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Assessment of Diagnostic Accuracy of Interleukins and Procalcitonin in Patients with Severe Illness and Suspected Sepsis

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ABSTRACT

The immune system's dysregulated response to infection, known as sepsis, is a severe potentially fatal illness that results in organ failure, tissue destruction, and systemic inflammation. **Objectives:** To explore the correlation between levels of interleukins and pro-calcitonin and the severity of sepsis and clinical outcomes. **Methods:** This cross-sectional study was conducted at Khairpur Medical College Civil Hospital Khairpur. The participants were n=200 including both male and female. The age range was 18-45 years. Procalcitonin levels were measured using enzyme-linked fluorescent assay and flow cytometry to elevate levels of different cytokines. Serum cytokine concentrations were compared between sepsis patients and healthy controls using the Mann-Whitney U-test for two-group comparisons. The diagnostic accuracy of cytokine levels at study entry was assessed through the area under the receiver operating characteristic curve derived from logistic regression analysis. **Results:** A positive culture report of microbial infectious disease was found in 100/200 (50%) of the patients after microbiological investigation. The male-to-female ratio in the investigated demographic was 3:2. Interleukin-6 levels were significantly higher, p<0.001 in the infectious diseases group than non-infectious Diseases Group. The area under the receiver operating characteristic value of interleukin-6 was found to be excellent and significantly higher (0.95(0.75-0.97)). **Conclusions:** Cytokines including Interleukin-6 and Interleukin-8 are strong biomarkers for diagnosing microbial infections in suspected sepsis cases, with interleukin-6 showing the highest diagnostic accuracy (AUC=0.912). Procalcitonin also demonstrated good discriminative power (AUC=0.895). While cytokines like Interleukin-2, tumor necrosis factor, and Interleukin-17A showed moderate value, interleukin-4 and interferon-gamma were less useful.

INTRODUCTION

Severe sepsis and septic shock, with a mortality rate of 40-50%, are major contributors to deaths in critically ill patients. Blood culture, the standard method for identifying infections in sepsis, is labor-intensive and can take days or weeks for results. Total leukocyte count (TLC) has also proven inconsistent for sepsis detection, highlighting the need for more sensitive and specific biomarkers [1, 2]. Scientists are increasingly exploring alternative markers that better differentiate between infectious and non-infectious states. Receptors like the lipopolysaccharide (LPS) receptor aid the immune system in distinguishing pathogens from non-harmful molecules, leading to the production of various cytokines, such as Interleukins (IL)-1, IL-6, IL-8, IL-12, IL-15, IL-18, IL-23, and tumor necrosis factor (TNF) [3]. For instance, TNF, the primary mediator in toxic shock and sepsis, triggers inflammation and vascular permeability, contributing to symptoms such as fever, anorexia, and hypotension in septic shock [4, 5]. Several cytokines, including pro-calcitonin (PCT), C-reactive protein (CRP), IL-6, and IL-8, have shown promise as biomarkers for sepsis. Procalcitonin, in particular, has demonstrated a strong

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correlation with infection severity, often outperforming traditional markers like TLC and CRP [6]. IL-6, a pro-inflammatory cytokine, plays a key role in the early response to infections and has potential as a guide for antibiotic therapy, as its levels correlate with infection severity [7]. Monitoring IL-6 may help differentiate bacterial infections from other inflammatory conditions and track disease progression or remission, making it a valuable tool for infection management [8]. IL-10, an anti-inflammatory cytokine, helps regulate immune responses by limiting tissue damage. Although it typically acts to moderate inflammation, high IL-10 levels in sepsis may indicate an excessive inflammatory reaction, often seen in worsening sepsis cases. Elevated IL-10 has been associated with severe sepsis and septic shock, reflecting the immune system's struggle to contain widespread inflammation [9]. Additionally, interferon-gamma (IFN-γ), primarily produced by T cells and Natural killer (NK) cells, plays a role in the immune response against infections and inflammation [10]. Technological advances now allow for the rapid detection of multiple interleukins through methods like the flow cytometric cytokine bead array (CBA) assay, which provides results within hours using a small sample volume [11]. Such advances could lead to more efficient and accurate sepsis diagnosis and monitoring, offering the potential for improved outcomes in critically ill patients.

