EVALUATION OF ROLE OF NOVEL BIOMARKERS SERUM FIBROBLAST GROWTH FACTOR 21 (FGF21) LEVEL AND NEUTROPHIL PARAMETERS {NEUTROPHIL-GRANULARITY-INTENSITY (NEUT-GI) AND NEUTROPHIL-REACTIVITY-INTENSITY (NEUT-RI)} IN DIAGNOSIS AND PROGNOSIS OF SEPSIS

THESIS

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DECLARATION

I hereby declare that the thesis titled "Evaluation of Role of Novel Biomarkers Serum Fibroblast Growth Factor 21 (FGF 21) Level and Neutrophil Parameters {Neutrophil-Granularity-Intensity (NEUT-GI) and Neutrophil-Reactivity-Intensity (NEUT-RI)} in Diagnosis and Prognosis of Sepsis" embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur.

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CERTIFICATE

This is to certify that the thesis titled "Evaluation of Role of Novel Biomarkers Serum Fibroblast Growth Factor 21 (FGF 21) Level and Neutrophil Parameters {Neutrophil-Granularity-Intensity (NEUT-GI) and Neutrophil-Reactivity-Intensity (NEUT-RI)} in Diagnosis and Prognosis of Sepsis" is bonafide work of Dr Vishwanath Jha carried out under our guidance and supervision in the department of General medicine, All India Institute of Medical Science, Jodhpur.

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"Alone we can do so little; together we can do so much"

-Helen Keller

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Dedicated to my family

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LIST OF ABBREVIATION

qSOFA	QUICK SEQUENTIAL ORGAN FAILURE ASSESSMENT
PCT	PROCALCITONIN
CBC	COMPLETE BLOOD COUNT
Hb	HEMOGLOBIN
PLT	PLATELET
HCT	HEMATOCRIT
MCV	MEAN CORPUSCULAR VOLUME
SGOT	SERUM GLUTAMIC OXALOACETIC TRANSAMINASE
SGPT	SERUM GLUTAMIC PYRUVIC TRANSAMINASE
ALP	ALKALINE PHOSPHATASE
LFT	LIVER FUNCTION TEST
KFT	KIDNEY FUNCTION TEST
HSCRP	HIGHLY SENSITIVE C-REACTIVE PROTEIN
ESR	ERYTHROCYTE SEDIMENTATION RATE
NEUT-RI	NEUTROPHIL REACTIVITY INTENSITY
NEUT-GI	NEUTROPHIL GRANULARITY INTENSITY
FGF21	FIBROBLAST GROWTH FACTOR 21
CLABSI	CENTRAL LINE ASSOCIATED BLOOD STREAM INFECTION
UTI	URINARY TRACT INFECTION

LRTI	LOWER RESPIRATORY TRACT INFECTION
CNS	CENTRAL NERVOUS SYSTEM
ICU	INTENSIVE CARE UNIT
MDR	MULTIDRUG RESISTANT
LMICs	LOW- AND MIDDLE-INCOME COUNTRIES
ELISA	ENZYME LINKED IMMUNOSORBENT ASSAY
SSC	SURVIVAL SEPSIS CAMPAIGN
KBL	KETOBUTYRATE COENZYME A LIGASE
SCM	SOCIETY OF CRITICAL CARE MEDICINE
IL	INTERLUEKIN
TNF	TUMOR NECROSIS FACTOR
APCs	ANTIGEN PRESENTING CELLS
TRRs	TOLL- LIKE RECEPTORS
PRRs	PATHOGEN RECOGNITION RECEPTORS
MAP	MEAN ARTERIAL PRESSURE
HRP	HORSERADISH PEROXIDASE
OD	OPTICAL DENSITY
NLR	NEUTROPHIL-LYMPHOCYTE RATIO
HIV	HUMAN IMMUNODEFICIENCY VIRUS
CLSI	CLINICAL AND LABORATORY STANDARDS INSTITUTE
SBP	SYSTOLIC BLOOD PRESSURE

DBP	DIASTOLIC BLOOD PRESSURE
PR	PULSE RATE
RR	RESPIRATORY RATE

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SUMMARY

Background - Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection frequently leading to septic shock and multiple organ dysfunction syndromes. Sepsis characteristically have elevated biomarkers i.e hsCRP, procalcitonin in early phase of infection.

Aims and objectives – To assess the sensitivity, specificity, positive predictive value of serum level of FGF21 and neutrophil parameters in the diagnosis and mortality correlation (early and late) in prognosis of sepsis with as well as assessment of comparative analysis of procalcitonin and hsCRP with serum FGF21 and neutrophil parameters for the diagnosis and prognosis in sepsis patients in a tertiary care Centre in Rajasthan.

Methods – This was a prospective observational study, in which all patients were ≥ 18 years of age attending patients services of the department of Medicine at AIIMS Jodhpur with diagnosis of sepsis and age and sex matched non-sepsis controls were included. Along with baseline hematological and biochemical parameters, FGF21 and neutrophil parameters (NEUT-RI and NEUT-GI) were measured in all patients. Follow-up of cases were done at day 7 and day 28 from the hospitalization.

Results – One hundred thirty-four patients were enrolled in this study. The mean age was 48.2±18.9 years and 82% were males. Among patients with sepsis the mean serum FGF21 level was significantly raised as compared to controls (469.6±298.45pg/ml vs 250.02±110.72pg/ml; p value < 0.0001). Similarly, NEUT-GI level was significantly raised in septic patients as compared controls (54.31±5.26 vs 46±3.93SI; p value < 0.0001). The sensitivity and specificity for serum FGF21 level were 75% and 63.8% respectively and similarly; the sensitivity and specificity of NEUT -GI at the time of diagnosis were and 81.6% and 84.5% respectively. Both serum FGF21 and NEUT-GI were significantly higher in non –survivor groups (p value <0.001). Early and delayed in-hospital mortality were 9.2% and 27.6% with overall mortality being 36% in this study. In multivariate analysis of predictors of delayed in-hospital mortality (day 28) were raised serum FGF21, higher qSOFA, MDR organism in culture and NEUT-GI (on day 7)

Conclusion- The level of novel biomarkers serum FGF21 and NEUT-GI have good diagnostic value in patients with sepsis. These biomarkers also helpful in prognostication of patients with sepsis. Incorporation of these biomarkers in algorithm of diagnosis along with

proven biomarkers would help in early diagnosis and selection of effective treatment of sepsis.

INTRODUCTION

INTRODUCTION

Sepsis is the life-threatening condition leading to multiple organ dysfunction caused by inadequate response from host against infections. The spectrum of sepsis is range from bacteremia to disseminated infections in all age groups. In modern world, sepsis continues to be the most common infectious cause of death globally. The exact burden of sepsis is difficult to estimate due to the heterogeneity of disease and lack of highly sensitivity tool for the diagnosis. World health organization has estimated the around 48.9 million cases of sepsis in 2017 with 11 million deaths related with the same.[1] Globally sepsis is accounted approximately 20% of all deaths and surprisingly among them 85% were belongs with low- and middle-income countries. The exact burden of sepsis in India is not extensively studied. However, the hospital-based studies have been suggested high incidence and mortality related to sepsis.[2,3,4]

Diagnosis of sepsis is challenging, especially in resource limited health care system, particularly in developing world. Delay in every hour of therapeutic intervention increases sepsis related mortality by approximately 8%. Therefore, early diagnosis and treatment is very important for sepsis related death prevention. Diagnostic insights and definition of sepsis is dynamic and initially sepsis-1 criteria were used in 1991 which was based on systemic inflammatory response syndrome (SIRS) in response to infection and defined sepsis according to severity classified as sepsis, severe sepsis, and septic shock.[5] Subsequently definition of sepsis-2 was introduced in 2001in which severe sepsis was redefined as sepsis complicated by organ dysfunction.[6] Sepsis-3 definition formulated in 2016 is most accepted definition of sepsis worldwide. The most recent accepted definition is - “sepsis is a life-threatening organ dysfunction caused by dysregulated response of the body to infection”.[7] Blood culture is considered gold standard for the confirmatory diagnosis of sepsis, but it is time consuming process. Procalcitonin, highly sensitive C-reactive protein (hs-CRP) and interleukin-6 (IL-6) are rapid diagnostic serum markers for assessing the need for antibiotics, severity, and prognosis of suspected sepsis. For early assessment of sepsis, quick sequential organ failure assessment (qSOFA) is used as a tool in clinically suspected patients. qSOFA score has three components – respiratory rate, mental status (GCS) and systolic blood pressure. qSOFA score ≥2 is associated with poor outcome in sepsis patient. qSOFA score is the simple clinical tool used for assessing the patient for diagnostic and therapeutic intervention. Glasgow coma score (GCS) <15, Systolic blood pressure<100mmHg, and

respiratory rate ≥22/min constellates qSOFA score to 3 which has high predictive value. Many retrospective studies and meta-analysis suggest low sensitivity and high specificity of qSOFA score in diagnosis of sepsis 3.[8-12] qSOFA score ≥2 has predictive validity is almost same as full SOFA score in non-ICU patients. qSOFA score ≥2 is an independent risk factor for in hospital mortality particularly in ICU patients.[1] SOFA score is considered more superior than qSOFA scoring system for prediction of sepsis in suspected patients. SOFA score is sequential organ failure assessment of suspected patients usually applied for in hospital ICU patients. SOFA score has six components – respiratory parameter (PaO2/FiO2), coagulation (platelets), liver function, cardiovascular (MAP and requirement of vasopressor), GCS and renal function (creatinine and urine output). SOFA score ≥2 suggests multiple organ dysfunction in sepsis and associated with poor prognosis and in hospital mortality of approximately 10%. SOFA is far more sophisticated tool than qSOFA and SIRS criteria. SOFA scoring system has more sensitivity and specificity than qSOFA for diagnosis and prognosis of sepsis. Therefore, SOFA has been considered as most accurate clinical tool for diagnostic and prognostic accuracy of sepsis patient.[2]

Various novel biomarkers are investigated and still the research being underway for early and effective diagnosis of sepsis. However, other than procalcitonin and CRP none has been approved in sepsis management protocol. Fibroblast growth factor-21 (FGF21) is subset of fibroblast growth factor encoded by FGF21 gene on chromosome 19. FGF21 is a member of fibroblast growth factors mainly consists of three factors namely - FGF23, FGF21, FGF19. FGF21 also have hormonal property with eccrine, paracrine, and endocrine functions. The main source of FGF21 are liver and adipose tissue, but pancreas, gonads, skeletal muscles, and heart also produces FGF21 in some quantity. Co-expression of KLB and FGF21 receptor is core component for tissue specific FGF signaling. Metabolic alteration of glucose and lipid is seen in patients with sepsis and inflammatory conditions. FGF 21 plays a very important role in regulating insulin resistance and glucose intolerance. FGF21 as a hormone induced by the peroxisome proliferator activated receptor α and γ which helps in maintaining tissue insulin sensitivity and cardioprotective effect. Increased serum level of FGF21 is also increased in type 2 diabetes mellitus, obesity, non-alcoholic fatty liver disease and dyslipidemia. Recently, role of FGF21 as early marker of sepsis is studied substantially.[13-15] Elevated baseline level of FGF21 is considered as poor prognostic marker in sepsis in critically ill patient. FGF21 is also used as biomarker for monitoring of sepsis patient in ICU.

According to studies, FGF21 is used for guiding the drug therapy and monitoring of antibiotics escalation in ICU patients.[3]

Neutrophil is important parameter for the innate immunity in patients with infection particularly sepsis patient. Neutrophil is the first cell to reach the site of infection and play a vital role in innate immunity. Neutrophils are activated by exogenous pathogens and cytokines released from inflammation. The key step for sepsis management is early diagnosis, localization of source, timely and adequate management in early hours of infection. NEUT-GI (neutrophil granularity intensity) and NEUT-RI (neutrophil reactivity intensity) are important neutrophilic parameter can be used novel biomarker for diagnostic and therapeutic use in sepsis patients.[16,17] NEUT-GI is a marker of neutrophil activation and measure of cytoplasmic granularity of neutrophil population, representing their response to inflammatory process and infectious disease. NEUT-GI has a unit specified in scatter intensity (SI). Normal reference range for NEUT-GI is 142.8 – 159.3 SI. NEUT-RI is defined as a measure of the fluorescence intensity of the neutrophil population representing their metabolic activity. Standard Unit used for NEUT -RI is florescence intensity (FI). NEUT -RI and NEUT- GI are evolving novel biomarker can be used for screening tool of early sepsis.[18] For early diagnosis and follow up of sepsis patient, serum markers are required both conventional and novel. This study was aimed to find the role of novel biomarkers FGF21, NEUT-GI and NEUT-RI for the diagnosis and prognosis of sepsis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Sepsis is a disease spectrum characterized by overt clinical symptoms that are difficult to treat. The incidence of sepsis has been growing at a rate of 1.5–1.8 percent each year, according to the most estimates. Sepsis and septic shock are two prevalent clinical conditions that are linked to high mortality rates and substantial medical expenses. Sepsis is caused by a malfunction of the host's immune system, which has been linked to organ tissue damage and even death. In low-and medium-income countries (LEDCs/MEDCs), the incidence rate of sepsis has reached approximately 288 people per 100,000 people per year, with a severe sepsis rate of 148 people per 100,000 people per year. The early identification of accurate treatment methods is crucial in the management of sepsis. Sepsis pathophysiology, epidemiology, and other factors are intricately linked to the disease treatment and prognosis.[19]

Sepsis was formally defined in 1992 as the presence of both probable infection and two of the four systemic inflammatory response syndrome (SIRS) criteria. Additional terminology has emerged on various occasions since then. "Severe sepsis" is defined as sepsis complicated by organ dysfunction, whereas "septic shock" is defined as sepsis complicated by hypotension refractory to adequate volume resuscitation in the absence of an alternate etiology. Despite improvement in health infrastructure, low- and middle-income countries (LMICs) are thought to have a disproportionately high rate of sepsis morbidity and mortality due to environmental degradation, widespread malnutrition, and increased rates of bacterial, parasitic, and HIV infection. The Surviving Sepsis Campaign (SSC) was founded in 2002 by the European Society of Intensive Care Medicine (ESICM), the International Sepsis Forum (ISF), and the Society of Critical Care Medicine in an effort to lower the risk of death from sepsis (SCM). Sepsis syndrome may include MODS characterized mainly by altered sensorium, hypoxemia, coagulopathy, oliguria, thrombocytopenia and hyperbilirubinemia. While sepsis is a significant cause of death worldwide, its mortality is believed to be disproportionately high in low- and middle-income countries (LMICs). In 2004, the SSC produced the “Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock,” one of the most recognized consensus statements regarding the treatment of sepsis recently updated in 2012. In the US alone, the incidence of severe sepsis is over 700,000 annually with an estimated 30% mortality. This is estimated to represent over 450,000 emergency Centre (EC) visits per year.[20]

Table 1: SIRS criteria

Presence of two or more of the following	
1. Temperature <36˚C	> 38˚C or
2. Heart rate	>90/min
3. Respiration or PaCO2 <32mmHg	Rate>20/min
4. White blood cell count >11000/µL	<4000/µL or

Source of sepsis

The respiratory, genitourinary, and gastrointestinal systems, as well as the skin and soft tissue, are the most commonly infected. These locations account for more than 80% of all sepsis cases. The most common presentation that leads to sepsis is pneumonia. Bacterial microbes are the most common pathogens (gram-positive bacteria account for 30 percent to 50 percent of total cases); however, a small percentage of patients may develop fungal, viral, or parasitic infections. People at the extremes of age are more likely to develop sepsis. Patients over the age of 65 are multiple times more likely to develop sepsis and have a twofold increased risk of death from sepsis, regardless of race, gender, comorbid conditions, or severity of illness. Malnutrition, chronic illness, immunosuppression, recent surgery or hospitalization, and indwelling catheters or other devices are additional risk factors.[21]

Pathogenesis of sepsis

A bacterial pathogen typically enters a sterile environment where resident cells detect the invader and initiate an inflammatory response. When only a small number of bacteria invade, the local defenses are sufficient to eliminate the pathogens. Macrophages phagocytoses bacteria and secrete a variety of proinflammatory cytokines that initiates the innate immune system's response to the bacterial pathogen. This process almost certainly occurred during patient's first few days of infection. When macrophages begin to produce interleukin (IL)-1, tumor necrosis factor (TNF), and IL-6, as well as chemokines such as IL-8, they are said to be polarized toward an M1 phenotype (CXCL8). Antigen-presenting cells (APCs) such as macrophages and dendritic cells, both resident and recruited, can alert the host to the presence of infection by recognizing pathogen-associated molecular patterns, which are conserved

microbial molecules found in a wide variety of bacteria, fungi, and viruses. Pathogen recognition receptors (PRRs) on APCs recognize such molecules and respond by secreting cytokines that contribute to the innate inflammatory response. Toll-like receptors (TLRs) are the most well-known PRRs, and they recognize a variety of bacterial cell-wall lipoproteins and lipopolysaccharides, as well as fungal-wall elements and bacterial and viral nucleic acids. The costimulatory receptors CD80 and CD86 are upregulated as part of the innate response after macrophage interactions with bacteria, and they participate in innate-adaptive immune interactions. The resident macrophages contain the initial release of bacteria from infective source in an optimal response to the invasion of bacteria into a sterile space. Occasionally bacteria overwhelm the first line of defense, the newly recruited neutrophil eradicates the bacteria.[22]

Additional cells are typically recruited to the site of inflammation to aid in pathogen eradication. Cytokines secreted by resident inflammatory cells stimulate the production of adhesion molecules on the surface of endothelial cells. Circulating white blood cells bind to endothelial cells transiently before being recruited through the vascular wall to the site of inflammation. MicroRNAs have also been linked to the control of adhesion molecules. Neutrophils, lymphocytes, and monocytes are among the cell types found in peripheral blood. Neutrophils are the most common cells in healthy individual, accounting for more than half of all blood cells. Because their nuclei can take on a variety of shapes, neutrophils are also known as polymorphonuclear leukocytes. Phagocytic cells include neutrophils and macrophages in the normal immunocompetent population. Both neutrophils and macrophages kill bacteria through a variety of mechanisms. Phagocytosis of the pathogen is the first step in the phagocytic-cell killing of bacteria. When bacteria enter the host, they are typically opsonized, or covered with host proteins such as antibodies and complement fragments. On the surface of the neutrophil, there are several different receptors that aid in phagocytosis by recognizing the opsonized proteins on the surface of the bacteria. These PRRs include complement receptors and receptors for the Fc portion of immunoglobulins.[22]

