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PhD COURSE IN TRANSLATIONAL MEDICINE

Department of Clinical Sciences and Community Health, Cardiovascular Section

PhD Thesis

Effects of Dapagliflozin on exercise capacity, respiratory function, biomarkers, sleep apnea, and left ventricular remodeling in patients with heart failure: a prospective, single center, non randomized study

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Abstract

Background:
Dapagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, has become a cornerstone in the management of heart failure with reduced ejection fraction (HFrEF). Its clinical efficacy is well-established, primarily attributed to its effects on diuresis and hemodynamic unloading. However, despite its widespread use and therapeutic success, the precise mechanisms through which dapagliflozin exerts its beneficial effects remain incompletely understood. Current research is focused on elucidating the broader physiological pathways involved. Given the complexity of its actions, a more comprehensive understanding of its multifaceted effects will require detailed, multidomain assessments, spanning metabolic, cardiovascular, and renal functions.

Methods:
A single prospective cohort of stable HFrEF patients was studied in two parallel, complementary investigations. The first focused on exercise capacity, cardiac remodelling, fluid status, and conventional biomarkers. The second investigated alveolar-capillary membrane function through surfactant protein-B (proSP-B), gas exchange, and sleep-disordered breathing. Evaluations were performed at baseline, 2–4 weeks, and after 6 months of treatment with dapagliflozin 10 mg/day.

Results:
Among 75 patients enrolled, 67 completed full follow-up. Dapagliflozin improved left ventricular ejection fraction (LVEF), reduced cardiac volumes and pulmonary artery pressures, and enhanced ventilatory efficiency (VE/VCO_2 slope). Haemoglobin increased significantly, while peakVO_2, natriuretic peptides, and spirometry remained unchanged. DLCO and its subcomponents (Dm and Vcap) were stable, yet a significant reduction in circulating proSP-B was observed, suggesting improved alveolar-capillary membrane integrity. Central sleep apnoea frequency decreased in affected patients, with no impact on obstructive events or total apnoea burden.

Conclusions:
In this multidimensional evaluation of a single HFrEF cohort, dapagliflozin showed concordant benefits on cardiac remodelling, ventilatory efficiency, haemoglobin, and alveolar-capillary membrane health, despite unchanged peakVO_2 and pulmonary function. These findings reinforce dapagliflozin's complex therapeutic profile, involving both hemodynamic and non-hemodynamic mechanisms.

Introduction

-Heart Failure

Heart failure (HF) is a syndrome caused by a functional and/or structural cardiac abnormality. It occurs when the heart is unable to pump enough blood forward to meet the metabolic demands of the entire body, or is able to do so only with high cardiac filling pressures [1]. Therefore, this impairment led to a reduced cardiac output and/or elevated intracardiac pressures at rest or during exercise.

To establish a diagnosis of HF, the European Society of Cardiology (ESC) guidelines warrant the presence of typical signs or symptoms (mainly decreased exercise tolerance with dyspnoea, fatigue, fluid retention and peripheral oedema), evidence of cardiac dysfunction (usually assessed by echocardiography), and a favourable response to specific treatment directed towards HF. HF aetiology can be different and to establish the underlying cardiac problems is important, mainly for therapeutic reasons. The most common causes of HF can be divided into three main categories:

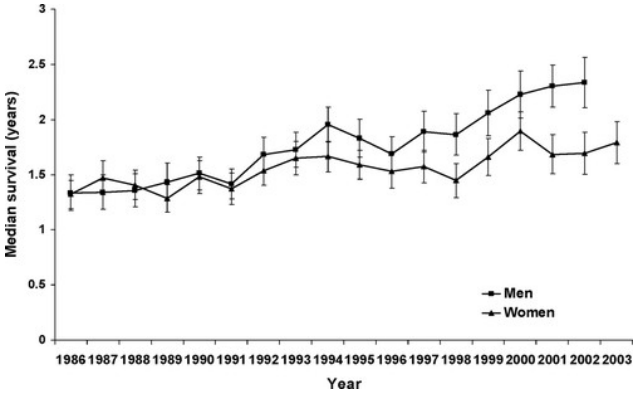
1) Myocardial disease caused by coronary heart disease, acute or chronic ischaemia, cardiomyopathies, immune-mediated and inflammatory damage (myocarditis), cardiotoxic substances, infiltration and hormonal or nutritional derangements.

2) Abnormal loading conditions caused by valvular disease, hypertension and pericardial or endomyocardial pathologies.

3) Arrhythmias in the form of tachyarrhythmias such as atrial fibrillation and ventricular arrhythmias or bradyarrhytmias.

Nevertheless, it is often difficult to discover the primary aetiology of HF in a patient with multiple potential causes also due to their complex interplay in the pathophysiology.

HF is a common disease, especially (but not only) in the elderly. It affects approximately 1–2% of the adult population in developed countries and both incidence and prevalence increase progressively with age [2] The number of HF patients is increasing, not only because of the greater life expectancy, but also as a result of interventions that prolong survival after damaging cardiac insults (i.e. post- myocardial infarction). Moreover, over the last few years, improvements in treatments and their implementation have increased survival and reduced the hospitalization rate in HF patients (Figure 1 and 2) [3].



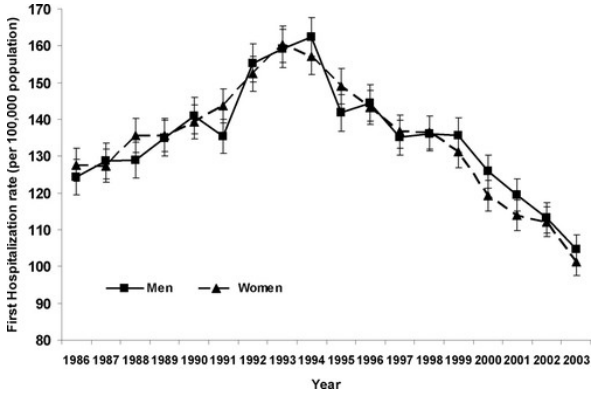


Figure 1. Trends in median HF survival according to sex and year of admission

Figure 2. Age-adjusted trends in first hospitalization for HF according to sex

However, HF remains a major public health problem associated with a high mortality rate, frequent hospitalizations and poor quality of life. HF is a chronic condition characterized by an acute exacerbation (index episode) and followed by increasing symptoms that lead to repeated hospitalizations and a significantly greater risk of premature death. Each year, 1.0 million patients are hospitalized due to HF in the US [4] and in Europe approximately 5% of all acute hospital admissions are HF-related [5]. From a prognostic point of view, approximately 40% of HF patients admitted to hospital will either die or be readmitted within 1 year and nearly 50% of HF patients die within 4 years of diagnosis [6]. The overall 5-year survival rate for HF is as poor as, or worse than, that for advanced cancer or stroke [4].

-Classification of heart failure

The main terminology used to describe HF is based on the measurement of the left ventricular ejection fraction (LVEF), the fraction of end-diastolic volume ejected from the ventricle during each systolic contraction. This is usually estimated using transthoracic echocardiography, or, less frequently, using a radionuclide technique or cardiac magnetic resonance. Accordingly, patients can be divided into two main groups: those with a reduced ejection fraction (typically considered as <40%; HF with reduced LVEF (HFrEF)), and those with normal LVEF (typically considered as ≥50%; HF with preserved LVEF (HFpEF)). The 2016 ESC guidelines have finally provided diagnostic criteria for a newly defined group of HF patients with a mild systolic dysfunction and a LVEF in the range of 40–49%, known as HF with mid-range LVEF (HFmrEF). This classification has been maintained in the most recent ESC guidelines [7]. A LVEF-based classification of HF patients is important due to the different underlying aetiologies, demographics, co-morbidities and response to therapies [8].

Another clinical approach is to classify patients according to the severity of symptoms, as per the New York Heart Association (NYHA) functional classification, based on the patient's impairment and exercise intolerance, and the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) classification, which describes stages of HF development based on structural changes and symptoms. The two classification systems are compared in Table 1.

Table 1. Comparison of NYHA functional class and ACCF/AHA stages of HF

<table>
<tr><th colspan="2">NYHA functional classification</th><th colspan="2">ACCF/AHA stages of heart failure</th></tr>
<tr><td colspan="2">None</td><td>A</td><td>At high risk of HF but without structural heart disease or symptoms of HF.</td></tr>
<tr><td>I</td><td>No limitation of physical activity. Ordinary physical activity does not cause undue breathlessness, fatigue, or palpitations.</td><td>B</td><td>Structural heart disease but without signs or symptoms of HF.</td></tr>
<tr><td>I</td><td>No limitation of physical activity. Ordinary physical activity does not cause undue breathlessness, fatigue, or palpitations.</td><td rowspan="2">C</td><td rowspan="2">Structural heart disease with prior or current symptoms of HF</td></tr>
<tr><td>II</td><td>Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in undue breathlessness, fatigue, or palpitations.</td></tr>
</table>

III	Marked limitation of physical activity. Comfortable at rest, but less than ordinary physical activities results in undue breathlessness, fatigue, or palpitations.		
IV	Unable to carry on a physical activity without discomfort. Symptoms at rest can be present.		
IV	Unable to carry on a physical activity without discomfort. Symptoms at rest can be present.	D	Refractory HF requiring specialized interventions.

Finally, HF can be defined according to its clinical presentation:

1. chronic HF refers to the long-term condition, characterized by the progressive reduction of cardiac performance, usually kept stable by the treatment of symptoms;

2. acute HF refers to the rapid onset or worsening of symptoms and/or signs of HF. Acute HF may present as a first occurrence or, more frequently, as a consequence of acute decompensation of chronic HF. It may be caused by primary cardiac dysfunction or it could be precipitated by extrinsic factors.

-Pathophysiological modifications in heart failure

In a healthy person, cardiac output is matched to the body's total metabolic need. The three major determinants of stroke volume – defined as the volume of blood ejected with each contraction - are preload, afterload and myocardial contractility (figure 3). An abnormality in one (or more) of these factors can lead to HF [9].

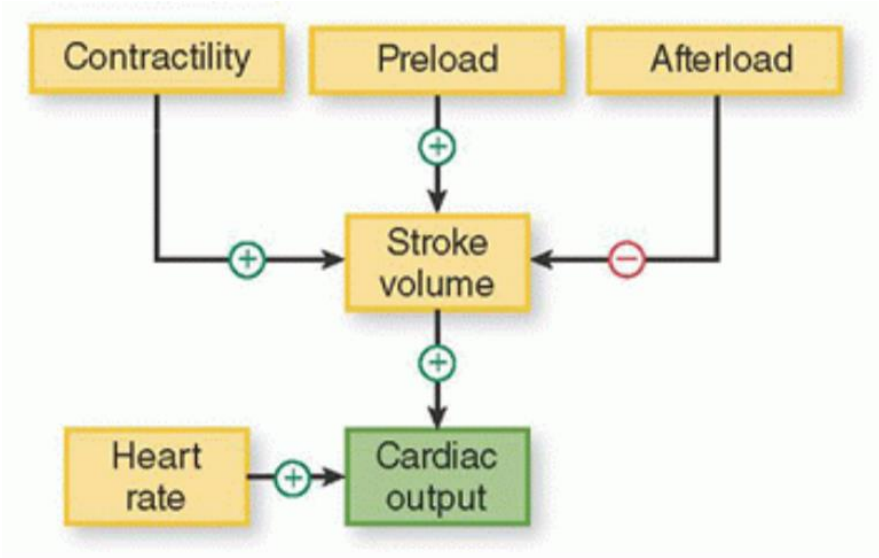


Figure 3. Key mediators of cardiac output = heart rate × stroke volume

-Preload

Preload is defined as the ventricular myocardial stretch at the end of diastole, caused by the blood filling the ventricle immediately before the contraction. Therefore, the preload corresponds to the end-diastolic volume. Within a physiologic range, the larger the ventricular volume during diastole, the more the fibers are stretched and the greater the force of the next contraction is. This is the basis of the Frank-Starling relationship, the observation that cardiac output increases in relation to the preload because stretching the muscle before stimulation optimizes the interaction of myosin and actin filaments and it also increases the sensitivity of the myofilaments to calcium, which further augments force development.

The Frank-Starling curve, also known as the ventricular function curve, is shown in the graph that relates preload, measured as left ventricular end-diastolic volume or pressure, to cardiac performance, measured as ventricular stroke volume or cardiac output (Figure 4).

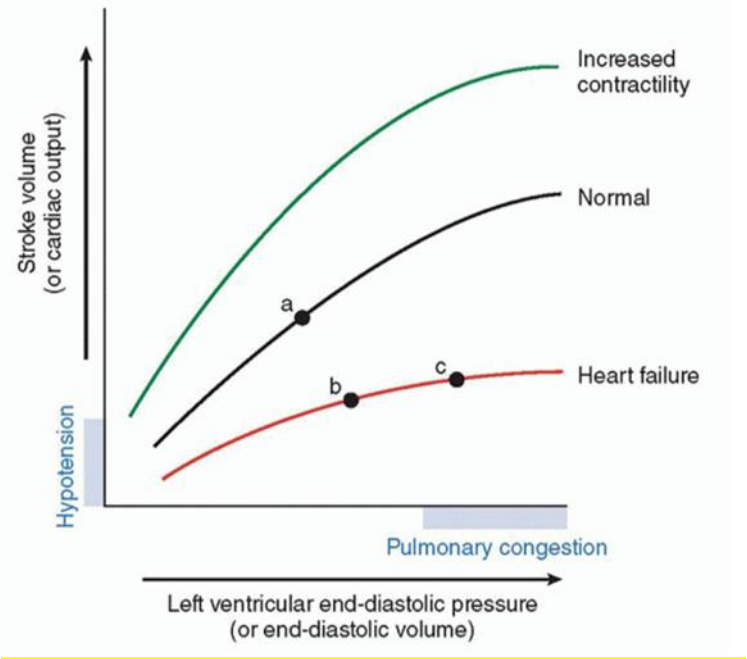


Figure 4. Frank-Starling curves. Point a is a normal subject at rest, point b is a HF patient with LV systolic dysfunction, point c is a patient with severe HF

On the normal heart curve (middle line), cardiac performance continuously increases as a function of preload. Point a is an example of a healthy person at rest. Conversely, point b represents the same person after developing systolic dysfunction and HF (e.g. after a large myocardial infarction). The impaired left ventricular contractile function shifts the performance curve downward and, at a given preload, stroke volume results decreased. The reduced left ventricular emptying causes the elevation of the end-diastolic volume, which serves a compensatory role by increasing the stroke volume in the subsequent systole.

