UNIVERSITY OF INSUBRIA

Department of Medicine and Surgery

PhD Course in Experimental and Translational Medicine

XXXVI cycle

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Interleukin-1 and Eosinophils in Heart Failure

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Academic year 2022/2023

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ABSTRACT

Background. Anakinra, an IL-1 inhibitor, temporarily increases eosinophil blood levels in patients with acute myocardial infarction and reduces heart failure (HF) events. Anakinra is also frequently associated with injection site reactions (ISR), and eosinophils have been shown to play a role. Whether anakinra is associated with changes in eosinophils in patients with HF and whether these changes are linked to a different response in terms of cardiorespiratory fitness (CRF) is unknown. Moreover, whether ISR represents an eosinophilic response to anakinra and is associated with a different response to treatment in patients with HF also remains unclear.

Aim. This study aims to explore the effect of anakinra on changes in eosinophils in patients with HF, and their correlation with CRF.

Methods. We measured eosinophils in 64 patients with HF (50% females), 55 [51–63] years of age, before and after treatment, and, in a subset of 41 patients, also after treatment cessation. We also evaluated CRF by measuring peak oxygen consumption (peak VO_2) with a treadmill test.

Results. Treatment with anakinra led to a significant and transient increase in eosinophils, from 0.2 [0.1–0.3] to 0.3 [0.1–0.4] $x10^3$ cells/μL ($p<0.001$), and from 0.3 [0.2–0.5] to 0.2 [0.1–0.3] $x10^3$ cells/μL, with suspension ($p<0.001$). Changes in eosinophils correlated with the changes in peak VO_2 (Spearman's Rho= +0.228, $p=0.020$) and predicted peak VO_2 (Rho= +0.265, $p=0.014$). Patients experiencing ISR (n=8, 13%) had higher levels of eosinophils (0.5 [0.4–0.6] vs. 0.2 [0.1–0.4] $x10^3$ cells/μL, $p=0.023$), and a greater increase in peak VO_2 (3.0 [0.9–4.3] vs. 0.3 [-0.6–1.8] $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, $p=0.015$).

Conclusion. Patients with HF treated with anakinra experience a transient increase in eosinophils, which is associated with ISR and greater improvement in peak VO_2.(1)

INTRODUCTION

1. HEART FAILURE

1.1 Definition

Heart failure (HF) is a clinical syndrome characterized by signs (e.g., elevated jugular venous pressure, pulmonary crackles, and peripheral edema) and symptoms (e.g., dyspnea, ankle swelling, and fatigue). Proper diagnosis of HF requires identifying the underlying cause, which can be structural or functional abnormalities of the heart, resulting in elevated intracardiac pressures and/or inadequate cardiac output at rest and/or during exercise.

HF has been historically categorized based on left ventricular ejection fraction (LVEF) measurements by transthoracic echocardiography (TTE).(**Table 1**) If TTE is not feasible, cardiac magnetic resonance or nuclear techniques can be employed.

The last European(2) and American(3) guidelines suggest classifying HF in:

- HF with reduced EF (HFrEF; LVEF ≤ 40% with symptoms ± signs);
- HF with mildly reduced EF (HFmrEF; LVEF from 41% to 49% with symptoms ± signs);
- HF with preserved EF (HFpEF; LVEF ≥ 50% with symptoms ± signs, and evidence of cardiac structural and/or functional abnormalities indicating LV diastolic dysfunction/raised LV filling pressures, including raised natriuretic peptides).

Table 1. Definition of the types of HF

Type of HF		HFrEF	HFmrEF	HFpEF
CRITERIA	1	Symptoms ± Signs	Symptoms ± Signs	Symptoms ± Signs
	2	LVEF ≤40%	LVEF 41-49%	LVEF ≥50%
	3			Evidence of cardiac structural and/or functional abnormalities indicating LV diastolic dysfunction/raised LV filling pressures, including raised natriuretic peptides

HF = heart failure; HFmrEF = heart failure with mildly reduced ejection fraction; HFpEF = heart failure with preserved ejection fraction; HFrEF = heart failure with reduced ejection fraction; LV = left ventricle; LVEF = left ventricular ejection fraction.
Adapted from McDonagh TA et al. Eur Heart J. 2021;42(36):3599-726 Doi: 10.1093/eurheartj/ehab368.

HF can also arise from right ventricular (RV) dysfunction due to pressure or volume overload (4). The main cause is pulmonary hypertension from LV dysfunction, but other contributors include myocardial infarction, arrhythmogenic right ventricular cardiomyopathy, and valve disease.(5)

HF is categorized into "chronic" (CHF), characterized by a longstanding diagnosis or gradual symptom onset, and "acute decompensated" (ADHF), with deteriorating CHF leading to potential hospitalization or outpatient intravenous diuretic treatment.

The New York Heart Association (NYHA) functional classification describes four classes of HF severity based on symptoms:(6)

- Class I: no physical activity limitations.
- Class II: no symptoms at rest, but ordinary activity causes symptoms.
- Class III: marked activity limitations with minimal activity (less than ordinary) causing discomfort.
- Class IV: any activity increases discomfort, with potential symptoms even at rest.

However, this classification relies only on symptoms, but even mild symptoms can indicate a high risk of severe outcomes, influencing decisions like cardiac transplantation and device therapies.

1.2 Epidemiology

HF remains the primary cause of early mortality among individuals with established cardiovascular (CV) disease.(7) The overall incidence of HF is increasing,(8) with an incidence in Europe of about 3/1000 person-years (all age groups) and a prevalence of 1-2% of adults.(9, 10) However, since these data account only for diagnosed HF cases, the prevalence is believed to be higher. The prevalence of heart failure increases with age, rising from 1% in those under 55 to over 10% in those 70 or older.(11) While about half of the hospitalized patients with HF have HFrEF, with the rest split between HFpEF and HFmrEF, the ESC Long-Term Registry shows a slightly different distribution among outpatients, indicating 60% with

HFrEF, 24% with HFmrEF, and 16% with HFpEF.(12) Notably, more than half of all HF patients are female (13), and there is a rising prevalence of HFpEF and HFmrEF in both developed and developing countries, possibly due to increased conventional CV risk factors in younger individuals(14) and the economic burden on healthcare systems.(15)

The most common causes of HF are coronary artery diseases, hypertension, valve disease, arrhythmias, cardiomyopathies, congenital heart diseases, infective, drug-induced, infiltrative, storage, endomyocardial, pericardial, metabolic, and neuromuscular diseases. In developed Western countries, the predominant causes are coronary artery disease (CAD) and hypertension.(16)

The prognosis of patients with HF has improved considerably over the years. Mortality rates are 20% at one year and from 53 to 67% at three years after diagnosis.(17, 18) Moreover, despite receiving less evidence-based treatment, women have better survival than men.(19) Lastly, HFmrEF and HFpEF patients have better survival than those with HFrEF.(12, 20)

After the initial diagnosis, HF patients are hospitalized once every year on average,(21) and the risk of hospitalization is 1.5 times higher in patients with diabetes. Moreover, atrial fibrillation, a higher body mass index (BMI), higher glycated hemoglobin, and a low estimated glomerular filtration rate (eGFR) are strong predictors of HF hospitalizations.(22)

1.3 Chronic Heart Failure (CHF)

Figure 1 shows the diagnostic algorithm for heart failure. Both symptoms/signs of HF and objective evidence of cardiac dysfunction are needed to diagnose CHF. Breathlessness, orthopnea, paroxysmal nocturnal dyspnea, fatigue, and ankle swelling are typical symptoms, while elevated jugular venous pressure, hepatojugular reflux, gallop rhythm, and laterally displaced apical impulse are more specific signs. **Table 2** shows typical and atypical signs and symptoms of HF. Notwithstanding, symptoms and signs alone are not enough for a diagnosis.(23, 24, 25, 26)

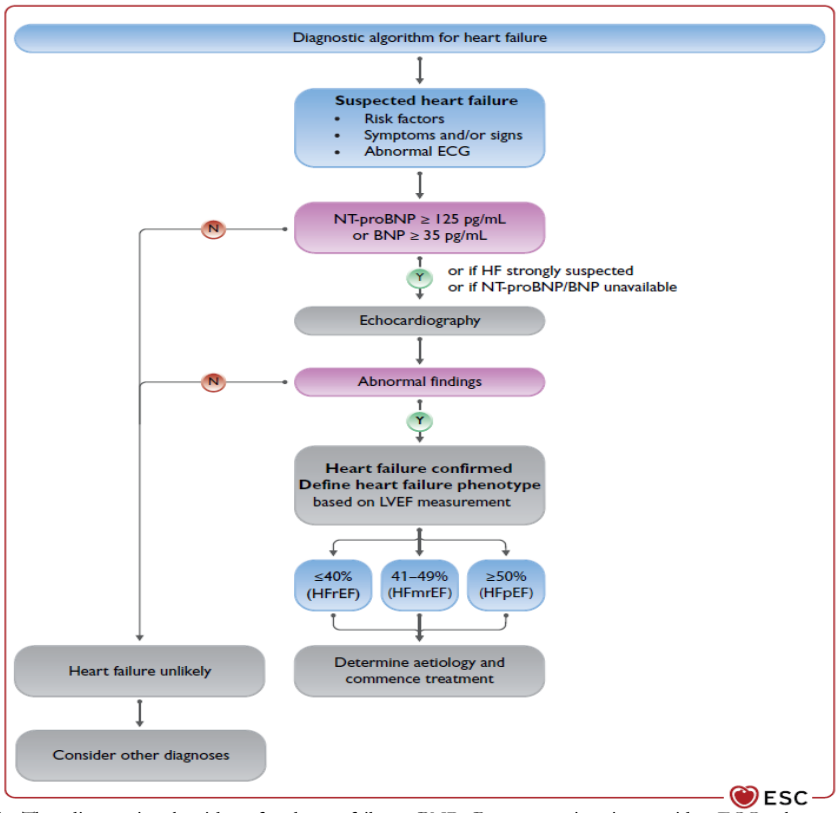


Figure 1. The diagnostic algorithm for heart failure. BNP=B-type natriuretic peptide; ECG=electrocardiogram; HFmrEF=heart failure with mildly reduced ejection fraction; HFpEF=heart failure with preserved ejection fraction; HFrEF=heart failure with reduced ejection fraction; LVEF= left ventricular ejection fraction; NT-proBNP=N-terminal pro-B type natriuretic peptide. Adapted from McDonagh TA et al. Eur Heart J. 2021;42(36):3599-726 Doi: 10.1093/eurheartj/ehab368.

Table 2. Symptoms and signs typical of heart failure.

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

Adapted from McDonagh TA et al. Eur Heart J. 2021;42(36):3599-726 Doi: 10.1093/eurheartj/ehab368.

Recommended diagnostic tests for suspected chronic HF include:

- Electrocardiogram (ECG): an abnormal ECG (atrial fibrillation, Q waves, left ventricular hypertrophy, wide QRS complex) can support the diagnosis and guide treatment. A normal ECG is unlikely to suggest HF.(23)
- Natriuretic peptides measurement: if the levels of specific peptides like B-type natriuretic peptide (BNP), N-terminal pro-B-type natriuretic peptide (NT-proBNP) or mid-regional pro-atrial natriuretic peptide (MR-proANP) are below specified thresholds (<35 pg/mL, <125 pg/mL and <40 pmol/L, respectively) the diagnosis of HF is unlikely.(27, 28)
- Basic blood investigations: serum urea, electrolytes, creatinine, complete blood count, liver and thyroid functions help distinguish HF from other conditions, in addition to offering prognostic information and informing treatment decisions.
- Echocardiography: essential for assessing LV and RV function, volumes, dimensions, wall motion abnormalities, pulmonary hypertension, valvular function, and presence of diastolic function.(29)
- Chest X-ray: helpful in identifying other causes of breathlessness and/or supporting HF diagnosis via signs of pulmonary congestion or cardiomegaly.

Regarding natriuretic peptides, they are recommended as primary diagnostic tests for patients showing symptoms of HF. Elevated levels support an HF diagnosis and are helpful for prognosis(30) and further cardiac investigations.(31) However, various factors can impact the diagnostic accuracy of natriuretic peptides, as noted in **Table 3.** Moreover, their levels might be unusually low in obese patients(32) and the current evidence does not advocate for routine measurement to guide therapy.

Table 3. Causes of elevated concentrations of natriuretic peptides.

CARDIAC	• Heart failure • ACS • Pulmonary embolism • Myocarditis • Left ventricular hypertrophy • Hypertrophic or restrictive cardiomyopathy • Valvular heart disease • Congenital heart disease • Atrial and ventricular tachyarrhythmias • Heart contusion • Cardioversion, ICD shock • Surgical procedures involving the heart • Pulmonary hypertension
NON-CARDIAC	• Advanced age • Ischaemic stroke • Subarachnoid haemorrhage • Renal dysfunction • Liver dysfunction (mainly liver cirrhosis with ascites) • Paraneoplastic syndrome • COPD • Severe infections (including pneumonia and sepsis) • Severe burns • Anaemia • Severe metabolic and hormone abnormalities

ACS = acute coronary syndrome; COPD = chronic obstructive pulmonary disease; ICD = implantable cardioverter-defibrillator.
Adapted from McDonagh TA et al. Eur Heart J. 2021;42(36):3599-726 Doi: 10.1093/eurheartj/ehab368.

To determine the underlying etiology of CHF, several diagnostic tests could be performed:

- Stress echocardiography: this method, either through physical or pharmacological stress, assesses inducible ischemia.(33)
- Cardiac Magnetic Resonance (CMR): especially when using techniques like late gadolinium enhancement (LGE), T1 mapping, and extracellular volume, it identifies myocardial fibrosis or scars. Scars can indicate ischaemic heart disease or dilated cardiomyopathy (DCM). Moreover, CMR can characterize conditions like myocarditis, amyloidosis, Chagas disease, etc.(34, 35)
- Computed Tomography Coronary Angiography (CTCA) is a valuable test to rule out CAD for patients with a low to intermediate risk of CAD or those with unclear non-invasive stress test results.(36)
- Single-Photon Emission CT (SPECT): it can assess myocardial ischemia, viability, inflammation, or infiltration.(37)
- Coronary angiography: recommended for patients with HF and chest pain or angina-like symptoms despite maximum tolerated medical therapy. Additionally, it can be considered for patients with HFrEF who have an

intermediate to high likelihood of CAD and might be candidates for coronary revascularization.

1.4 Acute Heart Failure (AHF)

AHF is characterized by a sudden or gradual onset of severe symptoms and/or signs of HF, leading to hospital admissions. It is a leading cause of hospitalizations in individuals over 65 and is associated with high in-hospital and post-discharge mortality rates.(38, 39, 40, 41, 42, 43)

AHF can represent the initial presentation of HF or an acute decompensation of CHF (named "ADHF"). Patients with new-onset AHF have higher in-hospital mortality but lower post-discharge mortality compared to those with ADHF.(39, 44, 45, 46)

Various factors can influence the severity of AHF. **Figure 2** shows the diagnostic workup for AHF.