Table 2: Clinical manifestations of sepsis and septic shock

Cardiac	Tachycardia, hypotension, poor capillary refill time, cold or clammy skin,
Neurological	Altered mental status, headache
Hematological	Anemia, leucopenia or leukocytosis, thrombocytopenia
Genitourinary	Increased frequency, dysuria, hematuria, costovertebral tenderness and genital discharge
Hepatic	Coagulopathy, jaundice
Pulmonary	Shortness of breath, tachypnea, hyperventilation

There are many scoring systems used for clinical assessment in suspected sepsis, namely the SOFA score, the modified qSOFA score, and the simplified acute physiology score II. SOFA is a scoring system that utilises oxygen levels (partial pressure of oxygen and fraction of inspired oxygen), platelet count, Glasgow Coma Scale score, bilirubin level, creatinine level (or urine output), and mean arterial pressure to determine major organ dysfunction (requirement of vasoactive agents). Multiple organ dysfunction in critically ill patients is routinely monitored using it in clinical and research practice. According to Sepsis-3 defining criteria, SOFA score is a valuable technique for assessing organ dysfunction. Patients with sepsis and a high risk of death can also be identified using a simpler assessment called the qSOFA score. qSOFA identifies severe organ dysfunction and predicts risk of death in sepsis, it needs careful interpretation for defining sepsis. SOFA is better clinical tool than modified qSOFA score in both diagnosis and treatment monitoring but SOFA is quite cumbersome to analyse without proper laboratory investigation.[23]

Septic shock is diagnosed using a complete blood count, which has been around for a long time. In 1992, for example, abnormalities in white blood cell count (either elevated or reduced) or a normal white blood cell count with > 10 percent bands were included in the initial definition of SIRS. Ironically, the two most popular parameters [white blood count (WBC) and bacteremia] may be among the least useful components of the complete blood count. Leukocytosis or leukopenia can occur as a result of septic shock. Many septic patients who have leukocyte count in between these two extremes have a normal WBC (such patients often develop delayed leukocytosis). For example, half of all bacteremia patients who present to the hospital may have normal WBC. As a result, a significantly abnormal WBC may

indicate the presence of infection but not always sepsis. A determination of the absolute neutrophil count must be made if the WBC is extremely low (the absolute number of mature neutrophils plus bands present). Neutropenia is defined as an absolute neutrophil count of less than 500/microliter or a decreasing count in the range of 500–1,000/microliter. Patients with neutropenia frequently fail to show focal signs of infection. For patients with neutropenia, a high index of suspicion for infection is required. For example, the mere presence of a fever generally indicates the need for broad-spectrum antibiotics.(24)

Blood cultures is the definitive diagnostic tool for sepsis, but they only detect bacteremia in about half of patients who are clinically suspected of having sepsis, and they have an even lower rate of positivity while on prior antibiotic therapy. Although the presence of a blood pathogen is a negative prognostic factor, the isolation of such a pathogen is critical for confirming the efficacy of antibiotic therapy, which has been shown to reduce morbidity and mortality. Furthermore, cultures of suspected infection sites do not always predict the results of blood cultures. On the other hand, false-positive bacterial contamination results, on the other hand, may result in unnecessary antibiotic therapy, longer hospital stays, and the selection of resistant microorganisms.(25)

Role of procalcitonin and hsCRP in diagnosis and prognosis of sepsis

Anand et al. in 2012 studied role of hsCRP as prognostic factor in elderly patients with sepsis. Prospective observational study conducted on 200 elderly patients suggests day 14 mortality of 20% in patients of sepsis. The mean serum level of hsCRP was higher (57.28±25.31) in mortality group than survivor group (33.42± 21.56) with p value of <0.001. This study concludes that increased serum level of hsCRP was associated with high mortality rate in old age group with sepsis.(28)

Henry E. Wang in 2013 studied the relation of hsCRP and risk of sepsis. Prospective observational study was done on 30,239 individuals with age >45 years. Baseline hsCRP and characteristics were documented at the commencement of observational study. 11,447 individuals had elevated baseline hsCRP and 974 individuals had sepsis. Conclusion of study shows that increased baseline hsCRP was associated with increased risk of sepsis.(29)

Shiferaw et al. in 2016 studied procalcitonin as a diagnostic marker of sepsis in critically ill patient via systemic review and meta-analysis. Meta-analysis on 30 studies suggests PCT has mean sensitivity of 77% and mean specificity of 79% with (95%) confidence interval.

Conclusion of the study was; PCT is very useful marker for early diagnosis of sepsis in critically ill patient.[26]

Zhang et al. in 2017 studied comparison between procalcitonin and hsCRP for diagnosis of sepsis and septic shock in old age. Prospective observational study was conducted on 70 patients aged 85 or above. Correlation was calculated using spearman's test for hsCRP and procalcitonin. In this study they found that hsCRP was a useful serum test to differentiate sepsis from non- sepsis patients with AUC of 0.819 (95% CI; 0.87- 0.93), sensitivity 78% and specificity 75% (cutoff value = 74.2mg/L).[27]

Stolz D et al. conducted study in 2017 for evaluation of safety and efficacy of PCT guidance compared to standard therapy for prescribed antibiotics in patients with severe infection. Study shows compared with standard treatment PCT guidance reduces the exposure of antibiotics with relative risk of 0.56 and 95% CI; 0.89-0.97.[30]

Schroeder et al. conducted a study in 2017 in ICU surgical unit patients with sepsis with two groups, PCT guided and control group. Drug therapy was given in accordance with microbiological profile. After sepsis resolution, procalcitonin level is decreased 35% of baseline value and antibiotics discontinued. Conclusion of the study was significantly decreased use of antibiotics based on PCT algorithm.[5]

G B Liu et al. conducted a prospective observational study in 2018 to study the role of hsCRP and PCT as early diagnostic modality in pneumonia with sepsis. 220 patients with pneumonia and sepsis were enrolled in study and divided into non-sepsis and sepsis group. It was found that serum hsCRP level was significantly lower in non-sepsis group with p value <0.05. The areas under the receiver operator curve of procalcitonin and hsCRP for sepsis with pneumonia were 0.841 and 0.817 respectively.[31]

Previsdomini et al. conducted a descriptive retrospective study for the prediction of blood culture positivity in critically ill patients. Study was conducted on 231 patients in 2-year period in ICU patients. In these patients; baseline procalcitonin, liver function test, SOFA score and simplified acute physiology score are documented. This study concludes that blood culture was positive in 20% cases and positive blood culture was associated with increased serum level of PCT, liver failure and higher severity score.[25]

Huadong Wang et al. conducted a prospective observational study in 2019 for predictive value of hsCRP and procalcitonin in patients with acute cerebral infarction complicated by

infection. 206 patients with acute cerebral infarction were enrolled. This study concludes that serum level of hsCRP have high predictive value for diagnosis of acute cerebral infraction with infection.(32)

Role of FGF-21 and other newer biomarker in the diagnosis and prognosis of sepsis

Karim Gariani et al. conducted a study in 2013 for the assessment of role of serum FGF21 in patient with sepsis and SIRS. Hospitalized ICU patient with age > 18yrs are selected for study and classified as severe sepsis, septic shock and non-sepsis (SIRS). This study concludes that serum level of FGF21 was significantly higher in patient with sepsis as compared to control.(14)

Xing li et al. conducted a prospective cohort study between January 2019 to December 2020 on 231 patients diagnosed with sepsis and patients were ≥18 years of age. All enrolled patients are categorized into sepsis only group and sepsis with ARDS group. Serum level of FGF21 were taken within 24 hours of diagnosis from both the groups and compared statistically. This study concludes that increase in serum FGF21 level in patients with sepsis with ARDS was associated with increased 28 day mortality rate.(13)

Role of Neutrophil parameters (NEUT- RI and NEUT-GI) in the diagnosis and prognosis of sepsis

Ustyantseva et al in 2019 conducted a prospective case-control study, a total of 40 patients were enrolled for the study. All enrolled patients were categorized into two categories sepsis group and non- sepsis group. Study concludes that elevated level of neutrophilic parameters (NEUT-RI) and (NEUT-GI) had significant association with increased risk of sepsis and these neutrophilic parameters had diagnostic as well as prognostic value.(88)

Study conducted by Kilercik et al. in 2021concluded that NLR (neutrophil: lymphocyte ratio) was significant predictor of sepsis mortality. Neutrophil: lymphocyte ratio >15 was associated with significant risk of developing septic shock particularly in elderly population with multiple risk factors.(89)

-A retrospective case-control study conducted by Wu et al. in 2021 in a secondary care Centre on a total of 21822 patients and patients were divided into survivor and non-survivor group. This study concluded that both low and high (>15) neutrophil: lymphocyte ratio were

associated with elevated mortality rates and inclusion of neutrophil: lymphocyte ratio improved the predictive power of the simplified acute physiology score II.[34]

Role of blood culture positivity in sepsis

In an observational study in 2021, Mallhammer et al. discovered that blood culture negativity was linked to previous antibiotic therapy. There was a similar decrease in the proportion of sterile sepsis patients without prior antibiotic therapy, from 43% to 22% of sterile sepsis patients with prior antibiotic therapy, and a similar decrease in the proportion of bacteremic sepsis patients without prior antibiotic therapy, from 63% to 37% of bacteremic sepsis patients with prior antibiotic therapy. As a result, antibiotic therapy appears to be a predictor of sepsis with no culture. Positive blood cultures were found to be an important predictor of increased mortality in sepsis patients.[25]

Scheer et al. in 2019 conducted a prospective cohort study and enrolled 559 patients and 1364 blood culture samples were sent. In this study, blood culture positivity was 50.6% among patients who had not received antibiotic therapy and 27.7% in those who had received prior antibiotics. (p value <0.001). Gram positive organisms (28.3%) and gram negative organisms (16.3%) were more prevalent in those who had not received antibiotics before culture.[35]

Management of sepsis

Early detection and treatment are critical in septic patients. The Surviving Sepsis Campaign (SSC) proposed a protocolized bundle therapy in 2005 to make it easier to implement at the bedside with a specific goal. In 2015, SSC bundles were reduced from six to three hours. According to 2018 guidelines, this resuscitation bundle treatment, known as the 1-h bundle, should be started within 1 hour of the emergency department (ED) triage time or the earliest chart annotation if presenting from another care venue. The 1-h bundle consists of five elements: measuring lactate, obtaining a blood culture prior to antibiotic administration, administering broad-spectrum antibiotics, initiating rapid administration of 30 mL/kg of crystalloid fluid for hypotension or lactate 4 mmol/L, and administering vasopressors if the patient is hypotensive during or after fluid resuscitation to maintain mean arterial pressure (MAP) at 65 mmHg within 1 h of sepsis.[36]

The roles of intravenous fluids (crystalloids), vasopressors and intravenous antibiotics are well known in the management of sepsis and septic shock. For sepsis-related hypotension (septic shock) or a lactate value of 4 mmol/L, guidelines indicate prompt administration of 30 ml/kg of crystalloid, which is a strong recommendation. Multiple organ dysfunction and overall mortality are reduced when intravenous fluids and vasopressors are used to treat septic shock.[37] Once a sepsis or septic shock diagnosis has been made, adequate intravenous antibiotic therapy should be started immediately, preferably within one hour of presentation and after cultures have been acquired. Antimicrobials should be chosen after considering the patient's history (including previous antibiotics), clinical context (community or acquired), suspected site of infection, presence of invasive devices, local prevalence, and resistance pattern. Early diagnosis of severe infection (sepsis) and prompt administration of suitable antibiotic medication are the most important factors affecting the efficacy of antimicrobial therapy in sepsis. The choice to attribute organ dysfunction to infection is difficult for clinicians, and it necessitates a balance of extensive clinical information and sound clinical judgement.[38]

METHODOLOGY

METHODOLOGY

Aims and Objectives of the study

To evaluate the role of novel biomarkers FGF21 and Neutrophil parameters (NEUT-RI and NEUT-GI) for the diagnosis and prognosis of sepsis.

Primary Objectives

1. To study the sensitivity, specificity, positive predictive value and negative predictive value of serum level of FGF21 in the diagnosis and prognosis of sepsis.
2. To study the sensitivity, specificity, positive predictive value and negative predictive value of neutrophil parameters (NEUT-RI and NEUT -GI) in the diagnosis and prognosis of sepsis.
3. To study the prognostic value of FGF21 and neutrophil parameters (NEUT-RI and NEUT-GI) to predict the day 7 and day 28 mortality in the sepsis.

Secondary Objective

1. To study the comparative analysis of procalcitonin and hs-CRP level with serum FGF21 and neutrophil parameters (NEUT-RI and NEUT-GI) for the diagnosis and prognosis of sepsis.

Study setting: Prospective Observational Study

Study Duration: From January 2020– July 2021

Study Participants:

This study was conducted in hospitalized patients at All India Institute of Medical Sciences Jodhpur. This was a prospective observational study, and no change was done in standard treatment of study subjects.

Inclusion Criteria

- Age ≥18 years
- All patient with clinical features of sepsis with qSOFA score 2 or 3.
- Patient willing to give informed consent.

Exclusion Criteria

- Age <18 years
- Known case of malignancy and autoimmune disease
- Received antibiotics in preceding 2 weeks

Data Collection:

The study was conducted after written informed consent from the study participants. On the first visit to the hospital, baseline assessment of various variables was done which includes

1. Socio-demographic details: Name, age, gender, locality
2. Clinical details: All patients meeting criteria for sepsis

Patients presenting to the emergency department or indoor patients were eligible for enrolment in patient with suspected sepsis (qSOFA≥2) as cases and healthy age and sex matched with no signs of infection were taken as control.

3. Investigations: All cases underwent the following investigations.

(a) Baseline hematological and biochemical assessment as per routine clinical care including Complete blood count (CBC), liver function test (LFT), kidney function test (KFT), Serum electrolytes, Fasting blood glucose, urine microscopy.

(b) Blood culture sensitivity, urine and other culture sensitivity were collected before antibiotics as per clinical need.

(c) Radiological investigations – Chest Xray and Ultrasound whole abdomen done as per history and clinical findings.

(d) Baseline Erythrocyte sedimentation rate (ESR), high sensitivity C- reactive protein (hs-CRP), procalcitonin, ferritin and fibrinogen were done on admission and for follow up on day 3.

(e) NEUT-RI and NEUT-GI were done using automated hematology analyzer.

(f) Sample for FGF21 was collected on the day of admission.

Treatment monitoring is done using hs-CRP and PCT in patient with sepsis. hs-CRP and procalcitonin were done on day 1 and day 3 of hospitalization.

FGF21

Detection of FGF21 was done using ELISA kit (catalogue no. E -EL- H0074)

Principle of test – The Sandwich-ELISA principle is used in this ELISA kit. The antibody specific to Human FGF21 has been pre-coated on the micro-ELISA plate included in this kit. Samples or Standards are mixed with the particular antibody in the micro-ELISA plate wells. After that, each microplate well is treated with a biotinylated detection antibody specific for Human FGF21 and an Avidin-Horseradish Peroxidase (HRP) conjugate. The free components are rinsed away. Each well receives the substrate solution. Only the wells containing Human FGF21, biotinylated detection antibody, and Avidin-HRP conjugate will be colored blue. The addition of solution stops the enzyme-substrate reaction, and the color changes to yellow. At a wavelength of 450 nm± 2 nm, the optical density (OD) is determined spectrophotometrically. The OD value is proportional to the amount of Human FGF21 in the sample.

Sample collection- blood samples were collected and allowed to clot for 10-20 minutes at room temperature and then serum was centrifuged at 2000-3000 RPM for 20 minutes. Centrifuged serum was stored at -80˚C.

Reagent preparation – All reagents were brought to room temperature (20-25˚C) before use for ELISA test.

Wash buffer- It was prepared by diluting wash buffer concentrate 30ml (25X) to 720ml of distilled water.

Standard – Centrifuge the standard at 10,000 g for 1 minute. Wait 10 minutes before adding 1.0 mL of reference standard and sample diluent, then gently rotate it several times. When it has entirely dissolved, thoroughly mix it using a pipette. This procedure produces a workable solution with a concentration of 2000 pg/mL. Add 1 mL of Reference Standard & Sample Diluent, let it settle for 1-2 minutes, and then thoroughly mix it with a low-speed vortex metre. By centrifuging at a low speed, the bubbles formed during the vortex could be removed. Then produces serial dilutions as needed. 2000, 1000, 500, 250, 125, 62.5, 31.25, and 0 pg/ml are the indicated dilution gradients. Dilution method: Fill 7 EP tubes with 500uL each of reference standard and sample diluent. To make a 1000 pg/mL working solution, pipette 500uL of the 2000 pg/mL working solution into the first tube was added and mix well. Following this procedure, pipette 500uL of the solution from the former tube into the latter transferred.

Assay procedure

1. All the specimens and reagent were brought to room temperature (20-25ºC).
2. Total 96 strips were placed into holder.
3. 6 standard dilutants and 90 samples was used.
4. Add 100µL standard or sample to the wells and incubate for 90 minutes at 37ºC.
5. Discard the liquid, immediately add 100µ L biotinylated detection antibody working solutions to each well. Then incubate it for next 60mins at 37ºC.
6. Aspirate and wash the plate for 3 times.
7. Add 100µL HRP conjugate working solutions, further incubate for 30 mins at 37ºC. Aspirate and wash for 5 times.
8. Add 90µL substrate reagent, incubate for next 15mins at 37ºC.
9. Add 50µL of the reaction terminator solution.
10. Read the plate at 450nm immediately

Calculation of the result

1. A standard curve was constructed by plotting the average OD for each standard for each standard on the vertical (Y) axis against the concentration on the horizontal.
2. Using the standard curve and OD of the sample's concentration of FGF21 is calculated.

Neutrophil parameters (NEUT-RI and NEUT-GI)

Detection of NEUT-RI and NEUT-GI were done using automated hematology analyzer.

Principle of the test- NEUT-RI and NEUT GI are considered as marker of neutrophil activation. Neutrophils are primary non- specific cells responsible for innate immunity. NUET-RI and NEUT-GI are measure of fluorescence intensity and cytoplasmic granularity. These parameters are calculated using forward scattering, backward scattering and granularity of the neutrophil population.

Sample collection and procedure - peripheral blood samples were collected from both case and controls. All the blood samples are collected in EDTA vials and processed within 2 hours on a sysmex XN series hematology analyzer.

Calculation of the results- Results for NEUT-RI and NEUT- GI will be calculated using automated analyzer using graphs.

