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Original Research Article

The Most Frequently Isolated Microorganisms and the Incidence of Positive Culture Report in Patients with Sepsis: A Hospital Based Prospective Study

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ABSTRACT

Background: There are many diagnostic tests to rule out the presence or absence of sepsis but blood culture remain the key test for estimating the presence of sepsis. Until recently no biomarker has been able to help differentiate bacterial infection from a viral or non-infectious inflammatory reaction. An ideal marker for bacterial infections should allow an early diagnosis, also inform about the course and prognosis of the disease and facilitate therapeutic decisions. The aim of present study is to establish the most frequently isolated microorganism and the incidence of positive blood culture in patients with sepsis admitted to the hospital.

Materials and Methods: The present prospective study was done as a part of research work at Department of Medicine, Dayanand Medical College and Hospital, Ludhiana. Patients admitted in emergency or Intensive Care Unit (ICU) and clinically suspected to have sepsis were included in the study. All the blood and body fluid sample were analyzed for microbiology profile in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana. 5- 10 ml of blood was withdrawn and bactec cultures were performed. Smears were prepared from positive blood culture bottles and examined after Gram's staining. Simultaneously all the positive bottles were sub-cultured on blood Agar and MacConkey's agar and the plates were then incubated at 37°C for 18-24 hours.

Results: In the present study 102 subjects were enrolled out of which 38.2% patients were of sepsis, 42.2% patients were of severe sepsis and 19.6% patients were of septic shock. The most common source of infection in the sepsis and severe sepsis group was lower respiratory infection; 61.54% and 35.00% respectively whereas in the septic shock group UTI (urinary tract infection) was more prevalent (34.88%). The pathogens isolated in blood culture sample are depicted in the table 4. Gram negative pathogens accounted for the majority (87.9%) of positive blood culture. We isolated fungal growth in 3 patients.

Conclusion: The most common cause of sepsis was lower respiratory tract infection and the most commonly isolated microorganisms were gram negative bacteria.

Keywords: Blood Culture, Procalcitonin, Respiratory Tract, Septic Shock.

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INTRODUCTION

Sepsis is the most frequently isolated condition amongst people worldwide. A study by Robert S. Munford shows that there are nearly 20-35% of patients with severe sepsis and 40-60% of patients with septic shock die within 30 days.[1] There are many diagnostic tests to rule out the presence or absence of sepsis but blood culture remain the key test for estimating the presence of sepsis. Until recently no biomarker has been able to help differentiate bacterial infection from a viral or non-infectious inflammatory reaction. But there are chances of blood contamination which represent upto one third of the positive blood cultures and these occur even in the absence of blood stream infection.[2]

Procalcitonin is one of the first to offer this possibility. An ideal marker for bacterial infections should allow an early diagnosis, also inform about the course and prognosis of the disease and facilitate therapeutic decisions.

Procalcitonin (PCT) increases 2-3 hours post induction by e.g. endotoxin. Levels then rise rapidly up to several hundred nanograms per ml in severe sepsis and septic shock, reaching a plateau after 6-12 hours. PCT concentrations remain high for up to 48 hours, falling to their baseline values within the following 2 days. If after successful treatment intervention the procalcitonin value decreases, this indicates a positive prognosis. Persistently high or increasing levels are indicators for poor prognosis.

In healthy volunteers injected with Escherichia coli endotoxin, procalcitonin levels, undetectable at baseline, started to increase 4 hours after endotoxin and became stable at 4ng/ml between 8-24 hours. TNF and IL-6 levels peaked 2-3 hours after endotoxin and were undetectable at baseline, started to increase 4 hours after endotoxin and became stable at 4ng/ml between 8-24 hours. TNF and IL-6 levels peaked 2-3 hours after endotoxin and were undetectable at 24 hours. That the same kinetics can be expected to occur in human septic shock has been recently described in a rare and interesting case. A hemodialysate of calf blood contaminated with acinetobacter baumanii was injected to a patient leading, within hours, to septic shock. TNF was detectable in serum at 1.5 hours, peaked at 3 hours, and decreased thereafter. Procalcitonin was first detectable at 3 hours, peaked 14 hours after the injection (300ng/ml), and remained increased for more than 24 hours.[3] Thus, in response to endotoxin or to live bacteria, increases in circulating procalcitonin levels occur shortly after cytokines have peaked. The aim of present study is to establish the most frequently isolated microorganism and the incidence of positive blood culture in patients with sepsis admitted to the hospital.

