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BIOCHEMISTRY AND MOLECULAR BIOLOGY FINAL DEGREE PROJECT

MENTION IN CLINICAL AND FORENSIC BIOCHEMISTRY

VARIABLES ASSOCIATED WITH READMISSION FOR HEART FAILURE IN PATIENTS IN THE GREY ZONE

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1. Abstract

Heart failure is a prevalent syndrome in the population over 65 years of age, characterised by complex clinical features and frequently non-specific symptoms. The key biomarker for diagnosis is NT-proBNP. This biomarker is interpreted using different pre-validated cut-off points. A value below 300 pg/ml (rule-out) allows the diagnosis to be ruled out. To confirm the diagnosis, age-adjusted cut-off points (rule-in) have been established: ≥450 pg/ml for patients under 50 years of age, ≥900 pg/ml for patients aged 50 to 75 years, and ≥1800 pg/ml for patients aged 50 to 75 years. If NT-proBNP concentrations are in the range where HF cannot be confirmed or ruled out (grey zone), the diagnosis depends on the clinical judgement of the treating physician. Knowledge of the clinical characteristics and prognosis of patients with NT-proBNP measurements in this grey zone has been little explored to date.

In order to characterise patients with NT-proBNP in the grey zone, an observational and retrospective study was conducted that included as the study group patients with NT-proBNP levels in the grey zone and as the control group patients with NT-proBNP levels below 300 pg/ml (rule-out). The clinical characteristics and factors associated with readmission for heart failure were analysed. The results showed that dyspnea, orthopnea, history of myocardial infarction, higher NT-proBNP levels (within the grey zone) and low CRP levels are variables associated with the risk of hospitalization. Specifically, NT-proBNP and CRP increase the discriminatory power of a clinical prognostic model in assessing the risk of adverse events during follow-up.

Keywords: heart failure, cardiovascular disease, cardiovascular syndrome, emergency department, NT-proBNP, gray zone, PCR, prognosis.

2. Introduction

2.1. Heart disease: Definition

Heart failure, also known as HF, is a clinical syndrome defined by the presence of symptoms such as dyspnea, orthopnea and edema in the lower limbs, as well as clinical signs such as pulmonary crackles or elevated jugular venous pressure, according to the European Society of Cardiology (ESC, 2016). This syndrome is caused by a structural or functional alteration of the heart, either due to an acute event, such as a myocardial infarction, or a chronic condition, such as ischemic heart disease or uncontrolled high blood pressure. [1]

HF, together with the alteration that causes it, leads to a reduction in stroke volume, i.e. the amount of blood that the heart can pump. In this situation, the body activates compensatory mechanisms to maintain oxygen supply to the tissues. Although initially effective, over time these mechanisms aggravate the patient's clinical condition, worsening congestion due to fluid accumulation, structural and functional alterations of the heart, and progressive involvement of other organs. In addition, some diseases can further worsen the problem by directly affecting the functioning of the heart. [2]

Consequently, HF affects more than one organ, transforming it into a systemic syndrome. Although the greatest dysfunction occurs in the heart, this same dysfunction leads to various systemic complications. [1]

2.2. Physiopathology

Heart failure

The functioning of the left ventricle (LV) depends on three elements: preload, the ability of the myocardial tissue to stretch during diastole; afterload, the resistance that must be overcome for the ventricle to eject blood; and the contractility of the cardiac tissue. [3]

Depending on the cause of HF, one of these elements is more affected than the others. In the case of ischemic disease or heart attack, functional myocardium is lost, affecting the contractility of the heart. In contrast, uncontrolled hypertension affects afterload, as it causes pressure overload. Volume overload due to valve incompetence affects preload. [3]

HF, together with the condition that causes it, leads to a reduction in stroke volume (SV), the amount of blood ejected by the ventricle per beat, which is usually 1 cc/kg or approximately 60–100 cc. This decrease is also often called a reduction in cardiac output (CO), which is the product of heart rate and SV, therefore a decrease in SV necessarily leads to a decrease in CO. This reduction causes hypoperfusion of blood to the entire system, which activates a series of compensatory mechanisms that initially help to maintain adequate perfusion but ultimately lead to complications in different organs. [3]

Consequently, HF affects more than one organ, transforming it into a systemic syndrome, although the greatest dysfunction occurs in the heart, and it is poor perfusion and compensatory mechanisms that cause complications throughout the body. [1]

Congestive heart failure

LV dysfunction causes a decrease in SV while maintaining blood volume within the ventricle, increasing end-systolic and end-diastolic volumes. This in turn leads to an increase in LV end-diastolic pressure (LVEDP), which causes elevations in left atrial pressures, which in turn lead to increases in capillary pressure in the lungs. This elevated pressure in the lungs expels fluid from the pulmonary capillaries and causes pulmonary congestion and the main clinical symptom of dyspnea. In addition, pulmonary congestion ultimately multiplies the pressure in the capillaries, causing the lymphatic system to overflow and generating edema and effusions. [3]

On the other hand, systemic congestion can be a cause or consequence of congestive heart failure (CHF), affecting multiple organs and aggravating organ dysfunction, especially if there is hypoperfusion. Congestion is not always caused by an increase in fluid volume but also by fluid redistribution. The latter is caused by stimulation of the sympathetic nervous system, which can lead to pulmonary congestion due to the redistribution of blood from the venous to the pulmonary circulation. [2,4]

Among systemic congestion, renal congestion is noteworthy, causing renal venous hypertension, tubular collapse and reduced filtration, aggravated by factors such as inflammation or medication. Therefore, monitoring creatinine would be a good way to control renal congestion, although clinical signs must be taken into account for a comprehensive approach. Similarly, hepatic congestion is noteworthy, where enzymes are altered and, in extremely severe cases, cause hepatic necrosis due to hypoperfusion. Splenic congestion must also be taken into account, which raises abdominal pressure, affecting the intestine and promoting intestinal inflammation. [4]

In addition, the chronic activation of compensatory hormonal systems characteristic of HF (such as the renin-angiotensin-aldosterone system and vasopressin), together with impaired renal function, promotes salt and water retention. For this reason, a diagnostic sign is edema of the lower limbs, and it is also vitally important to assess glomerular filtration rate, as this provides information on renal status. [2]

Energetic aspects in heart failure and overproduction of reactive oxygen species

Although these aspects are not usually taken into account when discussing the syndrome in question, they also influence it. On the one hand, heart contraction depends on the energy produced by oxidative phosphorylation and transport with creatine kinase. In HF, these processes can fail, for example, if the event that triggers HF is ischemia that deprives the heart of nutrients. In this situation, the availability of ATP (energy) is reduced, which affects the contraction of the organ. Similarly, the lack of energy prevents proper calcium management, worsening the relaxation of the heart. For this reason, treatments that improve the use of substrates and reduce mitochondrial damage are being studied, in addition to existing hormonal treatments. [1]

On the other hand, oxidative stress, i.e., an excess of free radicals, contributes to the development and worsening of HF by damaging the cells and function of the heart. In

addition, various studies in both animals and humans have shown that different risk factors such as hypertension, diabetes and obesity are associated with an increase in this stress. [1]

Oxidative damage occurs through pathways such as MAPK and NF-kB, which promote inflammation, apoptosis and cardiac remodelling. Similarly, compensatory systems increase the production of free radicals (ROS). To counteract these events, the body has antioxidant systems such as vitamins C and E and various enzymes. [1]

2.3. Compensation Mechanisms

As described above, a decrease in CO involves the activation of certain compensatory mechanisms. These mechanisms are actually activated by a decrease in mean arterial pressure (MAP), which is the product of CO and total peripheral resistance (TPR), the resistance that vessels place on blood flow, and by the hypoperfusion that this generates. Through various mechanisms such as Frank-Starling, neurohormonal activation, and ventricular remodelling, the body attempts to maintain adequate tissue perfusion. Although initially effective, over time these mechanisms aggravate the patient's clinical condition, worsening congestion due to fluid accumulation, structural and functional alterations of the heart, and progressive involvement of other organs. [1,3]

Frank-Starling mechanism

The Frank-Starling mechanism is the heart's ability to change its contractile force and thus increase stroke volume due to elevated preload. At the cellular level, it depends on the length-tension relationship, also called sarcolemma-force. This relationship can change depending on the HF situation and can also reach a plateau when the heart can no longer increase force after further stretching. Part of the Frank-Starling mechanism is also the cross-communication of right and left ventricular function through the shared myocardium. [3,5]

Neurohormonal activation

The neurohormonal mechanism increases MAP via two routes: increasing total blood volume by retaining sodium and water, thereby raising SV; and increasing TPR through vasoconstriction.

The neurohormonal mechanism is controlled by the sympathetic nervous system (SNS), which secretes catecholamines (adrenaline and noradrenaline) that act through β1, β2 and α1 receptors. Catecholamines increase heart rate, contractility and vasoconstriction, raising both CO and TRP. However, prolonged activation ends up being harmful because it induces arrhythmia, tachycardia, myocardial damage and reduced EF.

Simultaneously, the SNS activates the renin-angiotensin-aldosterone system (RAAS). In response to decreased renal perfusion, the kidneys secrete renin, which initiates a cascade that culminates in the formation of angiotensin II, a potent vasoconstrictor that also stimulates the release of aldosterone, vasopressin and more noradrenaline. These factors increase cardiac contractility and fluid retention, exacerbating volume overload, i.e., worsening congestion.

Apart from the neurohormonal mechanisms activated by the SNS, there are other processes such as natriuretic peptides: ANP (atrial), BNP (cerebral) and CNP. The most important is NT-proBNP, a molecule in the BNP formation pathway, because it is more stable and is the one determined in the laboratory; it is the main biomarker of HF. These peptides are secreted when the heart chambers are distended and promote the excretion of salt and water, inhibiting the effect of other neurohormones.

Endothelial vasoactive substances such as nitric oxide, bradykinin and prostacyclin also play a role as vasodilators. Finally, there is an increase in the release of pro-inflammatory cytokines (such as TNF-α, IL-1α, IL-6 and interferon-α), which decrease the contractile capacity of the heart and are associated with a worse clinical outcome in patients with HF. [3]

Remodelación ventricular

Chronic or acute heart injury, a precipitating factor, causes structural and functional changes in the heart. These changes can be adaptive, reversible, or pathological (such as fibrosis) and involve heart cells such as cardiomyocytes, fibroblasts, endothelium, and interstitium.

These changes include ventricular hypertrophy, dilation of the heart chambers, and cellular disorganization. Initially, the changes help maintain SV and GC, albeit with reduced EF. However, in the long term, remodelling causes increased stress on the ventricular wall, fibrosis, loss of contractility, cell death, and contraction desynchronization, which worsens heart function and creates a vicious cycle.

