.5 µM [5.0-8.5] respectively for CE, NW and S, *p for trend* <0.001). Approximately 24% of patients (n=531) reached the primary endpoint during a median follow-up of 21.5 [15.7-24.3] months. Mortality rate varied amongst regions [NW 26.7% (n=254), CE 22.0% (n=157), and S 21.1% (n=120), *p for trend*=0.019]. In a Cox model adjusted for confounders including BIOSTAT risk model factors, the CE group alone showed significant association of higher TMAO levels with mortality (HR: 2.40 (1.33-4.32); *p*=0.004) (Figure 1). When interaction between geographical differences with TMAO or NT-proBNP levels and outcome was investigated, a statistically significant interaction for TMAO alone was observed but not for NT-proBNP (*p interaction*= 0.033 and 0.586 respectively). Further, NT-proBNP levels were significantly associated with mortality after adjustment for confounders in all groups. However, TMAO levels significantly improved risk prediction when added to the BIOSTAT risk model in CE patients alone, as shown by changes in C-statistic (0.718 to 0.744, *p*=0.046), NRI (41.9 [21.2-62.7], *p*<0.001) and IDI (2.3 [0.9 – 3.7], *p*<0.001) (Supplementary table 1). In contrast, for NT-proBNP there was a statistically significant gain in C-statistic, NRI, and IDI in all groups, (Supplementary table 1). No associations of four previously identified genetic variants in the FMO3 gene with TMAO levels were observed (Supplementary table 2).

This is the first report on effects of regional differences on association between TMAO levels and mortality risk in HF. There are two main findings of the present analysis. First, TMAO levels of HF patients differed by region, even after adjustment for confounders. Second, there was a different association with outcome by region (i.e. mortality risk of patients with elevated TMAO levels was higher in CE patients than in NW and S patients). In addition to these main findings, in CE patients,

TMAO levels were predictive of mortality on C-statistic analysis, whereas BNP levels were not. Finally, known FMO3 gene variants were not associated with TMAO levels. Collectively, our findings demonstrate the different associations of TMAO with HF outcomes in a European population suggesting that geographical differences apart from measured demographic and associated comorbidities might represent at least one possible explanation for this [72,122,406]. The BIOSTAT-CHF cohort has two features that made it an ideal cohort to investigate regional discrepancies. First, patients from different European regions (NW, CE and S) were well represented in our cohort. Second, with more than 99% of patients being Caucasian, the role of ethnicity and the genetic pool was mitigated as possible confounders of underlying geographical differences [407]. There were several limitations in the present study, however. Although we estimated protein intake by the Maroni formula, we did not have any information regarding the actual dietary records, gut microbiota composition or intestinal permeability to confirm the impact of diet on TMAO levels.

In conclusion, geographical differences affect the levels and association of TMAO with heart failure mortality, regardless of main confounders. This finding suggests there may be other under-investigated factors that affect associations of TMAO and HF adverse outcomes.

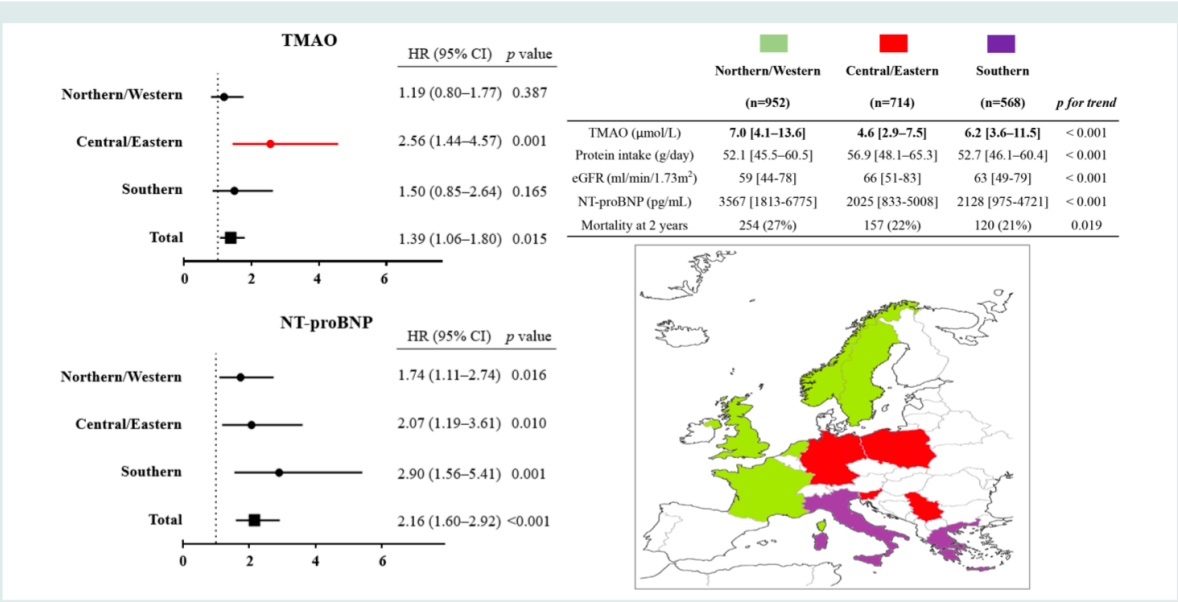


Figure 1 Association between trimethylamine N-oxide (TMAO)/N-terminal pro-B-type natriuretic peptide (NT-proBNP) and all-cause mortality at 2 years according to geographical groups. Forest plot of the hazard ratio (HR) of 2-year mortality risk per log TMAO/NT-proBNP increase. Whiskers represent 95% confidence intervals (CI). HRs adjusted for modified BIOSTAT-CHF full risk model factors (age, log urea, haemoglobin, use of beta-blocker at baseline, log NT-proBNP/log TMAO, ischaemic aetiology, chronic obstructive pulmonary disease, diastolic blood pressure, and sodium), estimated glomerular filtration rate (eGFR), body mass index, and gender.

Table 1 Patient characteristics regarding geographical groups

	Northern/Western ($n = 952$)	Central/Eastern ($n = 714$)	Southern ($n = 568$)	*P*-value for trend
Demographics				
Age	74 [64–80][a]	67 [59–75]	68 [59–76][a]	<0.001
Male	627 (66)[b]	548 (77)	479 (84)	<0.001
Body mass index (kg/m^2)	26.9 [23.7–30.9][b]	27.5 [24.7–31.1]	27.5 [24.4–29.8]	0.005
Current smoker	122 (13)	106 (15)	84 (15)	0.407
Ischaemic aetiology	477 (51)[b]	432 (62)	305 (56)	<0.001
Hypertension	516 (54)[b]	540 (76)	345 (61)[b]	<0.001
Diabetes mellitus	273 (29)	246 (35)	211 (37)	0.001
Atrial fibrillation	470 (49)[a]	293 (41)	243 (43)	0.002
COPD	180 (19)[a]	101 (14)	106 (19)	0.025
Previous HF hospitalization	260 (27)[b]	229 (32)	214 (38)	<0.001
NYHA class I/II/III/IV (%)	2/34/51/12[a]	1/39/53/7	5/33/45/17[a]	<0.001
LV ejection fraction (%)	30 [25–40][a]	30 [25–36]	30 [25–35]	<0.001
Clinical signs				
Pulmonary congestion	513 (57)†	325 (46)	311 (56)[a]	<0.001
Peripheral oedema	523 (69)[a]	358 (55)	222 (50)[b]	<0.001
Systolic blood pressure (mmHg)	120 [110–139][b]	125 [110–140]	120 [110–130][b]	<0.001
Diastolic blood pressure (mmHg)	71 [63–83][b]	78 [70–85]	70 [69–80][b]	<0.001
Heart rate (b.p.m.)	79 [68–95][a]	75 [66–84]	75 [66–86][a]	<0.001
Medication				
Beta-blocker	755 (79)[b]	629 (88)	479 (84)	0.017
ACE inhibitor or ARB	673 (71)[b]	569 (80)	396 (70)[b]	<0.001
MRA	379 (40)[b]	475 (67)	339 (60)	<0.001
Diuretics	950 (100)	714 (100)	568 (100)	0.260
Laboratory				
Haemoglobin (g/dL)	13.0 [11.7–14.4][b]	13.7 [12.3–14.7]	13.2 [11.8–14.4][b]	<0.001
Urea (mmol/L)	9.0 [6.7–13.1]	9.5 [6.9–15.0]	18.2 [12.5–26.1]	<0.001
Sodium (mmol/L)	139 [137–141][b]	140 [138–142]	139 [137–142][b]	<0.001
Outcomes (2 years)				
Mortality	254 (27)[a]	157 (22)	120 (21)	0.019

Data are expressed as median [interquartile range] for continuous variables, or *n* (%) for categorical values. *P*-values are quoted for Kruskal–Wallis tests for continuous variables and Chi-square tests for categorical variables.

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; COPD, chronic obstructive pulmonary disease; HF, heart failure; LV, left ventricular; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.

[a]Significantly higher compared to Central/Eastern.

[b]Significantly lower compared to Central/Eastern pairwise analyses.

4.5 Ethnic differences in association of outcomes with trimethylamine N-oxide in acute heart failure patients

Yazaki Y*, Aizawa K*, Israr MZ*, Negishi K, Salzano A, Saitoh Y, Kimura N, Kono K, Heaney LM, Cassambai S, Bernieh D, Lai F, Imai Y, Kario K, Nagai R, Ng LL, Suzuki T. Ethnic differences in association of outcomes with trimethylamine N-oxide in acute heart failure patients. ESC Heart Fail. 2020 Jun 29. doi: 10.1002/ehf2.12777 PMID: 32598563

Introduction

The oxidised gut microbiome-derived metabolite, trimethylamine N-oxide (TMAO), has been linked with outcomes in patients with heart failure (HF), with elevated circulating TMAO levels showing association with poor outcomes (mortality/rehospitalisation) [72,73,122,397,400,406,411,412]. However, these previous studies enrolled predominantly Caucasian patients, and ethnic influences on the association of TMAO with outcomes have not yet been investigated [72,73,122,397,400,406,411,412]. The aim of the current study was to investigate whether ethnic differences influence associations between TMAO levels and HF outcomes.

Methods

Study population

TMAO levels were measured in patients with acute HF at two sites (University Hospitals of Leicester, Glenfield Hospital, UK, and Jichi Medical University Hospital, Shimotsuke, Tochigi, Japan) with identical protocols for sample collection, TMAO measurements, and data management [122]. The UK Leicester cohort has been previously published [122], and consists of two main ethnic groups of Caucasian (n = 842, 87%) and South Asian (n = 129, 13%) patients, while the patients in the acute HF

cohort at Jichi Medical University Hospital were all Japanese. The study was approved by the local ethics committee at each participating centre and complied with the declaration of Helsinki.

Statistical analyses

The primary endpoint was a composite of all-cause mortality and/or rehospitalisation (mortality/HF) due to heart failure within 1 year post-admission. Demographic, laboratory, and clinical data were compared with ethnicity, using the Mann-Whitney U test for continuous variables and the chi-squared test for categorical variables. The Kruskal-Wallis test was used to compare group differences between the ethnic groups and TMAO levels. B-type natriuretic peptide (BNP) and N-terminal (NT)-pro-hormone BNP (NT-proBNP) levels were measured using different natriuretic peptide assays in the two cohorts and were therefore log normalised and then z-transformed (normalised to 1 standard deviation) along with TMAO levels for each cohort before analysis. A Cox proportional hazards regression model was used to analyse the associations between ZlogTMAO levels and study outcomes in each ethnic group after adjustment for age, sex, previous history of HF, ischemic heart disease, hypertension, diabetes mellitus, New York Heart Association class, systolic blood pressure, blood sodium levels, renal function by estimated glomerular filtration rate (eGFR), haemoglobin, and standardised natriuretic peptide (NT-proBNP or BNP). Interaction analysis was performed to assess whether the association between TMAO levels and mortality/HF differed amongst subgroups stratified according to baseline characteristics. Kaplan-Meier survival curves were generated and the Mantel-Cox log-rank test was used to compare the differences with survival of TMAO levels when stratified by the population median and ethnicity median.

Results

Patient demographics and clinical parameters associated with TMAO

In total, 1087 patients with acute HF were measured for TMAO levels; of these, 842 (77%) were classified as Caucasian, 129 (12%) as South Asian, and 116 (11%) as Japanese (Table 1). TMAO levels were significantly higher in Japanese patients compared to Caucasian or South Asian patients (median [inter-quartile range]: 9.9 μM [5.2-22.8], 5.9 μM [3.6-10.8], and 4.5 μM [3.1-8.4], respectively, for the Japanese, Caucasian and South Asian patients, p<0.001) (Figure 1). The Caucasian patients were significantly older than the Japanese and South Asian patients. The South Asian patients had higher prevalence of ischemic heart disease, hypertension, and diabetes mellitus, but lower prevalence of atrial fibrillation. The eGFR in Japanese patients was significantly higher than in Caucasian and South Asian patients (56 (ml/min/1.73m^2) [36-82], 48 (ml/min/1.73m^2) [35-64], and 51 (ml/min/1.73m^2) [37-68], respectively, p=0.026); however, there were no differences in the normalised natriuretic peptide distributions amongst the ethnic groups (Table 1).

TMAO levels as a predictor of mortality/HF at 1 year

The mortality rate at 1 year in Caucasian patients was the highest amongst the ethnic groups (p<0.001) whilst for mortality/HF rate at 1 year, no significant differences were observed between the ethnic groups (p=0.096; Table 1).

Higher TMAO levels were significantly associated with mortality/HF for univariate [hazard ratio (HR) 95% confidence interval (CI): 1.28 (1.17-1.40), p<0.001] and multivariable analyses in the entire population (unadjusted p<0.001, adjusted p=0.002) (Table 2). When ethnicity was considered as univariate model, there were no observed differences for associations with mortality/HF between Caucasian and South Asian patients (p=0.823), however there were differences with Japanese patients (p=0.042), with Japanese patients associated with a lower risk of mortality/HF. However, as multivariate models, there were no observed differences across the ethnic groups and associations with mortality/HF (unadjusted p=0.057, adjusted p=0.311) (Table 2). Kaplan-Meier survival analysis

also showed no significant differences for outcome between South Asian and Japanese patients (p=0.068), and between Caucasian and South Asian patients (p=0.822). However, a significant difference was observed between Caucasian and Japanese patients (p=0.041) (Figure 2).

Interactions between ethnicity group and TMAO levels were investigated and showed no significant interaction between the full population (ethnicity groups) and TMAO levels after adjustment (unadjusted p = 0.408, adjusted p=0.125). When each ethnic group was considered, the hazard ratios of TMAO with mortality/HF were similar across the three ethnic groups (HR unadjusted 1.15-1.38, adjusted 0.88-1.23) (Table 2).

Survival analysis showed that when the median TMAO level for the total population was considered, Caucasian patients showed increasing incidence of event for all cause death and/or rehospitalisation due to heart failure with elevated circulating levels ($p<0.001$), whereas Japanese and South Asian patients showed no associations between TMAO and survival ($p\geq0.444$) (Figure 3A). Furthermore, when TMAO was stratified by the median for each ethnic group, we again found Caucasian patients showed increasing incidence of event with elevated circulating levels ($p<0.001$), whereas Japanese and South Asian patients showed no associations between TMAO and mortality/HF event ($p\geq0.410$) (Figure 3B). Similar results were observed for mortality (Supplementary Figure 1A and Supplementary Figure 2) and rehospitalisation due to HF (Supplementary Figure 1B and Supplementary Figure 3).

Discussion

TMAO levels in patients with acute HF differed by ethnicity. Caucasian and non-Caucasian patients presented with a similar probability of survival for mortality/HF; however, when dichotomised by TMAO median, only Caucasian patients showed the ability to be stratified based on elevated TMAO levels but not in Japanese and South Asian patients. In conjunction with previous findings, TMAO

shows ethnicity-selective contributions with tailored risk selective to Caucasian patients demonstrating that TMAO measurements, at least in our investigated cohorts, are applicable for risk associations with adverse events in Caucasian but not in non-Caucasian patients.

TMAO levels are known to be affected by diet/lifestyle, with consumption of choline/carnitine rich foods (e.g. fish and red meat) being associated with higher TMAO levels [344,413], and vegetarians showing lower levels [414]. Although diet was not investigated in the present cohort, our findings might reflect cultural and ethnic differences in dietary contributions [415]. Different associations for cardiovascular disease between Caucasians and Asians have been previously reported [416], and our findings are consistent with these. A previous study has also shown that the association of TMAO levels with cardiovascular risk differed between Caucasian and Blacks in haemodialysis patients [417], with a linear increase in adverse events in Caucasian patients, but not in Blacks.

As limitations, we did not have any information regarding the dietary records and gut microbiota composition to investigate impact on TMAO levels which is a limitation of the present study. Differences in the standard of care between the UK and Japanese cohorts may have also contributed.

In conclusion, ethnic differences affect TMAO levels and their risk stratification with adverse outcomes in patients with acute HF. Our findings add to our present understanding of outcomes associated with acute HF through the identification of a hitherto unknown ethnicity-selective contribution of the gut microbiome through TMAO.

Table 1 Baseline patient characteristics according to ethnicity

	Total (n = 1087)	Caucasian (n = 842)	South Asian (n = 129)	Japanese (n = 116)	P value
TMAO (μmol/L)	5.9 (3.5–11.4)	5.9 (3.6–10.8)	4.5 (3.1–8.4)[b]	9.9 (5.2–22.8)[a]	<0.001
Demographics					
Age	77 (69–83)	79 (71–85)	71 (62–78)[b]	74 (67–81)[b]	<0.001
Male	594 (61%)	508 (60%)	86 (67%)	87 (67%)	0.207
Prior HF	364 (35%)	282 (34%)	44 (34%)	38 (35%)[a]	0.962
Ischaemic heart disease	322 (30%)	225 (27%)	58 (45%)[a]	39 (34%)	<0.001
Hypertension	641 (59%)	480 (57%)	86 (67%)[a]	75 (65%)	0.039
Diabetes mellitus	328 (34%)	261 (31%)	67 (52%)[a]	42 (36%)	<0.001
Dyslipidaemia	274 (25%)	202 (24%)	35 (27%)	37 (32%)	0.156
Atrial fibrillation	491 (45%)	418 (50%)	23 (18%)[b]	50 (43%)	<0.001
NYHA class IV	534 (54%)	451 (54%)	73 (59%)	35 (30%)[b]	<0.001
LV ejection fraction (%)	35 (25–48)	35 (26–48)	34 (23–48)	34 (26–49)	0.782
Clinical signs					
Systolic blood pressure (mmHg)	132 (115–150)	133 (115–150)	135 (116–155)	126 (105–150)	0.240
Diastolic blood pressure (mmHg)	75 (65–85)	74 (65–85)	74 (65–85)	81 (66–94)[a]	0.023
Heart rate (beat/min)	90 (74–106)	88 (74–106)	90 (73–102)	92 (76–112)	0.310
Medication					
Aspirin	458 (42%)	363 (43%)	64 (50%)	31 (28%)[b]	0.001
Beta-blocker	445 (41%)	345 (41%)	57 (45%)	43 (38%)	0.617
ACE inhibitor or ARB	587 (54%)	457 (54%)	70 (54%)	60 (54%)	0.990
Diuretics	647 (60%)	505 (60%)	81 (63%)	61 (55%)	0.371
Laboratory					
Urea (mmol/L)	8.9 (6.5–12.6)	9.0 (6.6–12.7)	8.1 (6.2–12.5)	8.9 (6.4–11.6)	0.383
eGFR (mL/min/1.73 m^2)[c]	49 (36–66)	48 (35–64)	51 (37–68)	56 (36–82)[a]	0.026
Sodium (mmol/L)	138 (135–141)	138 (135–141)	137 (135–140)	140 (137–142)[a]	0.001
Haemoglobin (g/dL)	12.3 (10.8–13.7)	12.4 (10.9–13.8)	11.9 (10.5–13.0)[b]	11.9 (10.7–13.4)	0.012
NT proBNP (pg/mL)	–	2123 (996–3946)	2103 (833–3454)	–	–
BNP (pg/mL)	–	–	–	654 (355–1095)	–
z-transformed log natriuretic peptide	0.17 (−0.43–0.62)	0.18 (−0.40–0.66)	0.18 (−0.53–0.56)	0.05 (−0.59–0.59)	0.460
Outcomes					
Mortality at 1 year	281 (26%)	243 (29%)	25 (19%)[b]	13 (11%)[b]	<0.001
Mortality/HF at 1 year	418 (39%)	332 (39%)	52 (41%)	34 (29%)	0.096

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; HF, heart failure; LV, left ventricular; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; TMAO, trimethylamine-N-oxide.
Data are expressed as median (interquartile range) for continuous variables or n (%) for categorical values. Categorical variables were analyzed with χ^2 tests. Continuous variables were analysed with Mann–Whitney U tests.
[a]Significantly higher compared with Caucasian.
[b]Significantly lower compared with Caucasian pairwise analyses.
[c]Estimated by Chronic Kidney Disease Epidemiology Collaboration formula.

Figure 1 TMAO levels in patients with acute heart failure according to ethnicity. Box and whisker plot to show the distribution of TMAO levels adjusted for age, sex, and estimated glomerular filtration rate across different ethnicity groups. Boxes indicate median and interquartile range, and whiskers indicate 10th and 90th percentiles. All P values were adjusted for multiple comparisons with Bonferroni correction. TMAO, trimethylamine-N-oxide.

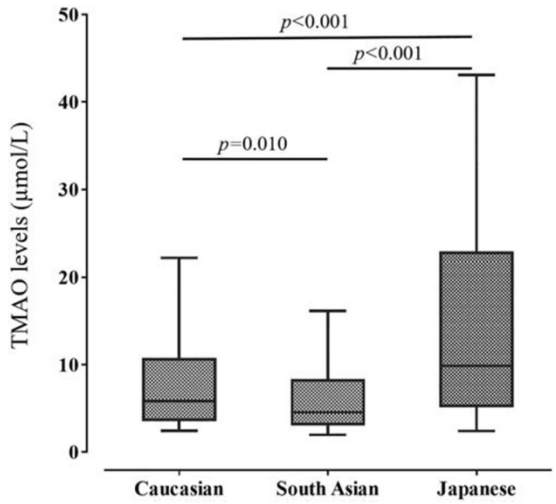


Table 2 Cox regression model for all-cause death and/or hospitalization due to heart failure

	Unadjusted		Adjusted[a]	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Univariate model				
logTMAO	1.28 (1.17–1.40)	<0.001		
Ethnic group				
Caucasian	Reference			
South Asian	1.03 (0.77–1.39)	0.823		
Japanese	0.69 (0.49–0.99)	0.042		
Multivariate model				
logTMAO		<0.001		0.002
Ethnicity group		0.057		0.311
Ethnic group*logTMAO		0.408		0.125
Caucasian*logTMAO	1.38 (1.24–1.53)		1.23 (1.08–1.40)	
South Asian*logTMAO	1.15 (0.86–1.55)		0.88 (0.61–1.25)	
Japanese*logTMAO	1.21 (0.93–1.57)		1.02 (0.77–1.35)	

CI, confidence interval; HR, hazard ratio; TMAO, trimethylamine-N-oxide.
[a]Adjusted for age, sex, previous history of heart failure, ischaemic heart disease, hypertension, diabetes, systolic blood pressure, New York Heart Association class, sodium, haemoglobin, estimated glomerular filtration rate, and standardized natriuretic peptide.

Figure 2 Kaplan–Meier curve showing the relationship of ethnicity and all-cause death and/or rehospitalization due to heart failure at 1 year. *Log rank *P* = 0.822; †*P* = 0.041; #*P* = 0.068. HF, heart failure.

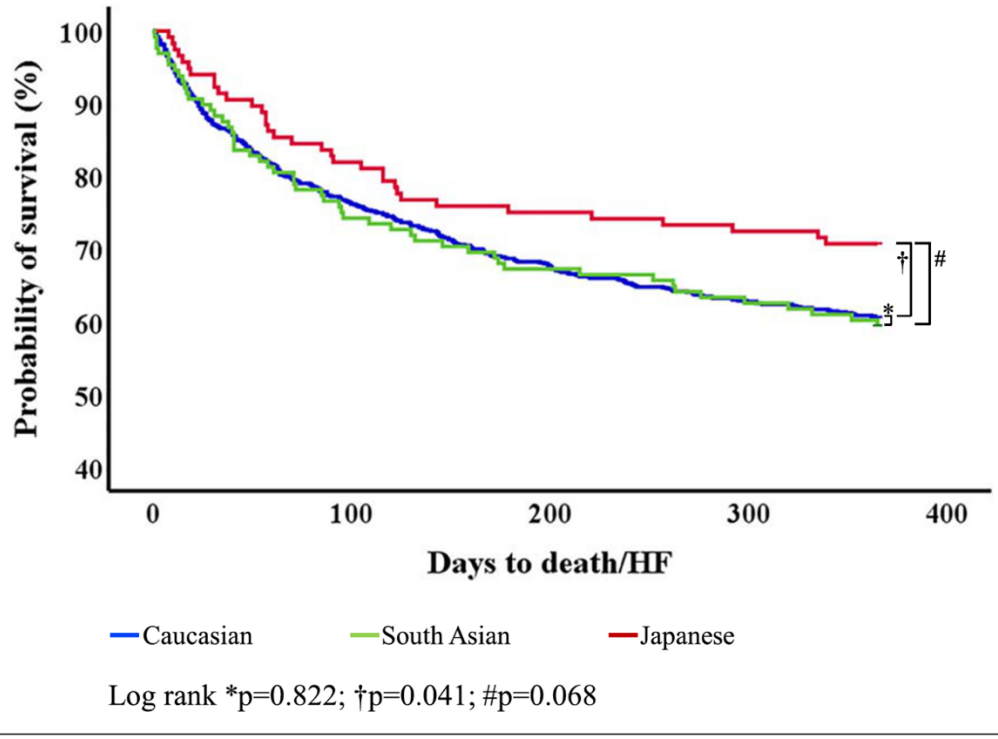
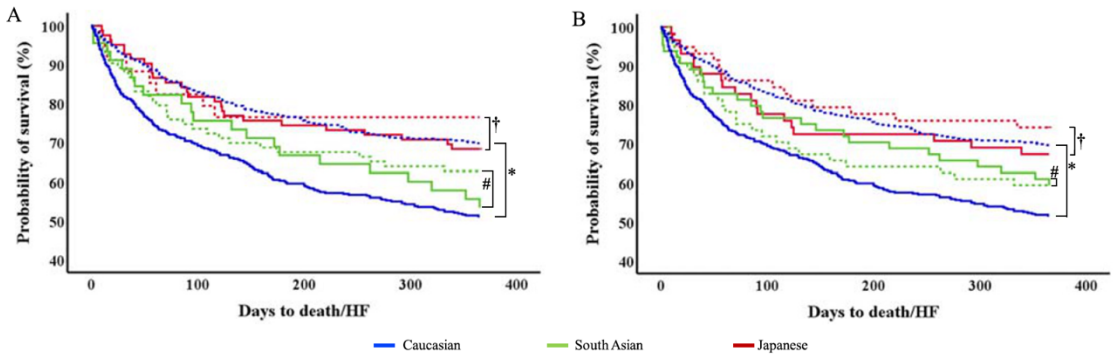


Figure 3 Kaplan–Meier curve showing the relationship of each ethnic group and all-cause death and/or rehospitalization due to heart failure at 1 year after stratification by TMAO median. HF, heart failure; TMAO, trimethylamine-N-oxide.



	Caucasian*		South Asian#		Japanese†	
	Chi-square	p Value	Chi-square	p Value	Chi-square	p Value
(A) Total cohort TMAO	33.295	<0.001	0.585	0.444	0.494	0.482
(B) Ethnicity-derived TMAO	31.046	<0.001	0.017	0.895	0.678	0.410

Solid lines represent above median and dashed lines represent below median.

Median TMAO levels for: total cohort 5.89 µmol/L; Caucasian 5.86 µmol/L; South Asian 4.53 µmol/L; Japanese 9.86 µmol/L

4.6 Combined use of trimethylamine N-oxide with BNP for risk stratification in heart failure with preserved ejection fraction: findings from the DIAMONDHFpEF study

Salzano A, Israr ZM, Yazaki Y, Heaney LM, Kanagala P, Singh A, Arnold JR, Gulsin G, Squire IB, McCann G, Leong LN, Suzuki T. Combined use of trimethylamine N-oxide with BNP for risk stratification in heart failure with preserved ejection fraction: findings from the DIAMONDHFpEF study. Eur J Prev Cardiol. 2019 Aug 14:2047487319870355. doi: 10.1177/2047487319870355. PMID: 31412713

Circulating levels of Trimethylamine N-oxide (TMAO), a gut microbiome-mediated metabolite related to Western diet [418,419], have been shown to be associated with risk stratification and outcome in patients with heart failure (HF) with reduced ejection fraction (HFrEF) [70,73,346,420]. The aim of the present study was to assess the associations between TMAO with outcomes in patients with HF with preserved ejection fraction (HFpEF).

To investigate the recently identified link between the gut and HF (namely: "gut hypothesis"), TMAO levels were measured in 118 patients with HFpEF, 38 patients with HFrEF, and 40 healthy volunteer participants (sex/age matched) with available baseline plasma samples from the Developing Imaging And plasMa biOmarkers iN Describing Heart Failure With Preserved Ejection Fraction (DIAMONDHFpEF) cohort, a prospective, observational, single-centre study aimed at developing imaging and plasma biomarkers of novel pathophysiological patterns in HFpEF [NCT03050593][421]. Plasma levels of TMAO were measured using liquid chromatography-tandem mass spectrometry, a high throughput, reproducible, and accurate method, as previously reported [70,346,422]. BNP was measured using a commercial immunoassay (Siemens, Erlangen, Germany). All patients had blood tests, transthoracic echocardiography (TTE, Philips iE33, Amsterdam, Netherlands), and cardiac magnetic resonance (CMR) imaging (Siemens Skyra Erlangen, Germany) during the same visit. Primary outcomes were defined as the composite endpoint of all-cause mortality or hospitalisation for HF at 18 months (short-term) and at 60 months (long-term).