MATERIALS AND METHODS

The present prospective study was done as a part of research work at Department of Medicine, Dayanand Medical College and Hospital, Ludhiana. Patients admitted in emergency or Intensive Care Unit (ICU) and clinically suspected to have sepsis were included in the study.

All the blood and body fluid sample were analyzed for microbiology profile in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana. This study was from 1st January 2013 to 1 February 2014 done and 102 consecutive patients were included in the study.

Ethical committee clearance was obtained from the hospital's ethical committee and all the patients or their guardians were informed and a written informed consent was obtained from all in their vernacular language. All the required details were obtained from the patients and noted. The details included their demographic data, any past treatments, their presenting signs and symptoms and any past illnesses.

Procedure: Approximately 5-10ml of blood was collected from anticubital vein under complete aseptic conditions by trained nursing personnel. The samples were collected in a sterile vial and send to department of microbiology for culturing. Bactec cultures were performed. Bottles were placed into receiving wells that were monitored once every 10 minutes. The voltage of current reading of diode was compared with the previous reading. If the voltage change exceeded a preset delta value, the microcomputer flagged the bottle as positive. Smears were prepared from positive blood culture bottles and examined after Gram's staining. Simultaneously all the positive bottles were sub-cultured on blood Agar and MacConkey's agar and the plates were then incubated at 37°C for 18-24 hours.

Principle - BACTEC 9240 blood culture system uses fluorescent sensor to monitor the presence and production of CO_2 that is dissolved in the culture medium. If microorganisms are present in the test sample, CO_2 is produced as the organisms metabolize the substrate in the culture medium. When growth of the microorganism produces CO_2, its sensor emits a fluorescent light that an emission filter on the way to a light sensitive didode.

Bac-T/Alert microbial detection system utilizes a calorimetric sensor and reflected light to monitor the presence and production of CO_2 that is dissolved in the culture medium. If microorganisms are present in the test sample, CO_2 is produced as the organisms metabolized the substrate in the culture medium. When growth of the microorganism produces CO_2, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow.

All the data was arranged in a tabulated form and analysed. SPSS software was used for analysis. Percentage was calculated and the most frequently organism was estimated.

Table 1: Categorisation of patients

Category	Percentage (%)
Sepsis	38.2
Severe Sepsis	42.2
Septic shock	19.6
Total	100.0

