"TO STUDY VOLUME, CONDUCTIVITY, SCATTER PARAMETERS OF NEUTROPHILS AS AN EARLY DIAGNOSTIC TOOL IN ACUTE BACTERIAL INFECTIONS AT A TERITIARY LEVEL CENTRE- A RETROSPECTIVE CASE CONTROL STUDY"

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
AMR	Antimicrobial Resistance
ANC	Absolute Neutrophil Count
AUC	Area Under the Curve
BMI	Body Mass Index
CBC	Complete Blood Count
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
DIC	Disseminated Intravascular Coagulation
EDTA	Ethylenediaminetetraacetic Acid
ESR	Erythrocyte Sedimentation Rate
G-CSF	Granulocyte Colony-Stimulating Factor
HIV	Human Immunodeficiency Virus
IL-6	Interleukin-6
IL-8	Interleukin-8
KOH	Potassium Hydroxide
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
MMV	Mean Monocyte Volume
MNC	Mean Neutrophil Conductivity

MNS	Mean Neutrophil Scatter
MNV	Mean Neutrophil Volume
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NDW	Neutrophil Distribution Width
NETs	Neutrophil Extracellular Traps
PAMPs	Pathogen-Associated Molecular Patterns
PCR	Polymerase Chain Reaction
PRRs	Pattern Recognition Receptors
ROC	Receiver Operating Characteristic
T3SS	Type III Secretion System
TLC	Total Leukocyte Count
TNF-α	Tumor Necrosis Factor-alpha
VCS	Volume, Conductivity, and Scatter
WBC	White Blood Cell

ABSTRACT

TO STUDY VOLUME, CONDUCTIVITY, SCATTER PARAMETERS OF NEUTROPHILS AS AN EARLY DIAGNOSTIC TOOL IN ACUTE BACTERIAL INFECTIONS AT A TERTIARY LEVEL CENTRE- A RETROSPECTIVE CASE CONTROL STUDY

ABSTRACT

Introduction

Acute bacterial infections are a leading cause of morbidity and mortality worldwide, with significant challenges in timely diagnosis due to the limitations of conventional methods such as microbiological cultures. Advances in haematology, particularly Volume, Conductivity, and Scatter (VCS) parameters, offer a rapid and efficient alternative for early detection. This study aimed to evaluate the utility of VCS parameters of neutrophils as an early diagnostic tool in acute bacterial infections.

Methodology

A retrospective case-control study was conducted at a tertiary care center in Belagavi from April 1, 2023, to March 31, 2024. The study included 124 participants (62 cases with confirmed bacterial infections and 62 healthy controls). VCS parameters—mean neutrophil volume (MNV), mean neutrophil conductivity (MNC), and mean neutrophil scatter (MNS)—were measured using an automated haematology analyser (Beckman Coulter DxH 900). Data were analysed using R version 4.2.2 and Microsoft Excel.

Result

Significant differences were observed in MNV between cases (157.8 ± 11.5) and controls (141.2 ± 4.7), with a p-value < 0.001. MNC was lower in cases (142.4 ± 5.3)

compared to controls (145.2 ± 5.6), while MNS showed no significant difference (p = 0.08). MNV was higher in cases with elevated WBC counts (>11,000/mm³) and neutrophil percentages (>85%). ROC curve analysis revealed an AUC of 0.99 for MNV, with a cutoff value of 148, demonstrating 96.8% sensitivity and specificity. In contrast, MNC and MNS exhibited lower diagnostic accuracy (AUC = 0.36 and 0.26, respectively).

Conclusion

MNV emerged as a highly sensitive and specific marker for early detection of acute bacterial infections. These findings highlight the potential of MNV as a rapid diagnostic tool, particularly in resource-limited settings.

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INTRODUCTION

Acute bacterial infections are caused by pathogenic bacteria that invade the body and trigger a rapid immune response. This response often manifests as general symptoms, including fever, chills, fatigue, localised pain, swelling, and redness. Depending on the site of infection, acute bacterial infections can present as, respiratory tract infections (cough, sputum production, breathlessness and chest pain), gastrointestinal tract infections (abdominal pain, diarrhoea, vomiting), urinary tract infections (dysuria, increased frequency of urination, urgency and haematuria), neurological infections (headache, neck stiffness, altered sensorium), skin infections (redness, warmth and swelling). Severe cases may progress to sepsis or septic shock, marked by hypotension, rapid heart rate, and organ failure.[1] The key modes of bacterial transmission include contact, airborne, droplet, vector-borne, and vehicular spread.[2,3]

Bacterial infections lead to a high number of morbidity and mortality worldwide. In 2019, bacterial infections led to 13.7 million infection-related deaths globally, with 7.7 million deaths linked to 33 pathogens across 11 syndromes. These accounted for 13.6% of all global deaths and 56.2% of sepsis-related deaths. Around 55% of these deaths were caused by common bacterial pathogens, including *Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, and Pseudomonas aeruginosa.* Among common bacterial pathogens, *S. aureus* was the leading cause of death globally, while *S. pneumoniae* was most lethal for children under five.[4] This substantial burden is further exacerbated by the growing threat of antimicrobial resistance (AMR). In 2021, AMR contributed to 4.71 million deaths (95% UI 4.23–5.19 million), with 1.14 million (1.00–1.28 million)

deaths directly from AMR. By 2050, AMR could cause 1.91 million deaths, with a total of 8.22 million linked deaths.[5]

Bacterial infections significantly contribute to patient admissions in tertiary care settings and often require prompt diagnostic and therapeutic interventions. Accurate and timely diagnosis is critical but frequently delayed due to reliance on traditional methods such as microbiological cultures, which use growth assays. It begins with proper sample collection and encounters issues, such as sample handling, culture challenges, misidentification, and problems with antibiotic susceptibility testing impacted by heteroresistance, biofilm-related tolerance, evolving breakpoints, and automated system limitations, leading to potential misinterpretation and treatment challenges. However, these conventional approaches require labour and frequently result in therapeutic delays.[6] Although these methods remain the gold standard for bacterial identification, their utility is limited by the time required for results and the dependence on specialised laboratory infrastructure.

Infections and sepsis induce numerical and morphological changes in leucocytes, particularly neutrophils, the first defence against bacterial pathogens.[7] Traditionally, assessing these changes has relied on manual examination of peripheral blood smears. However, advances in haematology have introduced Volume, Conductivity, and Scatter (VCS) parameters as sensitive predictors of acute bacterial infections and sepsis. VCS parameters are derived using automated haematology analysers, such as the Beckman Coulter DxH 900, which can analyse approximately 8,000 leucocytes within seconds.[8]

The technology utilises impedance to measure cell volume (V), radiofrequency opacity to assess internal conductivity (C), and laser beam scattering

to evaluate cytoplasmic granularity and nuclear structure (S).[9] A study by Bagdasaryan et al. found that the neutrophil VCS parameters, mean neutrophil volume (MNV), and NDW have higher sensitivity and specificity than manual band count, ANC, and C-reactive protein (CRP).[8] The clinical utility of VCS technology lies in the rapid turnaround time, often under 5 minutes enables its integration into routine complete blood count (CBC) workflows, requiring no additional blood beyond that used for CBC. This provides a practical and efficient diagnostic tool for early intervention.[10]

The necessity for this study arises from the increasing burden of bacterial infections and the limitations of existing diagnostic methods. While current practices, including microbiological cultures and biomarker assays like procalcitonin, are effective, they are either time-intensive or cost-prohibitive. The automated analysis of VCS parameters provides a promising alternative that delivers high specificity and sensitivity. Focusing on the morphological and numerical changes in neutrophils, this study seeks to validate the utility of VCS parameters as an early diagnostic tool, enabling timely intervention and better patient outcomes. The findings can improve early detection of bacterial infections, enhance patient outcomes, and reduce the overall burden on healthcare systems, especially in settings where timely and cost-effective diagnostic tools are needed.

AIMS AND OBJECTIVES

OBJECTIVE

- To study volume, conductivity and scatter of neutrophils as an early diagnostic tool in acute bacterial infections.

REVIEW OF LITERATURE

1. EPIDEMIOLOGY AND RISK FACTORS OF BACTERIAL INFECTIONS

Bacterial pathogens are transmitted through various routes, including air, water, food, and vectors, with transmission dynamics influenced by macro- and microenvironments.[11] Specific settings like healthcare facilities or overcrowded institutions may serve as hotspots for certain bacterial strains.[12]

Modes of Transmission of Bacterial Infections

The transmission of bacterial pathogens is closely tied to their mode of entry into the host.[1] Respiratory pathogens exploit damage to lung tissue to induce coughing, which aerosolises the bacteria, allowing them to be inhaled and colonise new hosts. Similarly, gastrointestinal pathogens often cause symptoms like diarrhoea, facilitating their spread through the faecal-oral route. This can occur directly or indirectly via contaminated food, water, or crops with infected waste. Understanding these transmission pathways is essential for implementing effective public health measures. Basic hygiene practices, such as hand washing, decontaminated shared surfaces, and social distancing, can significantly reduce transmission rates. Additionally, bacteriophages, viruses that infect bacteria, play a role in shaping bacterial pathogen dynamics, either by killing pathogens or transferring genetic material that may influence their virulence.[1]

Risk Factors for Bacterial Infections[1]

1. Travel History: It is important to consider both international and domestic travel when evaluating a patient. A recent return from a foreign country greatly expands the list of possible diagnosis. Domestic travel to areas like

California (risk of Coccidioides immitis) or Martha's Vineyard (risk of Francisella tularensis) can also expose individuals to uncommon pathogens. Activities during travel, such as consuming unsafe food or water, swimming in fresh water, or contact with animals, further increase the risk. Lack of pre-travel immunisations or prophylactic medications also contributes to infection.

2. Social and Behavioural Factors: High-risk behaviours, such as unsafe sexual practices or intravenous drug use, can predispose individuals to certain infections. Hobby-related exposures, such as gardening, or occupational exposures, such as working in funeral services (risk of M. tuberculosis), are important risk factors.

3. Dietary Habits: Consuming raw or undercooked meat increases the risk of infections such as Shiga toxin-producing E. coli and Toxoplasma gondii. Drinking unpasteurised milk can lead to Salmonella typhimurium, Listeria monocytogenes, or Mycobacterium Bovis infections. Unpurified water is associated with Leptospira, parasites, and enteric bacteria, while raw seafood can expose individuals to Vibrio species, norovirus, helminths, and protozoa.

4. Animal Exposures: Contact with animals, including pets, wild animals, or rodents, can transmit infectious diseases.

5. Host-Specific Factors: Immunocompromised individuals, due to underlying conditions like HIV, malignancy, or malnutrition or due to medications such as chemotherapy, glucocorticoids, or monoclonal antibodies, are at higher risk for opportunistic infections. Treatments like splenectomy, total body irradiation, and primary immunodeficiencies also increase susceptibility.

Additionally, inadequate immunisation against vaccine-preventable diseases further elevates the risk of infection.

6. Healthcare-Associated Factors: Invasive procedures, such as surgeries or the use of catheters, can provide entry points for bacteria.[13] Indwelling medical devices, like ventilators or urinary catheters, further increase the risk of infections. Prolonged hospital stays also raise the likelihood of healthcare-associated infections (HAIs), often caused by bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

7. Climate: Climate change, like rising global temperatures, altered precipitation patterns, and increased frequency of extreme weather events like hurricanes, influence the spread of infectious diseases, particularly vector-borne diseases, as warmer temperatures and shifting water availability affect the habitats and life cycles of disease-carrying insects.

8. Age and Demographics: *Streptococcus pneumoniae* commonly affects young children and the elderly[14], while *Neisseria meningitidis* is often seen in adolescents and young adults. Demographic factors, such as living conditions, access to healthcare, and socioeconomic status, can also play a significant role in infection rates.[1]

2. GLOBAL BURDEN OF BACTERIAL INFECTIONS

In 2019, bacterial infections led to 13.7 million (10.9–17.1) infection-related deaths worldwide. Of these, 7.7 million deaths (5.7–10.2) were linked to 33 bacterial pathogens across 11 infectious syndromes, encompassing both antimicrobial-resistant and susceptible cases. These pathogens were linked to 13.6% of global deaths and

56.2% of deaths related to sepsis. Five bacterial species—*Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*—were the most significant contributors, collectively responsible for 54.9% of deaths among the studied bacteria. The deadliest infectious syndromes and predominant pathogens varied across geographic locations and age groups. Globally, *S. aureus* emerged as the leading bacterial cause of mortality, ranking as the primary pathogen-associated death contributor in 135 countries and among individuals over 15 years old. Among children under five years, *S. pneumoniae* was identified as the pathogen responsible for the highest number of deaths. Three primary bacterial infectious syndromes were the leading contributors to mortality in 2019, with lower respiratory infections and bloodstream infections each accounting for over 2 million deaths and peritoneal and intra-abdominal infections responsible for more than 1 million deaths.[4]

The substantial burden of bacterial infections is further compounded by the escalating threat of antimicrobial resistance (AMR), which not only undermines the effectiveness of existing treatments but also exacerbates mortality and morbidity associated with these infections. In 2021, bacterial AMR contributed to an estimated 4.71 million deaths, with 1.14 million directly attributable to AMR. Globally, *Methicillin-resistant Staphylococcus aureus* (MRSA) saw the most significant rise in AMR-associated and attributable deaths, increasing from 261,000 in 1990 to 550,000 in 2021. Resistance to carbapenems among Gram-negative bacteria showed the highest growth, with associated deaths rising from 619,000 in 1990 to 1.03 million in 2021. Forecasts suggest that by 2050, 1.91 million deaths could be attributable to AMR, with 8.22 million associated deaths. The highest mortality rates are anticipated

in South Asia, Latin America, and the Caribbean. These projections highlight the importance of prioritising AMR interventions within the global health agenda.[5]

3. PATHOGENESIS OF ACUTE BACTERIAL INFECTIONS

Bacterial infections typically progress through several stages: colonisation, infection, disease, and transmission. Each stage is influenced by microbial virulence factors, host immune responses, and interactions with the host microbiota.[1]

Entry into the Human Host: Bacterial infections often begin when a live pathogen enters the human body, usually through breaks in the skin, mucous membranes of the respiratory, gastrointestinal, or genitourinary tracts, or via ingestion of contaminated food or water. Respiratory pathogens, such as *Mycobacterium tuberculosis* and influenza viruses, enter through inhalation of respiratory droplets or contact with contaminated surfaces. Gastrointestinal pathogens, like *Vibrio cholerae* and *Salmonella enterica*, are typically ingested via contaminated food or water. Genitourinary infections often arise from ascending fecal bacteria into the bladder or kidneys. Once inside the body, pathogens must establish themselves in their preferred niche to initiate infection.

