COMPARISON OF INFLAMMATORY MARKERS AS PREDICTORS OF BLOOD STREAM INFECTIONS IN POSITIVE BLOOD CULTURES OF COVID-19 PATIENTS IN A TERTIARY CARE HOSPITAL

Warda Furqan, Nadia Tayyab, Sakhawat Ali, Irfan Ali Mirza*, Arshad Naseem, Hafeez-Ud-Din**

Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Armed Forces Institute of Pathology/ National University of Medical Sciences (NUMS) Rawalpindi Pakistan, **Pak Emirates Military Hospital/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To determine the correlation of positive blood cultures in septicemic COVID-19 patients with significantly raised serum inflammatory markers C-reactive protein, lactate dehydrogenase and lactate levels.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Microbiology, Pak Emirates Military Hospital (PEMH) Rawalpindi from Apr 2020 to Jun 2020.
Methodology: This study included specimens from 69 adult COVID-19 hospitalized patients with moderate to severe infection. Blood cultures of cases with suspected blood stream infections were processed. Positive blood cultures were compared with markedly raised inflammatory markers.
Results: From a of total 69 blood culture specimen, 36 (52.17%) showed bacterial growth whereas 33 (47.82%) had no bacterial growth. The values of serum C-reactive proteins were in moderate to severe range (≥10mg/l) for 33 (91.66%) out of 36 positive blood cultures. The Lactate dehydrogenase values for 34 (94.44%) out of 36 positive blood cultures were in moderate to severe range (≥300U/L). Serum lactate values for 30 (83.33%) out of 36 patients with positive blood cultures were in moderate to severe range (≥2.21mmol/l). This result was statistically significant.
Conclusion: Serum C-reactive proteins, Lactate dehydrogenase and lactate were markedly high in patients with blood stream infections in patients with COVID-19. These inflammatory markers can be used, not only as early predictors of secondary bacterial infections in COVID-19 patients, but can also help to formulate empirical treatment.

Keywords: Blood cultures, Bloodstream infections, COVID-19, Inflammatory markers, Septicemia.

INTRODUCTION

Corona Virus Disease-19 (COVID-19) is caused by Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) which was first identified in Wuhan China in December 2019[1]. It is transmitted through respiratory droplets and can lead to viral pneumonia[2]. By June 12, 2020 WHO has reported 7,410,510 confirmed cases of COVID-19, with 418,294 deaths in 216 countries globally[3]. In Pakistan, more than 125,521 confirmed cases have been reported with death toll of 2,463 till 12th June[4].

During the pneumonia outbreaks due to other Corona viruses like the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), bacterial co-infections in hospitalized patients have resulted in poor clinical outcome and worst prognosis[5]. In these outbreaks, the data collected was insufficient regarding mortality rate due to secondary bacterial infections. In hospital settings, bacterial co-infections in critically ill patients of COVID-19 have not only resulted in increased mortality, but have also burdened the healthcare management system[6]. If these infections in hospitalized patients are diagnosed timely, initiation of an appropriate anti-microbial therapy can lead to better prognosis.

Infection due to SARS-CoV-2 causes an activation of the host immune response leading

Correspondence: Dr Warda Furqan, Department of Microbiology, Army Medical College Rawalpindi Pakistan
Email: warda_khan28@yahoo.com
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to rise in inflammatory mediators[7]. These markers are also elevated in bacterial infections[8]. Previous studies have shown that raised serum Lactate Dehydrogenase (LDH), serum Lactate and serum C-Reactive protein (CRP) are important indicators of bacterial infections[9,10].

In blood stream infections, blood culture remains the gold standard for isolation of bacteria; however, blood cultures take longer time for isolation of causative pathogens. Moreover, the yield of blood cultures becomes poor, if the blood culture specimens are collected after the initiation of antimicrobial therapy. Therefore, raised inflammatory biomarkers can be used as early predictors of secondary bacterial infections in COVID-19 cases before availability of blood culture results.