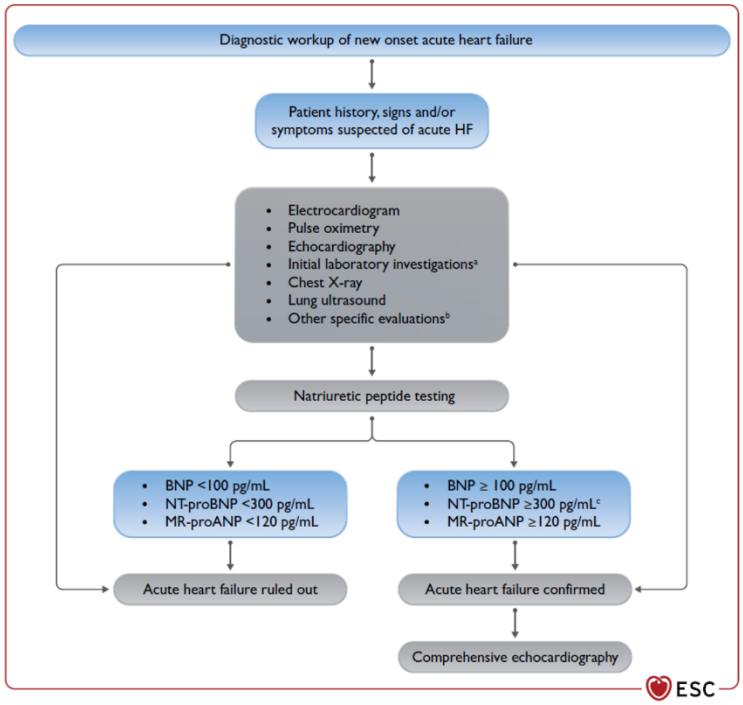


Figure 2. Diagnostic workup of new onset acute heart failure. ACS = acute coronary syndrome; BNP=B-type natriuretic peptide; CT=computed tomography; HF=heart failure; MR-proANP=mid-regional pro-atrial natriuretic peptide; NT-proBNP=N-terminal pro-B-type natriuretic peptide; TSH=thyroid-stimulating hormone. aInitial laboratory exams include troponin, serum creatinine, electrolytes, blood urea nitrogen or urea, TSH, liver function tests as well as D-dimer and procalcitonin when pulmonary embolism or infection are suspected, arterial blood gas analysis in case of respiratory distress, and lactate in case of hypoperfusion. bSpecific evaluation includes coronary angiography, in case of suspected ACS, and CT in case of suspected pulmonary embolism. cRule-in values for the diagnosis of acute HF: >450 pg/mL if aged <55 years, >900 pg/mL if aged between 55 and 75 years and >1800 pg/mL if aged >75 years. Adapted from McDonagh TA et al. Eur Heart J. 2021;42(36):3599-726 Doi: 10.1093/eurheartj/ehab368.

ECG, echocardiography, chest X-rays and plasma NP levels (BNP, NT-proBNP, or MRproANP) are the primary tools in the first assessment. Normal NP levels make AHF diagnosis unlikely. The diagnostic cutoffs to rule out acute HF are BNP <100 pg/mL, NT-proBNP <300 pg/mL, and MR-proANP <120 pg/mL.(47, 48, 49, 50) However, elevated NP levels are also associated with other cardiac and non-cardiac conditions.

Troponin levels are fundamental to detect acute coronary syndrome (ACS), although elevated levels are identified in most patients with AHF.(51, 52) Liver enzymes and thyroid-stimulating hormones should be measured to assess liver and thyroid function, which could precipitate AHF. Arterial blood gas analysis, lactate, and pH levels are functional in case of respiratory distress or cardiogenic shock. D-dimer is helpful for suspected pulmonary embolism, and procalcitonin may help diagnose pneumonia. Pulse oximetry is another routine tool for assessing patients with AHF.(47, 49)

There are four primary clinical presentations of AHF, which may sometimes overlap:

1. Acute Decompensated Heart Failure (ADHF): the most common form, often occurring in patients with a history of HF.(40, 41, 46) It is characterized by gradual fluid retention, causing systemic congestion and, in some cases, inadequate tissue perfusion.(40) Treatment aims to identify triggers, relieve congestion, and occasionally address tissue perfusion issues.
2. Acute Pulmonary Edema: characterized by lung congestion with symptoms of dyspnea, orthopnea, respiratory failure (hypoxemia-hypercapnia), and tachypnea.(53) Oxygen therapy, intravenous diuretics, and vasodilators in case of high blood pressure represent the therapy. In severe cases with low cardiac output, inotropes, vasopressors, and mechanical circulatory support may be necessary to restore organ perfusion.
3. Isolated Right Ventricular Failure: it leads to elevated RV and atrial pressures, causing systemic congestion and potentially impairing LV filling, reducing overall cardiac output due to ventricular interdependence.(54) Diuretics for congestion and inotropes like levosimendan or phosphodiesterase type III inhibitors represent the treatment in cases of low cardiac output and

hemodynamic instability. Inotropic agents may be combined with norepinephrine in case of arterial hypotension.(54)

4. Cardiogenic shock is a life-threatening condition characterized by primary cardiac dysfunction with low cardiac output and severe tissue hypoperfusion that could lead to multiorgan failure and death.(55, 56, 57) Characterizing clinical signs (e.g., cold, sweated extremities, oliguria, mental confusion, dizziness, narrow pulse pressure) and biochemical markers (e.g., elevated serum creatinine, metabolic acidosis, and elevated serum lactate) of hypoperfusion help with the diagnosis.(58) It is worth remembering that hypoperfusion may not always cause low BP. This is due to a compensatory vasoconstriction that can maintain BP but compromise tissue perfusion and oxygenation.(41) Treatment relies on the early address of the underlying cause, hemodynamics stabilization, and organ dysfunction management.

1.5 Biomarkers in Heart Failure (HF)

In recent years, the importance of using biomarkers to understand the underlying processes in HF has gained recognition. Still, their clinical utility may be limited due to the complex and multifaceted nature of HF, which varies with factors such as the cause, phenotype, and comorbidities. Different HF phenotypes (HFrEF, HFmrEF, HFpEF) have distinct progression patterns, and this highlights the importance of exploring potential biomarkers that can aid in the diagnosis and treatment of HF. These include factors related to myocardial stress, inflammation, fibrosis, neurohumoral activation, oxidative stress, endothelial function, and more. (**Figure 3**)

In the last 20 years, many biomarkers have emerged reflecting complex HF processes,(59) but their interpretation is challenging due to overlapping factors and inadequate predictive accuracy to guide HF management.

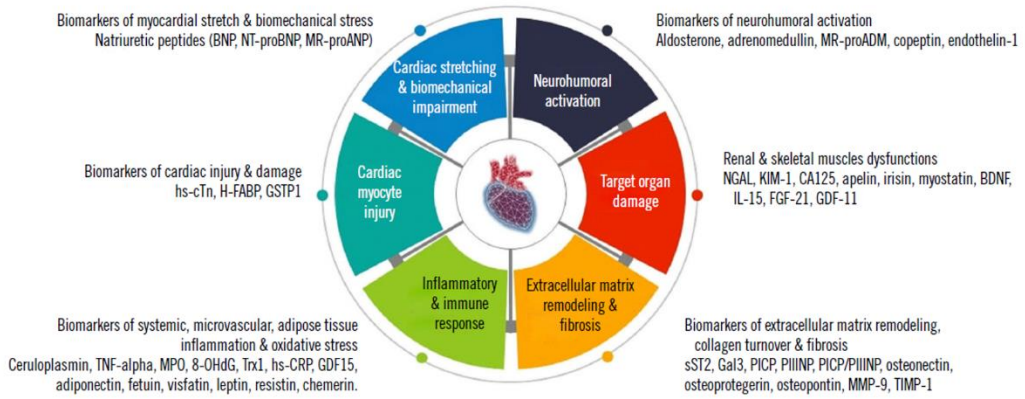


Figure 3. Circulating biomarkers of the most important conceptual clusters influencing the natural evolution of heart failure (HF). Abbreviations: BDNF, brain-derived neurotrophic factor; hs-cTn, high-sensitivity cardiac troponins; H-FABP, heart-type fatty acid-binding protein; FGF, fibroblast growth factor; Gal3, galectin-3; GDF, growth differentiation factor; GSTP1, glutathione transferase P1; IL, interleukin; KIM-1, kidney injury molecule-1; MR-proANP, mid-regional atrial natriuretic pro-peptide; MR-proADM, mid-regional pro-adrenomedullin; MPO, myeloperoxidase; sST2, soluble isoform of suppression of tumorigenicity 2; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; NT-proBNP, N-terminal brain natriuretic pro-peptide; NGAL, neutrophil gelatinase-associated lipocalin; PICP, procollagen type I carboxyterminal peptide; PIIINP, pro-collagen type III aminoterminal peptide; PICP/PIIINP; MMP-9, matrix metalloproteinase 9; TIMP-1, tissue inhibitor of matrix metalloproteinase; TNF, tumor necrosis factor; Trx1, thioredoxin 1. Adapted from Berezin AE et al. Doi: 10.3343/alm.2023.43.3.225

Table 4 and **Table 5** show the advantages and disadvantages of current and plausible biomarkers in current HF management.(28)

Biomarker	Correspondence to basic pathophysiological mechanism/triggers	Current role in HF	Advantages	Disadvantages
NPs	Biomechanical stress, ischemia/ necrosis/reperfusion damage, fluid overload	Rule out HF. Risk stratification of HF. Prediction of all-cause and CV mortality. POC management.	Independent predictor of high risk of HF, HF occurrence and progression, HF outcomes and death. Available for POC management and consequent measures in follow-up. Cost-effective diagnostic workup of newly suspected HF	Respectively high biological variability. Renal clearance. Different cut-off points for various HF populations depending on CV risk factor presentation, age, and gender. Dependence of diagnostic reliability from co-existing CV and non-CV conditions
hs-cTn	Myocardial necrosis	Prediction of HF occurrence. Prediction of all-cause and CV mortality.	Independent predictor of poor clinical outcomes. Available for continuous monitoring. Able to improve predictive value of NPs. Available for multiple-marker strategy for risk stratification.	No relation between an effect of OGBM and changes of hs-cTn. Optimal plasma cut-off point under question. Gender-specific effects.
H-FABP	Myocardial necrosis	Independent predictor of all-cause and CV mortality.	Peak concentrations independently predict HF occurrence.	No strong evidence in large clinical trials.
GSTP1	Myocardial necrosis, inflammation, apoptosis	Independent predictor of ACR.	Peak concentrations independently associated with susceptibility of cardiac dysfunction.	No strong evidence in large clinical trials.
Galectin-3	Extracellular fibrosis and inflammation	Alternative stratification at higher risk of CV death and HF manifestation.	Peak concentrations independently associated with elevated risk of all-cause mortality, CV mortality, and HF-related outcomes.	Lack of dynamics during therapy. Low diagnostic accuracy for HF. Predictive value for readmission lower than that of NT-proBNP. Cut-off depends on age and gender.
sST2	Extracellular fibrosis and inflammation	Alternative stratification at higher risk of all-cause mortality, CV death, and HF manifestation.	Peak concentrations independently associated with elevated risk of all-cause mortality, CV mortality, and HF-related outcomes. Available for serial measures and guided therapy.	The concentrations at discharge exert higher predictive potency than at admission.

Table 4. Advantages and disadvantages of current biomarkers in HF.
Abbreviations: ACR, adverse cardiac remodeling; HF, heart failure; CV, cardiovascular; hs-cTn, high-sensitivity cardiac troponin; H-FABP, heart-type fatty acid-binding protein; GSTP1, glutathione transferase P1; NPs, natriuretic peptides; NT-proBNP, N-terminal brain natriuretic pro-peptide; OGBM, optimal guide-based management; POC, point-of-care; sST2, soluble isoform of suppression of tumorigenicity 2. Adapted from Berezin AE et al. Doi: 10.3343/alm.2023.43.3.225

Biomarker	Correspondence to basic pathophysiological mechanism/triggers	Advantages	Disadvantages
Pro- or telopeptides of collagen type-I	ECM remodeling	Independent predictor of high risk of HF, HF outcomes, and death. Additive prognostic value when compared with the concentrations of NT-proBNP. Available for a multi-marker approach for risk stratification.	Unavailable for serial measures. Low diagnostic value
Bone-related proteins	ECM remodeling	Independent of NPs' predictive value for CV mortality, HF hospitalization, and arrhythmia. Available for a multi-marker approach for risk stratification.	Unavailable for serial measures. Low diagnostic value
GDF15	Inflammation	Predicts ischemia-induced HF and AF. Available for a multi-marker approach for risk stratification. Available for biomarker-guided therapy	Not available for prediction of newly diagnosed HF and non-ischemic cardiomyopathy.
Renal dysfunction biomarkers	Renal injury	Available for serial monitoring. Association of HF-related outcomes	No relation to change of HF management.
Biomarkers of neurohumoral activation	Neurohumoral activation	Available for mortality prediction regardless of HF phenotypes.	Strict similarity in predictive abilities with those of NPs. Available for acute HF rather than chronic HF.
Oxidative stress biomarkers	Mitochondrial injury	Relatively low-cost measures.	Low accuracy, predictive ability, reproducibility, and reliability.
Skeletal muscles dysfunction biomarkers	Muscles injury	Available for mortality prediction regardless of HF phenotypes. Association of HF-related outcomes. Available for biomarker-guided therapy.	No validated scores to use.

Table 5. Advantages and disadvantages of plausible biomarkers without proven value in current HF management.
Abbreviations: AF, atrial fibrillation; ECM, extracellular matrix; CV, cardiovascular; HF, heart failure; GDF-15, growth differentiation factor-15; NPs, natriuretic peptides. Adapted from Berezin AE et al. Doi: 10.3343/alm.2023.43.3.225

1.5.1 Natriuretic Peptides (NPs)

Natriuretic peptides (NPs) have emerged as vital tools for managing HF and are highlighted in current HF guidelines.(2, 3) NPs (including BNP, NT-proBNP, and MR-proANP) serve as circulating cardiac biomarkers reflecting myocardial stretch, biomechanical stress, and other factors.(2) These peptides are released due to myocardial stretching, elevated filling pressures, increased cardiac volumes, and fluid overload. However, inflammation, ischemia, hypoxia, infection, brain trauma, and more can influence NP production and clearance.(60)

NPs act as physiological antagonists to the sympathetic nervous system and the renin-angiotensin-aldosterone systems, affecting diuresis, electrolyte balance, blood pressure, and vasodilation.(60, 61) They also possess pleiotropic effects, including anti-inflammatory, antiapoptotic, tissue-protective, and angiopoietic properties.(61)

However, the clinical use of NPs (especially NT-proBNP) for HF diagnosis is complicated by age, sex, comorbidities, and various CV and non-cardiovascular conditions that can alter their levels.(27, 62) Consequently, different cutoff points for NPs are required for diagnostic purposes.(**Table 6**) Serial evaluation of NPs concentrations may enhance diagnostic accuracy, though its usefulness in all HF phenotypes is uncertain. Furthermore, the introduction of angiotensin receptor

neprilysin inhibitors (ARNIs) has raised questions about the role of BNP in guiding therapy, emphasizing the significance of NT-proBNP in HF therapy.(63)

NPs are promising indicators of HF risk, progression, and response to therapy, but their use in improving clinical outcomes is unclear.(64, 65) Despite these concerns, their integration into HF management can help identify high-risk patients, optimize care, assess prognosis, reduce mortality, and enhance cost-effectiveness.(66, 67)

Cut-off to make a decision	Cut-off points				
	BNP, pg/mL	NT-proBNP, pg/mL			MR-proANP, pmol/L
	All ages	Aged <50 yr	Aged 50–75 yr	Aged >75 yr	All ages
Acute/acutely decompensated HF					
Rule out HF	<100	<300	<300	<300	<40
Mild probability ("grey" zone)	100–400	300–450	300–900	300–1,800	40–120
Rule in HF	>400	>450	>900	>1,800	>120
Stable HF					
Rule out HF	<35	<125	<125	≥125	<40
Mild probability ("grey" zone)	35–150	125–600	125–600	125–600	40–120
Rule in HF	>150	>600	>600	>600	>120

Table 6. Recommended NP cut-offs for acute HF diagnosis.
Abbreviations: NP, natriuretic peptide; HF, heart failure; BNP, brain natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; MR-pro-ANP, mid-regional pro-atrial natriuretic peptide.
Adapted from Berezin AE et al. Doi: 10.3343/alm.2023.43.3.225

1.6 Inflammation and Heart Failure (HF)

Inflammation significantly affects the progression and severity of HFrEF and HFpEF.(68, 69, 70, 71, 72, 73) Myocardial injuries, chronic neurohumoral activation, and comorbidities instigate an inflammatory response via the activation of the NOD, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, and subsequent maturation and release of pro-inflammatory cytokines such as Interleukin(IL)-1β an IL-18.(74)(**Figure 4**) This leads to various adverse cardiac changes, such as cardiac leucocyte infiltration and collagen deposition, commonly seen in HF, especially after acute myocardial infarction.(75)(**Figure 5**)