Establishment of Infection: Bacterial pathogens exhibit tissue tropism, infecting specific tissues or organs. To establish infection, pathogens must adhere to host tissues, often through specific receptor-ligand interactions. Adhesins, such as pili, flagella, and autotransporter proteins, facilitate attachment to host cells. Once attached, pathogens may manipulate their niche to create a hospitable environment.

Attachment and Invasion: Pathogens employ a variety of adhesins, such as pili, flagella, and surface proteins, to bind to host cell receptors. These interactions not

only facilitate colonisation but also help pathogens evade host clearance mechanisms. Like, *Neisseria gonorrhoeae* uses type IV pili to adhere to epithelial cells and form microcolonies, promoting colonisation. Some pathogens, such as *Salmonella* and *Shigella spp.*, invade host cells using specialised secretion systems like the type III secretion system (T3SS). The T3SS delivers effector proteins into host cells, triggering actin polymerisation and membrane ruffling, which engulf the bacterium into a vacuole. Once inside, pathogens may either remain within the vacuole or escape into the cytosol to replicate.

Replicative Niche and Nutrient Acquisition: Bacterial pathogens must acquire nutrients to replicate and persist within the host. Extracellular pathogens, such as *Vibrio cholerae* and *Helicobacter pylori*, secrete enzymes to liberate nutrients from the extracellular environment. Intracellular pathogens, like *Salmonella* and *Listeria monocytogenes*, manipulate host cellular trafficking to redirect nutrients to their replicative niche. For example, *Salmonella* resides in a modified vacuole that recruits host vesicles to deliver nutrients. Pathogens also compete with the host microbiota for resources, highlighting the importance of nutrient acquisition in bacterial survival.

Avoidance of Host Immune Responses: To establish infection, bacterial pathogens must evade or subvert host immune defences. The innate immune system, which includes pattern recognition receptors (PRRs) like Toll-like receptors (TLRs), detects pathogen-associated molecular patterns (PAMPs) and initiates immune responses. Pathogens have evolved strategies to avoid detection, such as masking PAMPs or modifying surface structures. Pathogens also inhibit complement activation, a key component of humoral immunity, by degrading complement proteins or masking their surfaces with host proteins.

Inhibition of Inflammatory Responses: Bacterial pathogens often suppress host inflammatory responses to avoid elimination. The NF-κB signaling pathway, which regulates pro-inflammatory gene expression, is a common target of bacterial virulence factors. Additionally, pathogens may inhibit inflammasome activation, a process that triggers pyroptosis, a form of inflammatory cell death.

Tissue Damage and Dissemination: Bacterial infections often cause tissue damage, which can facilitate pathogen dissemination. Toxins produced by pathogens disrupt host cell functions and contribute to disease symptoms. Tissue damage also compromises epithelial barriers, allowing pathogens to access deeper tissues and the bloodstream.

Transmission to New Hosts: The ultimate goal of bacterial pathogens is to replicate and transmit to new hosts.

Role of Neutrophils in Bacterial Infections and Pathophysiology of Neutrophil Changes

Neutrophils serve as the first line of defence against bacterial infections. Upon bacterial invasion, neutrophils are mobilised through chemotactic signals such as interleukin-8 (IL-8), leukotriene B4, and N-formyl peptides, which guide their migration from the bloodstream into infected tissues. These cells exert antimicrobial activity through phagocytosis, generating reactive oxygen species via the respiratory burst and releasing proteolytic enzymes from azurophilic granules.

Additionally, neutrophils can trap bacteria in extracellular webs called neutrophil extracellular traps (NETs), composed of chromatin and antimicrobial peptides, which immobilise and kill pathogens while preventing their dissemination.[15]

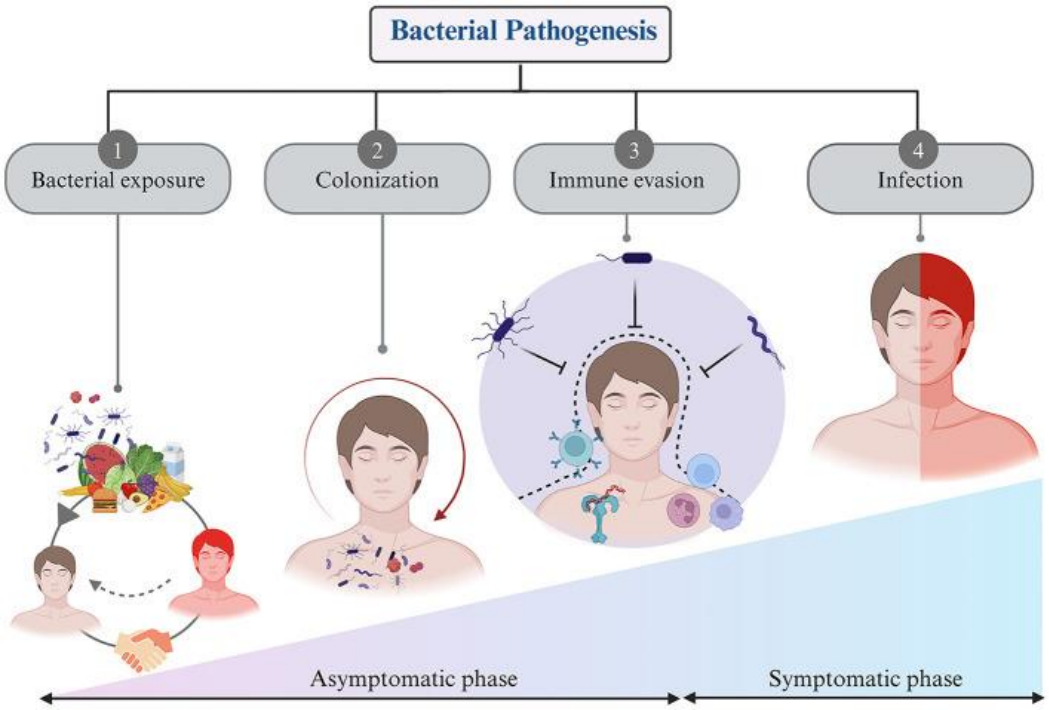

Figure 1: Steps in Bacterial Pathogenesis[11]

In bacterial infections, the pathophysiology of neutrophil responses is marked by dynamic changes in their numbers, function, and phenotype. Neutrophilia, or an increase in circulating neutrophil count, is a hallmark of acute bacterial infections and results from enhanced bone marrow production and release mediated by cytokines such as granulocyte colony-stimulating factor (G-CSF).

Neutropenia, characterised by reduced neutrophil counts, predisposes individuals to severe infections due to impaired pathogen clearance. Neutrophils undergo phenotypic and functional changes during infections, such as increased production of pro-inflammatory cytokines, improved degranulation, and upregulation of adhesion molecules like integrins. However, dysregulated neutrophil responses can exacerbate systemic inflammation and cause tissue damage, as in diseases like sepsis.[16,17]

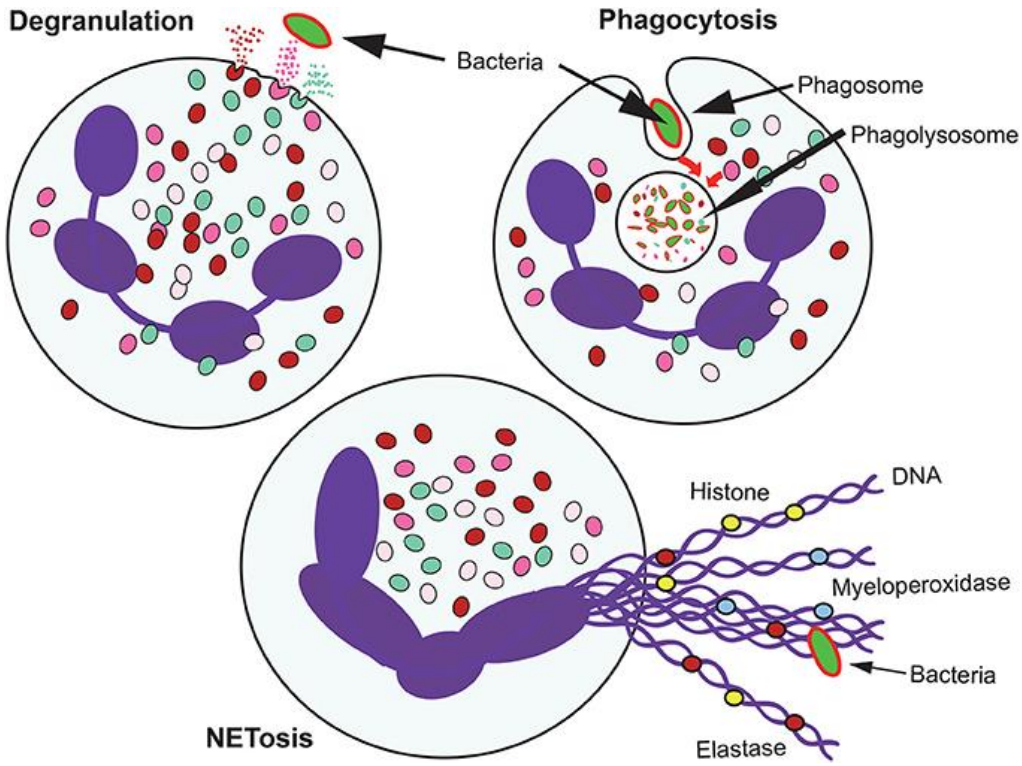

Figure 2: Antimicrobial mechanisms of neutrophils[15]

4. HAEMATOLOGICAL CHANGES IN ACUTE BACTERIAL INFECTIONS

Bacterial infections elicit distinct haematological changes that reflect both the immune response and the systemic effects of the infectious process. Among these, changes in white blood cell (WBC) counts and morphology are most prominent. Leucocytosis, frequently observed during bacterial infections, is typically accompanied by an increase in immature neutrophils. This neutrophilic response is a hallmark of bacterial infections, highlighting the critical role of neutrophils in bacterial clearance through mechanisms such as phagocytosis and reactive oxygen species generation.[18] However, in cases of severe infections or sepsis, leukopenia may develop, reflecting bone marrow suppression or excessive consumption of neutrophils.[19] Similarly, lymphopenia, a reduction in circulating lymphocyte levels, is commonly observed in systemic bacterial infections and is indicative of immune dysregulation and disease severity.[20]

During systemic bacterial infections, endotoxins and pro-inflammatory mediators interact with the RBC membrane, leading to alterations in its deformability and structural integrity. These changes include membrane stiffening, cell shrinkage, and phospholipid redistribution within the bilayer, which are characteristic of eryptosis and the suicidal death of erythrocytes. Eryptosis is triggered by a variety of inducers, including inflammatory cytokines, bacterial toxins such as sphingomyelinase and hemolysin, and oxidative stress. This process involves the translocation of phosphatidylserine to the outer membrane leaflet, signalling macrophages to engulf the affected cells. While eryptosis aids in the clearance of defective and infected RBCs, it also exacerbates systemic inflammation by releasing pro-inflammatory cytokines and contributing to blood clotting. Functionally, RBC deformability, crucial for traversing the microvasculature, is compromised during bacterial infections. This pathological deformability contributes to impaired tissue oxygenation and microvascular perfusion. Concurrently, changes in RBC indices, such as reduced mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV), reflect the inflammatory milieu and its effects on erythropoiesis. Hyperactivated platelets and RBC-platelet interactions further exacerbate the pathological rheology during bacterial infections. These interactions lead to RBC aggregation and entrapment in microthrombi, contributing to disseminated intravascular coagulation (DIC), a life-threatening complication characterised by widespread microvascular thrombosis and subsequent bleeding.[21]

Platelet counts in bacterial infections demonstrate a bidirectional response, varying with the stage and severity of the infection. Thrombocytosis, observed during the acute phase, is an adaptive response mediated by pro-inflammatory cytokines, particularly interleukin-6 (IL-6).[22,23] Thrombocytopenia is often indicative of severe

systemic involvement, such as sepsis, and may result from disseminated intravascular coagulation (DIC) or increased peripheral destruction of platelets. DIC, characterised by widespread activation of the coagulation cascade, manifests as microvascular thrombosis, consumption of clotting factors, and subsequent bleeding diathesis, often with significant prognostic implications.[24]

Advanced haematological parameters, such as VCS measurements obtained via automated haematology analysers, have gained attention for their utility in early diagnosis. These parameters assess changes in cell size, granularity, and internal complexity, providing valuable insights into the systemic inflammatory response during bacterial infections.

5. CLINICAL MANIFESTATIONS OF ACUTE BACTERIAL INFECTIONS

The clinical presentation of acute bacterial infections varies widely depending on the pathogen, the site of infection, and the host's immune response. While specific symptoms are associated with particular infections, there are general and site-specific features commonly observed in acute bacterial infections.[1]

General Clinical Features

- **Fever and chills:**

  i. Elevated body temperature is a hallmark of infection, with a core temperature ≥38.3°C (≥101°F) commonly defined as fever.

  ii. Rectal temperatures are the most accurate, being 0.4°C (0.7°F) higher than oral and 0.8°C (1.4°F) higher than axillary measurements.

- **Heart Rate Changes:**

  i. For every 1°C (1.8°F) increase in core temperature, the heart rate typically rises by 15–20 beats per minute.

  ii. Relative bradycardia (Faget's sign), where the heart rate is lower than expected for the degree of fever, may occur in certain infections (e.g., typhoid fever, brucellosis).

- **Lymphadenopathy:** Infections often cause lymph node enlargement or tenderness due to the body's immune response.

- **Malaise and Fatigue**: Patients frequently report a general sense of discomfort and tiredness.

- **Localized Symptoms**: Depending on the infection site, symptoms may include pain, swelling, redness, or impaired function of the affected area.

Site-Specific Manifestations

- **Respiratory Tract Infections**: Conditions like bacterial pneumonia present with cough, sputum, breathlessness and chest pain. Physical examination may reveal crackles, or decreased breath sounds over the affected lung areas.

- **Skin and Soft Tissue Infections**: Infections such as cellulitis or abscesses cause localized redness, warmth, swelling, and pain. Fever may accompany these infections, and severe cases can show signs of systemic involvement.

