The value of acute phase reactants and LightCycler® SeptiFast test in the diagnosis of bacterial and viral infections in pediatric patients

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ABSTRACT

***Introduction*:** This study was performed to investigate the value of acute phase reactants and LightCycler® SeptiFast test to differentiate bacterial and viral infections.

***Population and methods*:** Children with fever were enrolled to this prospective study. Peripheral white blood cell (WBC), C-reactive protein (CRP) and procalcitonin (PCT) were studied from all patients on day 1, 3 and 7. Blood culture and chest X-ray were also obtained on day 1. Blood samples for LightCycler® SeptiFast test were obtained in all patients to use them if there was uncertain diagnosis between bacterial or viral infection. The patients were divided into two groups as bacterial and viral infection.

***Results*:** A total of 94 children with fever were enrolled. The mean value of fever was significantly higher in bacterial group than viral group ($p < 0.001$). In bacterial infection group, 34 (72.3%) patients had negative blood culture. Of those, 12 (35.2%) had positive SeptiFast test. There were no positive blood culture in patients with viral infection group and all of them had negative SeptiFast test. The mean levels of CRP on the first day of admission were significantly higher in bacterial group than viral group ($p < 0.001$). CRP and PCT levels of day 3 and 7 were significantly higher in bacterial group ($p < 0.001$). The sensitivity and specificity levels of WBC, CRP and PCT were 63.8%, 44.7%, 74.5% and 78.7% ,68.1% and 100%, respectively.

***Conclusions*:** We found that acute phase reactants, especially PCT, and LightCycler® SeptiFast test may help to differentiate bacterial and viral infections.

Key words: *Infection, diagnosis, SeptiFast, child.*

http://dx.doi.org/10.5546/aap.2018.eng.35

To cite: Bozlu G, Tanriverdi H, Aslan G, et al. The value of acute phase reactants and LightCycler® SeptiFast test in the diagnosis of bacterial and viral infections in pediatric patients. *Arch Argent Pediatr* 2018;116(1):35-41.

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Funding:
None.

Conflict of interest:
None.

Received: 2-22-2017
Accepted: 8-14-2017

INTRODUCTION

Fever is one of the most common causes to visit doctors in childrens.[1] It is difficult to differentiate between serious bacterial infections and viral infections even for experienced doctors. Bacterial infections are frequent in childhood, and cause significant morbidity and mortality. Due to the lack of specific clinical symptoms, it can be confused with viral infections and non-infectious diseases. There is not a single laboratory indicator with high sensitivity and specificity and also with low cost that can help to distinguish bacterial and viral infections in early period.[2]

Currently, blood culture is still the gold standard method for the diagnosis of bacterial infections. However, blood culture has disadvantages including lack of rapidity and low diagnostic sensitivity.[3,4] It also takes time to get the results of the culture. In addition, early diagnosis and effective treatment is life-saving in the bacterial infection. On the other hand, unnecessary use of antibiotics in suspected severe bacterial infections can cause an extention of the duration of hospital stay, an increase of resistant bacteria in the community and an increase cost of simple disease to families and society. Therefore, the search for new markers have been made in the identification of infectious agents.

In daily practice, acute phase reactants are used extensively to distinguish bacterial and viral infections.[5] On the other hand, there is no cut-off value to reveal difference between these conditions. The most commonly used acute phase markers in clinical practice are leukocyte

count (WBC), absolute neutrophil count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels and procalcitonin levels (PCT).[5-7] LightCycler® SeptiFast Test MGRADE polymerase chain reaction (PCR) test helps to detect pathogens in 6 hours, it provides a rapid and early diagnosis of bacteremia.[8] This study was performed to investigate the value of acute phase reactants and LightCycler® SeptiFast test to differentiate bacterial and viral infections.

POPULATION AND METHODS

The study has been approved by the Ethics Committee of Mersin University Faculty of Medicine. A signed consent was obtained from all patients and/or individuals who are responsible for them to participate in the study. The children aged between 3 months and 12 years and hospitalized in Pediatric Infectious Diseases, Intensive Care Unit and Pediatrics Clinic of Mersin University Medical Faculty Hospital due to fever between March 2012 and October 2012, were enrolled to this prospective study. Inclusion criteria were fever > 38 °C at the admission, and not received any treatment two weeks before hospitalization. The patients with concomitant chronic disease (chronic renal failure, autoimmune diseases, or malignancies) and patients with fever linked to non-infectious reasons (such as connective tissue diseases) were excluded from the study.

Fever, respiratory rate, pulse rate, oxygen saturation on admission were recorded for all patients. A complete blood count (CBC), CRP, PCT and blood culture samples were taken on the first day.