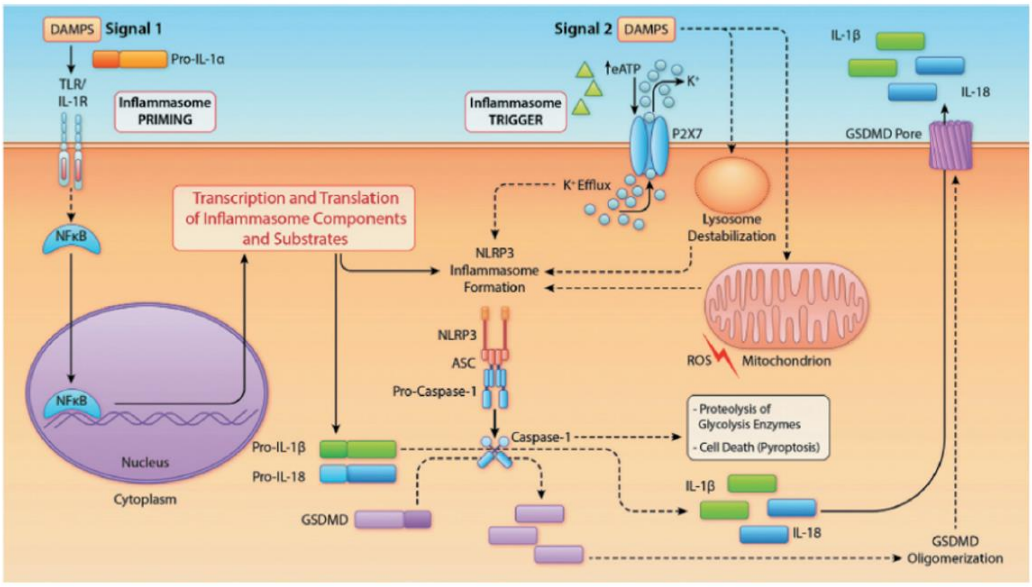


Figure 4. The formation of the inflammasome. In most cells, including macrophages resident cells, activation of the NLRP3 inflammasome activation requires 2 signals. Signal 1 (left) is a priming step. Tissue damage, such as ischemia-reperfusion injury, promotes the release of DAMPs (damage-associated molecular patterns), including the IL (interleukin)-1α precursor (pro-IL-1α) and extracellular ATP (eATP). The Signal 1 priming is initiated when DAMPs activate membrane receptors, including the TLRs (Toll-like receptors) or the IL-1R1 (IL-1 receptor type 1), leading to the translocation of NF-κB (nuclear factor-κB) into the nucleus. This event promotes the transcription and translation of several proinflammatory genes, particularly the precursors of IL-1β and IL-18, as well as components of the NLRP3 inflammasome. IL-1β and IL-18 precursors accumulate in the cytosol, and signal 1 does not directly result in NLRP3 activation. Signal 2 provides the trigger for activation of the inflammasome. This signal is promoted by eATP or intracellular DAMPs (e.g., mitochondrial molecules, like reactive oxygen species [ROS], or lysosomal content), which in most cases, involve the efflux of K+. The activation and assembly of the inflammasome (inhibited by colchicine) triggers the activation of caspase-1 that cleaves pro–IL-1β and pro–IL-18 into their mature and active forms. Adapted from Abbate et al. Interleukin-1 and the Inflammasome as Therapeutic Targets in Cardiovascular Disease. Circ Res. 2020;126(9):1260-80.

C-reactive protein (CRP) is a protein produced by the liver in response to IL-6, a secondary cytokine downstream of IL-1. Elevated CRP levels directly correlate with the more severe form and worse outcomes in HF.(76) Further evidence showed that even a minimally elevated CRP, measured with high-sensitivity assays (high-sensitivity CRP, hsCRP), has been linked to worse prognosis among patients hospitalized for HF.(77) Additionally, prolonged inflammation contributes significantly to cardiovascular diseases (CVDs). In this light, cumulative hsCRP, representing the summation of multiple hsCRP measurements over time, is emerging as a pivotal clinical marker for persistent inflammation, with a potential superiority over singular baseline hsCRP measurement in predicting worse outcomes.(77, 78, 79)

CRP indicates IL-1 activity, which in turn demonstrated direct adverse effects on cardiac cells and in animal models,(80, 81) and increased activity in patients with HF.(80) IL-1 has cardio-depressant characteristics, impacts heart rhythm and impairs myocardial relaxation by altering the sarcoplasmic reticulum phospholamban and

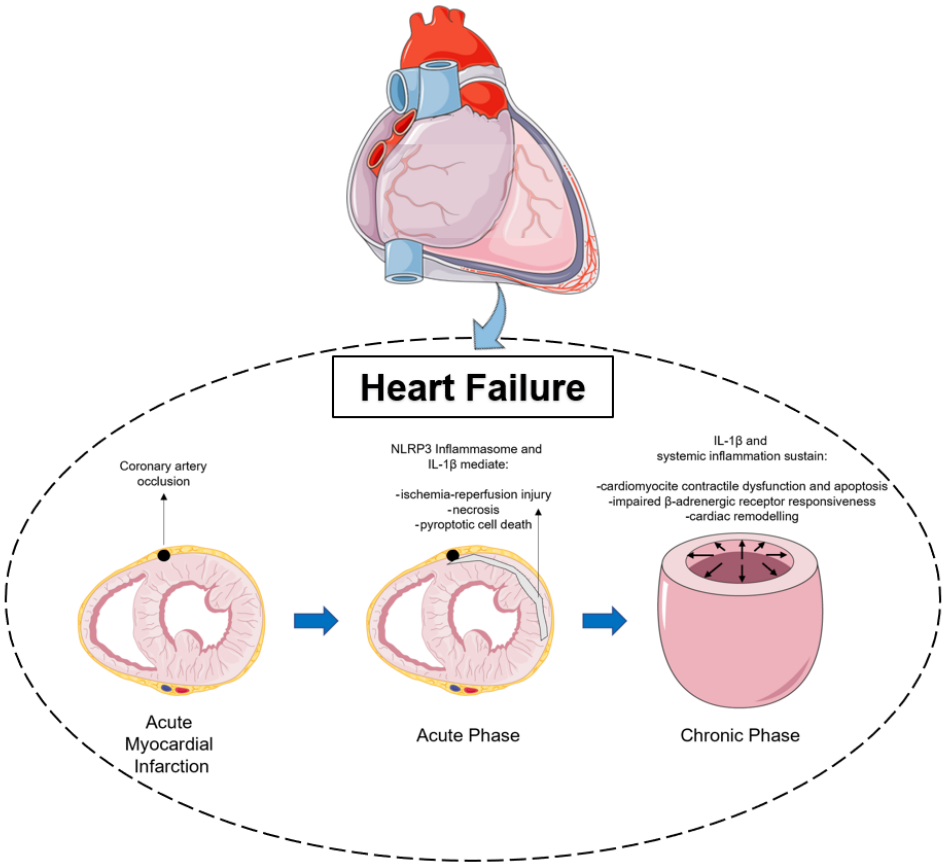


Figure 5. Acute Myocardial Infarction (AMI) and Heart failure: during the acute phase of an AMI, the activation of NLRP3 inflammasome and the subsequent IL-1β production are responsible of the ischemia-reperfusion injury, cardiomyocite necrosis and pyroptotic cell death; later, during the chronic phase, IL-1β and the systemic inflammation contribute to cardiomyocite contractile dysfunction and apoptosis, impaired β-adrenergic receptor responsiveness and cardiac remodeling leading to heart failure. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license. Adapted from Toldo S, Abbate A. The NLRP3 inflammasome in acute myocardial infarction. Nat Rev Cardiol 15, 203-214 (2018). https://doi.org/10.1038/nrcardio.2017.161

calcium-adenosine triphosphatase.(80, 81) Furthermore, IL-1 exacerbates the electrical instability in a failing heart, partly due to its effects on the stellate ganglia, leading to heightened cardiac sympathetic system activation. In rats with HF, elevated IL-1β levels in stellate ganglia have been observed, and reducing IL-1β, through macrophage depletion, can mitigate cardiac sympathetic hyperactivity and the occurrence of ventricular tachycardia/fibrillation.(82)

Several randomized trials have explored targeted approaches to counteract inflammation in HF patients, but the results have been inconsistent.(83, 84, 85) TNF inhibitors, once seen as promising, did not improve outcomes in HFrEF. A study using colchicine for six months in stable HFrEF patients successfully reduced inflammation

biomarkers but did not improve HF symptoms or outcomes.(86) Moreover, similar reductions in CRP and IL-6 were noted in the control group, complicating interpretations.

In contrast, more specific IL-1 signaling blockers have shown potential.(87)

The CANTOS trial indicated that canakinumab significantly decreased HF hospitalizations and related mortality.(73, 88) A sub-study revealed enhanced oxygen consumption and left ventricular ejection fraction (LVEF) in those treated with canakinumab.(72)

Anakinra, another IL-1 blocker, showed efficacy and safety in patients with HFrEF and HFpEF.(**Figure 6**) In stable HFrEF patients, it reduced CRP and IL-6 levels, enhancing cardiorespiratory fitness (i.e., peak oxygen consumption, peak VO_2) and quality of life (QoL).(80) Remarkably, its use in acute decompensated HfrEF patients, initiated within 24 hours of admission and administrated for 14 days, resulted in rapid inflammatory response reductions and significant LVEF recovery.(89) For patients recently hospitalized due to HFrEF, anakinra treatment started within two weeks from discharge and continued for 12 weeks improved peak oxygen consumption, quality of life, and NT-proBNP levels.(70) Another trial (REDHART2; NCT03797001) is ongoing, extending anakinra treatment to 24 weeks post-discharge in patients with recent ADHF.(90) In a small HFpEF study, anakinra improved cardiorespiratory fitness;(91) however, another study with a longer follow-up did not demonstrate a significant change in peak oxygen consumption, while there was a notable increase in exercise time and QoL, and a decrease in CRP and NT-proBNP.(71)

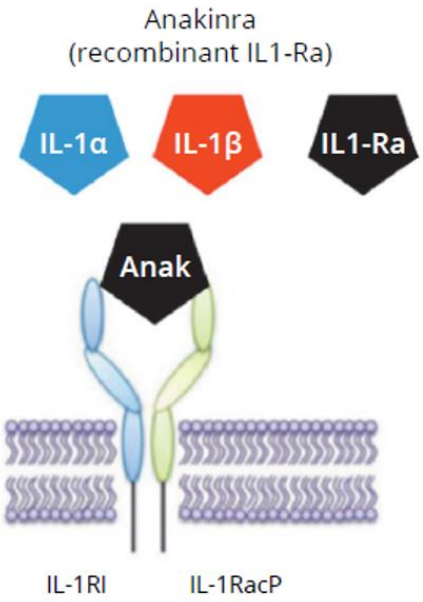


Figure 6. Mechanism of action of anakinra (Anak), which is a recombinant IL-1 receptor antagonist blocking either IL-1α or IL-1β. Abbreviations: IL1R1, IL-1 receptor 1 and IL-1RacP, IL-1 receptor accessory protein. Adapted from Imazio et al. [Anti-interleukin-1 agents: a new class of drugs for recurrent pericarditis. A practical guide for cardiologists]. G Ital Cardiol (Rome). 2021;22(10):833-43 and from Imazio et al. Anti-interleukin-1 agents for pericarditis: a primer for cardiologists. Eur Heart J. 2022;43(31):2946-57.

1.7 Exercise capacity/intolerance in Heart Failure (HF)

HF is a multifaceted clinical condition marked by shortness of breath, fatigue, and declining cardiac function.(3, 92) Reduced exercise capacity or exercise intolerance is defined as an impairment in the capacity to perform physical activities accompanied by symptoms of dyspnea and/or fatigue, and it is frequently observed in HF patients, considerably affecting their quality of life.(3) Key indicators of exercise capacity, like peak VO2 and minute ventilation and carbon dioxide production slope (VE/VCO2), are strong independent predictors of death and hospital admissions due to HF.(93, 94) Exercise intolerance in patients with HF derives from various pathophysiological factors, such as deficits in cardiac and pulmonary reserve, and reduced peripheral and respiratory skeletal muscle perfusion and/or function. Each of these elements can substantively influence the manifestation of HF.(95)

The diagnosis of exercise intolerance in HF is crucial for managing the patients. Furthermore, distinguishing between "exercise capacity" and "functional capacity" is

fundamental. The former denotes the "maximum amount of physical exertion a person can achieve", while the latter pertains to the "ability to perform activities of daily living that require sustained, submaximal aerobic metabolism".(96) Furthermore, cardiorespiratory fitness (CRF) is a term that includes cardiovascular, respiratory, and muscular reactions to physical activity.(97)

There are various methods to assess both exercise and functional capacity:

a) History-based systems
   - *New York Heart Association (NYHA) Functional Classification*: it is widely used for its simplicity and cost-effectiveness.(98) Patients are grouped into one of four categories (Class I, II, III, and IV). However, it has a low sensitivity to changes over time, an inability to discriminate exertional intolerance causes, and blurred distinctions between classes II and III.(99)
   - *HF QoL questionnaires*: Kansas City Cardiomyopathy and Minnesota Living With Heart Failure (MLWHF) offer an alternative to the NYHA functional classification, addressing some inherent limitations. These tools are commonly employed in clinical trials.(100) While temporal changes in QoL scores have shown correlations with clinical outcomes, interpreting them as indicative of exercise or functional capacity can be misleading, as they can evolve independently.(101) Duke Activity Status Index(102) and the EQ-5D(103) represent other valuable tools, providing additional perspectives but have unique challenges.

b) Quantitative Methods (**Table 7**)

- *6-Minute Walk Test (6MWT)*: a simple test where the distance covered by the patient walking for six minutes is recorded. It explores the performance of cardiovascular, pulmonary, and skeletal muscle systems without distinguishing them.(104) The result of this test provides information about disease severity, treatment efficacy, and could predict mortality and hospitalizations.(105, 106) Results are reported as meters and % of predicted according to age and sex.(107)

- *Exercise Testing with ECG*: typically employed for patients with suspected ischemic heart disease. Workload during the exercise is translated into an estimated oxygen uptake (VO_2) and expressed in metabolic equivalents (METS), and could tell the aerobic capacity of the patients.(108) Moreover, it is helpful to assess the chronotropic response by comparing the actual maximal heart rate (HR) with age-predicted maximal HR.(109, 110) Exercise time and a low maximal HR response have been shown to be strong predictors of poor outcomes in patients with HFrEF;(111) however, because the estimation of VO_2 is not precise, this test is not the best choice in HF.

- *Cardiopulmonary Exercise Testing (CPX)* is the gold standard to assess exercise capacity and CRF in HF.(97, 109, 112, 113) Several prognostic variables have been identified.(109) Moreover, the positive response by the CPX variables to various interventions underscores its importance in evaluating HF treatment.(114, 115, 116, 117) CPX is conducted on a cycle ergometer or treadmill, and it offers a comprehensive evaluation of exercise response by integrating conventional exercise parameters with a breath-by-breath analysis of ventilatory and respiratory gas metrics.(118) Pre-test spirometry measures lung function and defines the type and severity of ventilatory restrictions (vital capacity, the forced expiratory volume in 1 s, and vital capacity). Notably, peak oxygen consumption (peak VO_2) during a maximal effort CPX provides an independent estimate of prognosis for HF patients and is crucial for cardiac transplantation consideration.(119) Peak VO_2 can also be expressed as percentage predicted values compared to age- and sex (percent-predicted quantification of peak VO_2), representing a better quantification of the impairment of exercise capacity and, therefore, a better marker of prognosis in HF.(120, 121)

Table 7. Pros and Cons of the Different Methods Used to Quantify Exercise Intolerance in HF.

