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**Changes in Platelet Count and Mean Platelet Volume during Infectious and Inflammatory Diseases, and Their Correlation with ESR and CRP at Zewditu Memorial Hospital, Addis Ababa, Ethiopia**

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**Changes in Platelet Count and Mean Platelet Volume during Infectious and Inflammatory Diseases, and Their Correlation with ESR and CRP at Zewditu Memorial Hospital, Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Laboratory Management & Quality Assurance) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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## List of Abbreviations

AAHB	Addis Ababa Health Bureau
AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ANC	Absolute Neutrophil Count
AUC	Area under curve
CAM	Community Acquired Pneumonia
CD	Crohn's Disease
CRP	C - reactive protein
DRERC	Department Research and Ethics Review Committee
EDTA	Ethylene diamine tetra-acetic acid
ESR	Erythrocyte Sedimentation Rate
Fl	Femtoliter
g/dl	Gram per deciliter
HBSAg	Hepatitis B Surface Antigen
HIV	Human Immunodeficiency Virus
HS-CRP	High-Sensitivity C - reactive protein
ICU	Intensive Care Unit
IE	Infective Endocarditis
Mg/dl	Milligram per deciliter

Mg/l	Milligram per liter
Mm/h	Millimeter per hours
MPV	Mean Platelet Volume
NPV	Negative Predictive Value
PDW	Platelet Distribution Width
PI	Principal Investigator
PLT	Platelet
PNC	Pneumococcal Pneumonia
PPV	Positive Predictive Value
PTB	Pulmonary tuberculosis
PCT	Platelet-crit
RA	Rheumatoid Arthritis
RBC	Red Blood Cell
ROC	Receiver operating characteristic
SOP	Standard Operating Procedure
SST	Serum Separating Tube
TB	Tuberculosis
VCT	Voluntary Counseling and Testing
WBC	White Blood Cell
ZMH	Zewditu Memorial Hospital

## Abstract

**Background:** Infectious and inflammatory diseases have been among the major threats for public health. Many ways of diagnosing these diseases have been developed. However, there is still a need for better parameters that will help reduce the cost and increase the reliability of the diagnosis. Efforts have been undergoing to standardize the use of Platelet parameters with Erythrocyte Sedimentation Rate (ESR) and C - reactive protein (CRP).

**Objective:** To evaluate the changes in platelet count (PLT) and mean platelet volume (MPV), and the relation between each of those markers with erythrocyte sedimentation rate (ESR) and C - reactive protein (CRP) in infectious and inflammatory diseases in Zewditu Memorial Hospital, Addis Ababa, Ethiopia from July 2015 – May 2016.

**Methods:** A cross-sectional, hospital based study was conducted on 100 patients with infectious and inflammatory diseases and admitted in medical ward of Zewditu Memorial Hospital. PLT, MPV, ESR, and CRP were measured at hospitalization and discharge. Patients' demographic and clinical characteristics were collected using formats. Whole blood was collected and measured using Cell Dyn 1800 Hematological analyzer for Platelet parameters (PLT and MPV). The serum level of CRP and ESR were measured using semi quantitative agglutination test and Westergren methods, respectively. Values at admission and discharge were compared using paired t-test. In addition, Pearson correlation test was used to determine the relationship between the platelet count, MPV, ESR and CRP. Data was entered and analyzed using SPSS ver.21 statistical software and P-value less than 0.05 was considered as statistically significant.

**Result:** 48 male and 52 female adult patients with infectious and inflammatory conditions were involved in this study. Their mean PLT count changes from  $258.5 \pm 131.1$  ( $\times 10^9/l$ ) at admission to  $279.5 \pm 124.9$  ( $\times 10^9/l$ ),  $P = 0.001$ ) at discharge. MPV increased during the same period but the difference was not statistically significant ( $9.6 \pm 1.7$  fl vs.  $10.1 \pm 7.9$  fl,  $P < 0.58$ ). CRP and ESR decreased at discharge with clinical resolution of CRP  $6.6 \pm 9.7$  mg/l to  $2.8 \pm 5.9$  mg/l ( $p = 0.001$ ) and ESR  $46.0 \pm 40.2$  mm/h to  $31.5 \pm 30.9$  mm/h ( $p = 0.001$ ). PLT and MPV had a negative correlation at the time of admission ( $r = -0.371$ ,  $p = 0.001$ ) but not at discharge ( $r = 0.017$ ,  $p = 0.863$ ). ESR was not significantly correlated with PLT both at admission ( $r = 0.059$ ,  $p = 0.560$ ) and discharge ( $r = 0.077$ ,  $p = 0.447$ ). Besides, ESR had no significant correlation with MPV both at admission ( $r = -0.096$ ,  $p = 0.341$ ) and discharge ( $r = 0.019$ ,  $p = 0.849$ ). ESR and CRP had a significant positive correlation at the time of admission ( $r = 0.57$ ,  $p = 0.0001$ ) as well as discharge ( $r = 0.58$ ,  $p = 0.0001$ ).

**Conclusion:** The study demonstrated a change in PLT, ESR and CRP values of patients with infectious and inflammatory condition from the admission values. The platelet parameters were not well correlated with the known disease activity markers (CRP and ESR). Thus, further study is warranted addressing single disease so as to get convincing results before claiming them as reliable markers for assessment of infectious and inflammatory disease activity in our settings.

**Key words:** platelet count; mean platelet volume; C-reactive protein; erythrocyte sedimentation rate and disease activity

# 1.Introduction

## 1.1. Background

Infectious diseases have been threats for centuries and ranked with wars and famine as major challenges to human progress and survival. These human diseases have a profound effect in countries across the world, causing premature deaths, disability and morbidity. Against a constant background of established infections, epidemics of new and old infectious diseases periodically emerge, greatly magnifying the global burden of infections. Accurate case load numbers are difficult to determine, because so many of these diseases are endemic to developing countries, where many people do not have access to modern medical care. Approximately half of all deaths caused by infectious diseases each year can be attributed to just three diseases: tuberculosis, malaria, and AIDS. Those are thus the most common infectious diseases throughout the world today. Together, these diseases cause over 300 million illnesses and more than 5 million deaths each year (1).

Autoimmune and chronic inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease, diabetes mellitus, and multiple sclerosis, also cause considerable morbidity and early mortality (2). Infectious and inflammatory diseases are the most common diseases that delay the diagnosis and adequate treatment. As a result they can increase the mortality and morbidity as well as cause a lot of costs for national health services. Because of these problems, some kinds of diagnostic and examination approaches that can alleviate the problems are always undertaken. The platelet parameters can be considered as reliable markers for assessment of disease activity and response to treatment. In case of inflammatory stimulations, the first reaction step of this system is creating a local inflammation, in which the intrinsic immunity cells are called to be in the inflammation area. This response can be associated with the symptoms, including fever, fatigue, and anorexia (3, 4, 5).

Infectious as well as inflammatory processes are known to be associated with change in platelet indices (6). Platelets are the smallest blood cells and are associated with hemostasis and blood coagulation. There is growing clinical evidence suggesting that platelet play an important role in the inflammatory process. Multiple inflammatory mediators such as chemokine and cytokines

are secreted by platelets that can exacerbate the immune response, and also platelets size increase when they are activated (6, 7, 8).

The measurements of platelet count and mean platelet volume (MPV) are commonly available parameters nowadays. Mean platelet volume (MPV), which is commonly used as a measure of platelet size, indicates the rate of platelet production and platelet activation. It can be measured during a routine automatic whole blood count (3,4,9). The MPV has been shown to correlate with the function and activation of platelets. The importance of MPV has been emphasized as an inflammation marker in some chronic inflammatory disorders, such as inflammatory intestinal and a rheumatoid arthritis. An inverse correlation between disease activity and MPV has been demonstrated (10).

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are markers of inflammatory conditions and have been used extensively by clinicians both in outpatient and inpatient settings. These are thus two most commonly used laboratory tests for assessing inflammation are the erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP). Both ESR and CRP are known to lack specificity and sensitivity, and neither should be used by itself for diagnosing any infectious or inflammatory disorder. However, if used correctly and as an adjunct to a good clinical history and physical exam, they play an important role in clinical practice. One has to be careful in interpreting CRP results because CRP is reported either as mg/L or mg/dl, and it is important to be aware of the units used by a specific laboratory (11).

Erythrocyte sedimentation rate (ESR) is an inexpensive and simple test for evaluating the inflammatory or acute response. It is a useful test in clinical practice as an indicator of inflammation, infection, trauma or malignant disease. The ESR can also be an important prognostic factor in non-inflammatory conditions, such as coronary heart disease, stroke, heart failure and prostate cancer. There are several methods for measuring the ESR, but the International Committee on Standardization in Hematology Reference Procedure accepts the Westergren method developed in the early 20th century (12).

It is a common hematological test, to perform the test, the anti-coagulated blood was traditionally placed in an upright tube, known as a Westergren tube, and the rate at which the red blood cells fall was measured and reported in mm/h (11, 12).

An elevated concentration of C-reactive protein in the blood is an indicator of inflammation. The bulk of C-reactive protein tests are requested for the detection of inflammatory responses associated with microbes, autoimmune diseases and drug allergies (especially to antibiotics). C-reactive protein plays a key role in the host's defense against infection. It also neutralizes the pro-inflammatory platelet-activating factor and down-regulates polymorphs. C-reactive protein is predominantly made in the liver and is secreted in increased amounts within six hours of an acute inflammatory stimulus. The plasma concentration can double at least every eight hours, reaching a peak after about 50 hours. After effective treatments or removal of the inflammatory stimulus, concentrations can fall almost as rapidly as the 5–7 hour plasma half-life of labeled exogenous C-reactive protein. C-reactive protein responses may be reduced by severe hepato-cellular impairment, but renal dysfunction can elevate concentrations of C-reactive protein (13).

Levels of CRP increase very rapidly in response to trauma, inflammation and infection and decrease rapidly with the resolution of the condition. Since an elevated CRP level is always associated with pathological changes, determination of CRP is of great value in diagnosis, treatment and monitoring of inflammatory conditions. CRP is a more sensitive and reliable indicator of inflammatory processes than the ESR and the leucocytes count (11). The serum CRP concentrations increase faster than that of the ESR and when the condition subsides, CRP falls very quickly, reaching normal levels several days before the ESR normalises (11,14).

## **1.2. Statement of the problem**

Infectious and inflammatory diseases are among the most common diseases that threaten the public health (1). Although there are many ways of diagnosing these diseases, there is still a need for better parameters that will help reduce the cost and increase the reliability of the diagnosis. Among others, PLT, MPV, ESR and CRP are known to be good indicators of infectious and inflammatory diseases (2, 3, 4). However, these relatively simple markers have not commonly been included in diagnosis process routinely; as a result there is a problem of delay during treatment and misdiagnosis of patients. In addition, such studies have not been conducted in Ethiopian settings.

### **1.3. Significance of the study**

This study will add some information on the importance of platelet count and mean platelet volume as well as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in the diagnosis process of infectious and inflammatory diseases. MPV and other serological markers could be just of interest to address physicians toward the need of execute higher level diagnostic tools. Moreover, MPV can be a beneficial, time-saving, readily available marker without additional cost as it is one of the parameters already studied during routine CBC.

In addition, such kind of research was not conducted in Ethiopia and this study will show whether there are variations on results from Ethiopia. It can also be used as a baseline data for further study in our settings.

## 2. Literature review

Many Studies have shown the importance of determining platelet count (PLT) and mean platelet volume (MPV) to facilitate the diagnosis process of a number of diseases. As a result, these platelet indices as well as other markers, such as erythrocyte sedimentation rate and C-reactive protein, are becoming common parameters in the diagnosis process of infectious and inflammatory diseases.

