Research article

Blood culture and profile of inflammatory biomarkers among COVID-19 patients in an intensive care unit of a tertiary care hospital

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ABSTRACT

Introduction and Aim: This is a retrospective study to analyse the pathogens causing bacteraemia in COVID-19 patients and its correlation with inflammatory biomarkers such as procalcitonin (PCT), C-reactive protein (CRP), total WBC counts (TC) and D-dimer. The objectives of this study were to evaluate the blood culture positivity rate, to identify the pathogens causing bacteraemia, to analyse their antibiogram and to assess the significance of inflammatory markers along with patient outcomes.

Materials and Methods: This study included 165 COVID-19-positive patients admitted to the Intensive care unit (ICU) of a tertiary care hospital from June to September 2020. Blood culture, identification and antibiotic sensitivity testing (AST) were done using automated systems. Values of D-dimer, CRP, PCT and TC were obtained using immunoturbidimetric assay, chemiluminescent immunoassay, immunochromatographic testing and automated haematology analyser respectively.

Results: Among 143 blood culture samples obtained from 122 COVID-19 positive patients, 80 flagged positive. Out of the 80 isolates obtained, 53 (66.25%), 17 (21.25%) and 10 (12.5%) were gram-positive bacteria, gram-negative bacteria and candida respectively. The blood culture positivity rate was 25.4%. *Acinetobacter spp.* and *K. pneumoniae* showed high levels of antibiotic resistance. Among 16 patients with elevated PCT, 15 (93.7%) patients showed positive blood cultures. CRP of >5 mg/L and deranged total WBC counts were seen among all blood culture-positive patients. In 100 patients with elevated D-dimer, 54% (54/100) patients showed positive blood culture.

Conclusion: From this study, we conclude that early identification of pathogens and initiation of appropriate antimicrobial therapy is crucial for managing sepsis associated with COVID-19 infection. PCT, CRP, TC and D-dimer can help as biomarkers in the management of COVID-19 patients with secondary bloodstream infections (BSI).

Keywords: COVID-19; bloodstream infection; blood culture; CRP; procalcitonin.

INTRODUCTION

COVID-19 represents a spectrum of clinical severity ranging from asymptomatic to severe pneumonia, acute respiratory distress syndrome (ARDS), and even death. Monitoring the severity and effective early intervention are the fundamental measures for reducing mortality (1). Severe COVID-19 infections are often associated with bacterial co-infections and several studies suggest the use of empirical antibiotics in severely ill patients (2-4). Studies suggest that inflammatory markers are elevated in patients with severe disease admitted to the intensive care units (5-8). Therefore, it is important to identify the bacterial agents causing secondary bloodstream infections (BSI) and estimate the potential biomarkers for the effective and appropriate treatment of these patients.

As the prevalence, etiological agents, and antibiotic sensitivity profile of bacteria causing secondary BSI in COVID-19 patients is not very well understood, the present study was carried out with an aim to analyse the etiological agents causing bacteraemia in these patients and its correlation with inflammatory biomarkers such as procalcitonin (PCT), C-reactive protein (CRP), total WBC counts (TC) and D-dimer. The objectives of this study were to evaluate the blood culture positivity rate, to assess the distribution and antibiogram of pathogens obtained from blood cultures and to understand the association of blood culture positivity with inflammatory markers and disease outcome.

MATERIALS AND METHODS

This is a retrospective laboratory-based observational study that included 165 COVID-19-positive patients admitted to the ICU of tertiary care hospital in South India from June to September 2020. After obtaining due approval from the Institutional Ethical Committee, clinical and laboratory data of the patients was accessed from the hospital's electronic medical records archives. Blood culture samples were sent from 122 of these patients and incubated using an automated blood culture system (BD BACTEC 9120) for a maximum period of 5 days. Identification and antibiotic sensitivity testing (AST) of the bacterial isolates was

done using an automated ID/AST system (BD PHOENIX 100). D-Dimer values were determined using an immunoturbidimetric assay (Turbodyne SC) and values >500 ng/ml were considered significant. Procalcitonin levels were assessed by immuno-chromatographic testing (Thermo scientific) with the test values being interpreted as follows: Sepsis unlikely (<0.5 ug/l), possible sepsis (>0.5 & <2 ug/l), sepsis likely & high risk of progression to severe sepsis (>2 & <10 ug/l) and high likelihood of severe sepsis (10 ug/l).

