ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Role of Selected Hematologic Parameters and C-Reactive Protein as predictors of sepsis at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

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A research Thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Hematology and Immunohematology)

May, 2023

Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences

This is to certify that the thesis prepared by Nardos Gosaye, entitled:
Role of Some Hematologic Parameters and CRP as predictors of sepsis at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Acknowledgement

First of all, I would like to thank my Almighty GOD who helps and support me in all aspects of my life, then my advisors Prof. Aster Tsegaye, Elias Bisrat, Dr. Ananya Abate and Rahel Alemu and my collaborator Wondimu W/Yohanes for their great advice and support. Addis Ababa University, College of Health Sciences, Medical Laboratory Science Department, Tikur Anbesa Hospital, staffs of; laboratory, ICU and emergency are gratefully acknowledged for their support. Also, I would like to thank the study participants for their kind collaboration, finally I thank you so much my family and friends specially Muluken Gosaye.

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Abbreviations

ARF	Acute renal failure
CBC	Complete blood count
CRP	C-reactive protein
AER	Adult Emergency
I: T	Immature to total neutrophil ratio
ICU	Intensive care unit
NLR	Neutrophil to Lymphocyte ratio
NPV	Negative Predictive Value
PLT	Platelet
PPV	Positive Predictive Value
ROC	Receiver operating curve characteristic
SBP	Systolic blood pressure
SOFA	Sequential organ failure assessment
TLC	Total leukocyte count
WBC	White blood cell
WHO	World Health Organization

Abstract

Background: Sepsis resulted when our body reacts to infection in exaggerated manner. If left untreated, multiple organs can fail, septic shock, and even death can occur. Blood culture facility is highly limited in resource constrained settings like Ethiopia and hence searching for additional simple parameters is essential.

Objective: To determine the role of some hematological parameters and C-reactive protein as a predictor of sepsis at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Methods: In this cross-sectional study, 152 patients with sepsis suspecting were included from Adult ICU and Emergency of Tikur Anbessa Hospital from April to November 2021. Absolute neutrophil count (ANC), platelet count, and total WBC count were measured using Beckman coulter DXH800 hematology analyzer. For calculating the ratio of immature to total neutrophils, peripheral smear was done and neutrophil to lymphocyte ratio was derived from total neutrophil and lymphocyte count. Blood culture was performed in the microbiology department to confirm bacterial infection and CRP was determined semi quantitatively. SPSS version 26 was used for data entry and analysis. ROC curve analysis and descriptive statistics were used.

Result: Of a total of 152 sepsis suspected cases, 61were culture positive (40.1%). The area under the curve gives the probability that lower value of Absolute neutrophil count [AUC=0.636 (0.546-0.726) was able to identify sepsis with optimal cutoff value of $\leq 9.45 \times 10^9$/L having 65.6% sensitivity, 53.8% specificity, 48.8% PPV and 70.0% NPV. NLR was able to identify sepsis with [AUC=0.611(0.518-0.704) and cutoff value of ≤7.41; and CRP [AUC=0.468 (0.373-0.563) with optimal cutoff value of ≥ 14 mg/dl. The respective sensitivity for WBC, platelet, NLR, CRP, and Immature to total neutrophil ratio were: 62.3%, 60.7%, 60.7%, 52.5%, 54.1% while specificity was 51.6, 58.2, 59.3, 44.0, and 48.4% respectively.

Conclusion: Monitoring of changes in hematological parameters (ANC, Platelets count, WBC and NLR) and CRP, and combining with other clinical indicators can be useful in early prediction of sepsis i.e., before the appearance of blood culture results.

Keywords: Hematological parameters, WBC, platelet, NLR, I:T ratio, ICU, CRP, Culture, Sepsis, Ethiopia

1. Introduction

1.1 Background

Sepsis is a dysregulated immune response to infection that is a serious concern for global public health and is primarily linked to acute organ dysfunctions [1,2]. Despite the fact that there are very few sepsis treatments available, it is one of the leading causes of mortality in intensive care units (ICUs) worldwide [3,4].

According to the WHO 2020 report, there were 49 million cases and 11 million deaths related to sepsis worldwide in 2017. This represented nearly 20% of all deaths worldwide, and out of every five fatalities, one was caused by sepsis or a case related to it [5]. When a patient has sepsis, their immune system is downregulated as a result of the exaggerated (hyper-inflammatory) response. Both the onset of sepsis and the activation and regulation of subsequent antigen-specific adaptive immune responses depend heavily on innate immunity. Immune dysfunction that has already occurred seems to be a significant risk factor. For instance, immunosuppressed patients and elderly patients (who typically have some degree of immunodeficiency) have a higher incidence of sepsis [6, 7].

Finding evidence to support a decision is extremely challenging because there isn't a single gold standard marker for this disease [8]. Sepsis can only be treated effectively and promptly with an accurate and timely diagnosis [9]. Early sepsis treatment after diagnosis can lower sepsis-related morbidity and mortality, but many sepsis patients do not receive this potentially life-saving treatment early [10]. Culturing blood sample to identify the microorganism is the gold standard method for diagnosis. The low growth is caused by a number of factors, including the sample's low inoculum, the lab's inability to isolate all the organisms, and prior antibiotic use. Additionally, getting the laboratory results takes longer time than expected. After the blood sample had been incubated for 48 hours, the earliest result was possible. For the clinician to begin any beneficial treatment during this time frame may be too late [11,12].

Clinical characteristics and/or a positive septic screen should be taken into consideration before starting antibiotics. However, when the blood culture test becomes positive, it determines how long antibiotic therapy will last in area where culture facility is available [13].

Making an early diagnosis of sepsis is crucial because prompt initiation of antibiotic therapy improves outcome. In an effort to achieve this, the empiric use of antibiotics has grown, fostering the emergence of bacterial resistance [14]. The Surviving Sepsis Campaign, in contrast, advises that antibiotics be given within an hour of the onset of septic shock. The mortality rate for septic shock has been shown to increase by 6 to 7.6% for every hour that antibiotic administration is postponed [9,11]. Total Leukocyte Count (TLC), Immature to Total Neutrophil Ratio (I: T Ratio), C-Reactive Protein (CRP), and platelet count are sepsis screen parameters [15]. The CRP level can be used as a marker to determine how long an antibiotic course should last. CRP level, in contrast to blood culture, is unaffected by prior antibiotic medication, making it potentially beneficial in Sub-Saharan Africa [16]. Thrombocytopenia may be a sign of pathologic activation of coagulation, which adds to the risk of multiple organ failure. The intensive care unit (ICU) mortality can be predicted by it independently. Death can be predicted by a 30% or greater decline in platelet numbers [27]. Neutrophilia, thrombocytopenia, and the discharge of immature neutrophils into the bloodstream are frequently symptoms of sepsis [13].

The diagnosis of sepsis can be made with excellent accuracy using Thrombocytopenia, lymphocytopenia and also increased band cells counts [28]. In this study, those selected hematological parameters like WBC, absolute neutrophil, platelet, and serological test CRP, morphologically calculating I: T (immature to total neutrophil ratio) and neutrophil to lymphocyte ratio were examined as a predictive of sepsis.

1.2 Statement of the Problem

In 2018, there were notable regional differences in sepsis incidence and mortality; globally, low- and middle-income countries accounted for 85.0% of sepsis cases and sepsis-related fatalities. According to estimates, there are more than 19 million cases of sepsis (formerly known as severe sepsis) per year, and 5 million people die from sepsis-related causes [29,30]. Over 2 million estimated fatalities per year in Africa are attributed to sepsis, which is the most significant preventable cause of death in the continent. Therefore, the decision as to which definitions to use for clinical diagnosis for the continent is urgent [31].

Laboratory investigations that include hematological markers as a sepsis prediction are urgently needed, otherwise increases mortality and morbidity especially for immuno-compromised patients and elderly are at increased risk in developing countries as the population ages increase. But there is no specific rapid diagnosis and treatment. Microbiology facilities in developing countries like Ethiopia are not readily available and most antibiotic treatment is based on syndromic management, to assess the severity and course of sepsis as well as the difficulties in managing, such studies are necessary [6,32].

In Addis Ababa, Ethiopia, there were 26.5 cases of sepsis and septic shock in ICU for every 100 patients. The average length of stay in an intensive care unit was five days, with a mortality rate of 41.8% and 50.9% at 28 days, respectively. On day 1 of ICU admission, the Modified Sequential Organ Failure Assessment score was less than 10 [32]. The prevalence of sepsis in adult at the emergency department of Tikur Anbesa hospital was 1.35% and due to the complication, the prevalence of sepsis mortality rate was 56.7% [8].

Even though blood culture is the method of choice for diagnosing sepsis, it needs at least 2-3 days in area where it is available, which does not allow getting results early and increases the mortality rate. The mortality rate for septic shock has been observed to increase by 6% to 7.6% for every hour that antibiotic therapy is postponed [9,11]. So, the selected hematological parameters are very important and easy to perform; because they are demonstrated to be rapid, simple and easily affordable screening tool for better identifying patients and treat them early and effectively especially in areas where culture is not possible and results are delaying.

Other infectious diseases are prioritized over sepsis in Ethiopian Health Sector Development Program. The risk group and standard diagnosis of sepsis in Ethiopia is poorly understood and has not been given enough attention [8]. Consequently, this research will be beneficial to identify additional simple tests in the absence of rapid culture facilities and fill the gaps.

1.3 Significance of the Study

In this study a subset of hematological parameters including neutrophil, WBC and platelet counts, neutrophil to lymphocyte ratio (NLR), serological test CRP and immature to total neutrophil ratio were identified to play a role to predict sepsis within a few hours. In the absence of culture facility and when available in case of culture result delayance, if the identified hematological and CRP test result is indicative of absence of sepsis, the patient could leave the hospital sooner than expected, stop antibiotic early, and shortening hospital stays can reduce costs associated with diagnosis, treatment, and family stress as well as hospital acquired infections. In opposite, if the tests result shows the presence of sepsis, the patient can start antibiotics and also the physicians can know early their patient status as a result of this will help them for decision in the patient management practice. The results can also serve as baseline data for future research and might be an input to revise sepsis management guideline in our country.

2. Literature Review

Blood culture is the mainstay of identifying infectious agents causing sepsis. However, in resource limited settings access to culture facility and delayance of results availability in facilities where it is available necessitates to look for alternative parameters including hematological parameters to clinically manage patients [9, 11]. As a result, several studies in these settings tried to identify different alternative laboratory markers.