Modality	Pros	Cons
NYHA functional classification	Easy and rapid to perform, cost-free, prognostic value	Lack of reproducibility, low discriminatory power, no data about the mechanisms of EI
Health-related quality of life instruments*	Easy to perform, cost-free, prognostic value	Patient-derived measures, no data about the mechanisms of EI
ECG stress testing	Easy to perform, negligible cost, detection of CI	Inaccuracy in estimation of exercise capacity in patients with HF, incomplete understanding of exercise limitations due to lack of expired gas analysis, submaximal effort
6MWT	Simplicity, feasibility, negligible cost, prognostic value of the distance covered and changing overtime	Submaximal effort, no data about the mechanisms of EI
CPX	Provide insights in the understanding of the mechanism of exercise intolerance, reproducibility, high prognostic value, monitoring of therapeutic response, detection of CI, quantification of patient effort, it can be paired with cardiac imaging or invasive monitoring	Time-consuming, expensive, specialized personnel, complexity

*Such as Minnesota Living with Heart Failure Questionnaire, Kansas City Cardiomyopathy Questionnaire, Duke activity status index, EQ-5D. 6MWT = 6-min walk test; CI = chronotropic incompetence; CPX = cardiopulmonary exercise testing; ECG = electrocardiography; EI = exercise intolerance; HF = heart failure; NYHA = New York Heart Association.
Adapted from Del Buono MG et al. Exercise Intolerance in Patients With Heart Failure: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019;73(17):2209-25.

CPX measures differentiate between cardiac and non-cardiac exercise causes of exercise limitation,(112, 113) and reveal cardiac abnormalities not found with cardiac imaging or invasive hemodynamics studies.(113)

2. EOSINOPHILS

2.1 General biology and function

Eosinophils (EOS) are granulocytic white blood cells involved in health and disease.(1) They develop and differentiate in the bone marrow, guided by the influence of the transcription factor GATA-1, IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF).(122) EOS secrete granules filled cytokines (IL-4, IL-5, IL-10, IL-13), and chemokines (CCL-3, CCL-5, CCL-11) that influence cell interactions, immunity, tissue regeneration and repair, angiogenesis, fibrosis, and metabolic homeostasis. Additionally, they have specific proteins, including cationic protein (ECP), EOS-derived neurotoxin (EDN), EOS peroxidase, and the major basic protein.(123)

Average levels in peripheral blood range between 0 and 0.5 x 10^9/L and increase in various diseases.(124, 125)

A decrease in EOS (called "eosinopenia") can result from fever, bacterial and viral infections, and systemic glucocorticoid therapy.

A total absence of EOS, although extremely rare, has been noted in patients with thymoma (Good's syndrome).(126)

EOS have been considered end-stage effector cells mainly characterized by releasing granule-derived cytotoxic proteins and lipids. Still, they play a critical role in modulating innate and adaptive immune responses.(127)

2.2 The Versatility Role of Eosinophils in the Immune System

Innate Immunity

EOS, initially thought to phagocytize pathogens, instead release their mitochondrial DNA to contain bacterial infections in the gastrointestinal mucosa.(128) Their DNA trap formation post-cytolysis releases cell-free granules in an NADPH oxidase-dependent manner, highlighting their pathogen-fighting capabilities.(129) They also possess antiparasitic activity, as supported by multiple studies noting the deposition of EOS granule proteins around parasites.(130, 131) Gene deletion in mice showed increased worm burdens upon disrupting EOS peroxidase or MBP.(132) They possess functional receptors like TLRs that aid in pathogen-associated molecular pattern (PAMP) recognition and inflammation.(133, 134, 135) The expression of TLR by human EOS varies in relation to atopic and eosinophilia status.(136) EOS also plays roles in viral defense (such as against HIV and respiratory syncytial virus).(137, 138)

Adaptive Immunity

EOS play a central role in lymphocyte recruitment and homeostasis. Th2 cells produce IL-5 and IL-13, which are responsible for producing specific eotaxins by the EOS. However, recent studies have revealed a dual interaction between EOS and lymphocytes. In an eosinophil-deficient mouse model, Th2 cytokine and T-cell recruitment were reduced, but the reintroduction of EOS restored this deficit.(139) EOS also support lymphocyte homeostasis, as seen in eosinophil-deficient ΔdblGATA-1 mice with reduced Peyer's patch development and Th cell cytokine production.(139, 140, 141) EOS in the bone marrow assists plasma cell maturation and survival, affecting various B-cell processes.(140, 141, 142) EOS promotes B-cell proliferation, with a positive correlation between blood EOS and B-cell counts.(143)

EOS also emerged as potential antigen-presenting cells. There is evidence that EOS treated with GM-CSF can stimulate antigen-specific T-cell proliferation.(144)

After allergen exposure, they express major histocompatibility complex class II (MHC-II), CD80, CD86, CD9, CD28, and CD40.(145, 146) EOS can also migrate to lymph nodes, promoting T-cell proliferation.(147, 148)

EOS can drive a Th2 response through the production of Th2-specific cytokines (149, 150) and can regulate Th2 differentiation via high indoleamine 2,3-dioxygenase (IDO) levels in asthmatic patients.(151) EOS has been shown to play a pivotal role in the lung Th2 inflammation through dendritic cells (DCs).(148) They suppress Th17 and Th1 responses through DC modulation and interact with DCs, emphasizing their role in antigen presentation. EOS and DCs physically interact, leading to DC maturation.(152) EOS-derived granule protein affects DCs, acting as a Th2 adjuvant and influencing immunocyte maturation.(153, 154, 155, 156)

EOS is also present in the thymus, especially in childhood.(157) Thymic EOS have distinct phenotypes and display signs of activation, expressing various markers and Th2 cytokines.(158) They may also contribute to T-cell negative selection and potentially induce T-cell apoptosis. Thymic EOS are IDO-positive (151) and are responsible for regulating the balance between Th1/Th2 in the thymus.

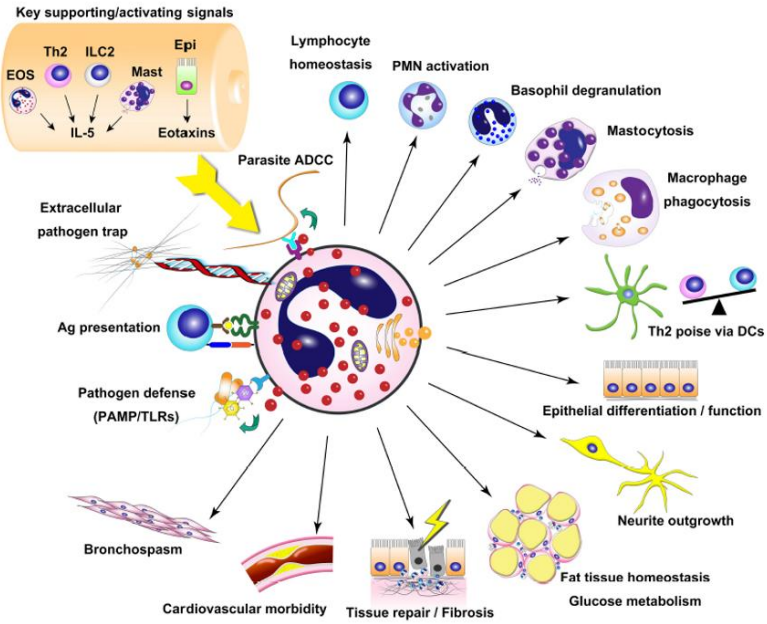


Figure 7. Schematic summary of eosinophil-tropic signaling and eosinophil cellular and humoral regulatory functions. EOS, eosinophils; Mast, mast cells; Epi, epithelium; ADCC, antibody-dependent cell-mediated cytotoxicity; Ag, antigen; PMN, polymorphonuclear leukocyte (neutrophil). Adapted from Wen T, Rothenberg ME. The Regulatory Function of Eosinophils. Microbiol Spectr. 2016;4(5).

2.2 Role of Eosinophils in Cardiovascular Diseases (CVDs)

Various preclinical and clinical studies have demonstrated the involvement of EOS in CVDs.

Preclinical data

Yang et al. investigated the role of EOS in cardiac hypertrophy in both a preclinical model and a retrospective human study. Although there was a positive correlation between EOS and LV mass in humans, the authors found that EOS-derived IL-4 and cationic proteins had a cardioprotective role in this setting.(159)

Another group led by Toor examined the role of EOS following acute MI using eosinophil deficiency and reconstitution models.(160) They highlighted that EOS is protective and mitigates LV remodeling post-experimental MI. They also found that the genetic absence of EOS in ddblGATA4 mice and/or a pharmacological depletion (using Siglec F antibody) led to increased infarct sizes, LV dilation, and reduced systolic function. EOS showed to play a role in collagen maturation, though not in the initial infarct area or coronary angiogenesis. A significant observation was the potential interaction of EOS with cardiac macrophages and monocytes, influencing their anti-inflammatory properties. IL-4 is a cytokine produced by EOS with potential implications for macrophage behavior. The study showed that exogenous IL-4 signaling could counteract the effects of genetic EOS deficiency post-MI. However, while these results were intriguing, they were not definitive, as the IL-4 agonist construct might have unexpected signaling pathways. The findings suggest that IL-4 signaling is possibly a primary mechanism through which EOS interacts with native cardiac macrophages and infiltrates monocyte-derived macrophages, emphasizing the central role of IL-4 in cardiac repair mechanisms.

Another study by Liu et al.(161) delved into the cardioprotective role of EOS in mouse hearts subjected to ischemia. In the mouse models used in this study, MI led to increased blood and heart EOS accumulation, suggesting that high EOS levels post-MI could be a compensatory response to protect the heart from ischemia injury. This study highlights that EOS has beneficial effects in: 1) protecting cardiomyocytes from H_2O_2 or hypoxia-induced cell death, a primary event in ischemic hearts(162, 163, 164) 2) reducing TGF-β-induced cardiac fibroblast activation and collagen synthesis, crucial

for cardiac function(165, 166) and 3) limiting the adhesion of neutrophils and other specific inflammatory cells involved in cardiac repair mechanisms.(167, 168)

Clinical data

The role of EOS in CVDs is not entirely understood, and it is also unclear whether a low EOS count (eosinopenia) or an increase in EOS is associated with worse outcomes.

Higher blood EOS counts were found in patients with acute myocardial infarction (AMI) compared to those without. Furthermore, those with non-ST-elevation ACS or stable angina had elevated plasma ECP levels, indicating enhanced EOS activation.(169) Elevated ECP levels were also observed in the early stages after infarction.(170) A possible role of ECP in inflammation, especially its influence on endothelial adhesion molecule expression and monocyte recruitment to the myocardium, is suggested.(171, 172)

Among 3,742 patients who underwent coronary angiography, an elevated blood EOS count was linked to major cardiovascular risk factors and the prevalence of CVD.(173)

In a 7-year study of 478,259 individuals from the England Biobank, those who died from CVD had notably higher blood EOS counts.(174)

In another study of 200 patients who underwent drug-eluting stent implantation, high plasma EOS cationic protein levels were associated with coronary atherosclerosis and predicted clinical events.(175)

Other studies suggest a protective role of EOS in CVDs. Güner et al. found that in patients with ST-segment elevation myocardial infarction (STEMI) who underwent primary PCI, a lower EOS percentage (EOS%) upon admission was associated with a higher likelihood of major adverse cardiac events (MACE), suggesting EOS% be a potential biomarker for assessing risk in these patients.(176)

In a study of 331 patients who underwent a percutaneous coronary intervention (PCI) for STEMI, a lower EOS-to-leucocyte ratio within 24 hours post-admission predicted MACE within a year.(177) The identical result was observed also in another study.(178)

In the England CALIBER program involving 775,231 individuals, a notable correlation was found between low EOS count, the risk of HF, coronary death, ventricular arrhythmia/sudden cardiac death, and subarachnoid hemorrhage over six months of follow-up.(179)

Finally, in CAD patients with low to intermediate risk who underwent PCI,(180) an increase in EOS was linked to decreased mortality in the initial six months and increased mortality afterward.

In summary, there is not a well-defined role for EOS in CVDs. Additionally, no data have been reported about the role of EOS in the initiation, progression, and outcomes of HF.

2.3 Eosinophils and Interleukin-1 (IL-1)

An interplay exists between EOS and IL-1.

IL-1 can increase the expression of various ligands (E- and P-selectins, ICAM-1, and VCAM-1) necessary for EOS recruitment and migration to the site of inflammation.(181) Furthermore, members of IL-1 family showed to increase EOS survival and activity.(182)

On the other side, EOS can produce and release IL-1, particularly upon activation in the tissue, amplifying the inflammatory response.(183) However, IL-4 produced by EOS inhibits the production of IL-1β from monocytes.(184) Furthermore, a subset of EOS has been shown to secrete high levels of IL-1 receptor antagonist (IL-1Ra), a natural inhibitor of IL-1β.(185)

To summarize, EOS can amplify or dampen the inflammation depending on the context (tissue or blood) and timing. The balance between these two phenotypes of EOS can determine their role in inflammation.

Changes in peripheral EOS have been reported in patients with rheumatoid arthritis receiving IL-1 blockade with anakinra(186), but the underlying mechanisms are still unclear.

In STEMI patients, anakinra reduced leukocyte count, with a relative reduction in neutrophils in favor of an increase in EOS but within the normal range.(187) In patients with STEMI, anakinra also reduced HF-related events.(1, 188, 189, 190, 191)

Injection site reactions (ISR) are very common side effects in patients treated with anakinra, consisting of swelling, erythema, pruritus, and pain. The average time of onset is 7-14 days after the initiation of the treatment, is frequently mild and self-limited, and could be mediated by EOS.(1, 192, 193)

Whether the beneficial effects of anakinra in dampening the inflammatory response and reducing the HF risk are mediated by an effect on EOS is still unknown. Moreover, it is also unknown if there are changes in EOS in patients with HF treated with anakinra, and whether these are associated with a different response in systemic inflammation, cardiorespiratory fitness, cardiac systolic and diastolic function.

3. HYPOTHESES

- The first hypothesis is that patients with HF treated with IL-1 blocker anakinra experience a transient increase in EOS.

-The second hypothesis is that changes in EOS in patients with HF treated with anakinra are associated with a greater response in terms of cardiorespiratory fitness.

- The third hypothesis is that patients experiencing ISR during treatment with anakinra have a higher peripheral level of EOS than those without ISR, and a greater response in terms of cardiorespiratory fitness.

4. AIMS

a) The primary aim of this study was to evaluate the role of the changes in EOS in patients with HF treated with anakinra.

b) The secondary aim was to determine whether changes in EOS correlated with clinical or functional parameters in patients with HF.

c) The third aim was to assess if changes in EOS correlate with the incidence of ISR and a different response in terms of cardiorespiratory fitness.

.

MATERIALS AND METHODS

1. Patient Population

We analyzed data from patients with HF who were treated with anakinra (Kineret®, Swedish Orphan Biovitrum, Waltham, MA, USA), and underwent blood sampling at baseline and during treatment. (1)

We performed a transthoracic echocardiogram (TTE), cardiopulmonary exercise testing (CPX), and quality of life (QoL) assessments at each visit.

In some patients, these assessments were also repeated after discontinuing the treatment.(1)

We included patients from the active treatment arms of four clinical trials: Anakinra in Heart Failure (AIR-HF)(80), Anakinra in Heart Failure With Preserved Ejection Fraction (D-HART)(91), D-HART2 trial (71), and Recently Decompensated Heart Failure Anakinra Response Trial (REDHART)(70).

The pilot study of the safety and efficacy of Anakinra in Heart Failure (AIR-HF) (80) was a phase II, open-label, single-arm pilot trial that enrolled seven patients with HFrEF and hsCRP >2 mg/L, baseline blood sampling, and CPX. Patients were treated with anakinra 100 mg subcutaneously daily for 14 days, with repeat testing at the end of the treatment.(**Figure 8**)

The pilot feasibility study of the safety and efficacy of Anakinra in Heart Failure With Preserved Ejection Fraction (D-HART)(91) was a phase II pilot crossover trial that randomly assigned 12 patients with stable HFpEF and with significant symptoms (NYHA class II or III) and hsCRP >2 mg/L to receive either anakinra 100 mg daily for 14 days or placebo. Blood sampling and CPX were performed at the end of each 14-day treatment course and two weeks after completing an experimental treatment course.(**Figure 9**)

The D-HART2 trial(71) was another phase II trial that randomized 31 patients with HFpEF and hsCRP (≥2 mg/L) to either anakinra 100 mg daily (n = 21) or placebo (n = 10) for 12 weeks. Blood sampling and CPX were performed at 4, 12, and 24 weeks from enrollment.(**Figure 10**)

The Recently Decompensated Heart Failure Anakinra Response Trial (REDHART)(70) enrolled patients with HFrEF and elevated CRP (>2 mg/L), within 14 days from discharge after hospitalization for HF. In this phase II trial, patients were randomized to receive either anakinra 100 mg daily for 12 weeks (n = 20), anakinra 100 mg daily for two weeks (n = 20), or placebo (n = 20). Patients underwent blood sampling and CPX at 2, 4, 12, and 24 weeks.(1)(**Figure 11**)

The original trials excluded patients with acute infections, severe asthma or chronic pulmonary obstructive diseases.(1)

All studies were conducted according to the Declaration of Helsinki and Good Clinical Practice and approved by the Virginia Commonwealth University Institutional Review Board: AIR-HF(80)(IRB approval on 12 May 2011), DHART(91)(IRB approval on 18 October 2012), DHART-2(71)(IRB approval on 19 September 2014), and REDHART(70)(IRB approval on 8 August 2013).