Baseline patient demographics, blood chemistry, echocardiographic, and CMR measurements are shown in Table 1. Patients with HFpEF had higher body mass index (BMI), higher prevalence of systemic hypertension and atrial fibrillation, and less coronary artery disease. HFpEF patients had lower BNP levels as compared to HFrEF patients [140 (55-252) pg/mL vs. 345 (167-605) pg/mL,

p<0.001]. HFrEF patients showed higher end-diastolic [140.8 (107.8-164.9) ml/m^2 vs 77.2 (65.9-91.9) ml/m^2] and end-systolic [97.8 (68.7-128.5) ml/m^2 vs 33.1 (27.4-41.9) ml/m^2] volumes on CMR (p<0.01), and higher LV filling pressures (E/e' ratio) [13.1 (11.5-19.9) vs. 11.5 (9.1-16.6), p=0.023] on TTE (Table 1). HF patients showed elevated circulating TMAO levels when compared to sex/age matched healthy controls [HFpEF 6.6 (4.3-12.2) µmol/L, adj. p=0.003; HFrEF 8.4 (3.7-13.8) µmol/L, adj. p=0.006; vs. control 4.0 (3.2-5.3) µmol/L], but no differences were observed between HF phenotypes. TMAO was positively correlated with age, BMI, E/e', and BNP levels, and negatively correlated with eGFR (Table 1). A total of 27 events (13 deaths and 14 HF hospitalisation) were recorded in the HFpEF cohort over a follow-up of 18 months (short-term), and 55 events (32 deaths and 23 hospitalisation) over a follow up of 60 months (long-term). For short-term outcomes, Cox proportional hazards regression showed that HFpEF patients with a circulating TMAO level exceeding a cut-off of 5 µmol/L (derived from the upper quartile of the control population in this study as a whole integer) showed a 4-fold increase in risk of an event [HR (95% CI) 3.82 (1.15-12.69), p=0.029]. TMAO remained significantly associated with outcome when adjusted for age, sex, BMI, and eGFR [3.66 (1.03-13.04) p=0.045]. However, TMAO was not significantly associated with events as a continuous, univariate marker (p=0.109). For long-term outcomes, as a dichotomised variable at the cut-off point of 5 µmol/L, TMAO was significantly associated with outcomes [2.74 (1.34-5.61) p=0.006], and showed associations when adjusted for confounders [2.45 (1.12-5.35) p=0.025]. As a continuous variable, TMAO was associated with outcome as a univariate marker [1.98 (1.11-3.55) p=0.021], but did not retain this when adjusted for confounders. When mortality was investigated, as a continuous variable, TMAO was significantly associated with outcome both as a univariate marker [2.40 (1.11-5.18) p=0.025], and after adjustment [3.53 (1.34-9.33) p=0.11]. As elevation of BNP levels is less pronounced in HFpEF patients, Kaplan-Meier survival analyses were performed to compare the use of TMAO as a predictor of outcome in HFpEF patients with low BNP levels (cut-off value of 140 pg/mL and 250 pg/mL, derived from the ESC guidelines [1]) and compared to those with more pronounced elevations of circulating BNP (≥140 pg/mL and ≥250 pg/mL) (Figure 1). When all HFpEF patients were stratified by TMAO and BNP levels, those with lower levels of both biomarkers reported the greatest survival, with a gradual increase of risk when one or both biomarkers were elevated for short- (log-rank test, p≤0.004, Figure 1A and 1C) and long-term outcomes (log-rank test, p≤0.010, Figure 1B and 1D). When compared to patients with lower levels of both biomarkers, BNP alone, when stratified at 140 pg/mL, showed significant changes in survival for short-term (p=0.04), but not long-term (p=0.11) outcomes, whereas TMAO alone, when stratified at 5 µmol/L, showed

significant changes in survival for both short- and long-term outcomes (p≤0.04, Figure 1A and 1B). When stratified at 250 pg/mL, both BNP alone and TMAO alone showed significant changes in survival for both outcomes (p≤0.03, Figure 1C and 1D).

The present study indicates that TMAO levels were elevated to a similar extent in HFpEF and HFrEF patients in comparison to cardiovascular disease-free participants, in line with a previous report [72]. Furthermore, our findings suggest the possible use of TMAO for risk stratification of long-term mortality. In addition, our results support the hypothesis that TMAO levels could aid in stratifying HFpEF patients who would otherwise be classified as low-risk based on BNP levels. Indeed, when a cut-off level was used for TMAO [upper quartile of the normal range (5 µmol/L)], associations with adverse outcomes in HFpEF patients in which BNP, the 'gold standard' biomarker in HFrEF, was less elevated were observed. TMAO levels also increased with higher LV filling pressure and BNP levels, suggesting association with worsening of HF with progressive diastolic dysfunction, in line with a previous report showing association between TMAO levels, HF severity, and diastolic dysfunction [345]. To date, only one study has investigated the prognostic value of TMAO in HFpEF [72], with inconclusive results on how TMAO may benefit risk stratification of patients with this condition, perhaps because of the enrolment criteria (only patients referred for coronary angiography have been enrolled). The present report suggests that TMAO could have a role in clinical management of HFpEF patients, allowing a better risk stratification, not achievable with BNP alone and that still remains an unmet need in clinical practice [423,424]. Further, therapeutic intervention to the gut microbiome might be potential additive treatments for HF. Notably, our data support the idea that the integration of multiple biomarkers, in particular if circulating and imaging biomarkers related to different pathophysiological patterns are combined, seems to be the most promising strategy in HF [8]. As study limitations, patients were recruited from a single centre with a relatively small sample size that does not allow extensive prediction modelling to be performed. In addition, data regarding dietary intake and antibiotic treatment that could influence TMAO levels were not available.

In conclusion, as natriuretic peptides are not as highly elevated in HFpEF compared to HFrEF, elevated circulating levels of TMAO may provide utility in risk stratification of HFpEF where this and other biomarkers show equivocal levels. Therefore, the combined use of BNP and TMAO may be useful in patients with HFpEF.

Table 1. Patient demographics.

	HF ($n=156$)	HFpEF ($n=118$)	HFrEF ($n=38$)	Control ($n=40$)	HFpEF vs. HFrEF	Correlation with TMAO full population (RS)	*P* value
Demographics							
Age (years)	73 (67–78)	73 (65–78)	72 (62–77)	72 (69–77)	ns	0.154	0.031
Male (%)	49	49	50	53%	ns	–	–
Body mass index (kg/m^2)	32.2 (26.4–38.5)	33.8 (28.4–39.7)	27.5 (24.7–32.3)	–	<0.001	0.158	0.027
NYHA (I/II/III/IV)	53/59/40/4	37/46/31/4	16/13/9/0	–	ns	–	–
Hypertension (%)	81	91	54	–	<0.001	–	–
Coronary artery disease (%)	29	21	50	–	<0.001	–	–
Diabetes mellitus (%)	46	49	39	–	ns	–	–
Atrial fibrillation (%)	38	79	24	–	0.017	–	–
eGFR (mL/min/1.73m^2)	68 (48–85)	69 (50–85)	65 (48–78)	–	ns	−0.527	<0.001
CMR							
LVEDVi (ml/m^2)	85.9 (69.6–106.8)	77.2 (65.9–91.9)	140.8 (107.8–164.9)	80.4 (72.34–89.6)	<0.001	0.028	0.736
LVESVi (ml/m^2)	39.2 (29.7–57.6)	33.1 (27.4–419)	97.8 (68.7–128.5)	32.6 (29.2–37.8)	<0.001	0.005	0.953
LVEF (%)	52.8 (41.8–58.6)	55.4 (51.8–60.1)	28.3 (20.7–36.4)	57.3 (55.4–59.77)	<0.001	−0.001	0.991
Echocardiography							
E/e′	12.3 (9.7–19.9)	11.5 (9.1–16.6)	13.1 (11.5–19.9)	–	0.023	0.287	<0.001
Biomarkers							
BNP (pg/mL)	170 (80–336)	140 (55–252)	345 (167–605)	–	<0.001	0.221	0.006
TMAO (μmol/L)	7.0 (4.2–12.5)	6.6 (4.3–12.2)	8.4 (3.7–13.8)	4.0 (3.2–5.3)	ns	–	–

HF: heart failure; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; TMAO: trimethylamine N-oxide; BNP: B-type natriuretic peptide; NYHA: New York Heart Association functional class; eGFR: estimated glomerular filtration rate; LVEDVi: left ventricular end diastolic volume indexed; LVESVi: left ventricular end systolic volume indexed; LVEF: left ventricular ejection fraction; E/e′: peak modal transmitral flow velocity in early diastole/pulse wave tissue Doppler imaging peak modal velocity in early diastole; r_s: Spearman's rho.
Data expressed as median (interquartile range) for continuous variables and percentage of total for categorical variables.

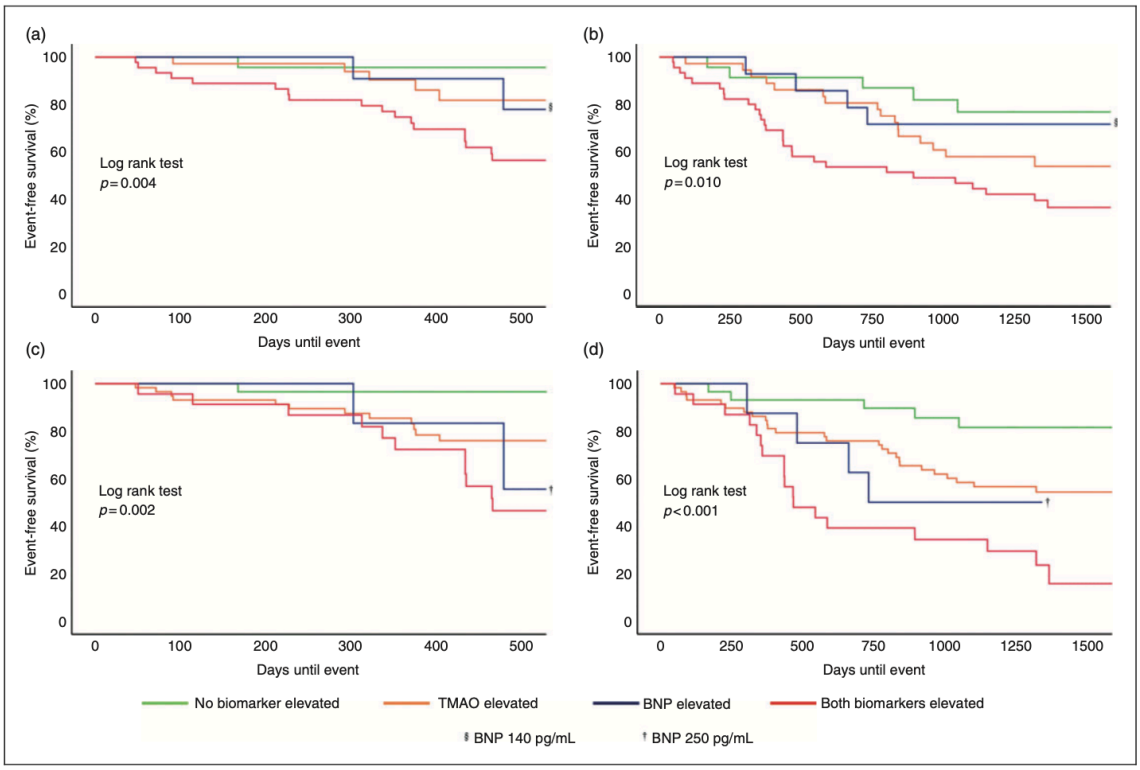


Figure 1. Kaplan–Meier analysis of survival at 18 months (short term), (a) and (c), and 60 months (long-term), (b) and (d), for all-cause mortality or heart failure hospitalisation in patients with preserved ejection fraction (HFpEF) with neither biomarker elevated, only trimethylamine N-oxide (TMAO) elevated (5 μmol/l), only B-type natriuretic peptide (BNP) elevated (>140 pg/ml in (a) and (b) or >250 pg/ml in (c) and (d)) or both biomarkers elevated.

5. Chapter 5. Novel biomarkers in heart failure

5.1 Salzano A, Israr MZ, Fernandez Garcia D, Middleton L, D'Assante R, Marra AM, Arcopinto M, Yazaki Y, Bernieh D, Cassambai S, Page K, Rengo G, Bossone E, Cittadini A, Shaw JA, Suzuki T. Circulating cell-free DNA levels are associated with adverse outcomes in heart failure: testing liquid biopsy in heart failure. Eur J Prev Cardiol. 2020 Mar 25:2047487320912375. doi: 10.1177/2047487320912375. PMID: 32212838

Circulating cell-free DNA (cfDNA) is genomic DNA released as result of cell death mechanisms (e.g. apoptosis, necrosis, and autophagy)[425]. Mostly investigated in oncology, cfDNA fragments have proven utility for early detection, treatment monitoring, and risk stratification of cancer and is known as the 'liquid biopsy' method [425-427]. More recently, investigations in the cardiovascular field [428-430] have shown associations of cfDNA with cardiovascular risk factors [431], cardiovascular disease status (acute myocardial infarction and atrial fibrillation) [430], and for the early diagnosis of heart transplant rejection [432-434]. However, despite knowing that cell death is one of the features of heart failure (HF) pathobiology, cfDNA has never been investigated in chronic HF. Aim of the present study was to assess, for the first time, the role of the liquid biopsy in HF, investigating cfDNA levels in HF patients and their associations with clinical status, morbidity, and mortality.

Seventy-one consecutive chronic stable HF patients with ejection fraction (EF) <50% were enrolled from our HF unit and compared with 64 healthy volunteers enrolled as controls from a preventive medicine programme. Inclusion criteria comprised no history of recent acute HF decompensation in the last 6 months, severe liver (cirrhosis CHILD B-C) or kidney disease (serum creatinine >2.5 mg/dL); further, patients had to be on stable HF medications for at least 3 months. Cell-free DNA was extracted from plasma using the QIAamp Blood DNA Mini Kit (Qiagen). Each sample was quantified in triplicate relative to a standard curve, generated from a serially diluted human genomic DNA template (Roche) of known concentration and an in-house locus-specific TaqMan assay targeting a 96bp intronic sequence in the housekeeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [426] . Primers were designed to target GAPDH as described previously [427]. B-type natriuretic peptide (BNP) levels were measured with the RapidPIA™ BNP kit (Sekisui Medical Co). The primary endpoint was defined as a composite of all-cause mortality and/or HF hospitalisation (death/HF) at 30 months. The associations between analysed variables and outcome were

investigated using Cox proportional hazard regression analyses. Kaplan–Meier survival curves were generated and the Mantel–Cox log-rank test was used to compare event-free survival after stratification of cfDNA levels by the median. Outcome prediction accuracies were assessed by calculating the area under the curve (AUC) for receiver operating characteristic (ROC) curves analyses and by using net reclassification index (NRI) for cfDNA and BNP levels, alone and/or combined, as adjusted for the multivariable linear model.

Patient demographics, blood chemistry measurements, and clinical characteristics are described in Table 1A. When cfDNA levels were compared, approximately 6-fold higher cfDNA levels were observed in HF patients (median [interquartile range]; HF: 19.4 [11.1-41.7], healthy: 3.4 [2.04-6.06], $p<0.001$), with a significant correlations between cfDNA levels and New York Heart Association (NYHA) class (r_s = 0.294, p=0.017) and biochemistry markers (albumin, calcium, creatine phosphokinase, and Beta-2 globulin) (r_s = -0.267-0.298, $p\leq0.048$). No correlations were found with age, sex nor echocardiographic parameters. For outcome analyses, cfDNA levels were not significantly associated with outcome as a continuous variable. However, when cfDNA levels were dichotomised according to the median value of the HF population (19.4 ng/mL) and also using the Youden's Index for optimal cut off point from the ROC curve (20.8 ng/mL), with both methods of applying a cut off value yielding identical categories (n=34 patients with elevated cfDNA levels), univariate analysis showed that cfDNA levels were associated with death/HF hospitalisation at 30 months [hazard ratio (95% confidence interval) p value; 2.57 (1.36-4.87) p=0.004]. When adjusted for main clinical parameters (age, NYHA class, sex, aetiology, BNP, and estimated glomerular filtration rate), cfDNA retained prediction abilities [2.12 (1.04-4.30) p=0.038] (Table 1B). ROC curves using the same multivariable model, with and without BNP and cfDNA levels, showed that the combination of BNP and cfDNA gave a consistent gain in C-statistic for associations with outcome when compared to the same model with either BNP or cfDNA alone [0.750 (0.619-0.881), 0.752 (0.632-0.872) and 0.796 (0.687-0.905) for BNP alone, cfDNA alone, and combination of both biomarkers respectively, $p<0.001$] (Table 1C). For net reclassification analysis, when added to the clinical variables for the composite endpoint of death/HF, the combination of cfDNA and BNP [55.8 (10.3-101.3) p=0.016] showed an improvement over BNP alone [52.9 (8.63-97.3) p=0.019]; however cfDNA alone was superior [63.8 (17.7-109.8) p=0.006] suggesting that cfDNA levels is the weighing factor (Table 1C). Kaplan-Meier survival analyses showed that HF patients with cfDNA levels above the median value have reduced survival (chi-square: 9.32; log rank test p=0.002) (Figure 1A). In addition, when patients were categorised according to median levels of cfDNA and BNP (Figure 1B), patients with elevations

in both biomarkers showed the lowest survival, significantly different to those with normal levels of both biomarkers (chi-square 17.83, log rank test $p<0.001$) or only BNP elevated (chi square 4.52, $p<0.030$). No differences were found when compared to patients with only cfDNA elevated which suggests that elevations in cfDNA levels is the weighing factor.

The present investigation showed, for the first time, that circulating cfDNA levels are elevated in HF patients compared to healthy subjects, similar to other cardiovascular diseases[430,435,436]. In addition, cfDNA is associated with clinical status. When combined with BNP, cfDNA levels added to patient risk stratification, with cfDNA as the weighing factor, as shown by the Kaplan-Meier and net reclassification analyses. Despite the precise mechanisms of cfDNA in HF pathophysiology being unknown, it is plausible to envisage a combinatorial mechanism involving myocardial injury, chronic inflammation, and thrombosis/platelet aggregation. Indeed, cfDNA is released from the heart upon reperfusion after prolonged ischemia, and plays a critical role in ischemia-reperfusion injury through a receptor for advanced glycation end products (RAGE)-toll like receptor 9 [437]. Additionally, in cardiovascular diseases, cfDNA has been implicated in hyperactivation of polymorphonuclear cells which promote platelet aggregation and chronic thrombosis [428,430]. Higher cfDNA levels have also been reported to be associated with systemic low-grade inflammation markers [e.g. Interleukin (IL)-6, IL-8] [431]. Further, association of cfDNA has been reported with increased neutrophil extracellular trap formation, which impacts the efficiency of fibrinolysis with enhanced peak thrombin generation, impaired fibrinolysis, and decreased clot permeability [438]. As study limitations, patients were recruited from a single centre, with a relatively small sample size that does not allow extensive prediction modelling to be performed. However, the present report is hypothesis generating, and further large-scale studies are warranted.

In conclusion, cfDNA levels are associated with morbidity and mortality in HF, suggesting that liquid biopsy may offer additional methods to monitor and stratify the risk of outcome in HF patients.

Table 1. Patient demographics.

Patient demographics	Total cohort ($n = 71$)	Low[a] cfDNA ($n = 37$)	High[a] cfDNA ($n = 34$)	p Value
Age (years)	63.5 ± 12.5	63.9 ± 12.9	62.9 ± 12.2	0.700
Sex (M/F)	60/11	32/4	27/7	0.300
NYHA (I–II/III–IV)	49/16	29/6	21/10	0.600
Aetiology (% ischaemic)	60	66	55	0.700
Systolic blood pressure (mm hg)	126.8 ± 14.6	128.5 ± 13.8	125 ± 15.4	0.300
Diastolic blood pressure (mm hg)	75.9 ± 9.5	78.7 ± 7.7	73.1 ± 10.5	0.010[b]
Heart rate (bpm)	66.8 ± 11.1	68.4 ± 11.9	65.1 ± 9.9	0.200
BMI (kg/m^2)	29.1 ± 4.7	29.4 ± 4.2	28.7 ± 5.2	0.600
Urea	50.5 ± 32	47.2 ± 22.8	54.8 ± 39.6	0.400
eGFR (CKD-EPI formula, ml/min)	89.6 ± 37.1	90.9 ± 38.4	88.3 ± 36.2	0.700
BNP (pg/ml)	55.6 (14.1–121.75)	37 (12.5–94.5)	76.5 (31.1–149)	0.200
cfDNA (ng/ml)	19.4 (11.1–41.7)	11.1 (5.6–13.9)	43.1 (28.5–81.2)	<0.001[b]
Left ventricular EF (%)	38.4 ± 8	37.8 ± 6.1	39.1 ± 9.7	0.500
VO_2 peak (ml/min/kg)	16.6 ± 5.22	16.1 ± 6.3	17.3 ± 3.6	0.600
Medication (%)				
Beta-blocker	87	92	91	0.900
ACE-I/ARBs	80	86	80	0.600
MRA	60	63	62	0.900
Diuretics	80	87	83	0.500

ACE-I: angiotensin-converting enzyme inhibitor; ARBs: angiotensin II receptor blockers; BMI: body mass index; BNP: B-type natriuretic peptide; cfDNA: cell-free DNA; CKD-EPI: chronic kidney disease-epidemiology collaboration; EF: ejection fraction; eGFR: estimated glomerular filtration rate; MRA: mineralocorticoid receptor antagonist; NYHA: New York Heart Association; VO_2: oxygen consumption.
[a]cfDNA levels dichotomised by the median 19.4 ng/ml; [b]p value < 0.05.

Table 2. Cox proportional hazard regression analyses to establish association between analysed variables and outcome.

Multivariate Cox model	HR	95% (CI)	p Value
cfDNA dichotomised[a]	2.12	1.04–4.30	0.038[b]
Age	0.99	0.96–1.04	0.920
Sex	0.67	0.76–2.15	0.350
NYHA class	1.28	0.23–1.98	0.470
Aetiology	1.17	0.63–2.14	0.630
BNP	1.01	1.00–1.01	0.020[b]
eGFR	0.99	0.98–1.01	0.300

BNP: B-type natriuretic peptide; cfDNA: cell-free DNA; CI: confidence interval; eGFR: estimated glomerular filtration rate; HR: hazard ratio; NYHA: New York Heart Association.
[a]cfDNA levels dichotomised by the median 19.4 ng/ml; [b]p value < 0.05.

Table 3. Reclassification analysis using continuous reclassification of adding B-type natriuretic peptide (BNP), cell-free DNA (cfDNA) or both to the multivariable predictive model.

Mortality/HF at 30 months	C statistic	*p* Value	NRI	*p* Value
BNP	0.750 (0.619–0.881)	0.001[a]	52.9 (8.63–97.3)	0.019[a]
cfDNA	0.752 (0.632–0.872)	0.001[a]	63.8 (17.7–109.8)	0.006[a]
BNP + cfDNA	0.796 (0.687–0.905)	0.001[a]	55.8 (10.3–101.3)	0.016[a]

HF: heart failure; NRI: Net Reclassification Index.
Mortality/HF is a composite endpoint of total mortality and/or HF hospitalisation.
[a]*p* Value < 0.05.

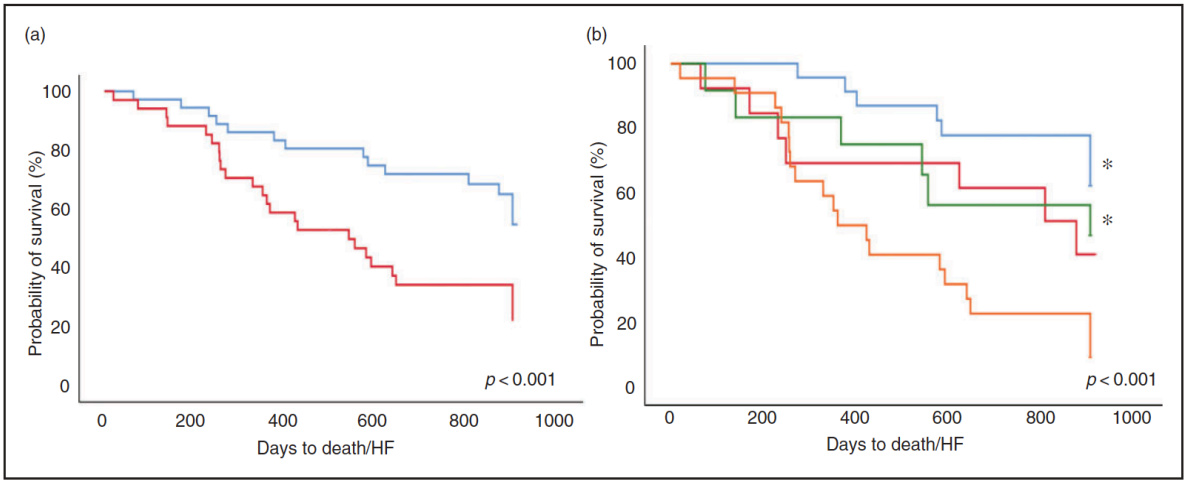


Figure 1. (a) Kaplan-Meier survival analysis for death and/or rehospitalisation due to heart failure (HF) stratified by cell-free DNA (cfDNA) median. Light blue: above the median; Red: under the median. (b) Kaplan-Meier survival analysis for death and/or rehospitalisation due to heart failure (death/HF) stratified by cfDNA and B-type natriuretic peptide (BNP) median. Blue: low cfDNA and low BNP; green: low cFDNA and high BNP; red: high cfDNA and low BNP; orange: high cfDNA and high BNP. *$p < 0.05$ when compared to high cfDNA and high BNP.

5.2 Israr MZ*, **Salzano A***, Yazaki Y, Voors AA, Ouwerkerk W, Anker SD, Cleland JG, Dickstein K, Metra M, Samani NJ, Ng LL, Suzuki T; on behalf of BIOSTAT-CHF consortium. Implications of serial measurements of natriuretic peptides in heart failure: insights from BIOSTAT-CHF **Eur J Heart Fail.** 2020. 10.1002/ejhf.195

Natriuretic peptides [NP, including B-type natriuretic peptide (BNP) and amino-terminal prohormone of BNP (NT-proBNP)] are the gold-standard biomarkers in heart failure (HF) management[1], with NP levels at presentation/admission routinely used for diagnostic and prognostic purposes[439]. NP levels at discharge/follow-up also show association with outcomes[440], and NP levels following HF treatment add further value to tailoring risk[441]. However, the usefulness of NP serial measurements beyond conventional HF treatment in clinical practice still remains a matter of controversy[440,442]. A cohort with current HF guideline-based treatment would provide an ideal setting to revisit usefulness of NP serial measurements in risk stratification of HF patients, including the role of recently identified BNP molecular forms[443]. The European multi-national BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF) provides an opportunity for the aforementioned analysis, being a European cohort in which serial sampling of NP was done before and after titration of HF medications according to current European guidelines in a multi-centre, observational, real-world setting[397].

Aims of the present study were to investigate the association with HF outcomes, effects of HF-guideline treatment, and the implications of NP serial measurement in the BIOSTAT-CHF cohort[397].

From the total cohort, 757 patients with available plasma samples at baseline (V1) and at follow-up (V2-approximately nine months apart) were measured for BNP and BNP molecular form, BNP 5-32[443] (see Table 1 for methods on measurements). NT-proBNP measurement was only available at baseline (V1); therefore, analyses related to this peptide were limited to baseline (V1). The primary endpoints were all-cause mortality and a composite of mortality with HF rehospitalisation (mortality/HF) at 3 years, and overall from baseline (V1). Changes in dosage titrations and response of peptide levels were investigated by splitting the population into two groups based on treatment up-titration, as previously reported (see Supplementary)[397].

Demographics and clinical measurements are described in Table 1. At baseline (V1), NP levels (BNP, NT-proBNP, and BNP 5-32) were strongly correlated with each other (r_s = 0.635-0.904,

p<0.001). Cox regression modelling showed baseline BNP levels to be associated with mortality [HR 1.99 (95% CI 1.23-3.23) p=0.005] and mortality/HF [1.72 (1.25-2.37) p=0.001]. NT-proBNP and detection of BNP 5-32 were similarly associated with mortality [≥1.85 (1.15-3.20) p≤0.012] and mortality/HF [≥1.54 (1.14-3.22) p≤0.015] after adjustment for the BIOSTAT compact model[397] (Table 2A). All three NPs retained their associations with outcomes after further adjustment with additional NP confounders (Table S1.A). With regard to effect of HF treatment, significantly reduced levels of BNP were observed only when at least one medication was up-titrated, whereas BNP 5-32 was reduced regardless of drug up-titration (Table 2B). A general linear model analysis for repeated measures confirmed these findings (Table S1.B). For serial measurements (Table 2C), when BNP baseline (V1) and follow-up (V2) levels were compared, follow-up (V2) measurements were more strongly associated with all cause-mortality than baseline (V1) (chi-square: 67.1 vs 16.0). However, even if the combination of baseline (V1) and follow-up (V2) measurements were significant (chi-square: 66.7, p<0.001), there was no added value to the follow-up (V2) measurement alone, as the role of the baseline (V1) measurement was not preponderant (p=0.878). Similarly, follow-up BNP 5-32 measurement showed a stronger association with all-cause mortality than the baseline value (chi-square: 64.3 vs 18.8); however, the combination of baseline (V1) and follow-up (V2) measurements was significantly better (chi-square: 69.8), with the baseline (V1) level providing additional value (chi-square: 5.5, p=0.017) to follow-up (V2) measurement alone. Furthermore, in patients that did not achieve ≥50% dose treatment but still showed BNP 5-32 to decrease from detectable to undetectable levels (or high-low for BNP) exhibited better outcomes than those who displayed increased levels at follow-up (Supplementary Figures 1 and 2).

There are three main findings of the present investigation. Firstly, baseline NP levels were independently associated with adverse outcomes, with comparable results for BNP, NT-proBNP, and BNP 5-32. Secondly, response to HF-guideline treatment up-titration was associated with a decrease in both BNP and BNP 5-32 levels. Finally, even if both BNP and BNP 5-32 showed stronger association with all-cause mortality at follow-up measurement compared to baseline, combination of baseline and follow-up measurements did not add value for BNP beyond follow-up alone, whereas BNP 5-32 did.

The recent North American GUIDE-IT trial[444] showed guideline-directed medical therapy (GDMT) guided by NT-proBNP levels was not superior to GDMT alone and that GDMT intensity was associated with lower NT-proBNP levels and further that low NP levels at follow-up (NT-proBNP levels ≤1,000 pg/ml during GDMT) were associated with better outcomes[445]. Consistent with this, the

present study based on a European real-world cohort showed that follow-up values after guideline-based treatment were more associated with outcomes for both BNP and BNP 5-32 (Supplementary Figures 3 and 4). In this context, analysis of the NP response in the BIOSTAT-CHF cohort, with medications optimised according to HF-guidelines, confirmed association of baseline NP levels (BNP, NT-proBNP and BNP 5-32) with adverse outcomes, and follow-up levels after treatment to show better association with adverse outcomes when compared to baseline levels, consistent with previous reports[1, 2, 441,446,447]. This is in line with a previous finding in another real-world cohort conducted in the UK in which the measurement of follow-up NT-proBNP, after optimisation of pharmacotherapy, although preceding current guidelines, provided more value than baseline measurements alone[446]. The difference in added value of combined use of baseline and follow-up measurements for association with mortality observed for BNP and BNP 5-32 in the present study may reflect different responses to treatment, with BNP levels being affected by treatment but not BNP 5-32 levels as a result of differential peptide processing in HF patients. BNP molecular forms may provide a more treatment-independent outcome biomarker. In the era of peptidase inhibitors (i.e. sacubitril/valsartan, DPP-IV inhibitors), monitoring NPs including molecular forms might allow further insight into NP processing that appear to be altered in HF.

As limitations, BIOSTAT-CHF was a non-randomised observational study, therefore it is not possible to infer causality to our findings or provide a mechanistic explanation. This study involved only European centres, and 99% of patients were Caucasian; therefore, the findings of this study may not be representative of HF patients at a global level.

In conclusion, findings from the BIOSTAT-CHF study, as a real-world cohort, supports the role of serial measurement of natriuretic peptides in clinical practice, with follow-up BNP and BNP 5-32 levels adding value to risk-stratification in HF patients. Future studies are needed in cohorts with NP-modulating treatment (i.e. peptidase inhibitors).

Table 1 **Patient characteristics**

	Patients with follow-up visit ($n = 757$)		
	Visit 1	**Visit 2**	***P*-value**
Age, years	69 (60–77)		
Male sex	76%		
Current smoker	14%		
Ischaemic aetiology	54%		
Diabetes mellitus	31%		
COPD	18%		
Previous HF hospitalisation	29%		
NYHA class			<0.001*
I	3%	16%	
II	42%	59%	
III	47%	24%	
IV	8%	1%	
LV ejection fraction (%)	30 (25–36)	35 (28–43)	<0.001*
Pulmonary congestion	49%	11%	<0.001*
Peripheral oedema	49%	24%	<0.001*
Systolic blood pressure (mmHg)	122 (110–140)	123 (110–140)	0.654
Diastolic blood pressure (mmHg)	75 (68–85)	75 (66–80)	0.011*
Heart rate (bpm)	75 (65–88)	70 (61–80)	<0.001*
Beta-blocker	85%	93%	<0.001*
ACEi or ARB	74%	89%	<0.001*
Haemoglobin (g/dL)	13.4 (12.1–14.5)	13.3 (12.1–14.3)	0.030*
Urea (mmol/L)	9.4 (6.8–14.3)	10.3 (7.1–15.7)	<0.001*
eGFR[a] (mL/min/1.73 m^2)	66 (49–82)	61 (46–79)	<0.001*
Sodium (mmol/L)	140 (137–142)	139 (137–142)	0.209
BNP (pg/mL)	202 (85–406)	134 (49–349)	0.001*
NT-proBNP (ng/L)	2236 (971–4654)	–	–
BNP 5–32[a]	50% [0.2 (0–0.5)]	25% [0 (0–0)]	<0.001*
Endpoints			
2 years			
Death	83		
Death/HF	219		
3 years			
Death	97		
Death/HF	230		

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; BP, blood pressure; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration formula); HF, heart failure; LV, left ventricular; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association.