- **Gastrointestinal Infections**: Bacterial GI infections often lead to abdominal pain, diarrhoea (which may be bloody), nausea, and vomiting. Severe cases can result in dehydration, especially if diarrhoea and vomiting are prolonged.

- **Urinary Tract Infections (UTIs)**: Symptoms include dysuria (painful urination), increased urinary frequency and urgency, and haematuria (blood in the urine).

- **Central Nervous System Infections**: Bacterial meningitis presents with severe headache, neck stiffness, photophobia, altered mental status, and fever.

Systemic Manifestations

- **Sepsis and Septic Shock**: Severe bacterial infections can trigger sepsis, characterised by a systemic inflammatory response. Clinical features include hypotension, tachycardia, tachypnoea, altered mental status, and multi-organ dysfunction. Without prompt treatment, sepsis can progress to septic shock, a life-threatening condition.

6. DIAGNOSIS

The diagnosis of acute bacterial infections is approached through a combination of clinical evaluation and targeted diagnostic testing.[1]

i. **Clinical Evaluation:** A thorough history is essential for diagnosing infectious diseases. This includes an exposure history, and social history should explore high-risk behaviours, such as IV drug use or unsafe sexual practices, as well as hobbies like gardening or animal contact and occupational exposures. Dietary habits, including the consumption of raw or undercooked meat, unpasteurized milk, or unpurified water, should be documented, as they may indicate exposure to specific pathogens. Travel history, including activities like freshwater swimming or consuming local food and water, is critical for identifying region-specific infections. Additionally, host-specific factors, such as immune status (e.g., underlying conditions like HIV or malignancy, medications like chemotherapy or

glucocorticoids) and immunisation history, should be assessed to evaluate susceptibility to infections and ensure protection against vaccine-preventable diseases.

Vital signs need to be monitored closely, with particular attention to fever, defined as a core temperature ≥38.3°C (101°F), and relative bradycardia, which may occur in infections such as typhoid fever. Lymph nodes should be evaluated for size, tenderness, and consistency, noting whether lymphadenopathy is localised or generalised. A full skin examination is essential to identify rashes, ulcers, or lesions, such as splinter haemorrhages or Janeway lesions, which may suggest conditions like endocarditis. IV lines, surgical drains, should be assessed, as these can serve as potential sites of infection.

ii. Laboratory Investigations[1]

Complete Blood Count: It evaluates the levels of different blood cells. Leucocytosis, particularly with a left shift (increased immature neutrophils), often suggests a bacterial infection. The WBC differential is important, as bacterial infections typically increase polymorphonuclear neutrophils (including bands), viral infections increase lymphocytes, and parasitic infections may elevate eosinophils.

Inflammatory Markers: Markers such as CRP and erythrocyte sedimentation rate (ESR) are nonspecific indicators of inflammation. Elevated levels may support the presence of an infectious process but are not definitive for bacterial aetiology.

- **C-Reactive Protein**: CRP is produced by the liver in response to inflammation. In healthy individuals, CRP levels are typically less than 0.3 mg/Dl. A range of 0.3 to 1.0 mg/dL indicates minor elevation, often linked to obesity, pregnancy, depression, diabetes, common colds, gingivitis, and

genetic factors. Moderate elevation (1.0 to 10.0 mg/dL) is associated with systemic inflammation and conditions like autoimmune diseases and myocardial infarction. Levels above 10.0 mg/dL indicate marked elevation, often due to acute infections or trauma. Severe elevation (more than 50.0 mg/dL) is primarily linked to acute bacterial infections.[25] CRP synthesis begins 6 to 8 hours after infection onset. Its levels peak between 36 to 50 hours. It's half-life is approximately 19 hours and is cleared by the liver.[26]

- **Procalcitonin**: In healthy adults, levels remain below 0.05 μg/L. A range of 0.05 to 0.5 μg/L suggests that systemic infection is unlikely, though localised infections may be present. Levels between 0.5 and 2 μg/L indicate a possible systemic infection but can also rise due to major trauma, recent surgery, or severe cardiogenic shock. When levels reach 2 to 10 μg/L, systemic infection is likely, and values ≥10 μg/L strongly suggest severe bacterial sepsis or septic shock. PCT becomes detectable 3 to 4 hours after infection begins, following TNF-α release at 90 minutes and IL-6 at 3 hours. It peaks within 6 to 12 hours and has a half-life of about 24 hours.[27]
- **Erythrocyte Sedimentation Rate (ESR)**: The ESR measured using the Westergren method, varies by age and gender. In males under 50 years, normal ESR is ≤15 mm/hr, while in females under 50 years, it is ≤20 mm/hr. For males over 50 years, the normal range extends to ≤20 mm/hr, and for females over 50 years, it is ≤30 mm/hr. In children, ESR is typically ≤10 mm/hr. Extremely high ESR levels exceeding 100 mm/hr are highly specific for conditions such as infection, malignancy, or arteritis. ESR begins to rise 24–48 hours after the onset of inflammation and declines gradually as the condition resolves. Unlike other inflammatory markers, in response to infection ESR

increases more slowly and remains elevated for a prolonged period as long as excess fibrinogen persists in the serum.[28]

Cerebrospinal Fluid (CSF) Analysis:

Key components include opening pressure, cell counts, glucose, protein, Gram stain, and culture.

- Lymphocytic pleocytosis with low glucose suggests infections such as *Listeria*, *M. tuberculosis*, or fungal infections, or non-infectious conditions like neoplastic meningitis.
- Bacterial antigen tests, such as latex agglutination for Haemophilus influenzae type b, Streptococcus agalactiae (group B Streptococcus), Streptococcus pneumoniae, and Neisseria meningitidis, are not recommended for routine screening since their sensitivity is similar to that of Gram's stain.
- Antigen tests for *Cryptococcus* and serologic tests for Treponema pallidum or Coccidioides are highly sensitivity.

Cultures:

- Cultures of infected tissue or fluids, such as blood, urine, sputum, or pus, are essential for identifying pathogens and determining antimicrobial susceptibility.
- Specimens should ideally be collected before starting antimicrobial therapy. Microscopic examination, such as Gram stain or KOH preparation, is crucial if cultures are delayed.
- While cultures provide essential diagnostic information, distinguishing between clinically significant results and contamination can be complex. This

determination requires consideration of the patient's immune status, prior exposures, and normal microbial flora. Serial cultures may be needed to confirm organism clearance.

Pathogen-Specific Testing:

- Serologic, antigen, and PCR tests are widely available and provide rapid diagnostics for specific pathogens.
- Some tests, such as universal PCRs, detect organisms that are difficult to culture.

Radiology:

- Imaging techniques, such as CT, MRI, or ultrasound, are used to evaluate lymphadenopathy in inaccessible regions like the mediastinum or abdomen and to assess internal organs for infection.

7. **VOLUME, CONDUCTIVITY, AND SCATTER PARAMETERS:**

The VCS technology of the automated haematology analyser (e.g., Beckman Coulter DxH 900) utilises direct current impedance to measure cell volume (V) and size, radiofrequency opacity to assess conductivity (C), and laser beam light scatter (S) to analyse the internal structure of cells. This system evaluates over 8,000 WBCs to provide an average value for the sample, classifying cells into neutrophils, lymphocytes, monocytes, eosinophils, or basophils, thereby generating an automated differential count. Additionally, the analyser can detect morphological changes in reactive neutrophils and monocytes associated with sepsis by analysing alterations in volume, conductivity, and scatter parameters. An increase in cell volume and size

reflects reactive neutrophils and neutrophilic left shift, while increased cytoplasmic granularity is indicated by changes in conductivity and scatter.[29]

8. VCS PARAMETERS IN THE EARLY DIAGNOSIS OF INFECTIONS

Automated haematology analysers measure Volume (V), Conductivity (C), and Scatter (S) (VCS parameters) to assess neutrophil activation, morphological changes, and function.

During bacterial infections, neutrophils undergo structural and functional alterations, which are reflected in VCS parameters as follows:

Neutrophil Volume: Neutrophil volume increases due to activation, cellular swelling, and the presence of immature neutrophils released in response to infection.[30]

Mechanisms Behind Increased Neutrophil Volume

Cytokine activation plays a significant role in neutrophil volume changes. Bacterial infections stimulate the release of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. These cytokines activate neutrophils, leading to increased metabolic activity and structural changes, which result in cell enlargement.[31]

Toxic granulation refers to the accumulation of azurophilic (primary) granules within neutrophils, containing antibacterial enzymes such as myeloperoxidase, elastase, and defensins. The increased number of granules raises the overall intracellular content, thereby increasing cell volume.[32]

Neutrophil swelling due to increased water content is another contributing factor. Activation leads to intracellular water retention, causing neutrophils to swell.

This is partially driven by ion influx (Na+, K+), which disrupts osmotic balance and promotes swelling.[33]

The presence of immature neutrophils, also known as a left shift, further contributes to the increased volume. In response to bacterial infections, the bone marrow releases immature neutrophils (bands and metamyelocytes) into circulation. These immature neutrophils are larger than mature segmented neutrophils, leading to an overall increase in MNV.[34]

Neutrophil Conductivity: Neutrophil conductivity, which measures cellular density, membrane permeability, and internal homogeneity, decreases due to toxic granulation, vacuolization, and nuclear swelling. These structural alterations disrupt the uniformity of the cell's internal composition, leading to lower conductivity.

Mechanisms Behind Decreased Neutrophil Conductivity

Toxic granulation and internal structural changes result in reduced conductivity. The increased presence of granules (toxic granulation) disrupts the even distribution of cytoplasmic components. Since conductivity is measured by how electrical signals pass through the cell, this heterogeneity reduces conductivity values. Vacuolisation due to phagocytosis also lowers conductivity. Activated neutrophils engulf bacteria, forming intracellular vacuoles. These vacuoles create regions of differing density, leading to uneven electrical conduction and reduced conductivity.[35]

Membrane permeability and neutrophil activation further decrease conductivity. Inflammatory cytokines increase neutrophil membrane permeability, allowing ion exchange necessary for activation. This disrupts ionic balance, further reducing conductivity.[36]

Apoptosis and membrane damage in severe infections also contribute to reduced conductivity. Neutrophils undergo apoptosis after completing their immune function. Apoptotic cells lose membrane integrity, causing cytoplasmic leakage and further decreasing conductivity.[37]

Neutrophil Scatter: Scatter is a measure of cytoplasmic granularity and nuclear complexity.

Mechanisms Behind Change in Neutrophil Scatter

The bone marrow stimulatory mechanisms that produce neutrophilia also leads to a release of immature granulocytes (left shift), the decreased MNS in infected patients is due to the lower nuclear complexity seen in such cells.[38]

The presence of toxic granules may lead to an increased scatter value.[39]

However, the complete mechanism behind the change in Neutrophil Scatter is not clear.

RELEVANT STUDIES ON VCS PARAMETERS IN BACTERIAL INFECTIONS

A study conducted by Suresh et al. in 2016 included 94 patients with infections (36 systemic and 58 localized) and 46 control subjects. The findings revealed a significant increase in the MNV (158.3 vs. 137.2) and MMV (177.8 vs. 161.7) in patients with infections compared to controls. However, no significant difference was observed in MNV between systemic and localized infections. The authors concluded that VCS parameters, particularly MNV and MMV, are sensitive markers and could serve as rapid diagnostic indicators for acute bacterial infections.[29]

A study conducted by Chaves et al. in 2005 peripheral blood samples from 69 patients with positive blood cultures for bacteria and 35 control subjects were examined. The study found a significant increase in the MNV in septic patients (156) compared to controls (143), $p<0.001$. Additionally, a significant decrease in the mean channel of neutrophil light scatter was observed in patients. An elevated MNV was correlated with a higher white blood cell count and neutrophil percentage, and this elevation was noted even in patients without leukocytosis or neutrophilia. Using a cutoff value of 150 for MNV, the study achieved a specificity of 91% and a sensitivity of 70%. The authors concluded that MNV, as a quantitative, objective, and sensitive parameter, has the potential to serve as an additional indicator for acute bacterial infections.[38]

A study conducted by Purohit et al. in 2015 analyzed Peripheral blood samples from 162 patients with positive blood cultures for bacteria and 40 healthy controls. The study found a significant increase in the MNV in septic patients compared to control subjects (156 vs. 143, $p<0.001$). An elevated MNV was correlated with a higher white blood cell count and neutrophil percentage, even in patients without leukocytosis or neutrophilia. Using a cutoff value of 149 for MNV, the study achieved a specificity of 91.4% and a sensitivity of 88.7%. The authors concluded that MNV, as a quantitative, objective, and sensitive parameter, has the potential to serve as an additional diagnostic indicator for acute bacterial infections.[39]

Arora et al. (2019) studied the usefulness of VCS parameters from the Coulter LH 750 Hematology Analyzer to detect sepsis early and monitor treatment response. The study included 134 blood culture-positive sepsis patients and 100 healthy controls, with measurements taken on day 0 (culture positivity), day 3, and day 7. The MNV and MMV were higher in sepsis patients compared to controls. Treatment led to

a decrease in MNV and MMV, along with changes in scatter and conductivity parameters. A cutoff of 150.2 for MNV showed 79.1% sensitivity, 95% specificity, and an AUC of 92.3%. For MMV, a cutoff of 168.3 showed 80.6% sensitivity, 77.5% specificity, and an AUC of 83%.[40]

Goyal et al. (2023) conducted a prospective case-control study to evaluate the utility of VCS parameters as early markers of sepsis in critically ill patients. A total of 80 patients were included in the study, with 40 sepsis cases and 40 healthy controls. The MNV and mean MMV were significantly higher in the sepsis group compared to controls. While neutrophil conductivity and scatter were lower in the sepsis group and higher in the control group, the difference was not statistically significant. Even with a low total leukocyte count (TLC), the mean neutrophil volume remained higher in sepsis patients, indicating its usefulness as a predictive marker for sepsis. The study concluded that the mean neutrophil volume and monocyte volume (MMV) are sensitive markers for predicting sepsis, even when TLC is low in sepsis patients. These parameters can help clinicians detect sepsis at an early stage, offering valuable practical implications in clinical settings.[41]

Vaswani et al. (2022) conducted a cross-sectional study to evaluate the role of automated VCS parameters of neutrophils as indicators of sepsis and their potential to differentiate sepsis from other inflammatory disorders. The study included 225 patients with culture-proven or clinically suspected sepsis, alongside an equal number of healthy controls. Additionally, 138 patients with non-infective inflammatory conditions. The results revealed that MNV was significantly higher ($p < 0.0001$), while Mean Neutrophil Scatter (MNS) was significantly lower ($p < 0.0001$) in sepsis patients compared to the control group. However, Mean Neutrophil Conductivity (MNC) values were comparable between the sepsis and control groups ($p = 0.4735$).

