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STUDY OF THE PATHOGENESIS AND TREATMENT OF HEART FAILURE THROUGH THE HARMONIZATION OF EVIDENCE-BASED CLINICAL DATA, ARTIFICIAL INTELLIGENCE AND ANIMAL MODELS

PhD Student: Marco MELE

Coordinator: Prof. Nicola COLABUFO

Supervisor: Prof.ssa Antonella LIANTONIO

Co-supervisor: Prof. Nicola AMOROSO

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Sommario

1. ABSTRACT

Background and aims. Heart failure (HF) is a major cause of death and hospitalization, especially in diabetic patients. The cornerstone of medical treatment for HF is represented by sodium-glucose cotransporter type 2 inhibitors (SGLT2i), gliflozins, especially in heart failure with reduced left ventricular ejection fraction (HFrEF). Nevertheless, the effects of SGLT2i on ventricular remodelling and function have not been completely understood yet. Explainable artificial intelligence represents an unprecedented explorative option to clinical research in this field. Based on echocardiographic evaluations, our first aim was to identify some key clinical responses to gliflozins by employing a machine learning (ML) approach. Renal dysfunction is common in HF and hypertension and is associated with increased risk of mortality. Nevertheless, the exact mechanisms underlying the pathological development of this chronic state are poorly clarified although a main role was attributed to inflammation. Thus, in parallel with the data analysis data regarding HF patients, we aimed to explore the role of inflammation in cardio-renal syndrome and the possible impact of dapagliflozin in a HF animal model. In the setting of HF, atrial fibrillation (AF) represents an extremely frequent arrhythmia. Oral anticoagulant therapy (OAT) for managing AF encompasses vitamin K antagonists (VKAs) and direct-acting oral anticoagulants. Due to the lower risk of major bleeding associated with DOACs, anticoagulant switching is a common practice in AF patients. Nevertheless, there are issues related to OAT switching that still need to be fully understood, especially for patients in whom AF and HF coexist. As second aim, we therefore sought to assess the real positive and pleiotropic effects mediated by DOACs in addition to their anticoagulant activity. In particular, we aimed at the effective impact of the therapeutic switching from warfarin to DOACs in HF patients with AF by a ML analysis of a clinical database. **Methods**. For the first aim, we assessed 78 consecutive diabetic outpatients followed for HFrEF, using a random forest classification. A single subject analysis was performed to define the profile of patients treated with gliflozins. Moreover, an explainability analysis using Shapley values was used to outline clinical parameters that mostly improved after gliflozin therapy and machine learning runs highlighted specific variables predictive of gliflozin response. Studies on HF animal model aimed to assess markers of

inflammation, endothelial function, renal function and fibrosis and the efficacy of dapagliflozin treatment were conducted by using Dahl rats fed with a high salt diet. For the second aim, 42 consecutive outpatients with HFrEF and AF in OAT for at least one year were enrolled. The k-means clustering method and the Random Forest learning algorithm were adopted in order to evaluate how switching from warfarin to DOACs may affect the clinical progression of the patients. **Results.** The five-fold cross-validation analyses showed that gliflozins patients can be identified with a 0.70 ± 0.03% accuracy. The most relevant parameters distinguishing gliflozins patients were Right Ventricular S'-Velocity, Left Ventricular End Systolic Diameter and E/e' ratio. In addition, low Tricuspid Annular Plane Systolic Excursion values along with high Left Ventricular End Systolic Diameter and End Diastolic Volume values were associated with lower gliflozin efficacy in terms of anti-remodeling effects. Kidney of HF animal model showed inflammation, endothelial dysfunction, fibrosis, increased oxidative stress. After treatment with dapagliflozin, renal function resulted ameliorated. In patients with AF and HFrEF, at the baseline, 75% of patients were correctly separated. At the follow-up, after the switch to DOACs, this accuracy decreased to 64%. The baseline model is more accurate, achieving an average accuracy of 78%. At follow-up, the accuracy decreases to 58%. The accuracy loss of about 20% is statistically significant and suggests a fundamental loss of the features' discriminative power. N-terminal pro-brain natriuretic peptide (NTproBNP)brought a fundamental contribution in discriminating the clinical cohorts. **Discussion and Conclusions.** A ML analysis on a population of diabetic patients with HFrEF showed that SGLT2i treatment improved left ventricular remodeling. This cardiovascular response may be predicted with an explainable artificial intelligence approach, suggesting a lower efficacy in case of advanced stages of cardiac remodeling. Dapagliflozin showed a positive effect on inflammation, endothelial disfunction, fibrosis and renal function of HF animal model. Our ML analysis in patients with AF and HFrEF demonstrated that, when warfarin-treated patients switched to DOACs, they were no longer differentiable. This means that DOACs somehow modify the considered features which have specific clinical significance.

These results have been reported in the following articles and abstracts:
Peer-reviewed journal article

I. Mele M, Mele A, Imbrici P, Samarelli F, Purgatorio R, Dinoi G, Correale M, Nicolotti O, De Luca A, Brunetti ND, Liantonio A, Amoroso N. Pleiotropic Effects of Direct

Oral Anticoagulants in Chronic Heart Failure and Atrial Fibrillation: Machine Learning Analysis. Molecules. 2024 Jun 4;29(11):2651.

II. Mele M, Imbrici P, Mele A, Togo MV, Dinoi G, Correale M, Brunetti ND, Nicolotti O, De Luca A, Altomare CD, Liantonio A, Amoroso N. Short-term anti-remodeling effects of gliflozins in diabetic patients with heart failure and reduced ejection fraction: an explainable artificial intelligence approach. Front Pharmacol. 2023 Jun 9;14:1175606.

III. Urbanek K, Cappetta D, Bellocchio G, Coppola MA, Imbrici P, Telesca M, Donniacuo M, Riemma MA, Mele E, Cianflone E, Naviglio S, Conte E, Camerino GM, Mele M, Bucci M, Castaldo G, De Luca A, Rossi F, Berrino L, Liantonio A, De Angelis A. Dapagliflozin protects the kidney in a non-diabetic model of cardiorenal syndrome. Pharmacol Res. 2023 Feb;188:106659.

Abstract

1) 'The effectiveness of gliflozins in heart failure: an eXplainable Artificial Intelligence approach.' XXVII National Meeting in Medicinal Chemistry/14th Young Medicinal Chemists' Symposium. Bari , Palazzo del Prete, September 11-14 2022.
2) 'Cardiorenal protection of SGLTS inhibitors in heart failure: a multidisciplinary approach from animal model to patient and back'. 41° Congresso SIF; 16-19 novembre 2022.
3) 'Short-term antiremodeling effects of gliflozins diabetic patients with heart failure and reduced ejection fraction: an eXplainable Artificial Intelligence approach'. Congresso Nazionale della Società Italiana di Cardiologia. Roma, 15-18 dicembre 2022.
4) Mele M., Mele A., Imbrici P., Conte E., Coppola M.A., Brunetti N.D., Correale M., De Angelis A., Berrino L., Nicolotti O., Altomare C.D., De Luca A., Amoroso N., Liantonio A. Cardiorenal protection of SGLT2 inhibitors in heart failure: a multidisciplinary

approach from animal model to patient and back. 41°Congresso Società Italiana di Farmacologia, Roma 16 19 novembre 2022.

5) 4. Mele A, Mele M, Imbrici P, Samarelli, Purgatorio R, Dinoi G, Correale M, Nicolotti O, De Luca A, Brunetti ND, Amoroso N, Liantonio A. A machine learning analysis reveals pleiotropic effects of direct oral anticoagulants in patients with chronic heart failure and atrial fibrillation. 42° Congresso Nazionale della Società Italiana di Farmacologia, Sorrento, 13-16 novembre 2024.

2. INTRODUZIONE

2.1. Heart Failure: definition and classifications

Heart failure (HF) is a syndrome resulting from structural and/or functional abnormality of the heart that results in elevated intracardiac pressures and/or inadequate cardiac output at rest and/or during exercise (1). HF has traditionally been sub-categorized according to left ventricle ejection fraction (LVEF) when defining recommended treatments in clinical practice guidelines (2-4). In order to standardize terminology and LVEF cut-points used the most important cardiology international societies, including European Society of Cardiology and American Heart Association, proposed the following definitions and EF ranges as their most recent recommendations: (i) HFrEF: HF with LVEF $\leq$ 40%; (ii) HFpEF: HF with LVEF $\geq$ 50%; (iii) HFmrEF: HF with LVEF > 40% and LVEF < 50% (1-5). The dichotomization of LVEF of above or below 40% has been helpful to apply therapies that have been shown to work in patients with reduced EF (7-10). Post-hoc analyses of certain HF trials have suggested that standard therapy for HFrEF may be effective and extended to patients with HFmrEF (7-10). The characteristics of HFmrEF overlap with HFrEF and HFpEF, straddling either category, sometimes one more than the other depending on the clinical circumstance or patients studied (10-13, 15-17).

An alternative classification of HF from American College of Cardiology (ACC)/American Heart Association (AHA) is based on symptoms and the presence/absence of structural heart disease (**Table 1**). The stages of this classification are applicable to HFrEF, HFmrEF and HFpEF (2, 3). The definitional progression along the ACC/AHA stages A through D is a unidirectional path with little appreciation of a possibility to revert to a lower stage with appropriate guideline-directed medical therapy (**Table 1**) (2, 3, 18). The original ACC/AHA definition of stages of HF (18) has been ubiquitously adapted throughout other HF guidelines globally (2-4). If the HF process was to be defined as a continuum from Stage A through D, the highest number of patients would be in Stage A or Stage B. (19-22). This is due to the fact that the prevalence of hypertension, diabetes, coronary artery disease, obesity/metabolic syndrome – the main risk factors for development of HF – are present in approximately one-third of the population (23). By population-based registries, more than 40 to 50% of the adult population has been categorized to be in Stages A or B (19-21).

Table 1. HF stages according to American College of Cardiology classification.

Stage	Description	Examples
A	Patients at risk of developing HF because of the presence of conditions that are strongly associated with the development of HF. Such patients have no identified structural or functional abnormalities of the pericardium, myocardium or cardiac valves and have never shown symptoms or signs of HF	Systemic hypertension; coronary artery disease; diabetes mellitus; history of cardiotoxic drug therapy or alcohol abuse; personal history of rheumatic fever; family history of cardiomyopathy
B	Patients who have developed structural heart disease that is strongly associated with the development of HF but who have never shown signs or symptoms of HF	Left ventricular hypertrophy or fibrosis; left ventricular dilatation of hypocontractility; asymptomati asymptomatic valvular heart disease; previous myocardial infarction
C	Patients who have current or prior symptoms of HF associated with underlying structural heart disease	Dyspnea or fatigue due to left ventricular systolic dysfunction; asymptomatic patients who are undergoing treatment for prior symptoms of HF
D	Patients with advanced structural heart disease and marked symptoms of HF at rest despite maximal medical therapy and who require specialized interventions	Patients who are frequently hospitalized for HF or cannot be safely discharged from the hospital; patients in the hospital awaiting heart transplantation; patients at home receiving continuous treatment

2.2. Heart Failure: epidemiology

HF affects an estimated 6.5 million adults in United States and accounts for an estimated 1 million hospitalizations annually, of which approximately 50% are caused by HFrEF, with the

balance caused by HF with midrange or preserved ejection fraction (24-25). The incidence and prevalence of HF are increasing: data from the National Health and Nutrition Examination Survey show that between 2009-2012 and 2013-2016, the prevalence of HF among US adults increased from 5.7 million to 6.2 million, while data from the Atherosclerosis Risk in Communities study has shown the annual incidence of HF among US adults older than 55 years increased from 870 000 cases in 2005-2011 to 1 million cases in 2014 (25-26). In a study from the UK, while the age-standardized incidence of HF decreased by 7% (from 358 per 100 000 person-years to332 per 100 000 person-years) between 2002 and 2014, the absolute number of incident HF cases increased by 12% (from 170 727 to 190 798 cases), and prevalent HF increased by 23% (from 750 127 to 920 616 cases) (27). This increase in the absolute number reflects an aging population, improved survival from myocardial infarction and other cardiovascular diseases, and the increasing prevalence of predisposing risk factors such as diabetes and obesity (28).

2.3. Diagnosis of Heart Failure

The diagnosis of HF requires the presence of symptoms and/or signs of HF associated with objective evidence of cardiac dysfunction. Typical symptoms include breathlessness, fatigue, and ankle swelling (**Figure 1**). Symptoms and signs lack sufficient accuracy to be used alone to make the diagnosis of HF (29-32). The diagnosis of HF is made more likely in patients with a history of myocardial infarction, blood hypertension, coronary artery disease, diabetes mellitus, alcohol misuse, chronic kidney disease (CKD), cardiotoxic chemotherapy, and in those with a family history of heart disease or sudden death. The following diagnostic tests are recommended for the assessment of patients with suspected chronic HF:

(a) electrocardiogram (ECG). A normal ECG makes the diagnosis of HF unlikely (29). The ECG may reveal abnormalities such as AF, Q waves, LV hypertrophy (LVH), and a widened QRS complex that increase the likelihood of a diagnosis of HF and also may guide therapy;

(b) measurement of NPs is recommended, if available. A plasma concentration of B-type natriuretic peptide (BNP) < 35 pg/mL, N-terminal pro-B-type natriuretic peptide (NT-proBNP)

< 125 pg/ mL, or mid-regional pro-atrial natriuretic peptide (MR-proANP) < 40 pmol/L (33) make a diagnosis of HF unlikely (34-35);

(c) basic investigations such as serum urea and electrolytes, creatinine, full blood count, liver and thyroid function tests are recommended to differentiate HF from other conditions, to provide prognostic information and to guide potential therapy;

(d) echocardiography is recommended as the key investigation for the assessment of cardiac function. As well as the determination of the LVEF, echocardiography also provides information on other parameters such as chamber size, eccentric or concentric LVH, regional wall motion abnormalities (that may suggest underlying CAD, Takotsubo syndrome, or myocarditis), RV function, pulmonary hypertension, valvular function, and markers of diastolic function (36, 37).

(e) a chest X-ray is recommended to investigate other potential causes of breathlessness (e.g. pulmonary disease). It may also provide supportive evidence of HF (e.g. pulmonary congestion or cardiomegaly).

Symptoms	Signs
Typical	**More specific**
Breathlessness	Elevated jugular venous pressure
Orthopnoea	Hepatojugular reflux
Paroxysmal nocturnal dyspnoea	Third heart sound (gallop rhythm)
Reduced exercise tolerance	Laterally displaced apical impulse
Fatigue, tiredness, increased time to recover after exercise	
Ankle swelling	
Less typical	**Less specific**
Nocturnal cough	Weight gain (>2 kg/week)
Wheezing	Weight loss (in advanced HF)
Bloated feeling	Tissue wasting (cachexia)
Loss of appetite	Cardiac murmur
Confusion (especially in the elderly)	Peripheral oedema (ankle, sacral, scrotal)
Depression	Pulmonary crepitations
Palpitation	Pleural effusion
Dizziness	Tachycardia
Syncope	Irregular pulse
Bendopnea[a]	Tachypnoea
	Cheyne-Stokes respiration
	Hepatomegaly
	Ascites
	Cold extremities
	Oliguria
	Narrow pulse pressure

Figure 1 Symptoms and signs of HF.

Occasionally an LVEF of < 40% is detected incidentally in individuals who are truly asymptomatic this is not HF by definition and is termed asymptomatic left ventricular systolic dysfunction (LVSD), equivalent to ACC/AHA stage B. Once a patient has been identified as having HFrEF, the underlying aetiology of their LVSD should be sought. The main causes, principal presentations and specific investigations are resumed in **Figure 2**. Most commonly this will relate to ischaemic heart disease or an idiopathic dilated cardiomyopathy (38). Patients with chronic impairment of LV systolic function may well have complete resolution of symptoms and LVEF with appropriate therapy but be at risk of recurrent decompensation and continuing medical therapy is supported by experience and randomized trial evidence (39). More rarely, patients may present with a reversible underlying cause of LVSD such as Takotsubo cardiomyopathy, tachycardia-related cardiomyopathy, thyrotoxicosis and others. Critical ischemia corrected by revascularization may reverse HFrEF in carefully selected patients but identifying individuals in this category is challenging and the literature would not support routine revascularization in the absence of anginal symptoms.

The key to understanding the aetiology underlying HFrEF is a careful history and physical examination combined with appropriate investigations. Cardiac MRI is increasingly used to identify specific appearances related to various potential aetiologies, according to patterns of myocardium seen following the administration of gadolinium contrast (40). However, according to the specific cause of HFrEF different clinical presentations and instrumental tests must be considered (**Figure 3**) (1).

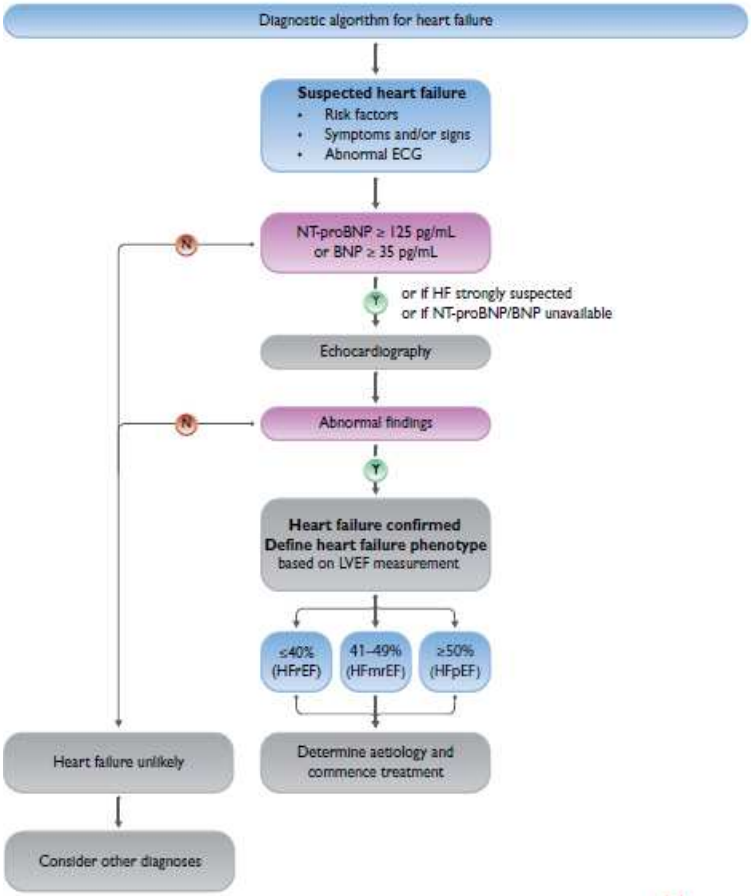

Figure 2 Diagnosis of HFrEF.

Cause	Examples of presentations	Specific investigations
CAD	Myocardial infarction Angina or "angina-equivalent" Arrhythmias	Invasive coronary angiography CT coronary angiography Imaging stress tests (echo, nuclear, CMR)
Hypertension	Heart failure with preserved systolic function Malignant hypertension/acute pulmonary oedema	24 h ambulatory BP Plasma metanephrines, renal artery imaging Renin and aldosterone
Valve disease	Primary valve disease e.g., aortic stenosis Secondary valve disease, e.g. functional regurgitation Congenital valve disease	Echo – transoesophageal/stress
Arrhythmias	Atrial tachyarrhythmias Ventricular arrhythmias	Ambulatory ECG recording Electrophysiology study, if indicated
CMPs	All Dilated Hypertrophic Restrictive ARVC Peripartum Takotsubo syndrome Toxins: alcohol, cocaine, iron, copper	CMR, genetic testing Right and left heart catheterization CMR, angiography Trace elements, toxicology, LFTs, GGT
Congenital heart disease	Congenitally corrected/repaired transposition of great arteries Shunt lesions Repaired tetralogy of Fallot Ebstein's anomaly	CMR
Infective	Viral myocarditis Chagas disease HIV Lyme disease	CMR, EMB Serology
Drug-induced	Anthracyclines Trastuzumab VEGF inhibitors Immune checkpoint inhibitors Proteasome inhibitors RAF+MEK inhibitors	
Infiltrative	Amyloid Sarcoidosis Neoplastic	Serum electrophoresis and serum free light chains, Bence Jones protein, bone scintigraphy, CMR, CT-PET, EMB Serum ACE, CMR, FDG-PET, chest CT, EMB CMR, EMB
Storage disorders	Haemochromatosis Fabry disease Glycogen storage diseases	Iron studies, genetics, CMR (T2* imaging), EMB α-galactosidase A, genetics, CMR (T1 mapping)
Endomyocardial disease	Radiotherapy Endomyocardial fibrosis/eosinophilia Carcinoid	CMR EMB 24 h urine 5-HIAA
Pericardial disease	Calcification Infiltrative	Chest CT, CMR, right and left heart catheterization
Metabolic	Endocrine disease Nutritional disease (thiamine, vitamin B1 and selenium deficiencies) Autoimmune disease	TFTs, plasma metanephrines, renin and aldosterone, cortisol Specific plasma nutrients ANA, ANCA, rheumatology review
Neuromuscular disease	Friedreich's ataxia Muscular dystrophy	Nerve conduction studies, electromyogram, genetics CK, electromyogram, genetics

Figure 3 Main causes, clinical presentation and investigations of HFrEF.

2.4. Pathophysiology of Heart Failure

HFrEF is characterized by a LVEF ≤40%, reflecting impaired myocardial contractility and systolic dysfunction. The syndrome arises from a complex interplay of structural, molecular, and systemic factors that progressively impair cardiac output and drive compensatory mechanisms, which, over time, become maladaptive (1). At the core of HFrEF is myocardial injury, often due to ischemic heart disease, myocardial infarction, hypertension, or cardiomyopathies. This initial insult leads to the loss of functional myocytes and alterations in myocardial architecture, a process termed adverse cardiac remodeling. Ventricular dilatation, wall thinning, and interstitial fibrosis impair the heart's ability to contract effectively, reducing stroke volume and ejection fraction. These structural changes increase wall stress and oxygen demand, perpetuating myocardial dysfunction (1,17,16). A critical feature of HFrEF is neurohormonal activation, triggered by reduced cardiac output. The activation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS) plays a central role. The RAAS promotes vasoconstriction, sodium retention, and fluid accumulation to increase preload and maintain perfusion pressure. Simultaneously, SNS stimulation raises heart rate and myocardial contractility to compensate for the reduced stroke volume. However, chronic activation of these systems becomes maladaptive (1,2). Prolonged RAAS stimulation leads to increased afterload due to vasoconstriction and fluid overload, exacerbating ventricular wall stress and promoting further remodeling. Aldosterone additionally contributes to myocardial fibrosis and vascular stiffening, worsening systolic dysfunction. Similarly, sustained sympathetic overdrive increases myocardial oxygen consumption, accelerates apoptosis of cardiomyocytes, and contributes to arrhythmogenesis, further impairing cardiac output. Another significant compensatory mechanism is the release of natriuretic peptides, such as B-type natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) (1, 2, 16). These hormones act to counterbalance RAAS and SNS activation by promoting natriuresis, vasodilation, and inhibition of fibrosis. Despite their beneficial effects, the natriuretic peptide system becomes insufficient to fully counteract the maladaptive neurohormonal activity as HF progresses. Hemodynamic alterations are central to the clinical manifestations of HFrEF. The reduced left ventricular contractility decreases stroke volume and cardiac output, leading to elevated end-diastolic pressure. This rise in pressure is transmitted backward to the pulmonary circulation, causing pulmonary congestion and symptoms such as dyspnea and orthopnea. Systemically, reduced cardiac output results in

impaired perfusion of vital organs, contributing to fatigue, exercise intolerance, and activation of further compensatory mechanisms. Systemic inflammation and oxidative stress also play a key role in the progression of HFrEF. Myocardial injury induces the release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukins, which contribute to myocyte apoptosis, fibrosis, and endothelial dysfunction (1-4). Oxidative stress from reactive oxygen species (ROS) further damages cellular structures, worsening both myocardial and vascular function. In addition to cardiac changes, peripheral adaptations significantly contribute to the clinical syndrome. Skeletal muscle perfusion is reduced due to vasoconstriction and endothelial dysfunction, leading to muscle atrophy and decreased exercise capacity. Furthermore, abnormalities in skeletal muscle metabolism and mitochondrial dysfunction amplify the sensation of fatigue and dyspnea during physical activity. As the disease progresses, compensatory mechanisms eventually fail to maintain sufficient perfusion and homeostasis. This results in worsening fluid retention, increased pulmonary congestion, and reduced systemic perfusion. Repeated episodes of acute decompensation are common, often triggered by ischemia, arrhythmias, or increased afterload. These exacerbations accelerate the downward trajectory of cardiac function, leading to recurrent hospitalizations and increased mortality. In summary, the pathophysiology of HFrEF involves a vicious cycle of myocardial injury, adverse remodeling, neurohormonal activation, and systemic dysfunction. These processes interact dynamically, driving both the clinical symptoms and progressive decline in cardiac function. On the other hand, HFpEF is characterized by the absence of systolic dysfunction of left ventricle. The basic mechanisms of HFpEF pathophysiology are well-understood. HFpEF is a multifactorial disease associated with an impaired diastolic function of the heart (41-43). Risk factors such as arterial hypertension, ischemic heart disease, diabetes mellitus and obesity are common to HFrEF. However, obesity, metabolic dysfunction, and physical inactivity appear to specifically predispose to HFpEF (41). The primary mechanistic hypothesis of HFpEF pathology was based on diastolic dysfunction caused by left ventricle remodelling-induced hypertension in older patients (44, 45). In the last 20 years, epidemiological data highlight the HFpEF frequency in patients with comorbidities, such as obesity metabolic disorders and diabetes, suggesting a major focus on systemic inflammation, endothelial dysfunction and cardiac structural and functions alterations. One of the mechanisms underlying the HFpEF is the increase of filling pressure in the chamber of LV leading to ventricular remodelling and dysfunction. A consequence of this chronic event is the development of atrial fibrillation (AF) due to the impairment function spanned towards the left atrial chamber. The AF is considered as a HFpEF biomarker, because

the alterations in left atrium are more predictive of adverse outcome than those of left ventricle contributing to discriminate HFpEF from non-cardiac causes of dyspnea (46,47).