Most of the available studies are not on adult rather carried out in neonates and children. For example, research done in Iraq with cross sectional study design, sets of tests were done. These include semi-quantitative latex agglutination test of C-reactive protein (CRP), White Blood Cells Count (WBC), Absolute Neutrophil Count (ANC), Platelets count (thrombocytopenia), and Immature to Total Neutrophil Ratio (I/T ratio), were used to evaluate a total of 100 neonates with clinical features of sepsis (Group A) and 100 normal asymptomatic neonates (Group B). CRP had 93% specificity; it was positive in 82.4% of the cases and 81.8% of asymptomatic neonates. ANC ranked second most sensitive parameter; with 61.8% sensitivity and 86% specificity. Sensitivity values were 55.9%, 29.4%, 17.6%, and 26.5% for platelet count, WBC, I/T ratio, and ESR, respectively, while specificity values were 91.0%, 89.0%, 92.0%, and 81.0%, respectively. This finding is as opposed to group-B, which had specificities of 91.0%, 89.0%, 92.0%, and 81.0% and sensitivities of 42.0%, 33.3%, 15.2%, and 22.7%, respectively [14]

In India a cross sectional study was performed with a sum of 238 clinically suspected cases of early onset neonatal sepsis. Blood cultures and numerous sepsis screening tests, including those for CRP, micro-erythrocyte sedimentation rate, total WBC, immature to total neutrophil ratio, and thrombocyte count, were performed on the patients. All the parameters' sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and P value were computed. 90 cases (37.8%) had positive blood cultures, with *Klebsiella pneumoniae* (45.71%) being the most common organism. The most helpful tests for sepsis included positive CRP and Micro ESR, with specificities of 91.89% and 90.54%, respectively; however, none of them had an adequate sensitivity. The CRP, micro-ESR, and I: T ratio's negative predictive values vary from 90.07% to 81.99%. Hematological markers were found to have changed in a way that was statistically significant in relation to sepsis (P value 0.05) [15].

Another study conducted in India from 2013 to 2015 with a cross sectional study design enrolling 191clinical suspected cases. The CBC was performed along with CRP, ESR, direct bilirubin, and differential leukocyte count on a field-stained peripheral smear, absolute neutrophil, and immature to total neutrophil ratio. Of the patients, 91 of the 191 investigated cases tested positive for cultures. The best sensitivity was demonstrated by CRP (84.6%), while the highest specificity was demonstrated by absolute neutrophil count (99.0%), direct bilirubin (93.0%), and adjusted total leukocyte count (93.0%). CRP (84.8%) and the absolute neutrophil count (97.5%) had the highest positive predictive values [17].

More evidence on the use of selected hematological tests was reported from Pakistan. In their study published in 2010, Shirazi *et al* recruited 138 suspected cases in their cross sectional study of whom 48 were confirmed septic cases using blood cultures. Their finding showed the selected hematological tests had below 60% sensitivity. Nonetheless, the specificities were >70%. When they analyze their data by combining parameters, they find it to be a good marker to rule out the neonatal sepsis. Of the 48 culture confirmed septic patients, 11/48(23%) had positive CRP. On the other hand, 14/90(16%) of the probable sepsis cases had positive CRP. Low platelet count showed 61% sensitivity and 82% specificity [18].

In Pakistan, a cross-sectional study that was undertaken in 2017 tested fifty individuals for sepsis. To get a quick diagnosis of newborn sepsis, five tests were used: Total Leukocyte Count (TLC), Absolute Neutrophil Count, I/T. ratio, Platelet count, and CRP. The sensitivity and specificity of CRP and absolute neutrophil count were around 60% and 50%, respectively. White blood cell count was 93% specific but only 14% sensitive [12].

Another cross-sectional study including 120 newborns divided into three groups was carried out in Riyadh from March to September 2019. Depending on the Tollner score, newborns were categorized as being proven to have neonatal sepsis, suspected cases, and healthy group. Each infant underwent a blood culture and hematologic profiling. Platelet count/mm3 was lower in newborns with proven sepsis (458914± 110305) and higher in neonates with suspected sepsis (425,891 ±141,258) compared to the control group (325,148± 810,250). Leukocyte count/mm3 was similarly greater for newborns with proven sepsis (18,912±9,541) and for neonates with suspected sepsis (10,912±2,451) than for the control group (6,417±213). All patients with proven

sepsis that could be shown had positive blood cultures. The sensitivity and specificity of CRP were, respectively, 72% and 61% [19].

In a cross-sectional study conducted in India in 2014, 110 neonates in total were enrolled. Acute phase reactant creative protein (CRP), which has the benefit of having low serum levels in healthy newborns, a quick rise after 12 to 24 hours of sepsis, a large spike thereafter for as long as inflammatory stimuli persist, and an immediate reduction in serum level once inflammation subsides, is also advantageous. The ratio of immature to total neutrophils (I: T) among hematological markers provides an appreciable prognostic value for early identification of newborn septicemia. Their study aims to advance current understanding of early newborn sepsis diagnosis in order to improve patient care for this population [20].

A cross-sectional investigation of 150 patients was undertaken in Nepal in 2017; 72 of those cases were positive for culture. The maximum sensitivity was seen in CRP (77.8%) and immature:total neutrophil ratio (73%) ratios. The highest specificity was seen in CRP (66.7%), I/T ratio (61.5%), and micro ESR (60.2%). CRP (68.2%) had the best positive predictive value, followed by I/T ratio (63.8%) and adjusted total leukocyte count (56.2%) [21].

A total of 145 ICU patients with sepsis and 143 patients without sepsis served as the control group in a 2013 study done in Turkey. About 49 (33.8%) individuals with sepsis and 4 (2.8%) patients in the control group both had thrombocytopenia (p=0.05). 88 (60.7%) of the 145 patients had a diagnosis of severe sepsis. Thirteen (22.8%) patients with non-severe sepsis and 36 (40.9%) patients with severe sepsis both showed thrombocytopenia (p=0.01); although there was no statistically significant difference between individuals with sepsis and those with severe sepsis in terms of demographics [25].

In Nigeria, retrospective research done which included 420 neonates found that 196 (46.7%) of them had elevated CRP and 181 (43.1%) had positive blood cultures. The sensitivity, specificity, positive and negative predictive values of CRP were 74.0%, 74.1%, 68.4% and 79.0%, respectively [16].

A study was carried out in Egypt by *El-Shafie et al* in 2017 with a total of 31 patients admitted to El-Sahel Teaching Hospital (median age 60 years, 16 males). The investigators studied 18 patients

with sepsis and 13 patients who had non-infectious systemic inflammatory response syndrome (SIRS). The measured serum CRP levels when the sepsis patients were admitted was 64 (50–73.25) mg/dL. Whereas in the SIRS category, the CRP value was low, 55 (45–65) mg/dL, but it did not reach to statistically significant level (P = 0.2) [33].

In a study conducted in Sudan, in 2013 recruited 70 babies admitted for sepsis, there were 27 females and 43 males. Of all the kids, 39 (58.6%) had suspected sepsis (negative blood culture + indicators of sepsis), while 29 (41.4%) were verified sepsis cases (positive blood culture plus symptoms of sepsis). Only 6 infants had suspected sepsis, while 17 infants with verified sepsis tested positive for CRP. Blood culture and CRP values were significantly correlated (P=0.001). At a cut-off value of 6 mg/L, CRP showed sensitivity, specificity, PPV, and NPV of 63%, 85.36%, 73.9%, and 73.9%, respectively [34].

From July 2016 to May 2018, five hospitals in Ethiopia, including Tikur Anbessa Specialized Hospital, Gandhi Memorial Hospital, St. Paul Hospital, Millennium Medical College, Jimma University Medical Center, and Gondar University Hospital were included in a cross-sectional study. CBC and other tests were conducted on preterm infants who were less than 7 days old. From the 4919 preterms, 3852(78.3%) of them were hospitalized in critical care unit. CBC was analyzed for 68.3% of them. Their Hb, WBC, and thrombocyte counts had respective mean values of 17.9mg/dL, 12,685 cells/cumm, and 159,340 cells/cumm. For 8.8%, 9.0%, and 11.1% of infants with Early-onset Neonatal Sepsis (EONS), asphyxia, and Respiratory distress syndrome (RDS), respectively, the WBC count was less than 5000cells/cumm. The thrombocyte count was less than 50,000 cells/cumm in 16.8% EONS, 17.7% asphyxia, and 19.8% RDS patients [22].

Another study conducted in Gonder, Ethiopia 2012, with cross sectional design recruited 181 suspected patients in the study. Of them, 89 (54.7%) were male. The neonates were 4.1 days old on average, with a 5.4 days standard deviation. With a standard deviation of 4 days, the average hospital stay was 5.6 days long. Based on clinical parameters, 148 (81.8%) had early while 33 (18.2%) had late onset neonatal sepsis [23].

The study from Shashemene which was carried out in 2017 studied 244 patients from two government hospitals' neonatal intensive care units. The study's overall prevalence of newborn

sepsis was 77.9%. From this, 65% experienced early and 35%late onset neonatal sepsis [24]. This indicated how sepsis is a critical issue in neonatal ICUs.

The study from Asella, Ethiopia in 2018 involved 303 newborns who had clinical sepsis. In the study's participants, 136 (45%) reported having positive CRP and 99 (32.7%) had abnormal WBC; 88 of the study patients (29.4%) had positive blood cultures. The sensitivity, specificity, PPV, and NPV of the WBC count were 59.5%, 79.6%, 52%, and 64.5%, respectively, while that of the CRP were 65.6%, 78%, 42%, and 91%. The sensitivity, specificity, PPV, and NPV increase to 78.5 %, 83 %, 60 %, and 93 %, respectively, when WBC and CRP are combined. While elevated WBC counts were more frequently observed in gram-positive sepsis than gram-negative (OR 4.8, (95% CI 1.45-15.87, P 0.01)), CRP positivity rates were equivalent in both groups [26].

As reviewed in the studies above different performance rates for selected hematological parameters and CRP is shown. Most studies available are on neonates and the prediction potential of the suggested markers in adults is not well investigated, a gap which this study is attempting to discourse. Providing more evidence from different settings will help to strengthen the body of literature in the prediction potential of the suggested simple parameters.

3. Objectives

3.1 General Objective

To investigate the utility of some hematologic parameters and CRP as predictor of sepsis case among Adult ICU and Emergency patients at Tikur Anbessa Hospital, 2021.

3.2 Specific Objectives

- To determine the diagnostic predictive value of WBC, ANC, PLT, I: T neutrophil ratio and NLR in sepsis
- To investigate the diagnostic predictive potential of CRP in sepsis
- To compare some hematological parameters between blood culture positive and negative patients

4. Materials and Methods

4.1 Study area

The study area was Tikur Anbessa Specialized Hospital located in Addis Ababa, the capital city of Ethiopia. It is the largest specialized hospital in Ethiopia, and serves as a training center for undergraduate and postgraduate health science students of the College of Health Sciences of Addis Ababa University. The hospital is staffed with about 929 academic staff, 825 nurses, 70 medical laboratorians, 74 pharmacists, 69 midwives, 39 anesthetists, 14 physiotherapists, 37 radiology technologists, 15 biomedical professionals, 6 environmental health, 500 medical doctors, 15 others and 891 administrative staff. It has different types of clinics in for out-patients like hematology, oncology, dermatology, neurology, surgical, gynecology, orthopedics, diabetics, antenatal, Emergency, GI, chest, staff clinic, etc. The hospital has more than 700 beds and from these 12 beds are in Adult ICU which means in surgical and in medicine site and approximately 25-40 surgical patients and 10-25 Medical patients (in average total 51-65) was admitted per a month in this department. And also, in adult emergency there are 40 beds and 1500 patients admitted per a month.