Over time, remodeling not only loses its compensatory effectiveness but also accelerates the progression of HF, reaching a terminal phase where blood flow is directed only to vital organs, compromising the patient's survival. [1,3]

Calcium management and contractility

Under normal conditions, following the action potential, calcium (Ca2+) enters cardiomyocytes through L-channels, which are voltage-regulated channels. This influx of calcium triggers a massive release of calcium from the sarcoplasmic reticulum. This calcium binds to troponin C, promoting cardiac muscle contraction. Then, during diastole, calcium is released from troponin C and is captured by the SERCA pump, which reintroduces it into the sarcoplasmic reticulum or expels it from the cytoplasm via the Na+/Ca2+ pump. [1]

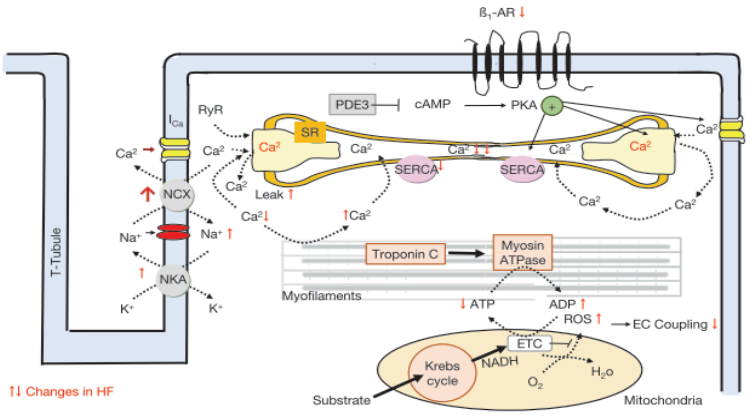

Figure 1. Contraction coupling in nonfailing cardiac myocytes and changes (red arrow) in ion concentration as well as alterations in expression/function of transporters/receptors in human heart failure.[1]

However, in the condition that concerns us, the contractility of cardiomyocytes is increased thanks to compensatory mechanisms. Contractility increases by raising the concentration of calcium in the cytoplasm. This contractility can increase through three cellular pathways: β-adrenergic receptors, the Frank-Starling mechanism, and the Bowditch effect. These prolonged pathways cause stiffness in the LV and deteriorate preload, decreasing blood flow.

Contractility can be increased by activating Ca2+ entry after stimulation of β-adrenergic receptors. After the binding of catecholamines, especially adrenaline, β-adrenergic receptors in cardiomyocytes are activated, triggering a cascade of intracellular signalling mediated by Gs protein. This protein activates adenylate cyclase, which increases the concentration of cAMP, which in turn activates PKA, which phosphorylates L channels. These allow Ca2+ to enter, increasing the calcium concentration above normal levels and hindering the Ca2+ efflux necessary for myocyte relaxation. [3]

Although it can also be increased by increasing the force with the stimulation frequency (Bowditch effect). The Bowditch effect is the increase in the force of contraction of the heart when the heart rate increases. This effect is due to calcium management, mainly through the functioning of proteins such as SERCA. In HF, reduced calcium uptake, caused by poor SERCA function, is associated with a reduced response to increased frequency. [6]

2.4. Epidemiological and Geographic Information

HF is a syndrome that affects 1–2% of the global population, especially among people over 65 years of age. In developed countries, HF has become a major public health problem, affecting 2% of the adult population, and the number of hospital admissions related to HF has tripled since the 1990s. It is estimated that one in five men over the age of 40 will suffer from HF, and this risk increases with age. [1,2]

In the US and Europe, there are over one million hospitalizations for HF per year, with very high readmission rates: 4% within 30 days, 30% within 3 months and 50% within 6 months. Comorbidities and psychosocial factors also influence readmissions and worsening of the condition. [2]

The increase in age at admission suggests that preventive treatments are delaying the development of HF, although the burden on health services represents approximately 2% of healthcare expenditure. In addition, patients with HF have a lower quality of life than those with other chronic diseases. [7]

In addition to being a significantly common syndrome, it carries considerable mortality. This is indicated by the INTER-CHF study, one of the largest registries, which reported on 5,823 patients with HF from 108 centres in six geographical regions. The overall 1-year mortality rate was 16.5%, with the highest mortality in Africa (34%) and India (23%), approximately average mortality in Southeast Asia (15%), and the lowest mortality in China (7%), South America (9%), and the Middle East (9%). [2]

On the other hand, several studies, including the Framingham study, indicate that mortality is much higher: between 30% and 40% of patients die within one year of diagnosis, and between 60% and 70% within five years. Most die due to worsening HF or ventricular arrhythmia. Mortality is even higher in patients requiring hospitalization, exceeding that of many cancers, although some recent studies suggest that the prognosis has improved. [7]

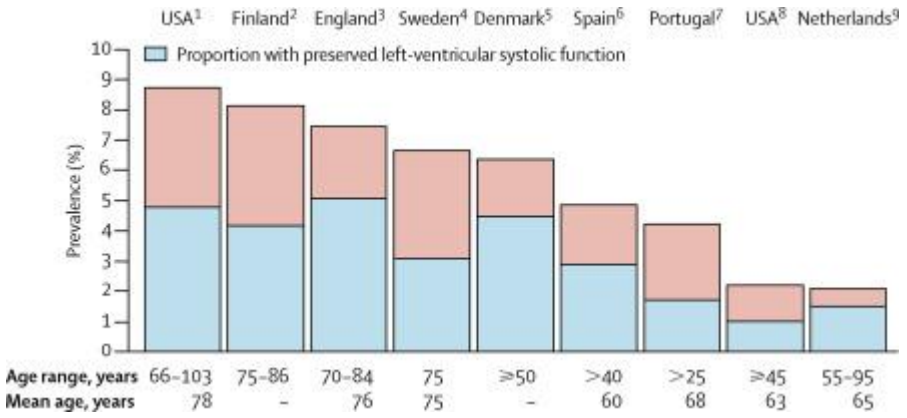

Figure 2. Prevalence of heart failure in cross-sectional population echocardiographic studies and proportion of patients with preserved left ventricular systolic function. [7]

In population-based echocardiographic studies, the prevalence of symptomatic HF with reduced and preserved systolic function has been estimated, with no significant differences observed in the classification by LVEF, or left ventricular ejection fraction. Even so, asymptomatic left ventricular systolic dysfunction and diastolic dysfunction remain an enigma for the scientific community. [7] 5.

2.5. Risk Factors and Comorbidities

The main risk factors for HF include coronary artery disease, hypertension, diabetes mellitus, family history, obesity, chronic lung disease, chronic inflammation or infection, metabolic diseases, cardiotoxic agents (such as cocaine, anthracyclines, or trastuzumab), and alcohol abuse. [1]

A systematic review of global risk factors for HF found that ischemic heart disease was the main contributor to AHF, acute heart failure, (50%). Hypertension is also a fairly prevalent precipitating factor for the syndrome (17%). In contrast, in East Asia and Africa, rheumatic heart disease and cardiomyopathy are also prominent. In high-income countries with a high level of medical development, patients with AHF tend to have a median age of over 75 years, while in regions such as Latin America and sub-Saharan Africa, the median age is up to 20 years younger, possibly due to poorly controlled hypertension and untreated ischemic or rheumatic heart disease. [7]

On the other hand, there are different diseases that frequently coexist with HF, known as comorbidities. The most frequent and studied comorbidities of these diseases are: anaemia, common in women, the elderly and diabetics; kidney disease, the most common and difficult to control due to its broad etiology, including cardiorenal syndrome, dysglycaemia and

diabetes. However, there are also other comorbidities or clinical manifestations that are being studied in relation to HF: osteoarthritis, gout, cachexia, COPD, neurological disorders, cerebrovascular accidents, and cancer, among others. In the case of cancer, it is a causal comorbidity, but not because of the disease itself, but rather because of the treatment, i.e., therapies such as anthracyclines, anti-HER2 and tyrosine kinase inhibitors, which have similar effects to other cardiomyopathies. These patients can continue their cancer treatment if cardiac therapy is optimised. [1,2,3,7]

These diseases are particularly relevant when analysing the clinical manifestations and treatment of HF, as they can interfere with treatments. Despite this, drugs have been found that do not cause interference and have good results, such as SGLT2 inhibitors in cases of diabetes and ferric carboxymaltose in cases of anaemia. [7,8]

2.6. Classification

Left ventricular dysfunction can be divided into two categories: systolic dysfunction, which is an alteration in ventricular contraction and ejection, and diastolic dysfunction, which is an alteration in ventricular relaxation and filling. Most patients suffer from systolic HF, although it can coexist with diastolic dysfunction.

The clinical functional classification refers to the ejection fraction (EF), which is defined as the amount of blood pumped from the ventricle in one heartbeat. If the EF is less than 40%, it corresponds to systolic HF, whereas if it is greater than 40%, it is diastolic HF.[3] The first type, HFrEF, HF with reduced EF, is associated with coronary artery disease, valvular heart disease, or hypertension. This condition involves a structural alteration called eccentric remodelling accompanied by ventricular dilation, volume overload and a loss of cardiomyocytes. [1]

The second type, HFpEF, is HF with preserved EF. This type is characterised by concentric hypertrophy of cardiomyocytes and increased ventricular stiffness, which alters organ relaxation and causes pressure overload. It is commonly associated with hypertension, diabetes, obesity, renal failure and other chronic diseases. There is no treatment for this type of HF that has been shown to improve prognosis. [1]

Secondly, HF can present acutely de novo or as decompensation of chronic HF. Acute HF develops suddenly as an acute event commonly caused by a heart attack or hypertensive crisis. Although emergency treatments are somewhat effective, oral medications upon discharge must be improved to enhance patient prognosis, which is the focus of sacubitril/valsartan. [1,8]

Chronic HF crises, commonly caused by congestion, are the main reason for emergency room visits and hospitalizations. In this type of HF, it has been shown that triggering factors have prognostic value, being worse in the presence of cardiovascular or infectious causes. [8]

The management of patients with chronic HF is complex and varies from case to case. The main objective of treatment is to relieve symptoms, especially extreme dyspnea, and in critical patients, to provide haemodynamic support, either with oxygen, intravenous opioids,

diuretics, or nitrates. In cases of severe hypotension or hypoperfusion, an inotropic agent such as dobutamine may be used. [7]

Thirdly, classification according to the side of the heart affected: there is left-sided and right-sided HF. If the left side is affected, the patient will experience pulmonary congestion, causing dyspnea and orthopnea; if the right side is affected, blood will accumulate in the systemic veins, causing edema in the lower limbs or jugular venous distension. [1]

Fourthly, we can classify HF according to the pathophysiological factor. The failure may be pressure-induced, where HF is caused by excessive pressure that the heart must overcome in order to pump blood; or volume-induced, where HF is due to excessive blood volume. [1,2,7]

All of these classifications can help guide both the diagnosis and treatment of HF, as therapeutic approaches may vary depending on the underlying cause and type of HF. [1]

2.7. Diagnostic Methods

The determination of cardiac dysfunction together with the identification of the pathophysiological mechanism leading to HF are crucial for choosing the appropriate therapeutic options and are therefore of great importance for diagnosis. A number of factors are taken into account in order to diagnose HF: clinical presentation, biomarkers, and additional tests. [1]

Clinic presentation

The most common symptoms include dyspnea, orthopnea, fatigue, and reduced exercise tolerance; symptoms are often accompanied by clinical signs such as peripheral edema, jugular vein distension, and the presence of a third heart sound. In patients with chest pain, differentiating between AHF and acute coronary syndrome can be difficult. In these cases, coronary syndrome should be ruled out if the patient does not present with cardiogenic shock. Cardiogenic shock is identified by signs of hypoperfusion such as cold skin, confusion, and low urine output. [9]

Diagnosis study

The clinical diagnosis of HF is not sufficiently reliable on its own, so it is necessary to determine the characteristic biomarker of HF, NT-proBNP.

Brain natriuretic peptide (BNP) is a 32-aa protein that is synthesized in the myocardium and secreted when cardiac tissue is distended. The starting molecule in the BNP synthesis pathway is a 108 aa peptide, this molecule is broken down into two fragments, BNP (32 aa) and NT-proBNP (76 aa). NT-proBNP is widely used as a diagnostic and prognostic biomarker of HF. [10]

Although it is the main biomarker of HF, it should be complemented with clinical evaluation and complementary tests. It is mandatory to take into account renal function, if it is severely affected, renal failure, the biomarker concentration may be altered. Only 25% of the biomarker is eliminated via the kidneys, therefore in cases of severe renal insufficiency the

NT-proBNP concentration will be overestimated in relation to the real concentration. It should also be taken into account in these cases if the patient is undergoing dialysis [11].