Although the role of interleukins and procalcitonin in assessing sepsis is well-established, the sensitivity and specificity of these biomarkers when used in combination for early diagnosis, especially in critically ill patients with ambiguous clinical symptoms, remains underexplored. Current studies often rely on one marker or lack a comprehensive comparison with clinical presentation. This study aims to fill the gap by evaluating the diagnostic accuracy of both biomarkers together, potentially leading to improved early detection and management strategies for sepsis.

This study aims to evaluate the diagnostic accuracy of procalcitonin (PCT) with interleukins in patients and to determine, and compare the most useful biomarkers for sepsis diagnosis.

METHODS

This cross-sectional study was conducted at Khairpur Medical College Civil Hospital, Khairpur Mirs, for six months (December 2023–May 2024). The study included 200 participants, both male and female, aged 20–45 years, admitted to the Intensive Care Unit (ICU) with suspected infection. Inclusion criteria encompassed clinical signs of infection, such as fever and tachycardia, while exclusion criteria included pregnancy, lactation, chronic autoimmune diseases, immunosuppressant use, and refusal to consent. Participants were divided into three groups: Infectious (microbiologically confirmed infections, Microbial-Infectious diseases (MDI)-positive), Non-Infectious (symptoms of infection but MDI-negative), and Healthy Controls (no signs of infection). A convenience sampling method was employed, with a sample size determined using the formula n= Z2×p×(1–p) /E2: Z-value (Z): assuming a 95% confidence level (Z=1.96), an estimated proportion (p) of 0.5, and a margin of error (E) of 0.0693. Microbiological testing, including cultures from various sources, was conducted. Plasma samples, collected before antibiotic administration, were stored at -80°C for cytokine analysis. Cytokines (IL-2, IL-4, IL-6, IL-10, TNFα, IFN-γ, and IL-17A) were measured using a BDTM Cytometry bead array (CBA) Cytokine Kit and flow cytometry, and procalcitonin (PCT) was measured using ELFA stands for Enzyme-Linked Fluorescent Assay. Data were analyzed using SPSS version 24.0, with cytokine concentrations compared between groups via the Mann-Whitney U test and diagnostic accuracy assessed by the area under the Receiver operating characteristic (ROC) curve from logistic regression analysis. A p-value of <0.005 was considered significant. Institutional Review Board approval was obtained (KMC/RERC/76) from Khairpur Medical College Civil Hospital, Khairpur Mirs and informed consent was secured from all participants.

RESULTS

The mean age of participants was 32.5 years and a standard deviation of 7.5 years, consisting of 120 male (60%) and 80 female (40%). Among the participants, 22.5% had hypertension, 25% had heart disease, and 27.5% had diabetes, while smoking status was nearly evenly split, with 50.5% smokers and 49.5% non-smokers. The participants were classified into three groups: 100 (50%) in the Microbial-Infectious diseases group (MID positive), 50 (25%) in the MID negative group, and 50 (25%) in the healthy control group, facilitating a comparative analysis of health outcomes across conditions (Table 1).

Table 1: Demographic characteristics of the study population

Characteristics	Total Number of Participants n= 200
Age	
20-45 Years	32.5±7.5 years
Gender	
Male	120 (60%)
Female	80 (40%)
Comorbidities	
Hypertension	45 (22.5%)
Heart Disease	50 (25%)
Diabetes	55 (27.5%)
Smoking Status	
Yes	101 (50.5%)

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No	99 (49.5%)
Sepsis Group	
Microbial-Infectious Diseases Group	100 (50%)
Microbial Non-Infectious Diseases Group	50 (25%)
Healthy Group	
Healthy control Group	50 (25%)

MID (Microbial infectious diseases) refers to participants with active infections, Non-infectious (MID negative) refers to those with microbial-related conditions that are not infectious

The analysis reveals that IL-6 and IL-8 levels are significantly elevated in the infectious group compared to the non-infectious and healthy control group, suggesting their potential as key biomarkers for identifying infection. IL-2, TNF, and IL-17A also show moderate differences, with higher levels in the infectious group, indicating some utility in distinguishing infection from non-infection. However, IL-4 and IFN-γ do not vary significantly across groups, suggesting limited diagnostic relevance in this context. These findings highlight IL-6 and IL-8 as primary indicators for infection, with other cytokines providing supplementary diagnostic value (Table 2).

