ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

Assessment of selected hematological parameters of Congestive Heart Failure patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Bekalu Yirga, entitled:

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Contents

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List of Tables and Figures

Abbreviations

ACC----------American College of Cardiology

ACCF/AHA------American College of Cardiology Foundation/American Heart Association

ADHF----------Acute decompensated heart failure

AHA----------American heart association

ALC---------- Absolute lymphocyte count

Baso----------Basophils

BNP----------B-type natriuretic peptide

CAD----------Coronary artery disease

CBC---------- Complete blood count

CHF----------Congestive heart failure

ECG----------Electrocardiogram

Eos----------Eosinophil

ESC----------European Society of Cardiology

Hct. ----------Hematocrit

HF----------Heart failure

HFpEF----------Heart failure with preserved ejection fraction

HFrEF----------Heart failure with reduced ejection fraction

Hgb----------Hemoglobin

Lymph----------Lymphocyte

JVP---------- Jugular venous pressure

LVSD---------- Left Ventricular Systolic Dysfunction

LVEF----------Left ventricular ejection fraction

LVESV----------Left ventricular end-systolic volume

MCH----------Mean cell hemoglobin

MCHC----------Mean cell hemoglobin concentration

MCV----------Mean cell volume

NLR---------- Neutrophil to Lymphocyte Ratio

NT-pro BNP----------N-terminal pro hormone BNP

NYHA----------New York Heart Association functional classification

PCV----------Packed cell volume

PLR---------- Platelet to Lymphocyte Ratio

RBC----------Red blood cell

RDW----------Red blood cell distribution width

SPHMMC----------St. Paul's Hospital Millennium Medical College

WBC----------White blood cell

Abstract

Background: Hematological parameters have been proposed as useful adjuncts in the clinical evaluation of patients with congestive heart failure (CHF), offering potential insight into disease severity when interpreted alongside other diagnostic tools.

Objectives*:* To assess selected hematological parameters in adult patients with CHF across different New York Heart Association (NYHA) functional classes.

Methods: A cross-sectional study was conducted among 206 adult patients with CHF attending the Cardiology Department at St. Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, in February 2022. Peripheral venous blood samples (5 mL) were collected in EDTA tubes and analyzed using the DxH 800 Beckman Coulter hematology analyzer. Data were processed using SPSS® version 26.0. A p-value of < 0.05 was considered statistically significant.

Result: Significant differences in hematocrit (Hct), lymphocyte count (Lymph), and red cell distribution width (RDW) were observed between NYHA functional classes. Specifically, the mean values of Hct, Lymph, and RDW differed significantly between NYHA class II and class IV (Hct: $p = 0.035$; 95% CI: −10.567 to −0.284; Lymph: $p = 0.035$; 95% CI: 0.027 to 1.096; RDW: $p = 0.002$; 95% CI: −2.926 to −0.489). Additionally, comparisons between NYHA class I and class III revealed significant differences in Lymph ($p = 0.010$; 95% CI: 0.127 to 1.279) and RDW ($p = 0.046$; 95% CI: −2.796 to −0.016). Receiver operating characteristic (ROC) curve analysis demonstrated that RDW had an AUC of 0.663 (95% CI: 0.549–0.777; $p = 0.009$) and Lymph had an AUC of 0.657 (95% CI: 0.543–0.771; $p = 0.012$) in distinguishing NYHA class I from class III, with cut-off values of 13.5% for RDW and 1.718×10^9/L for Lymph. When comparing NYHA class I and class IV, RDW showed an AUC of 0.708 (95% CI: 0.608–0.808; $p = 0.001$) and Lymph an AUC of 0.622 (95% CI: 0.514–0.729; $p = 0.035$), with corresponding cut-off values of 15.9% and 1.95×10^9/L, respectively**.**

Conclusion: Significant differences in hematological parameters, including Hct, RDW, and Lymph, were observed across NYHA functional classes, particularly in later stages of CHF. These findings may support their further investigation as potential markers in the clinical assessment of disease progression.

Keywords: Congestive heart failure, hematological parameters, NYHA classification, red cell distribution width, lymphocyte count, hematocrit.

1. Introduction

1.1 Background

Heart failure is a serious medical issue that affects an estimated 26 million people globally, and it accounts for more than 1 million hospital admissions annually in the US and Europe alone (1). Both the European Society of Cardiology (ECS) as well as American Heart Association (AHA)/American College of Cardiology (ACC) define heart failure (HF) as a clinical syndrome characterized by typical symptoms (e.g., shortness of breath, ankle swelling, and fatigue) and signs (e.g., elevated jugular venous pressure, pulmonary crackles, and peripheral edema) caused by a structural and/or functional cardiac abnormality, resulting in decreased cardiac output and/or elevated intracardiac pressures at rest or with stress (2,3). Although multiple disorders of respiratory and renal function can also contribute to some of these signs and symptoms, an overarching inability of the heart to maintain adequate pump function without utilizing reserve mechanisms is fundamental for the diagnosis of heart failure. The reduction in the pump function of the heart may occur as the consequence of any of a multitude of abnormalities that distort the tightly coordinated aspects of the normally highly efficient cardiac performance (4).

The pathophysiology of heart failure is a highly complex phenomenon that involves changes in; cardiac function, neurohumoral status, systemic vascular function, blood volume, and integration of cardiac and vascular change (5). Left ventricular (LV) dysfunction can be divided into two categories: systolic dysfunction (impaired ventricular contraction and ejection) and diastolic dysfunction (impaired relaxation and ventricular filling) (6). Overall, the changes in cardiac function associated with heart failure result in a decrease in cardiac output, that in turn is the direct consequence of a decline in stroke volume due to systolic dysfunction, diastolic dysfunction, or a combination of the two (5). Most HF patients with LV dysfunction have systolic dysfunction (about 70%) compared with diastolic dysfunction (about 30%) (6). Moreover, patients with systolic dysfunction also have a component of diastolic dysfunction. Whether or not a patient with HF has, systolic or diastolic dysfunction depends on the ejection fraction (EF). The other type of heart failure is a diastolic failure, which is caused by impaired ventricular filling. Diastolic failure can be caused by either decreased ventricular compliance or impaired relaxation (5). A number of factors can raise the risk of heart failure, the most important ones being age, family history, lifestyle habits, genetics, sex, race, or ethnicity (7).

Many factors can raise one's risk of heart failure, the most important ones being; age, family history, lifestyle habits, genetics, sex, race, or ethnicity. People 65 years or older have a higher risk of heart failure because aging can weaken and stiffen the heart (7). Older adults are also more likely to have other health conditions that cause heart failure. In other instances, the risk of heart failure is higher if people in one's family have been diagnosed with heart failure. Certain gene mutations can also raise the risk. These mutations make the heart tissue weaker or less flexible. Concerning lifestyle habits, an unhealthy diet, smoking, using cocaine or other illegal drugs, heavy alcohol use, and lack of physical activity can raise the risk of heart failure. Any heart or blood vessel condition, serious lung disease, or infection such as HIV or SARS-CoV-2 may also raise one's risk of heart failure (7). Long-term health conditions such as obesity, high blood pressure, diabetes, sleep apnea, chronic kidney disease, anemia, thyroid disease, or iron overload also raise the risk of heart failure (7). Atrial fibrillation, a common type of irregular heart rhythm, can also cause heart failure. Other major risk factors like race or ethnicity also increase one's likelihood of getting HF, African Americans are more likely to have heart failure than people of other races (7). They often have more serious cases of heart failure at a younger age. On the other hand, even though heart failure is common in both men and women, men often develop heart failure at a younger age than women do (7).

Since the diagnosis of heart failure covers such a broad range of severity from the compensated ambulatory fully functional to the bedridden mechanically supported patient, more refinement in categorization is needed. The New York Heart Association functional classification (NYHA) is the oldest, time-honored system that was introduced in 1928 to describe a person's physical limitations during the performance of daily activities (4). The NYHA classification has consistently proven its value as a major instrument to assess disease severity and provide important information regarding prognosis. The deceptively simple classification continues to be an extremely useful and powerful tool to communicate disease severity for both the individual patient as well as cohorts in clinical trials, epidemiologic surveys, and administrative databases (4,8). The NYHA classification system makes an important part in all of the major stratification scores systems of heart failure prognosis and continues as the main severity categorization for the most recent European Society of Cardiology (ESC) guidelines for the diagnosis and treatment of acute and chronic heart failure (3). The NYHA classification has consistently proven its value as a major instrument to assess disease severity and provide important information regarding prognosis (4).

The American Heart Association/American College of Cardiology (AHA/ACC) guideline for the diagnosis and management of heart failure in adults, introduced in 2001, provided a new contextual framework to characterize this spectrum of heart failure and its treatments. This system was introduced in the setting of heart failure practice guidelines to improve the utilization of evidence-based approaches to prevent as well as manage heart failure (9). This four-stage (A, B, C, D) classification underscores the progressive nature of heart failure and additionally provides an important stage-specific framework to consider opportunities for therapeutic interventions to lower the odds of both developing heart failure or progressing to the higher morbidity and mortality of heart failure (4).

The laboratory diagnosis of CHF generally involves the evaluation of selected hematologic, biochemical, and biomarker parameters. The initial evaluation of patients with CHF should include Compete blood count (CBC), urinalysis, serum electrolytes, glycohemoglobin, blood lipids, thyroid function test (thyroid-stimulating hormone), and renal and hepatic function test (9). Biochemical tests are the other important component of the whole test scheme and it mainly involves BUN (blood urea nitrogen), Albumin, C-reactive protein (CRP), uric acid (UA), estimated glomerular filtration rate (eGFR), creatinine, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), sodium, calcium and potassium (10–13). The other type of test, which is the least common and requires an advanced laboratory setting, is the biomarker test. The test includes; soluble toll-like receptor-2 (ST2), troponin I (Tnl), myeloperoxidase (MPO), high-sensitivity C-reactive protein (hsCRP), soluble fms-like tyrosine kinase receptor-1(sFlt-1), Growth differentiation factor-15 (GDF-15), Highly sensitive troponin T(hsTnT), Mid-regional pro adrenomedullin (MR-proADM), Mid-regional pro atrial natriuretic peptide (MR-proANP) and galectin-3 (16–19).

1.2 Statement of the Problem

Advancements in the fundamental understanding of the etiology, pathophysiology, and hemodynamics of Heart failure have opened the door for better therapy and treatment options, that span from effective new drugs to non-pharmacological interventions, including heart transplantation, that improved significantly the quality of life and prolonged survivability of patients (16, 17). The accurate diagnosis of HF is the highest priority to deliver the right treatment regimen for the patient.

Overreliance on clinical signs and symptoms alone for HF diagnosis could pose significant risks to patients, potentially leading them into serious difficulties. Research indicates that misdiagnosis is alarmingly common in both primary and secondary healthcare settings, with up to half of all cases being inaccurately diagnosed with HF(20). Though much of the burden of heart failure can be prevented or deferred, the reality is that symptomatic heart failure continues to be the dominant cardiovascular problem in elderly people. In Ethiopia, similar to other sub-Saharan African countries, the challenge lies in finding affordable diagnostic techniques suitable for use in the most basic healthcare facilities without sacrificing accuracy. Given that the country is very resource-limited in providing sophisticated, cutting-edge diagnostic tools and medical equipment that are necessary for the accurate diagnosis of HF, other easily accessible and available means should be explored to tackle this issue (21). The problem is most noticeable among primary and secondary healthcare facilities where patients first encounter General practitioners or other less qualified healthcare professionals that heavily relied on physical examination for the diagnosis of HF, and that accelerates the odds of misdiagnosis by far and large (22). Hematological tests can potentially be game changers here, as they are abundantly available even in the lowest-tier of health establishments, as primary healthcare facilities, and they can change the way we diagnose HF.

The primary aim of this research is to tap into the significant potential harbored within certain hematological parameters to enhance our comprehension of CHF diagnosis as a whole. More crucially, it seeks to improve the accuracy of predicting the disease's prognosis.

1.3 Significance of the Study

The primary beneficiaries of this research will be healthcare institutions operating at the lower tiers of the national medical system, particularly primary and secondary care facilities in Addis Ababa. The findings are expected to be applicable to similar settings across the country, especially where there is limited access to specialized personnel and advanced diagnostic technologies necessary for the accurate assessment and prognostication of congestive heart failure (CHF). By evaluating the utility of basic hematological parameters, this study addresses a critical diagnostic gap in these resource-constrained environments. The results may support clinical decision-making and risk stratification and contribute to the growing body of evidence on the role of routine laboratory tests in assessing CHF severity and progression.

2. Literature review

The Laboratory diagnosis of HF is a very important segment in the overall evaluation of the disease, along with other diagnostic tools it very much assists in understanding the cause and type of the disease, the most probable course it might take, or its level of severity. Among other tests, hematological assays are generally considered the most important ones since they present with the best assessment tools like red blood cell distribution width (RDW), neutrophil to lymphocyte ratio (NLR), serum ferritin, transferrin saturation, hemoglobin concentration (Hb), etc. that best predict the prognosis of the disease (11,23–25).

The initial evaluation of patients suspected of HF, as highlighted by Jessup M et al., includes a history and physical examination that involves chest radiography, electrocardiography, and laboratory assessment. On the other hand, according to the legendary Framingham diagnostic criteria, which mostly involves physical examination, for the patient to qualify as an HF patient one must fulfill two major or one major and two concurrent minor criteria (26). In almost all cases though, Echocardiography is used to confirm the diagnosis (9). There is such a strong consensus among various studies on the significance of BNP (B-type natriuretic peptide), NT-proBNP (N-terminal-pro hormone BNP), and ECG, in the diagnosis of Left ventricular systolic dysfunction (LVSD) (9,27,28). Madhok V et al. and Kelder J et al. claimed that a displaced cardiac apex, a third heart sound, pulmonary venous congestion, and interstitial edema are diagnostically more important to rule in LVSD. Contrastingly, Madhok V et al. argued that findings from the clinical history and examination alone are insufficient and misleading to "rule in" or "rule out" a diagnosis of LVSD, thus necessitating their integration with laboratory tests (28,29).

