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Master's thesis

Clinical and Experimental Evaluation of Soluble ST2 as a Biomarker in Acute Heart Failure

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1. Abstract

Acute heart failure is a complex clinical syndrome associated with high morbidity and mortality, particularly in elderly and comorbid patients admitted to the Emergency Department. Although natriuretic peptides are well established biomarkers for the diagnosis and prognostic assessment of heart failure, they mainly reflect haemodynamic stress and ventricular wall stretch. However, acute heart failure is a biologically complex syndrome that also involves myocardial injury, inflammation, fibrosis, neurohormonal activation and adverse cardiac remodelling. In this context, soluble suppression of tumorigenicity-2 (sST2) has emerged as a promising biomarker with potential prognostic value, as it may provide additional information on myocardial stress and remodelling processes.

The aim of this thesis was to evaluate the prognostic role of circulating ST2 concentrations in patients admitted at the Emergency Department of the "Maggiore della Carità" University Hospital in Novara with acute heart failure, with particular focus on 30-day mortality. The study population consisted of 137 patients enrolled for newly diagnosed acute heart failure or acute decompensation of chronic heart failure. At baseline, venous blood samples were collected, and plasma ST2 levels were measured using the Critical Diagnostics Presage® ST2 Assay, a quantitative sandwich monoclonal ELISA. Demographic, clinical, laboratory and follow-up data were collected. Survival analysis was performed using the Kaplan-Meier estimator, while multivariable logistic regression was used to identify independent predictors of 30-day mortality. The study population was predominantly elderly, with a median age of 81 years, and showed a high burden of comorbidities and advanced symptomatic status at admission. Most patients were classified as NYHA functional class III or IV. During the 30-day follow-up period, overall survival was approximately 86%. Patients who died during follow-up showed significantly higher ST2 concentrations compared with survivors. In particular, ST2 levels were markedly increased in non-survivors and were significantly associated with 30-day mortality. In the multivariable logistic regression analysis, ST2 remained independently associated with mortality after adjustment for clinically relevant variables, including age, estimated glomerular filtration rate, heart rate, diabetes mellitus and NYHA functional class.

These findings support the potential role of ST2 as an independent prognostic biomarker in patients admitted with acute heart failure. ST2 may provide complementary information to natriuretic peptides by reflecting pathophysiological mechanisms related to myocardial stress, inflammation and fibrosis. Although further studies in larger and multicenter cohorts are needed, the integration of ST2 into clinical and biomarker-based risk stratification could contribute to a more accurate identification of patients at higher short-term risk.

2. Introduction

2.1. Heart failure

Heart failure (HF) is a clinical syndrome traditionally described as a condition in which the heart is unable to pump sufficient blood and/or fill properly. It can also be defined as a structural or functional abnormality of the heart that results in either inadequate cardiac output, or a seemingly adequate output maintained only through compensatory neurohormonal mechanisms and elevated left ventricular filling pressures. HF is a multifactorial syndrome associated with a broad spectrum of risk factors that contribute to its onset and progression through complex pathophysiological mechanisms. Among the primary determinants are cardiovascular diseases such as hypertension, coronary artery disease, and prior myocardial infarction, which induce structural damage, myocardial ischemia, and progressive ventricular remodeling. Long-standing hypertension, in particular, leads to left ventricular hypertrophy and increased myocardial stiffness, predisposing to both systolic and diastolic dysfunction[1]. Metabolic conditions, including diabetes mellitus and obesity, further exacerbate HF risk by promoting systemic inflammation, oxidative stress, insulin resistance and endothelial dysfunction, all of which contribute to myocardial fibrosis and impaired cardiac performance[2]. In addition, comorbidities such as chronic kidney disease and chronic obstructive pulmonary disease are strongly associated with HF, as they enhance volume overload, neurohormonal activation, and systemic inflammation. Lifestyle-related factors, including cigarette smoke , sedentary behavior, and excessive alcohol intake, also significantly increase the likelihood of developing HF by accelerating atherosclerosis and impairing myocardial function[3]. Aging represents a major non-modifiable risk factor, as the prevalence of HF rises markedly with advancing age due to cumulative cardiovascular damage, reduced regenerative capacity, and the higher burden of comorbidities. Furthermore, genetic predisposition and sex-related differences have been increasingly recognized, with certain cardiomyopathies and molecular pathways contributing to individual susceptibility[4]. Overall, the interplay of these risk factors promotes adverse cardiac remodeling, neurohormonal activation, and progressive deterioration of cardiac function, ultimately leading to the clinical manifestation of heart failure[5].

HF is characterized by symptoms and signs caused by structural and functional cardiac abnormalities and its diagnosis is supported by elevated natriuretic peptide (NP) levels and objective evidence of pulmonary or systemic congestion. NPs are a group of closely related hormones involved in cardiovascular regulation. Atrial Natriuretic Peptide (ANP) is mainly produced by the atria of the heart, whereas B-type Natriuretic Peptide (BNP) is released predominantly from the ventricles. ANP contributes to the control of blood pressure and helps prevent enlargement of the heart muscle through

both local and systemic signaling pathways. In contrast, BNP mainly acts within ventricular tissue, where it plays a role in limiting fibrotic remodeling[6]. Based on left ventricular ejection fraction (EF), HF is categorized into three groups[7]:

- Heart failure with reduced EF (HFrEF), defined as EF ≤ 40%
- Heart failure with mildly reduced EF (HFmrEF), defined as EF 41-49%
- Heart failure with preserved EF (HFpEF), defined as EF ≥ 50%

HFpEF is characterized by diastolic dysfunction so consequently a heart damage due to comorbidities such as obesity, chronic kidney disease and pulmonary disease, while HFrEF shows systolic dysfunction, secondary to direct heart damage, a valve disease or a cardiomyopathy[8]. The chronic activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) plays a central role in disease progression[9]. Although the release of NPs increases as a compensatory response, this mechanism is not sufficient to counteract the persistent neurohormonal stimulation. The resulting imbalance promotes adverse cardiac remodeling, characterized by progressive myocardial hypertrophy and dilation of the cardiac chambers[10]. These structural changes progressively impair cardiac contractile function, contributing to the onset of clinical symptoms and increasing the risk of arrhythmias and abnormalities in cardiac electrical conduction[11].

In addition to the classification based on left ventricular ejection fraction, heart failure severity can also be described using the New York Heart Association (NYHA) functional classification. This system stratifies patients according to the degree of limitation in physical activity caused by symptoms such as dyspnea, fatigue or palpitations. NYHA class I includes patients with no limitation of ordinary physical activity; class II identifies patients with slight limitation, who are comfortable at rest but develop symptoms during ordinary activity; class III includes patients with marked limitation, in whom less than ordinary physical activity causes symptoms; and class IV refers to patients with symptoms even at rest or inability to perform any physical activity without discomfort. Although the NYHA classification is based on symptoms and may therefore be partly subjective, it remains widely used in clinical practice because it provides a simple assessment of functional impairment and disease severity in patients with heart failure[7].

2.2. Acute heart failure

As these structural and functional alterations in HF progress, they can culminate in acute heart failure (AHF), which encompasses a diverse group of clinical conditions associated with high morbidity and mortality. Heart failure is broadly classified into chronic heart failure (CHF) and AHF which represent different stages of the same disease continuum. CHF is a long-term, progressive condition characterized by gradual neurohormonal activation, ventricular remodeling, and a relatively stable but declining clinical status[12]. In contrast, AHF is defined by a rapid onset or worsening of symptoms and signs of heart failure, often requiring urgent medical intervention. While CHF is typically managed in an outpatient setting with long-term pharmacological and device-based therapies[13], AHF represents a state of acute decompensation with immediate hemodynamic instability and organ congestion. AHF encompasses a diverse group of clinical conditions that are linked to high morbidity and mortality. Although acute heart failure syndromes (AHFS) occur frequently, detailed information regarding their presentation, patient profiles and clinical outcomes remain limited. Acute heart failure is generally described as the sudden development of new symptoms or the rapid worsening of existing symptoms of heart failure, these symptoms are for example shortness of breath and difficulty breathing when lying flat. Physical signs include raised jugular venous pressure, pulmonary congestion and swelling of lower extremities [5]. AHF is frequently a life-threatening condition that requires urgent hospitalization. Initial treatment primarily focuses on relieving fluid congestion and stabilizing hemodynamic status. AHF is a term that includes both patients' experiencing heart failure for the first time (De Novo AHF) and those with an acute deterioration of previously diagnosed cardiomyopathy, commonly referred to as acute decompensated heart failure. De Novo AHF may result from a wide range of mechanisms, not solely intrinsic pump failure, it typically presents with signs of reduced perfusion and pulmonary congestion[15]. Although myocardial ischemia represents one of the most common triggers, AHF may also be caused by non-ischemic myocardial injury. This includes inflammatory conditions, such as myocarditis[16], and toxic damage, including drug-induced cardiotoxicity, for example related to trastuzumab treatment[17]. In some cases, hospitalization for AHF may be the first clinical presentation of previously unrecognized chronic heart failure, as acute myocardial injury can result in persistent myocardial dysfunction. The most frequent cause of AHF is myocardial ischemia, in which partial or complete coronary artery occlusion compromises blood flow to a portion of the myocardium, leading to decreased contractility[18]. AHF has been described as a two-stage process: an "initiation phase" sparked by specific triggers, such as myocardial ischemia, infection, uncontrolled hypertension, arrhythmias, this phase is characterized by an abrupt increase in cardiac filling pressures and early hemodynamic deterioration, followed by an "amplification phase" where various neurohormonal pathways, including the sympathetic nervous system and the

RAAS, together with systemic inflammation, endothelial impairment, and increasing fluid accumulation. These mechanisms reinforce each other in a vicious cycle, progressively deteriorating cardiac function and contributing to dysfunction across multiple organ systems. To navigate this complexity, Troponin I (TnI) and Troponin T (TnT) are utilized to decode the underlying pathophysiology and guide clinics management[19]. Troponins are structural proteins of the cardiac contractile apparatus and are released into circulation when cardiomyocytes undergo injury or necrosis. In the context of AHF, elevated troponin levels are thought to result from multiple mechanisms, including myocardial strain due to increased wall stress, subendocardial ischemia, neurohormonal activation, and inflammatory processes. Several studies have demonstrated that increased concentrations of cTnI or cTnT are associated with worse clinical outcomes, including higher in-hospital mortality, longer hospitalization, and increased risk of rehospitalization[20]. The decrease in cardiac reserve is an alarming sign of AHF[19]. Troponin concentration are therefore considered highly specific biomarkers of myocardial injury, as their presence in the circulation reflect disruption of cardiomyocyte membrane integrity when they undergo injury or necrosis and this way cytosolic and structural pools of troponins leak into the bloodstream, making them sensitive indicators of myocardial injury[21]. Managing AHF remains a significant hurdle in modern cardiology, primarily due to the clinical heterogeneity inherent in the syndrome[22].

2.3. Epidemiology of acute heart failure

Heart failure affects approximately 64 million people worldwide. Despite medical advances, mortality remains high: approximately 50% of patients die within 5 years of diagnosis. As life expectancy increases, the incidence is rising, making heart failure the leading cause of hospitalization over the age of 65[23]. HF persists as a clinical challenge, distinguished by its high prevalence and significant rates of mortality and disability. It is a multisystem condition encountered by a broad spectrum of medical and surgical specialists across both primary and secondary care. Over the past two decades its epidemiological patterns and underlying pathophysiology has evolved dramatically, paired with a rapid and ongoing transformation in diagnostic and therapeutic strategies[9].

In high income countries, the epidemiology of symptomatic heart failure has been extensively characterized. Overall, heart failure affects approximately 1-2% of the adult population (Figure 1). However, its prevalence increases substantially with age, with around 6-10% of individuals older than 65 years of living with the condition. The lifetime probability of developing heart failure is estimated

to be about 20% for a 40-year-old individual. The overall prevalence is believed to be rising, likely reflecting population ageing and improved survival[24].

Several registries have examined AHFS as a distinct clinical condition. Among the most relevant there are: the Americal registry ADHERE, the Euro Heart Failure Surveys, and national registries conducted in France, Finland and Italy. In Europe, the Euro Heart Failure Survey I described the clinical features and management of patients hospitalized with heart failure in 115 hospitals across 24 countries. However, acute heart failure was the primary reason for admission in only about 40% of cases. The Italian registry enrolled almost 3,000 patients in 206 cardiology centers with intensive coronary care units. Across these studies, patients admitted with AHFS shared similar demographic and clinical characteristics. The population was generally elderly, with a mean age of 70 and 75 years, and a nearly balanced sex distribution. A high burden of cardiovascular risk factors and comorbidities was consistently observed. Ischemic heart disease was present in 46-68% of patients, arterial hypertension in 53-73%, diabetes in 27-42% and arrythmias in 21-42%[25].

In the United States, more than 1 million hospital admissions each year are primarily due to heart failure, with an additional 3 million hospitalizations in which heart failure is recorded as a secondary or tertiary diagnosis. Among individuals over 65 years of age, heart failure represents the most common reason for hospital admission. Early rehospitalization is frequent, with rates reaching approximately 35% within 60 days of discharge[26].