Table 2: Different Cytokine Levels among study participants

Biomarker	Sepsis Group		Healthy Group	p-value (Kruskal-Wallis)
	Infectious Group (MDI Positive) (n=100) Median (IQR)	Non-Infectious Group (MDI Negative) (n=50) Median (IQR)	Healthy Control Group (n=50) Median (IQR)	
IL-2	5.7 (0-32)	5.0 (0-12)	3.4 (0-4)	0.03*
IL-4	3.9 (0-28)	3.0 (0-16)	3.7 (0-4)	0.07
IL-6	1677.3 (4-5000)	901.0 (4-5000)	4 (4-4)	<0.001**
IL-8	145.8 (0-1764)	110.4 (4-1372)	3.7 (0-4)	<0.001**
TNF	6.9 (0-48)	4.2 (0-20)	3.7 (0-4)	0.04*
IFN-γ	14.1 (0-148)	6.8 (0-28)	16.0 (0-36)	0.09
IL-17A	12.8 (0-92)	9.8 (0-44)	31.1 (4-48)	0.02*

Statistical; Mann-Whitney U test each cytokine comparison between two groups of sepsis (MDI positive, MDI negative) with a healthy group

With the greatest Area under the curve (AUC) value of 0.912 (95% CI: 0.853-0.971), according to the ROC analysis, IL-6 performed exceptionally well in differentiating between infected and non-infectious situations. Procalcitonin's AUC of 0.895 (p<0.001) demonstrated its great discriminative potential as well. The moderate AUC values of other cytokines, such as TNF, IL-2, IL-4, and IL-10, ranged from 0.678 to 0.756, indicating differing levels of efficacy in detecting infections. The clinical significance of these biomarkers in diagnostic contexts is further demonstrated by the sensitivity and specificity measurements (Table 3).

Table 3: Function of Cytokines with Procalcitonin

Cytokine/ Marker	AUC (95% CI)	p-value	Cut-off Value	TPR (Sensitivity, %)	FPR (1-Specificity, %)
IL-2	0.678 (0.578-0.778)	<0.001	5.5	70.0	20.0
IL-4	0.689 (0.600-0.778)	0.002	4.0	65.0	25.0
IL-6	0.912 (0.853-0.971)	<0.001	1000	85.0	10.0
IL-8	0.712 (0.618-0.806)	0.005	150	68.0	23.0
TNF	0.678 (0.578-0.778)	0.012	6.0	60.0	30.0
Pro-calcitonin	0.895 (0.820-0.970)	<0.001	0.5	90.0	15.0

The moderate AUC values of other cytokines, such as TNF, IL-2, IL-4, and IL-10, ranged from 0.678 to 0.756, indicating differing levels of efficacy in detecting infections (Figure 1).

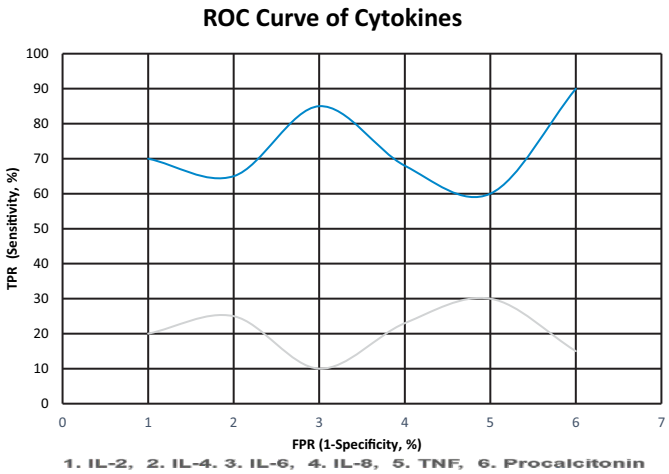

Figure 1: ROC Curves of the Activity of Cytokines

To visualize the ROC curve, plot FPR (x-axis) versus TPR (y-axis) for each cytokine/marker: X-axis: FPR (False Positive Rate, or 1 - Specificity). Y-axis: TPR (True Positive Rate, or Sensitivity). The ROC curve illustrates the diagnostic performance of cytokines and procalcitonin, with curves closer to the top left corner indicating better discriminative ability. IL-6 has the highest AUC at 0.912, followed closely by procalcitonin at 0.895. In contrast, other cytokines like IL-2, IL-4, IL-8, and TNF demonstrate lower AUC values, signifying reduced effectiveness in differentiating between groups.