### 2.1. Platelet (PLT) count and mean platelet volume (MPV)

The MPV and PLT are important parameters for diagnosis of some diseases as they have shown to correlate with the function and activities of platelets. A direct correlation of PLT and an inverse correlation of MPV to disease activities have been demonstrated by many studies (3, 9, and 10). However, there are significant variations among study results on the actual values of the parameters before and after treatments, which has to be worked more on it to reach a conclusive remark (6).

A retrospective study conducted in Turkey in 2013 showed the diagnostic value of mean platelet volume in the diagnosis of patients either with acute or chronic cholecystitis. Here, MPV values were found to be significantly lower in acute cholecystitis group when compared to those in chronic cholecystitis and control groups ( $p < 0.05$ ). MPV values were negatively correlated to WBC and CRP values, whereas it was positively correlated to platelet counts. WBC and CRP values were found to be significantly higher in acute cholecystitis group than those in chronic cholecystitis and control groups ( $p < 0.05$ ). The study has thus demonstrated the use of MPV as quick diagnostic tool of acute or chronic cholecystitis (15).

A similar study was also conducted in Turkey in 2014 on 72 patients with active disease and 48 patients under remission. The ESR, CRP, MPV, platelet-crit (PCT), and platelet distribution width (PDW) scores were all statistically different among groups. In the data, PCT was found to be a positive acute phase reactant and others were negative acute phase reactants for patients with rheumatoid arthritis (RA). The authors of this study claim that platelet indices as cheap, widely available, and useful parameters for routine clinical rheumatology practice (16).

Another cross-sectional study in Iran, in the year 2014 showed the use of MPV and PLT as promising parameters to diagnose some infectious and inflammatory diseases. Mean platelet count increased in the patients at the time of admission in the hospital compared to the recovery and discharge time (mean  $430,820 \pm 134,643/\mu\text{l}$  vs.  $350,970 \pm 99,374/\mu\text{l}$ ,  $P < 0.001$ ). However, MPV decreased significantly during the same period ( $8.2 \pm 1.1$  fl vs.  $8.7 \pm 0.9$ ,  $P < 0.001$ ). Platelet count was directly correlated with CRP (mean  $6.4 \pm 0.3$  mg/l), ( $r = 0.49$ ,  $P < 0.001$ ) and ESR (mean  $10.9 \pm 1.1$  mm/hr), ( $r = 0.32$ ,  $P = 0.003$ ). On the other hand, MPV was inversely correlated with CRP ( $r = 0.39$ ,  $P < 0.001$ ) and ESR ( $r = -0.24$ ,  $P = 0.034$ )(3).

Similarly a study in India in 2012 suggested MPV as an inexpensive test for the diagnosis of Brucellosis and for other inflammatory diseases as well. A total of 39 brucellosis patients (male/female: 15/24) and 40 healthy controls (male/female: 23/17) were enrolled in this study. Brucellosis diagnosis was based on clinical, serological and bacteriological data. MPV and inflammatory markers were measured at the time of diagnosis and at the end of the treatment. MPV levels of patients in acute phase were found to be significantly lower than control cases ( $p < 0.001$ ). Overall accuracy of MPV in determining acute brucellosis was 75.2% with sensitivity, specificity, NPV and PPV of 74.4%, 75%, 75% and 74.4% respectively (AUC: 0.774)(17).

Further, studies in Turkey, in 2010 have shown that MPV can reflect the disease activity of ulcerative colitis. Eleven patients were in remission (26.8%) and 30 had active disease (73.2%). The mean values were; age:  $44.58 \pm 15.08$  year (20 women, 21 men), CRP:  $36.80 \pm 32.90$  mg/L, ESR:  $52.29 \pm 31.23$  mm/h, albumin:  $3.43 \pm 0.65$  gm/dl, platelets  $400780 \pm 161196$  K/mm<sup>3</sup>, MPV:  $7.41 \pm 1.04$  femtoliter (fl), Prothrombine time:  $13.32 \pm 1.05$  second, respectively. Correlation analysis revealed a negative significant relation between CRP and MPV ( $p < 0.05$ ). The mean MPV values of the 11 patients in remission were  $8.62 \pm 1.15$  fl. The mean MPV values of the 30 patients who had active disease were  $6.97 \pm 0.53$  fl. The patients having active ulcerative colitis had lower MPV values when compared with the patients who had inactive disease (in remission) ( $p < 0.001$ ). There were negative significant relations between MPV and clinical, pathological and colonoscopic activity indices ( $p < 0.001$ ). However there was no relation between the extent of the disease involvement and MPV (18).

According to another study conducted in Turkey, the Mean platelet volume predicts embolic complications and prognosis in infective endocarditis suggesting that MPV can be used as an activity criterion in infective endocarditis, like ESR and high-sensitivity C-reactive protein (hs-CRP). These authors further suggested that high MPV is associated with a poor prognosis and adverse outcomes, and predicts complications including embolic events. There were 27 cases of native valve endocarditis and 13 of prosthetic valve endocarditis. While 31 patients were treated medically, an operation was performed in nine patients because of unsuccessful medical therapy. On admission, mean MPV was 10.8 -1.1 fl, ESR was 82 - 26 mm/h, and hs-CRP was 110 - 72 mg/l. Seven patients died: one intra-operatively, three patients postoperatively, and three patients during medical treatment. With the exception of these seven patients, ESR and hs-CRP were significantly reduced in all patients at discharge compared to levels at hospitalization (ESR 82 - 26 to 32 -22,  $p = 0.001$  and hs CRP 110 - 72 to 25 -15,  $p = 0.001$ ). Similarly, they detected a significant decrease in MPV from hospitalization to discharge, i.e., from the active period of the disease to recovery (10.8 -1.1 to 9.7 - 0.8 fl,  $p = 0.002$ ). In addition, MPV was found to be significantly higher in patients with observed embolic complications (11.5 vs. 10.3 fl,  $p = 0.001$ ), other complications (11.0 vs. 10.2 fl,  $p = 0.001$ ), and death (11.1 vs. 10.4 fl,  $p = 0.005$ ) (19).

Furthermore, study in China have shown the decline of MPV in Crohn's diseases (CD) patients compared with healthy subjects, and suggested MPV as the best accuracy in determination of CD patients and healthy controls. A significant decrease in MPV was noted in patients with CD compared with healthy controls ( $P < 0.0001$ ), but statistical difference was not found between active and inactive CD groups. In CD, no significant correlation was found between MPV and other inflammatory markers. The overall accuracy of MPV (cutoff: 10.35 fl), CRP (cutoff: 4.85 mg/dl) and ESR (cutoff: 8.5 mm/hour) in differentiating CD patients from healthy controls was 76.6%, 65.8% and 72.1% respectively. The overall accuracy of CRP (cutoff: 4.95 mg/dl) and ESR (cutoff: 16.5 mm/hour) in determination of active CD was 80.3% and 73.8% (20).

However, a number of other studies in Turkey, 2014 have shown insignificant variations in the values of platelets indices before and after treatment. The MPV was  $7.74 \pm 1.33/ \mu\text{l}$  in the PTB group and  $8.20 \pm 1.13/ \mu\text{l}$  in the control group ( $p = 0.005$ ). The blood platelet count, CRP level, and ESR were significantly higher in the active PTB group than in the control group ( $p < 0.0001$ ).

In the PTB group, CRP levels ( $r = 0.26$ ,  $p = 0.003$ ) and ESR ( $r = 0.39$ ,  $p = 0.003$ ), but not MPV ( $p = 0.80$ ), were significantly correlated with the radiologic extent of the disease (10).

Another cross-sectional study was conducted in Iran in 2014. In this study, a total of 60 patients with acute pyelonephritis and 60 health people as a control group were included. MPV and Platelet distribution width (PDW) were significantly different between patients and control group ( $p = 0.005$ ). Platelet count in patients was higher than control group but the difference was not significantly in response to treatment ( $< 0.001$  and  $p = 0.007$ , respectively (6). The above two consecutive studies demonstrated a small difference of values of these parameters before and after treatment showing the importance of these parameters for the diagnosis of inflammation due to pulmonary TB and acute pyelonephritis's questionable (6, 10)

Another study in Turkey in 2013 suggested that even though MPV is a useful predictor for the diagnosis of community-acquired pneumonia (CAP), it had low specificity and negative predictive value (NPV) rates which may lead to the false-negative diagnosis. In this study, a total of 196 patients were diagnosed with CAP during the study period, of which 108 (55.1%) had severe disease, which required hospitalization (Group 1a), while the remaining 88 (44.9%) were followed-up as outpatients (Group 1b). The control group consisted of 100 healthy children (Group 2). Patients with CAP had lower MPV values than their healthy counterparts ( $7.1 \pm 0.68$  vs.  $8.31 \pm 1.2$  fl;  $p < 0.001$ ). MPV value was significantly higher in hospitalized CAP patients compared to outpatients ( $7.32 \pm 0.71$  vs.  $6.83 \pm 0.5$  fl;  $p = 0.012$ ). ROC curve analysis suggested that MPV level cut-off point for making a diagnosis of CAP was 8.1 fl, with a sensitivity, specificity, positive predictive value (PPV), and NPV of 91%, 51%, 80.8% and 70.5%, respectively (21).

## **2.2. C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR)**

C-reactive protein (CRP) is one of the acute phase reaction proteins, which is synthesized from liver and increases during inflammatory reactions. Elevated erythrocyte sedimentation rate (ESR) is another marker of inflammatory reactions (11). ESR and CRP are thus widely used by the physicians in order to estimate the presence and severity of infectious and inflammatory diseases. On these regard, a number of studies have shown the importance of these parameters in

diagnosis test of many diseases (11, 13). However, studies have also shown that CRP and ESR having similar or different diagnostic efficiencies.

Study conducted in Nigeria in 2013 showed that the mean ESR value among the Acid Alcohol Fast Bacilli (AFB) positive patients was  $53.16 \pm 4.92$  mm/hr while the CRP value was  $0.273 \pm 0.035$  mg/L. Gender and age were not found to have any influence on the ESR and CRP values. No relationship was found to exist between ESR and CRP ( $r = 0.17$ ;  $p = 0.235$ ) and age ( $r = 0.125$ ;  $p = 0.388$ ). The study has also shown a moderately elevated ESR and low CRP values at the early diagnosis of tuberculosis. These authors also showed that there was no correlation between ESR and CRP at the onset of tuberculosis and they conclude that CRP cannot be used as a screening tool for early diagnosis of tuberculosis (22).

A descriptive-analytical study in Iran in 2013 indicated that ESR, CRP, PLT, and WBC in peri-sepsis were significantly higher than those in pre- and post-sepsis ( $p < 0.05$ ). Comparing PCT mean level in peri- and post-sepsis (lowest level) showed a significant difference, while no significant difference was seen between pre- and peri sepsis ( $p < 0.05$ ). C3 biomarker was also significantly higher in peri-sepsis than pre- and post-sepsis ( $p < 0.05$ ). Although changes in PCT and C3 have a high diagnostic value in early stages of sepsis and are used as guides for antibiotic therapy in suspected sepsis cases, significant difference was noted in ESR, CRP, and WBC in peri-sepsis compared to pre- and post-sepsis. They have thus shown these tests as simple and less expensive, having moderate diagnostic value for the diagnosis of sepsis in intensive care unit (ICU) admission (23).