The laboratory evaluation along with the history of the patient and physical examination provide clues on the type of heart failure, its cause, and any comorbidity involved. According to the American College of Cardiology (ACC) and American Heart Association (AHA) guidelines for the evaluation and management of chronic heart failure, once the structural abnormalities underlying the development of heart failure have been identified, clinical attention should be directed toward comprehensive and continuous patient assessment. Ongoing evaluation of the patient’s clinical status is integral to the appropriate selection, titration, and monitoring of therapeutic strategies (30). In accordance with these guidelines, Hunt et al. recommend that laboratory evaluation should include a complete blood count (CBC), urinalysis, serum electrolytes

(including calcium and magnesium), blood urea nitrogen, serum creatinine, blood glucose, liver function tests, and thyroid-stimulating hormone (TSH) levels (30). Antinuclear antibodies (ANA) and rheumatoid factor (RF) assays are done in conditions where connective tissue disease is suspected, whereas metanephrines are considered if pheochromocytoma is suspected, others like serum ferritin and liver function tests are important parameters when examining hemochromatosis. Kavsak et al. further reaffirmed the prognostic value of B-type natriuretic peptide (BNP) and NT-proBNP, associating elevated levels with advanced disease severity and poorer outcomes (31).

A growing body of evidence supports the prognostic role of hematological parameters in HF (24,32–34). Two independent studies by Azab et al. and Uthamalingam et al. examined patients with distinct cardiac conditions, highlighting the prognostic value of the neutrophil-to-lymphocyte ratio (NLR). Azab et al. analyzed 619 patients with non–ST-elevation myocardial infarction (NSTEMI) admitted to Staten Island University Hospital between September 2004 and September 2006. In contrast, Uthamalingam et al. investigated 1,212 consecutive patients admitted with acute decompensated heart failure (ADHF) to the New England Heart Institute at the Catholic Medical Center, Manchester, New Hampshire, from January 2006 to December 2008. Both studies concluded that NLR is a strong and independent predictor of both short-term and long-term survival. (32,35).

Another major study undertaken by Liu S et al., to assess the prognosis significance of RDW, that involves 179 CHF patients who were divided into four categories based on the NYHA classification system as class I (n=44), II (n=39), III (n=41), and IV (n=55), finds that RDW was markedly elevated in the mortality group compared with the survival group (13.7 ± 1.7 vs. 15.8 ± 1.8, $p < 0.01$) (33). Even though the predictive value of RDW was lower than that of NT-ProBNP, it was comparable to a white blood cell, neutrophil, lymphocyte, and neutrophil/lymphocyte ratio (N/L) for mortality during hospitalization, with the area under ROC curve (AUCs) of 0.837, 0.939, 0.858, 0.891, 0.885, and 0.885, respectively (33). Based on its findings the study concluded that elevated RDW is an independent risk factor for mortality a claim that is corroborated by Dai Y et al. (36). Another important element in the prognosis of chronic heart failure is anemia, it's the most common experience among HF patients and is associated with increasing disease severity and mortality (37). A study that involves a systematic review and meta-analysis of eleven studies has also shown that anemia is associated with an increased risk of

mortality and rate of hospitalization for heart failure (38). Both studies have indicated that anemia is an independent risk factor for adverse outcomes in patients (38,39).

In a 2010 study by Westenbrink B. et al., conducted at the University Medical Center Groningen, The Netherlands, bone marrow samples were collected from 20 patients undergoing coronary artery bypass graft surgery. The study revealed that chronic heart failure (CHF) is associated with significant and widespread bone marrow dysfunction, affecting multiple hematopoietic lineages. Notably, CD34+ cells from CHF patients generated approximately half the number of BFU-E colonies compared to controls ($p = 0.02$) (40).

The resistance to EPO was associated with markedly increased apoptosis during erythroid differentiation in CHF patients compared with controls [5.3% (2.9–8.1%) vs. 1.5% (0.8–3.4%), $p = 0.01$]. Moreover, the number of CFU-G and CFU-M colonies was also two-fold lower in CHF patients compared with controls (both $p < 0.01$) (40). These findings highlight the importance of evaluating hematologic parameters in understanding HF pathophysiology.

In the Sub-Saharan African context, most CHF studies have focused on disease etiology and the prevalence of anemia. For example, work by Khatibzadeh et al. and Gallagher et al. reported that cardiomyopathies and hypertension are among the leading causes of HF in the region (21,41). Akintunde AA and Aworanti OW conducted a study involving 140 heart failure patients recruited from the cardiology clinics of two teaching hospitals in southwest Nigeria—Ladoke Akintola University of Technology and Bowen University Teaching Hospitals—which revealed a high prevalence of anemia among Nigerian HF patients (43). Similarly, a study by Ikama M et al., conducted between January 1 and December 31, 2010, at Brazzaville University Hospital in Congo, that include 139 male and 142 female CHF patients also demonstrated a high prevalence of anemia (42). Both studies highlight the significant burden of anemia among CHF patients in the region. Both studies reported a high prevalence of anemia and noted its deleterious effect on prognosis (42,43). However, most research in the region has been limited to identifying etiological factors or assessing the impact of anemia, without evaluating how HF progression influences other hematologic parameters.

The bulk of the literature reviewed so far has pointed out that hematological parameters are good predictors of the prognosis of CHF. Studies have also revealed that the prognosis of CHF has a strong link with some specific hematologic parameters, like NLR and RDW (32,33,35). Although the prognostic value of hematologic parameters has been widely recognized, the effect of HF progression on these parameters across disease stages remains underexplored. This study aims to address this gap by evaluating the association between selected hematological indices and the severity of HF based on NYHA classification.

Conceptual Framework

The conceptual framework guiding this study is grounded in the understanding that heart failure (HF) is a complex, multifactorial syndrome wherein clinical progression and outcomes are influenced by a dynamic interplay of physiological, hematologic, and demographic variables. The model (Figure 1) illustrates the hypothesized relationships between patient characteristics, heart failure severity, hematologic parameters, and clinical prognosis.

At the foundation of this framework are key demographic and clinical modifiers—anemia status, age, and sex—which are known to influence both the severity and progression of HF. These factors contribute to the determination of New York Heart Association (NYHA) functional classification, a widely accepted metric of symptom burden and exercise limitation in HF. Comorbidities, including hypertension, diabetes mellitus, and chronic kidney disease, also interact with NYHA class, forming a bidirectional relationship wherein comorbid conditions may exacerbate HF severity, and vice versa.

NYHA classification in turn is hypothesized to influence hematologic parameters, including red cell distribution width (RDW), hematocrit (HCT), and lymphocyte count. These parameters are not only reflective of systemic inflammation, anemia, and nutritional status, but also serve as accessible, cost-effective biomarkers for disease severity.

The combined influence of hematologic markers, NYHA classification, and baseline demographic variables is expected to shape the clinical prognosis of HF patients—an implied outcome that encompasses survival, risk of hospitalization, and disease progression. In this framework, hematologic parameters are positioned as intermediate variables that mediate the relationship between heart failure severity and eventual clinical outcomes.

This model aligns with existing literature that highlights the prognostic relevance of hematologic parameters in HF, yet it underscores the need to explore these relationships more systematically across the spectrum of NYHA classifications, particularly in resource-constrained settings.

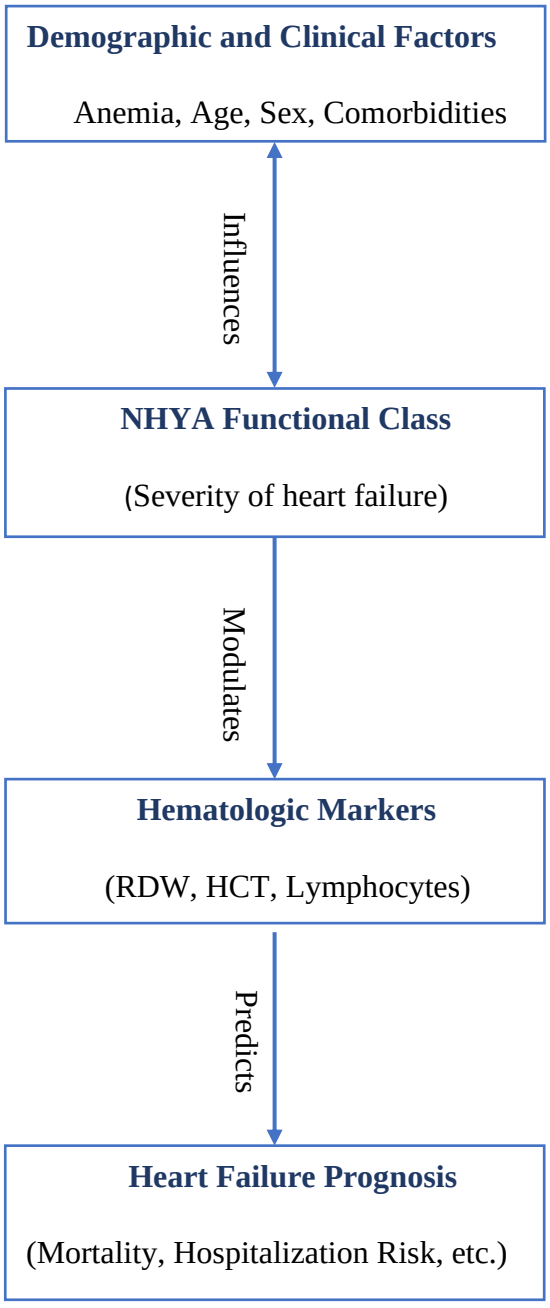

Figure 1. *Conceptual framework illustrating the relationships among demographic and clinical factors, NYHA functional class, hematologic markers, and heart failure prognosis. Arrows indicate directional influences, with a bidirectional arrow between NYHA class and demographic/clinical factors reflecting mutual exacerbation.*

3. Objective

3.1 General Objective

- To assess selected hematological parameters in patients with congestive heart failure (CHF) at St. Paul's Hospital Millennium Medical College.

3.2 Specific Objectives

- To compare selected hematological parameters across the New York Heart Association (NYHA) functional classes in patients with CHF.

- To evaluate the predictive value of selected hematological parameters for determining NYHA functional class in patients with CHF.

4. Materials and Methods

4.1 Study Area

The study was conducted primarily at SPHMMC. It's among a handful of tertiary-level referral hospitals in the country, with an inpatient capacity of more than 700 beds. It's also one of the largest and most prestigious teaching hospitals in the country, with an annual admission capacity of well over hundreds of thousands of patients from all over the country. SPHMMC has more than 2800 clinical, academic, administrative, and support staff, and sees a daily influx of over 1200 emergency and outpatient clients. In general, the hospital admits roughly more than 8 CHF cases per week and more than 400 annually.

4.2 Study Design and Period

A cross-sectional study design has been used to study 206 participants, who have been admitted with CHF cases at the inpatient and outpatient department of SPHMMC, over the period of six months from February 2022 to June 2022.

4.3 Population

4.3.1 Source Population

The source population for this study comprises all patients diagnosed with congestive heart failure (CHF) who have been admitted to either the inpatient or outpatient departments of SPHMMC.

4.3.2 Study Population

The study population includes CHF patients managed at SPHMMC during the study period who fulfilled the predefined inclusion criteria.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria:

Participants were eligible for inclusion if they met all of the following criteria, as identified by treating physicians or confirmed through medical record review:

- A confirmed diagnosis of congestive heart failure (CHF)
- Age ≥ 18 years
- At least New York Heart Association (NYHA) functional class I at the time of assessment

4.4.2 Exclusion Criteria

Patients were excluded if they met any of the following criteria:

- Major surgical procedure within the three months preceding enrollment
- Presence of comorbid conditions known to significantly alter hematological parameters, including active infection, end-stage renal disease requiring dialysis, advanced hepatic disease, malignancy, or primary hematologic disorders
- History of blood transfusion within the six months prior to enrollment

4.5 Study Variables

4.5.1 Dependent Variables: Hematological parameters.

4.5.1 Independent Variables: New York Heart Association (NYHA) functional class, and comorbid cardiovascular diseases (CVD).

4.6 Measurement and Data Collection

4.6.1 Sample Size Calculation and Sampling Method

All patients referred to SPHMMC with a diagnosis of congestive heart failure (CHF) during the study period were considered for inclusion, provided they met the eligibility criteria.

The sample size was calculated based on the following formula as follows;

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where n: sample size

z: standard normal variate (at 5% type I error it is 1.96)

p: expected prevalence

(The prevalence is estimated at approximately 16%, based on previous studies conducted in hospital settings (44))

d: precision (5%)

Therefore,

$$n = \frac{1.96^2 \times 0.16(1-0.16)}{0{,}05^2}$$

$$n = \frac{0.51456}{0.0025} = 205.82 \approx 206$$

$$n = 206$$

4.6.2 Data collection procedures

The PI selected highly experienced laboratory technologists and phlebotomists as data collectors for the purpose of sample collection. These individuals possessed extensive hands-on experience in conducting hematological tests, as well as substantial expertise in phlebotomy techniques acquired over many years of practice. Therefore, they required only a brief refresher on the protocols to be followed, ensuring consistency and reliability throughout the sample collection process.