In patients with HFpEF, left atrium dysfunction is a consequence of a LV dysfunction, but it has been revealed that, in some cases, the reduced cardiac output is caused by left atrium myopathy due to the increased endo-cavitary pressure and declined left ventricle filling (41). The respiratory system is the first district that is sensitive to the abnormalities of cardiac structure and functions. Indeed, roughly 80% of HFpEF patients developed pulmonary hypertension as the result of left atrium pressure increase (48,49). During this chronic state, the combination of remodelling and vasoconstriction of the pulmonary veins, capillaries and small arteries causes a worsening of the pulmonary hypertension, finally leading to pulmonary vascular disease (PVD) (50,51). In some patients, coronary artery disease may contribute to right ventricle dysfunction in concert with left ventricle dysfunction (52).

Importantly, recent studies have shown that inflammatory, metabolic and ischemic insults induce simultaneously atrial and biventricular dysfunctions as a common response (41). The pathophysiology of HFpEF is complex and may include numerous non cardiac mechanisms involving lung, kidney, skeletal muscle and adipose tissue (53). Understanding the role of the contributors in progression of HFpEF is important for planning the adequate strategy for efficacious pharmacological treatments. A common feature in patients with HFpEF is the tissue congestion due to hemodynamic abnormalities (1). However, there is no single mechanism associated with hemodynamic abnormalities. It has been proposed that HF is a result of haemodinamic abnormalities induced by multiple mechanisms combining to form the pathophysiological phenotype (41).

2.5. Cardiorenal syndrome

As previously described, HF is a disease frequently diagnosed in elderly people. The success of therapeutical strategies and the improvement of quality of life make survival possible after the diagnosis. As a consequence, many patients with HF may experience the worsening of the disease accentuated by new onset of other comorbidities. Among the comorbidities coexisting with HF, the renal defects are the most common and difficult to manage (54, 55). Indeed, about

40-50% of HF patients showed a reduced glomerular filtration rate (GFR), suggesting the presence of the CKD as a strong independent risk factor for HF, and the worsening of cardiovascular diseases is strongly correlated with the degree of renal injury. The CKD is defined as a persistent reduction of glomerular filtration rate (GFR) ranging < 60 ml/min/1,73 m^2 (55).
Cardiac and renal systems are tightly interconnected in a complex and bi-directional pathway (56,57). Alterations in this pathway lead to a particular clinical condition characterized by both organ dysfunctions also known as cardiorenal syndrome (58–60). In order to understand the patho-mechanism underlying the cardiorenal syndrome and to compare different clinical data, a classification has been established (Figure 3) (55). The cardio-renal syndromes comprise five subtypes according to the direction of the effect and whether the initiating insult is acute or chronic. Currently, the key factors contributing to the development and progression of cardiorenal syndrome are:

- haemodynamic mechanisms
- neuro-hormonal mechanisms
- inflammation

Kidneys receive about 25% of cardiac output. In both HF subtypes the cardiac defects affect the ability of the heart to pump blood reducing the volume of cardiac output implying alterations in GFR. However, renal autoregulatory and tubule-glomerular feedback mechanisms act in tandem to maintain constant GFR (61). If these mechanisms are not sufficient, the sensitivity of renal cells to hypoxia induces acute tubular necrosis contributing to the renal dysfunction. Moreover, hypoperfusion triggers baroreceptors, juxtaglomerular renin release as well as RAAS activation inducing vasoconstriction in glomerulus and tubular apparatus (Figure 4) (55, 62). Recent evidence indicates that the decline of renal function correlates with an increased central venous pressure, without affecting cardiac output (63, 64). In the HF settings the central venous pressure is typically increased as well as associated with renal venous hypertension. In this sense, renal venous pressure induces a decline of GFR, thus, reducing renal blood flow. Chronic state of renal venous hypertension contributes to the progression of renal injury characterized by hypertrophy, tubulointerstitial and intraglomerular fibrosis.
Since the early 1980s, the important role of the activation of RAAS and the SNS in the pathogenesis of chronic HF and CKD has been established (23). At early stages of cardiovascular disease, the blood pressure can be maintained in control values by directly RAAS activation, inducing vasoconstriction, as well as indirectly by SNS activity, promoting renal sodium retention (Figure 4). However, during chronic state, the two main RAAS players, i.e. angiotensin

II (ang II) and aldosterone, have deleterious effects on both organs (65, 66). Furthermore, the organs degeneration was caused by cardiac and renal remodeling induced by stimulation of macrophage-derived galectin-3 (67). Moreover, large quantities of natriuretic peptides, such as ANP and BNP, are released in order to contrast the anti-natriuretic effect induced by the RAAS activation. However, this effect was not sufficient, and the resulting imbalance leads to substantial urinary sodium and water retention as well as oedema formation. In addition, the persistent RAAS activation promotes reactive ROS formation. The resulting oxidative injury leads to endothelial dysfunction considered as hallmark of cardiorenal syndrome (68). The HF individuals with declined renal function display increased levels of catecholamines probably due to SNS activation and reduced clearance. The SNS system acts in the same direction of RAAS activation producing vasoconstriction and fluids overload.

Despite the role of innate immune system in HF is controversial (69), numerous evidence supports the main contribution of inflammatory pathways in cardiovascular disease. Since 1960s, the involvement of immune system in hypertension has been described. Several hypotheses have been postulated in relation to mechanisms underlying the involvement of inflammatory mediators on HF.

Firstly, the cardiac injury leads to systemic venous congestion that affects the mesenteric system (70). The resulting oedema contributes to the impairment permeability of the tissue promoting bacterial translocation, endotoxin release and the stimulation of immune system. The second concerns the ability of the defected heart to produce cytokines during the pathology progression (71). The third regards the activation of immune system following the tissue hypoxia caused by the declined of cardiac output (72).

Particularly, the immune cells release a wide range of components with low molecular weight. These proteins, also known as the pro-inflammatory cytokines, which resulted to have high levels of expression, are tumour necrosis factor-α (TNFα), inteleukin-1β(IL-1β), IL-6, IL-17 and INF-γ, all promoting renal and vascular damage (73-75).

Given the interplay between heart and kidney in the progression of the cardiorenal syndrome, it is also important to keep in mind the pro-inflammatory mechanisms following the interactions between hormones and other paracrine or autocrine substances with their targets localized on renal tissue.

Moreover, high concentrations of IL-10 and altered ratio of IL-6 to IL-10 reflect the declining of renal function. Other biomarkers of renal injury are the expression levels of C-reactive protein and pentraxin-3 (76). An accumulation of monocyte/macrophages was observed in renal arteries

and arterioles due to the increased concentrations of renin, ang II, aldosterone and catecholamines promoting the infiltration of effector like T cells (77,78).

Recently, Liu et al supported the involvement of immune system mediators in the salt-sensitive hypertension pathogenesis (79). In particular, they demonstrated the accumulation of T-cells in a specific area of renal tubule in the kidneys of an animal model of salt-sensitive hypertension. The infiltration of immune system cells (T lymphocytes, macrophages and monocytes) in distal convoluted tubule (DCT) cells results from renal hypoperfusion that is a common feature in HF. As theoretical mechanism, the authors proposed that CD8+ T-cells, directly interacting with DCT cells, induce the increase of intracellular ROS levels, which in turn induce Src kinase (Src) activation promoting sodium chloride reabsorption.

2.6. Heart Failure treatment

Patients with HFrEF should be engaged with a disease management program. According to European Society of Cardiology Guidelines, because of a wide range of treatment options, patients should be regularly reviewed to determine their eligibility for each of these (1). HF medical therapies have traditionally focused on addressing the maladaptive neurohormonal response to primary cardiac injury, that is, inhibition of the renin-angiotensin-aldosterone and adrenergic systems with angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), mineralcorticoid receptor antagonists (MRAs) and beta blockers (**Figure 4**). These agents work to counteract maladaptive systemic responses including vasoconstriction (which increases cardiac afterload), fluid and salt retention (leading to congestion) and sinus tachycardia (which increases myocardial oxygen demand and impairs myocardial perfusion). Two recent advances have revolutionized the medical treatment of HF: the addition of a neprilysin inhibitor to ARNI (angiotensin receptor and neprilysin inhibitor) and sodium-glucose cotransporter-2 (SGLT2) inhibition. Neprilysin is an enzyme which is responsible for the breakdown of a range of vasoactive and other peptides, including the natriuretic peptides, which have favourable cardiovascular effects counteracting the negative effects of the maladaptive neurohumoral activation which occurs in HF. Inhibition of neprilysin

activity thus augments these cardioprotective peptides. SGLT2 inhibitors inhibit renal glucose reabsorption by blocking the SGLT2 cotransporters in the proximal tubules, causing glucosuria, diuresis and natriuresis; however, studies have revealed myriad other cardioprotective and nephroprotective actions of these medications (80).

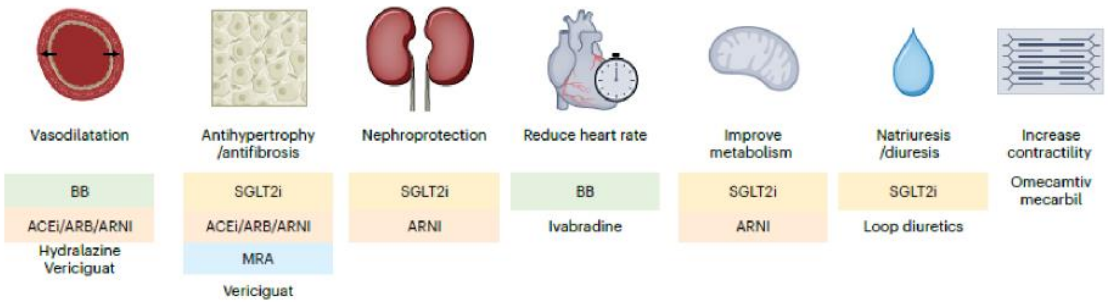

Figure 4 Indications and mechanisms of action of HF medications. Coloured boxes represent the four drug classes that are the pillars of current HF treatment.

2.6.1. Pharmacological treatment for Heart Failure with reduced Ejection Fraction

Pharmacotherapy is the cornerstone of treatment for HFrEF and should be implemented before considering device therapy, and alongside non-pharmacological interventions. There are three major goals of treatment for patients with HFrEF: (i) reduction in mortality, (ii) prevention of recurrent hospitalizations due to worsening HF, and (iii) improvement in clinical status, functional capacity, and QOL (81-83).

2.6.2. General principles of treatment

Modulation of the RAAS and sympathetic nervous systems with ACE-I or an ARNI, beta-blockers, and MRA has been shown to improve survival, reduce the risk of HF hospitalizations,

and reduce symptoms in patients with HFrEF. These drugs serve as the foundations of pharmacotherapy for patients with HFrEF. The triad of an ACE-I/ARNI, a beta-blocker, and an MRA is recommended as cornerstone therapies for these patients, unless the drugs are contraindicated or not tolerated (84-86). At the basis of the role of these drugs there are counter-regulatory mechanisms of the sympathetic nervous system and RAAS (**Figure 5**). Beta-blockers, ACE-I/ARNI, MRA should be up-titrated to the doses used in the clinical trials (or to maximally tolerated doses if that is not possible). The canonical HF guideline still recommends the use of ARNI as a replacement for ACE-I in suitable patients who remain symptomatic on ACE-I, beta-blocker, and MRA therapies; however, an ARNI may be considered as a first-line therapy instead of an ACE-I (87,88). ARBs still have a role in those who are intolerant to ACE-I or ARNI. The SGLT2i dapagliflozin and empagliflozin added to therapy with ACE-I/ARNI/betablocker/MRA reduced the risk of CV death and worsening HF in patients with HFrEF (89,90). Unless contraindicated or not tolerated, dapagliflozin or empagliflozin are recommended for all patients with HFrEF already treated with an ACE-I/ARNI, a beta-blocker, and an MRA, regardless of whether they have diabetes or not.

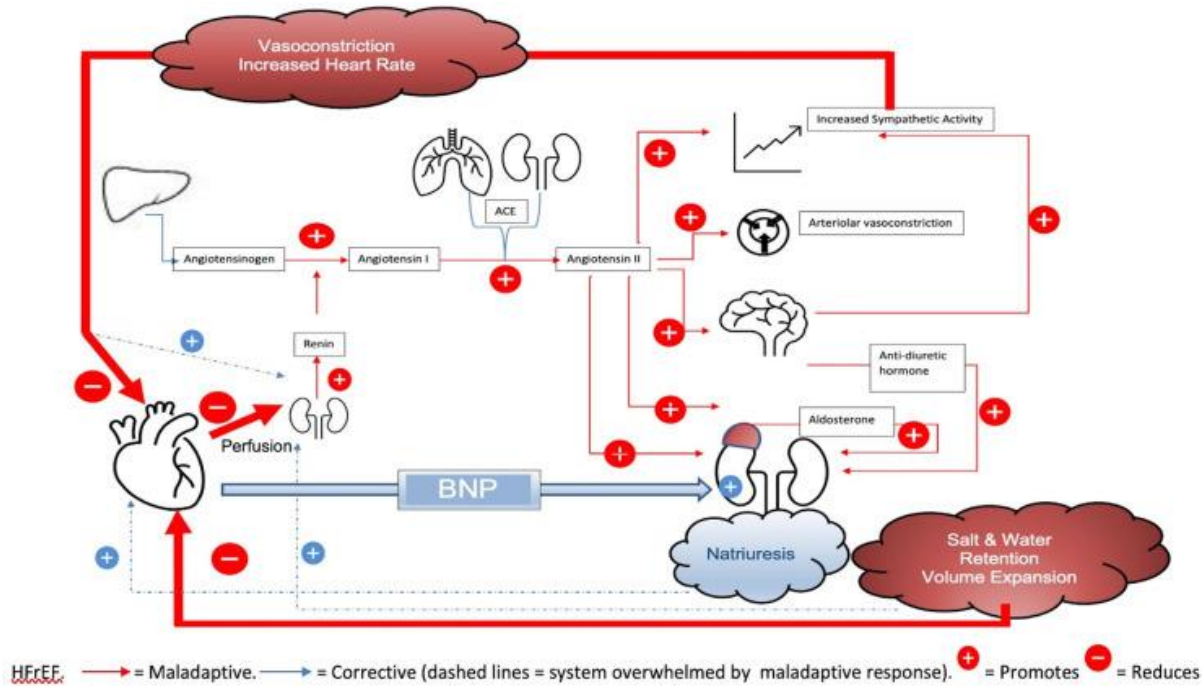

Figure 5. Schematic representation of counter-regulatory pathways in HFrEF and sites of action of drugs. ACE: angiotensin converting enzyme; BNP: brain natriuretic peptide.

2.6.3. Angiotensin-converting enzyme inhibitors

Angiotensin-converting enzyme (ACE) inhibitors decrease peripheral resistance and reduce the load on the failing myocardium by inhibiting the conversion of angiotensin I to angiotensin II, thus preventing vasoconstriction and causing relaxation of the vasculature (**Figure 6**). ACE inhibitors were the first class of drugs shown to reduce mortality and morbidity in patients with HFrEF (93-94). They have also been shown to improve symptoms (92). They are recommended in all patients unless contraindicated or not tolerated. They should be up titrated to the maximum tolerated recommended doses.

The efficacy of ACE inhibitors has been proven over several decades. Major trials analyzing ACE inhibitors in HFrEF have utilized them in addition to standards of care such as digoxin, vasodilators, loop diuretics, potassium-sparing diuretics, and beta-blockers (91). The CONSENSUS trial, which compared enalapril with placebo in addition to standard of care, showed that enalapril reduced overall mortality risk by 27% and significantly decreased the number of patients with HFrEF progression (92) The SOLVD trial demonstrated that, compared with placebo, treatment with enalapril over the course of three years prevented 50 premature deaths and 350 hospitalizations per 1,000 patients (95). Collectively, these trials suggest that ACE inhibitors, when taken concurrently with other HFrEF medications, provide significant reductions in morbidity and mortality. These benefits have been shown to remain clinically significant throughout long courses of therapy (91-93).

Contraindications to ACE inhibitor therapy include hypersensitivity, previous angioedema from ACE inhibitor use, or concomitant use with aliskiren. Adverse effects to monitor for in patients using ACE inhibitors include headache, cough, diarrhoea, dizziness, and fatigue; most of these effects are transient and mild. More serious events include reversible increases in serum creatinine (SCr) and symptomatic hypotension, both related to the hemodynamic effects of ACE inhibitors (91-94). While the exact number is not agreed upon, an SCr increase of up to 30% is regarded as acceptable and does not warrant stopping ACE inhibitor therapy. In trials, small but significant increases in serum potassium were observed (91). Caution should be exercised in patients with pre-existing hypotension, those with baseline hyperkalaemia (potassium greater

than 5 mEq/L), and those receiving concomitant potassium supplements or potassium-sparing diuretics (91)

The usual dosing strategy for ACE inhibitors is to initiate at a low dose and double the dose every one to two weeks, if tolerated, up to the prespecified target dose. Monitor patients for hypotension, potassium levels, and decreased renal function during the titration period to assess tolerability. Patients with pre-existing conditions that put them at a higher risk for side effects (sodium levels less than 130 mEq/L, creatinine clearance [CrCl] less than 30 mL/min, an increase in diuretic dose in the past week, or treatment with a potassium-sparing diuretic) may be initiated at a lower dose (91).

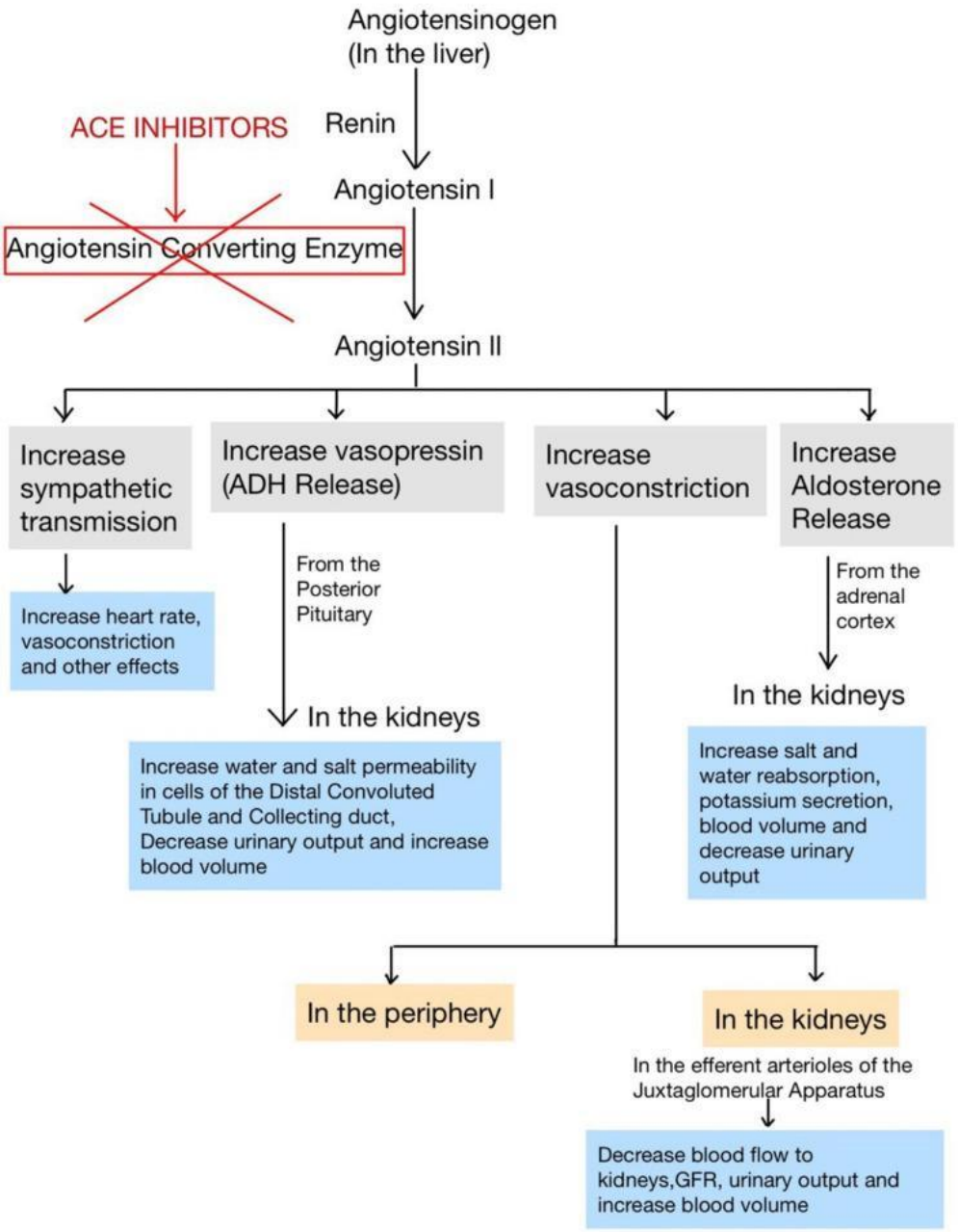

Figure 6: Mechanism of action of Angiotensin Converting Enzyme Inhibitors. GFR: glomerular Filtration Rate. ADH: Anti-Diuretic Hormone.

2.6.4. Beta-blockers

The beneficial effect of beta blockade in HFrEF has been documented for more than 40 years (91) Since 1975, data have shown that the use of bisoprolol, carvedilol, or sustained-release metoprolol succinate reduces morbidity and mortality in patients with HFrEF (91). These are the only beta-blockers tested in large clinical trials to show a mortality benefit, which led to their inclusion in the HF guidelines as first-line agents in all patients with HFrEF to reduce morbidity and mortality unless contraindicated (96-98). These three agents share a common pathway: they all block the β_1-adrenergic receptor located on the heart. HFrEF stimulates the RAAS and sympathetic system in order to compensate for the reduced EF. However, this activation may accelerate ventricular remodelling. By blocking β_1 receptors, these beta-blockers prevent ventricular remodelling promoted by the stimulated RAAS and sympathetic system. While metoprolol and bisoprolol are selective for the β_1 receptor, carvedilol also blocks the β_2 and α_1 receptors, leading to vasodilation (97-99). The COPERNICUS study had patients double their dose of carvedilol until a mean dose of 37 mg per day was achieved, showing an all-cause mortality of 11.4% versus 18.5% in the placebo group ($P = 0.00013$) (100). Bisoprolol was evaluated in the CIBIS-II trial, leading to all-cause mortality of 8.8% versus 13.2% in the placebo group ($P < 0.0001$) (101-103) Finally, the MERIT-HF trial compared metoprolol succinate with placebo in patients on baseline ACE-inhibitor and diuretic therapy to evaluate all-cause mortality (7.2% versus 11%; $P = 0.00009$) and all-cause mortality plus all-cause hospitalization (32% versus 38%; $P < 0.001$) (96).

Beta-blockers should be initiated at low doses and titrated slowly to target doses if tolerable. Adverse events include fluid retention and worsening HFrEF, fatigue, bradycardia or heart block, and hypotension. The fluid retention or worsening HFrEF associated with beta-blockers do not generally warrant the permanent withdrawal of treatment (91,104-105). Beta-blocker-induced bradycardia is generally asymptomatic and thus requires no treatment; however, if the bradycardia is accompanied by dizziness, light-headedness, or second- or third-degree heart block, the dose of the beta blocker should be decreased. Patients should be monitored closely for changes in vital signs and symptoms during this titration period. If the target doses are not tolerated, the highest tolerated dose should be continued (91).

Beta-blockers have been shown to reduce mortality and morbidity in patients with HFrEF, in addition to treatment with an ACE inhibitors and diuretic (96-102). They also improve symptoms (103). There is consensus that ACE inhibitors and beta-blockers can be commenced together as

soon as the diagnosis of symptomatic HFrEF is established. There is no evidence favouring the initiation of a beta-blocker before an ACE inhibitor and vice versa (104). Beta-blockers should be initiated in clinically stable, euvolemic, patients at a low dose and gradually up-titrated to the maximum tolerated dose. In patients admitted with acute HF, beta-blockers should be cautiously initiated in hospital, once the patient is hemodynamically stabilized. An individual patient data meta-analysis of all major beta blocker trials in HFrEF has shown no benefit on hospital admissions and mortality in the subgroup of patients with HFrEF with AF (105). However, since this is a retrospective subgroup analysis, and because beta-blockers did not increase risk, the guideline committee decided not to make a separate recommendation according to heart rhythm.