Subgroup analysis of sepsis patients showed significant differences in MNV ($p = 0.0009$) and MNS ($p = 0.0210$) among patients with leukopenia, normal TLC, and leucocytosis. The study concluded that MNV is an inexpensive and useful parameter that can be accessed from routine CBC data. It has the potential to serve as an early indicator of sepsis and can complement clinical diagnosis in suspected cases.[9]

A-Jin Lee and Sang-Gyung Kim (2013) evaluated the diagnostic significance of VCS parameters among 85 patients, categorized as control (n=29), localized infection (n=38), and sepsis (n=18). The results showed that the MNV and MMV were significantly higher in the sepsis group compared to both the localized infection and control groups (P=0.000 for both). Additionally, the mean cell conductivity and low median angle light scatter of neutrophils were significantly lower in the sepsis group than in the localized infection and control groups (P=0.029 and P=0.022, respectively). With a cut-off of 156.5, MNV demonstrated a sensitivity of 83.3% and specificity of 78% in predicting sepsis.[42]

Kannan et al. (2017) analyzed CBC and VCS reports for 110 cases and 110 control cases without the infection. The results showed differences a significant difference in MNV even when neutrophil count was <85% or WBC count was <11,000/cumm. ROC curve analysis for MNV revealed a criterion value of >129.3, with a sensitivity of 92.7%. For MMV, the criterion value was >157.4, with a sensitivity of 47.27%. In conclusion, the study demonstrated significant differences in VCS parameters of neutrophils and monocytes between acute bacterial infections and controls.[43]

Celik et al. (2011) investigated the value of VCS parameters in the diagnosis and treatment efficacy of neonatal sepsis using the Coulter LH780 hematology analyzer. The study included peripheral blood samples from 304 newborns, with 206 in the sepsis group (76 with proven sepsis and 130 with clinical sepsis) and 98 in the control group. The results showed significant increases in MNV, alongside significant decreases in MNC and MNS in septic newborns. At the end of treatment, MNV was decreased, while MNC and MNS increased, indicating recovery. Gram-negative sepsis was associated with higher MNV and VDW compared to Gram-positive sepsis.[44]

D Mardi et al. (2010) conducted a study which collected blood samples from patients with sepsis (n = 37), nonsystemic bacterial infections (n = 39), and controls (n = 48). MNV and MMV were significantly increased in the sepsis group. At a cut-off point of 250 pg/ml, IL-6 demonstrated the best predictive ability for sepsis, with a sensitivity of 93% and specificity of 76%. MNV, with a cut-off value of 150, exhibited comparable sensitivity and specificity to CRP (at a cut-off of 60 mg/dl) and was identified as the most predictive VCS parameter. The findings suggest that MNV and MMV may serve as potential parameters for differentiating between sepsis and nonsystemic infections, offering valuable diagnostic tools for clinicians in the rapid detection of severe infections.[45]

Bagdasaryan et al. (2007) analyzed data from 242 adult patients. They were randomized into three groups, one with no apparent clinical evidence of infection, another with localized infection, and last with severe infection. The results found that in group 1 and 3, the total WBC counts, neutrophils, ANC, band counts, MNV, and NDW were elevated. Along with this, MNV and ANC and band counts, as well as between NDW and ANC and band counts were significantly correlated. The study

concluded that the neutrophil VCS parameters, MNV and NDW, have superior sensitivity and specificity compared to manual band count, ANC, and CRP.[8]

Abiramalatha et al. (2016) studied the utility of neutrophil Volume, Conductivity, and Scatter parameters as a screening tool for neonatal sepsis. A total of 600 neonates were included, with 240 in the sepsis group and 360 in the control group. Most parameters had an area under the curve (AUC) of >0.6. The five most significant parameters—MNV, median angle light scatter (MALS), lower median angle light scatter (LMALS), MNV-DW, and ALL-DW—had sensitivity and specificity ranging from 65% to 75%. A combination of leukopenia, thrombocytopenia, MNV, and LMALS yielded a likelihood ratio (LR+) of 15.3 and LR- of 0.17. With a pre-test probability of 40%, post-test probabilities increased to 91% for a positive result and decreased to 10% for a negative result. A prospective validation study with 60 additional babies confirmed the robustness of the cutoffs. The study concluded that neutrophil VCS parameters, though not sufficient as stand-alone tests, can complement other hematological screening tests to enhance the diagnostic.[46]

Bharti et al. (2024) conducted a study among total of 41 sepsis cases and 43 healthy controls. The results revealed no significant difference in MNV between cases and controls, $p>0.05$. However, MNC and MNS were significantly higher in cases, $P < 0.001$. MNV had an Area Under the Curve of 0.54, indicating poor discriminatory ability, while MNC had AUC as 0.98 and MNS (AUC 0.95) demonstrated strong discriminatory power in diagnosing sepsis. The study concluded that while MNV failed as a standalone biomarker for sepsis, MNC and MNS showed significant diagnostic and discriminatory accuracy in identifying sepsis in hospitalized individuals in the emergency setting.[47]

MATERIALS AND METHODS

Study Design

The study was a retrospective case-control design to compare VCS parameters of neutrophils among patients with confirmed bacterial infections and healthy controls with normal complete blood counts (CBC).

Study Setting

The study was conducted at a tertiary care center in Belagavi.

Study Period

The study period spanned from April 1, 2023, to March 31, 2024, covering a full year of hospital admissions and bacterial culture-positive cases during this time frame.

Sampling Technique

Convenient sampling was employed for selecting participants. All patients who met the inclusion criteria during the study period were included.

Sample Size

The sample size was determined using a formula based on a two-group comparison:

$$n = \frac{2\,(Z\alpha/2+Z\beta)^2}{d^2}$$

Where: **d** (the difference to detect) $= \frac{(\mu 1-\mu 2)}{\sigma}$

where, μ is mean of the first group, μ2 is mean of the second group, σ2 is the common error variance, Zα/2 value is 1.96 for 95% confidence level and Zβ value is 1.2816 for 90% power. Mean of MNS among cases and control were 141.4 ± 7.6 and 136.8 ± 8.0 respectively. Considering similar result at 5% level of significance and 90% power, the minimum sample size required is 62 subjects per group. Hence, the total sample size is 62 x 2 = 124 subjects. As sample size increases, the accuracy of result also increases.

Study Population

Inclusion and Exclusion Criteria

Inclusion Criteria for Cases:

i. Age >18years
ii. Bacterial culture-positive patients

Inclusion criteria for controls:

i. Age >18 years
ii. BMI matched subjects with CBC within normal limits

Exclusion Criteria for cases:

i. Pregnant females
ii. Bacterial Culture negative subjects with history of fever and infection
iii. Chronic infectious patients
iv. Chronic inflammation patients- like rheumatoid arthritis, systemic lupus erythematosus

Exclusion criteria for controls:

i. Pregnant females
ii. Bacterial Culture negative subjects with history of fever and infection.
iii. Chronic infectious patients

iv. Chronic inflammation patients like rheumatoid arthritis, systemic lupus erythematosus

Data Collection Tool and Method

Data were collected from hospital records, including demographic details, clinical history, laboratory investigations, and imaging findings. The following investigations were included:

1. Complete Blood Count (CBC): Performed on EDTA samples to evaluate neutrophil counts and VCS parameters using an automated haematology analyser (e.g., Beckman Coulter DxH 900).
2. VCS Parameters of Neutrophils: Measured to analyse cell volume, conductivity, and scatter.
3. Culture and Sensitivity Testing: Blood or site-specific samples collected for bacterial culture.

Data processing was performed using Microsoft Excel and R version 4.2.2 for statistical analysis.

Study Protocol

1. Hospital records were reviewed to identify patients meeting the inclusion criteria.
2. VCS parameters of neutrophils from EDTA blood samples taken on the day of admission for cases were traced.
3. Matched control samples were identified based on BMI and normal CBC results.
4. Comparison of VCS parameters was made between cases and controls.

Data Analysis:

Data will be analysed using R version 4.2.2 and Microsoft Excel for statistical analysis and data processing. Categorical Variables will be represented by frequencies and percentages. Continuous Variables will be represented by Mean ± Standard Deviation (SD) or Median (Min, Max), depending on the distribution of the data.

The normality of continuous variables will be assessed using:

- Shapiro-Wilk test for normality.
- QQ plot for visual inspection of data distribution.

- If the data is found to be normally distributed, parametric tests will be applied.
- If the data is not normally distributed, non-parametric tests will be used.

Statistical Tests:

- **Chi-Square Test**: Used to assess the association between categorical variables (e.g., gender, infection type).
- **Two-Sample T-Test**: Used to compare the means of normally distributed continuous variables (e.g., VCS parameters) between two independent groups (cases vs controls).
- **Mann-Whitney U Test**: Used to compare the distributions of non-normally distributed continuous variables between two independent groups.

Predictive Modelling:

- **Logistic Regression**: Applied to assess the relationship between VCS parameters of neutrophils and the likelihood of acute bacterial infection. This will help in identifying significant predictors of infection based on neutrophil characteristics.
- **Receiver Operating Characteristic (ROC) Curve Analysis**: Conducted to evaluate the performance of VCS parameters as diagnostic tools.

- The Area Under the Curve (AUC) will be calculated to assess the overall accuracy of the VCS parameters.
- The cut-off values for the VCS parameters will be determined by optimizing both sensitivity and specificity, ensuring the best balance for predicting bacterial infections.

Statistical Significance:

- A P-value less than or equal to 0.05 will indicate statistical significance for all tests.
- Appropriate tables and figures (e.g., histograms, box plots, ROC curves) will be generated to visualize the data and results.

Ethical Considerations

Ethical approval was obtained from the institutional ethics committee prior to study initiation. Data confidentiality was maintained by anonymizing patient identifiers. As this was a retrospective study, direct informed consent was waived; however, records were only accessed following institutional guidelines.

RESULTS

Table 1. Age distribution among the cases and controls (N=124)

Age (in years)	Group 1- Cases n (%)	Group 2- Controls n (%)
18-30	5 (8.1)	13 (20.9)
31-50	18 (29.0)	25 (40.3)
51-70	26 (41.9)	19 (30.7)
71-90	13 (21.0)	5 (8.1)
Total	**62 (100)**	**62 (100)**

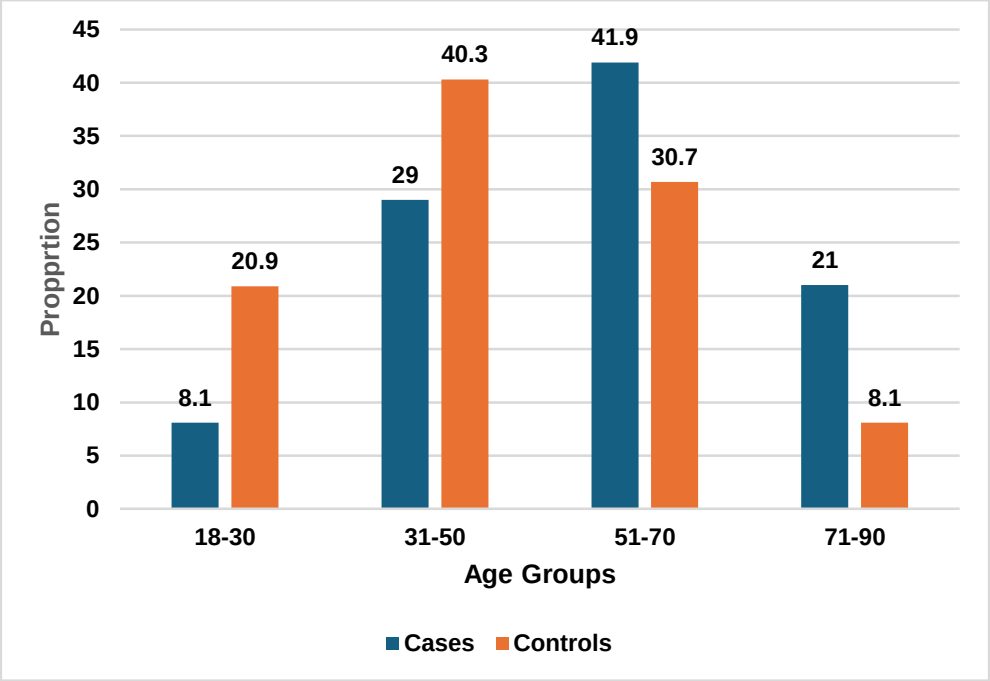

Figure 3. Age distribution among the cases and controls

Table 1 presents the age distribution of 124 individuals, divided equally into cases and controls (62 each). Cases were defined as patients with a positive bacterial culture, while controls were patients with normal complete blood count (CBC) results, matched for body mass index (BMI) with the cases. Among cases, the largest proportion falls within the 51-70 age group (41.9%), followed by 31-50 years (29.0%), 71-90 years (21.0%), and 18-30 years (8.1%). In contrast, controls are predominantly in the 31-50 age group (40.3%), followed by 51-70 years (30.7%), 18-30 years (20.9%), and 71-90 years (8.1%). Overall, cases have a higher representation in older age groups (51-90 years), while controls are more concentrated in younger age groups (18-50 years).