Generally, a venous blood sample of 5ml was collected in EDTA tubes from adult patients with strict adherence to Standard Operating Procedures and established guidelines. Additionally, sociodemographic and clinical data have been collected using a data extraction format specifically adapted for this study from the patient's medical record. (Refer to the annex at the very back)

4.6.3 Hematological analysis

In addition to the main sample collection unit of the laboratory, samples were collected in the emergency and cardiology departments of the hospital. After they have passed all the labeling criteria and sample integrity testing, it was then carefully delivered to the hematological department of the hospital, where it was analyzed with the 5-part DxH 800 Beckman Coulter Hematologic Analyser. This instrument operates based on Coulter Principle, according to this method, as a particle passes through the sensing zone when the liquid is drawn from the container; a volume of the electrolyte equivalent to the immersed volume of the particle is displaced from the sensing zone. This causes a short-term change in the resistance across the aperture. This resistance change can be measured as either a voltage or current pulse. By measuring the number of pulses and their amplitudes, it is possible to have information about the number of particles and the volume of each particle. (For all the quality control and test procedures, refer to the annex)

4.7 Data Quality Assurance

To ensure the quality of the tests we have taken all the necessary steps required, including implementing the quality control tools, like the one provided by the manufacturers of the machines. Overall, to verify the linearity of the machine, three types of quality control materials (normal, low abnormal, and high abnormal) have been run before examining patient samples. In accordance with this commitment, the PI and his associates ensured strict adherence to all relevant guidelines,

protocols, and SOPs throughout the entire process, from specimen collection and labeling to transportation, processing, and test result issuance. Moreover, during data entry, the PI verified the integrity of the recorded data by reviewing the original documents and cross-referencing them with the data in the system

4.8 Data Analysis and Interpretation

All data, including hematological, demographic, and clinical variables, were initially recorded in Microsoft Excel 2016 and subsequently imported into IBM® SPSS® Statistics for Windows, Version 26.0 for statistical analysis. Tables and figures have been employed to further illustrate statistical data, and one way ANOVA test have been used to compare mean values in the four stages (NYHA classes I through IV) of the disease. Generally, a p-value less than 0.05 has been considered statistically significant.

4.9 Ethical considerations

This study was conducted in full accordance with ethical research principles involving human participants. Prior to data and sample collection, informed written consent was obtained from all participants after a clear explanation of the study's objectives, procedures, potential risks, and benefits. Participation was entirely voluntary, and no patient was coerced, pressured, or unduly influenced to take part in the study. Confidentiality and anonymity were strictly maintained throughout the research process. Personal identifiers were removed from all data sets, and access to patient information was restricted exclusively to the principal investigator. All electronic data were stored in a password-protected account, created specifically for the study, and were not shared with third parties. Ethical approval for the study was obtained from the Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University, prior to commencement of the research.

4.10 Dissemination of the Result

The findings of this research will be submitted to St. Paul's Millennium Medical College (SPMMC) and the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. The results will be disseminated through both local and international channels, including academic seminars, conferences, and electronic publications.

4.11 Operational Definitions

- **Heart failure**: the term is interchangeably used with congestive heart failure throughout this paper.
- Throughout this paper, the terms "**Cases**," "**Study Subjects**," and "**Patients**" are used interchangeably to refer to individuals with congestive heart failure (CHF).
- **Framingham diagnostic criteria for heart failure**: The diagnosis of heart failure, in the Framingham heart failure study, required two ***major*** or one ***minor*** and two concurrent ***minor*** criteria.
- **Minor criteria:** cannot be attributed to another medical condition.
- **Major criteria**: includes acute pulmonary edema, Paroxysmal nocturnal dyspnea or orthopnea, Neck-vein distention, Rales, S_3 gallop, Abdominojugular reflux, Cardiomegaly on chest x-ray, increased venous pressure (> 16cm H_2O), Weight loss > 4.5 kg 5 days into treatment.
- **Minor criteria**: includes Dyspnea on exertion, Night cough, Tachycardia (> 120 beats/min), Pleural effusion, Hepatomegaly, Ankle edema, vital capacity decrease ($^1/_3$ from max), Weight loss > 4.5 kg 5 days into treatment.
- **HF-rEF:** an ejection fraction of less than or equal to 40% (LVEF ≤ 40%).
- **HF-pEF**: an ejection fraction greater than or equal to 50% (LVEF ≥ 40%).
- **HFmrEF**: LVEF 41–49%.
- **The New York Heart Association (NYHA) functional classification:**
  - **Class I —** No symptoms and no limitation in ordinary physical activity, e.g., shortness of breath when walking, climbing stairs etc.
  - **Class II—** Slight limitation of physical activity (ordinary physical activity results in fatigue, dyspnea, palpitations, or angina.), Comfortable at rest.
  - **Class III—** Marked limitation of physical activity (less than ordinary physical activity results in fatigue, dyspnea, palpitations, or angina), Comfortable at rest.
  - **Class IIIa—** No dyspnea at rest.
  - **Class IIIb—**Recent dyspnea at rest.
  - **Class IV — S**evere limitations. Inability to carry on any physical activity without discomfort, Symptoms present even at rest

5. Results

5.1 Sociodemographic Characteristics

The study population comprised 206 patients with chronic heart failure (CHF), with a near-equal sex distribution (52.9% male, 47.1% female). The mean age of the population was 48.1 years (SD: 17.2), with males being slightly older than females (50.2 vs. 45.9 years, respectively). Age distributions were right-skewed, as evidenced by medians (overall: 46.0 years; males: 47.0; females: 45.0) lower than means, and bimodal peaks at 40 and 60 years (overall mode). The age range was broad (18–85 years), with males having a narrower range (18–82 years) compared to females (21–85 years).

Table 1. Sociodemographic Characteristics of Patients with Chronic Heart Failure (n=206).

Characteristic	Overall Population	Male (n=109, 52.9%)	Female (n=97, 47.1%)
Age (years)			
Mean ± SD	48.1 ± 17.2	50.2 ± 16.7	45.9 ± 17.8
Median (IQR)	46.0 (33.0–62.0)	47.0 (35.0–63.0)	45.0 (32.0–60.0)
Range (min–max)	18–85	18–82	21–85
Mode	40, 60	40	60

5.2 Clinical Characteristics

The NYHA classes which comprise NYHA Class I, NYHA Class II, NYHA Class III, and NYHA Class IV have recruited 40 (19.4%), 49 (23.8%), 48 (23.3%), 69 (33.5%) patients respectively (Table 2). On the other hand, diabetic patients accounted for 25.7% of the total CHF patients whereas, regular smokers and heavy drinkers constitute 43(20.9%) and 62(30.1%) respectively. Other clinical findings include hypertensive patients, which constitute 128 (62.1%), whereas patients with other CVD diseases were 68 (33%), and patients with anemia and renal failures had been 30 (14.6%) and 45 (21.8%), respectively.

Table 2. Clinical Characteristics of Patients with Chronic Heart Failure (n=206)

Characteristic	Category	Frequency (n)	Percentage (%)
NYHA Functional Class	Class I	40	19.4
	Class II	49	23.8
	Class III	48	23.3
	Class IV	69	33.5
Diabetes Mellitus	Yes	53	25.7
	No	152	73.8
Hypertension	Yes	128	62.1
	No	78	37.9
Other CVDs	Present	68	33.0
	Absent	138	67.0
Anemia	Present	30	14.6
	Absent	176	85.4
Renal Dysfunction	Present	45	21.8
	Absent	161	78.2
Smoking Status	Current Smoker	43	20.9
	Non-Smoker	163	79.1
Alcohol Consumption	Heavy Drinker	62	30.1
	Non-Drinker	144	69.9

NYHA Class: "New York Heart Association functional classification of heart failure severity."
Heavy alcohol consumption*: "Defined as >14 drinks/week for men or >7 drinks/week for women (per AHA guidelines).*

Table 3 shows that most CHF patients' systolic blood pressure lies either in the <120 mmHg (33.83%) or ≥ 130 (39.32%) category. Whereas two scenarios were used to explain the left ventricular ejection fraction, the first was among the male population, where the majority of them (35.62%) lie in the range of 52% to 72% (normal range). The other scenario was among the female population, where the vast majority of patients (38.03%) have their LVEF lying in the 54% to 74% region, which is again in the normal range.

Table 3. Frequency Distribution of Systolic Blood Pressure (SBP) and Left Ventricular Ejection Fraction (LVEF) in Patients with Chronic Heart Failure (n=206).

A. Systolic Blood Pressure (SBP)			
SBP Category (mmHg)	**Frequency (n)**		**Percentage (%)**
<120	80		38.8
120–129	45		21.8
≥130	81		39.3
B. Left Ventricular Ejection Fraction (LVEF)			
LVEF Category (%)	**Male (n=109)**	**Female (n=97)**	**Total (n=206)**
<30	10 (4.9%)	6 (2.9%)	16 (7.8%)
30–40	13 (6.3%)	7 (3.4%)	20 (9.7%)
41–51 (M) / 41–53 (F)	6 (2.9%)	6 (2.9%)	12 (5.8%)
52–72 (M) / 54–74 (F)	80 (38.8%)	78 (37.9%)	158 (76.7%)

Table 4 presents the frequency distribution of cardiovascular comorbidities among patients with chronic heart failure admitted to SPHMMC (n=206). The data are summarized as both absolute frequencies (n) and percentages (%). Of the total 206 patients, 138 (67.5%) had no additional cardiovascular comorbidities (non-CV patients). Among those with cardiovascular comorbidities, hypertensive heart disease was the most common, observed in 33 patients (16.0%), followed by valvular heart disease in 12 patients (5.8%). Ischemic heart disease was identified in 6 patients (2.9%), while myocardial infarction and stroke each occurred in 4 patients (1.9%). Arrhythmia heart failure was noted in 2 patients (1.0%). Less frequent comorbidities included dilated cardiomyopathy, chronic rheumatic valvular disease, arterial thrombosis, mitral regurgitation, left bundle branch block, and eclampsia, each affecting 1 patient (0.5%). The total frequency of all conditions sums to 206, representing 100% of the study population.

Table 4. Frequency Distribution of Cardiovascular Comorbidities Among Patients with Chronic Heart Failure Admitted to SPHMMC (n=206).

Cardiovascular Comorbidity	Frequency (n)	Percentage (%)
Non-CVD Patients	138	67.5
Hypertensive Heart Disease	33	16.0
Valvular Heart Disease	12	5.8
Ischemic Heart Disease	6	2.9
Myocardial Infarction	4	1.9
Stroke	4	1.9
Arrhythmia Heart Failure	2	1.0
Dilated Cardiomyopathy	1	0.5
Chronic Rheumatic Valvular	1	0.5
Arterial Thrombosis	1	0.5
Mitral Regurgitation	1	0.5
Left Bundle Branch Block	1	0.5
Eclampsia	1	0.5
Total	206	100.0

5.3 Analysis of Selected Hematologic Parameters in Chronic Heart Failure Patients.

5.3.1 Statistical Analysis

Table 5 presents the descriptive statistics of red blood cell (RBC) indices among patients with chronic heart failure. The mean hemoglobin level was 13.1 ± 3.7 g/dL, with a corresponding hematocrit of 39.3 ± 11.2%. Red cell distribution width (RDW), a marker of erythrocyte size variability, had a mean of 15.9 ± 3.3%. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were within expected ranges, though wide variability was observed, particularly in RDW and hematocrit values. These findings suggest a heterogeneous burden of anemia and erythropoietic dysregulation within the study population.

Table 5. Red Blood Cell Indices in Patients with Chronic Heart Failure (n=206)

Parameter	Mean ± SD	Median (IQR)	Range (Min–Max)
Hgb (g/dL)	13.1 ± 3.7	13.7 (11.0–15.8)	3.0–21.0
Hct (%)	39.3 ± 11.2	40.9 (32.1–47.2)	8.1–66.4
RDW (%)	15.9 ± 3.3	14.8 (13.5–16.5)	11.6–32.7
MCV (fL)	87.1 ± 6.4	87.2 (83.0–91.0)	69.5–107.7
MCH (pg)	29.1 ± 2.7	29.0 (27.5–30.8)	22.0–38.0
MCHC (g/dL)	33.4 ± 1.7	33.4 (32.3–34.5)	27.7–43.8

Abbreviations: SD = standard deviation; IQR = interquartile range; RDW = red cell distribution width; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

Table 6 summarizes leukocyte profiles in patients with chronic heart failure, expressed as mean ± standard deviation, median (interquartile range), and absolute range. The mean total WBC count was elevated at 8.4 ± 4.7 $\times 10^9$/L, with neutrophils constituting the dominant fraction (6.03 ± 4.46 $\times 10^9$/L), suggestive of a pro-inflammatory state. Lymphocyte counts (1.47 ± 1.06 $\times 10^9$/L) were relatively suppressed, consistent with patterns of lymphopenia described in advanced heart failure.

Eosinophil and basophil counts were low in absolute terms, with substantial skewness indicated by wide ranges (eosinophils: 0–9.54 $\times 10^9$/L; basophils: 0–1.42 $\times 10^9$/L). Monocyte levels demonstrated notable heterogeneity (0.69 $\pm$ 1.12 $\times 10^9$/L; range 0.02–16.00 $\times 10^9$/L), potentially reflecting monocyte-driven inflammatory activation pathways implicated in myocardial remodeling. Collectively, these hematologic indices underscore the pervasive immune dysregulation and systemic inflammatory burden characterizing the CHF population under study.

Table 6. White Blood Cell Parameters in Patients with Chronic Heart Failure (n = 206)

Parameter	Mean ± SD	Median (IQR)	Range
Total WBC ($\times 10^9$/L)	8.4 ± 4.7	7.3 (4.2)	0.9–29.4
Neut ($\times 10^9$/L)	6.03 ± 4.46	5.07 (4.5)	0.46–27.90
Lymph ($\times 10^9$/L)	1.47 ± 1.06	1.36 (1.0)	0.12–12.58
Eos ($\times 10^9$/L)	0.22 ± 0.71	0.10 (0.2)	0–9.54
Baso ($\times 10^9$/L)	0.04 ± 0.10	0.02 (0.02)	0–1.42
Mono ($\times 10^9$/L)	0.69 ± 1.12	0.56 (0.3)	0.02–16.00

Abbreviations: WBC = white blood cells; Neut = Neutrophil; Lymph = Lymphocyte; Eos = Eosinophil; Baso = Basophil; Mono = Monocyte; SD = standard deviation; IQR = interquartile range.