Statistical Analysis

Statistical analysis was done using a statistical package -SPSS 20.0. Descriptive statistics were presented as mean with standard deviation or median with interquartile range in case of continuous variables and percentage were used for categorial variables. Student t test were used to calculate difference of mean for normality distributed variables and Kruskal-wallis test was applied for skewed data. Chi square test was used for calculation of difference in categorial variables. Receiver operator curves were drawn for calculation of sensitivity and specificity of the novel biomarker FGF21 and NEUT-GI for diagnosis of sepsis. Prognostic indicators of outcome were calculated by using multivariate analysis in general linear model. P value <0.05 was considered as statistically significant.

RESULTS

RESULTS

This was a prospective observational study conducted on hospitalized patients in tertiary care center in western Rajasthan. A total of 180 subjects undergone screening for study and total 134 study subjects were enrolled after written informed consent, among them 76 were cases and 60 were age and sex matched healthy controls. Distribution of mean age, sex and locality are depicted in table 3 and figure 1.

Table 3: Baseline characteristics of the study population

Baseline characteristics	Total	Cases	Control	P Value
Gender	134	76 (56.7%)	58 (43.3%)	0.36
Male	110 (82%)	60 (44.8%)	50 (37.2%)	
Female	24 (18%)	16 (11.9%)	8 (6.1%)	
Age in years (Mean ± SD)	47.3±14.6	48.2±18.9	45.5±12.8	0.35
Locality				0.32
Rural	100 (74.6%)	54 (40.3%)	46 (34.3%)	
Urban	34 (25.4%)	22 (16.4%)	12 (9%)	

Figure 1: Gender distribution in the study population

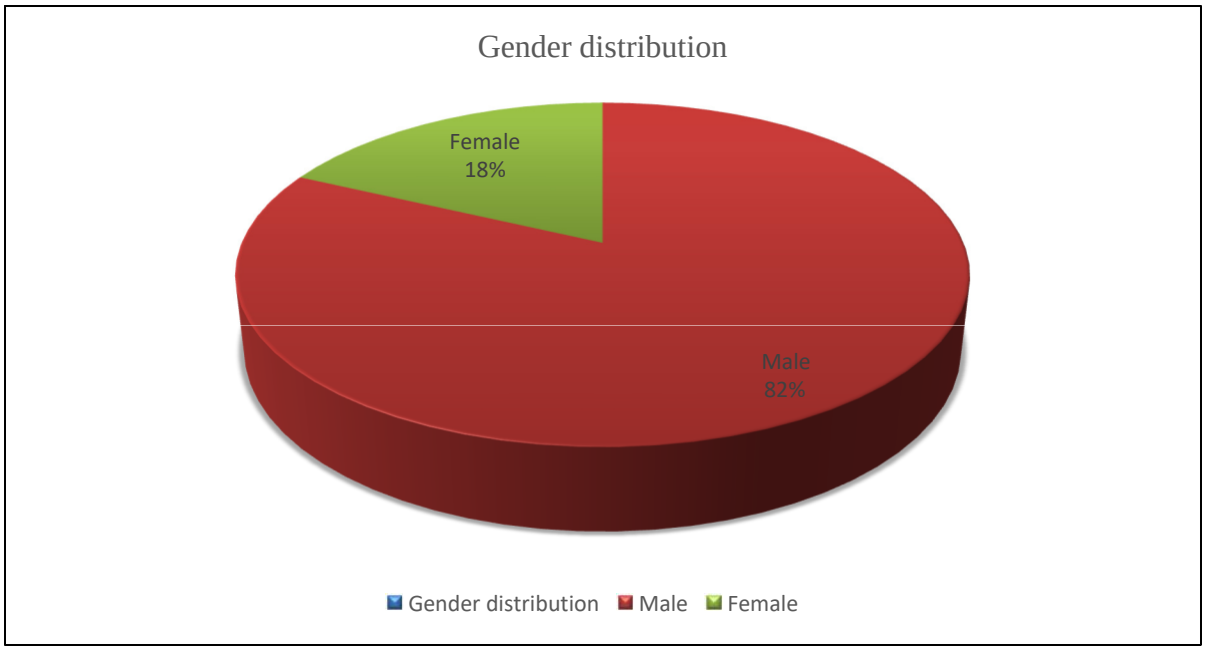
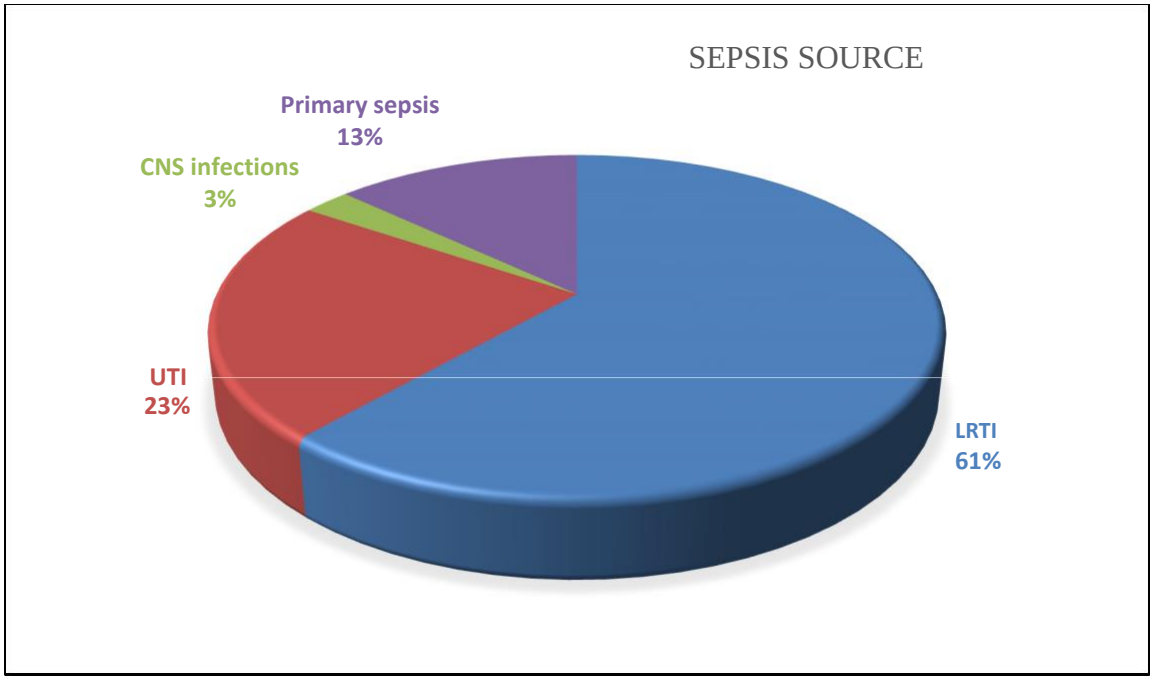


Figure 2: Disease distribution among study population according to sepsis source

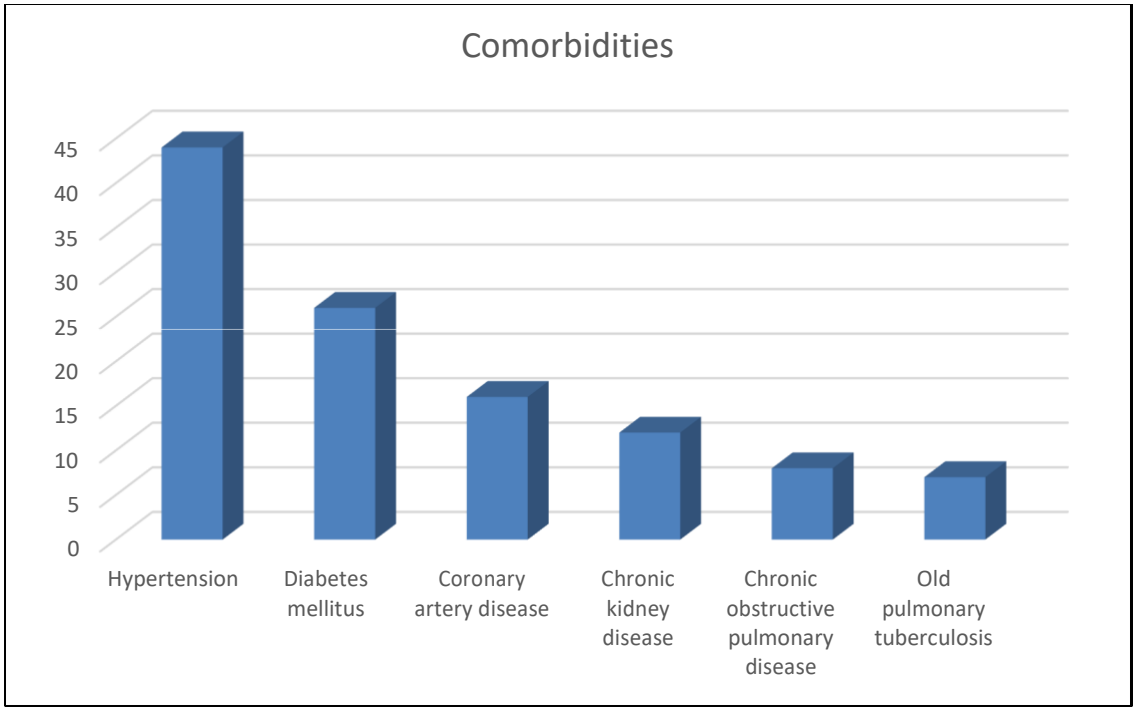


Source of sepsis was identified by careful history taking, clinical examination and laboratory examination. In our study, majority of cases are LRTI (61%) followed by UTI (23%), primary sepsis (13%) and CNS infections (3%). The distribution of the sepsis presentation are depicted in figure 2.

Table 4: Distribution of comorbidities among the study population

Comorbidities	Number of cases (percentage)
Hypertension	44(52.6%)
Diabetes mellitus	26(34.2%)
Coronary artery disease	16(21%)
Chronic kidney disease	12(15.7%)
Chronic obstructive pulmonary disease	8(10.5%)
Old pulmonary tuberculosis	7(9.2%)

Figure 3: Distribution of comorbidities among the study population



The most common comorbidity in sepsis patient was hypertension 44 (52.6%) followed by diabetes mellitus 26 (34.2%). Other comorbid conditions were coronary artery disease 16 (21%), chronic kidney disease 12 (15.7%), chronic obstructive pulmonary disease 8 (10.5%) and old pulmonary tuberculosis 7 (9.2%). The distributions of comorbidities are depicted in figure 3 and table 4.

Table 5: Symptomatology in the sepsis patients

Symptoms	**Number (%)**
Fever	75 (56%)
Cough	67 (50%)
Dyspnea	34 (26%)
Pain abdomen	20 (15%)
Burning micturition	20 (15%)
Altered sensorium	13 (10%)

Table 6: Clinical signs in the sepsis patients

Signs	Number (%)
Pallor	40 (52.6%)
Edema	14 (18.4%)
Abdominal tenderness	10 (13.1%)
Hepatosplenomegaly	8 (10.5%)
Icterus	8 (10.5%)
Clubbing	5 (6.5%)
Cyanosis	2 (2.6%)
Lymphadenopathy	2 (2.6%)

All sepsis patients underwent for detailed clinical history and physical examination. Fever (56%) was most common symptom among sepsis patients. Other symptoms were cough (50%), dyspnea (26%), pain abdomen (15%), burning micturition (15%) and altered sensorium (10%). Pallor (52.6%) was most clinical sign followed by edema (18.4%), abdominal tenderness (13.1%), hepatosplenomegaly (10.5%), icterus (10.5%), clubbing (6.5%), lymphadenopathy (2.6%) and cyanosis (2.6%). (Table 5 and 6) (Figure 5 and 6).

Figure 4: Symptomatology distribution in the study population

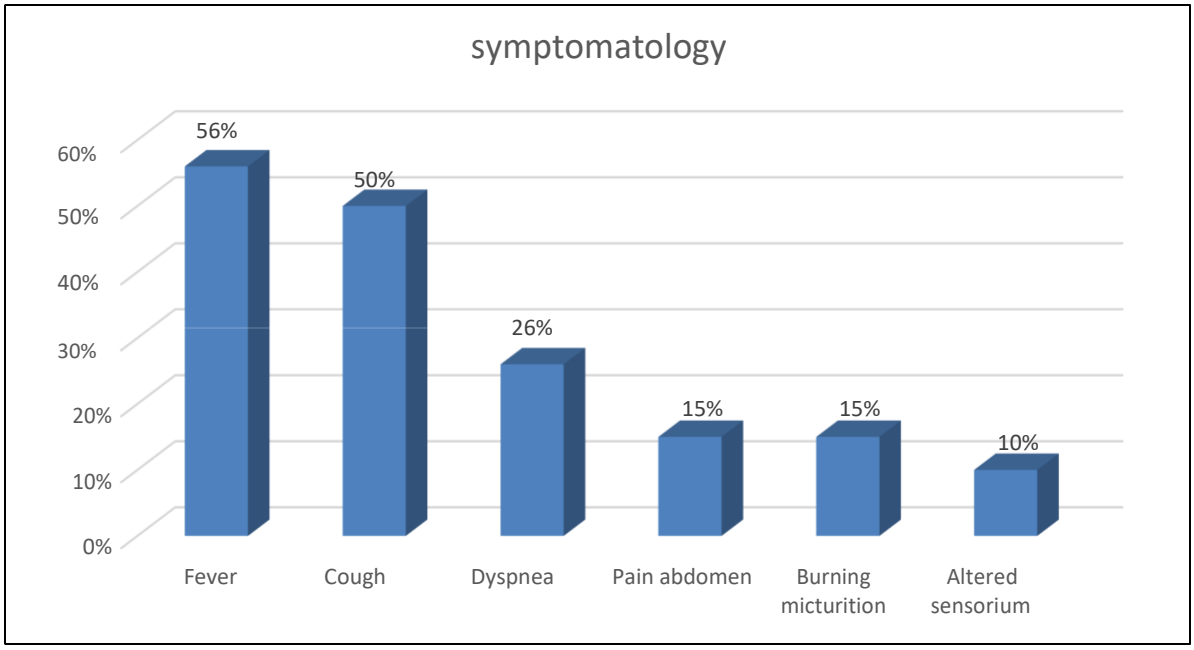


Figure 5: Clinical signs in the sepsis patient

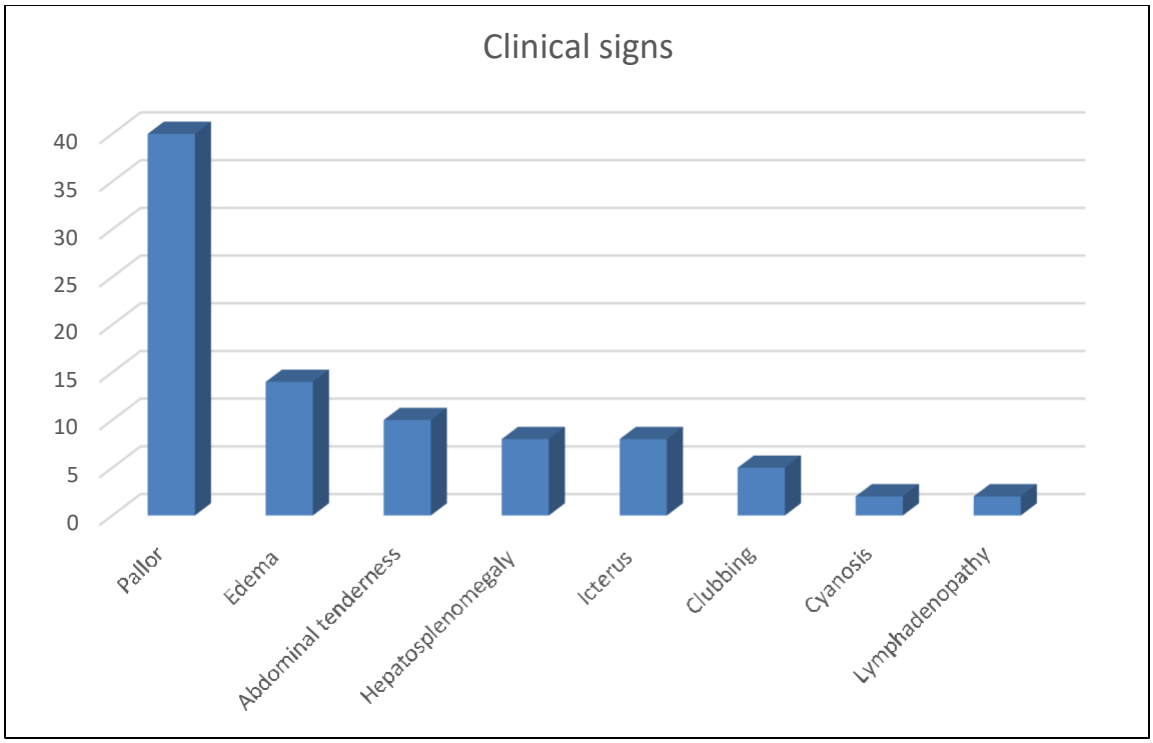


Figure 6: Percentage of patients with blood culture positivity in sepsis patients