2.6.5. Mineralcorticoid receptor antagonists

Aldosterone receptor antagonists (also called mineralocorticoid receptor antagonists, MRAs) are recommended for NYHA class II–IV HF patients with an EF of 35% or less, GFR of at least 30 mL/min/1.73 m^2, and a potassium level of 5.0 mEq/dL or lower. Studies have demonstrated that aldosterone receptor antagonists (when given in conjunction with ACE inhibitors and beta blockers) reduce the risk of morbidity and mortality in patients with NYHA class III-IV HFrEF with an EF of 35% or less (91). Further studies found similar benefits in NYHA class II HFrEF patients with an EF of 35% or less.

Spironolactone is a nonselective aldosterone antagonist, while eplerenone is selective to the aldosterone receptor (91). Aldosterone is an endogenous steroid hormone that increases sodium retention and facilitates magnesium/potassium loss. Aldosterone may ultimately cause myocardial fibrosis, vascular injury, direct vascular damage, and baroreceptor dysfunction leading to the development and progression of HFrEF (106). The use of MRAs may slow HF progression and prevent or reverse cardiac remodelling and the development of arrhythmias (107), Although ACE inhibitors block aldosterone, evidence indicates that this effect is only transient (107). There is little data comparing the efficacy of spironolactone versus eplerenone, but both have proven effective in placebo-controlled trials (91, 106).

The initial and maximum doses of aldosterone antagonists should be adjusted based on renal function. Spironolactone, which is chemically similar to progesterone, increases peripheral

oestradiol formation, potentially leading to adverse events, including gynecomastia or amenorrhea. These adverse events are not seen with eplerenone because it is selective to the aldosterone receptor (91). Furthermore, although ACE inhibitors and aldosterone antagonists are often used concomitantly for patients with HFrEF, concurrent use of these agents can cause life-threatening hyperkalaemia. Due to the risk of elevated potassium levels, potassium supplements should be discontinued (or reduced and carefully monitored in those with a history of hypokalaemia) when initiating aldosterone antagonist therapy in a patient already receiving an ACE inhibitor. Careful monitoring of potassium levels and renal function should be performed at initiation and closely checked within two to three days and again at seven days after initiation (91). Patients should subsequently be monitored monthly for the first three months and every three months thereafter. More frequent monitoring may be appropriate for patients who have fluctuating potassium levels, renal function, or fluid status, as well as patients who have had recent changes in their ACE inhibitor/ARB dosing regimens. Additional monitoring parameters include daily measures of blood pressure and weight (91).

MRAs (spironolactone, eplerenone) are recommended, in addition to an ACE inhibitor and a beta-blocker, in all patients with HFrEF to reduce mortality and the risk of HF hospitalization (106,107). They also improve symptoms (106). MRAs block receptors that bind aldosterone and, with different degrees of affinity, other steroid hormones (e.g. corticosteroid and androgen) receptors. Eplerenone is more specific for aldosterone blockade and, therefore, causes less gynaecomastia. Caution should be exercised when MRAs are used in patients with impaired renal function and in those with serum potassium concentrations >5.0 mmol/L.

2.6.6. Angiotensin receptor-neprilysin inhibitors

Angiotensin receptor-neprilysin inhibitors (ARNIs) are a new class of medications that may have a growing role in HF treatment (1). Sacubitril/valsartan is a novel therapy approved in July 2015 to reduce the risk of cardiovascular death and hospitalization for patients with HFrEF (NYHA class II–III). Sacubitril/valsartan consists of the neprilysin inhibitor sacubitril and the ARB valsartan. Neprilysin is a neutral endopeptidase that metabolizes endogenous vasoactive peptides, including natriuretic peptides, bradykinin, and substance P into their inactive metabolites. Inhibition of neprilysin increases the levels of these substances and decreases

vasoconstriction, sodium retention, abnormal growth, and remodeling (91). However, angiotensin II is also a substrate of neprilysin. Thus, the addition of an ARB to the neprilysin inhibitor is necessary to prevent activation of the RAAS.

Previous studies, such as OVERTURE, investigated the combination of a neprilysin inhibitor with an ACE inhibitor (91). Although the combination was shown to reduce mortality and hospitalization in chronic HF, it was not more effective than ACE inhibition alone and was associated with a higher rate of angioedema. Alternatively, new trials, as PARADIGM-HF investigated the combination of the neprilysin inhibitor sacubitril and the ARB valsartan. PARADIGM-HF aimed to study the long-term effects of sacubitril/valsartan 200 mg twice daily on mortality and hospitalization compared with enalapril 10 mg twice daily in patients with HFrEF (108-113). To be considered for trial inclusion, patients were required to tolerate a stable dose of a beta-blocker and an ACE inhibitor or ARB equivalent of at least 10 mg of enalapril daily for at least four weeks prior to trial screening. At baseline, of the 4,187 patients in the sacubitril/valsartan group, 78% were using an ACE inhibitor, 22.2% were on ARBs, 93.1% utilized a beta blocker, and 54.2% were taking an MRA. The study was stopped early (after the third interim analysis) due to a clear statistical and clinical advantage for sacubitril/valsartan; median follow-up was 27 months. The HR for sacubitril/valsartan for composite death from cardiovascular causes or first hospitalization for worsening HF was 0.80 (95% CI, 0.73–0.87; $P < 0.001$). Furthermore, when comparing sacubitril/valsartan with enalapril, the absolute risk reductions for death from cardiovascular cause and first hospitalization for worsening HF were found to be 3.2% ($P < 0.001$) and 2.8% ($P < 0.001$), respectively (109, 110). The total daily strength of the combination product used in the trial offered bioavailability similar to 320 mg valsartan. Although this is the desired target dose of valsartan according to the ACCF/AHA heart failure guidelines, the comparator (enalapril) was not pushed to its desired target dose. While less than the desired target dose, the studied dose of enalapril in PARADIGM-HF is reflective of the doses used in previous trials, such as CONSENSUS and SOLVD (108). Therefore, this new ARB and neprilysin inhibitor combination offers an additional option for patients who have optimized current guideline-supported therapies.

Special consideration should be given when determining the appropriate dose of sacubitril/valsartan. Clinical trials, such as PARADIGM-HF, studied Entresto 200 mg, which includes sacubitril 97 mg and valsartan 103 mg. Available preparations now include a range of sacubitril and valsartan strengths, including the dose studied in PARADIGM-HF, as well as doses that were not studied in the trial, including sacubitril 24 mg/valsartan 26 mg and sacubitril 49

mg/valsartan 51 mg. The valsartan component in the combination product is more bioavailable than valsartan in other marketed formulations. Valsartan strengths of 26 mg, 51 mg, and 103 mg in sacubitril/valsartan offer a similar bioavailability to valsartan 40 mg, 80 mg, and 160 mg, respectively, in other marketed formulations (91).

During the single-blind run-in period with enalapril and sacubitril/valsartan in PARADIGM-HF, 12.0% of the patients withdrew because of an adverse event (108). Adverse reactions to ARNIs include hypotension, hyperkalaemia, increased serum creatinine, angioedema, cough, and renal failure. Although there were fewer incidences of angioedema in clinical trials with ARNIs than with the combined ACE and neprilysin inhibition, PARADIGM-HF showed that the risk of angioedema was still a concern (108). Angioedema occurred in 19 patients in the sacubitril/valsartan group and 10 patients in the enalapril group ($P = 0.13$) (108). However, only 5% of the patients enrolled were African-American. Because African-Americans have a relatively higher risk of angioedema with ACE inhibitors and ARBs, the optimal agent for this high-risk population remains unclear. Monitoring parameters for ARNIs include baseline and periodic serum potassium, renal function, and blood pressure. ARNIs should be used with caution in patients with aortic/mitral stenosis, renal artery stenosis, or renal/hepatic impairment. Medications that work on the RAAS system (including ARNIs) should be discontinued as soon as pregnancy is detected because these agents can cause injury or death to the developing foetus. The 2021 ESC Guidelines focused update recommends use of ARNIs in patients with chronic symptomatic HFrEF NYHA class II or III as a replacement for an ACE inhibitor to reduce morbidity and mortality in conjunction with beta-blocker therapy. These guidelines caution not to administer ARNIs with ACE inhibitors or within 36 hours of the last dose of an ACE inhibitor due to the increased risk of angioedema. ARNIs should also not be administered in patients with a history of angioedema (1).

2.6.7. Sodium-glucose co-transporter 2 inhibitors

Recently, the use of sodium-glucose co-transporter 2 inhibitors (SGLT2i) appeared one of the first-in line therapies for glucose control in patients with HFpEF/HFrEF and diabetes. Indeed, the administration of these drugs reduced the hospitalizations and cardiovascular-related death

in patients with and without diabetes. The anti-diabetic properties of SGLT2 inhibitors are attributable to interrupting of renal glucose reabsorption thereby lowering blood pressure as well as weight loss (**Figure 7**) (91). Indeed, SGLT2 is a cotransporter predominantly expressed in the early portion of proximal tubule where participates to reabsorption of 80-90% of glucose filtered in kidney glomerulus. Clinically available SGLT2 inhibitors are dapagliflozin, empagliflozin, canagliflozin and ertugliflozin (91).

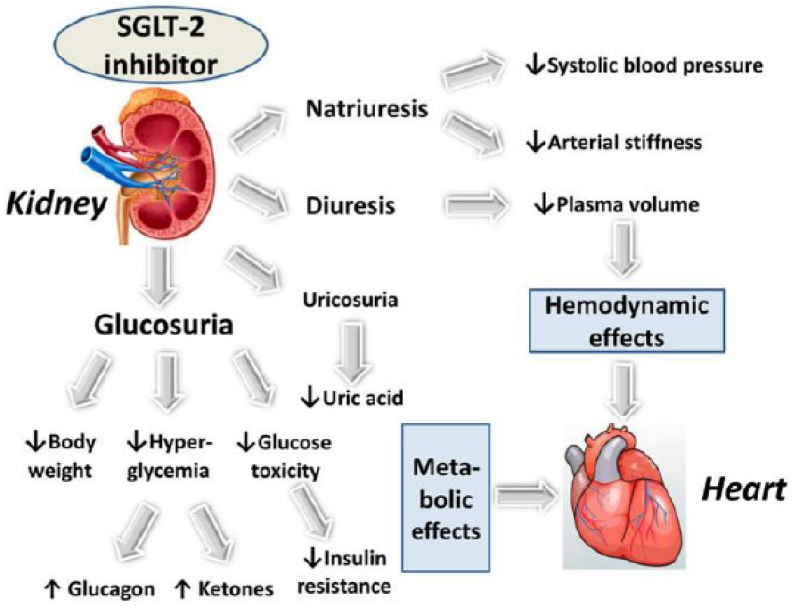

Figure 7. Primary mechanisms of SGLT2 inhibitors. *(From Scheen, 2018* (114)*)*

The DAPA-HF trial investigated the long-term effects of dapagliflozin compared to placebo in addition to optimal medical therapy (OMT), on morbidity and mortality in patients with ambulatory HFrEF (115). Patients participated in the trial if they were in NYHA class III-V and had an LVEF < 40% despite OMT. Patients were also required to have an elevated plasma NT-proBNP and an eGFR > 30 mL/min/1.73 m2 (115). Therapy with dapagliflozin resulted in a 26% reduction in the primary endpoint: a composite of worsening HF or CV death. Both of these components were significantly reduced. Moreover, dapagliflozin reduced all-cause mortality, alleviated HF symptoms, improved physical function and QOL in patients with symptomatic HFrEF (115-118). Benefits were seen early after the initiation of dapagliflozin, and the absolute

risk reduction was large. Survival benefits were seen to the same extent in patients with HFrEF with and without diabetes, and across the whole spectrum of HbA1c values (117-119). Subsequently, the EMPEROR-Reduced trial found that empagliflozin reduced the combined primary endpoint of CV death or HF hospitalization by 25% in patients with NYHA class IIIV symptoms, and an LVEF < 40% despite OMT (115-116). This trial included patients with an eGFR > 20 mL/min/1.73 m2 and there was also a reduction in the decline in eGFR in individuals receiving empagliflozin. It was also associated with an improvement in QOL. Although there was not a significant reduction in CV mortality in the EMPEROR-Reduced trial, a recent meta-analysis of the DAPA-HF and EMPEROR-Reduced trials found no heterogeneity in CV mortality (115-116). Therefore, dapagliflozin or empagliflozin are recommended, in addition to OMT with an ACE-I/ARNI, a beta-blocker and an MRA, for patients with HFrEF regardless of diabetes status. The diuretic/natriuretic properties of SGLT2 inhibitors may offer additional benefits in reducing congestion and may allow a reduction in loop diuretic requirement (118). The combined SGLT-1 and 2 inhibitor sotagliflozin, has also been studied in patients with diabetes who were hospitalized with HF. The drug reduced CV death and hospitalization for HF (120). Therapy with SGLT2 inhibitors may increase the risk of recurrent genital fungal infections. A small reduction in eGFR following initiation is expected and is reversible and should not lead to premature discontinuation of the drug.

2.6.8. Diuretics

Although no data have shown that they reduce mortality or hospital readmission, diuretics are the only agents that can adequately control the fluid retention associated with HF*r*EF. Unless contraindicated, diuretics are recommended in all HFrEF patients with fluid retention to improve symptoms. Diuretic use is generally combined with moderate dietary sodium restriction (91).
Loop diuretics, such as furosemide, are the preferred diuretic agents for most HFrEF patients (91). Loop diuretics work at the thick ascending limb of the loop of Henle to inhibit sodium and chloride reabsorption (91). In comparison, thiazide diuretics are less potent and thus have a less significant effect on fluid retention/oedema (121-123). Thiazides work at the renal distal convoluted tubule to inhibit the sodium chloride cotransporter. Due to their antihypertensive

effects, thiazide diuretics may be the preferred diuretic agents for HF*r*EF patients with concurrent hypertension and mild fluid retention (122). Some HFrEF patients may remain volume-overloaded despite the use of maximal loop diuretic therapy (91). Such loop diuretic resistance may be overcome by intravenous administration of loop diuretics or by the addition of a thiazide diuretic (1, 91, 121).

Adverse effects of diuretics include fluid depletion, hypotension, azotaemia, and depletion of sodium, potassium, magnesium, chloride, and calcium. Typical monitoring parameters for these agents include daily weight and blood pressure measurements, and periodic monitoring of renal function. Because loop and thiazide diuretics may increase uric acid, patients utilizing these agents should be monitored for changes in uric acid levels as well as signs and symptoms of gout. The presence of orthopnoea and B-type natriuretic peptide levels should be followed daily if possible, during inpatient admissions (91).

Diuretic therapy is initiated at low doses and is titrated up as needed and as tolerated. Adequate treatment is not determined by reaching a set target dose, but rather by looking for an increase in urine output and a 0.5-kg to 1.0-kg decrease in daily weight (91). These clinical markers should be monitored closely to determine appropriate patient-specific diuretic doses.

Loop diuretics are recommended to reduce the signs and/or symptoms of congestion in patients with HFrEF. The quality of the evidence regarding diuretics is poor and their effects on morbidity and mortality have not been studied in RCTs. However, it should also be remembered that the major disease-modifying treatment trials for HFrEF were conducted with a high background use of loop diuretic therapy. One meta-analysis has shown that in patients with HFrEF, loop and thiazide diuretics appear to reduce the risk of death and worsening HF compared with a placebo, and compared with an active control, diuretics improve exercise capacity (121-123). Loop diuretics produce a more intense and shorter diuresis than thiazides, although they act synergistically (sequential nephron blockade) and the combination may be used to treat diuretic resistance. However, adverse effects are more likely, and these combinations should only be used with care. Of note, ARNI, MRAs, and SGLT2 inhibitors may also possess diuretic properties (121, 122). The aim of diuretic therapy is to achieve and maintain euvolemia with the lowest diuretic dose. In some euvolemic/hypovolemic patients, the use of a diuretic drug might be reduced or discontinued (123). Patients should be trained to self-adjust their diuretic dose based on monitoring of symptoms/signs of congestion and daily weight measurements.

2.6.9. Angiotensin II type 1 receptor blockers

Angiotensin receptor blockers (ARBs) inhibit the RAAS by blocking the binding of angiotensin II to its receptor, which in turn leads to vasoconstriction and prevents the release of aldosterone. Although their mechanism of action is similar to that of ACE inhibitors, ARBs do not cause an inhibition of kininase, which reduces the incidence of cough in comparison with ACE inhibitors (91). The place of ARBs in the management of HFrEF has changed over the last few years. The 2021 ESC guidelines recommend that ARBs be used to reduce morbidity and mortality in patients who are intolerant of ACE inhibitors or ARNI because of cough or angioedema, or in patients who are tolerating ARBs for another indication (1). In addition, the 2021 guidelines recommend that ARBs be used with caution in patients with a history of angioedema with ACE inhibitors because of the risk of cross-reaction. For patients with HFrEF NYHA class II or III, the guidelines recommend replacing ARB therapy with an ARNI (1).

Placebo-controlled trials have shown that the use of ARBs reduces hospitalization and mortality. The 2003 Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM Alternative) study evaluated whether candesartan could improve cardiovascular outcomes compared with placebo, including the composite endpoint of cardiovascular death or hospital admission in patients with symptomatic HF with an EF of 40% or less who were intolerant of ACE inhibitors (124). The primary outcome of cardiovascular death or hospitalization for HF occurred in 33% of candesartan patients versus 40% of placebo patients (124). However, no ARB has reduced all-cause mortality in any trial (124,125).

When initiating ARB therapy, start with a low dose and titrate up as tolerated by doubling the dose to the target. Baseline renal function and serum potassium should be established prior to initiating ARB therapy. ARBs can cause hyperkalaemia due to the inhibition of aldosterone, often in combination with other predisposing factors such as combination medications or physiological conditions that have reduced serum aldosterone concentrations. It is important to monitor these assays regularly to identify abnormalities because modifications of the patient's drug therapy or dietary intake of potassium may be required (91).

2.6.10. Soluble guanylate cyclase (sGC) stimulator

Vericiguat, a novel soluble guanylate cyclase (sGC) stimulator, is approved for the treatment of HFrEF. Decreased nitric oxide (NO) availability, sGC desensitization to NO, sGC deficiency, and reduced cyclic guanosine monophosphate (cGMP) signalling are potential contributing factors for HF disease progression. Vericiguat works via stimulation of sGC in the critical NO-sGC-cGMP pathway. Vericiguat is primarily metabolized by glucuronidation via uridine diphosphate-glucuronosyltransferase (UGT) isoforms UGT1A1 and UGT1A9. Urinary excretion and renal clearance of vericiguat are low. No intrinsic factor had a clinically relevant effect on vericiguat exposure (126). Vericiguat has low drug-drug interaction potential with no clinically relevant pharmacokinetic or pharmacodynamic interactions observed with warfarin, digoxin, aspirin, or sacubitril/valsartan. The global phase III study VICTORIA included patients with HFrEF who had a recent HF hospitalization or intravenous diuretic treatment for HF (127). Treatment with vericiguat on top of standard of care resulted in a 10% relative reduction in the primary composite outcome of death from cardiovascular causes or first hospitalization for HF. Vericiguat was well-tolerated with low incidence of symptomatic hypotension and syncope compared to placebo. Given its positive benefit–risk profile, vericiguat is an important option for high-risk patients with HFrEF who are already on guideline-directed medical therapy and had recent worsening of HF (91).

2.6.11. Cardiac myosin activator

Cardiac myosin modulators work by directly interacting with the myosin heads in cardiac muscle fibers, influencing the contractile process of the heart. Myosin is a pivotal protein in muscle contraction, playing a crucial role in converting chemical energy from ATP into mechanical force. This force generation is essential for the proper pumping action of the heart. By modulating the activity of myosin, these drugs can enhance or inhibit the contraction strength,

thus directly affecting cardiac output (91) The GALACTIC-HF study assessed the efficacy and safety of the cardiac myosin activator, omecamtiv mecarbil, in HFrEF patients, enrolling patients in both the inpatient and outpatient settings. The primary endpoint of a first HF event or CV death was reduced by 8%. There was no significant reduction in CV mortality. Currently, this drug is not licensed for use in HF. However, in the future it may be able to be considered, in addition to standard therapy for HFrEF to reduce the risk of CV mortality and hospitalization for HF (127).

2.7. Comorbidities in Heart Failure: Atrial Fibrillation

Atrial fibrillation (AF) is a frequent arrhythmia consisting of a complete disorganization of atrial electrical activity (128). AF causes a reduction of cardiac output due to the absence of atrial contribution to ventricular filling during diastole. Moreover, AF is characterized by the absence of mechanical activity of atria with a consequent higher risk of left atrial thrombosis (86,87). AF and HF frequently coexist (128,129). They can cause or exacerbate each other through mechanisms such as structural cardiac remodelling, activation of neurohormonal systems, and rate-related LV impairment (128-132). The proportion of patients with HF who develop AF increases with age and HF severity. When AF causes HF the clinical course seems more favourable than with other causes of HF (so called tachycardiomyopathy) (133). In contrast, development of AF in patients with chronic HF is associated with worse prognosis, including stroke and increased mortality (134,135). The management of patients with concomitant HF and AF includes: 1. identification and treatment of possible causes or triggers of AF; 2. management of HF; 3. prevention of embolic events; 4. rate control; 5. rhythm control. Potential causes or precipitating factors such as hyperthyroidism, electrolyte disorders, uncontrolled hypertension, mitral valve disease, and infection should be identified and corrected. Worsening congestion due to AF should be managed with diuretics. Congestion relief may reduce sympathetic drive and ventricular rate and increase the chance of spontaneous return to sinus rhythm. The presence of AF may reduce or abolish the prognostic benefits of beta-blockers and render ivabradine ineffective (10). Some treatments for HF decrease the risk of developing AF, including ACE inhibitors, slightly, and CRT, probably (136, 137). Unless contraindicated, an oral, long-term

anticoagulant is recommended in all patients with HF and paroxysmal, persistent, or permanent AF. Direct-acting oral anticoagulants (DOACs) are preferred for the prevention of thromboembolic events in patients with AF and without severe mitral stenosis and/or mechanical valve prosthesis, as they have similar efficacy to vitamin K antagonists (VKAs) but a lower risk of intracranial haemorrhage (138). LA appendage closure can be considered in patients with HF and AF who have a contraindication to oral anticoagulation though data from randomized trials have not included patients with contraindications to oral anticoagulants (139,140). Data regarding rate control are not conclusive for the patients with AF and HF. A strategy of lenient rate control, defined by a resting heart rate <110 b.p.m., was compared to a strategy of strict rate control, defined by a heart rate <80 b.p.m. at rest and <110 b.p.m. during moderate exercise, in RACE II and in a pooled analysis of RACE and AFFIRM (141). The studies showed no differences in outcome between the two strategies. Beta-blockers can be used for rate control in patients with HFrEF or HFmrEF because of their established safety in these patients (136,142,143). Digoxin or digitoxin can be considered when the ventricular rate remains high, despite beta-blockers, or when beta-blockers are contraindicated or not tolerated (144,145). It may therefore be considered also an alternative to beta-blockers. For patients with NYHA class IV and/or haemodynamic instability, i.v. amiodarone can be considered to reduce ventricular rate (146). AV node ablation can be considered in patients with poor ventricular rate control despite medical treatment not eligible for rhythm control by catheter ablation or in patients with biventricular pacing (136, 147-149). Urgent electrical cardioversion is recommended in the setting of acute worsening HF in patients presenting with rapid ventricular rates and haemodynamic instability, after consideration of the thromboembolic risk. Cardioversion should be considered also to improve symptoms in patients who have persistent and symptomatic AF, despite optimal pharmacological management. In patients who do not receive chronic therapy with oral anticoagulant and with AF onset >48 h, at least 3 weeks of therapeutic anticoagulation or a transoesophageal echocardiography is needed before cardioversion (94). When pharmacological cardioversion is preferred, amiodarone is the drug of choice as other antiarrhythmic drugs (i.e. propafenone, flecainide, dronedarone) are associated with worse outcomes in HFrEF (150-153). Amiodarone can help maintain HF patients in SR after cardioversion (154-155). Overall, there is insufficient evidence in favour of a strategy of rhythm control with antiarrhythmic drugs vs. rate control in patients with HF and AF (156-163).

2.8. Artificial Intelligence, Machine Learning and Heart Failure

HF is one of the most expensive healthcare conditions to manage in high-income countries. Patients receive numerous diagnostic tests, invasive procedures, and therapies over the course of their illness, generating large amounts of data that can be aggregated into registries or other institutional databases to evaluate healthcare utilization, quality and cost of care, and disease progression (164). The size, complexity, and dynamic nature of these 'big data' can be challenging for traditional analytical methods to make sense of Machine Learning (ML) encompasses computational techniques that can extract patterns from data, acquire knowledge, and apply this knowledge to tasks such as risk prediction (165,166). ML methods can handle temporal, large-volume, and multi-modality data [e.g., sound, language, tabular electronic health record (EHR), imaging, and metabolomic data] (167). In HF, delivering the right care to the right patient is challenged by diagnostic uncertainty, variation in treatment and safety response due to suboptimal generalizability of clinical trial results, complexity in risk stratification, and limited integration of information at the point of care. ML can play an important role in bridging these gaps in HF and has important advantages over traditional human-derived models.