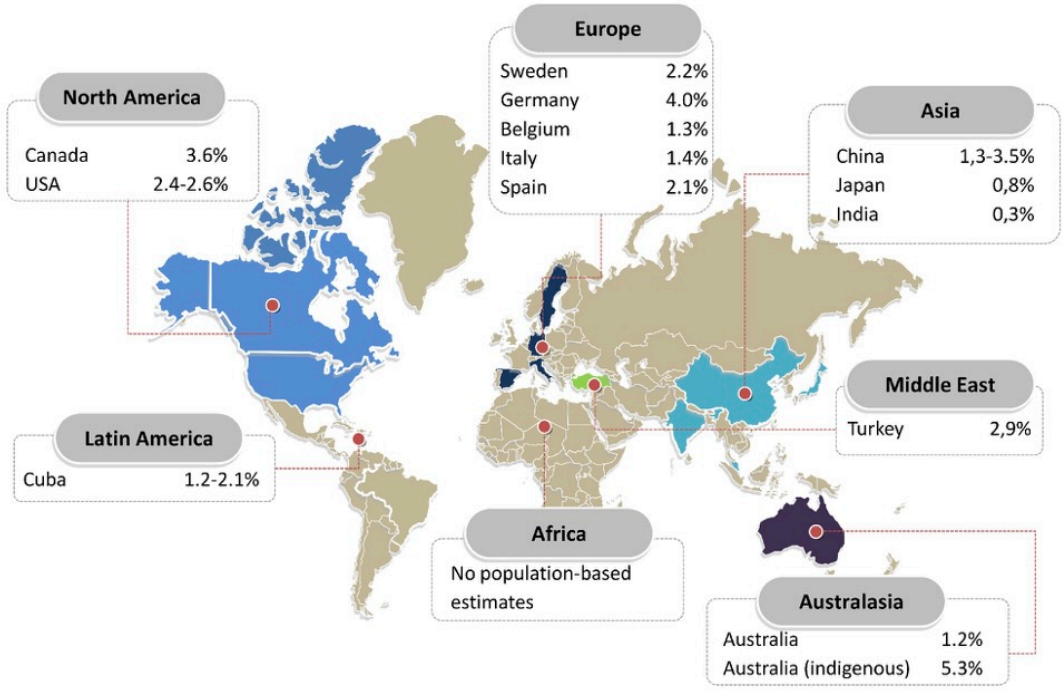

Figure 1: Worldwide heart failure prevalence[27].

In addition to its clinical burden, heart failure imposes a substantial economic and healthcare system impact worldwide. The worldwide economic burden of heart failure is estimated to exceed 100 billion dollars annually, with the majority of expenses driven by hospitalizations and long-term management. Notably, nearly 70-80% of total costs are attributable to inpatient care, reflecting the recurrent nature of decompensation episodes. Significant geographic disparities exist in both incidence and outcomes. In low and middle-income countries, patients tend to present at a younger age and often with more advanced disease, frequently related to untreated hypertension, rheumatic heart disease, or cardiomyopathies of infectious origin[28]. In contrast, in high-income regions, heart failure is more commonly associated with ischemic heart disease and aging populations. Temporal trends also show a shift in heart failure phenotypes, with an increasing prevalence of HFpEF, particularly among elderly patients and women. Despite improvements in survival due to advances in pharmacological and device-based therapies, the overall burden of disease continues to rise, driven by demographic changes and the growing prevalence of comorbidities such as obesity, diabetes, and chronic kidney disease. These evolving patterns highlight the need for tailored prevention strategies and optimized healthcare resource allocation [29].

2.4. Therapeutic strategies

In recent decades, the prognosis of patients with heart failure has improved considerably due to major therapeutic advances.

Several pharmacological strategies have been shown to reduce morbidity and mortality, including beta-adrenergic blockers which are a class of drugs that act within the cardiovascular system, including the heart and blood vessels exerting their effects by blocking beta-adrenergic receptors, leading to a reduction in heart rate and blood pressure[30]; angiotensin-converting enzyme inhibitors that act by interfering with RAAS, reducing the production of angiotensin II, a molecule responsible for vasoconstriction and fluid retention and as a result, they lower systemic blood pressure, decrease peripheral vascular resistance, and improve overall cardiac function[31]; and sodium-glucose co-transporter 2 (SGLT2) inhibitors which act primarily at the renal level by blocking the SGLT2 in the proximal tubule, thereby reducing glucose and sodium reabsorption[32].

In addition, device-based therapies, such as implantable cardioverter-defibrillators (ICDs) and cardiac resynchronization therapy (CRT), have further contributed to improving survival in patients with heart failure[7].

2.5. Multi-organ dysfunction in heart failure

Multiorgan dysfunction in HF is a frequent and prognostically relevant consequence. It is not only the heart that is compromised: reduced cardiac flow, increased venous pressures and neurohormonal activation (RAAS, sympathetic nervous system, vasopressin) determine systemic involvement. Circulating biomarkers such as natriuretic peptides, high-sensitivity troponins, galectin-3 and soluble Suppression of Tumorigenicity-2 (sST2) reflect not only myocardial stress but also systemic remodeling processes. An AHF episode often triggers a series of systemic insults, ranging from hepatic and cognitive impairment to endothelial dysfunction. This multi-organ involvement creates a self-perpetuating cycle that further destabilizes the failing heart, complicating both the treatment and the long term prognosis of the patient[19].

AHF frequently leads to renal dysfunction, traditionally attributed to reduced cardiac output and consequent renal hypoperfusion; renal injury in AHF is now recognized to be more strongly associated with venous congestion, which increases renal interstitial pressure, reduces glomerular filtration gradient, and impairs tubular function[33]. Renal involvement in HF is linked to increased central venous pressure, reduced renal perfusion pressure and intrarenal RAAS activation[34]. Emerging biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) may help detect early tubular injury before an overt creatinine rise, reflecting the structural component of renal damage in AHF[13].

Pulmonary involvement primarily results from elevated left ventricular filling pressures, which increase pulmonary capillary hydrostatic pressure and lead to interstitial and alveolar edema. Manifestations are also pleural effusion and stress and rest dyspnea[35].

Hepatic dysfunction in HF, often referred to as congestive hepatopathy, arises from chronic systemic venous congestion, particularly in right-sided HF. The liver is a remarkably vascularized organ, accounting for nearly 25% of the total cardiac output through a unique dual supply system. While the hepatic artery provides approximately 25% of the total flow with highly oxygenated blood, the portal vein supplies the remaining 75%, albeit with deoxygenated blood. This robust vascular architecture provides a natural defense, rendering the liver highly resilient to ischemic injury. A primary example of this resilience is the hepatic artery buffer response, a compensatory mechanism characterized by

the up-regulation of arterial flow in response to a reduction in portal supply. This autoregulatory process can offset up to a 60% decline in portal blood flow. Impaired hepatic perfusion and hypoxic injury contribute to biochemical abnormalities such as hyperbilirubinemia and elevated transaminases. Long standing congestion may evolve into cardiac cirrhosis. Cardiac cirrhosis represents an advanced consequence of chronic congestive hepatopathy. It develops when prolonged venous congestion, usually caused by right-sided heart failure or constrictive pericarditis, exposes the liver to persistently increased venous pressure. Over time, this condition may promote hepatic fibrosis, particularly around the central veins, leading to progressive impairment of liver structure and function[36].

Cerebral hypoperfusion represents another clinically relevant consequence of HF. A significant correlation exists between neurocognitive impairment and a spectrum of cardiovascular disorders, including chronic hypertension, ischemic heart disease, and atrial tachyarrhythmias. Reduced cardiac output and impaired cerebrovascular autoregulation have been associated with white matter lesions, cognitive impairment and increased risk of ischemic stroke. Neuroinflammation and endothelial dysfunction may further contribute to cognitive decline in heart failure patients[37]. Although cognitive impairment is more extensively described in chronic heart failure, acute heart failure may also be associated with transient cerebral dysfunction, particularly in hospitalized and elderly patients. In the acute setting, abrupt reductions in cardiac output and systemic blood pressure can impair cerebral perfusion, especially in individuals with compromised cerebrovascular autoregulation. In parallel, acute elevations in central venous pressure may reduce cerebral venous outflow, further contributing to impaired oxygen delivery. Importantly, in AHF, cognitive impairment is typically acute and potentially reversible, differing mechanistically from the progressive structural brain changes observed in chronic HF. Therefore, cerebral involvement in AHF is best interpreted as a consequence of acute hemodynamic instability and systemic inflammatory activation rather than as a primary neurodegenerative process. From a biomarker perspective, systemic inflammatory mediators and markers of endothelial dysfunction may indirectly reflect cerebral vulnerability during acute decompensation[38].

Systemic inflammation is frequently present in AHF and contributes to its pathophysiology. In patients with acute heart failure, activation of inflammatory pathways is commonly observed, even in the absence of infection. Elevated levels of inflammatory mediators such as high-sensitivity C-reactive protein, tumor necrosis factor-α (TNF-α), interleukin-6, interleukin-1, soluble suppression of tumorigenicity-2 (sST2), and galectin 3 have been detected and are implicated in disease progression and adverse outcomes. These biomarkers are associated with worse prognosis and may reflect systemic inflammatory responses intertwined with the heart failure process. Systemic inflammation

is a central feature of AHF[39]. Acute congestion may also impair intestinal barrier integrity, promoting endotoxin (lipopolysaccharide, LPS) translocation and Toll-like receptor 4 activation, thereby amplifying systemic inflammatory responses[40].

In AHF, multi-organ dysfunction therefore emerges from the interplay between hemodynamic stress (congestion and hypoperfusion) and acute molecular responses involving neurohormonal activation, endothelial dysfunction, mitochondrial impairment, and inflammatory signaling. Early biomarker profiling is increasingly recognized as a key tool for risk stratification and mechanistic characterization of systemic involvement in AHF. Figure 2 illustrates how non-cardiac organ failure impacts on patients' prognosis. As shown in the picture, the development of the failure of one organ (beyond heart) is associated to increased mortality; in-hospital mortality is further increased in case of ≥2 organs involved[41].

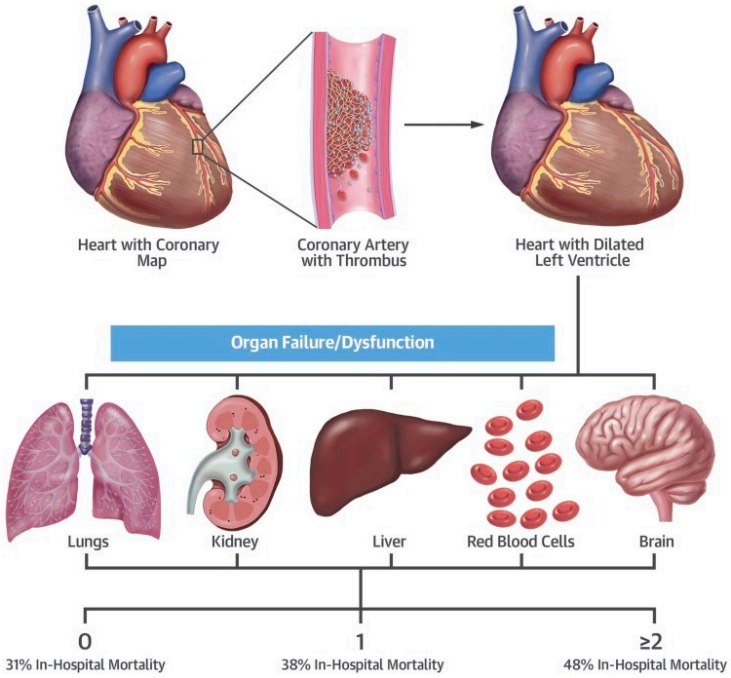

*Figure 2: Organ failure in acute myocardial infraction with cardiogenic shock. Patients are stratified into three groups: **0** indicates no additional non-cardiac organ failure, with dysfunction mainly confined to the heart; **1** indicates the presence of one additional failing organ; and **≥2** indicates multiple non-cardiac organ failures, reflecting a more severe systemic involvement[41].*

2.6. Innate Immune Activation and Cytokine Storm in Acute Heart Failure

Acute heart failure is increasingly recognized not only as a hemodynamic disorder but also as a condition characterized by rapid activation of the innate immune system. Myocardial stress, ischemia, and tissue injury promote the release of damage-associated molecular patterns (DAMPs), which

activate pattern recognition receptors such as Toll-like receptors on cardiomyocytes, endothelial cells, and immune cells. This triggers downstream Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling and the production of pro-inflammatory cytokines including interleukin IL-1β, IL-6, and TNF-α[42]. In the acute setting, this amplified inflammatory response may resemble a "cytokine storm," characterized by markedly elevated circulating cytokines that exert direct negative inotropic effects, impair β-adrenergic signaling, and promote nitric oxide overproduction, contributing to myocardial depression. TNF-α and IL-1β can reduce calcium handling efficiency in cardiomyocytes[42]. Beyond the myocardium, systemic cytokine release induces endothelial activation, increased vascular permeability, and microvascular dysfunction, facilitating tissue edema and contributing to multi-organ impairment, including renal and hepatic dysfunction[43]. Elevated circulating cytokines correlate with disease severity and adverse outcomes, suggesting that inflammation is not merely a bystander but an active driver of cardiac dysfunction and systemic complications. Inflammatory signaling amplifies neurohormonal activation and oxidative stress, creating a self-perpetuating cycle that worsens cardiac remodeling and organ cross-talk[7]. The liver plays a central role in the systemic response to cytokine storm, acting both as a target organ and an active contributor to inflammation. Elevated circulating cytokines such as IL-6, TNF-α, and IL-1β directly affect hepatic function by altering hepatocyte metabolism and promoting the acute-phase response. IL-6, in particular, drives the synthesis of acute-phase proteins, including C-reactive protein, fibrinogen and serum amyloid A, thereby amplifying systemic inflammation. At the same time, reduced cardiac output and venous congestion impair hepatic perfusion, leading to hypoxic injury and the clinical entity often referred to as congestive hepatopathy[44]. This results in hepatocellular dysfunction, cholestasis, and elevated liver enzymes, which are frequently observed in patients with acute decompensated heart failure. Hepatic dysfunction further exacerbates systemic inflammation by impairing the clearance of circulating cytokines and endotoxins, contributing to a self-perpetuating cycle of immune activation. Recent evidence suggests that liver injury markers, such as transaminases and bilirubin, are not only indicators of organ damage but also correlate with disease severity and worse clinical outcomes. This highlights the importance of the heart-liver axis in acute heart failure and underscores how cytokine storm contributes to multi-organ dysfunction beyond the cardiovascular system[45 46].