However, this beneficial compensatory mechanism has its limits. In the case of severe HF with marked depression of contractility (point c), the curve appears nearly flat at higher diastolic volumes, reducing the augmentation of cardiac output achieved by the increased chamber filling. Concurrently, in such a circumstance, marked elevation of the end-diastolic volume and pressure (which is transmitted retrograde to the left atrium, pulmonary veins, and capillaries) may result in pulmonary congestion and pulmonary oedema.

-Afterload

Afterload is the ventricular wall tension during contraction, reflecting the resistance that the ventricle must overcome to empty its contents. According to the Laplace relationship, ventricular wall stress rises in response to a higher-pressure load (e.g. hypertension) or an increased chamber size (e.g. a dilated left ventricle). Conversely, hypertrophy increases wall thickness, serving as a compensatory mechanism in reducing wall stress, because in this way the force is distributed over a greater mass of ventricular muscle.

Just like preload, an increase in afterload shifts the Frank-Starling curve down and to the right (Fig. 4), decreasing stroke volume and, at the same time, increasing left ventricular end-diastolic pressure. The reason is a decrease in fiber shortening velocity that reduces the rate of volume ejection and consequently increases end-systolic volume. This residual volume is added to the venous return into the ventricle and this augments end-diastolic volume. This increase in preload activates the Frank-Starling mechanism to partially compensate for the reduction in stroke volume caused by the increase in afterload.

-Myocardial contractility

Myocardial contractility is defined as the property of heart muscle to change the strength of contraction, independent of the preload and afterload. Contractility reflects chemical or hormonal influences on the force of contraction.

In the Frank-Starling curve, a change in contractility shifts the entire curve in an upward or downward direction. Thus, when contractility is enhanced, the ventricular performance curve is displaced upward so that at any given preload, the stroke volume is increased. Conversely, when the ventricle's contractile function is impaired (as in certain types of HF), the curve shifts downward, leading to reductions in stroke volume and cardiac output at any given preload.

-Compensatory mechanisms

Several natural compensatory mechanisms are called into action in patients with HF to balance the fall in cardiac output and help preserve a sufficient blood pressure to perfuse the vital organs. These compensations include: the Frank-Starling mechanism, neurohormonal mechanisms, and myocardial hypertrophy with ventricular remodelling.

-Neurohormonal activation

The first mechanism to come into action is the sympathetic nervous system, activated by the baroreceptors and chemoreceptors located in the carotid sinus and aortic arch, which sense the fall in cardiac output. This causes an increase in heart rate and ventricular contractility that directly increase cardiac output. In addition, the vasoconstriction triggered by the stimulation of α-receptors on the systemic veins and arteries redistributes blood flow to vital organs, particularly heart and brain, at the expense of the skin, splanchnic viscera, and kidneys.

Another compensatory mechanism is the activation of the renin-angiotensin-aldosterone system (RAAS), mediated by the increased renin secretion from the juxtaglomerular cells of the kidney. The main stimuli for renin release in HF patients include renal hypoperfusion, secondary to low cardiac output, and the direct stimulation of juxtaglomerular β-receptors by the activated adrenergic nervous system. Renin is an enzyme that converts angiotensinogen into angiotensin I, which is then rapidly cleaved by angiotensin-converting enzyme (ACE) to form angiotensin II, a potent vasoconstrictor. In addition, angiotensin II

stimulates the secretion of aldosterone, a hormone that promotes sodium and water reabsorption into the circulation, thus augmenting intravascular volume. The rise in blood return to the heart increases left ventricular preload and thereby improves cardiac output via the Frank-Starling mechanism in patients on the ascending portion of the ventricular performance curve [10].

Other neuroendocrine responses are the release of vasoconstrictor substances like endothelin that increases peripheral resistances and distribution of cardiac output and vasopressin, which is also an antidiuretic peptide. Finally, there is an increase in the release of cytokines like the tumor necrosis factor (TNF-α), leading to cachexia in the last stages of HF.

-Myocardial hypertrophy with ventricular remodelling

Ventricular hypertrophy and remodelling are important compensatory processes that develop over time in response to hemodynamic burdens in order to improve contractility. A sustained wall stress, along with neurohormonal and cytokine alterations, stimulates the development of myocardial hypertrophy and the deposition of extracellular matrix. This increased mass of muscle fibers serves as a compensatory mechanism to help maintain contractile force and, therefore, cardiac function. However, because of the increased stiffness of this wall, these benefits come at the expense of higher diastolic ventricular pressures, which are transmitted to the left atrium and pulmonary vasculature. Therefore, hypertrophy and remodelling help to reduce wall stress and maintain contractile force, by increasing cardiomyocyte size and thickening the ventricular walls, but ultimately, leading to a progressive decline in cardiac function. The excessive hemodynamic burden on the contractile units produces, in the end, progressive HF symptomatology [11].

-Downsides of the compensatory mechanisms

Although the acute effects of these compensatory mechanisms are beneficial, their chronic activation often ultimately proves detrimental to the failing heart and contributes to a progressive downhill course [12]. In fact, vasoconstriction increases afterload, which may then impede cardiac output, while the excess fluid retention contributes to peripheral oedema and pulmonary congestion. In addition, the increased heart rate augments the metabolic demand on the failing heart. Continuous sympathetic activation results in down-regulation of cardiac β-adrenergic receptors, contributing to a decrease in the myocardial sensitivity to circulating catecholamines and a reduced inotropic response. Moreover, chronically elevated levels of angiotensin II lead to the production of cytokines and stimulate fibroblasts, resulting in fibrosis and adverse remodelling of the failing heart.

Because the undesired consequences of chronic neurohormonal activation eventually outweigh the benefits, much of the current pharmacological therapy of HF aims to moderate these compensatory mechanisms.

-Natriuretic Peptides

As opposed to the ultimately adverse consequences of the neurohormonal alterations previously described, the natriuretic peptides are natural “beneficial” hormones secreted in HF in response to increased intracardiac pressures.

The natriuretic peptides are a family of similar but genetically distinct peptides, which include the atrial, brain-type, and C-type natriuretic peptides (ANP, BNP, and CNP, respectively) [13].

ANP is stored in atrial cells and is released in response to chamber distention [14, 15]. BNP is not normally detected in healthy hearts but is produced in response to volume overload and therefore to an increased tension of the cardiac chambers [16]. Contrary to ANP, which originates mainly from atrial tissue, ventricular myocytes constitute the major source of BNP and cardiac fibroblasts have also been recently shown to produce BNP [17, 18]. While ANP is secreted as the active hormone [19], BNP is synthesized as a preprohormone of 132 amino acids that is then processed to a 108-amino acid precursor protein. In the circulation the pro-peptide is cleaved into the biologically active 32 amino acid carboxy-terminal fragment and an inactive 76 amino acid N-terminal fragment, NT-proBNP [20, 21]. As regards CNP, the major sites of its expression are the nervous system and endothelial cells. Heart tissue contains little CNP and only small amounts are found in the plasma [22, 23].

To exert their physiological actions, natriuretic peptides interact with the natriuretic peptide receptors type A (NPR-A) and type B (NPR-B), which are coupled to guanylyl cyclase. The resulting elevation of the intracellular second messenger cyclic guanosine monophosphate (cGMP) may exert diverse physiological effects through activation of cGMP-dependent protein kinase [24, 25]. Their multiple biologic effects depend on the location of the receptor:

1) in the cardiovascular system, natriuretic peptides act on both venous and arterial smooth muscle causing vasodilation, reducing peripheral resistance and lowering blood pressure. Moreover, they increase the permeability of the endothelium promoting the flow of fluids towards the extravascular compartment. Therefore, they reduce both the preload and the afterload. Alongside these effects, natriuretic peptides seem to have antifibrotic and antiproliferative effects on cardiac myocytes [26-28];

2) in the kidneys, they exert a diuretic and natriuretic effect resulting in the increase of electrolytes and water excretion by antagonizing the RAAS; finally they limit water reabsorption thanks to the inhibition of vasopressin [29];

3) in the neuroendocrine system, besides interfering with the RAAS, natriuretic peptides decrease plasma levels of endothelin, a potent vasoconstrictor, and act on the sympathetic nervous system reducing catecholamine release and suppressing the arterial baroreceptor response [30, 31];

4) in the immune system, natriuretic peptides reduce the production of TNF-α in the macrophages and prevent the synthesis of cytokines and chemokines. This action blocks both the recruitment of leucocytes and the activation of the inflammatory response.

A third receptor, NPR-C, binds all natriuretic peptides with equal affinity and probably serves a primary role as a clearance receptor [32]. Natriuretic peptides are also cleared from plasma through proteolysis by neutral endopeptidase 24.11, also known as neprilysin (NEP) [33]. This is a zinc-dependent enzyme that hydrolyses peptides on the amino side of their hydrophobic residues [34]. NEP is widely expressed in kidney, lung, and endothelial cells, vascular smooth muscle cells, cardiac myocytes, fibroblasts, neutrophils, adipocytes, testes, and brain, with the highest concentrations being present in the renal proximal tubule [35-37].

NEP is also involved in the degradation of bradykinin, endothelin and angiotensin II [38-41]. Since many substrates for NEP are peptides with vasoactive, diuretic and natriuretic actions, NEP is a potentially useful therapeutic target in HF [42].

Besides, NEP does not hydrolyse NT-proBNP, therefore the latter is a useful cardiac biomarker to assess therapeutic effect and prognosis in patients treated with NEP inhibitors [26].

Given the marked augmentation of circulating natriuretic peptides in clinical conditions characterized by left ventricular dysfunction, the measurement of their plasma concentration can be a helpful diagnostic tool in discriminating symptoms of HF, like dyspnoea, from other non-cardiac causes [43].

Among all the natriuretic peptides, BNP has been demonstrated to be a powerful predictor of functional status deterioration and a marker for prognosis and risk stratification in the setting of HF [44, 45].

Multiple systematic reviews have confirmed BNP and NT-proBNP levels as a useful biomarker in the diagnosis of HF [22, 46]. According to the most recent ESC guidelines, the upper limit of normality in the non-acute setting for BNP is 35 pg/mL and 125 pg/mL for NT-proBNP, whereas in the acute setting, higher values should be used (BNP 100 pg/mL and NT-proBNP 300 pg/ mL). BNP and NT-proBNP also have a strong negative predictive value in patients with HF [47]. Indeed, patients with high circulating levels of BNP have a higher probability of deterioration of their functional status and hospital readmission or death, as compared with those with only moderately increased levels [48].

Surfactant binding proteins

Surfactant protein B (SP-B) is vital for normal lung function, and its complete deficiency leads to lethal, neonatal respiratory distress syndrome, which is characterized by a virtual absence of lung compliance, highly disorganized lamellar bodies, and greatly diminished levels of surfactant protein C (SP-C) mature peptide [49].

Previous research strengthens the role of the circulating immature SP-B forms (proSP-B) as the most reliable lung-specific circulating marker for alveolar-capillary membrane dysfunction (carbon monoxide lung diffusion (DLCO)) and for overall clinical status (NYHA class, peak oxygen consumption (VO2), minute ventilation (VE)/carbon dioxide production (VCO2) slope, etc.) of HF [50]. Notably, in terms of HF hospitalization, proSP-B overwhelms the prognostic role of other most frequently used parameters related to lung dysfunction such as DLCO, VE/VCO2 slope and spirometric data. With respect to other proteins proposed as possible markers of lung damage [51], immature circulating SP-B has some peculiarities that render it a potential specific marker for alveolar-capillary membrane dysfunction, such as: its essential role in the assembly of pulmonary surfactant; its predominant pulmonary synthesis, which differs from other surfactant proteins (i.e. surfactant protein A (SP-A) and surfactant protein D (SP-D)); its multistep pulmonary-cell-specific proteolytic maturation, which yields many immature intermediates with different molecular masses (from ~40 to ~20 kDa); and, its storage with surfactant phospholipids in lamellar bodies, the contents of which are released into the bloodstream only in case of alveolar–capillary barrier damage.

SP-B biosynthesis is a complex process involving both post-translational and proteolytic events. Prepro-SP-B is modified by glycosylation and signal peptide cleavage resulting in the proSP-B within the endoplasmic reticulum. Extensive studies demonstrated that the initial proteolytic cleavage of the N-terminal propeptide occurs in the medial Golgi with a subsequent C-terminal cleavage in the trans-Golgi, whereas a final N-terminal cleavage event occurs in a post-Golgi compartment, possibly in the multivesicular body, resulting in the mature form of SP-B in the lamellar body [49].

SP-B has a strong hydrophobic character [52], it is water-insoluble, co-isolates with lipids during the extraction of surfactant with organic solvents, and, consisting of amphipathic α-helices connected by highly apolar loops, preferably interacts with anionic phospholipids. In vitro, addition of SP-B to liposomes, composed of synthetic phospholipids, leads to membrane binding, destabilization, and fusion, ultimately resulting in dramatic rearrangement of the membrane structure; two properties, fusion and destabilization, that are likely important for the transition of surfactant phospholipids from the intracellular stores to the extracellular surfactant film [53].

Several reports studied surfactant proteins as markers of alveolar capillary membrane function either in physiological conditions, such as during high altitude hypobaric hypoxia exposure, or in case of respiratory and cardiovascular diseases such as HF [54-56]. Indeed, De Pasquale et al. reported a rapid SP-B reduction

after clinical improvement in acute HF. Accordingly, SP-B may in the near future become a marker to clinically follow alveolar capillary membrane damage and restoring processes

-Clinical manifestations of heart failure

The clinical manifestations of HF result from impaired forward cardiac output and/or elevated venous pressures relating to ventricular dysfunction.