Artificial intelligence (AI), the imitation of human cognition by technology, can be used to guide clinical care and decision-making without human involvement in the process. One sub-field of AI is ML, which provides computers with the capacity to evaluate data beyond programmatic algorithms, identifying patterns within data, mapping learned patterns to unseen data, and improving the performance of computational tasks beyond human capabilities (168).

ML and traditional statistical approaches have several unique as well as overlapping capabilities. ML methods are well-equipped to handle high-dimensional datasets with a very large number of variables that make traditional statistical approaches such as regression challenging. ML can also handle correlated or collinear data points and assess complex interactions between predictors. ML does not typically isolate the 'effect' of a single variable and does not require that predictor variables be selected a priori (169). ML can also generate dynamic models, where the training data are continuously updated to account for temporal changes in data. For example, 'baseline' characteristics such as haemodynamic, laboratory values, and comorbidities may evolve during

a study. The evolution of these characteristics may be important in predicting outcomes, but traditional statistical approaches are often not equipped to handle them. ML algorithms allow for higher performing, accurate computation of non-linear relationships, but the higher accuracy comes at the cost of interpretability (170) (**Figure 8**).

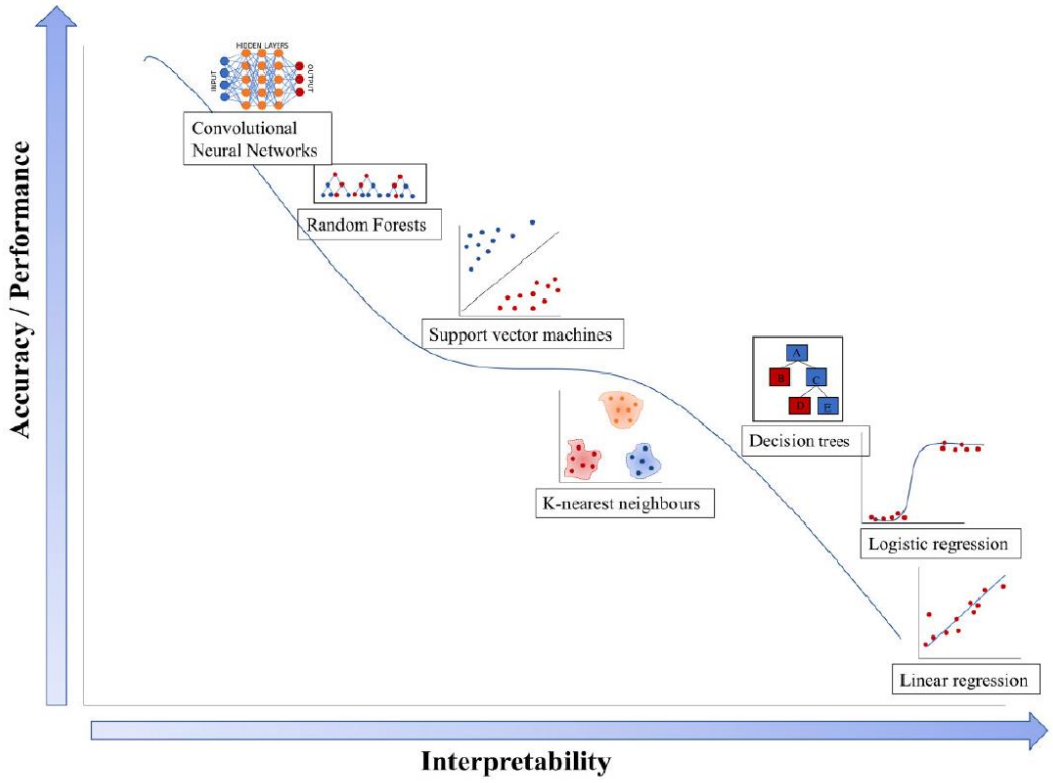

Figure 8. Comparison of the accuracy and interpretability of statistical vs. machine learning models. Traditional regression models demonstrate poor accuracy but are easier to interpret. Machine learning models with non-linear relationship offer superior accuracy but are harder to interpret. Adapted from Stewart 2020.

ML can be supervised or unsupervised. Supervised ML uses human-labelled data to learn the underlying patterns in a process called 'model training'. Data labelling of outcomes - for example, 'hospitalized' or 'not hospitalized'- requires human input. The algorithms then learn the relationships between variables and outcomes. As new input data is fed into the model,

weights are adjusted until the model has been fitted appropriately. Using this method, a model can be trained to predict events (e.g. hospitalization) in new datasets.

In contrast to supervised learning, unsupervised ML algorithms are exploratory and discover patterns without human-labelled data (169). By recognizing clinically relevant patterns or phenotypes that may not be evident to the clinician, ML can unearth disease mechanisms and improve the accuracy of diagnosis, management, and risk prediction in cardiovascular medicine (169). Deep learning (DL) is a subset of ML that uses multiple layers of artificial neural networks to identify patterns or make predictions of patterns (171). There is a hierarchy in the arrangement of layers, from learning simple representations of data to more complex relationships as the data is passed through deeper layers. DL is particularly useful with big data, as it does not require variable selection or rely as much on feature engineering to learn from big data sources such as EHRs (172). For example, DL models can predict incident HF by examining temporal relations amongst a large number of evolving variables (i.e., comorbidities, physiologic measures, laboratory indices, medication prescriptions, invasive procedures) (173).

2.8.1. Machine learning applications in Heart Failure

2.8.1.1. Prediction of Heart Failure

ML algorithms can identify risk factors for incident HF. In a prospective cohort of over 500 000 individuals in the United Kingdom, a supervised ML model confirmed leg bioimpedance as a major risk factor in addition to known risk factors for HF; lower leg bioimpedance values were associated with HF incidence during the 9.8-year follow-up (174). The resulting ML model, comprising leg bioimpedance, age, sex, and self-reported myocardial infarction provided a highly accurate prediction of incident HF without the variables being prespecified. This demonstrates that ML can identify novel HF risk factors for HF that may not otherwise be considered. A further application of ML algorithms may be the prediction of disease in populations that are not represented in registries or trials in which traditional clinical prediction models were derived or validated; utilizing a large set of unspecified variables to predict disease instead of limiting variables to those generated from homogenous research populations may mitigate historical structural biases and research inequities (175,176).

In a prospective study of patients enrolled in the Action to Control Cardiovascular Risk in Diabetes trial, a risk score for 5-year HF incidence was created using ML techniques. (177) The supervised ML model demonstrated better discrimination than a traditional Cox-proportional hazards model in predicting incident HF within the cohort in the 4.9 years of study follow-up. (177). Models that predict HF tend to treat race or ethnicity as a covariate, rather than developing race-specific models, which may be more appropriate due to variations in risk factors across racial or ethnic groups. (178). In a retrospective pooled analysis of cohort studies, ML was used to develop a race-specific model to predict HF incidence, (178) and it outperformed a traditional, non-race specific model. (178).

2.8.1.2. Diagnosis of Heart Failure

ML algorithms could assist physicians in early diagnosis of HF in at-risk patients. An electrocardiogram (ECG) is a non-invasive, widely available tool that can be used for early diagnosis of HF (179). A DL algorithm for ECG-based HF identification (DEHF) was developed and validated for this purpose, (179) using data including demographic information and ECG features from EHRs (179). The DEHF algorithm was superior in detecting HF with reduced ejection fraction (HFrEF) (C-statistic 0.843, 95% CI 0.840–0.845) compared to logistic regression (C-statistic 0.800, 95% CI 0.797–0.803) and random forest ML algorithms (C-statistic 0.807, 95% CI 0.804–0.810) (180).

AI when combined with expert knowledge may be superior to AI alone. An AI-Clinical Decision Support System that was developed using expert knowledge combined with a ML approach for the diagnosis of HF with reduced, mildly reduced, and preserved ejection fraction improved diagnostic accuracy over an expert-driven or ML approach alone (180). The variables in the ML model included left ventricular ejection fraction, left atrial volume index, left ventricular mass index, ECG features, clinical features and physical exam features.

Earlier recognition and improved diagnostic accuracy of HF may allow for more timely investigations for the underlying aetiology and earlier initiation of guideline-indicated therapy to delay disease progression. The use of ML as a diagnostic aid in HF is a nascent field, however.

In the absence of external validation and prospective testing of interventions based on ML outputs, the clinical impact remains to be realized.

2.8.1.3. Classification of Heart Failure phenotypes

ML may improve the current classification of HF. Compared to HFrEF, the underlying phenotypic heterogeneity is more complex in HF with preserved ejection fraction (HFpEF) (181-183). A prospective study of 397 ambulatory patients with HFpEF performed phenotype mapping using ML algorithms with data from EHRs (182). This technique resulted in a novel classification method for HFpEF, which clusters study participants into phenotypes according to clinical characteristics, ECG and echocardiographic parameters, invasive haemodynamic, and outcomes (182). These findings are important, as the improved classification of HFpEF may facilitate recruitment of patients most likely to benefit from a given intervention in randomized trials (182). Another unsupervised ML analysis of 1693 patients hospitalized with HF across the left ventricular ejection fraction (LVEF) spectrum revealed 6 discrete pheno-groups based on common comorbidities: coronary artery disease, valvular heart disease, atrial fibrillation, chronic obstructive pulmonary disease, obstructive sleep apnoea, or few comorbidities (184). Pheno-groups were LVEF-independent, with each group encompassing a wide range of LVEF. The groups stratified risk of composite all-cause death or hospitalization as well as a composite cardiovascular death or HF hospitalization at 6- and 12-months post-discharge more effectively than LVEF (184).

Similarly, unsupervised ML can be used to establish clinical pheno-groups with predictive values based on transcriptomic or metabolomic profiles 31 (185). Such pheno-groups or subgroups may have differential response to therapies (186), but this needs to be proven in prospective studies.

2.8.1.4. Prediction of outcomes following Heart Failure diagnosis

Existing HF prediction models are underused among cardiologists due to their complexity, lack of integration with workflow, and limited knowledge on how risk prediction can be used to improve outcomes (187). Risk prediction models aim to identify patients who are at risk of adverse events or who may benefit from closer follow-up and post-discharge services. From a systems level, risk stratification is important considering the readmission penalties imposed by

the Medicare Hospital Readmissions Reduction Programme (188). However, accurate risk prediction remains an unmet need which may be met through ML. The ML algorithm for predicting mortality of patients with acute HF, is a risk stratification model for predicting in-hospital and long-term mortality. Evidence from a large retrospective cohort study in Korea demonstrated the ability of acute HF to outperform mortality risk prediction models for HF such as Get with the Guidelines-Heart Failure Score (GWTG) and Meta-Analysis Global Group for Heart Failure (MAGGIC) (189). The DAHF predicted in-hospital through to 36-month mortality with greater discrimination than the GWTG risk score for in-hospital mortality (C-statistic 0.880; 95% CI 0.876–0.884 vs. 0.728; 95% CI 0.720–0.737) and the MAGGIC risk score for 36-month mortality (C-statistic 0.813; 95% CI 0.810–0.816 vs. 0.729; 95% CI 0.726–0.733) (189). This may be because DL algorithms do not limit the number of input predictive factors, preventing the unintended loss of data that comes from restricting analyses to known associations (189).

Data from cardiac monitoring, either external or implantable, can be used in real time to develop algorithms for risk prediction. The Multisensor Non-invasive Remote Monitoring for Prediction of Heart Failure Exacerbation (LINK-HF) study examined the accuracy of a remote monitoring system using a ML algorithm to predict hospitalization (unplanned non-trauma hospitalization) (190). The platform was able to predict precursors to hospitalization with a median alert time of 6.5 days in advance of the readmission (190). Implantable haemodynamic monitoring systems of pulmonary artery pressures (PAP) have demonstrated conflicting effects on clinical endpoints in the CardioMEMS Heart Sensor Allows Monitoring of Pressures to Improve Outcomes in New York Heart Association (NYHA) Functional Class III Heart Failure Patients (CHAMPION) and the Haemodynamic-guided Management of Heart Failure (GUIDE-HF) trials (191,192). Unlike the CHAMPION trial in which implantable haemodynamic-guided HF therapy decreased the rate of HF hospitalizations, PAP monitoring did not reduce the primary composite endpoint or component endpoints of all-cause mortality or HF hospitalization in the GUIDE-HF trial (192). In a sensitivity analysis of GUIDE-HF, a significant treatment effect was observed with PAP-guided HF therapy prior to, but not during, the COVID-19 pandemic (192). Event rates decreased during the COVID-19 pandemic and there was no longer a difference in guideline-directed medical therapy (GDMT) changes in the treatment relative to control groups during the pandemic; this, along with changes in patient behaviour (e.g. improved medication adherence, better nutrition, and hospital avoidance) may have attenuated the estimated treatment effect of PAP-guided care during the pandemic in GUIDE-HF (192). While the breadth of evidence does not favour the use of PAP monitoring overall, ML algorithms with haemodynamic and EHR data

from PAP-monitored patients may help determine which patients benefit the most from this intervention.

The HeartLogic Index™, a proprietary algorithm derived from Boston Scientific Cardiac resynchronization-defibrillator (CRT-D) device data, is an example of applied ML (193). The algorithm-using heart sounds, thoracic impedance, respiratory rate, tidal volume, heart rate and patient activity-delivers an alert when a certain threshold is reached. This index was demonstrated to predict HF events (hospitalization or need for IV diuretics) (193) and can potentially also be used to detect subclinical decompensation (194). At a cut-point of 16, the HeartLogic Index provides clinicians with an alert signalling an increased risk of HF hospitalization (modest performance with sensitivity 70% and positive predictive value 11.3%). As in the case of non-ML models, there is a paucity of randomized clinical trial evidence on the effect of care pathways guided by these alerts. Thus, the impact of the HeartLogic Index on clinical outcomes has yet to be established.

2.8.1.5. Optimization of medical therapy

A majority of patients do not receive target doses of evidence-based medical therapies in HF, possibly due to under-prescribing by clinicians, barriers to access, or intolerance to medications (1). ML algorithms have been used to improve HF medical management by assessing for heterogeneity in response to HF therapies. For example, ML methods were applied to EHRs of 44 886 patients in the Swedish HF Registry assess for heterogeneity in response to HF pharmacotherapy across propensity-matched clusters (195). Four clusters, based on demographic, NYHA class, LVEF, comorbidities and lab indices, were identified with marked differences in 1-year survival and response to therapies (196). Thus, ML may be used to better classify HF patients into high- and low-risk subgroups and identify those most likely to derive benefit with the least side effects in GDMT.

ML can potentially be used to optimize GDMT prescription in HF and identify patients at risk for adverse drug reactions. By extracting data from EHRs, ML algorithms could be used to provide recommendations to clinicians regarding optimal sequencing and dosing of evidence-based therapies (197). This approach could help reach a wider range of patients who may not

otherwise have access to multidisciplinary HF clinics that are often concentrated in urban centres.

2.9. Animal models of Heart Failure and evaluation of drug treatments

Cardiovascular diseases have a strong impact on public health due to high morbidity and mortality. Additionally, the pathophysiology of many disorders involving cardiovascular system is not well understood yet. Therefore, the identification of new therapeutic approaches to effectively treat these pathologies is one of the major challenges in scientific research world. In this context, the study of animal models resembling the phenotypic features of cardiovascular disease is very useful for the identification of new pharmacological targets and the evaluation of the drug efficacy. The main experimental animal models used for HF and hypertension studies include spontaneously hypertensive rats (SHR) and salt-sensitive Dahl (ss-Dahl) rats as well as rats treated with an acute or chronic salt diet overload or with desoxycorticosterone acetate (DOCA).

2.9.1. Cardiac and renal characteristics of the Dahl animal model of Heart Failure with preserved Ejection Fraction (HFpEF)

A useful non-diabetic animal model is the ss-Dahl rat, reproducing the aspects of the multimorbid chronic condition associated with HF. Several studies demonstrated that the NaCl overload induced acute as well as chronic proinflammatory and profibrotic effects in heart and in kidney. Indeed, 5 weeks of high salt diet (8% NaCl) in Dahl rats (7 weeks old) led to spontaneous hypertension (74, 75, 198). Treatment with the same high-salt diet continued for 8 weeks induces the HF subtype with HFpEF). The heart of this HF animal model shows alterations in diastolic function, increased circulating brain natriuretic peptide concentrations, cardiomyocyte hypertrophy and massive accumulation of fibrotic tissue (74). The observed cardiorenal disorder is also accompanied by inflammatory processes, oxidative stress and endothelial dysfunction. In particular, the upregulation of NF-κB promotes the transcriptions of several pro-inflammatory

mediators including TNFα, IL-1β, IL-6, and monocyte chemoattractant protein-1 (MCP-1) (74). The infiltration of immune system cells led to an avid sodium retention and increase of vascular resistance. As it is well known, the chronic renal disease frequently coexists with cardiac dysfunctions as consequence as well as cause (55, 56, 57). In the ss-Dahl rats fed with high salt diet the signs including increase of blood pressure, serum creatinine levels as well as increase of albuminuria and UAC ratio were associated with kidney injury (75). Moreover, the high salt intake promotes glomerulosclerosis and tubulointerstitial fibrosis confirming the presence of remarkable renal fibrosis (75). Furthermore, within renal compartment, this animal model showed a markedly increased expression of Nox4, the main NADPH isoform to produce ROS, and an increase of the levels of 8-OhdG, a marker of DNA damage (75). The increased levels of E-selectin associated with a dramatic reduction of eNOS levels, indicating vascular endothelium dysfunction, further underline the cardiorenal disorders phenotype characterizing this HF animal model (75).

2.9.2. Beneficial effects of SGLT2 inhibitor dapagliflozin on failed cardiac function of the Dahl animal model of HFpEF

Several clinical and preclinical studies highlighted the cardiac positive effects following gliflozins administration associated with an improvement of diastolic function. Recently, Cappetta et al (74) demonstrated the cardiac positive effects of dapagliflozin, using the non-diabetic HF animal model represented by the ss-Dahl rat fed with a high salt diet. In this work, the authors focused on the dapagliflozin effects mediated on the diastolic function. Moreover, the results showed that dapagliflozin may influence the expression of proteins involved in the sodium and calcium homeostasis. Sodium and calcium ions are important for the cardiomyocyte excitation-contraction coupling. The increased level of CaMKII in the diseased heart suggested an impaired regulation of ion handling proteins. Following chronic treatment with dapagliflozin, the lowering of diastolic sodium and calcium overload is closely associated with improvement of the compromised diastolic function. Being HFpEF a multimorbid syndrome frequently characterized by the promotion of pro-inflammatory processes one of the commonest signs can be observed in the coronary vascular endothelium dysfunction. Moreover, the upregulation of

VCAM1 and E-selectin as well as the downregulation of eNOS were observed in the coronary endothelium of the HFpEF animal model, confirmed the progression of cardiac dysfunction. The treatment with dapaglifozin partially restored the VCAM1, E-selectin and eNOS levels confirming its positive role in the amelioration of endothelial status (74). The endothelium dysfunction is strictly linked to inflammatory state generating a vicious loop involving oxidative stress, as indicated by the marked increase of NF-κB, IL-6 and MPC-1 characterizing HFpEF Dahl animal model (**Figure 9**). Consistently, dapagliflozin interferes with macrophages infiltration and reduced cytokines production (**Figure 9**).

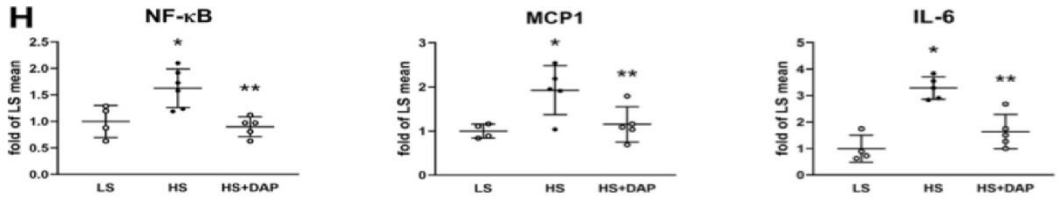

Figure 9. Effect of dapagliflozin. Bar graphs of NF- κB, MCP1 and IL-6 protein expression *(From Cappetta et al, 2020* (74)*)*

Finally, animals with HFpEF presented increased levels of the profibrotic biomarker, i.e. galactin-3, resulting reduced after the treatment with dapagliflozin (74).

In summary, the cardioprotection induced by dapagliflozin is due to the lowering of proinflammatory state and endothelium dysfunction as well as fibrosis. To gain insights into the mechanism involved in the dapagliflozin-induced protective effect on cardiac endothelium improving diastolic parameters, the evaluation of SGTL1 and NHE1 expression levels was detected (74). Indeed, given the lack of SGTL2 isoform expression in cardiac tissue as well as the increase of cardiac SGTL1 isoform associated with cardiovascular disorders (28), it is reasonable to hypothesized that dapagliflozin induce its positive effect on myocardium by directly interacting with SGTL1. Moreover, recent findings showed the binding between dapagliflozin and the extracellular domain of NHE1 (74). Importantly, hearts of HFpEF Dahl animal showed an up-regulation of NHE1 and SGTL1, which assume control level after dapagliflozin treatment (**Figure 10**) (74).

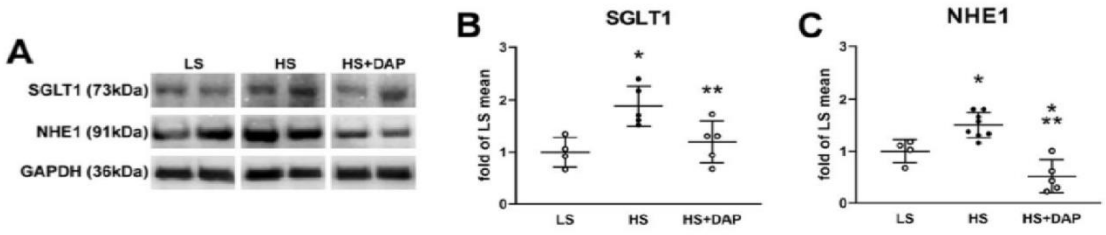

Figure 10. Effect of dapagliflozin. Western blotting gel and bar graphs show protein level expression of SGLT2 and NHE1 *(From Cappetta et al, 2020* (74)*)*

These results confirm the protective role of dapagliflozin in HF in ameliorating diastolic function by exerting a plethora of beneficial effects.

3. RATIONALE AND AIMS

3.1. Aim 1a: to evaluate the short-term anti-remodeling effect of SGLT2 inhibitors in HF patients by a ML approach

Rationale. As aforementioned, gliflozins, which act as inhibitors of SGLT2, are a new class of blood glucose-lowering medications that block renal glucose reabsorption in the proximal tubule, thereby increasing urinary glucose excretion and improving glycaemic control (116,117). Initially approved for the treatment of type 2 diabetes mellitus, SGLT2i provided a reduction in cardiovascular (CV) outcomes (116-118). The EMPA-REG OUTCOME trial with empagliflozin, the CANVAS Program with canagliflozin, the DECLARE-TIMI 58 with dapagliflozin, and real-life data from the CVD-Real Study demonstrated that SGLT2i may reduce CV events and improve both mortality and hospitalization rates in diabetic patients (116-121). More recently, results from the EMPEROR-Reduced trial with empagliflozin and results from DAPA-HF trial confirmed the reduction of CV death, HF hospitalization, and worsening in patients with HFrEF (LVEF $\leq$ 40%), regardless of the presence or absence of diabetes (119-121).

For these reasons, in the HF 2021 European Society of Cardiology Guidelines, gliflozins have been introduced for the treatment of patients affected by HFrEF independently of diabetes (1). Despite robust evidence for the CV benefit of SGLT2i, some important questions remain unanswered. Among them, the effect on cardiac remodeling and function, in particular with regards to the right ventricle (RV), has not been completely established. Moreover, the best timing to start gliflozin treatment and parameters possibly predicting clinical response have not been sufficiently evaluated.

Specific aims. We, therefore, evaluated the short-term CV effect of gliflozins in diabetic patients with HFrEF in terms of LV and RV remodelling and function by applying an ML approach in order to identify variables effective useful in predicting the CV response to gliflozins.

3.2. Aim 1b: to investigate the renoprotective effects mediated by dapagliflozin in HF-induced kidney dysfunction by using an animal model of HFpEF

Rationale. During pathological chronic state, such as HF, the inflammatory component plays a pivotal role in the development of renal dysfunction. Indeed, in a recent preclinical study employing the ss-Dahl rats as animal model of HF, Cappetta et al demonstrated a marked increase of inflammatory mediators in the renal tissues of the hypertensive animals in which chronic kidney dysfunction is established (75). In particular, an upregulation of TNF-α, IL-1β, IL-6, TGF-β expression was observed. As it is well known, inflammatory response, immune system and oxidative stress are tightly interconnected in an auto-reinforcing loop. Intriguingly, it has been postulated that the inflammatory components may contribute to the sodium retention modulating the expression profile of several renal transporters.

Specific aims. We therefore explored the inflammatory status associate with HF-induced kidney dysfunction and assessed the impact of dapagliflozin treatment in this setting by using an animal model of HF.