A retrospective study in Turkey in 2013 was conducted on 503 patients in the acute appendicitis group and 121 patients in the control group, making up a total of 624 subjects. The median MPV levels were  $7.92 \pm 1.68$  fl in the acute appendicitis group, while  $7.43 \pm 1.34$  fl in the control group. CRP, leukocyte count, and MPV level were significantly higher in the acute appendicitis group ( $P < 0.001$ ). MPV, leukocyte count, and CRP had a sensitivity and specificity of 66% and 51%; 91% and 74%; and 97% and 41%, respectively. No correlation was found between MPV, CRP, and leukocyte count. MPV level was higher in patients with acute appendicitis. MPV may guide the diagnostic process of acute appendicitis. However, the authors suggested that leukocyte

count and CRP were more sensitive and specific than MPV in the diagnosis of acute appendicitis (24).

A similar study in Romania in 2012 was conducted to evaluate the usefulness of leukocytes, CRP and ESR as identifications of invasive bacterial infections in children. The results show that the Mean values for leukocytes were  $12536/\text{mm}^3 \pm 6642$  for invasive bacterial infections versus  $11263/\text{mm}^3 \pm 5285$  for localized bacterial infections ( $p < 0.03$ ). Mean ESR for invasive infections was  $31.13 \text{ mm/hr} \pm 29.11$  versus  $25.94 \text{ mm/hr} \pm 22.93$  for localized infections ( $p < 0.08$ ). Mean CRP for invasive infections was  $25.45 \text{ mg/dl} \pm 42.57$  versus  $13.58 \text{ mg/dl} \pm 24.10$  ( $p < 0.04$ ) for localized bacterial one. This study suggest that CRP, ESR and leukocytes are good indicators in diagnose of invasive bacterial infections (25).

A prospective multicenter study in Israel, in 2011 of CRP and ESR showed that the best thresholds to differentiate quiescent, mild, moderate and severe disease activity, were  $b_{23}$ , 23–29, 30–37,  $N_{37}$  mm/h for ESR, and  $b_{2.5}$ , 2.5–5, 5.01–9,  $N_9$  mg/L for CRP (area under the ROC curves 0.70–0.81). Correlation of endoscopic appearance with CRP and ESR were 0.55 and 0.41, respectively ( $P \leq 0.001$ ). Both CRP and ESR may be completely normal in 34% and 5–10% of those with mild and moderate-severe disease activity, respectively. Elevated CRP in the presence of normal ESR or vice versa was noted in 32%, 38%, 30% and 17% of those with quiescent, mild, moderate and severe disease activity. Over time, the utility of CRP and ESR in reflecting disease activity remained stable in 70–80% of cases. This study of Turner et al. in Israel revealed that both parameters gave similar results (in about 60% of the cases), which means both CRP and ESR values reflect disease activity to a similar degree, and the authors suggested that either CRP or ESR may be sufficient. However, the authors claimed that CRP has shown slight superiority of over ESR, and CRP is more closely correlated with endoscopic appearance (26).

A prospective study conducted in Sweden, in 1997 was to evaluate the sensitivity of CRP elevation compared to erythrocyte sedimentation rate, Leucocytes count and thrombocytes count in the diagnosis of infective endocarditic (IE). The results indicate that Median CRP concentration was found to be 90(range 0-357) mg/dl with only 4% having normal values. Episodes involving native valves had higher CRP than episodes occurring with prosthetic valves. Staphylococcal origin, Short duration of symptoms, short duration of fever and highest recorded

temperature all correlated to higher CRP levels. The CRP response was also prominent among patients greater than 70 years old. Among non-responders, a few cases with simultaneous cirrhosis were noted. ESR was less sensitive than CRP, with a normal level in 28% of the episodes. These authors concluded that CRP determination is superior to ESR, leucocytes count and thrombocyte count in the diagnosis of infective endocarditis (27).

Another prospective study in Finland, 1997, evaluated the applicability of C-reactive protein, erythrocyte sedimentation rate, white blood cell count (WBC), and absolute neutrophil count (ANC), in the screening of pneumococcal (PNC) pneumonia in children. The results showed that CRP and ESR were significantly higher in patients with alveolar (n=53) than in those with interstitial (n=108) pneumonia. CRP, ESR and ANC were significantly higher in PNC (n=29) than in viral (n=23) pneumonia. The values in mixed PNC and viral infections (n=17) were approximately midway between PNC and viral cases. All cases with serologic evidence of *S. pneumoniae* an etiology were combined (n=46) for calculation of diagnostic parameters. When a cut-off limit of  $60 \text{ mg} \cdot \text{L}^{-1}$  was used, CRP had a sensitivity of 26% and a specificity of 83% in the screening of PNC pneumonia. However, there are studies, which have shown the limitations of these parameters in diagnosis of some diseases. The authors concluded that ESR and CRP have a limited capacity to differentiate between pneumococcal and non-pneumococcal pneumonia. CRP is recommended as the first-line method of screening (28).

In conclusion, a number of studies have been conducted to show the efficiency and specificity of platelet indices and other markers like ESR and CRP for the diagnosis of various diseases. Generally, most studies suggested that these parameters should be requested by the physicians and be used as they are simple and cost effective. However, some studies have also shown the limitations of these parameters. Therefore, there is a need for continued researches on various diseases using these parameters thereby strengthening either their usability or limitation in the diagnosis of diseases.

## **3. Objectives**

### **3.1. General objective**

- To evaluate the changes in platelet count and mean platelet volume (MPV) and their correlation with erythrocyte sedimentation rate (ESR) and C - reactive protein (CRP) in infectious and inflammatory diseases at admission and discharge in Zewditu Memorial Hospital, Addis Ababa.

### **3.2. Specific objectives**

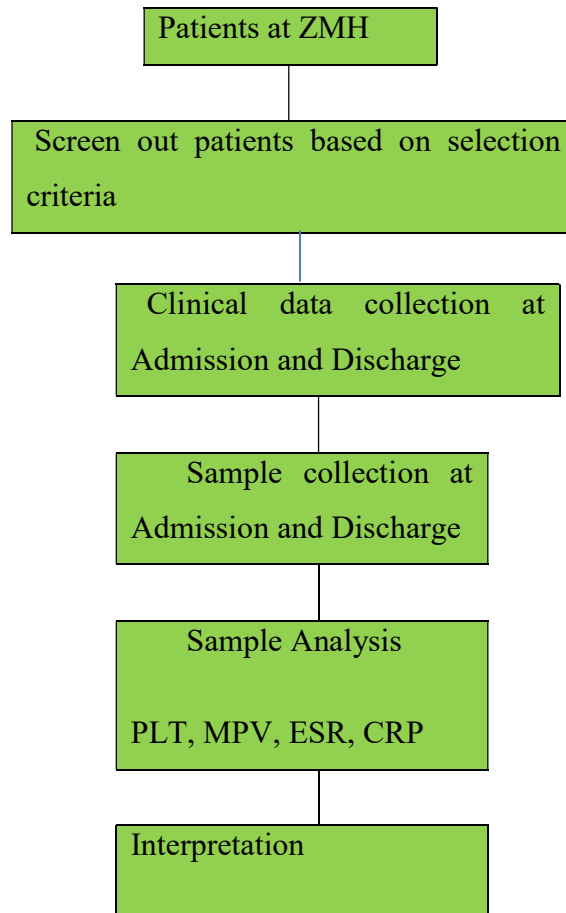
- To determine platelet count and mean platelet volume values of patients with infectious and inflammatory diseases at admission and discharge.
- To determine ESR and CRP values of patients with infectious and inflammatory diseases at admission and discharge.
- To determine the correlation between platelet counts, mean platelet volume, erythrocyte sedimentation rate and C - reactive protein at admission and discharge.
- To describe the type and frequency of infectious and inflammatory diseases.

## 4. Materials and Methods

### 4.1. Study Design

A cross-sectional hospital based study was conducted.

#### Study Design Flow Chart



## 4.2. Study Setting

The study was conducted in Addis Ababa, which is the capital city of Ethiopia, in Zewditu Memorial Hospital under the city administration of Health Bureau. Zewditu Memorial Hospital is found in Addis Ababa city in Kirkose Sub City Woreda7, which locally named Fillweha/Ambassador, and lies on estimated 32,946 square meters. The hospital starts its service in 1963 E.C by the help of American Missionaries. It provides different medical services like Surgical (General Surgery, Plastic Surgery, Neurosurgery), Internal Medicine (Medical), Gynecology and Obstetrics, Maternity, Emergency, Special clinics (Dermatology, Psychiatric, Neurology), Modern VCT and TB/HIV Clinics, Ambulance, Pediatrics, Pharmacy and Diagnostic services.

The hospital has a total of 750 staff members and around 400 up to 500 clients visit per day to access these different services from Addis Ababa and other regions. Zewditu Memorial hospital laboratory is equipped with fully automated hematology analyzer and participate in external quality control assessment schemes; additionally the laboratory has implements laboratory quality management system and is awarded four stars according to the WHO AFRO SLIPTA checklist.

## 4.3. Study Period

The study was conducted from July 2015 – February2016.

## 4.4. Populations

### 4.4.1. Source of populations

The source populations were all hospital clients or patients who have visited Zewditu Memorial Hospital during the study period.

### 4.4.2. Study population

The study population was all admitted patients with their case of infectious and inflammatory disease during the study period.

## 4.5. Sample size

A total of 100 patients sample was collected by convenient sampling technique.

## 4.6. Study Variables

### 4.6.1. Dependent variables

- ✓ Platelet count, Mean platelet volume, erythrocyte sedimentation rate and C - reactive protein.

### 4.6.2. Independent variables

- ✓ Socio-demographic characteristics (age, sex)
- ✓ Type and frequency of disease

## 4.7. Inclusion and Exclusion criteria

### 4.7.1. Inclusion criteria

- All admitted patients 18years and older at the time of the study and volunteering to participate in the study period.

### 4.7.2. Exclusion criteria

- Those patients who are taking anti-inflammatory therapy upon admission.

## 4.8. Sampling Technique

All patients who fulfilled the inclusion and exclusion criteria and diagnosed with any infectious or inflammatory disease were enrolled in the study. Convenient sampling technique was used to collect patients sample to address the stated objectives. The study was carried out on different days in the hospital and a total of 100 admitted patients in the ward were contacted during admission and at discharge.

## **4.9. Data collection and measurement**

Structured and proper data collection form was used to collect demographic information of the patients, contact number, residential place, disease name, and physical examination or the preclinical findings. At patient admission or registration center, effort was made to improve triage of patients to ensure that all eligible suspects are recruited. All adult patients with infectious or inflammatory diseases and admitted at the time of the study were involved in the sample collection process.

Platelet parameters including platelet count and Mean platelet volume was measured automatically using Cell Dyn1800 Hematological analyzer. The serum level of C-reactive protein (CRP) was calculated using semi quantitative agglutination on latex kit. Erythrocyte sedimentation rate (ESR) was measured using Westergren methods.

### **4.9.1. Whole blood collection**

After having the ethical clearance, about 5 milliliter of whole blood was collected from the participant patients during the admission and recovery time for platelet parameters, CRP and ESR investigations using EDTA and SST tube by trained laboratory personnel by following the standard venous sample collection procedure. Thus, purposive sample collection was continued until the achievement of the expected sample size within the given study period. The standard sample collection procedure is found in the Annex.

### **4.9.2. Laboratory testing Principles and procedures**

#### **Platelet and Mean Platelet Volume Analysis**

**Principle:** -CELDYN 1800 Hematology analyzer employ the Electrical impedance principle for determining WBC, RBC, and PLT count. As the cells pass through the aperture of the van Behrens transducer/transducer bath, a change in electrical resistance occurs generating an equivalent voltage pulse. The number of pulses sensed during each cycle corresponds to the number of WBC, RBC and PLT cell counted. The amplitude of each pulse is essentially proportional to the cell volume (29). The standard testing procedure is found in the Annex.