The most prominent manifestation of chronic left ventricular failure is dyspnoea on exertion. The elevation of the end-diastolic volume and pressure leads to interstitial oedema, as a consequence of imbalanced hydrostatic and osmotic pressures between capillaries and alveoli. The resulting reduced pulmonary compliance and the excess fluid in the interstitial space, increase the resistance to airflow and require greater effort of respiration. Interstitial oedema can then evolve into pulmonary oedema when fluids leak into the alveoli impairing gas exchange and worsening breathing problems. Some pulmonary vascular changes, in response to the increased hydrostatic capillary pressure, counteract the oedema formation: increased capillary membrane thickness and capillary dilatation, intimal thickening and circumferential fibrosis of the arteries and veins, thickening of the alveolar wall and compression of the peripheral airways by increased connective tissue. All these structural changes prevent pulmonary oedema, but on the other hand, they encourage the pulmonary restrictive syndrome typical of HF, which contributes to the reduced exercise capacity [57]. HF patients can also suffer from dyspnoea even without pulmonary congestion, because of the reduced blood flow to the overloaded respiratory muscles and accumulation of lactic acid. HF may initially cause dyspnoea only on exertion, but more severe dysfunction results in symptoms at rest as well.

Another congestive manifestation of HF includes orthopnoea, the sensation of labored breathing while lying flat which is relieved by sitting upright. The degree of orthopnoea is generally assessed by the number of pillows on which the patient sleeps to avoid breathlessness. Sometimes, orthopnoea is so significant that the patient may try to sleep upright in a chair.

Peripheral oedema, especially up to the ankles, also reflects increased hydrostatic venous pressures. Because of the effects of gravity, it tends to worsen during the day and is often improved by the morning after lying supine at night. Even before peripheral oedema develops, the patient may note an unexpected weight gain resulting from the accumulation of interstitial fluid.

Low forward cardiac output in HF may also cause impaired urine output because of the decreased renal perfusion. Whereas diuresis impairment is worse during the day, urinary frequency may often increase at night (nycturia) when, while supine, blood flow is redistributed to the kidney, promoting renal perfusion and diuresis. However, in advanced chronic HF, renal hypoperfusion becomes steady with oliguria.

Reduced perfusion also affects skeletal muscles where anaerobic metabolism and lactic acid production result increased. Therefore, HF patients often present symptoms like asthenia and weakness during physical activity.

-Heart failure diagnosis

There is no single diagnostic test for HF; therefore, diagnosis is based on a clinical evaluation requiring a history, physical examination, and laboratory testing confirming HF, determining its potential causes and identifying comorbid illnesses.

Whereas HF signs and symptoms may be typical (decreased exercise tolerance with dyspnoea, fatigue, generalized weakness and fluid retention, with peripheral or abdominal swelling and possibly orthopnoea), they are often non-specific and, as such, do not help discriminate between HF and other problems. As a result, clinical history and physical examination are useful to evaluate for alternative or reversible causes of these symptoms. More objective tests can help confirm the suspicion of HF [1]:

1. echocardiography is the most widely accepted and available method to identify systolic and diastolic dysfunction and therefore assist in the diagnosis of HF. In fact, echocardiography can assess LVEF, left ventricular size, wall thickness, valve function, and left atrial pressure [58];

2. electrocardiography (ECG) is useful to identify abnormalities such as left bundle branch block, left ventricular hypertrophy, acute or previous myocardial infarction, or atrial fibrillation, which could provide further information on the aetiology of HF [59];

3. chest radiography can identify pulmonary causes of dyspnoea other than HF and allows the assessment of pulmonary congestion and interstitial oedema. Other findings, such as pleural effusion or cardiomegaly, may also increase the likelihood of HF.

Besides these instrumental diagnostic tests, there are other parameters that make the diagnosis more accurate and also have a prognostic value:

1. plasma concentrations of natriuretic peptides, BNP and NT-proBNP in particular, can be used to evaluate patients with dyspnoea for HF since, as previously assessed, they are secreted in response to stretching or increased wall tension.;

2. exercise testing helps evaluate exercise tolerance and symptoms that appear on exertion, like dyspnoea and asthenia;

3. pulmonary function tests can confirm or exclude other respiratory causes of breathlessness and for assessing concomitant pulmonary diseases.

-Cardiopulmonary exercise test

Cardiopulmonary exercise test (CPET) is an important clinical tool to evaluate exercise capacity in patients with HF and is far more reliable than conventional exercise testing [60]. CPET, in fact, provides the performance and cardiac parameters traditionally measured during exercise testing, such as heart rate, blood pressure, work rate and exercise duration, as well as respiratory parameters, like gas exchanges. Therefore, it assesses the whole exercise responses involving the pulmonary, cardiovascular, and skeletal muscle systems, and thus provides a non-invasive, dynamic physiological overview of the individual's functional capacity and impairment (Figure 5) [61].

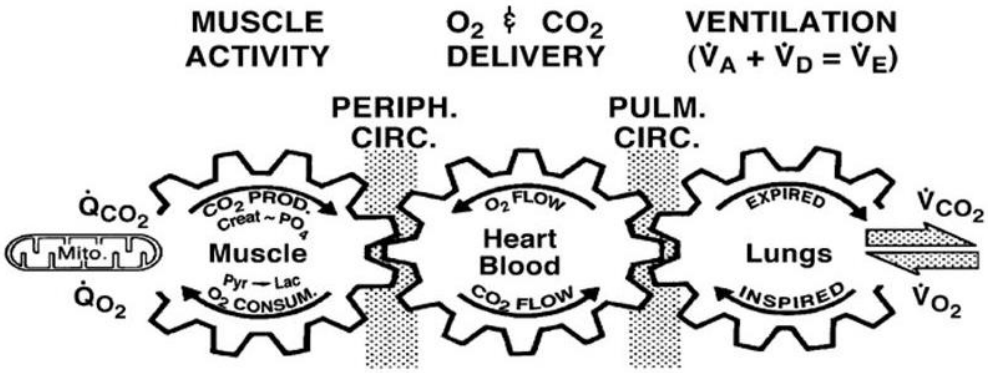


Figure 5. Integrative exercise responses involving pulmonary, cardiovascular and muscle system

Because of its importance in HF prognosis and diagnosis, CPET is being increasingly used and the information it provides has direct impact on clinical decisions [62]. CPET offers various parameters that could help understand if the cause of the limited exercise capacity is mainly pulmonary (ventilation or diffusion problem) rather than cardiogenic or peripheral (oxygen transport or its peripheral extraction).

CPET evaluates peak exercise capacity, defined as the maximum ability of the cardiovascular system to deliver oxygen to exercising skeletal muscle and of the exercising muscle to extract oxygen from the blood [63]. Therefore, exercise tolerance is determined by three factors: pulmonary gas exchange, cardiovascular performance, and skeletal muscle metabolism.

At rest, the organism needs to provide all the organs with the energy to carry out their functions. This energy is obtained from the oxidation of metabolic substrates (carbohydrates, proteins, lipids). The more oxygen our organism can use, the more it is efficient because the aerobic metabolism provides the major source of energy in the most inexpensive way. Therefore, the measurement of oxygen consumption permits to quantify the functional capacity of an individual.

During exercise, an increase in oxygen consumption is essential to generate energy aerobically and to sustain the physical activity. The organism implements some adaptive responses to tackle this request: the elevated tidal volume and respiratory rate are followed by an increase in cardiac output and heart rate. These reactions lead to higher levels of CO2 that lungs have to remove and this causes an increased ventilation.

Patients with HF cannot increase cardiac output adequately with the increased oxygen uptake required during exercise. This results in an inadequate perfusion to the exercising muscles, which can cause early anaerobic metabolism and muscle fatigue with a reduced exercise tolerance.

Oxygen uptake (VO2) is measured throughout the test and can be defined as:

VO2 = CO x C(a-v)O2

where CO=cardiac output and C(a-v)O2= arteriovenous oxygen content difference.

One of the key parameters measured during CPET is peak oxygen uptake (peakVO2), the highest volume of VO2 achieved at maximal effort during the incremental exercise. This value is matched with predicted peakVO2, normalized per age, sex and height, but not per weight. Obese patients therefore require an adjustment in the formula because oxygen uptake from adipose tissue is one third of that of the muscle

tissue [64]. PeakVO2 has been identified as the most important predictor of prognosis in HF patients and, more specifically, the lower the values, the higher the mortality risk [65, 66]. Emphasizing the prognostic role of exercise testing parameters, Mancini et al. Identified a peakVO2 cut-off value of 14 ml/min/kg to refer patients with symptomatic HF and severe ventricular dysfunction to heart transplantation [67].

Another important parameter measured with CPET is the anaerobic threshold that marks the shift from the aerobic metabolism to one with an added anaerobic component [68]. This occurs during the latter half of exercise, because the oxygen supply cannot meet the increasing metabolic requirements of exercising muscles. At the same time, there is a significant increase in lactic acid production in the muscles as lactate is the primary by-product of the anaerobic glycolysis. As the exercise intensity rises, the blood lactate concentration increases because its production exceeds its removal rate. Because of its low pK, lactic acid is almost completely dissociated and the hydrogen ion (H+) generated is buffered by bicarbonate, in order to avoid acidosis. The rapid conversion of the bicarbonate (H2CO3) produced leads to CO2 production:

$HCO3^- + H^+ \leftrightarrow H2CO3 \leftrightarrow CO2 + H2O$

The excess CO2 produced above the threshold stimulates chemoreceptors that, in response, increase ventilation. Therefore, the anaerobic threshold is the point where ventilation increases disproportionately to VO2 and it is lower in patients with heart disease because oxygen consumption at the anaerobic threshold depends on factors that affect oxygen delivery to the tissues (i.e. the reduced cardiac output in patients with HF). The ability to achieve the anaerobic threshold can help distinguish cardiac and non-cardiac (pulmonary or musculoskeletal) causes of exercise limitation: a failure to reach this threshold strongly suggests poor motivation or non-cardiovascular problems [69]. However, a failure to reach the anaerobic threshold is also detected in a large number of patients with HF and this in associated with a significantly worse prognosis [70].

There are three methods to determine the anaerobic threshold [61]:

1) anaerobic threshold is the point where the ventilatory equivalent for O2 (VE/VO2) begins to increase systematically without an immediate increase in the ventilatory equivalent for CO2 (VE/VCO2);

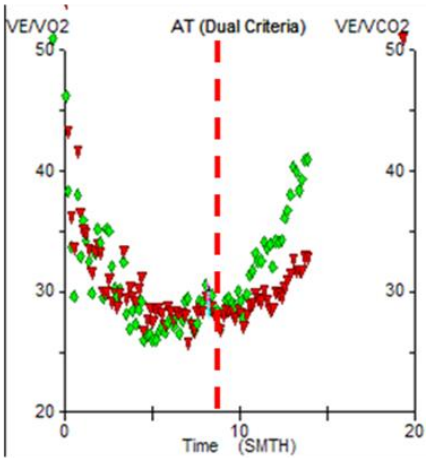


Figure 6. Anaerobic threshold VE/VO2 – VE/VCO2

2) anaerobic threshold corresponds to the point where end-tidal oxygen tension (PETO2) increases systematically without an immediate increase in the end-tidal CO2 tension (PETCO2);

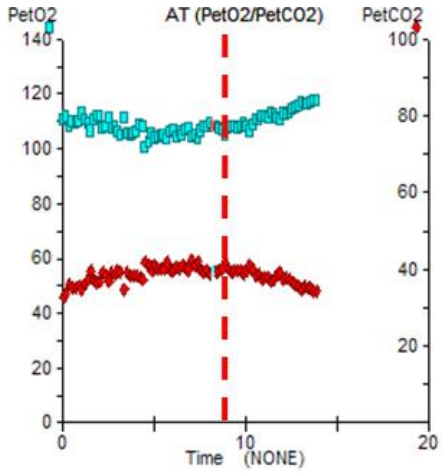


Figure 7. Anaerobic threshold: PETO2/PETCO2

3) anaerobic threshold is the point where VCO2 undergoes an increase in steepness not equally followed by V O2 in the plot VO2/VCO2.

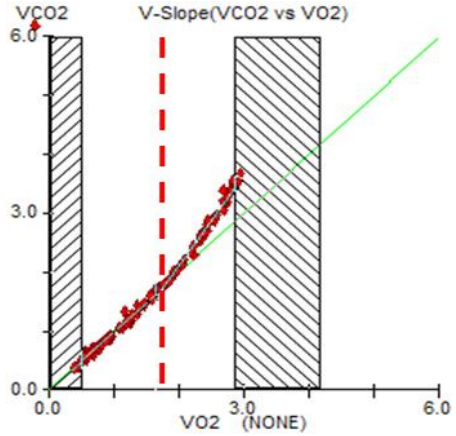


Figure 8. Anaerobic threshold VCO2 vs VO2

Finally, there is another parameter derived from CPET with a very important prognostic role in patients with HF: the relationship between minute ventilation (VE) and CO2 production (VCO2), known as VE/VCO2 slope. This value represents the ventilatory efficiency of the organism, showing the amount of air that must be ventilated to eliminate 1 l of CO2. Normally the VE/VCO2 slope is < 30, independently from age and gender, but it can be higher in particular pathological conditions. A higher-than-normal VE/VCO2 slope during exercise is associated with a respiratory or cardiac disease that induces a mismatch of ventilation to perfusion. In patients with HF, the rising slope value is a prognostic marker of the disease, with the degree of the slope elevation reflecting disease severity [71]. The VE/VCO2 slope is an even more appropriate strategy for prognosis stratification when combined with peakVO2 [72]. In fact, alongside ventilation-perfusion mismatching in the lungs, multiple factors lead to a high VE/VCO2 slope in HF: chemoreceptor and ergoreceptor heightened sensitivities due to the increased activity of the sympathetic nervous system, increased anatomical and physiological dead space, reduced cardiac output and alveolar-capillary conductance and pulmonary oedema. Lastly, the VE/VCO2 slope is a prognostic marker independent from symptoms and age as opposed to peakVO2 [66].