3.3. Aim 2: to evaluate the impact of therapeutic switching from warfarin to DOACs in patients with HFrEF and AF by ML approach

Rationale. In a recent large observational study, oral anticoagulants proved to reduce mortality in AF patients in the setting of HF (1). Moreover, when compared with VKA-based therapy, the use of DOACs was associated with a better outcome in AF hospitalized patients with HF (199) and a lower risk of all-cause mortality in elderly HF patients with AF and renal dysfunction (200). Interestingly, the switch from VKAs to DOACs in patients suffering from HFrEF and AF was associated with improved endothelial function and C-Reactive Protein levels (201). Regarding the latter effects, it is conceivable that the actions of DOACs can potentially extend beyond their conventional role in anticoagulation as inhibitors of the blood coagulation factors thrombin (thr) and factor Xa (fXa). Indeed, it is well established that these proteases mediate several (patho)physiological processes such as inflammation, atherothrombosis and angiogenesis (202, 203) by triggering the activation of proteinase-activated receptors (PARs). The ability of DOACs to regulate PAR responses provides new insights into their actions beyond anticoagulation. Indeed, several preclinical studies demonstrated that DOACs exhibit pleiotropic actions on endothelial cells such as anti-inflammatory, anti-atherosclerotic and anti-fibrotic effects, as well as the preservation of endothelial integrity (204-207). It has been established that DOACs block some pro-inflammatory processes, regulating the expression of some key cytokines in a plethora of in vitro cell systems (207) as well as in animal models of atherosclerotic lesions (208,209). Furthermore, the decrease in endothelial permeability and reduction in ROS generation have been reported as the main mechanisms underlying the capability of DOACs to enhance endothelial barrier integrity (210,211). In this context, the relationship between DOAC-mediated pleiotropic effects and clinical efficacy still requires investigation. Moreover, some controversies exist on the use of DOACs in HF patients depending on the single different clinical scenario, and a further in-depth analysis of real word clinical data is highly desirable in this context (212).

Specific aims. Based on the above observations, DOACs may have a positive impact on the prognosis of HF patients with AF, regardless of their anticoagulant effect. Herein, using a ML approach, we aim at evaluating the impact of therapeutic switching from warfarin, as representative of VKAs, to DOACs (i.e., dabigatran etexilate, apixaban, rivaroxaban, edoxaban)

in patients with HFrEF and AF in terms of cardiac remodeling, clinical status, endothelial function and inflammation biomarkers.

4. MATERIALS AND METHODS

4.1. Aim 1a-Data Source

From 28 January 2019 to 9 March 2021, we enrolled 78 consecutive diabeti outpatients followed up for HFrEF at Policlinico Riuniti University Hospital (Foggia, Italy). Each patient received evidence-based pharmacological treatment according to the European Society of Cardiology guidelines for treatment of chronic heart failure (1), clinical decision, individual tolerance, and contraindications. Thirty-eight patients were also treated with SGLT2i. Clinical data, echocardiographic, biochemical, and pharmacological parameters (for a total of 66 parameters) were recorded from all patients at baseline and at 4–6 month follow-up (Figure 11). Based on the European Society of Cardiology Guidelines for the Management of HF (1), such a range of time is considered appropriate to assess the short-term efficacy associated with drug administration. Echocardiographic data analysis was performed by the same fully accredited operator using ultrasound device Philips EPIQ 7c (Philips, Amsterdam, Netherlands). Patients without diabetes and with preserved or mid-reduced left ventricular ejection fraction were excluded from the analysis. The informative content provided by the available features was then exploited by means of a random forest (RF) classifier and a SHAP (SHapley Additive exPlanations) explainability analysis. The framework is schematically presented in **Figure 6**.

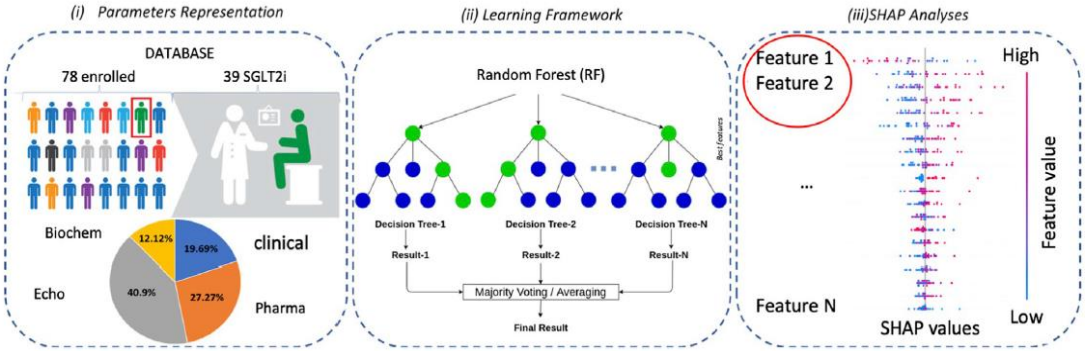

Figure 11. Overview of the methodological approach: (i) the enrolled patients are represented by a set of clinical parameters; (ii) a Random Forest (RF) classifier is then used for classification purposes; and (iii) finally, an explainability analysis is carried out by means of a SHAP analysis.

4.2. Aim 1a-Data Analysis

To evaluate the informative content of the available clinical features, a RF classifier was employed (213). This supervised learning algorithm exploited an ensemble of tree classifiers, each one grown with different features and diverse partitioning of the training set, to achieve a statistically robust classification. In this respect, a subset of the available training examples was first randomly selected to set the model parameters, while those left out, namely, out-of-bag observations, were used instead to evaluate the model performances and minimize overfitting; the square root of available features was randomly selected to increase the branching of the decisional tree at each split, and the operation was repeated until all available examples were assigned to a given class. This procedure was optimized by using Gini's index, a metric assessing the node purity, i.e., the separation between the classes. The goal was to determine the optimal cuts by discriminating against the classes on the basis of the available features. In this respect, several different tree classifiers were trained to achieve statistical robustness, with this task intrinsically related to the number of observations and of the randomly selected features. Final decisions were taken by majority vote, across all generated trees.

The number of trees within the forest and the number of features to pick represented the most important parameters to be tuned for the RF model. The internal validation provided by out-of-bag observations, along with the ease of tuning, made this modeling approach a first choice for many different applications. Moreover, it was worth saying that RF was capable of achieving

state-of-the-art performances that were often comparable even with more sophisticated and computationally demanding algorithms, such as neural networks. Finally, RF provided an embedded way to measure feature importance, thus providing a basic but very effective way to understand which features best contributed to the model's accuracy. Each time a feature was used to split a node, Gini importance measured the purity of the node, i.e., how well that feature was able to separate the available classes. This measure was averaged across the forest and used to rank the importance of each feature based on its impact on node purity. However, such an approach returned an overall view of the feature contribution to the model accuracy, and no information could be derived about a single decision. Noteworthy, the XAI paradigm was employed to make the classifier's decision more transparent and desirable. The Shapley paradigm was adopted (214) to derive classification models that are explainable and easy to interpret. Such an approach was based on the idea that all available features behaved like the players of a team (i.e., the classifier), whose final score summarized the goals of each player. Likewise, the contribution of each feature was modeled through a linear relationship so that the final score assigned by the model to each prediction could be explained as the sum of the contributions of all the features. Thus, this framework not only provided a global feature importance evaluation (as RF already did) but, more importantly, gave the option to "locally" inspect the model decisions and explain how they were reached. According to the SHAP (SHAP website: https://shap.readthedocs.io/en/latest/index.html) package implemented in Python, we evaluated for each feature *j* the *SHAP* value:

$$SHAP_j(\boldsymbol{o}) = \sum_{c:\ j \in c} \left[|c| \times \binom{F}{|c|} \right]^{-1} \left[p_c(\boldsymbol{o}) - p_{c-j}(\boldsymbol{o}) \right],$$

with *F* being the total number of input features, *c* a subset of the features, and |*c*| their number; an observation was a vector **o** whose components were the input features, *p c* (**o**) the prediction yielded by the features in *c,* and *p c-j* (**o**) the prediction obtained without the *j* feature. Thus, the importance of each feature on the model prediction was evaluated by averaging all possible differences.The presented machine learning and explainability analyses were carried out using cross-validation to ensure unbiased performance and Shapley value estimates. In particular, we adopted a repeated five-fold cross-validation for analyses concerning the whole set of patients to assess the SGLT2i effectiveness. For this classification task, the classes to predict were the one

of patients treated with gliflozins against the one including patients undergoing standard treatment. Instead, a leave-one-out approach was used for the cohort of only treated patients whose size was limited; here, the labels distinguished the patients treated with gliflozins from those who responded to from treatment in a different way from those who were standardly treated. To this aim, we performed several cross-validation rounds of the previous task and counted the number of times when patients treated with gliflozins were correctly classified and when they were misclassified; this measure yielded an operative definition of "responders" and "not-responders."

Classification performance was evaluated in terms of accuracy, sensitivity, and specificity:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

$$Sensitivity = \frac{TP}{TP + FN}$$

$$Specificity = \frac{TN}{TN + FP}$$

While accuracy globally evaluated the model performance, sensitivity and specificity characterized the model behavior with respect to the positive class (patients treated with gliflozins) and the negative class (patients not treated with gliflozins). Cross-validation analyses allowed us to estimate an average value for each adopted metric, while the related uncertainties were reported in terms of standard deviations.

4.3. Aim 1b-Animal model and dapagliflozin treatment

The *in vivo* experiments were conducted on six-week-old male Dahl salt-sensitive rats (Charles River Laboratories). The animals were fed laboratory chow containing 8% NaCl (high-salt diet) or 0.3% NaCl (low- salt diet). The first batch of samples was obtained after five weeks from animals on high-salt (HS 11 weeks; n = 5) and low-salt diet (LS 11 weeks; n = 5). Functional

analysis and molecular biology assays were performed to characterize early-onset functional and molecular modifications prior to dapagliflozin treatment (first time-point). Remaining animals on the high-salt diet were randomized into two groups and treated with dapagliflozin (0.1 mg/kg/day; HS+DAP; n = 10) or vehicle (HS 17 weeks; n = 10) for the following six weeks by oral gavage (second time-point). Rats maintained on a low-salt diet served as age-matched control (LS 17 weeks; n = 5) (Figure 12). Analysis at this time-point served to assess the effects of dapagliflozin treatment on renal condition highlighting significant differences to untreated animals. Systolic and diastolic blood pressures were measured in conscious animals by the tail-cuff method (BP-2000; Visitech Systems). Urinary proteins, creatine and electrolyte values were determined by the Architect ci8200 Chemistry Analyzer (Abbott Diagnostics). Euthanasia was done by an overdose of anaesthetic agents [intraperitoneal injection of ketamine (300 mg/kg) and medetomidine (0.75 mg/kg)]. In vivo procedures were carried out in accordance with the National ethical guidelines and the guidelines from Directive 2010/63/EU of the European Parliament. The present study complies with the ARRIVE guidelines and has been approved by the Ministry of Health (protocol n. 582/2015-PR) and by the local ethics committee.

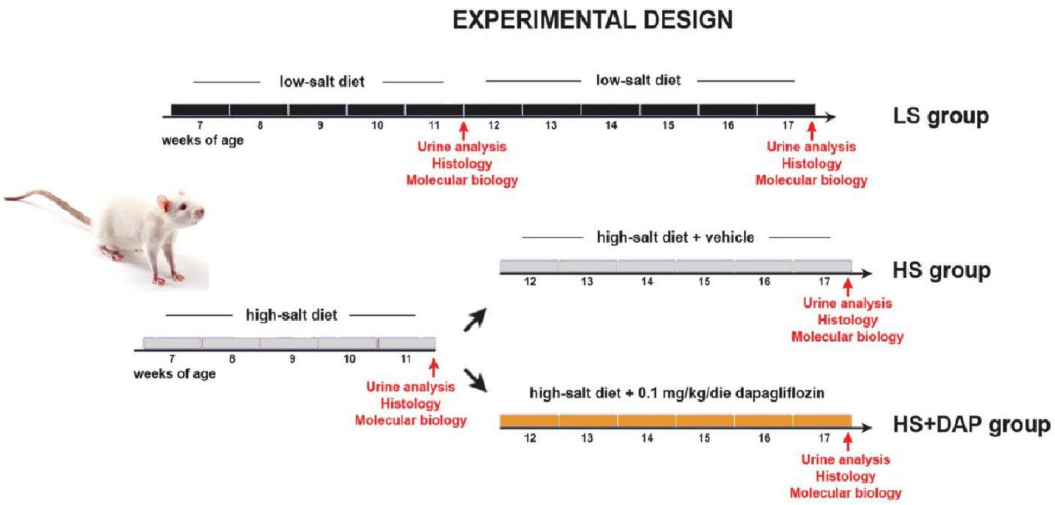

Figure 12. Experimental protocol regarding high salt diet and dapagliflozin treatment of Dahl rats.

4.4. Aim 1b-*Ex-vivo* experiments

For histology, kidneys were embedded in optimal cutting temperature (OCT) compound, frozen in liquid nitrogen and stored at -80° C; 10 mm thick tissue sections were cut with a CM3050 S cryostat (Leica Microsystems) (252). Sample preparation of cryosections was carried out to evaluate fibrosis, glomerulosclerosis and oxidative stress. For molecular biology, samples were snap-frozen in liquid nitrogen and stored at -80°C.

Histology: Masson's trichrome staining was used to detect fibrosis. The extent of glomerulosclerosis was determined by counting sclerotic glomeruli and expressed as a percentage of sclerotic glomeruli over the total number of glomeruli. To evaluate oxidative stress, ROS levels were examined with dihydroethidium, damage at membrane level was determined with 4- hydroxynonenal antibody (Abcam), peroxynitrite formation was assessed by 3-nitrotyrosine immunostaining (Merck Millipore). NF-κB phosphorylation on Ser536 was analyzed (Sigma-Aldrich). Fluorescein isothiocyanate-conjugated secondary antibodies were used (Jackson ImmunoResearch). Nuclei were counterstained with 4′ ,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). Samples were analyzed with a LSM700 confocal microscope (Zeiss).

RNA isolation and real-time PCR: Total RNA from the kidney (separated into cortex and inner medulla) was isolated in TRIzol reagent according to the manufacturer's recommendations (Ambion, Life Technologies). Real-time PCR analysis was performed as previously described [21]. Samples were loaded in triplicate using the real-time PCR 7500 Fast system (Applied Biosystems). Normalization for RNA quantity was performed to the best house- keeping gene selected from 2-beta-microglobulin (2Bm) and glyceraldehyde-3-phosphate dehydrogenase (Gapdh). TaqMan hydrolysis primer and probe gene expression assays were obtained from Life Technologies with the following assay IDs: BSND: Rn00564503_m1; Kcnj10: Rn04219568_m1; Kcnj16: Rn01465215_m1; Src Kinase: Rn00676848_m1; Clcnk2: Rn00687467_m1; ClcnK1: Rn00677135_m1; Clcn5: Rn00567357_m1; 2Bm: Rn00560865_m1; Gapdh: Rn01775763_g1. Absolute quantification was performed using the standard curve method. The real-time PCR protocols were performed in line with the guidelines for qPCR (215).

Western blotting: Tissue was lysed in ice-cold RIPA buffer (Santa Cruz Biotechnology), and protein concentration was determined with Bradford assay (Bio-Rad Laboratories). Protein

extracts were separated by 6%− 12% SDS- polyacrylamide gel electrophoresis, and transferred onto a polyvinylidene fluoride membrane (216). Membranes were probed with primary antibodies against IL-1β, IL-6, total nuclear factor-κB (NF-κB), vascular cell adhesion molecule-1 (VCAM-1), e-selectin, in order to evaluate inflammatory process and endothelial activation. NADPH oxidases 2 and 4 (NOX2 and NOX4) and antioxidant enzymes [catalase and manganese superoxide dismutase (MnSOD)] were measured to establish the involvement of oxidative stress. Pro-fibrotic pathway was analyzed through TGF-β. Classical and non-classical renal RAAS was assessed by measuring ACE, angiotensin II type 1 and type 2 receptor (AT1R and AT2R), and MAS1 receptor. Loading conditions were determined with GAPDH. Antibody binding was visualized by chemiluminescence, and images were collected and analyzed using a Chemidoc Imaging System (Bio-Rad Laboratories).

***Zymography**:* was performed according to the manufacturer's protocol (Bio-rad Laboratories). Frozen tissue was homogenized in lysis buffer including protease inhibitors. Equal amounts of non-denatured total cellular proteins were separated by electrophoresis. Gel was incubated in an appropriate buffer, stained with 0.5% Coomassie Blue R-250 in 30% methanol and 10% acetic acid and successively destained in the same solution without Coomassie Blue. Area of matrix metalloproteinase-2 (MMP2) gelatinolytic activity was shown as white bands against a blue background.

Statistical analysis: Results were presented as fold of control mean ± standard error of the mean unless stated otherwise. Each value has been normalized to the mean value of the control group, dividing the raw value (from each experimental group) by the value of the mean of the control values. This generates a Gaussian dataset that can be analysed by parametric statistics, allowing to show the standard error in the control group as well. Data were analyzed by using GraphPad Prism Software. Significance between two comparisons was determined by Student's t-test and, for multiple comparisons, by one-way ANOVA and Bonferroni's post-test. A P-value < 0.05 was considered statistically significant.

4.5. Aim 2-Data Source

Forty-two consecutive outpatients with CHF and AF in OAT (warfarin or DOACs) for at least one year, enrolled in the Daunia Heart Failure Registry, were followed up between June 2019 and November 2019. At the baseline, within the cohort of DOAC-treated patients, 27.7% were taking dabigatran etexilate, 22.2% apixaban, 33.3% rivaroxaban and 16.6% edoxaban. Patients shifting from warfarin to DOAC therapy, because of poor patient compliance or time in therapeutic range (TTR), were compared with those already in treatment with DOACs. In the cohort of patients shifted to DOACs, 22.2% took dabigatran etexilate, 44.4% apixaban, 22.2% rivaroxaban and 11.1% edoxaban. All patients underwent an evaluation of clinical status, endothelial function, inflammatory state and cardiac remodeling at the beginning and after 5 months of follow-up (**Figure 13**).

Figure 13. Scheme of patients' treatment. Blue: DOACs treatment; Red: Warfarin treatment. DOACs: direct oral anti-coagulants.

4.6. Aim 2-Data analysis

A correlation analysis was performed to evaluate to what extent the clinical descriptors could be considered independent. To this aim, Pearson's pairwise correlation coefficients were calculated, and the results are shown in **Figure 14**.

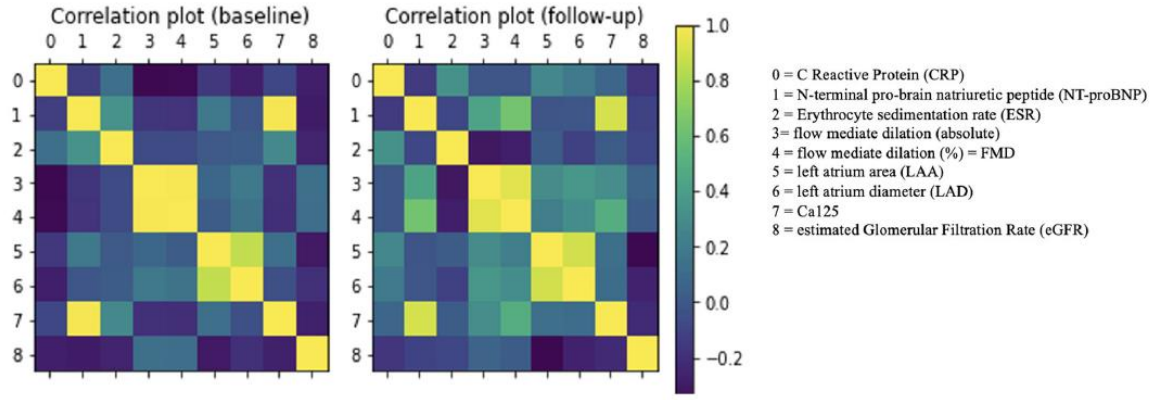

Figure 14. Squared matrix correlation plots of available clinical features. No substantial redundancy can be detected. Max. correlation did not exceed 0.9.

The correlation analysis ensured that no variable had to be excluded; in fact, no correlation exceeded the 0.9 value which is commonly adopted as a threshold value to identify high-correlated variables. 4.2.1. Clustering Analyses To evaluate how switching from warfarin to DOACs may affect the clinical progression of the patients, we carried out two separate analyses. The case query is whether or not changing therapy has possible drawbacks or, on the contrary, it can even trigger an improvement in patients' clinical conditions. First of all, carrying out a clustering analysis, the existence of patterns distinguishing the clinical cohorts was examined. In fact, clustering allows us to determine if, according to the examined clinical features, it is possible to classify the patients in different groups. Of course, given the underlying assumption that switching from one treatment to another should not be detrimental for patients, this analysis should assess whether a substantial overlap between the cohorts exist or not. The fundamental idea behind clustering techniques is that each patient can be seen as a point in the multidimensional space defined by the examined clinical features; accordingly, it is possible to measure the pairwise distances among all patients enrolled and define the so-called similarity/dissimilarity matrix. Then, several approaches can be adopted to separate the data points in different classes. Hierarchical clustering techniques outline the possibility to segregate data in a varying number of classes; thus, they are particularly useful when it is not possible to establish the number of classes a priori or in cases where multiple classifications can coexist. Here, a centroid-based approach was adopted because the number of desired classes is already

known, i.e., the class of patients treated with DOACs and the class of patients switching from warfarin to DOACs.

Herein, the k-means clustering method was adopted (217). According to this specific analysis, two random points, representing the centroids of clusters, were randomly initialized in the features' space, and then the k-means algorithm proceeded to assign each data point, i.e., each patient, to one class or another according to the distance from the centroids. Of course, this clustering approach is highly sensitive to the initialization, and therefore, we performed one hundred different simulations to establish the average clusters. Then, to visualize the clustering results, a data reduction technique was adopted. As no assumption can be made about the clinical data, a standard Principal Component Analysis (PCA) was performed. The first two components were suitably used to visualize the clustering results and ultimately evaluate if these clusters reproduce the clinical cohorts. This approach may provide only qualitative evaluations. Therefore, a second analysis, based on machine learning, was carried out to quantify the possible overlapping between the patients treated with DOACs and those switching from warfarin. For the presented analyses, the RF standard configuration with 100 trees was adopted; moreover, for the sake of reproducibility, a random seed of 0 was set. Given the limited sample size, a leave-one-out (loo) cross-validation framework was adopted. Although loo (leave-one-out) cross-validation has some issues, the most important one being the risk of overestimating the model's predictive performance, it remains the best choice when the data amount is limited. In fact, in this framework, all but one observation is used to learn the model, while the one left out is used for validation purposes. In this way, especially when the amount of data is limited, the number of observations used to learn the model is maximized. Here, the adoption of machine learning is aimed at providing a measure of the separation between the available patients' classes, and therefore, the possible performance overestimation, which could cause problems when testing the model on unseen data, does not represent an issue.

5. RESULTS

5.1. Aim 1a: evaluation of the short-term anti-remodeling effect of SGLT2 inhibitors by ML approach

The main demographics, clinical, and biochemical characteristics, as well as treatments, of the patients under investigation are listed in **Table 2**. By using an RF model, we initially evaluated, on the available cohort of 78 patients and based on clinical, echocardiographic, biochemical, and pharmacological parameters, if patients treated with gliflozins could be distinguished from non-treated ones at baseline and at 4-6 months follow-up. We, therefore, trained two different RF models to discriminate patients treated with gliflozins (positive class "1") from patients undergoing standard treatment (negative class "0"): the first reporting baseline features (i.e., before treatment); the second concerned with follow-up (i.e., after treatment with gliflozins). A synoptic view is shown in **Figure 15**.