As indicated in Table 7 the mean platelet count of 228.55 (97.16) suggests a population-level tendency toward normal thrombocytic levels, though the substantial standard deviation (97.16) and range (6–478) indicate significant interindividual variability, potentially reflecting divergent clinical states such as thrombocytopenia or reactive thrombocytosis. The median (222) closely approximates the mean, implying a roughly symmetric distribution.

MPV, a marker of platelet activation and production kinetics, exhibits minimal variability (mean: 8.7 fL$\pm$1.15), with the median (8.7) and mode (8.6) underscoring a tightly clustered distribution. The range (6.10–11.70 fL) aligns with physiological norms, though outliers may

signify underlying megakaryocytic activity shifts. PLR and NLR, as inflammatory and prognostic indices, demonstrate pronounced heterogeneity. The PLR mean (216.20) is skewed by extreme values (range: 5.27–1346.77), evidenced by the median (158.09) lying substantially below the mean—a hallmark of right-skewed data. Similarly, NLR's mean (6.74) vastly exceeds its median (3.47), with a formidable standard deviation (9.83) and range (0.21–87.30), indicative of a non-normal distribution.

Table 7. Hematological Profiling in Chronic Heart Failure: Platelet Indices and Systemic Inflammation Biomarkers (n=206)

Statistic	Platelets ($\times 10^9$/L)	MPV (fL)	PLR	NLR
Mean ± SD	228.5 ± 97.2	8.7 ± 1.2	216.2 ± 186.4	6.7 ± 9.8
Median (IQR)	222 (158–289)	8.7 (7.9–9.5)	158.1 (96.4–276.3)	3.5 (2.1–6.8)
Range	6–478	6.1–11.7	5.3–1346.8	0.2–87.3

Abbreviations: CHF = chronic heart failure; MPV = mean platelet volume; PLR = platelet-to-lymphocyte ratio; NLR = neutrophil-to-lymphocyte ratio; SD = standard deviation; IQR = interquartile range.

Table 8 outlines a detailed summary of various hematological parameters measured across different NYHA functional classes (I to IV) in heart failure patients, with a total sample size of 206. For each parameter, the table displays the number of patients (N), mean value, standard deviation (SD), and the corresponding 95% confidence interval (CI) of the mean, both within each NYHA class and for the overall cohort. For hemoglobin (g/dL), mean levels ranged from 12.0 in NYHA Class II to 13.9 in Class I, with the overall cohort averaging 13.1. Standard deviations varied slightly between classes, with the highest variability observed in Class II (3.9) and Class IV (3.5). The 95% confidence intervals reflected this spread, with Class II ranging from 10.9 to 13.2 g/dL, and Class I from 12.9 to 14.9 g/dL. White blood cell (WBC) counts ($\times 10^9$/L) were generally similar across classes, with means ranging from 7.6 in Class III to 9.2 in Class II, and a total mean of 8.4. The standard deviation was consistently around 4.0 to 5.4, and the confidence intervals remained within the typical physiological range. For red blood cells (RBC, $\times 10^{12}$/L), the mean values were relatively stable, ranging from 4.3 to 4.7 across the groups. Class I showed the highest mean (4.7) with a standard deviation of 1.0, while other groups had slightly lower values with

similar variability. Hematocrit (%) displayed more apparent differences across classes. NYHA Class I had the highest mean hematocrit (42.0%), while Class II had the lowest (35.9%). The standard deviation was notably larger in Class II (11.7), indicating greater spread in values within this group. The mean corpuscular hemoglobin (MCH, pg) values ranged narrowly between 28.8 and 30.0 across NYHA classes, with small standard deviations (2.1–2.7), suggesting consistent measurements across patients. Mean corpuscular hemoglobin concentration (MCHC, g/dL) values were also similar across groups, with means ranging from 33.3 to 33.9. Standard deviations were below 2.1 in all groups, and the 95% CIs remained tight, showing little within-group dispersion. Mean platelet volume (MPV, fL) showed uniformity across classes, with mean values between 8.5 and 8.8 and small standard deviations. Neutrophil counts ($\times 10^9$/L) exhibited slightly more variation, with the lowest mean (5.0) observed in Class II and the highest (6.0) in Class IV. Red cell distribution width (RDW, %) showed a wider range across classes, from 14.6% in Class I to 16.4% in Class II. Class II also had the highest standard deviation (4.2), reflected in the broader CI (15.2–17.6). This parameter demonstrated the greatest between-group variability among red cell indices. Monocyte and eosinophil counts ($\times 10^9$/L) remained nearly identical across all NYHA classes, with means of 0.7 and 0.2, respectively, and very narrow confidence intervals. Basophil counts were reported as 0.0 (rounded) for all groups, indicating either uniformly low or negligible levels among the patients.

Lastly, the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) both demonstrated wider variation across groups. NLR increased from 4.3 in Class I to 7.9 in Class III, while PLR showed a broader spread from 167.6 in Class I to 269.4 in Class III. These parameters had the largest standard deviations among the hematological ratios—10.2 for NLR in Class III and over 263.0 for PLR in the same class—suggesting substantial within-group variability.

Table 8. Hematological Parameters Across NYHA Classes in Heart Failure Patients.

Parameter	Group	N	Mean	SD	95% CI [Lower, Upper]
Hemoglobin (g/dL)	NYHA Class I	40	13.90	2.90	[12.9, 14.9]
	NYHA Class II	49	12.00	3.90	[10.9, 13.2]
	NYHA Class III	48	12.70	3.50	[11.7, 13.8]
	NYHA Class IV	69	13.70	3.50	[12.9, 14.6]
	Total	206	13.10	3.70	[12.6, 13.6]
WBC (10^9/L)	NYHA Class I	40	8.30	3.70	[7.1, 9.5]
	NYHA Class II	49	9.20	5.40	[7.6, 10.7]
	NYHA Class III	48	7.60	4.30	[6.3, 8.8]
	NYHA Class IV	69	8.50	4.00	[7.5, 9.4]
	Total	206	8.40	4.30	[7.8, 9.0]
RBC (10^{12}/L)	NYHA Class I	40	4.70	1.00	[4.4, 5.0]
	NYHA Class II	49	4.20	1.50	[3.7, 4.6]
	NYHA Class III	48	4.30	1.50	[3.9, 4.7]
	NYHA Class IV	69	4.50	1.30	[4.2, 4.8]
	Total	206	4.40	1.30	[4.2, 4.6]
Hematocrit (%)	NYHA Class I	40	42.00	8.50	[39.2, 44.7]
	NYHA Class II	49	35.90	11.70	[32.6, 39.3]
	NYHA Class III	48	37.50	12.70	[33.9, 41.1]
	NYHA Class IV	69	41.20	10.20	[38.8, 43.7]
	Total	206	39.30	11.20	[37.8, 40.9]
MCH (pg)	NYHA Class I	40	30.00	2.10	[29.3, 30.7]

	NYHA Class II	49	28.80	2.70	[28.0, 29.5]
	NYHA Class III	48	29.80	2.70	[29.0, 30.5]
	NYHA Class IV	69	29.00	2.40	[28.5, 29.6]
	Total	206	29.40	2.50	[29.0, 29.7]
MCHC (g/dL)	NYHA Class I	40	33.90	1.70	[33.4, 34.5]
	NYHA Class II	49	33.30	1.40	[32.9, 33.7]
	NYHA Class III	48	33.50	2.10	[32.9, 34.1]
	NYHA Class IV	69	33.70	1.50	[33.3, 34.1]
	Total	206	33.60	1.60	[33.4, 33.8]
MPV (fL)	NYHA Class I	40	8.70	1.20	[8.3, 9.1]
	NYHA Class II	49	8.80	1.20	[8.5, 9.2]
	NYHA Class III	48	8.50	1.20	[8.1, 8.8]
	NYHA Class IV	69	8.80	1.20	[8.5, 9.1]
	Total	206	8.70	1.20	[8.5, 8.8]
Neutrophil (10^9/L)	NYHA Class I	40	5.90	3.70	[4.7, 7.1]
	NYHA Class II	49	5.00	3.90	[3.9, 6.1]
	NYHA Class III	48	5.30	3.60	[4.2, 6.3]
	NYHA Class IV	69	6.00	4.20	[5.0, 7.0]
	Total	206	5.60	3.90	[5.1, 6.1]
RDW (%)	NYHA Class I	40	14.60	2.00	[13.9, 15.2]
	NYHA Class II	49	16.40	4.20	[15.2, 17.6]
	NYHA Class III	48	15.90	2.20	[15.3, 16.6]
	NYHA Class IV	69	15.50	2.50	[14.9, 16.1]
	Total	206	15.60	2.90	[15.2, 16.0]
Monocytes (10^9/L)	NYHA Class I	40	0.70	0.30	[0.6, 0.8]
	NYHA Class II	49	0.80	0.40	[0.7, 0.9]
	NYHA Class III	48	0.70	0.30	[0.6, 0.8]
	NYHA Class IV	69	0.70	0.30	[0.6, 0.8]

	Total	206	0.70	0.30	[0.7, 0.8]
Eosinophil (10^9/L)	NYHA Class I	40	0.20	0.20	[0.1, 0.2]
	NYHA Class II	49	0.20	0.20	[0.1, 0.2]
	NYHA Class III	48	0.20	0.20	[0.1, 0.2]
	NYHA Class IV	69	0.20	0.20	[0.1, 0.2]
	Total	206	0.20	0.20	[0.1, 0.2]
Basophil (10^9/L)	NYHA Class I	40	0.0	0.0	[0.0, 0.1]
	NYHA Class II	49	0.0	0.0	[0.0, 0.1]
	NYHA Class III	48	0.0	0.0	[0.0, 0.1]
	NYHA Class IV	69	0.0	0.0	[0.0, 0.1]
	Total	206	0.0	0.0	[0.0, 0.1]
NLR	NYHA Class I	40	4.30	4.20	[3.0, 5.7]
	NYHA Class II	49	6.50	6.20	[4.8, 8.3]
	NYHA Class III	48	7.90	10.20	[4.9, 10.8]
	NYHA Class IV	69	6.70	9.40	[4.5, 9.0]
	Total	206	6.40	8.0	[5.3, 7.5]
PLR	NYHA Class I	40	167.60	103.30	[134.6, 200.6]
	NYHA Class II	49	214.90	166.50	[167.1, 262.7]
	NYHA Class III	48	269.40	263.30	[193.1, 345.7]
	NYHA Class IV	69	210.80	166.60	[170.8, 250.8]
	Total	206	216.00	191.00	[189.8, 242.2]

Data represent hematological parameters in heart failure patients (N = 206) across NYHA classes (I: n = 40, II: n = 49, III: n = 48, IV: n = 69). Differences across NYHA classes were assessed using one-way ANOVA. WBC = white blood cell count; RBC = red blood cell count; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MPV = mean platelet volume; RDW = red cell distribution width; NLR = neutrophil to lymphocyte ratio (unitless); PLR = platelet to lymphocyte ratio (unitless); NYHA = New York Heart Association.

Table 9 displays the results of a one-way ANOVA comparing hematological parameters across NYHA functional classes (I–IV) in patients with heart failure (n = 206). Statistically significant differences were observed for hemoglobin ($F = 3.54$, $p = 0.016$), hematocrit ($F = 3.35$, $p = 0.020$),

and lymphocyte count (F = 4.01, p = 0.008). These parameters showed varying mean values across the NYHA classes, indicating differences in group-level distributions. Red blood cell (RBC) count (F = 2.22, p = 0.087), mean corpuscular volume (MCV; F = 1.28, p = 0.284), mean corpuscular hemoglobin (MCH; F = 2.08, p = 0.104), and mean corpuscular hemoglobin concentration (MCHC; F = 2.01, p = 0.114) did not show statistically significant differences. Among these, RBC count approached significance. The between-group and within-group mean square values are reported for each parameter, reflecting the relative variance attributable to NYHA classification versus intra-group variability.

Table 9. Comparison of Hematological Parameters Across NYHA Classes in Patients with Heart Failure.

Parameter	Between-Groups Mean Square	Within-Groups Mean Square	*F*-statistic	*p*-value
Hemoglobin	45.59	12.90	3.54	**0.016***
Hematocrit	407.16	121.59	3.35	**0.020***
Lymphocytes	4.32	1.08	4.01	**0.008***
RBC	3.76	1.70	2.22	0.087
MCV	51.80	40.58	1.28	0.284
MCH	14.96	7.20	2.08	0.104
MCHC	5.67	2.82	2.01	0.114

*Data represent means of hematological parameters across NYHA classes (I–IV) in heart failure patients (n = 206). Comparisons were made using one-way ANOVA. *p < 0.05 indicates statistical significance. RBC = red blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MPV = mean platelet volume; NYHA = New York Heart Association.*

Table 10 presents a comparison of selected hematological parameters across NYHA functional classes in patients with heart failure, using Welch's ANOVA to account for unequal variances. A statistically significant difference across NYHA classes was observed for red cell distribution width (RDW; test statistic = 5.46, p = 0.002). No significant differences were found for white

blood cell count (WBC; p = 0.440), basophil count (Baso; p = 0.080), neutrophil-to-lymphocyte ratio (NLR; p = 0.050), or platelet-to-lymphocyte ratio (PLR; p = 0.060), although the p-values for NLR and PLR were close to the conventional threshold for significance. These data represent the distribution of values across the four NYHA classes (I–IV) in a sample of 206 patients.

Table 10. Comparison of Selected Hematological Parameters Across NYHA Classes in Heart Failure Patients.