4.2 Study design and Period

-Across sectional study was conducted from April to November 2021.

4.3 Population

4.3.1 Source population

Patients that visited Adult ICU and ER of Tikur Anbessa Specialized Hospital were the source population.

4.3.2 Study population

Patients with suspected sepsis that visit Tikur Anbessa Specialized Hospital admitted at Adult ICU and Emergency department from April to November 2021 and met the eligibility requirement.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

Volunteering patients at Adult ICU and ER suspected of sepsis during the study period. TASH adult ER and ICU admit patients.

4.4.2 Exclusion criteria

Patients those who have other hematological disorders, malignancy, managing with radiation and chemo therapy and pregnant women.

4.5 Study variables

4.5.1 Dependent variables

WBC, PLT, Neutrophil, I:T ratio, NLR, blood culture and CRP

4.5.2 Independent variables

Socio demographic characteristics, history of admission, site of infection, history of infection, immunocompromised patients

4.6 Measurement and Data collection

4.6.1 Sample size determination

Using a single population proportion formula, the sample size is calculated and the proportion of sepsis among adult patients admitted in Egypt prevalence WBC and PLT is 0.1, there is no study conducted in Ethiopia which is similar with this study title so, we can use Egyptian p value. The sample size was estimated by considering 95% confidence interval (CI) and a 5% margin of error as follows:

Where $n = (Z\ ^{a}/_{2})^{2}\ p\ (1 - p)/\ d^{2}$

n= required sample size Z= the standard normal deviation at 95% confidence interval =1.96

P= proportion of sepsis among adult critically ill patients admitted in Egypt is 0.1 (p=0.1) [33].

d= margin of error that can be tolerated, 5% (0.05)

1-p = proportion of population that do not possess the character of interest (q=0.9) [33].

Therefore, $n = (Z^{a}/2)^2 p (1 - p) /d^2 = (1.96)^2 (0.1) (1-0.1)/ (0.05)^2 = 138$

By considering nonresponse rate 10% (14), the final sample size was 152 sepsis cases. n=152

4.6.2 Sampling method

Convenient sampling techniques was applied.

4.6.3 Data collection procedure

All patients who fulfill the eligibility criteria in adult ICU and emergency department, from April to November 2021 was selected and also picked from patients who were admitted to TASH hospital and developed sepsis after admission. After identifying patients with suspected for sepsis, could give the consent him/her self. If they were unconscious patients, getting from the guardian. Then demographic information and medical history was collected by assigned nurse by using information sheet which is prepared by PI. After eligibility was confirmed, 10ml of venous blood was collected using aseptic technique and transferred 5ml to culture bottle, two ml in to serum separator tube and 3ml into an EDTA tube. The blood sample was transported to the central laboratory for those test analysis.

Unicel DxH 800 (Beckman Coulter USA) automated hematology analyzer was used to analyze WBC, Absolute neutrophil, platelet count and NLR. Wright-stained blood smear was examined by experienced laboratory technologist to calculate immature total neutrophil ratio. Semi-quantitative, membrane-based immunoassay was employed for the detection of CRP and blood culture was performed in the microbiology department.

Principle of automated hematology analyzer DxH 800 Beckman Coulter

The Coulter VCS Technology and Impedance principle; Performance Specifications and Characteristics of the analyzer: Fluorescent Flow-Cytometry for WBC-Diff and DC-Sheath for Flow: RBC, HCT, PLT and Cyanide-free SLS-Method for HGB Diagnostic. There are 27 parameters that the analyzer performs, WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, RDWSD, PLT, MPV, NE, NE#, LY, LY#, MO, MO#, EO, EO#, BA, BA#, NRBC, NRBC#, RET, RET#, MRV, IRF by using 5 different reagents like diluent, cell Lyse, diff Pack, Erythrolytic

reagent and retic pack. After aspirated 165 microliter whole blood two dilution of a blood sample are made.

Those are: -One is delivered to the RBC bath for the RBC count and thrombocyte analysis; the other is delivered to the WBC bath for the leucocyte and Hb estimation. WBC and Platelet coulter principle: WBC Measure directly & multiply by calibration factor, WBC = N x 10^3 cells/ µl and for thrombocyte the number of thrombocytes derived from the platelet histogram multiply by a calibration factor, PLT = N x 10^3 cells/ml and then release in to the computer (LIS) and interpret the result.

Principle of Peripheral Blood Morphology

A thin smear was prepared from EDTA blood samples, air dry and stained by wright stain to calculate immature cells to total neutrophil ratio (I/T ratio). Eosin and methylene blue are the components of the Wright's stain which is a polychromatic stain. Multiple colors are detected based on the ionic charge of the stain and the various constituents of the cell. The negatively charged eosin ions stain the basic structures giving the cell components an orange to pink color. Whereas, the positively charged methylene blue ions stain the acidic structures giving an appearance of varying shades of blue. The neutral structures of the cell give variable colors by taking up both the acidic and basic components of the stain. Sepsis is suggested to occur if the I/T ratio is 0.2 and above; the opposite is true if it is less than 0.2[37].

Test Principle of Blood Culture

Culturing the blood of patients is the gold standard method for diagnosis of sepsis. It is a requirement that all suspects undergo blood culture and sensitivity test before commencing antibiotics treatment. As a result, strict adherence to pre-analytic, analytic and post-analytics steps are vital. Specimens should be collected aseptically. About 5 cm in diameter over the proposed vein-puncture site should be cleansed thoroughly with 70% isopropyl alcohol, followed by povidone-iodine in concentric circles moving out ward from the center. Then with the same cleaning strategy the area should be cleaned again by alcohol and air dried for at least 1 minute. Five-mL sample of blood would be adequate for a blood culture bottle containing 5-10 mL of culture media. Aseptically collected blood samples are inoculated into aerobic BACTEC bottle.

Then it is incubated in the BACTEC instrument at 35-37°C for 5 days or until microbial growth is detected. At least 72 hours observation is needed before they are reported as sterile. The improved bacteriological technique (BACTEC) blood culture system allows detection of bacterial growth within 12-24 hours. The advanced methods can detect bacteria at a concentration of 1-2 colony-forming unit (CFU) per milliliter. Positive bottles are removed from the BACTEC blood culture system and inoculated on chocolate agar plates, blood agar plates and MacConkey plate agar. If there is no growth until five days, the test is reported as negative/no growth. After gram stain, biochemical test for gram negative isolates likes indole, urea, TSI, citrate, motility, whereas catalase, slide and tube coagulase for gram positive bacteria should be done. Enterococcus specious are identified by using PYR and bile esculin. Antibiotics susceptibility is done by using Kirby-Bauer disc diffusion technique on Muller Hinton agar based on clinical laboratory standards institute (CLSI) 2020 guidelines.

Principle of CRP serological test: C-reactive protein is a semi-quantitative, membrane-based immunoassay test for the detection of CRP in whole blood, serum or plasma specimens. The membrane is pre-coated with anti-CRP antibodies on the test line region. During testing, specimen reacts with the particles coated with anti-CRP antibodies. The mixture migrates upward on the membrane by capillary action to react with anti-CRP antibodies on the membrane and generate a colored line. If the intensity of the test line (T) is weaker than reference line 2 (R2), it indicates that the CRP level in the specimen is between 1-3 mg/L. If the intensity of the test line (T) is weaker than reference line 1 (R1) but stronger than reference line 2 (R2), it indicates that the CRP level in the specimen is between 3-10 mg/L. If the intensity of the test line (T) is stronger than the reference line (R1), it indicates that the CRP level is above 10 mg/L. To serve as a procedural control, reference line 1 and 2 (R1 and R2) will always appear in reference line region indicating that proper volume of specimen has been added and membrane wicking has occurred. The test device contains anti-CRP antibodies conjugated to colored particles and anti-CRP antibodies coated on the membrane [38].

Principle of clinically diagnosing sepsis

Sepsis is a term used to describe a systemic illness brought on by microbial invasion of normally sterile body regions. It covers a wide spectrum of illnesses, from minor signs and symptoms to

shock and organ dysfunction. The virulence of the pathogen, the portal of entry, the susceptibility and response of the host, and the temporal evolution of the condition all have an impact on the sepsis signs and symptoms. The association of a variety of non-specific inflammatory reactions with proof or a suspicion of a microbial origin is necessary for the clinical diagnosis of sepsis.

This develops into "severe sepsis" when there is also evidence of hypoperfusion or dysfunction in at least one organ system. The term "septic shock" is used when severe sepsis is accompanied by hypotension or the need for vasopressors despite adequate fluid resuscitation. The mortality rate rises with increasing severity, from 25–30% for severe sepsis to 40–70% for septic shock. This terminology includes "septicemia," which encompasses sepsis, severe sepsis, and septic shock.

Some of sepsis associated inflammation are: core temperature >38°c or <36°c, Heart rate >90/min (or >2 SD above normal for age), Tachypnoea altered mental status, significant oedema or positive fluid balance (>20 ml/kg in 24 hours), blood glucose >7.7 mmol/l in absence of diabetes, when adult SBP is greater than 40 mm Hg, cardiac index is greater than 3.5 l/min/m2, arterial hypoxemia, capillary refill is reduced or there is mottling, etc. [35].

Current sepsis definition

Based on sepsis-3- trial currently Sepsis is defined as a potentially fatal organ malfunction brought on by an unbalanced host response to an infection. Organ dysfunction, can be detected by a sudden change of 2 points in the SOFA score overall as a result of the infection [36]

In this study clinical sepsis (suspected/probable) is considered when a treating physician using the above definitions diagnose sepsis and sought for supportive investigation and or culture and document it in the patient chart. This will further be correlated with some hematologic parameters and CRP test to describe the role in diagnosing sepsis.

4.7 Data Quality Assurance

The data collection sheet for data collection was checked for completeness by Advisors and principal investigator. It was cleaned, coded, then entered and analyzed using SPSS version 26. The sample collectors were experienced nurse and principal investigator. The professionals involved in this study was participated in data confidentiality, safety and precautions to follow in collecting, transporting, analyzing the sample.