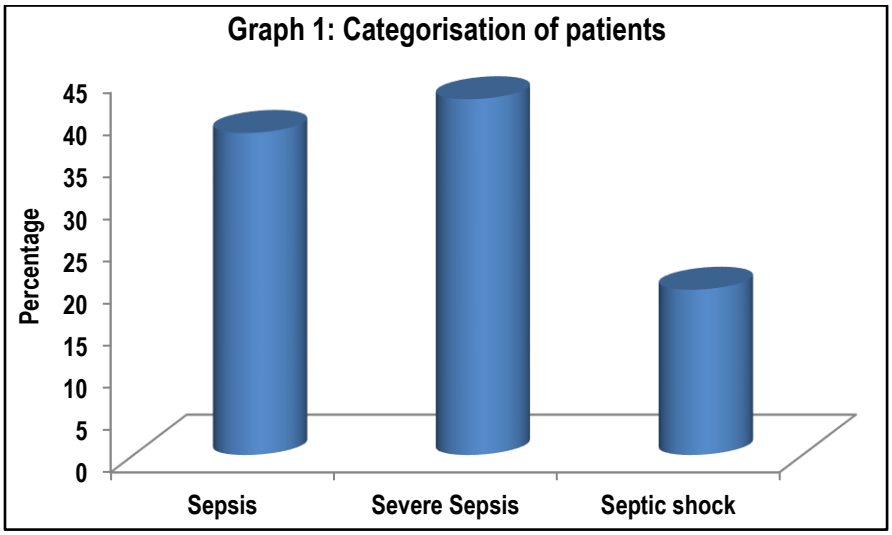

Table 2: Type of infection in all three groups of sepsis

Type of infection (system)	Category						Total		P value
	Sepsis		Severe Sepsis		Septic Shock				
CNS	4	10.26%	3	15.00%	2	4.65%	9	8.82%	0.004
GIT	5	12.82%	2	10.00%	11	25.58%	18	17.65%	Sig.
LRTI	24	61.54%	7	35.00%	12	27.91%	43	42.16%	
SKIN	2	5.13%	5	25.00%	3	6.98%	10	9.80%	
UTI	4	10.26%	3	15.00%	15	34.88%	22	21.57%	
TOTAL	39	100.00%	20	100.00%	43	100.00%	102	100.00%	

Table 3: Percentage of Blood Culture positive

Blood Culture	Number	Percentage
No growth	69	67.6%
Growth	33	32.4%
Total	102	100.0

Table 4: Type of growth in positive blood culture

Type of growth in Blood Culture	Number	Percentage
Gram Negative Bacteria	29	87.9
Fungal	3	9.1
Gram Positive Bacteria	1	3.0
Total	33	100.0

RESULTS

In the present study 102 subjects were enrolled out of which 38.2% patients were of sepsis, 42.2% patients were of severe sepsis and 19.6% patients were of septic shock.(Table 1,Graph 1)

The patients were categorized according to the type of infection as depicted in table 2. The most common source of infection in the sepsis and severe sepsis group was lower respiratory infection; 61.54% and 35.00% respectively whereas in the septic shock group UTI (urinary tract infection) was more prevalent (34.88%). This was statistically significant with p value of 0.004.

The percentage of patients who had a positive blood culture is depicted in table 3. A total of 33 patients (32.4%) had positive blood cultures in our study. A total of 69 patients ie 67.6% showed no growth of any microorganism.

The pathogens isolated in blood culture sample are depicted in the table 4. Gram negative pathogens accounted for the majority (87.9%) of positive blood culture. We isolated fungal growth in 3 patients. These 3 patients did not have a positive blood culture for bacteria. Only 1 patient (3%) had a positive blood culture for gram positive bacterial.

DISCUSSION

Blood cultures are the main method to determine the causes of a BSI because they are highly sensitive, simpler to perform and reliable. The sensitivity of blood cultures chiefly depends upon the volume of the sample. For adults, generally up to 20 mL of blood is used to inoculate two bottles ie one for aerobic microorganisms and one for anaerobic microorganisms. Before initiating any antibacterial treatment two to four blood cultures are required to detect a causative agent in 80% to 96% of bacteremias.[4,5]

In our study, based on the definition given by ACCP patients were classified as sepsis, severe sepsis and septic shock. Accordingly, 39 patients (38.2%) were in the group of sepsis; 43 patients (42.2%) were in the group of severe sepsis and 20 patients (19.6%) were in the group of septic shock. Similarly, in a study by Sudhir U et al, where out of the 100 study patients, groups of sepsis, severe sepsis and septic shock had 52, 25 and 23 patient's respectively.[6]

In our study, the most common focus of sepsis was lung LRTI (lower respiratory tract infection) in 43 patients (42.16%) followed by UTI in 22 patients (21.57%). Respiratory sepsis included lobar and bronchopneumoniae, infective exacerbation of COPD and some of the patients had ARDS. Urinary tract infections included mostly complicated infections like pyelonephritis. This is probably due to the higher incidence of diabetes mellitus in our study. In the study conducted by Sudhir et al respiratory tract infection was (40%) the most common source of sepsis.27 cases had lobar pneumonia and 12 cases had lobar pneumonia and 12 cases had bronchopneumonia. In 20 % of patients, the primary source could not be identified. UTI was the second most common focus.[6] According to Calandra et al six common causes of sepsis were identified were pneumonia, blood stream infections including infective endocarditis, intravascular catheter related sepsis, intra-abdominal infections, urosepsis and surgical wound infections.[7]