2.7. The role of biomarkers

Given the central role of innate immune activation and inflammatory mediators in the pathophysiology of acute heart failure, circulating biomarkers may provide a valuable link between

the underlying biological processes and their clinical expression. Biomarkers represent an essential diagnostic tool for the detection and characterization of myocardial insults, as well as the subsequent maladaptive remodeling observed in the acute heart failure population. Furthermore, the strategic application of these molecular indicators can improve diagnostic accuracy and allow more precise risk stratification, ultimately enhancing the prognostic evaluation of these patients.

Acute heart failure is not only a hemodynamic syndrome but also a condition characterized by systemic and myocardial inflammation, in which pro-inflammatory cytokines play a fundamental role in disease progression, impairment of cardiac function, and adverse clinical outcomes[47]. These inflammatory mediators are released from immune cells (macrophages, monocytes), activated endothelial cells, and stressed cardiomyocytes, contributing to local myocardial dysfunction as well as systemic effects on vascular tone, neurohormonal activation, and multi-organ interaction.

Clinical guidelines emphasize the measurement of NP plasma levels as a primary diagnostic instrument for patients presenting with symptoms indicative of heart failure, facilitating the confirmation or exclusion of the diagnosis. In modern emergency department settings, NPs are essential components of the standard diagnostic protocol for dyspnea due to their immediate availability and they are considered the "gold standard"[48]. Elevated concentrations are critical for both diagnosis and prognostic assessment, their accuracy can be compromised by various physiological and clinical modifiers, such as advanced age, obesity, renal impairment, atrial fibrillation and sepsis[49]. B-type natriuretic peptide (BNP) and its inactive cleavage fragment N-terminal pro-B-type natriuretic peptide (NT-proBNP) are cardiac neurohormones predominantly synthesized and released by ventricular cardiomyocytes in response to increased wall stress, volume overload, and pressure overload. BNP is generated from the precursor molecule proBNP, which is cleaved into the biologically active 32-amino acid BNP and the biologically inactive NT-proBNP fragment[50]. While BNP exerts physiological effects including natriuresis, diuresis, vasodilation, and inhibition of the RAAS, NT-proBNP does not have direct biological activity but is released in equimolar amounts and has a longer plasma half-life, making it particularly useful for laboratory measurement[51]. Clinically, both BNP and NT-proBNP are established biomarkers for the diagnosis, risk stratification, and prognostic assessment of heart failure. The circulating levels of natriuretic peptides are tightly regulated by enzymatic systems that control both their activation and degradation. The biological activity of natriuretic peptides is regulated by enzymes involved in their activation and degradation. Corin promotes the conversion of pro-ANP and pro-BNP into their active forms, whereas neprilysin contributes to their enzymatic degradation, thereby limiting their effects.[52]. This balance influences the circulating levels and biological activity of BNP and related peptides in heart failure[53].

BNP and NT-proBNP concentrations correlate with ventricular filling pressures and myocardial dysfunction, and elevated levels are associated with an increased risk of hospitalization and mortality in both acute and chronic heart failure[54]. Due to their high negative predictive value, especially in acute dyspnea, normal BNP or NT-proBNP levels can effectively exclude heart failure as the underlying cause[7].

In addition to haemodynamic stress, inflammation plays an important role in the pathophysiology of AHF[55]. Several inflammatory mediators, including TNF-α, IL-1β and IL-6, have been implicated in myocardial dysfunction, fibroblast activation, extracellular matrix remodeling and adverse clinical outcomes. These cytokines may interact with neurohormonal pathways, oxidative stress and endothelial dysfunction, contributing to disease progression and multi-organ involvement[56]. Other biomarkers, such as growth differentiation factor-15 (GDF-15) and IL-18, have also been associated with inflammation, oxidative stress, tissue injury and worse outcomes in acute heart failure. However, in the context of this thesis, particular attention is given to sST2, which provides prognostic information related to myocardial stress, inflammation, fibrosis and adverse cardiac remodeling [57] [47].

Beyond classical cytokines, sST2 has emerged as a robust biomarker of myocardial stress and fibrosis. sST2 is a member of the interleukin-1 receptor family and represents the circulating isoform of the ST2 protein, which exists in two main forms: a transmembrane receptor (ST2L) and a soluble decoy receptor[58]. ST2 is the receptor for IL-33, a cytokine that exerts cardioprotective effects by reducing myocardial fibrosis, hypertrophy, and apoptosis through activation of intracellular anti-remodeling signaling pathways (Figure 3) [59]. In the setting of myocardial stress and mechanical strain, cardiomyocytes and cardiac fibroblasts upregulate sST2 production. Elevated circulating sST2 acts as a decoy receptor for IL-33, preventing its binding to ST2L and thereby attenuating its cardioprotective effects, ultimately promoting fibrosis and adverse ventricular remodeling. Importantly, sST2 has demonstrated strong and independent prognostic value in AHF, associated with increased short-term mortality and risk of rehospitalization, independently of natriuretic peptides and traditional clinical risk factors[59]. From a clinical perspective, a commonly used prognostic cut-off for sST2 is 35 ng/mL[60]. Values above this threshold have been associated with worse prognosis in patients with heart failure, and the International ST2 consensus Panel recommended sST2 ≥35 ng/mL as a threshold for identifying patients with AHF at higher risk of adverse outcomes. However, in AHF, some studies have suggested that higher cut off-values may improve prognostic specificity, reflecting the particularly elevated biomarker levels observed during acute decompensation[61]. sST2 reflects activation of the IL-33/ST2 signaling pathway and is strongly associated with mortality and rehospitalization in both acute and chronic heart failure settings[62]. Importantly, sST2 provides

prognostic information independent of natriuretic peptides and is less affected by age, renal function, or body mass index (BMI) compared to BNP or NT-proBNP[63].

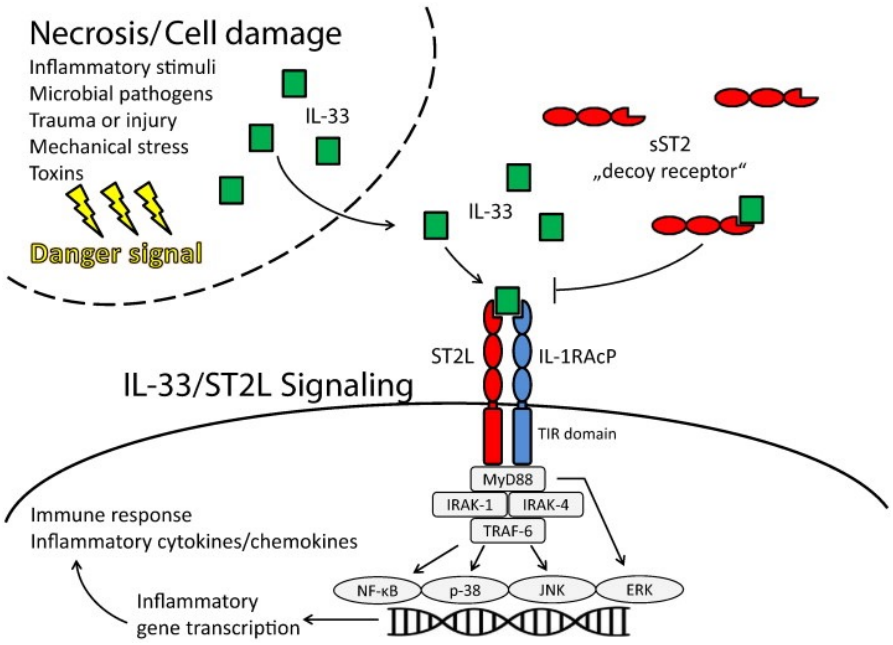

Figure 3:Interleukin-33/ST2L signaling pathway[64].

3. Objective of the thesis

The primary objective of this thesis was to evaluate the association between plasma ST2 concentrations and 30-day mortality in patients admitted to the Emergency Department for AHF. In particular, the study aimed to assess whether ST2 levels differed between survivors and non-survivors and whether ST2 could independently predict short term mortality after adjustment for clinically relevant variables. The secondary objectives were to describe the distribution of ST2 concentrations according to clinical severity, particularly NYHA functional class, and to compare the prognostic information provided by ST2 with that of traditional clinical and laboratory parameters, including NT-proBNP, renal function, heart rate and comorbidities. Overall, this thesis aimed to explore the potential contribution of ST2 to early risk stratification in patients with AHF.

4. Materials and methods

4.1. Study design and population

The present work involved the recruitment of patients presenting to the Emergency Department of the "Maggiore della Carità" University Hospital in Novara with either newly diagnosed acute heart failure or acute decompensation of chronic heart failure. This was a retrospective observational study approved by the local ethics committee (Study 371/CE , protocol number: E00116-2026). The patient cohort analyzed for this thesis was collected between January 24th 2018, and March 3rd 2020 .

4.1.1. Patients

Patients were enrolled based on the following inclusion criteria:

- Age ≥ 18 years,

- Expression and signature of informed consent

- Admission to the Emergency Department for Acute Heart Failure based on the following:

- Dyspnea with minimal exertion or at rest that has worsened in the last 2 weeks

- Clinical and radiological signs of HF according to the Framingham Criteria

Among the major criteria: pulmonary edema, cardiomegaly, hepatojugular reflux, jugular distension, paroxysmal nocturnal dyspnea or orthopnea, pulmonary rales, S3 tone and weight loss greater than 4.5 kg in 5 days in response to diuretic treatment. While, ankle edema, dyspnea on exertion, hepatomegaly, nocturnal cough, pleural effusion and heart rate > 120 bpm were considered minor criteria.

While, the exclusion criteria chosen were:

- Under 18 years of age;

- Refusal or inability to give informed consent;

- Severe hemodynamic instability (cardiogenic shock);

- Active acute coronary syndrome (Non-STEMI and STEMI);

- Pregnant women;

- Patient previously enrolled in the study.

Patient enrollment took place at the time of presentation to the Emergency Department, after obtaining written signed informed consent. For each patient, demographic data, medical history, home therapy, vital and instrumental parameters, and laboratory data were collected at baseline (T0, time of enrollment) and 30 days after admission to the emergency department.

The final dataset consisted of samples collected from 137 patients. To ensure patient confidentiality, all patient data and identifying information were coded and anonymized within Research Electronic Data Capture (REDCap) platform.

4.2. Samples and Laboratory analysis

At time zero (T0), corresponding to the time of enrollment, a venous blood sample from the patients was collected in EDTA tubes, and the tubes were subsequently centrifuged at 3000 rpm for 10 minutes at 25°C to allow plasma separation. The obtained plasma was then aliquoted into Eppendorf-type tubes. After being labeled with an anonymous patient code, the samples were stored at -80°C until ELISA analysis was performed.

The laboratory procedures were conducted inside the laboratory of Medical Clinic at the University of Piemonte Orientale inside the department of Translational Medicine.

4.3. Enzyme-Linked Immunosorbent Assay (ELISA)

The Critical Diagnostics Presage® ST2 Assay (3030 Bunker Hill St. Suite 117A San Diego, CA 92109) EIA Test Kit REF# BC-1065E which, is a quantitative sandwich monoclonal ELISA, was used to measure plasma ST2 levels according to the manufacturer's instructions. Absorbance was measured at 450 nm using Perkin Elmer Victor X4 Multimode Microplate Reader.

The 137 samples were initially tested at a 1:50 dilution. Samples with values falling outside the standard curve were subsequently retested at a 1:200 dilution.

ST2 concentrations were calculated by plotting the standard curve and performing linear interpolation of the data using GraphPad PRISM 11 (GraphPad Software, La Jolla, California, USA).Statistical analysis.

4.4. Statistical analysis

The statistical analysis was performed using Stata Statistical Software, version 19.5 (StataCorp LLC, College Station, TX, USA).

Continuous variables were expressed as median and interquartile range (IQR) or as mean ± standard deviation (SD), according to data distribution, whereas categorical variables were reported as frequencies and percentages. Comparisons between independent groups were performed using the Mann-Whitney U test for continuous variables and the Pearson chi-square test or Fisher's exact test for categorical variables, as appropriate.

Correlations between continuous variables of interest, including ST2 and NT-proBNP concentrations, were evaluated using Spearman's rank correlation coefficient (Spearman's rho). Correlation analyses were performed to investigate the relationship between biomarkers associated with acute heart failure severity.

Survival analysis was performed using the Kaplan-Meier estimator to evaluate 30-day overall survival in the study population. Survival curves were generated to describe the probability of survival during the follow-up period. To identify independent predictors of 30-day mortality, multivariable logistic regression analysis was performed including clinically relevant variables associated with mortality in univariate analyses. Results were expressed as odds ratios (ORs) with corresponding 95% confidence intervals (95% CI). Graphical analyses, including box plots of ST2 concentrations according to mortality and NYHA functional class, were generated to visually assess biomarker distribution and variability across clinical groups. For the stratification analysis, patients were divided according to the median ST2 concentration in order to obtain two numerically comparable groups. Survival curves between ST2 groups were compared using the log-rank test.