Clinical	
Age (years)	67.9 ± 1
Male (%)	84.6
Body weight (kg)	85.4 ± 2
Time from diabetes mellitus diagnosis (years)	9.1 ± 0.6
SBP (mmHg)	118.7 ± 2.4
DBP (mmHg)	71.5 ± 1.2
Heart rate (bpm)	69.7 ± 1.4
NYHA class ≥2 (%)	96.1
Medical history	
Arterial hypertension (%)	75.6
Anemia, Hb < 11 (%)	6.4
COPD (%)	21.8
Atrial fibrillation/flutter (%)	39.7
Medications	
ACEi/ARB/ARNI (%)	89.7
Beta-blockers (%)	92.3
MRA (%)	47.4
Diuretics (%)	89.7
Statins/fibrate (%)	88.5
Allopurinol (%)	37.2
Ivabradine/ranolazine (%)	47.4
Digoxin (%)	15.4
Amiodarone (%)	10.2
Antiplatelet drugs (%)	28.2
Anticoagulant drugs (%)	51.3
Warfarin/acenocoumarol (%)	16.6
Apixaban (%)	12.8
Dabigatran etexilate (%)	7.7
Rivaroxaban (%)	12.8
Edoxaban (%)	1.2
Other antidiabetic drugs (%)	62.8
Insulin (%)	38.5
Laboratories	
HbA1c (%)	8.0 ± 0.2
SCr (mg/dL)	1.1 ± 0.03
MDRD-based eGFR (mL/min)	72.5 ± 2.3
CRP (mg/L)	2.9 ± 0.3
Ca-125 (U/mL)	22.4 ± 5.4

Echocardiographic	
LVEDD (mm)	56.7 ± 0.8
LVESD (mm)	47.6 ± 0.9
IVS (mm)	12.0 ± 0.2
LVPW (mm)	10.8 ± 0.2
LV Mass (g)	271.8 ± 8.3
RWT (ratio)	0.4 ± 0.01
LVMI (g/m^2)	139.1 ± 3.8
LVEF (%)	38.9 ± 0.9
EDV (mL)	151.9 ± 7.6
ESV (mL)	97.9 ± 6
MR ≥ 2 (%)	33.3
LAD (mm)	44.3 ± 0.8
LA Area (mm^2)	22.7 ± 0.7
LAVI (mL/m^2)	36.8 ± 1.6
LAV (mL)	72.8 ± 3.5
TR ≥ 2 (%)	19.2
TAPSE (mm)	18.7 ± 0.4
RV S' (cm/s)	10.7 ± 0.3
sPAP (mmHg)	30.5 ± 1.0
E/A	1.7 ± 0.3
E/è	14.2 ± 1
E wave (cm/s)	80.2 ± 3.6
A wave (cm/s)	86.6 ± 3.6
EDT (ms)	206.1 ± 11.9
è (cm/s)	7.1 ± 0.2
a' (cm/s)	7.6 ± 0.6
LV GLS (%)	−10.4 ± 0.3
NT-pro-BNP (pg/mL)	13480.6 ± 334.5
CKD stage ≥2 (%) eGFR (mL/min)	91.02 72.5 ± 2.3
ESR (mm/h)	21.37 ± 2.7

Table 2. List of patient features included in the database. *Abbreviations:* Systolic blood pressure- SBP, Diastolic blood pressure- DBP, beats per minute- BPM, New York Heart Association- NYHA, chronic obstructive pulmonary disease- COPD, angiotensin-converting enzyme inhibitors- ACEi, angiotensin II, receptor blockers- ARB, angiotensin receptor-neprilysin inhibitors- ARNI, mineralcorticoid receptor antagonist- MRA, glycated hemoglobin- HbA1c, serum creatinine- SCr, Modification of Diet in Renal Disease- MDRD, estimated glomerular filtration rate- eGFR, C-reactive protein- CRP, plasma N-terminal pro-brain natriuretic peptide- NT-pro-BNP, chronic kidney disease- CKD, erythrocyte sedimentation rate- ESR, left ventricular end diastolic diameter- LVEDD, left ventricular end-systolic diameter- LVESD, inter-ventricular septum thickness- IVS, left ventricular posterior wall thickness- LVPW, left ventricular mass- LV, mass, relative wall thickness- RWT, left ventricular mass index- LVMI, left ventricular ejection fraction- LVEF, end diastolic volume- EDV, end systolic volume- ESV, mitral regurgitation- MR, left atrial diameter- LAD, left atrium area- LA area, left atrium volume index- LAVI, left atrium volume- LAV, tricuspid regurgitation- TR, tricuspid annular plane systolic excursion- TAPSE, right ventricular systolic excursion velocity- RV S′, systolic pulmonary artery pressure- sPAP, E wave deceleration rate- EDT, left ventricular global longitudinal strain- LV GLS.

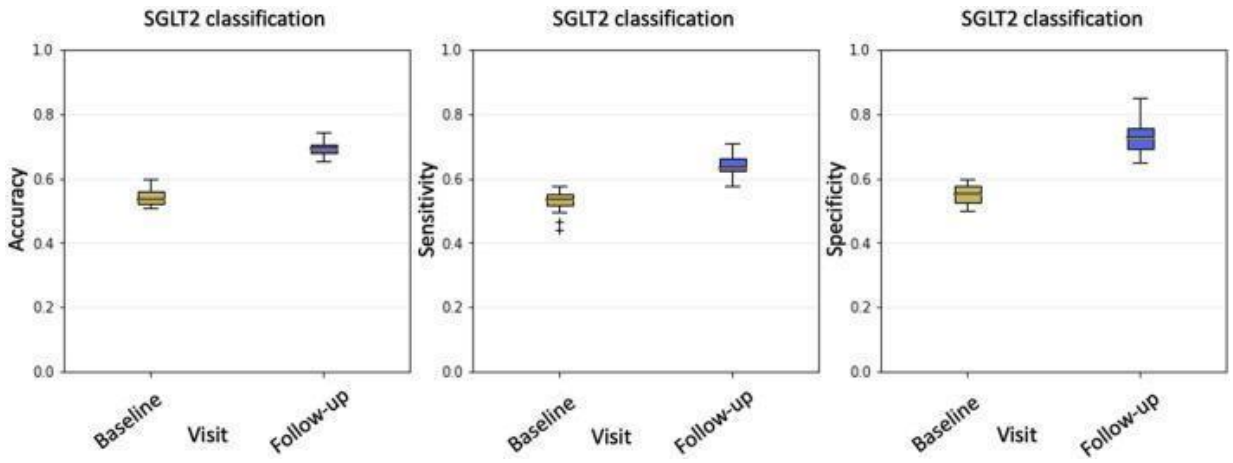

Figure 15. Accuracy, sensitivity and specificity at baseline and follow up according to RF analysis. From left to right: accuracy, sensitivity, and specificity boxplots comparing baseline and follow-up classification performance. For each metric, the results obtained through 100 repeated five-fold cross-validations are reported. According to all three metrics, after gliflozin treatment, patients who were indistinguishable at baseline show significant differences.

At the baseline, classification accuracy was 0.52±0.03, a value comparable with chance for a binary classifier. The same considerations held true for sensitivity 0.53±0.03 and specificity 0.52±0.03. As far as follow-up was concerned, classification performance showed a significant improvement for all the metrics: accuracy 0.70±0.03, specificity 0.73±0.05 and sensitivity 0.65±0.04. Therefore, according to the three metrics, after gliflozins treatment, patients that were undistinguishable at the baseline showed significant differences. As the only difference between the two cohorts at the follow-up was the clinical treatment, it is reasonable to conclude that gliflozins affected the patients' conditions differently from standard treatments. The unbalancing between sensitivity and specificity suggests that the classification model does not equally perform on the two classes; in particular, higher specificity values should indicate that the majority of misclassified examples belong to the class of treated patients. A visual confirmation was obtained by examining the histogram of the average cross-validation scores shown in **Figure 16**.

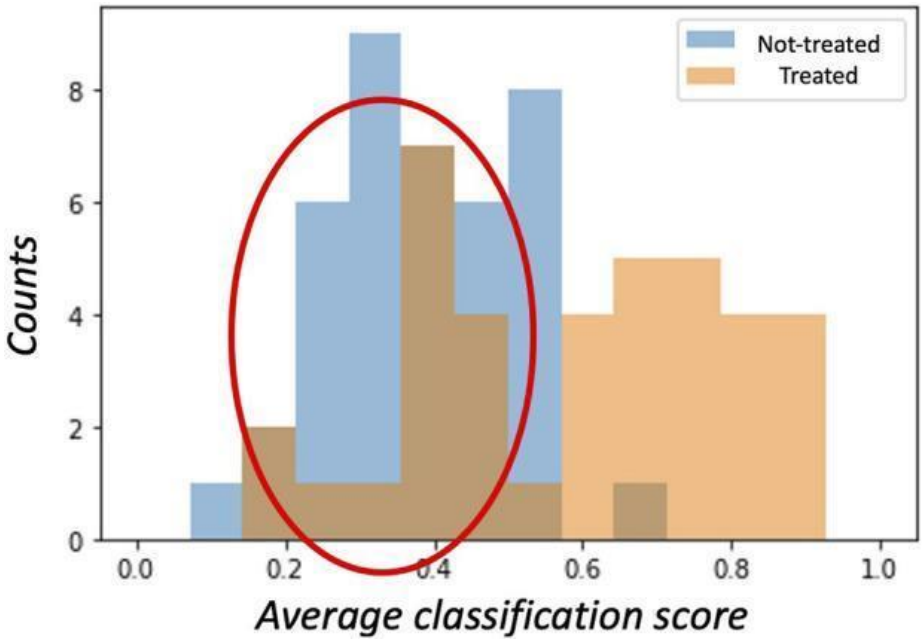

Figure 16. The histogram of average cross-validation scores shows how, on average, treated patients (class '1') have scores distributed towards the right hand-side 1 limit, while non-treated (class '0') patients have scores distributed towards the left hand-side 0 limit. Interestingly, treated patients are often misclassified (red circle) as having scores lower than 0.5. These patients are treated with gliflozins but are erroneously assigned to the '0' class.

A subject belonging to the '1' class (treated) is correctly classified if the model assigns a score greater than 0.5; analogously, a score lower than 0.5 is assigned to a patient in the '0' class (not treated). Thus, for a perfect model, the score distributions should ideally be separated and show the so-called bathtub distribution. In our case, the distribution of scores among treated patients showed a huge tail below the 0.5 threshold. Accordingly, within the SGLT2i treated group, we labelled the 16 patients responding to treatment as 'responders' to distinguish them from the remaining 22 called 'not-responders.' Conversely, only few examples of the non-treated patients presented scores exceeding the threshold on average.

Previous results demonstrated that the gliflozin treatment made patients much easier to distinguish from baseline to follow-up. Thus, we wondered which features were driving the classifier's decisions. A first answer to this question could be given from a global perspective by inspecting the average SHAP values of the classification model (**Figure 17**). In our model, the most important features able to discriminate between treated and untreated patients were the echography-related ones, listed in order of relevance in **Figure 17**. Global explainability ranks

the features according to their average importance in the model's decisions. As a difference to classical feature importance rankings, the SHAP paradigm allows one to directly interpret how features contribute. For example, the most important feature for the RF model was the 'RV systolic excursion velocity' (RV S') variable. High values of this feature are strong predictors of survival for HFrEF patients. Interestingly, by visual inspection of the SHAP graph, it was evident that high RV S' values characterized treated patients. On the contrary, the second important variable, left ventricular end systolic diameter (LVESD), had low values for treated patients and high values for non-treated ones, as expected by an efficacious HF treatment.

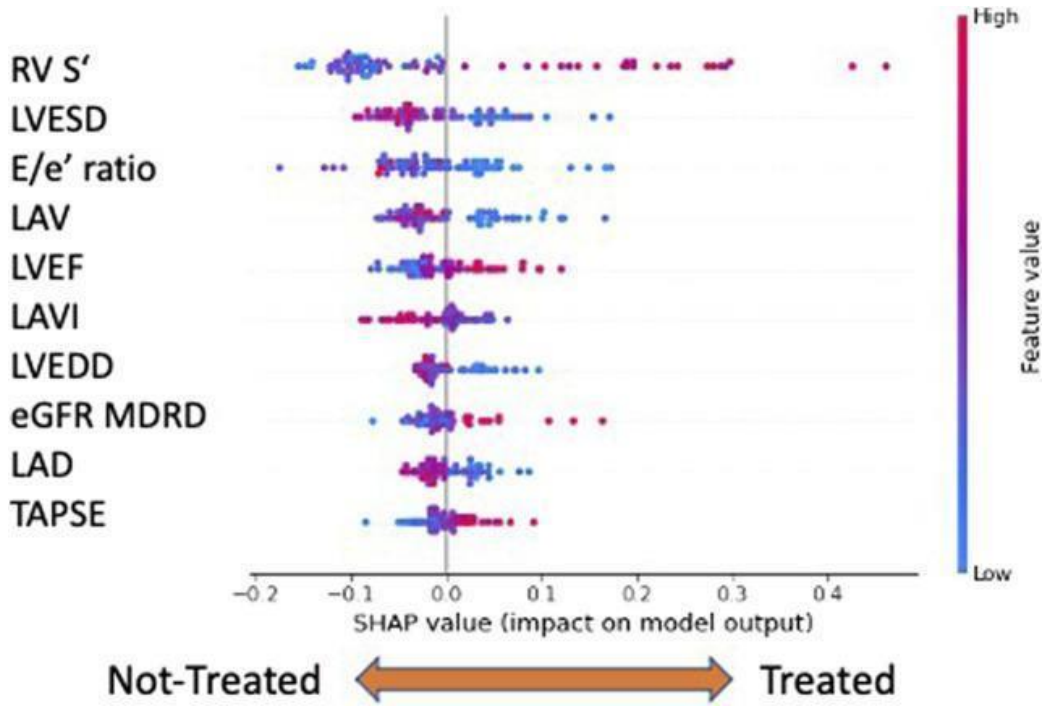

Figure 17. Global explainability: the top ten features are ranked (from top to bottom) according to their importance. The horizontal axis evaluates the feature impact on the model decisions: right positive SHAP values contribute to assigning observations to the class of treated patients '1', left negative values to non-treated ones. In addition, high/low feature values are color coded. For example, high 'RV S' values and low 'LVESD' characterize treated patients. Legend: RV S′, right ventricular systolic excursion velocity; LVESD, left ventricular end-systolic diameter; E/e', E velocity/e' velocity ratio; LAV, left atrium volume; LVEF, left ventricular ejection fraction; LAVI, left atrium volume index; LVEDD, left ventricular end-diastolic diameter; eGFR MDRD, estimated glomerular filtration rate MDRD (Modification of Diet in Renal Disease); LAD, left atrial diameter; and TAPSE, tricuspid annular plane systolic excursion.

Previous analyses demonstrated that, based on clinical features, treated patients could be distinguished by RF. Nevertheless, there were some misclassified cases (labeled as non-responders) especially among the treated patients. Thus, we considered only the 38 treated patients representing the '1' class and distinguished those misclassified among the patients on average correctly classified (responders) (average classification score greater than 0.5). As the number of such cohorts was limited, for the subsequent analyses, we adopted a leave-one-out cross-validation framework. We observed that using only baseline features, it was possible to accurately (74%) predict which subjects would have successfully responded to gliflozins treatment. Even in this case, we performed a global SHAP explanation of the model (**Figure 18**).

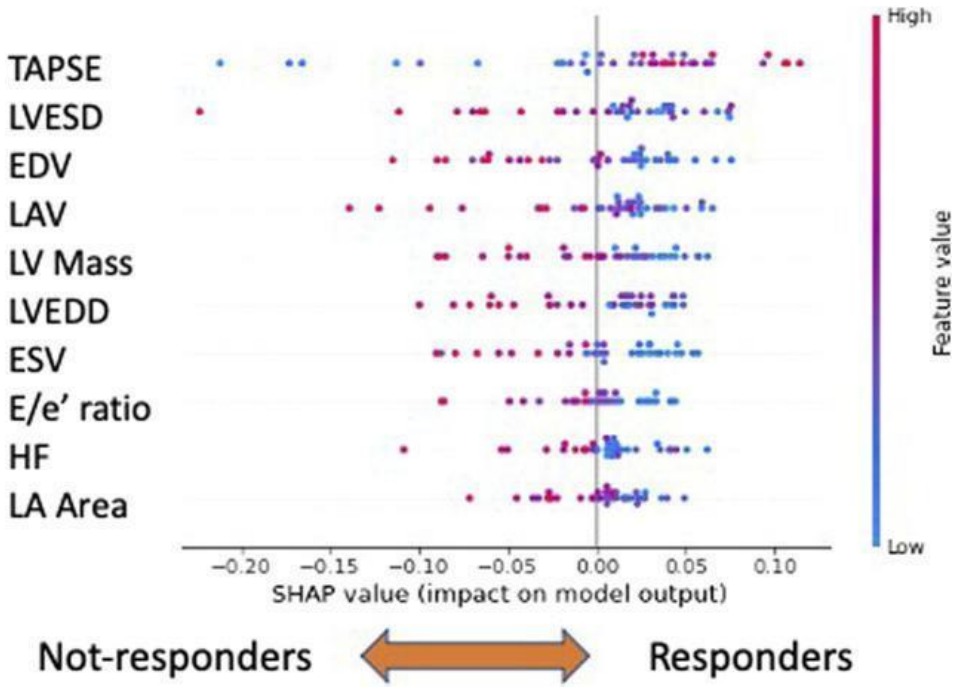

Figure 18. Explainability analysis of responder *versus* non-responder classification. In this case, the ten most important features are shown. The top three performers are "TAPSE," "LVESD,"and "EDV": low values of "TAPSE" and high values of the remaining features characterize not-responders. Legend: TAPSE, tricuspid annular plane systolic excursion; LVESD, left ventricle end systolic diameter; EDV, end diastolic volume; LAV, left atrium volume; LV Mass, left ventricular mass; LVEDD, left ventricle end diastolic diameter; ESV, end systolic volume; E/e', E velocity/e' velocity ratio; HR, heart rate; and LA area, left atrial area.

Within the global feature importance evaluation, the most important feature to distinguish responders from non-responders was tricuspid annular plane systolic excursion (TAPSE), a reliable index of the right ventricle systolic function (1). Concerning the other variables, among the top ten, echocardiographic variables kept playing a relevant role. In general, low values of 'TAPSE' and high values of the remaining features, such as heart rate (HR), left ventricular end systolic diameter, end diastolic volume (EDV), left ventricular mass (LV Mass), and left atrial volume (LAV), characterize non-responders.

5.2. Aim 1b: investigation of the renoprotective effects mediated by dapagliflozin in HF-induced kidney dysfunction by using an animal model of HFpEF

During pathological chronic state, such as HF, the inflammatory component plays a pivotal role in the development of renal dysfunction. Indeed, in a recent preclinical study employing the ss-Dahl rats as animal model of HF, Cappetta et al demonstrated a marked increase of inflammatory mediators in the renal tissues of the hypertensive animals in which chronic kidney dysfunction is established (75). In particular, an upregulation of TNF-α, IL-1β, IL-6, TGF-β expression was observed. As it is well known, inflammatory response, immune system and oxidative stress are tightly interconnected in an auto-reinforcing loop.

Given the involvement of chronic inflammation and endothelial dysfunction in the worsening of the clinical status of CKD patients, at this first time-point, we evaluated the levels of inflammatory markers and enzymes producing ROS in the kidneys of HS rats after five weeks of high-salt diet.

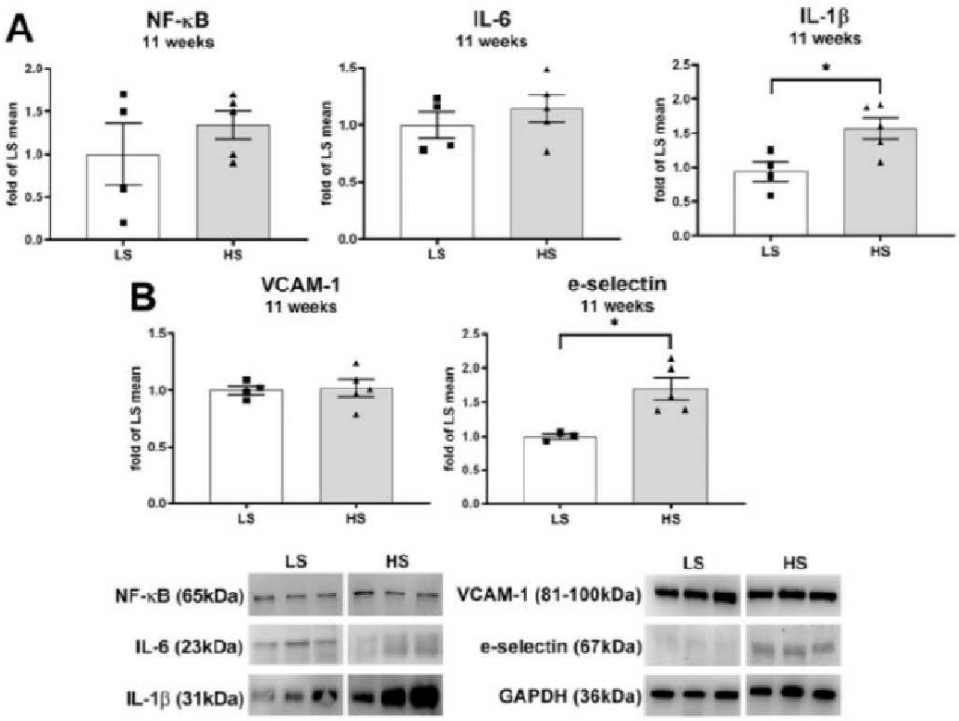

Figure 19. Evaluation of endothelial function, inflammation and oxidative stress at 11 weeks of age. Western blotting analysis of (A) total nuclear factor-κB (NF-κB), interleukin-6 (IL-6), IL-1β, (B) vascular cell adhesion molecule-1 (VCAM-1), e-selectin. Western blotting data from 3 to 4 LS and 5 HS kidney samples. Western blotting gel bands represents 3 selected biological samples. * $P < 0.05$.

The first step of renal injury is the activation of inflammation and endothelial dysfunction. Indeed, the results show a slight increase of all investigated inflammatory markers that was significant for IL-6 and IL-1β together with a significant increase of e-selectin (**Figure 19**).

To evaluate the possible renoprotective role of dapagliflozin in a non-diabetic model of salt-induced hypertension and cardiorenal syndrome, the feeding with high-salt diet continued for the following six weeks with or without treatment with dapagliflozin.

The impact of cardiorenal disease is evident on well-being of the animal model. Indeed, in HS rats at 17 weeks of age a significant decrease of body weight is observed compared to control LS. Notably, in the treatment group with dapagliflozin the body weight was preserved (Table 4). The protective effect of dapagliflozin on general animal health is confirmed by restoration trend in kidney weight and kidney weight over tibia length ratio that were higher in HS than in LS rats as the results show (**Table 3**).

Groups	LS 17 weeks	HS 17 weeks	HS+DAP 17 weeks
Body weight (g)	396 ± 12	342 ± 18 **	365 ± 14°
Kidney weight (g)	1.46 ± 0.21	1.86 ± 0.15 **	1.70 ± 0.14
Kidney weight/tibia lenght (g/cm)	0.348 ± 0.050	0.448 ± 0.048 **	0.412 ± 0.025

Table 3. Effects of dapagliflozin on body and kidney weight. Results are expressed as mean ± standard deviation. ** $p < 0.0005$ vs LS (17 weeks); c $p < 0.05$ vs HS (17 weeks).

To test the functionality of cardiovascular and renal systems, we determined the blood pressure and urine concentrations of kidney damage markers. In HS rats the blood pressure continued to rise. However, after six weeks the treatment with dapagliflozin significantly reduced systolic and diastolic pressures, although the rats remained severely hypertensive (**Figure 20, A**). Moreover, in HS rats elevated proteinuria, albuminuria and UAC *ratio* was observed compared to LS rats. Once again, six weeks of dapagliflozin treatment partially restored albuminuria levels and UAC *ratio* preserving renal integrity (**Figure 20, B**). However, dapagliflozin has no effect on modulation of urinary the elevated concentrations of sodium, chloride and calcium. Potassium and magnesium remained unchanged (**Figure 20, C**).

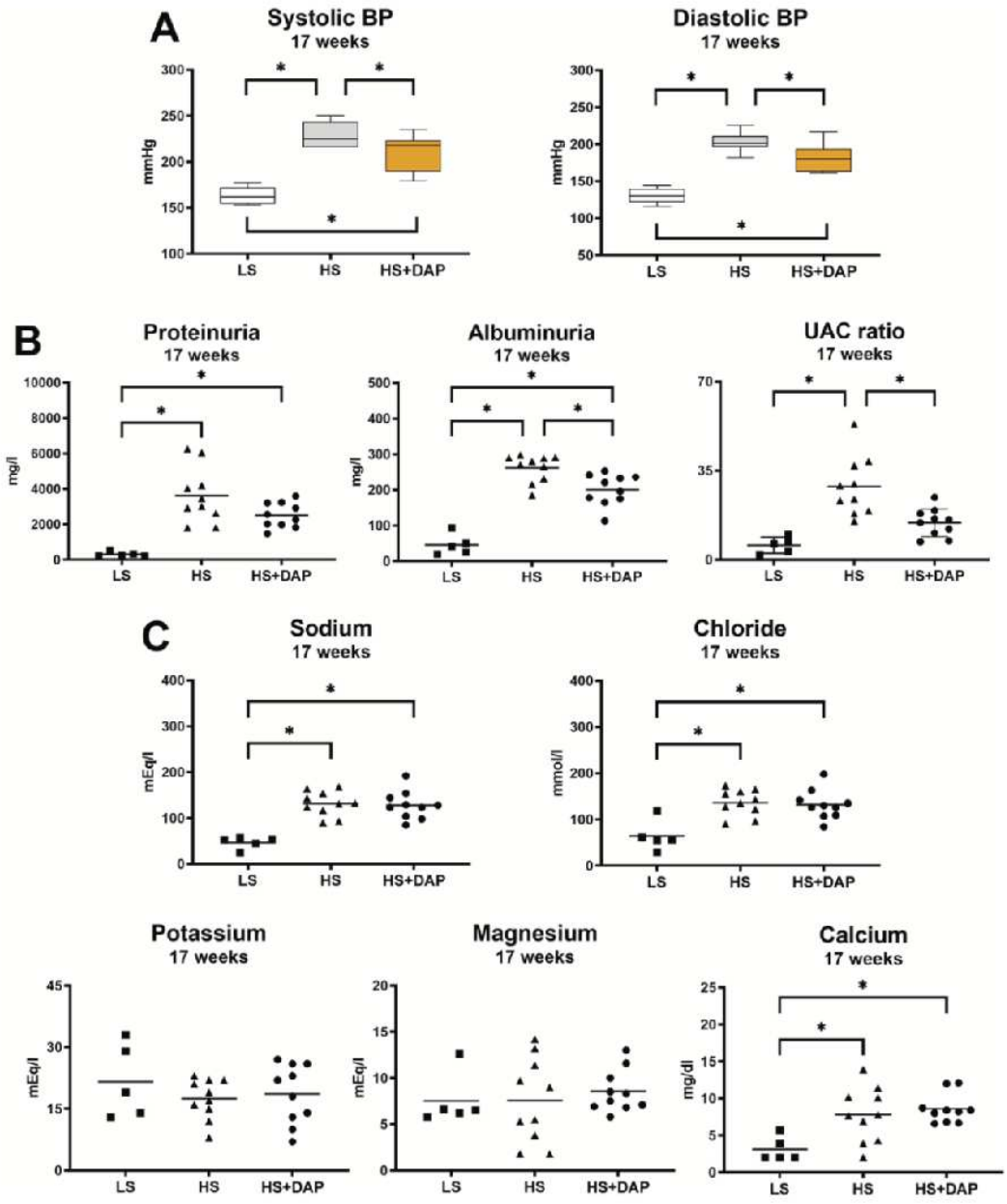

Figure 20. Effect of dapagliflozin on blood pressure and renal function. A) Systolic and diastolic blood pressure. Analysis of **B)** proteinuria, albuminuria, urine albumin- to-creatinine (UAC) *ratio,* **C)** sodium, chloride, potassium, magnesium and calcium concentration. Data from 5 LS, 10 HS and 10 HS+DAP rats. * $P < 0.05$.