In addition, the interpretation of NT-proBNP concentration limits are adjusted by age to diagnose HF: ≥450 pg/mL for patients younger than 50 years, ≥900 pg/mL for patients between 50-75 years and ≥1800 pg/mL for patients older than 75 years. In contrast, the lower limit is maintained in all age ranges; values <300 pg/mL rule out HF. The adjustment for age, already takes into consideration the renal deterioration characteristic of age. [12]

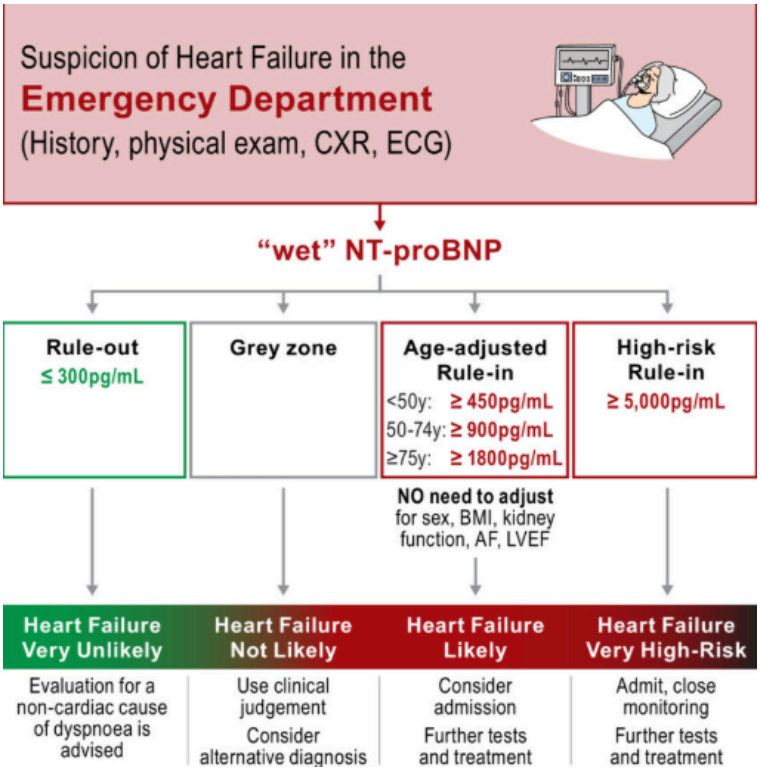

Figure 3. NT-proBNP for diagnosis of heart failure in the emergency department. AF, atrial fibrillation/flutter; BMI, body mass index; CXR, chest x-ray; ECG electrocardiogram; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

These limits imply a major limitation that is the protagonist of this study, there is a range of concentrations that do not rule out or confirm the diagnosis of HF, they are an inconclusive, indeterminate result. The main biomarker of the syndrome has a considerable range where it does not give any diagnostic information [12].

Additional tests

In addition to symptoms and biomarkers, the medical team can perform a series of additional tests in order to gather more information and identify the pathology suffered by the patient. Through the chest X-ray the healthcare professionals can detect physical signs of pulmonary congestion such as interstitial edema and pleural effusions, among others. [13]

Another test that plays an important role is echocardiography, although this is a very old test, recently great advances have been made that have allowed the development of 3D echocardiography. It is the main imaging test used in HF because it can assess cardiac

structure and function. It is also able to calculate LVEF, which is essential to choose the best treatment and to follow up with the patient. Moreover, it is the most widely used test because of its availability, safety and low cost [14].

2.8. Treatments

The fundamental treatment in HF is diuretics, although they do not directly treat cardiac deterioration but rather mitigate congestion due to their fundamental role in HF. The diuretics used are loop diuretics, such as furosemide or thiazides such as hydrochlorothiazide, which compete with Cl to bind to the Na-K-2Cl cotransporter in the ascending part of the loop of Henle, inhibiting the reabsorption of sodium and chloride. In this way it reduces the tonicity of the interstitial space causing water excretion. However, it must be taken into account that blocking the cotransporter causes a loss of potassium, calcium and magnesium ions. [15,16]

Although diuretics are essential drugs for the treatment of HF, resistance to them is a major limitation. This resistance is defined as an alteration of sensitivity to diuretics that generates reduced natriuresis and diuresis, limiting their effect. The pathophysiology of this resistance is multifactorial and is still under study but it is currently believed that the SNS, the renin-angiotensin-aldosterone system, and nephron remodeling are involved. [15]

3. Objective

As has been explained so far, HF is a syndrome that affects a large part of the world's population. It is a very complex syndrome, containing different pathophysiological factors that activate various compensatory mechanisms that end up affecting the whole organism. It is the mechanisms themselves that cause the clinical signs and symptoms.

As a consequence of the complexity of the syndrome, the diagnosis of HF is also complex due to the variety of symptoms. Moreover, these symptoms are not specific to HF but can be caused by other pathologies, which is why the main diagnostic tool in the emergency department is the determination of NT-proBNP. But this biomarker also has limitations, the so-called grey zone, i.e., in a part of the population this biomarker does not provide any diagnostic help.

That is why the aim of the project is to carry out a clinical characterisation and establish the prognosis of patients treated in the emergency department with NT-proBNP levels that are in the grey zone. That is why the aim of the project is to carry out a clinical characterisation and establish the prognosis of patients treated in the emergency department with NT-proBNP levels that are in the grey zone.

This project is based on activities carried out during internships at the Clinical Laboratory of the ICS Camp in Tarragona under the supervision of Sergio Pardo Granell.

4. Material and Methods

4.1. Study Design

This is an observational, retrospective, single-center cohort study. All patients of legal age who attended the emergency department of the Hospital Universitari Joan XXIII during the period 1/3/2022 to 31/8/2022 were included in this study. The inclusion criteria used were patients of legal age with NT-proBNP natriuretic peptide quantification analyzed in the emergency department of the Clinical Laboratory of the ICS Camp de Tarragona.

On the other hand, the exclusion criteria were the presence of a history of HF and NT-proBNP values above the upper age-adjusted limit (above 450 pg/mL in patients <50 years, above 900 pg/mL in patients between 50 and 75 years, or above 1800 pg/mL in persons older than 75 years). Non-resident patients were also excluded because they could not be followed up.[8]

In the same way, patients were divided into two groups according to the NT-proBNP quantification result: patients in the so-called Rule out group had values below 300 pg/mL, while patients in the grey zone group had values between 300 pg/mL and the upper age-adjusted limit previously mentioned.

4.2. Quantification of analytical parameters

Hemoglobin

This anemia marker was determined by the Sysmex XN-1000 automated hematology analyzer for hematology. The measurement method is photometric, which uses cyanide-free sodium lauryl sulfate (SLS) as the main reagent. The procedure begins by introducing EDTA whole blood samples into the equipment, using racks from which the equipment aspirates a small aliquot of blood. SLS is added to this aliquot in order to lyse the red blood cells; once the hemoglobin is released, the SLS itself oxidizes the heme group. In this way, the hydrophilic groups of SLS can bind to the modified heme group to form a new compound, SLS-HGB, a chromophore derivative, which allows us to carry out a photometric analysis; specifically, at 555nm, the emission measured by a photosensor is inversely proportional to the hemoglobin concentration of the sample. [17,18,19]

Creatinine

Creatinine determination was performed on an Atellica instrument using the Atellica CH enzymatic assay (Siemens Healthcare Diagnostics, Atellica Creatinine_3 (ECre_3), ref. 11537216).

The enzymatic assay is based on a series of coupled enzymatic reactions: the test starts by breaking down sarcosine and endogenous creatine in the sample via the enzymes creatine kinase and sarcosine oxidase. Next, creatinine is hydrolyzed to cretinine by creatininase and transformed to sarcosine by creatine oxidase. Next, sarcosine is oxidized using sarcosine oxidase producing hydrogen peroxide. Finally, peroxidase produces the oxidative

condensation of 4-aminoantipyrine and N-ethyl-N-(3-methylphenyl)-N'-succinyl-ethylenediamine, generating a reddish-purple pigment, the absorbance of which is measured at 545/694 nm.

This technique can be used with serum, plasma with lithium heparin or plasma with dipotassium EDTA. The system automatically dispenses 50 µL of primary sample and 200 µL of Atellica CH diluent into a dilution cuvette. It then dispenses 85 µL of Reagent 1 (creatinase, sarcosine oxidase, creatininase), 1.5 µL of special reagent water and 8.5 µL of prediluted sample into a reaction cuvette. The absorbance is measured after the sample is added. Next, the analyzer dispenses 28.4 µL of Reagent 2 (peroxidase), mixes and incubates the reaction cuvette at 37°C. Finally, the system measures the absorbance and reports the results. The total duration of the test is 10 minutes.

The measuring range is 0.15 - 30.00 mg/dL (13-2652 µmol/L). The different detection limits are: blank limit: 0.03 mg/dL, limit of detection (LOD9: 0.08 mg/dL and limit of quantification (LOQ): 0.15 mg/dL.

The creatinine determination kit contains everything necessary to carry out the quantification: Atellica CH Diluent (ref. 11099300), Atellica CH Wash (ref. 11099301), Atellica CH Conditioner (ref. 11099302), Atellica CH Cleaner (ref. 11099303), Atellica CH Reagent Probe Cleaner 1 (ref. 11099312), Atellica CH Reagent Probe Cleaner 2 (ref. 11099313) Atellica CH Reagent Probe Cleaner 4 (ref. 11099309), Atellica CH Lamp Coolant (ref. 11099307) and Atellica CH Water Bath Additive (ref. 11099308). [20]

NT-proBNP

NT-proBNP was analyzed on the Atellica kit via chemiluminescent immunoassay technique (Siemens Healthcare Diagnostics, Atellica IM NT-proBNP (PBNP), ref. 11200588 for 100 tests or ref. 11200589 for 500 tests).

The Atellica IM PBNP assay is a quantitative immunological test that operates in a fully automated fashion. It employs streptavidin-coated magnetic particles as a solid phase, together with fixed amounts of two types of antibodies:

1. A biotinylated, anti-human, ovine monoclonal anti-human antibody specific for NT-proBNP (first antibody), which binds to the streptavidin particles.
2. An $F(ab')_2$ fragment of an acridinium ester-labeled ovine monoclonal anti-human antibody specific for PBNP (second antibody).

The quantification kit contains everything needed to perform the determination: Atellica IM PBNP ReadyPack: primary reagent package containing Lite reagent, solid phase, and auxiliary well reagent (ref. 11200588), Atellica IM Multi-Diluent 1 (ref. 10995637), Atellica IM PBNP MCM (ref. 11200590), Atellica IM Wash (ref. 11098501), Atellica IM Acid (ref. 11417929) and Atellica IM Base (ref. 11417930).

It also contains the necessary materials for equipment calibration, such as the Atellica IM PBNP Master Curve and Test Definition (ref. 11200588) and Atellica IM PBNP CAL Low and High Calibrators (ref. 11200588). In addition to the analyzer cleaning solutions: Atellica IM Cleaner (ref. 11098502).

As far as the procedure is concerned, it only requires 20 µL of any of the three sample types: serum, plasma with dipotassium EDTA or plasma with lithium heparin. These 20 µL are automatically dispensed into a well along with 200 µL of the solid phase reagent and 75 µL of the reagent in a cuvette.

That cuvette is incubated, 3 minutes at 37°C, before 5 µL of Lite reagent is dispensed. This is followed by a second incubation, 9 minutes at 37°C, at the end of the incubation the separator aspirates and washes the cuvette with Atellica IM Wash, and then dispenses 300 µL of Atellica IM Acid and 300 µL of Atellica IM Base. Next, the chemiluminescent reaction is started, finally, the results are reported automatically.