Table 2. Gender distribution among the cases and controls (N=124)

Gender	Group 1- Cases n (%)	Group 2- Controls n (%)
Male	43 (69.3)	46 (74.2)
Female	19 (30.7)	16 (25.8)
Total	**62 (100)**	**62 (100)**

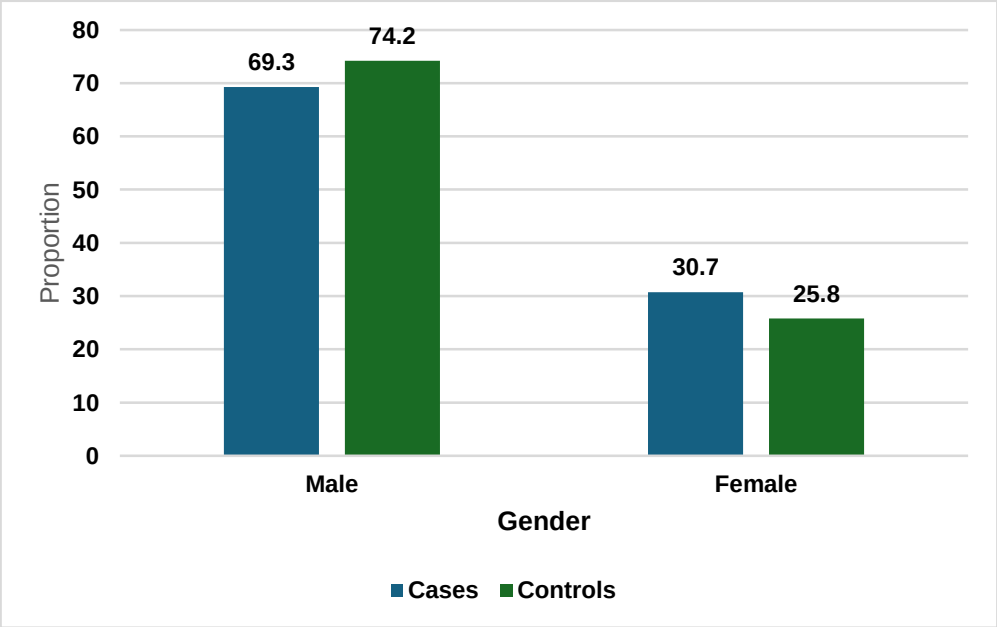

Figure 4. Gender distribution among the cases and controls

Table 2 illustrates the gender distribution among 124 participants, evenly divided into cases and controls (62 each). Among cases, 69.3% are male, and 30.7% are female, whereas among controls, 74.2% are male, and 25.8% are female. Overall, both groups are predominantly male, with a slightly higher proportion of males in the control group compared to the cases.

Table 3. Comparison between the Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter among the cases and controls (N=124)

Parameters	Groups	Number of cases	Mean	Standard Deviation	Median	IQR	P value*
Mean Neutrophil Volume	Group 1- Cases	62	157.8	11.5	155	150-160	**<0.001**
	Group 2- Control	62	141.2	4.7	142	138-145	
Mean Neutrophil conductivity	Group 1- Cases	62	142.4	5.3	142	139-147	**<0.001**
	Group 2- Control	62	145.2	5.6	145	141-149	
Mean Neutrophil Scatter	Group 1- Cases	62	138.2	7.5	140.5	135-142	0.08
	Group 2- Control	62	142.4	17.3	144	141-148	

**Independent t test*

Table 3 compares the MNV, MNC, and MNS between cases and controls among 124 participants (62 cases and 62 controls).

For Mean Neutrophil Volume, the cases had a significantly higher mean (157.8 ± 11.5) compared to controls (141.2 ± 4.7). The median MNV was also higher in cases (155) than in controls (142), with an interquartile range (IQR) of 150-160 for cases and 138-145 for controls. This difference was statistically significant, with a P-value <0.001.

Regarding Mean Neutrophil Conductivity, controls exhibited a slightly higher mean (145.2 ± 5.6) than cases (142.4 ± 5.3). The median MNC for controls was 145, compared to 142 for cases, with overlapping IQRs (141-149 for controls and 139-147 for cases). The difference in conductivity was also statistically significant (P-value <0.001).

For Mean Neutrophil Scatter, the mean values were comparable, with cases having a mean of 138.2 ± 7.5 and controls slightly higher at 142.4 ± 17.3. The median values were close, at 140.5 for cases and 144 for controls, with overlapping IQRs (135-142 for cases and 141-148 for controls). This difference was not statistically significant, as indicated by a P-value of 0.08.

Table 4. Comparison between the Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter among the cases with WBC count ≤ 11,000, count > 11,000 mm^3 and controls (N=124)

Parameters	Groups	Number of cases	Mean	SD	Median	IQR	P value*			
							All groups	1 vs. 3	2 vs. 3	1 vs. 2
Mean Neutrophil Volume	Group 1- Cases with WBC count ≤ 11,000 mm^3	32	155.4	9.1	152.5	149.5-157.5	**<0.001**	**<0.001**	**<0.001**	0.07
	Group 2- Cases with WBC count > 11,000 mm^3	30	160.5	13.2	156.5	152				
	Group 3- Control	62	141.2	4.7	142	138-145				
Mean Neutrophil conductivity	Group 1- Cases with WBC count ≤ 11,000 mm^3	32	142.8	5.9	141.5	139-148	**0.01**	0.14	**0.02**	1.00
	Group 2- Cases with WBC count > 11,000 mm^3	30	141.9	4.6	142	138-144				
	Group 3- Control	62	145.2	5.6	145	141-149				
Mean Neutrophil Scatter	Group 1- Cases with WBC count ≤ 11,000 mm^3	32	139.8	6.6	141	137-143.5	0.13	1.00	0.14	0.97
	Group 2- Cases with WBC count > 11,000 mm^3	30	136.4	8.1	139	132-142				
	Group 3- Control	62	142.4	17.3	144	141-148				

**One-way ANOVA for common p value and Bonferroni post hoc test for inter-group p value*

Table 4 compares the MNV, MNC, and MNS across three groups: cases with WBC counts ≤ 11,000/mm³, cases with WBC counts > 11,000/mm³, and controls.

In MNV, cases with WBC counts greater than 11,000/mm³ (Group 2) exhibited the highest mean value (160.5 ± 13.2), followed by cases with WBC ≤ 11,000/mm³ (Group 1) with a mean of 155.4 ± 9.1. Controls (Group 3) had the lowest mean at 141.2 ± 4.7. Statistically significant differences were found across all groups ($P < 0.001$), with pairwise comparisons revealing significant differences between Group 2 and both Group 1 and Group 3 ($P < 0.001$ for both), but no significant difference between the two case groups ($P = 0.07$). Median MNV values followed the same trend, with the cases showing higher values than controls.

For MNC, the controls had the highest mean (145.2 ± 5.6), followed by cases with WBC ≤ 11,000/mm³ (142.8 ± 5.9) and cases with WBC > 11,000/mm³ (141.9 ± 4.6). The overall difference among the groups was statistically significant ($P = 0.01$), with pairwise comparisons revealing significant differences between the controls and both case groups ($P = 0.02$ for both). However, no significant difference was found between the two case subgroups ($P = 1.00$). The median MNC values also reflected this trend, with controls having higher values than the two case groups.

In MNS, controls again had the highest mean (142.4 ± 17.3), followed by cases with WBC ≤ 11,000/mm³ (139.8 ± 6.6) and cases with WBC > 11,000/mm³ (136.4 ± 8.1). Despite the differences in means, the overall difference across the groups was not statistically significant ($P = 0.13$). Pairwise comparisons showed no significant differences between any of the groups, with P-values of 1.00 for Group 1 vs. Group 3, 0.14 for Group 2 vs. Group 3, and 0.97 for Group 1 vs. Group 2.

Overall, the study found significant differences in MNV and MNC across the groups, with higher values in cases with WBC > 11,000/mm³ compared to controls and cases with WBC ≤ 11,000/mm³. However, no significant differences were observed in MNS across the groups. The results highlight the distinction in neutrophil volume and conductivity between cases with varying WBC counts and controls, while scatter values did not show marked variation.

Table 5. Comparison between the Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter among the cases with Neutrophil <85 %, >85 %and controls (N=124)

Parameters	Groups	Number of cases	Mean	SD	Median	IQR	P value*			
							All groups	1 vs. 3	2 vs. 3	1 vs. 2
Mean Neutrophil Volume	Group 1- Cases with Neutrophil <85 %	45	154.2	7.9	152	149-156	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Group 2- Cases with Neutrophil >85 %	17	167.6	13.8	167	159-170				
	Group 3- Control	62	141.2	4.7	142	138-145				
Mean Neutrophil conductivity	Group 1- Cases with Neutrophil <85 %	45	143.1	5.3	142	140-147	**<0.001**	0.16	**<0.001**	0.26
	Group 2- Cases with Neutrophil >85 %	17	140.5	4.8	140	138-143				
	Group 3- Control	62	145.2	5.6	145	141-149				
Mean Neutrophil Scatter	Group 1- Cases with Neutrophil <85 %	45	139.7	6.8	141	137-144	0.07	0.90	0.07	0.42
	Group 2- Cases with Neutrophil >85 %	17	134.1	8.0	137	132-139				
	Group 3- Control	62	142.4	17.3	144	141-148				

**One-way ANOVA for common p value and Bonferroni post hoc test for inter-group p value*

Table 5 compares the MNV, MNC, and MNS across three groups: cases with neutrophil counts <85%, cases with neutrophil counts >85%, and controls.

In MNV, cases with Neutrophil >85% (Group 2) exhibited the highest mean value (167.6 ± 13.8), followed by cases with Neutrophil <85% (Group 1) with a mean of 154.2 ± 7.9. Controls (Group 3) had the lowest mean at 141.2 ± 4.7. Statistically significant differences were observed across all groups ($P < 0.001$), with pairwise comparisons showing that Group 2 had significantly higher MNV than both Group 1 and Group 3 ($P < 0.001$ for both). Similarly, Group 1 also had significantly higher MNV than Group 3 ($P < 0.001$). The median values followed the same trend, with Group 2 having the highest median and Group 3 the lowest.

For MNC, controls had the highest mean (145.2 ± 5.6), followed by cases with Neutrophil <85% (143.1 ± 5.3) and cases with Neutrophil >85% (140.5 ± 4.8). The overall difference was statistically significant ($P < 0.001$), with pairwise comparisons revealing significant differences between the controls and both case groups ($P = 0.16$ for Group 1 vs. Group 3, $P < 0.001$ for Group 2 vs. Group 3). However, no significant difference was found between the two case subgroups ($P = 0.26$). The median values also followed this pattern, with controls having the highest MNC.

In MNS, controls had the highest mean (142.4 ± 17.3), followed by cases with Neutrophil <85% (139.7 ± 6.8) and cases with Neutrophil >85% (134.1 ± 8.0). However, the overall difference across the groups was not statistically significant ($P = 0.07$). Pairwise comparisons showed no significant differences between any of the groups, with P-values of 0.90 for Group 1 vs. Group 3, 0.07 for Group 2 vs. Group 3, and 0.42 for Group 1 vs. Group 2.

`In conclusion, significant differences were found in MNV and MNC across the groups, with cases with Neutrophil >85% showing higher values compared to both controls and cases with Neutrophil <85%. However, no significant differences were found in MNS, indicating that scatter values remain similar across the groups.

Table 6. Comparison between the Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter among the cases with systemic infections, localized infections and controls (N=124)

Parameters	Groups	Number of cases	Mean	SD	Median	IQR	P value*			
							All groups	1 vs. 3	2 vs. 3	1 vs. 2
Mean Neutrophil Volume	Group 1- Cases with systemic infections	21	160.8	12.7	158	153-163	**<0.001**	**<0.001**	**<0.001**	0.18
	Group 2- Cases with localized infections	41	156.4	10.6	155	150-158				
	Group 3- Control	62	141.2	4.7	142	138-145				
Mean Neutrophil conductivity	Group 1- Cases with systemic infections	21	140.9	4.7	141	137-143	**0.006**	**<0.001**	0.19	0.40
	Group 2- Cases with localized infections	41	143.1	5.5	142	140-147				
	Group 3- Control	62	145.2	5.6	145	141-149				
Mean Neutrophil Scatter	Group 1- Cases with systemic infections	21	136.8	9.1	139	132-142	0.18	0.31	0.57	1.00
	Group 2- Cases with localized infections	41	138.8	6.6	141	137-142				
	Group 3- Control	62	142.4	17.3	144	141-148				

**One-way ANOVA for common p value and Bonferroni post hoc test for inter-group p value*

Table 6 compares the MNV, MNC, and MNS among cases with systemic infections, cases with localized infections, and controls.

In MNV, cases with systemic infections (Group 1) had the highest mean value (160.8 ± 12.7), followed by cases with localized infections (Group 2) with 156.4 ±

10.6. Controls (Group 3) had the lowest mean at 141.2 ± 4.7. Statistically significant differences were observed across all groups ($P < 0.001$), with pairwise comparisons showing that both Group 1 and Group 2 had significantly higher MNV than Group 3 ($P < 0.001$ for both), but no significant difference was found between Group 1 and Group 2 ($P = 0.18$). The median values followed a similar pattern, with Group 1 having the highest median and Group 3 the lowest.

For MNC, the controls had the highest mean (145.2 ± 5.6), followed by cases with localized infections (143.1 ± 5.5) and cases with systemic infections (140.9 ± 4.7). The overall difference was statistically significant ($P = 0.006$), with pairwise comparisons showing significant differences between controls and both case groups ($P < 0.001$ for both). However, no significant difference was found between the two case subgroups ($P = 0.40$). The median values reflected this trend, with controls having the highest MNC.

In MNS, controls had the highest mean (142.4 ± 17.3), followed by cases with localized infections (138.8 ± 6.6) and cases with systemic infections (136.8 ± 9.1). However, the overall difference across the groups was not statistically significant ($P = 0.18$), with no significant differences observed in pairwise comparisons. The P-values for the pairwise tests were 0.31 for Group 1 vs. Group 3, 0.57 for Group 2 vs. Group 3, and 1.00 for Group 1 vs. Group 2, indicating that MNS remained relatively consistent across the groups.

In conclusion, significant differences were observed in MNV and MNC between the groups, with cases having higher values than controls. However, no significant differences were found in MNS, suggesting that scatter values do not vary substantially across different infection types or controls.

Table 7. Determination of cut-offs, specificity, sensitivity, and area under the curve for the Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter

	Area under the curve	95% CI	Cut-off point	Sensitivity (%)	Specificity (%)
Cases vs. Controls					
Mean Neutrophil Volume	0.99	0.98-1.00	>148	96.8	96.8
Mean Neutrophil conductivity	0.36	0.27-0.46	>144	38.7	35.5
Mean Neutrophil Scatter	0.26	0.17-0.34	>142	37.1	29.0

Table 7 presents the diagnostic performance of MNV, MNC, and MNS in differentiating cases from controls, as assessed by the area under the curve (AUC), cut-off points, sensitivity, and specificity.

For MNV, the AUC was 0.99 (95% CI: 0.98-1.00), indicating excellent diagnostic accuracy. The optimal cut-off point for distinguishing cases from controls was >148, achieving high sensitivity and specificity, both at 96.8%.

In contrast, MNC showed poor diagnostic performance, with an AUC of 0.36 (95% CI: 0.27-0.46). The cut-off point of >144 yielded low sensitivity (38.7%) and specificity (35.5%), suggesting limited utility in distinguishing cases from controls.