In cardiorenal syndrome, activation of the RAAS pathway plays a key role in maintaining arterial blood pressure and sodium and water balance leading to a strong AT1 activation as well as fibrosis. In order to investigate the presence of fibrotic tissue in the kidneys we performed a histological study and TGF-βgene expression analysis.

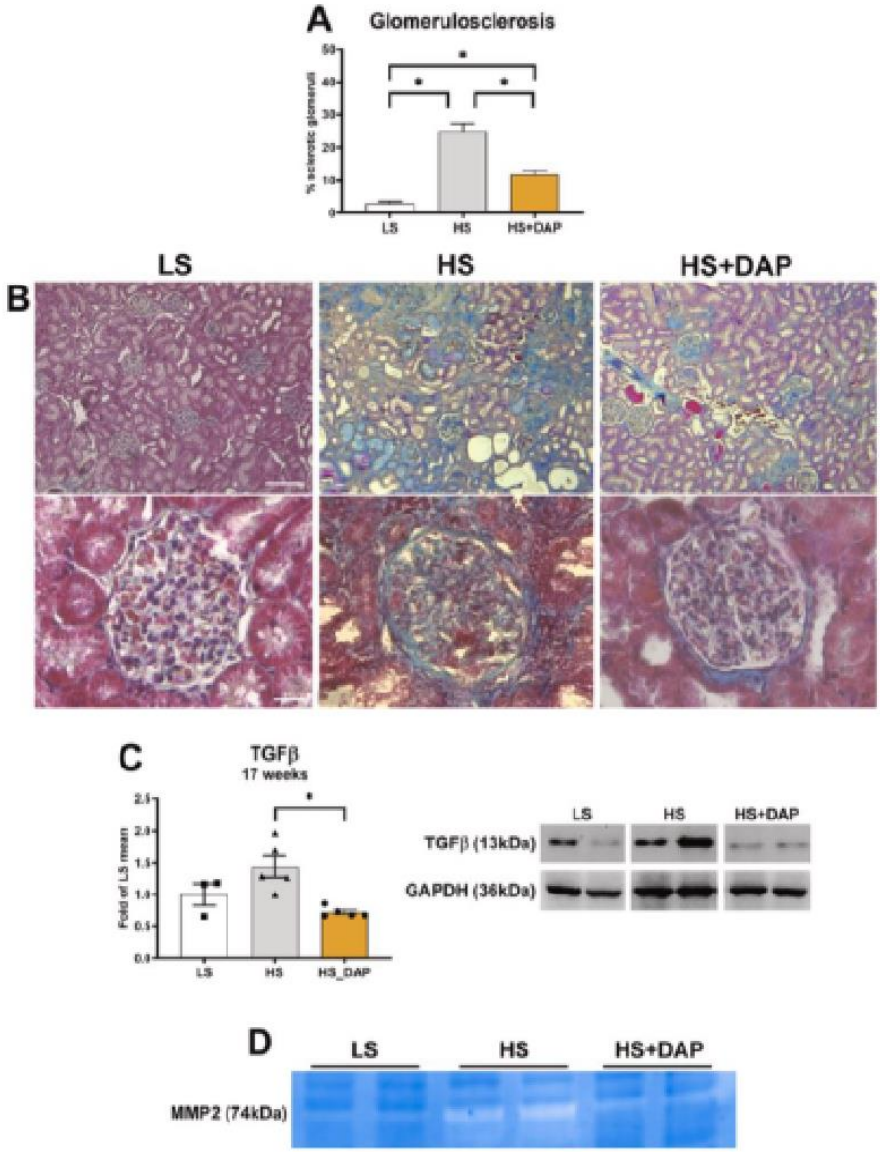

Figure 21. Effect of dapagliflozin on fibrosis. A) Percentage of sclerotic glomeruli, determined by counting sclerotic glomeruli over the total number of glomeruli. **B)** Masson's trichrome staining showing collagen deposition (blue) and sclerotic glomeruli. Scale bars: upper panels 200 µm, lower panels 25 µm. **C)** Protein expression analysis by western blotting of transforming growth factor β(TGFβ). Western blotting data from 3 LS, 5 HS and 5 HS+DAP kidney samples. Western blotting gel bands represents 2 selected biological samples. **D)** Matrix metalloproteinase-2 (MMP2) gelatinase activity shown by gel zymography assay. * $P < 0.05$.

These histological findings reveal tubulointerstitial and glomerular fibrosis in kidneys of HS rats. A strongly reduced extension of glomerulosclerosis was observed after treatment with dapagliflozin compared to the HS group (**Figure 21, A-B**). The protective effect induced by

dapagliflozin is confirmed in the important down regulation of TGF-βas well as in the reduced MMP2 activity which were higher in HS rats compared to control group LS (**Figure 21, C-D**). Notably, the massive fibrotic remodeling was not previously detectable and this evidence highlights the renoprotective effect of dapagliflozin. At this second time-point, given the complicated inflammatory profile due to an upregulation of proinflammatory cytokines in HS rats, we decided to verify if dapagliflozin could modulate the expression of these components by western blotting analysis. In contrast to previous data, in this case all cytokines under investigation were significantly elevated in the kidneys of HS rats. Interestingly, administration of dapagliflozin partly reduced the levels of IL-6 and IL-1β whereas it significantly downregulated NF-κBexpression (**Figure 22, A**).

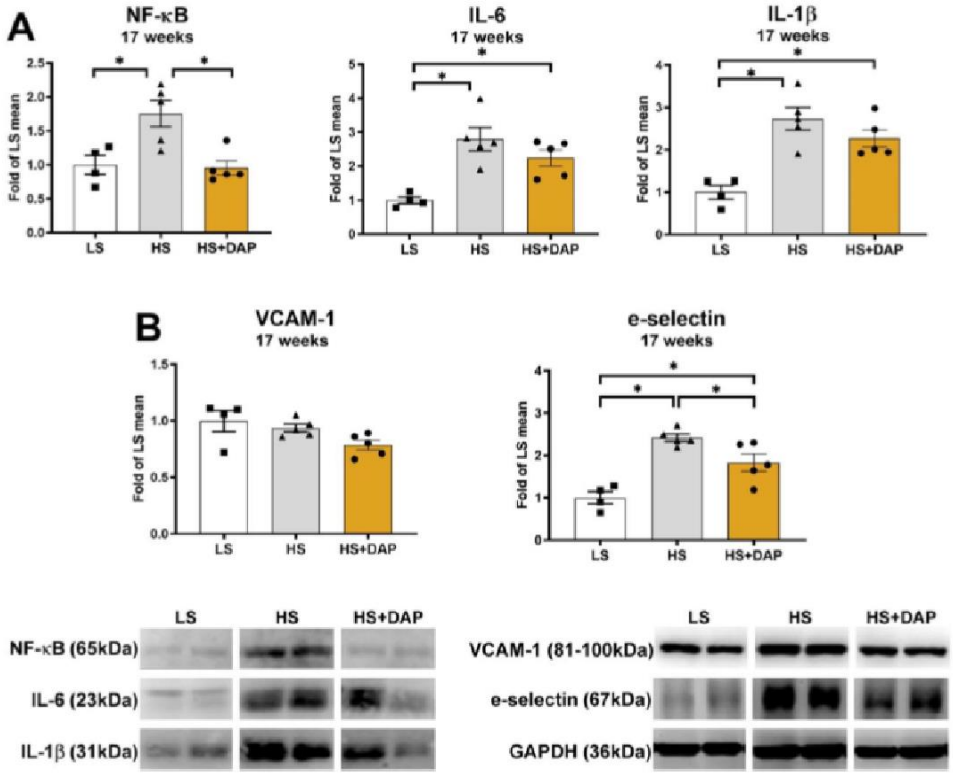

Figure 22. Effect of dapagliflozin on inflammation and endothelial activation. Protein expression analysis by western blotting of **(A)** total nuclear factor-κB (NF-κB), interleukin-6 (IL-6), IL-1β, **(B)** vascular cell adhesion molecule-1 (VCAM-1), e-selectin. Western blotting data from 4 LS, 5 HS and 5 HS+DAP kidney samples. Western blotting gel bands represents 2 selected biological samples. *P < 0.05.

Consistently, the positive effect of dapagliflozin on the vascular endothelium is demonstrated by the restored basal levels of e-selectin that was significantly higher in the HS group (**Figure 22, B**).

Given the role of RAAS activation in the decline of renal and cardiovascular systems, the study was extended to evaluate the levels of the components involved in this pathway. Consistently, in the HS rats group the RAAS components which negatively play in the cross-talk between heart and kidney, such as ACE and AT1R, are significantly higher compared to the LS control group (Figure 35A), whereas AT2R and MAS1, two reno-protective proteins are significantly downregulated (Figure 35B). Following dapagliflozin treatment, a decrease of ACE, but not for AT1R was observed in the kidneys of the treated group (**Figure 23, A**). In parallel, dapagliflozin show a reno-protective effect by raising the healthy AT2R and MAS1 levels in the treated group (**Figure 23, B**).

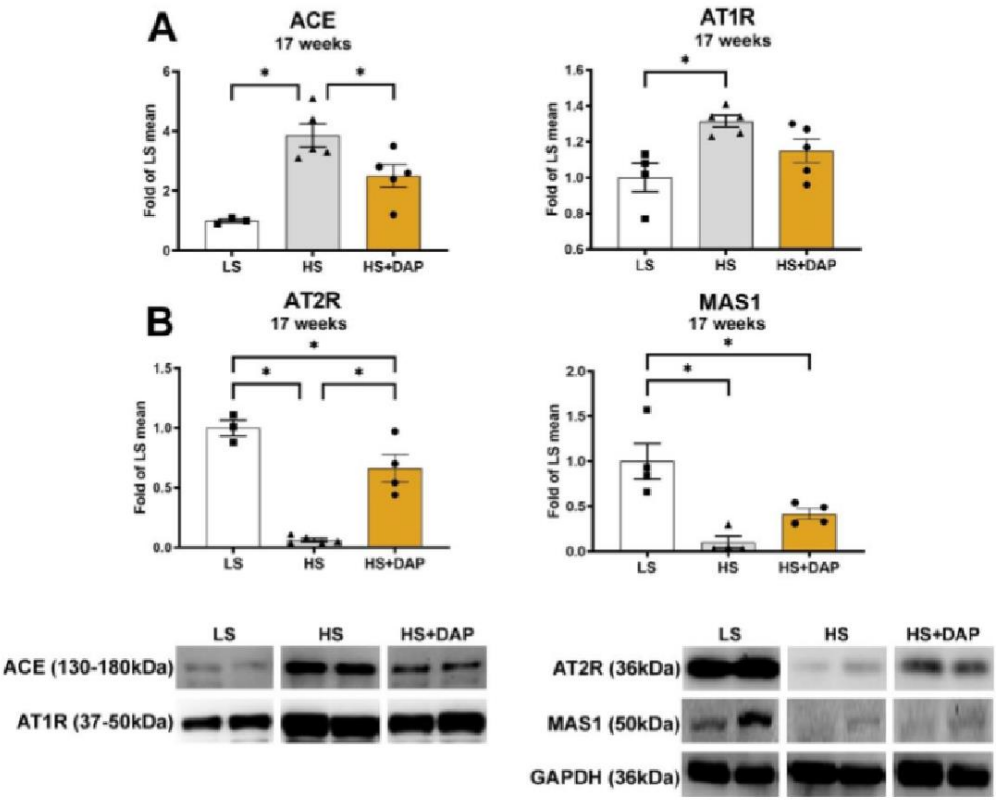

Figure 23. Effect of dapagliflozin on kidney renin-angiotensin-aldosterone system signaling. Protein expression analysis by western blotting of **A)** angiotensin- converting-enzyme (ACE), angiotensin II type 1 receptor (AT1R), **B)** angiotensin II type 1 receptor (AT2R), MAS1. Western blotting data from 3 to 4 LS, 5 HS and 5 HS+DAP kidney samples. Western blotting gel bands represents 2 selected biological samples. * $P < 0.05$.

Therefore, the anti-inflammatory and anti-fibrotic mechanisms could be actively potentiated by promoting alternative RAAS pathway.

5.3. Aim 2: evaluation of the impact of therapeutic switching from warfarin to DOACs in patients with HFrEF and AF by ML approach

The main clinical characteristics, as well as treatments, of these patients are summarized in **Table 4**. By using a clustering analysis, we initially evaluated, in the available cohort of 42 patients and based on clinical, biochemical and echocardiographic parameters listed in **Table 5**, the existence of specific patterns distinguishing the clinical cohorts. Then, by using an RF model, we further investigated the features possibly responsible for the revelation of these two patterns.

Clinical Features		Medications	%
Age (years)	70.6 ± 1.5	ACEi/ARB/ARNI	92.8
Male (%)	64.3	Beta-blockers	78.5
Body weight (kg)	74 ± 1.5	MRA	40.5
SBP (mmHg)	110 ± 2.0	Diuretics	54.7
DBP (mmHg)	66 ± 1.7	Ivabradine	2.4
Heart rate (bpm)	64 ± 1.1	Digoxin	9.7
LVEF (%)	47 ± 1.8	Amiodarone	31.7

Table 4. List of clinical characteristics and treatments of patients included in this study. *Abbreviations:* angiotensin-converting enzyme inhibitor, ACEi; angiotensin II receptor blocker, ARB; angiotensin receptor–neprilysin inhibitor, ARNI; beats per minute, bpm; diastolic blood pressure, DBP; left ventricular ejection fraction, LVEF; mineralocorticoid receptor antagonist, MRA; systolic blood pressure, SBP.

Clinical Significance	Feature	Value
Index of inflammation		
state of systemic inflammation	CRP (mg/L)	1.7 ± 0.1
	ESR (mm/h)	30.0 ± 1.9
Index of clinical status		
congestion/fluid overload	NTproBNP (pg/mL)	652 ± 190
	Ca125 (U/mL)	34.0 ± 11.6
kidney function	eGFR (mL/min)	62.8 ± 3.3
Index of endothelial function		
vasodilation in response to increased blood flow	FMD (%)	11.8 ± 1.78
Index of cardiac remodeling		
left atrial size	LAD (mm)	44.6 ± 1.0
	LAA (mm^2)	24.0 ± 0.7

Table 5. List of clinical, biochemical and echocardiographic parameters used for clustering and RF analysis. The value reported refers to baseline characteristics. Abbreviations: C-Reactive Protein, CRP; Erythrocyte Sedimentation Rate, ESR; estimated Glomerular Filtration Rate, eGFR; Flow-Mediated Dilation, FMD; left atrial area, LAA; left atrial diameter, LAD; plasma N-terminal pro-brain natriuretic peptide, NT-pro-BNP.

The first question addressed here concerns the existence of specific patterns distinguishing the clinical cohorts. To this aim, a clustering analysis was performed. The k-means algorithm was able to partially reproduce the clinical cohort. In fact, by inspecting the patient assigned to both classes, at the baseline, 75% of patients were correctly separated. At the follow-up, this accuracy decreased to 64%. Using the first two components of PCA, which account for 55% of the variance, a graphical representation of clusters is presented in **Figure 24**.

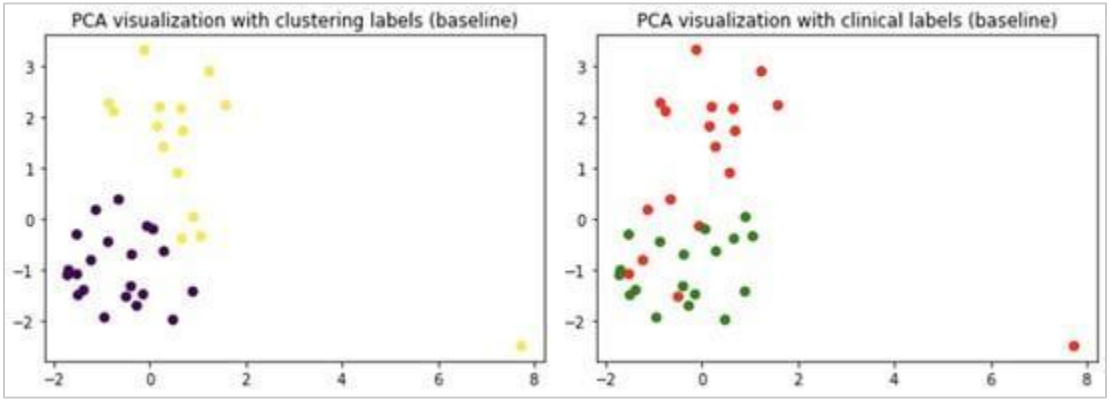

Figure 24. (left panel) A PCA visualization of the cluster results: purple and yellow denote the two cluster colors. (right panel) Clinical labels: patients who change therapy are in green, DOACs in red. The first two components account for 28% and 27% of the explained variance, respectively.

While PCA was used for visualization purposes, it is worth mentioning that the third component accounted for about 16% of the explained variance. The scores' plot shows at the baseline how the two clusters can be revealed with a limited overlap (left panel). However, inspecting the clinical labels, one can see that these clusters can only yield a partial representation of the clinical cohorts which result in a significant superimposition. To obtain further insight into the informative content provided by PCA, the variance explained was evaluated. In fact, the first two components accounted for 58% of the explained variance. Albeit quite low, the explained variance of the first two PCs highlights a reasonable patient clustering. Moreover, evaluating to what extent the clusters were able to reproduce the clinical labels afforded a cluster purity of about 75%.

An analogous analysis was carried out at follow-up, and the results are shown in **Figure 25**.

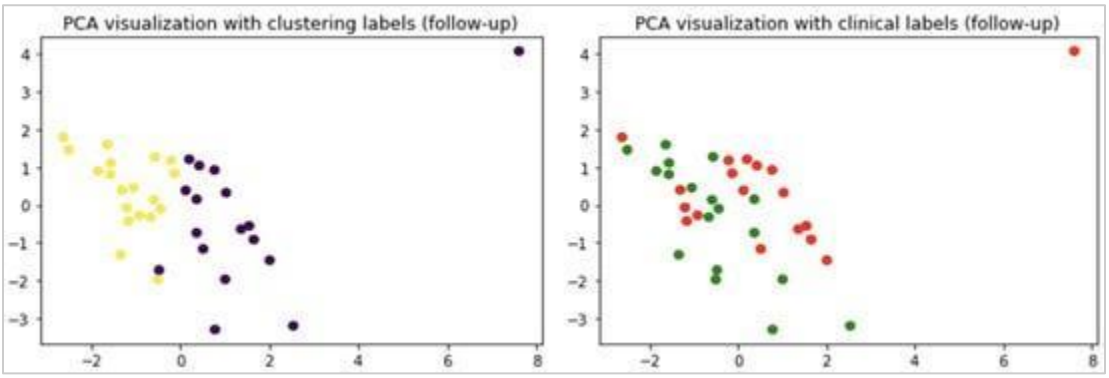

Figure 25. (left panel) A PCA visualization of the cluster results: purple and yellow denote the two cluster colors. (right panel) Clinical labels: patients who change therapy are in green, DOACs in red. The first two components account for 35% and 23% of the explained variance, respectively.

In this case, the separation in two clusters appeared less clear. Again, the representation can be considered reasonable as the first two PCs account for about 58% with the third one accounting for about 16% of the explained variance. The clinical cohorts appear overlapped, and this is reflected in the purity measure which decreases to 64%. While at the baseline the clinical cohorts seem to show two distinguished patterns, at the follow-up, these patterns appear to fade.

Unsupervised clustering revealed the presence, at least at the baseline, of two patterns partially distinguishing the two clinical cohorts. To further inspect this behavior and outline which feature(s) were able to reveal the two patterns, a supervised classification framework was adopted. An RF classifier was used within a loo cross-validation framework to evaluate to what extent the two cohorts were distinguishable. The classification contingency matrices, for both baseline and follow-up, are shown in **Figure 26**.

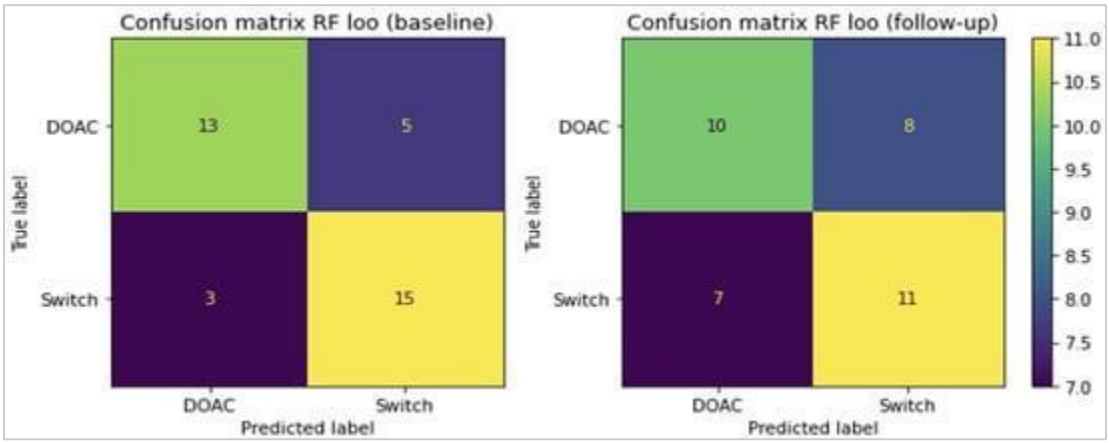

Figure 26. Contingency table for baseline classification (left panel). Contingency table for follow-up classification (right panel).

The baseline model is more accurate, achieving an average accuracy of 78%. At follow-up, the accuracy decreases to 58%. The accuracy loss of about 20% is statistically significant and suggests a fundamental loss of the features' discriminative power. To further assess the models' robustness, cross-validation analyses were repeated 20 times randomly subsampling 30 observations: in this way, we were able to evaluate a 2.5% accuracy uncertainty for both baseline and follow-up results. A feature importance analysis was then performed (**Figure 27**).

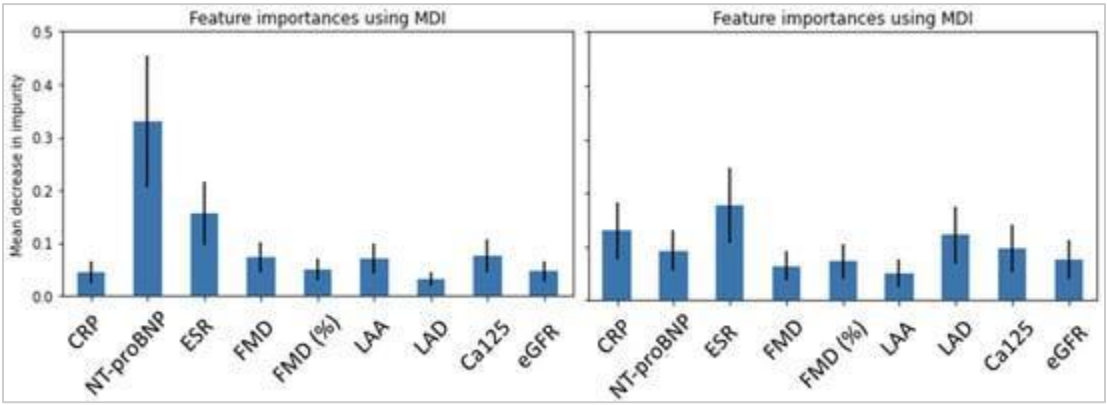

Figure 27. Feature importance measured in terms of mean decrease in impurity (MDI) for both baseline (left panel) and follow-up (right panel).