Therefore, CPET can help stratify the prognosis in HF patients, although it needs to be integrated into clinical practice. Parameters derived from CPET have been combined with demographic data, medical

history and laboratory values in multivariable prognostic risk scores. In particular, Agostoni et al. developed a new risk score for HF: the Metabolic Exercise test data combined with Cardiac and Kidney Indexes (MECKI) score. This score has been suggested to identify the risk of cardiovascular mortality and urgent heart transplantation and it relies on six simple variables: haemoglobin, sodium, kidney function by means of Modification of Diet in Renal Disease (MDRD), LVEF, percentage of predicted peakVO2 and VE/VCO2 slope [73, 74]. Recently, it has been demonstrated that the prognostic accuracy of the MECKI score is superior to other prognostic scores in chronic HFrEF [75].

-Pulmonary function testing

As previously exposed, patients with HF may develop pulmonary function abnormalities such as restrictive, obstructive or diffusive defects [76]. The most common pulmonary function testing that could help identify lung defects is spirometry, the measurement of the lung volumes mobilized with inspiratory or expiratory manoeuvres.

A restrictive ventilatory defect is expressed by a reduction of total lung capacity (TLC) and forced vital capacity (FVC), the maximal volume of gas exhaled as forcefully as possible after full inhalation. This reduction of lung volumes is the result of accumulation of liquid in the interstitial and alveolar space, cardiomegaly and pleural effusion. This evidence is often combined with an increase in the residual volume (RV), the air remaining in the lungs at the end of maximal expiration, because not all portions of the lungs are affected by HF [77, 78].

An obstructive defect in HF is caused by airway narrowing, evidenced by a reduced forced expiratory volume in 1 second to FVC ratio (FEV1/FVC). The main reason for the airway caliber reduction is the compression caused by the alveolar fluid accumulation and the increase in bronchial wall thickness induced by the congestion. An increase in the RV is also common in case of an obstructive defect, because the airway closure during expiration causes air trapping [79, 80].

HF patients may also show a reduction of respiratory muscle strength, caused by the hypoperfusion of respiratory muscles due to the impaired cardiac output. The increased work of breathing of these under perfused and weak respiratory muscles is one of the main reasons for the development of dyspnoea on exertion [81, 82].

Moreover, in patients with HF, the alveolar-capillary membrane undergoes a remodelling process characterized by fibrosis, deposition of connective tissue and micro thrombosis. These structural changes negatively affect the physiological functions of the membrane, such as gas exchange or pulmonary fluids homeostasis. These biological functions are possible because of the peculiar anatomic configuration of the blood-gas barrier, composed of three layers: the alveolar epithelium, the interstitial space and the capillary endothelium [83].

Gas exchange through the alveolar-capillary membrane is commonly assessed using inert gases such as carbon monoxide (CO) and nitric oxide (NO). Among these gases, CO is the most used because, after inhalation, it transits through the airways towards the alveoli, reaches the blood by crossing the alveolar-capillary membrane and binds to haemoglobin with a 240-fold greater affinity than O2. In clinical practice, the diffusing capacity of the lung for CO (DLCO) is commonly evaluated, and in particular the diffusion through the alveolar-capillary membrane is measured [84]. DLCO may be partitioned into its two subcomponents: Dm which is the conductance of CO across the alveolar–capillary membrane, and Vc which represents the pulmonary capillary blood volume.

In HF, the remodelling of the alveolar-capillary membrane causes a reduction in both Dm and Vc. According to recent findings, the greater the severity of HF, the lower DLCO and Dm [85, 86]. Furthermore, Vc

decreases in parallel with DLCO and Dm and this reduction may be related to pulmonary vascular resistance, local thrombosis, micro embolism, low blood flow due to the reduced cardiac output and, in some patients, low circulatory volume induced by high-dose diuretic therapy [86]. The alveolar-capillary membrane is well known for being a target of some treatments for HF. Specifically, ACE inhibitors [87] and aldosterone antagonists [88] improve gas diffusion through the alveolar-capillary membrane, while other categories of drugs like angiotensin receptor antagonists [89, 90] have no effect, likely because the improvement seems to be dependent on the over-expression of the bradykinin-prostaglandin pathway.

-Pharmacological treatment of heart failure

The key treatment goals in patients with HF are to improve their clinical status, functional capacity and quality of life, prevent hospital admission and reduce mortality.

Three neurohormonal antagonists (ACE inhibitors, beta-blockers and aldosterone antagonists) have been shown to improve survival in patients with HF and are recommended for the treatment of patients with HFrEF, unless contraindicated or not tolerated. Ivabradine, which reduces the elevated heart rate often seen in patients with HFrEF, has also been found to improve outcomes, and should be considered when appropriate. The above medications should be used in conjunction with diuretics in patients with symptoms and/or signs of congestion. The use of diuretics should be modulated according to the patient's clinical status.

1. Angiotensin-converting enzyme inhibitors: ACE inhibitors have been shown to prevent hospitalization for worsening HF and to reduce the mortality rate by 15-25% when compared to placebo in patients with chronic congestive HF [91]. ACE inhibitors are therefore recommended by all major international HF treatment guidelines as first-line treatment for all patients with HFrEF, unless intolerant.

Angiotensin-receptor blockers (ARB) are an alternative for those who are unable to tolerate ACE inhibitors although evidence for a mortality reduction in HF is inconsistent [92] and deemed less robust [93].

2. Beta-blockers: they reduce mortality and morbidity in symptomatic patients with HFrEF [94-96] already being treated with an ACE inhibitor and, in most cases, a diuretic. The addition of a beta-blocker to standard therapy with a diuretic and an ACE inhibitor is recommended because of the concomitant inhibition of the potential harmful effects of two compensatory mechanisms, the RAAS and the sympathetic activity. As a result, cardiac work and energy consumption are decreased by the unloading obtained by ACE inhibitors and diuretics, heart rate is slowed by beta-blockers, and blood pressure is lowered with both.

3. Mineralocorticoid/aldosterone receptor antagonists: MRAs block receptors that bind aldosterone and, with different degrees of affinity, other steroid hormone receptors. They are recommended in all symptomatic patients, in addition to treatment with ACE inhibitor and beta-blocker, to reduce mortality and HF hospitalization [97, 98].

4. Diuretics: diuretics are recommended to reduce the signs and symptoms of congestion in patients with HFrEF. Loop diuretics produce a more intense and shorter diuresis than thiazides, although they act synergistically and their combination may be used to treat resistant oedema and relieve dyspnoea. The aim of diuretic therapy is to achieve and maintain euvolemia with the lowest achievable dose. Although the effects of diuretics on mortality and morbidity have not been studied in patients with HF, a Cochrane meta-analysis has shown that loop and thiazide diuretics appear to reduce the risk of death and worsening of HF compared with placebo, and compared with an active control, diuretics appear to improve exercise capacity [99].

Despite these guideline-recommended HF treatments, mortality and morbidity rates remained high [100, 101] and there was a high unmet medical need for new therapies with different mechanisms of action, which could further reduce mortality and morbidity and improve quality of life compared to the standard of care.

-Angiotensin receptor neprilysin inhibitors

Neurohormonal pathways are of critical importance in the pathogenesis and progression of HF. Previous HF therapies mainly focused on blocking the detrimental effects of long-term neurohormonal activation (ACE inhibitors, ARBs, beta-blockers and MRAs) and largely ignored the physiological compensatory effect of the natriuretic peptide system and other endogenous vasodilator mechanisms. On the other hand, the inhibition of NEP results in an increase in the activity of natriuretic peptides and other vasoactive peptides that potentially exert favourable long-term compensatory effects. However, NEP is also involved in the degradation of angiotensin II, therefore its inhibition leads to an increase of this oligopeptide, which is a major mediator of HF development and progression [38]. Accordingly, the full compensatory benefit of NEP inhibition can only be leveraged if both the RAAS and NEP system are inhibited simultaneously [42].

An early example of a dual ACE/NEP inhibitor, omapatrilat, was no more effective than an ACE inhibitor alone in reducing the risk of death and HF hospitalization in the OVERTURE study; a possible cause could have been its once-daily dosing, which did not provide 24-hour NEP and ACE-inhibition [102]. In addition, omapatrilat was associated with an increased incidence of serious angioedema with airway compromise requiring mechanical support [103]. Even if the compound was never marketed, these studies confirmed the correct approach in the simultaneous inhibition of the RAAS and NEP systems leading to the development of a new therapeutic class: the angiotensin receptor neprilysin inhibitors (ARNI).

The first in class is LCZ696, a fixed dose combination of an ARB (valsartan) and a NEP inhibitor (sacubitril), which has recently shown superiority to an ACE inhibitor (enalapril) in reducing the risk of death and of hospitalization for HF in the PARADIGM-HF trial [104]. Sacubitril/valsartan is therefore recommended in the outpatient setting as an alternative to ACE inhibitors in HFrEF patients who remain symptomatic despite optimal therapy with an ACE inhibitor, a beta-blocker and a MRA.

LCZ696 exhibits the novel mechanism of action of an ARNI by simultaneously inhibiting NEP via sacubitrilat, the active metabolite of the pro-drug sacubitril, and by blocking the angiotensin II type-1 (AT1) receptor via valsartan. As a result, the cardiovascular benefits of LCZ696 in HF patients are attributed to the enhancement of protective peptides that are degraded by NEP, such as natriuretic peptides, and to the simultaneous inhibition of the deleterious effects of angiotensin II.

Natriuretic peptides exert their effects by activating guanylyl cyclase-coupled receptors (NPR-A and -B), resulting in increased concentrations of the second messenger cGMP. Therefore, the latter could be used as a biomarker indicative of NEP inhibition, alongside plasma and/or urinary ANP and BNP levels in patients with HF. Pharmacodynamics studies also assessed markers of RAAS blockade: valsartan, by blocking the activity of angiotensin II at its receptor AT1, reduces the normal feedback inhibition of renin release from the kidneys and therefore increases renin concentration, plasma renin activity (PRA), and angiotensin II levels.

Pharmacodynamics assessments in healthy subjects demonstrated a significant increase in plasma cGMP levels following multiple-dose administration of LCZ696, consistently with the inhibition of NEP activity. The increase in cGMP peaked at 4 and 12 hours after dosing, shortly after the maximum plasma concentrations of sacubitrilat were reached. The cGMP response returned to baseline at 24 hours post dose, suggesting the need for a twice daily administration of LCZ696 to sustain the cGMP response throughout the dosing

interval. Following the high strength dose administration, urinary ANP excretion was significantly increased [105]. A prospective comparison of ARNI with an ARB in HFpEF patients also showed a greater reduction in plasma concentrations of NT-proBNP, a strong marker of left ventricular wall stress, in patients assigned to LCZ696. Because NT-proBNP is not a NEP substrate, it could be a suitable biomarker of HF in patients treated with LCZ696. This result was associated with improvements in some echocardiographic parameters, such as left atrial dimension and volume, consistent with the hypothesis that LCZ696 reduced left ventricular pressures and wall stress [106]. Finally, plasma endothelin levels were measured in both healthy and HF patients, showing a reduction that demonstrates the NEP inhibition.

Moreover, LCZ696 stimulates significant, dose-dependent increases in renin concentration, PRA, and angiotensin II levels, indicative of blockade of the AT1 receptor. Notably, the high strength dose increased renin concentration by 3.1-fold, PRA by 4.9-fold, and angiotensin II by 3.7-fold relative to placebo. These increases are comparable with those previously observed with the administration of valsartan 320 mg in healthy participants. Significant increases in all RAAS biomarkers were sustained 24 hours after LCZ696 administration, in accord with the observed long plasma half-life of valsartan (15-22 hours). Even though sacubitril/valsartan increases the circulating angiotensin II levels, the overall effect is a dose-dependent decrease in blood pressure due to the simultaneous NEP inhibition that prolongs the beneficial actions of natriuretic peptides [107]. LCZ696, in fact, results in a transient larger natriuretic and diuretic effect in HF patients compared to valsartan, consistent with the expected effects of natriuretic peptides. They also exert their inhibitory effects on aldosterone release, explaining the decrease in aldosterone plasma concentration measured after administration of sacubitril/valsartan [108].

-SGLT-2 inhibitors

Recent clinical trials have led to the introduction of new medications for the treatment of heart failure with reduced ejection fraction (HFrEF), which provide additional prognostic benefits [7, 109, 110]. Among these, sodium-glucose cotransporter 2 inhibitors (SGLT2-i) have become a cornerstone of contemporary HFrEF treatment strategies[111]. Dapagliflozin is a molecule belonging to this class of drugs. Initially used in the treatment of diabetes mellitus, SGLT2-i have demonstrated significant clinical and prognostic benefits over the past several years in patients with HFrEF, even in the absence of diabetes mellitus[112, 113]. Current heart failure guidelines have incorporated this evidence by recommending the use of SGLT2-i therapy in patients with HFrEF. More recently, this drug class has also shown significant prognostic improvement in patients with heart failure with preserved and mildly reduced systolic function as well as in chronic kidney disease[114].