Combined data are shown as median (interquartile range) for continuous variables and as a % for categorical variables. *P*-values for visit 1 vs. visit 2 are quoted for Wilcoxon matched-pair signed-rank tests for continuous variables and McNemar test for categorical variables. BNP 5–32 is reported as a ratio of molecular form signal intensity against an internal reference standard.

BNP was measured using Luminex multiplexed bead-based immunoassays (Alere, San Diego, CA, USA) and validated in a small subset using a commercial assay [RapidPIA®, Sekisui Medical Co.; $r^2 = 0.825$)]. NT-proBNP measured using the Roche NT-proBNP assay (Roche Diagnostics, Risch-Rotkreuz, Switzerland). BNP 5–32 was measured using matrix-assisted laser desorption ionisation-time of flight-mass spectrometry (MALDI-ToF-MS).[6] BNP 4–32 and BNP 3–32 were also detected in the same assay as BNP 5–32 but were not as sensitive and not comparable to BNP and NT-proBNP, and therefore omitted from analyses.

[a] Values recorded as % detection [median (interquartile range)].

*$P < 0.05$.

Table 2 **Independent prediction abilities of baseline natriuretic peptides for overall outcomes of death and death/heart failure**

Multivariate Cox model	**Mortality**			**Mortality/HF**		
	HR	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
BNP[b]	1.99	1.23–3.23	0.005*	1.72	1.25–2.37	0.001*
BNP 5–32[a]	2.01	1.26–3.20	0.003*	1.54	1.14–2.08	0.005*
NT-proBNP[b]	1.85	1.15–2.99	0.012*	2.33	1.69–3.22	<0.001*

BNP; B-type natriuretic peptide; CI, confidence interval; HF, heart failure; HR, hazard ratio; NT-proBNP, N-terminal pro B-type natriuretic peptide.

The compact risk model for mortality adjusted for age, haemoglobin, blood urea and use of beta-blocker at baseline. The compact risk model for mortality/HF included age, previous HF hospitalisation, peripheral oedema, systolic blood pressure, haemoglobin, sodium and use of beta-blocker at baseline.

[a] Dichotomised according to detection or no detection of the peak.

[b] Values were log transformed.

Table 3 Response to guideline-based treatment for B-type natriuretic peptide (BNP) and BNP 5–32

Dose up-titration		BNP (pg/mL)			BNP 5–32[a]		
	n	V1	V2	*P*-value	V1	V2	*P*-value
ACEi							
<50%	325	228 [100–467]	161 [69–420]	0.359	0.3 [0.0–0.5]	0.0 [0.0–0.3]	<0.001*
≥50%	432	169 [77–344]	114 [39–283]	0.001*	0.0 [0.0–0.5]	0.0 [0.0–0.0]	<0.001*
Beta-blocker							
<50%	424	183 [85–390]	142 [54–382]	0.389	0.2 [0.0–0.5]	0.0 [0.0–0.3]	<0.001*
≥50%	333	208 [88–413]	125 [43–291]	<0.001*	0.0 [0.0–0.5]	0.0 [0.0–0.0]	<0.001*
Both drugs							
Both <50%	684	200 [85–408]	141 [56–382]	0.362	0.2 [0.0–0.5]	0.0 [0.0–0.3]	<0.001*
Both ≥50%	73	206 [86–391]	121 [37–251]	<0.001*	0.0 [0.0–0.5]	0.0 [0.0–0.0]	<0.001*

ACEi, angiotensin-converting enzyme inhibitor; BNP, B-type natriuretic peptide; V1, visit 1 (enrolment); V2, visit 2 (9-month follow-up) <50% less than 50% of optimal recommended dosage, ≥50% of optimal recommended dosage.
Values are reported as median [interquartile range].
[a]BNP 5–32 values reported as a ratio of the mass spectral peak signal intensity against adrenocorticotropic hormone (internal reference standard).

Table 4 Cox models of baseline B-type natriuretic peptide (BNP) 5–32, follow-up BNP 5–32, and combination of BNP 5–32 detection to illustrate whether their combination can better explain all-cause mortality

Serial measurement	Model chi-square	Chi-square if term removed	HR for all-cause mortality (95% CI)	*P*-value
BNP[a] (V1) only	16.0	16.0	2.04 (1.44–2.90)	<0.001*
BNP[a] (V2) only	67.1	67.1	4.03 (2.88–5.65)	<0.001*
BNP[a] (V2) +	66.7	75.1	4.00 (2.71–5.91)	<0.001*
BNP[a] (V1)		0.4	1.04 (0.67–1.60)	0.878
BNP 5-32[b] (V1) only	18.8	18.8	2.14 (1.50–3.04)	<0.001*
BNP 5-32[b] (V2) only	64.3	64.3	3.77 (2.66–5.34)	<0.001*
BNP 5-32[b] (V2) +	69.8	52.2	3.28 (2.28–4.73)	<0.001*
BNP 5-32[b] (V1)		5.5	1.61 (1.09–2.37)	0.017*

BNP, B-type natriuretic peptide; CI, confidence interval; HR, hazard ratio; V1, visit 1; V2, visit 2.
Univariate Cox regression analysis was performed initially for (i) baseline measurement, then for (ii) follow-up measurement, and finally for (iii) baseline + follow-up generating a chi-square for the overall model and also chi-square values for the contribution of the individual variables to the overall model, hence chi-square if term removed.
[a]Values were log transformed.
[b]Dichotomised according to detection or no detection of the peak.

6 Chapter 6. Miscellaneous

6.1 Salzano A[§,] Suzuki T, Squire IB, Cittadini A. Are heart failure observational studies still useful? 'No need to argue'. Eur J Prev Cardiol. 2020 Jun 24:2047487320932258. doi: 10.1177/2047487320932258. PMID: 32580568

Despite a general improvement in prevention, epidemiology, and management of cardiovascular diseases [448], heart failure (HF) still remains a major healthcare issue, affecting approximately 35 million subjects worldwide [449]. Because of its increasing prevalence, an unmet need in public health is to find novel strategies capable of slowing disease progression and reducing its high rate of mortality, still about 50% at 5 years. These necessities led to a great valuable effort in HF community, with the implementation in the last decade of an even larger amount of clinical research studies aimed at further understanding pathophysiological models, HF phenotypes, and role of comorbidities, as well as investigating the possible effects of novel treatments.

As result, a huge burden of new evidence has become available in the last few years, with some improvement in the general knowledge of the disease. However, as downside, this great accessibility of research data from several sources makes HF a field in which physicians have to disentangle themselves, in the difficult attempt to translate research findings into the clinical practice. Further, this massive amount of information paradoxically leads to the risk of misinterpreting them, with an even more spread of the simplistic view that wrongly consider as valuable only data derived from randomised control trials (RCTs), making data from observational studies (OS) neglected as 'children of a lesser God'. Testifying these concerns, recently a European Society of Cardiology (ESC) workgroup published a statement that pointed out strengths and weaknesses of both methods [450], to provide physicians with a possible guide in interpreting research results.

Although RCTs can be considered as the most powerful studies in clinical research in providing data on treatment effects, mostly thanks to random sampling (that limits the possibility to have differences in study groups characteristics, distributing known and unknown confounders between control and active arms), several limitations need to be acknowledged[451]. For instance, because of enrolment criteria, too often some categories of patients are underrepresented (e.g. elder people, ethnic minorities, advanced disease stages, patients with more comorbidities, low education level); further, because of the study design, patients enrolled in clinical trials have the risk to be over-

medicated and invited to attend a lot of follow-up visits, not reflecting the real clinical practice; in parallel, RCTs habitually enrol more motivated patients, and exclude patients who cannot strictly follow the study design dictates and/or attend to all controls scheduled, introducing an important selection bias difficult to delete; for example, it has been demonstrated that the patient response rate to a RCT screening invitation is generally pretty poor. In addition, usually RCTs have not enough duration to show long-term effects, nor enough sample size to identify potential rare adverse drugs effects. For these reasons, results from clinical trials need to be confirmed in the 'real world' scenario, where findings from RCTs could be occasionally confuted or reduced in importance when tested in larger population. Finally, there are contexts in which RCTs do not perform at best (i.e. rare diseases).

On the other hand, OS are able to solve several of these points. Indeed, subjects enrolled in OS are usually more similar to patients genuinely observed in everyday practice; further, the easier design allows to have a high participation rate, including categories of patients that are excluded by RCTs. In addition, higher numerosity and longer follow-up provide information that are often not reachable from RCTs[450]. Notably, small observational studies can also be useful in investigating, for the first time, novel hypotheses, as proof of concept studies of future more wide investigation[452].

Therefore, there is '*no need to argue*' that large OS provide data that are still necessary; indeed, they are more appropriate to describe natural history of a disease, to suggest novel risk factors, and generate new hypotheses; in addition, large OS can be used to have data about evaluation of drug safety; finally, observational studies can be used to have a snapshot of the performance of health systems (e.g. adherence to evidence-based guideline treatment) and to provide possible solutions to implement better health programs. However, it is necessary to be careful to claim that OS can address all limitation of RCTs. The truth lies somewhere in the middle, and both instruments are fundamental tools in clinical research, able to provide high quality research data, with different roles in different stages of the research process (Figure 1).

In the recent years, several are the examples of OS that provided (or are supposed to provide in the very next future) very useful information in HF, that changed or will change our HF management. For example, in the context of the ESC Eurobservational Research Programme (EORP), a series of cardiovascular registries launched in 2009, the EORP-HF registry[453], currently in the phase III, will assess contemporary patterns of HF management, prevalence of HF clinical profiles, phenotypes, and clusters across Europe. Another successful example is the Swedish Heart Failure Registry (SwedeHF), a nationwide continuous health quality and research registry, founded in 2000

throughout Sweden, that showed for the first time that enrolment in a HF registry improves outcome in HF patients[454].

A further evolution of OS, recently developed, is the possibility of collect blood samples, building biobanks available for future analyses; this allows to test supplementary hypotheses, often different from the main hypotheses of the principal study aims, further supporting the versatility of OS[455]. In addition, this possibility allows also the develop of multiparametric biomarkers (i.e. based on demographic data, blood and imaging biomarkers). For instance, the BIOSTAT-CHF (BIOlogy Study to TAilored Treatment in Chronic Heart Failure)[399,456], a multicentre, multinational, prospective, observational study from 11 European countries, aimed to characterize biological pathways related to response/no-response to guideline-recommended treatment for HF, in last years provided a great amount of data, that might have a strong impact on HF management (e.g. showing a sex difference in needed doses of HF drugs, with women who might need lower doses when compared to men)[456], with the possibility of further investigating in the future several different aspects, thanks to the availability of a wide biobank[399]. Another interesting example is the DIAMONDHFpEF (Developing Imaging And plasMa biOmarkers iN Describing Heart Failure With Preserved Ejection Fraction), a prospective, observational, cohort study aimed at developing imaging and plasma biomarkers in HF with preserved ejection fraction (HFpEF) [400], that is providing useful data to further define HFpEF phenotype, linking blood biomarkers with imaging. Finally, the T.O.S.CA. registry, a prospective multicentre observational study, has been specifically designed to investigate the prognostic impact of hormone deficiencies in HF[49], with serial blood samples and imaging parameters available over the follow-up, and that is expected to provide first results in the next months.

In this context, in the current issue of the European Journal of Preventive Cardiology, Göbel S and co-workers presented rationale, design, and baseline characteristics of the MyoVasc, a prospective, observational, cohort study, investigating development and progression of HF in a cohort of subjects enrolled from the University Medical Centre Mainz, Germany[457]. A preliminary information to keep in mind approaching the reading of the present article, is that MyoVasc investigators, by protocol, used the American College Cardiology/American Heart Association HF classification (which comprises 4 progressive and inviolate stages of HF), rather than ESC classification; as result, in the MyoVasc study have been enrolled not only subjects with overt HF (HF patients following ESC guidelines are equivalent to stage C and D of American guidelines), but also subjects at high risk of HF but without structural heart disease or HF symptoms (stage A), and subjects with structural heart disease, but without HF signs or symptoms of HF (stage B). In total, from January

2013 to April 2018, 3289 subjects have been enrolled. Of these, 1741 were overt HF subjects, with preserved ejection fraction (HFpEF) as the prevalent phenotype (37.1%), whereas 19.7% were HF with reduced ejection fraction (HFrEF) and 23% were HF mid-range (or HFpEF with borderline EF). In addition, 1253 individuals were not HF patients (considered as stage A/B following American guidelines).

Several are the particular features of the MyoVasc study. Firstly, the presence of stage A/B HF will allow to observe the natural history of the development of HF, from risk factor to overt HF; notably, recently have been published results of the Characteristics and Course of Heart Failure Stages A–B and Determinants of Progression (STAAB) cohort study, aimed to identify the frequency and characteristics of individuals at risk of HF in the general population of a representative sample of residents of Würzburg, Germany[458]; it will be of great interest to compare these results with the MyoVasc study, considering the geographical proximity between the two cohorts; secondly, the presence of all the three HF phenotypes will allow to investigate the featuring of HF across ejection fraction; finally, the biobank, with blood sampling each 2 years for 6 years of follow-up, will provide valuable resource for future analyses.

In conclusion, in the intricate but even more fascinating scenario of HF, observational data still remains necessary, even in the era of the big trials, in particular if biobanks are collected. The continued interplay between observational and randomised control trials is the road map to success in HF research. 'There's no need to argue, anymore'*.

*The Cranberries – No need to argue-Island Records – CID 8029, Island Records – 524050-2, Island Records – 524 050-2 -Release data 04 oct 1994

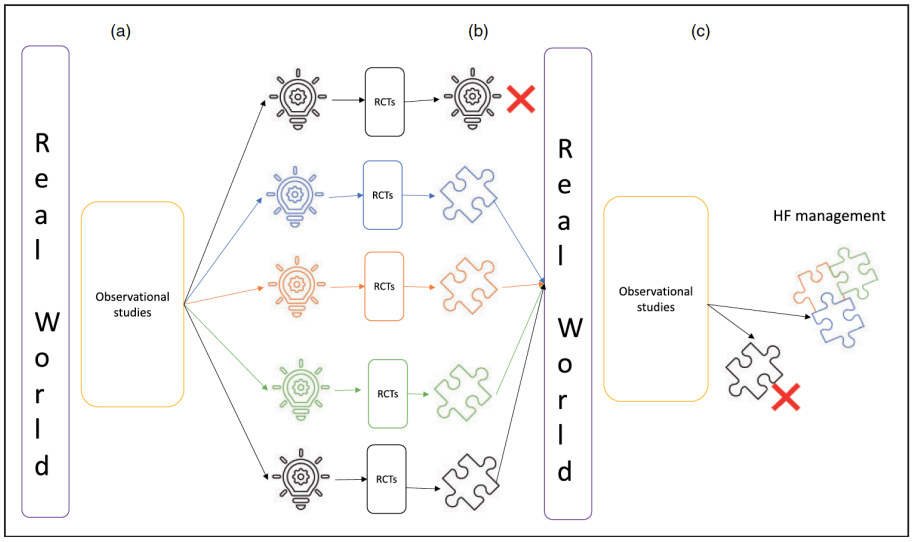


Figure 1. (a) From the real-world experience, observational studies (Oss) allow us to make new hypotheses (light bulb), that need to be tested in randomised control trials (RTCs). (b) RCTs can be confirmative of these hypotheses, providing useful results (puzzle piece), or can reject them. (c) Useful results from RCTs, when applied in the real world, can be validated in large OSs, and enter into clinical practice (puzzle pieces together), or be rejected.

6.2 **Salzano A**, D'Assante R, Stagnaro FM, Valente V, Crisci G, Giardino F, Arcopinto M, Bossone E, Marra AM, Cittadini A. Heart failure management during COVID-19 outbreak in Italy. Telemedicine experience from a heart failure university tertiary referral centre. **Eur J Heart Fail.** 2020 May 28:10. doi: 10.1002/ejhf.1911. PMID: 32463534

A few weeks after the first Italian case of person-to-person transmission of severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) [459], Italian government promulgated a Decree-Law imposing a general lockdown, aimed at reducing the spread of coronavirus disease of 2019 (COVID-19). Among restrictions affecting healthcare system, outpatient clinics and day-services have been suspended, and in-ward access has been granted only for urgent procedures. If on the one hand this reduced the risk of in-hospital COVID-19 spread, on the other hand led to marked decrease in the clinic assistance of chronic diseases -i.e. heart failure (HF).

Aims of the present report were to investigate whether a telemedicine service (TMS), expressly set up by our HF university tertiary referral centre[49] during the COVID-19 outbreak, impacts on HF outcomes (i.e. composite of HF hospitalisation/death), and to compare outcomes with the same period (11th March -4th May) of the previous year, when a TMS was not available.

Our TMS included two phone numbers, active 24/7, with the possibility of chat- and videoconference-services available with most popular smartphone applications; an email address was available too. Telemedicine visits were not scheduled, and accesses were all on voluntary basis by the patients, who were advised to use the TMS for all clinical necessity. However, if the attending physician deemed a further access necessary, patients were encouraged to contact us again. All patients were advised to follow all measures to prevent COVID-19 transmission (e.g. social distance, frequent handwashing, the use of face masks in public places, self-isolating). With regard to HF-treatment, we recommended the prosecution of all drugs as medical standard[460]. Notably, after a few days, European Society of Cardiology published a statement against the discontinuation of ACEi/ARBs/ARNI[461]; further, recent evidence from literature showed the lack of negative effects of ACEi/ARBs/ARNI for COVID-19 infection and severity [462]. At the end of the study period, all patients were contacted, to get information about outcome.

One hundred and three patients participated in the present study; outcomes were compared with data from 104 HF patients attending our unit in the same period of 2019 (Table 1). From 11th March to 4th May 2020, 58% of patients made at least one TMS access, mostly by phone call (64.2%),

followed by chat-service (33.6%). Overall, 51% of contacts led to a clinical decision (adjustment of diuretic doses, change of blood pressure drugs, rate controls, anticoagulants management, others) (Figure 1). With regard to primary outcome, 5 patients experienced the primary endpoint. Specifically, 3 patients were hospitalised (one for NSTEMI-ACS, one for pulmonary oedema, and one for change of defibrillator battery), whereas 2 patients died (both were at terminal stage of HF before the lockdown – NYHA IV- and died for sudden cardiac death). Notably, none of our HF patients got COVID-19. Pearson's chi square and Fisher exact tests showed that patients in 2019 cohort (when TMS was not available) were more likely than cohort with access to TMS to experienced primary outcome [X^2 (degree of freedom 1, cases = 207) 10.699, p=0.001]. Specifically, a significant difference was observed for HF hospitalisation (p=0.001) whereas no differences were observed with regard to deaths.

The present investigation represents the first about the utility of telemedicine during the lockdown due to COVID-19 outbreak in Italy; using commonly available technologies (analogic phones, smartphones, apps) our team was able to offer a continuous service for all our HF patients.

It has been described a decrease of acute coronary syndrome-related hospitalisation rates in Italy during the COVID-19 outbreak[463,464], suggesting that several patients experienced a poor outcome because they did not access to health system. We provided a TMS that allows our patients to have direct access to health system, observing a significant reduction of primary endpoint when compared to the same period of the previous year, supporting its use to increase the value of health care[465]. Further, our finding supports the recent statement from Heart Failure Society of America, in which the use of the telemedicine for HF management during COVID-19 outbreak is strongly suggested[466], in line with European Society of Cardiology advice[461]. Notably, recently it has been stated that '*it is a great shame that home telemonitoring was not already routine before the pandemic struck'* [467].

To avoid possible social disparities (e.g. accessibility of service only to people with available technologies and/or capacities to use the service), on purpose we based our telemedicine system mostly on phone calls.

In conclusion, our telemedicine service allows our HF patients to be follow-upped also during COVID-19 lockdown, with a positive impact on HF outcome; the present report confirms telemedicine as a valuable tool in HF-management and shows for the first time its feasibility during COVID-19 outbreak.

Table 1 Demographic characteristics at baseline, telemedicine data, and outcomes

Variables	2020 Cohort ($n = 103$)	2019 Cohort ($n = 104$)	
Demographics			
Age (years)	68 ± 12.7	68 ± 11.4	NS
Sex (male/female)	84/19	84/20	NS
NYHA class (I–II/III–IV)	66/37	65/39	NS
Aetiology (ischaemic) (%)	53	51	NS
Years of disease	6 [1–12]	5 [1–13]	NS
Systolic blood pressure (mmHg)	123 ± 15.6	122 ± 6.4	NS
Diastolic blood pressure (mmHg)	78 ± 9.8	79 ± 7.5	NS
BMI (kg/m^2)	29.8 ± 6.3	29.7 ± 5.2	NS
eGFR (mL/min)	80 [53–118]	81[49–112]	NS
ICD (%)	45.8	43.9	NS
CRT (%)	14.6	13.4	NS
LVEF (%)	34.1 [28.8–39.3]	34.8 [29.2–39.8]	NS
LVEDVi (mL/m^2)	88.5 [74.3–114.1]	87.6 [72.8–111.9]	NS
LVESVi (mL/m^2)	57.9 [46.9–79.5]	57.3 [46.7–77.3]	NS
NT-proBNP (pg/mL)	536 [180–1621]	600 [201–1815]	NS
Medication (%)			
Beta-blockers	92.3	91.7	NS
ACEi/ARB/ARNI	89	88	NS
MRA	49	50	NS
Loop diuretics	74.7	75.1	NS
Telemedicine			
Total no. of accesses	127	–	–
Patients with at least 1 access, *n* (%)	60 (58)	–	–
Type of access, *n* (%)			
Phone call	82 (64.2)	–	–
Chat service	43 (33.6)	–	–
Video	3 (2.2)	–	–
Email	0 (0)	–	–
Patients needing at least one clinical intervention, *n* (%)	31 (52)	–	–
No. of clinical interventions, *n* (%)	65 (51)		
Type of clinical intervention, *n*			
Loop diuretic dose change	23	–	–
Blood pressure management	10	–	–
Rate control	6	–	–
Anticoagulation management	6	–	–
Other[a]	20	–	–
Outcome, *n*			
Composite HF hospitalization/death	5	21	0.001*
HF hospitalizations	3	18	0.001*
Deaths	2	3	NS

Data are expressed as mean ± standard deviation, or median [interquartile range] unless otherwise specified.
ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor–neprilysin inhibitor; BMI, body mass index; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration formula); HF, heart failure; ICD, implantable cardioverter-defibrillator; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume index; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association.
*$P < 0.01$.
[a] Other interventions: antibiotics management, pain management, general advise.

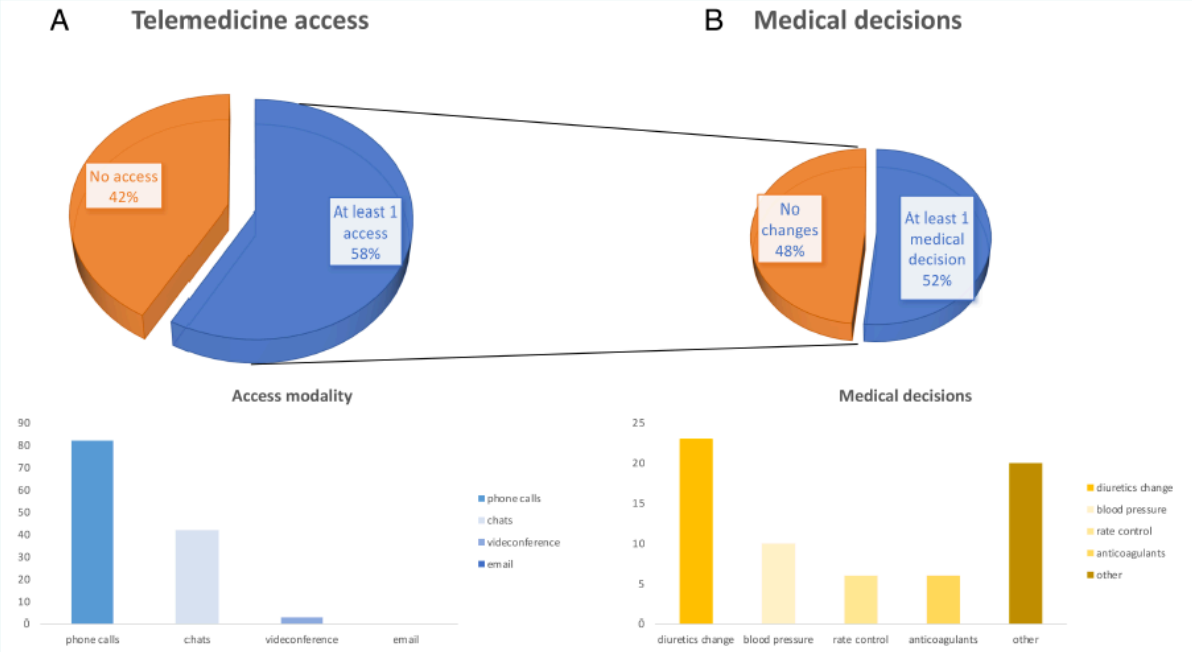


Figure 1 (*A*) Patients who accessed to the telemedicine service and access modality. (*B*) Outcome of the accesses performed and medical decisions.

7. Conclusions

Despite the number of biomarkers investigated in HF is dramatically increasing, to date relatively few of them showed ability in modifying decision making in the clinical arena and may therefore be termed 'clinically useful'.

Natriuretic peptides (BNP and NT-proBNP) represent the gold-standard circulating biomarkers in HF, whereas transthoracic echocardiography represents the indispensable imaging tool in the management of patients with HF for its safety, low cost, and availability.

Biomarkers may derive from the blood, the urine, genetic studies, imaging, physiological tests, and tissue-specimen biopsies. The multi-biomarkers strategy, in particular if biomarkers are combined and information from different pathophysiological pathways are provided, appears the most promising strategy that goes beyond the limits of the current management of HF. However, biomarkers can be also useful in showing new pathophysiologic pathway, representing possible novel therapeutic target. Valuable examples are the hormone deficiencies and the gut-axis, which demonstrated a role not only as biomarker of disease, but also as index of worse cardiovascular performance and poor outcome.

In conclusions, biomarkers can be considered as powerful tools in the clinical management of heart failure; however, it is important to know the proper role and the intrinsic characteristics of the single biomarker, with the possibility of combine different biomarkers as strategy to fill the current gap in evidence in HF management.

In this context, as the final result of the investigation at the basis of the present thesis, the most valuable approach seems to be the multi-parameter approach, specifically when different types of biomarkers (e.g. circulating and imaging) and biomarkers from different pathophysiological pathways are combined. Further, herein it has been spotlighted that several biomarkers have different potential roles, identifying for the first time the concept of a 'biomarker continuum' in HF[468].

Unexplored lands open up on our horizon and, unarguably, in the very next future novel biomarkers will be added in the everyday clinician quiver.

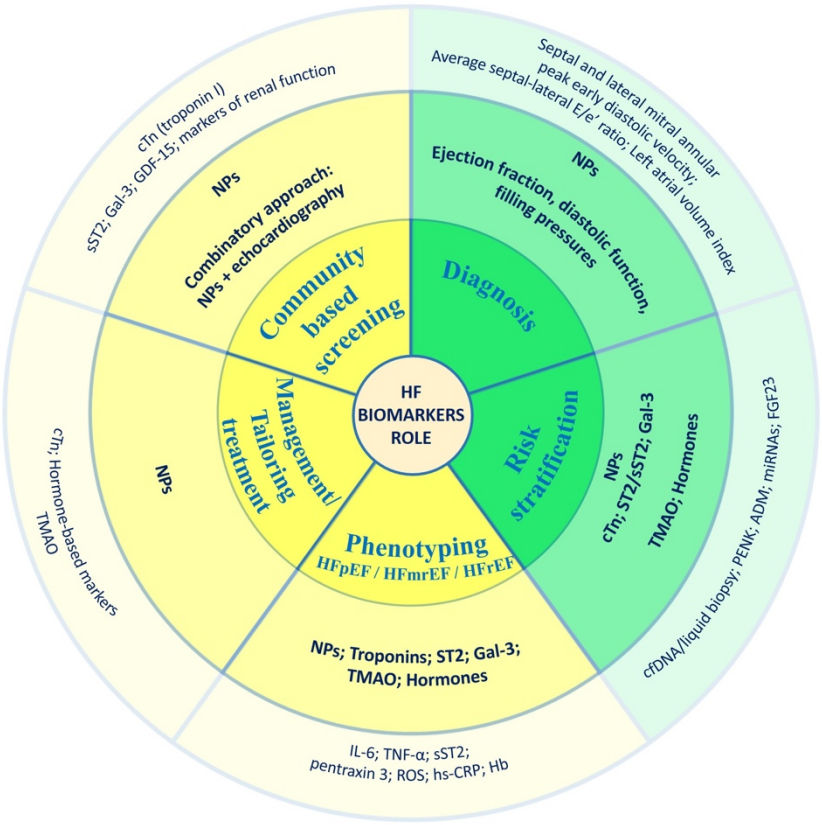


Figure. Identified roles for biomarkers in Heart Failure. Green box: well established and supported by data. Yellow box: promising but not yet recommended.

From: Salzano A et al. Heart Fail Clin. 2021 Apr;17(2):223-243. doi: 10.1016/j.hfc.2021.01.002.

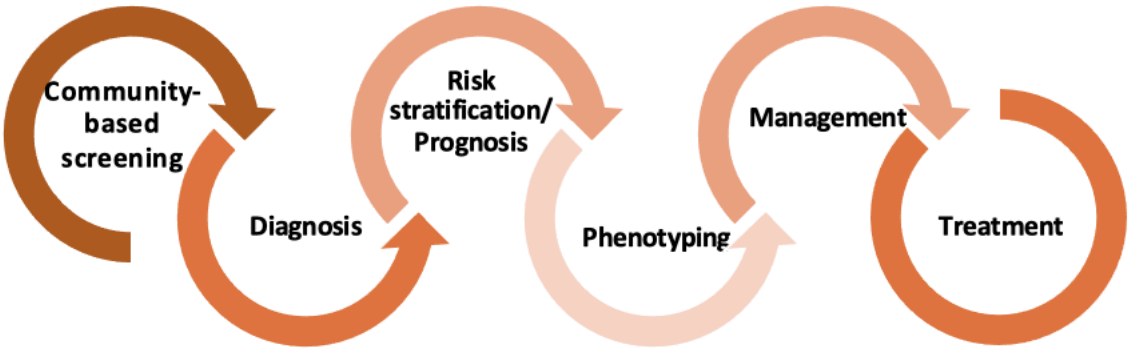


Figure. The continuum of the biomarker clinical utility in heart failure.

From: Salzano A et al. Heart Fail Clin. 2021 Apr;17(2):223-243. doi: 10.1016/j.hfc.2021.01.002.