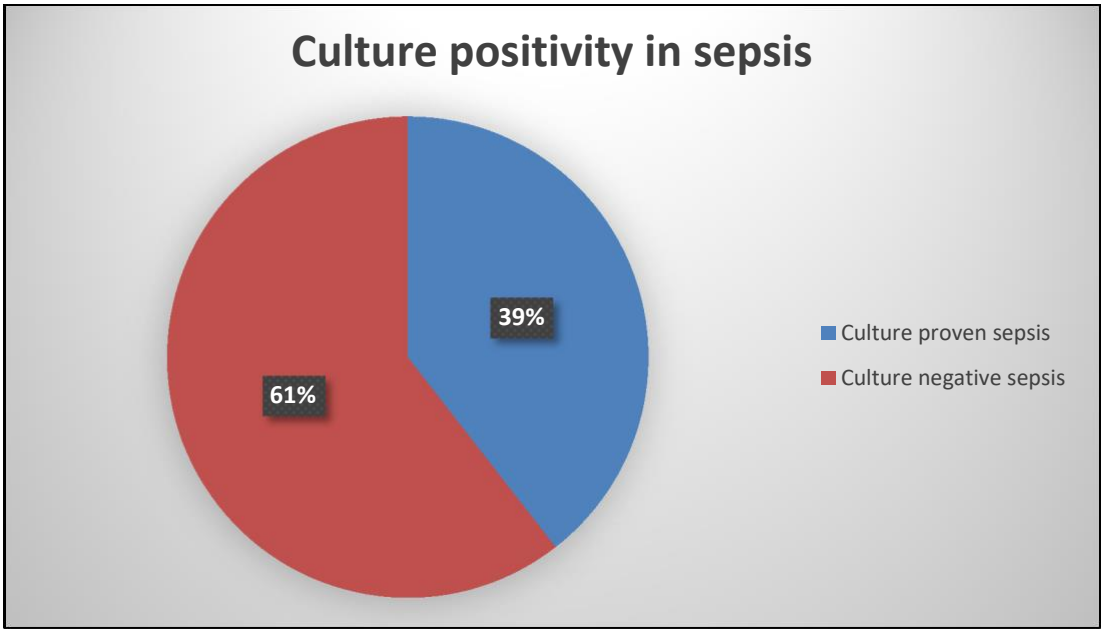
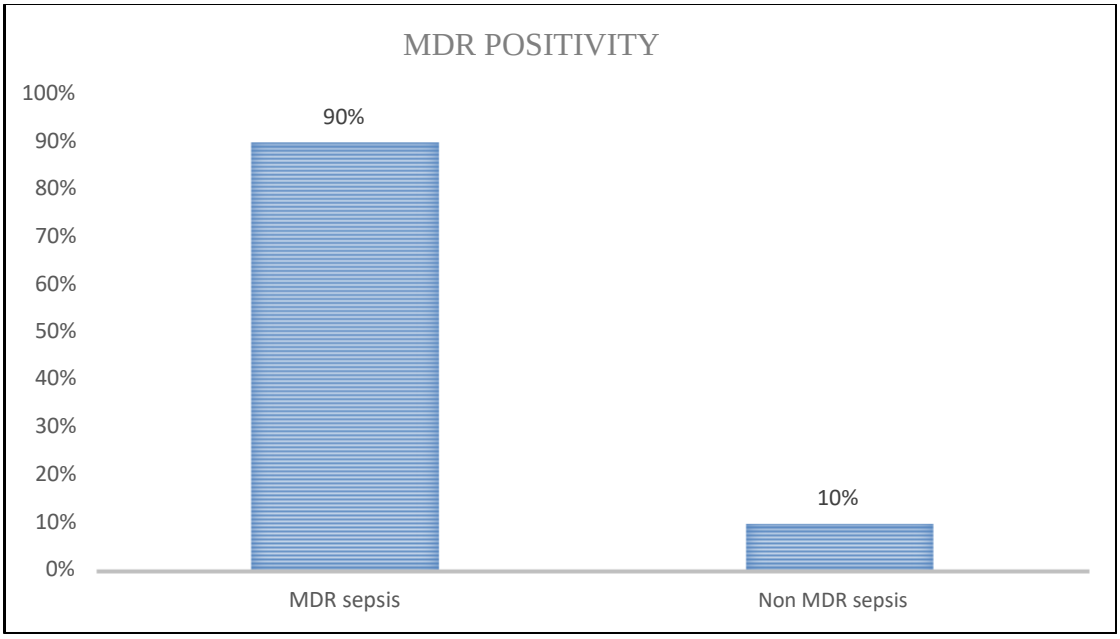


Figure 7: MDR positive organism in culture proven sepsis



Blood culture, and other all relevant cultures were sent for all the enrolled cases in this study. Antibiotic sensitivity was performed with CLSI guidelines in all positive cultures. Majority of cases are culture negative 46 (61%) while culture proven sepsis cases were 30 (39%). Among the culture proven sepsis, the percentage of multidrug resistant cases were 27 (90%)

and non-MDR were 3 (10%). The blood culture positivity and MDR are well illustrated in figure 6 and 7.

Table 7: Vitals examination findings in the study population

Vital Parameters	**(Mean ±SD)**
Temperature (°F)	100.3±1.6
qSOFA (score out of 3)	2.2±0.4
SBP (mmHg)	99.4±17.9
DSP (mmHg)	66.7±12.8
Pulse (beat per minute)	96.4±14.8
Resp rate (per minute)	24.3±4.3
GCS (max score 15)	12.3±2.4

Diagnosis of all patients were done by calculating the qSOFA score for all the patient enrolled in the study. Patients with clinical features of sepsis and qSOFA score ≥2 were eligible for enrollment in the study. In our study we have mean of qSOFA 2.21± 0.41. Vital parameters including GCS were taken for every patient of the study group. The mean GCS was 12.25±2.35. The mean systolic and diastolic blood pressure was 99.42±17.87 and 66.68±12.83 mmHg respectively in the sepsis group. The mean for pulse rate and respiratory rate were 96.42±14.83 bpm and 24.34±4.276 per minute in our study (Table 7).

Table 8: Laboratory values of sepsis cases on day 1 and day 3 of hospitalization

Lab parameter	Mean ±SD (DAY1)	Mean ±SD (DAY 3)	p-value (PAIRED T-TEST)
Hb (gm/dl)	11.47±3.11	10.18±2.73	<0.001
WBC (/µL)	12.58±7.20	11.94±6.19	0.65
Platelet (10^3/µL)	226.37±141.61	216.37±133.61	0.26
Urea (mg/dl)	68.97±63.20	61.63±42.20	0.55
Creatinine (mg/dl)	2.33±2.67	2.95±2.85	0.67
SGOT (U/L)	145.46±57.95	112.36±25.87	0.22
SGPT (U/L)	122.09±47.25	105.09±31.25	0.26
Albumin (gm/dl)	3.00±0.69	2.63±0.70	0.01
Sodium (mmol/L)	133.6±7.90	131.6±7.60	0.49
Potassium (mmol/L)	4.47±0.96	4.01±0.71	0.003

All cases were investigated according with sepsis protocol of institute. Among laboratory investigation, significant renal and hepatic dysfunction were found in cases. Total leukocyte counts are slightly increased from upper normal limit. The mean for urea and creatinine was 68.97±63.20 and 2.33±2.67 mg/dl respectively. Derangement in liver function was characterized by increased transaminase level. The mean value for SGOT and SGPT were 145.46±578.95 and 122.09±479.25 U/L. There was significant difference in mean Hb, albumin and serum potassium level as depicted in Table 8

Table 9: Proven biomarkers in diagnosis of sepsis

Proven biomarkers	(Mean ± SD) (Day 1)	Mean ± SD (Day 3)	p-value (PAIRED T-TEST)
Hs-CRP (mg/dl)	104.50 ± 65.36	102.44 ± 58.13	0.34
ESR (mm/hr)	49.97 ± 5.39	53.48 ± 33.55	0.54
Procalcitonin (ng/ml)	8.27 ± 2.62	23.64 ± 5.25	0.52
Ferritin (µg/L)	1001.13 ± 949.41	922.24 ± 113.35	0.19
Lactate (mmol/L)	2.66 ± 1.01	1.90 ± 1.02	0.001

Table 10: Novel biomarkers in diagnosis and prognosis of sepsis

Novel biomarkers	Cases	Controls*	P Value
FGF21 (pg/ml)	469.6±298.45	250.02±110.72	<0.0001
NEUT-RI day1 (FI)	155.7±38.23	150.14±4.89	0.273
NEUT-GI day1(SI)	54.31±5.26	46.90±3.93	<0.0001
NEUT-RI day3 (FI)	148.7±3.95	150.14±4.89	0.071
NEUT-GI day3(SI)	52.04±4.92	46.90±3.93	0.0001
NEUT-RI day7 (FI)	146.9±4.47	150.14±4.89	<0.0001
NEUT-GI day7(SI)	48.54±.93	46.90±3.93	0.04

*****Neutrophil activation parameters (NEUT-RI and GI) of day 3 and day 7 were compared with day 1 parameters of controls.

All sepsis patients enrolled in study were evaluated by both proven novel biomarkers of sepsis. Proven biomarkers were tabulated in table 10, which showed that levels of all proven biomarkers were raised significantly in patients with sepsis (Table 9). However, controls were not evaluated for proven biomarkers.

Among novel biomarkers serum FGF21 was measured on day 1 of diagnosis of sepsis and neutrophil activation parameters (NEUT-RI and NEUT-GI) were measured on day 1, 3 and 7 after admission, while control groups were tested for both biomarkers only at day 1 of their recruitment. Level of serum FGF21 level and NEUT-GI (day1, day 3 and day7) were found to be significantly higher than the controls (p value <0.0001, <0.0001, 0.0001 and 0.004 respectively) (Table 10). The mean for FGF21 at the time of diagnosis was 469.6±298.45 pg/ml, however for controls the value was 250.02±110.72 pg/ml. Similarly, the NEUT -GI level at day1, day 3 and day 7 for cases were 54.31±5.26, 52.04±4.92 and 48.54±.93 in SI and in control the level was 46.90±3.93 SI. There was significant difference in mean of serum lactate at day 1 and day 3 of admission. The level of NEUT-GI showed the decreasing trend with the duration of illness and treatment. (Table 10)

Figure 8: Receiver operating characteristic (ROC) curve of serum FGF21 for the diagnosis of sepsis.

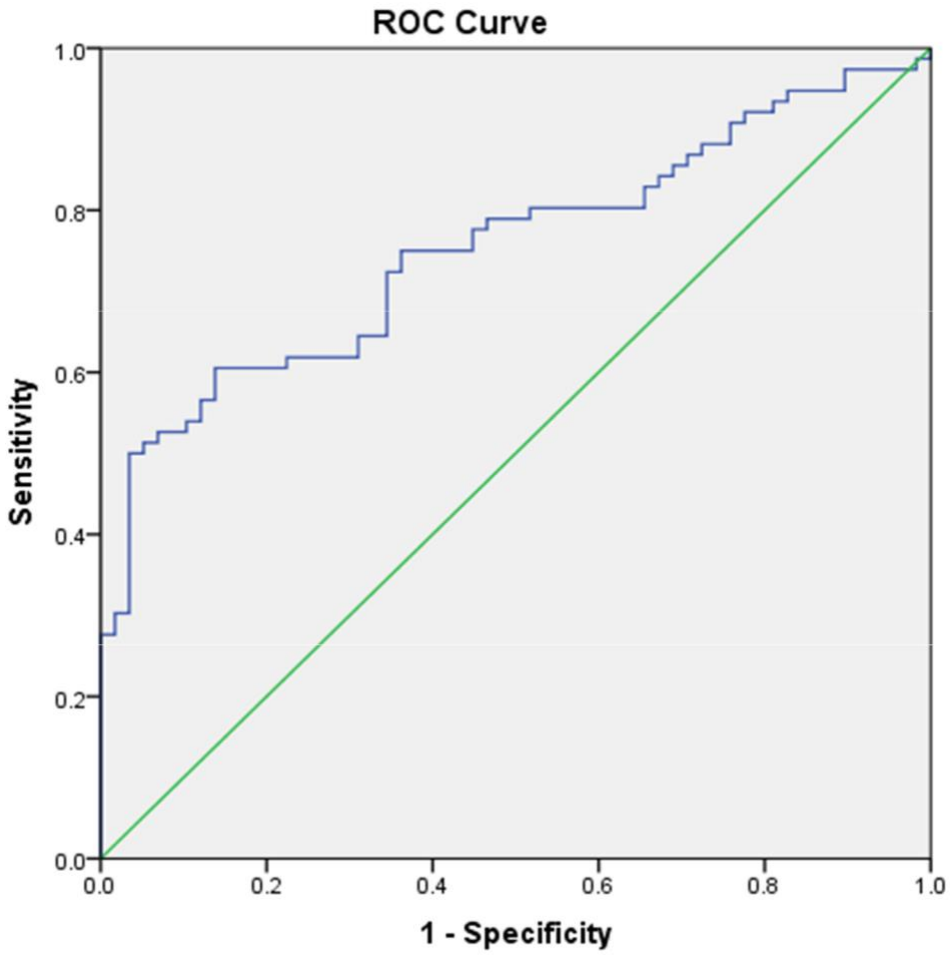


Table 11: Area under curve (AUC) of serum FGF21 level for the diagnosis of sepsis.

Area under curve	95% confidence interval	P value
0.75	0.67 – 0.83	<0.0001

Figure 9: Receiver operating characteristic (ROC) curve of NEUT-GI on day 1 of hospitalization for the diagnosis of sepsis.

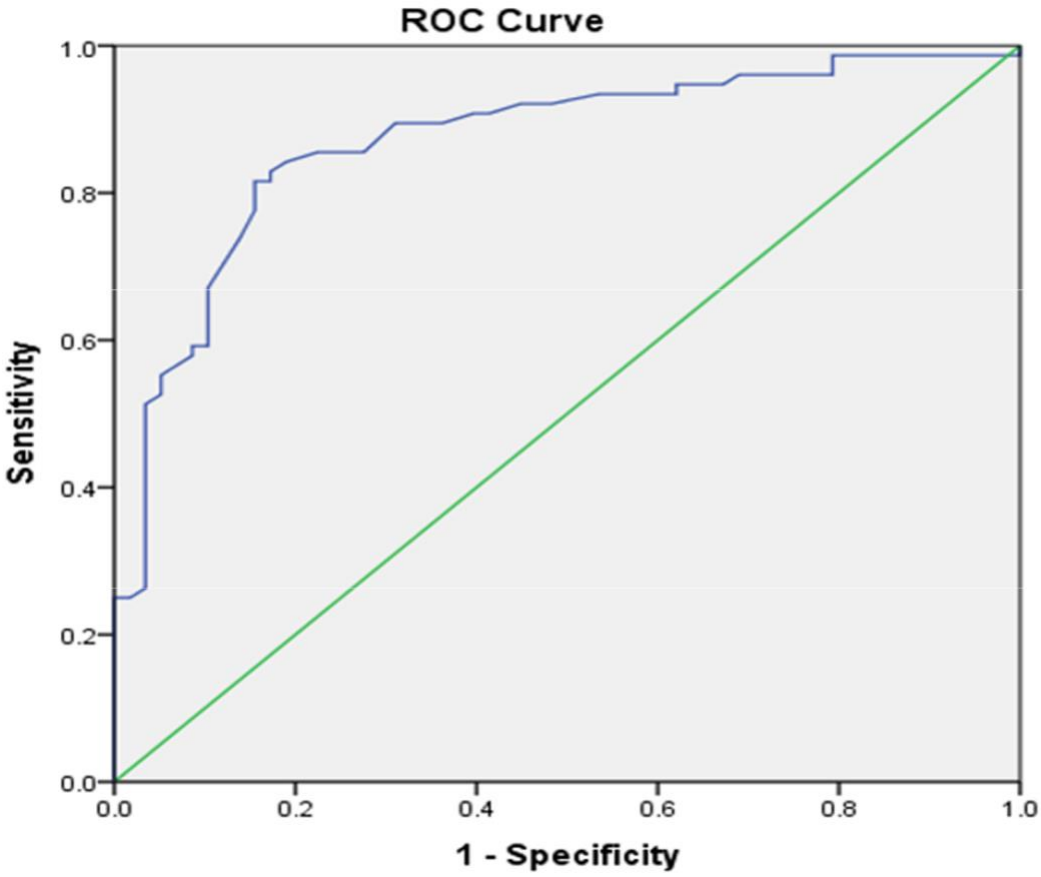


Table 12: AUC of NEUT-GI on day1 of hospitalization for the diagnosis of sepsis.

Area under curve	95% confidence interval	P value
0.87	0.80 – 0.93	<0.0001

Figure 10: Receiver operating characteristic (ROC) curve of NEUT-GI on day 3 of hospitalization for the diagnosis of sepsis.

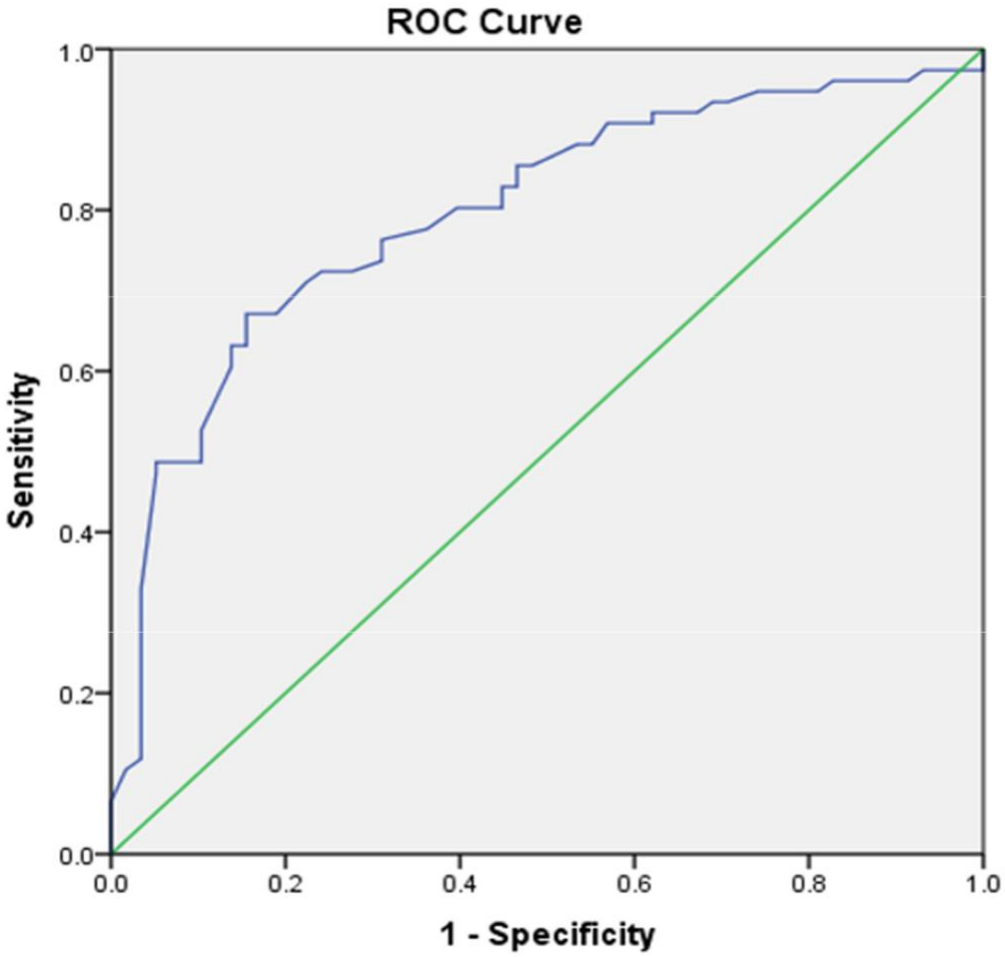


Table 13: AUC of NEUT-GI on day 3 of hospitalization for the diagnosis of sepsis.

Area under curve	95% confidence interval	P value
0.79	0.72 – 0.87	<0.0001

Figure 11: Receiver operating characteristic (ROC) curve of NEUT-GI on day 7 of hospitalization for the diagnosis of sepsis.