METHODOLOGY

This cross-sectional study was conducted at the Pak Emirates Military Hospital (PEMH) Rawalpindi which is an eleven hundred (1100) bedded facility now dedicated exclusively for COVID-19 patients. In this study we collected specimens from 69 hospitalized patients from 1st April to 9th June, 2020. Sampling technique used was non-probability consecutive sampling.

Inclusion Criteria

Patients in ICU with suspected blood stream infections along with positive real time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for SARS-CoV-2 along with typical findings of viral pneumonia on high resolution computed tomography (HRCT) of chest.

Exclusion Criteria

Patients with mild symptoms of COVID-19 and admitted patients of other wards. Repeated blood samples from same patients. Blood cultures and serum samples for inflammatory biomarkers from patients with suspected septicemia on the basis of clinical presentation were simultaneously obtained. Permission was obtained from the ethics review committee (ERC approval certificate number ERC/ID/35) and informed consent was taken from all the patients. Data and patient identification (ID) included in this study remains confidential.

All the blood culture specimens received in microbiology department PEMH Rawalpindi were processed according to standard microbiological guidelines. Eight to ten milliliter blood was collected aseptically and inoculated in blood culture bottles. These culture bottles were incubated in automated blood culture system BacT/ALERT (Biomerieux microbial identification system USA). Specimens were subcultured on 5% sheep blood agar and MacConkey agar plates when bottles flagged positive. These agar plates were then incubated overnight at 37°C in ambient atmospheric conditions. After incubation, plates were examined for bacterial growth. Bacterial isolates were identified by colony morphology and Gram stain.

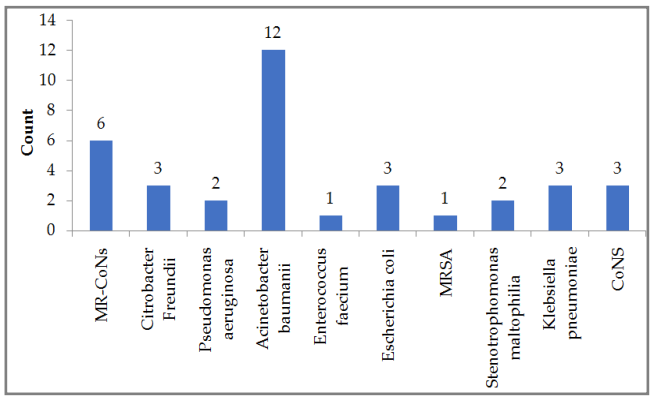

Figure-1: Bacterial isolates frequency in positive blood cultures.

For Gram positive cocci, catalase and coagulase tests were performed and organisms were further identified by standard biochemical reactions using Analytical Profile Index (API Staph and API 20 Strep). For Gram negative rods, oxidase test was done and organisms were further identified by standard biochemical reactions on Analytical Profile Index for Enterobacteriaceae (API20E) and non Enterobacteriaceae (API20NE).

Antimicrobial susceptibility testing was done according to the modified Kirby Bauer Disc Diffusion method on Muller Hinton Agar (Oxoid,

UK) for all antimicrobials, except polymyxin E/ colistin, for which colistin agar dilution method as per protocol was used. Results were interpreted according to Clinical & Laboratory Standards Institute (CLSI) guidelines 2020[11]. Inflammatory markers as serum LDH, serum lactate and serum CRP processed on Chemistry Analyzer COBAS 501 and 601.

The results were statistically analyzed and groups were compared by chi-square test using SPSS version 22. Differences between groups were considered significant if *p*-value ≤0.05.

RESULTS

Blood culture specimens from 69 patients with suspected blood stream infection were processed. Specimens from the same patients were also analyzed for inflammatory markers. Data was collected from 1st April to 9th June, 2020 (2 months and 8 days). Hospitalized patients were categorized into moderate and severe disease based on clinical presentation, laboratory parameters and radiological findings[12].