Similarly, MNS performed poorly, with an AUC of 0.26 (95% CI: 0.17-0.34). A cut-off point of >142 resulted in low sensitivity (37.1%) and specificity (29.0%), highlighting its minimal diagnostic value.

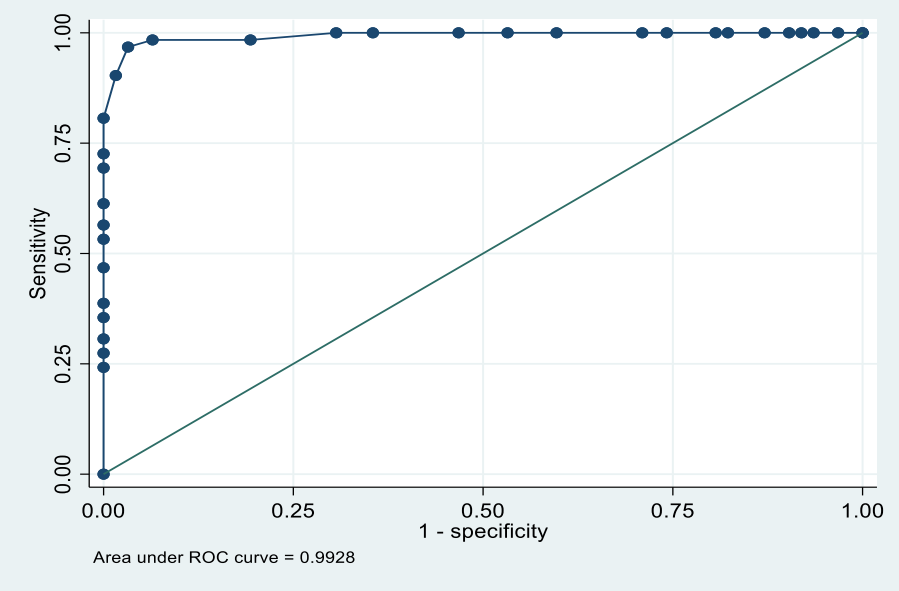

Figure 5. Receiver operating characteristic (ROC) curve representing the cut-off point of Mean Neutrophil Volume in prediction of cases and controls

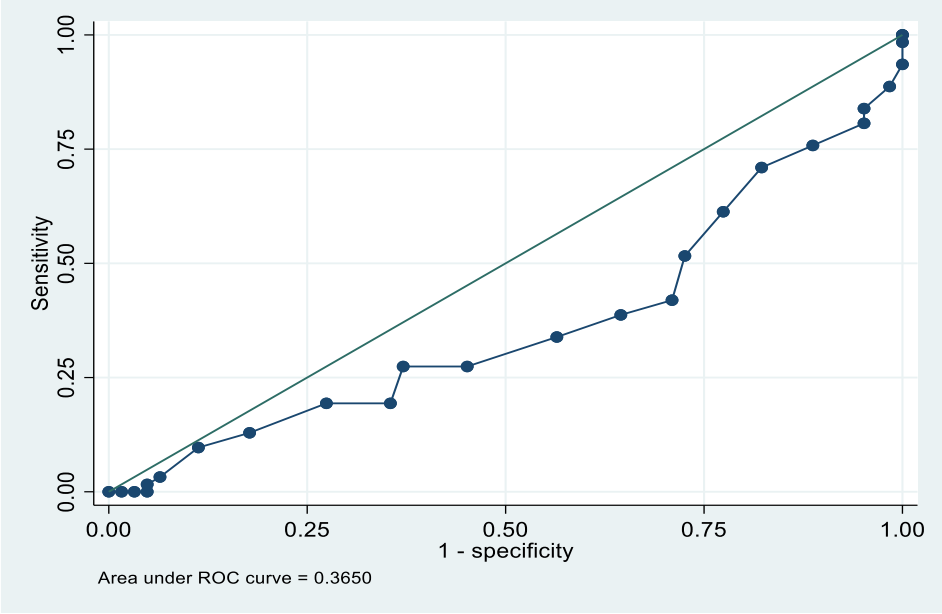

Figure 6. Receiver operating characteristic (ROC) curve representing the cut-off point of Mean Neutrophil conductivity in prediction of cases and controls

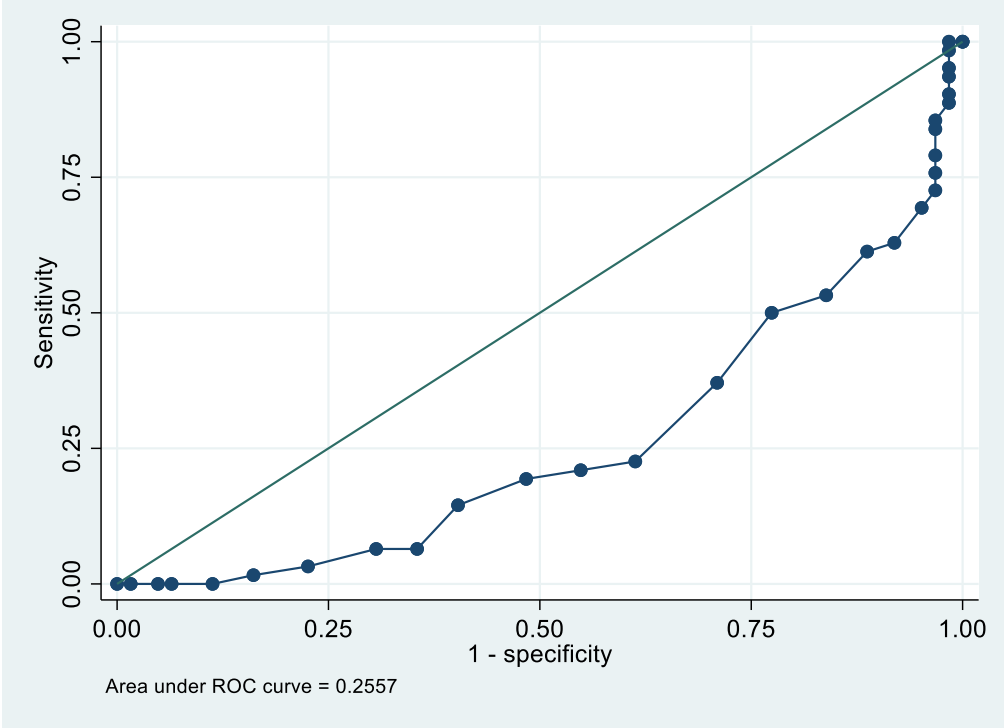

Figure 7. Receiver operating characteristic (ROC) curve representing the cut-off point of Mean Neutrophil Scatter in prediction of cases and controls

DISCUSSION

The study was a retrospective case-control design conducted at a tertiary care center in Belagavi from April 2023 to March 2024. It aimed to compare the VCS parameters of neutrophils between 62 patients with confirmed bacterial infections and 62 BMI-matched healthy controls with normal CBC. Data were collected retrospectively from hospital records, including demographics, clinical history, laboratory findings, and imaging results. VCS parameters were measured using an automated haematology analyser, while bacterial culture and sensitivity tests confirmed infections.

Descriptive Parameters

In our study, 124 individuals were included, equally divided into cases (patients with positive bacterial cultures) and controls (individuals with normal CBC results, matched for BMI). The age distribution revealed that cases were primarily older, with 41.9% in the 51-70 age group and 21.0% in the 71-90 age group. This was consistent with the results of a study conducted by Natalie R. Wodniak et al. They found that bacterial infections were independently associated with several factors, including age ≥50 years, with an adjusted odds ratio of 4.18.[48] Similar findings were reported in a study conducted by Vaswani et al.; the mean age of patients with clinically and culture-proven sepsis was 54.1 ± 16.9 years.[9] These findings suggest bacterial infections are more prevalent in older populations, potentially due to factors such as declining immunity or comorbidities. Gender distribution showed a male predominance in our study in both groups, with 69.3% males among cases and 74.2% among controls. In line with this, a study by Vaswani et al. had 151 males and 74 females, with a male-to-female ratio of 2:1 for patients with clinically and culture-

proven sepsis. Consistent findings were also reported in a study by Arora et al., where patients with positive bacterial blood cultures had a male-to-female ratio of 69:65, showing a slightly higher number of males than females.[40] In contrast, a study by Natalie R. Wodniak et al.[48] reported a higher prevalence of bacterial infections in females, though this difference was not statistically significant.

Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter In our study, we compared the MNV, MNC, and MNS between cases and controls among 124 participants (62 cases and 62 controls). For Mean Neutrophil Volume, the cases had a significantly higher mean (157.8 ± 11.5) than the controls (141.2 ± 4.7). This difference was statistically significant, with a P-value <0.001. In line with our study, Suresh et al. reported a significant increase in the MNV in patients with infections (158.3± 13.7) compared to controls (137.2 ± 4.3).[29] Similarly, a study by Chaves et al. found a significant increase in the MNV in septic patients (156 ± 13.5) compared to controls (143 ± 4.8).[38] Another study by Purohit et al. also found a significant increase in the MNV in septic patients compared to control subjects.[39] Vaswani et al. also reported that MNV was significantly higher ($p < 0.0001$) in sepsis patients compared to the control group.[9]

Regarding Mean Neutrophil Conductivity in our study, controls exhibited a slightly higher mean (145.2 ± 5.6) than cases (142.4 ± 5.3). This difference was also statistically significant (P-value <0.001). Similar findings were reported by Celik et al., which found significant decreases in MNC in septic newborns.[44] However, in a study by Vaswani et al., the MNC showed a mean ± SD of 147.1 ± 5.5 in the control group and 148.9 ± 8.5 in the sepsis group, with the difference not being statistically significant ($P = 0.4735$).[9] Bharti et al. also reported that MNC was significantly higher in sepsis cases compared to controls (both $P < 0.001$).[47]

For Mean Neutrophil Scatter, the mean values in cases were 138.2 ± 7.5 and controls slightly higher at 142.4 ± 17.3 in this study. However, this difference was not statistically significant, as indicated by a P-value of 0.08. The findings are in line with a study by Vaswani et al., which reported that MNS was significantly lower ($p < 0.0001$) in sepsis patients (130.9 ± 9.2) compared to the control group (138.7 ± 5.7).[9] However, in a study by Bharti et al. MNS were significantly higher in sepsis cases compared to controls (both $P < 0.001$).[47] Overall, our findings suggest that while MNV holds promise as a diagnostic marker, MNC and MNS may not be as reliable for early bacterial infection detection, requiring further research for validation.

Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter among the cases with WBC count ≤ 11,000, count > 11,000 mm3 and controls

Our study observed that MNV was highest in cases with WBC > 11,000/mm³ (160.5 ± 13.2), followed by cases with WBC ≤ 11,000/mm³ (155.4 ± 9.1) and controls (141.2 ± 4.7), with statistically significant differences between all groups except between the two case subgroups. For MNC, controls exhibited the highest mean (145.2 ± 5.6), followed by cases with WBC ≤ 11,000/mm³ (142.8 ± 5.9) and cases with WBC > 11,000/mm³ (141.9 ± 4.6), with significant differences observed between controls and both case subgroups but not between the two case subgroups. Similarly, MNS was highest in controls (142.4 ± 17.3), followed by cases with WBC ≤ 11,000/mm³ (139.8 ± 6.6) and cases with WBC > 11,000/mm³ (136.4 ± 8.1), showing significant differences between controls and both case subgroups but not between the case subgroups.

These findings are consistent with a study by Purohit et al., they reported that an elevated MNV was associated with higher white blood cell counts.[39] In a study by Kannan et al., similar results were reported; for MNV, cases with a WBC count >11,000 showed the highest mean (140.5 ± 9.4), followed by cases with a WBC count ≤11,000 (136.8 ± 8.3), and controls (132.0 ± 7.4), with statistically significant differences observed between all groups (P < 0.01). For MNC, controls had a mean of 158.5 ± 5.0, cases with a WBC count ≤11,000 had 158.2 ± 5.4, and cases with a WBC count >11,000 had 157.7 ± 6.3, with no significant differences between the groups (P = 0.79 for cases ≤11,000 vs. control, P = 0.30 for cases >11,000 vs. control). For MNS, cases with a WBC count ≤11,000 had the highest mean (142.0 ± 7.7), followed by cases with a WBC count >11,000 (140.4 ± 7.7), and controls (136.8 ± 8.0), with significant differences between controls and both case groups (P < 0.01).[43] These findings suggest that MNV is a promising diagnostic marker for acute bacterial infections, particularly in cases with elevated WBC counts, as it reflects neutrophil activation and infection response. In contrast, MNC and MNS appear less sensitive for distinguishing varying severities of infection.

Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter among the cases with Neutrophil <85 %, >85 %and controls

In this study, for MNV, cases with neutrophil counts >85% had the highest mean (167.6 ± 13.8), followed by cases with counts <85% (154.2 ± 7.9) and controls (141.2 ± 4.7), with significant differences between all groups (P < 0.001). For MNC, controls had the highest mean (145.2 ± 5.6), followed by cases with neutrophil counts <85% (143.1 ± 5.3) and >85% (140.5 ± 4.8), with significant differences between controls and both case groups (P < 0.05). For MNS, controls showed the highest mean (142.4 ± 17.3), followed by cases with neutrophil counts <85% (139.7 ± 6.8) and

>85% (134.1 ± 8.0), with significant differences observed across all groups (P < 0.001).