In the present study 33 patients (32.4%) had positive blood culture. Out of this 29 patients (87.9%) had growth of gram negative bacteria, 3 patients (9.1%) had fungal growth and only 1 patient (3%) had growth of gram positive bacteria. In a multicentric prospective study conducted by Naoki Aikawa et al in 2005 the clinical significance of procalcitonin for discriminating between bacterial infectious diseases and non-bacterial infectious diseases (SIRS) was compared with the significance of enotoxin, beta –d-glucan, interleukin-6 and CRP. The concentration of PCT in patients with systemic bacterial infection and those with localized bacterial infection were significantly higher than the concentration in patients without sepsis.[8]

A Study was conducted by Amal Abd el-Azeem et al in 2013 to assess the value of PCT as a rapid and sensitive marker for diagnosis, prognosis, and therapy of lower respiratory tract bacterial infections necessitating antimicrobial treatment and comparing this marker with other markers of infections including CRP and WBC. A total of 60 patients were enrolled in the study and were subjected to complete history taking, physical examination, laboratory investigations including complete blood count, blood gases, blood chemistry, bacteriological culture for sputum and blood, serology for atypical, and PCR for respiratory viruses, Serum C-reactive protein (CRP) and PCT levels were

also measured. The patients were divided into two groups, group 1 included 26 patients who were culture negative for bacterial infection and group 2 included 34 patients who were culture positive. Group 2 patients were given antibiotic therapy according to the culture sensitivity. The results revealed that, there was no significant difference between group 1 and group 2 patients as regards age, sex, clinical manifestations, final diagnosis, white blood cell counts, blood gases, intensive care unit admission and length of hospital stay. A significant increase of PCT and CRP levels was detected in group 2 compared to group 1 at initial diagnosis. At cut-off value >0.5 ng/ml, PCT gave a sensitivity of 94.1%, specificity of 88.4%, positive predictive value (PPV) of 91.4%, negative predictive value (NPV) of 92% and diagnostic efficiency of 91.6% for diagnosis of respiratory tract bacterial infections. After antibiotic therapy PCT and CRP levels dropped in group 2 patients as compared to their pre-treatment levels. In their opinion PCT assessment reduces side-effects of unnecessary antibiotic, lowers costs, and in the long-term may lead to diminishing drug resistance.[9]

Similarly a study was conducted by Rahim Raoofi et al , in 2014 in which 60 patients aged 59-68 yrs ,including 35 men and 25 women who fulfilled the criteria of sepsis were taken. Blood culture, peripheral blood smear at first time, serum procalcitonin levels at first hour and 72 hours after admission were taken. A total of 9 patients had positive blood cultures. Peripheral blood smear was positive in 38 patients, (29 patients: gram stain positive, 6 patient's gram stain negative and 3 patients: both gram positive and gram negative). At first time 76.8 % had positive procalcitonin (>0.5 ng/ml) and after 72 hrs 65% of patients had positive procalcitonin. Sensitivity, specificity, positive and negative predictive value of procalcitonin at first time was 100, 16, 16 and 100% and for procalcitonin after 72 hrs were 75.35,15 and 90%. This study showed that sensitivity of procalcitonin level can be used for diagnosis of sepsis. Procalcitonin increased as severity of sepsis increased, high levels of procalcitonin after 72 hrs indicated poor outcome.[10]

CONCLUSION

The most common cause of sepsis was lower respiratory tract infection and the most commonly isolated microorganisms were gram negative bacteria. With the advent of newer technologies and advancements there has been a significant improvement in blood culture techniques leading to lesser incidence of false positive and false negative results.

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