8. References

1. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail 2016;18(8):891-975. DOI: 10.1002/ejhf.592.
2. Yancy CW, Jessup M, Bozkurt B, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. Journal of cardiac failure 2017;23(8):628-651. DOI: 10.1016/j.cardfail.2017.04.014.
3. Braunwald E. Heart failure. JACC Heart Fail 2013;1(1):1-20. DOI: 10.1016/j.jchf.2012.10.002.
4. Benjamin EJ, Virani SS, Callaway CW, et al. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. Circulation 2018;137(12):e67-e492. DOI: 10.1161/CIR.0000000000000558.
5. Suzuki T. Cardiovascular diagnostic biomarkers: the past, present and future. Circ J 2009;73(5):806-9.
6. Suzuki T, Bossone E. Biomarkers of Heart Failure: Past, Present, and Future. Heart Fail Clin 2018;14(1):ix-x. DOI: 10.1016/j.hfc.2017.08.012.
7. Nymo SH, Aukrust P, Kjekshus J, et al. Limited Added Value of Circulating Inflammatory Biomarkers in Chronic Heart Failure. JACC Heart Fail 2017;5(4):256-264. DOI: 10.1016/j.jchf.2017.01.008.
8. Salzano A, Marra AM, D'Assante R, et al. Biomarkers and Imaging: Complementary or Subtractive? Heart Fail Clin 2019;15(2):321-331. (In eng). DOI: 10.1016/j.hfc.2018.12.008.
9. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69(3):89-95. DOI: 10.1067/mcp.2001.113989.
10. Naylor S. Biomarkers: current perspectives and future prospects. Expert Rev Mol Diagn 2003;3(5):525-9. DOI: 10.1586/14737159.3.5.525.
11. Ibrahim NE, Gaggin HK, Konstam MA, Januzzi JL, Jr. Established and Emerging Roles of Biomarkers in Heart Failure Clinical Trials. Circ Heart Fail 2016;9(9). DOI: 10.1161/CIRCHEARTFAILURE.115.002528.
12. Januzzi JL, Jr. Will Biomarkers Succeed as a Surrogate Endpoint in Heart Failure Trials? JACC Heart Fail 2018;6(7):570-572. DOI: 10.1016/j.jchf.2018.02.008.
13. Ghashghaei R, Arbit B, Maisel AS. Current and novel biomarkers in heart failure: bench to bedside. Curr Opin Cardiol 2016;31(2):191-5. DOI: 10.1097/HCO.0000000000000254.
14. Drucker E, Krapfenbauer K. Pitfalls and limitations in translation from biomarker discovery to clinical utility in predictive and personalised medicine. EPMA J 2013;4(1):7. DOI: 10.1186/1878-5085-4-7.
15. Mayeux R. Biomarkers: potential uses and limitations. NeuroRx 2004;1(2):182-8. DOI: 10.1602/neurorx.1.2.182.
16. Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. Circulation 2007;115(8):949-52. (In eng). DOI: 10.1161/CIRCULATIONAHA.106.683110.

17. Braunwald E. Biomarkers in heart failure. N Engl J Med 2008;358(20):2148-59. DOI: 10.1056/NEJMra0800239.
18. Ahmad T, Fiuzat M, Felker GM, O'Connor C. Novel biomarkers in chronic heart failure. Nat Rev Cardiol 2012;9(6):347-59. DOI: 10.1038/nrcardio.2012.37.
19. Magnussen C, Blankenberg S. Biomarkers for heart failure: small molecules with high clinical relevance. J Intern Med 2018;283(6):530-543. DOI: 10.1111/joim.12756.
20. Yasue H, Yoshimura M, Sumida H, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994;90(1):195-203.
21. Iwanaga Y, Nishi I, Furuichi S, et al. B-type natriuretic peptide strongly reflects diastolic wall stress in patients with chronic heart failure: comparison between systolic and diastolic heart failure. J Am Coll Cardiol 2006;47(4):742-8. DOI: 10.1016/j.jacc.2005.11.030.
22. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016;37(27):2129-2200. DOI: 10.1093/eurheartj/ehw128.
23. Januzzi JL, Jr., Camargo CA, Anwaruddin S, et al. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. Am J Cardiol 2005;95(8):948-54. DOI: 10.1016/j.amjcard.2004.12.032.
24. Seronde MF, Gayat E, Logeart D, et al. Comparison of the diagnostic and prognostic values of B-type and atrial-type natriuretic peptides in acute heart failure. Int J Cardiol 2013;168(4):3404-11. DOI: 10.1016/j.ijcard.2013.04.164.
25. Fonarow GC, Peacock WF, Phillips CO, et al. Admission B-type natriuretic peptide levels and in-hospital mortality in acute decompensated heart failure. J Am Coll Cardiol 2007;49(19):1943-50. DOI: 10.1016/j.jacc.2007.02.037.
26. Cheng V, Kazanagra R, Garcia A, et al. A rapid bedside test for B-type peptide predicts treatment outcomes in patients admitted for decompensated heart failure: a pilot study. J Am Coll Cardiol 2001;37(2):386-91.
27. O'Brien RJ, Squire IB, Demme B, Davies JE, Ng LL. Pre-discharge, but not admission, levels of NT-proBNP predict adverse prognosis following acute LVF. Eur J Heart Fail 2003;5(4):499-506.
28. Januzzi JL, Jr., Rehman SU, Mohammed AA, et al. Use of amino-terminal pro-B-type natriuretic peptide to guide outpatient therapy of patients with chronic left ventricular systolic dysfunction. J Am Coll Cardiol 2011;58(18):1881-9. DOI: 10.1016/j.jacc.2011.03.072.
29. Felker GM, Anstrom KJ, Adams KF, et al. Effect of Natriuretic Peptide-Guided Therapy on Hospitalization or Cardiovascular Mortality in High-Risk Patients With Heart Failure and Reduced Ejection Fraction: A Randomized Clinical Trial. JAMA 2017;318(8):713-720. DOI: 10.1001/jama.2017.10565.
30. Pfisterer M, Buser P, Rickli H, et al. BNP-guided vs symptom-guided heart failure therapy: the Trial of Intensified vs Standard Medical Therapy in Elderly Patients With Congestive Heart Failure (TIME-CHF) randomized trial. JAMA 2009;301(4):383-92. DOI: 10.1001/jama.2009.2.
31. Sanders-van Wijk S, van Asselt AD, Rickli H, et al. Cost-effectiveness of N-terminal pro-B-type natriuretic-guided therapy in elderly heart failure patients: results from TIME-CHF (Trial of Intensified versus Standard Medical Therapy in Elderly Patients with Congestive Heart Failure). JACC Heart Fail 2013;1(1):64-71. DOI: 10.1016/j.jchf.2012.08.002.

32. Masson S, Anand I, Favero C, et al. Serial measurement of cardiac troponin T using a highly sensitive assay in patients with chronic heart failure: data from 2 large randomized clinical trials. Circulation 2012;125(2):280-8. DOI: 10.1161/CIRCULATIONAHA.111.044149.
33. Horwich TB, Patel J, MacLellan WR, Fonarow GC. Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure. Circulation 2003;108(7):833-8. DOI: 10.1161/01.CIR.0000084543.79097.34.
34. Omland T, de Lemos JA, Sabatine MS, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. N Engl J Med 2009;361(26):2538-47. DOI: 10.1056/NEJMoa0805299.
35. Watson CJ, Gupta SK, O'Connell E, et al. MicroRNA signatures differentiate preserved from reduced ejection fraction heart failure. Eur J Heart Fail 2015;17(4):405-15. DOI: 10.1002/ejhf.244.
36. Masson S, Batkai S, Beermann J, et al. Circulating microRNA-132 levels improve risk prediction for heart failure hospitalization in patients with chronic heart failure. Eur J Heart Fail 2018;20(1):78-85. DOI: 10.1002/ejhf.961.
37. Bayes-Genis A, Lanfear DE, de Ronde MWJ, et al. Prognostic value of circulating microRNAs on heart failure-related morbidity and mortality in two large diverse cohorts of general heart failure patients. Eur J Heart Fail 2018;20(1):67-75. DOI: 10.1002/ejhf.984.
38. Krack A, Sharma R, Figulla HR, Anker SD. The importance of the gastrointestinal system in the pathogenesis of heart failure. Eur Heart J 2005;26(22):2368-74. DOI: 10.1093/eurheartj/ehi389.
39. Anand IS, Latini R, Florea VG, et al. C-reactive protein in heart failure: prognostic value and the effect of valsartan. Circulation 2005;112(10):1428-34. DOI: 10.1161/CIRCULATIONAHA.104.508465.
40. Alonso-Martinez JL, Llorente-Diez B, Echegaray-Agara M, Olaz-Preciado F, Urbieta-Echezarreta M, Gonzalez-Arencibia C. C-reactive protein as a predictor of improvement and readmission in heart failure. Eur J Heart Fail 2002;4(3):331-6.
41. Siirila-Waris K, Lassus J, Melin J, et al. Characteristics, outcomes, and predictors of 1-year mortality in patients hospitalized for acute heart failure. Eur Heart J 2006;27(24):3011-7. DOI: 10.1093/eurheartj/ehl407.
42. Marra AM, Arcopinto M, Salzano A, et al. Detectable interleukin-9 plasma levels are associated with impaired cardiopulmonary functional capacity and all-cause mortality in patients with chronic heart failure. Int J Cardiol 2016;209:114-7. DOI: 10.1016/j.ijcard.2016.02.017.
43. Januzzi JL, Jr., Peacock WF, Maisel AS, et al. Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. J Am Coll Cardiol 2007;50(7):607-13. DOI: 10.1016/j.jacc.2007.05.014.
44. Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR, Januzzi JL. Serum levels of the interleukin-1 receptor family member ST2, cardiac structure and function, and long-term mortality in patients with acute dyspnea. Circ Heart Fail 2009;2(4):311-9. DOI: 10.1161/CIRCHEARTFAILURE.108.833707.
45. Sharma UC, Pokharel S, van Brakel TJ, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. Circulation 2004;110(19):3121-8. DOI: 10.1161/01.CIR.0000147181.65298.4D.

46. de Boer RA, Lok DJ, Jaarsma T, et al. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. Ann Med 2011;43(1):60-8. DOI: 10.3109/07853890.2010.538080.
47. Sharma A, Stevens SR, Lucas J, et al. Utility of Growth Differentiation Factor-15, A Marker of Oxidative Stress and Inflammation, in Chronic Heart Failure: Insights From the HF-ACTION Study. JACC Heart Fail 2017;5(10):724-734. DOI: 10.1016/j.jchf.2017.07.013.
48. Sacca L. Heart failure as a multiple hormonal deficiency syndrome. Circ Heart Fail 2009;2(2):151-6. DOI: 10.1161/CIRCHEARTFAILURE.108.821892.
49. Bossone E, Arcopinto M, Iacoviello M, et al. Multiple hormonal and metabolic deficiency syndrome in chronic heart failure: rationale, design, and demographic characteristics of the T.O.S.CA. Registry. Intern Emerg Med 2018;13(5):661-671. (In eng). DOI: 10.1007/s11739-018-1844-8.
50. Marra AM, Bobbio E, D'Assante R, et al. Growth Hormone as Biomarker in Heart Failure. Heart Fail Clin 2018;14(1):65-74. DOI: 10.1016/j.hfc.2017.08.008.
51. Marra AM, Arcopinto M, Bobbio E, Salzano A, Sacca L, Cittadini A. An unusual case of dilated cardiomyopathy associated with partial hypopituitarism. Intern Emerg Med 2012;7 Suppl 2:S85-7. DOI: 10.1007/s11739-011-0649-9.
52. Bossone E, Limongelli G, Malizia G, et al. The T.O.S.CA. Project: research, education and care. Monaldi Arch Chest Dis 2011;76(4):198-203. DOI: 10.4081/monaldi.2011.177.
53. Jankowska EA, Biel B, Majda J, et al. Anabolic deficiency in men with chronic heart failure: prevalence and detrimental impact on survival. Circulation 2006;114(17):1829-37. DOI: 10.1161/CIRCULATIONAHA.106.649426.
54. Jankowska EA, Rozentryt P, Ponikowska B, et al. Circulating estradiol and mortality in men with systolic chronic heart failure. JAMA 2009;301(18):1892-901. DOI: 10.1001/jama.2009.639.
55. Salzano A, Marra AM, Ferrara F, et al. Multiple hormone deficiency syndrome in heart failure with preserved ejection fraction. Int J Cardiol 2016;225:1-3. DOI: 10.1016/j.ijcard.2016.09.085.
56. Pasquali D, Arcopinto M, Renzullo A, et al. Cardiovascular abnormalities in Klinefelter syndrome. Int J Cardiol 2013;168(2):754-9. DOI: 10.1016/j.ijcard.2012.09.215.
57. Marra AM, Improda N, Capalbo D, et al. Cardiovascular abnormalities and impaired exercise performance in adolescents with congenital adrenal hyperplasia. J Clin Endocrinol Metab 2015;100(2):644-52. (In eng). DOI: 10.1210/jc.2014-1805.
58. Sacca F, Puorro G, Marsili A, et al. Long-term effect of epoetin alfa on clinical and biochemical markers in friedreich ataxia. Mov Disord 2016;31(5):734-41. DOI: 10.1002/mds.26552.
59. Salzano A, Arcopinto M, Marra AM, et al. Klinefelter syndrome, cardiovascular system, and thromboembolic disease: review of literature and clinical perspectives. Eur J Endocrinol 2016;175(1):R27-40. (In eng). DOI: 10.1530/eje-15-1025.
60. Salzano A, D'Assante R, Heaney LM, et al. Klinefelter syndrome, insulin resistance, metabolic syndrome, and diabetes: review of literature and clinical perspectives. Endocrine 2018;61(2):194-203. DOI: 10.1007/s12020-018-1584-6.
61. Arcopinto M, Salzano A, Giallauria F, et al. Growth Hormone Deficiency Is Associated with Worse Cardiac Function, Physical Performance, and Outcome in Chronic Heart Failure: Insights from the T.O.S.CA. GHD Study. PLoS One 2017;12(1):e0170058. DOI: 10.1371/journal.pone.0170058.

62. Arcopinto M, Isgaard J, Marra AM, et al. IGF-1 predicts survival in chronic heart failure. Insights from the T.O.S.CA. (Trattamento Ormonale Nello Scompenso CArdiaco) registry. Int J Cardiol 2014;176(3):1006-8. DOI: 10.1016/j.ijcard.2014.07.003.
63. Arcopinto M, Salzano A, Bossone E, et al. Multiple hormone deficiencies in chronic heart failure. Int J Cardiol 2015;184:421-3. DOI: 10.1016/j.ijcard.2015.02.055.
64. Cittadini A, Saldamarco L, Marra AM, et al. Growth hormone deficiency in patients with chronic heart failure and beneficial effects of its correction. J Clin Endocrinol Metab 2009;94(9):3329-36. DOI: 10.1210/jc.2009-0533.
65. Cittadini A, Marra AM, Arcopinto M, et al. Growth hormone replacement delays the progression of chronic heart failure combined with growth hormone deficiency: an extension of a randomized controlled single-blind study. JACC Heart Fail 2013;1(4):325-330. DOI: 10.1016/j.jchf.2013.04.003.
66. Salzano A, Marra AM, D'Assante R, et al. Growth Hormone Therapy in Heart Failure. Heart Fail Clin 2018;14(4). DOI: 10.1016/j.hfc.2018.05.002.
67. Arcopinto M, Salzano A, Isgaard J, Cittadini A. Hormone replacement therapy in heart failure. Curr Opin Cardiol 2015;30(3):277-84. DOI: 10.1097/HCO.0000000000000166.
68. Nagatomo Y, Tang WH. Intersections Between Microbiome and Heart Failure: Revisiting the Gut Hypothesis. Journal of cardiac failure 2015;21(12):973-80. DOI: 10.1016/j.cardfail.2015.09.017.
69. Troseid M, Ueland T, Hov JR, et al. Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure. J Intern Med 2015;277(6):717-26. DOI: 10.1111/joim.12328.
70. Suzuki T, Heaney LM, Bhandari SS, Jones DJ, Ng LL. Trimethylamine N-oxide and prognosis in acute heart failure. Heart 2016;102(11):841-8. DOI: 10.1136/heartjnl-2015-308826.
71. Tang WH, Wang Z, Kennedy DJ, et al. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. Circ Res 2015;116(3):448-55. DOI: 10.1161/CIRCRESAHA.116.305360.
72. Schuett K, Kleber ME, Scharnagl H, et al. Trimethylamine-N-oxide and Heart Failure With Reduced Versus Preserved Ejection Fraction. J Am Coll Cardiol 2017;70(25):3202-3204. DOI: 10.1016/j.jacc.2017.10.064.
73. Tang WH, Wang Z, Fan Y, et al. Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. J Am Coll Cardiol 2014;64(18):1908-14. DOI: 10.1016/j.jacc.2014.02.617.
74. Albert CL, Tang WHW. Metabolic Biomarkers in Heart Failure. Heart Fail Clin 2018;14(1):109-118. DOI: 10.1016/j.hfc.2017.08.011.
75. Sengupta PP, Kramer CM, Narula J, Dilsizian V. The Potential of Clinical Phenotyping of Heart Failure With Imaging Biomarkers for Guiding Therapies: A Focused Update. JACC Cardiovasc Imaging 2017;10(9):1056-1071. DOI: 10.1016/j.jcmg.2017.07.001.
76. Bristow MR, Kao DP, Breathett KK, et al. Structural and Functional Phenotyping of the Failing Heart: Is the Left Ventricular Ejection Fraction Obsolete? JACC Heart Fail 2017;5(11):772-781. DOI: 10.1016/j.jchf.2017.09.009.
77. Tischler MD, Ashikaga T, LeWinter MM. Relation between left ventricular shape and Doppler filling parameters in patients with left ventricular dysfunction secondary to coronary artery disease. Am J Cardiol 1995;76(8):553-6.
78. Geyer H, Caracciolo G, Abe H, et al. Assessment of myocardial mechanics using speckle tracking echocardiography: fundamentals and clinical applications. J Am Soc Echocardiogr 2010;23(4):351-69; quiz 453-5. DOI: 10.1016/j.echo.2010.02.015.

79. Hasselberg NE, Haugaa KH, Sarvari SI, et al. Left ventricular global longitudinal strain is associated with exercise capacity in failing hearts with preserved and reduced ejection fraction. Eur Heart J Cardiovasc Imaging 2015;16(2):217-24. DOI: 10.1093/ehjci/jeu277.
80. Tsang TS, Abhayaratna WP, Barnes ME, et al. Prediction of cardiovascular outcomes with left atrial size: is volume superior to area or diameter? J Am Coll Cardiol 2006;47(5):1018-23. DOI: 10.1016/j.jacc.2005.08.077.
81. Santos AB, Kraigher-Krainer E, Gupta DK, et al. Impaired left atrial function in heart failure with preserved ejection fraction. Eur J Heart Fail 2014;16(10):1096-103. DOI: 10.1002/ejhf.147.
82. Marra AM, Benjamin N, Eichstaedt C, et al. Gender-related differences in pulmonary arterial hypertension targeted drugs administration. Pharmacol Res 2016;114:103-109. DOI: 10.1016/j.phrs.2016.10.018.
83. Marra AM, Bossone E, Salzano A, et al. Biomarkers in Pulmonary Hypertension. Heart Fail Clin 2018;14(3):393-402. DOI: 10.1016/j.hfc.2018.03.005.
84. Ferrara F, Gargani L, Armstrong WF, et al. The Right Heart International Network (RIGHT-NET): Rationale, Objectives, Methodology, and Clinical Implications. Heart Fail Clin 2018;14(3):443-465. DOI: 10.1016/j.hfc.2018.03.010.
85. Pleister A, Kahwash R, Haas G, Ghio S, Cittadini A, Baliga RR. Echocardiography and heart failure: a glimpse of the right heart. Echocardiography 2015;32 Suppl 1:S95-107. DOI: 10.1111/echo.12678.
86. Thenappan T, Roy SS, Duval S, Glassner-Kolmin C, Gomberg-Maitland M. beta-blocker therapy is not associated with adverse outcomes in patients with pulmonary arterial hypertension: a propensity score analysis. Circ Heart Fail 2014;7(6):903-10. DOI: 10.1161/CIRCHEARTFAILURE.114.001429.
87. Puwanant S, Priester TC, Mookadam F, Bruce CJ, Redfield MM, Chandrasekaran K. Right ventricular function in patients with preserved and reduced ejection fraction heart failure. Eur J Echocardiogr 2009;10(6):733-7. DOI: 10.1093/ejechocard/jep052.
88. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2015;28(1):1-39 e14. DOI: 10.1016/j.echo.2014.10.003.
89. Salzano A, Sirico D, Golia L, et al. [The portopulmonary hypertension: an overview from diagnosis to treatment]. Monaldi Arch Chest Dis 2013;80(2):66-8. DOI: 10.4081/monaldi.2013.81.
90. Pellicori P, Carubelli V, Zhang J, et al. IVC diameter in patients with chronic heart failure: relationships and prognostic significance. JACC Cardiovasc Imaging 2013;6(1):16-28. DOI: 10.1016/j.jcmg.2012.08.012.
91. Papolos A, Narula J, Bavishi C, Chaudhry FA, Sengupta PP. U.S. Hospital Use of Echocardiography: Insights From the Nationwide Inpatient Sample. J Am Coll Cardiol 2016;67(5):502-11. DOI: 10.1016/j.jacc.2015.10.090.
92. Peterzan MA, Rider OJ, Anderson LJ. The Role of Cardiovascular Magnetic Resonance Imaging in Heart Failure. Card Fail Rev 2016;2(2):115-122. DOI: 10.15420/cfr.2016.2.2.115.
93. Kim RJ, Chen EL, Lima JA, Judd RM. Myocardial Gd-DTPA kinetics determine MRI contrast enhancement and reflect the extent and severity of myocardial injury after acute reperfused infarction. Circulation 1996;94(12):3318-26.
94. Assomull RG, Prasad SK, Lyne J, et al. Cardiovascular magnetic resonance, fibrosis, and prognosis in dilated cardiomyopathy. J Am Coll Cardiol 2006;48(10):1977-85. DOI: 10.1016/j.jacc.2006.07.049.

95. Bello D, Fieno DS, Kim RJ, et al. Infarct morphology identifies patients with substrate for sustained ventricular tachycardia. J Am Coll Cardiol 2005;45(7):1104-8. DOI: 10.1016/j.jacc.2004.12.057.
96. Abbasi SA, Ertel A, Shah RV, et al. Impact of cardiovascular magnetic resonance on management and clinical decision-making in heart failure patients. J Cardiovasc Magn Reson 2013;15:89. DOI: 10.1186/1532-429X-15-89.
97. Larose E, Ganz P, Reynolds HG, et al. Right ventricular dysfunction assessed by cardiovascular magnetic resonance imaging predicts poor prognosis late after myocardial infarction. J Am Coll Cardiol 2007;49(8):855-62. DOI: 10.1016/j.jacc.2006.10.056.
98. Haykowsky MJ, Nicklas BJ, Brubaker PH, et al. Regional Adipose Distribution and its Relationship to Exercise Intolerance in Older Obese Patients Who Have Heart Failure With Preserved Ejection Fraction. JACC Heart Fail 2018;6(8):640-649. DOI: 10.1016/j.jchf.2018.06.002.
99. van Woerden G, Gorter TM, Westenbrink BD, Willems TP, van Veldhuisen DJ, Rienstra M. Epicardial fat in heart failure patients with mid-range and preserved ejection fraction. Eur J Heart Fail 2018. DOI: 10.1002/ejhf.1283.
100. Arvidsson PM, Toger J, Pedrizzetti G, et al. Hemodynamic forces using 4D flow MRI: an independent biomarker of cardiac function in heart failure with left ventricular dyssynchrony? Am J Physiol Heart Circ Physiol 2018. DOI: 10.1152/ajpheart.00112.2018.
101. Lum YH, McKenzie S, Brown M, Hamilton-Craig C. Impact of Cardiac MRI in Heart Failure Patients referred to a Tertiary Advanced Heart Failure Unit: Improvements in Diagnosis and Management. Intern Med J 2018. DOI: 10.1111/imj.14087.
102. Cohn JN, Levine TB, Olivari MT, et al. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 1984;311(13):819-23. DOI: 10.1056/NEJM198409273111303.
103. Carrio I, Cowie MR, Yamazaki J, Udelson J, Camici PG. Cardiac sympathetic imaging with mIBG in heart failure. JACC Cardiovasc Imaging 2010;3(1):92-100. DOI: 10.1016/j.jcmg.2009.07.014.
104. Al Badarin FJ, Wimmer AP, Kennedy KF, Jacobson AF, Bateman TM. The utility of ADMIRE-HF risk score in predicting serious arrhythmic events in heart failure patients: incremental prognostic benefit of cardiac 123I-mIBG scintigraphy. J Nucl Cardiol 2014;21(4):756-62; quiz 753-55, 763-5. DOI: 10.1007/s12350-014-9919-z.
105. Ketchum ES, Jacobson AF, Caldwell JH, et al. Selective improvement in Seattle Heart Failure Model risk stratification using iodine-123 meta-iodobenzylguanidine imaging. J Nucl Cardiol 2012;19(5):1007-16. DOI: 10.1007/s12350-012-9603-0.
106. Wessler BS, Udelson JE. Neuronal dysfunction and medical therapy in heart failure: can an imaging biomarker help to "personalize" therapy? J Nucl Med 2015;56 Suppl 4:20S-24S. DOI: 10.2967/jnumed.114.142778.
107. Verschure DO, de Groot JR, Mirzaei S, et al. Cardiac (123)I-mIBG scintigraphy is associated with freedom of appropriate ICD therapy in stable chronic heart failure patients. Int J Cardiol 2017;248:403-408. DOI: 10.1016/j.ijcard.2017.08.003.
108. Dimitriu-Leen AC, Scholte AJ, Jacobson AF. 123I-MIBG SPECT for Evaluation of Patients with Heart Failure. J Nucl Med 2015;56 Suppl 4:25S-30S. DOI: 10.2967/jnumed.115.157503.
109. Cruz MC, Abreu A, Portugal G, et al. Relationship of left ventricular global longitudinal strain with cardiac autonomic denervation as assessed by (123)I-mIBG scintigraphy in patients with heart failure with reduced ejection fraction submitted to cardiac resynchronization therapy : Assessment of cardiac autonomic denervation by GLS in

patients with heart failure with reduced ejection fraction submitted to CRT. J Nucl Cardiol 2017. DOI: 10.1007/s12350-017-1148-9.
110. Maisel AS, Duran JM, Wettersten N. Natriuretic Peptides in Heart Failure: Atrial and B-type Natriuretic Peptides. Heart Fail Clin 2018;14(1):13-25. DOI: 10.1016/j.hfc.2017.08.002.
111. Richards AM. N-Terminal B-type Natriuretic Peptide in Heart Failure. Heart Fail Clin 2018;14(1):27-39. DOI: 10.1016/j.hfc.2017.08.004.
112. McKie PM, Burnett JC, Jr. NT-proBNP: The Gold Standard Biomarker in Heart Failure. J Am Coll Cardiol 2016;68(22):2437-2439. DOI: 10.1016/j.jacc.2016.10.001.
113. Alba AC, Agoritsas T, Jankowski M, et al. Risk prediction models for mortality in ambulatory patients with heart failure: a systematic review. Circ Heart Fail 2013;6(5):881-9. DOI: 10.1161/CIRCHEARTFAILURE.112.000043.
114. Grande D, Leone M, Rizzo C, et al. A Multiparametric Approach Based on NT-proBNP, ST2, and Galectin3 for Stratifying One Year Prognosis of Chronic Heart Failure Outpatients. J Cardiovasc Dev Dis 2017;4(3). DOI: 10.3390/jcdd4030009.
115. Doumouras BS, Lee DS, Levy WC, Alba AC. An Appraisal of Biomarker-Based Risk-Scoring Models in Chronic Heart Failure: Which One Is Best? Curr Heart Fail Rep 2018;15(1):24-36. DOI: 10.1007/s11897-018-0375-y.
116. Gong FF, Campbell DJ, Prior DL. Noninvasive Cardiac Imaging and the Prediction of Heart Failure Progression in Preclinical Stage A/B Subjects. JACC Cardiovasc Imaging 2017;10(12):1504-1519. DOI: 10.1016/j.jcmg.2017.11.001.
117. Kalogeropoulos AP, Georgiopoulou VV, deFilippi CR, Gottdiener JS, Butler J, Cardiovascular Health S. Echocardiography, natriuretic peptides, and risk for incident heart failure in older adults: the Cardiovascular Health Study. JACC Cardiovasc Imaging 2012;5(2):131-40. DOI: 10.1016/j.jcmg.2011.11.011.
118. Lupon J, de Antonio M, Galan A, et al. Combined use of the novel biomarkers high-sensitivity troponin T and ST2 for heart failure risk stratification vs conventional assessment. Mayo Clin Proc 2013;88(3):234-43. DOI: 10.1016/j.mayocp.2012.09.016.
119. Kosmala W, Przewlocka-Kosmala M, Rojek A, Marwick TH. Comparison of the Diastolic Stress Test With a Combined Resting Echocardiography and Biomarker Approach to Patients With Exertional Dyspnea: Diagnostic and Prognostic Implications. JACC Cardiovasc Imaging 2018. DOI: 10.1016/j.jcmg.2017.10.008.
120. Girerd N, Seronde MF, Coiro S, et al. Integrative Assessment of Congestion in Heart Failure Throughout the Patient Journey. JACC Heart Fail 2018;6(4):273-285. DOI: 10.1016/j.jchf.2017.09.023.
121. Metra M, Teerlink JR. Heart failure. Lancet 2017;390(10106):1981-1995. DOI: 10.1016/S0140-6736(17)31071-1.
122. Suzuki T, Heaney LM, Bhandari SS, Jones DJL, Ng LL. Trimethylamine N-oxide and prognosis in acute heart failure. Heart 2016;102(11):841-848. (In English). DOI: 10.1136/heartjnl-2015-308826.
123. Christensen D. Scientists divided on relevance of race in medical research. Nat Med 2004;10(12):1266. (In eng). DOI: 10.1038/nm1204-1266a.
124. Freeman HP. Commentary on the meaning of race in science and society. Cancer Epidemiol Biomarkers Prev 2003;12(3):232s-236s. (In eng).
125. Yudell M, Roberts D, DeSalle R, Tishkoff S. SCIENCE AND SOCIETY. Taking race out of human genetics. Science 2016;351(6273):564-5. (In eng). DOI: 10.1126/science.aac4951.
126. Amos W, Manica A. Global genetic positioning: evidence for early human population centers in coastal habitats. Proc Natl Acad Sci U S A 2006;103(3):820-4. (In eng). DOI: 10.1073/pnas.0507991103.

127. Foster MW, Sharp RR. Race, ethnicity, and genomics: social classifications as proxies of biological heterogeneity. Genome Res 2002;12(6):844-50. (In eng). DOI: 10.1101/gr.99202.
128. Johnson JA. Ethnic differences in cardiovascular drug response: potential contribution of pharmacogenetics. Circulation 2008;118(13):1383-1393.
129. Exner DV, Dries DL, Domanski MJ, Cohn JN. Lesser response to angiotensin-converting-enzyme inhibitor therapy in black as compared with white patients with left ventricular dysfunction. N Engl J Med 2001;344(18):1351-7. (In eng). DOI: 10.1056/NEJM200105033441802.
130. Preston RA, Materson BJ, Reda DJ, et al. Age-race subgroup compared with renin profile as predictors of blood pressure response to antihypertensive therapy. Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. JAMA 1998;280(13):1168-72. (In eng). DOI: 10.1001/jama.280.13.1168.
131. Saunders E, Weir MR, Kong BW, et al. A comparison of the efficacy and safety of a beta-blocker, a calcium channel blocker, and a converting enzyme inhibitor in hypertensive blacks. Arch Intern Med 1990;150(8):1707-13. (In eng).
132. Bahrami H, Kronmal R, Bluemke DA, et al. Differences in the incidence of congestive heart failure by ethnicity: the multi-ethnic study of atherosclerosis. Arch Intern Med 2008;168(19):2138-45. (In eng). DOI: 10.1001/archinte.168.19.2138.
133. Spanakis EK, Golden SH. Race/ethnic difference in diabetes and diabetic complications. Curr Diab Rep 2013;13(6):814-23. (In eng). DOI: 10.1007/s11892-013-0421-9.
134. Gardener H, Leifheit EC, Lichtman JH, et al. Racial/Ethnic Disparities in Mortality Among Medicare Beneficiaries in the FL - PR CR eSD Study. J Am Heart Assoc 2019;8(1):e009649. (In eng). DOI: 10.1161/JAHA.118.009649.
135. Simon P. Collecting ethnic statistics in Europe: a review. Ethnic and Racial Studies 2012;35(8):1366-1391.
136. Yazaki Y, Salzano A, Nelson CP, et al. Geographical location affects the levels and association of trimethylamine N-oxide with heart failure mortality in BIOSTAT-CHF: a post-hoc analysis. European journal of heart failure 2019.
137. Hackler EI, Lew J, Gore MO, et al. Racial Differences in Cardiovascular Biomarkers in the General Population. Journal of the American Heart Association 2019.
138. Victor RG, Haley RW, Willett DL, et al. The Dallas Heart Study: a population-based probability sample for the multidisciplinary study of ethnic differences in cardiovascular health. Am J Cardiol 2004;93(12):1473-80. (In eng). DOI: 10.1016/j.amjcard.2004.02.058.
139. Howard G, Prineas R, Moy C, et al. Racial and geographic differences in awareness, treatment, and control of hypertension: the REasons for Geographic And Racial Differences in Stroke study. Stroke 2006;37(5):1171-8. (In eng). DOI: 10.1161/01.STR.0000217222.09978.ce.
140. LaBounty TM, Bach DS, Bossone E, Kolias TJ. Effect of Race on Echocardiographic Measures of Cardiac Structure and Function. Am J Cardiol 2019 (In eng). DOI: 10.1016/j.amjcard.2019.05.049.
141. Folsom AR, Basu S, Hong CP, et al. Reasons for Differences in the Incidence of Venous Thromboembolism in Black Versus White Americans. Am J Med 2019 (In eng). DOI: 10.1016/j.amjmed.2019.03.021.
142. Williams DR, Priest N, Anderson NB. Understanding associations among race, socioeconomic status, and health: Patterns and prospects. Health Psychol 2016;35(4):407-11. (In eng). DOI: 10.1037/hea0000242.