A two-sided p value < 0.05 was considered statistically significant for all analyses.

5. Results

5.1. Baseline characteristics of the study Population

The study population consisted of 137 patients admitted to the ED because of acute heart failure. Patients were predominantly elderly, with a median age of 81 years, and showed a slight male predominance. A high burden of comorbidities was observed, reflecting the clinical complexity typically associated with acute heart failure. Most patients presented with advanced symptomatic status at admission, as demonstrated by NYHA functional class III-IV. Atrial fibrillation or flutter was highly prevalent within the cohort, together with alterations in vital parameters such as increased heart rate and respiratory rate.

Renal dysfunction was frequently observed, although patients with severe impairment of glomerular filtration represented a minority of the study population. Biomarker analysis demonstrated elevated levels of both NT-proBNP and ST2 in a substantial proportion of patients, supporting the presence of significant myocardial stress and advanced heart failure severity. Overall, the baseline characteristics describe a frail and clinically complex cohort of patients admitted with acute decompensated heart failure.

Table 1:Baseline characteristics of the study population.

Continuous variables are presented as median (IQR), while categorical variables are expressed as frequencies (%).

Variable	Total Patients N=137
Age, years	81 (78-89)
Male/Female, N.	74 (54)/63 (46)
Body mass index (BMI), kg/m²	27 (24-30)
Charlson Comorbidity Index	
2-4	11 (19)
5-7	25 (44)
≥8	21 (37)
NYHA class at admission, N.	
NYHA II	19 (14)
NYHA III	61 (45)
NYHA IV	56 (41)
Cardiac rhythm at admission, N.	
Sinus rhythm	61 (45)
Atrial fibrillation/flutter	68 (50)
Other arrhythmia	8 (6)

Systolic blood pressure, mmHg	140 (120-160)
Heart rate, bpm	88 (73-105)
Respiratory rate, rpm	24 (22-30)
Peripheral oxygen saturation, %	92 (85-94)
Hemoglobin, g/dL	12.2 (10.0-14.0)
White blood cells, $\times 10^9$/L	9.8 (7.6-11.8)
Platelets, $\times 10^9$/L	210 (191-275)
eGFR, mL/min	49 (37-58)
eGFR <30 mL/min, N.	8 (14)
NT-proBNP, ng/mL	119 (96-157)
ST2, ng/mL	83 (52-140)

NYHA = New York Heart Association; eGFR = estimated glomerular filtration rate; NT-proBNP = N-terminal pro-B-type natriuretic peptide.

Table 1 summarizes the main demographic, clinical, and laboratory characteristics of the study population.

5.2. ST2 Concentrations according to NYHA Functional class

ST2 concentrations were compared across NYHA functional classes. Considering NYHA classes II–IV, median ST2 values were 84.47 ng/mL in NYHA II, 73.87 ng/mL in NYHA III, and 114.79 ng/mL in NYHA IV patients (Kruskal-Wallis $p = 0.027$). Patients with more advanced NYHA classes tended to exhibit higher ST2 concentrations, suggesting a relationship between elevated ST2 levels and greater clinical severity of acute heart failure. In particular, patients classified as NYHA IV demonstrated higher median ST2 concentrations and greater variability of values compared with patients presenting with lower NYHA classes. Furthermore, several elevated outliers were observed among patients with advanced NYHA classes, supporting the hypothesis that ST2 reflects both myocardial stress and disease severity in acute heart failure.

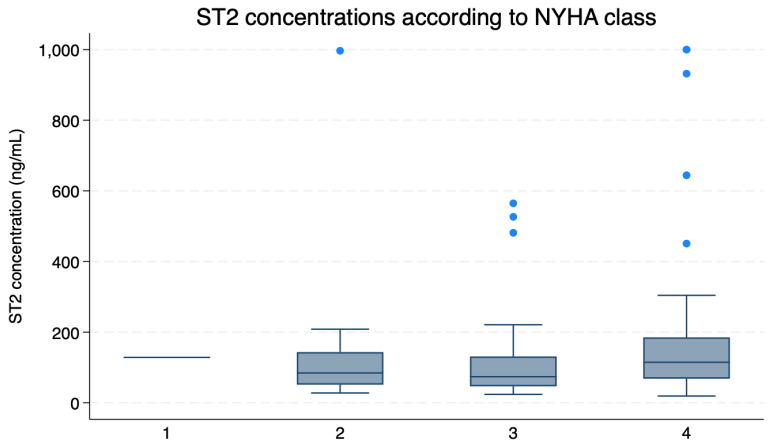

Figure 4: Distribution of ST2 concentrations according to NYHA functional class.

The distribution of ST2 concentrations according to NYHA functional class is illustrated in Figure 4.

5.3. Thirty-day survival analysis

Kaplan-Meier analysis demonstrated a progressive decline in survival probability during the 30-day follow-up period among patients admitted with acute heart failure. Although survival probability remained relatively high throughout the observation period, a gradual reduction over time was observed, corresponding to the occurrence of mortality events during follow-up. The estimated overall 30-day survival rate in the analyzed cohort was approximately 86%. 19 out of 137 patients died. These findings reflect the prognostic burden associated with acute heart failure and highlight the importance of early risk stratification using clinical and laboratory biomarkers.

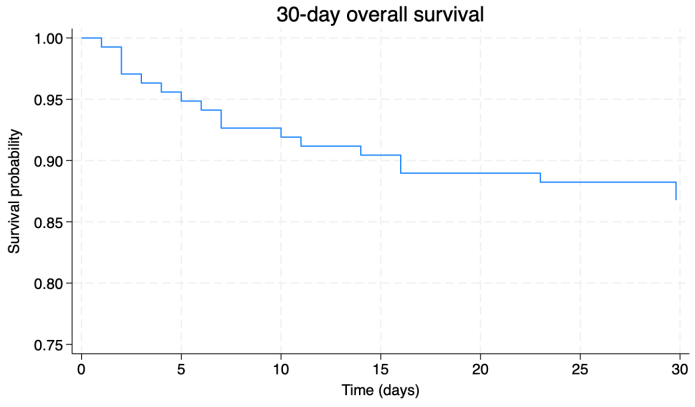

Figure 5: Kaplan-Meier survival curve showing 30-day overall survival in the study population.

Thirty-day overall survival was evaluated using Kaplan-Meier survival analysis, as shown in Figure 5.

5.4. Comparison between survivors and non-survivors

Patients died during follow-up presented significantly higher ST2 concentrations compared with survivors (285.767 vs 116.966 ng/mL, $p < 0.001$). Non-survivors also exhibited significantly higher heart rate values and a higher prevalence of chronic obstructive pulmonary disease (COPD).

Although systolic blood pressure tended to be lower among non-survivors, the difference did not reach statistical significance. Non-survivors were significantly older. No statistically significant differences were observed for BMI, creatinine, NT-proBNP, eGFR, diabetes mellitus, obesity, smoking status, or atrial fibrillation.

Overall, these findings support the potential prognostic role of ST2 in patients admitted with AHF.

Table 2: Comparison between survivors and non-survivors

*Continuous variables are presented as mean (SD), whereas categorical variables are expressed as frequencies (%). P values refer to comparison between survivors. NT-proBNP * was measured in a subgroup of 57 patients put of the total study population of 137 patients.*

Variable	**Survivors N=118**	**Non-survivors N=19**	**P value**
Age, years	80.65 (9.13)	84.32 (6.44)	**0.01**
BMI	27.19 (5.08)	26.62 (4.57)	0.65
Creatinine (mg/dL)	1.25 (0.63)	1.47 (0.89)	0.17
NT-proBNP *	122.67 (42.60)	141.75 (48.57)	0.16
ST2	116.97 (140.35)	285.77 (290.58)	**<0.001**
Heart rate	88.80 (21.57)	101.95 (26.18)	**0.018**
Systolic blood pressure	143.59 (26.33)	131.105 (23.48)	0.05
eGFR (mL/min)	52.91 (21.22)	49.32 (29.53)	0.52
Sex	0.55 (0.50)	0.53 (0.51)	0.53
Diabetes mellitus	0.41 (0.63)	0.26 (0.65)	0.33
Atrial fibrillation	0.48 (0.50)	0.58 (0.51)	0.42
COPD	0.12 (0.40)	0.47 (0.51)	**0.008**
Obesity	0.35 (0.48)	0.368 (0.50)	0.90
Smoking	0.96 (0.94)	0.79 (0.97)	0.47

The main clinical and laboratory characteristics of survivors and non-survivors at 30 days are summarized in Table 2.

5.5. Distribution of ST2 levels according to thirty-day mortality

The box plot illustrates the distribution of ST2 concentrations among survivors and non-survivors at 30-day follow-up. Patients died during follow-up presented substantially higher ST2 levels compared with survivors. The non-survivor group demonstrated greater variability and dispersion of ST2 values, with the presence of several elevated outliers. Notably, the median ST2 concentration in both groups was above the commonly reported prognostic reference threshold of 35 ng/mL, indicating that the majority of patients were at increased risk according to current evidence. However, ST2 levels were markedly higher among non-survivors, suggesting a greater degree of myocardial stress, inflammation, and adverse cardiac remodelling. These graphical findings are consistent with the statistical analysis of the study, in which ST2 was significantly associated with 30-day mortality ($p < 0.001$). Overall, the figure further supports the potential prognostic role of ST2 as a biomarker in patients admitted with AHF and highlights its ability to identify patients at particularly high risk of short-term mortality.

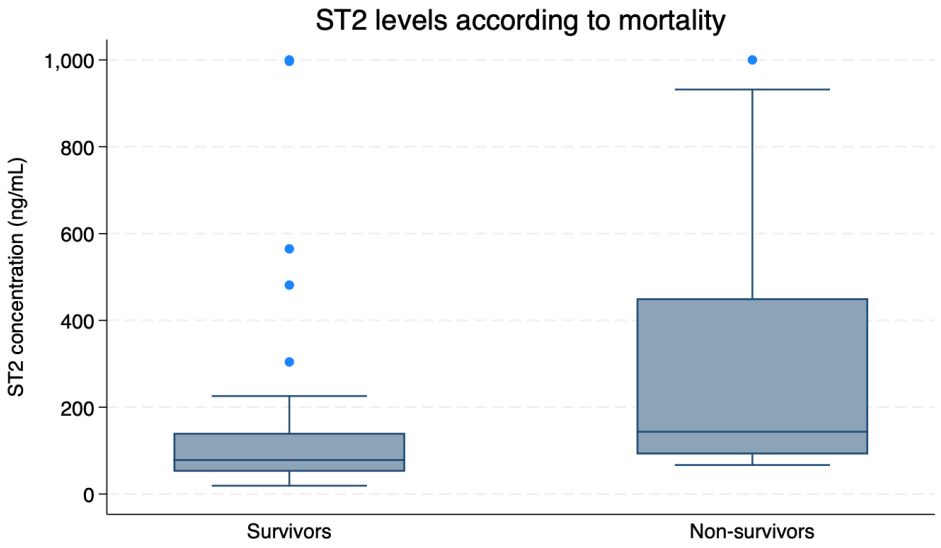

Figure 6: Distribution of ST2 concentrations according to 30-day mortality.

Figure 6 illustrates the distribution of ST2 concentrations according to 30-day mortality status.

5.6. *Multivariable logistic regression analysis*

Multivariable logistic regression analysis was performed to identify independent predictors of 30-day mortality among patients admitted with AHF. Among the analyzed variables, ST2 remained independently associated with mortality risk after adjustment for clinically relevant covariates (OR 1.003, 95% CI 1.001-1.006, p = 0.007).

Heart rate showed a borderline association with mortality, whereas age, eGFR, diabetes mellitus, and NYHA functional class were not independently associated with the outcome in the multivariable model. These findings support the potential prognostic role of ST2 as an independent biomarker in patients admitted with AHF.

Results are summarized in Table 3.

Table 3: Multivariable logistic regression analysis for 30-day mortality

Variable	**Odds Ratio (OR)**	**95% Confidence Interval**	**p-value**
ST2	1.003	1.001-1.006	**0.007**
Age	1.046	0.963-1.137	0.286
eGFR (mL/min)	1.002	0.972-1.034	0.876
Heart rate	1.022	0.999-1.046	0.052
Diabetes mellitus	0.708	0.269-1.865	0.485
NYHA class at admission	1.674	0.711-3.944	0.239

OR = Odds Ratio; CI = Confidence Interval. Variables with $p < 0.05$ were considered statistically significant.

5.7. *Stratification analysis according to median ST2 levels*

Patients were stratified according to the median ST2 concentration in order to obtain two numerically comparable groups. The median ST2 value was 83.19 ng/mL; therefore, the low-ST2 group included patients with ST2 values ≤83.19 ng/mL (n = 69), whereas the high-ST2 group included patients with ST2 values >83.19 ng/mL (n = 68). Clinical and biomarker characteristics of the two groups are summarized in Table 4.

Patients with ST2 concentrations above the median were significantly older and showed lower BMI compared with patients in the low-ST2 group. NT-proBNP concentrations tended to be higher in the high-ST2 group, although this difference did not reach statistical significance (p = 0.061). This finding should be interpreted with caution, since NT-proBNP values were available only for a subgroup of 57 patients.

Atrial fibrillation was significantly more frequent among patients with ST2 values above the median (p = 0.003), whereas COPD and smoking status did not significantly differ between groups. Obesity was more frequent in the low-ST2 group (p = 0.011). Regarding clinical outcomes, 30-day mortality was significantly higher in patients with ST2 concentrations above the median (p = 0.007). Overall, these findings suggest that elevated ST2 may identify a subgroup of patients with a more severe clinical profile and higher short-term mortality risk.