CRP values were determined by CLIA (VITROS 5600 integrated system) with a normal range of 0-5 mg/L. Total WBC counts were estimated using automated haematology analyser (WBC Sysmex) with a normal range of 4000-11000/ul. SARS-CoV-2 RT-PCR testing was performed in-house using commercially available kits. The data was transcribed onto a Microsoft Office Excel worksheet and percentages were calculated for categorical variables. To find significant association appropriate statistical tests were used. The Chi-square test was used to compare two groups. *P-value* <0.05 was considered statistically significant.

RESULTS

Out of the 165 COVID-19 patients admitted to the ICU during the study period, 143 blood cultures were sent to the laboratory from 122 patients. Out of the 143 blood cultures processed, 80 samples flagged positive and yielded 70 bacterial isolates and 10 fungal isolates which were identified as Candida species. Blood culture positivity rate was 48.3% (59/122) considering only one isolate per patient. Excluding the single CONS isolate, the blood culture positivity rate was 25.4% (31/122). Among the 59 patients whose blood culture was positive, 72% (40/59) were male and 28% (19/59) were female. A gender-wise ratio of 2.1:1 was observed. The highest rate of blood culture positivity was seen in the age group of >60 years (42%) as shown in Table 1.

Table 1: Age wise distribution of blood culture positivity

Age	Number of patients (%)
20-29	1 (2%)
30-40	2 (3%)
40-49	11 (19%)
50-59	20 (34%)
>60	25 (42%)

Out of the 70 bacterial isolates, 53 (75.7%) were identified as Gram-positive and 17 (24.3%) as Gram-negative. The predominant microbial isolate was Coagulase-negative Staphylococcus (CONS) in 52.5% (42/80) of the positive blood cultures. *S. aureus* was isolated in 8% (6/80), and *Enterococcus spp*. in 6.25% (5/80) of the positive cultures. Among the Gram-negative bacterial isolates, *Acinetobacter spp*. 6.25% (5/80) was the most predominant, followed by *E.coli*, 5% (4/80); *K. pneumoniae*, 4% (3/80); and *P. aeruginosa*, 4% (3/80). Candidemia was seen in 10 patients (12.5%) as shown in Fig. 1.

Antibiotic sensitivity testing results showed that all the isolates of *S. aureus* and *Enterococcus spp.* were sensitive to daptomycin, linezolid and teicoplanin. However, these isolates showed higher rates of resistance to commonly used antibiotics like penicillin, ampicillin, co-trimoxazole and erythromycin (Table 2). Two isolates of *S. aureus* isolates (33%) were found to be methicillin-resistant. *Acinetobacter spp.* and *K. pneumoniae* showed very high rates of resistance to majority of the antibiotics tested (Fig. 2). Among the gram-negative bacilli 29% (5/17) were ESBL producers and 24% (4/17) were potential carbapenemase producers.