All patients provided written informed consent.

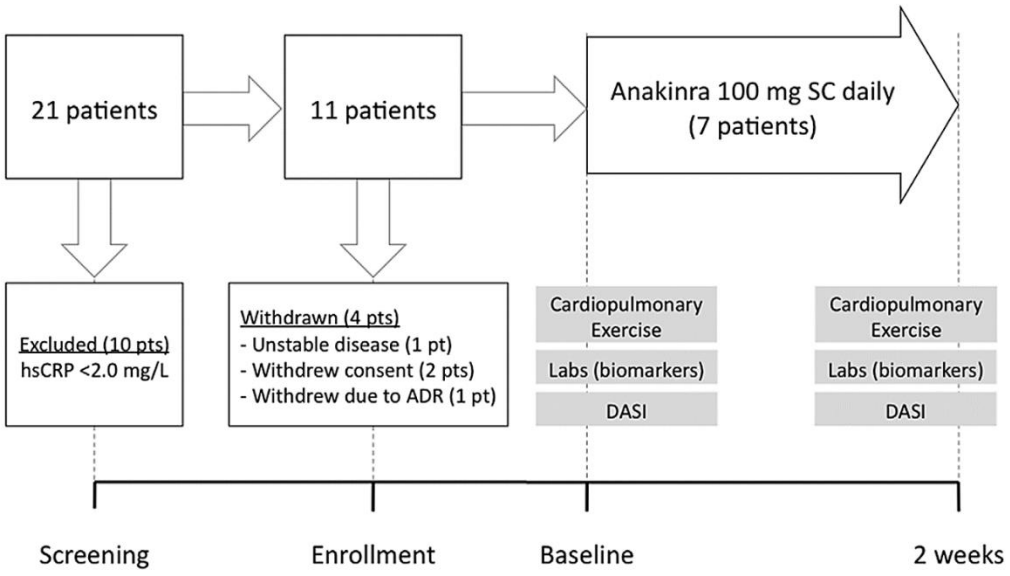


Figure 8. Anakinra in Heart Failure (AIR-HF) trial.
Adapted from Van Tassell BW et al. Enhanced interleukin-1 activity contributes to exercise intolerance in patients with systolic heart failure. PLoS One. 2012;7(3):e33438.

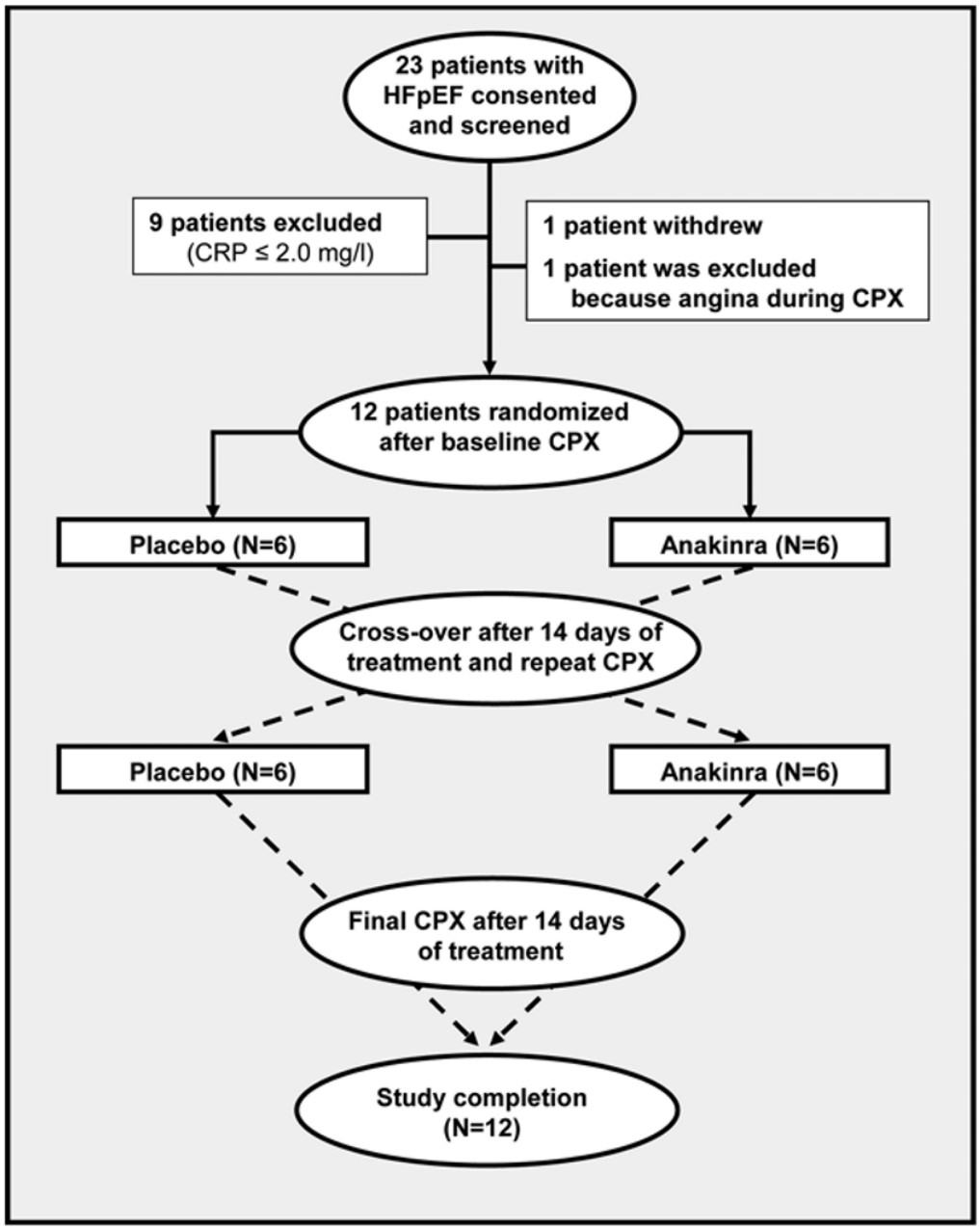


Figure 9. Diastolic Heart Failure Anakinra Response Trial (D-HART). Adapted from Van Tassell BW et al. Effects of interleukin-1 blockade with anakinra on aerobic exercise capacity in patients with heart failure and preserved ejection fraction (from the D-HART pilot study). Am J Cardiol. 2014;113(2):321-7.

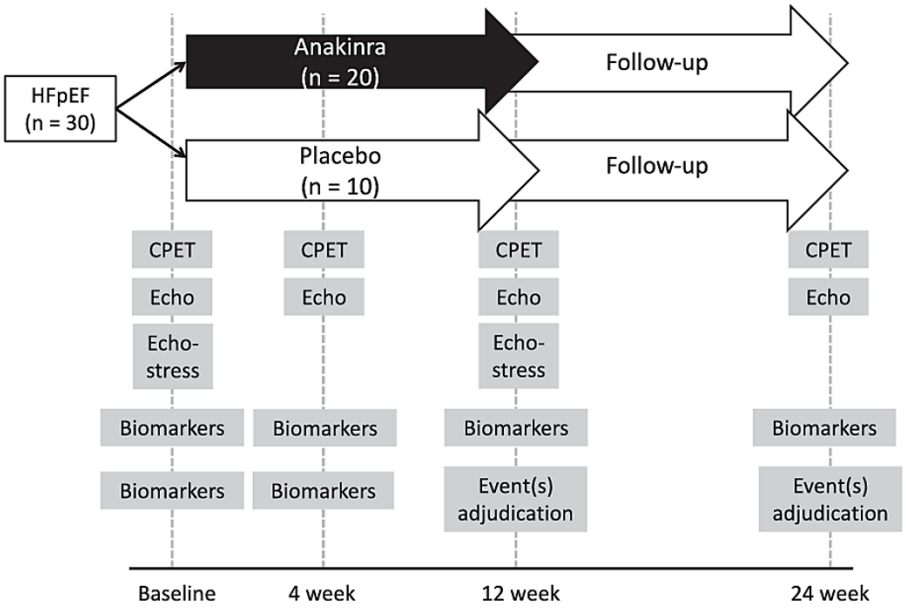


Figure 10. Diastolic Heart Failure Anakinra Response Trial 2 (D-HART2).
Adapted from Van Tassell BW, Trankle CR, Canada JM, Carbone S, Buckley L, Kadariya D, et al. IL-1 Blockade in Patients With Heart Failure With Preserved Ejection Fraction. Circ Heart Fail. 2018;11(8):e005036.

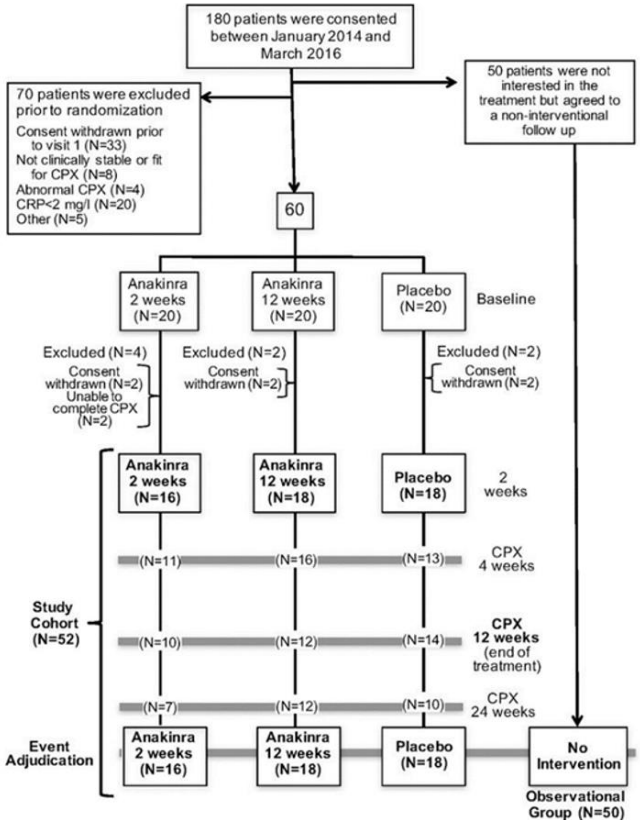


Figure 11. Recently Decompensated Heart Failure Anakinra Response Trial (REDHART).
Adapted from Van Tassell BW et al. Interleukin-1 Blockade in Recently Decompensated Systolic Heart Failure: Results From REDHART (Recently Decompensated Heart Failure Anakinra Response Trial). Circ Heart Fail. 2017;10(11).

2. Laboratory Data

We obtained a complete blood cell count (CBC) with differential count at baseline, during treatment with anakinra, and again after discontinuation of the treatment.(1)

We used a hematology analyzer to calculate white blood cell count (WBC), lymphocyte (LYM), neutrophil (NEU), eosinophil (EOS), and basophil (BAS) counts. We also calculated the leukocyte-to-eosinophil ratio (LER) and the neutrophil-to-eosinophil ratio (NER), as an expression of preferential eosinophilic maturation and increase.(1)

High-sensitivity CRP, chosen as a surrogate for IL-1 activity, and NT-proBNP, a biomarker of myocardial strain, were also measured at each visit.

We used the data from the last laboratory test conducted while on anakinra for the on-treatment analysis and the data from the last available laboratory test after the suspension of the treatment for the off-treatment analysis.

3. Cardiorespiratory Fitness

We performed a maximal aerobic cardiopulmonary exercise test (CPX) using a conservative ramping treadmill protocol to assess cardiorespiratory fitness.(70, 71, 80, 91, 194, 195, 196) We used a gas analyzer capable of breath-by-breath determination of O_2 and CO_2 concentrations in inspired and expired air during exercise. We also monitored ECG and blood pressure throughout the entire exercise.

We measured peak oxygen consumption (peak VO_2), minute ventilation to carbon dioxide production (VE/VCO_2) slope, exercise time, peak respiratory exchange ratio (peak RER), and the oxygen uptake efficiency slope (OUES).(1)

Peak VO_2 represents the gold standard for assessing exercise capacity (97), and it was determined by the highest 10-second average value of VO_2 measured during the last 30 seconds of exercise and was expressed in $mLO_2 \cdot kg^{-1} \cdot min^{-1}$.(197)

Minute ventilation (VE) and carbon dioxide production (VCO_2) were acquired in 10-second interval averages throughout the entire exercise period to calculate the VE/VCO_2 slope via the least squares linear regression formula ($y = mx + b$; m = slope).

We also measured exercise time, defined as the maximum duration from rest to the peak of the exercise expressed in seconds.

Peak RER is the ratio between VCO_2 and VO_2, and was measured to quantify the effort during CPX. A peak RER ≥ 1.0 value was considered a minimally acceptable threshold to indicate the maximal effort of a patient during CPX.(118, 198) Patients were excluded if they had a peak RER <1.0, angina, abnormal blood pressure or heart rate response, or ECG changes suggestive of coronary ischemia.

The oxygen uptake efficiency slope (OUES) was calculated using the formula:

$$VO_2\,(L/min) = m\,(\log 10\ \text{ventilation [VE]}) + b,$$

where m = OUES.

4. Cardiac Function

According to the study protocols, cardiac systolic and diastolic function was assessed through a resting TTE during the same outpatient visit as the laboratory data and prior to CPX.(1, 70) The LVEF was measured using the modified Simpson method. LV diastolic function was evaluated using trans-mitral diastolic flow tracings assessed with pulsed-wave Doppler from an apical four-chamber view with early (E)-wave and late (A)-wave velocity measurements, pulsed-wave tissue Doppler early diastolic mitral annular velocity (e') averaged between the lateral and septal annulus, and calculation of the average E/e' ratio.

5. Quality of Life

Symptom burden and quality of life were assessed at each visit with two different QoL questionnaires: the Duke Activity Status Index (DASI)(199) and the Minnesota Living with Heart Failure (MLHFQ) questionnaire.(1, 200)

The DASI is a twelve-item "yes/no" questionnaire related to ordinary activities of daily living that calculates perceived functional capacity. The answers are weighted, and, when summed up, they estimate the aerobic capacity and functional health. A lower DASI score reflects impaired perceived functional capacity.(**Table 8**)

The MLHFQ consists of 21 questions, with each item scored from 0 (no impact) to 5 (high impact), designed to assess the physical, emotional, and socio-economic

impacts of HF on the QoL of the patients; the total score ranges from 0 to 105, with higher scores indicating greater HF symptom burden and worse QoL.(**Table 9**)

Can you…	No	Yes
1. Take care of yourself (i.e. eating, dressing, bathing, using the toilet)?	0	2.75
2. Walk indoors, such as around the house?	0	1.75
3. Walk a block or two on level ground?	0	2.75
4. Climb a flight of stairs or walk up a hill?	0	5.50
5. Run a short distance?	0	8.00
6. Do light work around the house like dusting or washing dishes?	0	2.70
7. Do moderate work around the house like vacuuming, sweeping floors, or carrying in groceries?	0	3.50
8. Do heavy work around the house like scrubbing floors, or lifting or moving heavy furniture?	0	8.00
9. Do yard work around the house like raking leaves, weeding, or pushing a power mower?	0	4.50
10. Have sexual relations?	0	5.25
11. Participate in moderate recreational activities like golf, bowling, dancing, doubles tennis, or throwing a baseball or football?	0	6.00
12. Participate in strenuous sports like swimming, single tennis, football, basketball, or skiing?	0	7.50

Table 8. Duke Activity Status Index (DASI) questions and scoring.
The values of each questions checked "yes" are summed to derive a total score. The maximum possible score is 58.2.
Adapted from Hlatky MA et al. A brief self-administered questionnaire to determine functional capacity (the Duke Activity Status Index). Am J Cardiol. 1989;64(10):651-4.