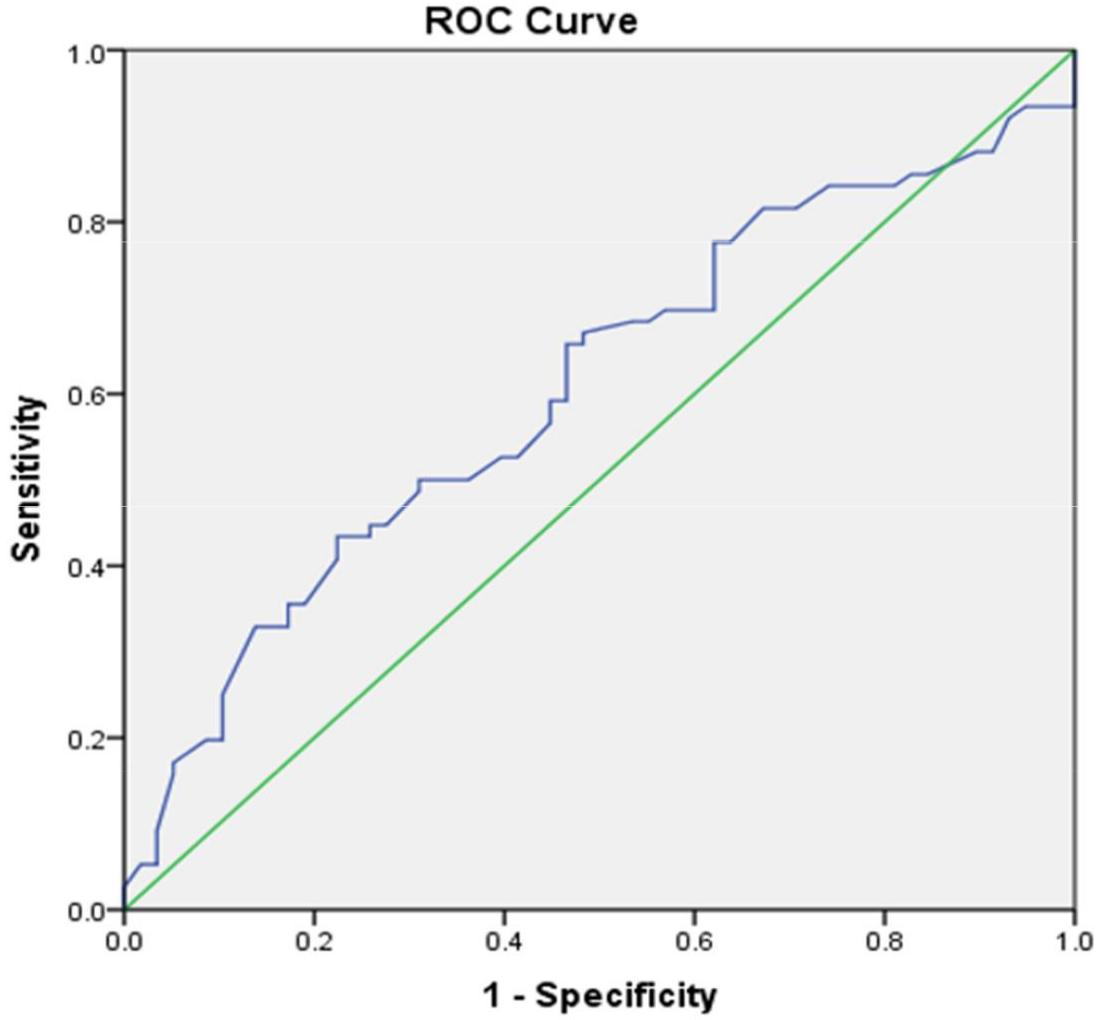


Table 14: AUC of NEUT-GI on day 3 of hospitalization for the diagnosis of sepsis.

Area under curve	95% confidence interval	P value
0.61	0.51 – 0.70	<0.0001

Figure 12: Combined ROC curve of serum FGF 21 and NEUT-GI on day 1 of hospitalization for the diagnosis of sepsis.

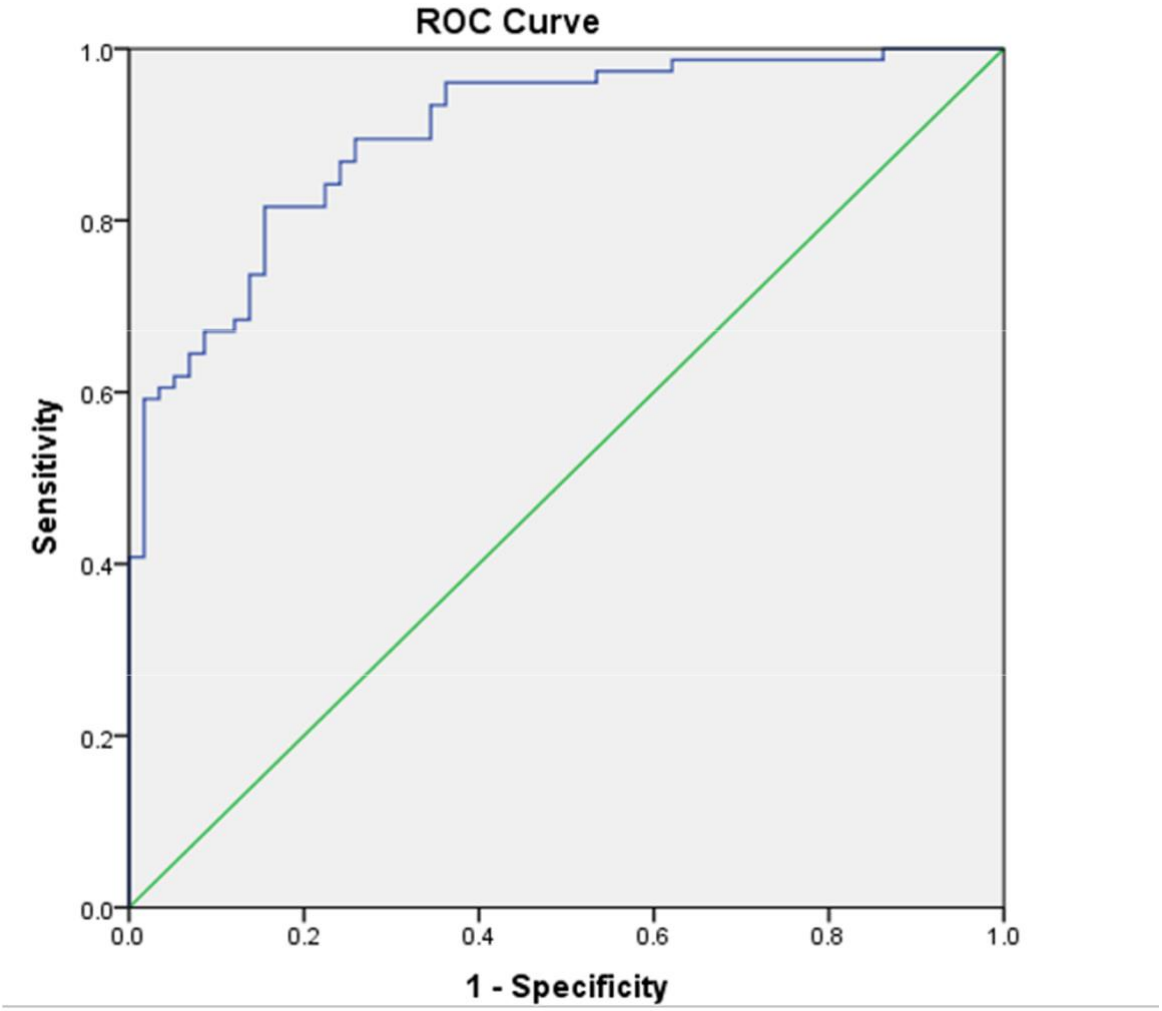


Table 15: AUC of Combined serum FGF 21 and NEUT-GI on day 1 of hospitalization for the diagnosis of sepsis.

Area under curve	95% confidence interval	P value
0.90	0.85 – 0.95	<0.0001

Table 16: Sensitivity, specificity, positive and negative predictive value of serum FGF21 and NEUT-GI level for the diagnosis of sepsis

Novel Biomarkers	Sensitivity	Specificity	Cutoff value	Positive predictive value	Negative predictive value
FGF- 21	75%	63.8%	267.84 pg/ml	73.42%	79.46%
NEUT-GI 1	81.6%	84.5%	50.95 SI	89.47%	86.21%
NEUT-GI 3	76.3%	69.6%	48.25 SI	75.68%	78.56%
NEUT -GI 7	67.1%	51.7%	46.10 SI	69.88%	89.67%
Combined serum FGF21 and NEUT-GI 1	89.5%	74.1%	_	88.67%	78.89%

The diagnostic utility of novel biomarkers serum FGF21 and NEUT-GI were calculated by Receiver operating characteristic (ROC) curve. Serum FGF21 level on day 1 showed sensitivity of 75%, specificity of 66.8% with 89.47% PPV and 86.21% NPV for the diagnosis of sepsis. AUC for serum FGF21 was 0.75 with 95 CI of 0.67 – 0.83. (Figure 9, Table 11, and Table 16). Among the NEUT-GI level, day 1 level was found to be most useful for diagnosis of sepsis with sensitivity of 81.6%, specificity of 84.5% with 89.47% PPV and 86.21% NPV (Table 16). We also evaluated the ROC for combined use of serum FGF21 and NEUR-RI on day 1, which found 89.5% sensitivity, 74.1% specificity, 91.67% of PPV and 78.89% of NPV for the diagnosis of sepsis (Table 10).

Table 17: Mortality in different subgroups of sepsis patients at day 7

		Non-survivor (7)	Survivor (49)
Age	>60yrs <60yrs	4(9.5%) 3 (10%)	32 (69.5%) 17 (56.6%)
Gender	Male Female	5 (9.2%) 2 (8.9%)	36 (62.5%) 13 (62.4%)
Locality	Rural Urban	5 (9.1%) 2 (9%)	34 (66.1%) 15 (60.1%)

Table 18: Mortality in different subgroups of sepsis patients at day 28

		Non-survivor (20)	Survivor (49)
Age	>60yrs <60yrs	10 (26%) 10 (26.7%)	32 (69.5%) 17 (56.6%)
Gender	Male Female	16 (27%) 4 (28.9%)	36 (62.5%) 13 (62.4%)
Locality	Rural Urban	16 (30%) 4 (28.1%)	34 (66.1%) 15 (60.1%)

Table 19: Overall Mortality in different subgroups of sepsis patients

		Non-survivor (27)	Survivor (49)
Age	>60yrs <60yrs	14 (35.8%) 13 (37.6%)	32 (69.5%) 17 (56.6%)
Gender	Male Female	22 (36.5%) 5 (38.6%)	36 (62.5%) 13 (62.4%)
Locality	Rural Urban	21 (33.9%) 6 (39.7%)	34 (66.1%) 15 (60.1%)

There was a total of 27 patients who died of sepsis among the cases. While, overall in-hospital mortality were 36% in this study.

Table 20: Antibiotics used for treatment of sepsis.

Source of sepsis	**Antibiotics (empirical as initial therapy)**	**Antibiotics (definitive therapy after culture and sensitivity reports)**
LRTI	Intravenous ceftriaxone plus macrolide	Intravenous meropenem
UTI	Intravenous piperacillin + tazobactam	Intravenous meropenem
CNS infections	Intravenous vancomycin, and ceftriaxone	
Primary sepsis	Intravenous piperacillin + tazobactam/meropenem	Intravenous meropenem

Selection of antibiotics for treatment of sepsis has been done according to institutional treatment guidelines, which is based on Infectious disease society of America and Surviving sepsis guidelines. Table 17 is showing the antibiotics used as empirical or definitive

commonly, however the individualized therapy had also given according to the culture sensitivity reports considering patients factors.

Table 21: Univariate analysis of factors predicting early in-hospital mortality (day 7) and delayed in-hospital mortality (day 28) in sepsis.

Variables	Early in-hospital mortality (day 7) P- value	Delayed in-hospital mortality (day 28) P – value
Gender	0.46	0.51
Age > 60 (years)	0.98	0.97
Locality	0.22	0.11
Comorbidities	0.13	0.38
TLC > 11000(/µL)	0.07	0.009
Hb <12 (gm/dl)	0.38	0.76
Serum Lactate (mmol/L)	0.95	0.82
Hs-CRP (mg/dl)	0.48	0.50
ESR (mm/hr)	<0.0001	0.70
Serum Procalcitonin (ng/dl)	<0.0001	0.88
Serum Ferritin (µg/L)	0.04	0.43
Blood culture proven sepsis	0.56	0.56
Multidrug resistant organism	0.47	<0.001
qSOFA	0.29	0.02
Serum FGF21(pg/ml)	0.01	0.05
NEUT-RI 1(FI)	0.73	0.63
NEUT-RI 3(FI)	0.58	0.50
NEUT-RI 7(FI)	0.28	0.63
NEUT-GI 1(SI)	0.49	0.80
NEUT-GI 3(SI)	0.43	0.30
NEUT-GI 7(SI)	-	0.001

Univariate analysis of predictors of mortality in sepsis found that raised ESR, raised serum procalcitonin, raised serum ferritin, raised serum FGF21 and higher NEUT-GI at day 7 were associated with early in-hospital mortality (day 7) in sepsis (Table 18). Delayed in-hospital mortality (day 28) was significantly associated with higher qSOFA during hospitalization, culture positive MDR organism, raised serum FGF21 level, leukocytosis and NEUT-GI on day 7 as shown in table 18. Overall, there were a total of 21 non –survivor and 55 survivors in the sepsis patients. Early and delayed in-hospital mortality was 9.2% and 27.6% in this study.

Table 22: Multivariate analysis of factors predicting early in-hospital mortality (day 7)

Variables	**Early in-hospital mortality (day 7)** **P- value**
ESR	<0.0001
Serum Procalcitonin	<0.0001
Serum Ferritin	0.004
Serum FGF21	0.003

Multivariate analysis of predictors of early in-hospital mortality (day 7) in sepsis found that raised ESR, raised serum procalcitonin, raised serum ferritin, raised serum FGF21 were independent risk factors as shown in table 19.

Table 23: Multivariate analysis of factors predicting late in-hospital mortality (day 28)

Variables	**Delayed in-hospital mortality (day 28)** **P- value**
Serum FGF21	<0.0001
MDR organism in culture	<0.0001
Higher qSOFA	0.004
NEUT-GI (on day 7)	0.003

Multivariate analysis of predictors of delayed in-hospital mortality (day 28) in sepsis found that raised serum FGF21, higher qSOFA, MDR organism in culture and NEUT-GI (on day 7) were independent risk factors as depicted in table 19.

DISCUSSION

DISCUSSION

Sepsis is disease spectrum of dysregulated host response and multiple organ dysfunction caused by infective organisms. This is one of the most common causes of mortality among infectious diseases worldwide despite paramount improvement in medical science. Culture sensitivity is gold standard test till date, but time consumption is major disadvantage. Delay in the diagnosis of sepsis leads to significant increase in mortality and morbidity even after adequate treatment. In cases of septic shock mortality rate may approach to 50% due to delayed diagnosis and even further in developing countries due to inadequate health facility. There are many studies have been done to find the role of biomarkers in early and effective diagnosis of sepsis[26,30,38,91]. Among all only few biomarkers like hs-CRP, serum procalcitonin have proved their role in diagnosis of sepsis. Moreover, there still a great lacuna in effective and early diagnostic modalities in sepsis, especially in resource constrained settings. This study has focused on novel markers like serum FGF21 and NEUT-GI, and these markers are showed promising results in the early diagnosis and prognosis of sepsis through some observational studies[14,15,101]. However, there effective role in management of sepsis is yet to be established.

Demography of sepsis

In this prospective observational study, a total of 134 patients were analyzed. The mean age of study subjects was 48.2±18.9 years. In contrast to our study by Arvind Anand et al., showed mean age of sepsis patients was 67.62 years ± 6.69 years and most of them were elderly (range 60-69 years).[28] Another study by Wang et al., also found age of patients with sepsis were ≥45 years.[38] Similarly, Zhang et al., shows that sepsis population in their study was 85 years or above.[27] However, similar to the present study, Previsdomini et al., found that blood culture positive sepsis common in younger age group.[25] Study conducted by Pregernig et al., also showed that sepsis is common in younger age group.[39] While, study conducted by Chin et al., showed that there were no difference in incidence based on age of patients admitted in ICU with sepsis.[40] Majority of studies had included older population in their study, few studies were done on adults and younger populations. In our study, we have taken study population ≥18 years of age.

Most of the cases in this study were male (82%). Similar results were observed in studies done earlier in sepsis patients. Study by Sakr et al, on influence of gender on the

epidemiology and outcome from severe sepsis in 2013 shows prevalence of sepsis in female was lower than male but increased mortality in female patients.[41] Male gender was found to be an independent risk factor for the development of sepsis and septic shock according a study.[42]

Among total cases 71% were belonged to the rural locality, while only 29% cases were from the urban locality. Incidence of sepsis and related mortality was more in rural patients with low socio-economic status according to a recent ecological study done by Rose et al.[43] In urban area, the incidence of sepsis was comparatively lower in higher economic class compared with low economic classes.[44] In our study, incidences of sepsis is higher in rural population and the probable explanation would be lack of effective diagnostic modalities at the interior area of our geographical region and most of the suspected patient are referring to tertiary care center.

