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Prospective Comparison of Eubacterial PCR and Measurement of Procalcitonin Levels with Blood Culture for Diagnosing Septicemia in Intensive Care Unit Patients[▽]

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Rapid identification of infection has a major impact on the clinical course, management, and outcome of critically ill intensive care unit (ICU) patients. We compared the results of PCR and procalcitonin with blood culture for ICU patients suspected of having septicemia. Ninety patients (60 patients meeting the criteria for sepsis and 30 patients not meeting the criteria for sepsis) were evaluated. Compared with blood culture as the gold standard, the sensitivity, specificity, and positive and negative predictive values for PCR were 100%, 43.33%, 46.87%, and 100%, respectively, and for procalcitonin were 100%, 61.66%, 56.6%, and 100%, respectively. The average times required to produce a final result were as follows: PCR, 10 h; blood culture, 33 h; procalcitonin, 45 min. Both PCR and procalcitonin may be useful as rapid tests for detecting septicemia but compared with blood cultures lacked specificity.

A rapid and reliable system to detect bacteria in the bloodstream would be clinically useful as it could guide early appropriate antibiotic treatment and result in improved patient survival (14). The gold standard for the diagnosis of infection is the isolation and identification of organisms by culture (27). This process usually requires 24 h or more. A large proportion of patients suspected of having septicemia have negative blood cultures (3) due to either previous antibiotic treatment, samples of small volume, transient bacteremias, or sepsis of nonbacterial origin (8, 30). Given the slowness and low sensitivity of blood culture, there is a need for more-rapid and more-sensitive techniques. PCR, which amplifies characteristic genes of microorganisms, is one such technique. In clinical conditions with diverse etiological agents in sterile sites, e.g., blood in sepsis, a broad-range bacterial PCR which uses a primer pair aimed at highly conserved DNA coding regions on bacterial rRNA can be used (8, 10, 11, 20). This is described as eubacterial PCR as well as broad-range bacterial PCR as it detects an rRNA gene component present in all bacteria. PCR cannot differentiate DNA sequences from viable and nonviable bacteria. The value of this test may be enhanced if it is coupled with a host response biomarker indicative of infection and systemic inflammation. Procalcitonin is one such marker and is gaining increasing importance in identification of sepsis (1, 15, 16). Procalcitonin levels are undetectable in healthy individuals but increase in patients with bacterial sepsis and correlate well with the severity of the illness (5, 19, 29).

The aim of this study was to compare the results for eubacterial PCR and procalcitonin with blood culture in intensive care unit (ICU) patients suspected of having septicemia.

MATERIALS AND METHODS

This prospective case control study was done with ICU patients of a tertiary referral hospital. The study involved patients admitted to the ICU from January to April 2004.

Definition. According to the American College of Chest Physician/Society for Critical Care Medicine (4), sepsis was defined as the systemic inflammatory response to infection. The diagnosis of sepsis requires that at least two systemic inflammatory response syndrome (SIRS) criteria be met, as well as an infection. Signs of infection include an inflammatory response to the presence of microorganisms or the invasion of a normally sterile host tissue by those organisms.

SIRS. The systemic inflammatory response to wide variety of severe insults involves two or more of the following conditions: temperature >38°C or <36°C, heart rate >90 beats/min, respiratory rate >20 breaths/min or partial CO_2 pressure <32 mm Hg, and white blood cell (WBC) count >12,000/μl or <4,000/μl or >10% immature (band) WBC forms.

Study population. The study population included consecutive clinically diagnosed patients with sepsis admitted to the ICU of our hospital. The patients included in the study were diagnosed to have clinical sepsis by a consultant intensivist, and those satisfying American College of Chest Physician/Society for Critical Care Medicine criteria for the diagnosis of sepsis/SIRS and who had a clinically suspected infection were included in the study (4). The study population consisted of four groups of patients. Group I comprised 30 consecutive clinically septic patients with positive blood cultures, group II comprised 30 consecutive clinically septic patients with negative blood cultures, group III comprised 15 consecutive clinically nonseptic ICU medical patients, and group IV comprised 15 consecutive clinically nonseptic ICU postoperative patients. The patients in groups III and IV were considered nonseptic by the consultant intensivist, and samples for blood cultures were withdrawn only for study purposes. The study was not performed blinded since the patients were included in groups I and II only after the blood culture results were available.