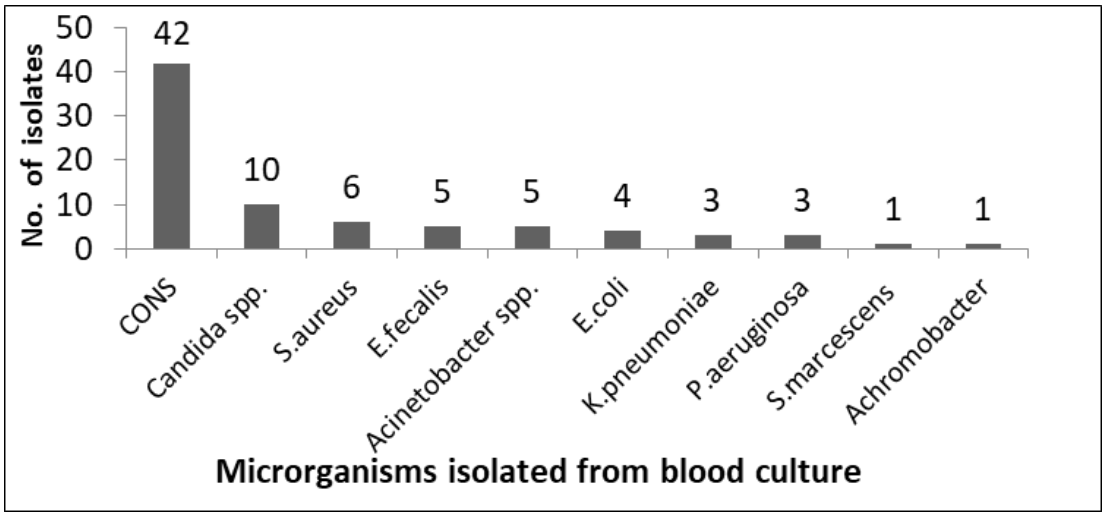

Fig. 1: Distribution of microorganisms isolated from positive blood cultures obtained from COVID-19 positive patients admitted in the ICU (n=80)

Table 2: AST pattern of gram-positive bacterial isolates (n=11)

Antibiotics	*S. aureus*	*E. faecalis*	Total percentage	
			Sensitive	Resistant
PEN	0/5	1/5	1/5 (10%)	4/5(90%)
AMP	0/3	0/5	0/8 (0%)	8/8 (100%)
VAN	6/6	1/5	7/11 (63.6%)	4/11 (36.4%)
TEI	3/3	NT	3/3 (100%)	0/3 (0%)
GEN	4/6	0/4	4/10 (40%)	6/10 (60%)
TE	4/5	0/5	4/10 (40%)	6/10 (60%)
ERY	3/6	0/4	3/10 (30%)	7/10 (70%)
CD	5/6	IR	5/6 (83.3%)	1/6 (16.7%)
LZ	6/6	5/5	11/11 (100%)	0/11 (0%)
SXT	2/6	NT	2/6 (33.33%)	4/6 (66.67%)
DAP	5/5	5/5	10/10(100%)	0/10 (0%)

(PEN: Penicillin, AMP: Ampicillin, VAN: Vancomycin, TEI: Teicoplanin, GEN: Gentamicin, TE: Tetracycline, ERY: Erythromycin CD: Clindamycin, LZ: Linezolid, SXT: Trimethoprim-sulfamethoxazole DAP: Daptomycin, IR: Intrinsic resistance, NT: Not tested)

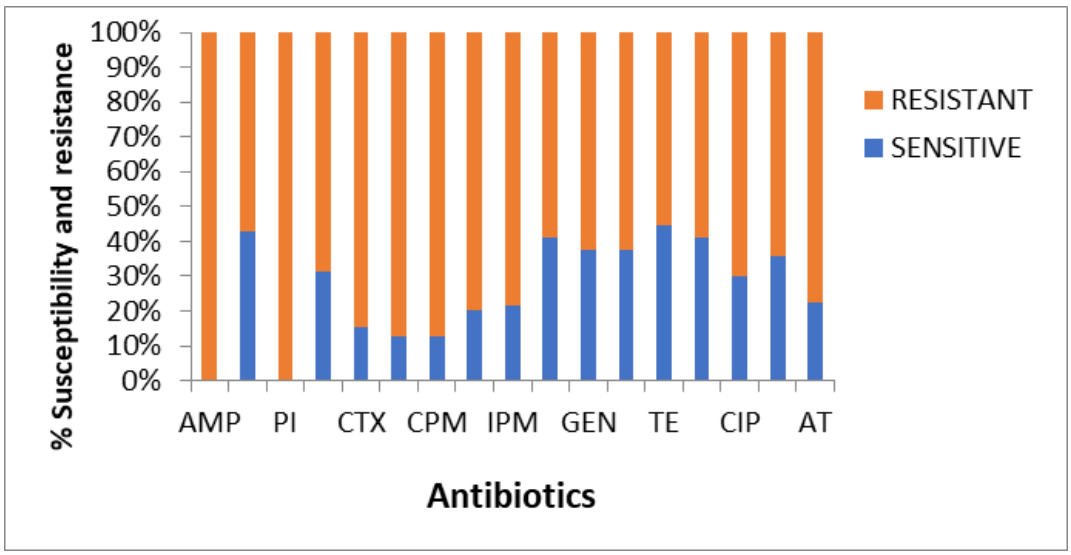

Fig. 2: AST pattern of gram-negative bacterial isolates (n=17)