143. Vinther JL, Jacobsen RK, Jørgensen T. Current European guidelines for management of cardiovascular disease: Is medical treatment in nearly half a population realistic? European journal of preventive cardiology 2018;25(2):157-163.
144. Royal CD, Dunston GM. Changing the paradigm from 'race' to human genome variation. Nat Genet 2004;36(11 Suppl):S5-7. (In eng). DOI: 10.1038/ng1454.
145. Virani SS, Alonso A, Benjamin EJ, et al. Heart Disease and Stroke Statistics-2020 Update: A Report From the American Heart Association. Circulation 2020;141(9):E139-E596. DOI: 10.1161/cir.0000000000000757.
146. Berry C, Doughty RN, Granger C, et al. The survival of patients with heart failure with preserved or reduced left ventricular ejection fraction: an individual patient data meta-analysis. European Heart Journal 2012;33(14):1750-1757. DOI: 10.1093/eurheartj/ehr254.
147. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European Journal of Heart Failure 2016;18(8):891-975. (Article) (In English). DOI: 10.1002/ejhf.592.
148. Yancy CW, Jessup M, Bozkurt B, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. Circulation 2017;136(6):E137-+. DOI: 10.1161/cir.0000000000000509.
149. Sacca L. Heart Failure as a Multiple Hormonal Deficiency Syndrome. Circulation-Heart Failure 2009;2(2):151-156. DOI: 10.1161/circheartfailure.108.821892.
150. Jankowska EA, Biel B, Majda J, et al. Anabolic deficiency in men with chronic heart failure - Prevalence and detrimental impact on survival. Circulation 2006;114(17):1829-1837. (Article) (In English). DOI: 10.1161/circulationaha.106.649426.
151. Arcopinto M, Salzano A, Bossone E, et al. Multiple hormone deficiencies in chronic heart failure. International Journal of Cardiology 2015;184:421-423. DOI: 10.1016/j.ijcard.2015.02.055.
152. Salzano A, Marra AM, Ferrara F, et al. Multiple hormone deficiency syndrome in heart failure with preserved ejection fraction. International Journal of Cardiology 2016;225:1-3. DOI: 10.1016/j.ijcard.2016.09.085.
153. Marra AM, Arcopinto M, Salzano A, et al. Detectable interleukin-9 plasma levels are associated with impaired cardiopulmonary functional capacity and all-cause mortality in patients with chronic heart failure. International Journal of Cardiology 2016;209:114-117. DOI: 10.1016/j.ijcard.2016.02.017.
154. Suskin N, McKelvie RS, Burns RJ, et al. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. European Heart Journal 2000;21(16):1368-1375. DOI: 10.1053/euhj.1999.2043.
155. Doehner W, Rauchhaus M, Ponikowski P, et al. Impaired insulin sensitivity as an independent risk factor for mortality in patients with stable chronic heart failure. Journal of the American College of Cardiology 2005;46(6):1019-1026. DOI: 10.1016/j.jacc.2005.02.093.
156. Chandramouli C, Teng THK, Tay WT, et al. Impact of diabetes and sex in heart failure with reduced ejection fraction patients from the ASIAN-HF registry. European Journal of Heart Failure 2019;21(3):297-307. DOI: 10.1002/ejhf.1358.

157. McMurray JJV, Solomon SD, Inzucchi SE, et al. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. New England Journal of Medicine 2019;381(21):1995-2008. DOI: 10.1056/NEJMoa1911303.
158. Arcopinto M, Salzano A, Giallauria F, et al. Growth Hormone Deficiency Is Associated with Worse Cardiac Function, Physical Performance, and Outcome in Chronic Heart Failure: Insights from the TOSCA. GHD Study. Plos One 2017;12(1). DOI: 10.1371/journal.pone.0170058.
159. Arcopinto M, Bobbio E, Bossone E, et al. The GH/IGF-1 Axis in Chronic Heart Failure. Endocrine Metabolic & Immune Disorders-Drug Targets 2013;13(1):76-91. (Article) (In English). DOI: 10.2174/1871530311313010010.
160. Arcopinto M, Isgaard J, Marra AM, et al. IGF-1 predicts survival in chronic heart failure. Insights from the TOSCA. (Trattamento Ormonale Nello Scompenso CArdiaco) registry. International Journal of Cardiology 2014;176(3):1006-1008. DOI: 10.1016/j.ijcard.2014.07.003.
161. Isgaard J, Wahlander H, Adams MA, Friberg P. INCREASED EXPRESSION OF GROWTH-HORMONE RECEPTOR MESSENGER-RNA AND INSULIN-LIKE GROWTH-FACTOR-I MESSENGER-RNA IN VOLUME-OVERLOADED HEARTS. Hypertension 1994;23(6):884-888. (Article; Proceedings Paper) (In English). DOI: 10.1161/01.hyp.23.6.884.
162. Niebauer J, Pflaum CD, Clark AL, et al. Deficient insulin-like growth factor I in chronic heart failure predicts altered body composition, anabolic deficiency, cytokine and neurohormonal activation. Journal of the American College of Cardiology 1998;32(2):393-397. (Article) (In English). DOI: 10.1016/s0735-1097(98)00226-5.
163. Spies M, Nesic O, Barrow RE, Perez-Polo JR, Herndon DN. Liposomal IGF-1 gene transfer modulates pro- and anti-inflammatory cytokine mRNA expression in the burn wound. Gene Therapy 2001;8(18):1409-1415. (Article) (In English). DOI: 10.1038/sj.gt.3301543.
164. Arcopinto M, Salzano A, Isgaard J, Cittadini A. Hormone replacement therapy in heart failure. Current Opinion in Cardiology 2015;30(3):277-284. DOI: 10.1097/hco.0000000000000166.
165. Bossone E, Arcopinto M, Iacoviello M, et al. Multiple hormonal and metabolic deficiency syndrome in chronic heart failure: rationale, design, and demographic characteristics of the TOSCA. Registry. Internal and Emergency Medicine 2018;13(5):661-671. (Article) (In English). DOI: 10.1007/s11739-018-1844-8.
166. Arcopinto M, Salzano A, Giallauria F, et al. Growth Hormone Deficiency Is Associated with Worse Cardiac Function, Physical Performance, and Outcome in Chronic Heart Failure: Insights from the T.O.S.CA. GHD Study. PLoS One 2017;12(1):e0170058. (In eng). DOI: 10.1371/journal.pone.0170058.
167. Campbell KS, Sorrell VL. Cell- and molecular-level mechanisms contributing to diastolic dysfunction in HFpEF. J Appl Physiol (1985) 2015;119(10):1228-32. (In eng). DOI: 10.1152/japplphysiol.01168.2014.
168. Rengo G, Lymperopoulos A, Leosco D, Koch WJ. GRK2 as a novel gene therapy target in heart failure. J Mol Cell Cardiol 2011;50(5):785-92. (In eng). DOI: 10.1016/j.yjmcc.2010.08.014.
169. Hasenfuss G, Reinecke H, Studer R, et al. Relation between myocardial function and expression of sarcoplasmic reticulum Ca(2+)-ATPase in failing and nonfailing human myocardium. Circ Res 1994;75(3):434-42. (In eng). DOI: 10.1161/01.res.75.3.434.
170. Hamdani N, Paulus WJ, van Heerebeek L, et al. Distinct myocardial effects of beta-blocker therapy in heart failure with normal and reduced left ventricular ejection fraction. Eur Heart J 2009;30(15):1863-72. (In eng). DOI: 10.1093/eurheartj/ehp189.

171. Engelhardt S, Bohm M, Erdmann E, Lohse MJ. Analysis of beta-adrenergic receptor mRNA levels in human ventricular biopsy specimens by quantitative polymerase chain reactions: Progressive reduction of beta(1)-adrenergic receptor mRNA in heart failure. Journal of the American College of Cardiology 1996;27(1):146-154. DOI: 10.1016/0735-1097(95)00425-4.
172. Fukuta H, Goto T, Wakami K, Ohte N. The effect of beta-blockers on mortality in heart failure with preserved ejection fraction: A meta-analysis of observational cohort and randomized controlled studies. International Journal of Cardiology 2017;228:4-10. DOI: 10.1016/j.ijcard.2016.11.239.
173. Das S, Frisk C, Eriksson MJ, et al. Transcriptomics of cardiac biopsies reveals differences in patients with or without diagnostic parameters for heart failure with preserved ejection fraction. Scientific Reports 2019;9. DOI: 10.1038/s41598-019-39445-2.
174. Bornfeldt KE, Arnqvist HJ, Enberg B, Mathews LS, Norstedt G. REGULATION OF INSULIN-LIKE GROWTH FACTOR-I AND GROWTH-HORMONE RECEPTOR GENE-EXPRESSION BY DIABETES AND NUTRITIONAL STATE IN RAT-TISSUES. Journal of Endocrinology 1989;122(3):651-656. DOI: 10.1677/joe.0.1220651.
175. Faxen UL, Hage C, Benson L, et al. HFpEF and HFrEF Display Different Phenotypes as Assessed by IGF-1 and IGFBP-1. Journal of Cardiac Failure 2017;23(4):293-303. DOI: 10.1016/j.cardfail.2016.06.008.
176. D'Assante R, Napoli R, Salzano A, et al. Human heart shifts from IGF-1 production to utilization with chronic heart failure. Endocrine 2019;65(3):714-716. (In eng). DOI: 10.1007/s12020-019-01993-y.
177. Palmieri EA, Benincasa G, Di Rella F, et al. Differential expression of TNF-alpha, IL-6, and IGF-1 by graded mechanical stress in normal rat myocardium. American Journal of Physiology-Heart and Circulatory Physiology 2002;282(3):H926-H934. DOI: 10.1152/ajpheart.00436.2001.
178. Mann DL, Bristow MR. Mechanisms and models in heart failure: the biomechanical model and beyond. Circulation 2005;111(21):2837-49. DOI: 10.1161/CIRCULATIONAHA.104.500546.
179. Salzano A, Demelo-Rodriguez P, Marra AM, Proietti M. A Focused Review of Gender Differences in Antithrombotic Therapy. Curr Med Chem 2017;24(24):2576-2588. DOI: 10.2174/0929867323666161029223512.
180. Bossone E, Limongelli G, Malizia G, et al. The T.O.S.CA. Project: research, education and care. Monaldi Arch Chest Dis 2011;76(4):198-203. (In eng). DOI: 10.4081/monaldi.2011.177.
181. Arcopinto M, Salzano A, Ferrara F, et al. The Tosca Registry: An Ongoing, Observational, Multicenter Registry for Chronic Heart Failure. Transl Med UniSa 2016;14:21-7.
182. Salzano A, Marra AM, Ferrara F, et al. Multiple hormone deficiency syndrome in heart failure with preserved ejection fraction. Int J Cardiol 2016;225:1-3. (In eng). DOI: 10.1016/j.ijcard.2016.09.085.
183. Arcopinto M, Salzano A, Giallauria F, et al. Growth Hormone Deficiency Is Associated with Worse Cardiac Function, Physical Performance, and Outcome in Chronic Heart Failure: Insights from the T.O.S.CA. GHD Study. PLoS One 2017;12(1):e0170058. (In eng). DOI: 10.1371/journal.pone.0170058.
184. Napoli R, Salzano A, Bossone E, Cittadini A. Hormonal Therapy in the Treatment of Chronic Heart Failure A2 - Vasan, Ramachandran S. In: Sawyer DB, ed. Encyclopedia of Cardiovascular Research and Medicine. Oxford: Elsevier; 2018:508-516.

185. Bossone E, Arcopinto M, Iacoviello M, et al. Multiple hormonal and metabolic deficiency syndrome in chronic heart failure: rationale, design, and demographic characteristics of the T.O.S.CA. Registry. Intern Emerg Med 2018. DOI: 10.1007/s11739-018-1844-8.
186. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. European heart journal 2016;37:2893-2962. DOI: 10.1093/eurheartj/ehw210.
187. Pagan J, Seto T, Pagano M, Cittadini A. Role of the ubiquitin proteasome system in the heart. Circ Res 2013;112(7):1046-58. DOI: 10.1161/CIRCRESAHA.112.300521.
188. Suskin N, McKelvie RS, Burns RJ, et al. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. European heart journal 2000;21(16):1368-75. DOI: 10.1053/euhj.1999.2043.
189. Arcopinto M, Bobbio E, Bossone E, et al. The GH/IGF-1 axis in chronic heart failure. Endocr Metab Immune Disord Drug Targets 2013;13(1):76-91.
190. Petretta M, Colao A, Sardu C, et al. NT-proBNP, IGF-I and survival in patients with chronic heart failure. Growth Horm IGF Res 2007;17(4):288-96. DOI: 10.1016/j.ghir.2007.01.020.
191. Niebauer J, Pflaum CD, Clark AL, et al. Deficient insulin-like growth factor I in chronic heart failure predicts altered body composition, anabolic deficiency, cytokine and neurohormonal activation. J Am Coll Cardiol 1998;32(2):393-7.
192. Opasich C, Pacini F, Ambrosino N, et al. Sick euthyroid syndrome in patients with moderate-to-severe chronic heart failure. Eur Heart J 1996;17(12):1860-6.
193. Iervasi G, Pingitore A, Landi P, et al. Low-T3 syndrome: a strong prognostic predictor of death in patients with heart disease. Circulation 2003;107(5):708-13.
194. Pingitore A, Iervasi G. Thyroid (dys)function in heart failure: is it a potential target for medical treatment? Vasc Health Risk Manag 2005;1(2):97-100.
195. Caminiti G, Volterrani M, Iellamo F, et al. Effect of long-acting testosterone treatment on functional exercise capacity, skeletal muscle performance, insulin resistance, and baroreflex sensitivity in elderly patients with chronic heart failure a double-blind, placebo-controlled, randomized study. J Am Coll Cardiol 2009;54(10):919-27. DOI: 10.1016/j.jacc.2009.04.078.
196. Iellamo F, Volterrani M, Caminiti G, et al. Testosterone therapy in women with chronic heart failure: a pilot double-blind, randomized, placebo-controlled study. J Am Coll Cardiol 2010;56(16):1310-6. DOI: 10.1016/j.jacc.2010.03.090.
197. Doehner W, Rauchhaus M, Ponikowski P, et al. Impaired insulin sensitivity as an independent risk factor for mortality in patients with stable chronic heart failure. J Am Coll Cardiol 2005;46(6):1019-26. DOI: 10.1016/j.jacc.2005.02.093.
198. Wong AK, Symon R, AlZadjali MA, et al. The effect of metformin on insulin resistance and exercise parameters in patients with heart failure. European journal of heart failure 2012;14(11):1303-10. DOI: 10.1093/eurjhf/hfs106.
199. Cittadini A, Napoli R, Monti MG, et al. Metformin prevents the development of chronic heart failure in the SHHF rat model. Diabetes 2012;61(4):944-53. DOI: 10.2337/db11-1132.
200. Meta-analysis Global Group in Chronic Heart F. The survival of patients with heart failure with preserved or reduced left ventricular ejection fraction: an individual patient data meta-analysis. Eur Heart J 2012;33(14):1750-7. DOI: 10.1093/eurheartj/ehr254.
201. Plesner LL, Dalsgaard M, Schou M, et al. The prognostic significance of lung function in stable heart failure outpatients. Clin Cardiol 2017. DOI: 10.1002/clc.22802.
202. Maggioni AP, Anker SD, Dahlstrom U, et al. Are hospitalized or ambulatory patients with heart failure treated in accordance with European Society of Cardiology guidelines?

Evidence from 12,440 patients of the ESC Heart Failure Long-Term Registry. Eur J Heart Fail 2013;15(10):1173-84. DOI: 10.1093/eurjhf/hft134.
203. Voors AA, Anker SD, Cleland JG, et al. A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. Eur J Heart Fail 2016;18(6):716-26. DOI: 10.1002/ejhf.531.
204. Iervasi G, Landi P, Raciti M, et al. Low-T3 syndrome - A strong prognostic predictor of death in patients with heart disease. Circulation 2003;107(5):708-713. DOI: 10.1161/01.cir.0000048124.64204.3f.
205. Jankowska EA, Rozentryt P, Ponikowska B, et al. Circulating Estradiol and Mortality in Men With Systolic Chronic Heart Failure. Jama-Journal of the American Medical Association 2009;301(18):1892-1901. DOI: 10.1001/jama.2009.639.
206. Anker SD, Volterrani M, Pflaum CD, et al. Acquired growth hormone resistance in patients with chronic heart failure: Implications for therapy with growth hormone. Journal of the American College of Cardiology 2001;38(2):443-452. DOI: 10.1016/s0735-1097(01)01385-7.
207. Pugh PJ, Jones RD, West JN, Jones TH, Channer KS. Testosterone treatment for men with chronic heart failure. Heart 2004;90(4):446-447. DOI: 10.1136/hrt.2003.014639.
208. Malkin CJ, Pugh PJ, West JN, van Beek EJR, Jones TH, Channer KS. Testosterone therapy in men with moderate severity heart failure: a double-blind randomized placebo controlled trial. European Heart Journal 2006;27(1):57-64. DOI: 10.1093/eurheartj/ehi443.
209. Caminiti G, Volterrani M, Iellamo F, et al. Effect of Long-Acting Testosterone Treatment on Functional Exercise Capacity, Skeletal Muscle Performance, Insulin Resistance, and Baroreflex Sensitivity in Elderly Patients With Chronic Heart Failure A Double-Blind, Placebo-Controlled, Randomized Study. Journal of the American College of Cardiology 2009;54(10):919-927. DOI: 10.1016/j.jacc.2009.04.078.
210. Oh JY, Barrett-Connor E, Wedick NM, Wingard DL. Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo Study. Diabetes Care 2002;25(1):55-60. DOI: 10.2337/diacare.25.1.55.
211. Pingitore A, Galli E, Barison A, et al. The acute effects of triiodothyronine replacement therapy in patients with chronic heart failure and low T3 syndrome: a randomised, placebo-controlled study. European Heart Journal 2007;28:648-648.
212. Swan JW, Anker SD, Walton C, et al. Insulin resistance in chronic heart failure: Relation to severity and etiology of heart failure. Journal of the American College of Cardiology 1997;30(2):527-532. DOI: 10.1016/s0735-1097(97)00185-x.
213. Cittadini A, Saldamarco L, Marra AM, et al. Growth Hormone Deficiency in Patients with Chronic Heart Failure and Beneficial Effects of Its Correction. Journal of Clinical Endocrinology & Metabolism 2009;94(9):3329-3336. DOI: 10.1210/jc.2009-0533.
214. Cittadini A, Marra AM, Arcopinto M, et al. Growth Hormone Replacement Delays the Progression of Chronic Heart Failure Combined With Growth Hormone Deficiency An Extension of a Randomized Controlled Single-Blind Study. Jacc-Heart Failure 2013;1(4):325-330. DOI: 10.1016/j.jchf.2013.04.003.
215. Salzano A, Marra AM, Arcopinto M, et al. Combined effects of growth hormone and testosterone replacement treatment in heart failure. Esc Heart Failure 2019;6(6):1216-1221. DOI: 10.1002/ehf2.12520.
216. Salzano A, Suzuki T, Squire IB, Cittadini A. Are heart failure observational studies still useful? 'No need to argue'*. European Journal of Preventive Cardiology. DOI: 10.1177/2047487320932258.

217. Sirico D, Salzano A, Celentani D, et al. [Anti remodeling therapy: new strategies and future perspective in post-ischemic heart failure: Part I]. Monaldi Arch Chest Dis 2014;82(4):187-94. DOI: 10.4081/monaldi.2014.63.
218. Salzano A, Sirico D, Arcopinto M, et al. [Anti remodeling therapy: new strategies and future perspective in post-ischemic heart failure. Part II]. Monaldi Arch Chest Dis 2014;82(4):195-201. DOI: 10.4081/monaldi.2014.64.
219. Alghamdi F, Chan M. Management of heart failure in the elderly. Curr Opin Cardiol 2017;32(2):217-223. DOI: 10.1097/HCO.0000000000000375.
220. Salzano A, Cittadini A, Bossone E, Suzuki T, Heaney LM. Multiple hormone deficiency syndrome : a novel topic in chronic heart failure. Future Sci OA 2018:3. DOI: 10.4155/fsoa-2018-0041
221. Salzano A, D'Assante R, Heaney LM, et al. Klinefelter syndrome, insulin resistance, metabolic syndrome, and diabetes: review of literature and clinical perspectives. Endocrine 2018. DOI: 10.1007/s12020-018-1584-6.
222. Salzano A, Rocca A, Arcopinto M, et al. Bowel Angiodysplasia and Myocardial Infarction secondary to an ischaemic imbalance: a case report. Open Med (Wars) 2015;10(1):543-548. (In eng). DOI: 10.1515/med-2015-0092.
223. Marsh JD, Lehmann MH, Ritchie RH, Gwathmey JK, Green GE, Schiebinger RJ. Androgen receptors mediate hypertrophy in cardiac myocytes. Circulation 1998;98(3):256-61.
224. Perusquia M, Stallone JN. Do androgens play a beneficial role in the regulation of vascular tone? Nongenomic vascular effects of testosterone metabolites. Am J Physiol Heart Circ Physiol 2010;298(5):H1301-7. DOI: 10.1152/ajpheart.00753.2009.
225. Tirabassi G, Cutini M, Beltrami B, Delli Muti N, Lenzi A, Balercia G. Androgen receptor GGC repeat might be more involved than CAG repeat in the regulation of the metabolic profile in men. Intern Emerg Med 2016;11(8):1067-1075. DOI: 10.1007/s11739-016-1479-6.
226. Brouillette J, Rivard K, Lizotte E, Fiset C. Sex and strain differences in adult mouse cardiac repolarization: importance of androgens. Cardiovasc Res 2005;65(1):148-57. DOI: 10.1016/j.cardiores.2004.09.012.
227. Cavasin MA, Tao ZY, Yu AL, Yang XP. Testosterone enhances early cardiac remodeling after myocardial infarction, causing rupture and degrading cardiac function. Am J Physiol Heart Circ Physiol 2006;290(5):H2043-50. DOI: 10.1152/ajpheart.01121.2005.
228. Gupta V, Bhasin S, Guo W, et al. Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes. Mol Cell Endocrinol 2008;296(1-2):32-40. DOI: 10.1016/j.mce.2008.08.019.
229. Filippi S, Vignozzi L, Morelli A, et al. Testosterone partially ameliorates metabolic profile and erectile responsiveness to PDE5 inhibitors in an animal model of male metabolic syndrome. J Sex Med 2009;6(12):3274-88. DOI: 10.1111/j.1743-6109.2009.01467.x.
230. Kelly DM, Akhtar S, Sellers DJ, Muraleedharan V, Channer KS, Jones TH. Testosterone differentially regulates targets of lipid and glucose metabolism in liver, muscle and adipose tissues of the testicular feminised mouse. Endocrine 2016;54(2):504-515. DOI: 10.1007/s12020-016-1019-1.
231. Oh JY, Barrett-Connor E, Wedick NM, Wingard DL, Rancho Bernardo S. Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. Diabetes Care 2002;25(1):55-60.
232. Brand JS, Rovers MM, Yeap BB, et al. Testosterone, sex hormone-binding globulin and the metabolic syndrome in men: an individual participant data meta-analysis of observational studies. PLoS One 2014;9(7):e100409. DOI: 10.1371/journal.pone.0100409.

233. Pugh PJ, Jones TH, Channer KS. Acute haemodynamic effects of testosterone in men with chronic heart failure. Eur Heart J 2003;24(10):909-15.
234. Pugh PJ, Jones RD, West JN, Jones TH, Channer KS. Testosterone treatment for men with chronic heart failure. Heart 2004;90(4):446-7.
235. Malkin CJ, Pugh PJ, West JN, van Beek EJ, Jones TH, Channer KS. Testosterone therapy in men with moderate severity heart failure: a double-blind randomized placebo controlled trial. Eur Heart J 2006;27(1):57-64. DOI: 10.1093/eurheartj/ehi443.
236. Pugh PJ, Jones RD, Malkin CJ, et al. Physiologic testosterone therapy has no effect on serum levels of tumour necrosis factor-alpha in men with chronic heart failure. Endocr Res 2005;31(4):271-83.
237. Malkin CJ, Morris PD, Pugh PJ, English KM, Channer KS. Effect of testosterone therapy on QT dispersion in men with heart failure. Am J Cardiol 2003;92(10):1241-3.
238. Schwartz JB, Volterrani M, Caminiti G, et al. Effects of testosterone on the Q-T interval in older men and older women with chronic heart failure. Int J Androl 2011;34(5 Pt 2):e415-21. DOI: 10.1111/j.1365-2605.2011.01163.x.
239. Dharmarajan K, Rich MW. Epidemiology, Pathophysiology, and Prognosis of Heart Failure in Older Adults. Heart Fail Clin 2017;13(3):417-426. DOI: 10.1016/j.hfc.2017.02.001.
240. Panjrath G, Ahmed A. Diagnosis and Management of Heart Failure in Older Adults. Heart Fail Clin 2017;13(3):427-444. DOI: 10.1016/j.hfc.2017.02.002.
241. Mirdamadi A, Garakyaraghi M, Pourmoghaddas A, Bahmani A, Mahmoudi H, Gharipour M. Beneficial effects of testosterone therapy on functional capacity, cardiovascular parameters, and quality of life in patients with congestive heart failure. Biomed Res Int 2014;2014:392432. DOI: 10.1155/2014/392432.
242. Stout M, Tew GA, Doll H, et al. Testosterone therapy during exercise rehabilitation in male patients with chronic heart failure who have low testosterone status: a double-blind randomized controlled feasibility study. Am Heart J 2012;164(6):893-901. DOI: 10.1016/j.ahj.2012.09.016.
243. Dos Santos MR, Sayegh AL, Bacurau AV, et al. Effect of Exercise Training and Testosterone Replacement on Skeletal Muscle Wasting in Patients With Heart Failure With Testosterone Deficiency. Mayo Clin Proc 2016;91(5):575-86. DOI: 10.1016/j.mayocp.2016.02.014.
244. Marra AM, Biskup E, Raparelli V, group Iw. The Internal Medicine and Assessment of Gender Differences in Europe (IMAGINE): The new European Federation of Internal Medicine initiative on sex and gender medicine. Eur J Intern Med 2018;51:e30-e32. DOI: 10.1016/j.ejim.2018.02.003.
245. Salzano A, Proietti M, D'Assante R, Saldamarco L, Cittadini A, Paladino F. Bleeding related to non-vitamin K antagonist oral anticoagulants in emergency department: A "Real-world" snapshot from Southern Italy. On behalf of MIRC-NOAC study group. Eur J Intern Med 2017 (In eng). DOI: 10.1016/j.ejim.2017.10.018.
246. Bossone E, Lyon A, Citro R, et al. Takotsubo cardiomyopathy: an integrated multi-imaging approach. Eur Heart J Cardiovasc Imaging 2014;15(4):366-77. DOI: 10.1093/ehjci/jet167.
247. Toma M, McAlister FA, Coglianese EE, et al. Testosterone supplementation in heart failure: a meta-analysis. Circ Heart Fail 2012;5(3):315-21. DOI: 10.1161/CIRCHEARTFAILURE.111.965632.
248. Fazio S, Palmieri EA, Affuso F, et al. Effects of growth hormone on exercise capacity and cardiopulmonary performance in patients with chronic heart failure. J Clin Endocrinol Metab 2007;92(11):4218-23. DOI: 10.1210/jc.2007-1189.

249. Cittadini A, Monti MG, Castiello MC, et al. Insulin-like growth factor-1 protects from vascular stenosis and accelerates re-endothelialization in a rat model of carotid artery injury. J Thromb Haemost 2009;7(11):1920-8. DOI: 10.1111/j.1538-7836.2009.03607.x.
250. Hartupee J, Mann DL. Neurohormonal activation in heart failure with reduced ejection fraction. Nat Rev Cardiol 2017;14(1):30-38. DOI: 10.1038/nrcardio.2016.163.
251. Colao A. The GH-IGF-I axis and the cardiovascular system: clinical implications. Clin Endocrinol (Oxf) 2008;69(3):347-58. DOI: 10.1111/j.1365-2265.2008.03292.x.
252. Alvarez-Nava F, Lanes R. GH/IGF-1 Signaling and Current Knowledge of Epigenetics; a Review and Considerations on Possible Therapeutic Options. Int J Mol Sci 2017;18(10). DOI: 10.3390/ijms18101624.
253. Orru S, Nigro E, Mandola A, et al. A Functional Interplay between IGF-1 and Adiponectin. Int J Mol Sci 2017;18(10). DOI: 10.3390/ijms18102145.
254. Nicholls AR, Holt RI. Growth Hormone and Insulin-Like Growth Factor-1. Front Horm Res 2016;47:101-14. DOI: 10.1159/000445173.
255. Ranke MB. Insulin-like growth factor binding-protein-3 (IGFBP-3). Best Pract Res Clin Endocrinol Metab 2015;29(5):701-11. DOI: 10.1016/j.beem.2015.06.003.
256. Himpe E, Kooijman R. Insulin-like growth factor-I receptor signal transduction and the Janus Kinase/Signal Transducer and Activator of Transcription (JAK-STAT) pathway. Biofactors 2009;35(1):76-81. DOI: 10.1002/biof.20.
257. Isgaard J, Arcopinto M, Karason K, Cittadini A. GH and the cardiovascular system: an update on a topic at heart. Endocrine 2015;48(1):25-35. DOI: 10.1007/s12020-014-0327-6.
258. Hjalmarson A, Isaksson O, Ahren K. Effects of growth hormone and insulin on amino acid transport in perfused rat heart. Am J Physiol 1969;217(6):1795-802.
259. Stromer H, Palmieri EA, De Groot MC, et al. Growth hormone- and pressure overload-induced cardiac hypertrophy evoke different responses to ischemia-reperfusion and mechanical stretch. Growth Horm IGF Res 2006;16(1):29-40. DOI: 10.1016/j.ghir.2005.09.002.
260. Cittadini A, Monti MG, Iaccarino G, et al. Adenoviral gene transfer of Akt enhances myocardial contractility and intracellular calcium handling. Gene Ther 2006;13(1):8-19. DOI: 10.1038/sj.gt.3302589.
261. Broglio F, Benso A, Gottero C, et al. Patients with dilated cardiomyopathy show reduction of the somatotroph responsiveness to GHRH both alone and combined with arginine. Eur J Endocrinol 2000;142(2):157-63.
262. Kontoleon PE, Anastasiou-Nana MI, Papapetrou PD, et al. Hormonal profile in patients with congestive heart failure. Int J Cardiol 2003;87(2-3):179-83.
263. Anwar A, Gaspoz JM, Pampallona S, et al. Effect of congestive heart failure on the insulin-like growth factor-1 system. Am J Cardiol 2002;90(12):1402-5.
264. Bolger A, Doehner W, Anker SD. Insulin-like growth factor-I can be helpful towards end of life. BMJ 2001;322(7287):674-5.
265. Anker SD, Volterrani M, Pflaum CD, et al. Acquired growth hormone resistance in patients with chronic heart failure: implications for therapy with growth hormone. J Am Coll Cardiol 2001;38(2):443-52.
266. Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Vance ML, Endocrine S. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96(6):1587-609. DOI: 10.1210/jc.2011-0179.
267. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. Growth Horm IGF Res 2008;18(1):1-16. DOI: 10.1016/j.ghir.2007.07.004.