Did your heart failure prevent you from living as you wanted during the last month by:	*No*					*Very much*
		Very little				
1. Causing swelling in your ankles, legs, etc?	0	1	2	3	4	5
2. Making you sit or lie down to rest during the day?	0	1	2	3	4	5
3. Making your walking about or climbing stairs difficult?	0	1	2	3	4	5
4. Making your working around the house or yard difficult?	0	1	2	3	4	5
5. Making your going places away from home difficult?	0	1	2	3	4	5
6. Making your sleeping well at night difficult?	0	1	2	3	4	5
7. Making your relating to or doing things with your friends or family difficult?	0	1	2	3	4	5
8. Making your working to earn a living difficult?	0	1	2	3	4	5
9. Making your recreational pastimes, sports or hobbies difficult?	0	1	2	3	4	5
10. Making your sexual activities difficult?	0	1	2	3	4	5
11. Making you eat less of the foods you like?	0	1	2	3	4	5
12. Making you short of breath?	0	1	2	3	4	5
13. Making you tired, fatigued, or low on energy?	0	1	2	3	4	5
14. Making you stay in a hospital?	0	1	2	3	4	5
15. Costing you money for medical care?	0	1	2	3	4	5
16. Giving you side effects from medications?	0	1	2	3	4	5
17. Making you feel you are a burden to your family or friends?	0	1	2	3	4	5
18. Making you feel a loss of self-control in your life?	0	1	2	3	4	5
19. Making you worry?	0	1	2	3	4	5
20. Making it difficult for you to concentrate or remember things?	0	1	2	3	4	5
21. Making you feel depressed?	0	1	2	3	4	5

Table 9. Minnesota Living With Heart Failure Questionnaire (MLHFQ).
The values of each questions are summed to derive a total score. The maximum possible score is 105.
Adapted from Rector TS et al. Validity of the Minnesota Living with Heart Failure questionnaire as a measure of therapeutic response to enalapril or placebo. Am J Cardiol. 1993;71(12):1106-7.

6. Statistical Analyses

We combined individual patient data into one data set and analyzed it.(1) We used the Kolmogorov–Smirnov test to assess continuous variables for normal distribution and we reported baseline characteristics as number (percentage) or median

[interquartile range]. We used Spearman's rank correlation coefficient to assess the correlation between continuous variables. When indicated, we used the chi-square or Fisher's exact test to compare categorical variables and the Wilcoxon signed-rank test to compare the within-group paired difference. A 2-sided *p*-value of less than 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics 26 (IBM, Armonk, NY, USA).

RESULTS

We identified 64 patients with HF who had an available CBC with differential before and during treatment with anakinra (on-treatment analysis). Among those, 41 (64%) also repeated the assessment after discontinuing the treatment (off-treatment analysis).

The median age of the patients included in the on-treatment analysis (N=64) was 55 [51-63] years; 32 (50%) were biological female, and 47 (73%) self-identified as Black/African-American. Body mass index (BMI) was 38 [32–44] kg/m^2, most of which were classified as NYHA class III. Regarding CV risk factors, 90% were hypertensive, 59% diabetic, and 66% dyslipidemic.(**Table 10**)

The median duration of the treatment with anakinra was 4 [2-12] weeks, whereas the time from discontinuation of anakinra to the latest assessment was 12 [4–12] weeks. There were no statistically significant differences in clinical characteristics between the patients with on-treatment and off-treatment analyses (all $p>0.05$).(1)(**Table 10**)

Effects of Anakinra on EOS and White Blood Cells

Treatment with anakinra was associated with a significant increase in EOS (from 0.2 [0.1–0.3] $x10^3$ cells/μL to 0.3 [0.1–0.4] $x10^3$ cells/μL, $p<0.001$) and this change was reverted after the discontinuation of the treatment (from 0.3 [0.2–0.5] $x10^3$ cells/μL to 0.2 [0.1–0.3] $x10^3$ cells/μL, $p<0.001$).(**Table 11** and **Figure 12)** A decrease in NER (from 21.0 [14.3-36.8] to 9.5 [6.4-20.9], $p<0.001$) and LER (from 38.0 [21.7-56.0] to 19.0 [12.9-40.1], $p<0.001$) during treatment was observed that was reversed after suspension (from 8.0 [5.3-13.0] to 18.5 [10.7-35.0] for NER, $p<0.001$ and from 16.0 [11.5-27.7] to 31.0 [19.5-64.0] for LER, $p<0.001$).(**Table 11**)

Comparing the patients treated for 2-4 weeks (N=35) and patients treated for 12 weeks (N=29), we found no significant changes in EOS in patients treated with anakinra for 2-4 weeks (from 0.2 [0.1-0.3] $x10^3$ to 0.2 [0.1-0.3] $x10^3$ cells/μL, $p=0.18$), and a statistically significant increase in EOS (from 0.2 [0.1-0.3] to 0.4 [0.2-0.5] $x10^3$ cells/μL, $p<0.001$) in patients treated with anakinra for 12 weeks (absolute change in

EOS was 0 [0-0.1] and 0.2 [0-0.3] $x10^3$ cells/μL, for patients treated for 2-4 and 12 weeks, respectively, p<0.01)(**Figure 13**). These data showed a greater increase in EOS with a longer duration of treatment.(1)

Treatment with anakinra was also associated with a significant decrease in white blood cells (WBC), neutrophils and basophils counts (from 6.5 [5.6–8.2] to 5.8 [4.5–6.9] $x10^3$ cells/μL, p<0.001, for WBC; from 4.1 [3.2–5.5] to 2.9 [2.0–4.0] $x10^3$ cells/μL, p<0.001, for neutrophils and from 0.1 [0–0.1] to 0 [0–0.1] $x10^3$ cells/μL, p=0.028, for basophils).

Effects of Anakinra on Biomarkers

Anakinra significantly reduced CRP (from 6.2 [3.0–15.4] to 1.4 [0.8–3.7] mg/L, p<0.001); however, after his suspension, CRP levels significantly increased (from 1.4 [1.0–3.7] to 5.9 [1.5–13.6] mg/L, p=0.002). NT-proBNP also significantly decreased during treatment (from 572 [163–1576] to 346 [85–1101] pg/mL, p=0.004) with no significant changes after suspension of the treatment.(**Table 11**)

Effects of Anakinra on Cardiopulmonary Exercise Test

Peak VO_2 and predicted peak VO_2 improved during treatment with anakinra (from 14.2 [11.6–16.9] to 15.2 [12.9–17.3] $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, p=0.006; and from 49 [39–53] to 50 [43–58] %, p=0.05, respectively) and both decreased after suspension (from 16.1 [13.8–18.7] to 14.5 [11.5–17.4] $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, p=0.006; and from 53 [45–62] to 48 [43–59] %, p=0.007).(**Table 12**) Similarly, VE/VCO_2, exercise time, and OUES improved upon anakinra introduction, but no changes were observed after suspension of the treatment except for OUES, which tended to worsen.(**Table 12**) Of note, the change in CRP was significantly negatively correlated to the change in pVO2 (Spearman's Rho = -0.267, p=0.006).

Effects of Anakinra on resting Transthoracic Echocardiography

Regarding echocardiographic parameters, no differences in LVEF were detected during and after treatment with anakinra.(**Table 13**) The early mitral inflow velocity/early diastolic mitral annular velocity (E/e') decreased during treatment (from 14.2 [9.7–19.9] to 11.6 [8.6–14.9], p=0.004) and increased after treatment suspension (from 11.6 [8.7–14.4] to 11.9 [8.5–17.5], p=0.029).(**Table 13**)

Effects of Anakinra on Quality-of-Life assessment

The DASI score and the MLHFQ significantly improved during treatment with anakinra (the first increased from 24.2 [15.5–37.3] to 31.7 [20.9–42.7], p=0.001, and the second decreased from 51 [35.5–67.3] to 41 [14–62], p=0.002), reflecting a better perceived functional capacity and reduced HF burden. No changes were observed after the suspension of the treatment.(**Table 14**)

Correlations between EOS and other parameters

Of note, the changes in EOS over time were significantly positively correlated to the changes in peak VO_2 (Spearman's Rho= +0.228, p=0.020), predicted peak VO_2 (Rho= +0.265, p=0.014), DASI score (Rho= +0.261, p=0.015) and negatively with changes in CRP (Rho= -0.297, p=0.002), NT-proBNP (Rho= -0.222, p=0.041), and the E/e' ratio (Rho= -0.288, p=0.011). No significant correlations were found for changes in the other parameters (VE/VCO_2 slope, Rho= -0.040, p=0.690; exercise time, Rho= +0.185, p=0.059; Peak RER, Rho= +0.160, p=0.11; OUES, Rho= +0.069, p=0.849; LVEF, Rho= -0.029, p=0.795; MLHFQ, Rho= -0.132, p=0.227).(**Table 15** and **Figure 14**) Notably, no significant correlations were found regarding neutrophils or basophils with these parameters.(1)

Both changes in NER and LER correlated negatively with the changes in peak VO_2 (Rho= -0.303, p=0.002 and Rho= -0.344, p<0.001, respectively) and DASI sore (Rho= -0.358, p=0.001 and Rho= -0.360, p=0.001, respectively), and positively with changes in CRP (Rho= +0.525, p<0.001 and Rho= +0.504, p<0.001, respectively). On the other hand, only LER correlated with MLHFQ score (Rho= +0.251, p=0.026). No correlations were observed regarding the other parameters and biomarkers analyzed.(1)

Injection Site Reactions

Injection site reactions (ISR) were characterized by swelling, erythema, pruritus, and pain around the injection site,(**Figure 15**) and were reported by 8 (13%) patients (median age of 51 [45-59] years, of which 4 (50%) were biological females and 4 (50%) self-identified as Black/African-American). All the ISR were considered to be mild, expected and none of these required drug discontinuation.

There were no statistically significant differences in clinical characteristics between the patients with and without ISR nor in baseline biomarkers and CRF parameters (all p>0.05).(1)(**Table 16**) On the other hand, patients with ISR had higher on-treatment EOS counts (0.5 [0.4-0.6] vs. 0.2 [0.1-0.4] $x10^3$ cells/μL, p=0.023), higher peak VO_2 (18.5 [14.5-25.2] vs. 14.9 [12.3-17.2] $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, p=0.044), higher predicted peak VO_2 (59 [53–77] vs. 48 [41–58] $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, p=0.009), larger change from baseline to on-treatment in peak VO_2 (3.0 [0.9-4.3] vs. 0.3 [-0.6-1.8] $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, p=0.015) and predicted peak VO_2 (10 [6–16] vs. 0 [-3–6] $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, p=0.034), longer on-treatment exercise time (650 [490-830] vs. 500 [370-583] seconds, p=0.016), and lower on-treatment NT-proBNP levels (81 [13-120] vs. 445 [95-1187] pg/mL, p=0.003).(**Table 16 and Figure 16**)

Table 10. Clinical characteristics of patients in the on-treatment and off-treatment analyses.

	On-treatment analysis (N=64)	**Off-treatment analysis (N=41)**
Clinical variables		
Age (years)	55 [51–63]	54 [51–59]
Sex – F (%) / M (%)	32 (50) / 32 (50)	20 (49) / 21 (51)
Race – Black (%) /White (%)	47 (73) / 17 (27)	30 (73) / 11 (27)
BMI (kg/m^2)	38 [32–44]	38 [34–44]
NYHA class		
NYHA class II	24 (38)	20 (49)
NYHA class III	40 (63)	21 (51)
CAD	15 (23)	9 (22)
DM	38 (59)	22 (54)
HTN	58 (90)	35 (85)
HLP	45 (66)	28 (68)
ACEi/ARB	49 (77)	32 (78)
Beta-blocker	57 (89)	37 (90)
MRA	30 (47)	22 (54)

Data are expressed as median [interquartile range] or number (%).

Abbreviations: F, female; M, male; AA, African-American; BMI, body mass index; NYHA, New York Heart Association; CAD, coronary artery disease; DM, diabetes mellitus; HTN, hypertension; HLP, hyperlipidemia; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockad e; MRA, mineralocorticoid receptor antagonist.

(Adapted from Golino et al. Change in Eosinophil Count in Patients with Heart Failure Treated with Anakinra. Cells. 2023;12(8).)

Table 11. Biomarkers and complete blood cell count in the on-treatment and off-treatment analyses.

	On-treatment analysis (N=64)			**Off-treatment analysis (N=41)**		
Complete blood cell count	*Baseline*	*On-Treatment*	*P*	*On-treatment*	*Off-Treatment*	*P*
WBC (x10^3 cells/μL)	6.5 [5.6–8.2]	5.8 [4.5–6.9]	**<0.001**	5.7 [4.7–7.0]	6.6 [5.4–8.2]	**<0.001**
Lymphocytes (x10^3 cells/μL)	1.9 [1.6–2.3]	1.9 [1.6–2.4]	0.322	1.9 [1.6–2.4]	1.9 [1.6–2.2]	0.420
Neutrophils (x10^3 cells/μL)	4.1 [3.2–5.5]	2.9 [2.0–4.0]	**<0.001**	2.9 [2.0–4.0]	4.1 [2.9–5.4]	**<0.001**
Eosinophils (x10^3 cells/μL)	0.2 [0.1–0.3]	0.3 [0.1–0.4]	**<0.001**	0.3 [0.2–0.5]	0.2 [0.1–0.3]	**<0.001**
Basophils (x10^3 cells/μL)	0.1 [0–0.1]	0 [0–0.1]	**0.028**	0 [0–0.1]	0.1 [0–0.1]	0.285
Neutrophil-to-eosinophil ratio (NER)	21.0 [14.3–36.8]	9.5 [6.4–20.9]	**<0.001**	8.0 [5.3–13.0]	18.5 [10.7–35.0]	**<0.001**
Leukocyte-to-eosinophil ratio (LER)	38.0 [21.7–56.0]	19.0 [12.9–40.1]	**<0.001**	16.0 [11.5–27.7]	31.0 [19.5–64.0]	**<0.001**
Biomarkers	*Baseline*	*On-Treatment*	*P*	*On-treatment*	*Off-Treatment*	*P*
CRP (mg/L)	6.2 [3.0–15.4]	1.4 [0.8–3.7]	**<0.001**	1.4 [1.0–3.7]	5.9 [1.5–13.6]	**0.002**
NT-proBNP (pg/mL)	572 [163–1576]	346 [85–1101]	**0.004**	346 [91–1009]	242 [77–1003]	0.22

Data are expressed as median [interquartile range].

Abbreviations: CRP, C-reactive protein; NT-proBNP, N-terminal pro b-type natriuretic peptide; WBC, white blood cells.

(Adapted from Golino et al. Change in Eosinophil Count in Patients with Heart Failure Treated with Anakinra. Cells. 2023;12(8).)

Table 12. CPX parameters in the on-treatment and off-treatment analyses.