(AMP: Ampicillin, AMX: Amoxicillin, PI: Piperacillin, PTZ: Piperacillin-tazobactam, CTX: Cefotaxime, CAZ: Ceftazidime, CPM: Cefepime, MEM: Meropenem, IPM: Imipenem, AK: Amikacin, GEN: Gentamicin, CM: Chloramphenicol, TE: Tetracycline, LE: Levofloxacin, CIP: Ciprofloxacin, SXT: Trimethoprim-sulfamethoxazole, AT: Aztreonam)

In this study, among 122 patients whose blood culture was sent, 100 patients showed elevated D-dimer values (Table 3). Fifty four blood culture-positive patients (54%) showed D-dimer values of >500 ng/ml and 46 blood culture-negative patients (46%) showed elevated D-dimer values of >500 ng/ml. Among 16 patients with elevated PCT levels, 15 (93.7%) patients had a positive blood culture and 1 (6.3%) patient's blood culture was negative. These 16 patients were categorized into 3 groups according to the interpretation criteria of the test kit. Four patients had values of >0.5 and <2 ug/l, suggesting possible sepsis, 9 patients had values of >2 and <10 ug/l, suggesting likely sepsis and a high risk of progression to severe sepsis and 3 patients had a high likelihood of severe sepsis with PCT values of >10 ug/l.

Elevated CRP values of >5 mg/l and deranged WBC counts of <4000 or >11,000/ul were seen among all culture-positive patients (Table 3). It was observed that 57/111 (51%) and 53/105 (50.5%) of blood culture-negative patients also showed deranged CRP and WBC counts respectively. The outcome of only 61 patients out of the 165 included in the study was available at the time of completion of this study and is shown in table 3. Blood culture results were available for all of these 61 patients. Out of 58 culture-positive patients, 42 (72.4%) expired and 16 (27.6 %) were discharged on improvement. Out of 3 culture-negative patients, only one expired (33.3%) and 2 (66.6%) were discharged on improvement.

Table 3: Procalcitonin, CRP, total WBC count, D-dimer values and outcomes in COVID-19 patients and its association with blood culture results

Parameter studied	Blood culture positive	Blood culture negative	*P-value*
Blood culture positivity rate including single CONS isolate from blood culture (n=122)	59 (48.3%)	63 (51.7%)	-
Blood culture positivity rate excluding single CONS isolate from blood culture (n=122)	31 (25.4%)	91 (74.6%)	-
PCT* (n=16)	15 (94%)	1 (6%)	0.000 **
CRP* (n=111)	54 (49%)	57 (51%)	0.776
Total WBC count* (n=105)	52 (49.5%)	53 (50.5%)	0.992
D-dimer* (n=100)	54 (54%)	46 (46%)	0.424
OUTCOME Deceased during hospital stay (n=43)	42 (68.9 %)	1 (1.6%)	0.000 **

*Highest deranged value during ICU stay; ** Significant p-value *of* <0.005

DISCUSSION

At the beginning of the second wave of COVID-19 pandemic in India, there was a surge in the number of critically ill COVID patients who required admission into the intensive care unit. This study was conducted to identify the prevalence, etiological agents, antimicrobial sensitivity pattern and clinical outcomes in COVID-19 patients with BSI. The study population included 165 patients across different age groups admitted to the ICU of a tertiary care hospital and the blood culture positivity rate among them was 25.4%. In a study done by Patel *et al.*, from March 2020 to October 2020 on 166 patients admitted to the COVID ICU, the blood culture positivity rate of 22.28% was reported (9). Another study by Palanisamy *et al.*, with 750 patients admitted in the COVID ICU, blood culture positivity rate of 8.5% was reported (10). The number of subjects allotted is more when compared to the present study, which may be the reason for the low culture positivity rate. Microbial pathogens isolated from blood cultures in this study are very similar to the findings reported by Sepulveda *et al.*, wherein a large number of CONS were isolated (11). It is challenging to consider CONS as a causative agent of bloodstream infections when only a single blood culture was sent.