Sample collection. The samples were collected for groups I and II in the first 24 h after the onset of sepsis. Blood was collected from clinically septic patients until 30 consecutive patients with positive blood cultures were identified. These 30 patients constituted group I. The 30th patient with a positive blood culture was identified after a total of 167 patients were enrolled; the 30 patients in group II were selected from the remaining 137 patients with negative blood cultures. For groups III and IV, samples were collected consecutively during the same period. Blood samples were collected between 2 and 4 h after arrival at the ICU for nonseptic postoperative patients. Blood cultures and eubacterial PCR and

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procalcitonin estimations were performed for consecutive ICU septic and nonseptic patients and healthy controls. Blood specimens for blood culture (in BD Bactec standard and Plus aerobic medium), PCR, and procalcitonin determination (in a Becton Dickinson Vacutainer EDTA tube) were collected at the same time after skin disinfection following triple rounds of cleaning with isopropyl alcohol, povidone-iodine (Povidine), and isopropyl alcohol. Blood collection (both venous and arterial) was not performed through a catheter site. For blood cultures 7 ml of blood was inoculated into a blood culture bottle. For PCR 2 ml was collected in a Vacutainer tube containing EDTA and the tube was wrapped thoroughly to prevent external-source contamination. Samples were then aliquoted for PCR in volumes of 200 to 300 μl and stored at −70°C. For procalcitonin estimation another sample was collected in EDTA and plasma was aliquoted and stored at −20°C. Initially blood samples were set up for cultures and then processed for eubacterial PCR and determination of procalcitonin levels after 8 to 10 days, when the blood culture results were available.

Blood culture. Blood culture bottles (either Plus aerobic or standard aerobic) inoculated at bedside were monitored using a Bactec 9050 instrument (Becton Dickinson) for 5 days. The positive blood culture vials were processed by routine microbiological subculture and identification methods using biochemicals and antisera. The time to positivity was calculated as the time from inoculation to the first subculture after the positive signal.

Control measures for eubacterial PCR. Measures such as the use of dedicated areas of work and equipment were taken to reduce the possibility of contamination at all times (18). Only molecular-biology-grade reagents and consumables that were DNase, RNase, pyrogen, DNA, and RNA free were used. PCR studies were done with nucleic acid-free PCR vials (Eppendorf). Every batch of PCR mixture included reagent controls for extraction reagents and PCR reagents, negative controls, and samples from healthy controls along with patient samples.

As the PCR system we used was an open system, blood samples from 15 healthy controls with no signs of infection or history of antibiotic use in the previous 2 weeks were also evaluated to control for amplicon contamination.

Eubacterial PCR. A eubacterial PCR targeting the 16S DNA sequence, present in all bacteria, was used (9). Bacterial DNA was extracted from 300 μl of patient blood specimens using a PureGene DNA extraction kit. A 241-bp fragment of the bacterial 16S DNA sequence was amplified using forward primer UNI-1 (5′-GAGGAAGGTGGGGTGACG-3′) and reverse primer UNI-2 (5′-TGGTGTGACGGGCGGTGTG-3′). The PCR master mixture (40 μl) consisted of 3.5 mM Mg^{2+}, 25 pmol of each primer, 1.25 U of HotStar *Taq* DNA polymerase (Qiagen) (stock: 5 U/μl), 200 μM (each) deoxynucleoside triphosphates, 1× PCR buffer, 5× Q solution, water, and 8-methoxypsoralen (8-MOP). We standardized eubacterial PCR and decontaminated the master mix using a new method to remove contaminating endogenous DNA present in PCR reagents, especially *Taq* polymerase (21), and subjected the mixture to the following amplification protocol: 95°C for 15 min (for activation of *Taq* DNA polymerase), followed by 35 cycles of 95°C for 45 s and 60°C for 30 s and a final extension at 72°C for 2 min (with or without the addition of the target DNA).

PCR inhibition. To check for the presence of PCR inhibitors, PCR was used to coamplify a β-globin gene with the eubacterial PCR on the specimens. A 291-bp fragment of the β-globin gene was amplified using forward primer BGF (5′ TGA ACG TGG ATG AAG TTG GTG GTG 3′) and reverse primer BGR (5′ ACT TTC TTG CCA TGA GCC TTC ACC TT 3′). The PCR mixture consisted of 2 mM Mg^{2+}, 20 pmol of each primer, 1 U of HotStar *Taq* DNA polymerase (Qiagen), 200 μM (each) deoxynucleoside triphosphates, and 1× PCR buffer. The cycling conditions were same as for eubacterial PCR.

PCR validation. The analytical sensitivity was determined by using dilutions of 50 organisms/ml and 100 organisms/ml of standard strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. DNA extraction and eubacterial PCR were performed as described above. For analytical specificity the eubacterial PCR primers were checked only with standard fungal strains *Aspergillus fumigatus* ATCC 13073 and *Candida albicans* ATCC 10231 since these are commonly cultured laboratory isolates.

Post-PCR analysis. Aliquots of amplified (10-μl) samples were electrophoresed through 3% high-resolution agarose (Sigma Aldrich) gels, visualized by UV fluorescence after ethidium bromide staining, and compared with a pUC mix marker.