Chest x-ray was taken on hospital admission in all patients. Radiological assessment has been interpreted by an experienced radiologist blinded to the clinical characteristics of the patients. Radiological findings have been evaluated as consolidation, interstitial infiltrate, peribronchial infiltrates, pleural effusion, pneumatoceles and/or air bronchogram. The result of these findings were classified as due to bacterial or viral respiratory tract infection.

A throat, urine, cerebrospinal fluid, pleural fluid, joint fluid cultures were taken if necessary. CBC, CRP and PCT tests have been reevaluated on day 3 and 7 admission to evaluate the response to treatment.

Blood samples for LightCycler® SeptiFast test were obtained in all patients in order to use them if there was uncertain diagnosis between bacterial or viral infection. A 2 mL blood taken to tube with EDTA and stored at -80 °C for SeptiFast PCR analysis.[8]

The DNA of microorganisms in whole blood samples taken from patients was obtained using DNA extraction kit (High Pure PCR Template Preparation Kit, Roche). The LightCycler® SeptiFast Test MGRADE kit was used as foreseen by the manufacturer for rapid detection of microorganisms causing bacteremia (*Table 1*).[8] The LightCycler SeptiFast Test is a commercial diagnostic test utilizing real-time multiplex PCR. The diagnostic probes for PCR target the internal transcribed sequences situated between 16S and 23S bacterial ribosomal RNA as well as between 18S and 5.8S fungal ribosomal RNA.[8]

Patients were divided into 2 groups as bacterial and viral infection. The patients with bacterial infection group were included according to the following criteria; the patients with fever ≥ 39 °C, WBC ≥ 15 x10/μL, and dominance of neutrophils in the peripheral blood smear, elevated CRP levels (>5 mg/L), the signs suggesting bacterial lower respiratory tract infections in the chest radiograph (such as pleural effusion, consolidation, lobar involvement), the presence of positive blood cultures, other culture positivity, SeptiFast PCR positivity and the impaired general condition

TABLE 1. *Pathogens detectable by SeptiFast*[8]

Gram negative	Gram positive	Fungi
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella (pneumoniae/oxytoca)	CNS (Coagulase negative *Staphylococci,*	*Candida tropicalis*
Serratia marcescens	*S. epidermidis, S. haemolyticus)*	*Candida parapsilosis*
Enterobacter (cloacae/aerogenes)	*Streptococcus pneumoniae*	*Candida krusei*
Proteus mirabilis	*Streptococcus spp. (S. pyogenes, S. agalactiae, S. mitis)*	*Candida glabrata*
Pseudomonas aeruginosa	*Enterococcus faecium*	*Aspergillus fumigatus*
Acinetobacter baumannii	*Enterococcus faecalis*	
Stenotrophomonas maltophilia		

(petechiae, confusion and poor circulation). The patients with viral infection group were included according to the following criteria; fever < 39 °C, WBC <15 x10/μL and the dominance of lymphocytes in the peripheral blood smear, not-elevated CRP levels, normal chest x-ray or the presence of patterns suggestive of viral lower respiratory tract infections (interstitial infiltrate and peribronchial infiltrates), the absence of positive blood cultures, other culture negativity, SeptiFast PCR negativity and good general condition.

SeptiFast PCR test were studied in order to distinguish the 50 patients who can not be differentiated based on the above criteria.

Statistical analysis

Estimated number of patients in each group was calculated as 47 with 13% estimated error and 95% CI. The normal distribution eligibility checks the parameters were tested with Shapiro-Wilks test in bacterial and viral infection groups. All continuous variables are expressed as mean ± standard deviation. Student's t test, Mann-Whitney U test and receiver operating characteristic (ROC) curve were used for statistical analysis. The area under curves were calculated for the parameter. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 94 children with fever were included in the study. The mean age of patients was 47.65 ± 40.23 months. There was no statistically significant difference between the groups in terms of mean age, gender, respiratory rate and oxygen saturation ($p > 0.05$) (*Table 2*).

The average value of fever was statistically significant higher in bacterial group than viral group ($p <0.001$). The mean levels of CRP, PCT and absolute neurophil count were significantly higher in the children with bacterial infection group ($p <0.001$), but no statistically significant difference was found in WBC levels between the bacterial and viral infection groups ($p >0.05$) (*Table 2*). The diagnostic distribution of the patients in the bacterial and viral infection groups are shown in *Table 3*.