## **C - reactive protein**

**Principle:** CRP-Latex/Determination of C- reactive protein slide test is a rapid slide agglutination procedure based on a modification of the latex fixation method developed for the direct detection and semi-quantization of C-reactive protein (CRP) in serum. The assay is performed by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum. The presence of a visible agglutination indicates an increase of the CRP level above the upper limit of the reference interval in the samples tested (30). The standard testing procedure is found in the Annex.

## **Erythrocyte sedimentation rate**

**Principle:** When anti-coagulated blood is allowed to stand undisturbed, the red blood cells will normally settle out to the bottom of the tube. This principle is the basis for the erythrocyte sedimentation rate (ESR). By definition, the ESR is the distance, in millimeters, that the red cells fall (in millimeters) per unit of time, which is usually 1 hour. Various factors will affect the ESR, such as the size and shape of the red cells, plasma fibrinogen, and globulin levels, as well as mechanical and technical factors.

The ESR is directly proportional to the red cell mass and inversely proportional to the plasma viscosity. In normal whole blood red blood cells do not form rouleaux; the red cell mass is small and therefore the ESR is decreased (cells settle out slowly). In abnormal conditions when red cells can form rouleaux, the red cells mass is greater, thus increasing the ESR (cells settle out faster). The recommended and well accepted Westergren method was used in this study (31). In brief whole blood was filled in a standard Westergren tube and set on Westergren rack for one hour. The distance the red cells settled was measured in millimeter and the result reported as mm/hr. The standard testing procedure is found in the Annex.

#### **4.10. Data management and analysis**

The completed checklist was checked for completeness, consistency and was coded by the principal investigator. Data cleanup was performed to check for accuracy and consistencies. Any error identified was corrected immediately. For electronic records, password protected databases was created and maintained throughout the study period and back up was picked using compact discs.

The platelet counts and MPV of admission and recovery times was compared using paired t-test. Additionally Pearson correlation test was used in order to determine the relationship between the platelet count and MPV with ESR and CRP. P-value less than 0.05 were considered as statistically significant. A statistical analysis was performed using SPSS statistics version 21.

#### **4.11. Data quality control and assurance**

The data collectors were instructed to check the completeness of the data collecting format at the end of each data collection. The principal investigator has also checked during submission. Internal Quality control was performed continuously to ensure precise and reliable operational results by the CELL DYN 1800 hematological instruments and other parameter of the study. All samples were analyzed after the daily control running. All reagents were stored at 2-8°C without freezing, since frozen reagents could change the functionality of the test. Reagent and Controls are stable until the expiry date stated on the test kit. The most practical way of controlling ESR tests is to follow standard operating procedures exactly. Data entry quality was maintained by entering into SPSS version 21, software and verified its quality against the collected hard copied data during entry. Finally, all the necessary data was kept in a locked place.

#### **4.12. Ethical consideration**

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences. Ethical clearance was also obtained from Addis Ababa Health Bureau. A formal letter of cooperation was requested to Addis Ababa Health Bureau, Zewditu Memorial Hospital.

The study was carried out after obtaining informed consent and confidentiality of data was obtained throughout the study by locking hard copies and password protection of electronic data.

#### **4.13. Dissemination of results**

This study on completion could serve as a reference material to physicians or any health professionals, researchers, experts and policy makers for any appropriate interventions. To reach these bodies, the finalized paper was submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences. So it can serve as a reference in the library. In addition, a copy of this material will be given to Addis Ababa Health Bureau and Zewditu Memorial Hospital. Additional effort will also be made to present on conferences to reach the medical/scientific community and publish the article on reputable Journals after the final reports.

## 5. Result

A total of 100 patients aged 18 to 77 years, 48% Male and 52% Female, who were diagnosed for infectious and inflammatory diseases and admitted in medical ward at Zewditu Memorial Hospital were enrolled in this study. The mean ( $\pm$  SD) age of the patients was  $37.5 \pm 12.5$  years and Median and IQR was 35, [28.5-45] years respectively. Majority (87%) of the patients were urban dwellers. Table 1 summarizes the demographic characteristics of study participants.

Table 1. Demographic characteristic of patients with infectious and inflammatory diseases at Admission and Discharge in Zewditu Memorial Hospital, Addis Ababa, July 2015-February 2016 (n= 100)

Variables	Frequency	
	Number	%
<b>Gender</b>		
Male	48	48
Female	52	52
<b>Age group</b>		
18-34	41	41
35-54	50	50
55 and above	9	9
<b>Residence</b>		
Urban	87	87
Rural	13	13

The most common disease types were as follows: there were 24 patients with infectious and inflammation of respiratory system, 18 patients with respiratory system infection, 13 patients with gastrointestinal infection, 10 patients with central nervous system infection, 10 patients with dermal infection (aspergillus), 6 patients with Rheumatoid arthritis, 4 pelvic inflammation. Frequency and type of infectious and inflammatory disease is summarized in Table 2.

Table 2. Frequency and type of infectious and inflammatory diseases in patients admitted in Zewditu Memorial Hospital, Addis Ababa, July 2015-February 2016(n=100)

<b>Type of diseases</b>	<b>Frequency</b>	<b>%</b>
Infections and inflammation of respiratory system	24	24
Respiratory system infection	18	18
Gastrointestinal infection	13	13
Central Nervous system infection	10	10
Joint infection and rheumatoid arthritis	1	1
Dermal infection (Aspergillus)	10	10
Hepatitis	1	1
Central Nervous System and Respiratory system infection	2	2
Rheumatoid arthritis	6	6
Typhoid fever	1	1
Pneumonia	3	3
Pelvic inflammation	4	4
Central nervous system and gastrointestinal infection	3	3
Bone and Joint infection	3	3
Hepatitis and typhoid fever	1	1
Total	100	100

Overall 10% of the patients had a platelet count more than 450,000/ $\mu$ l and 22% of them had a platelet count less than 150,000/ $\mu$ l at the time of admission. At discharge, 7% of them had above normal range and 11% below normal value.

The mean  $\pm$  standard deviation of platelet count, MPV, CRP and ESR are presented in Table 3. As shown in the table, at the time of hospitalization, the mean platelet count was  $258.5 \pm 131.1$  ( $\times 10^9/l$ ) and at discharge period the result was  $279.5 \pm 124.9$  ( $\times 10^9/l$ ) ( $p=0.04$ ). The MPV of the patients' results at the time of admission was  $9.6 \pm 1.7$ fl, CRP was  $6.6 \pm 9.7$ mg/l and ESR was  $46.0 \pm 40.2$  mm/hr.

CRP and ESR results decreased after the treatment or during the discharge time with clinical resolution of CRP  $6.6 \pm 9.7$ mg/l to  $2.8 \pm 5.9$  mg/l ( $p=0.001$ ) and ESR  $46.0 \pm 40.2$  mm/h to  $31.5 \pm 30.9$  mm/hr ( $p=0.001$ ) respectively.

Table 3. Comparison of PLT, MPV, CRP, and ESR (Mean  $\pm$  SD) at Admission and Discharge of patients with infectious and inflammatory diseases in Zewditu Memorial Hospital, Addis Ababa, July 2015-February 2016 (n = 100)

Parameter	Admission	Discharge	P value
PLT ( $\times 10^9/l$ )	$258.5 \pm 131.1$	$279.5 \pm 124.9$	0.040
MPV (fl)	$9.7 \pm 1.7$	$10.1 \pm 7.9$	0.580
CRP (mg/l)	$6.6 \pm 9.7$	$2.8 \pm 5.9$	0.001
ESR (mm/hr)	$46.0 \pm 40.2$	$31.5 \pm 30.9$	0.001

The proportion of patients with abnormal ESR and CRP values were also compared at admission and discharge (Figure 1). The proportions of patients with abnormal ESR, as determined by ESR value of greater than 15 mm/hr for males and 20 mm/hr for females, were 64% at admission and reduced to 56% at discharge. As depicted in Figure 1, the change in CRP values at time of discharge of patients was remarkable. Elevated serum CRP level above the cutoff 6mg/l was recorded in 41% of the patients during admission while this proportion was reduced to 20% during the discharge time (Figure 1). The change was statistically significant ( $P = 0.001$ ). The ESR and CRP gave discordant result in 35.94% of patients in which patients with an elevated ESR have normal CRP vales. On the other hand, both parameters were elevated in 64.06% of patients (concordant); the discrepancy rate was much higher at discharge. The possible reason was CRP shows a rapid response to infection and inflammation: increasing within hours of stimulus, returning rapidly to normal following resolution.

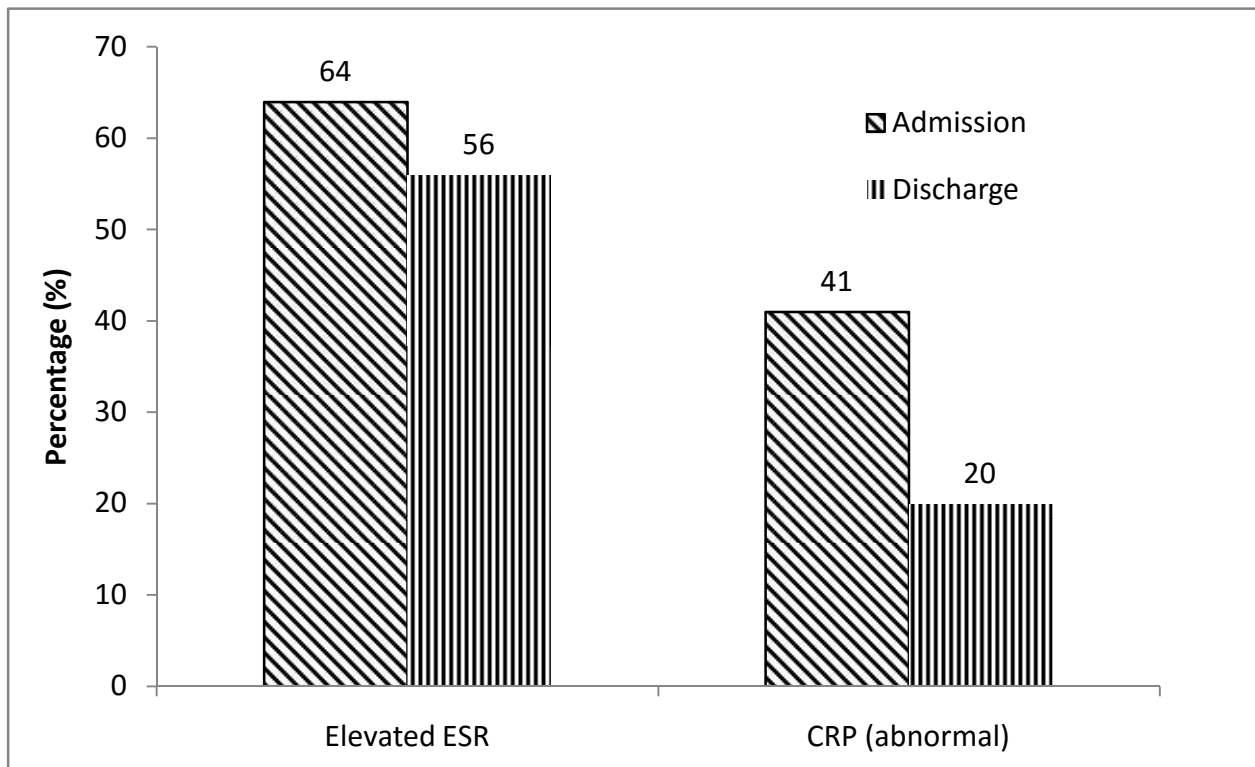


Figure1. Proportion of patients with abnormal ESR and CRP values at admission and discharge of patients at Zewditu Memorial Hospital, Addis Ababa, July 2015-February2016(n=100).