268. Yuen KC, Tritos NA, Samson SL, Hoffman AR, Katznelson L. American Association of Clinical Endocrinologists and American College of Endocrinology Disease State Clinical Review: Update on Growth Hormone Stimulation Testing and Proposed Revised Cut-Point for the Glucagon Stimulation Test in the Diagnosis of Adult Growth Hormone Deficiency. Endocr Pract 2016;22(10):1235-1244. DOI: 10.4158/EP161407.DSCR.
269. Gordon MB, Levy RA, Gut R, Germak J. Trends in Growth Hormone Stimulation Testing and Growth Hormone Dosing in Adult Growth Hormone Deficiency Patients: Results from the Answer Program. Endocr Pract 2016;22(4):396-405. DOI: 10.4158/EP14603.OR.
270. Prodam F, Pagano L, Corneli G, et al. Update on epidemiology, etiology, and diagnosis of adult growth hormone deficiency. J Endocrinol Invest 2008;31(9 Suppl):6-11.
271. Pena-Bello L, Seoane-Pillado T, Sangiao-Alvarellos S, et al. Oral glucose-stimulated growth hormone (GH) test in adult GH deficiency patients and controls: Potential utility of a novel test. Eur J Intern Med 2017;44:55-61. DOI: 10.1016/j.ejim.2017.06.004.
272. Rahim A, Toogood AA, Shalet SM. The assessment of growth hormone status in normal young adult males using a variety of provocative agents. Clin Endocrinol (Oxf) 1996;45(5):557-62.
273. Junnila RK, Strasburger CJ, Bidlingmaier M. Pitfalls of insulin-like growth factor-i and growth hormone assays. Endocrinol Metab Clin North Am 2015;44(1):27-34. DOI: 10.1016/j.ecl.2014.10.003.
274. Colao A, Di Somma C, Cuocolo A, et al. The severity of growth hormone deficiency correlates with the severity of cardiac impairment in 100 adult patients with hypopituitarism: an observational, case-control study. J Clin Endocrinol Metab 2004;89(12):5998-6004. DOI: 10.1210/jc.2004-1042.
275. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 1990;336(8710):285-8.
276. Bhandari SS, Narayan H, Jones DJ, et al. Plasma growth hormone is a strong predictor of risk at 1 year in acute heart failure. Eur J Heart Fail 2016;18(3):281-9. DOI: 10.1002/ejhf.459.
277. Fonarow GC, Adams KF, Jr., Abraham WT, et al. Risk stratification for in-hospital mortality in acutely decompensated heart failure: classification and regression tree analysis. JAMA 2005;293(5):572-80. DOI: 10.1001/jama.293.5.572.
278. Wang CY, Chung HW, Cho NY, et al. Idiopathic growth hormone deficiency in the morphologically normal pituitary gland is associated with perfusion delay. Radiology 2011;258(1):213-21. DOI: 10.1148/radiol.10100504.
279. Yoshida T, Tabony AM, Galvez S, et al. Molecular mechanisms and signaling pathways of angiotensin II-induced muscle wasting: potential therapeutic targets for cardiac cachexia. Int J Biochem Cell Biol 2013;45(10):2322-32. DOI: 10.1016/j.biocel.2013.05.035.
280. Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 1998;19(6):717-97. DOI: 10.1210/edrv.19.6.0353.
281. Napoli R, Salzano A, Bossone E, Cittadini A. Hormonal Therapy in the Treatment of Chronic Heart Failure: Elsevier, 2017.
282. Yang R, Bunting S, Gillett N, Clark R, Jin H. Growth hormone improves cardiac performance in experimental heart failure. Circulation 1995;92(2):262-7.
283. Duerr RL, McKirnan MD, Gim RD, Clark RG, Chien KR, Ross J, Jr. Cardiovascular effects of insulin-like growth factor-1 and growth hormone in chronic left ventricular failure in the rat. Circulation 1996;93(12):2188-96.

284. Ryoke T, Gu Y, Mao L, et al. Progressive cardiac dysfunction and fibrosis in the cardiomyopathic hamster and effects of growth hormone and angiotensin-converting enzyme inhibition. Circulation 1999;100(16):1734-43.
285. Cittadini A, Ishiguro Y, Stromer H, et al. Insulin-like growth factor-1 but not growth hormone augments mammalian myocardial contractility by sensitizing the myofilament to Ca2+ through a wortmannin-sensitive pathway: studies in rat and ferret isolated muscles. Circ Res 1998;83(1):50-9.
286. Cittadini A, Isgaard J, Monti MG, et al. Growth hormone prolongs survival in experimental postinfarction heart failure. J Am Coll Cardiol 2003;41(12):2154-63.
287. Cittadini A, Grossman JD, Napoli R, et al. Growth hormone attenuates early left ventricular remodeling and improves cardiac function in rats with large myocardial infarction. J Am Coll Cardiol 1997;29(5):1109-16.
288. Fazio S, Sabatini D, Capaldo B, et al. A preliminary study of growth hormone in the treatment of dilated cardiomyopathy. N Engl J Med 1996;334(13):809-14. DOI: 10.1056/NEJM199603283341301.
289. Frustaci A, Gentiloni N, Russo MA. Growth hormone in the treatment of dilated cardiomyopathy. N Engl J Med 1996;335(9):672-3; author reply 673-4. DOI: 10.1056/NEJM199608293350915.
290. Isgaard J, Bergh CH, Caidahl K, Lomsky M, Hjalmarson A, Bengtsson BA. A placebo-controlled study of growth hormone in patients with congestive heart failure. Eur Heart J 1998;19(11):1704-11.
291. Osterziel KJ, Strohm O, Schuler J, et al. Randomised, double-blind, placebo-controlled trial of human recombinant growth hormone in patients with chronic heart failure due to dilated cardiomyopathy. Lancet 1998;351(9111):1233-7. DOI: 10.1016/S0140-6736(97)11329-0.
292. Genth-Zotz S, Zotz R, Geil S, Voigtlander T, Meyer J, Darius H. Recombinant growth hormone therapy in patients with ischemic cardiomyopathy : effects on hemodynamics, left ventricular function, and cardiopulmonary exercise capacity. Circulation 1999;99(1):18-21.
293. Spallarossa P, Rossettin P, Minuto F, et al. Evaluation of growth hormone administration in patients with chronic heart failure secondary to coronary artery disease. Am J Cardiol 1999;84(4):430-3.
294. Jose VJ, Zechariah TU, George P, Jonathan V. Growth hormone therapy in patients with dilated cardiomyopathy: preliminary observations of a pilot study. Indian Heart J 1999;51(2):183-5.
295. Smit JW, Janssen YJ, Lamb HJ, et al. Six months of recombinant human GH therapy in patients with ischemic cardiac failure does not influence left ventricular function and mass. J Clin Endocrinol Metab 2001;86(10):4638-43. DOI: 10.1210/jcem.86.10.7832.
296. Perrot A, Ranke MB, Dietz R, Osterziel KJ. Growth hormone treatment in dilated cardiomyopathy. J Card Surg 2001;16(2):127-31.
297. Napoli R, Guardasole V, Matarazzo M, et al. Growth hormone corrects vascular dysfunction in patients with chronic heart failure. J Am Coll Cardiol 2002;39(1):90-5.
298. Adamopoulos S, Parissis JT, Paraskevaidis I, et al. Effects of growth hormone on circulating cytokine network, and left ventricular contractile performance and geometry in patients with idiopathic dilated cardiomyopathy. Eur Heart J 2003;24(24):2186-96.
299. Acevedo M, Corbalan R, Chamorro G, et al. Administration of growth hormone to patients with advanced cardiac heart failure: effects upon left ventricular function, exercise capacity, and neurohormonal status. Int J Cardiol 2003;87(2-3):185-91.

300. van Thiel SW, Smit JW, de Roos A, et al. Six-months of recombinant human GH therapy in patients with ischemic cardiac failure. Int J Cardiovasc Imaging 2004;20(1):53-60.
301. Frustaci A, Gentiloni N, Corsello SM, Caldarulo M, Russo MA. Reversible dilated cardiomyopathy due to growth hormone deficiency. Chest 1992;102(1):326-7.
302. Cuneo R, Salomon F, Wilmshurst P, Lowy C, McGauley G, Sonksen P. Reversible dilated cardiomyopathy due to growth hormone deficiency. Am J Clin Pathol 1993;100(5):585-6.
303. Cittadini A, Ines Comi L, Longobardi S, et al. A preliminary randomized study of growth hormone administration in Becker and Duchenne muscular dystrophies. Eur Heart J 2003;24(7):664-72.
304. Le Corvoisier P, Hittinger L, Chanson P, Montagne O, Macquin-Mavier I, Maison P. Cardiac effects of growth hormone treatment in chronic heart failure: A meta-analysis. J Clin Endocrinol Metab 2007;92(1):180-5. DOI: 10.1210/jc.2006-1313.
305. Tritos NA, Danias PG. Growth hormone therapy in congestive heart failure due to left ventricular systolic dysfunction: a meta-analysis. Endocr Pract 2008;14(1):40-9. DOI: 10.4158/EP.14.1.40.
306. Spielhagen C, Schwahn C, Moller K, et al. The benefit of long-term growth hormone (GH) replacement therapy in hypopituitary adults with GH deficiency: results of the German KIMS database. Growth Horm IGF Res 2011;21(1):1-10. DOI: 10.1016/j.ghir.2010.10.005.
307. Bozkurt B, Colvin M, Cook J, et al. Current Diagnostic and Treatment Strategies for Specific Dilated Cardiomyopathies: A Scientific Statement From the American Heart Association. Circulation 2016;134(23):e579-e646. DOI: 10.1161/CIR.0000000000000455.
308. Wang W, Jiang T, Li C, et al. Will testosterone replacement therapy become a new treatment of chronic heart failure? A review based on 8 clinical trials. J Thorac Dis 2016;8(5):E269-77. DOI: 10.21037/jtd.2016.03.39.
309. Fazio S, Palmieri EA, Biondi B, Cittadini A, Sacca L. The role of the GH-IGF-I axis in the regulation of myocardial growth: from experimental models to human evidence. Eur J Endocrinol 2000;142(3):211-6.
310. Piccioli L, Arcopinto M, Salzano A, et al. The impairment of the Growth Hormone/Insulin-like growth factor 1 (IGF-1) axis in heart failure: A possible target for future therapy. Monaldi Arch Chest Dis 2018;88(3):975. DOI: 10.4081/monaldi.2018.975.
311. Gareri P, De Fazio P, Cotroneo A, et al. Anticholinergic drug-induced delirium in an elderly Alzheimer's dementia patient. Arch Gerontol Geriatr 2007;44 Suppl 1:199-206. DOI: 10.1016/j.archger.2007.01.027.
312. Brankovic M, Akkerhuis KM, Mouthaan H, et al. Cardiometabolic Biomarkers and Their Temporal Patterns Predict Poor Outcome in Chronic Heart Failure (Bio-SHiFT Study). J Clin Endocrinol Metab 2018;103(11):3954-3964. DOI: 10.1210/jc.2018-01241.
313. Salzano A, Marra AM, Bossone E, Suzuki T, Cittadini A. Letter to the Editor: "Cardiometabolic biomarkers and their temporal patterns predict poor outcome in chronic heart failure (Bio-SHiFT study)". J Clin Endocrinol Metab 2018. DOI: 10.1210/jc.2018-02043.
314. Brankovic M, Akkerhuis KM, Umans V, Boersma E, Kardys I. Response to Letter: Cardiometabolic biomarkers and their temporal patterns predict poor outcome in chronic heart failure (Bio-SHiFT study). J Clin Endocrinol Metab 2018. DOI: 10.1210/jc.2018-02149.
315. Salzano A, Marra AM, D'Assante R, et al. Growth Hormone Therapy in Heart Failure. Heart Fail Clin 2018;14(4):501-515. DOI: 10.1016/j.hfc.2018.05.002.
316. Sacca L, Napoli R, Cittadini A. Growth hormone, acromegaly, and heart failure: an intricate triangulation. Clin Endocrinol (Oxf) 2003;59(6):660-71. DOI: 10.1046/j.1365-2265.2003.01780.x.

317. Lapauw B, Goemaere S, Zmierczak H, et al. The decline of serum testosterone levels in community-dwelling men over 70 years of age: descriptive data and predictors of longitudinal changes. Eur J Endocrinol 2008;159(4):459-68. DOI: 10.1530/EJE-07-0873.
318. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 2005;26(6):833-76. DOI: 10.1210/er.2004-0013.
319. Bhasin S, Cunningham GR, Hayes FJ, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2010;95(6):2536-59. DOI: 10.1210/jc.2009-2354.
320. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103(5):1715-1744. DOI: 10.1210/jc.2018-00229.
321. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR, Baltimore Longitudinal Study of A. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab 2001;86(2):724-31. DOI: 10.1210/jcem.86.2.7219.
322. Haring R, Volzke H, Steveling A, et al. Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20-79. Eur Heart J 2010;31(12):1494-501. DOI: 10.1093/eurheartj/ehq009.
323. Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. J Clin Endocrinol Metab 2008;93(1):68-75. DOI: 10.1210/jc.2007-1792.
324. Anker SD, Chua TP, Ponikowski P, et al. Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia. Circulation 1997;96(2):526-34.
325. Moriyama Y, Yasue H, Yoshimura M, et al. The plasma levels of dehydroepiandrosterone sulfate are decreased in patients with chronic heart failure in proportion to the severity. J Clin Endocrinol Metab 2000;85(5):1834-40. DOI: 10.1210/jcem.85.5.6568.
326. Malkin CJ, Jones TH, Channer KS. Testosterone in chronic heart failure. Front Horm Res 2009;37:183-96. DOI: 10.1159/000176053.
327. Malkin CJ, Channer KS, Jones TH. Testosterone and heart failure. Curr Opin Endocrinol Diabetes Obes 2010;17(3):262-8. DOI: 10.1097/MED.0b013e328339543e.
328. Volterrani M, Rosano G, Iellamo F. Testosterone and heart failure. Endocrine 2012;42(2):272-7. DOI: 10.1007/s12020-012-9725-9.
329. Ohlsson C, Labrie F, Barrett-Connor E, et al. Low serum levels of dehydroepiandrosterone sulfate predict all-cause and cardiovascular mortality in elderly Swedish men. J Clin Endocrinol Metab 2010;95(9):4406-14. DOI: 10.1210/jc.2010-0760.
330. Enina T, Kuznetsov V, Soldatova A, et al. Association of sex hormones level and effect of cardiac resynchronisation therapy in men with congestive heart failure. Disease Markers 2018;2018.
331. D'Assante R, Piccioli L, Valente P, et al. Testosterone treatment in chronic heart failure. Review of literature and future perspectives. Monaldi Arch Chest Dis 2018;88(3):976. DOI: 10.4081/monaldi.2018.976.
332. Navarro-Penalver M, Perez-Martinez MT, Gomez-Bueno M, et al. Testosterone Replacement Therapy in Deficient Patients With Chronic Heart Failure: A Randomized Double-Blind Controlled Pilot Study. J Cardiovasc Pharmacol Ther 2018;23(6):543-550. DOI: 10.1177/1074248418784020.
333. Pedretti RFE, Fattirolli F, Griffo R, et al. Cardiac Prevention and Rehabilitation "3.0": From acute to chronic phase. Position Paper of the Italian Association for Cardiovascular

Prevention and Rehabilitation (GICR-IACPR). Monaldi Arch Chest Dis 2018;88(3):1004. DOI: 10.4081/monaldi.2018.1004.
334. Marra AM, Salzano A, Arcopinto M, Piccioli L, Raparelli V. The impact of gender in cardiovascular medicine: Lessons from the gender/sex-issue in heart failure. Monaldi Arch Chest Dis 2018;88(3):988. DOI: 10.4081/monaldi.2018.988.
335. De Fazio SR, Warner CM. Activation of T cells by cross-linking Qa-2, the ped gene product, requires Fyn. Am J Reprod Immunol 2007;58(4):315-24. DOI: 10.1111/j.1600-0897.2007.00503.x.
336. Salzano A, D'Assante R, Lander M, et al. Hormonal Replacement Therapy in Heart Failure: Focus on Growth Hormone and Testosterone. Heart Fail Clin 2019;15(3). DOI: 10.1016/j.hfc.2019.02.007.
337. Blackman MR, Sorkin JD, Munzer T, et al. Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. JAMA 2002;288(18):2282-92.
338. Sattler FR, Castaneda-Sceppa C, Binder EF, et al. Testosterone and growth hormone improve body composition and muscle performance in older men. J Clin Endocrinol Metab 2009;94(6):1991-2001. DOI: 10.1210/jc.2008-2338.
339. Schroeder ET, He J, Yarasheski KE, et al. Value of measuring muscle performance to assess changes in lean mass with testosterone and growth hormone supplementation. Eur J Appl Physiol 2012;112(3):1123-31. DOI: 10.1007/s00421-011-2077-y.
340. Bozkurt B, Colvin M, Cook J, et al. Current Diagnostic and Treatment Strategies for Specific Dilated Cardiomyopathies: A Scientific Statement From the American Heart Association. Circulation 2016;134(23):e579-e646. DOI: doi:10.1161/CIR.0000000000000455.
341. Sandek A, Bjarnason I, Volk HD, et al. Studies on bacterial endotoxin and intestinal absorption function in patients with chronic heart failure. Int J Cardiol 2012;157(1):80-5. DOI: 10.1016/j.ijcard.2010.12.016.
342. Pasini E, Aquilani R, Testa C, et al. Pathogenic Gut Flora in Patients With Chronic Heart Failure. JACC Heart Fail 2016;4(3):220-7. DOI: 10.1016/j.jchf.2015.10.009.
343. Baker JR, Chaykin S. The biosynthesis of trimethylamine-N-oxide. J Biol Chem 1962;237:1309-13.
344. Cassambai S, Salzano A, Yazaki Y, et al. Impact of acute choline loading on circulating trimethylamine N-oxide levels. Eur J Prev Cardiol 2019:2047487319831372. DOI: 10.1177/2047487319831372.
345. Tang WH, Wang Z, Shrestha K, et al. Intestinal microbiota-dependent phosphatidylcholine metabolites, diastolic dysfunction, and adverse clinical outcomes in chronic systolic heart failure. Journal of cardiac failure 2015;21(2):91-6. DOI: 10.1016/j.cardfail.2014.11.006.
346. Suzuki T, Yazaki Y, Voors AA, et al. Association with outcomes and response to treatment of trimethylamine N-oxide in heart failure (from BIOSTAT-CHF). Eur J Heart Fail 2018. DOI: 10.1002/ejhf.1338.
347. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19(5):576-85. DOI: 10.1038/nm.3145.
348. Wang Z, Roberts AB, Buffa JA, et al. Non-lethal Inhibition of Gut Microbial Trimethylamine Production for the Treatment of Atherosclerosis. Cell 2015;163(7):1585-95. DOI: 10.1016/j.cell.2015.11.055.
349. Bennett BJ, de Aguiar Vallim TQ, Wang Z, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. Cell Metab 2013;17(1):49-60. DOI: 10.1016/j.cmet.2012.12.011.

350. Mayatepek E, Flock B, Zschocke J. Benzydamine metabolism in vivo is impaired in patients with deficiency of flavin-containing monooxygenase 3. Pharmacogenetics 2004;14(11):775-7.
351. Fennema D, Phillips IR, Shephard EA. Trimethylamine and Trimethylamine N-Oxide, a Flavin-Containing Monooxygenase 3 (FMO3)-Mediated Host-Microbiome Metabolic Axis Implicated in Health and Disease. Drug Metab Dispos 2016;44(11):1839-1850. DOI: 10.1124/dmd.116.070615.
352. Ufnal M, Zadlo A, Ostaszewski R. TMAO: A small molecule of great expectations. Nutrition 2015;31(11-12):1317-23. DOI: 10.1016/j.nut.2015.05.006.
353. Christodoulou J. Trimethylaminuria: an under-recognised and socially debilitating metabolic disorder. J Paediatr Child Health 2012;48(3):E153-5. DOI: 10.1111/j.1440-1754.2010.01978.x.
354. Zou Q, Bennion BJ, Daggett V, Murphy KP. The molecular mechanism of stabilization of proteins by TMAO and its ability to counteract the effects of urea. J Am Chem Soc 2002;124(7):1192-202.
355. Welch WJ. Role of quality control pathways in human diseases involving protein misfolding. Semin Cell Dev Biol 2004;15(1):31-8. DOI: 10.1016/j.semcdb.2003.12.011.
356. Leandro P, Gomes CM. Protein misfolding in conformational disorders: rescue of folding defects and chemical chaperoning. Mini Rev Med Chem 2008;8(9):901-11.
357. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472(7341):57-63. DOI: 10.1038/nature09922.
358. Suzuki T, Heaney LM, Jones DJ, Ng LL. Trimethylamine N-oxide and Risk Stratification after Acute Myocardial Infarction. Clin Chem 2017;63(1):420-428. DOI: 10.1373/clinchem.2016.264853.
359. Sala-Vila A, Guasch-Ferre M, Hu FB, et al. Dietary alpha-Linolenic Acid, Marine omega-3 Fatty Acids, and Mortality in a Population With High Fish Consumption: Findings From the PREvencion con DIeta MEDiterranea (PREDIMED) Study. J Am Heart Assoc 2016;5(1). DOI: 10.1161/JAHA.115.002543.
360. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N Engl J Med 2002;346(15):1113-8. DOI: 10.1056/NEJMoa012918.
361. Cheung W, Keski-Rahkonen P, Assi N, et al. A metabolomic study of biomarkers of meat and fish intake. Am J Clin Nutr 2017;105(3):600-608. DOI: 10.3945/ajcn.116.146639.
362. Zhu W, Gregory JC, Org E, et al. Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk. Cell 2016;165(1):111-124. DOI: 10.1016/j.cell.2016.02.011.
363. Chen K, Zheng X, Feng M, Li D, Zhang H. Gut microbiota-dependent metabolite trimethylamine N-oxide contributes to cardiac dysfunction in western diet-induced obese mice. Frontiers in physiology 2017;8:139.
364. Boutagy NE, Neilson AP, Osterberg KL, et al. Probiotic supplementation and trimethylamine-N-oxide production following a high-fat diet. Obesity (Silver Spring) 2015;23(12):2357-63. DOI: 10.1002/oby.21212.
365. Zhang H, Meng J, Yu H. Trimethylamine N-oxide supplementation abolishes the cardioprotective effects of voluntary exercise in mice fed a western diet. Frontiers in physiology 2017;8:944.
366. Rasmussen LG, Winning H, Savorani F, et al. Assessment of the effect of high or low protein diet on the human urine metabolome as measured by NMR. Nutrients 2012;4(2):112-131.

367. Bergeron N, Williams PT, Lamendella R, et al. Diets high in resistant starch increase plasma levels of trimethylamine-N-oxide, a gut microbiome metabolite associated with CVD risk. British Journal of Nutrition 2016;116(12):2020-2029.
368. Janeiro M, Ramírez M, Milagro F, Martínez J, Solas M. Implication of Trimethylamine N-Oxide (TMAO) in Disease: Potential Biomarker or New Therapeutic Target. Nutrients 2018;10(10):1398.
369. Wang Z, Bergeron N, Levison BS, et al. Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women. European heart journal 2018.
370. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 2015;26:26191. DOI: 10.3402/mehd.v26.26191.
371. Yin J, Liao SX, He Y, et al. Dysbiosis of Gut Microbiota With Reduced Trimethylamine-N-Oxide Level in Patients With Large-Artery Atherosclerotic Stroke or Transient Ischemic Attack. J Am Heart Assoc 2015;4(11). DOI: 10.1161/JAHA.115.002699.
372. Organ CL, Otsuka H, Bhushan S, et al. Choline Diet and Its Gut Microbe-Derived Metabolite, Trimethylamine N-Oxide, Exacerbate Pressure Overload-Induced Heart Failure. Circ Heart Fail 2016;9(1):e002314. DOI: 10.1161/CIRCHEARTFAILURE.115.002314.
373. Randrianarisoa E, Lehn-Stefan A, Wang X, et al. Relationship of Serum Trimethylamine N-Oxide (TMAO) Levels with early Atherosclerosis in Humans. Sci Rep 2016;6:26745. DOI: 10.1038/srep26745.
374. Sun G, Yin Z, Liu N, et al. Gut microbial metabolite TMAO contributes to renal dysfunction in a mouse model of diet-induced obesity. Biochem Biophys Res Commun 2017;493(2):964-970. DOI: 10.1016/j.bbrc.2017.09.108.
375. Gan XT, Ettinger G, Huang CX, et al. Probiotic administration attenuates myocardial hypertrophy and heart failure after myocardial infarction in the rat. Circ Heart Fail 2014;7(3):491-9. DOI: 10.1161/CIRCHEARTFAILURE.113.000978.
376. Martin FP, Wang Y, Sprenger N, et al. Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. Mol Syst Biol 2008;4:157. DOI: 10.1038/msb4100190.
377. Savi M, Bocchi L, Bresciani L, et al. Trimethylamine-N-Oxide (TMAO)-Induced Impairment of Cardiomyocyte Function and the Protective Role of Urolithin B-Glucuronide. Molecules 2018;23(3). DOI: 10.3390/molecules23030549.
378. Borges NA, Stenvinkel P, Bergman P, et al. Effects of Probiotic Supplementation on Trimethylamine-N-Oxide Plasma Levels in Hemodialysis Patients: a Pilot Study. Probiotics Antimicrob Proteins 2018. DOI: 10.1007/s12602-018-9411-1.
379. Salzano A, Marra AM, Proietti M, Raparelli V, Heaney LM. Biomarkers in Heart Failure and Associated Diseases. Dis Markers 2019;2019:8768624. DOI: 10.1155/2019/8768624.
380. Streng KW, Nauta JF, Hillege HL, et al. Non-cardiac comorbidities in heart failure with reduced, mid-range and preserved ejection fraction. Int J Cardiol 2018;271:132-139. DOI: 10.1016/j.ijcard.2018.04.001.
381. Hayashi T, Yamashita T, Watanabe H, et al. Gut Microbiome and Plasma Microbiome-Related Metabolites in Patients With Decompensated and Compensated Heart Failure. Circ J 2018;83(1):182-192. DOI: 10.1253/circj.CJ-18-0468.
382. Schiattarella GG, Sannino A, Toscano E, et al. Gut microbe-generated metabolite trimethylamine-N-oxide as cardiovascular risk biomarker: a systematic review and dose-response meta-analysis. Eur Heart J 2017;38(39):2948-2956. DOI: 10.1093/eurheartj/ehx342.

383. Thursby E, Juge N. Introduction to the human gut microbiota. Biochemical Journal 2017;474(11):1823-1836.
384. Gill SR, Pop M, DeBoy RT, et al. Metagenomic analysis of the human distal gut microbiome. science 2006;312(5778):1355-1359.
385. Rodríguez JM, Murphy K, Stanton C, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microbial ecology in health and disease 2015;26(1):26050.
386. Salzano A, Cassambai S, Yazaki Y, et al. The Gut Axis Involvement in Heart Failure: Focus on Trimethylamine N-oxide. Heart failure clinics 2020;16(1):23-31.
387. Janeway Jr CA, Travers P, Walport M, Shlomchik MJ. The mucosal immune system. Immunobiology: The Immune System in Health and Disease. 5th edition: Garland Science; 2001.
388. Ascher S, Wilms E, Pontarollo G, et al. The gut microbiota restricts NETosis in acute mesenteric ischemia-reperfusion injury. Arteriosclerosis Thrombosis and Vascular Biology 2020.
389. Souza DG, Vieira AT, Soares AC, et al. The essential role of the intestinal microbiota in facilitating acute inflammatory responses. The Journal of Immunology 2004;173(6):4137-4146.
390. Derikx JP, Matthijsen RA, de Bruïne AP, et al. Rapid reversal of human intestinal ischemia-reperfusion induced damage by shedding of injured enterocytes and reepithelialisation. PLoS One 2008;3(10):e3428.
391. Grootjans J, Lenaerts K, Derikx JP, et al. Human intestinal ischemia-reperfusion–induced inflammation characterized: experiences from a new translational model. The American journal of pathology 2010;176(5):2283-2291.
392. Kinross J, Warren O, Basson S, et al. Intestinal ischemia/reperfusion injury: defining the role of the gut microbiome. 2009.
393. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Science translational medicine 2009;1(6):6ra14-6ra14.
394. Tang WW, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. The Journal of clinical investigation 2014;124(10):4204-4211.
395. Yang S, Li X, Yang F, et al. Gut microbiota-dependent marker TMAO in promoting cardiovascular disease: inflammation mechanism, clinical prognostic, and potential as a therapeutic target. Frontiers in Pharmacology 2019;10.
396. Suzuki T, Heaney LM, Bhandari SS, Jones DJ, Ng LL. Trimethylamine N-oxide and prognosis in acute heart failure. Heart 2016;102(11):841-848.
397. Suzuki T, Yazaki Y, Voors AA, et al. Association with outcomes and response to treatment of trimethylamine N-oxide in heart failure: results from BIOSTAT-CHF. European journal of heart failure 2019;21(7):877-886.
398. Suzuki T, Heaney LM, Jones DJ, Ng LL. Trimethylamine N-oxide and risk stratification after acute myocardial infarction. Clinical chemistry 2017;63(1):420-428.
399. Yazaki Y, Salzano A, Nelson CP, et al. Geographical location affects the levels and association of trimethylamine N-oxide with heart failure mortality in BIOSTAT-CHF: a post-hoc analysis. Eur J Heart Fail 2019;21(10):1291-1294. (In eng). DOI: 10.1002/ejhf.1550.
400. Salzano A, Israr MZ, Yazaki Y, et al. Combined use of trimethylamine N-oxide with BNP for risk stratification in heart failure with preserved ejection fraction: findings from the DIAMONDHFpEF study. Eur J Prev Cardiol 2019:2047487319870355. (In eng). DOI: 10.1177/2047487319870355.