Consistent with our results, a study by Kannan et al. found that MNV was highest in cases with neutrophil percentages >85% (142.7 ± 11.8), followed by those <85% (138.7 ± 8.4), with significant differences between controls (131.9 ± 7.4) and both case groups (P < 0.01). For MNC, the values were similar across all groups, with no significant differences (P = 0.235 for cases <85%, P = 0.672 for cases >85%). For MNS, cases with neutrophil percentages <85% had the highest mean (141.9 ± 7.0), followed by those >85% (138.8 ± 9.5), and controls (136.8 ± 8.0), with significant differences between controls and cases <85% (P < 0.01). No significant difference was found between controls and cases >85% (P = 0.32).[43] Similar findings were reported in a study by Chaves et al., patients were divided into three groups based on their WBC counts: group 1 (1,700–11,000/μL), group 2 (>11,000–<15,000/μL), group 3 (15,000–39,200/μL). For MNV, the mean was highest in group 3 (161 ± 9.1), followed by group 2 (157 ± 15.8), group 1 (152 ± 13.5), and control (143 ± 4.8), with significant differences (P = .0001 for control vs group 3, P = .015 for control vs group 1, P = .0001 for control vs group 2). For MNC, values were similar across groups, with group 2 (142 ± 3.1) slightly higher than control (142 ± 2.6), group 3 (141 ± 3.8), and group 1 (141 ± 4.4), with no significant differences (P > .05). For MNS, control had the highest value (146 ± 7.3), followed by group 2 (143 ± 6.9), and both groups 1 (139 ± 12.7) and 3 (139 ± 7.2) had the lowest, with a significant decrease in group 1 (P = .021 vs control).[38]

Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter among the cases with systemic infections, localized infections and controls

In this study, for MNV, cases with systemic infections had the highest mean (160.8 ± 12.7), followed by localized infections (156.4 ± 10.6) and controls (141.2 ± 4.7), with significant differences between controls and both infection groups ($P < 0.001$), but no difference between the infection groups ($P = 0.20$). For MNC, controls had the highest mean (145.2 ± 5.6), followed by localized infections (143.1 ± 5.5) and systemic infections (140.9 ± 4.7), with significant differences between controls and systemic infections ($P < 0.001$) and marginal significance with localized infections ($P = 0.05$). The difference between infection groups was not significant ($P = 0.05$). For MNS, controls had the highest mean (142.4 ± 17.3), followed by localized infections (138.8 ± 6.6) and systemic infections (136.8 ± 9.1), with significant differences between controls and both infection groups ($P < 0.001$), but no difference between the infection groups ($P = 0.32$).

This was consistent with the results of a study by Suresh et al., which reported that MNV was highest in the systemic infection group (160.5), followed by localised infections (156.8) and controls (137.2), with significant differences observed between the control group and both infection groups ($P < 0.001$), but no significant difference between the systemic and localised infection groups ($P = 0.402$).[29] Similar findings were observed in a study by Lee and Kim, with MNV was highest in the sepsis group (138–203) compared to the localized infection (135–190) and control groups (130–169) with significant differences ($P = 0.003$ and $P < 0.001$). MNC was lower in the sepsis group (135–154) than in non-infected patients (143–155; $P = 0.048$), with no significant difference from the localised infection group (137–157; $P = 0.847$).

Neutrophil scatter was reduced in the sepsis group (116–145) compared to non-infected patients (120–146; P = 0.015), but no difference was observed with the localised infection group (111–148; P = 0.123).[42] In conclusion, systemic infections were associated with higher MNV.

Cut-offs, specificity, sensitivity, and area under the curve for the Mean Neutrophil Volume, Mean Neutrophil conductivity and Mean Neutrophil Scatter

In this study, for MNV, the AUC was 0.99 (95% CI: 0.98-1.00), indicating excellent diagnostic accuracy. The optimal cut-off point for distinguishing cases from controls was >148, achieving high sensitivity and specificity, both at 96.8%. In contrast, MNC had an AUC of 0.36 (95% CI: 0.27-0.46). The cut-off point of >144 resulted in low sensitivity (38.7%) and specificity (35.5%), suggesting limited utility in differentiating cases from controls. Similarly, MNS showed minimal diagnostic value, with an AUC of 0.26 (95% CI: 0.17-0.34). A cut-off point of >142 resulted in low sensitivity (37.1%) and specificity (29.0%), indicating that MNS also lacks sufficient discriminatory power for clinical use in this study.

Similarly, in a study by Kannan et al. ROC curve analysis for MNV revealed a criterion value of >129.3, with a sensitivity of 92.7%.[43] A study by Purohit et al. also had consistent findings. It reported a cut-off value of 149 for MNV, which achieved a sensitivity of 88.7% and a specificity of 91.4%.[39] In a study by Arora et al., a cutoff value of 150.2 for MNV was found to have a sensitivity of 79.1% and a specificity of 95%, with an area under the curve (AUC) of 92.3%.[40]

In a study by Goyal et al., MNV also demonstrated strong diagnostic performance, with an AUC of 0.80, similar to our findings, indicating its potential as a reliable parameter for distinguishing between cases and controls. On the other hand,

the MNC and MNS had lower diagnostic accuracies, with AUC values of 0.33 and 0.17, respectively, reinforcing their limited utility in differential diagnosis, as observed in our study.[41] In a study by Celik et al., for MNV, the cutoff was >157.15 au, with 79% sensitivity and 82% specificity. For MNC, the cutoff was <159.3 au, with 66% sensitivity and 64% specificity. For MNS, the cutoff was <127.5 au, with 60% sensitivity and 55% specificity. Logistic regression analysis identified MNV as an independent risk factor for sepsis.[44] Overall, our findings highlight that while MNV shows promise as a diagnostic tool, MNC and MNS have limited diagnostic utility for distinguishing cases from controls.

STRENGTHS

- Reduced Inter-Observer Bias: The use of the Beckman Coulter DxH 900 ensures precise, standardized, and reproducible measurements of VCS parameters, minimizing inter-observer variability and improving data reliability.
- Enhanced Internal Validity: The retrospective case-control design allows for direct comparisons between patients with confirmed bacterial infections and healthy controls, strengthening the study's internal validity.
- Minimized Misclassification Bias: By including only bacterial culture-positive cases, the study ensures that observed differences in VCS parameters are attributable to bacterial infections, minimizing the risk of misclassification bias.

LIMITATIONS

The retrospective nature of the study may have led to biases. The sample size was relatively small, and the study was conducted at a single center, limiting the generalizability of the findings. Another limitation is the lack of follow-up data, which prevents the assessment of long-term outcomes associated with neutrophil parameters in bacterial infections.

CONCLUSION

In this retrospective case-control study, significant differences were observed between cases and controls, particularly in MNV, which was higher in the infection group (157.8 ± 11.5) compared to the control group (141.2 ± 4.7), with a p-value < 0.001. In contrast, MNC was lower in the infection group (142.4 ± 5.3 vs. 145.2 ± 5.6 in controls), with a p-value < 0.001. MNS showed no significant difference between the groups (p = 0.08), indicating it has limited diagnostic value. The analysis also found that higher MNV values were associated with elevated WBC counts and neutrophil percentages, particularly in patients with WBC > 11,000/mm^3 and neutrophil percentages > 85%. The diagnostic accuracy assessment showed that MNV had an AUC of 0.99, with a cutoff value of 148, yielding a sensitivity of 96.8% and specificity of 96.8%. MNC and MNS exhibited lower AUC values. Overall, MNV emerges as a promising tool for early detection of bacterial infections, while MNC and MNS have limited diagnostic utility. Further studies are needed to validate these findings and evaluate their practical implementation in clinical practice.

SUMMARY

The study was conducted as a retrospective case-control design at a tertiary care center in Belagavi from April 2023 to March 2024. The primary objective was to explore the use of volume, conductivity, and scatter of neutrophils as an early diagnostic tool for acute bacterial infections. The study compared VCS parameters between 62 patients with confirmed bacterial infections and 62 BMI-matched healthy controls, all with normal complete blood count (CBC). The study included 124 individuals, evenly divided between cases (patients with positive bacterial cultures) and controls (individuals with normal CBC results, matched for BMI). The age distribution revealed that the majority of cases were older, with 41.9% in the 51-70 age group and 21.0% in the 71-90 age group. Regarding gender distribution, a male predominance was observed in both cases (69.3%) and controls (74.2%).

The MNV was significantly higher in the cases (157.8 ± 11.5) compared to the controls (141.2 ± 4.7), with a P-value of <0.001. For MNC, the controls had slightly higher values (145.2 ± 5.6) than the cases (142.4 ± 5.3), with a statistically significant difference (P-value <0.001). For MNS, the cases had a mean value of 138.2 ± 7.5, while the controls had a slightly higher value of 142.4 ± 17.3, but this difference was not statistically significant (P-value = 0.08).

The study also examined the VCS parameters among cases with WBC counts ≤ 11,000/mm³, WBC counts > 11,000/mm³, and controls. For MNV, the cases with WBC > 11,000/mm³ had the highest mean (160.5 ± 13.2), followed by cases with WBC ≤ 11,000/mm³ (155.4 ± 9.1), and controls (141.2 ± 4.7). Statistically significant differences were observed between the groups, except between the two case subgroups. MNC showed higher values in controls (145.2 ± 5.6) compared to both

case subgroups, with a statistically significant difference. Similarly, MNS was highest in controls (142.4 ± 17.3), followed by cases with WBC ≤ 11,000/mm³ (139.8 ± 6.6), and cases with WBC > 11,000/mm³ (136.4 ± 8.1), with significant differences between controls and both case subgroups, but no significant difference between the case subgroups.

The study examined the VCS parameters among cases with neutrophil percentages <85%, >85%, and controls. For MNV, cases with neutrophil counts >85% had the highest mean (167.6 ± 13.8), followed by cases with neutrophil counts <85% (154.2 ± 7.9), and controls (141.2 ± 4.7). Statistically significant differences were observed between all groups. For MNC, controls had the highest mean (145.2 ± 5.6), followed by cases with neutrophil counts <85% (143.1 ± 5.3) and >85% (140.5 ± 4.8), with significant differences between controls and both case groups. For MNS, controls showed the highest mean (142.4 ± 17.3), followed by cases with neutrophil counts <85% (139.7 ± 6.8) and >85% (134.1 ± 8.0), with significant differences observed across all groups.

Finally, the study also evaluated the diagnostic accuracy and found the AUC for MNV was found to be 0.99. A cutoff value of 148 for MNV was identified, yielding a sensitivity of 96.8% and specificity of 96.8%. However, MNC and MNS showed lower AUC values, with MNC exhibiting an AUC of 0.36 and MNS showing an AUC of 0.26.

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ANNEXURE – I - INFORMED CONSENT FORM

KAHERs JNMC
BELAGAVI
INFORMED CONSENT FORM

TO STUDY VOLUME, CONDUCTIVITY,SCATTER PARAMETERS OF NEUTROPHILS AN EARLY DIAGNOSTIC TOOL IN ACUTE BACTERIAL INFECTIONS AT A TERITIARY LEVEL CENTRE- A RETROSPECTIVE CASE CONTROL STUDY

Name of Student/Principal Investigator:]

Name of Guide/Co Investigators:

Introduction:

- Infection and sepsis are known to produce numerical and morphological changes in the leucocytes. Bloodstream infection is a major cause of morbidity and mortality. Sepsis is the most common cause of death in hospitalized patients worldwide, especially in a developing country like India.
- Morphological assessment of leucocytes by peripheral blood smear examination is time consuming and more subjective. The V (Volume), C (Conductivity), and S (Scatter) of neutrophils are emerging as a sensitive predictors of acute bacterial infection and sepsis even in the absence of leucocytosis and neutrophilia.
- Volume, Conductivity and Scatter parameters (VCS) of leucocytes are obtained after analysis of about 8,000 leucocytes in a few seconds, using impedance to measure cell volume (V), radio frequency opacity to characterize conductivity or internal composition of each cell and laser beam to measure light scatter (S) for cytoplasmic granulations and nuclear structure.
- The change in the morphology and in the number of these cells which are reflected in the VCS parameters of neutrophils proves to be very accurate and sensitive method than the manual method.

Need for study:

- The necessity of a rapid, accurate and highly specific diagnostic method for a very common and greatly prevalent disease such as acute bacterial infections is clear. The current routines and gold standard methods used in diagnosis of this disease are effective but have their own drawbacks. This study, along with many, done in the wake of the Beckman Coulter Haematology Analyser Dxh 900,compare and establish the study of the VCS parameters of neutrophils as an early diagnostic tool

- The reactive left shift in neutrophils can be assessed by increase in volume (V) & size of the cells as well as the increased cytoplasmic granularity can be assessed by conductivity (C) & scatter (S). However, this use of the VCS technology to evaluate morphologic changes in cell population during acute bacterial infection has never been studied well .Hence an attempt to study the VCS changes in acute bacterial infection is made here, so as to evaluate the usefulness of these parameters as an early diagnostic tool of infection in clinical practice.

Explanation of procedure:

- An informed consent is obtained from all thc subjects.
- Patients above the age of 18 years are included in the study.
- The subjects with bacterial culture positive are taken as cases .
- The EDTA sample taken for CBC and VCS parameters of the cases on the day of admission will be traced and compared with EDTA sample taken from BMI matched subjects with CBC within normal limits who are taken as controls.