Primary sources of sepsis

In present study, the most common primary source of sepsis was LRTI (61%), followed by urinary tract infection (23%), primary sepsis (13%) and CNS infections (3%). Similar to our result, study done by Vijay et al., showed that the incidence of LRTI induced sepsis along with MDR organism were emerging in the general population.[45] Another study conducted by Xiaoying et al., showed LRTI had a higher incidence as a primary source of sepsis.[46] Study conducted by Purba et al. in 2020 concluded that multifocal infection and lower respiratory tract infection were the leading source of sepsis.[47] However, contrary to our results, study conducted by Hsiao et al., showed that urinary tract infection is the leading cause of sepsis among adults and frequently leads to hospitalization.[48] Similarly, another study in 2020 also found urinary tract infection as emerging risk factor for septic shock particularly in old age population.[49] Incidence of urinary tract infection was 44.1% followed by primary bacteremia (39.8%) and lower respiratory tract infection (23%) in a study conducted by alexander et al. in 2014.[50] A study conducted by Robertson et al., concluded that the incidence of central nervous system infection leading to sepsis is increasing in developing world.[51]

Comorbidities associated with sepsis

In our study, hypertension (52.6%), followed by diabetes mellitus (34.2%), were the major comorbid conditions associated with sepsis patients. Other comorbid conditions were

coronary artery disease (21%), chronic kidney disease (15.7%), chronic obstructive pulmonary disease (10.5%) and old pulmonary tuberculosis (9.2%). A longitudinal cohort study conducted in 2012 concluded that any chronic medical conditions, i.e., diabetes mellitus, coronary artery disease, or chronic kidney disease, increases the risk of sepsis.[54] Another study on the relationship between chronic disease and sepsis risk was done by Tran et al. also found increased incidences of sepsis with longstanding comorbid conditions.[55] In a systemic review and meta-analysis done in 2020, concluded that higher incidences of septic shock and AKI were associated with patients had hypertension, diabetes mellitus and blood culture positivity.[52] Another retrospective study showed that multiple comorbid conditions increase the incidence of septic shock in ICU patients.[57] There are multiple risk factors that increase the incidence of sepsis and mortality associated with the sepsis spectrum. A study conducted by Frydrych et al. in 2017 shows that diabetes mellitus was an important risk factor for the development of sepsis.[48] A similar study shows that uncontrolled diabetes mellitus is a well-documented independent risk factor for the development of sepsis.[49] Another study shows that neutropenia was associated with an increased risk of sepsis in an observational study.[50] There was increased risk of sepsis in chronic liver disease patients in a study conducted by in 2020.[53] Williams er al., also found increased risk and mortality of sepsis in hospitalized patients with malignancy.[54] In a prospective observational study conducted by Bohnen et al., concluded that use of steroids was associated with increased risk of sepsis.[55]

Clinical features of sepsis

The most common symptom in this study was fever (56%) followed by cough (50%). Other symptoms were dyspnoea (26%), pain abdomen (15%), burning micturition (15%) and altered sensorium (10%). Similar to this study, the common presentations of sepsis were fever and altered mental status.[56] Fever and chills along with hypotension were the main presentation in patients with urosepsis.[57] Patients presented with complaints of urosepsis in which 80% cases are associated with obstructive uropathy in a study conducted by Dreger et al.[58] The presenting manifestation of primary sepsis were altered mental status, delirium, malaise and urinary incontinence in a study by Nasa et al.[59] Likewise, altered sensorium, delirium, restlessness and altered sleep wake pattern were the presenting symptoms in elderly with septic encephalopathy.[60] Genga et al., also reported that altered mental state along with hypotension were the early signs of sepsis in elderly.[61] Study in ICU setting by Pascale et al., found that fever and dyspnoea were major clinical presentation hospital acquired

pneumonia.[62] Fever with dyspnoea were major presenting complaints in patients with severe pneumonia with sepsis in a study by wood et al.[63] In a study done by Michael et al., approximately 20% of patients developed hypotension in sepsis, which also increased the hospitalization rates.[64] The most common sign in this study was pallor (52.6%) in followed by edema (18.4%), abdominal tenderness (13.1%), hepatosplenomegaly (10.5%), icterus (10.5%), clubbing (6.5%), cyanosis (2.6%) and lymphadenopathy (2.6%). Pallor was manifestations of anaemia which was mainly due to nutritional deficiency, pre-existing chronic illness or sepsis related. Many studies had been conducted for evaluation of anaemia in sepsis patients. Incidence of sepsis related anaemia was significant in males but comparably equal in both cases and controls in a retrospective study.[65]

Scoring system in sepsis

The qSOFA model (affected mental status, respiration rate greater than or equal to 22 breaths/min, and systolic blood pressure less than or equal to 100 mmHg) was developed and validated as a method to detect sepsis in patients with suspected infection who were not admitted to the ICU. As per Sepsis-3, the Surviving Sepsis Campaign suggested that qSOFA with at least two out of three factors may be used as a supplementary screen to identify individuals at risk for clinical deterioration in patients who have screened positive for infection.[66] We used qSOFA scoring ≥2 for the screening of patients with suspected sepsis and found that patients with higher qSOFA values had an increased incidence of sepsis.

Proven biomarkers in diagnosis of sepsis

The mean value for hs-CRP was significantly raised in cases of this study as compare to reference range of general population. In our study, the mean value for hsCRP on day 1 was 104.50 ± 65.36 mg/dl and day 3 was 102.44 ± 58.13mg/dl. Our institutional guideline for management of sepsis is using the hsCRP as an important marker in diagnosis as well as prognosis of sepsis. Similar to our results, the mean value for hsCRP was 91.68± 73.53 mg/dl in study by Youssef et al.[67] Recent study by Ma et al., also found hsCRP was significantly raised (129.93± 73.53 mg/dl) in sepsis as well as septic shock.[68] Study by Wang et al in 2013 concluded that elevated serum level of hsCRP was associated with sepsis.[29]

Serum procalcitonin mean value was raised in cases of this study, similar to the elevated hs-CRP level. Procalcitonin was useful for the diagnosis of bacteraemia in sepsis and have significant diagnostic value when combined with blood culture in a study by Nakamura et al.

in 2009.[69] In a systemic review and meta-analysis by Hoeboer et al., concluded that procalcitonin had a fair diagnostic efficacy in hospitalised adults.[70] Procalcitonin was a good diagnostic marker and was used for monitoring of antibiotics therapy in adults according to a study conducted by Vijayan et al in 2017.[30] A retrospective cohort study conducted by Tsui et al. in 2021 in which ICU patients of regional hospital were enrolled. This study concluded that procalcitonin based score performed well in diagnosis of sepsis with significant AUC, sensitivity and specificity.[71] We are also using the serum procalcitonin level as an early marker for diagnosis of sepsis, which helps us in initiation of effective antibiotic therapy. Moreover, serum procalcitonin level also guide the optimization regarding de-escalation and duration of antibiotics in sepsis.

In a similar study, which was conducted by Jain et al., in 2014 concluded that serum procalcitonin level ≥7ng/ml correlates with increased risk of sepsis.[72]

Table 24: Studies assessing serum procalcitonin concentration level in sepsis patients with diagnostic and prognostic values

Author	Year	Results
Nakamura et al.	2009	Procalcitonin was useful for the diagnosis of bacteraemia in sepsis and have significant diagnostic value when combined with blood culture
Jain et al.	2014	Serum procalcitonin level ≥7ng/ml correlates with increased risk of sepsis and sepsis associated early mortality
Hoeboer et al.	2015	Procalcitonin had a fair diagnostic efficacy in hospitalised adults
Vijayan et al.	2017	PCT was used for monitoring of antibiotics therapy
Tsui et al.	2021	PCT based scoring have good diagnostic efficacy with significant sensitivity and specificity

A prospective observational study conducted on comparison of hsCRP and procalcitonin for the diagnosis of sepsis by Zang et al. in 2017 shows that hsCRP was non-inferior to procalcitonin for diagnosis of sepsis particularly in old patient.[27] A study conducted by Nargis et al. in 2014 for comparison of diagnostic efficacy of hsCRP and procalcitonin concluded that PCT has greater diagnostic value as compared to hsCRP and more effective in assessing the severity of sepsis.[73] A study conducted by Nargis et al. in 2014 for

comparison of diagnostic efficacy of hsCRP and procalcitonin concluded that PCT has greater diagnostic value as compared to hsCRP and more effective in assessing the severity of sepsis.[73] A retrospective analysis conducted by Sui et al. in 2020 concluded that procalcitonin had higher diagnostic value as compared to hsCRP.[74] In our study, both PCT and hsCRP were significantly raised in sepsis patients.

Table 25: Studies assessing the comparison between hsCRP and PCT for diagnosis in sepsis

Author	Year	Results
Nargis et al.	2014	PCT had greater diagnostic value than hsCRP in sepsis
Zang et al.	2017	hsCRP was comparable to PCT for sepsis diagnosis especially in elderly population
Sui et al.	2020	Serum level of PCT had greater diagnostic value than hsCRP

Novel biomarkers for diagnosis of sepsis

In this study, the mean value of serum FGF21 was significantly higher in patients with sepsis compare to healthy controls. The sensitivity and specificity of serum FGF21 was 75% and 63.8% respectively with a cutoff 267.48 pg/ml in diagnosis of sepsis with qSOFA ≥ 2. PPV and NPV were 73.42% and 79.46% respectively. The FGF21 is secreted from multiple sources from the body and its blood level increased significantly in patients of sepsis.[75] Previous study also found that, plasma level of FGF21 was found to be significantly higher (approximately 10 times) in sepsis group compared to controls.[14] Similarly, study by Siahanidou et al., showed that serum level of FGF21 was significantly high in neonatal sepsis as compared to control groups.[15] The mean value of serum FGF21 in patients with sepsis was 496.6±298.45pg/ml, while in healthy controls was 250.02±110.72 pg/ml in this study. Among literature search, the data on diagnostic utility of serum FGF21 are sparse.

Neutrophils are major cells in innate immunity and activation of these cells occurred as early as initiation of infection in human body. There are multiple neutrophil activation parameters evaluated in various studies in patients with sepsis. Role of automated neutrophil parameters, NEUT-X, NEUT-Y and NEUT-Z have been studied by Luo Y ei al., especially in patients with malignancy and sepsis. This study found the usefulness of these parameters for rapid diagnosis of sepsis.[76] Neutrophil volume, conductivity and scatter parameters have also studied and found to be raised in sepsis but the study focused only neonates.[77] The present study has evaluated the role of neutrophil activation parameters (NEUT-RI and NEUT-GI) in

diagnosis of sepsis, which can be easily calculated in automated hematology analyzer. We found that NEUT-GI was significantly higher in patients with sepsis when compared to healthy controls. NEUT-GI level on the day of hospitalization was found to be a good sensitivity, specificity, PPV and NPV of more than 80% in sepsis with qSOFA ≥ 2. Recent study also found that elevated level of neutrophilic parameters (NEUT-RI) and (NEUT-GI) had significant association with increased risk of sepsis and these neutrophilic parameter had diagnostic as well as prognostic value.[78] NEUT-RI level is not found to be significantly different in sepsis and healthy controls in our study. However, previous study has showed that, NEUT -RI was significantly elevated in sepsis as compared to non-sepsis and strongly correlates with other biomarkers (PCT and CRP) of sepsis.[79] There are dearth of data regarding the utility if NEUT-RI and NEUT-GI for diagnosis of sepsis.

Table 26: Study assessing novel neutrophil parameters in sepsis patients with diagnostic and prognostic value

Author	Year	Results
Ustyantseva et al.	2019	NEUT-RI was significantly elevated in sepsis patients

Predictors of mortality in sepsis

Sepsis-associated mortality continues to be an important concern despite in advancement in diagnosis and invent of newer antibiotics. Sepsis related mortality was dependent on various factors. Early and delayed in-hospital mortality were 9.2% and 27.6% in this study. A univariate analysis of predictors of early in-hospital mortality showed that raised ESR, raised serum procalcitonin, raised serum ferritin, raised serum FGF21 were associated with significant mortality in sepsis patients. Higher qSOFA during hospitalization, culture positive MDR organism, raised serum FGF21 level, and NEUT-GI on day 7 were all associated with delayed in-hospital mortality (day 28). Many studies conducted have been conducted to find various predictors of mortality in sepsis.

In present study sepsis related mortality was equal in male and female as depicted in univariate analysis of predictors of mortality. Similar to a previous study, the mortality rate was found to be higher among females admitted to the ICU.(80) Similar to the our study, study by Nachtigall et al., concluded that there was no significant difference in mortality outcome based on gender in sepsis. Likewise, study by Papathanassoglou et al., showed that

there was no significant gender-dependent mortality among the sepsis.[81] In contrast to our study, Nosheen Nasir et al., found that mortality was higher male as compared to female.[82] Another study conducted on mortality in ICU patients and its relationship with gender found that women have more risk of death as compared to male.[83] Similarly, study conducted by Soumitra R et al., concludes that female sex is independent risk factor of increased mortality in critically ill patients.[83]

Association between extremes of age and sepsis related mortality were well established in various. While, in our study we did not found association between age and sepsis associated mortality. In contrast to this study; a prospective multicenter study concluded that, patients aged 80 or over had higher hospital mortality compared to patients aged 65–79 years. Similarly, age was found to be an important risk factor in the elderly sepsis patients.[84] Another similar study conducted in 2021 concluded that mortality rate was significantly high in elderly patients with sepsis.[85] Neonates have higher mortality rate as compared to older children in sepsis.[86]

All the enrolled patients with sepsis were also categorized into rural and urban based on locality, and the results were analyzed to correlate locality with sepsis related mortality. Previously, many studies conducted to establish the correlation between locality and mortality in sepsis. The difference in sepsis mortality have been decreased between urban and rural areas and elderly males were at increased risk of sepsis in a study conducted by Chen et al in 2015.[87] Another study concluded that sepsis related mortality was higher in urban area with high population density than low population density.[44] Risk of mortality in sepsis increased in patients who come directly to tertiary care centre without seeking any treatment in primary Centre according to a observational cohort study conducted by Mohr et al. in 2016.[88] There was no association of sepsis mortality and locality in this study.

A cohort study done in 2011 by Prebil et al., concludes that presence of comorbidities were not associated with increased mortality in sepsis.[89] Comorbidities were one of the most important predictor of sepsis related mortality according to a retrospective cohort study conducted by Yong yang et al in 2010.[90] Patients with preexisting COPD was strongly associated with mortality in sepsis group, a study conducted by Chen et al. in 2018.[91] We have not found any association between sepsis related mortality and comorbidity in our study.

Study conducted by kopanitsa et al., in 2021 concludes that pregnancy as a risk factor increases the mortality in sepsis.[92] A study conducted by Singh et al in 2016 concluded that

use of glucocorticoids was associated with increased risk of sepsis mortality.[93] Age was independent risk factor associated with high mortality as per prospective cohort study done by martin et al on 2019.[84] Mortality outcome in sepsis increases in people living with HIV-AIDS in a study conducted by Japaissu et al in 2010.[94] High mortality was associated with sepsis in post renal-transplant patients according to study conducted by de Carvalho et al in 2014.[54] We have not included the above factors in our study as per selection criteria.

A study conducted by Xiaoying et al., showed LRTI had a higher incidence as a primary source of sepsis in critically ill patients.[46] Study conducted by Purba et al. on 2020 concluded that multifocal infection and lower respiratory tract infection were associated with high mortality.[47] In our study, we found that mortality associated with LRTI and UTI were 33% and 27% respectively.

Leukocytosis and leukopenia both are important factors predisposing the risk of sepsis and leads to increased sepsis related mortality. In the same view; a retrospective cohort study conducted by Belok et al in 2021 concluded leukopenia was associated with higher mortality risk as compared to controls.[95] Liver dysfunction was associated with long term mortality in septic shock in a study conducted by Nesseler et al. in 2013.[96] We had renal dysfunction, hepatic dysfunction, and thrombocytopenia as part of sepsis associated MODS in our prospective observational study. A prospective observational study conducted by Jung et al in 2019 concluded that low haemoglobin level <9gm/dl was present in 20% cases of septic shock and was associated with high mortality rate in septic shock.[97] Moderate anaemia was significantly associated with mortality in sepsis patients according to recent study by Ten et al.[98] Elevated level of serum bilirubin within 72 hours of the admission increases the risk of mortality in septic shock.[99] In our study leucocytosis was found to be an important predictor of delayed in-hospital mortality (day 28).

The elevated serum hsCRP level was not significantly associated with mortality among sepsis patients in this study. In contrast, elevated level of hsCRP was associated with high mortality in sepsis patients in a study conducted by Anand et al., in 2013.[28] Similarly, increased level of hsCRP was associated with high mortality in study by Oh et al,. in 2017.[100] In comparison of hsCRP, Presepsin was an early predictor of in-hospital mortality in a study conducted by Hasan et at. in 2019.[101] Also, we have significant association between increased procalcitonin and sepsis mortality at day 7 of hospital admission in present study. Similarly a study by Jain et al. in 2014 concluded that serum procalcitonin level ≥7ng/ml correlates with

sepsis associated early mortality.[72] Serum concentration of both biomarkers of sepsis (hsCRP and PCT) was significantly higher in non-survivor group admitted in ICU but sensitivity of PCT and procalcitonin were 94.64% and 83.63% respectively according to a study conducted by Suhua et al. in 2017.[18] Another study conducted by Cui et al. in 2019 concluded that both hsCRP and procalcitonin had similar prognostic efficacy to predict mortality in sepsis.[102] The mean of FGF21 were 3574.7 ng/ml and 986.6 ng/ml in non-survivor and survivor group in sepsis patients respectively in a recent study.[13] In a recent study conducted by Kilercik et al., concluded that neutrophil – lymphocyte ratio was an important predictor of mortality.[103] In this study, NEUT-GI have both diagnostic and prognostic value. NEUT-GI at day 7 have statistically significant correlation with early in-hospital (day 7) and delayed in-hospital (day 28) mortality.

Table 27: Assessment of various biomarkers and their relation to mortality

Author	Year	Results
Anand et al.	2013	Elevated level of hsCRP was associated with high mortality in sepsis
Oh et al.	2017	Increased serum hsCRP was associated with significantly high mortality
Suhua et al.	2017	Serum concentration of both biomarkers of sepsis (hsCRP and PCT) was significantly higher in non-survivor group
Cui et al.	2019	hsCRP and procalcitonin had similar prognostic efficacy to predict mortality in sepsis
Hasan et al.	2019	Presepsin was an early predictor of in-hospital mortality as compared to hsCRP
Ebrahimi et al.	2019	elevated levels of serum FGF21 and 30-day mortality in patients with severe pneumonia.
Kilercik et al.	2021	NLR was an early predictor of mortality
Li et al.	2021	significant association between elevated level of FGF21 and day 28 mortality in sepsis patient hospitalized in ICU

CONCLUSION

CONCLUSION

This prospective observational study found that novel biomarkers like serum FGF21 and NEUT-GI have both diagnostic and prognostic value in sepsis. There is a significant correlation between mortality (at day 7 and day 28 of admission) and novel markers (FGF21 and NEUT-GI) level. Along with novel biomarkers, proven biomarkers like serum procalcitonin and hs-CRP were also significantly raised in patients with sepsis. Along with novel biomarkers, raised markers on inflammation (ESR, serum ferritin) and MDR organism in culture and sensitivity were also found to be important predictors of mortality in sepsis. Incorporation of these novel biomarkers in diagnostic algorithm of sepsis would be helpful in effective management of sepsis.

REFERENCES

REFERENCE

1. 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 Lond Engl. 2020 Jan 18;395(10219):200–11.

2. Chatterjee S, Bhattacharya M, Todi SK. Epidemiology of Adult-population Sepsis in India: A Single Center 5 Year Experience. Indian J Crit Care Med Peer-Rev Off Publ Indian Soc Crit Care Med. 2017 Sep;21(9):573–7.