Moderate Disease

1. Respiratory distress, RR ≥30 times/ minute.
2. Oxygen Saturation ≤93% at Room Air
3. Oxygen partial pressure (PaO2)/oxygen concentration (FiO2) in arterial blood ≤300 mmHg more than 50% lung involvement on HRCT chest.

Severe Disease

1. Respiratory failure and requirement of mechanical ventilation
2. Shock
3. Multi organ failure and requiring treatment in ICU.

Out of 69 samples, 58 (84.1%) were male, and remaining 11 (15.9%) were from female patients. Blood cultures of 35 (50.73%) patients with severe disease were received and 34 (49.27%) from patients with moderate disease.

Out of 69 blood cultures, 36 (52.2%) showed bacterial growth, whereas 33 (47.8%) had no bacterial growth. Three positive blood culture samples were excluded from the study as they yielded growth of coagulase negative *staphylococcus* (CoNS) from single blood culture specimen. If blood cultures were not collected with due aseptic precautions, CoNS were considered as part of normal skin flora or contaminant in the absence of clinical signs and symptoms of bacteremia[13]. All remaining 33 bacterial pathogens were isolated from more than one blood culture.

The isolated organisms from 33 positive blood culture were mostly Gram negative rods, including 12 (36.36%) *Acinetobacter baumannii*, 3 (9.09%) *Klebsiella pneumoniae*, 3 (9.09%) *Citrobacter freundii*, 3 (9.09%) *Escherichia coli*, 2 (6.06%) *Pseudomonas aeruginosa* and 2 (6.06%) *Stenotropho-*

Table-I: Ranges of inflammatory markers.

Inflammatory Markers	Range	Remarks
C-Reactive Protein	Less than 6.00mg/L	Normal
	6.01-9.99mg/L	Minor Elevation
	10.00-100.00mg/L	Moderate Elevation
	100.01 and Above mg/L	Severe Elevation (Indicates Acute bacterial infections)
Serum Lactate	0.5-2.20mmol/L	Normal Value
	2.21-3.99mmol/L	Moderate elevation
	More than 4.00mmol/L	Severe Elevation (Lactic Acidosis)
Lactate Dehydrogenase	125-220 U/L	Normal
	221-299 U/L	Mild Elevation
	300-449 U/L	Moderate Elevation
	450 and above	Severe Elevation

monas maltophilia. Gram positive *cocci* include 1 (3.03%) *Methicillin-resistant Staphylococcus aureus* (MRSA), 6 (18.18%) *Methicillin resistant coagulase negative staphylococci* (MR-CoNS) and 1 (3.03%) *Enterococcusfaecium* (fig-1). Range for the inflammatory markers with categorization as normal, mild, moderate and severe[14,15] is shown in table-I. Our study showed that in positive blood cultures showing bacterial growth, there was moderate to severe elevation in serum LDH, lactate and CRP levels as elaborated in table-II. The C-Reactive Protein, Serum Lactate, Lactate Dehydrogenase are statistically significant in relation to blood culture results.

Table-II: Categorization of inflammatory markers in COVID-19.

Parameters				No. of Patients (n=69)
Blood Cultures		Inflammatory Markers		
Blood Culture Result	Negative	C-Reactive Protein	Normal	1 (3.03%)
			Minor Elevation	3 (9.09%)
			Moderate Elevation	21 (63.6%)
			Severe Elevation	8 (24.2%)
		Serum Lactate	Normal	21 (63.6%)
			Moderate Elevation	9 (27.2%)
			Severe Elevation	3 (9.09%)
		Lactate Dehydrogenase	Normal	2 (6.06%)
			Minor Elevation	8 (24.2%)
			Moderate Elevation	17 (51.5%)
			Severe Elevation	6 (18.1%)
	Total			33 (47.8%)
	Positive	C-Reactive Protein	Normal	1 (2.77%)
			Minor Elevation	2 (5.55%)
			Moderate Elevation	18 (50.0%)
			Severe Elevation	15 (41.6%)
		Serum Lactate	Normal	6 (16.6%)
			Moderate Elevation	24 (66.6%)
			Severe Elevation	6 (16.6%)
		Lactate Dehydrogenase	Normal	1 (2.77%)
			Minor Elevation	1 (2.77%)
			Moderate Elevation	17 (47.2%)
			Severe Elevation	17 (47.2%)
	Total			36 (52.1%)

Table-III: Blood culture results x inflammatory markers chi-square test.