The pre analytical method quality was ensured by carefully taking the venous blood from the right patient following the guide line of standard operative procedure. The tube will be properly labeled with unique code and transported to the testing site. In the analytical phase, properly Performed daily quality control and properly mixed the sample before running up on the analyzer and make the data double checked to be sure that, the result is reliable and taken directly from printed out put on analyzer for WBC, PLT, and ANC parameters and calculate Neutrophil to Lymphocyte ratio from the CBC. Staining reagents for smear preparation (I: T) and CRP reagents were checked their quality and expire date. The smears were examined under the supervision and checking of Hematology and Immunohematology clinical laboratory science specialist (one of the advisors). In post analytical phase results were recorded and interpreted based on the reference range and handled appropriately and in secured way.

4.8 Data analysis and interpretation

The data was entered and analyzed using SPSS version 26 (SPSS INC, Chicago, IL USA) and presented in tables and figures. Descriptive statistics, chi-square, independent sample t test, ROC curve analysis were used to describe WBC, PLT, neutrophil, NLR and I: T ratio, CRP to relate with blood culture result in the entire study groups where appropriate. From the ROC curve analysis at various cutoff values, sensitivity, specificity, negative and positive predictive values (NPV and PPV) were calculated. Statistics were considered significant for P values less than 0.05.

4.9 Ethical considerations

This study was ethically reviewed and approved by the departmental research and ethics review committee (DRERC) of the department of Medical Laboratory Science, College of Health Science, Addis Ababa University. In addition, official permission from study site was obtained. Written informed consent was obtained from study participants and parents or guardians for those under

18 with assent after explaining the aim of the study. Data confidentiality was maintained by locking hardcopies of results and password protection for electronic files.

4.10 Operational Definitions

Sepsis: Dysregulated host response to infection (inflammation plus evidence of or suspicion of microbial process).

Sever sepsis: Sepsis + organ dysfunction.

Septic shock: sepsis + hypotension despite adequate volume resuscitation.

Receiver operating characteristic (ROC) curves: The diagnostic performance of a test, or the accuracy of a test to separate sepsis cases from non-septic cases.

Leukopenia means WBC <4000, Leukocytosis means WBC>12,000; Neutropenia means Neut<1500 Neutrophilia means >8000, Thrombocytopenia means platelet<100,000, Thrombocytosis means PLT>450,000, high Immature/Total neutrophil ratio means increase the no of released immature neutrophile than the mature one (I/T≥0.245), C-reactive protein as best predictor meansCRP≥14mg/dl.

5. Results

5.1 Socio demographic characteristics and clinical history of study subjects

In this study 152 study participants were enrolled with clinically suspected sepsis; 85 (55.9%) were females. The mean age of study participant was 45.34 ±19.33 years; the majority (30.9%) were above 50 years of age. There is no statistically significant difference in the mean age of sepsis positive and sepsis negative patients (p=0.44). The majority (73%) were urban residents; 44 study participants had completed high school. Among the study participants 63% had a history of hospital admission, 83% had infection at the past and 21.7% of them had wound focus infection. History of infection was associated with culture positive result (p<0.001), whereas history of admission had no significant association with culture positive result (p=0.81) (Table 1).

Table 1: Sociodemographic characteristics and clinical history of sepsis suspected pt in ICU and ER at TASH, Addis Ababa, Ethiopia, 2021 (n=152).

Variables	Category	Frequency (%)
Age	<20	24 (15.8)
	20-29	29 (19.1)
	30-39	23 (15.1)
	40-49	29 (19.1)
	≥50	47 (30.9)
Sex	Female	85 (55.9)
	Male	67 (44.1)
Residence	Urban	111 (73)
	Rural	41 (27)
Level of education	Cannot read & write	19 (12.5)
	Read & write	25 (16.4)
	Primary school	42 (27.6)
	Secondary school	44 (28.9)
	Diploma and above	22 (14.5)
History of Admission	No	55 (36.2)
	Yes	97 (63.8)
History of infection	No	69 (45.4)
	Yes	83 (54.6)
Site of infection	Wound focus	33 (21.7)
	Chest focus	15 (9.9)
	GI focus	18 (11.8)
	UTI focus	17 (11.2)

5.2 Categorical frequency of culture result and types of microorganism

In this study, blood culture revealed pathogenic growth in 61 cases; most common were Coagulase-negative staphylococci (CONS) (12.5%), *E. coli* (7.9%), followed by, Acinetobacter (4.6%) with Klebsiella (3.9%) (Table 2).

Table 2: Blood culture result and the frequency of isolated microorganism in ICU and ER sepsis suspected patients at TASH, Addis Ababa, Ethiopia, 2021.

Parameters	**Category**	**Frequency (%)**
Blood culture	Positive	61 (40.1)
	Negative	91 (59.9)
Causative Organisms	E. coli	12 (7.9)
	Actinobacteria spp	7 (4.6)
	K.pneumonia	6 (3.9)
	S.aureus	2 (1.3)
	Bacillus spp	2 (1.3)
	Enterococci spp	2 (1.3)
	S. lugdunensis	2 (1.3)
	Yeast cell	2 (1.3)
	Enterobacter spp	1 (0.7)
	Pseudomonas spp	1 (0.7)
	Aspergillus spp	1 (0.7)
	Citrobacter spp	1 (0.7)
	CONS	19 (12.5)

5.3 Comparison of WBC, ANC, PLT, NLR, I:T ratio and CRP between culture positive and negative results

The mean± SD value of WBC for culture positive results were 11.76±12.9 x 10^9/Land for culture negative were 16.48±13.51. Independent sample t test showed that there was statistically significant difference in the WBC value (p=0.032), ANC (p=0.002) and NLR (p=0.029) between culture positive and negative results. Comparisons of test variables between culture positive and negative results are summarized in Table 3.

Table 3: Comparison of WBC, ANC, PLT, NLR, I:T ratio and CRP between culture positive and negative results in ICU and ER at TASH, Addis Ababa, Ethiopia, 2021.

Variables	Culture result	Mean	Std. Deviation	P value
White blood cell (x 10^9/L)	Positive	11.763	12.9258	**0.032**
	Negative	16.485	13.5140	
Absolute neutrophil (x 109/L)	Positive	8.436	6.6811	**0.002**
	negative	12.666	10.2529	
Platelet (x 109/L)	positive	213.08	185.418	0.432
	negative	235.04	138.847	
Neutrophil to Lymphocyte ratio	positive	7.5014	6.76575	**0.029**
	negative	10.2928	8.80665	
Immature to Total neutrophil ratio	positive	0.2723	0.14660	0.905
	negative	0.2753	0.15774	
C-reactive protein	positive	12.08	3.938	0.282
	negative	12.74	3.186	

5.4. Sensitivity, specificity, PPV and NPV of WBC, ANC, PLT, NLR, I:T ratio and CRP in predicting sepsis

The Sensitivity, specificity, PPV and NPV of WBC, ANC, PLT, NLR, I:T ratio and CRP in predicting sepsis were determined using ROC analysis and are summarized in Figure 1 to 3, and Tables 4 and 5). The area under the curve gives the probability that patients with sepsis had lower value of WBC, ANC, PLT, NLR. WBC [AUC=0.630 (0.540-0.720)] was able to identify sepsis with optimal cutoff value of ≤11.95x10^9/L having 62.3% sensitivity, 51.6% specificity, 46.3% PPV and 67.1 % NPV. PLT [AUC= 0.588 (0.492-0.684)] was able to identify sepsis with optimal cutoff value of ≤200.5 x10^9/L having sensitivity of 60.7%, specificity of 58.2%, 49.3 % PPV and 68.8 % NPV [Figure 1].

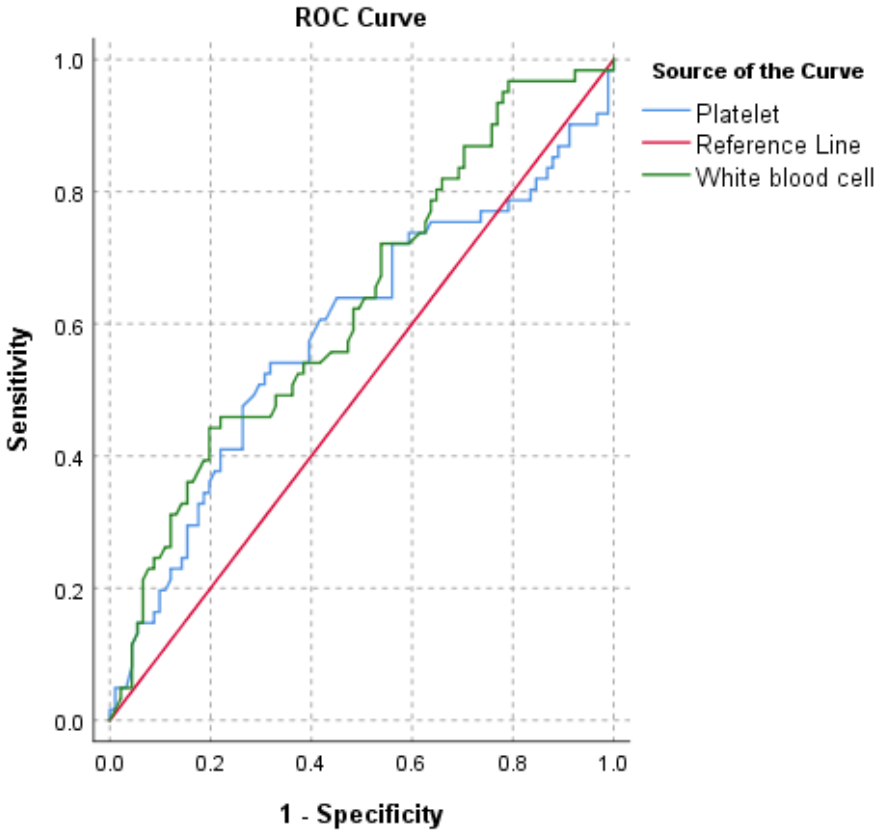

Figure 1: Receiver operating characteristic (ROC) curves of PLT and WBC with blood culture.

The area under the curve gives the probability that patients with sepsis had lower value of ANC [AUC=0.636 (0.546-0.726) was able to identify sepsis with optimal cutoff value of ≤9.45x10^9/L having 65.6% sensitivity, 53.8% specificity, 48.8 % PPV and 70.0% NPV. For NLR [AUC=0.611(0.518-0.704) was able to identify sepsis with optimal cutoff value of ≤7.41 having 60.7 % sensitivity, 59.3% specificity, 50 % PPV and 69.2 % NPV [Figure 2].