The measurement range of the assay is 35 to 35,000 pg/mL (4.13-4130 pmol/L). The limits established by the manufacturer are: limit of blank (LoB): ≤13 pg/mL (2.36 pmol/L), limit of detection (LoD): ≤20 pg/mL (2.36 pmol/L) and limit of quantification (LoQ): ≤18 pg/mL (4.13 pmol/L). In addition, the manufacturer demonstrates that the area under the curve is 0.953 and the overall reproducibility is estimated with the coefficient of variation which in this case is ≤9%.[20]

C-reactive protein

C-reactive protein (CRP) quantifications were performed using the same latex particle-enhanced turbidimetric immunoassay Siemens Healthcare Diagnostics, Atellica CH C-Reactive Protein_2 (CRP_2) (ref. 11097631), on an Atellica analyzer.

The assay requires: Pack 1 (ref. 11097631), containing main reagent solution and buffer solution; Pack 2 (ref. 11097631), containing main reagent, latex with anti-PCR antibody (rabbit) and sodium azide; Atellica CH CRP_2 CAL (ref. 11099430), specific calibrator and additional system fluids: diluent, wash solutions, cleaners, water bath additive, lamp coolant, among others.

The assay starts with the transfer of 50 µL of sample and 200 µL of diluent into a dilution cuvette. Then, 4 µL of pre-diluted sample are introduced into a reaction cuvette together with 40 µL of Reagent 1. Finally 40 µL of Reagent 2 are added, mixed and the cuvette is incubated at 37°C. During such incubation the latex particles coated with anti-PCR antibody rapidly agglutinate in the presence of the analyte, causing an increase in turbidity, which is measured at 571 nm. The intensity of scattered light is proportional to the PCR concentration.

Some relevant data on the assay are: the measurement range: 0.4-30.4 mg/dL (4-304 mg/L), blank and detection limit: 0 mg/L, the limit of quantification: 0.3 mg/dL and the coefficient of variation (CV), which is ≤ 8% for plasma and ≤ 5% for serum.[20]

4.3. Physical exploration

Blood pressure monitoring

As stated at the beginning of this document, hypertension is a known and studied risk factor because it can cause pathophysiological changes such as left ventricular hypertrophy, diastolic dysfunction or myocardial stiffness. These structural alterations trigger or worsen

HF. Specifically, hypertension causes pressure overload, there is concentric structural remodeling, a thickening of the left ventricular wall. [21,22]

There are two main methods of measuring blood pressure, the auscultatory (with phonendoscope and manometer) and the oscillometric (automated with electronic sensors). Although the auscultatory method is more accurate, validated automated devices are reliable and easy to use. In all cases, blood pressure is considered normal when it is less than 120/80 mmHg; if it is higher than 130/80 mmHg repeatedly, it is hypertension.[23]

The auscultatory method consists of inflating a cuff placed on the patient's arm until the brachial artery collapses, which prevents the passage of blood and eliminates any sound. Then the pressure exerted on the artery is decreased and the return of blood flow generates Korotkoff sounds, which are detected to measure blood pressure. In contrast, in the oscillometric method, although pressure is also exerted on the branchial artery and the oscillations of the arterial wall are measured instead of the Korotkoff noises [24].

Pulse Oximetry

Oxygen saturation by pulse oximetry is commonly used in critically ill patients in the emergency department, especially in cases of COPD, asthma or other exacerbations of respiratory disease. In contrast, in our study it is used to assess peripheral arterial saturation (SpO2) and heart rate in patients with dyspnea or other symptoms typically associated with HF. At sea level normal oxygen levels are between 96-100% and hypoxemia is considered with an SpO2<90%. [25,26]

Pulse oximetry is a test where the pulse oximeter measures oxygen levels through light. The device emits two wavelengths, a red wavelength (660nm) that is absorbed by deoxygenated hemoglobin and an infrared wavelength absorbed by oxygenated hemoglobin. Then, the light detector of the equipment measures how much light of each wavelength passes through the tissue. With this, the difference in wavelength absorption and the ratio between the red and infrared light allow the amount of oxygenated hemoglobin to be determined. Although this is a non-invasive, fast and easy-to-use test, it has limitations in case of hypoperfusion as in the case of HF or in cases of severe anemia [27].

In addition, the equipment is capable of measuring heart rate, and the measurement of heart rate at rest is a clear prognostic indicator, an indicator of severity and mortality due to cardiac events, including HF, as has been demonstrated in different studies, such as Aaronson, Poole-Wilson or GESICA. [28]

Under normal conditions heart rate depends on age, but in adults it is estimated that between 60 and 100 beats per minute are normal. Continuously high rates are called tachycardias, while those below normal values are called bradycardias. These alterations are indicators of the severity of the patient and of different cardiovascular diseases, in particular tachycardia has been related to HF as in the study of the Study of Survival in HF in Argentina [28,29].

Chest auscultation

Chest auscultation is a clinical technique that consists of listening to the sounds produced by the heart and lungs using a stethoscope that selectively filters these sounds. With this

method, heart sounds such as murmurs or gallops or respiratory sounds such as wheezing or crackles can be heard.

Auscultation allows the identification of noises caused by the complication of HF. Related to the latter, one of these lung sounds is: crackles, fine bubbling sounds that indicate pulmonary congestion due to fluid accumulation in the alveoli, typical of left HF. Decreased breath sounds are also suggestive of pleural effusion secondary to HF.

There are also heart sounds related to the syndrome that concerns us, such as the third heart sound, S3, which indicates abnormal filling associated with systolic dysfunction, or heart murmurs that may indicate valvular heart disease, which may be causes or consequences of HF.

Although it does not replace complementary studies such as echocardiography or pulse oximetry, it provides immediate clinical clues about the presence and severity of cardiac decompensation. [30,31,32]

Chest X-RAY

Chest radiography is a diagnostic imaging test in which ionizing radiation (X-rays) is used, in small proportion, to obtain images of the inside of the chest. This quick, painless and non-invasive procedure allows observing the state of organs such as the lungs and heart as well as structures such as ribs, airways, blood vessels, among others. [33]

There are several physical signs associated with HF that can be detected through a chest X-ray. For example, cardiomegaly, an increase in the size of the heart with respect to the size of the thoracic cage (ICT), this increase is a clear sign of HF, either aortic, which is usually caused by a ventricular or ventricular only [13].

Radiography not only allows the identification of physical signs associated with the syndrome in question but can also provide valuable information on the stage of the disease, as occurs in the case of congestive HF.[9] The pathology begins in the redistribution phase, where we can observe that the blood vessels of both lobes are equal in number and size, when under normal conditions those of the lower lobes are larger than the vessels of the upper lobes. [13]

The next phase is interstitial edema, characterized by the appearance of Kerley lines. These, which can be classified into different types, occur due to a thickening of the interlobular septa caused by the outflow of fluid into the interlobular interstitium caused in turn by the increase in pressure in the pulmonary capillaries. Finally, there is the alveolar phase, characterized by the appearance of alveolar edema and pleural effusions, caused by the chronification of the outflow of fluid into the interlobular interstitium. [13]

Electrocardiogram

The electrocardiogram (ECG) records the rhythm and electrical activity of the heart. In addition to being noninvasive, it is very sensitive in detecting HF. This method is used to look for irregularities that may cause HF; the normality of an ECG usually rules out HF. On the

other hand, the presence of abnormalities in such test is an indication of the existence of HF, as a cause or as a consequence of it. [34]

An example of those abnormalities detected in the ECG is atrial fibrillation (AF), this is shown in the ECG as the absence of a P wave and irregular RR intervals. Sometimes there is also the presence of F waves, irregular oscillations in configuration, amplitude and frequency.[35]

AF and HF have a bidirectional cause-consequence relationship that aggravates each other. Up to 40% of patients with HF have AF and vice versa, and patients with both pathologies have a higher risk of mortality, hospitalizations and cardiovascular complications compared to those with only one of them. [36]

Another relevant electrocardiographic abnormality is left bundle branch block, an alteration in the electrical conduction of the heart that specifically affects the left ventricle. This cardiac event manifests mainly with a QRS ≥ 120 ms on electrocardiograms.

There are studies that claim that the presence of LBBB in patients with HF is associated with a significant increase in 1-year mortality. [37] In contrast, an analysis of three independent cohorts (EAHFE, RICA and BASEL-V) showed that the prevalence of LBBB in patients with HF was not associated with increased mortality.[38] Therefore, the relationship between HF and left bundle branch block is not yet well defined and requires further study.

Echocardiogram

The echocardiogram is a non-invasive diagnostic test that uses ultrasonic waves to create images of the heart that allows you to know the state of cardiac function and structure in real time. There are different types and equipment of echocardiograms such as transthoracic echocardiogram, the most common, transesophageal, Doppler echocardiogram, 3D or stress echocardiogram. [39]

This method also allows us to measure LVEF, the capacity of the left ventricle to pump blood. This measurement allows us to classify patients with HF into patients with preserved or reduced LVEF and this classification allows us to know the severity of the patient's condition and to guide the treatment and monitoring to follow. [40]

4.3. Statistical analysis

All statistical analyses were carried out using version 13 of the STATA program. First, the normality of the variables was determined using the Shapiro-Wilk test and the Q-Q graph. The t-Student test was used for normally distributed variables and the Mann-Whitney U test was used for non-normally distributed variables. The parameters studied expressed as numbers and percentages were analyzed by means of chi-square tests. On the other hand, those expressed with means and standard deviations were studied thanks to t-Student or Mann-Whitney U test, as explained above.

For the analysis of the criterion of readmission for HF, a competitive risk regression was performed using the Fine and grey subdistribution model, considering death as a competitive event. Variables with a p-value < 0.05 in the univariate analysis were included in the

multivariate models. For the analysis of the combined event of all-cause mortality and hospitalization for HF, Cox proportional hazards regression was used in univariate and multivariate analyses. The validity of the proportional hazards assumption will be verified by evaluating the consistency of the log-log curves and Schoenfeld residuals. Variables with non-normal distribution will be log-transformed before inclusion in the statistical models.

Finally, to determine the diagnostic value of the different clinical models, that is, to determine the area under the curve of the different clinical models, taking or not taking into account the biochemical biomarkers, a Harrell's C analysis was performed.

4.4. Information collection

All data collection was performed using available ED discharge records, electronic medical records, electronic test records and mortality records. Baseline patient characteristics such as age, sex, medication and patient history were collected. The reason for consultation (symptoms or physical signs), laboratory test results NT-proBNP, creatinine, C-reactive protein and hemoglobin, although glomerular filtration was calculated with the CKD-EPI equation) were also compiled. In addition, from the reports of complementary examinations (ECG and chest X-ray) their results were known and recorded.

On the other hand, cardiology department consultations, hospitalizations and under which department the patients were admitted and the emergency department diagnosis were also documented. A three-year follow-up was also carried out to record readmissions for HF or death of the patient.

5. Results

5.1. Clinical profile of the cohort

Although the main characteristic that differentiates patients in the two groups of the cohort is the quantification of Nt-proBNP: Rule out (RO) group, NT-proBNP less than 300 pg/mL; grey zone (GZ) group, NT-proBNP between 300 pg/mL and the upper age-adjusted limit; there may be other differential characteristics between the groups (**Table 1**). For this reason, characteristics from different clinical settings were analyzed by T-test and Chi2: history, symptoms, physical signs, electrocardiographic and analytical parameters, and results of thoracic radiographs.