Analysis of titer changes from admission CRP values also revealed a significant reduction in titer after treatment as evidenced by the CRP titer values at discharge,  $p=0.009$  (Figure 2)

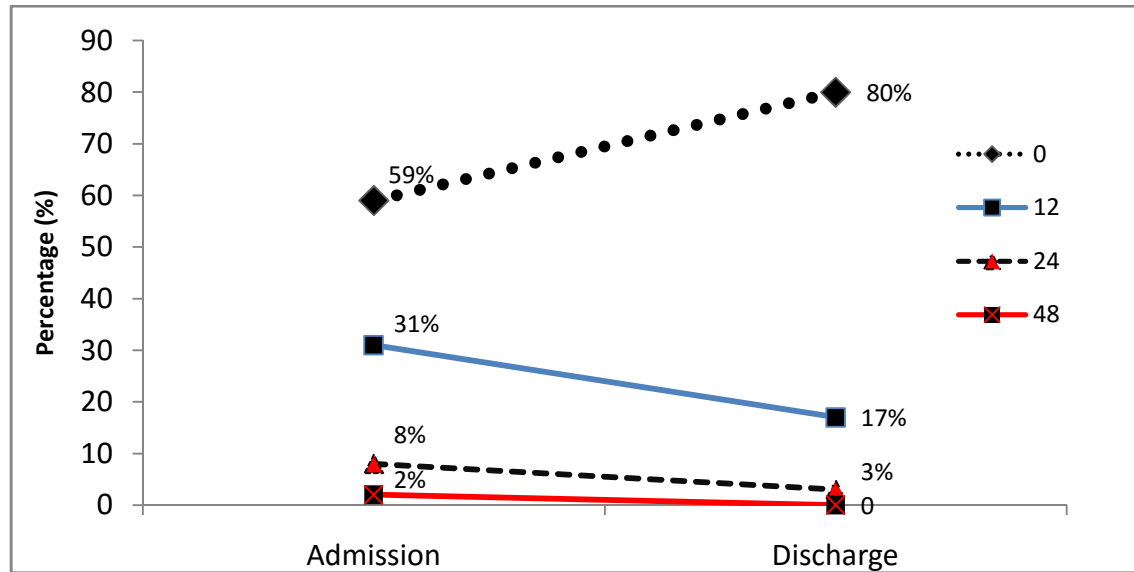


Figure 2. Distribution of CRP titer values at Admission and Discharge of patients at Zewditu Memorial Hospital, Addis Ababa, July 2015-February 2016 (n=100)

The study also checked if there are any correlations between the parameters. CRP had no statistically significant correlation with platelet count at time of admission ( $r = -0.004$ ,  $p = 0.972$ ) as well as discharge ( $r = 0.062$ ,  $p = 0.538$ ). Similarly, CRP had no statistically significant correlation with mean platelet volume during admission time ( $r = -0.094$ ,  $p = 0.352$ ) and discharge time ( $r = 0.139$ ,  $p = 0.167$ ).

The study revealed a negative correlation between platelet count and mean platelet volume at the time of admission ( $r = -0.371$ ,  $p = 0.001$ ). In contrast, at the time of discharge, platelet count and mean platelet volume had no statistically significant correlation ( $p = 0.863$ ,  $r = 0.017$ ).

Moreover, ESR was not significantly correlated with platelet count during admission ( $p = 0.560$ ,  $r = 0.059$ ) as well as discharge time ( $p = 0.447$ ,  $r = 0.077$ ). Besides, ESR had no significant correlation with mean platelet volume at admission ( $r = -0.096$ ,  $p = 0.341$ ) as well as discharge ( $r = 0.019$ ,  $p = 0.849$ ).

As shown in Figure 3a and 3b, ESR and CRP had a significant positive correlation at the time of admission ( $r = 0.57$ ,  $p = 0.0001$ ) and at the time of discharge ( $r = 0.58$ ,  $p = 0.0001$ ).

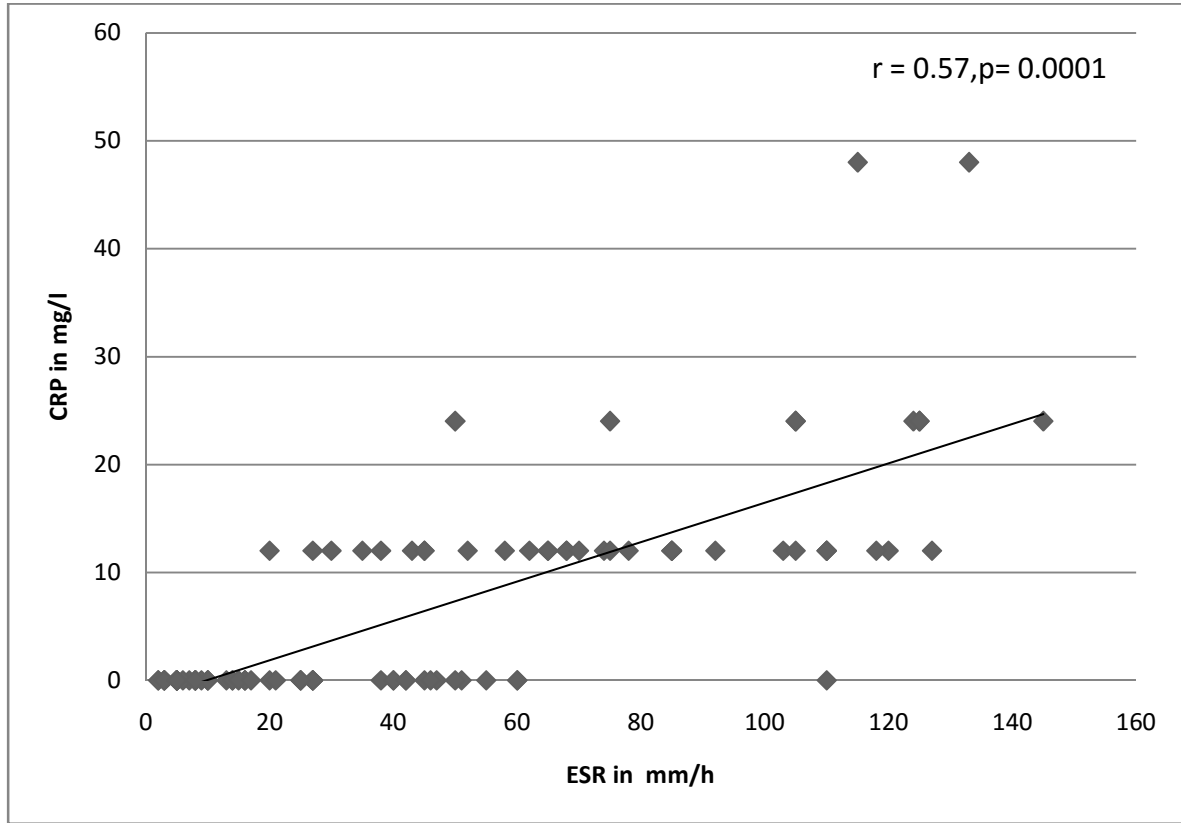


Figure 3a. Correlation between CRP and ESR at Admission of patients at Zewditu Memorial Hospital, Addis Ababa, July 2015-February2016(n=100)

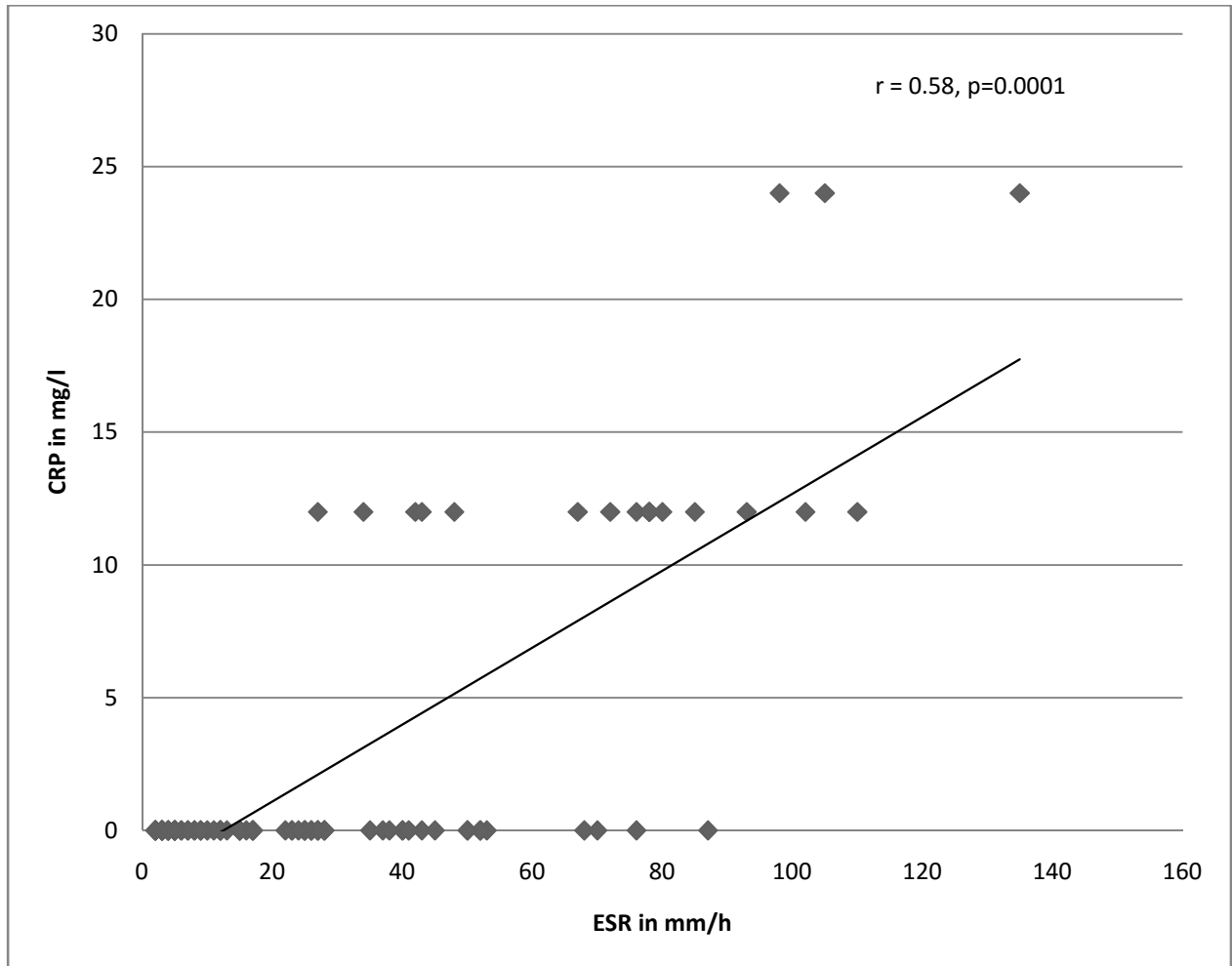


Figure 3b. Correlation between CRP and ESR at Discharge of patients at Zewditu Memorial Hospital, Addis Ababa, July 2015-February 2016 (n=100)

## 6. Discussion

This cross-sectional study was aimed to assess the changes in platelet count (PLT) and mean platelet volume (MPV) among patients attending Zewditu Memorial Hospital (Ethiopia) with infectious and inflammatory disorders between admission and discharge. Moreover, this study tried to show the degree of correlation of the PLT and MPV with erythrocyte sedimentation rate (ESR) and C - reactive protein (CRP). The study participants were adult females and males predominantly urban dwellers. We noted that the major causes of admission of the patients included in this study were infection and inflammation of Respiratory system, Respiratory system infection, gastrointestinal infection and central nervous system infections and some other diseases.