401. Yazaki Y, Aizawa K, Israr MZ, et al. Ethnic differences in association of outcomes with trimethylamine N-oxide in acute heart failure patients. ESC Heart Failure.
402. Alm JS, Swartz J, Lilja G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. The Lancet 1999;353(9163):1485-1488.
403. Wang B, Huang Q, Zhang W, Li N, Li J. Lactobacillus plantarum prevents bacterial translocation in rats following ischemia and reperfusion injury. Digestive diseases and sciences 2011;56(11):3187.
404. Salim S, Young P, Lukowski C, et al. VSL# 3 probiotics provide protection against acute intestinal ischaemia/reperfusion injury. Beneficial microbes 2013;4(4):357-365.
405. Lam V, Su J, Koprowski S, et al. Intestinal microbiota determine severity of myocardial infarction in rats. The FASEB journal 2012;26(4):1727-1735.
406. Troseid M, Ueland T, Hov JR, et al. Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure. J Intern Med 2015;277(6):717-726. (In English). DOI: 10.1111/joim.12328.
407. Ferreira JP, Girerd N, Rossignol P, Zannad F. Geographic differences in heart failure trials. Eur J Heart Fail 2015;17(9):893-905. DOI: 10.1002/ejhf.326.
408. Voors AA, Ouwerkerk W, Zannad F, et al. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. Eur J Heart Fail 2017;19(5):627-634. DOI: 10.1002/ejhf.785.
409. Ferreira JP, Metra M, Mordi I, et al. Heart failure in the outpatient versus inpatient setting: findings from the BIOSTAT-CHF study. Eur J Heart Fail 2019;21(1):112-120. DOI: 10.1002/ejhf.1323.
410. Elmadfa I, Meyer A, Nowak V, et al. European Nutrition and Health Report 2009. Forum Nutr 2009;62:1-405. DOI: 10.1159/000242367.
411. Yazaki Y, Salzano A, Nelson CP, et al. Geographical location affects the levels and association of trimethylamine N-oxide with heart failure mortality in BIOSTAT-CHF: a post-hoc analysis. Eur J Heart Fail 2019. DOI: 10.1002/ejhf.1550.
412. Salzano A, Cassambai S, Yazaki Y, et al. The Gut Axis Involvement in Heart Failure: Focus on Trimethylamine N-oxide. Heart Fail Clin 2020;16(1):23-31. (In eng). DOI: 10.1016/j.hfc.2019.08.001.
413. Cho CE, Taesuwan S, Malysheva OV, et al. Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. Mol Nutr Food Res 2017;61(1). DOI: 10.1002/mnfr.201600324.
414. Wu WK, Chen CC, Liu PY, et al. Identification of TMAO-producer phenotype and host-diet-gut dysbiosis by carnitine challenge test in human and germ-free mice. Gut 2018. DOI: 10.1136/gutjnl-2018-317155.
415. Jaacks LM, Kapoor D, Singh K, et al. Vegetarianism and cardiometabolic disease risk factors: Differences between South Asian and US adults. Nutrition 2016;32(9):975-84. DOI: 10.1016/j.nut.2016.02.011.
416. Zaman MJ, Philipson P, Chen R, et al. South Asians and coronary disease: is there discordance between effects on incidence and prognosis? Heart 2013;99(10):729-36. DOI: 10.1136/heartjnl-2012-302925.
417. Shafi T, Powe NR, Meyer TW, et al. Trimethylamine N-Oxide and Cardiovascular Events in Hemodialysis Patients. J Am Soc Nephrol 2017;28(1):321-331. DOI: 10.1681/ASN.2016030374.

418. Wang Z, Bergeron N, Levison BS, et al. Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women. Eur Heart J 2018. DOI: 10.1093/eurheartj/ehy799.
419. Cassambai S, Salzano A, Yazaki Y, et al. Impact of acute choline loading on circulating trimethylamine N-oxide (TMAO) levels. Eur J Prev Cardiol 2019.
420. Yazaki Y, Salzano A, Nelson PC, et al. Geographical location affects the levels and association of trimethylamine N-oxide with heart failure mortality: a post-hoc analysis of BIOSTAT-CHF Eur J Heart Fail 2019.
421. Kanagala P, Cheng ASH, Singh A, et al. Relationship Between Focal and Diffuse Fibrosis Assessed by CMR and Clinical Outcomes in Heart Failure With Preserved Ejection Fraction. JACC Cardiovascular imaging 2019. DOI: 10.1016/j.jcmg.2018.11.031.
422. Heaney LM, Jones DJ, Mbasu RJ, Ng LL, Suzuki T. High mass accuracy assay for trimethylamine N-oxide using stable-isotope dilution with liquid chromatography coupled to orthogonal acceleration time of flight mass spectrometry with multiple reaction monitoring. Analytical and bioanalytical chemistry 2016;408(3):797-804. DOI: 10.1007/s00216-015-9164-6.
423. O'Neal WT, Sandesara PB, Samman-Tahhan A, Kelli HM, Hammadah M, Soliman EZ. Heart rate and the risk of adverse outcomes in patients with heart failure with preserved ejection fraction. European journal of preventive cardiology 2017;24(11):1212-1219. DOI: 10.1177/2047487317708676.
424. Sato T, Yoshihisa A, Kanno Y, et al. Cardiopulmonary exercise testing as prognostic indicators: Comparisons among heart failure patients with reduced, mid-range and preserved ejection fraction. European journal of preventive cardiology 2017;24(18):1979-1987. DOI: 10.1177/2047487317739079.
425. Husain H, Velculescu VE. Cancer DNA in the Circulation: The Liquid Biopsy. JAMA 2017;318(13):1272-1274. DOI: 10.1001/jama.2017.12131.
426. Page K, Shaw JA, Guttery DS. The liquid biopsy: towards standardisation in preparation for prime time. Lancet Oncol 2019;20(6):758-760. DOI: 10.1016/S1470-2045(19)30310-9.
427. Shaw JA, Brown J, Coombes RC, et al. Circulating tumor cells and plasma DNA analysis in patients with indeterminate early or metastatic breast cancer. Biomarkers in medicine 2011;5(1):87-91.
428. Arroyo AB, de Los Reyes-Garcia AM, Rivera-Caravaca JM, et al. MiR-146a Regulates Neutrophil Extracellular Trap Formation That Predicts Adverse Cardiovascular Events in Patients With Atrial Fibrillation. Arterioscler Thromb Vasc Biol 2018;38(4):892-902. DOI: 10.1161/ATVBAHA.117.310597.
429. Sanchis J, Garcia-Blas S, Ortega-Paz L, et al. Cell-free DNA and Microvascular Damage in ST-segment Elevation Myocardial Infarction Treated With Primary Percutaneous Coronary Intervention. Rev Esp Cardiol (Engl Ed) 2019;72(4):317-323. DOI: 10.1016/j.rec.2018.03.005.
430. Xie J, Yang J, Hu P. Correlations of Circulating Cell-Free DNA With Clinical Manifestations in Acute Myocardial Infarction. Am J Med Sci 2018;356(2):121-129. DOI: 10.1016/j.amjms.2018.04.007.
431. Jylhava J, Lehtimaki T, Jula A, et al. Circulating cell-free DNA is associated with cardiometabolic risk factors: the Health 2000 Survey. Atherosclerosis 2014;233(1):268-71. DOI: 10.1016/j.atherosclerosis.2013.12.022.
432. De Vlaminck I, Valantine HA, Snyder TM, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci Transl Med 2014;6(241):241ra77. DOI: 10.1126/scitranslmed.3007803.

433. Macher HC, Garcia-Fernandez N, Adsuar-Gomez A, et al. Donor-specific circulating cell free DNA as a noninvasive biomarker of graft injury in heart transplantation. Clin Chim Acta 2019;495:590-597. DOI: 10.1016/j.cca.2019.06.004.
434. Khush KK, Patel J, Pinney S, et al. Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. Am J Transplant 2019. DOI: 10.1111/ajt.15339.
435. Cui M, Fan M, Jing R, et al. Cell-free circulating DNA: a new biomarker for the acute coronary syndrome. Cardiology 2013;124(2):76-84.
436. Chang CP-Y, Chia R-H, Wu T-L, Tsao K-C, Sun C-F, Wu JT. Elevated cell-free serum DNA detected in patients with myocardial infarction. Clinica chimica acta 2003;327(1-2):95-101.
437. Tian Y, Charles EJ, Yan Z, et al. The myocardial infarct-exacerbating effect of cell-free DNA is mediated by the high-mobility group box 1-receptor for advanced glycation end products-Toll-like receptor 9 pathway. J Thorac Cardiovasc Surg 2019;157(6):2256-2269 e3. DOI: 10.1016/j.jtcvs.2018.09.043.
438. Bryk AH, Prior SM, Plens K, et al. Predictors of neutrophil extracellular traps markers in type 2 diabetes mellitus: associations with a prothrombotic state and hypofibrinolysis. Cardiovasc Diabetol 2019;18(1):49. DOI: 10.1186/s12933-019-0850-0.
439. Mueller C, McDonald K, de Boer RA, et al. Heart Failure Association of the European Society of Cardiology practical guidance on the use of natriuretic peptide concentrations. Eur J Heart Fail 2019;21(6):715-731. (In eng). DOI: 10.1002/ejhf.1494.
440. Januzzi JL, Troughton R. Are serial BNP measurements useful in heart failure management? Serial natriuretic peptide measurements are useful in heart failure management. Circulation 2013;127(4):500-7; discussion 508. (In eng). DOI: 10.1161/CIRCULATIONAHA.112.120485.
441. Latini R, Masson S, Wong M, et al. Incremental prognostic value of changes in B-type natriuretic peptide in heart failure. The American journal of medicine 2006;119(1):70. e23-70. e30.
442. Desai AS. Are serial BNP measurements useful in heart failure management? Serial natriuretic peptide measurements are not useful in heart failure management: the art of medicine remains long. Circulation 2013;127(4):509-16; discussion 516. (In eng). DOI: 10.1161/CIRCULATIONAHA.112.120493.
443. Suzuki T, Israr MZ, Heaney LM, Takaoka M, Squire IB, Ng LL. Prognostic Role of Molecular Forms of B-Type Natriuretic Peptide in Acute Heart Failure. Clin Chem 2017;63(4):880-886. (In eng). DOI: 10.1373/clinchem.2016.265140.
444. Felker GM, Anstrom KJ, Adams KF, et al. Effect of Natriuretic Peptide-Guided Therapy on Hospitalization or Cardiovascular Mortality in High-Risk Patients With Heart Failure and Reduced Ejection Fraction: A Randomized Clinical Trial. JAMA 2017;318(8):713-720. (In eng). DOI: 10.1001/jama.2017.10565.
445. Januzzi JL, Ahmad T, Mulder H, et al. Natriuretic Peptide Response and Outcomes in Chronic Heart Failure With Reduced Ejection Fraction. J Am Coll Cardiol 2019;74(9):1205-1217. (In eng). DOI: 10.1016/j.jacc.2019.06.055.
446. Kubánek M, Goode KM, Lánská V, Clark AL, Cleland JG. The prognostic value of repeated measurement of N-terminal pro-B-type natriuretic peptide in patients with chronic heart failure due to left ventricular systolic dysfunction. Eur J Heart Fail 2009;11(4):367-77. (In eng). DOI: 10.1093/eurjhf/hfp003.
447. Zile MR, Claggett BL, Prescott MF, et al. Prognostic Implications of Changes in N-Terminal Pro-B-Type Natriuretic Peptide in Patients With Heart Failure. J Am Coll Cardiol 2016;68(22):2425-2436. (In eng). DOI: 10.1016/j.jacc.2016.09.931.

448. Piepoli MF, Abreu A, Albus C, et al. Update on cardiovascular prevention in clinical practice: A position paper of the European Association of Preventive Cardiology of the European Society of Cardiology. Eur J Prev Cardiol 2020;27(2):181-205. (In eng). DOI: 10.1177/2047487319893035.
449. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol 2016;13(6):368-78. (In eng). DOI: 10.1038/nrcardio.2016.25.
450. Bowman L, Baras A, Bombien R, et al. Understanding the use of observational and randomized data in cardiovascular medicine. Eur Heart J 2020 (In eng). DOI: 10.1093/eurheartj/ehaa020.
451. Frieden TR. Evidence for Health Decision Making - Beyond Randomized, Controlled Trials. N Engl J Med 2017;377(5):465-475. (In eng). DOI: 10.1056/NEJMra1614394.
452. Salzano A, Israr MZ, Garcia DF, et al. Circulating cell-free DNA levels are associated with adverse outcomes in heart failure: testing liquid biopsy in heart failure. Eur J Prev Cardiol 2020:2047487320912375. (In eng). DOI: 10.1177/2047487320912375.
453. Crespo-Leiro MG, Anker SD, Maggioni AP, et al. European Society of Cardiology Heart Failure Long-Term Registry (ESC-HF-LT): 1-year follow-up outcomes and differences across regions. Eur J Heart Fail 2016;18(6):613-25. (In eng). DOI: 10.1002/ejhf.566.
454. Lund LH, Carrero JJ, Farahmand B, et al. Association between enrolment in a heart failure quality registry and subsequent mortality-a nationwide cohort study. Eur J Heart Fail 2017;19(9):1107-1116. (In eng). DOI: 10.1002/ejhf.762.
455. Littlejohns TJ, Sudlow C, Allen NE, Collins R. UK Biobank: opportunities for cardiovascular research. Eur Heart J 2019;40(14):1158-1166. (In eng). DOI: 10.1093/eurheartj/ehx254.
456. Santema BT, Ouwerkerk W, Tromp J, et al. Identifying optimal doses of heart failure medications in men compared with women: a prospective, observational, cohort study. Lancet 2019;394(10205):1254-1263. (In eng). DOI: 10.1016/S0140-6736(19)31792-1.
457. Göbel S, Prochaska JrHT, Sven-OliverPaniva-Noeva, MarinaEspinola-Klein, CrhistineMichal, MatthiasLackner, Karl J.Gori, TommasoMünzel, ThomasWild, Philipp S. Rationale, design and baseline characteristics 1 of the MyoVasc Study -A prospective cohort study investigating development and progression of heart failure Eur J Prev Cardiol 2020.
458. Morbach C, Gelbrich G, Tiffe T, et al. Prevalence and determinants of the precursor stages of heart failure: results from the population-based STAAB cohort study. Eur J Prev Cardiol 2020:2047487320922636. (In eng). DOI: 10.1177/2047487320922636.
459. Spina S, Marrazzo F, Migliari M, Stucchi R, Sforza A, Fumagalli R. The response of Milan's Emergency Medical System to the COVID-19 outbreak in Italy. Lancet 2020;395(10227):e49-e50. (In eng). DOI: 10.1016/S0140-6736(20)30493-1.
460. Seferovic PM, Ponikowski P, Anker SD, et al. Clinical practice update on heart failure 2019: pharmacotherapy, procedures, devices and patient management. An expert consensus meeting report of the Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail 2019;21(10):1169-1186. (In eng). DOI: 10.1002/ejhf.1531.
461. European Society of Cardiology (ESC) Guidance for the Diagnosis and Management of CV Disease during the COVID-19 Pandemic. 2020.
462. Tomasoni D, Italia L, Adamo M, et al. COVID 19 and heart failure: from infection to inflammation and angiotensin II stimulation. Searching for evidence from a new disease. Eur J Heart Fail 2020 (In eng). DOI: 10.1002/ejhf.1871.
463. De Filippo O, D'Ascenzo F, Angelini F, et al. Reduced Rate of Hospital Admissions for ACS during Covid-19 Outbreak in Northern Italy. N Engl J Med 2020 (In eng). DOI: 10.1056/NEJMc2009166.

464. Piccolo R, Bruzzese D, Mauro C, et al. Population Trends in Rates of Percutaneous Coronary Revascularization for Acute Coronary Syndromes Associated with the COVID-19 Outbreak. Circulation 2020 (In eng). DOI: 10.1161/CIRCULATIONAHA.120.047457.
465. Fraiche AM, Eapen ZJ, McClellan MB. Moving Beyond the Walls of the Clinic: Opportunities and Challenges to the Future of Telehealth in Heart Failure. JACC Heart Fail 2017;5(4):297-304. (In eng). DOI: 10.1016/j.jchf.2016.11.013.
466. Gorodeski EZ, Goyal P, Cox ZL, et al. Virtual Visits for Care of Patients with Heart Failure in the Era of COVID-19: A Statement from the Heart Failure Society of America. J Card Fail 2020 (In eng). DOI: 10.1016/j.cardfail.2020.04.008.
467. Cleland JG, Clark RA, Pellicori P, Inglis SC. Caring for people with heart failure and many other medical problems through and beyond the COVID-19 pandemic; the advantages of universal-access to home telemonitoring. Eur J Heart Fail 2020 (In eng). DOI: 10.1002/ejhf.1864.
468. Salzano A, D'Assante R, Israr MZ et al. Biomarkers in Heart Failure: clinical insights. Heart Fail Clin 2021 (in press).

9. Brief Curriculum Vitae

ANDREA SALZANO
MD, MRCP (London)

Curriculum Vitae

Contact details

A: Cardiovascular Research Centre, John and Lucille van Geest Biomarker Facility
Department of Cardiovascular Sciences, University of Leicester,
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A: Heart Failure Clinical Research Unit
Internal Medicine and Cardiometabolic Rehabilitation Unit
Department of Translational Medical Sciences, Federico II University,
Via S Pansini 5, 80131 Napoli, Italy
A: IRCCS SDN, Diagnostic and Nuclear Research Institute
Vie E Gianturco 113, 80143 Naples, Italy

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E (university): andrea.salzano@leicester.ac.uk; andrea.salzano@unina.it
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Specialist qualifications

British General Medical Council Specialist Register: General (Internal) Medicine

British General Medical Council: registration number 7635538

Italian order of Physician: registration number NA 33955

Current Appointment

- Consultant Physician, Covid-19 Unit, General Internal Medicine, S Maria di Loreto Nuovo Hospital, Napoli, Italy (August 2020 to present).
- Honorary Consultant, Department of Cardiology, Glenfield Hospital, University Hospitals of Leicester. Groby Rd, Leicester LE3 9QP, Leicester, UK (March 2019 to present)
- Honorary Research Fellow in Cardiovascular Sciences (March 2019 to present)
  Department of Cardiovascular Sciences, University of Leicester, Leicester, UK
  University Hospitals of Leicester, Glenfield Hospital Groby Road Leicester LE3 9QP U.K

- Senior Clinical Research Fellow, IRCCS SDN, Diagnostic and Nuclear Research Institute, Via E Gianturco, 80143, Naples, Italy (September 2019 to the present)
- International PhD student (November 2017 to the present)
  International PhD Programme in Cardiovascular Pathophysiology and Therapeutics (Federico II University Naples, Italy - University of Bern, Switzerland - Cardiovascular Research Centre Aalst, Belgium)

Skills and competencies

- Expertise in clinical cardiology in chronic setting (e.g. Heart Failure, Atrial Fibrillation, Arterial Hypertension), cardiac rehabilitation, and general internal medicine
- Expertise in non-invasive cardiovascular diagnostic tests (e.g. echocardiography, exercise stress test, cardiopulmonary exercise test, 24-hours EKG)
- Expertise in the management of Direct Oral Anticoagulant therapy
- Expertise in cardiovascular evaluation of systemic disease (e.g. Klinefelter Syndrome, Wilson Disease, Friedriech's Ataxia)
- Expertise in Cardio-Endocrinology, in particular in Hormonal treatment in cardiovascular disease and effect of endocrine disease on cardiovascular system
- I have been involved in the opening of Heart Failure Unit (Federico II University, Napoli, Italy,)
- Highly effective in working in multidisciplinary teams with individuals from different educational and cultural backgrounds and nationalities
- Very capable in managing multiple tasks, including experimental and clinical research, clinical activities, academic activity, scientific activity, mentoring, teaching, training, and administrative tasks
- Take extreme care in the details and accuracy required for preparation of clinical and research protocols
- Understand the importance of identifying problems and finding the most appropriate solutions
- Extensive scientific background in both basic experimental and clinical research
- Member of the Editorial Board and Reviewer of international medical journals
- Fluent in English (written and spoken), with Italian as mother language; basic German
- Advanced written and oral communication skills (see list of articles and lectures)

Academic training and appointments

September 2019- to present: Senior Clinical Research Fellow, IRCCS SDN, Diagnostic and Nuclear Research Institute, Naples, Italy

March 2019-to present: Honorary Research Fellow, Department of Cardiovascular Sciences, University of Leicester, Leicester, UK

September 2018- March2019: Research Fellow, Department of Cardiovascular Sciences,

University of Leicester, Leicester, UK

September 2017- August 2018: Honorary Research Fellow, Department of Cardiovascular Sciences, University of Leicester, Leicester, UK

July 31, 2018: Degree of Specialist in General Internal Medicine *cum laude* from the Federico II University, Napoli, Italy

July 26, 2012: Master's degree in medicine and Surgery *cum laude* from the Medical School of the Federico II University, Napoli, Italy

Clinical professional appointments

August 2020- to present: Consultant Physician, Covid-19 Unit, General Internal Medicine, S Maria di Loreto Nuovo Hospital, Napoli, Italy (August 2020 to present)

March 2019-to present: Honorary Consultant, Department of Cardiology, Glenfield Hospital, Groby Rd, Leicester LE3 9QP, Leicester, UK

August 2013-July 2018: Specialty training in General Internal Medicine, Federico II University, Napoli, Italy

July 2015-July 2017
and August 2019 to December 2019: Consultant in General Internal Medicine and Cardiology at S.DO.P. sud, Corso Secondigliano, 236, 80144 Napoli, Italy

Additional Professional qualifications

June 2015: Master Class in Cardiac Rehabilitation – Federico II University, Napoli, Italy

November 2013: Certificate of Prehospital Trauma Care (PTC) - International Resuscitation Council (IRC)/European Resuscitation Council (ERC), Napoli, Italy

October 2012: Certificate of Basic Life Support- Defibrillation (BLS-D) – International Resuscitation Council (IRC)/European Resuscitation Council (ERC), Napoli, Italy

Journal Board Memberships

- ESC Heart Failure (IF. 3.94) – Associate Editor
- Open Medicine (IF 1.204) – Section Editor Cardiology
- Open Medicine (IF 1.204) – Section Editor General Internal Medicine
- Lead Guest Editor special issue Disease and Markers (IF 2.949)
- Lead Guest Editor special issue Cardiology Research and Practice (IF 2.140)
- Journal of Cardiovascular Medicine and Cardiology
- Edorium Journals of Disability and Rehabilitation
- Annals of Anaesthesia and Pain Medicine
- International Journal of Cardiology and Cardiovascular Medicine
- Journal of Cardiovascular Diseases and Cardiac Surgery

Peer-reviewer for the following journals:

- European Journal of Heart Failure (IF 11.6)
- European Journal of Preventive Cardiology (5.8)
- ESC Heart Failure (3.9)
- JACC Basic to Translational Science
- Journal of Clinical Medicine (5.688)
- Plos One (2.343)
- Disease Markers (2.949)
- Medicine (1.870)
- Cancers (6.162)
- Pharmacological Research (5.574)
- Archives of Medical Sciences (2.380)
- Cardiology Research and Practice (2.140)
- Endocrine, Metabolic & Immune Disorders – Drug Targets (2.013)
- Journal of Diabetes Research (2.885)
- Biomolecules (4.964)
- International Journal of Molecular Sciences (4.183)
- Clinical Interventions in Aging (3.023)

Membership of Medical Societies

- Royal College of Physician – RCP – Collegiate member
- European Society of Cardiology - ESC
- Heart Failure Association of the ESC- HFA-ESC – Silver member
- European Association of Preventive Cardiology – EAPC – Silver member
- European Federation of Internal Medicine – EFIM
- Endocrine Society (ES)
- Italian Society of Internal Medicine (SIMI) – Board panel of young Internists
- Italian Society of Diabetology (SID)
- Italian Society of Geriatric Medicine and Gerontology (SIGG)
- National Association of out-of-hospital Cardiology (ANCE)

Invited Lectures

1. Echocardiography and thoracic ultrasound in emergency medicine – Department of Advanced Biomedical Sciences – Federico II University, 11 December 2019 Aula Didattica Biotecgnologie, Via Pansini 5, Napoli.
   Title: Echocardiographic evaluation of the right heart.
2. National conference of Italian Society of Internal Medicine, Young Physician group- "Giornate Itineranti del gruppo Giovani Internisti", Italian Society of Internal Medicine, 30 November 2019, Sala Falk, Fondazione Culturale Ambrosianeum, Milan, Italy
   Title: 'Diabetes and heart: a dangerous liason'
3. Joint Meeting Association of Medical Endocrinologists (AME) and American Association of Clinical Endocrinologists, 9 November 2019 Ergife Conference Palace, Via Aurelia, 619 - 00165 Roma
   Title: "Growth hormone replacement treatment in chronic heart failure"
4. 18th National conference of Association of Medical Endocrinologists (AME) –Update in Clinical endocrinology – 8 November 2019 Ergife Conference Palace, Via Aurelia, 619 - 00165 Roma
   Title: "Growth hormone replacement treatment in chronic heart failure"
5. Regional Conference of Italian Society of Study on Atherosclerosis (SISA)- 24 October 2019, Aula Magna Ceinge, Via G Salvatore, 80131, Napoli (IT).

Title: ‘ST2 and D-dimer: rule out biomarkers’

6. Applied Cardiology, 4 October 2019, Sala convegni Hotel dei Congressi, Viale Puglia 45, 80053, Castellamare di Stabia (NA).
   Title: “Biomarkers in Heart Failure”
7. Mediterranean School of Cardiovascular Sciences: II focus on Heart Failure and Comorbidity, 27 September 2019, Aula Magna, Faculty of Biotechnological Sciences, Federico II University of Naples.
   Title: “Combined effects of growth hormone and testosterone replacement treatment in heart failure”
8. Cardiovascular research day, 22 May 2019, Aula Magna, Faculty of Biotechnological Sciences, Federico II University of Naples.
   Title: “The T.O.S.CA. project”
9. Echocardiography Course in General Internal Medicine, 30 March 2019, Centro Studi GE-Medisol, Via Ponza 7, Complesso Delta 2, Casoria, Napoli
   Title : “Geometria e funzione del Ventricolo destro”.
10. Congresso Regionale Associazione Italiana di Cardiologia Clinica, Preventiva e Riabilitativa, 29 March 2019, Centro Congressi Palazzo Caracciolo, Via Carbonara 112, Napoli.
    Title: “Cardiologia riabilitativa e nuovi biomarkers”
11. Congresso Nazionale “Giornate Itineranti del gruppo Giovani Internisti”, Societa’ Italiana di Medicina Interna Aula Magna Universita’ Vanvitelli, 28 September 2018
    Title: “Monoterapia vs Terapia combinata nell’ipertensione arteriosa”
12. Mediterranean school of cardiovascular sciences. III international meeting. 13 April 2018. Hotel Royal Continental Via Partenope 38/44. 80121 Napoli (NA), Italy.
    Title: "Multiple hormonal and metabolic deficiency syndrome in chronic heart failure: Rationale, design, and preliminary data of the T.O.S.CA. Registry"
13. Congresso Il progetto T.O.S.CA. come modello di Ricerca Traslazionale. 11 April 2018. Hotel Royal ContinentalVia Partenope 38/44. 80121 Napoli (NA), Italy.
    Title: “T.O.S.CA. Registry. Stato dell'arte"
14. Congresso Associazione Nazionale Cardiologi Extraospedalieri. Multimorbidita e complessita terepeutica: nuove sfide nel paziente cardiopatico. 3 Febbraio 2017. Sala Convegni Novotel, Via S. Allende. Salerno (Sa)
    Title: " Vitamina D e cuore: dalla genetica alla fisiopatologia"
15. V Congresso Nazionale della Societa di Scienze Mediche (SISMED). 11 Dicembre 2016. Sala Convegni Grand Hotel Salerno, Lungomare Tafuri, Salerno (SA).
    Title: "Sarcopenia: dalla diagnosi alla definizione di nuove strategie terapeutiche".
16. Le giornate Modenesi del GIS. Up to Date in tema di Scompenso Cardiaco. 2 Dicembre 2016. Centro Servizi Didattici – AOU di Modena Policlinico, Via del Pozzo 71, Modena (MO). Responsabile: Prof. A. Pietrangelo, Prof. F. Perticone.
    Title: " Scompenso Cardiaco e balance ormono-metabolico".
17. Aggiornamento in tema di Scompenso Cardiaco. 5 Novembre 2016. Fondazione Ebris, Via Salvatore De Renzi 3, Salerno. Responsabile: Prof. Carlo Vigorito
    Title: "Deficit ormonali multipli: registro T.O.S.CA."
18. Aggiornamento in tema di Scompenso Cardiaco. 4 Novembre 2016. Fondazione Ebris, Via Salvatore De Renzi 3, Salerno. Responsabile: Prof. Carlo Vigorito
    Title: "Vitamina D e patologie cardiovascolari"
19. Mediterranean school of cardiovascular sciences. 20 Ottobre 2016. Lloyd's Baia Hotel, Vietri sul Mare (Sa). Responsabile: Prof. Eduardo Bossone, Prof. Antonio Cittadini.
    Title: "T.O.S.CA. Network – Growth Hormone and Heart Failure: Ready for Prime Time?"
20. Corso di Formazione ANCE Campania. Gestione integrata del paziente affetto da cardiopatia ischemica post-acuta. 11 Giugno 2016. Responsabile: Dott. Filomeno Covelluzzi, Dott. Vincenzo Aulitto. Hotel De La Ville, Avellino
    Title: "Beta-bloccanti, ivabradina ed attivita fisica: antagonisti o complici nel controllo della frequenza cardiaca?"
21. III Corso di Formazione ANCE Campania. Gestione integrata del paziente affetto da cardiopatia ischemica post-acuta. 12 Marzo 2016. Responsabile: Dott. Annibale Parente, Dott. Vincenzo Aulitto. Hotel Plaza, Viale Lamberti, Caserta
    Title: "Effetti di un nuovo farmaco bradicardizzante: il training fisico."
22. Simposio "Registro TOSCA: dati preliminari". 20 Febbraio 2015, Sala Convegni Hotel Holiday Inn – Centro Direzionale Isola e/6, Via Domenico Ausilio, Napoli.

