

A SPECTRUM OF BACTERIAL SEPSIS IN COVID -19 POSITIVE AND NON COVID -19 PATIENTS – AN INDIAN MONOCENTER RETROSPECTIVE STUDY

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(Received 15 June, 2022; Accepted 4 August, 2022)

Key words: *Coronavirus, Bacterial sepsis, Acute respiratory distress syndrome, Hematological biomarkers.*

Abstract–This study aims to determine spectrum of bacterial infection in patients with severe acute respiratory syndrome coronavirus-2 infection at the time of hospital admission and identify changes in hematological biomarkers of COVID19 severity. A retrospective study was conducted in blood cultures from patients suspected to have sepsis were included in the study. Patients were grouped based on SARS-CoV-2 RT-PCR result as positive, negative, or not tested. For the purposes of classifying blood cultures by SARS-CoV-2 RT-PCR status, hematological parameters were analyzed. Out of 825 blood sample, 466 samples were positive for blood culture identified by conventional and Automatic blood culture system – Vitek 2. Among these 466 patients, 211(45.2%) were positive for SARS-CoV2 virus and 255 (54.7%) were negative for SARS-CoV2 virus by RT -PCR. Regarding CRP Total number of CRP positive samples was 654, CRP negative samples was 84 and CRP was not done for 87samples. The total number of IL6 positive samples was 83 and IL6 negative samples were not evaluated for 125 patients. The concern of Bacterial sepsis in COVID-19 patients due to the above organisms based on our study over the period of one year. Consequently, it is important to pay attention to bacterial co-infections in critical patients diagnosed for COVID-19.

INTRODUCTION

Corona viruses can cause an array of respiratory conditions, ranging from common cold to severe acute respiratory syndrome (SARS) to the Middle East respiratory syndrome (MERS). In December 2019, cases of acute respiratory illness with unknown etiology was detected in Wuhan city in the Hubei province of China, related to Huanan seafood market was identified, which is now known as severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) (Guan *et al.*, 2019). It is the third in the line of corona viruses that have emerged among the human population over the last two decades, the other two being severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak in 2002-2003 and the Middle East respiratory syndrome coronavirus

(MERS-CoV) outbreak in 2012-2013. In March 2020, the World Health Organization declared the outbreak to be a pandemic, with more than 35 million infections worldwide and still counting.

SARS-CoV-2 are newly emerged β coronaviruses belonging to the Coronaviridae family has four major structural proteins, i.e spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins. Spike (S) protein binds to angiotensin-converting enzyme-2 (ACE-2) receptor and mediates subsequent fusion between the envelope and host cell membranes to aid viral entry into the host cell. The presence of glutamine, asparagine, leucine, phenylalanine and serine amino acids in SARS-CoV-2 enhances ACE2 binding [3]. The nasal epithelial cells have the highest expression of ACE-2 receptors in the respiratory tree hence it used for detection of

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viral RNA from the nasopharyngeal swabs.

The main clinical symptoms of SARS-CoV-2 patients are fever, cough, fatigue or myalgia, sputum production, headache and diarrhea were less common symptoms. About 50% patients developed dyspnea, among which one third were admitted to ICU (Huang *et al.*, 2020), while the severely ill patients often develop dyspnea within a week, which rapidly progress to acute respiratory distress syndrome (ARDS), sepsis and multiple organ dysfunction (MODS) (Chen *et al.*, 2020). Epidemiological studies have shown that severity of the disease is higher in patients with co-morbidities like Diabetes mellitus, hypertension, etc. and also at the extremes of age. Patients with severe disease showed lymphopenia, increased plasma concentrations of proinflammatory cytokines produced by the infected lung epithelial cells, including IL-6, IL-10, etc. SARS-CoV-2 infects the pulmonary capillary endothelial cells and triggers an influx of monocytes and neutrophils, infiltrating the air spaces causing diffuse thickening of the walls of the alveoli (Xu *et al.*, 2020) leading to sepsis.

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer *et al.*, 2016). Sepsis can be caused by a variety of pathogens but bacteria are the most common cause. The lungs have a rich network of blood vessels enveloping the capillaries and a large surface area which are in constant contact with the environment via the air we breathe in. This makes the organ highly susceptible to the air-borne pathogens. Micro thrombi formation occurs as a consequence of the inflammation of lung tissues and pulmonary endothelial cells.