Parameter	Test Statistic	*p*-value
WBC (10^9/L)	0.91	0.440
RDW (%)	5.46	**0.002***
Baso (10^9/L)	2.27	0.080
NLR	2.67	0.050
PLR	2.62	0.060

*Data represent means of hematological parameters across NYHA classes (I–IV) in heart failure patients (N = 206; I: n = 40, II: n = 49, III: n = 48, IV: n = 69). Comparisons were made using Welch's ANOVA due to unequal variances. *p < 0.05 indicates statistical significance. Abbreviations: WBC = white blood cell count; RDW = red cell distribution width; NLR = neutrophil to lymphocyte ratio (unitless); PLR = platelet to lymphocyte ratio (unitless); NYHA = New York Heart Association.*

Detailed pairwise comparisons of hemoglobin levels across NYHA functional classes are presented in Table 11, based on Tukey's Honestly Significant Difference (HSD) post hoc test. This analysis was performed following a significant overall ANOVA to identify specific group differences. For each comparison, the table reports the mean difference (I–J), corresponding p-value, and 95% confidence interval.

The comparison between NYHA Class I and Class II yielded a mean difference of 1.96 g/dL (p = 0.05), with a 95% confidence interval ranging from –0.02 to 3.94. Similar p-values of 0.05 were observed for comparisons between Class I and Class IV (mean difference = 1.72) and between Class II and Class IV (mean difference = 1.72). Despite p-values at the threshold of statistical significance, all associated confidence intervals included zero. The remaining pairwise

comparisons produced non-significant p-values, ranging from 0.19 to 0.99, with mean differences varying between –1.96 and 1.54 g/dL. This table outlines the distribution of hemoglobin differences between NYHA classifications, as detected by post hoc analysis.

Table 11. Post Hoc Analysis of Hemoglobin Across NYHA Classes in CHF Patients Using Tukey HSD (n=206).

Dependent Variable	NYHA Classification (I)	NYHA Classification (J)	Mean Difference (I–J)	P-Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Hemoglobin (g/dL)	NYHA Class I	NYHA Class II	1.96	0.05	-0.02	3.94
		NYHA Class III	1.54	0.19	-0.45	3.53
		NYHA Class IV	0.25	0.99	-1.60	2.09
	NYHA Class II	NYHA Class I	-1.96	0.05	-3.94	0.02
		NYHA Class III	-0.42	0.94	-2.31	1.47
		NYHA Class IV	-1.72	0.05	-3.45	0.02
	NYHA Class III	NYHA Class I	-1.54	0.19	-3.53	0.45
		NYHA Class II	0.42	0.94	-1.47	2.31
		NYHA Class IV	-1.29	0.22	-3.04	0.45
	NYHA Class IV	NYHA Class I	-0.25	0.99	-2.09	1.60
		NYHA Class II	1.72	0.05	-0.02	3.45
		NYHA Class III	1.29	0.22	-0.45	3.04

*Tukey HSD, Tukey Honestly Significant Difference post hoc test following ANOVA. (I) and (J) represent the NYHA classes being compared. *P < 0.05 indicates statistical significance. Values are rounded to 2 decimal places for mean differences and confidence intervals, and 3 decimal places for P-values.*

To assess variation in hematocrit levels across New York Heart Association (NYHA) functional classes, a one-way analysis of variance (ANOVA) was performed. Post hoc analysis using Tukey's Honest Significant Difference (HSD) test revealed a statistically significant difference in hematocrit between NYHA Class II and NYHA Class IV (mean difference = -5.620, p = 0.035;

95% CI = -10.957 to -0.284). The inverse comparison (NYHA Class IV vs. Class II) yielded the same result (mean difference = 5.620, p = 0.035; 95% CI = 0.284 to 10.957). No other pairwise comparisons between NYHA classes reached statistical significance. For instance, differences between Class I and Class II (p = 0.112), Class I and Class III (p = 0.374), and Class III and Class IV (p = 0.195) were not significant. All corresponding 95% confidence intervals included zero, indicating a lack of statistically meaningful difference. These results demonstrate a statistically significant reduction in hematocrit levels between specific NYHA classifications, specifically between Class II and Class IV, while other intergroup differences were not significant.

Table 12. Post-hoc comparisons of hematocrit levels across NYHA classifications using Tukey's HSD test.

Dependent Variable	NYHA Classification (I)	NYHA Classification (J)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Hematocrit	NYHA Class I	NYHA Class II	5.299	0.112	-0.787	11.386
		NYHA Class III	3.805	0.374	-2.309	9.921
		NYHA Class IV	-0.321	0.999	-5.998	5.355
	NYHA Class II	NYHA Class I	-5.299	0.112	-11.386	0.787
		NYHA Class III	-1.493	0.909	-7.294	4.307
		NYHA Class IV	-5.620*	**0.035**	-10.957	-0.284
	NYHA Class III	NYHA Class I	-3.805	0.374	-9.921	2.309
		NYHA Class II	1.493	0.909	-4.307	7.294
		NYHA Class IV	-4.127	0.195	-9.496	1.241
	NYHA Class IV	NYHA Class I	0.321	0.999	-5.355	5.998
		NYHA Class II	5.620*	**0.035**	0.284	10.957
		NYHA Class III	4.127	0.195	-1.241	9.496

*Note: * The mean difference is statistically significant at the 0.05 level.*

The differences in lymphocyte levels across New York Heart Association (NYHA) functional classes were analyzed using a one-way ANOVA. Post hoc pairwise comparisons using Tukey's Honestly Significant Difference (HSD) test identified several statistically significant differences between groups. NYHA Class I was associated with significantly higher lymphocyte levels compared to Class II (mean difference = 0.620, 95% CI = 0.047 to 1.194; p = 0.028), Class III (mean difference = 0.703, 95% CI = 0.127 to 1.279; p = 0.010), and Class IV (mean difference = 0.562, 95% CI = 0.027 to 1.096; p = 0.035). The reciprocal comparisons confirmed these differences as statistically significant. For example, Class III had significantly lower lymphocyte

levels than Class I (mean difference = –0.703, 95% CI = –1.279 to –0.127; p = 0.010), and Class IV showed lower lymphocyte levels compared to Class I (mean difference = –0.562, 95% CI = –1.096 to –0.027; p = 0.035). No significant differences were observed between Classes II, III, and IV in any other combination (all p > 0.05), with 95% confidence intervals including zero. These findings suggest that individuals classified as NYHA Class I exhibit significantly elevated lymphocyte counts compared to those with more advanced functional impairment (Classes II–IV), while differences among Classes II–IV were not statistically meaningful.

Table 13. Post-hoc comparisons of lymphocyte levels between NYHA functional classes (Tukey's HSD).

Dependent Variable	NYHA Classification (I)	NYHA Classification (J)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Lymphocytes	NYHA Class I	NYHA Class II	0.620*	**0.028**	0.047	1.194
		NYHA Class III	0.703*	**0.01**	0.127	1.279
		NYHA Class IV	0.562*	**0.035**	0.027	1.096
	NYHA Class II	NYHA Class I	-0.620*	**0.028**	-1.194	-0.047
		NYHA Class III	0.082	0.98	-0.463	0.628
		NYHA Class IV	-0.058	0.99	-0.561	0.443
	NYHA Class III	NYHA Class I	-0.703*	**0.01**	-1.279	-0.127
		NYHA Class II	-0.082	0.98	-0.628	0.463
		NYHA Class IV	-0.141	0.887	-0.646	0.364
	NYHA Class IV	NYHA Class I	-0.562*	**0.035**	-1.096	-0.027
		NYHA Class II	0.058	0.99	-0.443	0.561
		NYHA Class III	0.141	0.887	-0.364	0.646

*Note: * The mean difference is statistically significant at the 0.05 level.*

The one-way ANOVA test was performed on the selected twelve hematologic parameters and two ratios. The test compares the variances of each parameter's means both within and between groups (NYHA classes) and we evaluate the significance of the test at the very end column of the table to see if the difference between groups is statistically significant enough (i.e., p < 0.05). Beforehand, though, we have run the ANOVA test; each parameter has gone through a Levene's test of homogeneity of variance to assess the significance of each test, and we picked all those Levene's tests that have rejected the null hypothesis of equal population variance (i.e., p > 0.05)

for the one-way ANOVA. Levene's test showed that the variances for Hgb, RBC, Hct, MCV, MCH, MCHC, MPV, Neut, Mono, Plt, Lymph, and Eos, were not equal: [F (3, 202) = 2.18, p = 0.091], [F (3,202) = 2.04, p = 0.110], [F (3, 202) = 2.12, p = 0.098], [F (3,202) =1.60, p = 0.189], [F (3,202) = 0.583, p = 0.627], [F (3, 202) = 0.861, p = 0.462], [F (3,202) = 2.471, p = 0.063], [F (3,202) = 2.530, p = 0.058], [F (3, 202) = 2.532, p = 0.57], and [F (3,202) = 2.089, p = 0.103], respectively. The one-way ANOVA test for the above parameters has been shown in Table 9, and as can be seen, only three parameters have statistical significance (i.e., $p < 0.05$). One-way ANOVA revealed statistically significant differences across groups for hemoglobin (Hgb) [F (3,202) = 3.536, p = 0.016], hematocrit (Hct) [F (3,202) = 3.349, p = 0.020], and lymphocyte count (Lymph) [F (3,202) = 4.323, p = 0.008].

Analysis of red cell distribution width (RDW) values across NYHA functional classifications using the Games-Howell post-hoc test revealed significant differences between patient groups. Compared to NYHA Class I patients, RDW levels were significantly elevated in both Class III (mean difference = 1.406, p = 0.046, 95% CI: 0.016 to 2.796) and Class IV patients (mean difference = 1.707, p = 0.002, 95% CI: 0.489 to 2.926). While RDW values in Class II patients showed a trend toward being higher than in Class I (mean difference = -1.821), this difference did not reach statistical significance (p = 0.071, 95% CI: -3.752 to 0.109). Comparisons between other NYHA classes demonstrated no significant differences in RDW values. The mean differences between Class II and Class III (p = 0.952), Class II and Class IV (p = 0.999), and Class III and Class IV (p = 0.944) were all non-significant, with confidence intervals crossing zero in each case. The Games-Howell test was selected for these analyses due to its robustness when comparing groups with potentially unequal variances or sample sizes.

Table 14. Post-hoc comparisons of red cell distribution width (RDW) levels across NYHA functional classes using Games-Howell test.

Parameter	NYHA Classification (I)	NYHA Classification (J)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RDW	NYHA Class I	NYHA Class II	-1.821	0.071	- 3.752	0.109
		NYHA Class III	-1.406*	**0.046**	-2.796	-0.016
		NYHA Class IV	-1.707*	**0.002**	-2.926	-0.489
	NYHA Class II	NYHA Class I	1.821	0.071	-0.109	3.752
		NYHA Class III	0.415	0.952	-1.639	2.469
		NYHA Class IV	0.113	0.999	-1.836	2.063
	NYHA Class III	NYHA Class I	1.406*	**0.046**	0.016	2.796
		NYHA Class II	-0.415	0.952	-2.469	1.639
		NYHA Class IV	-0.301	0.944	-1.717	1.114
	NYHA Class IV	NYHA Class I	1.707*	**0.002**	0.489	2.926
		NYHA Class II	-0.113	0.999	-2.063	1.836
		NYHA Class III	0.301	0.944	-1.114	1.717

*Note: * The mean difference is statistically significant at the 0.05 level.*

5.3.3 Receiver operating characteristic (ROC) curve analysis of RDW, hematocrit, and lymphocyte counts across statistically significant NYHA classes.

The ROC curve analysis (Youden index (*J*) method) of the three statistically significant parameters (RDW, Hct, and Lymph) revealed that the cut-off value for hematocrit was statistically insignificant both for the NYHA class III and class IV. As shown below in Table 15, figs.1A and 1B, the analysis of hematological parameters for distinguishing disease progression from NYHA class I to NYHA class III revealed significant findings. Red cell distribution width (RDW) demonstrated moderate diagnostic accuracy with an AUC of 0.663 (95% CI: 0.549–0.777; p = 0.009). At the optimal cut-off value of 13.5%, RDW exhibited high sensitivity (87.5%) but relatively low specificity (40%), suggesting its potential utility as a screening tool despite limitations in confirming disease progression.

In contrast, hematocrit (Hct) showed poor discriminatory ability, with an AUC of 0.412 (95% CI: 0.306–0.518; p = 0.106), indicating no statistically significant predictive value for NYHA class progression. Although the cut-off of 59.15% yielded very high specificity (97.1%), its low sensitivity (42%) further underscored its limited clinical applicability in this context.

Lymphocyte count emerged as another significant predictor, with an AUC of 0.657 (95% CI: 0.543–0.771; p = 0.012). The optimal cut-off of 1.718 × 10^9/L provided moderate sensitivity (50%) and specificity (79.2%), positioning it as a balanced biomarker that may complement RDW in monitoring disease progression as CHF advanced from NYHA I to NYHA III the two statistically significant parameters were Lymph and RDW. These two hematologic parameters were found to have cut values of 1.718 [AUC, 0.657; 95% CI, 0.543 - 0.771; p = 0.011] and 13.5 [AUC, 0.663; 95% CI, 0.549 - 0.777); p = 0.001], respectively, with the sensitivity and specificity of [87.5%, 40%] and [50%, 79.2%]. On the other hand, as indicated in Table 16, figs. 2A and 2B, the progression of the disease from NYHA I to NYHA IV, the ROC analysis of RDW and Lymph shows that the cut-off points lie at 15.9 [AUC, 0.708; 95% CI, 0.608 - 0.808; p = 0.0001] and 1.95 [AUC, 0.622; 95% CI, 0.514 - 0.729; p = 0.035], respectively. The sensitivity and specificity of RDW and Lymph in this NYHA class transition were [53.6%, 42.5%] and [42.5%, 78%], respectively. On the contrary, ROC analysis of Hct revealed that it was statistically insignificant (p = 0.106).