First of all, the most statistically significant difference between groups is age, it has the lowest p-value, 0.0001, there is a 15-years difference. Differences in the clinical history of the two groups were evaluated, the Rule out group had more smoking at the time of admission than the grey zone group. While the grey zone group had significantly more hypertension, stroke, COPD and atrial fibrillation, which is four times more frequent in the group with higher NT-proBNP quantification. Other clinical antecedents such as diabetes, dyslipidemia, myocardial infarction or peripheral vascular disease did not show significant differences.

Continuing with the time of admission to the emergency department, there was a significant difference in the number of patients taking ARA II, Angiotensin II Receptor Antagonists, 10% more grey zone patients were medicated with this treatment. Secondly, variations in symptoms and physical signs between the groups were analyzed. Rule out patients had significantly more orthopnea and chest pain, a difference of 5% in the presence of orthopnea and 10% in the case of chest pain. Studies were also carried out on the signs studied through physical examinations where higher blood pressure, presence of crackles and edema were observed in the grey zone group. The presence of edema stands out, since it is twice as high in GZ than in RO.

Thirdly, variations in the different types of parameters were examined. In the case of electrocardiographic parameters, the results showed that patients in the GZ group had four times more atrial fibrillation. In contrast, no significant evidence was found in the presence of the left bundle branch block. The analytical parameters, all those taken into account in this study; hemoglobin, creatinine/glomerular filtration, NT-proBNP and C-reactive protein; show relevant differences. While filtration is much lower in GZ than in RO, the subtraction of parameters is the reverse. We highlight NT-proBNP which is practically six times higher in GZ and CPR is almost twice as high as in RO.

Finally, a study was made of the findings in the chest X-rays of the patients, through which we can affirm that patients in the GZ group suffer substantially more interstitial edema, pleural effusions, cardiomegaly and pneumonia. Above all, cardiomegaly stands out, which is more than three times more frequent in GZ than in RO.

Tabla 1. Comparison of profiles of the court groups

Variable	Rule out (%)	grey zone (%)	P-value
Male sex	121 (53.5)	126 (55.2)	0.712
Age (years)	64.89 ± 14.6	79.87 ± 9.8	**0.0001** *
Clinical history			
Active smoking	36 (15.9)	22 (9.6)	**0.045** *
High blood pressure	130 (57.5)	165 (72.4)	**0.001** *
Diabetes mellitus	58 (25.6)	76 (29.5)	0.073
Dyslipidemia	94 (41.6)	114 (50.0)	0.072
COPD	36 (15.9)	58 (25.4)	**0.012***
Myocardial infarction	22 (9.7)	31(13.6)	0.2
Stroke/TIA	10 (4.4)	25 (11.0)	**0.009***
Peripheral vascular disease	24 (10.6)	16 (7.0)	0.176
Atrial fibrillation	9 (4.0)	47 (20.6)	**0.001***
Previous treatment			
ACE inhibitors	66 (29.2)	57 (25.0)	0.314
ARBs	30 (13.3)	53 (23.2)	0**.006***
Beta blockers	52 (23.0)	66 (28.9)	0.149
Aldosterone antagonist	3 (1.3)	5 (2.2)	0.483
Diuretic	65 (28.7)	84 (36.8)	0.067
Symptoms			
Progressive dyspnea	106 (46.9)	111 (48.7)	0.704
Paroxysmal nocturnal dyspnea	4 (1.8)	5 (2.2)	0.746
Orthopnea	20 (8.8)	7 (3.1)	**0.009***
Lower limb edema	31 (13.7)	44 (19.3)	0.109
Cough	31 (13.7)	42 (18.4)	0.172
Fever	29 (12.8)	31 (13.6)	0.810
Chest pain	73 (32.3)	49 (21.5)	**0.009***
Other symptoms	86 (38.0)	98 (43.0)	0.285
Physical examination			
Heart rate, bpm	83.9 ± 20.5	85.1 ± 21.2	0.539
Systolic blood pressure, mmHg	133.8 ± 21.4	140.0 ± 26.9	**0.013***
Oxygen saturation, %	96.2 ± 5.0	95.4 ± 4.8	0.106
Crackles	57 (25.2)	91 (39.9)	**0.001***
Edemas	33 (14.6)	64 (28.1)	**0.001***
Wheezing	34 (15.0)	37 (16.2)	0.728
Murmur	5 (2.2)	10 (4.4)	0.195
Jugular venous distention	0 (0.0)	2 (0.9)	0.158
Electrocardiogram			
Atrial fibrillation	3 (2.2)	37 (21.9)	**0.001***
LBBB	5 (3.7)	8 (4.7)	0.669
Analytical parameters			
Hemoglobin, g/dL	13.4 ± 1.8	12.49 ± 1.9	**0.0001***
eGFR, mL/min/1.73m2	91.4 ± 36.4	78.0± 34.3	**0.0001***
NT-proBNP, pg/mL	112.5 (55-195)	624 (440-1057)	**0.0001***
CRP	1.1 (0.4-3.7)	1.95 (0.4-6.8)	**0.0002***
Chest X-ray			
Performed	195 (86.3)	203 (89.0)	0.373
Interstitial edema	12 (5.3)	57 (28.1)	**0.0001***
Pleural effusion	6 (2.6)	17 (8.4)	**0.009***
Cardiomegaly	14 (6.2)	48 (23.6)	**0.001***
Pneumonia	3 (1.3)	16 (7.9)	**0.001***

ACE: angiotensin-Converting Enzyme; **ARB:** Angiotensin II Receptor Blocker; **COPD:** Chronic Obstructive Pulmonary Disease; **CRP**: C-reactive protein; **eGRF**: estimated Glomerular Filtration Rate; **LBBB**: Left Bundle Branch Block; **TIA:** Transient Ischemic Attack; *Significant difference (p<0.05).

5.2. Diagnosis upon discharge

In order to obtain more information and accurately determine the patient's condition, the health care staff carried out a series of tests and consultations (**Table 2**). During the statistical study, Chi2, the performance of echocardiograms and consultation with the cardiology service were especially considered, and no significant differences were found between the two groups in these aspects.

The severity of the clinical condition and the individual needs of each patient were also considered evaluating the need for hospital admission. In this regard, in the statistical analyses, a notable difference was observed between the groups: approximately half of the patients in the GZ group required hospitalization (42.5%), twice as many as in the RO group. Likewise, the hospital service to which they were admitted was recorded, paying special attention to the cardiology service, where no relevant differences were found between the two groups.

After evaluating the symptoms, physical signs and results of various cardiac tests, radiographs and clinical analyses, the healthcare personnel determined the pathology presented by each patient (**Table 3**). Among the diseases analyzed, significant differences were observed in the prevalence of HF, respiratory infection or COPD exacerbation, myocarditis and other conditions. In the GZ group, patients presented a sevenfold higher frequency of HF, as well as a higher incidence of respiratory infections or COPD exacerbations, compared to the RO group. In contrast, more cases of myocarditis and other pathologies were identified in the RO group.

Tabla 2. Hospital admission statistics

Variable	Rule out	grey zone	P-value
Assessment by cardiology	42 (18.6)	34 (14.9)	0.295
Echocardiogram Performed	28 (12.4)	20 (8.7)	0.210
Hospital Admission	53 (23.4)	97 (42.5)	**0.0001***
Admission Service:			
Cardiology	8 (15.1)	11 (11.3)	0.141
Internal Medicine	26 (49.0)	44 (45.3)	
Pulmonology	11 (20.7)	12 (12.4)	
Nephrology	0 (0.0)	2 (2.0)	
ICU (Intensive Care Unit)	2 (3.7)	1 (1.0)	
Gastroenterology	1 (1.9)	1 (1.0)	
Other Service	5 (9.4)	26 (26.8)	

Two additional columns have been established to identify statistically significant differences between patients who had a Rule out NT-proBNP quantification and who had a grey zone quantification.Results are displayed as “mean (percentage of the group)”

Tabla 3. Primary diagnosis at ED discharge

Diagnosis	Rule out	grey zone	P-value
Heart Failure	5 (2.2)	32 (14.0)	**0.0001***
Respiratory Infection/Exacerbated COPD	57 (25.2)	88 (38.6)	**0.002***
Pulmonary Thromboembolism	14 (6.2)	12 (5.2)	0.669
Tachyarrhythmia	4 (1.7)	10 (4.4)	0.107
Acute Coronary Syndrome	4 (1.7)	8 (3.5)	0.248
Hypertensive Crisis	1 (0.4)	2 (0.9)	0.568
Myocarditis	7 (3.1)	0 (0.0)	**0.007***
Stroke	2 (0.9)	0 (0.0)	0.155
Other	117 (51.7)	92 (40.3)	**0.015***

Results are displayed as “number of patients diagnosed with this condition (percentage of the cohort)”.

5.3. Healthcare Events During the Monitoring Period

As part of the study, patients were followed up clinically, during which various events were recorded: readmission for HF, death during hospitalization, total mortality, and a composite event including both death and readmission for HF (**Table 4**). These events were examined by Chi2 analysis. Significant differences were observed between the two groups, with the GZ group presenting higher readmission and mortality rates compared to the RO group, 14 and 2. Among the events analyzed, readmission for HF was especially noteworthy, which was eleven times more frequent in the GZ group.

As can be seen in **Table 4** and **Figure 4**, the composite event during the three years of follow-up, the RO group presented 7.5% of patients who were readmitted or died, while in the affected group 50% of individuals suffered the event under study.

Tabla 4. Clinical findings during patient follow-up

Clinical event	Rule out	grey zone	P-value
In-hospital death	8 (3.5)	32 (14.0)	**0.0001***
Readmission for HF	5 (2.2)	55 (24.1)	**0.0001***
Total mortality	12 (5.3)	83 (36.4)	**0.0001***
Composite event (Death + Readmission for HF)	17 (7.5)	114 (50.0)	**0.0001***

HF: Heart Failure. Results are displayed as “number of patients diagnosed with this condition (percentage of the cohort)”. *Significant difference ($p<0.05$).

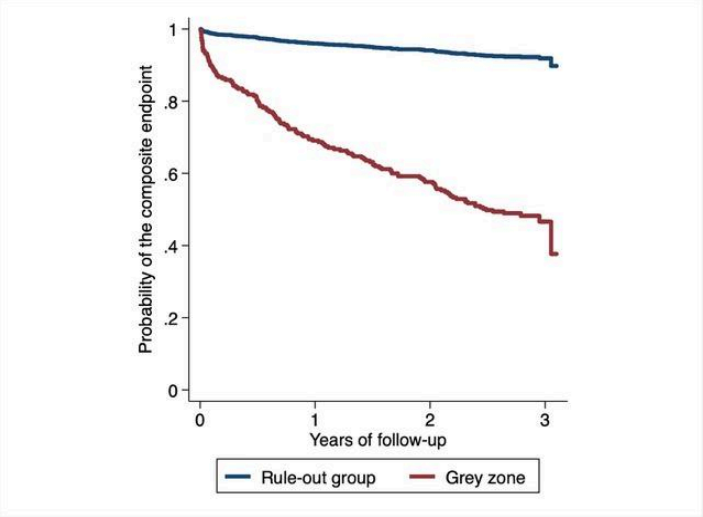

Figure 4. Survival rate Grey zone vs Rule out during the follow-up.

5.3.1. Heart failure-related readmission throughout follow-up

Within hospital readmission, we analyzed the variables collected at the time of first admission (**Table 1**) that were related to the occurrence of the event (**Table 5**). Two types of analysis were performed with these data: a univariate and a multivariate analysis using the Fine and Grey risk subdistribution model.

On the one hand, as a result of the study of independent variables, different hazards ratios with statistical significance were identified. One of the relevant variables is age (HR: 1.02); on the other hand, the sex of the patient does not show a significant association. As for clinical history, having had diabetes increases the probability of readmission for HF by 1.7 times. Another important factor is hypertension, which more than doubles (HR: 2.71) the risk of the event occurring. Likewise, having suffered a myocardial infarction almost doubles (HR: 1.94) the probability of a new hospitalization.