Title: "Alterazione dell'asse gonadotropo nell'Insufficienza Cardiaca Cronica: dalla fisiopatologia alle implicazioni cliniche"

23. Progetto di Formazione dalle Linee Guida E.S.C. alla Pratica Clinica 2014 Diagnosi e Cura delle Malattie CardiovascolariMeta - Modello di Continuita Assistenziale Ospedale – Territorio-SCOMPENSO CARDIACO. Divisione di Cardiologia, Ospedale "Santa Maria Immacolata dell'Olmo" Cava de' Tirreni, Via Enrico De Marinis 8- 29 Novembre 2014
    Title: "Oltre le linee Guida: Sindrome da deficit ormonali multipli. Registro TOSCA"
24. Congresso "Cardiologia Aperta", sponsorizzato Associazione Nazionale Medici Cardiologi Ospedalieri (ANMCO) e ASL Napoli 3 sud - 12 Ottobre 2013, Sala convegni Hotel i Gigli di Nola-Nola (Na)
    Title: " Il progetto TOSCA"
25. Congresso "L'asse GH/IGF-1 nel paziente adulto affetto da Insufficienza Cardiaca Cronica", 23 Maggio 2013, Hotel Holiday Inn – Centro Direzionale Isola E/6 Via Domenico Aulisio, Napoli.
    Title: " Terapia del deficit di GH nell'adulto affetto da Insufficienza Cardiaca Cronica: from bench to bedside"

Oral communications

1. 120° Congresso Nazionale della Società Italiana di Medicina Interna (SIMI), 20 Ottobre 2019 sala Botticelli-Hotel Cavalieri Waldorf Astoria-Via Alberto Cadlolo 101 Roma
   Title: 'Evaluation of the mRNA expression of myocardial genes involved in anabolic/catabolic axis and adrenergic system in endomyocardial biopsies of patients affected with chronic heart failure
2. 117° Congresso Nazionale della Società Italiana di Medicina Interna (SIMI), 16 Ottobre 2016 sala Botticelli-Hotel Cavalieri Waldorf Astoria-Via Alberto Cadlolo 101 Roma
   Title: "Multiple hormone deficiency syndrome in heart failure with preserved ejection fraction"
3. Congresso Regionale della Società Italiana di Medicina Interna (SIMI), Sezione Campania, 13 Giugno 2014, aula grande edificio 5 dell'AOU Federico II di Napoli, Via Pansini n 5
   Title: "Full Metal Patient"
4. Congresso Regionale della Società Italiana di Medicina Interna (SIMI), Sezione Campania, 13 Giugno 2013, aula grande edificio 2 dell'AOU Federico II di Napoli, Via Pansini n 5.
   Title: " Why is the heart suffering?"
5. 73° Congresso Nazionale Società Italiana di Cardiologia (SIC), 15 Dicembre 2012, sala Rodi- Hotel Cavalieri Waldorf Astoria- Via Alberto Cadlolo 101 Roma
   Title: "New evidence in CHF: disruption of the physiological age-related decline of serum"
6. 73° Congresso Nazionale Società Italiana di Cardiologia (SIC), 15 Dicembre 2012, sala Rodi- Hotel Cavalieri Waldorf Astoria- Via Alberto Cadlolo 101.
   Title: " Cardiovascular abnormalities in Klinefelter Syndrome"
7. 113° Congresso Nazionale della Società Italiana di Medicina Interna (SIMI), 21 Ottobre 2012 sala Botticelli-Hotel Cavalieri Waldorf Astoria-Via Alberto Cadlolo 101 Roma
   Title: "Chronic Heart Failure and Testosterone: new evidence about disruption of the physiological age-related decline of serum testosterone"
8. Congresso Regionale della Società Italiana di Medicina Interna (SIMI), Sezione Campania, 5 Luglio 2012, Aula Magna dell'Università di Napoli Federico II- Via Partenope 36, Napoli
   Title: "Long term growth hormone replacement therapy in chronic heart failure"

Peer-Reviewed Articles as main author (Indexed for Medline)

1. D'Assante R, Arcopinto M, Rengo G, **Salzano A**, Walser M, Gambino G, Monti MG, Bencivenga L, Marra AM, Aberg DN, De Vincentis C, Ballotta A, Bossone E, Isgaard J, Cittadini A. Myocardial expression of somatotropic axis, adrenergic, and calcium handlings genes in HFpEF and HFrEF. **ESC Heart Failure**. 2020 (in press)
2. Israr MZ, **Salzano A**, Suzuki T. Gut Feeling: the role of gut microbiota in immunomodulation of ischemia-reperfusion injury. **Arterioscler Thromb Vasc Biol** 2020 Sep;40(9):1967-1969. doi: 10.1161/ATVBAHA.120.314941 PMID: 32845773
3. Israr MZ*, **Salzano A***, Yazaki Y, Voors AA, Ouwerkerk W, Anker SD, Cleland JG, Dickstein K, Metra M, Samani NJ, Ng LL, Suzuki T; on behalf of BIOSTAT-CHF consortium. Implications of serial measurements of natriuretic

peptides in heart failure: insights from BIOSTAT-CHF **Eur J Heart Fail**. 2020 Jul 14. doi: 10.1002/ejhf.1951. PMID: 32666670

4. **Salzano A**, D'Assante R, Stagnaro FM, Valente V, Crisci G, Giardino F, Arcopinto M, Bossone E, Marra AM, Cittadini A. Heart failure management during COVID-19 outbreak in Italy. Telemedicine experience from a heart failure university tertiary referral centre. **Eur J Heart Fail.** 2020 May 28:10. doi: 10.1002/ejhf.1911. PMID: 32463534
5. **Salzano A**[§,] Suzuki T, Squire IB, Cittadini A. Are heart failure observational studies still useful? 'No need to argue'. **Eur J Prev Cardiol.** 2020 Jun 24:2047487320932258. doi: 10.1177/2047487320932258. PMID: 32580568
6. Yazaki Y*, Aizawa K*, Israr MZ*, Negishi K, **Salzano A**, Saitoh Y, Kimura N, Kono K, Heaney LM, Cassambai S, Bernieh D, Lai F, Imai Y, Kario K, Nagai R, Ng LL, Suzuki T. Ethnic differences in association of outcomes with trimethylamine N-oxide in acute heart failure patients. **ESC Heart Fail.** 2020 Jun 29. doi: 10.1002/ehf2.12777 PMID: 32598563
7. Demelo-Rodriguez P, Galeano-Valle F, **Salzano A**, Biskup E, Vriz O, Cittadini A, Falsetti L, Ranieri B, Russo V, Stanziola AA, Bossone E, Marra AM. Pulmonary embolism: A practical guide for the busy clinician. **Heart Fail Clin** 2020, Jul;16(3):317-330. Doi: 10.1016/j.hfc.2020.03.004
8. **Salzano A**, Israr MZ, Fernandez Garcia D, Middleton L, D'Assante R, Marra AM, Arcopinto M, Yazaki Y, Bernieh D, Cassambai S, Page K, Rengo G, Bossone E, Cittadini A, Shaw JA, Suzuki T. Circulating cell-free DNA levels are associated with adverse outcomes in heart failure: testing liquid biopsy in heart failure. **Eur J Prev Cardiol**. 2020 Mar 25:2047487320912375. doi: 10.1177/2047487320912375. PMID: 32212838
9. Israr MZ, Bernieh D, **Salzano A**, Cassambai S, Yazaki Y, Suzuki T. Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry (MS): basics and clinical application. **Clin Chem Lab Med.** 2020 Mar 25:/j/cclm.ahead-of-print/cclm-2019-0868/cclm-2019-0868.xml. doi: 10.1515/cclm-2019-0868. PMID: 32229653
10. **Salzano A**, Cassambai S, Yazaky Y, Israr ZI, Wong M, Suzuki T. The gut axis involvment in heart failure. Focus on Trimethylamine N-oxide **Heart Fail Clin** 2020 Jan;16(1):23-31. doi: 10.1016/j.hfc.2019.08.001.PMID: 31735312
11. Napoli R, D'Assante R, Miniero M, **Salzano A**, Cittadini A. Anabolic deficiencies in Heart Failure: ready for prime time? **Heart Fail Clin** Heart Fail Clin. 2020 Jan;16(1):11-21. doi: 10.1016/j.hfc.2019.08.012. PMID: 31735309
12. **Salzano A**, Marra AM, Arcopinto M, D'Assante R, Triggiani V, Coscioni E, Pasquali D, Rengo G, Suzuki T, Bossone E, Cittadini A. Combined effect of growth hormone and testosterone replacement treatment in heart failure. A hypothesis generating pilot study **ESC Heart Fail**. 2019 Nov 7. doi: 10.1002/ehf2.12520. PMID: 31696666
13. Suzuki T, Israr ZM, **Salzano A**. Biomarkers in cardiovascular disease: the dilemma of racial differences. **J Am Heart Assoc** 2019;8:e014295 doi: 10.1161/JAHA.119.014295
14. **Salzano A**, Israr ZM, Yazaki Y, Heaney LM, Kanagala P, Singh A, Arnold JR, Gulsin G, Squire IB, McCann G, Leong LN, Suzuki T. Combined use of trimethylamine N-oxide with BNP for risk stratification in heart failure with preserved ejection fraction: findings from the DIAMONDHFpEF study. **Eur J Prev Cardiol**. 2019 Aug 14:2047487319870355. doi: 10.1177/2047487319870355. PMID: 31412713
15. Yazaki Y*, **Salzano A***, Nelson CP, Voors A, Anker S, Cleland JG, Lang C, Metra M, Samani N, Leong LN, Suzuki T. Geographical location affects the levels and association of trimethylamine N-oxide with heart failure mortality: a post-hoc analysis of BIOSTAT-CHF. **Eur J Heart Failure** 2019 Jul 28. doi: 10.1002/ejhf.1550. PMID: 31353762
16. D'Assante R*, Napoli R*, **Salzano A**, Pozza C, Marra AM, Arcopinto M, Perruolo G, Milano S, Formisano P, Saldamarco L, Cirillo P, Cittadini A. Human heart shifts from IGF-1 production to utilisation with chronic heart failure. **Endocrine**. Endocrine. 2019 Sep;65(3):714-716. doi: 10.1007/s12020-019-01993-y. Epub 2019 Jul 2 PMID: 31267324
17. Marra AM, Proietti M, Bossone E, **Salzano A**, Cittadini A. Risk stratification in hospitalized heart failure patients: do the RIGHT thing! **Intern Emerg Med**. 2019 Oct;14(7):1021-1023. doi: 10.1007/s11739-019-02121-4. Epub 2019 PMID: 31214885
18. **Salzano A**, D'Assante R, Lander M, Arcopinto M, Bossone E, Suzuki T, Cittadini A. Hormonal replacement therapy in heart failure: focus on growth hormone and testosterone. **Heart Fail Clin**. 2019 Jul;15(3):377-391. doi: 10.1016/j.hfc.2019.02.007. Epub 2019 Apr 6. Review. PMID: 31079696.
19. Arcopinto M, Schiavo A, **Salzano A**, Bossone E, D'Assante R, Marsico F, Demelo-Rodriguez P, Baliga RR, Cittadini A, Marra AM. Metabolic Syndrome in Heart Failure: Friend or Foe?. **Heart Fail Clin**. 2019 Jul;15(3):349-358. doi: 10.1016/j.hfc.2019.02.004. Epub 2019 Apr 5. Review. PubMed PMID: 31079693.
20. **Salzano A**, Marra AM, D'Assante R, Arcopinto M, Bossone E, Suzuki T, Cittadini A. Biomarkers and imaging: complementary or subtractive? **Heart Fail Clin**. 2019 Apr;15(2):321-331. doi: 10.1016/j.hfc.2018.12.008. PMID: 30832821
21. Accardo G, Amoresano Paglionico V, Di Fraia R, Cittadini A, **Salzano A**, Esposito D, De Bellis A, Pasquali D. Management of cardiovascular complications in Klinefelter syndrome patients. **Expert Rev Endocrinol Metab**.

2019 Feb 16:1-8. doi: 10.1080/17446651.2019.1584036. PMID: 30793993
22. **Salzano A**§, Marra AM, Proietti M, Raparelli V, Heaney LM. Biomarkers in Heart Failure and Associated Diseases. **Dis Markers**, vol. 2019, Article ID 8768624, 2 pages, 2019. https://doi.org/10.1155/2019/8768624
23. Cassambai S, **Salzano A**, Yazaki Y, Bernieh D, Wong M, Israr MZ, Heaney LM, Suzuki T. Impact of acute choline loading on circulating trimethylamine N-oxide (TMAO) levels. **Eur J Prev Cardiol**. 2019 Feb 18:2047487319831372. doi: 10.1177/2047487319831372 PMID: 30776913
24. Marra AM, **Salzano A**, Arcopinto M, Piccioli L, Raparelli V. The impact of gender in cardiovascular medicine: Lessons from the gender/sex-issue in heart failure. **Monaldi Arch Chest Dis**. 2018 Oct 11;88(3):988. doi: 10.4081/monaldi.2018.988. PubMed PMID: 30375811
25. **Salzano A**, Marra AM, Bossone E, Suzuki T, Cittadini A. Letter to the Editor: "Cardiometabolic biomarkers and their temporal patterns predict poor outcome in chronic heart failure (Bio-SHiFT study)". **J Clin Endocrinol Metab**. J Clin Endocrinol Metab. 2019 Mar 1;104(3):734-735. doi: 10.1210/jc.2018-02043 PubMed PMID: 30339197.
26. **Salzano A**, Marra AM, D'Assante R, Arcopinto M, Suzuki T, Bossone E, Cittadini A. Growth Hormone Therapy in Heart Failure. **Heart Fail Clin**. 2018 Oct;14(4):501-515. doi: 10.1016/j.hfc.2018.05.002. Epub 2018 Aug 17. Review. PubMed PMID: 30266359.
27. Piccioli L, Arcopinto M, **Salzano A**, D'Assante R, Schiavo A, Stagnaro FM, Lombardi A, Panicara V, Valente P, Vitale G, Sarullo FM, Giallauria F, Marra AM. The impairment of the Growth Hormone/Insulin-like growth factor 1 (IGF-1) axis in heart failure: A possible target for future therapy. **Monaldi Arch Chest Dis**. 2018 Sep 5;88(3):975. doi: 10.4081/monaldi.2018.975. PubMed PMID: 30183158.
28. D'Assante R, Piccioli L, Valente P, Stagnaro FM, Schiavo A, Lombardi A, Panicara V, Arcopinto M, Vitale G, Sarullo FM, Giallauria F, Marra AM, **Salzano A**§. Testosterone treatment in chronic heart failure. Review of literature and future perspectives. **Monaldi Arch Chest Dis**. 2018 Sep 5;88(3):976. doi: 10.4081/monaldi.2018.976. PubMed PMID: 30183157.
29. **Salzano A**§, Cittadini A, Bossone E, Suzuki T, Heaney LM. Multiple hormone deficiency syndrome: a novel topic in chronic heart failure. **Future Sci OA**. 2018 Apr 16;4(6):FSO311. doi: 10.4155/fsoa-2018-0041. eCollection 2018 Jul. PubMed. PMID: 30057788; PubMed Central PMCID: PMC6060397.
30. Marra AM, Bossone E, **Salzano A**, D'Assante R, Monaco F, Ferrara F, Arcopinto M, Vriz O, Suzuki T, Cittadini A. Biomarkers in Pulmonary Hypertension. **Heart Fail Clin**. 2018 Jul;14(3):393-402. doi: 10.1016/j.hfc.2018.03.005. Review. PubMed PMID: 29966636.
31. Bossone E, Arcopinto M, Iacoviello M, Triggiani V, Cacciatore F, Maiello C, Limongelli G, Masarone D, Perticone F, Sciacqua A, Perrone-Filardi P, Mancini A, Volterrani M, Vriz O, Castello R, Passantino A, Campo M, Modesti PA, De Giorgi A, Monte I, Puzzo A, Ballotta A, Caliendo L, D'Assante R, Marra AM, **Salzano A**, Suzuki T, Cittadini A; TOSCA Investigators. Multiple hormonal and metabolic deficiency syndrome in chronic heart failure: rationale, design, and demographic characteristics of the T.O.S.CA. Registry. **Intern Emerg Med**. 2018 Aug;13(5):661-671. doi: 10.1007/s11739-018-1844-8. Epub 2018 Apr 4. PubMed PMID: 29619769.
32. **Salzano A**, D'Assante R, Heaney LM, Monaco F, Rengo G, Valente P, Pasquali D, Bossone E, Gianfrilli D, Lenzi A, Cittadini A, Marra AM, Napoli R. Klinefelter syndrome, insulin resistance, metabolic syndrome, and diabetes: review of literature and clinical perspectives. **Endocrine**. 2018 Aug;61(2):194-203. doi: 10.1007/s12020-018-1584-6. Epub 2018 Mar 23. Review. PubMed PMID: 29572708.
33. Buonomo AR, Scotto R, Nappa S, Arcopinto M, **Salzano A**, Marra AM, D'Assante R, Zappulo E, Borgia G, Gentile I. The role of the curcumin in liver diseases. **Arch Med Sci**. 2018 Feb 20 Arch Med Sci 2019; 15 (6): 1608–1620 doi.: 10.5114/aoms.2018.73596
34. Schiavo A, Stagnaro FM, **Salzano A**, Marra AM, Bobbio E, Valente P, Grassi S, Miniero M, Arcopinto M, Matarazzo M, Napoli R, Cittadini A. Pregabalin-induced first degree atrioventricular block in a young patient treated for pain from extrapulmonary tuberculosis. **Monaldi Arch Chest Dis**. 2017 Sep 28;87(3):838. doi: 10.4081/monaldi.2017.838. PubMed PMID: 29424197.
35. Quarto G, Amato B, Serra R, Benassai G, Monti MG, **Salzano A**, D'Assante R, Furino E. The effects of Crenotherapy and exercise in peripheral arterial occlusive disease. A comparison with simple exercise training. **Ann Ital Chir**. 2017;88:469-477. PubMed PMID: 29339594.
36. Marra AM, Bobbio E, D'Assante R, **Salzano A,** Arcopinto M, Bossone E, Cittadini A. Growth Hormone as Biomarker in Heart Failure. **Heart Fail Clin**. 2018 Jan;14(1):65-74. doi: 10.1016/j.hfc.2017.08.008. Review. PubMed PMID: 29153202.
37. **Salzano A**, Proietti M, D'Assante R, Saldamarco L, Cittadini A, Paladino F.Bleeding related to non-vitamin K antagonist oral anticoagulants in emergency department: A "Real-world" snapshot from Southern Italy. On behalf of MIRC-NOAC study group. **Eur J Intern Med**. 2018 Feb;48:e21-e24. doi: 10.1016/j.ejim.2017.10.018. Epub 2017 Nov 1. PubMed PMID: 29102387.
38. Rocca A, Aprea G, Surfaro G, Amato M, Giuliani A, Paccone M, **Salzano A**, Russo A, Tafuri D, Amato B.

Prevention and treatment of peritoneal adhesions in patients affected by vascular diseases following surgery: a review of the literature. Open Med (Wars). 2016 Jun 23;11(1):106-114. doi: 10.1515/med-2016-0021. eCollection 2016. PubMed PMID: 28352777;

39. Arcopinto* M, Salzano A*, Giallauria F, Bossone E, Isgaard J, Marra AM, Bobbio E, Vriz O, Åberg DN, Masarone D, De Paulis A, Saldamarco L, Vigorito C, Formisano P, Niola M, Perticone F, Bonaduce D, Saccà L, Colao A, Cittadini A; T.O.S.CA.(Trattamento Ormonale Scompenso CArdiaco) Investigators. Growth Hormone Deficiency Is Associated with Worse Cardiac Function, Physical Performance, and Outcome in Chronic Heart Failure: Insights from the T.O.S.CA. GHD Study. PLoS One. 2017 Jan 17;12(1):e0170058. doi: 10.1371/journal.pone.0170058. eCollection 2017. PubMed PMID: 28095492; PubMed Central PMCID: PMC5240983.
40. Marra AM, Benjamin N, Eichstaedt C, Salzano A, Arcopinto M, Gargani L, D Alto M, Argiento P, Falsetti L, Di Giosia P, Isidori AM, Ferrara F, Bossone E, Cittadini A, Grünig E. Gender-related differences in pulmonary arterial hypertension targeted drugs administration. Pharmacol Res. 2016 Dec;114:103-109. doi: 10.1016/j.phrs.2016.10.018. Epub 2016 Oct 19. Review. PubMed PMID: 27771466.
41. Salzano A, Demelo-Rodriguez P, Marra AM, Proietti M. A Focused Review of Gender Differences in Antithrombotic Therapy. Curr Med Chem. 2017;24(24):2576-2588. doi: 10.2174/0929867323666161029223512. Review. PubMed PMID: 27804874.
42. Salzano A, Marra AM, Ferrara F, Arcopinto M, Bobbio E, Valente P, Polizzi R, De Vincentiis C, Matarazzo M, Saldamarco L, Saccà F, Napoli R, Monti MG, D'Assante R, Isidori AM, Isgaard J, Ferrara N, Filardi PP, Perticone F, Vigorito C, Bossone E, Cittadini A; T.O.S.CA. investigators. Multiple hormone deficiency syndrome in heart failure with preserved ejection fraction. Int J Cardiol. 2016 Dec 15;225:1-3. doi: 10.1016/j.ijcard.2016.09.085. Epub 2016 Sep 26. PubMed PMID:27689528.
43. Arcopinto M*, Salzano A*, Ferrara F, Bobbio E, Marra AM, Abete R, Stagnaro F,Polizzi R, Giallauria F, Illario M, Menditto E, Vigorito C, Bossone E, Cittadini A. The Tosca Registry: An Ongoing, Observational, Multicenter Registry for Chronic Heart Failure. Transl Med UniSa. 2016 May 16;14:21-7. eCollection 2016 May. PubMed PMID: 27326392; PubMed Central PMCID: PMC4912335.
44. Aprea G, Rocca A, Salzano A, Sivero L, Scarpaleggia M, Ocelli P, Amato M,Bianco T, Serra R, Amato B. Laparoscopic single site (LESS) and classic video-laparoscopic cholecystectomy in the elderly: A single centre experience. Int J Surg. 2016 Sep;33 Suppl 1:S1-3. doi: 10.1016/j.ijsu.2016.05.059. Epub 2016 May 30. PubMed PMID: 27255133.
45. Marra AM, Arcopinto M, Salzano A, Bobbio E, Milano S, Misiano G, Ferrara F, Vriz O, Napoli R, Triggiani V, Perrone-Filardi P, Saccà F, Giallauria F, Isidori AM, Vigorito C, Bossone E, Cittadini A. Detectable interleukin-9 plasma levels are associated with impaired cardiopulmonary functional capacity and all-cause mortality in patients with chronic heart failure. Int J Cardiol. 2016 Apr 15;209:114-7. doi: 10.1016/j.ijcard.2016.02.017. Epub 2016 Feb 3. PubMed PMID:26889593.
46. Saccà F, Puorro G, Marsili A, Antenora A, Pane C, Casali C, Marcotulli C, Defazio G, Liuzzi D, Tatillo C, Cambriglia DM, Schiano di Cola G, Giuliani L, Guardasole V, Salzano A, Ruvolo A, De Rosa A, Cittadini A, De Michele G, Filla A. Long-term effect of epoetin alfa on clinical and biochemical markers in friedreich ataxia. Mov Disord. 2016 May;31(5):734-41. doi: 10.1002/mds.26552.Epub 2016 Feb 16. PubMed PMID: 26879839.
47. Salzano A, Arcopinto M, Marra AM, Bobbio E, Esposito D, Accardo G, Giallauria F, Bossone E, Vigorito C, Lenzi A, Pasquali D, Isidori AM, Cittadini A. Klinefelter syndrome, cardiovascular system, and thromboembolic disease: review of literature and clinical perspectives. Eur J Endocrinol. 2016 Jul;175(1):R27-40. doi: 10.1530/EJE-15-1025. Epub 2016 Feb 5. Review. PubMed PMID: 26850445.
48. Salzano A, Rocca A, Arcopinto M, Amato B, Marra AM, Simonelli V, Mozzillo P, Giuliani A, Tafuri D, Cinelli M. Bowel Angiodysplasia and Myocardial Infarction secondary to an ischaemic imbalance: a case report. Open Med (Wars). 2015 Dec 17;10(1):543-548. doi: 10.1515/med-2015-0092. eCollection 2015. PubMed PMID: 28352752; PubMed Central PMCID: PMC5368879.
49. Salzano A, Sirico D, Arcopinto M, Marra AM, Guerra G, Rocca A, Grieco A, Giallauria F, Vigorito C. [Anti remodeling therapy: new strategies and future perspective in post-ischemic heart failure. Part II]. Monaldi Arch Chest Dis.2014 Dec;82(4):195-201. Review. Italian. PubMed PMID: 26562985.
50. Sirico D*, Salzano A*, Celentani D, Arcopinto M, Marra AM, Bobbio E, Russo A, Giallauria F, Vigorito C. [Anti remodeling therapy: new strategies and future perspective in post-ischemic heart failure: Part I]. Monaldi Arch Chest Dis. 2014 Dec;82(4):187-94. Review. Italian. PubMed PMID: 26562984.
51. Arcopinto M, Salzano A, Isgaard J, Cittadini A. Hormone replacement therapy in heart failure. Curr Opin Cardiol. 2015 May;30(3):277-84. doi:10.1097/HCO.0000000000000166. Review. PubMed PMID: 25807222.
52. Arcopinto M, Salzano A, Bossone E, Ferrara F, Bobbio E, Sirico D, Vriz O, De Vincentiis C, Matarazzo M, Saldamarco L, Saccà F, Napoli R, Iacoviello M, Triggiani V, Isidori AM, Vigorito C, Isgaard J, Cittadini A. Multiple hormonedeficiencies in chronic heart failure. Int J Cardiol. 2015 Apr 1;184:421-3. doi:

10.1016/j.ijcard.2015.02.055. Epub 2015 Feb 24. PubMed PMID: 25755056.

53. Marra AM, Improda N, Capalbo D, **Salzano A**, Arcopinto M, De Paulis A, Alessio M, Lenzi A, Isidori AM, Cittadini A, Salerno M. Cardiovascular abnormalities and impaired exercise performance in adolescents with congenital adrenal hyperplasia. **J Clin Endocrinol Metab**. 2015 Feb;100(2):644-52. doi: 10.1210/jc.2014-1805. Epub 2014 Nov 18. PubMed PMID: 25405496.
54. Arcopinto M, Cella CA, Wesolowski R, **Salzano A**, Bossone E, Cittadini A, Baliga RR. Primary prevention of cancer-related thrombosis: special focus on ambulatory patients. **Int J Cardiol**. 2014 May 15;173(3):583-4. doi: 10.1016/j.ijcard.2014.03.109. Epub 2014 Mar 20. PubMed PMID: 24698248.
55. Cittadini A, Marra AM, Arcopinto M, Bobbio E, **Salzano A**, Sirico D, Napoli R, Colao A, Longobardi S, Baliga RR, Bossone E, Saccà L. Growth hormone replacement delays the progression of chronic heart failure combined with growth hormone deficiency: an extension of a randomized controlled single-blind study. **JACC Heart Fail**. 2013 Aug;1(4):325-330. doi: 10.1016/j.jchf.2013.04.003. Epub 2013 Aug 5. PubMed PMID: 24621936.
56. **Salzano A**, Sirico D, Golia L, Faga V, Flora M, Bossone E, Cittadini A. [The portopulmonary hypertension: an overview from diagnosis to treatment]. **Monaldi Arch Chest Dis**. 2013 Jun;80(2):66-8. Review. Italian. PubMed PMID: 24494409.
57. Pasquali D, Arcopinto M, Renzullo A, Rotondi M, Accardo G, **Salzano A**, Esposito D, Saldamarco L, Isidori AM, Marra AM, Ruvolo A, Napoli R, Bossone E, Lenzi A, Baliga RR, Saccà L, Cittadini A. Cardiovascular abnormalities in Klinefelter syndrome. **Int J Cardiol**. 2013 Sep 30;168(2):754-9. doi: 10.1016/j.ijcard.2012.09.215. Epub 2012 Oct 23. PubMed PMID: 23092857.
58. Marra AM, Arcopinto M, Bobbio E, **Salzano A**, Saccà L, Cittadini A. An unusual case of dilated cardiomyopathy associated with partial hypopituitarism. **Intern Emerg Med**. 2012 Sep;7 Suppl 2:S85-7. doi: 10.1007/s11739-011-0649-9. Epub 2011 Jun 29. PubMed PMID: 21713548.

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Peer-Reviewed Articles as collaborator-author

1. Rossio R, Ardoino I, Franchi C, Nobili A, Mannuccio Mannucci P, Peyvandi F; REPOSI Investigators Patterns of infections in older patients acutely admitted to medical wards: data from the REPOSI register. **Intern Emerg Med**. 2019 Aug 23. doi: 10.1007/s11739-019-02178-1. PMID: 31444740
2. Carlotta F, Raffaella R, Ilaria A, Alessandro N, Mannuccio MP; REPOSI Collaborators. Prevalence of use and appropriateness of antidepressants prescription in acutely hospitalized elderly patients. **Eur J Intern Med**. 2019 Aug 9. pii: S0953-6205(19)30258-4. doi: 10.1016/j.ejim.2019.07.025. PMID: 31405773
3. Proietti M, Agosti P, Lonati C, Corrao S, Perticone F, Mannucci PM, Nobili A, Harari S. Hospital Care of Older Patients With COPD: Adherence to International Guidelines for Use of Inhaled Bronchodilators and Corticosteroids. **J Am Med Dir Assoc**. 2019 Mar 6; doi: 10.1016/j.jamda.2019.01.132. PMID: 30852169
4. Mannucci PM, Nobili A, Pasina L; REPOSI Collaborators (REPOSI is the acronym of REgistro POliterapie SIMI, Società Italiana di Medicina Interna). Polypharmacy in older people: lessons from 10 years of experience with the REPOSI register. **Intern Emerg Med.** 2018 Aug 31. doi: 10.1007/s11739-018-1941-8. [Epub ahead of print] Review. PubMed PMID: 30171585.
5. Paciullo F, Proietti M, Bianconi V, Nobili A, Pirro M, Mannucci PM, Lip GYH, Lupattelli G; REPOSI Investigators. Choice and Outcomes of Rate Control versus Rhythm Control in Elderly Patients with Atrial Fibrillation: A Report from the REPOSI Study. **Drugs Aging**. 2018 Apr;35(4):365-373. Doi 10.1007/s40266-018-0532-8. PubMed PMID: 29564755.
6. Raparelli V, Pastori D, Pignataro SF, Vestri AR, Pignatelli P, Cangemi R,Proietti M, Davì G, Hiatt WR, Lip GYH, Corazza GR, Perticone F, Violi F, Basili S; ARAPACIS Study Collaborators. Major adverse cardiovascular events in non-valvular atrial fibrillation with chronic obstructive pulmonary disease: the ARAPACIS study. **Intern Emerg Med**. 2018 Aug;13(5):651-660. doi: 10.1007/s11739-018-1835-9. Epub 2018 Mar 26. Erratum in: Intern Emerg Med. 2018 Aug 3; PubMed PMID: 29582316.
7. Bertolotti M, Franchi C, Rocchi MB, Miceli A, Libbra MV, Nobili A, Lancellotti G, Carulli L, Mussi C; REPOSI Investigators. Prevalence and Determinants of the Use of Lipid-Lowering Agents in a Population of Older Hospitalized Patients: the Findings from the REPOSI (REgistro POliterapie Società Italiana di Medicina Interna) Study. **Drugs Aging**. 2017 Apr;34(4):311-319. doi:10.1007/s40266-017-0448-8. PubMed PMID: 28299634.
8. Basili S, Loffredo L, Pastori D, Proietti M, Farcomeni A, Vestri AR, Pignatelli P, Davì G, Hiatt WR, Lip GY, Corazza GR, Perticone F, Violi F; in collaboraton with ARAPACIS Study Investigators. Carotid plaque detection improves the predictive value of CHA2DS2-VASc score in patients with non-valvular atrial fibrillation: The ARAPACIS Study. **Int J Cardiol**. 2017 Mar 15;231:143-149. doi: 10.1016/j.ijcard.2017.01.001. Epub 2017 Jan 4. PMID: 28087176

9. Bossone E, Limongelli G, Malizia G, Ferrara F, Vriz O, Citro R, Marra AM, Arcopinto M, Bobbio E, Sirico D, Caliendo L, Ballotta A, D'Andrea A, Frigiola A, Isgaard J, Saccà L, Antonio C; T.O.S.CA. Investigators. The T.O.S.CA. Project: research, education and care. **Monaldi Arch Chest Dis**. 2011 Dec;76(4):198-203.PubMed PMID: 22567736.

Book Chapters

1. Colao A, Marsico F, **Salzano A**, D'Assante R, Cittadini A. Cuore e malattie sistemiche in Perrone Filardi P. **Cardiologia per studenti e medici di medicina generale**. Idelson Gnocchi. 2020. EAN 9788879477079
2. Arcopinto M, D'Assante R, **Salzano A**, Marra AM, Cittadini A, Growht hormone in chronic heart failure. In Mancini A **Adult growth hormone deficiency. Physiopathological and clinical aspects. Minerva Medica. 2019. EAN 9788877119544**
3. Napoli R, **Salzano A**, Bossone E, Cittadini A. Hormonal Therapy in the treatment of chronic heart failure. Pages 508-516. In Vasan RS, Sawyer DB. **Encyclopedia of Cardiovascular Research and Medicine**. Elsevier. 2018. EAN 978-0-12-805154-2

Grants

1. Grant of Federico II University, Naples, Italy, Department of Translational Medical Sciences and Merck Serono- "Insufficienza cardiaca cronica nei pazienti affetti da deficit di ormone della crescita. Analisi dei dati, interpretazione delle caratteristiche basali della popolazione arruolata con particolare focus sui biomarcatori cardiometabolici" – GGI Trattamento del deficit di GH associato ad insufficienza cardiaca cronica – Dicembre 2019 (5000€).
2. Grant of Federico II University, Naples, Italy, Department of Translational Medical Sciences and Merck Serono- "Dosaggio di biomarcatori implicati nell' insufficienza cardiaca cronica" – GGI Trattamento del deficit di GH associato ad insufficienza cardiaca cronica – December 2018 (3500€).
3. Travel Grant of European Federation of Internal Medicine to participate to European School of Internal Medicine Winter 2018- 22-26 January Levi, Finland. (1000 €)
4. Grant of Federico II University, Centro di Servizio di Ateneo per il Coordinamento di Progetti Speciali e l'Innovazione Organizzativa (CONOIR)- Sostegno Territoriale alle aree di Ricerca (STAR)- Linea 2- Mobilità Giovani Ricercatori – January 2018. (9500 €)

Bibliometric indices (from Scopus)

- Total impact factor: 180
- H-index: 16
- Total citations: 712

29th of September 2020