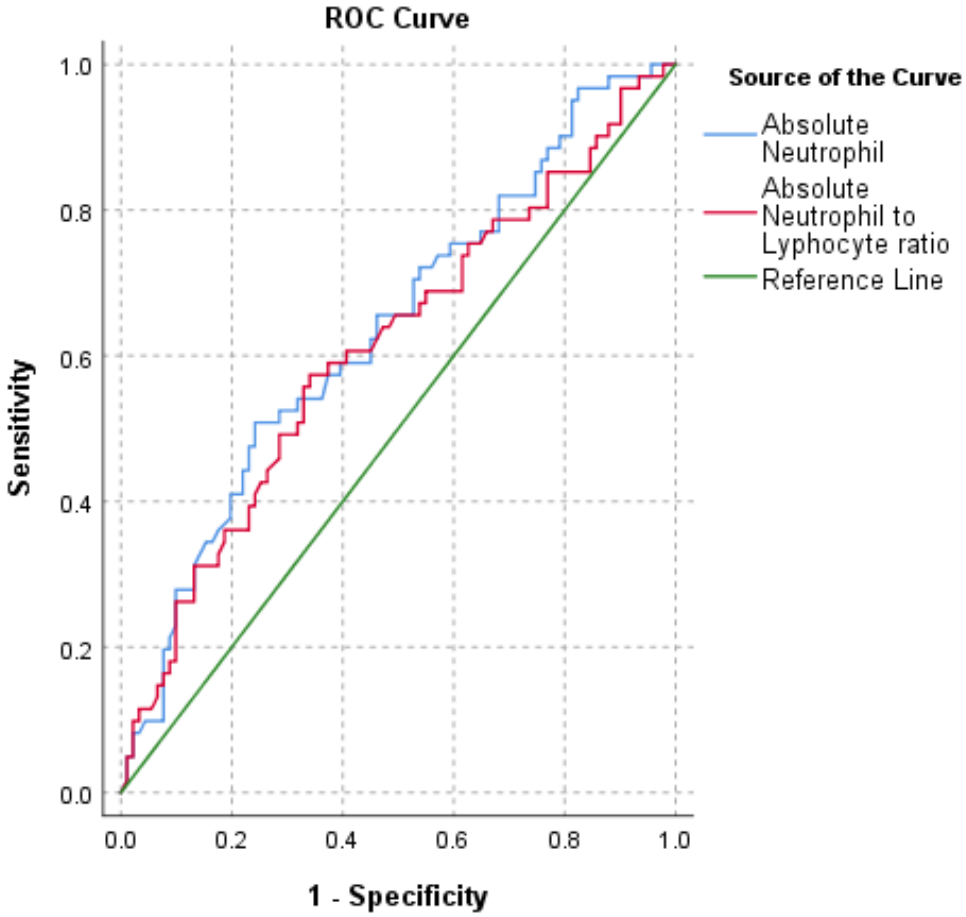

Figure 2: Receiver operating characteristic (ROC) of ANC and NLR with blood culture test result.

The area under the curve gives the probability that patients with sepsis had upper value of CRP [AUC=0.468 (0.373-0.563) was able to identify sepsis with optimal cutoff value of ≥ 14 mg/dl having 52.5% sensitivity, 44% specificity, 38.6% PPV and 58% NPV. I: T neutrophil ratio [AUC=0.501 (0.406-0.597) was able to identify sepsis with optimal cutoff value of ≥ 0.245 having 54.1% sensitivity, 48.4% specificity, 41.3% PPV and 61.1% NPV [Figure 3].

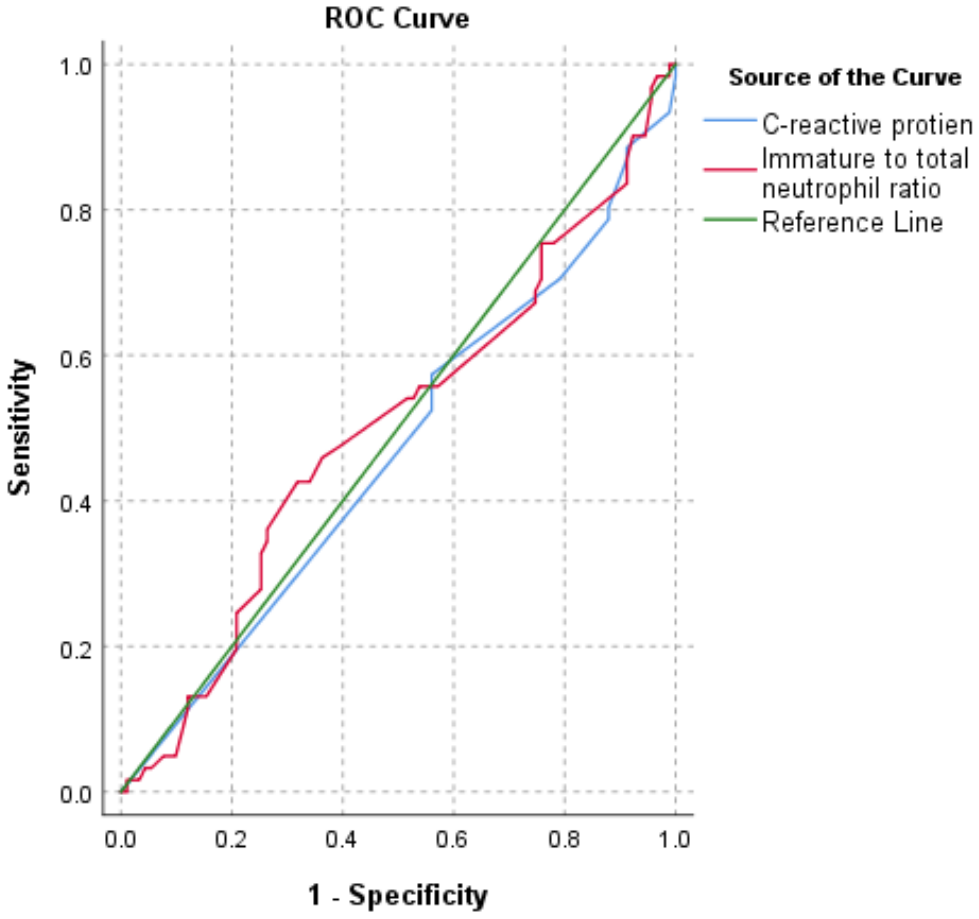

Figure 3: Receiver operating characteristic (ROC) curves of CRP and immature to total neutrophil ratio with blood culture.

Table 4 summarizes the sensitivity, specificity, PPV and NPV values of WBC, ANC, PLT, NLR, CRP and I: T ratio. Whereas Table 5 summarizes the various cutoffs for these parameters with their respective sensitivity and specificity values which helped to determine the optimum cutoffs balancing the sensitivity and specificities.

Table 4: Sensitivity, Specificity, PPV and NPV of WBC, ANC, PLT, NLR, I:T ratio and CRP in predicting sepsis, in ICU and ER at TASH, Addis Ababa, Ethiopia, 2021.

Parameters	Cut off value	Sensitivity	specificity	PPV	NPP
WBC	≤11.95	62.3%	51.6%	46.3%	67.1%
ANC	≤9.45	65.6%	53.8%	48.8%	70%
PLT	≤200.5	60.7%	58.2%	49.3%	68.8%
NLR	≤7.41	60.7%	59.3%	50%	69.2%
CRP	≥14	52.5%	44%	38.6%	58%
I: T	≥0.245	54.1%	48.4%	41.3%	61.1%

Abbreviations: CRP, C-reactive protein; I:T, immature to total neutrophil ratio; TLC, total leukocytes count; NLR, neutrophile to lymphocyte ratio

Table 5: Sensitivity and specificity of WBC, PLT, ANC, NLR, I: T and CRP for the prediction of sepsis at different cutoff points from ROC curve at Tikur Anbessa specialized hospital, 2021.

WBC	Sensitivity	Specificity		**ANC**	Sensitivity	Specificity
≤9.75	49.2%	63.7%		≤8.75	60.7%	54.9%
≤10.15	52.5%	62.6%		≤9.15	62.3%	53.8%
≤12.6	65.6%	47.3%		≤10.25	67.2%	47.3%
≤12.9	68.9%	46.2%		≤10.65	70.5%	46.2%
PLT				**N: L ratio**		
≤194	55.7%	60.4%		≤6.63	55.7%	67%
≤197	57.4%	60.4%		≤7.05	59%	62.6%
≤208.5	62.3%	56%		≤7.75	62.3%	53.8%
≤215	63.9	51.6%		≤7.85	63.9%	52.7%
CRP				**I: T ratio**		
≥9.5	78.7%	12.1%		≥0.225	55.7%	46.2%
≥11.0	70.5%	20.9%		≥0.260	47.5%	60.4%
≥ 12.50	57.4%	44%		≥0.275	45.9%	63.7%

Abbreviations: CRP, C-reactive protein; I:T, immature to total neutrophil ratio; NLR, neutrophile to lymphocyte ratio; TLC, total leukocytes count

5.5 Antimicrobial Susceptibility test (AST)

5.5.1 Antimicrobial Susceptibility test for gram positive pathogen bacteria

Gram positive pathogens such as S. *aureus*, S. *lugdunensis* and enterococcus species were subjected for antimicrobial susceptibility test. The result was interpreted based on CLSI 2021. All S. *aureus* isolates were fully sensitive for ciprofloxacin and oxacillin. All S. *lugdunensis* and S. *aureus* isolates were 100 % resistance for penicillin. Two isolates of Enterococcus species were sensitive for ciprofloxacin (Table 6).

Table 6: **AST pattern of gram-positive isolates from blood culture of sepsis patients at Tikur Anbessa Specialized Hospital, 2021.**

Antimicrobial drugs	**Bacterial isolates**			
		S. *aureus*	S.*lugidinase*	Enterococcus species
Gentamycin	Sensitive	1(50%)	1(50%)	-
	Resistance	1(50%)	1(50%)	-
Ciprofloxacin	Sensitive	2(100%)	0(0)	2(100%)
	Resistance	0(0)	2(100%)	0(0)
Vancomycin	Sensitive	-	-	1(50%)
	Resistance	-	-	1(50%)
Clindamycin	Sensitive	1(50%)	0(0)	-
	Resistance	1(50%)	2(100%)	-
Erythromycin	Sensitive	1(50%)	0(0)	-
	Resistance	1(50%)	2(100%)	-
Oxacillin	Sensitive	2(100%)	0(0)	-
	Resistance	0(0)	2(100%)	-
Penicillin	Sensitive	0(0)	0(0)	-
	Resistance	2(100%)	2(100%)	-

5.5.2 **Antimicrobial Drugs Susceptibility pattern for gram negative pathogen bacteria**

E. coli isolates were 33.3% sensitive for gentamycin and ceftazidime, 50% sensitive for cefotaxime and ceftriaxone, 91.7% sensitive for amikacin. Whereas, 85.7% of Acinetobacter species were sensitive for Meropenem, amikacin and cefepime. Amikacin has the least resistance antimicrobial drugs compared to other drugs used in AST determination of gram-negative bacteria, i.e., 8.3% for *E. coli*, 0(0) for Pseudomonas, Enterobacter and Citrobacter species, 14.3% for Acinetobacter, 16.7% for *K. pneumoniae* (Table 7).