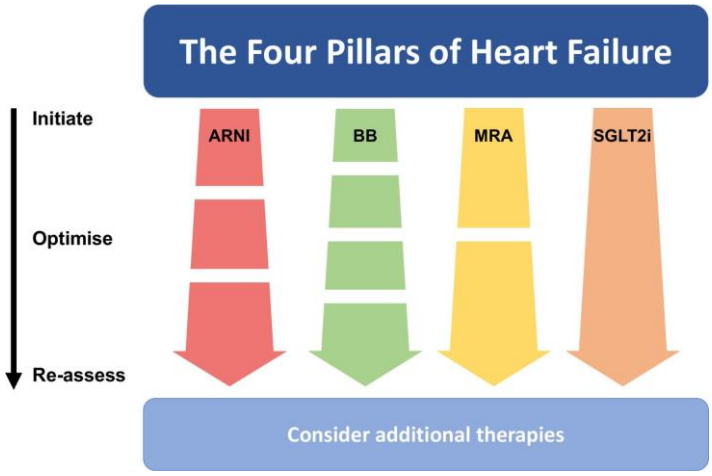


Figure 9. The “four pillars” of HF treatment

While their positive effects on prognosis, cardiac remodelling, functional capacity, and natriuretic peptides are well-established[115, 116], the mechanisms driving these benefits remain largely unclear. Indeed, either hemodynamic or non-hemodynamic pathways have been proposed, but none with convincing evidence[117]. Given the recent introduction of the drug into clinical practice, direct real-world evaluation is crucial to refine clinical management of patients treated with SGLT2-i and to better understand the mechanisms underlying its clinical benefits.

To date, several potential targets of SGLT2-i have been poorly assessed, such as the alveolar-capillary membrane function and sleep disturbances. The former is frequently impaired in HFrEF patients and can be evaluated through lung diffusion capacity for carbon monoxide (DLCO) or by detecting the abnormal presence of surfactant proteins in peripheral blood. In particular, the immature form of surfactant-derived protein type B (proSP-B) has emerged as a novel biomarker of alveolar-capillary membrane function and an indicator of overall HFrEF status. Both DLCO and circulating proSP-B levels have demonstrated correlation with HF severity, possess strong prognostic value, and have been shown to respond to specific acute and/or chronic treatments for HFrEF, such as inotropic infusions (e.g., levosimendan) or HFrEF drugs (e.g., sacubitril/valsartan) [49, 50, 118-121].

In parallel, emerging biomarkers such as soluble interleukin-1 receptor-like 1 (ST-2) have provided complementary prognostic insights, reflecting not only the hemodynamic state of HF patients but also their inflammatory and pro-fibrotic responses[122, 123]. Furthermore, ventilatory abnormalities are commonly associated with an increased prevalence of sleep disorders in HF [124]. Sleep apnoea is particularly prevalent among HFrEF patients, significantly contributing to patients' poor quality of life, disease progression, and mortality [125-129]. Of note, central sleep apnoea (CSA) is primarily linked to reduced cardiac output, while obstructive sleep apnoea (OSA) is associated with intrathoracic fluid accumulation[130].

Indeed, in some small preliminary studies conducted in patients with HFrEF, SGLT2-i, when added to otherwise optimized medical therapy, have been shown to be effective in improving left ventricular ejection fraction (LVEF) and other echocardiographic parameters of ventricular remodelling [115, 116]. However, a comprehensive assessment of the potential effects of SGLT2-i therapy on overall body function—including exercise capacity measured by the gold-standard cardiopulmonary exercise test (CPET), pulmonary function, body fluid homeostasis, and biomarkers—has not yet been reported in patients with HFrEF.

Aim of the study

The present study was therefore designed to evaluate the effects of dapagliflozin—one of the two SGLT2-i currently approved for HFrEF therapy—on CPET-derived parameters, pulmonary function, echocardiographic indicators of left ventricular systolic function, cardiac biomarkers, fluid homeostasis, alveolar-capillary membrane integrity (including circulating surfactant proteins), heart failure severity and fibrosis biomarkers, sleep apnoea, and quality of life (QoL) in a single-center cohort of patients with HFrEF (NYHA functional class II–III)

Methods:

At the HF Unit of the Centro Cardiologico Monzino, IRCCS in Milan, we enrolled a cohort of consecutive stable HFrEF patients who were referring to the HF Unit outpatient clinic and were eligible for treatment

with SGLT2-i. The inclusion criteria were as follows: age >18 years; ability to sign the study informed consent; stable clinical condition defined as absence of heart failure exacerbations in the past 3 months, i.e., no hospitalizations for heart failure requiring intravenous diuretic administration; LVEF ≤40% (echocardiography); diagnosis of HFrEF; and eligibility for treatment with SGLT2-i according to the most recent guidelines. In addition, they had to be able to undergo CPET and provide signed informed consent to participate in the study. The exclusion criteria included contraindications to SGLT2-i, moderate-to-severe chronic obstructive pulmonary disease (COPD), or an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2 according to Modified Diet in Renal Disease (MDRD) criteria [131].

Patients who met the study's inclusion and exclusion criteria and signed the study agreement form underwent an initial evaluation (T0) that included:

- A clinical examination
- Kansas City Cardiomyopathy Questionnaire (KCCQ-12) to assess quality of life (QoL)
- Blood sample: complete blood count, creatinine, urea nitrogen, sodium, potassium, glycated haemoglobin (Hb), N-terminal BNP (NT-proBNP), suppression of tumorigenicity 2 (ST-2), high-sensitivity C-reactive protein (hsCRP), and high-sensitivity troponin I (hs-TNI)
- Standard spirometry and DLCO, with diffusion subcomponents (membrane diffusion [Dm] and capillary volume [Vcap]) assessed by the Roughton and Forster method [132], and alveolar volume measured by CH4 decay slope during the single-breath constant expiratory flow measurement [133]
- Maximal ramp-protocol CPET on cycle ergometer
- Transthoracic echocardiogram
- Bioelectrical impedance vector analysis (BIVA)
- Nocturnal cardiorespiratory monitoring

Subsequently, patients started treatment with dapagliflozin at a dose of 10 mg/day, as per the guidelines-directed HFrEF treatment. Between 2 and 4 weeks after starting the therapy (T1), a safety evaluation was performed, including a clinical evaluation, blood sample collection, and lung function tests. All parameters evaluated at T0 were reassessed 6 months after the start of treatment (T2). The study synopsis is shown below.

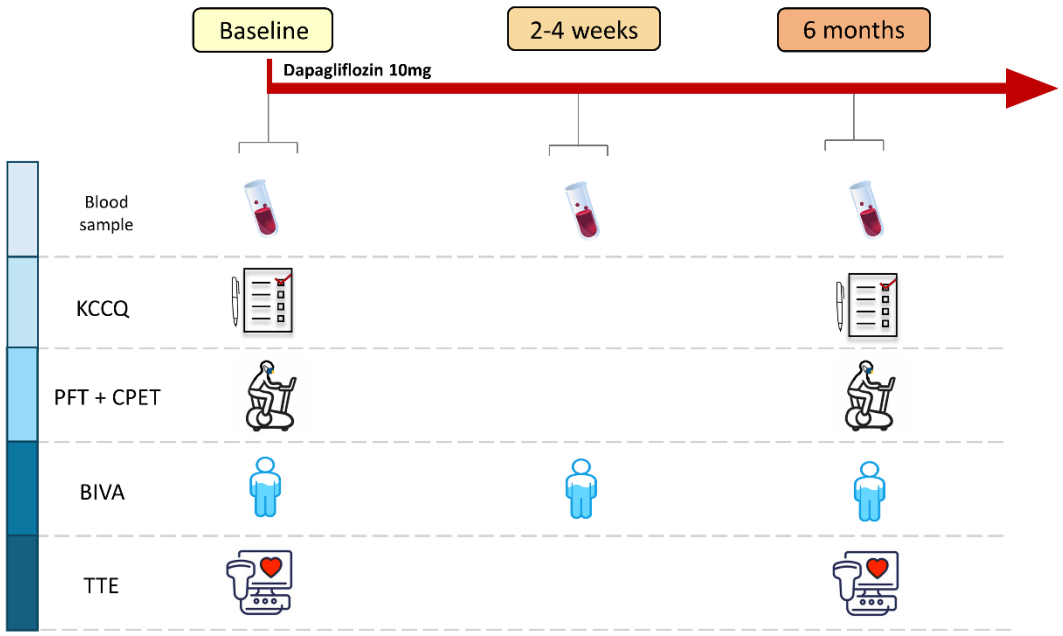


Figure 10. study synopsis

-Kansas City Cardiomyopathy Questionnaire analysis

QoL was evaluated using the KCCQ-12 at baseline and at 6 months, administrated before any other assessment. The KCCQ-12 was analysed combining the reported Physical Limitation, Symptom Frequency, QoL, and Social Limitation scales into the Summary Score, calculated as the average of the four scores. To calculate the summary score, at least one of the four scale scores must be present [134, 135]. Only scales available at both T0 and T2 were considered.

-Cardiopulmonary exercise testing

CPET was performed on an electronically braked cycle ergometer using a personalized ramp protocol set to reach peak exercise in 10 ± 2 min [136] at T0 and applied unchanged at T2. CPET was performed and analysed as standard [137]. Specifically, in the absence of clinical events, tests were self-interrupted by patients when they reported the maximal effort. Patients wore a mask to measure ventilation (VE) and respiratory gases breath by breath. During the test, heart rate and a 12-lead ECG were continuously monitored, Hb O_2 saturation was recorded by an oximeter, and blood pressure was monitored with a cuff sphygmomanometer at rest and every 2 min. Peak VO_2 was calculated as the 30 s average of the highest VO_2 recorded, while the VE/VCO_2 slope was calculated based on the linear relationship between VE and VCO_2, starting from 1 min after the initiation of loaded exercise until the end of the isocapnic buffering period. This value was also expressed as a percentage of the predicted value [138]. Predicted peak VO_2 was calculated using the Hansen and Wasserman equation as (height – age) × 20 for men and (height – age) × 14 for women [63]. The anaerobic threshold (AT) was measured using a V-slope analysis of VO_2 and VCO_2 [139]. The VO_2/work relationship was measured through the entire exercise protocol. Other data are reported as the 20 s average. The MECKI score, including six relevant prognostic parameters (Hb, LVEF, MDRD, Na, Peak VO_2, and VE/VCO_2 slope), was calculated as previously described [73].

-Lung function tests

Standard spirometry and DLCO measurements were performed at rest according to the American Thoracic Society and the European Respiratory Society criteria (ATS/ERS guidelines) [84, 140]. DLCO measurements were obtained while subjects were comfortably seated, with the single-breath constant expiratory flow technique (exhalation rate = 0.5 L/sec), (Quark PFT Cosmed, Rome, Italy). DLCO measurements were corrected for haemoglobin (Hb) as previously described [141]

-Venous blood sample collection, specimen handling and assay

Blood samples were drawn after assuring that any intense physical effort was avoided in the previous 3 hours and after 5 minutes of rest in sitting position. ProSP-B determination was performed as follows: fresh blood (5 mL) was drawn into Vacutainer tubes containing citrate 0.129 mol/L as an anticoagulant. Plasma was immediately prepared by means of centrifugation at 1,500×g for 10 minutes at 4°C, divided into aliquots and frozen at −80°C until assayed. The immature form of proSP-B was assayed as previously described [142]. Briefly, equal amounts of plasma proteins (50 μg) were separated by one-dimensional SDS-PAGE on 15% polyacrylamide gels using a Tris-Tricine buffer system in non-reducing conditions [143]. The protein concentration was evaluated by the method of Bradford [144]. Immunoblotting was performed using the primary antibody against SP-B (rabbit anti-human SP-B H300; Santa Cruz Biotechnology, Santa Cruz, CA, US) diluted at 1:200 in 5% (w/v) non-fat milk in TBS-T; followed by an incubation with a secondary goat anti-rabbit antibody conjugated to horseradish peroxidase (Bio-Rad, Milan, Italy). Following transfer, membranes were stained with MemCode reversible protein stain (Pierce Biotechnology, Cramlington, UK) according to the manufacturer's instructions to ensure equivalent loading of protein. Immunoreactive bands ranging from 42 kDa to 17 kDa detected by ECL were quantified by densitometry of exposed film using image analysis software (QuantityOne version 4.5.2; Bio-Rad, Milan, Italy). For each subject, data are reported as the ratio of band volume, after local background subtraction, versus the volume of the total

proteins loaded and stained with MemCode. The values were also normalized versus the band volume of pooled plasma, loaded as control on each gel, and they are expressed as arbitrary units (AU). Inter-assay coefficient of variation was 12.1 ± 2.9%.

-Echocardiography

Transthoracic echocardiography (TTE) examinations were conducted using an Epiq CVx ultrasound machine (Philips Medical Systems, Andover, MA, USA) equipped with an X5-1 probe. A comprehensive standard 2D TTE analysis was performed, with left chamber volumes and LVEF measured from four-chamber and two-chamber views using the biplane Simpson's method [145]. All echocardiograms were conducted by highly trained operators. Pulmonary artery systolic pressure (PAP) was calculated by quantifying the peak velocity of tricuspid regurgitation and then adding the estimated pressure in the right atrium [146].

-Nocturnal cardiorespiratory monitoring

Nocturnal cardiorespiratory monitoring was recorded by SOMNOtouch™ RESP device (SOMNOmedics, Germany) before starting dapagliflozin and after six months of treatment. The SOMNOtouch™ RESP device is composed of a nasal cannula, a pulse oximeter, two respiratory sensors positioned at the level of the manubrium and abdomen, and three thoracic electrodes for ECG recording. Apnoea was identified as a reduction in the amplitude of the respiratory flow signal, defined as a respiratory flow amplitude <10% of the preceding baseline value for at least 10 seconds, while hypopnea was defined as a reduction of respiratory flow <50% of the baseline for at least 10 seconds. Guidelines also recommend to use oxygen desaturation >3% as a criterion to detect hypopnea [147]. Apnoea were considered of central origin (CSA) when the interruption in respiratory flow was associated with the absence of thoracic and abdominal respiratory effort; obstructive (OSA) if respiratory thoracic activity or abdominal activity were present during a cessation in respiratory flow, and mixed when an initially CSA turned into OSA in its final phase. Apnoea and hypopnea indexes (AHI) were calculated as the number of apnoea and hypopneas per hour of estimated or measured sleep time, respectively. The AHI is the sum of apnoea and hypopneas per hour of sleep.