Generally, this study revealed significant changes between admission and discharge for PLT, ESR, and CRP values. Thus, these results strengthen the growing evidences that claim the suitability of these indices for the diagnosis of inflammatory and infectious diseases. The MPV, however, showed no significant difference at the two time points, which may suggest that this index may not help in diagnosing such diseases in our settings, if confirmed by a study addressing a single disease. In line with this study, others have also shown insignificant variations in the values of MPV. For example, a study by Gunluoglu et al.(2014)in Turkey demonstrated insignificant variations of MPV values in patients with active pulmonary tuberculosis and healthy controls (10). Moreover, Sert et al.(2012) from Turkey showed insignificant difference in MPV values between patients with acute rheumatic fever and controls (9).

In contrast to the current study, Zareifar et al (2014), in their retrospective study conducted in 2013 in Turkey, demonstrated that mean platelet volume as an important tool in the diagnosis of patients either with acute or chronic cholecystitis (6).Additionally, Nassaji et al. (2014) showed the significantly lower MPV values in patients with acute pyelonephritis than control groups (4).In addition, a number of other studies have shown that MPV can be cheap and efficient marker in diagnosing infections and inflammatory diseases (3,6,15-18,19,20).For example, studies done by Abidinet et al, in Turkey in 2012,Serek et al., in Turkey in 2013,by Zareifar et al in Iran, in the year 2014 all claim MPV as an inexpensive test for the diagnosis of some

infectious and inflammatory diseases. Nevertheless, even though many studies suggest MPV as a cheap and quick diagnosis tool (6), other studies, including ours, did not support well. Therefore, more studies at varying settings, methods and conditions are needed to resolve such inconsistencies and so as to give better explanations.

Another seemingly controversial observation of this study was that mean value of PLT has increased during discharge of the patients. For instance, Zareifar et al. observed an increased platelet count at admission (3). As far as my literature search goes, there is no study that showed similar observation. To resolve such uncommon findings, both indices should be evaluated considering only a single type of diseases to specifically show their usefulness for the diagnosis of diseases in our setting.

Regarding ESR and CRP, there was a significant decrease after treatment, which generally agrees with a number of studies. For example, the study conducted by Rastabi et al. in Iran in 2013 reported a significant decrease in mean of ESR and CRP after treatment (23). They indicated that ESR and CRP in pre-sepsis were significantly higher than those in post-sepsis ( $p < 0.05$ ). They have, thus, shown these tests as simple and less expensive, having moderate diagnostic value for the diagnosis of sepsis in intensive care unit (ICU) admission. This study further showed that serum CRP level and ESR can be applied to assess the disease activity and treatment response.

A study conducted in Konya, Turkey, showed that MPV values were negatively correlated with ESR and platelet counts before treatment in patients with ARF (3). The current study has also generated similar finding that MPV values were inversely correlated with platelet count before treatment of inflammatory and infectious diseases, though it did not reach to a statistically significant level. The difference can be accounted to various reasons. One possible explanation can be the inclusion of patients of different diseases, which may affect the level of MPV and its correlation with ESR.

In contradiction to the current study, a study conducted in Iran has shown that Platelet count was directly correlated with CRP and ESR. On the other hand, MPV was inversely correlated with CRP and ESR (9). Additional study conducted in Turkey indicated that MPV values were

inversely correlated to CRP values, whereas it was positively correlated to platelet counts (15).Further, a study done in Turkey showed that there was a negative significant relation between CRP and MPV (18).But this study showed that MPV values had no correlation with CRP and it indicated that MPV was negatively correlated with platelet count at admission time.

According to a study conducted in India, a negative correlation was found between ESR and MPV values in acute patients (17) a finding which contradicts to the current study. As described above, the variation might be attributed to patient, disease type and other unseen factors.

In this study, ESR was well correlated with CRP at both time points, which is in agreement with other studies. For example, a study by Zavarache et al. in Romania in 2012 reported a strong positive correlation between CRP and ESR showing that these indices could be good indicators in diagnosis of invasive bacterial infections (25). These indices are therefore suggested as useful tools in diagnosis of the diseases across various settings. However, a study conducted by Jeremiah et al in Nigeria in 2013 reported a moderately elevated ESR and low CRP values at the early diagnosis of tuberculosis. They also reported that there was no correlation between ESR and CRP at the onset of tuberculosis; hence they questioned the use of CRP as a screening tool for early diagnosis of tuberculosis (22). Such inconsistent finds need to be clarified by further studies employing varies approaches.

This study also tried discordant rate of CRP and ESR at admission and discharge. Accordingly, a remarkable discordance between the two parameters particularly at discharge was noted. The possible explanation is that the ESR may remain elevated long after the CRP has returned towards normal after patient is treated for the underlying diseases (32-34).

## **7. Strength and Limitation of the Study**

### **7.1. Strength**

- ✓ The study tried to investigate the usefulness of simple markers (platelet parameters, ESR and CRP) for patients with infection and inflammatory diseases at admission and discharge. These markers have never been investigated in our setting.

### **7.2. Limitation**

- ✓ The study was including any type of infectious and inflammatory disease, which means the study by itself was not focused on one specific type of disease.
- ✓ CRP was determined using semi-quantitative methods
- ✓ Patients were evaluated for a short time due to limited hospital length of stay; this reduced our ability to assess long term effect.

## 8. Conclusions and Recommendation

### 8.1. Conclusions

- ✓ The most common disease types were infectious and inflammation of respiratory system, respiratory system infection, gastrointestinal infection, central nervous system infection, dermal infection (aspergillus), Rheumatoid arthritis, and pelvic inflammation.
- ✓ There was a change between admission and discharge values of PLT ( $258.5 \pm 131.1$  (x 10<sup>9</sup>/l) to  $279.5 \pm 124.9$  (x 10<sup>9</sup>/l) ( $p=0.04$ ) and MPV ( $9.6 \pm 1.7$ fl to  $10.1 \pm 7.9$ fl), though not statistically significant for MPV( $p=0.580$ )
- ✓ CRP and ESR results decreased at discharge time with clinical resolution of CRP  $6.6 \pm 9.7$ mg/l to  $2.8 \pm 5.9$  mg/l ( $p=0.001$ ) and ESR  $46.0 \pm 40.2$  mm/h to  $31.5 \pm 30.9$  mm/hr ( $p=0.001$ ).
- ✓ The proportion of patients with abnormal ESR and CRP declined at discharge; the decline was remarkable and statistically significant for CRP (from 41% at admission to 20% at discharge,  $p=0.001$ ; ESR from 64% to 56% ,  $p>0.05$ )
- ✓ Analysis of titer changes from admission CRP values also revealed a significant reduction in titer after treatment as evidenced by the CRP titer values at discharge ( $p = 0.009$ )
- ✓ Among the four parameters studied (PLT, MPV, ESR, CRP):
  - the correlation between ESR and CRP was significant (both at admission  $r=0.57$ , and discharge,  $r=0.58$ ,  $p=0.0001$ )
  - There was a negative correlation between PLT and MPV at the time of admission ( $r=-0.371$ ,  $p=0.001$ ); no statistically significant correlation at discharge ( $r= 0.017$ ,  $p=0.863$ ).
  - No statistically significant correlation between the platelet markers and ESR/CRP
- ✓ ESR and CRP gave discordant result in 35.94% of patients in which patients with an elevated ESR have normal CRP vales. Both parameters were elevated in 64.06% of patients (concordant); the discrepancy rate was much higher at discharge.

### 8.2. Recommendations

This study generates some useful information on the importance of platelet count and mean platelet volume as well as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in

the diagnosis process of infectious and inflammatory diseases in the context of Ethiopia. The following recommendation is forwarded based on the findings.

- Since platelet count is part of the CBC printout, there is a need to pay attention of their and utilize this parameter, with no additional cost, for non-hematological conditions like inflammation and infectious diseases.
- Routine monitoring of patients with inflammatory and infectious conditions using CRP and ESR needs to be considered.
- Since the possible explanation for some of the inconsistency between the results of this study and previous ones could be the diseases type considered, studies to evaluate the efficiencies of PLT, MPV, ESR and CRP considering single diseases is recommended.

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## 10. Annexes

### 10.1. Data collection format

Date \_\_\_/\_\_\_/\_\_\_

Patient Name \_\_\_\_\_ Code No. \_\_\_\_\_

Hospital No \_\_\_\_\_ Address \_\_\_\_\_ (Tele.) \_\_\_\_\_

Age \_\_\_\_\_ Sex \_\_\_\_\_

Living Area: Urban \_\_\_\_\_ Rural \_\_\_\_\_

Infectious or Inflammatory disease Type

Infectious; please specify \_\_\_\_\_

Inflammatory; please specify \_\_\_\_\_

Treatment History (specify treatment):  \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

### 10.2. Laboratory data

Date of sample collection \_\_\_\_\_ day \_\_\_\_\_ Month \_\_\_\_\_ year

Time of sample collection \_\_\_\_\_ before treatment  after treatment

Total no of sample received \_\_\_\_\_

Results:

a) Completed                       b) Incomplete                       c) Excluded

Action taken for the incomplete data \_\_\_\_\_

Rejected sample

Clotted

Others

\_\_\_\_\_

Unlabeled

Insufficient

Test Results

By principal investigator:

PLT \_\_\_\_\_

MPV \_\_\_\_\_

ESR \_\_\_\_\_

CRP \_\_\_\_\_

Date and signature of laboratory technician \_\_\_\_\_

Comment: \_\_\_\_\_

If you have any question you can ask the following individuals

**Asnake Setu**

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Department of Medical Laboratory Sciences

Cell phone: +251-911-860057

Email: [-setuasnake@gmail.com](mailto:-setuasnake@gmail.com)

### 10.3. Consent form or Information sheet for patient/study subjects (English version)

**Principal Investigator: Asnake Setu**

**Addis Ababa University College of Health Sciences**

**Purpose:** The purpose of this study is to evaluate the changes in platelet count and mean platelet volume (MPV) and the relation between each of those markers with erythrocyte sedimentation rate (ESR) and C - reactive protein (CRP) in infectious and inflammatory disease in Addis Ababa City Administration Health Bauru at Zewditu Memorial Hospital.

**Procedures to be carried on:** you are invited to participate in the study after giving your consent by giving blood samples

**Risks associated with the study:** There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

**Benefits of the study:** There was no financial benefit to you. But the result of the study was used for your clinical care as well as plays a role in the infectious and inflammatory disease managing and control program. There was no compensation for using your blood sample.

**Confidentiality of your information:** The results of the laboratory findings were kept confidential and could only be accessed by the researcher and the responsible physician. There was no personal information to be attached to your data.

**Termination of the study:** We will respect your decision if you later on change your mind.

Your withdrawal of consent will not affect your right to receive medication.