Plasma procalcitonin estimation. A semiquantitative PCT-Q kit was used to measure procalcitonin (B.R.A.H.M.S. Diagnostica GmbH, Berlin, Germany). The PCT-Q test uses a monoclonal mouse anticatacalcin antibody conjugated with colloidal gold (tracer) and a polyclonal sheep anticalcitonin antibody (solid phase). When the plasma sample is applied to a test strip, the tracer binds to the procalcitonin in the sample, forming a labeled antigen-antibody complex. On passing the test band region, the labeled antigen-antibody complex binds to the anticalcitonin antibody immobilized on the solid phase and forms a sandwich complex. At procalcitonin concentrations of >0.5 ng/ml, this sandwich complex is seen as a reddish band. The color intensity of the band is directly proportional to the procalcitonin concentration of the sample. Procalcitonin concentrations of <0.5, 0.5, >0.5, 2, >2, 10, and >10 ng/ml were recorded according to the color intensity of the test band in the reference card (PCT-Q kit insert; B.R.A.H.M.S.). A value of greater than 2 ng/ml was considered a positive result.

Statistics. Sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) for the PCR and procalcitonin were calculated and compared to those for blood culture as the gold standard. In a second analysis, the discordant "false-positive" PCR and procalcitonin results were resolved, using the blood culture result as the gold standard, as "true-positive" results if the patients from which these samples were obtained fulfilled the criteria for sepsis. Healthy controls were not included in the calculations.

The study protocol was reviewed and approved by the Institutional Review Board, and signed informed consent forms in English and two regional languages were collected from patients and controls.

RESULTS

The clinical details for patients and controls at the time of sample collection are given in Table 1. The clinical diagnosis at the time of blood collection and results of blood culture, eubacterial PCR, and plasma procalcitonin determinations for group I, group II, group III, and group IV patients are shown in Tables 2, 3, and 4, respectively.

Blood culture. Of the 30 culture-positive samples, 24 grew gram-negative bacilli, 4 grew gram-positive cocci, 1 grew both gram-negative bacilli and gram-positive cocci, and 1 grew a *Candida* species (Table 2). All 30 samples were positive by PCR; an additional 34 samples were also positive by PCR. Eighteen patients had procalcitonin levels of >10 ng/ml, 7 had a level of 10 ng/ml, and 5 had levels of ≥2 ng/ml.

PCR. Of 90 patients tested in the first PCR analysis, all 30 that were blood culture positive were PCR positive, 34 that were blood culture negative were PCR positive, and 26 that were blood culture positive were PCR negative. The sensitivity, specificity, PPV, and NPV of PCR were observed to be 100%, 43.33%, 46.87%, and 100%, respectively, compared with blood culture. Low specificity and PPV values were observed due to the 34 patients who had negative results for blood cultures but positive results for PCR. Twenty-two of these 34 patients fulfilled the criteria for sepsis and therefore in the second analysis were counted as true positives. For this second analysis, sepsis was present in 52 patients who were PCR positive and none who were PCR negative and absent in 12 patients who were PCR positive and 26 who were PCR negative. The sensitivity, specificity, PPV, and NPV for PCR were 100%, 68.42%, 81.25%, and 100%, respectively.

All sepsis patients ($n = 30$) with positive blood cultures (group I) were positive by PCR (Table 2). PCR was also positive even for the sample growing *Candida* spp. As previously indicated, among 30 patients with sepsis but negative blood cultures (group II) 22 were PCR positive, supporting the bacterial nature of sepsis (Table 3). Seventeen of these 22 PCR-positive samples also showed significantly elevated levels of procalcitonin (six patients had procalcitonin levels ≥10 ng/ml, three had a level of 10 ng/ml, and eight had levels >2 ng/ml). PCR was also positive for 2 of 15 nonseptic ICU medical patients (group III); none of these patients had positive procalcitonin results (Table 4). PCR was positive for 10/15 nonseptic ICU postoperative patients (group IV); six of these PCR-positive patients also had elevated procalcitonin levels (Table 4).