Next, we studied how the presence of certain symptoms influences the appearance of HF during follow-up. The symptoms that were significant, $p<0.05$, were dyspnea and orthopnea, dyspnea more than doubled the probability of readmission (HR: 2.71) but orthopnea more than doubled the probability of readmission (HR: 6.76). Then, physical signs were analyzed, while the appearance of pulmonary crackles (HR: 1.97) or the presence of edema (HR: 1.77) increased the risk of reentry significantly.

Also, data from the tests performed were analyzed, in the case of ECG's neither in atrial fibrillation nor left bundle branch block are unremarkable. On the other hand, in the analysis of biochemical parameters, there were relevant statistical changes in both NT-proBNP (HR: 2.35) and CRP (HR: 0.8); in the case of natriuretic peptide, the GZ group had a higher quantification of this biomarker and twice the probability of readmission to the hospital for HF. In contrast, in CRP, the higher the quantification of this biomarker, the lower the risk of readmission, decreasing by up to 20%.

On the other hand, a multivariate determination was made of the variants that gave relevant values in the univariate study. One variant that was significant in the univariate study but not

in the multivariate study was age; while in the study of independent variables it had a p value of 0.03, in the multivariate study the significance was 0.581. With respect to history, the variables that stood out in the previous study were HT and myocardial infarction; in this data evaluation only diabetes and infarction maintain their significance. Diabetes maintains the hazard ratio while myocardial infarction slightly increases it (HR:2.39), and the same occurs in the symptoms with statistically significant results, specifically dyspnea and orthopnea. However, in the multivariate study of physical signs no significant variable was found, while the analytical parameters studied, NT-proBNP and CRP, were significant and maintained the hazard ratio practically unchanged.

In order to represent the results more visually, we created graphs showing the hazard ratio of the significant biomarkers, NT-proBNP and CRP. The graphs show how the probability of being hospitalized for HF varies during follow-up. In **Figure 5**, we observed that as natriuretic peptide increased, always remaining in the grey Zone, the probability of readmission increased. In contrast, in the case of CRP, the opposite is true: the higher the quantification of the biomarker in the emergency department, the lower the risk of readmission (**Figure 6**).

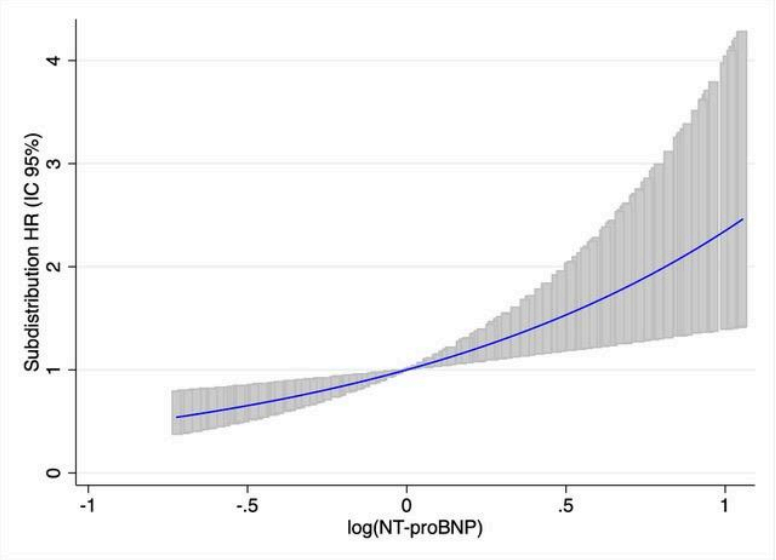

Figure 5. Hazard ratio of NT-proBNP for readmission due to HF during follow-up

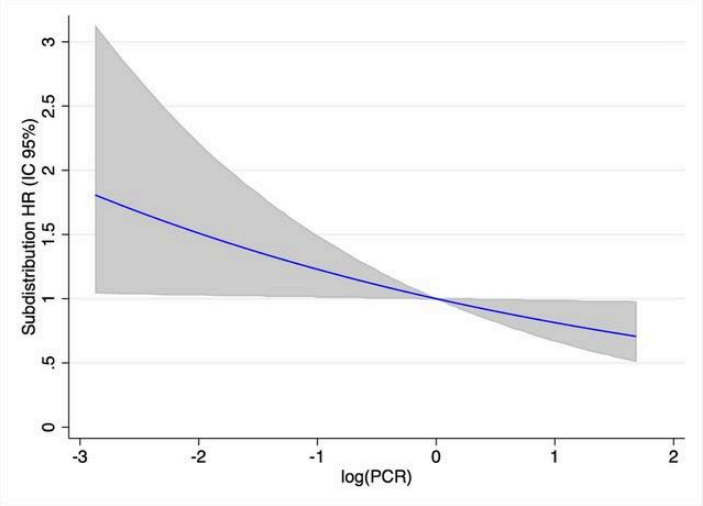

Figure 6. Hazard ratio of PCR for readmission due to HF during follow-up.

Tabla 5 Factors associated with readmission for HF during follow-up

	UNIVARIABLE		MULTIVARIABLE	
Variable	**HR (CI 95%)**	**P-value**	**HR (CI 95%)**	**P-value**
Age, years	1.02 (1.0 - 1.05)	**0.03***	1.01 (0.97 - 1.05)	0.581
Male sex	1.11 (0.65 - 1.89)	0.701		
Clinical history				
Active smoking	0.49 (0.16 - 1.5)	0.209		
Diabetes	1.7 (1.0 - 2.89)	**0.048***	1.77 (0.92 - 3.42)	0.088
High blood pressure	2.71 (1.21 - 6.05)	**0.015***	1.38 (0.57 - 3.33)	0.468
Dyslipidemia	1.20 (0.71 - 2.03)	0.499		
COPD	1.52 (0.88 - 2.64)	0.135		
Myocardial infarction	1.94 (1.0 - 3.73)	**0.047***	2.39 (1.25 - 4.55)	**0.008***
Peripheral vascular disease	0.69 (0.22 - 2.20)	0.535		
Atrial fibrillation	1.62 (0.91 - 2.88)	0.102		
Symptoms				
Progressive dyspnea	2.71 (1.53 - 4.78)	**0.0006***	2.46 (1.23 - 4.92)	**0.011***
Paroxysmal nocturnal dyspnea	3.33 (0.50 - 22.0)	0.211		
Orthopnea	6.76 (1.95 - 23.4)	**0.0026***	5.67 (1.51 - 21.28)	**0.01***
Lower limb edema	0.99 (0.51 - 1.93)	0.982		
Cough	0.49 (0.21 - 1.13)	0.094		
Fever	0.91 (0.42 - 1.96)	0.806		
Chest pain	1.26 (0.69 - 2.29)	0.444		
Other symptoms	0.62 (0.35 - 1.08)	0.093		
Physical Examination				
Heart rate, bpm	1.0 (0.99 - 1.02)	0.318		
Systolic blood pressure, mmHg	1.0 (0.99 - 1.01)	0.359		
Oxygen saturation, %	1.02 (0.97 - 1.08)	0.354		
Crackles	1.97 (1.16 - 3.36)	**0.012***	1.65 (0.91 - 2.99)	0.096
Edema	1.77 (1.03 - 3.05)	**0.038***	1.38 (0.73 - 2.61)	0.323
Wheezing	0.88 (0.42 - 1.83)	0.733		
Murmur	0.88 (0.20-3.84)	0.864		
Jugular venous distention	0.0	**0.0001***		
Electrocardiogram				
Atrial fibrillation	1.74 (0.9 - 3.36)	0.1		
LBBB	1.17 (0.26 - 5.19)	0.83		
Analytical Parameters				
Hemoglobin, g/dL	0.91 (0.81 - 1.03)	0.141		
eGFR, mL/min/1.73m2	0.99 (0.98- 1.0)	0.132		
NT-ProBNP, pg/mL	2.35 (1.39 - 3.97)	**0.001***	2.29 (1.26 - 4.18)	**0.007***
CRP (n=213)	0.80 (0.65 - 0.98)	**0.035***	0.81 (0.65 - 1.0)	**0.05***

ACE: Angiotensin-Converting Enzyme; **ARB**: Angiotensin II Receptor Blocker; **CI 95%**: Confidence interval of the 95%; **COPD:** Chronic Obstructive Pulmonary Disease; **CRP**: C-reactive protein; **eGRF**: estimated Glomerular

Filtration Rate; **LBBB**: Left Bundle Branch Block; **TIA**: Transient Ischemic Attack; **HR:** Hazard ratio. *Significant difference ($p<0.05$). Results are displayed as “Hazard ratio (95% Confidence interval)”. The statically significant variables identified in the univariate analysis were further examined in a multivariate analysis, in which only the most relevant variables are selected. As a result, these result variables are not marked with an asterisk, as this study includes only those with a previous significant p-value.

5.3.2. Determinants of the combined event: Mortality and HF Readmission

Composite events are frequently used as a prognostic indicator in clinical studies. In the present study, the composite event consists of hospital readmission due to HF or patient death (**Table 6**). A Cox proportional hazards model was carried out on this event, which included a univariate study, evaluating whether a single variant is associated with the event, and a multivariate study, where several parameters are evaluated at the same time, which allows us to determine the independent and significant effect of these indicators.

First of all, and as the most relevant factor in both the univariate and multivariate studies, is age. Although the hazard ratio is not very exaggerated, 1.05, i.e. for each year the possibility of the study event occurring increases by 5%, the significance of this parameter is of great importance, 0.00001 in the univariate study and 0.0001 in the multivariate study. On the other hand, neither the patient's clinical history nor his or her sex presented a significant p-value.

On the other hand, the only symptom with statistical value is dyspnea (HR:1.71), which, if a GZ patient had dyspnea at the time of admission to the emergency room, increased the probability of that patient being readmitted for HF or dying by 70%. Furthermore, in the multivariate study, it maintained significance and only slightly decreased the HR. Next, physical signs were evaluated, which did not show any statistical significance, as did electrocardiographic findings.

On the contrary, within the analytical parameters, hemoglobin seems to reflect a trend in the multivariate study where it shows that as its quantification increases, the risk of occurrence of the event studied decreases. NT-proBNP is relevant in a different way, its importance resides in the univariate study, in which it is shown that increasing the quantification of natriuretic peptide increases the possibility of readmission or death. Finally, treatment at patient discharge is relevant if the patient is on diuretics, which are associated with a 70% increase in the likelihood of the composite event occurring.