In patients with COVID-19, endothelial barrier of the lung is disrupted, oxygen diffusion capacity (13) is also impaired and additional exposure to bacterial pathogens that reach via other routes like blood or airway, contributing to increased morbidity and mortality of patients. These further complications land the patient in the hospital environment a prolonged period which increases the chances of a hospital acquired infection. High incidence of thrombotic complications, such as deep venous thrombosis, pulmonary embolism, and thrombotic arterial complications (e.g., limb ischemia, ischemic stroke, myocardial infarction) in critically ill patients is a result of this micro thrombification (Tang *et al.*, 2020; Thachil *et al.*, 2020). The development of viral sepsis, by a dysregulated host response to infection, may further contribute to multiorgan

failure. Cumulative evidence from studies infer that a subgroup of patients with severe COVID-19 have a cytokine storm syndrome. Respiratory failure from acute respiratory distress syndrome (ARDS) is the leading cause of mortality (Ruan *et al.*, 2020). Secondary haemophagocytic lymphohistiocytosis (sHLH), characterized by fatal hypercytokinaemia with multiorgan failure in adults, triggered by viral infections occurs in 3.7–4.3% cases of sepsis (Ramos-Casals *et al.*, 2014; Karakike and Giamarellos-Bourboulis, 2019). Pulmonary involvement (including ARDS) occurs in almost 50% of cases (Seguin *et al.*, 2016). Similar to sHLH, increased interleukin (IL)-2, IL-7, granulocyte colony stimulating factor, interferon- γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1- α , and tumor necrosis factor- α are observed in severe COVID-19 (Huang *et al.*, 2020). Considering all this study aimed to compare the difference in hematological parameters of patients diagnosed with bacterial sepsis in COVID-19 positive and COVID-19 negative patients.

MATERIALS AND METHODS

Study design

After obtaining institutional ethical committee's approval, a study was conducted on patients with blood cultures performed at Saveetha Medical College and Hospital located at Thandalam, Kanchipuram district from 1 May 2020 to 31st December 2021. Records were extracted and various details patients based on SARS-CoV-2 reverse transcription-PCR (RT-PCR) result, blood culture result, organism(s) identified, blood culture collection date and time, IL-6 results, CRP and total leukocyte count were collected.

Laboratory methods

Blood cultures were incubated on Bactec instrument (Figure 1) for a maximum of 5 days and those samples which indicated positivity were subjected to microscopic examination after gram staining, followed by inoculation into blood agar, lysed blood agar and Mac-conkey agar (Figure 2). The isolates were identified and their antibiotic susceptibility was analysed by either automated method using Vitek 2 or by biochemical tests and Kirby-Bauer disk diffusion method for antibiotic susceptibility testing. SARS-CoV-2 RT-PCR testing was performed in house or in a laboratory accredited by NABL and ICMR. Complete hemogram, CRP levels and



Fig. 1. Bactec instrument (Automated Blood Culture System utilizes fluorescent technology in detecting the growth of organisms in the blood culture bottles)



Fig. 2. Colonies of different bacteria in culture medium plate

interleukin-6 (IL-6) levels of the patients was analysed.

Sample size

A total of 825 blood cultures from patients suspected to have bacterial blood stream infections were included in the study. Patients were grouped based on SARS-CoV-2 RT-PCR result as positive, negative,

or not tested. For the purposes of classifying blood cultures by SARS-CoV-2 RT-PCR status, we used the following criteria: Blood cultures were labeled SARS-CoV-2 status positive if they were performed within 2 days of a positive SARS-CoV-2 RT-PCR result and considered positive for all subsequent blood cultures after a positive SARS-CoV-2 RT-PCR result.

Blood cultures were labeled SARS-CoV-2 status negative if they were performed within 2 days of a negative SARS-CoV-2 RT-PCR result and considered negative for all subsequent blood cultures unless the patient had a subsequent positive SARS-CoV-2 RT-PCR result, at which point the status was changed to positive for any blood cultures performed within 2 days of the positive SARS-CoV-2 RT-PCR result. The two-day interval was used to account for turnaround time from test ordering to test results for SARS-CoV-2 RT-PCR tests keeping in mind that it may have taken up to 2 days for the SARS-CoV-2 RT-PCR result to be available.

RESULTS

Out of 825 blood sample, 466 samples were positive for blood stream infections in culture (Figure 3).