A high rate of antimicrobial resistance was observed among isolates of *Acinetobacter spp.* and *K. pneumoniae*. Isolates of *S. aureus* also showed high rates of resistance to penicillin, ampicillin, erythromycin, and co-trimoxazole and 33.3% of them were methicillin-resistant. Among the Gram negative bacilli, 5 were extended-spectrum beta-lactamase (ESBL) producers and 4 were potential carbapenemase producers. A study by Vijay *et al.*, that analysed retrospective data of hospitalized COVID-19 patients from June to August 2020, showed almost similar rates of antibiotic resistance among isolates of *A. baumannii* and *K. pneumoniae* (12). Although candidemia was seen in only 12.5% (10/80) of the total cases of BSIs in our study, higher rates of candidemia have been reported among COVID-19 patients. A retrospective study with 209 patients in COVID-19 ICU, by Niyas *et al.*, reported that candidemia accounted for 18.18% of the total BSIs. Several factors such as underlying comorbidities, prolonged hospital stay and extensive use of broad spectrum antibiotics and corticosteroids could contribute towards the higher rates of candidemia seen among critically ill COVID-19 patients (13). To prevent severe illness and death due to candidemia, early diagnosis, and appropriate antifungal therapy based on antifungal susceptibility testing should be performed.

The present study supports the fact that blood cultures are an essential tool for the early diagnosis and management of BSIs among COVID-19 patients suspected to have sepsis since both COVID-19 and secondary BSIs have several overlapping clinical and metabolic features. Hence biomarkers play a vital role by facilitating risk assessment and prognosis of severity in COVID-19 (14, 15). Out of the 100 patients for whom D-dimer test was done, 54 (54%) showed values of >500 ng/m in culture positive and 46 (46%) showed >500 ng/ml in culture negative. Elevated D-dimer level in COVID-19 patients is associated with increased mortality as reported by Yao *et al.*, wherein significantly higher D-dimer levels were detected in non-survivors versus survivors (16). In this study elevated PCT levels showed significant association with positive blood culture results (*p-value*: 0.000). Cheng *et al.*, observed that bacterial infection rate in COVID-19 patients with elevated PCT was 4.92 times more than that in those with normal levels (17). Thus PCT may be used as an indicator of disease severity in patients with COVID-19 (18).

It is known that COVID-19 patients with increased WBC count and CRP levels are more likely to develop Systemic inflammatory response syndrome (19). In this study elevated CRP levels and WBC counts were observed among all the blood culture-positive patients.

However, low WBC counts especially lymphopenia was associated with poor outcomes among these critically ill patients. The role of inflammatory markers in COVID-19-infected patients is multifunctional. Results of our study showed that higher levels of inflammatory markers are associated with increased disease severity and could be used to prognosticate the outcome. Out of 58 culture-positive patients, 42 (72.4.9%) expired and 16 (27.6%) showed improvement, which shows the significant impact of blood culture positivity in COVID-19 patients (*p-value*: 0.000). Pourajam *et al.*, noted the mortality among COVID-19 patients who acquired BSI was 83% against an overall mortality of 38.1% in total admitted COVID-19 patients (20). This study clearly shows that COVID-19 patients in the ICUs with secondary BSIs had poor outcomes, even after antibiotic therapy was initiated according to AST reports.

This study has a few limitations since it was retrospective in nature and restricted to a short duration. Details of pre-existing comorbidities, intake of inappropriate prophylactic antibiotics during the pandemic and their impact on developing secondary BSIs with MDR bacteria were not correlated. Cause of death in critically ill COVID-19 patients was often multifactorial due to ARDS, multi-organ dysfunction etc. Hence secondary BSI could not be taken as the sole cause for morbidity. A large multi-centric study will be required to substantially prove the association of positive blood cultures with the outcomes in critically ill COVID-19 patients.

CONCLUSION

Even though COVID-19 infection is associated with mild symptoms and favourable outcomes in a majority of the infected cases, a small subset of critically ill patients can develop complications due to ARDS, bacterial sepsis, multi-organ dysfunction etc. leading to death. From this study, we conclude that early identification of sepsis, the identification of appropriate causative microorganisms and initiation of antimicrobial therapy guided by AST report is crucial in managing secondary bacterial infections in these patients. PCT, CRP, WBC counts, and D-dimer can help as biomarkers in indicating the disease severity and monitoring the outcome.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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