	On-treatment analysis (N=64)			**Off-treatment analysis (N=41)**		
Variables	*Baseline*	*On-Treatment*	*P*	*On-treatment*	*Off-Treatment*	*P*
Peak RER	1.13 [1.04–1.19]	1.14 [1.05–1.20]	0.39	1.15 [1.06–1.20]	1.10 [1.06–1.15]	**0.04**
Peak VO_2 ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$)	14.2 [11.6–16.9]	15.2 [12.9–17.3]	**0.006**	16.1 [13.8–18.7]	14.5 [11.5–17.4]	**0.006**
Predicted peak VO_2 (%)	49 [39–53]	50 [43–58]	0.05	53 [45–62]	48 [43–59]	**0.007**
VE/VCO_2 slope	31.1 [26.9–35.2]	30.7 [26.1–32.4]	**0.004**	30.7 [25.9–33.1]	30.8 [27.6–33.9]	0.052
Exercise time (seconds)	465 [340–583]	510 [372–610]	**<0.001**	530 [435–675]	570 [390–665]	0.68
OUES	1.88 [1.49–2.20]	1.97 [1.66–2.36]	**0.027**	2.05 [1.70–2.36]	1.83 [1.57–2.24]	**0.027**

Data are expressed as median [interquartile range]. Abbreviations: CPX, cardiopulmonary exercise test; Peak RER, peak respiratory exchange ratio; Peak VO_2, peak oxygen consumption; VE/VCO_2, minute ventilation to carbon dioxide production slope; OUES, oxygen uptake efficiency slope.

(Adapted from Golino et al. Change in Eosinophil Count in Patients with Heart Failure Treated with Anakinra. Cells. 2023;12(8).)

Table 13. Echocardiographic parameters in the on-treatment and off-treatment analyses.

	On-treatment analysis (N=64)			**Off-treatment analysis (N=41)**		
Variables	*Baseline*	*On-Treatment*	*P*	*On-treatment*	*Off-Treatment*	*P*
LVEF (%)	49 [33–60]	45 [36–58]	0.71	44 [35–56]	50 [37–56]	0.44
E/e'	14.2 [9.7–19.9]	11.6 [8.6–14.9]	**0.004**	11.6 [8.7–14.4]	11.9 [8.5–17.5]	**0.029**

Data are expressed as median [interquartile range]. Abbreviations: LVEF, left ventricular ejection fraction.

Table 14. Measures of Quality of Life.

	On-treatment analysis (N=64)			**Off-treatment analysis (N=41)**		
Variables	*Baseline*	*On-Treatment*	*P*	*On-treatment*	*Off-Treatment*	*P*
DASI	24.2 [15.5–37.3]	31.7 [20.9–42.7]	**0.001**	32.2 [22.9–40.1]	30.8 [19.7–50.2]	0.77
MLHFQ	51 [35.5–67.3]	41 [14–62]	**0.002**	38 [14–58]	20 [8–54]	0.15

Data are expressed as median [interquartile range]. Abbreviations: DASI, Duke Activity Status Index; MLHFQ, Minnesota Living with Heart Failure Questionnaire.

Table 15. Correlations between changes in EOS and changes in biomarkers, CRF, echo, and QoL parameters in patients with HF treated with anakinra.

Variables		**CRP**	**NT-proBNP**	**Peak VO_2**	**Predicted peak VO_2**	**VE/VCO_2 slope**	**Exercise time**	**Peak RER**	**OUES**	**LVEF**	**E/e'**	**DASI score**	**MLHFQ**
Changes in EOS	*Spearman's Rho*	- 0.297	- 0.222	+ 0.228	+ 0.265	- 0.040	+ 0.185	+ 0.160	+ 0.069	- 0.029	- 0.288	+ 0.261	- 0.132
	P value	**0.002**	**0.041**	**0.020**	**0.014**	0.690	0.059	0.11	0.849	0.795	**0.011**	**0.015**	0.227

Abbreviations: EOS, eosinophils; CRF, cardiorespiratory fitness; QoL, quality of life; HF, heart failure; CRP, C-reactive protein; NT-proBNP, N-terminal pro b-type natriuretic peptide; VE/VCO_2, minute ventilation/carbon dioxide production; RER, respiratory exchange ratio; OUES, oxygen uptake efficiency slope; LVEF, left ventricle ejection fraction; DASI, Duke Activity Status Index; MLHFQ, Minnesota Living with Heart Failure Questionnaire.

(Adapted from Golino et al. Change in Eosinophil Count in Patients with Heart Failure Treated with Anakinra. Cells. 2023;12(8).)

Table 16. Clinical characteristics, biomarkers, and cardiorespiratory fitness parameters in patients with and without injection site reactions.

	Patients with injection site reactions (N=8)	**Patients without injection site reactions (N=56)**	***P value***
Clinical variables			
Age (years)	51 [45–59]	55 [52–64]	0.10
Sex – F (%) / M (%)	4 (50) / 4 (50)	28 (50) / 28 (50)	1.00
Race – Black (%) /White (%)	4 (50) / 4 (50)	393 (70) / 17 (30)	0.11
BMI (kg/m^2)	36 [35–41]	38 [32–45]	0.45
NYHA class			
NYHA class II	4 (50)	20 (36)	0.43
NYHA class III	4 (50)	36 (64)	0.43
CAD	2 (25)	13 (23)	0.91
DM	6 (75)	32 (57)	0.34
HTN	6 (75)	52 (93)	0.11
HLP	4 (50)	41 (73)	0.18
ACEi/ARB	7 (88)	42 (75)	0.44
Beta-blocker	8 (100)	49 (88)	0.29
MRA	6 (75)	24 (43)	0.09
Biomarkers			
CRP (mg/L)			
- *Baseline*	2.8 [2.3–13.3]	6.5 [3.6–15.5]	0.12
- *On-treatment*	1.3 [0.5–7.2]	1.4 [0.9–3.7]	0.81
- *Off-treatment*	2.1 [0.6–9.7]	6.8 [2.9–13.6]	0.11
NT-proBNP (pg/mL)			
- *Baseline*	74 [13–219]	634 [200–1774]	**0.002**
- *On-treatment*	81 [13–120]	445 [95–1187]	**0.003**
- *Off-treatment*	57 [20–120]	383 [121–1094]	**0.001**
Complete blood cell counts with differential			
WBC (x10^3 cells/μL)			
- *Baseline*	6.1 [5.2–7.8]	6.6 [5.6–8.3]	0.50
- *On-treatment*	6.1 [4.4–7.4]	5.6 [4.5–6.7]	0.44
- *Off-treatment*	6.2 [5.4–7.3]	6.9 [5.4–8.3]	0.48
Neutrophils (x10^3 cells/μL)			
- *Baseline*	3.5 [2.5–5.1]	4.4 [3.2–5.7]	0.27
- *On-treatment*	2.9 [1.9–5.1]	2.9 [2.0–4.0]	0.75
- *Off-treatment*	3.7 [2.7–4.3]	4.4 [3.1–5.7]	0.34
Eosinophils (x10^3 cells/μL)			
- *Baseline*	0.3 [0.1–0.4]	0.2 [0.1–0.3]	0.21
- *On-treatment*	0.5 [0.4–0.6]	0.2 [0.1–0.4]	**0.023**
- *Off-treatment*	0.2 [0.2–0.4]	0.2 [0.1–0.3]	0.34
CPX			
Peak VO_2 ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$)			
- *Baseline*	16.8 [13.2–19.4]	14.0 [11.6–16.5]	0.16
- *On-treatment*	18.5 [14.5–25.2]	14.9 [12.3–17.2]	**0.044**
- *Off-treatment*	15.0 [13.7–25.5]	14.8 [11.4–17.5]	0.19
- *Delta baseline-on treatment*	3.0 [0.9–4.3]	0.3 [-0.6–1.8]	**0.015**
- *Delta on-off treatment*	-1.5 [-2.1–0.7]	-0.3 [-2.1–0.6]	0.62
Predicted peak VO_2 (%)			
- *Baseline*	51 [46–64]	47 [37–52]	0.09

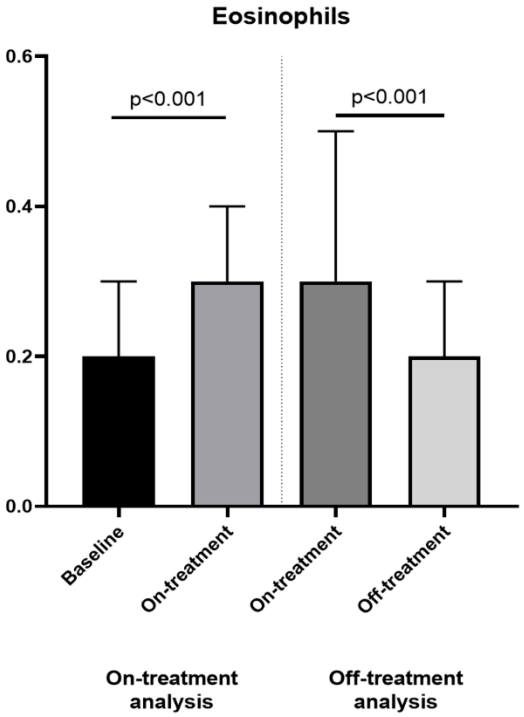
- *On-treatment*	59 [53–77]	48 [41–58]	**0.009**
- *Off-treatment*	51 [47–75]	46 [41–56]	0.16
- *Delta baseline-on treatment*	10 [6–16]	0 [-3–6]	**0.034**
- *Delta on-off treatment*	-5 [-8–3]	-1 [-11–2]	0.69
Exercise time (seconds)			
- *Baseline*	595 [505–725]	455 [290–548]	**0.007**
- *On-treatment*	650 [490–830]	500 [370–583]	**0.016**
- *Off-treatment*	730 [580–870]	510 [375–630]	**0.026**

Data are expressed as median [interquartile range] or number (%).

Abbreviations: F, female; M, male; AA, African-American; BMI, body mass index; CAD, coronary artery disease; DM, diabetes mellitus; HTN, hypertension; HLP, hyperlipidemia; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockade; MRA, mineralocorticoid receptor antagonist; LVEF, left ventricular ejection fraction; CPX, cardiopulmonary exercise test; VO_2, oxygen consumption; CRP, C-reactive protein; NT-proBNP, N-terminal pro b-type natriuretic peptide.

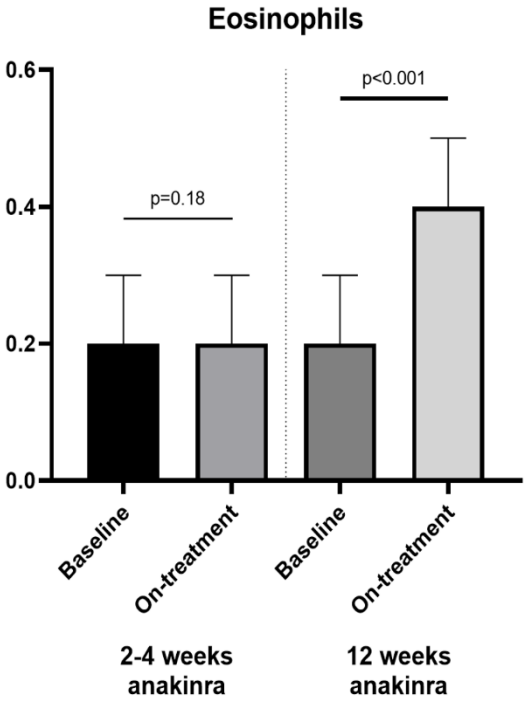
(Adapted from Golino et al. Change in Eosinophil Count in Patients with Heart Failure Treated with Anakinra. Cells. 2023;12(8).)

Figure 12. Changes in Eosinophils - *on* and *off-treatment analyses.*



On-treatment analysis included 64 patients. Off-treatment analysis included 41 patients.
(Adapted from Golino et al. Change in Eosinophil Count in Patients with Heart Failure Treated with Anakinra. Cells. 2023;12(8).)

Figure 13. Changes in Eosinophils based on the treatment duration.



35 patients were treated for 2-4 weeks, while 29 patients were treated for 12 weeks.
(Adapted from Golino et al. Change in Eosinophil Count in Patients with Heart Failure Treated with Anakinra. Cells. 2023;12(8).)

Figure 14. Correlations between changes in Eosinophils and peak VO2, predicted peak VO2, DASI score, C-reactive protein, NT-proBNP, E/e'.