Thirteen patients had positive blood culture in the bacterial infection group. In 34 patients with negative blood culture, 12 of them were positive with SeptiFast. Coagulase negative *Staphylococcus* (CNS) was detected in 3 patients, *Enterococcus*

TABLE 2. *Characteristics of patients (N: 94)*

	Bacterial infection group (n= 47)	**Viral infection group (n= 47)**	***p***
Female/male	28 (59.6%)/19 (40.4%)	33 (70.2%)/14 (29.8%)	>0.05
Age (month) (range)	49.87 ± 44.13 (3-144)	45.43 ± 36.28 (3-144)	>0.05
Fever (range)	39.11 °C (39-39.4)	38.8 °C (38.4-38.9)	<0.001
Respiratory rate	30 (24-38)	28 (24-38)	>0.05
Oxygen saturation (%)	97(94-99)	97(95-98)	>0.05
WBC (x10/μL) Day 1/3/7	13.2/10/10.3	12.6/9.1/9.9	>0.05
CRP (mg/L) Day 1/3/7	75/41.1/6.3	14.9/6.1/2.5	<0.001
PCT (ng/ml) Day 1/3/7	2.4/0.6/0.1	0.2/0.1/0.1	<0.001
ANC	10 418 ± 13 542	6954 ± 4466	<0.001

WBC, White blood cell; CRP, C-reactive protein; PCT, Procalcitonin; ANC, Absolute neurophil count.

TABLE 3. *Diagnosis of the patients (N: 94)*

	Bacterial infection group n (%)	**Viral infection group n (%)**
Upper respiratory tract infection	4 (8.5)	16 (34)
Lower respiratory tract infection	26 (55.3)	27 (57.4)
Meningitis	3 (6.4)	3 (6.4)
Endocarditis	1 (2.1)	-
Septic arthiritis	2 (4.3)	-
Sepsis	5 (10.6)	-
Brucellosis	2 (4.3)	-
Lymphadenitis	2 (4.3)	1 (2.1)
Others	2 (4.3)	-

cloacae in 2 patients, *Pseudomonas aeruginosa* in 1 patient, *Staphylococcus aureus* in 1 patient, *Acinetobacter baumannii* in 1 patient, *Klebsiella Pneumoniae* and *Enterococcus cloacae* in 2 patients, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were detected in 1 patient. There was no positive blood culture in patients with viral infection group. Of those, 16 had high CRP and WBC levels and fever, therefore SeptiFast PCR was performed. CNS was detected in one patient and was considered as a contaminant.

Sensitivity, specificity, PPV and NPV rates for WBC, CRP and PCT are shown in *Table 4*.

The appropriate cutoff points determined by maximum sum of sensitivity and specificity for WBC, CRP and PCT were 9.95x10/μL, 22 mg/L and 1.07 ng/ml, respectively. According to the ROC curve analysis, the values of CRP and PCT to distinguish bacterial and viral infections was found statistically significant (p <0.001). We found a correlation between CRP and PCT levels (*Table 5*).

In logistic regression analysis performed to determine important parameters for differentiation of viral and bacterial infections, every 1 ng/ml increase of PCT was found to be 6.6-fold increases the risk of being bacterial infection.

DISCUSSION

Currently, early diagnosis of infections still poses a major challenge for clinicians. Bacteria and viruses are responsible for the majority of infections. Because the same symptoms, it is difficult to determine whether the patients have bacterial or viral infections. In contrast to the viral etiology, bacterial infections can cause life-threatening outcomes if untreated. The distinction between these two conditions required different approaches, however, the complaints of the patients were similar in both groups.

The main objective of laboratory diagnosis of infectious diseases is growth of the causative organisms in the culture.[3,9] However, it is known that culture techniques may not be sufficient in some cases such as long growth time for some types of bacteria, expensive tissue culture procedures for viruses, negative culture of certain microorganisms, taking culture samples after starting antibiotics or sent to the laboratory in unsuitable conditions. The diagnosis is difficult because of the lack of high sensitivity and specificity of other laboratory markers.[2] However, delay in the treatment may be an important cause of morbidity and mortality in bacterial infections. In addition, bacterial resistance problem may arise in connection with the use of unnecessary antibiotics in viral infections. Therefore, the search for new markers has been made in the identification of infectious agents.

We found that 13 patients had positive blood culture in the bacterial infection group. In patients with negative blood culture, 12 of them were positive with SeptiFast. It is commercially available for the detection of bacteria and fungi directly from blood.[8] The assay can be performed in less than 6 hours and it identifies the 25 most important pathogens causing bloodstream infections. More recently, there have been several studies to focus on the diagnostic feasibility and potential clinical utility of SeptiFast.[10,11] On the other hand, fewer studies have evaluated this assay in routine clinical practice regarding impact on therapy.

Today, there are so many markers to differentiate bacterial and viral infections, but

Table 5. *Correlation analysis of WBC, CRP, and PCT*

Parameters	Correlation coefficient (r)	P
CRP-PCT	0.547	<0.001
CRP-WBC	0.118	0.258
PCT-WBC	0.179	0.084

WBC, White blood cell; CRP, C-reactive protein; PCT, Procalcitonin.