3. Ghanshani RV, Gupta R, Sood S, Bansal A, Joad SHK, Khedar RS. Epidemiology of infections in a medical ICU in India. Intensive Care Med. 2014 Mar;40(3):456–7.

4. Ghanshani R, Gupta R, Gupta BS, Kalra S, Khedar RS, Sood S. Epidemiological study of prevalence, determinants, and outcomes of infections in medical ICU at a tertiary care hospital in India. Lung India Off Organ Indian Chest Soc. 2015 Oct;32(5):441–8.

5. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992 Jun;101(6):1644–55.

6. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med. 2003 Apr;31(4):1250–6.

7. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016 Feb 23;315(8):801-10. doi: 10.1001/jama.2016.0287. PMID: 26903338; PMCID: PMC4968574.

8. Williams J, Greenslade J, McKenzie J, Chu K, Brown A, Lipman J. SIRS, qSOFA and organ dysfunction: insights from a prospective database of emergency department patients with infection. Chest. 2016 Nov 1;151.

9. Song J-U, Sin CK, Park HK, Shim SR, Lee J. Performance of the quick Sequential (sepsis-related) Organ Failure Assessment score as a prognostic tool in infected patients

outside the intensive care unit: a systematic review and meta-analysis. Crit Care Lond Engl. 2018 Feb 6;22(1):28.

10. Guirgis FW, Puskarich MA, Smotherman C, Sterling SA, Gautam S, Moore FA, et al. Development of a Simple Sequential Organ Failure Assessment Score for Risk Assessment of Emergency Department Patients With Sepsis. J Intensive Care Med. 2020 Mar;35(3):270–8.

11. Szakmany T, Pugh R, Kopczynska M, Lundin RM, Sharif B, Morgan P, et al. Defining sepsis on the wards: results of a multi-centre point-prevalence study comparing two sepsis definitions. Anaesthesia. 2018 Feb;73(2):195–204.

12. Serafim R, Gomes JA, Salluh J, Póvoa P. A Comparison of the Quick-SOFA and Systemic Inflammatory Response Syndrome Criteria for the Diagnosis of Sepsis and Prediction of Mortality: A Systematic Review and Meta-Analysis. Chest. 2018 Mar;153(3):646–55.

13. Li X, Shen H, Zhou T, Cao X, Chen Y, Liang Y, et al. Does an increase in serum FGF21 level predict 28-day mortality of critical patients with sepsis and ARDS? Respir Res. 2021 Jun 22;22(1):182.

14. Gariani K, Drifte G, Dunn-Siegrist I, Pugin J, Jornayvaz FR. Increased FGF21 plasma levels in humans with sepsis and SIRS. Endocr Connect. 2013;2(3):146–53.

15. Siahanidou T, Margeli A, Bourika V, Papassotiriou I. Association of fibroblast growth factor 21 plasma levels with neonatal sepsis: preliminary results. Clin Chem Lab Med CCLM. 2019 May 1;57(5):e83–5.

16. P S, Rao PS, R SRA, Anil M, Benny A, I S. Diagnostic Utility of Cell Population Data (CPD) In Sepsis Using Automated Hematology Analysers. Ann Pathol Lab Med. 2019 May 30;6(5):A284-288.

17. Purohit AH, Kumar P, Sharma S, Kapil A, Gupta A, Mukhopadhyay AK. Volume, conductivity, and scatter parameters as diagnostic aid to bacterial sepsis: A tertiary care experience. Indian J Pathol Microbiol. 2015 Oct 1;58(4):459.

18. Suhua Z, Lefeng Z, Qingli C, Yueying W. The prognostic value of serum PCT, hs-CRP, and IL-6 in patients with sepsis. Open Life Sci. 2017 Jan 1;12(1):425–8.

19. Xu C, Wang Z, Lu K, Jin H. Hotspot Analysis of Sepsis Literature. Med Sci Monit Int Med J Exp Clin Res. 2018 Aug 5;24:5427–36.

20. Tupchong K, Koyfman A, Foran M. Sepsis, severe sepsis, and septic shock: A review of the literature. Afr J Emerg Med. 2014 Aug 1;31.

21. Gauer R. Early Recognition and Management of Sepsis in Adults: The First Six Hours. Am Fam Physician. 2013 Jul 1;88(1):44–53.

22. Stearns-Kurosawa DJ, Osuchowski MF, Valentine C, Kurosawa S, Remick DG. The Pathogenesis of Sepsis. Annu Rev Pathol. 2011;6:19–48.

23. Dugar S, Choudhary C, Duggal A. Sepsis and septic shock: Guideline-based management. Cleve Clin J Med. 2020 Jan 1;87(1):53–64.

24. Farkas JD. The complete blood count to diagnose septic shock. J Thorac Dis. 2020 Feb;12(Suppl 1):S16–21.

25. Previsdomini M, Gini M, Cerutti B, Dolina M, Perren A. Predictors of positive blood cultures in critically ill patients: a retrospective evaluation. Croat Med J. 2012 Feb 15;53(1):30–9.

26. Shiferaw B. The Role of Procalcitonin as a Biomarker in Sepsis. J Infect Dis Epidemiol. 2016 Apr 30;2(1).

Available from: https://clinmedjournals.org/articles/jide/journal-of-infectious-diseases-and-epidemiology-jide-2-006.php?jid=jide

27. Zhang H, Wang X, Zhang Q, Xia Y, Liu D. Comparison of procalcitonin and high-sensitivity C-reactive protein for the diagnosis of sepsis and septic shock in the oldest old patients. BMC Geriatr. 2017 Aug 1;17(1):173.

28. Anand A, Kumar N, Gambhir I, Kishore D, Varshney A, Tiwari A. Role of serum HsCRP as prognostic markers in septicemia in elderly population. Int J Med Sci Public Health. 2013;2(2):290.

29. Wang HE, Shapiro NI, Safford MM, Griffin R, Judd S, Rodgers JB, et al. High-sensitivity C-reactive protein and risk of sepsis. PloS One. 2013;8(7):e69232.

30. Vijayan AL, Vanimaya null, Ravindran S, Saikant R, Lakshmi S, Kartik R, et al. Procalcitonin: a promising diagnostic marker for sepsis and antibiotic therapy. J Intensive Care. 2017;5:51.

31. Liu GB, Cui XQ, Wang ZB, Wen L, Duan HL. Detection of serum procalcitonin and hypersensitive C-reactive protein in patients with pneumonia and sepsis. J Biol Regul Homeost Agents. 2018 Oct;32(5):1165–9.

32. Wang H. [Predictive value of serum procalcitonin and hypersensitive C-reactive protein levels in patients with acute cerebral infarction complicated with infection]. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2019 Aug;31(8):962–6.

33. Wu J, Hu L, Zhang G, Wu F, He T. Accuracy of Presepsin in Sepsis Diagnosis: A Systematic Review and Meta-Analysis. PLOS ONE. 2015 Jul 20;10(7):e0133057.

34. Wu X, Luo Q, Su Z, Li Y, Wang H, Liu Q, et al. Neutrophil-to-lymphocyte ratio as a predictor of mortality in intensive care unit patients: a retrospective analysis of the Medical Information Mart for Intensive Care III Database. BMJ Open. 2021 Nov 11;11(11):e053548.

35. Scheer CS, Fuchs C, Gründling M, Vollmer M, Bast J, Bohnert JA, et al. Impact of antibiotic administration on blood culture positivity at the beginning of sepsis: a prospective clinical cohort study. Clin Microbiol Infect. 2019 Mar 1;25(3):326–31.

36. Ko BS, Choi S-H, Shin TG, Kim K, Jo YH, Ryoo SM, et al. Impact of 1-Hour Bundle Achievement in Septic Shock. J Clin Med. 2021 Feb 2;10(3):527.

37. Is D, Pm A, Ka C, Mc E, Lg F, Al H, et al. Fluid Response Evaluation in Sepsis Hypotension and Shock: A Randomized Clinical Trial. Chest [Internet]. 2020 Oct [cited 2022 Jan 4];158(4). Available from: https://pubmed.ncbi.nlm.nih.gov/32353418/

38. Wang HE, Donnelly JP, Yende S, Levitan EB, Shapiro NI, Dai Y, et al. Validation of the REGARDS Severe Sepsis Risk Score. J Clin Med. 2018 Dec 11;7(12):536.

39. Pregernig A, Müller M, Held U, Beck-Schimmer B. Prediction of mortality in adult patients with sepsis using six biomarkers: a systematic review and meta-analysis. Ann Intensive Care. 2019 Nov 8;9(1):125.

40. Chen C-M, Cheng K-C, Chan K-S, Yu W-L. Age May Not Influence the Outcome of Patients with Severe Sepsis in Intensive Care Units. Int J Gerontol. 2014 Mar 1;8(1):22–6.

41. Sakr Y, Elia C, Mascia L, Barberis B, Cardellino S, Livigni S, et al. The influence of gender on the epidemiology of and outcome from severe sepsis. Crit Care. 2013;17(2):R50.

42. Angele MK, Pratschke S, Hubbard WJ, Chaudry IH. Gender differences in sepsis. Virulence. 2014 Jan 1;5(1):12–9.

43. Rose N, Matthäus-Krämer C, Schwarzkopf D, Scherag A, Born S, Reinhart K, et al. Association between sepsis incidence and regional socioeconomic deprivation and health care capacity in Germany – an ecological study. BMC Public Health. 2021 Sep 7;21(1):1636.

44. Lewis JM, Abouyannis M, Katha G, Nyirenda M, Chatsika G, Feasey NA, et al. Population Incidence and Mortality of Sepsis in an Urban African Setting, 2013-2016. Clin Infect Dis Off Publ Infect Dis Soc Am. 2020 Dec 17;71(10):2547–52.

45. Vijay S, Dalela G. Prevalence of LRTI in Patients Presenting with Productive Cough and Their Antibiotic Resistance Pattern. J Clin Diagn Res JCDR. 2016 Jan;10(1):DC09-DC12.

46. Gu X, Zhou F, Wang Y, Fan G, Cao B. Respiratory viral sepsis: epidemiology, pathophysiology, diagnosis and treatment. Eur Respir Rev. 2020 Sep 30;29(157):200038.

47. Purba AKR, Mariana N, Aliska G, Wijaya SH, Wulandari RR, Hadi U, et al. The burden and costs of sepsis and reimbursement of its treatment in a developing country: An observational study on focal infections in Indonesia. Int J Infect Dis. 2020 Jul 1;96:211–8.

48. Hsiao C-Y, Yang H-Y, Chang C-H, Lin H-L, Wu C-Y, Hsiao M-C, et al. Risk Factors for Development of Septic Shock in Patients with Urinary Tract Infection. BioMed Res Int. 2015;2015:717094.

49. Hsiao CY, Chen TH, Lee YC, Hsiao MC, Hung PH, Wang MC. Risk factors for uroseptic shock in hospitalized patients aged over 80 years with urinary tract infection. Ann Transl Med. 2020 Apr;8(7):477. doi: 10.21037/atm.2020.03.95. PMID: 32395521; PMCID: PMC7210120.

50. Infectious Disease New. Fall patients frequently present with UTI, bacteremia, LRTI.

Available from: https://www.healio.com/news/infectious-disease/20151009/fall-patients-frequently-present-with-uti-bacteremia-lrti

51. Robertson FC, Lepard JR, Mekary RA, Davis MC, Yunusa I, Gormley WB, et al. Epidemiology of central nervous system infectious diseases: a meta-analysis and systematic review with implications for neurosurgeons worldwide. J Neurosurg. 2018 Jun 1;1–20.

52. Liu J, Xie H, Ye Z, Li F, Wang L. Rates, predictors, and mortality of sepsis-associated acute kidney injury: a systematic review and meta-analysis. BMC Nephrol. 2020 Jul 31;21(1):318.

53. Philips CA, Ahamed R, Rajesh S, George T, Mohanan M, Augustine P. Update on diagnosis and management of sepsis in cirrhosis: Current advances. World J Hepatol. 2020 Aug 27;12(8):451–74.

54. Williams MD, Braun LA, Cooper LM, Johnston J, Weiss RV, Qualy RL, et al. Hospitalized cancer patients with severe sepsis: analysis of incidence, mortality, and associated costs of care. Crit Care. 2004 Jul 5;8(5):R291.

55. Bohnen JMA, Mustard RA, Oxholm SE, Schouten BD. APACHE II Score and Abdominal Sepsis: A Prospective Study. Arch Surg. 1988 Feb 1;123(2):225–9.

56. Vincent J-L. The Clinical Challenge of Sepsis Identification and Monitoring. PLOS Med. 2016 May 17;13(5):e1002022.

57. Kalra OP, Raizada A. Approach to a Patient with Urosepsis. J Glob Infect Dis. 2009;1(1):57–63.

58. Dreger NM, Degener S, Ahmad-Nejad P, Wöbker G, Roth S. Urosepsis—Etiology, Diagnosis, and Treatment. Dtsch Ärztebl Int. 2015 Dec;112(49):837–48.

59. Nasa P, Juneja D, Singh O. Severe sepsis and septic shock in the elderly: An overview. World J Crit Care Med. 2012 Feb 4;1(1):23–30.

60. Gofton TE, Young GB. Sepsis-associated encephalopathy. Nat Rev Neurol. 2012 Oct;8(10):557–66.

61. Genga KR, Russell JA. Update of Sepsis in the Intensive Care Unit. J Innate Immun. 2017;9(5):441–55.

62. Pascale GD, Ranzani OT, Nseir S, Chastre J, Welte T, Antonelli M, et al. Intensive care unit patients with lower respiratory tract nosocomial infections: the ENIRRIs project. ERJ Open Res. 2017 Oct;3(4).

63. Wood J, Butler CC, Hood K, Kelly MJ, Verheij T, Little P, et al. Antibiotic prescribing for adults with acute cough/lower respiratory tract infection: congruence with guidelines. Eur Respir J. 2011 Jul 1;38(1):112–8.

64. Marchick MR, Kline JA, Jones AE. The significance of non-sustained hypotension in emergency department patients with sepsis. Intensive Care Med. 2009 Jul;35(7):1261–4.

65. Jansma G, de Lange F, Kingma WP, Vellinga NA, Koopmans M, Kuiper MA, et al. 'Sepsis-related anemia' is absent at hospital presentation; a retrospective cohort analysis. BMC Anesthesiol. 2015 Apr 24;15:55.

66. Hou PC, Seethala RR, Aisiku IP. qSOFA—welcome to the sepsis alphabet soup. J Thorac Dis. 2017 Apr;9(4):965–8.

67. Youssef HA, Nasseh Sh, Hafiz HA, Gawesh A. Evaluation of diagnostic and prognostic value of high sensitivity C reactive protein (Hs-CRP) in community acquired pneumonia. Egypt J Chest Dis Tuberc. 2013 Apr 1;62(2):301–4.

68. Ma H, Liu H, Wu C, Huang L. Diagnostic Value of Serum Heparin Binding Protein, Blood Lactic Acid Combined with hs-CRP in Sepsis and Its Relationship with Prognosis. Evid Based Complement Alternat Med. 2021 Nov 9;2021:e5023733.

69. Nakamura A, Wada H, Ikejiri M, Hatada T, Sakurai H, Matsushima Y, et al. Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. Shock Augusta Ga. 2009 Jun;31(6):586–91.

70. Hoeboer SH, van der Geest PJ, Nieboer D, Groeneveld ABJ. The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis. Clin Microbiol Infect. 2015 May 1;21(5):474–81.

71. Tsui T-L, Huang Y-T, Kan W-C, Huang M-S, Lai M-Y, Ueng K-C, et al. A novel procalcitonin-based score for detecting sepsis among critically ill patients. PLOS ONE. 2021 Jan 22;16(1):e0245748.

72. Jain S, Sinha S, Sharma SK, Samantaray JC, Aggrawal P, Vikram NK, et al. Procalcitonin as a prognostic marker for sepsis: a prospective observational study. BMC Res Notes. 2014 Jul 17;7:458.

73. Nargis W, Ibrahim M, Ahamed BU. Procalcitonin versus C-reactive protein: Usefulness as biomarker of sepsis in ICU patient. Int J Crit Illn Inj Sci. 2014;4(3):195–9.

74. Sui Y, Xin W, Feng L. Comparison of the clinical application values of PCT, hs-CRP and SAA detection in the early diagnosis of sepsis. Pak J Med Sci. 2020 Sep 23;36.

75. Gariani K, Drifte G, Dunn-Siegrist I, Pugin J, Jornayvaz FR. Increased FGF21 plasma levels in humans with sepsis and SIRS. Endocr Connect. 2013 Sep 17;2(3):146–53.

76. Luo Y, Lin J, Chen H, Zhang J, Peng S, Kuang M. Utility of neut-X, neut-Y and neut-Z parameters for rapidly assessing sepsis in tumor patients. Clin Chim Acta Int J Clin Chem. 2013 Jun 25;422:5–9.

77. Abiramalatha T, Santhanam S, Mammen JJ, Rebekah G, Shabeer MP, Choudhury J, et al. Utility of neutrophil volume conductivity scatter (VCS) parameter changes as sepsis screen in neonates. J Perinatol Off J Calif Perinat Assoc. 2016 Sep;36(9):733–8.

78. Ustyantseva I.M., Kulagina E.A., Aliev A.R., Agadzhanyan V.V. Relationship Between Extended Inflammatory Parameters of Hematologic Analysis (NEUT-RI, NEUT-GI, RE-LYMP, AS-LYMP) With Risk of Infection In Polytrauma. Polytrauma. 2019.

79. Ustyantseva M, Khokhlova OI, Agadzhanyan VV.Innovative Technologies in the Evaluation of the Neutrophil Functional Activity in Sepsis. Sysmex Journal International. 2019; 29:1.

80. Eachempati SR, Hydo L, Barie PS. Gender-Based Differences in Outcome in Patients With Sepsis. Arch Surg. 1999 Dec 1;134(12):1342–7.

81. Papathanassoglou E, Middleton N, Benbenishty J, Williams G, Christofi M-D, Hegadoren K. Systematic review of gender- dependent outcomes in sepsis. Nurs Crit Care. 2017;22(5):284–92.

82. Nasir N, Jamil B, Siddiqui S, Talat N, Khan FA, Hussain R. Mortality in Sepsis and its relationship with Gender. Pak J Med Sci. 2015;31(5):1201–6.

83. Pietropaoli AP, Glance LG, Oakes D, Fisher SG. Gender Differences in Mortality in Patients with Severe Sepsis and Septic Shock. Gend Med. 2010 Oct;7(5):422–37.