Table 15. Diagnostic Performance of Hematological Parameters in Differentiating NYHA Class I from Class III.

parameters	AUC (95%CI)	*P*-Value	Cut-off points	Sensitivity (%)	Specificity (%)
RDW (%)	0.663 (0.549, 0.777)	**0.009**[a]	13.5	87.5%	40%
Hct (%)	0.412 (0.306, 0.518)	0.106	59.15	42%	97.1%
Lymph(10^9/L)	0.657 (0.543, 0.771)	**0.012**[a]	1.72	50%	79.2%

[a] *Statistically significant difference (p < 0.05) Abbreviations: RDW = red cell distribution width; Hct = Hematocrit; Lymph = Lymphocyte.*

The diagnostic performance of various parameters for predicting disease progression from NYHA Class I to NYHA Class IV was evaluated using receiver operating characteristic (ROC) curve analysis. Table 16 summarizes the area under the curve (AUC), p-values, optimal cut-off points, sensitivity, and specificity for each parameter. The RDW (%) demonstrated an AUC of 0.708 (95% CI: 0.608–0.808) with a statistically significant p-value of 0.001 ($p < 0.05$). The optimal cut-off point for RDW was determined to be 15.9, yielding a sensitivity of 53.6% and a specificity of 82.5%. For Hct (%), the AUC was 0.520 (95% CI: 0.411–0.630), with a p-value of 0.725, indicating no statistical significance ($p > 0.05$). The optimal cut-off point was 40.35, with a sensitivity of 60.9% and a specificity of 56.3%. Lymph (10^9/L) had an AUC of 0.622 (95% CI: 0.514–0.729) and a statistically significant p-value of 0.035 ($p < 0.05$). The optimal cut-off point was 1.95, with a sensitivity of 42.5% and a specificity of 78%. These findings indicate that RDW and Lymph were statistically significant predictors of disease progression, while Hct did not show a significant association ($p > 0.05$, $p = 0.106$).

Table 16. Diagnostic Performance of Hematological Parameters in Stratifying NYHA Class I Through IV in Patients with Heart Failure.

parameters	AUC (95%CI)	p-Value	Cut-off points	Sensitivity (%)	Specificity (%)
RDW (%)	0.708 (0.608, 0.808)	**0.001**[a]	15.9	53.6%	82.5%
Hct (%)	0.520 (0.411, 0.630)	0.725	40.35	60.9%	56.3%
Lymph (10^9/L)	0.622 (0.514, 0.729)	**0.035**[a]	1.95	42.5%	78%

[a] *Statistically significant difference (p < 0.05)*

Table 17. Diagnostic Utility of Hematocrit in Distinguishing NYHA Class II from Class IV in Heart Failure Patients.

parameter	AUC (95%CI)	p-Value	Cut-off points	Sensitivity (%)	Specificity (%)
Hct (%)	0.588(0.482, 0.694)	0.106	40.35	60.9	56.3

Hematologic Predictors of Congestive Heart Failure Progression: ROC Curve Analysis of Absolute Lymphocyte Count and Red Cell Distribution Width.

Panels A and B display the receiver operating characteristic (ROC) curves evaluating the predictive performance of selected hematologic parameters for the progression of congestive heart failure (CHF) from New York Heart Association (NYHA) Class I to Class III. Panel A shows the ROC curve for absolute lymphocyte count, with an area under the curve (AUC) of 0.657 ($p = 0.012$), indicating moderate discriminatory ability. Panel B presents the ROC curve for red cell distribution width (RDW) levels, with an AUC of 0.663 ($p = 0.009$), suggesting a slightly higher predictive capacity. In both panels, the x-axis represents 1-specificity, and the y-axis represents sensitivity. The diagonal line denotes the line of no discrimination (AUC = 0.5). Statistical significance was determined using the DeLong test.

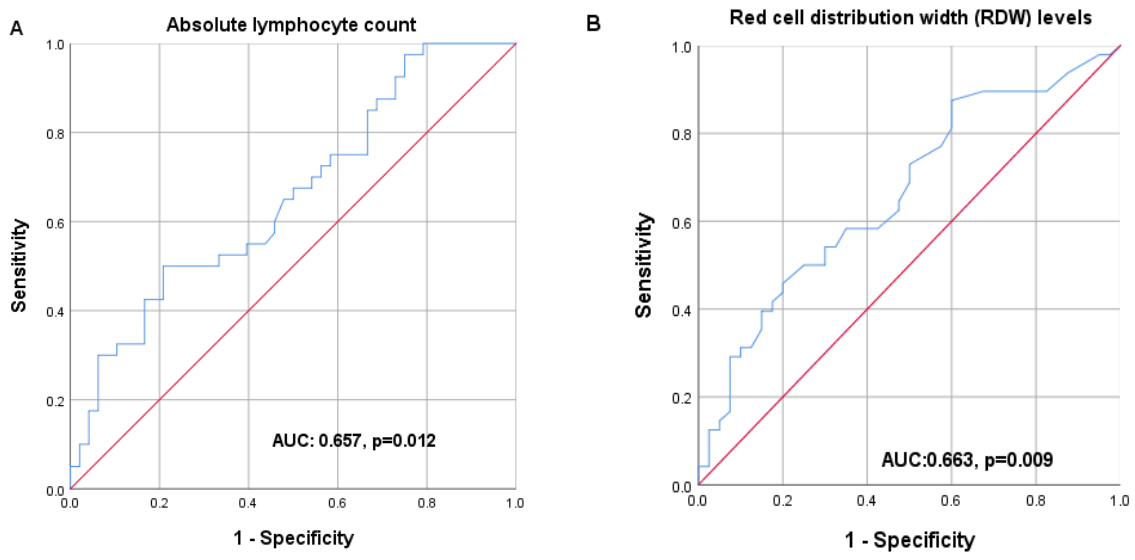

Figure 2: Predictive Value of Hematologic Parameters for Congestive Heart Failure Progression: ROC Curve Analysis of Absolute Lymphocyte Count (**A**) and Red Cell Distribution Width (**B**).

Figure 3 illustrates the Receiver Operating Characteristic (ROC) curves assessing the predictive ability of two hematologic parameters—(A) Absolute Lymphocyte Count (ALC) and (B) Red Cell Distribution Width (RDW)—in evaluating the progression of congestive heart failure (CHF) from New York Heart Association (NYHA) Class I to Class IV. The x-axis represents 1-Specificity (false positive rate), and the y-axis represents Sensitivity (true positive rate). The area under the curve (AUC) for each parameter is reported, along with its statistical significance (p-value). In Panel A, the AUC for ALC is 0.622 ($p = 0.035$), indicating moderate predictive performance. In Panel B, RDW shows an AUC of 0.708 ($p < 0.0001$), suggesting a stronger predictive capability. The diagonal red line in each panel represents the reference line of no discriminatory ability (AUC = 0.5). These findings highlight the potential of ALC and RDW as biomarkers for assessing CHF progression, with RDW demonstrating greater discriminatory power in this context.

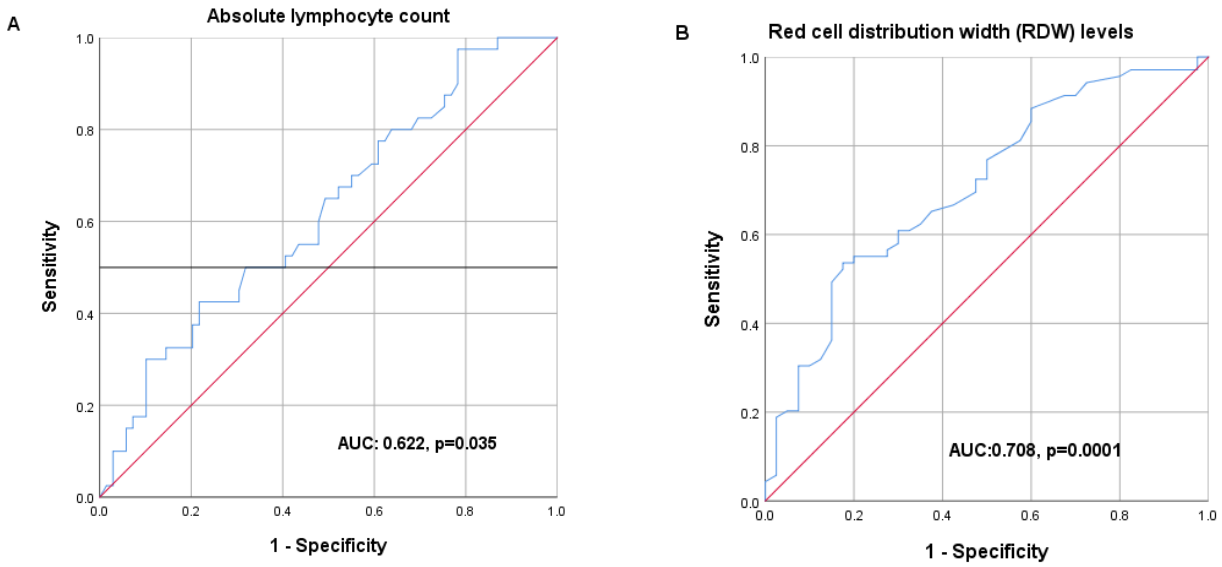

Figure 3: ROC Analysis of Absolute Lymphocyte Count and Red Cell Distribution Width in Predicting Congestive Heart Failure Progression Across NYHA Classes I to IV. **A**. absolute lymphocyte count **B**. Red cell distribution width (RDW).

6. Discussion

This study explored the prognostic relevance of selected hematological parameters—specifically red cell distribution width (RDW), lymphocyte count (Lymph), and hematocrit (Hct)—in relation to chronic heart failure (CHF) severity, as assessed by the New York Heart Association (NYHA) functional classification. Our findings suggest that RDW and Lymph may serve as accessible and cost-effective biomarkers with moderate predictive value, particularly in stratifying patients according to disease progression. The results support the hypothesis that routine hematological indices can reflect the underlying pathophysiological changes associated with CHF, including inflammation, immune dysregulation, and neurohormonal activation.

Among the parameters examined, RDW emerged as the most statistically significant predictor of CHF severity. RDW values were significantly elevated in patients with NYHA class III and IV compared to those in class I [$F(3,105.652) = 5.456$, $p = 0.002$], and ROC analysis further demonstrated its potential as a prognostic biomarker, with an area under the curve (AUC) of 0.708 ($p = 0.001$) and a cut-off point of 15.9. At this threshold, RDW achieved a high specificity (82.5%) but only moderate sensitivity (53.6%), indicating it may be more useful for excluding severe disease than for detecting it.

This finding aligns with several previous studies that have linked elevated RDW to adverse outcomes in heart failure and other cardiovascular conditions (10, 12, 33, 36). The biological plausibility of RDW as a prognostic marker stems from its association with chronic inflammation, oxidative stress, and impaired erythropoiesis. Inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) disrupt erythropoietin-mediated erythroid maturation, leading to increased heterogeneity in red cell size, a condition known as anisocytosis (57, 59, 60). RDW has also been found to correlate with acute phase reactants like high-sensitivity C-reactive protein (hs-CRP) and erythrocyte sedimentation rate (ESR), reinforcing its value as a surrogate marker of systemic inflammation (58, 59). Notably, the levels of these cytokines and inflammatory biomarkers have been shown to rise progressively with worsening NYHA functional class, supporting RDW's role as a dynamic indicator of disease progression (57, 58, 60, 61).

Lymphocyte count also demonstrated significant variation across NYHA classes, with absolute lymphocyte counts (ALC) declining progressively from class I through class IV. Statistically

significant differences were observed between class I and each of the higher classes: class II ($p = 0.028$; 95% CI: 0.047–1.194), class III ($p = 0.01$; 95% CI: 0.127–1.279), and class IV ($p = 0.035$; 95% CI: 0.027–1.096). ROC analysis showed an AUC of 0.622 ($p = 0.035$) at a cut-off value of 1.95, with specificity of 78% and sensitivity of 42.5%. While not as robust a predictor as RDW, Lymph still demonstrated utility in identifying lower-risk patients and may serve as a complementary marker in a multi-parametric prognostic model.

The decline in lymphocyte count in CHF patients—particularly those in advanced stages—is consistent with the well-documented phenomenon of stress-induced lymphopenia and immune dysregulation (45, 46, 48, 49). Several mechanisms have been proposed to explain this trend. Chronic activation of the sympathetic nervous system in heart failure leads to elevated levels of catecholamines, which stimulate β-adrenergic receptors on lymphocytes. This activation increases intracellular cAMP levels, modulating cytokine production and promoting lymphocyte apoptosis or redistribution (49, 54). Additionally, elevated cortisol levels and inflammatory cytokines such as IL-6 further contribute to lymphocyte suppression (50–53). Subset analysis in previous studies has shown that cytotoxic T cells (CD8+) and natural killer (NK) cells are particularly vulnerable, decreasing by up to 50%, while helper T cells (CD4+) remain relatively stable (49). This immunological shift may predispose patients to both poor cardiac outcomes and increased susceptibility to infections, highlighting the broader clinical relevance of lymphopenia in CHF.

In contrast to RDW and Lymph, hematocrit demonstrated limited prognostic value. Although group-level differences were statistically significant [$F(3,202) = 3.349$, $p = 0.02$], ROC analysis revealed poor discriminatory performance, with an AUC of 0.520 ($p = 0.725$), sensitivity of 60.9%, and specificity of 56.3%. The only notable difference was between NYHA class II and IV ($p = 0.035$; 95% CI: –10.957 to –0.284), suggesting that Hct may have some value in distinguishing patients at extreme ends of the disease spectrum but is otherwise limited as a prognostic tool.