Withdrawal from participation in the study: Participation in this study in voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will get any benefits by participating in this study. As early diagnosis will be helpful to determine further course of treatment. The data gathered will help population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Cost of investigations done during the course of study will be paid by the principal investigator.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact:

If you have any question or complaints with regard to your right as study participant you may contact:

Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "TO STUDY VOLUME, CONDUCTIVITY,SCATTER PARAMETERS OF NEUTROPHILS AS AN EARLY DIAGNOSTIC TOOL IN ACUTE BACTERIAL INFECTIONS AT A TERITIARY LEVEL CENTRE- A RETROSPECTIVE CASE CONTROL STUDY" My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURES – II PROFORMA

"TO STUDY VOLUME, CONDUCTIVITY, SCATTER PARAMETERS OF NEUTROPHILS AS AN EARLY DIAGNOSTIC TOOL IN ACUTE BACTERIAL INFECTIONS AT A TERITIARY LEVEL CENTRE- A RETROSPECTIVE CASE CONTROL STUDY"

PROFORMA

CASE NO	
NAME	
IP NO/OP NO	
AGE	YEARS
SEX	MALE FEMALE
ADDRESS	
OCCUPATION	

Complaints at presentation	
Past history	
Family History	
Personal History	

VITALS

Temperature	
Pulse	
Respiratory rate	
Blood pressure	

PHYSICAL EXAMINATION

	Yes	**No**
Pallor		
Icterus		
Lymphadenopathy		
Cyanosis		
Clubbing		
Edema		

SYSTEMIC EXAMINATION

C.V.S	
R.S.	
C.N.S.	
PER ABDOMEN	

INVESTIGATIONS

Hemoglobin	
Platelets	
Total count	
Neutrophils	
Mean Neutrophil Volume (MNV)	
Mean Neutrophil Counductivilty (MNC)	
Mean Neutrophil Scatter (MNS)	
Lymphocytes	
Eosinophils	
Monocytes	
Basophils	
Culture and sensitivity	

ANNEXURES – III MASTER CHART

CASES

SL NO	Ip/op no	Hemoglobin	WBC	Plt	Neu	Lym	Monocyte	Eos	Bas	MNV	MNC	MNS	Organism	Culture	Age	Sex
1	7169692	13.9	4.8	147	67	20	13	0	0	149	145	150	Gram negative	Blood - Acinetbacter Iwofii	32	Male
2	6189685	12.5	19.4	22	86	13	1	0	0	163	136	142	Gram negative	Blood - Acinetobacter Iwofii	74	Male
3	7604533	10.1	13.9	215	77	11	12	0	0	159	141	142	Gram negative	Blood- Enterobacter	59	Male
4	7605866	10	9.4	238	71	18	10	0	1	168	136	141	Gram positive	Blood-coag neg staphylococcus	48	M
5	10092538	12.1	27.3	229	93	4	3	0	0	161	142	132	Gram negative	Blood -Acinetobacter baumanii	79	M
6	10093488	9	18.7	206	81	6	13	0	0	153	142	145	Gram positive	Blood -Staph Hemolyticus	71	Male
7	10094348	11.7	23.2	277	92	4	3	1	0	158	137	141	Gram negative	Blood-Acinetobacter Iwofii	70	M
8	10094576	13.1	7.8	378	52	34	6	2	2	150	139	141	Gram positive	Blood-Staph Hemolyticus	19	M
9	10094743	10.6	9.3	291	42	46	11	2	0	151	141	148	Gram positive	Blood -Staph epidermidis	22	F
10	10094136	10.4	11.3	515	67	24	6	2	0	153	135	132	Gram positive	Blood -Staph Hemolyticus	50	M
11	10090619	10.7	5	228	62	24	11	2	0	154	141	135	Gram positive	Blood-staph species	58	F
12	10094375	11.5	20.7	201	88	7	5	0	0	170	138	139	Gram positive	Blood -staph epidermidis	54	F
13	10090446	9.1	11.9	167	81	14	5	0	0	158	140	146	Gram positive	Blood -Staph epidermidis	64	M
14	10067974	12.5	6.4	121	86	7	5	2	0	191	133	117	Gram negative	Blood-cedacea sp	53	M
15	10073648	14	6	232	59	34	5	1	1	148	150	136	Gram negative	Blood -E coli	83	M
16	7446862	12.2	7.5	205	66	26	0	8	0	157	147	128	Gram negative	Blood- Pesudomonas aeruginosa	75	M
17	10071281	15.5	7.5	121	64	19	7	10	0	149	151	144	Gram positive	Blood-staph epidermidis	25	M
18	10043607	9.9	19.6	180	80	5	1	5	0	194	143	116	Gram positive	Blood -Staph Hemolyticus	57	F
19	10063928	6.7	12.3	136	78	20	0	2	0	163	137	127	Gram negative	Blood-klebsiella pneumoniae	32	M
20	10076426	15.8	8.8	72	95	3	1	1	0	174	145	137	Gram positive	Blood-Staph epidermidis	64	M

21	10064082	10	8.2	59	85	10	0	2	0	153	141	135	Gram negative	Blood - pseudomonas aeruginosa	53	F
22	10064099	10.1	10	276	76	11	13	0	0	155	151	146	Gram negative	Urine -E coli	78	M
23	10094987	11	4.2	272	65	23	8	4	0	150	140	140	Gram negative	Urine-Ecoli	76	F
24	10093231	10	9.4	238	71	18	10	0	1	168	136	141	gram negative	Urine - Burkoldheria	48	M
25	7594652	14.1	11.7	236	67	25	8	10	0	150	144	146	Gram negative	Urine -E coli	50	M
26	10065620	11.7	10.3	129	73	17	0	10	0	154	150	141	Gram negative	Urine-Ecoli	77	M
27	10065466	11.7	10.6	167	86	6	0	8	0	160	139	136	Gram negative	Urine -E coli	79	M
28	10066411	14.2	16.6	369	84	10	0	6	0	147	149	146	Gram negative	Urie -Enterococcus sp	66	F
29	10089481	10.3	17.1	77	88	4	3	5	0	170	144	133	Gram negative	Urine-Klebsiella pneumoniae	70	F
30	10096778	10.7	10.6	354	75	18	7	0	0	149	141	142	gram positive	urine-staph epidermidis	65	F
31	10096672	10.3	9.2	685	73	20	4	2	1	148	139	142	gram negative	urine-proteus mirabilis	35	F
32	10096557	10.3	8.5	255	82	5	11	1	1	158	135	142	Gram negative	urine- klebsiella pneumoniae	73	M
33	10096654	13.4	10.3	279	80	13	7	0	0	152	149	139	Gram negative	urine-E coli	73	F
34	10096822	16.9	8.5	357	62	29	6	3	0	145	135	142	Gram negative	urine - proteus mirabilis	32	M
35	10097235	14.2	11.4	298	66	26	6	2	0	148	134	130	Gram negative	urine-E coli	77	M
36	10094375	11.5	20.7	201	88	7	5	0	0	170	138	139	Gram negative	Urine -E coli	54	F
37	10096618	8.7	5.6	292	58	29	10	2	1	156	134	141	Gram negative	urine - Acibetobacter	49	F
38	10096715	12.5	5	321	52	35	10	2	1	152	145	149	Gram negative	urine-E coli	55	M
39	10093281	14.5	5.7	292	45	43	7	4	1	155	151	146	Gram negative	urine- klebsiella pneumoniae	35	M
40	10096674	13	6	300	72	18	7	3	0	152	149	139	Gram negative	urine - proteus mirabilis	42	M
41	10093236	8.8	10.2	365	62	27	10	1	0	160	140	137	Gram negative	BAL-Enterobacter cloacae	44	F
42	10094840	9.2	10.6	237	74	13	11	2	0	156	142	143	Gram positive	sputum-MRSA	86	M
43	10092559	13.9	10.2	145	88	9	3	0	0	159	134	137	Gram negative	Sputum-E.coli	62	M
44	10076011	12.2	6.5	132	75	6	18	1	0	149	147	145	Gram negative	Sputum klebsiella pneumoniae	68	F
45	10075534	9.2	13.4	506	90	7	3	0	0	167	140	125	Gram negative	Sputum-Enterobacter cloacae	63	F
46	10075883	13	11.4	142	85	7	0	8	0	151	151	148	Gram negative	Sputum-klebsiella pneumoniae	69	M

47	10083159	10.5	11.1	143	84	11	5	0	0	149	147	137	Gram positive	sputum-strep bovis	23	F
48	1094295	11	5.6	321	88	8	4	0	0	150	147	145	Gram negative	Sputum klebsiella pneumoniae	49	M
49	10093235	10.8	16.4	275	83	13	4	0	0	152	141	142	Gram negative	pus - psedomonas	64	M
50	10094193	9.5	11.6	223	80	10	10	0	0	156	140	140	Gram negative	other-Acinetobacter	50	M
51	10074927	12.3	19.9	435	93	4	2	1	0	156	143	138	Gram negative	pus - proteus mirabilis	59	M
52	10064423	9.1	9.8	432	70	16	11	3	0	149	144	127	Gram negative	pus Klebsiella pneumonia	60	M
53	10072645	12.6	14.8	160	90	7	3	0	0	171	152	133	Gram positive	pus-staph hemolyticus	60	M
54	10066113	9.6	8.8	266	74	18	2	6	0	152	153	141	Gram negative	pus E.coli	50	M
55	10071614	10.9	18.2	414	72	18	9	1	0	148	145	141	Gram negative	pus-klebsiella pneumonia	33	M
56	10071384	11.1	13.9	369	85	7	1	7	0	157	147	132	Gram negative	pus-pseudomonas aeruginosa	64	M
57	10044499	12	13.8	162	89	3	0	8	0	167	138	130	Gram negative	pus-citrobacter	62	M
58	10089219	10	20.9	360	95	3	2	0	0	156	140	139	Gram positive	pus-MRSA	60	M
59	10073312	15	13.1	440	75	12	13	0	0	155	142	131	Gram negative	pus -E coli	47	M
60	7594655	14.5	22.3	342	85	8	7	0	0	150	142	142	Gram positive	pus- staph	48	M
61	10092959	11.1	17.8	319	75	17	8	0	0	155	149	142	Gram negative	pus-klebsiella pneumonia	62	M
62	10068129	10.8	12.3	150	91	5	0	4	0	207	142	117	Gram negative	other - Acibetobacter baumanii	42	F

CONTROLS

SL NO	Ip/op no	Hemoglobin	WBC	Plt	Neu	Lym	Monocyte	Eos	Bas	MNV	MNC	MNS	Organism	Culture	Age	Sex
1	10076217	12.8	9.6	260	73	23	4	0	0	146	154	140	-	-	20	M
2	10076892	12.4	9.5	325	72	19	8	0	0	148	156	137	-	-	48	F
3	10075816	13.6	9.5	292	75	16	9	0	0	138	146	138	-	-	45	M
4	10075813	13.4	4	276	55	36	9	0	0	136	151	149	-	-	18	M
5	10075110	13.6	7.4	291	72	18	9	1	0	130	151	141	-	-	41	M
6	10076644	14.3	4	171	54	36	10	0	0	143	152	144	-	-	24	M
7	10076574	15	5.6	163	65	25	9	0	1	141	148	130	-	-	28	M
8	6995474	14	6	321	54	34	9	3	0	144	149	137	-	-	74	M
9	10075134	13.8	6	224	51	36	6	7	0	132	150	136	-	-	35	M
10	10075708	14.6	10	274	72	13	1	4	0	131	150	140	-	-	56	M
11	10095372	13.3	7.6	183	73	18	7	1	1	138	140	151	-	-	33	F
12	10090885	12.4	7.4	296	53	35	9	3	0	145	143	149	-	-	35	M
13	10096220	14.6	4.8	201	52	41	4	2	1	130	145	156	-	-	32	M
14	10090744	13.8	8.7	356	46	40	9	4	1	140	144	144	-	-	36	M
15	10090811	14.1	10	316	64	29	4	2	1	146	145	152	-	-	50	M
16	10090866	12.3	6.3	367	62	25	10	2	1	145	140	146	-	-	62	M
17	10090616	14	7.4	228	58	23	13	6	1	143	141	146	-	-	63	M
18	10090862	13.8	9	265	71	16	10	2	0	131	149	150	-	-	73	M
19	10090834	14.9	9.5	293	50	37	6	6	1	138	141	145	-	-	40	M
20	10090654	14.3	9.9	421	60	33	6	1	0	146	149	142	-	-	29	M
21	10090863	15	8.5	289	61	27	10	2	0	135	143	147	-	-	41	M
22	10093545	13.2	8.2	316	49	36	7	7	1	145	140	138	-	-	46	F
23	10090845	13.8	9.7	227	74	14	10	2	0	145	145	153	-	-	62	M
24	10090817	14.8	9.1	185	73	19	6	2	0	138	145	14	-	-	65	M

25	10090835	14.2	9.2	326	58	32	8	2	0	143	148	148	-	-	74	M
26	10090861	12.2	6.2	244	50	38	10	2	0	145	145	143	-	-	54	M
27	10082245	15	5.3	371	54	41	2	2	0	140	145	151	-	-	60	M
28	10090864	12.5	4.4	296	75	21	4	0	0	140	144	151	-	-	30	F
29	6186522	14.2	10	377	66	24	4	5	1	147	146	143	-	-	24	F
30	10090665	12.5	10	325	75	21	4	0	0	144	141	150	-	-	24	F
31	24530512	14.3	9.9	421	60	33	6	1	0	146	149	142	-	-	29	M
32	10090870	13.6	10	188	70	20	9	1	0	134	148	148	-	-	62	M
33	10090755	14.4	4.8	247	59	32	7	1	1	146	147	142	-	-	25	M
34	10090729	12.9	6.3	300	66	22	7	4	1	143	146	145	-	-	71	F
35	10090802	12.6	8.2	245	61	32	6	1	0	142	144	145	-	-	61	F
36	10090783	12	9.1	349	61	32	5	1	1	135	145	147	-	-	52	F
37	10090794	12.2	10	381	75	19	6	0	0	136	146	145	-	-	70	F
38	6768748	13.9	8.6	239	65	25	8	2	0	142	151	153	-	-	46	M
39	5298730	12	7.2	296	55	31	7	7	0	143	146	141	-	-	42	F
40	7584784	13.9	5.9	234	55	37	6	2	0	146	143	148	-	-	47	M
41	7584558	15.3	7.2	232	49	41	8	2	0	145	142	147	-	-	55	M
42	7584694	12.9	7.1	252	49	33	8	8	1	145	144	150	-	-	65	M
43	7584650	13.7	7.2	263	53	34	9	3	1	137	144	149	-	-	66	M
44	7007464	13.9	5.9	207	70	15	10	5	0	142	143	148	-	-	72	M
45	10074567	12.5	10	219	75	20	5	0	0	136	150	145	-	-	33	F
46	10075938	14.2	10	393	68	17	10	4	1	140	150	140	-	-	30	M
47	10075205	10.5	9.6	235	69	20	2	9	0	144	148	140	-	-	63	F
48	10074334	14.3	7	302	75	22	1	2	0	143	169	139	-	-	60	M
49	10074398	14.4	8	253	66	28	0	6	0	140	149	139	-	-	58	M
50	10076668	14.2	3.4	151	61	26	8	5	0	142	149	144	-	-	45	M
51	10076628	12.9	9.6	207	69	22	0	9	0	141	148	142	-	-	45	F
52	7623387	14.7	5.5	292	45	42	8	4	1	139	139	142	-	-	45	M

53	7623255	12.8	4.4	304	75	18	5	1	0	143	135	139	-	-	62	M
54	7623289	14.1	7.1	359	48	40	6	5	1	141	139	142	-	-	48	M
55	7623271	12.1	7.7	364	57	34	5	3	1	146	139	146	-	-	48	F
56	7623414	13.8	6.2	201	46	42	10	2	0	139	138	148	-	-	50	M
57	7623443	13.8	8.2	315	60	34	4	1	1	147	138	143	-	-	34	M
58	7623498	15	8.5	257	46	41	10	2	1	146	136	141	-	-	40	M
59	10096864	15	6.9	220	50	35	9	5	1	140	138	149	-	-	19	M
60	10096594	11.5	9	195	73	17	6	3	1	149	138	144	-	-	27	F
61	7621986	15	9	205	48	40	7	4	1	141	139	143	-	-	39	M
62	7621902	13.7	4.3	233	49	40	8	1	1	140	136	141	-	-	56	M