Table 7: AST pattern of gram-negative isolates from sepsis patients at Tikur Anbessa Specialized Hospital, 2021.

Antimicrobial drug		Gram negative bacteria					
		E. coli	Pseudomonas species	Enterobacter species	Acinetobacter species	K.pneumonia	Citrobacter species
Gentamycin	**S**	4(33.3%)	1(100%)	1(100%)	1(14.3%)	1(16.7%)	1(100%)
	R	8(66.6%)	0(0)	0(0)	6(85.7%)	5(83.3%)	0(0)
SXT	**S**	5(41.7%)	-	1(100%)	3(42.9%)	3(50%)	0(0)
	R	7(58.3%	-	0(0)	4(57.1%)	3(50%)	1(100%)
Ceftazidime	**S**	4(33.3%)	0(0)	0(0)	4(57.1%)	1(16.7%)	1(100%)
	R	8(66.6%)	1(100%)	1(100%)	3(42.9%)	5(83.3%)	0(0)
Ciprofloxacin	**S**	8(66.6%)	1(100%)	1(100%)	2(28.6%)	3(50%)	1(100%)
	R	4(33.3%)	0(00	0(0)	5(71.4%)	3(50%)	0(0)
Cefotaxime	**S**	6(50%)	1(100%)	0(0)	3(42.9%)	4(66.7%)	0(0)
	R	6(50%)	0(0)	1(100%)	4(57.1%)	2(33.3%)	1(100%)
Ceftriaxone	**S**	6(50%)	-	0(0)	5(71.4%)	3(50%)	0(0)
	R	6(50%)	-	1(100%)	2(28.6%)	3(50%)	1(100%)
Amikacin	**S**	11(91.7%)	1(100%)	1(100%)	6(85.7%)	5(83.3%)	1(100%)
	R	1(8.3%)	0(0)	0(0)	1(14.3%)	1(16.7%)	0(0)
Cefepime	**S**	9(75%)	1(100%)	1(100%)	6(85.7%)	4(66.7%)	1(100%)
	R	3(25%)	0(0)	0(0)	1(14.3%)	2(33.3%)	0(0)
Meropenem	**S**	10(83.3%)	1(100%)	1(100%)	6(85.7%)	3(50%)	0(0)
	R	2(16.7%)	0(0)	0(0)	1(14.3%)	3(50%)	1(100%

Abbreviations: R=Resistance, S= Sensitive

6. Discussions

Many sepsis markers including selected hematological parameters and their derived ratios are immerging to help as a good predictor which is crucial to prevent sepsis serious outcome particularly in resource constrained settings where microbiology services are limited. Sepsis patients are a very diverse population, and the disease is frequently challenging to diagnose, especially in its early stages. It has been demonstrated that prompt diagnosis of sepsis patients and prompt beginning of proper medication improve outcomes; nonetheless, timely diagnosis and treatment of sepsis are daily challenges for doctors, particularly in emergency rooms and critical care units [43]. Cognizant of this challenge, this study aimed to investigate the predictive performance of six laboratory parameters namely, WBC, ANC, PLT, NLR, CRP and I: T ratio.

From the participants enrolled with clinically suspected sepsis; 85 (55.9%) were females and 67(44.1) were male. There is no statistically significant association between gender and culture result (p=.082). The study done in Sudan by Ahmed A *et al.* 2020, had 23 (46%) male, and 27(54%) were female in both study majority of the suspected participants were females. The mean age of study participant was 45.34 ±19.33; the majority (30.9%) were above 50 years of age [41]. However, Mustafić S and his colleagues, 2021 reported the mean age of patients with sepsis were slightly older (57.84±1.65 years). There is no statistically significant difference in the mean age of sepsis positive and sepsis negative patients (p=0.44) [43].

In this study, six variables (WBC count, neutrophil count, l:T ratio, platelet count, NLR, and CRP) were assessed for utility in the sepsis prediction. The mean± SD value of WBC for culture positive results were 11.76±12.9 and for culture negative were 16.48±13.51 and the mean± SD value of ANC for culture positive results were 8.436±6.6811 and for culture negative were 12.667±10.2529. Independent sample t test showed that there was statistically significant difference in the WBC, ANC and N:L ratio value between culture positive and negative results (p=0.032), (p=0.002) and (p=0.029), respectively. Yan et al. (2013), in contrast, showed no significant variation in total leukocyte count between patients with negative and positive blood cultures: WBC for culture positive result were 11.18.9x109 /L and for culture negative result were 10.6±7.9x10^9 /L (p=0.789) [45].

Similar study by Wang H *et al.* (2013) reported no a significant difference in number of WBC between patients with positive and negative blood cultures result (p=0.86) while they found significantly higher C-reactive protein and neutrophils in patients with sepsis [44]. Factors such as patients' selection might have contributed for the observed differences between the current study and others.

The percentage of each tests had different specificity, sensitivity, positive and negative predictive accuracy of the sepsis. This study documented a relatively higher sensitivity for ANC, WBC, platelet and neutrophil to lymphocyte ratio which were: 65.6%, 62.3%, 60.7 %, 60.7 % respectively, WBC was the second sensitive test in detection of sepsis next to ANC. Also, Soresa A. 2018 report agreed with the Sensitivity, Specificity, PPV and NPV of WBC count were 59.5 %, 79.6%, 52%, 64.5%, respectively [26]. While Jeyaganguli D and his colleague ,2018 reported lower sensitivity for ANC, Platelets and WBCs which were as follow: 61.8%, 29.4% and 55.9% respectively for sepsis [14].

In this study, CRP results have shown lowest specificity, sensitivity, PPV and NPV compared to other studies. The results were: 52.5%, 44 %, 38.6% and 58%, respectively. However, CRP has the highest sensitivity, specificity and high PPV and NPV which is 82.4%, 93.0%, 80.0% and 93.9% respectively according to a study by Jeyaganguli D, *et al* [14]. These results are similar to that of Bhale CP and his colleague, 2016 where CRP showed 84.6% sensitivity [17]. And also, in Assela, south East Ethiopia reported by Sorsa A, 2018 sensitivity, specificity, PPV and NPV of CRP were 65.6%, 78%, 42% and 91%, respectively [26]. The difference in sensitivity, specificity, NPV and PPV in different studies may be due to different methods of CRP estimation, host immune and diversity of patient group, including age difference (neonates and adults).

Immature to total neutrophil had the second lowest specificity, sensitivity, NPV and PPV which were:54.1%, 48.4%, 41.3% and 61.1%, respectively. Similar finding was documented by Jeyaganguli D and his colleague, 2018 where the I/T ratio sensitivity was 26.5%. However, according to results reported by Bhale CP and his colleague, 2016 Immature to total neutrophil ratio showed highest sensitivity of 75.8% [17].

This discrepancy in sensitivity and specificity of I/T ratio may be due to different cut off values used, as some studies consider I/T ratio ≥0.2 is significant while others consider it ≥ 0.3 or > 0.15[14]. However, in the current study we had cut off value I/T ratio of ≥ 0.250 which is significant for sepsis.

In this study the specificity for platelet and neutrophil to lymphocyte ratio, respectively were higher 58% and 59%. In line with this Salman Ali *et al*, 2005, has reported 99% specificity of Platelets and 92.0 % for WBC which was the highest value [41] while the specificity of WBC was discordant with this study. Similar study conducted by Bhale CP and his colleague, 2016 absolute neutrophil count (99.0%) and total leukocyte count (93.0%) showed the highest specificity [17].

Sepsis diagnosis requires clinical and microbiological correlation. In this study, of a total of 152 patients admitted in the AICU and ER unit who were classified as having probable infection with clinical evidence, 91(59.9%) cases lacked microbiological proof of infection while 61(40.1%) that were clinically suspected to have sepsis had a positive blood culture. This culture positive report was similar with Anwar et al, 2000 who have reported culture positivity rate of 42% [11]; 43.1% reported by West *et al*, 2012 [16] and also (41.4%) culture positive results reported in Sudan,2013 by Kheir AE, with his colleagues [34]. While in Iraq Jeyaganguli D and his colleague, 2018 has documented (34%) culture positive [14]; Krishnamurthy V et al, 2017 has documented 12% culture positive [12] and also, in Assela, south East Ethiopia reported by Sorsa A, 2018 was 29.4% blood culture positive [26]. This variation in blood culture positively may depend on the criteria of studied group, low level of bacteremia and volume sample and sampling site. In the current study it is not known why the 59.9% cases lacked microbiological proof of infection, it might be the reasons is as justified by Ter SK *et al* who demonstrated that molecular investigation of negative blood cultures can identify potential pathogens that will otherwise be missed by routine culture [40]. According to their study, from 190 sepsis suspected cases day-5 negative blood culture samples were studied; of these, 53 (27.9%) were positive by PCR for bacterial DNA.

Similar with this study which was done in Egypt by Morad EA and his colleagues, 2020 also, out of 19 negative blood culture result, 10 positive PCR samples were found, despite negative blood

culture could be due to administration of antibiotic, which influenced culture results and/or an inadequate amount of blood samples that allow the optimal detection of bacteria.

So, low level bacteremia would be difficult to be identified by any procedure based on the detection of bacterial growth or DNA, which raises the need for neonatal sepsis markers [42].

In this study, blood culture revealed pathogenic growth in 61 cases; most common were CONS 19(12.5%), E. coli 12(7.9%), followed by, Acinetobacter 7(4.6%) with Klebsiella 6(3.9%) was identified. CONS were predominant microorganism followed by E. coli. However, in other study conducted by Roberts T *et al*, 2021 reported as, among 53 positive cases *E. coli* 11 (26.1%) were predominant microorganism followed by *K. pneumoniae* 8 (21.7%) [40]. Similar study conducted in Egypt by Gabriel MG and his colleagues, 2020 with a total of 31 positive cases; the most common were *Klebsiella* (61.3%), *E. coli* (9.7%), followed by *CONS* (9.7%) and *Acinetobacter* were less common with 3.2% [42].

7. Strength and Limitations

Strength of the study

- It is the first study in Ethiopia on adult age group, thus it can be used as a base line for further study
- Findings can be used as an input for sepsis management guidelines
- Laboratory analysis done in nationally accredited laboratory at TASH

Limitation of the study

- The sample was drawn for diagnosis purpose (no follow up)
- Due to shortage of the literature that are done on adult sepsis, neonatal sepsis literatures included as a reference.
- Due to limited resource, we used semi quantitative CRP test.