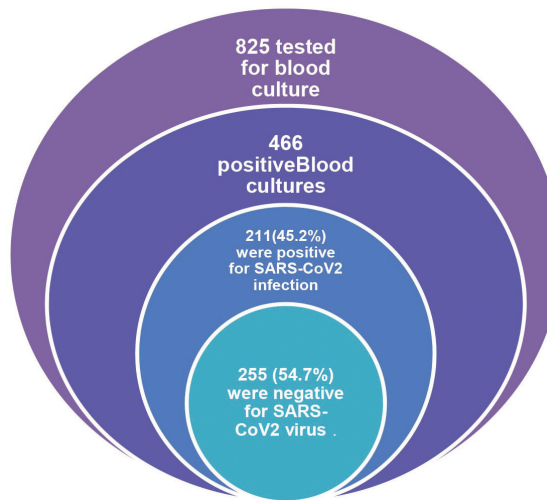


Fig. 3. Blood culture among patients was positive for SARS-CoV2 virus and negative for SARS-CoV2 virus.

Among these 466 patients, 211(45.2%) were positive for SARS-CoV2 virus and 255 (54.7%) were negative for SARS-CoV2 virus.

The total number of CRP positive samples was

654, CRP negative samples was 84 and CRP was not done for 87 samples. Most patients with positive covid would have crp done. The total number of IL6 positive samples was 83 and IL6 negative samples were not evaluated for 125 patients.

In the present study, a total of 466 patients with bacterial sepsis were included. Out of this, 360 patients were positive for SARS-CoV-2 by RT-PCR and 106 were negative. Among the 360 patients who had tested positive for Covid-19 with bacterial sepsis, 238 had elevated CRP levels, 92 were negative and CRP was not done for 30 patients. Among the 255 patients who were negative for

Table 1. CRP sample analysis

Total 654	CRP +ve	Crp -VE	CRP NOT DONE
Covid positive 360	238	92	30
Covid Negative 255	176	28	51

SARS-CoV-2, 176 showed increase in CRP levels, 28 had normal level of CRP and CRP were not tested for 51 patients.

Out of the 238 COVID-19 patients with elevated levels of CRP, 177 had increased levels of interleukin-6 ranging from 15.5 pg/ml to 4441 pg/ml. and 43 patients who were had low level of IL- 6 and IL- 6 was not done in 18 patients (Table 1).

Table 2. COVID results and analysis

	Covid Positive			Covid Negative		
	CRP Positive	Crp Negative	Crp Not Done	Crp Positive	Crp Negative	Crp Not Done
IL6 POSITIVE	177	43	18	-	-	6
IL6 NEGATIVE	43	-	0	-	-	0
IL-6 not done	18	2	21	16	4	59

Table 3. Isolated

Name of organism	Number	Percentage
<i>Acinetobacter Species</i>	70	31%
<i>MRSA (methicillin-resistant Staphylococcus aureus)</i>	31	14%
<i>Pseudomonas Species</i>	29	13%
<i>Burkholderia Species</i>	24	11%
<i>Klebsiella Pneumoniae</i>	23	10.4%
<i>Enterobacter Species</i>	21	9.5%
<i>MR CONS (Methicillin-resistant coagulase negative staphylococci)</i>	8	3.6%
<i>Escherichia Coli</i>	6	2.7%
<i>CONS (Coagulase negative staphylococci)</i>	3	1.3%
<i>Serratia Species</i>	3	1.3%
<i>Sreptococcus Species</i>	1	0.4%
<i>Aeromonas Species</i>	1	0.4 %

Among those 177 patients with elevated CRP Levels and IL-6 Levels the following bacterial organism has been isolated. 70 *Acinetobacter SP.* (31%), 31 *MRSA (Methicillin-resistant Staphylococcus aureus)* 14%, 29 *Pseudomonas SP.* 13%, 24 *Burkholderia Species* 11%, 23 *Klebsiella Pneumoniae* (10.4 %), 21 *Enterobacter species* (9.5%), 8 *MR CONS (Methicillin-resistant coagulase negative staphylococci)* 3.6%, 6 *Escherichia coli* 2.7%, 3 *CONS (Coagulase negative*

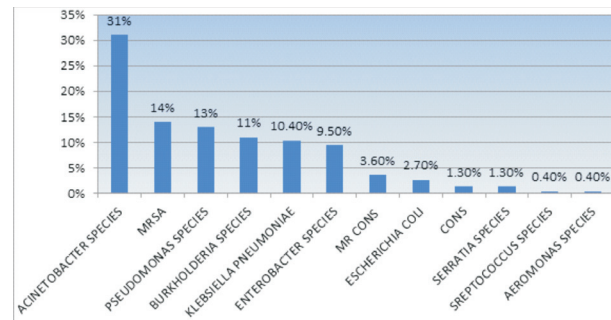


Fig. 4. Distrubution of bacterial microganism

staphylococci) 1.3%, 3 *SERRATIA SP.* 3%, 1 *Sreptococcus species* 0.4%, 1 *Aeromonas Species* 0.4 % (Figure 4).