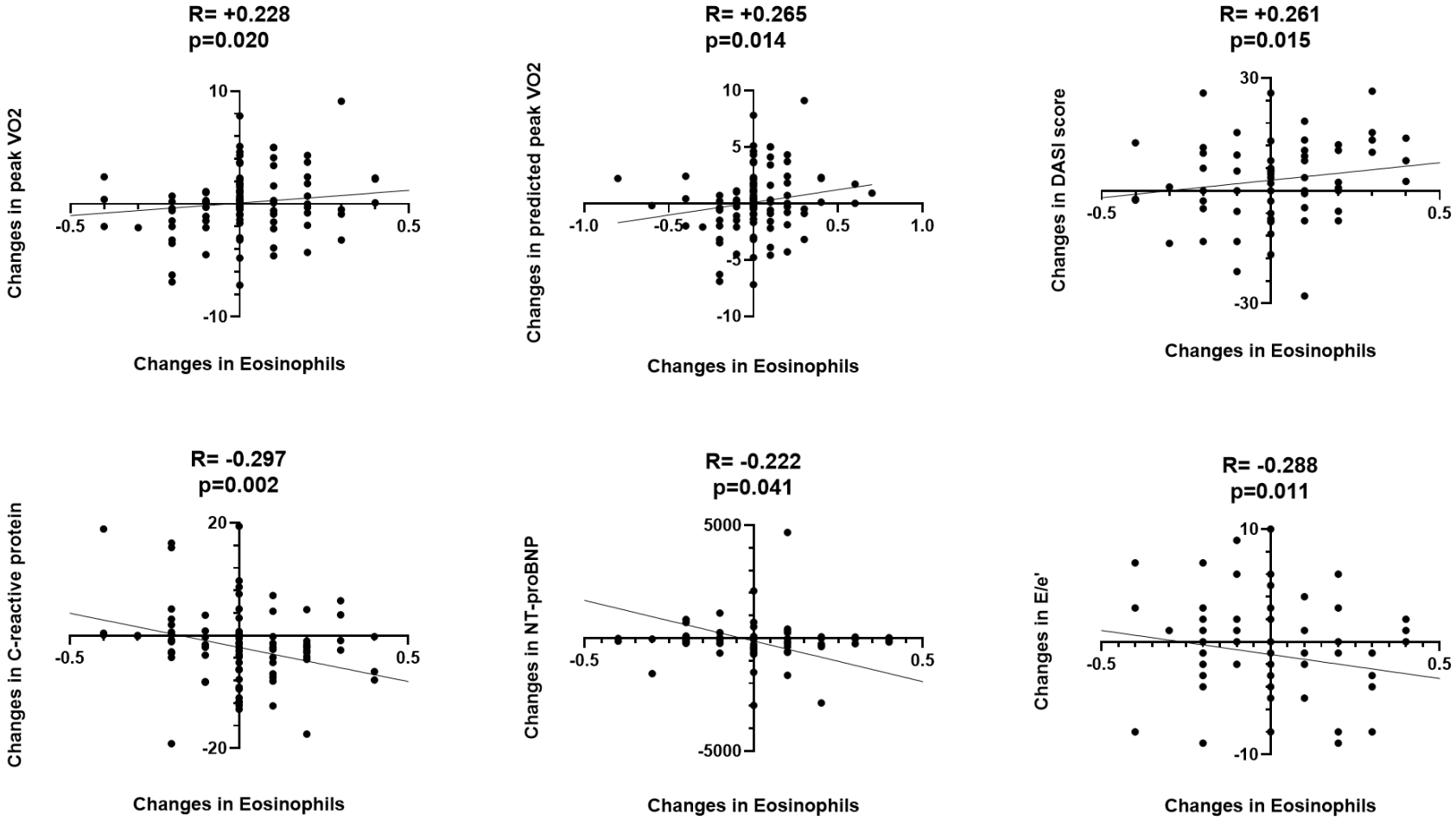
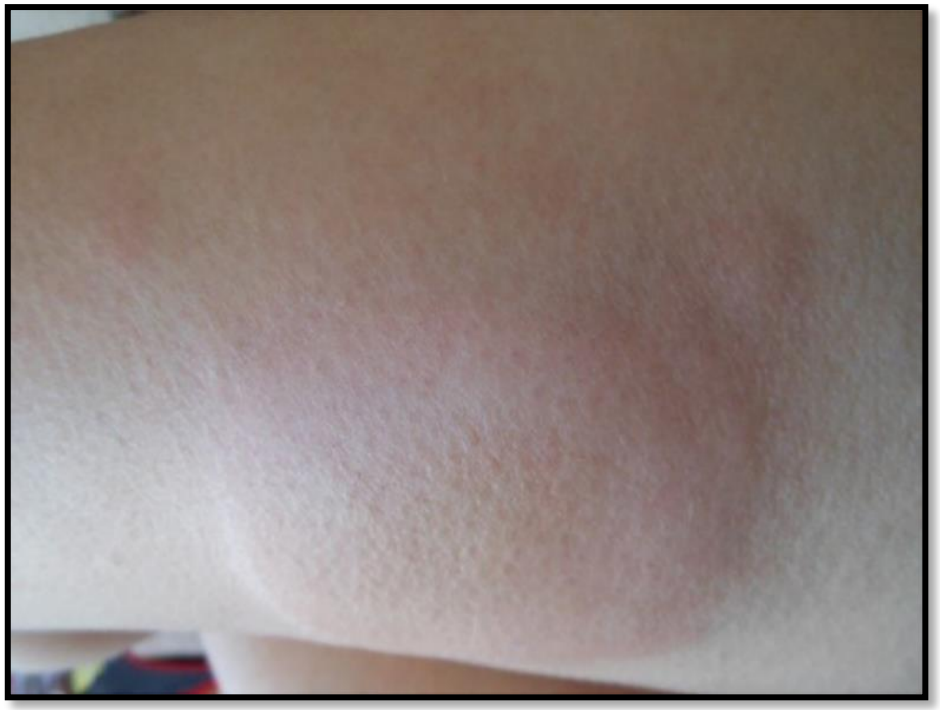
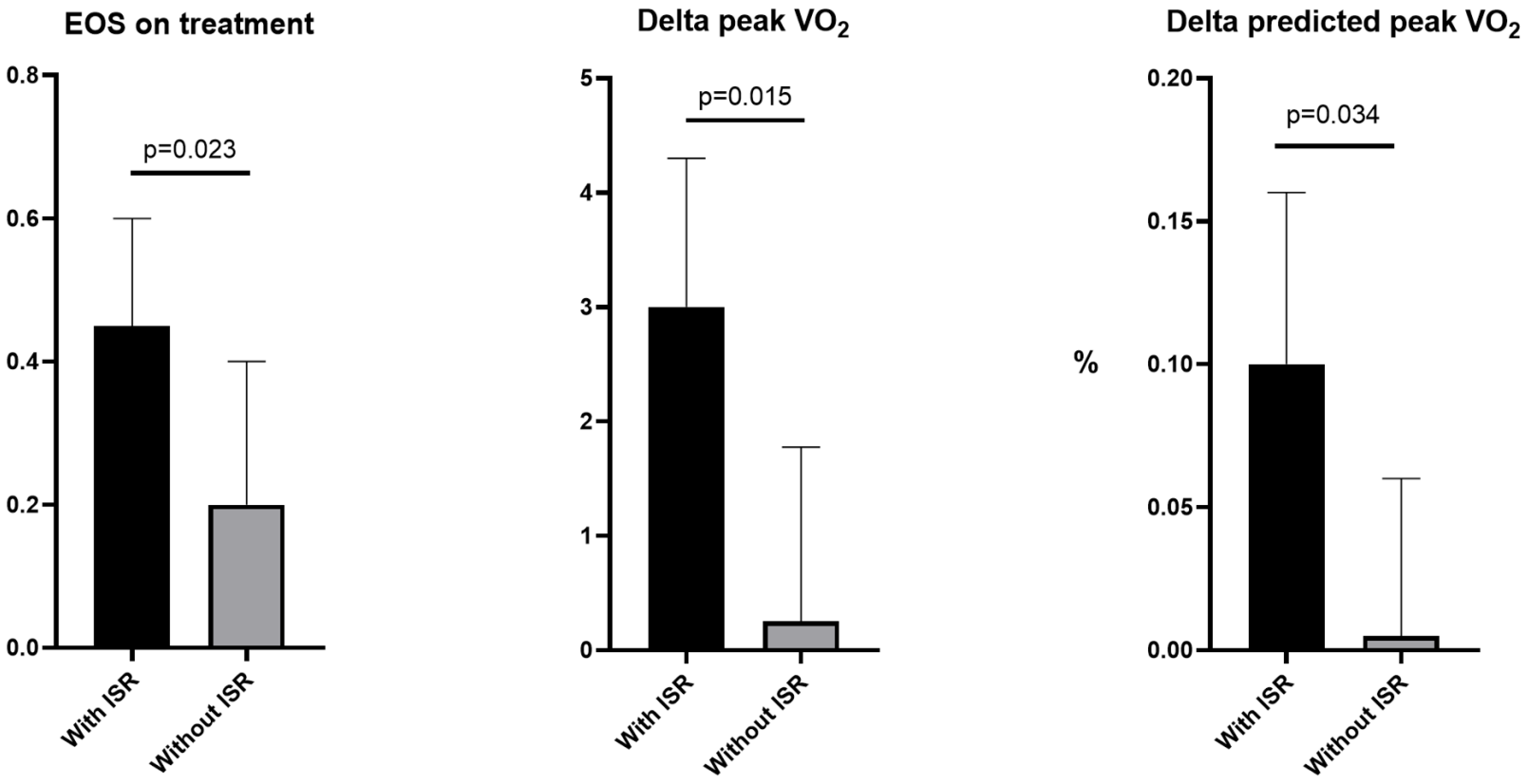


Figure 15. Example of an injection site reaction (ISR) with anakinra.



(Adapted from Hentgen V et al. The Use of Interleukine-1 Inhibitors in Familial Mediterranean Fever Patients: A Narrative Review. Front Immunol. 2020;11:971.)

Figure 16. Injection site reactions (ISR), eosinophils and peak VO_2 changes.



(Adapted from Golino et al. Change in Eosinophil Count in Patients with Heart Failure Treated with Anakinra. Cells. 2023;12(8).)

DISCUSSION

The tangled link between inflammation and HF has led to extensive research efforts over the past decade. By investigating the effects of IL-1 blockade via anakinra, this study addresses notable gaps in our understanding of the dynamic interplay of widespread inflammatory pathways and the subsequent cellular responses in patients with HF.

The study found that IL-1 blockade with anakinra led to a significant transient increase in EOS in patients with HF, although within the normal range. Notably, the increase in EOS was greater with a longer duration of treatment and was reversed upon withdrawal. In addition, the increase in EOS was inversely related to systemic inflammation, assessed through CRP, and directly associated with greater improvement in CRF, precisely peak VO_2 and predicted peak VO_2. This finding provides new insights into how IL-1 blockade might impact distinct WBC types in the context of HF and the possible consequent clinical benefits.

Historically, EOS have held the most attention in the contexts of allergic responses and specific infections. On the other side, eosinophilia after treatment with anakinra has been reported in as many as 9% of patients with rheumatoid arthritis,(186) although the exact mechanism and function are unknown.(1)

An increase in EOS above normal limits has been associated with severe CV manifestations such as myocarditis, endocarditis, thrombosis, endomyocardial fibrosis, valvular disease, and coronary artery disease.(125, 201) Few studies have investigated the protective role of EOS in CVD, especially within normal range levels; notably, EOS was pivotal in preclinical AMI models,(161) protecting cardiomyocytes from ischemia injury and death, regulating cardiac fibroblast activity and post-AMI inflammation. A reduction in EOS, named "eosinopenia", has been reported as a surrogate of hyper-inflammation, linked to adverse cardiac remodeling and function following AMI,(202) and associated with worse clinical outcomes over long-term follow-up.(1, 179, 203)

In preclinical models, Toor et al.(160) found that EOS and IL-4-driven EOS production reduced adverse remodeling of the LV following AMI. Mechanistically, they demonstrated this was achieved through IL-4 signaling because IL-4

supplementation rescued the adverse remodeling phenotype in the context of EOS deficiency. The role of EOS and IL-4 in cardiac health and disease now seems an important area for investigation.

The emergent observations of changes in EOS in the context of STEMI(176, 177, 178, 187) push the boundaries of our understanding of these cells. The effects of anakinra on EOS and its implications in patients with HF remain largely unexplored, and our study seeks to address this gap.

The current study reports a significant increase in EOS, and a reduction in NER and LER, in HF patients while on anakinra treatment. However, whether EOS counts may predict major adverse clinical events while on and off anakinra treatment is unknown. On this basis, the high EOS count seen during anakinra treatment in STEMI patients(187) might represent a potential mechanism by which anakinra reduces the innate immune inflammatory response to AMI and prevents HF post-AMI. This would then offer an opportunity to monitor the biological underpinnings of HF progression post-AMI.

Preclinical data showed that IL-1β, together with TNF-α, can upregulate the expression of E- and P-selectins, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 on the endothelial cells, necessary for the EOS adhesion and diapedesis. One possible explanation for the increase in EOS lies in the role of anakinra in blocking the IL-1 receptor and preventing the diapedesis of EOS, causing a prolonged presence in the peripheral blood and a greater increase in number.(181) However, the capacity for EOS to transmigrate through the endothelium is contingent upon their prior activation or preconditioning (a process known as "priming") through IL-3, IL-5, and GM-CSF.(204) These molecules are prevalently expressed in allergic and asthmatic individuals(205, 206) and could elucidate the variance observed in the peripheral EOS increase among patients treated with anakinra.

Another preclinical study has shown that IL-4 inhibits the production of IL-1β from monocytes.(184) In addition to T helper 2 lymphocytes, EOS are indeed a source of IL-4 and the enhanced clinical benefit in patients with an increase in EOS during treatment with anakinra may stem from a synergistic effect between the blockade of IL-1 activity and the inhibition of the production of IL-1 by the action of EOS-derived

IL-4. The inverse correlation between CRP (a surrogate for IL-1 activity) and EOS levels found in this study could provide evidence for this.

Finally, the simultaneous increase in EOS and decrease in absolute neutrophils, NER and LER during treatment with IL-1 blockade could be interpreted as a sign of potential action at the bone marrow level and a preferential maturation of the granulocyte-macrophage progenitor towards one lineage (eosinophilic) rather than another (neutrophilic). The results of the study by Vural et al.,(207) reporting that NER and LER could serve as predictors of 6-month mortality and MACE in a combined cohort of patients with ADHF with reduced EF, may add clinically beneficial evidence to this theory.

Injection-site reactions (ISR) are the most commonly reported side effects during treatment with anakinra, occurring in more than 70% of cases in some series. This finding is associated with increased circulating EOS and eosinophilic infiltrates into the dermal site.(193) For the first time, this study showed that patients with ISR had increased peripheral EOS counts and a greater response in CRF, measured by peak VO_2 and predicted peak VO_2. This suggests a correlative effect of an enhanced eosinophilic response to anakinra and clinical response. Accordingly, a positive correlation was found between EOS, NER, LER, and the changes in peak VO2, predicted peak VO2, and perceived functional capacity (DASI score). Whether the difference in improvement in CRF was related to the different baseline clinical characteristics of the study populations with and without ISR, or it represented the ability of EOS to serve as a biomarker of a more robust therapeutic response to anakinra cannot be determined from these data.

The inverse correlation found between EOS and markers of impaired cardiac diastolic function (NT-proBNP and E/e' ratio) and the direct correlation with CRF (peak VO2 and predicted peak VO2) and DASI score advocates a possible cardioprotective effect of EOS in HF.(1) The present study suggests that the increase in EOS may be one of the therapeutic mechanisms of anakinra in HF, also reflecting a biomarker of response, and EOS could be a direct therapeutic target or a target to modulate for additional benefits in this clinical setting. Additional studies are required to substantiate these findings.

These data also show that 1) treatment with IL-1 blockade with anakinra for a median of 4 weeks is associated with an improvement in CRF, measured by peak VO_2 and predicted peak VO_2; 2) the change in peak VO_2 with anakinra is significantly associated with the change in systemic inflammatory burden, estimated by plasma concentration of CRP; 3) the improvement in CRF with anakinra is not explained solely by improvement of congestion or an increase in baseline LVEF; 4) the improvement in peak VO_2 is reversed mainly upon discontinuation of treatment, in parallel with a loss of improvement in CRP values; 5) there is an improvement in QoL with anakinra.

Animal studies have shown that IL-1, produced both locally and systemically, can significantly impair myocardial contractility as well as relaxation, favoring adverse remodeling and HF.(208, 209) In the long term, IL-1 perpetuates myocardial dysfunction, causes impaired β-receptor responsiveness and leads to progression of HF.(209) Building on these observations, IL-1 has attracted much attention as a potential therapeutic target in HF. Notably, results from the Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) trial have highlighted that blocking IL-1 in patients who previously had an AMI reduced the risks of MI, stroke and cardiac death, as well as HF-related adverse events. This underscores the potential beneficial effects of IL-1 blockade in heart disease.(73, 88) Additionally, previous studies have shown that the modulation of systemic inflammation by inhibition of IL-1 with anakinra positively impacts exercise capacity in HF patients.(70, 71, 91) The current study expands previous findings, suggesting that IL-1 is an active and dynamic regulator of CRF in HF. While IL-1 blockade with anakinra improves peak VO_2, the benefits are lost once the treatment is stopped after about 12 weeks. This indicates that IL-1 blockade does not resolve or cure inflammation in HF, or at least that anakinra, given for a median duration of 4 weeks at a standard dose of 100 mg daily, is incapable of doing so. A new clinical trial is ongoing to explore the effects of 6-month therapy with anakinra in HF (REDHART2, clinicaltrials.gov identifier NCT03797001).(90) It is worth mentioning that changes in CRP observed during initiation and discontinuation of the treatment are paralleled to a reduction in total WBC count and especially circulating neutrophils. Similar changes in peripheral WBC have been recently described after anakinra administration in the setting of STEMI.(187) At the same time, however, a suspension of the treatment with anakinra does not result in a

rebound inflammatory response, which has been a concern in some previous studies.(210)

These results support the key role of systemic inflammation and IL-1 in influencing CRF in patients with HF. CRF significantly improved despite a small change in Doppler-derived estimates of cardiac filling pressure. However, no stress Doppler echocardiography was performed in these patients, so an improvement in cardiac reserve cannot be excluded. Indeed, IL-1-mediated inflammation has been shown to adversely impact exercise cardiac reserve in pre-clinical models of HF.(211, 212) An ongoing clinical trial aimed at exploring the role of IL-1 blockade on cardiac reserve after a STEMI will shed light and bring new insights.(Virginia-ART4, clinicaltrials.gov identifier NCT05177822) Furthermore, inflammation is deleterious to the peripheral determinants of exercise capacity, including peripheral vascular function and muscular metabolism,(213, 214) and peripheral hemodynamics may be relatively more important in determining exercise tolerance in patients with HF than healthy subjects.(215) Thus, IL-1 inhibition could potentially improve the peripheral component of VO_2 consumption even in a lack of data.

LIMITATIONS

Some limitations of this study should be noted:

1. First, the analysis was based on a small number of patients with HF selected from previous clinical trials and may not be generalizable to a broader population.
2. Second, the observation period was short, limiting the long-term implications of using anakinra.
3. Third, there was no available data on the EOS activity (protein secretion, IL-4, etc.) in the original studies, so it was impossible to say whether the observed increase in EOS corresponded to an increase in their activity.
4. Fourth, only 41 of the 64 patients had assessments after discontinuing the treatment and were included in the off-treatment analysis.

CONCLUSION

In conclusion, patients with HF and high systemic inflammation treated with IL-1 blockade with anakinra experience a significant increase in peak oxygen consumption and in peripheral EOS count within the normal range. These changes are reversed upon cessation of anakinra therapy, highlighting the role of IL-1 as an active modulator of CRF in HF.

Also important is the observation that patients who have ISR show a higher increase in EOS count and a greater improvement in peak oxygen consumption. These could represent a subset of patients with a greater response to anakinra. Moreover, these findings could also suggest EOS as a useful prognostic biomarker for IL-1 blockade and a potential therapeutic target in the setting of HF.(1)

All these observations thus support IL-1 blockade as an increasingly promising therapeutic strategy in HF patients and emphasize the importance of further research. A deeper understanding of the intricate interaction between IL-1 and EOS in the pathogenesis and progression of HF may lead to new directions for more specific and effective interventions.

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