8. Conclusions

Relatively better predictive value was displayed for the presence of sepsis by absolute neutrophil count at the lower cutoff value could be predicted with sensitivity of 65.6% and specificity of 53.8% and total leukocyte count is the second sensitive test which is 62.3% sensitivity with 51.6% specificity; while the specificity of platelet and neutrophil to lymphocyte ratio was higher when we compared with other parameters specificity (58% and 59%) respectively. However, CRP and immature to total neutrophil showed lowest sensitivity and specificity (there is discrepant with others). By monitoring changes in hematological parameters (ANC, Platelets count, WBC and N:LR) and combining with other clinical indicators, for patients with sepsis can be useful in early prediction of sepsis i.e., before the appearance of blood culture results.

Recommendations

We recommend using these hematological parameters of investigations, in situations where access to culture results is difficult or delayed, as they are readily available, inexpensive and rapid in prediction of sepsis, to manage patients early and reduce, prolonged staying in hospital (stop antibiotic therapy) and family anxiety play an important role to predict sepsis within a few hours. The management of 91 patients in the current study for whom a bacterial organism was not detected would have been affected, and it is possible that molecular analysis would have resulted in altered final outcome. Therefore, further studies may be required to validate our results, for routine use.

9. References

1. Schwarzbold AV, Georgala A, Paesmans M, Barette M, Aoun M. Sepsis in hematologic patients. Int J Infect Dis. 2006;10: S69. [Internet](2020) 10:273 Available from: https://doi.org/10.1038/s41598-019-57107-1
2. Choi JS, Trinh TX, Ha J, Yang MS, Lee Y, Kim YE, et al. Implementation of Complementary Model using Optimal Combination of Hematological Parameters for Sepsis Screening in Patients with Fever. Sci Rep. 2020;10(1):1–10.
3. Li X, Xu Z, Pang X, Huang Y, Yang B, Yang Y, et al. Interleukin-10/lymphocyte ratio predicts mortality in severe septic patients. PLoS One. 2017;12(6):1–10.
4. Flores C. Host genetics shapes adult sepsis survival. Lancet Respir Med [Internet]. 2015;3(1):7–8. Available from: http://dx.doi.org/10.1016/S2213-2600(14)70307-8
5. Ghebreyesus TA. WHO says sepsis causes 20 % of global deaths 2020;10–2.
6. Faix JD. Biomarkers of sepsis. Crit Rev Clin Lab Sci. 2013;50(1):23–36.
7. Minasyan H. Sepsis: Mechanisms of bacterial injury to the patient: Medical and Health Sciences Scand J Trauma Resusc Emerg Med. 2019;27(1):1–22.
8. Zewdu T. Sepsis in the emergency department in Tikur Anbessa specialized hospital Addis ababa ethiopia 2013;
9. Fan S, Miller NS, Lee J, Remick DG. Diagnosing Sepsis: The Role of Laboratory Medicine 2017;203–10.
10. Barbash IJ, Davis B, Kahn JM. National performance on the Medicare SEP-1 sepsis quality measure 2020;47(8):1026–32.
11. Anwer SK and Mustafa S. (Department of pediatrics, Abbasi Shaheed Hospital, Karachi. Rapid Identification of Neonatal Sepsis. Department of Pediatric, Abbasi Shaheed Hospital, Karach (Pakistan)JPMA 2000;50:94.
12. Krishnamurthy V, Thandaveshwar D, Doreswamy SM. Modified hematological sepsis score in early diagnosis of neonatal sepsis. Int J Res Med Sci. 2017;5(8):3573.
13. Merck Manual. Sepsis in Newborns. 2002;1–13. (https://www.merckmanuals.com/home/children-s-health-issues/infections-in-newborns/sepsis-in-newborns) Accessed 1/23/2023.

14. Jeyaganguli D, Velvizhi G, Sucilathangam G, Revathy C. Diagnostic Value of C - Reactive Protein and Hematological Markers in Neonatal Sepsis. Int J Curr Microbiol Appl Sci. 2018;7(03):722–7.
15. Samanta PK and Kumar A. Evaluation of Hematological Parameters in Early Onset Neonatal Sepsis. National Int J Med Re. 2013; 4(6) :29-34.
16. West BA, Peterside O, Ugwu RO, Eneh AU. Prospective evaluation of the usefulness of C-reactive protein in the diagnosis of neonatal sepsis in a sub-Saharan African region. Antimicrob Resist Infect Control. 2012;1(1):1-22
17. Bhale CP, Vasant Kale AU, Kale SS, Mahajan M and Smulay ST. Utility of sepsis screen in India JNMR 2016;4(3) id 2178
18. Shirazi H, Riaz S, Tahir R. Role of the Hematological Profile in Early Diagnosis of Neonatal Sepsis Haider Shirazi et al Ann. Pak Inst Med Sci. 2010;6(3):152–6.
19. Khater ES, Al-Hosiny TM. Presepsin as a New Marker for Early Detection Neonatal Sepsis in Al-Quwayiyah General Hospital Riyadh, KSA. J Adv Microbiol. 2020;20(September 2019):80–90.
20. Lokhande R, Gedam B, Shah Y, Tandon M, Bansod PY. Rationale for near total thyroidectomy in patients with nodular goitre. Int J Biomed Adv Res IJBAR Int J Biomed Adv Res J. 2015;6(605):427–30.
21. Lakhey A, Shakya H. Role of Sepsis Screening in early diagnosis of Neonatal Sepsis. J Pathol Nepal. 2017;7(1):1103–10.
22. Tigabu Kebede Z, Matebe YH, Demisse AG, Yimer MA, Mekasha A, Worku A, et al. Hematologic Profiles of Ethiopian Preterm Infants with Clinical Diagnoses of Early-Onset Sepsis, Perinatal Asphyxia, and respiratory distress syndrome. Glob Pediatr Heal. 2020;7.
23. Gebrehiwot A, Lakew W, Moges F, Moges B, Anagaw B, Unakal C, et al. Predictors of positive blood culture and death among neonates with suspected neonatal sepsis in Gondar University Hospital, Northwest Ethiopia. College of Medicine and Health Science. Euro. J. Exp. Bio. 2012;2(6):2212–18.
24. Getabelew A, Aman M, Fantaye E, Yeheyis T. Prevalence of Neonatal Sepsis and Associated Factors among Neonates in Neonatal Intensive Care Unit at Selected

Governmental Hospitals in Shashemene Town, Oromia Regional State, Ethiopia, 2017. Int J Pediatr. 2018; 2018:1–7.

25. Guclu E, Durmaz Y, Karabay O. Effect of severe sepsis on platelet count and their indices. Afr Health Sci. 2013;13(2):333–8.
26. Sorsa A. Diagnostic Significance of White Blood Cell Count and C-Reactive Protein in Neonatal Sepsis; Asella Referral Hospital, South East Ethiopia. Open Microbiol J. 2018;12(1):209–17.
27. de Stoppelaar SF, van 't Veer C, van der Poll T. The role of platelets in sepsis. Thromb Haemost. 2014;112(4):666–77.
28. Marik PE. Don't miss the diagnosis of sepsis! Crit Care. 2014;18(5):1–3.
29. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. /Lancet. 2020;395(10219):200-11.
30. Rudd KE, Kissoon N, Limmathurotsakul Di, Bory S, Mutahunga B, Seymour CW, et al. The global burden of sepsis: Barriers and potential solutions. Crit Care. 2018;22(1):123305059.
31. The Kampala Declaration: Commitment to improve care for sepsis and severely ill patients in Africa. Connect World Crit Care Nurs. 2017;11(3):47–8. Available from: https://www.wfccn.org
32. Mulatu HA, Bayisa T, Worku Y, Lazarus JJ, Woldeyes E, Bacha D, et al. Prevalence and outcome of sepsis and septic shock in intensive care units in Addis Ababa, Ethiopia: A prospective observational study. African J Emerg Med [Internet]. 2020;(May):0–1. Available from: https://doi.org/10.1016/j.afjem.2020.10.001
33. El-Shafie ME-S, Taema KM, El-Hallag MM, Kandeel AMA. Role of presepsin compared to C-reactive protein in sepsis diagnosis and prognostication. Egypt J Crit Care Med [Internet]. 2017;5(1):1–12. Available from: http://dx.doi.org/10.1016/j.ejccm.2017.02.001
34. Kheir AE, Jobara GA, Elhag KM, Karar MZ. Qualitative C-reactive protein as a marker of neonatal sepsis in a tertiary neonatal unit in Sudan. Health Low-resource Settings. 2013;1(2):21.

35. Lever A, Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. BMJ. 2007 Oct 27; 335(7625): 879–883.
36. Sinha S, Ray B. Sepsis-3: How useful is the new definition J Anaesthesiol Clin Pharmacol. 2018; 34(4): 542–543.
37. Saboohi E, Saeed F, Khan RN, Khan MA. Immature to total neutrophil ratio as an early indicator of early neonatal sepsis. Pak J Med Sci. 2019; 35(1):241-246. doi: 10.12669/pjms.35.1.99. PMID: 30881431; PMCID: PMC6408653.
38. Thompson D, Milford-Ward A, Whicher JT. The value of acute phase protein measurements in clinical practice. Ann. Clin Biochem; 1992; 29:123–31.
39. Chernecky CC, Berger BJ. Differential leukocyte count (diff) - peripheral blood. In: Chernecky CC, Berger BJ, eds. *Laboratory Tests and Diagnostic Procedures*. 6th ed. St Louis, MO: Elsevier Saunders; 2013:440-446.
40. Ter SK, Rattanavong S, Roberts T, Sengduangphachanh A, Sihalath S, Panapruksachat S, et al. Molecular detection of pathogens in negative blood cultures in the Lao People's Democratic Republic. Am J Trop Med Hyg. 2021;104(4):1582–5.
41. Ahmed Z, Ghafoor T, Waqar T, Mahmud S, Ali S, Aziz S, Mahmud S. Diagnostic value of CRP and hematological parameters in neonatal sepsis. JCPSP 2005; 15:152-56
42. Morad EA, Rabie RA, Almalky MA, Gebriel MG. Evaluation of Procalcitonin, C-Reactive Protein, and Interleukin-6 as Early Markers for Diagnosis of Neonatal Sepsis. Int J Microbiol. 2020;2020.
43. Mustafić S, Brkić S, Mujić-Ibralić A. Hematological Parameters and C-Reactive Protein in Prediction of Disease Severity and Mortality in Patients with Sepsis. Res Educ Rehabil. 2021;4(1):13–26.
44. Wang H, Yin F, Shen DX, Zhang YJ, Luo YP, Liu CJ, et al. Predictive value of procalcitonin for excluding bloodstream infection: results of a retrospective study and utility of a rapid, quantitative test for procalcitonin. J Int Med Res. 2013 Oct;41(5):1671-81. doi: 10.1177/0300060513497558.
45. Yan S, Bin D, Ying-Chun X, Xi R, Wei D, Yao W. Early changes of procalcitonin predict bacteremia in patients with intensive care unit-acquired new fever. Chin Med J 2013; 126:1832-7.