Tabla 6. Variables associated with the composite event Death + HF Readmission

	UNIVARIABLE		MULTIVARIABLE	
Variable	**HR (CI 95%)**	**P-value**	**HR (CI 95%)**	**P-value**
Age, years	1.05 (1.03 -1.07)	**0.00001***	1.04 (1.02 - 1.07)	**0.0001***
Male sex	0.79 (0.55 -1.15)	0.228		
Clinical history				
Active smoking	0.49 (0.23 - 1.06)	0.072		
Diabetes	1.03 (0.70 - 1.51)	0.878		
High blood pressure	0.91 (0.60 - 1.38)	0.668		
Dyslipidemia	1.18 (0.82 - 1.71)	0.374		
COPD	1.20 (0.79 - 1.82)	0.381		
Myocardial infarction	0.81 (0.46 - 1.41)	0.441		
Stroke/TIA	0.93 (0.52 - 1.65)	0.800		
Peripheral vascular disease	1.34 (0.70 - 2.56)	0.399		
Atrial fibrillation	0.88 (0.56 - 1.39)	0.584		
Symptoms				
Progressive dyspnea	1.71 (1.17 - 2.48)	**0.004***	1.59 (1.09 - 2.32)	**0.015***
Paroxysmal nocturnal dyspnea	1.30 (0.32 - 5.29)	0.719		
Orthopnea	2.40 (0.98 - 5.91)	0.091		
Lower limb edema	1.13 (0.72 - 1.76)	0.594	1.26 (0.85 - 1.87)	0.246
Cough	0.67 (0.40 - 1.13)	0.119		
Fever	1.32 (0.80 - 2.19)	0.287		
Chest pain	0.66 (0.41 - 1.07)	0.081		
Physical Examination				
Crackles	1.26 (0.87 - 1.82)	0.227		
Edema	1.54 (1.04 - 2.27)	0.033		
Wheezing	0.76 (0.44 - 1.28)	0.287		
Murmur	1.06 (0.43 - 2.60)	0.898		
Jugular venous distention	1.16 (0.16 - 8.31)	0.886		
Electrocardiogram				
Atrial fibrillation	1.25 (0.75 - 2.08)	0.394		
LBBB	0.92 (0.29 - 2.92)	0.889		
Analytical Parameters				
Hemoglobin, g/dL	0.87 (0.80 - 0.96)	0.005	0.91 (0.82 - 1.0)	**0.058***
eGFR, mL/min/1.73m2	1.0 (0.99 - 1.0)	0.884		
NT-ProBNP, pg/mL	1.7 (1.18 - 2.45)	**0.004***	1.21 (0.83 - 1.75)	0.311
CRP (n=213)	1.05 (0.92 - 1.19)	0.444		
Hospital Admission	0.90 (0.62 - 1.32)	0.613		
Discharge Treatment				
ACE inhibitors	0.69 (0.42 - 1.11)	0.116		
ARBs	0.97 (0.62 - 1.51)	0.895		
Beta blockers	0.79 (0.52 - 1.21)	0.283		
Aldosterone antagonist	1.73 (0.71 - 4.26)	0.266		
Diuretic	1.70 (1.17 - 2.46)	**0.006***		

CRP: C-reactive protein; **COPD:** Chronic Obstructive Pulmonary Disease; **eGRF**: estimated Glomerular Filtration Rate; **HR**: Hazard ratio; **TIA:** Transient Ischemic Attack. *Significant difference (p<0.05). Results are displayed as "Hazard ratio (95% Confidence interval)". The statically significant variables identified in the univariate analysis were further examined in a multivariate analysis, in which only the most relevant variables are selected.

5.4. Harrell's C Study

In order to determine the impact of adding the two biochemical biomarkers to a model with clinical variables in the estimation of the risk of readmission for HF, an estimation of Harrell's C statistic was performed. Specifically, three of these analyses were performed: one with the simplified clinical model, taking into account only the medical history and physical examination (**Table 7**); one with the previous clinical model and taking into account the CRP (**Table 8**); and one like the previous one but also adding the NT-proBNP (**Table 9**).

In the first clinical model (**Table 7**) , background (age, HT, diabetes and myocardial infarction), symptoms (dyspnea and orthopnea) and physical signs (crackles and edema) were analyzed. Of all these variants, the only ones that gave a relevant p-value were: diabetes, myocardial infarction and progressive dyspnea. Next, in the second clinical model and CRP (**Table 8**), the above variables were also relevant but in addition both orthopnea and CRP presented a significant p-value. Finally, in the third clinical model, with CRP and NT-proBNP (**Table 9**), the same results as in the previous model were repeated, adding to the significant results the logarithm of NT-proBNP. In Table 10, there is a notable progressive increase in the C statistic when adding CRP or NT-proBNP to the clinical model, with a progressive increase from 0.727 to 0.769.

Table 7. Harrell's C study clinical model

Variable	HR (CI 95%)	P-value
Age	1.02 (0.99 - 1.05)	0.215
High pressure blood	2.01 (0.88 - 4.60)	0.098
Diabetes	1.92 (1.12 - 3.31)	**0.018***
Myocardial infarction	2.22 (1.14 - 4.34)	**0.019***
Progressive disnea	2.57 (1.39 - 4.74)	**0.002***
Orthopnea	2.78 (0.78 - 9.93)	0.115
Crackles	1.50 (0.83 - 2.71)	0.171
Edema	1.33 (0.71 - 2.48)	0.365

*Significant difference (p<0.05). Results are displayed as "Hazard ratio (95% Confidence interval)".

Table 8. Harrell's C study clinical model + PCR

Variable	HR (CI 95%)	P-value
Age	1.02 (0.99 - 1.06)	0.159
High pressure blood	1.51 (0.64 - 3.57)	0.349
Diabetes	1.88 (0.99 - 3.55)	**0.051***
Myocardial infarction	2.27 (1.13 - 4.56)	**0.021***
Progressive disnea	2.52 (1.28 - 4.95)	**0.007***
Orthopnea	6.07 (1.78 - 20.75)	**0.004***
Crackles	1.54 (0.84 - 2.82)	0.160
Edema	1.35 (0.71 - 2.57)	0.361
log PCR	0.80 (0.64 - 1.01)	**0.061***

*Significant difference (p<0.05). Results are displayed as “Hazard ratio (95% Confidence interval)”.

Table 9. Harrell's C study clinical model + PCR + NT-proBNP

Variable	HR (CI 95%)	P-value
Age	1.01 (0.97 - 1.05)	0.581
High pressure blood	1.38 (0.57 - 3.33)	0.468
Diabetes	1.77 (0.92 - 3.41)	0.088
Myocardial infarction	2.39 (1.25 - 4.55)	**0.008***
Progressive disnea	2.46 (1.23 - 4.92)	**0.011***
Orthopnea	5.67 (1.51 - 21.28)	**0.010***
Crackles	1.65 (0.91 - 2.99)	0.096
Edema	1.38 (0.73 - 2.61)	0.323
log PCR	0.81 (0.65 - 1.0)	**0.057***
log BNP	2.29 (1.26 - 4.18)	**0.007***

*Significant difference (p<0.05). Results are displayed as “Hazard ratio (95% Confidence interval)”.

Table 10. Harrell’s C study comparative

Model type	Coefficient (CI 95%)	P-value
Clinical model	0.727 (0.65 - 0.80)	**0.0001***
Classic clinical model + PCR	0.738 (0.66 - 0.81)	**0.0001***
Classic clinical model + PCR + NT-proBNP	0.769 (0.7 - 0.83)	**0.0001***

*Significant difference (p<0.05). Results are displayed as “Hazard ratio (95% Confidence interval)”.

6. Discussion

6.1. Clinical profile of the Grey Zone

Demographic data and medical history

The first characteristic feature of the GZ group is the age of the patients (**Table 1**), which is significantly older than the RO group. The fact that patients with NT-proBNP are older may be due to two reasons: cardiac deterioration caused by HF or due to age itself [41].

Aging leads to a number of pathophysiological processes that enhance cardiac deterioration. As the body ages, telomeres are shortened and damaged and epigenetic modifications increase; these, among many other causes, lead to cellular senescence, mitochondrial dysfunction and cell death, also affecting the heart and leading to cardiac deterioration. [41,42]

On the one hand, normal wear and tear of the heart can increase NT-proBNP levels and for this reason, in order to make a correct interpretation of this biomarker, the critical values are adjusted with age. But on the other hand, NT-proBNP is a compensatory mechanism that is activated when the patient suffers HF. But how the medical team can differentiate whether the small increases, the so-called grey zone, are age-related or an onset of HF. This is the main objective of this study [43].

There are studies comparing patients in GZ who have HF with others in the GZ who do not have HF that also obtain as a result that people with HF and GZ are the oldest people, around 80 years of age. [41] So we can affirm that patients with GZ the older they are the more likely that the ailment they have is HF.

Another significant feature of the GZ group, these patients frequently present HT, stroke, atrial fibrillation and COPD in their clinical history. These results are quite consistent with current knowledge of the syndrome, HT is a clear precipitating factor of HF, AF is a clear comorbidity of HF, and both stroke and COPD are mentioned as risk factors.[1,3,7,8]

As a consequence of the fact that there are more patients with HT in the GZ, angiotensin receptor blockers are more frequent in the GZ than in the RO. It is also surprising that the same does not happen with ACE inhibitors; there is no study that relates treatment with ARBs to a higher risk of HF [44].

On the other hand, the RO group presents more smoking, which could be because although the Rule Out included patients suffering from many different pathologies, most patients were diagnosed with respiratory infections or COPD, of which smoking is a major risk factor. [45]

Physical signs and symptoms

The symptomatic differences between groups are much more complex, although orthopnea and chest pain are more significant in the RO group. The complexity of the symptoms is that

those that appear frequently in cases of HF are not specific to this syndrome, such as dyspnea, orthopnea or edema. Dyspnea is the main symptom of HF but dyspnea can have cardiac origin (HF, pulmonary hypertension, valvular heart disease, arrhythmias,etc.), respiratory origin (COPD, asthma, infections), metabolic origin (anemia, metabolic acidosis, hyperthyroidism) among others [46]. Something similar occurs with orthopnea, on the other hand, edema can have various origins such as HF, renal failure, liver problems [47]. On the other hand, the more specific symptoms of HF such as paroxysmal nocturnal dyspnea and jugular venous distension are less frequent, as can be seen in **Table 1**. Finally, the mean blood pressure of the GZ group is higher than that of RO, this is also due to the greater presence of HT in GZ.

Analytical parameters and additional tests

In turn, all the biochemical parameters studied present significant statistical p-values. The mean hemoglobin of GZ patients is lower than that of RO patients, despite this, both groups are within normality, taking into account that the lower limit of normal hemoglobin is 13.8 in men and 12.1 in women. [48] Likewise, the glomerular filtration rate, calculated thanks to the determination of creatinine. The normal rate in adults is 90-120 mL/min/1.73 m2, but it decreases with age, and the critical values are less than 60 mL/min/1.73 m2. [49] Therefore, the values shown in **Table 1** are within the normal range considering the situation of the cohort.

Continuing with the biochemical parameters, NT-proBNP obviously presents significant differences because it is the basis of the distinction between the two groups GZ and RO. In contrast, the CRP results of the two groups are within physiological values. [50] Despite this, the GZ group practically doubles the mean value of the RO group.

The medical team also performed ECG and chest X-rays when deemed appropriate. The results of these tests follow the trend of the rest. In the ECG the AF findings are considerable, GZ patients show more AF than RO patients; as mentioned above this could be related to the fact that AF is a risk factor for HF. All the pathological thoracic findings studied present significant statistical values.

The GZ group presents more cardiomegaly, interstitial edema, pleural effusion and pneumonia. Cardiomegaly and interstitial edema are closely related to HF; cardiomegaly may be a risk factor for HF if congenital or may be a consequence of HF. Interstitial edema is part of CHF, it is part of the redistribution phase of this syndrome. Pleural effusion has more to do with the alveolar phase of CHF. [13] But both interstitial edema and pleural effusion can occur as complications of exacerbations of COPD or acute respiratory infections, as can pneumonia. [52,53]

Hospital Admission and Primary diagnosis

Table 2 shows that only 15% of patients in the GZ group were evaluated by cardiology, despite the fact that NT-proBNP quantification does not rule out HF. It should be studied whether more cardiology consultations would increase the rate of diagnosis of HF, since the patients classified as other diagnoses in **Table 3** include inconclusive or imprecise diagnoses.

However, hospital admission (**Table 2**) does show a relevant statistical variation; practically half of the people in the GZ group were hospitalized. But there are no notable distinctions in the area of hospitalization, although the area that received the most patients is Internal Medicine, which usually attends patients with different types of pathologies including cardiological, respiratory, metabolic, infectious,etc. Many patients in the cohort were also hospitalized in Cardiology and Pneumology. This coupled with the fact that the majority of patients in the GZ group, excluding the “other diagnosis” classification, were diagnosed with HF or COPD/respiratory infections.