Prior studies have linked elevated Hct levels (> 45% in women and > 49% in men) to increased risk of developing heart failure (13, 47). One plausible mechanism is the reduced availability of nitric oxide due to its scavenging by hemoglobin, impairing vasodilation and contributing to vascular dysfunction (47). However, hematocrit is a nonspecific marker influenced by numerous extrinsic and intrinsic factors, including hydration status, anemia, renal function, and comorbid

conditions. These confounding variables likely contribute to the inconsistent prognostic performance observed in this and other studies.

The collective findings from this study support the use of RDW and lymphocyte count as adjunctive tools in the prognostic assessment of CHF. Their high specificity and ease of measurement make them attractive for use in resource-limited settings or as part of routine clinical evaluations. However, their modest sensitivity indicates that they should not be used in isolation for definitive risk stratification.

The integration of hematological biomarkers into existing clinical models could enhance early detection of disease progression, optimize therapeutic decision-making, and potentially improve outcomes. Further research is warranted to validate these findings in larger, multi-center cohorts with greater demographic and clinical diversity. Additionally, longitudinal studies assessing temporal changes in these markers in response to therapy could provide deeper insight into their dynamic relationship with disease trajectory.

Finally, mechanistic studies investigating the cellular and molecular pathways linking hematological changes to cardiac dysfunction would enrich our understanding of CHF pathophysiology and potentially unveil novel therapeutic targets. The modest yet consistent associations observed in this study underscore the complex interplay between cardiovascular, hematologic, and immune systems in the progression of heart failure.

7. Strengths and Limitations of the study

Strengths: A principal strength of this study is its rigorous and comprehensive analysis of hematologic parameters within the context of the New York Heart Association (NYHA) functional classification, enabling a nuanced evaluation of their clinical significance and interrelationships. Furthermore, this work establishes a robust foundation for future investigations, providing critical insights that will guide researchers in exploring this domain with greater depth and precision beyond the present study's scope.

Limitations: Due to resource constraints and time limitations, this study could not incorporate a control group within the designated timeframe. The inclusion of a control group could have provided additional comparative insights, potentially enriching the interpretation and generalizability of the findings.

8. Conclusion and Recommendations

Conclusion

This study provides compelling evidence that specific hematologic parameters—particularly red cell distribution width (RDW), lymphocyte count, and to a lesser extent hematocrit (Hct)—possess prognostic value in the context of chronic heart failure (CHF), as stratified by the New York Heart Association (NYHA) functional classification. Among these, RDW and lymphocyte count emerged as the most informative markers, showing significant associations with advancing NYHA class and offering moderate predictive accuracy for disease progression.

These results underscore the clinical utility of routinely available and cost-effective hematologic indices in the risk assessment and longitudinal monitoring of CHF. RDW, likely reflecting systemic inflammation and impaired erythropoiesis, and lymphocyte count, indicative of neurohormonal and immune dysregulation, may serve as practical adjuncts to existing prognostic models. While Hct showed statistical significance in some class comparisons, its limited predictive strength suggests a more supplementary role.

Importantly, the findings highlight the need for integrative risk prediction strategies that combine clinical, biochemical, and hematologic data to enhance prognostic precision. Future studies are warranted to validate these biomarkers in larger, multi-ethnic cohorts and to explore their temporal dynamics in response to therapeutic interventions.

In summary, RDW and lymphocyte count represent promising, accessible biomarkers that could be incorporated into standard heart failure management protocols, particularly in resource-limited settings. Their integration into multivariable prognostic frameworks may improve patient stratification, guide treatment intensification, and ultimately contribute to better clinical outcomes in chronic heart failure.

Recommendations

The findings of this study underscore the potential of the identified hematologic parameters—RDW, Lymph, and Hct—as valuable adjuncts in the laboratory diagnosis of congestive heart failure (CHF), particularly in resource-constrained settings such as lower-tier health facilities. These parameters offer a pragmatic approach to enhancing diagnostic accuracy in environments where access to advanced diagnostic technologies and specialized expertise may be limited. By leveraging these readily available hematologic markers, such facilities can optimize the use of existing resources, thereby enabling more precise and reliable assessments of disease progression.

The integration of these hematologic parameters into existing prognostic frameworks holds significant promise for improving clinical decision-making. This approach can empower clinicians and healthcare professionals to more effectively evaluate the trajectory of CHF, facilitating the timely identification of patients at risk of deterioration. Consequently, this may guide the formulation of tailored therapeutic strategies, ultimately improving patient outcomes. To fully realize the clinical utility of these markers, future research should focus on validating their prognostic value across diverse populations and establishing standardized protocols for their implementation in routine clinical practice. Such efforts could bridge critical gaps in CHF management, particularly in underserved healthcare settings.

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10. Annex

Annex I. Information sheet (English Version)

Title of the Research Project: Assessment of selected hematological parameters of congestive heart failure patients, at SPMMC, Addis Ababa, Ethiopia.

Principal Investigator: Bekalu Yirga (BSc, MSc candidate)
Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

You are invited to participate as a study subject in research conducted by MSc candidate, Bekalu Yirga from Addis Ababa University. Your participation is voluntary. The research teams will include one principal investigator, and two advisors; both from the Addis Ababa University Department of Hematology. Please take as much time as you need to read or listen to the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because your participation is of great value in our effort in figuring out an efficient, effective, and affordable hematological test that could best predict the prognosis of the disease.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimens collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staff as needed. First, you are requested to give your consent to the sample collector, then after garnering your permission the required clinical sample will be collected by the laboratory technician or phlebotomist of the hospital's laboratory.

Potential risks and Discomforts

There might be some, if any, pain during blood drawing, especially if you have sensitive skin, however, there will be no risk involved while taking your venous blood. Nevertheless, we will try to minimize the discomfort as much as possible.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer, that can only be accessed by the lead investigators. There will be no sensitive issue that you will be asked about your social desirability, but any information that is obtained in connection with this study that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial to deal with the disease (CHF) from the hematologic perspective, in order to better complement its clinical management. Hence, you are indirectly benefiting other patients and society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study, you can contact the author of this research through the following address.

E-mail: bkk1152@outlook.com

Annex **II**. Informed consent form (English version)

Card no…..……

I had been informed that the objective of this study is to assess the hematological profile of congestive heart failure patients. The results of this study have an important role in treating me and other patients and are to be used as input for the future development of diagnostic tools or treatment guidelines. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study, which would require my willingness to provide the required data that include a blood sample, and a filling questionnaire. Therefore, with the full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ (አማርኛ ቅጂ)

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ “Assessment of selected hematologic parameters of congestive heart failure patients”.

የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ። በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኳን በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም። በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል።

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል። ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል። ይሁን እንጂ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤ አድራሻና ስልክ ቁጥር የመሳሰሉትን መረጃዎች አይጨምርም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል። በተጨማሪም ስለእርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎታል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ናሙናዉንም ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

የህክምና መረጃ በሚሰጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው። ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት። በቆየ የልብ ህመም ምክንያት በጤናዎት ላይ የሚፈጠረዉን ቀዉስ ቀድሞ ለመተንበይ እና በሽታዉ ወደ ከፋ ደረጃ ከማምራቱ አስቀድሞ ለማስጠንቀቂያነት የሚረዱ የደም ምርመራ ዓይነቶችን ለይቶ ማመላከት የጥናቱ ዋነኛ ዓላማ ሲሆን በዚህ ጥናት ላይ በመሳተፍዎት እርስዎና በተመሳሳይ ችግር የተጠቁ ወገኖችዎት የተሻለ የህክምና አገልግሎት እንዲያገኙ ከማገዝዎትም በላይ ወደፊት ጥናቱን ተመርኩዘዉ ሊደረጉ የሚችሉ ተጨማሪ ጠቃሚ ምርምሮችንም ማበረታታት በመሆኑ አስተዋጽዎት የላቀ ዋጋ ይኖረዋል።

በዚህ ጥናት ተሳታፊ በመሆንዎ የሚያገኙዋቸዉ መብቶች ምንድን ናቸው?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም። ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት። የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ። በተጨማሪም እርስዎ የሚሰጡን መረጃ የችግሩን ስፋት ለመከላከልና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛና እዉነተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ። E-mail: bkk1152@outlook.com

Annex **III**. Informed consent form (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር _____

የተሳታፊዉ ስም _____

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “assessment of selected hematological parameters of congestive heart failure patients” **ጥናት ላይ በቂ ገለጻ ተደርጎልኛል። ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጾልኛል። የጥናቱንም አላማዎችም ተረድቻለሁ።**

በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል። በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል።

ስለዚህ ለዚህ ጥናት የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ። የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ።

በአጠቃላይ እኔ _____**ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።**

ፊርማ_____ **ቀን** _____/_____/_____

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም _____ **ፊርማ** _____

ቀን_____

Annex IV. Pre-analytical and post-analytical errors in automated hematoanalyzer

1. THE PRE-ANALYTICAL PHASE: from the patient to the laboratory bench

Pre-analytical errors are one of the major error types frequently seen in medical laboratories, accounting for 46%-68% of the overall errors committed. Some examples of this type of error include; Test ordering, patient identification, patient preparation, sample collection, sample quality, transportation, and storage.

Patient preparation

- Its strongly advised to avoid excessive or unaccustomed physical exercise for 24 hours before routine phlebotomy, as it produces an increase in plasma volume (PV), which may lead to a decrease in hemoglobin (Hb), red blood cells (RBC) and hematocrit (Hct.) in athletes in training programs.
- The status of the patient, therefore, needs to be understood in the interpretation of results against standard reference intervals.
- Since the physiological response to exercise will be affected by the physical fitness of the individual, it is important to distinguish between the effects of sustained training from episodes of unaccustomed, intense exercise in an otherwise sedentary individual.
- The EFLM recommendation is that patients should rest for fifteen minutes in a seated position before phlebotomy as a standardized approach to phlebotomy procedures.

Specimen quality and acceptance criteria

For the specimen to qualify for the test it must conform to the following quality criteria

- Clotted specimens must be rejected
- Clotted samples are mainly the result of poor phlebotomy and inadequate specimen mixing post collection
- Contamination with infusion fluids, when specimens are taken close to an infusion site may be a cause of spurious anemia and abnormal coagulation test results
- Venous stasis of just one to three minutes during venipuncture has an adverse effect on CBC results, leading to an increase in Hb, Hct, and RBC.
- The anticoagulant of choice for CBC analysis is the di- or tri-potassium salt of ethylenediaminetetraacetic acid (EDTA), with a preference for K_2EDTA although alternatives (e.g., magnesium sulphate, $MgSO_4$) may be better for some platelet parameters.
- The order of draw of specimen types may not affect automated counting results; however, there is a risk of contamination of chemistry and clotting specimens with EDTA and potassium if the CBC specimen is taken first.
- EDTA tubes have a fixed fill volume that gives the optimum concentration of anticoagulant and both under or overfilling can be a cause of erroneous CBC results.
- Overfilling the sample risks inadequate mixing before testing and may be a cause of a pseudopolycythemia, pseudothrombocytopenia and pseudoleucopenia, even though the sample is not clotted.
- Underfilled tubes will result in an increased concentration of EDTA, which may cause platelet volume changes and an excess of K_3EDTA has been suggested as a cause of spurious reduction in WBC.
- Underfilled specimens may also indicate a difficult venipuncture, which in itself may cause platelet activation, platelet swelling, and problems with coagulation testing.
- Lipemia, in a patient on parenteral nutrition, with lipid disorders or post a heavy meal, may affect the platelet and WBC counts as a result of the presence of lipid droplets and may

cause sufficient turbidity in the sample to interfere with the Hb_{20} (and some other tests, e.g., the sickle solubility test).

- Artificially induced, moderate-to-high hemolysis has been shown to produce a decrease in RBC and Hct and an increase in mean cell hemoglobin (MCH) and platelet count, related to the degree of hemolysis.
- A major cause of potential hemolysis during specimen collection arises from sample collection through intravenous (IV) catheters.
- Similar effects may be seen in patients with severe burns or other conditions with a significant increase in red cell fragmentation or microspherocytes.

Sample venous blood collection procedures

1. Assemble equipment

Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. The equipment required includes:

- a supply of laboratory sample tubes, which should be stored dry and upright in a rack
- bleeding pack (collapsible) if large quantities of blood are to be collected
- Well-fitting, non-sterile gloves.
- Needles (19G or 20G) and syringes (10cc).
- a tourniquet, alcohol hand rub, 70% alcohol swabs for skin disinfection, gauze or cotton-wool ball to be applied over the puncture site.
- laboratory specimen labels
- A puncture-resistant sharps container (safety box).

2. Identify and prepare the patient

Where the patient is adult and conscious, follow the steps outlined below.

- Introduce yourself to the patient, and ask the patient to state their full name.
- Check that the laboratory form matches the patient's identity (i.e., match the patient's Details with the laboratory form, to ensure accurate identification).
- Ask whether the patent has allergies, phobias, or has ever fainted during previous injections or blood draws.
- If the patient is anxious or afraid, reassure the person and ask what would make them more comfortable.
- Make the patient comfortable in a supine position (if possible).
- Place a clean paper or towel under the patient's arm.
- Discuss the test to be performed and obtain verbal consent. The patient has a right to refuse a test at any time before the blood sampling, so it is important to ensure that the patient has understood the procedure.

3. Select the site

- Extend the patient's arm and inspect the antecubital fossa or forearm.
- Locate a vein of a good size that is visible, straight, and clear. The median cubital vein lies between muscles and is usually the easiest to puncture. Under the basilic vein runs an artery and a nerve, so puncturing here runs the risk of damaging the nerve or artery and is usually more painful. DO NOT insert the needle where veins are diverting, because this increases the chance of a hematoma.
- The vein should be visible without applying the tourniquet. Locating the vein will help in determining the correct size of the needle.
- Apply the tourniquet about 4–5 finger widths above the venipuncture site and re-examine the vein.