Table 4: Clinical and biomarker characteristics according to median ST2 levels

Continuous variables are expressed as median (IQR), while categorical variables are expressed as number (%).

Variable	ST2 ≤ median (n=69)	ST2 > median (n=68)	p-value
Age, years	80.09 (72.31–86.06)	84.29 (78.86–89.61)	**0.01**
BMI, kg/m²	28.90 (23.50–31.10)	25.90 (22.50–29.47)	**0.02**
Creatinine, mg/dL	1.03 (0.85–1.43)	1.20 (0.84–1.68)	0.35
eGFR, mL/min	52.00 (39.00–73.00)	45.00 (32.75–61.25)	0.05
NT-proBNP, ng/mL*	107.87 (90.14–151.60)	136.13 (104.55–170.39)	0.06
Male sex	40 (58.0%)	34 (50.0%)	0.39
Diabetes mellitus			0.51
No diabetes	47 (68.1%)	47 (69.1%)	
Non-complicated diabetes	18 (26.1%)	14 (20.6%)	
Diabetes with end-organ damage	4 (5.8%)	7 (10.3%)	
Atrial fibrillation	25 (36.2%)	42 (62.7%)	**0.003**
COPD	18 (26.5%)	14 (20.6%)	0.54
Obesity	32 (46.4%)	16 (24.2%)	**0.01**
Smoking status			0.68
No	31 (44.9%)	34 (50.7%)	
Active smoker	9 (13.0%)	6 (9.0%)	
Ex-smoker	29 (42.0%)	27 (40.3%)	
30-day mortality	4 (5.8%)	15 (22.1%)	**0.007**

*Patients were stratified according to the median ST2 concentration: ST2 ≤ 83.19 ng/mL and ST2 > 83.19 ng/mL. P values were calculated using the Mann-Whitney U test for continuous variables and Fisher's exact test or chi-square test for categorical variables, as appropriate. *NT-proBNP values were available only for 57 patients. BMI = body mass index; eGFR = estimated glomerular filtration rate; NT-proBNP = N-terminal pro-B-type natriuretic peptide; COPD = chronic obstructive pulmonary disease.*

Kaplan-Meier survival curves showing 30-day survival according to median ST2 levels. Patients with ST2 concentrations above the median value of 83.19 ng/mL showed lower survival probability compared with patients with ST2 values at or below the median. The difference between the survival curves was statistically significant according to the log-rank test (p = 0.0065).

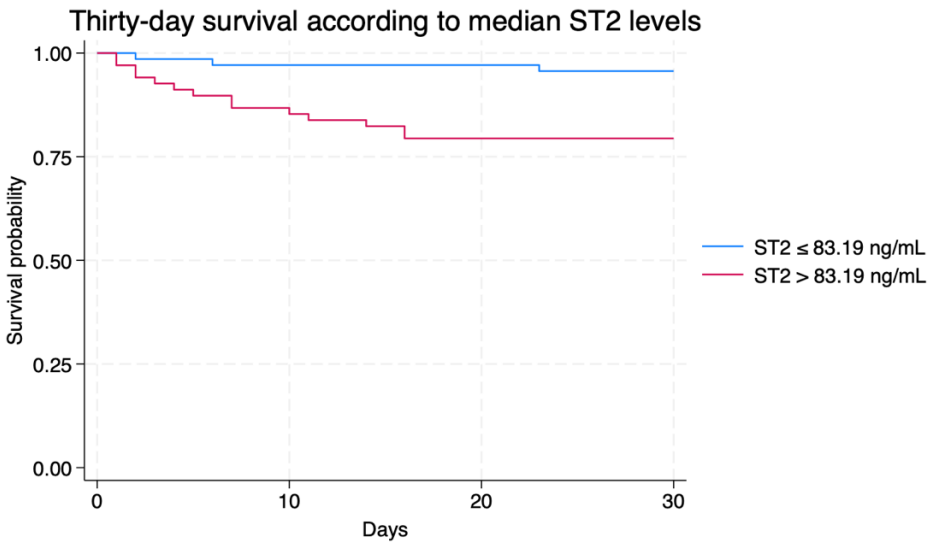

Figure 7: Kaplan-Meier survival curves according to median ST2 levels

Kaplan-Meier survival curves showing 30-day survival according to median ST2 levels. Patients with ST2 concentrations above the median value of 83.19 ng/mL showed lower survival probability compared with patients with ST2 values at or below the median. The difference between the survival curves was statistically significant according to the log-rank test (p = 0.0065).

6. Discussion

AHF is one of the most challenging clinical presentations in cardiovascular medicine. It is not only the abrupt worsening of cardiac pump function, but rather a systemic syndrome in which congestion, hypoperfusion, neurohormonal activation, inflammation, endothelial dysfunction and organ cross-talk interact with each other. For this reason, the prognosis of patients admitted with AHF remains unfavorable, especially during the early post-admission period, when the risk of death and rehospitalization is particularly high[7]. In this setting, early risk stratification is essential. Clinical evaluation remains the first step in patient assessment, but it may not fully capture the biological heterogeneity of AHF. This has encouraged the study of circulating biomarkers able to reflect different pathophysiological pathways and to provide prognostic information beyond standard clinical parameters[65 66]. The work evaluated the prognostic role of sST2 in patients admitted to the Emergency Department with AHF. The population analyzed in this study was clinically complex, elderly and highly comorbid, with a high prevalence of advanced NYHA functional class at admission. This profile is consistent with the real-world population typically hospitalized for AHF, in which frailty, cardiovascular comorbidities and non-cardiac diseases frequently coexist[67]. The main finding of the study was that circulating ST2 concentrations were markedly higher in patients who died during the 30-day follow-up compared with survivors. Furthermore, ST2 remained independently associated with mortality after adjustment for clinically relevant variables, supporting its potential value as a prognostic biomarker in this clinical setting.

This result is relevant because mortality in AHF is determined by multiple mechanisms that are not completely reflected by conventional biomarkers. NPs, particularly BNP and NT-proBNP, are established tools for the diagnosis and prognostic assessment of heart failure, and they remain central in current clinical practice[68]. However, they mainly reflect ventricular wall stress, volume overload and increased filling pressures[69]. Although these mechanisms are fundamental in heart failure, they do not fully describe other biological processes that become particularly important during acute decompensation, such as myocardial injury, inflammation, fibrosis, endothelial activation and adverse remodeling[70]. ST2 may add complementary information because it is linked to myocardial stress and inflammatory-fibrotic pathways, especially through the IL-33/ST2 axis[64]. The biological interpretation of ST2 is based on the presence of two main isoforms: the transmembrane receptor ST2L and the soluble circulating form, sST2. Under conditions of biomechanical stress, IL-33 binds to ST2L and activates protective pathways that can limit cardiomyocyte hypertrophy, fibrosis and apoptosis. Experimental studies have described this pathway as a cardioprotective fibroblast-cardiomyocyte signalling system activated in response to myocardial strain. By contrast, soluble ST2

acts as a decoy receptor. It binds IL-33 in the circulation and prevents its interaction with ST2L, thereby reducing the protective effect of IL-33/ST2L signalling[65]. In AHF, increased sST2 may therefore indicate a disruption of a protective pathway, with a shift toward fibrosis, inflammation and maladaptive remodeling. This mechanism provides a biologically plausible explanation for the association observed in our cohort between elevated ST2 levels and early mortality.

The prognostic relevance of ST2 has already been described in several studies involving both acute and chronic heart failure. In patients presenting with acute dyspnea, ST2 was shown to predict mortality and to provide prognostic information when considered together with NT-proBNP[71]. In patients with acute destabilized heart failure, increased plasma sST2 concentrations were associated with worse outcomes and higher mortality[72]. Manzano-Fernandez et al. reported that soluble ST2 concentrations predicted mortality in patients with acutely decompensated heart failure[73]; other studies also support the role of ST2 in multi-marker risk stratification, especially when combined with biomarkers reflecting different domains, such as NPs[74 75]. These data suggest that ST2 should not be interpreted as a replacement for established biomarkers, but rather as an additional tool able to describe a different component of risk. A particularly important aspect of the present study is the comparison between ST2 and NT-proBNP. In our cohort, NT-proBNP values were numerically higher in non-survivors than in survivors, but the difference did not reach statistical significance. In contrast, ST2 showed a strong association with 30-day mortality. This finding does not reduce the clinical importance of NT-proBNP, which remains one of the most validated biomarkers for heart failure diagnosis and prognosis[68 69]; it suggests that in this specific cohort ST2 may have captured a dimension of risk that was not fully represented by NT-proBNP. Several explanations may be considered. NT-proBNP is influenced by age, renal function, atrial fibrillation, body mass index and other conditions that are very common among patients admitted with AHF[7]. ST2, although not completely free from biological variability, appears to be less dependent on some of these confounders, particularly renal function and body mass index[76]. The interpretation of NT-proBNP in the present cohort requires caution, since this biomarker was available only in a subgroup of 57 patients out of the total population of 137. Although higher NT-proBNP concentrations in patients with elevated ST2 suggest a possible relationship between ST2 elevation and greater haemodynamic burden, the incomplete availability of NT-proBNP data limits the generalizability of this observation and reduces its statistical robustness. For this reason, the comparison between ST2 and NT-proBNP should be considered exploratory rather than definitive[61].The relationship between ST2 and NT-proBNP should therefore be considered complementary rather than competitive. NT-proBNP mainly reflects haemodynamic stress, whereas ST2 may better reflect the downstream consequences of cardiac stress, including inflammation, extracellular matrix activation and tissue remodeling[66]. This

distinction is clinically meaningful: two patients may have comparable natriuretic peptide concentrations but different degrees of myocardial injury, systemic inflammation, pulmonary disease, renal reserve and frailty. In such cases, ST2 may help to identify an additional layer of biological vulnerability. This interpretation is consistent with the growing interest in multi-marker approaches for AHF, in which each biomarker contributes information on a specific pathophysiological domain[74]. The concept of multi-marker risk stratification is particularly appropriate for AHF because the syndrome cannot be reduced to a single mechanism. NPs reflect congestion and wall stress; troponins indicate myocardial injury; inflammatory markers such as CRP or IL-6 reflect immune activation; renal biomarkers describe kidney involvement; and biomarkers such as ST2 are related to fibrosis and remodeling[77]. The results of this thesis are aligned with this view and support the idea that ST2 may contribute to a more complete biological characterization of patients admitted with AHF. The baseline profile of the present cohort confirms the clinical complexity of patients admitted with acute decompensated heart failure. Most patients were elderly, highly comorbid and frequently presented with advanced functional limitation at admission[78]. This pattern is consistent with the typical real-world population hospitalized for AHF, in which frailty, cardiovascular disease and non-cardiac comorbidities often coexist and may contribute to early clinical deterioration[79].

Another element that should be considered is the timing of ST2 measurement. In this study, blood samples were collected at baseline, at the time of presentation to the Emergency Department. This is a clinically relevant moment, because the first hours after admission represent a critical window in which physicians must rapidly estimate the patient risk and decide the intensity of monitoring and treatment. A biomarker measured at admission may be useful if it is able to identify biological stress before the full clinical trajectory becomes evident. In this perspective, elevated ST2 at presentation may indicate that the acute episode is not limited to hemodynamic congestion, but is accompanied by more intense systemic activation and adverse myocardial remodeling.

The marked difference in ST2 concentrations between survivors and non-survivors is therefore not only a statistical result, but also a clinically meaningful observation. The box plot distribution showed higher values and greater dispersion among patients who died during follow-up, suggesting that some non-survivors had extremely elevated ST2 concentrations. This variability is not surprising, because AHF is a heterogeneous syndrome that may arise from different underlying mechanisms, including ischemic disease, arrhythmias, uncontrolled hypertension, valvular disease, infection, renal dysfunction or poor therapeutic adherence. In this context, very high ST2 values may identify patients with a particularly severe biological phenotype, characterized by intense myocardial stress and systemic inflammatory-fibrotic activation. The independent association between ST2 and mortality in the multivariable logistic regression model further supports the relevance of this biomarker. ST2

remained significantly associated with mortality after adjustment for age, eGFR, heart rate, diabetes mellitus and NYHA class. This means that the prognostic information provided by ST2 was not fully explained by older age, renal dysfunction, clinical severity or metabolic comorbidity. Although the odds ratio per unit increase may appear numerically small, this is expected when a biomarker with a wide range of values is analyzed as a continuous variable. When ST2 concentrations increase substantially, as observed in several non-survivors, the cumulative effect on risk becomes more relevant. This point is important when interpreting biomarkers whose clinical impact becomes clearer across larger concentration differences.

The relationship between ST2 and NYHA functional class also deserves discussion. In our analysis, patients with more advanced NYHA class tended to show higher ST2 concentrations, particularly those classified as NYHA IV. NYHA class is based on symptoms and functional limitation, and it remains a useful clinical indicator of disease severity and prognosis[67]. The tendency toward higher ST2 values in patients with more severe NYHA class suggests that ST2 may reflect not only molecular remodeling, but also the clinical burden of disease. However, NYHA class did not remain independently associated with 30-day mortality in the multivariable model. This may be explained by the limited sample size, by the high proportion of patients already presenting with advanced NYHA classes, and by the partly subjective nature of symptom-based classification. In elderly and comorbid patients, dyspnea and exercise limitation may be influenced by COPD, anemia, obesity, frailty or deconditioning, and not only by heart failure severity. Heart rate was significantly higher in non-survivors and showed a borderline association with mortality in the multivariable model. This finding is clinically plausible. In AHF, tachycardia may reflect sympathetic activation, reduced stroke volume, respiratory distress, hypoxia, pain, anxiety, infection or arrhythmias. Initially, an increased heart rate may help maintain cardiac output when stroke volume is reduced. However, persistent tachycardia may become harmful by increasing myocardial oxygen demand, shortening diastolic filling time and reducing cardiac efficiency. In elderly patients, and particularly in those with atrial fibrillation, high heart rate may also indicate limited cardiovascular reserve. Previous studies have suggested that heart rate during hospitalization or at discharge may have prognostic relevance in acute decompensated heart failure, although this association is less established than in CHF[80].