The baseline feature importance shown in the left panel indicates a fundamental contribution in discriminating the clinical cohorts brought by a single feature which is N-terminal pro-brain natriuretic peptide (NTproBNP). This feature alone, at the baseline, results in a mean decrease in impurity of 0.32 with peaks reaching almost 0.5. Of course, it must be kept in mind that RF models combine the available features to fully exploit their informative content; hence, it is not reasonable to assume that one feature is able to distinguish the cohorts on its own; nevertheless, the importance of this feature is manifest. Interestingly, at follow-up, no feature prevails over the others, and the overall importance of all features decreases, not reaching even a 0.2 value of mean decrease in impurity. A "flat" feature importance plot often signals that classification cannot achieve a reliable distinction of the classes.

Finally, to ease clinical interpretation, a comparison of NTproBNP values at baseline and follow-up is provided in **Figure 28.**

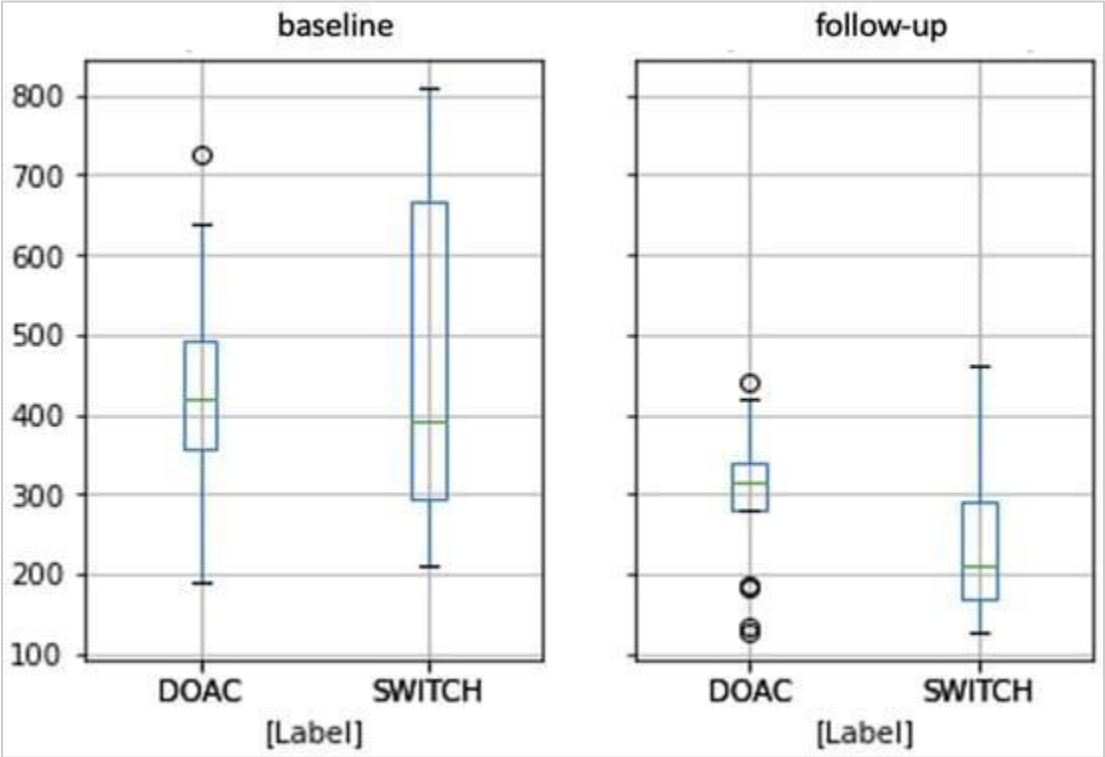

Figure 28. Boxplot representing NTproBNP values at baseline (left panel) and follow-up (right panel). The green lines represent the distributions' medians, the circles the statistical outliers.

As expected, NTproBNP values decrease for both clinical cohorts, thus demonstrating the efficacy of both treatments. Interestingly, the SWITCH cohort shows a stronger improvement; in fact, while patients treated with DOACs show a decrease in the median NTproBNP from about 420 to 310, the SWITCH cohort shows a decrease of about 200 points, from 400 to 200.

6. DISCUSSION AND CONCLUSIONS

SGLT2 inhibitors in HF patient and animal model. In this study, we aimed at assessing the short-term effect of gliflozins on cardiac remodeling and function and revealing possible predictors of clinical response using XAI algorithms in a population of diabetic patients with HFrEF. Beyond a positive effect on LV remodeling and performance, we detected a beneficial effect on RV systolic function.

In addition to glycemic control, several mechanisms have been hypothesized to explain the beneficial CV effects of gliflozins (218). Plausible assumptions included improvement in volume status, natriuresis, expansion of red blood cell mass, and myocardial energetics (218-220). Furthermore, a direct effect of gliflozins on LV remodeling, in particular on LV mass, volumes, and systolic and diastolic functions, has been reported (221,222). However, there was less convincing evidence on the impact of gliflozins on RV parameters. In a *post hoc* analysis, empagliflozin showed no significant impact on RV volumes or mass index in T2DM patients with coronary artery disease (223). On the other hand, in the randomized, multicenter, double-blind, placebo-controlled trial EMBRACE-HF, empagliflozin resulted in a reduction of pulmonary artery pressures regardless of the use of diuretics, although the proportion of diabetic patients was only 18% (224). Interestingly, our study provided evidence of the contribution of gliflozins to improving RV systolic function, as highlighted by the SHAP value obtained for RV S' velocity. As RV dysfunction was a strong predictor of survival for HFrEF (225, 226), RV systolic function improvement may contribute to the well-known reduction of mortality and hospitalization in diabetic patients with HFrEF (227-229). Distinct mechanisms of action and targets involving multiple biochemical and hemodynamic pathways have been proposed to explain the effects of gliflozins at the CV level and can also support an improvement of the RV systolic function (230). Direct myocardial effects such as a reduction in myocardial stretch and inhibition of Na^+-H^+ exchanger 1 or a reduction in oxidative stress and low-grade inflammation (231-234) could be relevant in this regard.

Regarding the potential influence on the remodelling and ejection fraction by other drugs concomitantly used, such as MRA and digoxin, it is worth noting that in our exploratory analysis all the available features were employed to train a classification model and never a classification accuracy different from chance was achieved; this led us to conclude that the other pharmacological treatments did not significantly affect the cohort.

The metabolic efficacy of SGLT2i in patients with T2DM has been investigated; the glucose-lowering effect appeared greater in patients with a shorter duration of T2DM, better renal function, and higher levels of HbA1c (235). These clinical factors may help to predict 'metabolic responders' to treatment with SGLT2i. On the other hand, poor data exist about markers predicting the response in terms of CV outcomes. Vaduganathan et al. demonstrated that higher levels of stress cardiac biomarkers, in particular troponin I, soluble suppression of tumorigenesis 2 protein, and insulin-like growth factor binding protein 7, were associated with a greater relative risk reduction in terms of CV events in the CANVAS study population, namely, in diabetic patients (236). LVEF appeared to poorly predict CV response, to gliflozins. According to a pooled analysis of the EMPEREOR-Reduced and EMPEREOR-Preserved trials, the beneficial effect of empagliflozin was similar in patients with LVEF <25% and <65% and produced a reduced response in patients with LVEF ≥65%, in terms of major adverse cardiovascular events and hospitalizations (237). Moreover, the use of algorithms failed to predict adequately CV and renal effects of gliflozins (238).

According to our observations, at a short-term follow-up, the population of gliflozins-treated patients was clearly distinguishable from non-treated patients, as evident in the histogram of average cross-validation scores. In addition, within the gliflozin-treated group, some patients were misclassified. The explainability analysis disclosed a pool of clinical variables that allowed us to distinguish the responder vs. non-responder patients at baseline with an accuracy of 74%. Actually, eight echocardiographic parameters and the presence of a high heart rate may predict the response to gliflozins in terms of anti-remodelling effects. In more detail, the presence of LV remodelling in advanced stages, high degree of RV dysfunction, and a higher heart rate may all together predict a lower probability of a beneficial response to gliflozins. Furthermore, TAPSE is considered a reliable index of the right ventricle's systolic function (1). Importantly, the right heart dysfunction may be a primitive disorder but is more often associated with left HF in advanced stages (239). Thus, low TAPSE values characterise patients with an advanced-stage HF and biventricular dysfunction. In other words, it appeared that patients with an overall lower TAPSE, higher LV mass and volumes, worse LV diastolic function, the presence of AF, and a higher HR could likely respond less to therapy with gliflozins.

Even if highly recommended as BB, ACEi/ARNI, or MRA for patients affected by HFrEF, including diabetic patients, there is no sound evidence about the best timing to start the gliflozins therapy. In general, gliflozins (e.g., dapagliflozin and empagliflozin) are highly recommended drugs in all patients with reduced LVEF, with a level of recommendation of IA (1). However, in

the main trials (DAPA-HF and EMPEREOR-Reduced) that led to the introduction of both dapagliflozin and empagliflozin in the guidelines for the treatment of HFrEF, gliflozins had been administered in patients with NHYA class > II, diabetic and not diabetic, in addition to optimal medical therapy with BB, ACEi/ARNI, and MRA. Gliflozins were, therefore, considered a 'second-line' treatment (214, 207). Moreover, a recent consensus from the American College of Cardiology suggests ARNI and BB as frontline therapies and MRA and SGLT2i as second-line treatments to be introduced in cases of persisting symptoms (240). Our results support the use of gliflozins as frontline treatment as soon as a diagnosis of HFrEF is made, as delayed treatment would act in more advanced stages of cardiac remodeling, resulting in a lower clinical efficacy. As it occurs for other pharmacological therapies, the need to compare drugs in terms of efficacy and order of administration requires a continuous re-evaluation of the algorithm to use.

This study confirms how XAI and ML algorithms have the potential to go beyond the simplistic phenotyping of HF, e.g., HF with reduced or preserved ejection fraction. To date, this is the first observation of simple clinical parameters to predict CV response to gliflozins. Whether these predicting parameters are applicable to a larger population deserves further investigation. Furthermore, as gliflozins are effective in diabetic patients with HFpEF as well as in HF irrespective of the diabetic state (233,241-243), it would be of interest to apply our analysis to additional populations.

In conclusion, an ML analysis of a population of diabetic patients with HFrEF shows that SGLT2i treatment results in a beneficial effect in terms of LV remodelling, LV diastolic function, and biventricular systolic function. This cardiovascular response may be predicted by routine echocardiographic and clinical parameters with an explainable ML approach. The results will be further validated in larger populations.

Renal and cardiovascular diseases share risk factors, such as hypertension and type 2 diabetes, and a strict pathophysiological and clinical relationship between kidney and heart constitutes the cardiorenal axis, whereby a dysfunction of one organ may induce a dysfunction in the other (244). Importantly, trials on diabetic and non-diabetic patients at risk of cardiovascular events have tested the efficacy of SGLT2i not only on cardiovascular outcomes but also on renal function, demonstrating that the beneficial effects were largely independent of the blood glucose-lowering action, and prompting dedicated studies evaluating renal outcomes (244-247). While experimental and clinical data on diabetic nephropathy has pointed out that dapagliflozin (and other SGLT2i) exerts protective effects on renal physiology by improving endothelial

dysfunction and inflammation (245-247), research on renal effects of SGLT2i in non-diabetic conditions are still limited and inconclusive (248-253).

In the non-diabetic model of salt-induced hypertension and cardiorenal syndrome, previously studied by Cappetta et al (74), the overexpression of several cytokines was observed, implying a large contribution of inflammatory components in the development of the pathology. Since the SGTL2i dapaglifozin induced a marked reduction of the inflammatory component, we aimed to investigate the expression profile of genes encoding for inflammatory mediators in non-diabetic salt-sensitive Dahl rats, which represent animal models of HFpEF. We also wanted to evaluate the effects of dapagliflozin on the progression of renal damage associated with the development of HFpEF.

In this study, dapagliflozin conferred benefits in diseased rats by partially preventing alteration in expression of several genes. Nevertheless, the partial restoration of gene expression is not sufficient to re-establish an electrolyte balance, as the sodium and chloride amounts remained elevated in the urine of HS animals. This could be because dapagliflozin increases glucose and sodium excretion without secondary effects on the expression and function of Na^+ channels and transporters along the nephron (254).

The major and novel findings are that dapagliflozin attenuated renal inflammation and endothelial activation and modulated RAAS by decreasing classic and enhancing non-classic pathways. These events translated into reduction of renal injury and highlighted a reno-protective role of this anti-diabetic drug in the absence of diabetes.

Chronic low-grade inflammation and endothelial dysfunction are two recognized determinants of renal dysfunction, characterized by a continuous production of pro-inflammatory cytokines that correlates with a worsening of clinical status of CKD patients (255-258). Accordingly, a progressive involvement of endothelial dysfunction and pro-inflammatory markers, limited at 11 weeks and significant at 17 weeks of age, was evident in our model. Of note, dapagliflozin significantly weakened the expression of adhesion molecule e-selectin and attenuated pro-inflammatory components.

RAAS signaling, a fundamental component of renal and cardiovascular pathophysiology, is essential in maintaining arterial blood pressure, and sodium and water balance (255-259). Furthermore, RAAS is strongly influenced by diet (260-261). In Dahl salt-sensitive rats or in hypertensive patients, the elevated sodium intake, leading to an increased glomerular ACE/ACE2 *ratio* or excessive AT1R activation, promote nitro-oxidative stress and proteinuria, and mediate deleterious effects by inducing vasoconstriction, vascular hypertrophy and fibrosis

(262,263). In the same scenario, the protective route, which *via* MAS1 activation leads to natriuresis and diuresis, seems to be suppressed. Indeed, non-classic RAAS is viewed as an essential element in counterbalancing the deleterious effects of angiotensin II.

In conclusion, our findings provide new insights into the understanding of the mechanisms involved in the pathogenesis of cardiovascular diseases, revealing inflammation and RAAS modification as important actors and the SGLT2i dapagliflozin as a positive impacting factor.

Anticoagulant therapy in HF patients. In this study, two groups of patients with AF and HF were analyzed at baseline and at a five-months follow-up: patients already in treatment with DOACs *vs* patients switching from warfarin to DOACs. In order to compare these groups, we took into consideration features not directly connected with the anti-thrombotic properties of DOACs but rather related to inflammation, clinical status, endothelial function and cardiac remodeling (**Table 3**).

Our study demonstrated that patients with AF and HF taking warfarin or DOACs for the prevention of embolic events were clearly distinguishable in terms of remodeling, clinical status, inflammation and endothelial function. Moreover, based on ML analysis, when warfarin-treated patients switched to DOACs, they were no longer differentiable. This means that DOACs somehow modify the considered features which have specific clinical significance (**Table 3**). Interestingly, a great contribution to this uniformization of VKA and DOACs groups is mainly due to NTproBNP, but the contribution of other features cannot be overlooked.

Brain natriuretic peptide (BNP) and NT-proBNP are secreted in equimolar concentrations into the blood stream after the cleavage of their precursor pro-BNP 13 (264). Specifically, NT-proBNP performs better than BNP for a longer half-life compared with BNP, and thus, the determination of NT-proBNP levels is today considered the best way to detect the activation of the natriuretic peptide (NP) system (64). Accordingly, NT-proBNP is a biomarker strictly related to the hemodynamic status of the patient as it is released in response to the stretching of atrial and ventricular walls: in other words, the release of NTproBNP depends on intracardiac pressure and fluid overload (265,266). Factors that cause the stretching of the heart wall can increase the blood level of NT-proBNP. Among the causes of increased heart wall tension, the following can be mentioned: systolic heart failure, the diastolic dysfunction of the heart, restrictive cardiomyopathy, acute coronary syndrome, valvular heart diseases, AF rhythm and amyloidosis (267).

Furthermore, BNP and NT-proBNP are also considered as markers of response to pharmacological treatment. During the treatment, their levels should be checked frequently, and

their downward trend indicates a suitable response to the treatment (268). The lack of a reduction during the treatment indicates an unstable condition and a poor prognosis in the patients. The reduction in NT-proBNP values for both clinical cohorts under investigation in our study also demonstrated the efficacy of DOAC treatment in switched patients.

In general, when a patient switches from VKAs to DOACs, the number of medical contacts is reduced. Thus, the hypothesis that a patient in DOAC treatment is better followed up could be excluded. Moreover, taking into account the features investigated and the observed reduction in NT-proBNP, a positive impact of DOACs, both thr- and fXa-selective inhibitors, on inflammation and endothelial function could be inferred. Indeed, preclinical evidence suggests that nonhemodynamic triggers for natriuretic peptide (NP) release exist, with inflammation increasing the levels of NTproBNP (269,270). Therefore, inflammatory conditions should be considered when interpreting NTproBNP levels, also in the case of patients with HF. NTproBNP could be considered a kind of crosstalk factor between clinical status and inflammatory status specifically associated with patient. Likewise, various indexes of endothelial dysfunction are associated with higher NTproBNP levels (271,272). Thus, it could be speculated that in our study, the reduction in NTproBNP levels observed in patients treated with DOACs at follow-up could be potentially related to anti-inflammatory effects and the amelioration of endothelial dysfunction mediated by DOACs.

Our study supports previous observations suggesting pleiotropic effects associated with DOACs besides their beneficial action in reducing embolic events and mortality in HF patients. As mentioned above, preclinical studies showed anti-inflammatory, antioxidant and anti-fibrotic effects on endothelial cells. Importantly, several studies highlighted DOAC-mediated effects on endothelial cells in terms of improvement in endothelial function and integrity and the inhibition of neo-angiogenesis (273,274). Although peculiar effects of a single molecule have not yet been determined, it is generally accepted that factor Xa inhibitors produce anti-angiogenic and anti-fibrotic effects beyond the anti-inflammatory action and stabilization of endothelial cells. In line with our study, the authors demonstrated a protective effect on endothelial function in patients with AF and HF switched from VKA to DOACs (157).

In conclusion, our ML analysis demonstrated that in a population of patients with HF and AF, patients treated with the VKA warfarin or DOACs are distinguishable according to features related to inflammation, endothelial function, cardiac remodeling and clinical status. Due to the clinical database composition, our multivariate analysis cannot distinguish the behaviour of a selective thrombin inhibitor (dabigatran etexilate) from that of selective inhibitors of factor Xa

(apixaban, rivaroxaban and edoxaban). It appeared quite clear, however, that the switch from warfarin to DOACs, regardless of their pharmacological target in the blood coagulation cascade and their pharmaceutical properties, aligned the features of the patients, particularly in relation to NTproBNP levels.

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9. RIASSUNTO

Introduzione e obiettivi. L'insufficienza cardiaca è una delle principali cause di morte e ospedalizzazione, soprattutto nei pazienti diabetici. Il cardine del trattamento per l'insufficienza cardiaca è rappresentato dagli inibitori del cotrasportatore sodio-glucosio di tipo 2 (SGLT2i), rappresentata dalla classe delle gliflozine, soprattutto nell'insufficienza cardiaca con frazione di eiezione ventricolare sinistra ridotta (HFrEF). Tuttavia, gli effetti di SGLT2i sul rimodellamento e sulla funzione ventricolare non sono ancora stati completamente compresi. L'intelligenza artificiale rappresenta un'opzione esplorativa senza precedenti per la ricerca clinica in questo campo. Sulla base delle valutazioni ecocardiografiche, il nostro primo obiettivo è stato quello di identificare alcune risposte cliniche chiave alle gliflozine in pazienti con scompenso cardiaco impiegando un approccio di apprendimento automatico. La disfunzione renale è comune nell'insufficienza cardiaca e nell'ipertensione ed è associata ad un aumento del rischio di mortalità. Tuttavia, i meccanismi esatti alla base dello sviluppo patologico di questo stato cronico sono scarsamente chiariti, sebbene un ruolo principale sia stato attribuito all'infiammazione. Pertanto, in parallelo all'analisi dei dati relativi ai pazienti con scompenso cardiaco, nell'ambito del primo obiettivo, abbiamo investigato il ruolo dell'infiammazione nella sindrome cardio-renale e il possibile impatto di dapagliflozin utilizzando un modello animale di scompenso cardiaco.

Nel contesto dell'insufficienza cardiaca, la fibrillazione atriale rappresenta un'aritmia estremamente frequente. La terapia anticoagulante orale per la gestione della fibrillazione atriale comprende antagonisti della vitamina K e anticoagulanti orali ad azione diretta. A causa del minor rischio di sanguinamento associato agli anticoagulanti diretti, lo switch terapeutico nell'ambito delle varie classi di anticoagulanti è una pratica comune nei pazienti con fibrillazione atriale. Tuttavia, ci sono problemi legati allo switch terapeutico che devono ancora essere pienamente compresi, soprattutto per i pazienti in cui la fibrillazione atriale e l'insufficienza cardiaca coesistono. Come secondo obiettivo, abbiamo quindi cercato di valutare gli effetti positivi e pleiotropici mediati dagli anticoagulanti diretti, oltre alla loro attività anticoagulante. In particolare, abbiamo valutato l'impatto dello switch terapeutico da warfarin agli anticoagulanti orali diretti in pazienti con fibrillazione atriale mediante la tecnologia del *Machine Learning*.

Metodi. Per il primo obiettivo, sono stati valutati 78 pazienti diabetici consecutivi seguiti ambulatorialmente per insufficienza cardiaca con ridotta frazione di eiezione del ventricolo sinistro, utilizzando una classificazione *Random Forest*. È stata eseguita un'analisi di un singolo

soggetto per definire il profilo dei pazienti trattati con gliflozine. Inoltre, è stata utilizzata una analisi di *explainability* con i valori *Shapley* per delineare i parametri clinici che sono migliorati maggiormente dopo la terapia con gliflozine; parallelamente, mediante *Machine Learning* ha evidenziato variabili specifiche predittive della risposta a gliflozine. Gli studi su un modello animale di insufficienza cardiaca volti a valutare i marcatori di infiammazione, funzione endoteliale, funzione renale e fibrosi ed efficacia del trattamento con dapagliflozin sono stati condotti utilizzando ratti Dahl alimentati con una dieta ricca di sale. Per il secondo obiettivo, sono stati arruolati 42 pazienti consecutivi seguiti ambulatorialmente con insufficienza cardiaca con riduzione della frazione di eiezione del ventricolo sinistro e fibrillazione atriale in terapia anticoagulante orale da almeno un anno. Il metodo *clustering k-means* e l'algoritmo di apprendimento *Random Forest* sono stati applicati al fine di valutare come lo switch terapeutico dal warfarin ai DOAC possa influenzare la progressione della malattia. **Risultati.** Le analisi di convalida incrociata hanno mostrato che i pazienti trattati con gliflozine possono essere identificati con una accuratezza dello 0,70 ± dello 0,03%. I parametri più rilevanti che contraddistinguono i pazienti trattati con gliflozine da i pazienti non trattati sono stati la velocità S del ventricolo destro, il diametro sistolico dell'estremità del ventricolo sinistro e il rapporto E/e'. Inoltre, bassi valori di escursione sistolica sul piano anulare tricuspide, insieme a valori elevati di diametro telesistolico del ventricolo sinistro e di volume telediastolico, sono stati associati a una minore efficacia della gliflozine in termini di effetti anti-rimodellamento. Il modello animale di insufficienza cardiaca utilizzato in questo studio, ha mostrato a livello renale la presenza di infiammazione, disfunzione endoteliale, fibrosi, aumento dello stress ossidativo. Dopo il trattamento con dapagliflozin, la funzionalità renale è risultata migliorata nei vari parametri considerati. Nello studio relativo alla fibrillazione atriale e insufficienza cardiaca, alla baseline il 75% dei pazienti è stato separato correttamente. Al follow-up, dopo il passaggio agli anticoagulanti diretti, questo parametro si è ridotto al 64%. Parallelamente, il modello al *baseline* è risultato più accurato, raggiungendo un valore medio del 78%. Al follow-up, l'accuratezza è risultata ridotta al 58%. La perdita di accuratezza di circa il 20% è statisticamente significativa e suggerisce una perdita del potere discriminante delle caratteristiche dei pazienti. I livelli di peptide natriuretico pro-cerebrale N-terminale giocano un ruolo chiave nella discriminazione delle coorti di pazienti considerate. **Discussione e Conclusioni.** Una analisi *Machine Learning* su una popolazione di pazienti diabetici con insufficienza cardiaca con riduzione della frazione di eiezione del ventricolo sinistro ha mostrato che il trattamento con gliflozine ha migliorato il rimodellamento ventricolare sinistro. Questa risposta cardiovascolare può essere prevista con

tecniche di intelligenza artificiale, suggerendo una minore efficacia in caso di stadi avanzati di rimodellamento cardiaco. Il trattamento con dapagliflozin ha esercitato un effetto positivo sull'infiammazione, la disfunzione endoteliale, la fibrosi e la funzione renale del modello animale di scompenso cardiaco. La nostra analisi *Machine Learning* in pazienti con fibrillazione atriale e insufficienza cardiaca con riduzione della frazione di eiezione del ventricolo sinistro ha dimostrato che, quando i pazienti trattati con warfarin subiscono uno switch terapeutico verso i DOAC, non risultano più differenziabili. Ciò significa che i DOAC modificano in qualche modo le caratteristiche cliniche considerate.