Based on the above information I agree to participate in the research

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name of Data collector \_\_\_\_\_ Signature \_\_\_\_\_

If you have any question you can ask the principal investigator

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#### 10.4. Informed consent form (Amharic version)

##### የተሳታፊዎች መረጃ ቅጽ

##### ጥናቱን የሚያጠናው፣ አስናቀ ሰጡ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

##### የጥናቱ አላማ

የጥናቱ አላማ ህመምተኞች ተላላፊ በሆኑ በሽታዎች በተያዙበት ወቅት የPILT እና የMPV ላቦራቶሪ ምርመራ በማድረግ ከህክምና በፊትና በኋላ ያለውን ልዩነት መለካትና ማወዳደር ሲሆን በተጨማሪም ከESR እና CRP ጋር ያላቸውን ግንኙነት ማነፃፀር አስፈላጊ ሆኖ በመገኘቱ ተጨማሪ ሀሳብ ለመስጠት ነው ፡፡ ጥናቱም በአዲስ አበባ ከተማ መስተዳድር ጤና ቢሮ በዘውዲቱ መታሰቢያ ሆስፒታል ውስጥ ይካሄዳል፡፡

በጥናቱ ወቅት ከእርስዎ የሚጠበቀው በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ የደም ናሙና መስጠት ነው፡፡

##### ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገር ግን ከጥናቱ የሚገኘው ውጤት ለእርስዎ ህክምና ተጨማሪ መረጃ ለማግኘትና ተላላፊ የሆኑ በሽታዎችን ለመቆጣጠር ይጠቅማል፡፡

##### በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት

በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ

ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ በጥናቱ ምክንያት የሚያሳክኑት ተጨማሪ ጊዜም አይኖርም፡፡

##### የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘው በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡

በጥናቱ ላይ ያለመሳተፍ መብት አለዎት።

ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል ። በመጨረሻም የጥናቱ ውጤት ለሚመለከተው አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል።

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ።

እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ።

ፊርማ -----

መረጃውን የሰበሰበው ግለሰብ ስም-----

ፊርማ -----

የዋና ተመራማሪው አድራሻ፤

- አስናቀ ሰጡ ፤ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርት መንት ፤ የጤና ሳይንስ ኮሌጅ ፤ አዲስ አበባ ዩኒቨርሲቲ- አዲስ አበባ፤ ኢትዮጵያ

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## 10.5. SOPs for Different Laboratory Procedures

### 10.5.1. SOPs for Blood Sample Collection

Identify and prepare the patient where it is adult and conscious follow the steps outlined below.

Compare the data with the information on the request form and report any major discrepancy to the responsible person in the area.

- ✓ Introduce yourself to the patient, and ask the patient to state their full name and demographic information
- ✓ The patients should have an order or requisition slip for the test to be performed
- ✓ Check the test order form, all the records must be completed.
- ✓ If the patient has any drug therapies it may affect the test, and the test ordered physician be responsible for advising his/her patient if the requirement to discontinue certain drugs.
- ✓ Ask whether the patient is latex sensitivity if so use non-latex supplies where appropriate
- ✓ Ask whether 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).
- ✓ Discuss the test to be performed and obtain verbal consent. The patient has a right to refuse a test at any time before the sampling, so it is important to ensure that the patient has understood the procedure.

#### **Patient Reassurance**

- Describe procedure to the patient
- Get oral consent from the patient
- Tell the patient it is going to be slightly painful
- Gain patient's confidence
- Refuses - Never force a patient.
- Describe the use of the study to the patients.

#### **Assemble supplies and position patient**

- Inspect all supplies for possible defect and applicable expiration dates.
- For patient safety, draw all specimens with the patient seated comfortably in an appropriate chair or by lying down.

Each specimen must be clearly labeled with the following:

- 2 patient identifiers, usually patient name and hospital or clinic number
- Type of specimen

**Note: labels should always be placed on the specimen bottle, tubes, etc., not on the lid**

Each specimen must be accompanied by a requisition form. The requisition form must include the following:

- 2 patient identifiers, usually patient name and hospital or clinic number
- Patient age, sex and address (hospital ward, clinic, etc.)
- Date and time collected
- Name or initials of collector
- Test requested
- Name of requestor
- Any antibiotics patient may be on
- Any other pertinent medical information

### **Apply Tourniquet**

- Tourniquet is used to increase intravascular pressure, which facilitates vein palpation and filling of the tube (s) or syringe.
- Tourniquet application should not exceed one minute as localized stasis with hemo-concentration and infiltration of blood into tissue can occur.
- If the patient has a skin lesion at the intended tourniquet location, consider an alternative draw site, or apply the tourniquet over the patient's gown (cloth)
- Wrap the tourniquet around the arm 3-4 inches (7.5-10 cm) above the puncture site.
- Ask the patient to form a fist, but avoid vigorous hand exercise.
- Collect blood from median capital (H pattern) and median (M pattern) veins because these veins are typically closer to the surface of the skin, more stationary, less painful upon needle insertion, and less likely to injure nerves if needle placement is not accurate.

### **Put On Gloves**

- The phlebotomist must put gloves on before the veni-puncture is performed with consideration for latex sensitivity as discussed.

### **Cleanse vein-puncture Site**

- Use a gauze pad with 70% isopropyl alcohol solution, or a commercially prepared alcohol pad.
- Cleanse the site with a circular motion from the center to the periphery.
- Allow the area to air dry.
- If the vein-puncture proves difficult and the vein must be touched again to draw blood, the site must be cleansed again.

### **Vein-puncture procedure using needle and syringe**

- Break the seal and look for any defects, check the plunger.
- Prepare the patient by informing him/her that the vein puncture is about to occur.
- With the bevel up, puncture the vein with the needle at angle of insertion of 30 degrees or less.
- Keeping the needle as stable as possible in the vein, slowly withdraw the desired amount of blood and ask the patient to open his arm.
- Release the tourniquet as soon as possible, after the blood begins to flow.
- Transfer the blood from the syringe to a venous blood collection tube by piercing the stopper with the needle. Allow the tube to fill without applying pressure to the plunger until flow ceases. This will help to maintain the correct ratio of blood to additive if an additive tube is being used.
- Mix the additive tubes by inversion. Do not shake tubes. Rubber stoppers should not be removed from venous blood collection tubes to transfer blood to multiple tubes.

### **Additional Considerations**

#### **Hemolysis**

To prevent hemolysis; allow the vein-puncture site to air dry after cleansing, never draw blood through a hematoma & make sure the needle is fitted securely on a syringe to avoid frothing.

## **Hematoma**

To prevent hematoma, the phlebotomist should make sure the needle fully penetrates the uppermost wall of the vein, remove the tourniquet before removing the needle, use the major superficial veins, apply small amount of pressure to puncture site.

## **Materials and Supplies required for the collection, storage and shipment of whole blood/Plasma/Serum**

1. 10% bleach (0.5% sodium hypochlorite) in spray bottle disinfectant
2. 70% ethanol in spray bottle or skin disinfectant alcohol wipes Alcohol resistant marker
3. Bench top biohazard waste bag and holder
4. Clean laboratory coats
5. Disposable powder-free, latex or non-latex gloves
6. EDTA ,Sodium citrate or heparin vacationer tube
7. Gauze or cotton wool
8. Lab forms/labels
9. Needle or Vacationer
10. Paper towels/absorbent pads
11. Pastor Pipette
12. Sharp disposal container for used Needle
13. 2 ml sterile 2 ml polypropylene screw-cap tube.

### **10.5.2. SOPs for Platelet and Mean Platelet Volume Analyses (CBC)**

**Principle:** -CELDYN 1800 Hematology analyzer are Electrical impedance method used for determining WBC, RBC, and PLT count. As the cells pass through the aperture of the van Behrens transducer/transducer bath, a change in electrical resistance occurs generating an equivalent voltage pulse. The number of pulses sensed during each cycle corresponds to the number of WBC, RBC and PLT cell counted. The amplitude of each pulse is essentially proportional to the cell volume.

**Primary sample system:**-Whole bloods specimen, collected in K<sub>2</sub>EDTA anticoagulant and Stability 24 hrs. at room temperature.

**Required equipment and reagents – Celldyn 1800 Diluents of 20 lit**

- Celldyn 1800 Detergent of 20 lit
- Celldyn 1800 Lyses of 3.8 or 5 lit
- Celldyn 1800 Enzymatic cleaner
- Celldyn 1800 Calibrator
- Celldyn 1800 tri- level control reagents

**Reagents preparation:** Reagents are commercially prepared.

**Reagents stability and storage:** - Cleaner, calibrator & control are stored at 2 – 8 °C.

Diluents, Detergent and Lyses are stored at room Temperature and all reagents are stable up to expiry date.

**Supplies–**

- Disposable glove
- Dry gauze
- Cotton wool
- 70% Ethanol alcohol or similar antiseptic
- Bleach

**Equipment**

- Celldyn1800 Hematology Analyzer
- Key Board
- Electrical blood mixer
- UPS
- Printer

**Calibration methods**

- ✓ Calibrator
- ✓ Fresh whole blood

## Standard Operational Procedure for hematological tests using cell dyne 1800

- ✓ Polystyrene microspheres(performed by an authorized person Abbott representative only)
  - ❖ Commercial calibrator procedure  
When at least one valid reference value has been entered in the calibrator calibration screen, READY appears in the status box. Then follow the direction given in the package insert.
  - ❖ Whole blood calibration procedure

Perform multiple analysis of each specimen using acceptable reference methodology.

- Calculate the mean reference value for each parameter.

### Frequency

- ✓ When there is reformulation of a vendor's reagent or when switching to a different reagent vendor.
- ✓ When indicated by quality control data
- ✓ Following major maintenance or service
- ✓ When directed by an Abbott communications
- ✓ At least every 6 months.

Calibration must be considered the last step in a troubleshooting sequence. Frequent unnecessary recalibrations can mask an underlying problem with the instrument's performance. Built-in Quality Control programmers on the CELL-DYN 1800 are designed to provide continual monitoring and confirmation of instrument calibration. Because the calibrator material is not available in the country most of the time, the calibration was performed based on the performance of the CELL-DYN 1800 system in these quality control programs.

### Quality control procedures

When to run QC

- After daily start up procedures are completed
- To confirm calibration
- When a reagent lot number has been changed
- After maintenance or component replacement

## **Commercial control**

- Cell-Dyne 1800 control provides 3 level of controls=Low, normal and high.
- If the commercial quality control expiry date is less than 2 months the quality control was monitored by the acceptable range. But if it is greater than two months the LJ was used as monitoring mechanism.

## **Procedural steps for sample run**

1. Prior to running patient specimens, perform DAILY Start –up procedure.
2. When the READY message is displayed on the RUN Screen, the instrument is ready to run specimens. To run patient specimens, proceed as follows:
3. With the cap tightly secured on the specimen tube, mix the sample using automatic mixer or slowly invert the tube 10 to 15 times.
4. Remove the cap from the pre-mixed specimen tube.
5. Place the tube under the aspiration probe and raise the tube so that the end of probe is deeply immersed in the specimen.
6. Press the Touch Plate to activate the run.
7. When the sample has been aspirated from the tube, the probe will move up through the Wash Block. Remove the specimen tube and Recap the tube.
8. After the cycle is completed, run results are displayed on screen and the aspiration probe moves into position to accept a new specimen. The current run data is saved to the data log.
9. Check that all results are record in the request paper, registration book and entered in to LIS or soft copy.
10. If not, go back to Cell Dyne machine and press [data log] -> [transmit data] by selecting each result.

### **10.5.3. SOPs for C - reactive protein/ CRP-Latex Determination of C- reactive protein slide test.**

#### **PRINCIPLE**

CRP-Latex Test is a rapid slide agglutination procedure based on a modification of the latex fixation method developed for the direct detection and semi-quantitation of C-reactive protein (CRP) in serum. The assay is performed by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum. The presence of a visible agglutination indicates an increase of the CRP level above the upper limit of the reference interval in the samples tested.

#### **REAGENT COMPOSITION**

**R- CRP-Latex Reagent** - Suspension of polystyrene latex particles coated with specific anti-human C-reactive protein antibodies in a buffered saline solution. Contains 0.95 g/L of sodium azide.