For Hospitalized patients

- In hospitalized patients, do not take blood from an existing peripheral venous access site because this may give false results. Hemolysis, contamination, and the presence of intravenous fluid and medication can all alter the results. Nursing staff and physicians may access central venous lines for specimens following protocols. However, specimens from central lines carry a risk of contamination or erroneous laboratory test results.
- The vein should be visible without applying the tourniquet. Locating the vein will help in determining the correct size of the needle.
- Apply the tourniquet about 4–5 finger widths above the venipuncture site and re-examine the vein.

4. Perform hand hygiene and put on gloves

- Perform hand hygiene; that is wash hands with soap and water, and dry with single-use towels; or if hands are not visibly contaminated, clean with alcohol rub – use 3 ml of alcohol rub on the palm, and rub it into fingertips, back of hands and all over the hands until dry.
- After performing hand hygiene, put on well-fitting, non-sterile gloves.

5. Disinfect the entry site

- Unless drawing blood cultures, or prepping for a blood collection, clean the site with a 70% alcohol swab for 30 seconds and allow it to dry completely (30 seconds).

Note: alcohol is preferable to povidone-iodine, because blood contaminated with povidone-iodine may falsely increase levels of potassium, phosphorus, or uric acid in laboratory test results.

- Apply firm but gentle pressure. Start from the center of the venipuncture site and work downward and outwards to cover an area of 2 cm or more.
- Allow the area to dry. Failure to allow enough contact time increases the risk of contamination.
- DO NOT touch the cleaned site; in particular, DO NOT place a finger over the vein to guide the shaft of the exposed needle. If the site is touched, repeat the disinfection.

6. Take blood

Select a sterile, disposable syringe with a capacity of 5 ml. Attach it to a 19 or 20G disposable needle. If the patient is an adult with small veins, use a 23G needle

- Anchor the vein by holding the patient's arm and placing a thumb BELOW the venipuncture site.
- Ask the patient to form a fist so the veins are more prominent.
- Enter the vein swiftly at a 30-degree angle or less, and continue to introduce the needle along the vein at the easiest angle of entry.
- Once sufficient blood has been collected, release the tourniquet before withdrawing the needle. Some guidelines suggest removing the tourniquet as soon as blood flow is established, and always before it has been in place for two minutes or more.
- Withdraw the needle gently and apply gentle pressure to the site with a clean gauze or dry cotton-wool ball. Ask the patient to hold the gauze or cotton wool in place, with the arm extended and raised. Ask the patient NOT to bend the arm, because doing so causes a hematoma.

7. Fill the laboratory sample tubes

- If a syringe needle set is used, the best practice is to place the tube into a rack before filling the tube. To prevent needle sticks, use one hand to fill the tube or use a needle shield between the needle and the hand holding the tube.
- Pierce the stopper on the tube with the needle directly above the tube using slow, steady pressure. Do not press the syringe plunger because additional pressure increases the risk of hemolysis.
- Where possible, keep the tubes in a rack and move the rack towards you. Inject downwards into the appropriate colored stopper (purple top). Do not remove the stopper because it will release the vacuum.
- If the sample tube does not have a rubber stopper, inject extremely slowly into the tube as minimizing the pressure and velocity used to transfer the specimen reduces the risk of hemolysis. DO NOT recap and remove the needle.

8. Clean contaminated surfaces and complete patient procedure

- Discard the used needle and syringe or blood-sampling device into a puncture-resistant sharps container (safety box).
- Check the label and forms for accuracy. The label should be written with the information required by the laboratory, which is typically the patient's first and last name, file number, date of birth, and the date and time when the blood was taken.
- Discard used items into the appropriate category of waste. Items used for phlebotomy that would not release a drop of blood if squeezed (e.g., gloves) may be discarded in the general waste.
- Perform hand hygiene again
- Recheck the labels on the tubes and the forms before dispatch.
- Inform the patient when the procedure is over.
- Ask the patient or donor how they are feeling. Check the insertion site to verify that it is not bleeding, then thank the patient and say something reassuring and encouraging before the person leaves.

Sample rejection criteria

1. Clotted Specimens
    - Clotted specimens, where appropriate should be discarded and a recollection performed.
2. Insufficient Specimen
    - EDTA tube - less than 1ml
    - Citrate tubes - less than 10% of stated tube volume (up to the mark)
3. Unlabeled Specimen
    - Specimens that do not have any patient details or the wrong patient details written on the tube, should be returned to Specimen Reception, who will organize the appropriate corrective action.
    - If a recollection is not done, a comment will be made on the report saying that the specimen was received unlabeled or wrongly labeled once the doctors/nurses accept the responsibility
4. Lipemic Samples
    - Grossly lipemic samples giving inaccurate results even after plasma replacement should be recollected.
5. Hemolyzed Samples
    - Grossly hemolyzed samples giving inaccurate results or unreadable blood films should be rejected and a recollection performed.
6. Aged Specimens
    - As a general rule EDTA samples up to 24 hours old are acceptable. However, if old samples are received and there are significant morphological changes in the white cells or red cells the specimen should be discarded and a recollection performed.
7. Leaked sample
    - If a larger volume of blood has leaked from the tube, the sample should be discarded as the results may be misleading.

Specimen transportation and preparation

- All CBC specimens ideally should be analyzed within 6 hours of collection, especially where blood cell morphology is required.
- Prolonged storage of CBC specimens is a well-recognized cause of an elevated MCV and will also result in morphological changes in the WBC and RBC.
- Excessive heat or freezing will render CBC specimens unsuitable for testing.
- Time-critical results are defined in hematology in the context of the patient's clinical background, for example, WBC and platelet counts in oncology patients, and Hb following major blood loss.
- If the analysis is delayed, samples are better stored at 2-8°C but this is not advised where the patient is known to have cold agglutinins, which will cause clumping of the red cells and a consequent false elevation of the MCV, reduced RBC and increased mean cell hemoglobin concentration (MCHC).
- It is not advised to warm the specimens after collection but to re-bleed the patient and keep the specimen warm from the time it is taken until it is analyzed to ensure an accurate result.

2. **THE POSTANALYTICAL PHASE**: from the laboratory bench to the requesting physician

Although erroneous hematology results usually arise from factors in the pre-analytical phase, they may only be detected at test validation in the post-analytical phase through a review of flags, histograms, and scatterplots before the release of the result.

- The use of delta checks as part of result validation is an important “safety net” that may detect errors in specimen identification and collection, for example, specimens diluted with infusion fluid, inadequate mixing due to sample overfilling, or with an undetected clot.

- MCV has been suggested as having the highest positive predictive value of several parameters for the identification of specimen mislabeling.

- The platelet count may be affected by factors in specimen collection (e.g., clotting, platelet activation) or related to the individual patient and their clinical condition.

- Once the latter is identified, then the laboratory can be aware of the phenomenon for future tests. In particular, the presence of EDTA-dependent pseudothrombocytopenia, platelet satellitism, platelet clumping, and the presence of giant platelets have been cited as a cause of fake thrombocytopenia and may also interfere with the provision of an accurate WBC.

- RBC fragments and microspherocytes, the presence of cytoplasmic fragments in acute leukemia, microorganisms in sepsis, cryoglobulins, and cryoprecipitate are potential causes of a falsely elevated platelet count, although the extent to which these factors may confound the result will depend on the counting technology used.

- polymorphonuclear cell clumping, which occurs in a small proportion of specimens taken into EDTA, will reduce the total WBC and the neutrophil count.

- A falsely elevated WBC may occur if platelet aggregates, large platelets, nucleated RBC, lysis-resistant RBC, and (as before) cryoglobulinemia or cryoprecipitate are present, as these may be counted as WBC.

- The Hb and RBC will be affected additionally by a very high WBC (e.g., greater than 100×10^9/L) and the RBC to a lesser extent by the presence of giant platelets.
- Because a number of reported red cell parameters are derived by calculation, an erroneous result in one of the directly measured analytes (Hb, RBC, Hct, and/or MCV, depending on the manufacturer) may affect the value of any calculated analyte.

Critical results reporting

- The failure to identify and report a critical result is a major post-analytical error.

Quality control procedures

1. At the beginning of each work shift, all parameters are tested with blood control.
2. The 3 levels include: Abnormal Low, Normal, Abnormal High
3. Controls are stored at 2-8ºC and brought to room temperature on a roller mixer before use.
4. Controls are gently inverted many times according to the manufacturer's instruction before use.
5. From the RUN screen, press [SPECIMEN TYPE].
6. Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal, or high) and press the [QC SPECIMEN] key.
7. Control values must be within three standard deviations, otherwise, the measurement has to be repeated. if the control is still out of range:
    - ❖ Check the operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
    - ❖ Check reagents for expiration dates and lot numbers. If this does not solve the problem, prepare new control(s) and try again, if the controls are still out, check the operator's manual, or recalibrate the instrument and if controls are still out, Contact servicing engineer.
8. All control data are managed using software that provides graphical reports (Levey Jennings graphs, and monthly cumulative histograms).

Analytical factors affecting the performance of an examination

Factors	Precautions
Mixing	Thorough but not violent mixing of blood with anti-coagulant must be carried out by gently inverting the tube at least three times, immediately on collection.
Hemolysis	Avoid mechanical trauma to red cells. Never inject blood through a syringe needle into a specimen collection tube. Avoid extremes of temperature.
Contamination	Do not take blood from the same limb being used for infusion of fluids or decant blood from one container to another. Collect tubes for hematology (EDTA tubes) AFTER samples for biochemistry (serum tubes).
Venous Constrictions	There must be no venous constriction (tourniquet) or active muscle movement during the collection of blood for the estimation of such constituents as calcium, protein, lactate, and electrolytes, as this can lead to considerable alteration in levels. If avoidance of constriction is not practicable, its duration must be kept to an absolute minimum.
Delay in Transport of Specimens to laboratory	Considerable changes in the concentration of some blood constituents may occur if the blood is allowed to stand for any length of time before analysis begins, or separation of serum or plasma occurs.
Interfering Substances	Previous administration of a substance or drug may cause interference in analysis. It is impossible to list all such potential interferences and advice should be sought from Laboratory staff when there is any doubt

Annex V Questionnaires (English and Amharic versions)

Questionnaire (English version)

1. Age: _____

2. Gender: Male ☐ Female ☐

3. What's the status of your heart failure (based on NYHA classification)?

   Class I ☐ Class II ☐ Class III ☐ Class IV ☐

4. Are you diabetic? Yes ☐ No ☐

5. Are you a smoker? Yes ☐ No ☐

6. Are you hypertensive? Yes ☐ No ☐

7. What was the etiology of your heart failure? (Based on the medical diagnosis)

   _____

   _____ .

8. Have you ever been diagnosed with any other cardiovascular diseases (CVD) previously other than your current condition (HF)? Yes ☐ No ☐

9. If the answer to question no. 8 is yes, please specify it.

   _____.

10. Are you a heavy drinker? Yes ☐ No ☐

11. Are you anemic? Yes ☐ No ☐

12. Do you have renal dysfunction or renal failure? Yes ☐ No ☐

13. B-type natriuretic peptide (BNP)_____

14. Systolic blood pressure (SBP)_____

15. Left ventricular ejection fraction (LVEF)_____

16. peak oxygen uptake (VO_2 peak) _____

17. Hemoglobin (Hb)_____

18. Hematologic tests (attach here)

መጠይቅ (የአማርኛ ቅጂ)

1. **እድሜ**_____

2. **ጾታ** **ወንድ** ☐ **ሴት** ☐

3. **የልብ ህመምዎ ደረጃ ስንት ነዉ?**

   ደረጃ አንድ ☐ **ደረጃ ሁለት** ☐ **ደረጃ ሶስት** ☐ **ደረጃ አራት** ☐

4. **የስኳር ህመም አለብዎት?** **አዎን አለብኝ** ☐ **የለብኝም** ☐

5. **ሲጋራ ያጨሳሉ?** **አዎ አጨሳለሁ** ☐ **አላጨስም** ☐

6. **ደም ግፊት አለብዎት (በህክምና የተረጋገጠ)?** **አዎ አለብኝ** ☐ **የለብኝም** ☐

7. **የበሽታዎት መንስዔ ምንድነዉ (የህክምና ምርመራዉን በማየት የሚወሰን)?**

   _____

   _____

   _____።

8. **ከዚህ በሽታ ባሻገር ሌላ ዓይነት የልብ እና የደም ባንቧዎች ሕመም አለብዎት?**

   አዎን አለብኝ ☐ **የለብኝም** ☐

9. **በተራ ቁ.8 ለተጠቀሰዉ ጥያቄ መልስዎት አዎን ከሆነ ችግሩን ቢገልጹልን?**

_____

_____።

10. ብዙ የአልኮል መጠጥ ይጠጣሉ (ከባድ ጠጪ ነዎት)?

አዎ እጠጣለሁ ☐ አይደለሁም ☐

11. በሕክምና የተረጋገጠ የደም ማነስ ችግር አለብዎት?

አዎ አለብኝ ☐ የለብኝም ☐

12. የኩላሊት መድከም ወይም ስራ ማቆም ችግር አለብዎት?

አዎ አለብኝ ☐ የለብኝም ☐

13. የቢ ኤን ፒ መጠን

14. የደም ግፊት መጠን (የላይኛዉ)_____

15. ኤል ቪ ኢ ኤፍ _____

16. ትልቁ አክስጅንን የመሳብ አቅም_____

17. የሄሞግሎቢን መጠን_____

18. ሙሉ የደም ምርመራ ዉጤቶች ይጠቀሱ/ይያያዝ.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been duly acknowledged.

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Date of submission: 25/06/2025

This thesis has been submitted with our approval as advisors.

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Date: _____

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Advisor: **Rahel Alemu (M.Sc.)**

Signature: _____

Date: _____

Place Addis Ababa, Ethiopia