Information sheet

1. Code number Age: Sex; Male ☐ Female ☐
2. Address: Rural ☐ Urban ☐
3. Level of Education: Cannot read and write ☐ Read and write ☐ Elementary ☐ high school ☐ Diploma ☐ Degree ☐ above first Degree ☐
4. What type of job you work?
5. Are you pregnant? (For women) Yes ☐ No ☐
6. Are your malignant patient Yes ☐ No ☐
7. Have you taken any antibiotics within this week? Yes ☐ No ☐
8. Do you have a previous exposure/ history of any infection? Yes ☐ No ☐
   If yes, which type of infection
9. Do you have a previous history of surgery? Yes No
10. Do you have a previous history of hospital admission? Yes ☐ No ☐

Annex I. Information sheet in English Version

Title of the Research Project: The role of some hematologic parameters and CRP as a predictor of sepsis at Tikur Anbessa Specialized hospital, Addis Ababa, Ethiopia.

Principal Investigator: Nardos Gosaye (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 or parent guardian in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include the principal investigator, one nurse, three advisors and one collaborator from Addis Ababa University department of Hematology. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assess the role of Hematologic parameters for prediction sepsis.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis) epidemiological surveillance as well as Research to understand the morbidity and mortality of a particular disease process. Even though the Hematologic parameters has a role as a predictor of sepsis. The physician and the lab technologist give a different view for it most of the time Blood culture is the prioritizes test orders when in sepsis suspecting. So as many researches done Hematologic profile has a great role of the p diagnostic effect and to early manage for the sepsis case.

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 specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by nurse/principal investigator in AICU department. Then, you are requested to give your consent to the sample collector. After consent, a blood sample will be taken from vein. Moreover, there will be a face-to-face interview for additional questions.

Procedures: The blood was collected from vein and transferred to EDTA, blood culture bottle and SST tube then mix the EDTA then within 2 hours the CBC was done by using Beckman coulter automated hematology analyzer and WBC, Neutrophil and platelet parameters were reported and N: L ratio was calculated for all CBC results. Blood smear was done to get I: T neutrophil and SST tube sample for CRP was performed by using a semi-quantitative, membrane-based immunoassay technique. Also blood culture (gold standard) done by following proper procedures to determine the patient is sepsis positive or not.

Potential risks and Discomforts

There will be minimal discomfort in giving blood samples. However, there might be some minimal risk and discomfort when we take venous blood. Nevertheless, we tried to minimize the discomfort as much as possible, the blood samples were taken by experienced laboratory professionals and PI.

Confidentiality

We respected your or your child privacy and confidentiality. Any information that identifies you was not shared with anyone else outside the study team. The information we were collect from you as part of the study kept in a locked file cabinet, or protected by a password on the computer only accessible to personnel who involved in the study. There was no sensitive issue that you were asked related with your social desirability but any information that was obtained in connection with this study and that could be identified with you were 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 for the early diagnosis of sepsis. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you or your child 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 to 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 following principal investigator for further information.

Name Phone: 09-22-08-35-36

E-mail: nardosgosaye29@gmail.com

Annex II የተሳታፊዎችፈቃድናመተማመኛቅፅ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስድግሪተማሪየመመረቂያየጥናትላይእዲሳተፉተጋብዘዋል፡፡እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘዉንምንባብበጥሞናያንብቡናግልጽያልሆነ ልዎትንማንኛዉምሃሳብይጠይቁ፡፡

መግቢያ

የጥናቱርዕስ “Role of Some Hematologic Parameters and CRP as a predictor of sepsis”.

የእርስዎወይምየልጅዎ
በዚህጥናትላይየሚኖርዎትተሳትፎሙሉበሙሉበበጎፈቃደኝነትላይየተመሰረተነዉ፡፡በዚህጥናትዉስጥላለመሳተፍወይምለመሳተፍከወሰኑበኃላለማቋረጥየሚወስኑቢሆንምእንኩዋበዚህሆስፒታልየሚሰጠዉማንኛዉምአገልግሎትአይቋረጥም፡፡በጥናቱለመሳተፍየሚስማሙከሆነየስምምነትቅጹላይበጹሁፍወይምበጣትፊርማማስቀመጥይጠበቅዎታል፡፡

የጥናቱተሳታፊለመሆንየሚጠበቅበዎትምንድንነው?

በዚህጥናትለመሳተፍየሚስማሙከሆነናሙናዎለጥናቱእንዲሚዉልመስማማትይጠበቅብዎታል፡፡ከተወሰደዉናሙናላይየሚገኙ መረጃዎችከዚህሆስፒታልዉጭለሚገኙናለስራዉአግባብነትላላቸዉሰዎችቢነገርየማይቃወሙመሆኑንመስማማትይጠበቅብዎ ታል፡፡ይሁንእንጅይህአይነቱመረጃየርስዎንማንነትየሚገልጡመረጃዎችንማለትምስም፤አድራሻናየስልክቁጥርየመሳሰሉትንመረጃ ዎቸንአይጨምርም፡፡ይልቁንምለዚህአገልግሎትብቻየሚዉልእርስዎንለማወቅየሚያስችልመለያቁጥርጥቅምላይእንዲዉልይደረ ጋል፡፡በተጨማሪምስለርስዎአጠቃላይየጤናሁኔታለሚቀርቡአንዳንድተጨማሪጥያቄዎችመልስመስጠትይኖርብዎትአል፡፡

በዚህጥናትመሳተፍየሚያስከትላቸዉቸግሮችምንድንናቸዉ?

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም፡፡ሆኖምግንናሙናዉንለመሰብሰብልምድያለዉባለሙያስለ ሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለሚወሰድየህመምስሜትአይኖርም፡፡

የህክምናመረጃበሚስጥርተጠብቆመቆየትየሚችለዉእንዴትነዉ?

ስለራስዎወይምልጅዎ
የሰጡትማንኛዉምመረጃናከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥናቱአላማብቻነዉ፡፡ይህንማህደርሊ ያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው፡፡ከዚያምበላይስለእርስዎያለውንማንኛውንምመረጃየተለየየይለፍቃልባ ለውየኮምፒውተርየመረጃማህደርውስጥእንዲቀመጥይደረጋል፡፡

በዚህጥናትመሳተፍየሚያስገኛቸውጥቅሞችምንድንናቸው ?

ይህጥናትየማስተርስዲግሪመመረቂያእንደመሆኑመጠንበዚህጥናትበመካፈልዎበገንዘብየሚያገኙትጥቅምባይኖርምከጥናቱበሚገ ኝውውጤትግንተጠቃሚነዎት፡፡የእርሶዎተሳትፎየእርስዎንናየወገንዎየደምዉስጥበክተሪያለማወቅናለማከምከፍተኛጥቅምይኖረ ዋል፡፡

በዚህጥናትተሳታፊየመሆንዎመብቶችምንድንናቸው ?

በዚህጥናትመሳተፍሙሉበሙሉበእርስዎፈቃደኝነትየተመሰረተበመሆኑበማንኛውምሰዓትናቢታየማቋረጥሙሉመብትየተጠበቀ ከመሆኑምበላይእራስዎንከጥናቱበማግለልዎምክንያትለእርሶምሆነለልጅዎየሚቀርብዎትምንምአይነትየሆስፒታልአገልግሎትአይ ኖርም፡፡ከዚህምበተጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄየመጠየቅናገለጻየማግኘትመብትአለብዎት፡፡የላብራቶሪ ምርመራውጤቱንምበነጻማግኘትይችላሉ፡፡ነገርግንእርስዎበሚሰጡንመረጃየችግሩንስፋትለመከላከልእናለመቆጣጠርጠቃሚስ ለሆነለሚቀርብልዎትጥያቄቀጥተኛመልስይሰጡንዘንድበታላቅአክብሮትእንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንንጥናትበተመለከተወይምከዚህጥናትጋርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋዎችወይምጥያቄካለዎትበሚመለከ ተውአድራሻይጠቀሙ፡፡

ሞባይል፡ +251-922083536

ኢሜል፡nardosgosaye29@gmail.com

Annex III Informed consent form in English version

Code no…..……

I had been informed that the objective of this study is to determine some Hematologic parameters and CRP as a predictor of sepsis, the results of this study have an importance to treat me and other

patients, and to be used as an input for the future development of strategies or guidelines for diagnosing and early treatment of sepsis. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood and filling questionnaire. Therefore, with 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 or my child if he/she provides assent.

Signature: _____ Date_____

Annex IV. Informed consent form in Amharic version

የተሳታፊዎችስምምነትማረጋገጫ

የሚስጥርቁጥር ----------

የተሳታፊዉስም ----------

እኔስሜከላይየተጠቀሰውተሳታፊ “Role of Some Hematologic Parameters and CRP as a predictor of sepsis”ጥናትላይበቂገለጻተደርጎልኛል፡፡ለጥናቱምደምናሙናእንደሚያስፈልግተገልጾልኛል፡፡የጥናቱንምአላማዎችምተረድቻለሁ፡፡

በቃለመጠይቁላይየገለጽኳቸውመረጃዎችበሙሉበሚስጥርየተጠበቁእንደሚሆኑተነግሮኛል፡፡በጥናቱላይያለመሳተፍናማንኛውንምመረጃያለመስጠትእንዲሁምበማንኛውምጊዜከጥናቱራሴንየማግለልመብቴየተጠበቀእንደሆነተገልጾልኛል፡፡

ስለዚህለዚህጥናትመረጃናየስምምነትቃሌንየሰጠሁትበአጠቃላይሁኔታውንበመረዳትናበፍጹምፍቃደኝነትነው፡በተጨማሪምጥያቄለመጠየቅተፈቅዶልኝለማወቅየፈለኩትንያህልማብራሪያአግኝቻለሁ፡፡የዚህጥናትተሳታፊበመሆኔየማገኘውጥቅምየሁሉንምምርመራውጤትበነጻማግኘትእንደሆነተረድቻለሁ፡፡

በአጠቃላይእኔከላይበመተማመኛቅፅየተጠቀሱትንሁሉበሚገባናበተረጋጋመንፈስአንብቤዋለሁኝ፡፡ስለዚህበዚህጥናትለመሳተፍፈቃደኛመሆኔንበፊርማዬአረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

(የስምምነትቅጹንማንበብለማይችሉተሳታፊዎች)

የአማካሪነርስስም ---------- ፊርማ ----------

ቀን----------

Annex V Consent form for patients

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. **I voluntarily consent/assent that I would participate in this study.**

To collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time.

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy) _____

Phone number_____

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

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.

M.Sc. candidate: Nardos Gosaye (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisors:

Dr Aster Tsegaye (PhD, Professor of Immuo-Hematology)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Elias Bisrat (MSc CLS, Hematology and Immunohematology specialty)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Dr. Ananya Abate (MD, Assistant Professor of Anesthesiology)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.