	Value	df	Asymptotic Significance (2-Sided)
Pearson Chi-Square	10.929a	3	0.012
Likelihood Ratio	11.889	3	0.008
Linear-by-Linear Association	8.853	1	0.003
No. of Valid Cases	69		

A. 4 cells (50.0%) have expected count less than 5. The minimum expected count is 1.43.

DISCUSSION

Secondary bacterial infections in hospitalized patients of viral pneumonia have resulted in increased mortality[16]. Accurate diagnosis of blood stream infections by isolation of bacterial pathogens through blood culture followed by subsequent initiation of appropriate antimicrobial therapy has a key role for better prognosis and survival in patients[17]. During COVID-19 pandemic, all health management resources are already over utilized with a significant burden on health care management system. However secondary bacterial infections have further added to this burden leading to prolong hospital stay, high

mortality, and increased workload of health care workers and draining of financial resources.

Blood culture is no doubt the gold standard for isolation of micro-organisms causing blood stream infections but it takes a minimum time period of 72 to 96 hours for identification of isolate and antimicrobial susceptibility[18]. In our study, we correlated positive blood cultures of COVID-19 hospitalized patients having blood stream infection with inflammatory markers, which included serum CRP, Lactate and LDH levels. Our study shows that organisms isolated from blood cultures causing blood stream infections were mainly *Acinetobacter baumannii*, *Klebsiella pneumoniae, E. Coli and Stenotrophomonas maltophilia*. A study was conducted in China in COVID19 patients to see co-infections due to various pathogens. Out of 243 patients with co-infections 236 (91.8%) had bacterial co-infections. Which included Gram positive and Gram negative bacteria among Gram negative bacteria most common isolates were *Klebsiella pneumoniae, Haemophilus influenzae, E. Coli* and *Acinetobacter baumannii*[19].

Previous studies have shown that inflammatory markers like LDH and CRP are raised in COVID-19 patients with moderate to severe disease. According to a study conducted in Wuhan China on patients with SARS-CoV2 pneumonia, 86% of 99 patients had raised CRP levels. LDH levels were 6 foldraised insevere COVID19 patients as referred from a study[2,20].

In this study inflammatory markers were also raised in all patients irrespective of secondary bacterial infections.However, in most of the patients with positive blood cultures, the values of serum LDH, CRP and serum lactate were significantly higher as compared to patients with negative blood cultures. All these values were in moderate to severe range as shown in table-IV. Therefore, rising trend in these inflammatory markers can be used as a predictor of blood stream infections in COVID-19 patients with suspected septicemia. Better outcome can be achieved by timely initiation of broad spectrum antimicrobial therapy according to local antimicrobial data and then de-escalation according to culture and sensitivity results.

This study also emphasizes on implementation of strictinfection prevention and control practices which include hand hygiene, use of appropriate personal protective equipment, environmental cleaning, disinfection of equipments or use of disposable equipments, safe injection practices and adherence to infection control bundles especially in critical care settings to restrict the secondary bacterial infection.

LIMITATION OF STUDY

Limitations of our study were that other inflammatory markers like the interleukin-6, procalcitonin, D-dimers, Tumor necrosis factor (TNF) alpha and Brain natriuretic peptide (BNP) were not included for comparison which would have guided us more. Secondly, multicenter studies on large scale are required for better clinical correlation.

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CONCLUSION

COVID 19 patients in our study had raised inflammatory markers, but this rise was significantly marked in patients who were found to have positive blood cultures. While culture results are pending, these inflammatory markers can help in clinical decision making for a better prognosis. This would be much helpful towards optimum antimicrobial stewardship.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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