84. Martin-Loeches I, Guia MC, Vallecoccia MS, Suarez D, Ibarz M, Irazabal M, et al. Risk factors for mortality in elderly and very elderly critically ill patients with sepsis: a prospective, observational, multicenter cohort study. Ann Intensive Care. 2019 Feb 4;9(1):26.

85. Bruno RR, Wernly B, Mamandipoor B, Rezar R, Binnebössel S, Baldia PH, et al. ICU-Mortality in Old and Very Old Patients Suffering From Sepsis and Septic Shock. Front Med. 2021;8:1034.

86. Turhan EE, Gürsoy T, Ovalı F. Factors which affect mortality in neonatal sepsis. Turk Arch Pediatr Pediatri Arş. 2015 Sep;50(3):170.

87. Chen X-C, Yang Y-F, Wang R, Gou H-F, Chen X-Z. Epidemiology and microbiology of sepsis in mainland China in the first decade of the 21st century. Int J Infect Dis. 2015 Feb 1;31:9–14.

88. Mohr N, Harland K, Shane D, Ahmed A, Fuller B, Ward M, et al. Rural Patients With Severe Sepsis or Septic Shock Who Bypass Rural Hospitals Have Increased Mortality: An Instrumental Variables Approach. Crit Care Med. 2016 Sep 8;45.

89. Prebil SE , Esper AM , Martin GS. American Journal of Respiratory and Critical Care Medicine 2011;183: A4676.

90. Yang Y, Yang KS, Hsann YM, Lim V, Ong BC. The effect of comorbidity and age on hospital mortality and length of stay in patients with sepsis. J Crit Care. 2010 Sep;25(3):398–405.

91. Chen C-H, Lai C-C, Wang Y-H, Wang C-Y, Wang H-C, Yu C-J, et al. The Impact of Sepsis on the Outcomes of COPD Patients: A Population-Based Cohort Study. J Clin Med. 2018 Oct 27;7(11):393.

92. Kopanitsa G, Metsker O, Paskoshev D, Greschischeva S. Identification of Risk Factors and Prediction of Sepsis in Pregnancy Using Machine Learning Methods. Procedia Comput Sci. 2021 Jan 1; 193:393–401.

93. Singh JA, Hossain A, Kotb A, Wells G. Risk of serious infections with immunosuppressive drugs and glucocorticoids for lupus nephritis: a systematic review and network meta-analysis. BMC Med. 2016 Sep 13;14(1):137.

94. Japiassú AM, Amâncio RT, Mesquita EC, Medeiros DM, Bernal HB, Nunes EP, et al. Sepsis is a major determinant of outcome in critically ill HIV/AIDS patients. Crit Care Lond Engl. 2010;14(4): R152.

95. Belok SH, Bosch NA, Klings ES, Walkey AJ. Evaluation of leukopenia during sepsis as a marker of sepsis-defining organ dysfunction. PLOS ONE. 2021 Jun 24;16(6):e0252206.

96. Nesseler N, Launey Y, Aninat C, White J, Corlu A, Pieper K, et al. Liver Dysfunction Is Associated with Long-Term Mortality in Septic Shock. :3.

97. Jung SM, Kim Y-J, Ryoo SM, Kim WY. Relationship between low hemoglobin levels and mortality in patients with septic shock. Acute Crit Care. 2019 May;34(2):141–7.

98. Tan SMY, Zhang Y, Chen Y, See KC, Feng M. Association of fluid balance with mortality in sepsis is modified by admission hemoglobin levels: A large database study. PLoS ONE. 2021 Jun 14;16(6): e0252629.

99. Patel JJ, Taneja A, Niccum D, Kumar G, Jacobs E, Nanchal R. The association of serum bilirubin levels on the outcomes of severe sepsis. J Intensive Care Med. 2015 Jan;30(1):23–9.

100. Oh J, Kim SH, Park K, Oh S, Kim Y, Kim HJ, et al. High-sensitivity C-reactive protein/albumin ratio as a predictor of in-hospital mortality in older adults admitted to the emergency department. Clin Exp Emerg Med. 2017 Mar 30; 4:19–24.

101. Hassan EA, Abdel Rehim AS, Ahmed AO, Abdullahtif H, Attia A. Clinical Value of Presepsin in Comparison to hsCRP as a Monitoring and Early Prognostic Marker for Sepsis in Critically Ill Patients. Medicina (Mex). 2019 Feb 2;55(2):36.

102. Cui N, Zhang H, Chen Z, Yu Z. Prognostic significance of PCT and CRP evaluation for adult ICU patients with sepsis and septic shock: retrospective analysis of 59 cases. J Int Med Res. 2019 Jan 18; 47:030006051882240.

103. Kilercik M, Demirelce Ö, Serdar MA, Mikailova P, Serteser M. A new haematocytometric index: Predicting severity and mortality risk value in COVID-19 patients. PLOS ONE. 2021 Aug 5;16(8): e0254073.

ANNEXURES

APPENDIX-1

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/0116 **Date: 01/01/2020**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/**1025

Project title: **"The Evaluation of role of novel biomarkers serum fibroblast growth factor 21 (FGF21) level and neutrophil parameters {Neutrophil--Granularity-intensity (NEUT-G1) and neutrophil-reactivity-intensity (NEUT-RI) in diagnosis and prognosis of sepsis"**

Nature of Project:	**Research Project**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr.Vishwanath Jha**
Guide:	**Dr. Mahendra Kumar Garg**
Co-Guide:	**Dr.Satyendra Khichar, Dr. Deepak Kumar, Dr.Abhishek Purohit & Dr. Purvi Purohit**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **17-01-2020** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure 1

Page 1 of 2

Annexure 1

Institutional Ethics Committee
All India Institution of Medical Sciences, Jodhpur

Meeting of Institutional Ethics committee held on **23-12-2019 at 10:00** AM at Committee Room, Admin Block AIIMS Jodhpur.

Following members were participated in the meeting:-

S/No.	Name of Member	Qualification	Role/Designation in Ethics Committee
1.	Dr. F.S.K Barar	MBBS, MD (Pharmacology)	**Chairman**
2.	Justice N.N Mathur	LLB	Legal Expert
3.	Dr. Varsha Sharma	M.A (Sociology)	Social Scientist
4.	Mr. B.S.Yadav	B.Sc., M.Sc. (Physics), B.Ed.	Lay Person
5.	Dr. K.R.Haldiya	MD (General Medicine)	Clinician
6.	Dr. Arvind Mathur	MBBS, MS (General Medicine)	Clinician
7.	Dr. Surajit Ghatak	MBBS, MS (Anatomy)	Basic Medical Scientist
8.	Dr. Vijaya Lakshmi Nag	MBBS, MD (Microbiology)	Basic Medical Scientist
9.	Dr. Sneha Ambwani	MBBS, MD (Pharmacology)	Basic Medical Scientist
10.	Dr. Kuldeep Singh	MBBS, MD (Paediatric), DM (General Medicine)	Clinician
11.	Dr. Abhinav Dixit	MBBS, MD (Physiology), DNB (Physiology)	Basic Medical Scientist
12.	Dr. Pradeep Kumar Bhatia	MBBS, MD (Anaesthesiology)	Clinician
13.	Dr. Tanuj Kanchan	MBBS, MD (Forensic Medicine)	Basic Medical Scientist
14.	Dr. Pankaj Bhardwaj	MBBS, MD (CM&FM)	Clinician
15.	Dr. Praveen Sharma	M.Sc., Ph.D. (Biochemistry)	Member Secretary

Dr. Praveen Sharma
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

APPENDIX-2

All India Institute of Medical Science

Jodhpur Rajasthan

Informed Consent Form

Title of Thesis/Dissertation: ROLE OF NOVEL BIOMARKERS SERUM FIBROBLAST GROWTH FACTOR 21 (FGF21) LEVEL AND NEUTROPHIL PARAMETERS {NEUTROPHIL-GRANULARITY-INTENSITY(NEUT-GI)ANDNEUTROPHIL-REACTIVITY-INTENSITY (NEUT-RI)} IN DIAGNOSIS AND PROGNOSIS IN SEPSIS
Name of PG Student : Dr. Vishwanath jha , Contact No. - 6202427871 Patient/Volunteer Identification No.: _____

I,_____S/o or D/o_____

R/o,_ _____give my full, free, voluntary consent to be a part of the study "ROLE OF NOVEL BIOMARKERS SERUM FIBROBLAST GROWTH FACTOR 21 (FGF21) LEVEL AND NEUTROPHIL PARAMETERS {NEUTROPHIL-GRANULARITY-INTENSITY(NEUT-GI)ANDNEUTROPHIL-REACTIVITY-INTENSITY (NEUT-RI)} IN DIAGNOSIS AND PROGNOSIS IN SEPSIS ", the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.
I understand that my participation is voluntary and am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individual from _____(Company Name) or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____ _____

Place: _____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date: _____ _____

Place: _____ Signature of PG Student

APPENDIX-3

अखल भारतीय आयुिवानसंथान
जोधपुर,राजथान

सूिचतसहमित प

थीिसस / शोध बंधका शीषक:"सीरम बायोमाक रफाइ"ो#ा$ %ोथ फै 'र21 (fgf21) (र और *ूट-ोिफलमापदंडों{*ुट-ोिफल-%े*ुलै1रटी-इंट2िसटी(*ुटी-जीआई) और *ूट-ोिफल-1रए'िवटी-इंट2िसटी(*ूट-री-)} की से5स के िनदान और पूवानुमानथापित करनेम2भूिमका" पीजी छा7 का नाम: डॉ। िव:नाथ झा, संपकनंबर– 6202427871

रोगी /

=यंसेवकपहचान सं@ाI:_____

मA,_____ S / o या D / o _____

R / o _____ R / o, _ _____ मेरी पूण,=तं7,=ैCकसहमित द2

अDयन का एक िहEा बननेकेिलए "सीरम बायोमाक रफाइ"ो#ा$ %ोथ फै 'र21 (fgf21) (र और *ूट-ोिफलमापदंडों{*ुट-ोिफल-%े*ुलै1रटी-इंट2िसटी(*ुटी-जीआई) और *ूट-ोिफल-1रए'िवटी-इंट2िसटी(*ूट-री-)} की से5स के िनदान और पूवानुमानथापित करनेम2भूिमका", िजस िFया और कृ ितसेमुझेअपनी भाषा म2अपनी पूणसंतुिHकेिलए समझाया गया है।मAपुिHकरता Iंिक मुझे सवाल पूछनेका अवसर िमला है।

मAसमझता Iंिक मेरी भागीदारी =ैCकहैऔर िबना िकसी कारण केिकसी भी समय अDयन से बाहर िनकलनेकेमेरेअिधकार सेअवगत Iं।

मAसमझता Iंिक मेरेऔर मेरेिकसी भी मेिडकल 1रकॉडके बारेम2एकि7त जानकारी को _____ (कं पनीका नाम) या िनयामक अिधका1रयोंकेिजJेदार KL Mारा देखा
जा सकता है।मAइन KLयोंको अपने1रकॉडतक पNंचनेकी अनुमितदेता Iं।

िदनांक:_____ _____

जगह: _____ ह(ाIOर / बाएंअंगूठेकािनशान

यह माणित करनेकेिलए िक मेरी उपथित म2उपरोL सहमित ाIR Nई है।

िदनांक:_____ _____

जगह: _____ पीजी छा7 केह(ाIOर

APPENDIX-4

PATIENT INFORMATION SHEET

Name of the patient:

Patient ID:

EVALUATION OF ROLE OF NOVEL BIOMARKERS SERUM FIBROBLAST GROWTH FACTOR 21 (FGF21) LEVEL AND NEUTROPHIL PARAMETERS {NEUTROPHIL-GRANULARITY-INTENSITY (NEUT-GI) AND NEUTROPHIL-REACTIVITY-INTENSITY (NEUT-RI)} IN DIAGNOSIS AND PROGNOSIS IN SEPSIS

1. Aim of the study: To establish role of novel biomarkers serum fibroblast growth factor 21 (FGF21) level and neutrophil parameters {neutrophil-granularity-intensity (NEUT-GI) and neutrophil-reactivity-intensity (NEUT-RI)} in diagnosis and prognosis in sepsis

2. Study site: In-patient services of Department of Internal Medicine, All India Institute of Medical Sciences, Jodhpur, Rajasthan.
3. Study procedure: Case control study by detailed clinical history and go for physical examination, laboratory investigations including markers in patients with sepsis. We will go for first FGF 21, neutrophil granularity intensity and neutrophil reactivity intensity on the diagnosis and follow up after 7 days and 28 days to correlate with trends.
4. Confidentiality: All the data collected from each study participant will be kept highly confidential.
5. Risk: Enrollment in above study poses no substantial risk to any of the study participant and if any point of time participant wants to withdraw himself/ herself, he/ she can do so voluntarily at any point of time during the study.

For further information / questions, the following personnel can be contacted:

Dr Vishwanath jha,

Junior Resident, Department of Internal Medicine, All India Institute of Medical Sciences, Jodhpur, Rajasthan. Ph 620242787

APPENDIX -5

रोगी की सूचना

सीरम बायोमाक रफाइ"ो#ा$ %ोथ फै 'र21 (FGF21) **(र और *ूट-ोिफलमापदंडों{*ुट-ोिफल-%े*ुलै1रटी-इंट2िसटी(*ुटी-जीआई) और *ूट-ोिफल-1रए'िवटी-इंट2िसटी(*ूट-री-)} की से5स के िनदान और पूवानुमानथाि पत करनेम2भूिमका**

रोगी का नाम: **रोगी आईडी:**

1. **अDयन का उSेT: सीरम बायोमाक रफाइ"ो#ा$ %ोथ फै 'र**21 (FGF21) **(र और *ूट-ोिफल मापदंडों{*ुट-ोिफल-%े*ुलै1रटी-इंट2िसटी(*ुटी-जीआई) और *ूट-ोिफल-1रए'िवटी-इंट2िसटी(*ूट-- री)} की से5स के िनदान और पूवानुमानथाि पत करनेम2भूिमका**

2. **अDयन थल: आंत1रकिचिकUा िवभाग, अखल भारतीय आयुिवसंानथान,जोधपुर,राजथान की रोगी सेवाएं ।**

3. **अDयन िFया: िव(ृतनैदाि नकइितहास Mारा केस िनयं7णअDयन और से5स केरोिगयोंम2 माक रसिहत शारी1रक जांच,योगशाला जांचकेिलए जाना। हम िनदान पर पहलेसीरम फाइ"ो#ा$ %ोथ फै 'र**21, ***ूट-ोिफल%ै*ुलै1रटीतीVता और *ूट-ोिफलितिFया तीVता केिलए जाएं गेऔर** 7 **िदनोंऔर** 28 **िदनोंकेबाद अनुवतWXझान केसाथ सहसंबंिधतकर2गे।**

4. **गोपनीयता: Yेक अDयन ितभागी सेएक7 िकए गए सभी डेटा को अYिधक गोपनीय रखा जाएगा।**

5. **जोखम: उपरोL अDयन म2नामांकनसेअDयन केिकसी भी ितभागी को कोई भारी जोखम नहींहोता हैऔर यिद कोई भी ितभागी =यंको =यंवापस लेना चाहता है,तो वह अDयन केदौरान िकसी भी समय =ेCा सेऐसा कर सकता है।**

अिधक जानकारी / \ोंकेिलए, िन]िलखत किमयोंसेसंपकिकया जा सकता है:

डॉ। िव:नाथ झा,

जूिनयररेिजड2ट,आंत1रकिचिकUा िवभाग, अखल भारतीय आयुिवसंानथान,जोधपुर,राजथान। Ph 6202427871

APPENDIX -6

CASE RECORD FORM AND DATA

Patient ID:

Name of patient **Age** **Gender**

Rural/Urban

Address

Contact number

CHIEF COMPLAINTS

qSOFA

BRIEF HOPI:

PAST HISTORY:

TREATMENT HISTORY:

FAMILY HISTORY:

PERSONAL HISTORY:

VITALS:

Date	Pulse rate	Blood pressure	Respiratory rate	Temp

GENERAL PHYSICAL EXAMINATION:

Date	Pallor	Icterus	Cyanosis	Clubbing	Edema	Lymphadenopathy

SYSTEMIC EXAMINATION:

CVS

CNS

R/S

P/A

INVESTIGATIONS:

CBC	Day 1	Day 3	Day 7
Hb			
TLC			
DLC			

PLT			
MCV			
HCT			

KFT	**Day 1**	**Day 3**	**Day 7**
Urea			
Creatinine			

LFT	**Day 1**	**Day 3**	**Day 7**
SGOT/SGPT/ALP			
TOTAL BILIRUBIN/DIRECT/INDIRECT			
TOTALPROTEIN/ ALBUMIN/GLOBULIN			

BLOOD GLUCOSE	ON ADMISSION
FASTING BLOOD GLUCOSE	
RANDOM GLUCOSE	
HbA1c	

Urine microscopy	Day 1	Day 3
Pus cells		
Ketone		
Glucose		
Protein		

Serum electrolyte

Date	**Day1**	**Day3**	**Day7**
Serum sodium			
Serum potassium			
Serum chloride			
Serum lactate			

Biomarkers for diagnosis and prognosis of sepsis

Date	**Day1**	**Day3**	**Day7**
Procalcitonin			
HsCRP			
ESR			
Ferritin			
Fibrinogen			

Culture sensitivity report		**Day 1**	**Day3**	**Day7**
	Organism			
	1st line sensitivity			
	2nd line sensitivity			
FGF21 titre	**Case**			

	Control	
Viral markers	**HIV**	
	HbsAg	
	HCV	

SOFA Score

PaO2/FIO2, mm Hg	**Day1**	**Day3**	**Day7**
Platelets ×103 /Ml			
Bilirubin, mg/dL (μmol/L)			
Cardiovascular			
Glasgow Coma Scale score			
Creatinine, mg/Dl			
Urine output, mL/d			
Final score			

qSOFA

Parameter	Score
GCS	
SBP	
RR	

NEUTROPHIL GRANULARITY INTENSITY and NEUTROPHIL REACTIVITY INTENSITY

Date	DAY 1	DAY 7	DAY 28
NUET -RI			
NUET- GI			

ECHOCARDIOGRAPHY REPORT

Date	
NAD	
ABNORMAL FINDING	

ARTERIAL BLOOD GAS ANALYSIS

PH	
PCO2	
PO2	
SpO2	
Na/ k/Cl /Ca	
Lactate	

PROVISIONAL DIAGNOSIS:	
FINAL DIAGNOSIS:	

TREATMENT GIVEN:	
TOTAL DURATION OF ILLNESS:	
DURATION OF HOSPITAL STAY:	
ANY COMPLICATIONS DURING HOSPITAL STAY:	