DISCUSSION

Cytokines, which are repetitively secreted proteins, play significant roles in development, differentiation, and immune activation. These proteins regulate and specify immune responses, guide immune cell movement, and support cellular organization within immune organs [12]. The current study found that elevated levels of IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, and IL-17A correlate with severe

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infection and systemic inflammation in septic patients, with values above 100–300 pg/mL often indicative of sepsis. However, present results showed low specificity for procalcitonin (PCT) in differentiating gram-positive from gram-negative bacteria and reduced sensitivity in predicting infection severity compared to IL-6 and IL-10 [13, 14]. To measure cytokine levels, two widely used methods are Enzyme-Linked Immunosorbent Assay (ELISA) and Cytometry Bead Array (CBA). ELISA typically measures individual cytokines, while CBA allows simultaneous evaluation of multiple cytokines in a single sample, providing a broader cytokine profile. CBA uses fluorescent beads coated with specific antibodies, mixed with the sample, and analyzed via flow cytometry to quantify fluorescence emitted by the beads [15]. This method is particularly useful for studying cytokine interactions, as it is faster and more cost-effective, requiring a smaller sample volume compared to multiple ELISAs. However, CBA may have lower sensitivity than individual ELISAs, with detection limits varying by kit and cytokine [16]. In the current study, IL-6 and IL-10 were found to be more accurate sepsis biomarkers than PCT, with peak AUC values of 0.95 (0.75–0.97) and 0.90 (0.72–0.94) for IL-6 and IL-10, respectively [17]. Cytokine-based therapies hold potential for autoimmune diseases, cancer, and infections by either amplifying or dampening immune responses. Administering cytokines in combination with other treatments can maximize efficacy while reducing toxicity. Present findings suggest that while IL-6 and IL-10 effectively diagnose sepsis, PCT and IL-6 are particularly effective for sepsis severity, with PCT helping assess bacterial infection severity [18, 19]. PCT showed a robust ability to differentiate between infectious and non-infectious cases, with high AUC values in septic individuals. Early PCT measurement may improve the detection and follow-up of sepsis, making it an invaluable biomarker for identifying bacterial infections [20]. Additionally, curent study found that TNF-α and IFN-γ levels were significant in predicting infection severity, with AUCs of 0.85 (0.63–0.90) and 0.80 (0.66–0.91), respectively. This correlation could make them valuable markers during septic shock [21]. Research also shows that reactive oxygen and nitrogen species, generated by macrophages in response to IFN-γ, are critical in destroying intracellular pathogens like Mycobacterium tuberculosis. IFN-γ has shown potential in enhancing T-cell responses and antigen presentation, essential in treating drug-resistant tuberculosis. While not a first-line treatment, IFN-γ may be effective in cases where traditional therapies fail, though its usage requires careful evaluation due to cost, accessibility, and possible side effects.

CONCLUSIONS

It was concluded that IL-6 and IL-8 are strong biomarkers for diagnosing microbial infections in suspected sepsis cases, with IL-6 showing the highest diagnostic accuracy (AUC=0.912). Procalcitonin also demonstrated good discriminative power (AUC = 0.895). While cytokines like IL-2, TNF, and IL-17A showed moderate value, IL-4 and IFN-γ were less useful. These findings highlight the importance of IL-6 and IL-8 in infection diagnosis, enhancing clinical decision-making and potentially improving patient outcomes.

Authors Contribution

Conceptualization: AHP
Methodology: AHP, MK, AA, AQM, MAC, SAP
Formal analysis: AQM, MAC
Writing review and editing: AA, SAP

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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