-Bioelectrical impedance vector analysis

Bioimpedance measurements were conducted using an impedance plethysmograph (BIA 101 BIVA; AKERN SRL, Pisa, Italy) with a 250 µA RMS 50 kHz sinusoidal output signal. The device was calibrated using the standard control circuit with a known impedance [resistance = 383 ohms; reactance (Xc) = 45 ohms]. Measurements were taken with participants in a supine position, with their arms and legs by their sides. Values were recorded after a minimum rest of 5 min. Before measurement, the skin was cleaned with an alcohol solution and four contact electrodes (BIATRODES; AKERN SRL, Pisa, Italy) were placed on the dorsal surface of the right hand and foot as per the manufacturer's instructions. KCCQ-12, CPET, spirometry, BIVA, and cardiac ultrasound were obtained and analysed by medical personnel who were blinded to the study timeline, meaning they were unaware of whether the assessments were performed at T0, T1, or T2, while patients and referring physicians were unblinded.

The present research protocol complies with the World Medical Association's Declaration of Helsinki and was approved by the Centro Cardiologico Monzino Ethical Committee (R 11637-22 CCM 1756). Each individual provided written informed consent to participate in the study. This study was registered on Clinicaltrials.gov (reference ID NCT05770167). Study data were collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at Centro Cardiologico Monzino IRCCS. REDCap is a secure, web-based software platform.

Statistical analysis

Continuous variables are described as mean ± standard deviation (SD) in case of normal distribution, and as median and interquartile range (IQR) in case of non-normal distribution. Categorical variables are expressed as numbers (percentages). For continuous variables, differences between T0 and T6 were assessed with a paired t-test or a non-parametric test as appropriate; alternatively, for the second analysis, differences between T0 and T2 were assessed with a paired T-test or a Wilcoxon-signed-rank test, as appropriate. Missing data at time-point T1 were imputed using the PROC MI (multiple imputation) procedure with the Markov Chain Monte Carlo (MCMC) method. When variables were measured at all three protocol-specified time points (T0, T1, and T2), a statistical analysis was conducted using repeated measures tests for normally distributed variables or the Friedman test for non-normally distributed variables; a repeated-measures ANOVA model was applied after log-transformation of variables with a skewed distribution. A sensitivity analysis was performed by repeating the analyses in patients with complete data for all three time points. For pairwise comparisons, a Bonferroni correction was used to account for multiple testing. The correlation between the variables was evaluated using Pearson's correlation coefficient or Spearman's non-parametric coefficient. A p-value <0.05 was considered statistically significant. Statistical analyses were performed with SAS software, version 9.4 (SAS Institute, Cary, NC, USA) and SPSS Statistics V.29 (SPSS Inc., Chicago, Ill., USA).

Results

A total of 75 patients were enrolled between January 2022 and July 2023. Eight patients (10.7%) were excluded from the final analysis because they discontinued the drug or the study for personal reasons (specifically: two participants interrupted the study treatment for personal decision; six individuals continued the study treatment but did not perform the follow-up evaluation), while none of the enrolled patients stopped the treatment for clinical reasons or drug-related complaints. All the remaining 67 HF patients (median age 66 years; age range 56–73 years) completed the evaluation at 6 months (T2), while 5 of 67 did not perform the safety evaluation at 2–4 weeks (T1). Table 1 reports the main parameters collected for the study population.

Table 1 Main variables at baseline (T0), 2–4 weeks (T1), and 6 months (T2).

Variable	*n*	T0	*n*	T1	*n*	T2	*p* T0 vs. T2	*p* Repeated meas.	Bonferroni *post-hoc* test		
									T2 vs. T0	T1 vs. T0	T1 vs. T2
Age (years)	67	66 [56–73]	67	66 [56–73]	66	66 [57–73]		0.604			
Weight (kg)	67	79.0 ± 14.3	61	79.1 ± 14.0	67	78.6 ± 14.2		0.016	–	0.020	–
Height (cm)	67	172 ± 8	67	172 ± 8	67	172 ± 8		1			
BMI (kg/m^2)	67	26.5 ± 3.5	61	26.5 ± 3.4	67	26.4 ± 3.4		0.014	–	0.019	–
LVEF (mL)	67	34.6 ± 7.8			67	37.5 ± 9.2	<0.001				
EDV (mL)	67	186 [145–232]			67	177 [129–225]	<0.001				
ESV (mL)	67	113 [87–163]			67	110 [76–145]	<0.001				
PAPs (mmHg)	62	27.0 [23.7–29.0]			58	25.0 [23.0–28.0]	0.046				
SBP (mmHg)	67	118.6 ± 16.1				61	109.3 ± 13.4	<0.001			
DBP (mmHg)	67	72.5 ± 9.5			60	68.4 ± 7.9	0.003				
Heart rate (bpm)	63	63.4 ± 11.3			67	63.3 ± 12.0	0.796				
Vital capacity (L)	65	3.74 ± 0.95	60	3.84 ± 0.89	61	3.79 ± 0.90		0.672			
FEV1 (L)	67	2.71 ± 0.74	61	2.74 ± 0.74	62	2.69 ± 0.69		0.226			
FEV1 (%)	67	87.34 ± 17.04	61	88.18 ± 16.22	62	87.20 ± 15.19		0.358			
FVC (L)	67	3.38 ± 0.91	61	3.45 ± 0.93	62	3.43 ± 0.87		0.476			
FVC (%)	67	84.36 ± 16.11	61	84.58 ± 18.08	62	85.51 ± 14.82		0.772			
FEV1/FVC	67	0.80 ± 0.06	61	0.80 ± 0.07	62	0.79 ± 0.07		0.366			
Total body water (L)	67	44.7 [39.8–51]	60	44.9 [40.23–9.75]	67	44.9 [39.5–50]		0.145			
Extracellular water (L)	67	21.2 [18.2–23.1]	60	21.0 [18.82–2.95]	67	20.5 [18.4–22.9]		0.145			
Hydration index (%)	67	73.6 [73.2–73.8]	60	73.5 [73.3–73.8]	67	73.5 [73.3–73.8]		0.552			

BMI, body mass index; LVEF, left ventricular ejection fraction; EDV, left ventricle end-diastolic volume; ESV, left ventricle end-systolic volume; PAPs, systolic pulmonary artery pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

Table 2. Main variables at baseline (T0), 2–4 weeks (T1), and 6 months (T2).

Variable	*n*	T0	*n*	T1	*n*	T2	*p* T0 vs. T2	*p* Repeated meas.	Bonferroni *post-hoc* test		
									T2 vs. T0	T1 vs. T0	T1 vs. T2
Age (years)	67	66 [56–73]	67	66 [56–73]	66	66 [57–73]		0.604			
Weight (kg)	67	79.0 ± 14.3	61	79.1 ± 14.0	67	78.6 ± 14.2		0.016	–	0.020	–
Height (cm)	67	172 ± 8	67	172 ± 8	67	172 ± 8		1			
BMI (kg/m^2)	67	26.5 ± 3.5	61	26.5 ± 3.4	67	26.4 ± 3.4		0.014	–	0.019	–
LVEF (mL)	67	34.6 ± 7.8			67	37.5 ± 9.2	<0.001				
EDV (mL)	67	186 [145–232]			67	177 [129–225]	<0.001				
ESV (mL)	67	113 [87–163]			67	110 [76–145]	<0.001				
PAPs (mmHg)	62	27.0 [23.7–29.0]			58	25.0 [23.0–28.0]	0.046				
SBP (mmHg)	67	118.6 ± 16.1				61	109.3 ± 13.4	<0.001			
DBP (mmHg)	67	72.5 ± 9.5			60	68.4 ± 7.9	0.003				
Heart rate (bpm)	63	63.4 ± 11.3			67	63.3 ± 12.0	0.796				
Vital capacity (L)	65	3.74 ± 0.95	60	3.84 ± 0.89	61	3.79 ± 0.90		0.672			
FEV1 (L)	67	2.71 ± 0.74	61	2.74 ± 0.74	62	2.69 ± 0.69		0.226			
FEV1 (%)	67	87.34 ± 17.04	61	88.18 ± 16.22	62	87.20 ± 15.19		0.358			
FVC (L)	67	3.38 ± 0.91	61	3.45 ± 0.93	62	3.43 ± 0.87		0.476			
FVC (%)	67	84.36 ± 16.11	61	84.58 ± 18.08	62	85.51 ± 14.82		0.772			
FEV1/FVC	67	0.80 ± 0.06	61	0.80 ± 0.07	62	0.79 ± 0.07		0.366			
Total body water (L)	67	44.7 [39.8–51]	60	44.9 [40.23–9.75]	67	44.9 [39.5–50]		0.145			
Extracellular water (L)	67	21.2 [18.2–23.1]	60	21.0 [18.82–2.95]	67	20.5 [18.4–22.9]		0.145			
Hydration index (%)	67	73.6 [73.2–73.8]	60	73.5 [73.3–73.8]	67	73.5 [73.3–73.8]		0.552			

BMI, body mass index; LVEF, left ventricular ejection fraction; EDV, left ventricle end-diastolic volume; ESV, left ventricle end-systolic volume; PAPs, systolic pulmonary artery pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

At enrolment, all patients were on optimized HF medical treatment, with 100% of patients taking ACEi (n = 6, 9%), ARBs (n = 7, 10.4%), or Sacubitril/Valsartan (n = 54, 80.6%), 64 (95.5%) patients taking a β-blocker, 56 (83.6%) taking an MRA, and 35 (52.2%) taking a loop diuretic. With regard to comorbidities, at enrolment there were 7 (10.4%) patients with diabetes, 40 (59.7%) with dyslipidaemia, 33 (49.3%) with

hypertension, and 1 (1.5%) with moderate COPD. In total, 12 (18%) patients were current smokers while 26 (39%) were former smokers.

After 6 months of dapagliflozin treatment, both systolic and diastolic blood pressure were lower than at T0, while heart rate remained unchanged. Echocardiographic evaluation confirmed its positive impact, showing an 8% increase in LVEF with a parallel reduction in LV volumes and a slight but significant decrease in PAPs. Moreover, a decrease in E/E' was also observed.

Table 3. Main changes in echocardiographic, pulmonary, and hematochimical values during the trial.

	N	T0	T1	T2	*p* TREND ANOVA	*p* Value Bonferroni Adjusted		
						T0 vs. T1	T0 vs. T2	T1 vs. T2
ProSP-B (AU)	64	32.65 ± 13.36	31.29 ± 12.43	30.86 ± 12.45	0.0092	0.0277	0.0209	1.0000
DLCO (mL/min/mmHg)	51	18.46 ± 5.4	18.75 ± 6.26	18.76 ± 5.3	0.4175	1.0000	1.0000	1.0000
DLCOHb (mL/min/mmHg)	51	18.65 ± 5.44	18.55 ± 5.58	18.85 ± 5.27	0.6241	1.0000	1.0000	1.0000
DLCO%	51	70 ± 16	69 ± 15	71 ± 15	0.6241	1.0000	1.0000	0.7748
Dm (mL/min/mmHg)	47	22.27 ± 8.11	21.97 ± 7.53	23.0 ± 6.82	0.1783	1.0000	1.0000	0.3640
FEV1 (L)	61	2.68 ± 0.69	2.7 ± 0.67	2.66 ± 0.64	0.5193	0.7952	1.0000	0.2881
FEV1%	61	87.02 ± 16.31	87.83 ± 15.99	86.9 ± 15.14	0.8521	0.6004	1.0000	0.7355
FCV (L)	61	3.36 ± 0.86	3.4 ± 0.84	3.39 ± 0.84	0.4112	0.5215	1.0000	1.0000
FVC%	61	84.3 ± 15.71	85.18 ± 14.72	85.22 ± 14.76	0.2642	0.8538	1.0000	1.0000
Hb (g/dL)	66	13.74 ± 1.55	13.93 ± 1.44	14.59 ± 1.7	<0.0001	0.1141	<0.0001	<0.0001
Creatinine (mg/dL) *	66	1.07 [0.91–1.33]	1.12 [0.94–1.39]	1.08 [0.89–1.36]	0.0123	0.0017	0.1352	0.9555
Potassium (mmol/L)	66	4.44 ± 0.39	4.52 ± 0.47	4.4 ± 0.37	0.7714	0.2833	1.0000	0.1098
NT-proBNP (ng/mL) *	64	852 [466–1858]	793 [298–1771]	944 [320–1854]	0.0717	0.0453	0.4037	1.0000
ST-2 (ng/mL)	61	27.28 ± 6.81	27.02 ± 6.48	28.08 ± 6.8	0.2628	1.0000	0.6841	0.5015
VA (L)	51	5.57 ± 1.32	5.57 ± 1.34	5.59 ± 1.32	0.9264	1.0000	1.0000	1.0000
Vcap (mL) *	43	98 [79–115]	101 [80–126]	94 [75–117]	0.7040	1.0000	1.0000	1.0000
EDV (mL)	66	186 [145–232]		177 [129–225]	<0.0001			
ESV (mL)	66	113 [87–163]		110 [76–145]	<0.0001			
LVEF (%)	66	34.60 ± 7.82		37.54 ± 9.20	<0.0001			
E/E′	60	8.5 [7.0–13.0]		8.0 [6.0–9.8]	0.0050			
PAPs (mmHg)	56	27 [24–29]		25 [23–28]	0.0460			
TAPSE (mm)	65	20.13 ± 5.72		19.34 ± 4.29	0.2010			

Medical treatment did not change during the course of the study, including the median dose of loop diuretics [25 mg/day (range 25–50) to 25 mg/day (range 25–25); p = 1.000]. None of the patients who were not on loop diuretics at baseline were prescribed them during the study, while one patient had loop diuretic therapy discontinued.