Another relevant finding was the higher prevalence of COPD among non-survivors. The coexistence of COPD and heart failure is common and clinically challenging. Both diseases can cause dyspnea, reduced exercise tolerance and hypoxemia, making clinical assessment more complex. COPD may worsen heart failure through chronic hypoxia, pulmonary hypertension, right ventricular overload, systemic inflammation and a higher risk of respiratory infections. COPD can complicate treatment, especially with regard to beta-blockers, bronchodilators, oxygen therapy and non-invasive

ventilation. Previous evidence has shown that COPD is associated with worse outcomes in patients hospitalized for acute decompensated heart failure[81]. In our cohort, COPD was significantly more frequent among non-survivors, suggesting that pulmonary vulnerability may have contributed to early mortality.

Atrial fibrillation was also frequent in the study population. This is consistent with the known relationship between atrial fibrillation and heart failure, two conditions that often coexist and worsen each other. Atrial fibrillation may precipitate acute decompensation by increasing heart rate, reducing atrial contribution to ventricular filling and worsening haemodynamic stability. At the same time, chronic pressure and volume overload in heart failure promotes atrial remodeling and favours the development of arrhythmias[82]. In our stratification analysis, atrial fibrillation was more frequent among patients with higher ST2 levels, which may reflect a more advanced structural and remodeling phenotype. However, atrial fibrillation was not significantly associated with mortality in the survivor versus non-survivor comparison, possibly because of its high overall prevalence in the cohort, reducing its ability to discriminate risk between groups. The stratification analysis provides an additional clinical interpretation of elevated ST2 levels. The stratification analysis provides an additional clinical interpretation of elevated ST2 levels. Patients with ST2 concentrations above the median showed a higher prevalence of atrial fibrillation and higher 30-day mortality, while COPD did not significantly differ between the two groups. This suggests that increased ST2 may identify a phenotype characterized by greater cardiac stress, arrhythmic burden and short-term vulnerability, rather than by pulmonary comorbidity alone. This suggests that increased ST2 may identify a phenotype characterized by more pronounced cardiac stress together with arrhythmic and pulmonary comorbidity. Such interpretation is coherent with the concept of AHF as a systemic syndrome, in which cardiac dysfunction, inflammation, pulmonary involvement and comorbid conditions interact and contribute to short-term vulnerability[61].

Age was higher among non-survivors but did not reach statistical significance. This may appear unexpected, since advanced age is one of the strongest predictors of adverse outcomes in HF[67]. However, the entire cohort was already elderly, with a median age above 80 years. In this context, the limited age difference between groups may have reduced the possibility of detecting a significant association. In older patients, chronological age alone may not be sufficient to explain prognosis. Frailty, comorbidity burden, nutritional status, cognitive function, renal reserve and inflammatory activation may be more informative than age considered as an isolated variable. This may explain why a biomarker such as ST2, which reflects biological stress and remodeling, remained associated with mortality even when age did not. Renal function is another key aspect in AHF. Cardiorenal interactions are central in the pathophysiology of decompensation, and renal dysfunction is usually

associated with worse outcomes[70]. In our study, eGFR did not differ significantly between survivors and non-survivors and was not independently associated with mortality. This finding should be interpreted with caution. A single baseline eGFR value may not fully reflect dynamic renal changes occurring during hospitalization. In AHF, venous congestion may impair renal function even in the absence of severe baseline renal disease, and creatinine-based measures may not capture early tubular injury. Serial measurements of renal function and the inclusion of tubular injury biomarkers could provide a more complete assessment of the relationship between renal impairment, ST2 and prognosis. The short term nature of the outcome evaluated in this thesis is also important. Thirty-day mortality is a clinically relevant endpoint because it reflects early vulnerability after an acute episode, death occurring within the first month may be related to persistent congestion, arrhythmias, respiratory complications, myocardial injury or failure to recover from the initial decompensation. Biomarkers measured at admission may be particularly informative for this endpoint, because they reflect the biological condition of the patient at the beginning of the acute event. At the same time, longer follow-up would be useful in future studies, because ST2 has also been associated with medium and long term outcomes, including cardiovascular mortality and rehospitalization[83]. The potential value of serial ST2 measurements should also be considered. In the present study, ST2 was measured only at baseline. This approach is practical and provides useful prognostic information, but it does not allow evaluation of biomarker kinetics during hospitalization. Previous studies suggest that changes in ST2 concentrations over time may improve prognostic accuracy and may reflect response to therapy or persistent biological stress[84]. A patient whose ST2 levels decrease during hospitalization may have a different prognosis from a patient whose levels remain elevated despite symptomatic improvement. This could be clinically relevant because standard parameters may improve rapidly after diuretic therapy, while molecular remodeling and inflammatory activation may persist. ST2 is not only a laboratory value, but the circulating expression of a specific biological pathway. Its measurement connects molecular mechanisms, including cytokine signalling, receptor decoy activity, inflammation and fibrosis, with clinical outcomes. This represents a clear example of translational medicine, in which a molecular biomarker may contribute to the interpretation of a complex clinical syndrome. In AHF, where patients are highly heterogeneous, biomarkers may help move from a purely symptom-based classification toward a more biologically informed risk assessment.

The occurrence of deaths within the first 30 days further emphasizes the unfavorable short-term prognosis associated with AHF. Although most patients survived during follow-up, early mortality in this setting reflects the high clinical vulnerability that characterizes acute decompensation[70]. This finding supports the need for early risk stratification based on both clinical variables and circulating

biomarkers, in order to identify patients who may require closer monitoring and more individualized management[7].

The ELISA-based measurement of ST2 also has practical implications. ELISA assays allow quantitative assessment of circulating proteins and are widely used in biomedical research. In this thesis, the use of the Presage ST2 assay allowed the measurement of plasma ST2 concentrations in a standardized experimental setting. However, for routine clinical implementation, biomarker assays must be reproducible, rapid and cost-effective. This is especially important in the Emergency Department, where turnaround time may influence clinical decisions. Although ELISA is appropriate for experimental and research purposes, the translation of ST2 into routine acute care would require platforms able to provide reliable results within a clinically useful timeframe[64].

The clinical implications of our findings should be interpreted with balance. ST2 could be incorporated into risk stratification models rather than used as a stand-alone marker. A patient with AHF, elevated ST2, advanced age, atrial fibrillation, high heart rate or advanced NYHA class may represent a particularly vulnerable phenotype, even when natriuretic peptide values do not fully capture the overall clinical risk. In our cohort, patients were stratified according to the median ST2 value, which allowed two numerically comparable groups to be obtained. However, this cut-off is data-driven and should therefore be interpreted as exploratory rather than as a validated clinical threshold. The marked difference in ST2 concentrations between survivors and non-survivors provides one of the strongest elements supporting its prognostic relevance in this cohort. The graphical distribution of ST2 according to 30-day mortality further reinforces this observation, since patients who died showed not only higher values but also wider dispersion, suggesting a heterogeneous but generally more severe biological profile. These findings are in line with previous studies showing that sST2 is associated with adverse outcomes in acute and chronic heart failure and may help identify patients at increased short-term risk[61]. This result was further supported by the Kaplan-Meier analysis performed according to median ST2 levels, which showed significantly lower 30-day survival in patients with ST2 concentrations above 83.19 ng/mL compared with those with lower values.

It is also important to avoid overinterpreting ST2 as a diagnostic biomarker. Natriuretic peptides remain superior for the initial diagnostic evaluation of suspected heart failure, particularly in patients presenting with acute dyspnea[69]. ST2 appears more useful for prognostic assessment once AHF has

been diagnosed[76]. This distinction is essential. The value of ST2 lies not in confirming the diagnosis, but in helping to identify patients at higher risk of adverse outcomes. Therefore, ST2 may be most useful when interpreted together with clinical presentation, echocardiographic parameters, natriuretic peptides, renal function, troponin and relevant comorbidities. Whether ST2 could guide therapy remains an open question. At present, evidence mainly supports ST2 as a prognostic biomarker rather than as a direct therapeutic guide. Some studies suggest that serial changes in ST2 may reflect treatment response, but interventional evidence is still limited[84]. Unlike natriuretic peptides, which have been more extensively investigated in therapy-guided strategies, ST2 guided management is not yet established in routine practice. Nevertheless, the identification of patients with markedly elevated ST2 could indirectly influence clinical management by encouraging careful optimization of guideline-directed therapy, closer follow-up and a more individualized discharge plan.

This study has several strengths. First, it was conducted in a representative population of patients (elderly, comorbid and clinically complex) admitted for AHF, making the findings clinically relevant. Indeed, in this type of population, risk stratification is often difficult, and biomarkers reflecting biological severity may be particularly valuable. Second, ST2 was measured using a quantitative ELISA-based method, which is appropriate for experimental biomarker evaluation. Third, the study focused on 30-day mortality, an endpoint of clear clinical importance in AHF. Fourth, the multivariable analysis included clinically meaningful covariates, allowing assessment of whether ST2 provided prognostic information beyond standard clinical variables. Importantly, the association between ST2 and mortality persisted after adjustment for clinically relevant variables. This supports the hypothesis that ST2 provides prognostic information beyond traditional clinical parameters and may reflect biological mechanisms not fully captured by age, renal function, heart rate, diabetes mellitus or NYHA functional class. Therefore, in this cohort, ST2 appears to behave as an independent biomarker of early adverse outcome in patients admitted with AHF[60].

Several limitations must be acknowledged. The first limitation is the single-center design, which may reduce the generalizability of the results. Patients were recruited from one hospital, and local characteristics related to patient population, admission pathways and clinical management may have influenced the findings. The second limitation is the relatively limited sample size, particularly the number of deaths during follow-up. This may reduce statistical power and increase uncertainty in the multivariable model. Therefore, the results should be interpreted as hypothesis-generating rather than definitive. The third limitation is the short follow-up period. Although 30-day mortality is clinically relevant, it does not provide information on longer term outcomes such as rehospitalization, cardiovascular mortality or one-year survival. Additional limitations should also be considered: ST2 was measured only at baseline, preventing analysis of biomarker changes during hospitalization or

after treatment. The study did not include a complete multi-marker panel including troponin, CRP, IL-6, which would have allowed a more detailed comparison between different biological pathways. Echocardiographic parameters, frailty measures, details of in-hospital therapy and discharge treatment were not fully integrated into the prognostic model. Despite these limitations, the results provide useful evidence supporting the prognostic role of ST2 in AHF. Some subgroup findings should be interpreted with particular caution: Some subgroup findings should be interpreted with caution. In the stratification analysis, patients were divided according to the median ST2 value in order to obtain two numerically comparable groups. This approach allowed a more balanced comparison than the conventional 35 ng/mL cut-off, which would have classified only a small number of patients as having low ST2 levels in this cohort. Patients with ST2 values above the median showed higher 30-day mortality compared with those with ST2 values at or below the median, and this finding was further supported by the Kaplan-Meier survival analysis. However, the use of a data-driven cut-off should be considered exploratory and requires external validation in larger cohorts. Similarly, associations involving NT-proBNP should be interpreted carefully, because NT-proBNP values were available only for a subgroup of patients rather than for the entire cohort. Overall, these elements do not weaken the main finding regarding ST2 and mortality, but they indicate that subgroup analyses require confirmation in larger and more complete datasets.

Future studies should validate these findings in larger and multicenter cohorts. It would be useful to evaluate ST2 together with NT-proBNP, high-sensitivity troponin, inflammatory markers and renal biomarkers, in order to build integrated risk models. Such an approach could clarify whether ST2 improves prognostic prediction beyond established scores and whether it identifies specific biological phenotypes of AHF. Serial measurements should be performed to determine whether changes in ST2 during hospitalization are associated with treatment response and post discharge outcomes. Finally, prospective studies would be needed to determine whether ST2 guided management can improve clinical prognosis.

In conclusion, the present study supports the prognostic relevance of soluble ST2 in patients admitted with AHF. ST2 concentrations were significantly higher in patients who died during follow-up and remained independently associated with mortality after adjustment for clinically relevant variables. These findings are consistent with the biological role of ST2 as a marker of myocardial stress, inflammation, fibrosis and adverse remodeling, and with previous evidence supporting its prognostic value in AHF[71]. Although NT-proBNP remains a cornerstone biomarker for diagnosis and risk assessment, ST2 may provide complementary prognostic information, particularly for early risk

stratification. The integration of ST2 into a broader clinical and biomarker-based approach may contribute to a more precise and individualized evaluation of patients with acute heart failure[76].

7. Bibliography

1. Virani, S.S., Alonso, A., Benjamin, E.J., Bittencourt, M.S., Callaway, C.W., Carson, A.P., Chamberlain, A.M., Chang, A.R., Cheng, S., Delling, F.N., et al. (2020). Heart Disease and Stroke Statistics—2020 Update: A Report From the American Heart Association. Circulation *141*. https://doi.org/10.1161/CIR.0000000000000757.

2. Tsutsui, H., Kinugawa, S., and Matsushima, S. (2011). Oxidative stress and heart failure. Am. J. Physiol.-Heart Circ. Physiol. *301*, H2181–H2190. https://doi.org/10.1152/ajpheart.00554.2011.