**CONTROL (positive)** - Human serum with a CRP concentration >6 mg/L. Contains 0.95 g/L of sodium azide.

**CONTROL (negative)**-Animal serum with a maximum concentration of human CRP of 1 mg/L. Contains 0.95 g/L of sodium azide.

**Precautions:** Components of different human origin have been tested and found to be negative for the presence of antibodies anti-HIV 1+2 and anti HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.

**Warning:** The reagents in this kit contain sodium azide. Do not allow contact with skin or mucous membranes.

#### **PACKAGING CONTENTS**

**REF 2410005**, kit 50 tests. 1 vial CRP-Latex Reagent, 1x1 ml Positive control, 1x1 ml Negative control, 3 Test cards and 1x50 disposable stirrers.

**REF 2410010**, kit 100 tests. 2 vials CRP-Latex Reagent, 1x1 ml Positive control, 1x1 ml Negative control, 3 Test cards and 2x50 disposable stirrers.

**STORAGE AND STABILITY** -Store at 2-8°C. Do not freeze. Frozen reagents could change the functionality of the test. Reagent and Controls are stable until the expiry date stated on the label.

**REAGENT PREPARATION** -Reagent and Controls are ready to use.

**SAMPLES** -Use fresh, clear serum collected by centrifuging clotted blood.

After the clear serum has been separated it may be stored at 2-8°C for up to one week or longer periods at -20°C or the sample must be frozen.

If the test cannot be carried out on the same day, the serum may be stored between 2 - 8°C for no longer than 72 hours after collection.

As in all serological tests, hemolytic or contaminated serum must not be used. **Do not use plasma!**

**MATERIAL REQUIRED** - Automatic pipettes.

- Saline solution (0.9% NaCl, only for semi-quantitation procedure).
- Mechanical rotator, adjustable at 100 r.p.m.
- Laboratory alarm clock.

## **PROCEDURE**

### **A. Qualitative Test**

1. Bring the test reagents and samples to room temperature (Note 1).
2. Suspend the Reagent vial gently. Aspirate dropper several times to obtain a thorough mixing.
3. Place 1 drop (50 µL) of the serum under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control serum into two additional circles.
4. Add 1 drop of CRP-Latex Reagent to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide means of a mechanical rotator (100 r.p.m.) for a period of 2 minutes (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination

**Reading**

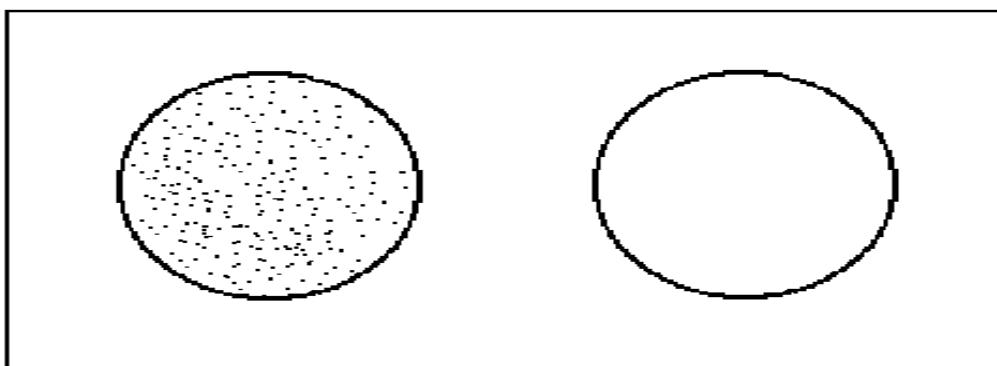
**Nonreactive:** Smooth suspension with no visible agglutination, as shown by negative control (Note 3).

**Reactive:** Any degree of agglutination visible macroscopically (Note 4).

**EVALUATION OF RESULTS**

**POSITIVE-** A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the CRP Negative Control.

**NEGATIVE-** A negative reaction is indicated by a uniform milky suspension with noagglutination as observed with the CRP Negative Control.



Positive

Negative

**Semi-quantitative test evaluation**

A positive reaction is indicated by any observable agglutination in the reaction mixture. Record the last dilution showing a positive reaction. The titer of the serum is the reciprocal of the highest dilution which exhibits a positive reaction. For example, if the last positive reaction is found in the 1:8 dilutions, the titer of the sample is 8.

**Test validity**

CRP Positive and Negative Control should be included in each test batch.

Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the CRP Negative Control and agglutination with large aggregates is observed with the CRP Positive Control.

#### **A. Semi-quantitative Test**

1. For each specimen to be tested place with an automatic pipette 50  $\mu$ L of 0.9% saline solution into each of the circles of a card. Do not spread diluents.
2. To circle one add 50  $\mu$ L of specimen to the saline solution and, using the same tip, mix the saline solution with the sample by repeated aspiration and expulsion of the fluid and transfer 50  $\mu$ L of the mixture to the saline solution in the second circle.
3. Continue with the 2-fold serial dilutions in a similar manner up to the sixth circle, and discard 50  $\mu$ L from this circle. Final sample dilutions will be: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64.(Test each dilution as described in steps 4-7 for the Qualitative)
4. Add 1 drop of CRP-Latex Reagent to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide means of a mechanical rotator (100 r.p.m.) for a period of 2minutes (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination.

#### ***Reading***

Same as in Qualitative Test. The titer of the specimen is reported as the highest dilution that shows reactivity. The next higher dilution should be negative. If the highest dilution tested is reactive repeat the test starting with a preliminary 1:16 dilution. Use a 1:50 dilution of negative control serum in 0.9% saline solution to the replace the 0.9% saline solution in the new 2-fold dilution series. The approximate CRP level (mg/L) present in the sample may be obtained multiplying the titer of the last positive dilution by the minimum detectable unit (analytical sensitivity).

#### **NOTES**

1. The sensitivity of the test may be reduced at low temperatures. The best results are achieved at 15-25°C.
2. Delays in reading the results may result in over-estimation of the CRP concentration.
3. When CRP contents of the serum is in excess, prozoning effect may result in false negative reactions with undiluted serum. The test may be repeated using 10  $\mu$ L of sample. In case of positivity, use the titration procedure above.

4. The strength of the agglutination reaction is not indicative of the CRP concentration in the samples tested.

### **QUALITY CONTROL**

Positive and negative controls should be run daily following the steps outlined in the Qualitative test, in order to check the optimal reactivity of the reagent. The positive control should produce clear agglutination. If the expected result is not obtained, do not use the kit.

### **EXPECTED VALUES**

While the C-reactive protein concentration is generally below 6 mg/L in the sera of healthy adults, in a number of disease states these values often exceeded within 4 to 8 hours after an acute event and reach levels up to 500 mg/L. Since an elevated CRP level is always associated with pathological changes, determination of CRP is of great value in diagnosis, treatment and monitoring of inflammatory conditions.

### **CLINICAL SIGNIFICANCE**

C-reactive protein is an acute phase protein present in normal serum, which increases significantly after most forms of tissue injuries, bacterial and virus infections, inflammation, and malignant neoplasia. CRP contributes to non-specific defense by complement activation and accelerating phagocytosis. CRP testing has a high diagnostic value on a tentative diagnosis made on the basis of case history and clinical findings.

### **LIMITATIONS OF PROCEDURE**

The presence of rheumatoid factors (RF) in a serum sample may cause false positive reactions. Weak or negative reactions may occur with marked antigen excess (prozone effect).

## **10.5.4. SOPs for Erythrocyte sedimentation rate**

### **PRINCIPLE:**

When anticoagulated blood is allowed to stand undisturbed, the red blood cells will normally settle out to the bottom of the tube. This principle is the basis for the erythrocyte sedimentation rate (ESR). By definition, the ESR is the distance in millimeters that the red cells fall (in millimeters) per unit of time, which is usually 1 hour. Various factors will affect the ESR, such as the size and shape of the red cells, plasma fibrinogen, and globulin levels, as well as mechanical and technical factors.

The ESR is directly proportional to the red cell mass and inversely proportional to the plasma viscosity. In normal whole blood red blood cells do not form rouleaux; the red cell mass is small and therefore the ESR is decreased (cells settle out slowly). In abnormal conditions when red cells can form rouleaux, the red cells mass is greater, thus increasing the ESR (cells settle out faster).

Historically, there have been two methods for the erythrocyte sedimentation rate - the Wintrobe method and the Westergren method. Several newer methods, including an automated method and modifications have been developed in recent years but have not been well-accepted. One newer modification of the ESR is capable of using smaller sample sizes; however, it has not been accepted as a standard procedure yet.

**Specimen:** Either venous blood collected directly into sodium citrate and tested within 2 hours, or EDTA anticoagulated blood diluted in sodium citrate can be used. If EDTA blood is used and kept refrigerated at 4–8dC, citrate dilution of the blood and testing can be delayed for up to 6 hours.

### **Equipment**

- Westergren ESR pipette
- Westergren ESR stands with leveling device
- Timer capable of timing accurately 1 hour
- Reusable ESR sucking plastic valve

**Reagent-** *Tri* -Sodium citrate, 32 g/l (3.2% W/v) anticoagulant

**Quality control** - The most practical way of controlling ESR tests is to follow the test method exactly.

### **TEST PROCEDURE**

1. Label the vial with a laboratory system label for proper identification of the sample.
2. Pipette 0.4 ml of sodium citrate anticoagulant into a small container.
3. Add 1.6 ml of venous blood or EDTA anticoagulated blood and mix well. Remove the cap of the container and place the sample in the ESR stand (make a note of the number in the patient's notes). Insert a Westergren pipette and ensure it is positioned vertically.
4. Using a safe suction method, draw the blood to the 0 mark of the Westergren pipette, avoiding air bubbles.

5. Check that the ESR stand is level by making sure that the bubble in the spirit level is central. If required, adjust the screws on the bottom of the stand. Re-check that the pipette is vertical.
6. Set the timer for 1 hour. Ensure the ESR stand and pipette will not be exposed to direct sunlight.
7. After exactly 1 hour, read the level at which the plasma meets the red cells in mm.
8. After reading the ESR, return the blood to its container, remove carefully the pipette and soak it in sodium hypochlorite (0.25%) disinfectant. Dispose of the blood safely and decontaminate the container before washing it.

. Normal Values: - Male: 0-15 mm/hour

- Female: 0-20 mm/hour

## 11. Declaration

I the undersigned agreed and accepted all responsibilities for the scientific and ethical conduct of the research project. I have provided timely progress report to my advisors and seek the necessary advice and approval from my advisors in the course of the research. I have communicated to my advisors and all stakeholders involved in the study including any source of funding for this research. I declare that, this thesis is my own original work.

Name of the candidate: Asnake Setu (BSc)

Signature \_\_\_\_\_

Place: Addis Ababa University School of Medical Laboratory Sciences, Ethiopia

Date of submission \_\_\_\_ / \_\_\_\_ / \_\_\_\_

This thesis has been submitted with our approval as university advisor.

Name of advisor: Aster Tsegaye (MSc, PhD, Associate Professor)

Signature \_\_\_\_\_

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission \_\_\_\_ / \_\_\_\_ / \_\_\_\_

Name of advisor: Binyam Teye (MPH, PhD, Assistant Professor)

Signature \_\_\_\_\_

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission \_\_\_\_ / \_\_\_\_ / \_\_\_\_

Name of advisor: Mistire Wolde (MSc, PhD, Assistant Professor ,)

Signature \_\_\_\_\_

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission \_\_\_\_ / \_\_\_\_ / \_\_\_\_