We observed a progressive increase in Hb values (13.8 ± 1.5, 13.9 ± 1.5, and 14.6 ± 1.7 g/dL at T0, T1, and T2, respectively; p < 0.001), and a modest but significant change in red cell distribution width (RDW) [13.7 (13.1–14.6), 13.8 (13.3–14.6), 13.8 (13.3–14.6); p < 0.019] (Figure 2). In contrast, biomarkers such as Na+ (140.3 ± 2.0, 140.1 ± 2.0, and 140.6 ± 2.2 at T0, T1, and T2, respectively; p = 0.192), NT-proBNP [from 852.0 pg/mL (455.3–1,845.3) at T0 to 916.5 pg/mL (301.7–1,831.0) at T2; p = 0.081], interleukin ST-2 [from 26.35 ng/mL (23.08–30.23) to 27.80 ng/mL (22.80–32.10); p = 0.829], hsCRP [from 1.07 mg/L (0.40–3.07) to 1.04 mg/L (0.46–2.82); p = 0.114], and hs-TNI [from 12.22 ng/L (7.31–25.53) to 9.49 ng/L (6.74–22.88); p = 0.201] did not significantly change during the study.

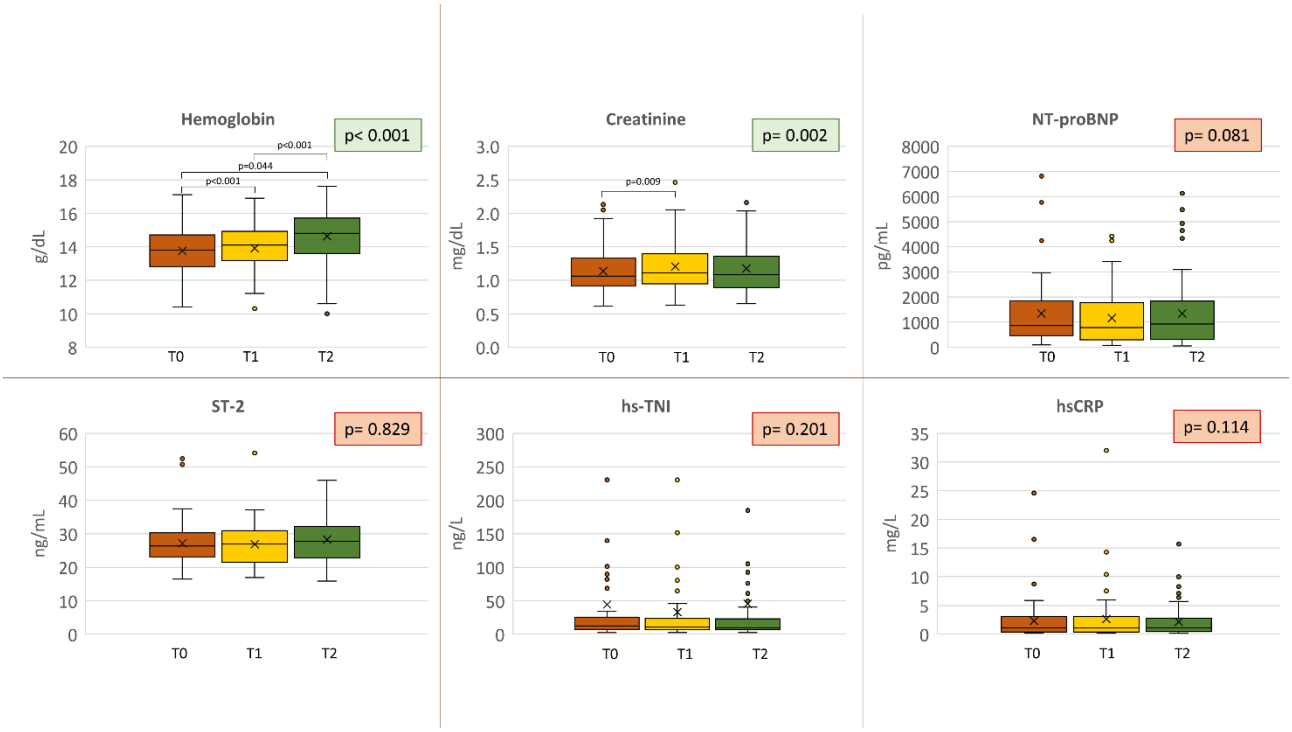


Figure 11. Biomarkers analysis. Biomarker variations at each study point. Orange = baseline; yellow = 2–4 weeks (T1); green = 6 months (T2). Nt-proBNP, N-terminal pro B-type natriuretic peptide; ST-2, suppression of tumorigenicity 2; hs-TNI, high-sensitivity troponin I; hsCRP, high-sensitivity C-reactive protein.

As expected, we observed a short-term worsening of creatinine at T1, with a complete recovery at T2 ($p = 0.009$ T0 vs. T1). The same trend was confirmed when renal function was analysed using MDRD (73.0 ± 22.8, 68.8 ± 21.3, and 70.2 ± 20.9 at T0, T1, and T2, respectively; $p < 0.01$). No changes in potassium values were observed (p for trend = 0.088).

Regarding CPET evaluation, no significant changes in peak VO_2 or other VO_2-derived parameters were detected at 6 months, while the VE/VCO_2 slope decreased both as absolute value and as percent of predicted. Two patients did not repeat the cardiopulmonary assessment at 6 months due to limitations unrelated to HF; therefore, they were excluded from this analysis.

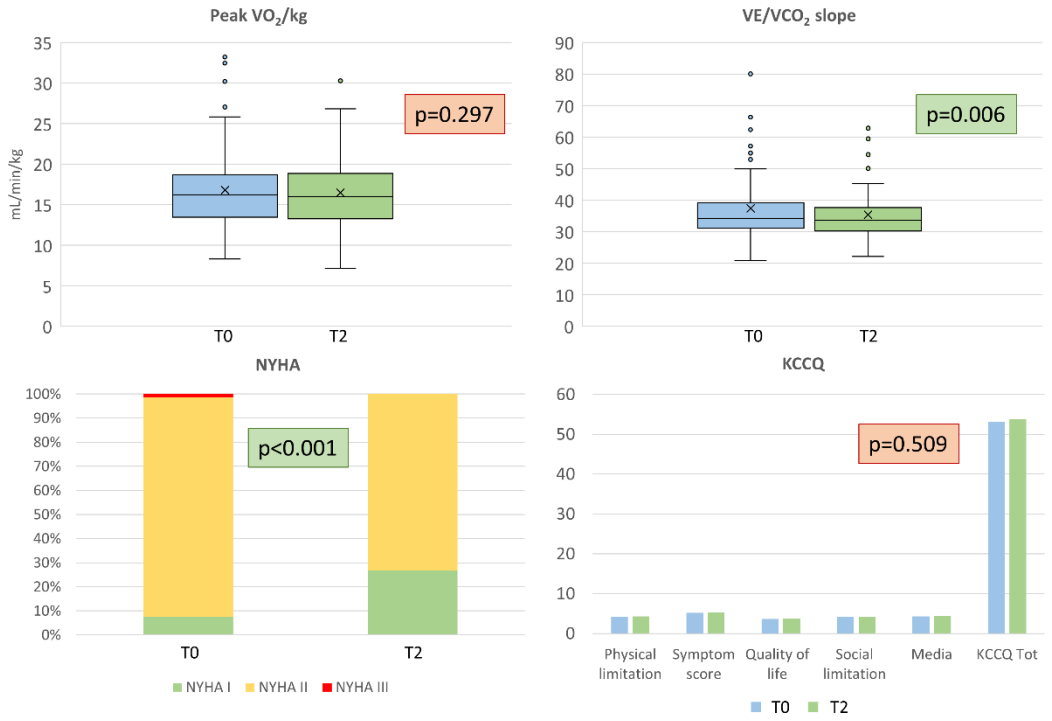


Figure 12. Functional and quality of life evaluation. Top: peak oxygen uptake (VO2/kg) on the left, and ventilatory efficiency (VE/VCO2 slope), on the right. Bottom: New York Heart Association (NYHA) class and Kansas City Cardiomyopathy Questionnaire results are reported on the left and right respectively. T0 = baseline, T2 = 6 months.

KCCQ did not reveal an improvement in the subjective perception of QoL. No significant differences with respect to the T0 evaluation were found in either the total score or in the analysed domains. VE/VCO_2 slope and Hb improvements were not correlated (R^2 for $\Delta VE/VCO_2$ vs. $\Delta Hb = 0.029$). NYHA class improved in 13 patients (from NYHA II or III at baseline to NYHA I at T2) ($p < 0.001$), while only one patient worsened from NYHA I to NYHA II. Regarding BIVA, we did not find any significant difference (Table 1). A significant improvement in the median MECKI score (18) from 3.3% (range 1.9–8.0) to 2.8% (range 1.2–5.7) was observed, suggesting a positive impact on prognosis at 2 years ($p < 0.001$).

On average, at T0, FEV_1, FVC, and DLCO were in the lower part of the normal range. No significant change in respiratory parameters, including FEV_1 and FVC, was observed. Similarly, no changes were detected in DLCO or its components Dm and Vcap, reflecting alveolar-capillary membrane function. Conversely, proSP-B showed a significant reduction over the course of the study (p for trend = 0.009)

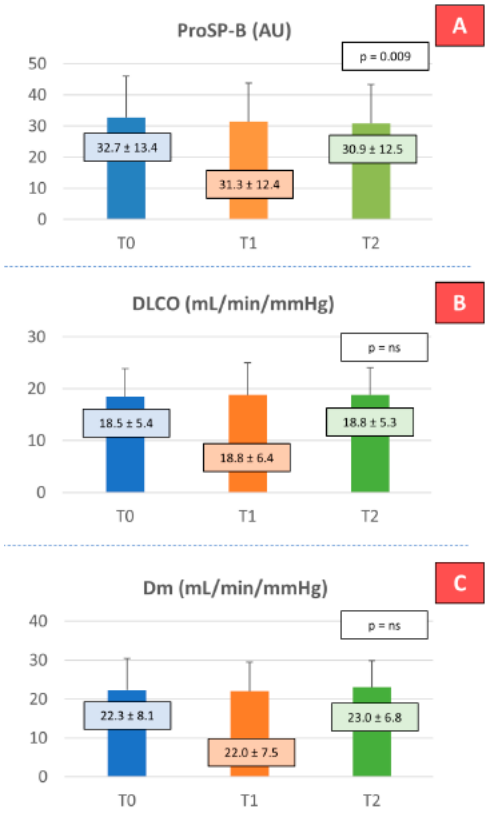


Figure 13. Effects of dapagliflozin on alveolar‑capillary membrane function. (A) ProSP-B: surfactantderived protein type B; (B) DLCO: lung diffusion capacity for carbon monoxide; (C) Dm: membrane diffusion; T0: initial evaluation; T1: 2‑4 weeks after starting the therapy; T2: after 6 months of treatment. To analyze these variables a repeated-measures ANOVA model was applied and a p-value < 0.05 was considered statistically significant. n = 64 for proSP-B, n = 51 for DLCO and n = 47 for Dm.

Nocturnal cardiorespiratory monitoring was performed in 59 out of 66 patients and revealed minor or absent abnormalities in sleeping characteristics at T0. At T2, no effect was observed on AHI, OSA, mixed apnoea, and hypopneas, while a significant reduction in the number of central sleep apnoea (CSA) was found in the 13 patients (22%) with at least one CSA at baseline (from 15 [3–48] at T0 to 0 [0–18.5] at T2; p = 0.017).

Table 4. Main changes in sleep monitoring values during the trial.

	N	T0	T1	T2	*p* TREND ANOVA	*p* Value Bonferroni Adjusted		
						T0 vs. T1	T0 vs. T2	T1 vs. T2
AHI (n)	59	5.0 [1.1–16.6]		6.2 [0.7–13.8]	0.3660			
Hypopneas (n)	49	8.0 [3.5–23.0]		10.5 [2.0–23.0]	0.8110			
CSA (n)	50	0.0 [0.0–2.3]		0.0 [0.0–1.0]	0.8090			

Abbreviation: ProSP-B: surfactant-derived protein type B; DLCO: Diffusing Capacity of the Lungs for Carbon monoxide; DLCOHb: DLCO corrected for heamoglobin level; DLCO%: DLCO expressed as percent of the predicted; Dm: Diffusing capacity of the alveolocapillary Membrane; FEV1: Forced Expiratory Volume in 1 s; FEV1%: FEV1 as percent of the predicted; FVC: Forced Vital Capacity; FVC%: FVC as percent of the predicted; Hb: Hemoglobin; NT-proBNP: N-terminal pro–B-type Natriuretic Peptide; ST-2: Soluble interleukin-1 receptor-like 1; Vcap: diffusing capacity for the pulmonary capillary blood volume; EDV: End-Diastolic Volume; ESV: End-Systolic Volume; LVEF: Left Ventricle Ejection Fraction; PAPs: Pulmonary Arterial Pressures; TAPSE: Tricuspid Annular Plane Systolic Excursion; AHI: The Apnea–Hypopnea Index; CSA: Central Sleep Apnea. * Results are presented for the log-transformed variables.

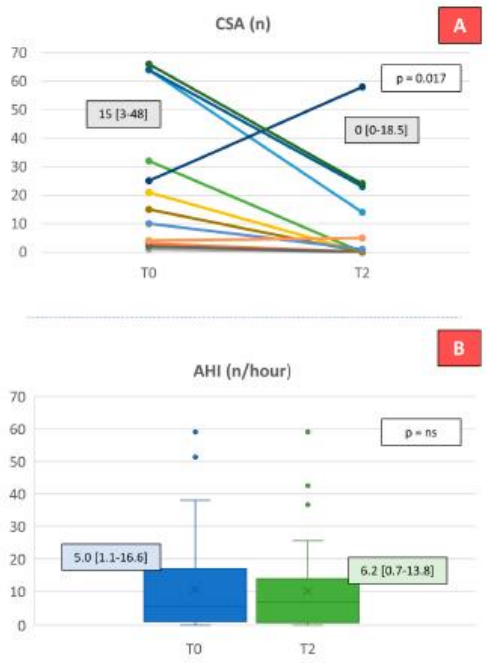


Figure 14. Dapagliflozin effects on sleep apnoea (n = 59). (A) CSA: central sleep apnea; (B) AHI: apnea hypopnea index; T0: initial evaluation; T2: after 6 months of treatment. To analyse these variables, a Wilcoxon-signed-rank test was performed. Panel (A): analysis conducted only on patients with CSA > 0 at baseline (n = 13).