3. Fair, J.M., and Berra, K. (1995). Life–style changes and coronary heart disease: The influence of nonpharmacologic interventions: J. Cardiovasc. Nurs. *9*, 12–24. https://doi.org/10.1097/00005082-199501000-00003.

4. Oneglia, A., Nelson, M.D., and Merz, C.N.B. (2020). Sex Differences in Cardiovascular Aging and Heart Failure. Curr. Heart Fail. Rep. *17*, 409–423. https://doi.org/10.1007/s11897-020-00487-7.

5. Lam, C.S.P., and Yancy, C. (2021). Universal Definition and Classification of Heart Failure: Is It universal? Does It Define Heart Failure? J. Card. Fail. *27*, 509–511. https://doi.org/10.1016/j.cardfail.2021.03.003.

6. Potter, L.R., Yoder, A.R., Flora, D.R., Antos, L.K., and Dickey, D.M. (2009). Natriuretic Peptides: Their Structures, Receptors, Physiologic Functions and Therapeutic Applications. In cGMP: Generators, Effectors and Therapeutic Implications Handbook of Experimental Pharmacology., H. H. H. W. Schmidt, F. Hofmann, and J.-P. Stasch, eds. (Springer Berlin Heidelberg), pp. 341–366. https://doi.org/10.1007/978-3-540-68964-5_15.

7. McDonagh, T.A., Metra, M., Adamo, M., Gardner, R.S., Baumbach, A., Böhm, M., Burri, H., Butler, J., Čelutkienė, J., Chioncel, O., et al. (2021). 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur. Heart J. *42*, 3599–3726. https://doi.org/10.1093/eurheartj/ehab368.

8. Pieske, B., Tschöpe, C., De Boer, R.A., Fraser, A.G., Anker, S.D., Donal, E., Edelmann, F., Fu, M., Guazzi, M., Lam, C.S.P., et al. (2019). How to diagnose heart failure with preserved ejection fraction: the HFA–PEFF diagnostic algorithm: a consensus recommendation from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC). Eur. Heart J. *40*, 3297–3317. https://doi.org/10.1093/eurheartj/ehz641.

9. McMurray, J.J., and Pfeffer, M.A. (2005). Heart failure. The Lancet *365*, 1877–1889. https://doi.org/10.1016/S0140-6736(05)66621-4.

10. Hartupee, J., and Mann, D.L. (2017). Neurohormonal activation in heart failure with reduced ejection fraction. Nat. Rev. Cardiol. *14*, 30–38. https://doi.org/10.1038/nrcardio.2016.163.

11. Rubattu, S., and Triposkiadis, F. (2017). Resetting the neurohormonal balance in heart failure (HF): the relevance of the natriuretic peptide (NP) system to the clinical management of patients with HF. Heart Fail. Rev. *22*, 279–288. https://doi.org/10.1007/s10741-017-9605-8.

12. Heidenreich, P.A., Bozkurt, B., Aguilar, D., Allen, L.A., Byun, J.J., Colvin, M.M., Deswal, A., Drazner, M.H., Dunlay, S.M., Evers, L.R., et al. (2022). 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation *145*. https://doi.org/10.1161/CIR.0000000000001063.

13. Asano, R., Mathai, S.C., Macdonald, P.S., Newton, P.J., Currow, D.C., Phillips, J., Yeung, W.-F., and Davidson, P.M. (2020). Oxygen use in chronic heart failure to relieve breathlessness: A systematic review. Heart Fail. Rev. *25*, 195–205. https://doi.org/10.1007/s10741-019-09814-0.

14. Arrigo, M., Jessup, M., Mullens, W., Reza, N., Shah, A.M., Sliwa, K., and Mebazaa, A. (2020). Acute heart failure. Nat. Rev. Dis. Primer *6*, 16. https://doi.org/10.1038/s41572-020-0151-7.

15. Bayes-Genis, A., Docherty, K.F., Petrie, M.C., Januzzi, J.L., Mueller, C., Anderson, L., Bozkurt, B., Butler, J., Chioncel, O., Cleland, J.G.F., et al. (2023). Practical Algorithms for Early Diagnosis of Heart Failure and Heart Stress Using NT-proBNP: A Clinical Consensus Statement from the Heart Failure Association of the ESC. Eur. J. Heart Fail. *25*, 1891–1898. https://doi.org/10.1002/ejhf.3036.

16. Ammirati, E., Frigerio, M., Adler, E.D., Basso, C., Birnie, D.H., Brambatti, M., Friedrich, M.G., Klingel, K., Lehtonen, J., Moslehi, J.J., et al. (2020). Management of Acute Myocarditis and Chronic Inflammatory Cardiomyopathy: An Expert Consensus Document. Circ. Heart Fail. *13*, e007405. https://doi.org/10.1161/CIRCHEARTFAILURE.120.007405.

17. Ewer, M.S., and Ewer, S.M. (2015). Cardiotoxicity of anticancer treatments. Nat. Rev. Cardiol. *12*, 547–558. https://doi.org/10.1038/nrcardio.2015.65.

18. Kurmani, S., and Squire, I. (2017). Acute Heart Failure: Definition, Classification and Epidemiology. Curr. Heart Fail. Rep. *14*, 385–392. https://doi.org/10.1007/s11897-017-0351-y.

19. Sabbah, H.N. (2017). Pathophysiology of acute heart failure syndrome: a knowledge gap. Heart Fail. Rev. *22*, 621–639. https://doi.org/10.1007/s10741-017-9651-2.

20. Peacock, W.F., De Marco, T., Fonarow, G.C., Diercks, D., Wynne, J., Apple, F.S., and Wu, A.H.B. (2008). Cardiac Troponin and Outcome in Acute Heart Failure. N. Engl. J. Med. *358*, 2117–2126. https://doi.org/10.1056/NEJMoa0706824.

21. Thygesen, K. (2018). What's new in the Fourth Universal Definition of Myocardial infarction? Eur. Heart J. *39*, 3757–3758. https://doi.org/10.1093/eurheartj/ehy655.

22. Metra, M., Felker, G.M., Zacà, V., Bugatti, S., Lombardi, C., Bettari, L., Voors, A.A., Gheorghiade, M., and Dei Cas, L. (2010). Acute heart failure: Multiple clinical profiles and mechanisms require tailored therapy. Int. J. Cardiol. *144*, 175–179. https://doi.org/10.1016/j.ijcard.2010.04.003.

23. Savarese, G., Becher, P.M., Lund, L.H., Seferovic, P., Rosano, G.M.C., and Coats, A.J.S. (2023). Global burden of heart failure: a comprehensive and updated review of epidemiology. Cardiovasc. Res. *118*, 3272–3287. https://doi.org/10.1093/cvr/cvac013.

24. Ho, K.K.L., Pinsky, J.L., Kannel, W.B., and Levy, D. (1993). The epidemiology of heart failure: The Framingham Study. J. Am. Coll. Cardiol. *22*, A6–A13. https://doi.org/10.1016/0735-1097(93)90455-A.

25. Alla, F., Zannad, F., and Filippatos, G. (2007). Epidemiology of acute heart failure syndromes. Heart Fail. Rev. *12*, 91–95. https://doi.org/10.1007/s10741-007-9009-2.

26. Teerlink, J., Alburikan, K., Metra, M., and Rodgers, J. (2014). Acute Decompensated Heart Failure Update. Curr. Cardiol. Rev. *11*, 53–62. https://doi.org/10.2174/1573403X09666131117174414.

27. Groenewegen, A., Rutten, F.H., Mosterd, A., and Hoes, A.W. (2020). Epidemiology of Heart Failure. Eur. J. Heart Fail. *22*, 1342–1356. https://doi.org/10.1002/ejhf.1858.

28. Cook, C., Cole, G., Asaria, P., Jabbour, R., and Francis, D.P. (2014). The annual global economic burden of heart failure. Int. J. Cardiol. *171*, 368–376. https://doi.org/10.1016/j.ijcard.2013.12.028.

29. Savarese, G., and Lund, L.H. (2017). Global Public Health Burden of Heart Failure. Card. Fail. Rev. *3*, 7–11. https://doi.org/10.15420/cfr.2016:25:2.

30. Do Vale, G.T., Ceron, C.S., Gonzaga, N.A., Simplicio, J.A., and Padovan, J.C. (2019). Three Generations of β-blockers: History, Class Differences and Clinical

Applicability. Curr. Hypertens. Rev. *15*, 22–31. https://doi.org/10.2174/1573402114666180918102735.

31. Momoniat, T., Ilyas, D., and Bhandari, S. (2019). ACE inhibitors and ARBs: Managing potassium and renal function. Cleve. Clin. J. Med. *86*, 601–607. https://doi.org/10.3949/ccjm.86a.18024.

32. Vallon, V., and Verma, S. (2021). Effects of SGLT2 Inhibitors on Kidney and Cardiovascular Function. Annu. Rev. Physiol. *83*, 503–528. https://doi.org/10.1146/annurev-physiol-031620-095920.

33. Ronco, C., McCullough, P., Anker, S.D., Anand, I., Aspromonte, N., Bagshaw, S.M., Bellomo, R., Berl, T., Bobek, I., Cruz, D.N., et al. (2010). Cardio-renal syndromes: report from the consensus conference of the Acute Dialysis Quality Initiative. Eur. Heart J. *31*, 703–711. https://doi.org/10.1093/eurheartj/ehp507.

34. Rangaswami, J., Bhalla, V., Blair, J.E.A., Chang, T.I., Costa, S., Lentine, K.L., Lerma, E.V., Mezue, K., Molitch, M., Mullens, W., et al. (2019). Cardiorenal Syndrome: Classification, Pathophysiology, Diagnosis, and Treatment Strategies: A Scientific Statement From the American Heart Association. Circulation *139*. https://doi.org/10.1161/CIR.0000000000000664.

35. Lindow, T., Quadrelli, S., and Ugander, M. (2023). Noninvasive Imaging Methods for Quantification of Pulmonary Edema and Congestion. JACC Cardiovasc. Imaging *16*, 1469–1484. https://doi.org/10.1016/j.jcmg.2023.06.023.

36. Fortea, J.I., Puente, Á., Cuadrado, A., Huelin, P., Pellón, R., González Sánchez, F.J., Mayorga, M., Cagigal, M.L., García Carrera, I., Cobreros, M., et al. (2020). Congestive Hepatopathy. Int. J. Mol. Sci. *21*, 9420. https://doi.org/10.3390/ijms21249420.

37. Cannon, J.A., Moffitt, P., Perez-Moreno, A.C., Walters, M.R., Broomfield, N.M., McMurray, J.J.V., and Quinn, T.J. (2017). Cognitive Impairment and Heart Failure: Systematic Review and Meta-Analysis. J. Card. Fail. *23*, 464–475. https://doi.org/10.1016/j.cardfail.2017.04.007.

38. Boulet, J., Sridhar, V.S., Bouabdallaoui, N., Tardif, J.-C., and White, M. (2024). Inflammation in heart failure: pathophysiology and therapeutic strategies. Inflamm. Res. *73*, 709–723. https://doi.org/10.1007/s00011-023-01845-6.

39. Garofalo, M., Corso, R., Tomasoni, D., Adamo, M., Lombardi, C.M., Inciardi, R.M., Gussago, C., Di Mario, C., Metra, M., and Pagnesi, M. (2023). Inflammation in acute heart failure. Front. Cardiovasc. Med. *10*, 1235178. https://doi.org/10.3389/fcvm.2023.1235178.

40. Tang, W.H.W., Kitai, T., and Hazen, S.L. (2017). Gut Microbiota in Cardiovascular Health and Disease. Circ. Res. *120*, 1183–1196. https://doi.org/10.1161/CIRCRESAHA.117.309715.

41. Vallabhajosyula, S., Dunlay, S.M., Prasad, A., Kashani, K., Sakhuja, A., Gersh, B.J., Jaffe, A.S., Holmes, D.R., and Barsness, G.W. (2019). Acute Noncardiac Organ Failure in Acute Myocardial Infarction With Cardiogenic Shock. J. Am. Coll. Cardiol. *73*, 1781–1791. https://doi.org/10.1016/j.jacc.2019.01.053.

42. Mann, D.L. (2015). Innate Immunity and the Failing Heart: The Cytokine Hypothesis Revisited. Circ. Res. *116*, 1254–1268. https://doi.org/10.1161/CIRCRESAHA.116.302317.

43. Capone, F., Vacca, A., Bidault, G., Sarver, D., Kaminska, D., Strocchi, S., Vidal-Puig, A., Greco, C.M., Lusis, A.J., and Schiattarella, G.G. (2025). Decoding the Liver-Heart Axis in Cardiometabolic Diseases. Circ. Res. *136*, 1335–1362. https://doi.org/10.1161/CIRCRESAHA.125.325492.

44. Alogna, A., Koepp, K.E., Sabbah, M., Espindola Netto, J.M., Jensen, M.D., Kirkland, J.L., Lam, C.S.P., Obokata, M., Petrie, M.C., Ridker, P.M., et al. (2023). Interleukin-6 in Patients With Heart Failure and Preserved Ejection Fraction. JACC Heart Fail. *11*, 1549–1561. https://doi.org/10.1016/j.jchf.2023.06.031.

45. Poelzl, G., Ess, M., Mussner-Seeber, C., Pachinger, O., Frick, M., and Ulmer, H. (2012). Liver dysfunction in chronic heart failure: prevalence, characteristics and prognostic significance. Eur. J. Clin. Invest. *42*, 153–163. https://doi.org/10.1111/j.1365-2362.2011.02573.x.