DISCUSSIONS

Co-infections are distinct clinical entities, although

used interchangeably in medical literature and clinical practice. Although both are present in COVID-19, the issue of secondary infection and multidrug resistance are of greater concern. Recent studies showed, sepsis with subsequent multiorgan dysfunction is one of the main causes of death in COVID-19 patients, and several previous studies have looked at the question of activation of the coagulation system in advanced or severe patients with SARS-CoV-2 infection (Bowcock *et al.*, 1988; Hirano *et al.*, 1990). The main aim of our study is to investigate pattern abnormalities in hematological parameters in COVID-19 Patients includes higher serum C-reactive protein levels, increase in lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase levels (Huang *et al.*, 2020). Lymphopenia (absolute lymphocyte count $<1.0 \times 10^9/L$) was observed more commonly in up to 83% of hospitalized patients and mild thrombocytopenia was observed in approximately 30% of patients with COVID-19.(25-29). However, most of these laboratory characteristics are nonspecific and are common in pneumonia.

Interleukin-6 (IL-6) is an important cytokine and its assay is to be interpreted by the treating clinician since IL6 is known to regulate immune responses, acute phase reaction, hematopoiesis and host defense mechanisms. Elevated levels of IL-6 can occur in acute bacterial, viral infections including intraamniotic infections and neonatal sepsis, besides severe burns, proliferative diseases like psoriasis and mesangial proliferative glomerulonephritis, several neoplastic diseases and diseases of the immune system (Thachil *et al.*, 2020; Bowcock *et al.*, 2020; Hirano *et al.*, 1990; Sallmann *et al.*, 2011; Koul *et al.*, 2013; Rodriguez-Morales *et al.*, 2020; Guan *et al.*, 2020; Huang *et al.*, 2020). In this study, we focus on Bacterial sepsis in covid positive and non covid patients using CRP and IL- 6 Markers. IL-6 is the primary trigger for cytokine storms. Yang *et al.* pointed out that peripheral blood IL-6 levels could be used as an independent factor to predict the progression of COVID-19, which is consistent with the results of this study; therefore, the role of IL-6 in this disease deserves special attention. *A. baumannii* was the most common organism followed by MRSA. In a study conducted in Shiraz, Iran, in 2009, Hassanzadeh *et al.* suggested that ICU-acquired infections were documented in 51.7% of ICU patients, with a mortality rate of 10.9% (Hassanzadeh *et al.*, 2009). In recent years, emerging

strains of both species that have acquired additional genetic features have shown to be commonly associated with hypervirulence and resistant to many types of antibiotics (Paczosa and Meccas, 2016; Sampredo and Wardenburg, 2017).

CONCLUSION

Mortality rates for COVID sepsis either caused by the virus or complicated by secondary infection is high. Our findings emphasize the concern of Bacterial sepsis in COVID-19 patients due to the above organisms based on our study over the period of one year. Consequently, it is important to pay attention to bacterial co-infections in critical patients positive for COVID-19. The best management of COVID-19 sepsis should not be focused only on respiratory failure but supportive care and monitoring of patients.

Limitations of the study

There were some limitations in our study. Firstly, this was a retrospective study, therefore, complete information was not available for all the patients. Secondly, though our study was based on the data of the single tertiary care centre in Tamil Nadu, a large-scale study involving other Tertiary hospitals are required.

Statement of Ethics

This study was approved by Ethics Committee of Saveetha Medical and Hospital. As this study was a retrospective study, there was no patient's privacy data such as patient name, ID number, telephone and address were involved. Only demographic information and laboratory testing data of patients were collected and analyzed in this study.

ACKNOWLEDGEMENT

I sincerely thank Saveetha Medical College and Hospital, Chennai, for their continued support in the procurement of the data. I also extend heartfelt gratitude to my research guide Dr. Aruna. We thank all patients who participated in this study. Thanks to all health care workers in our hospital for their efforts in caring and fighting against COVID19.

Competing Interests

Authors have declared that no competing interests exist.

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