Table 4. *Sensitivity, specificity, PPV and NPV rates for WBC, CRP and PCT*

Variable	AUC	Sensitivity %	Specificity %	PPV %	NPV %
WBC (>9.95 x10/μL)	0.519	63.8	44.7	53.6	55.3
CRP (>22 mg/L)	0.764	74.5	78.7	77.8	75.5
PCT (>1.07 ng/ml)	0.835	68.1	100	100	75.8

WBC, White blood cell; CRP, C-reactive protein; PCT, Procalcitonin.
AUC, area under curve; PPV, positive predictive value; NPV, negative predictive value.

the most commonly used are WBC, absolute neutrophil count, ESR, CRP, and PCT.[5-7] It has been reported that the proportion of patients with an increased WBC (>15x10/μL) was similar in bacterial and viral pneumonia.[12] Moulin et al. determined the sensitivity as 65.1% and specificity as 79.3% for WBC (>15x10/μL) to distinguish bacterial and viral pneumonia.[13] In current study, we determined 63.8% sensitivity and 44.7% specificity for WBC. Compared to the literature, relatively lower sensitivity and specificity levels in our study may be due to different bacterial infection from other studies. Our findings showed that WBC was not useful for distinction between bacterial and viral infections.

In our study, we determined the sensitivity as 74.5% and the specificity as 78.7% for CRP. CRP level is increasing in many cases with tissue damage, such as acute infections, rheumatic diseases, malignancies and acute myocardial infarction.[14] In general, high CRP value is detected in invasive acute bacterial infections, and its lower in viral infections.[15] It can be detected as high in adenovirus, cytomegalovirus, influenza, mumps, measles and other viruses related infections. In addition, the low level of CRP does not eliminate the possibility of bacterial infection. CRP level may be negative in the first 12 hours after onset of the illness. However, serial CRP measurements should be used if bacterial infection is clinically suspected.[16] Tayyil et al. reported that the sensitivity and specificity were 75% and 68.7%, respectively for CRP (>50 mg/L) to detect bacterial infection.[17] Ip et al. reported that the sensitivity and specificity of CRP (>10 mg/L) were 95% and 55%, respectively.[18] Yo et al. found that the sensitivity was 74% and the specificity was 76% for CRP (>9.83 mg/L) to detect severe bacterial infection.[19] As it is shown in the studies, the sensitivity and the specificity of different values are variable; and suggesting that it is difficult to give a certain amount for distinction between bacterial and viral infections.

A small amount of bacterial endotoxin injection stimulates production of PCT in healthy subjects.[20] The level of PCT rises and can be measured after 2-3 hours, rapidly increases within 6-8 hours, reaches a maximum level in 12 hours, and reduces to normal levels within two days. The half-life of PCT is about 20 to 24 hours. It is considered that PCT rises earlier than CRP and is a more useful indicator in identifying early infection.[20] Lopez et al. reported that PCT and CRP levels were significantly different between bacterial and viral infection groups.[21] Gendrel et al. showed that PCT and CRP were significantly higher in bacterial meningitis. In our study, sensitivity and specificity of PCT were 68.1% and 100%, respectively.[22]

It has been reported that serum PCT, CRP and WBC levels were significantly higher in bacterial meningitis group than viral group, and it continued to remain significantly higher on the 3rd day of treatment.[23] It was also reported that serum PCT, CRP and WBC levels were significantly reduced after 72 hours of treatment in bacterial meningitis group. Similar to the previous studies, we found that PCT and CRP levels were significantly decreased from first to seventh day of treatment in both bacterial and viral infection groups. Studies have reported that PCT and CRP showed a significant correlation when used in the management of patients with bacterial infection. In our study, we found a positive correlation between CRP and PCT.

Limitations of our study can be summarized as follows: a small number of patients, the data was not supported by viral PCR to prove the viral etiology, and PCT was not compared with various cytokines to distinguish bacterial infections and monitoring the response to antibiotic treatment. In the current study, SeptiFast PCR test were not studied from all the patients. However, this test were performed only to 50 patients who can not be differentiated based on the criteria. Our study has strength to the literature with the following issues: PCT, CRP and WBC were combined and cut-off level determined to distinguish bacterial and viral infections. LightCycler® SeptiFast also ensures that switching to appropriate antibiotic therapy early and prevent unnecessary use of broad-spectrum antibiotics.

CONCLUSIONS

We found that acute phase reactants, especially PCT, and LightCycler® SeptiFast test may help to differentiate bacterial and viral infections. We also found that PCT is an important diagnostic and prognostic marker to distinguish viral and bacterial etiology of childhood infections and monitoring the response to antibiotic treatment. In order to reduce costs, PCT only can be studied rather than multiple biochemical parameters. However, further studies are needed to distinguish bacterial and viral infections. ■

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