46. Biegus, J., Zymliński, R., Sokolski, M., Nawrocka, S., Siwołowski, P., Szachniewicz, J., Jankowska, E.A., Banasiak, W., and Ponikowski, P. (2012). Liver function tests in patients with acute heart failure. Pol. Arch. Intern. Med. *122*, 471–479. https://doi.org/10.20452/pamw.1413.

47. Hanna, A., and Frangogiannis, N.G. (2020). Inflammatory Cytokines and Chemokines as Therapeutic Targets in Heart Failure. Cardiovasc. Drugs Ther. *34*, 849–863. https://doi.org/10.1007/s10557-020-07071-0.

48. McKie, P.M., and Burnett, J.C. (2016). NT-proBNP. J. Am. Coll. Cardiol. *68*, 2437–2439. https://doi.org/10.1016/j.jacc.2016.10.001.

49. Fountoulaki, K., Ventoulis, I., Drokou, A., Georgarakou, K., Parissis, J., and Polyzogopoulou, E. (2022). Emergency department risk assessment and disposition of acute heart failure patients: existing evidence and ongoing challenges. Heart Fail. Rev. *28*, 781–793. https://doi.org/10.1007/s10741-022-10272-4.

50. Panagopoulou, V., Deftereos, S., Kossyvakis, C., Raisakis, K., Giannopoulos, G., Bouras, G., Pyrgakis, V., and W.Cleman, M. (2013). NTproBNP: An Important Biomarker in Cardiac Diseases. Curr. Top. Med. Chem. *13*, 82–94. https://doi.org/10.2174/1568026611313020002.

51. Maisel, A.S., Krishnaswamy, P., Nowak, R.M., McCord, J., Hollander, J.E., Duc, P., Omland, T., Storrow, A.B., Abraham, W.T., Wu, A.H.B., et al. (2002). Rapid Measurement of B-Type Natriuretic Peptide in the Emergency Diagnosis of Heart Failure. N. Engl. J. Med. *347*, 161–167. https://doi.org/10.1056/NEJMoa020233.

52. Lambertz, P., Theisen, L., Längst, N., Garvie, C.W., MacDonald, B.T., Yu, J., Elowe, N.H., Zubov, D., Kaushik, V.K., and Wunder, F. (2019). Development of a novel, sensitive cell-based corin assay. Biochem. Pharmacol. *160*, 62–70. https://doi.org/10.1016/j.bcp.2018.12.009.

53. Esser, N., and Zraika, S. (2019). Neprilysin inhibition: a new therapeutic option for type 2 diabetes? Diabetologia *62*, 1113–1122. https://doi.org/10.1007/s00125-019-4889-y.

54. Januzzi, J.L., Chen-Tournoux, A.A., and Moe, G. (2008). Amino-Terminal Pro–B-Type Natriuretic Peptide Testing for the Diagnosis or Exclusion of Heart Failure in Patients with Acute Symptoms. Am. J. Cardiol. *101*, S29–S38. https://doi.org/10.1016/j.amjcard.2007.11.017.

55. Ahmed, S.A., Ismail, H.M., Alahmedi, A.B., Alahmadi, F.B., Muhawish, A.F., Alsubhi, A.A., Almohammadi, Y.S., Alwusaidi, A.K., Alsaedi, A.S., Alhazmi, T.G., et al. (2025). Decoding the Inflammatory Pathway in Heart Failure: The Role of Interleukins and Tumor Necrosis Factor-Alpha in Disease Severity. J. Clin. Med. *14*, 6092. https://doi.org/10.3390/jcm14176092.

56. Perez, A.L., Grodin, J.L., Chaikijurajai, T., Wu, Y., Hernandez, A.F., Butler, J., Metra, M., Felker, G.M., Voors, A.A., Mcmurray, J.J., et al. (2021). Interleukin-6 and Outcomes in Acute Heart Failure: An ASCEND-HF Substudy. J. Card. Fail. *27*, 670–676. https://doi.org/10.1016/j.cardfail.2021.01.006.

57. Kempf, T., Von Haehling, S., Peter, T., Allhoff, T., Cicoira, M., Doehner, W., Ponikowski, P., Filippatos, G.S., Rozentryt, P., Drexler, H., et al. (2007). Prognostic Utility of Growth Differentiation Factor-15 in Patients With Chronic Heart Failure. J. Am. Coll. Cardiol. *50*, 1054–1060. https://doi.org/10.1016/j.jacc.2007.04.091.

58. Pascual-Figal, D.A., and Januzzi, J.L. (2015). The Biology of ST2: The International ST2 Consensus Panel. Am. J. Cardiol. *115*, 3B-7B. https://doi.org/10.1016/j.amjcard.2015.01.034.

59. Savarimuthu, S., Goel, P., and Harky, A. (2022). Soluble ST2: a valuable prognostic marker in heart failure. Heart Fail. Rev. *27*, 2155–2164. https://doi.org/10.1007/s10741-022-10258-2.

60. Januzzi, J.L., Mebazaa, A., and Di Somma, S. (2015). ST2 and Prognosis in Acutely Decompensated Heart Failure: The International ST2 Consensus Panel. Am. J. Cardiol. *115*, 26B-31B. https://doi.org/10.1016/j.amjcard.2015.01.037.

61. Riccardi, M., Myhre, P.L., Zelniker, T.A., Metra, M., Januzzi, J.L., and Inciardi, R.M. (2023). Soluble ST2 in Heart Failure: A Clinical Role beyond B-Type Natriuretic Peptide. J. Cardiovasc. Dev. Dis. *10*, 468. https://doi.org/10.3390/jcdd10110468.

62. Villacorta, H., and Maisel, A.S. (2015). Soluble ST2 Testing: A Promising Biomarker in the Management of Heart Failure. Arq. Bras. Cardiol. https://doi.org/10.5935/abc.20150151.

63. Mueller, T., and Dieplinger, B. (2013). The Presage® ST2 Assay: analytical considerations and clinical applications for a high-sensitivity assay for measurement of soluble ST2. Expert Rev. Mol. Diagn. *13*, 13–30. https://doi.org/10.1586/erm.12.128.

64. Dieplinger, B., and Mueller, T. (2015). Soluble ST2 in heart failure. Clin. Chim. Acta *443*, 57–70. https://doi.org/10.1016/j.cca.2014.09.021.

65. Sanada, S., Hakuno, D., Higgins, L.J., Schreiter, E.R., McKenzie, A.N.J., and Lee, R.T. (2007). IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. J. Clin. Invest. *117*, 1538–1549. https://doi.org/10.1172/JCI30634.

66. Kakkar, R., and Lee, R.T. (2008). The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat. Rev. Drug Discov. *7*, 827–840. https://doi.org/10.1038/nrd2660.

67. Shah, R.V., and Januzzi, J.L. (2010). ST2: A Novel Remodeling Biomarker in Acute and Chronic Heart Failure. Curr. Heart Fail. Rep. *7*, 9–14. https://doi.org/10.1007/s11897-010-0005-9.

68. Januzzi, J.L., Camargo, C.A., Anwaruddin, S., Baggish, A.L., Chen, A.A., Krauser, D.G., Tung, R., Cameron, R., Nagurney, J.T., Chae, C.U., et al. (2005). The N-terminal Pro-BNP Investigation of Dyspnea in the Emergency department (PRIDE) study. Am. J. Cardiol. *95*, 948–954. https://doi.org/10.1016/j.amjcard.2004.12.032.

69. Booth, R.A., Hill, S.A., Don-Wauchope, A., Santaguida, P.L., Oremus, M., McKelvie, R., Balion, C., Brown, J.A., Ali, U., Bustamam, A., et al. (2014). Performance of BNP and NT-proBNP for diagnosis of heart failure in primary care

patients: a systematic review. Heart Fail. Rev. *19*, 439–451. https://doi.org/10.1007/s10741-014-9445-8.

70. Wettersten, N. (2021). Biomarkers in Acute Heart Failure: Diagnosis, Prognosis, and Treatment. Int. J. Heart Fail. *3*, 81. https://doi.org/10.36628/ijhf.2020.0036.

71. Januzzi, J.L., Peacock, W.F., Maisel, A.S., Chae, C.U., Jesse, R.L., Baggish, A.L., O'Donoghue, M., Sakhuja, R., Chen, A.A., Van Kimmenade, R.R.J., et al. (2007). Measurement of the Interleukin Family Member ST2 in Patients With Acute Dyspnea. J. Am. Coll. Cardiol. *50*, 607–613. https://doi.org/10.1016/j.jacc.2007.05.014.

72. Mueller, T., Dieplinger, B., Gegenhuber, A., Poelz, W., Pacher, R., and Haltmayer, M. (2008). Increased Plasma Concentrations of Soluble ST2 are Predictive for 1-Year Mortality in Patients with Acute Destabilized Heart Failure. Clin. Chem. *54*, 752–756. https://doi.org/10.1373/clinchem.2007.096560.

73. Manzano-Fernández, S., Mueller, T., Pascual-Figal, D., Truong, Q.A., and Januzzi, J.L. (2011). Usefulness of Soluble Concentrations of Interleukin Family Member ST2 as Predictor of Mortality in Patients With Acutely Decompensated Heart Failure Relative to Left Ventricular Ejection Fraction. Am. J. Cardiol. *107*, 259–267. https://doi.org/10.1016/j.amjcard.2010.09.011.

74. Pascual-Figal, D.A., Manzano-Fernández, S., Boronat, M., Casas, T., Garrido, I.P., Bonaque, J.C., Pastor-Perez, F., Valdés, M., and Januzzi, J.L. (2011). Soluble ST2, High-Sensitivity Troponin T- and N-Terminal Pro-B-Type Natriuretic Peptide: Complementary Role for Risk Stratification in Acutely Decompensated Heart Failure. Eur. J. Heart Fail. *13*, 718–725. https://doi.org/10.1093/eurjhf/hfr047.

75. Lassus, J., Gayat, E., Mueller, C., Peacock, W.F., Spinar, J., Harjola, V.-P., Van Kimmenade, R., Pathak, A., Mueller, T., diSomma, S., et al. (2013). Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: The Multinational Observational Cohort on Acute Heart Failure (MOCA) study. Int. J. Cardiol. *168*, 2186–2194. https://doi.org/10.1016/j.ijcard.2013.01.228.

76. Aimo, A., Vergaro, G., Ripoli, A., Bayes-Genis, A., Pascual Figal, D.A., De Boer, R.A., Lassus, J., Mebazaa, A., Gayat, E., Breidthardt, T., et al. (2017). Meta-Analysis of Soluble Suppression of Tumorigenicity-2 and Prognosis in Acute Heart Failure. JACC Heart Fail. *5*, 287–296. https://doi.org/10.1016/j.jchf.2016.12.016.

77. Castiglione, V., Aimo, A., Vergaro, G., Saccaro, L., Passino, C., and Emdin, M. (2022). Biomarkers for the diagnosis and management of heart failure. Heart Fail. Rev. *27*, 625–643. https://doi.org/10.1007/s10741-021-10105-w.

78. Francesco Orso (2021). Acute heart failure in the elderly: setting related differences in clinical features and management. 老年心脏病学杂志（英文版） *18*, 407. https://doi.org/10.11909/j.issn.1671-5411.2021.06.003.

79. Crespo-Leiro, M.G., Anker, S.D., Maggioni, A.P., Coats, A.J., Filippatos, G., Ruschitzka, F., Ferrari, R., Piepoli, M.F., Delgado Jimenez, J.F., Metra, M., et al. (2016). European Society of Cardiology Heart Failure Long-Term Registry (ESC-HF-LT): 1-Year Follow-Up Outcomes and Differences Across Regions. Eur. J. Heart Fail. *18*, 613–625. https://doi.org/10.1002/ejhf.566.

80. Bahouth, F., Elias, A., Ghersin, I., Khoury, E., Bar, O., Sholy, H., Khoury, J., and Azzam, Z.S. (2022). The Prognostic Value of Heart Rate at Discharge in Acute Decompensation of Heart Failure with Reduced Ejection Fraction. ESC Heart Fail. *9*, 585–594. https://doi.org/10.1002/ehf2.13710.

81. Fisher, K.A., Stefan, M.S., Darling, C., Lessard, D., and Goldberg, R.J. (2015). Impact of COPD on the Mortality and Treatment of Patients Hospitalized With Acute Decompensated Heart Failure. Chest *147*, 637–645. https://doi.org/10.1378/chest.14-0607.

82. Gopinathannair, R., Chen, L.Y., Chung, M.K., Cornwell, W.K., Furie, K.L., Lakkireddy, D.R., Marrouche, N.F., Natale, A., Olshansky, B., Joglar, J.A., et al. (2021). Managing Atrial Fibrillation in Patients With Heart Failure and Reduced Ejection Fraction: A Scientific Statement From the American Heart Association. Circ. Arrhythm. Electrophysiol. *14*. https://doi.org/10.1161/HAE.0000000000000078.

83. Bayés-Genís, A., Núñez, J., and Lupón, J. (2017). Soluble ST2 for Prognosis and Monitoring in Heart Failure. J. Am. Coll. Cardiol. *70*, 2389–2392. https://doi.org/10.1016/j.jacc.2017.09.031.

84. Manzano-Fernández, S., Januzzi, J.L., Pastor-Pérez, F.J., Bonaque-González, J.C., Boronat-Garcia, M., Pascual-Figal, D.A., Montalban-Larrea, S., Navarro-Peñalver, M., Andreu-Cayuelas, J.M., and Valdés, M. (2012). Serial Monitoring of Soluble Interleukin Family Member ST2 in Patients with Acutely Decompensated Heart Failure. Cardiology *122*, 158–166. https://doi.org/10.1159/000338800.