In conclusion, the GZ group presents certain risk factors, symptoms, signs, findings in thoracic radiographs that are consistent with HF. There are other studies that have obtained the same results as this project [54], but in order to know the real statistical value of the similarities with HF, the study should be extended to include a group with NT-proBNP indicating HF. In this way, it could be determined whether the GZ group is more similar to the RO group or to the Rule in group.

On the other hand, the features that can confirm the diagnosis of HF are also consistent with a diagnosis of respiratory infections/COPD, and for this reason it would be very beneficial for the scientific and health community, and more importantly for patients, to find a biomarker that allows us to discern in this situation whether the patient is experiencing a respiratory pathology or HF.

6.2. Events during monitoring

During the beginning of the millennium some studies were done but during the last 20 years it has been very limited [41,54,55,56]. Specifically, the research by van Kimmenade RR states that people with HF and elevated NT-proBNP have a high mortality rate, while patients with RO have a much lower rate. On the other hand, people with NT-proBNP in grey zone have a higher mortality rate than RO but lower than people with HF.[41]

This study confirms these facts, **Table 4** shows that during the three-year monitoring of the patients in the cohort, GZ patients show higher HF readmission, hospital mortality, total mortality and composite event mortality (HF readmission or mortality). As we can see in **Figure X** 50% of the GZ patients were readmitted to the ED for HF or died during the three years of follow-up. In contrast, only 7% of those in the RO group suffered the composite event.

Due to the proven mortality and readmission rates due to HF, as shown by several studies, including this one, it is mandatory to carry out projects to improve diagnosis, in order to improve the situation of these patients. There is a lack of study of which factors are associated with a higher risk of readmission or mortality. In this way, it would be possible to carry out a conscientious follow-up of patients at higher risk and would allow the medical team to detect exacerbations early and protect patients from these events through preventive treatments [57].

6.3. HF readmission indicators

Univariate and multivariate Cox analyses were performed to determine the probability of readmission of patients with NT-proBNP in the grey zone at their first visit to the emergency department (**Table 5**). Another study analyzed the same and only found that women with AF had a higher risk of readmission [58]. In contrast, this study did obtain remarkable results.

Factors with some association with readmission

There are a series of results that have statistically relevant p-values in the study of independent variables, but lose significance in the multivariate study. This is the case of age, diabetes, hypertension, crackles, edema at the time of first admission to the emergency department. The fact that it is relevant in one study and in the multivariate study does not mean that these variables may have a certain correlation with readmission for HF, but this is not independent.

As already mentioned, age is a risk factor due to the cardiac deterioration inherent to the physiological decline of the organism over the years. Likewise, HT is a chronic precipitating event of HF because it creates pressure overload and antihypertensive drugs have been shown to prevent the development or worsening of HF [41]. Diabetes mellitus has comorbidity with HF; people with diabetes have 2 to 4 times more HF than the control population according to the American Diabetes Association report [59]. This is because uncontrolled diabetes leads to diabetic heart disease which involves insulin resistance leading to metabolic alterations cardiomyopathies, oxidative and inflammatory processes; diabetic cardiomyopathy where lipids accumulate in the myocardium producing lipotoxicity; and microvascular dysfunction affecting the coronary microcirculation [60].

Patients presenting with crackles or edema at the time of first admission to the emergency department have also been shown to be associated with readmission for HF, since these symptoms are clearly associated with HF but can be confused with other conditions such as renal failure in the case of edema and respiratory pathologies in the case of crackles. They may indicate that presenting congestion on admission to the emergency department may be an indicator that the patient will suffer HF in the near future [61]. Finally, jugular venous distension in the univariate study was significant, but considering the small population presenting this condition, 2 patients, we did not take this result into account.

Factors independently associated with HF readmission

Other factors show p-values < 0.05 in both analyses, such as having suffered a myocardial infarction, dyspnea, orthopnea, NT-proBNP and CRP. That a variable shows statistical value in the two analyses demonstrates that the factor is independently correlated with the study event.

Firstly, the fact that the patient has had a myocardial infarction increases the risk of readmission by 140%; in infarction, cardiomyocyte death occurs, causing systolic dysfunction [1,3]. Secondly, presenting dyspnea or orthopnea, these two symptoms that are clearly of HF syndrome, but are not specific to it, are also symptoms of respiratory pathologies [62]. This can lead to confusion in the first admission, and no signs of HF are seen, and after a while the patient is readmitted to the hospital. If the patient exhibits

dyspnea this increases the probability of the event twofold, but orthopnea increases it up to six times.

NT-proBNP has a hazard ratio of 2.29, that is, it doubles the risk of hospitalization for each unit of concentration, all within the grey zone adjusted for age. **Figure 5** shows that as NT-proBNP increases, within the grey zone, the risk of readmission increases. Although the increase in NT-proBNP can be caused by different pathologies such as renal failure, it is closely related to cardiac damage and therefore to HF, so the slightest increase is already indicative of cardiac involvement [63].

NT-proBNP can offer different characteristics that are typical of a good prognostic biomarker. This analysis demonstrates that the biomarker has clinical utility and incremental value that can improve clinical outcomes. It also has a good cost-effectiveness ratio since when a HF harvest is present, NT-proBNP is already measured, not requiring complementary measurements. Finally, it should be studied whether the biomarker has the capacity to discriminate between patients at risk or not through the evaluation of AUC [64].

In contrast, CRP has the same relevance as NT-proBNP but in a different way, i.e., it has a hazard ratio of 0.8; therefore, each unit of CRP concentration decreases the risk of HF by 20% (**Figure 6**). It should be taken into account that values lower than 0.3 mg/L are normal, up to 10 mg/L is a moderate increase and quantifications higher than this are pathological.

As we have observed during the results of the whole study, most of the GZ patients presented with COPD/respiratory infection or HF, CRP can help us in these cases since it is related to inflammation and infection. Moreover, it is a biomarker that is currently used to diagnose COPD exacerbations [66] and has already shown to have all the necessary characteristics to be a good biomarker like NT-proBNP [64].

In conclusion, presenting dyspnea and/or orthopnea, high NT-proBNP levels within the grey zone and low CRP levels are directly related to the patient's pro-HF readmission. But above all, both NT-proBNP and CRP are helpful because they are biomarkers and therefore objective, not dependent on the patient's or physician's perceptions. It could also have an impact on diagnosis, it could be a diagnostic biomarker, but it should be thoroughly investigated.

6.4. Indicators of compound event

As in HF readmission, a univariate and multivariate Cox analysis was performed (**Table 6**), where different statistically significant results were obtained. The univariate study shows that age, dyspnea, NT-proBNP and diuretics show statistically relevant results, that is, they show a possible association with the composite event (readmission or death). Despite this, a multivariate analysis was performed to know whether the variables are independently related to the outcome event or on the contrary whether their association with the composite event depends on other variables [67].

Both age and dyspnea present relevant statistical values in both types of analysis. Thus, age is actually independently associated with the incident, with each additional year increasing

the probability of occurrence by 5%. The same is true for dyspnea; a patient presenting this symptom increases the probability of the event by 60%.

On the other hand, hemoglobin, despite not having an outstanding value in the univariate assessment, but in the multivariate assessment, as the multivariate is a broader and more robust study, we can conclude that there is a correlation between hemoglobin and the HF event. The higher the quantification, the lower the risk of readmission to the emergency department for HF, with each g/dL of hemoglobin decreasing the risk by 10%. These results could be explained by the comorbidity of anemia with HF, the neurohumoral activation characteristic of HF contributes to the development of anemia [68], therefore, anemia may be a sign of HF and a normal hemoglobin concentration may be an indicator of the opposite.

In contrast, NT-proBNP and diuretics at discharge show a remarkable p-value in the univariable analysis but not in the multivariable analysis. In the univariate study, the variables are studied one by one as the name indicates, and that the p-value<0.05 means that there is a possible correlation between the variable and the outcome event, but when this same variable loses significance in the multivariate analysis it can be explained because its effect depends on other factors. Therefore, we cannot reliably assure that NT-proBNP in the grey zone and treatment with diuretics has any association with hospitalization for the study syndrome. Finally, we found no remarkable findings in the anamnesis, physical signs or ECG.

Finally, we can conclude that age, the presence of dyspnea and hemoglobin concentration may be prognostic indicators of the patient specifically after the composite event occurs. But in order to determine whether they are good prognostic indicators with certainty and can be used as such by the medical sector, more research with much larger cohorts should be done.

6.5. Comparison of clinical models

In view of the results obtained in **Table 5** and with the intention of testing the capacity of biomarkers to determine the development of the disease, three types of regression models were analyzed: a clinical model, a clinical model taking into account CRP and another taking into account CRP and NT-proBNP. Three types of regression models were analyzed: a clinical model, a clinical model taking CRP into account and another taking CRP and NT-proBNP into account.

In the first model (**Table 7**) diabetes, dyspnea and myocardial infarction have a significant hazard ratio, and within the regression model they are the most important factors in the probability of readmission for HF. On the other hand, in **Table 8**, in addition to these variables, orthopnea and CRP itself are added. Looking at **Table 9**, dyspnea, myocardial infarction, orthopnea, CRP and NT-proBNP become significant.

These factors have been shown to be important in HF-related readmission and mortality. The most frequent symptoms, dyspnea and orthopnea, are a precipitating factor in the development of the pathology and the most important biomarker, NT-proBNP, for the

diagnosis of the syndrome. And finally, a biomarker, CRP, which in this study has been shown to be statistically important in HF readmission.

Furthermore, an analysis was performed of the improvement in discriminatory capacity evidenced by including in a model with purely clinical variables the values of two biomarkers widely used in emergency departments: CRP and NT-proBNP (results derived from the estimation of Harrel's C statistic). Table 10 shows how there is a progressive increase in the C statistic when CRP or NT-proBNP is added to the clinical model, with a progressive increase from 0.727 to 0.769. Although all of them exceed the acceptable limit, since values below 0.7 are not very discriminative. These results allow us to infer that in the comprehensive clinical assessment in emergency departments and in the case of a patient with respiratory distress, the use of the aforementioned biomarkers should always be considered, as they help us in a certain way to perform a comprehensive clinical assessment.

Despite the low increase in discrimination, the implementation of these biomarkers in the evaluation of patients with intermediate NT-proBNP levels does not entail an added cost, as both parameters are routinely quantified upon the arrival of these patients at the emergency department.

In summary, NT-proBNP and CRP slightly increase the prognostic value of the clinical model for readmission due to HF in patients with suspected HF and NT-proBNP determination in the indeterminate zone, without the need for additional studies. Despite the apparent lack of importance, if we take into account that this is a population with complex diagnoses, the minimal increase becomes quite relevant.

7. Conclusion

The results of this study demonstrate that patients with NT-proBNP levels in the grey zone have a clinical profile similar to HF, with more frequent comorbidities and symptoms characteristic of this syndrome. This group has higher readmission and mortality rates, and half of these patients suffer the composite event at three years of follow-up. For this reason, this group should be considered a population with specific follow-up and diagnostic needs.

In addition, the analyses showed that certain factors such as dyspnea, orthopnea, history of myocardial infarction, and elevated NT-proBNP levels (within the indeterminate range) are independently associated with readmission for HF in these patients. These biomarkers have proven to be valuable for prognosis, improving the prognostic value and increasing the AUC of clinical models.

Given the above, it is imperative to expand the research with larger cohorts that include an additional group with a confirmed diagnosis of HF in order to more accurately assess the similarity of the GZ group to clinical extremes and confirm the clinical utility of these biomarkers in healthcare practice.

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