Discussion

The main and novel finding of this study is that, in our cohort of HFrEF patients, dapagliflozin therapy showed no impact on exercise capacity, as assessed by peak VO_2 and workload. Similarly, other relevant CPET parameters, including VO_2 at AT, VO_2/work, peak O_2-pulse, and peak heart and respiratory rates, remained unchanged, with patients achieving maximal or near-maximal effort. However, dapagliflozin improved exercise ventilatory efficiency, as demonstrated by a modest but significant reduction in the VE/VCO_2 slope (34.2 vs. 33.7; $p < 0.001$).

The neutral effect of the drug on peak VO_2 is somewhat unexpected, given previously reported data. The DAPA-VO_2 study documented a significant improvement in peak VO_2 after 1 and 3 months of treatment (+Δ 1.09 mL/kg/min and +Δ 1.06 mL/kg/min, respectively). Compared to that trial, our cohort had less advanced HF, as indicated by the relatively higher baseline peak VO_2 (16.2 mL/kg/min vs. 13.4 mL/kg/min), LVEF (34.6% vs. 33.7%), and lower NT-proBNP levels (774 vs. 1,085 pg/mL). Therefore, a smaller effect on peak VO_2 in our population may have been anticipated. The absence of peak VO_2 improvement remains surprising, especially considering the significant increases in LVEF and Hb. O_2 delivery is closely linked to cardiac output (CO) and Hb levels. In this study, the former was possibly unchanged, as the improvement in LVEF occurred in parallel with the reduction in LV volumes, while Hb was significantly increased by dapagliflozin (from 13.8 to 14.6 g/dL; $p < 0.001$), consistent with previous reports.

We hypothesize two potential explanations. First, the reduction in LV volumes might have resulted in a relevant decrease in peak CO regardless of the improvement in LVEF, potentially influencing exercise-induced blood flow redistribution. This phenomenon, which we recently reported, is rarely considered in clinical practice but may explain the blunted changes in peak VO_2 despite significant hemodynamic improvements. As HF severity increases, blood flow distribution during exercise is progressively directed toward working muscles, leading to differences in arteriovenous O_2 content ($\Delta a\text{-}vCO_2$). However, as HF

improves and CO rises, the percentage of blood flow to the muscles decreases, reducing $\Delta a\text{-}vCO_2$ and affecting peak VO_2 measurements. This may explain the discrepancy observed between peak VO_2 and LVEF/Hb changes. Therefore, the possibility that dapagliflozin improved exercise hemodynamic cannot be ruled out by the unchanged peak VO_2 observed.

The reduction of the VE/VCO_2 slope, indicating improved ventilatory efficiency during exercise, is another notable finding. This parameter is prognostically significant in HFrEF and other cardiomyopathies, with predictive value comparable to peak VO_2. Consequently, it is included in heart transplant screening guidelines and in HF prognostic scores such as the MECKI score. The VE/VCO_2 slope depends on chemoreflex-mediated ventilatory regulation and ventilation-perfusion mismatch at the pulmonary level. While no data exist regarding direct effects of SGLT2 inhibitors on chemo- or metaboreceptor activity, the absence of changes in $PetCO_2$ at rest, during exercise, and at key points (AT, respiratory compensation point, and peak exercise) suggests alterations in reflex effects on ventilation during exercise. An improvement in ventilation-perfusion mismatch seems plausible, given the significant reduction in pulmonary pressures observed by echocardiography. This improvement might be due to the well-recognized diuretic effect of dapagliflozin, which helps reduce pulmonary pressures and interstitial oedema. However, our data do not indicate a relevant diuretic effect, as weight loss was negligible (−0.4 kg), and NT-proBNP and BIVA remained unchanged. Notably, BIVA does not assess thoracic fluid status. Thus, although resting hemodynamic effects appear limited, improvement during exercise remains possible.

In addition to exercise-related findings, we observed favourable LV reverse remodelling, with a small but statistically significant reduction in EDV and ESV, along with improved LVEF. These results are consistent with previous studies and confirm the beneficial impact of SGLT2 inhibitors on LV geometry and HF progression. Prevention and reversal of adverse cardiac remodelling is a key mechanism through which SGLT2 inhibitors exert cardiovascular benefits, involving molecular, cellular, and interstitial adaptations related to apoptosis, necrosis, autophagy impairment, and altered energy metabolism. However, in this study, these positive structural changes were not accompanied by significant improvements in cardiac biomarkers such as NT-proBNP, hs-TNI, or ST-2.

When contextualizing these results with existing data, several aspects should be noted. Most patients were non-diabetic, in NYHA class II at baseline, and showed only mild impairment in QoL (KCCQ), peak VO_2, and NT-proBNP, reflecting a non-severe HF phenotype. Importantly, our cohort demonstrated a high level of therapeutic optimization, with a greater proportion on disease-modifying therapies compared to major trials. For example, in DAPA-HF, only 10% of patients were on sacubitril/valsartan versus 81% in our population. This likely contributed to the mild baseline impairment and reduced potential for further improvement. Furthermore, while DAPA-HF observed a significant NT-proBNP reduction, this occurred from higher baseline levels. Other studies in specific HF phenotypes, such as amyloidosis, have confirmed dapagliflozin's positive impact on NT-proBNP. Thus, the drug's effect on biomarkers appears influenced by baseline severity and follow-up duration.

In our population with moderate HF and optimized therapy, dapagliflozin did not provide additional benefits over sacubitril/valsartan in terms of peak VO_2 or cardiac biomarkers—parameters considered pivotal for HF treatment evaluation. Nevertheless, these mechanistic findings should not discourage SGLT2 inhibitor use. Even in a stable, low-severity, well-treated population, dapagliflozin improved overall prognostic balance, as indicated by a significant MECKI score improvement. This underscores the need for a holistic approach to HF assessment, rather than focusing solely on single parameters such as peak VO_2. Furthermore, dapagliflozin exhibited excellent tolerability without significant adverse effects. Given the progressive nature of HF, where worsening of key parameters over time is expected, stabilization or prevention of decline—as seen in this study—should also be considered a positive outcome.

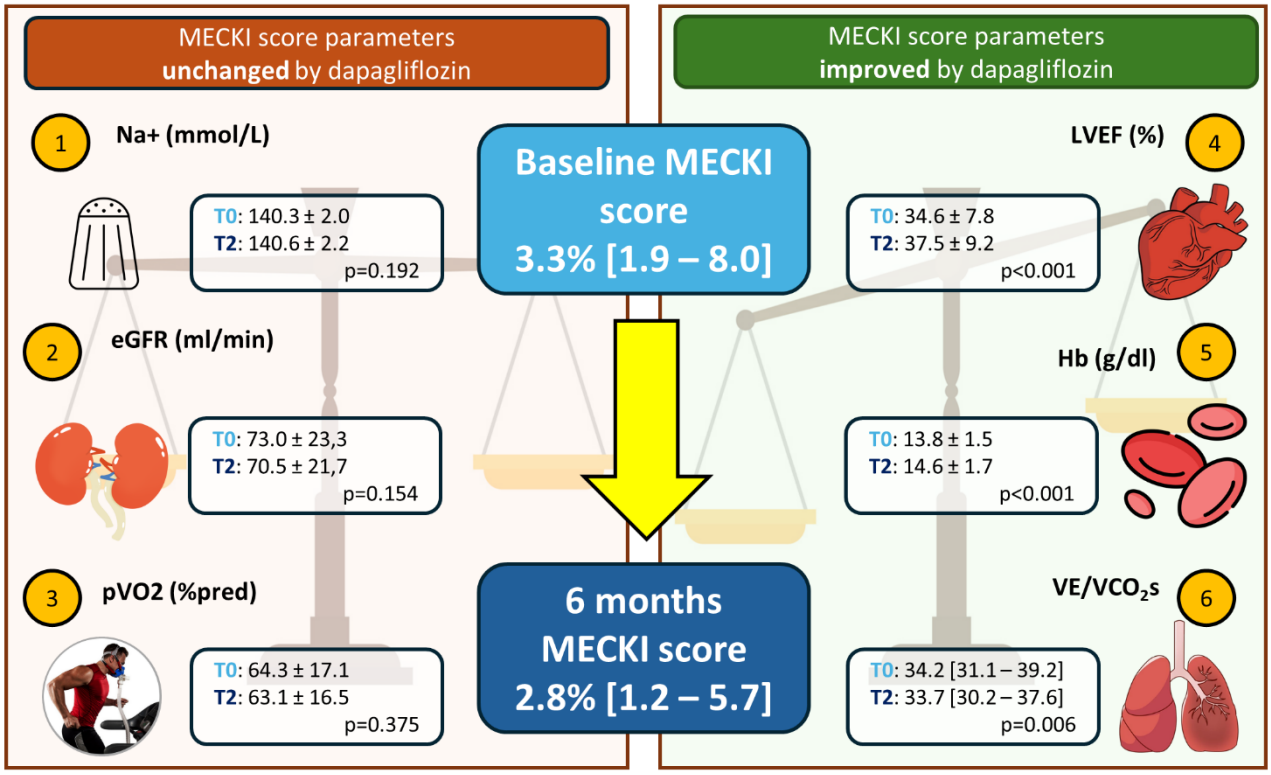


Figure 15. Effect of dapagliflozin on prognosis estimation by MECKI score. Schematic representation of the six variables constituting the MECKI score, which assesses prognosis. Left: variables that remained unchanged in our study; right: variables that significantly improved after 6 months with dapagliflozin. Na+, sodium; eGFR, estimated glomerular filtration rate calculated by modification of diet in renal disease (MDRD) formula; MECKI, Metabolic Exercise test data combined with Cardiac and Kidney Index; pVO2 (%pred), peak oxygen uptake expressed as% of predicted; LVEF, left ventricular ejection fraction; Hb, haemoglobin; VE/VCO2s, ventilatory efficiency.

Another novel and clinically relevant finding of this study is that dapagliflozin therapy was associated with a significant reduction in circulating proSP-B, despite no concomitant improvement in DLCO or pulmonary function tests. The reduction of proSP-B was evident soon after treatment initiation and persisted throughout follow-up. This protein, produced exclusively by alveolar cells, is typically elevated in HFrEF patients, correlates with DLCO, and decreases in response to therapeutic interventions such as inotropes or HF medications.

The absence of DLCO changes alongside proSP-B reduction is not unexpected for several reasons. First, our patients had preserved DLCO at baseline. Second, they were clinically stable, a setting in which alveolar-capillary fluid accumulation is uncommon, unlike acute HF. Indeed, DLCO remains unchanged after ultrafiltration in stable patients but improves in acute settings alongside reductions in proSP-B following levosimendan. In this context, DLCO abnormalities are often non-fluid dependent, whereas ultrafiltration primarily improves lung mechanics without altering DLCO.

The progressive reduction in proSP-B supports an improved alveolar-capillary membrane health status. While underlying mechanisms remain unclear, potential explanations include favourable hemodynamic effects or modulation of receptors and ionic pumps at the alveolar-capillary interface. Although a possible antifibrotic effect of SGLT2 inhibitors has been hypothesized, our study found no evidence of improved membrane diffusion or fibrosis biomarkers. However, the absence of DLCO abnormalities at baseline and the relatively short observation period may have limited detection of changes. Indeed, patients with baseline impaired DLCO (<80% predicted; n = 38) showed a trend toward improvement at 6 months (p = 0.063).

The modest hemodynamic impact of SGLT2 inhibitors—less pronounced than that of sacubitril/valsartan—has been previously documented, with reductions in blood pressure and wedge pressure both at rest and during exercise. In our results, proSP-B may represent an earlier and more sensitive marker of pulmonary microvascular health compared to traditional biomarkers of congestion such as NT-proBNP. This is particularly relevant because dapagliflozin demonstrated no effect on DLCO, OSA, or spirometry, and had a neutral impact on NT-proBNP under stable diuretic dosing.

The effect on sleep disturbances warrants discussion. In this cohort, sleep-disordered breathing was generally mild, and no significant change in AHI was observed after 6 months of dapagliflozin therapy. OSA remained unchanged, while a modest but significant reduction in CSA was noted among patients who had CSA at baseline. These results likely reflect the low prevalence and severity of sleep apnoea in this population. In patients with more advanced HF, SGLT2 inhibitors may exert more evident benefits on sleep-disordered breathing, as suggested by prior studies and confirmed by recent randomized trials.

Limitations

This study has some limitations. First, due to ethical considerations aligned with recent HF guideline recommendations, it was not randomized; therefore, a direct comparison of interventions is not possible. Second, the monocentric nature and relatively small sample size of the study limit the generalizability of the findings to other populations. Third, most patients were in NYHA class II, with relatively stable and non-advanced heart failure, so the effects of dapagliflozin in more severe HF populations warrant investigation in dedicated trials. Fourth, the study was designed to detect short-term changes during the initial months of therapy in patients with reduced LVEF; thus, further studies are needed to explore long-term outcomes and the effects in other HF phenotypes, including those with mid-range or preserved LVEF. Finally, only dapagliflozin was evaluated, and no other SGLT2 inhibitors (such as empagliflozin) were included, so it remains unclear whether similar results would be achieved with different molecules.

Conclusions

In conclusion, our trial, conducted in a well-treated population of clinically stable patients with moderate HFrEF, demonstrated a beneficial impact of dapagliflozin on several key parameters. These include improvements in VE/VCO_2 slope, haemoglobin, LV volumes, and ejection fraction, along with favourable changes in alveolar-capillary unit markers such as proSP-B and a reduction in CSA. These effects occurred despite a neutral impact on peak VO_2, cardiac biomarkers, lung diffusion, and pulmonary function tests. Taken together, these findings contribute to a better understanding of the mechanisms and expected benefits of this cornerstone HF therapy, even in a population already optimized on guideline-directed medical treatment. Furthermore, the results underscore the potential of proSP-B as an early and sensitive biomarker for assessing therapeutic response.

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