TABLE 1. Clinical details of patients and controls at the time of sample collection

Variable	Value for group[a]:				
	I (n = 30)	II (n = 30)	III (n = 15)	IV (n = 15)	V (n = 15)
Mean age (yr) (range)	62.63 (22–85)	53.03 (15–76)	61.53 (33–79)	52.42 (35–74)	33.66 (24–42)
No.					
Male	19	18	10	11	10
Female	11	12	5	4	5
No. with temp (°C) of:					
>38	19	20	3	1	0
37	11	9	12	13	15
<37	0	1	0	1	0
No. with pulse rate (beats/min) of:					
>90	27	28	8	8	ND[c]
90	1	0	1	0	ND
<90	2	2	6	7	ND
Respiratory rate (breaths/min)					
No. with >20	12	13	9	10	ND
No. with 20	3	2	2	3	ND
No. with <20	11	8	4	2	ND
No. on respirator	4	7	0	0	
WBC count (/mm^3)					
No. with <4,000 or >12,000)	22	22	3	4	ND
No. with 4,000–12,000	6	6	11	10	ND
No. not tested[b]	2	2	1	1	
No. on an antibiotic(s) at sample collection	21	25	5	15	0

[a] Groups I to IV are as defined in Materials and Methods. Group V comprised healthy controls.
[b] Not tested on the day of sample collection.
[c] ND, not done.

All samples were analyzed for PCR inhibitors, and none of the negative samples showed the presence of PCR inhibitors.

Procalcitonin. Of 90 patients tested in the first procalcitonin analysis, all 30 that were blood culture positive were procalcitonin positive, 23 that were blood culture negative were procalcitonin positive, and 37 that were blood culture positive were procalcitonin negative. The sensitivity, specificity, PPV, and NPV of procalcitonin were observed to be 100%, 61.66%, 56.6%, and 100%, respectively, compared with blood culture. The specificity and PPV values were low because of the 23 patients who had negative blood cultures and positive results for procalcitonin. Seventeen of these 23 patients fulfilled the criteria for sepsis and therefore in the second analysis were counted as true positives. For this second analysis, sepsis was present in 47 patients who were procalcitonin positive and none who were procalcitonin negative and absent in 6 patients who were procalcitonin positive and 37 who were procalcitonin negative. The sensitivity, specificity, PPV, and NPV for procalcitonin were 100%, 86.05%, 88.68%, and 100%, respectively. The concordance between PCR and procalcitonin results was observed to be 88.88%. Of 90 patients, 54 that were PCR positive were procalcitonin positive, 16 who were PCR positive were procalcitonin negative, none who were PCR negative were procalcitonin positive, and 26 who were PCR negative were procalcitonin negative. In all groups, none of the PCR-negative samples had detectable plasma procalcitonin levels.

All the healthy controls showed no growth of bacteria in culture, and PCR and procalcitonin were also negative.

DISCUSSION

Rapid identification of infection has a major impact on the clinical course, management, and outcome of critically ill ICU patients. We compared the results of eubacterial PCR and procalcitonin determination with blood culture for diagnosing septicemia in ICU patients. Though blood culture is considered the gold standard test method for diagnosing septicemia, this method lacks sensitivity and is time-consuming, and contamination by skin microorganisms can be problematic. PCR, because of enhanced sensitivity, may be potentially more useful for detecting septicemia. However, PCR methods can result in false-positive results due to amplicon contamination, and PCR cannot differentiate between viable and nonviable bacteria. Procalcitonin has been recognized as a sensitive and specific biomarker of the host response to sepsis (1, 5, 15, 16, 19, 29). For the current study we used semiquantitative estimation of procalcitonin as a quantitative analysis was not available at the time the study commenced. The results of the current study showed excellent sensitivity for both the procalcitonin and PCR assays compared to the results for culture; however, both methods lacked specificity.

In our study, blood cultures yielded a predominance of gram-negative bacteria. These results are similar to those reported previously for an ICU setting on the Indian subcontinent (13). Among gram-positive bacteria, three *Staphylococcus epidermidis* strains and one *Corynebacterium* sp. were isolated from single blood cultures. They were regarded as significant by the treating physician because, in two urosepsis patients, the organisms were also grown in urine and because the other two patients were sufficiently immunocompromised for these organisms to be considered significant. The ability of blood cultures to enable diagnosis of bloodstream infections is enhanced if an increased volume of blood is cultured and more consecutive cultures are obtained over a 24-h period (7, 12). The limited volume of blood (7 to 8 ml per bottle) collected from

TABLE 2. Blood culture, eubacterial PCR, and procalcitonin results for group I sepsis patients with positive blood cultures

Patient no.	Clinical diagnosis[a]	Blood culture isolate(s)	Result for:	
			Eubacterial PCR	Procalcitonin (ng/ml)
1	Malaria, MODS, and sepsis	*Candida albicans*	+	>10
2	Leptospirosis, MODS, and sepsis	*Klebsiella pneumoniae*	+	>10
3	Cirrhosis, GI bleeding, and sepsis	*Klebsiella pneumoniae*	+	>2
4	Cirrhosis, hepatorenal syndrome, GI bleeding, and sepsis	*S. epidermidis*	+	>2
5	Diabetic infected foot and renal failure	*Klebsiella pneumoniae*	+	>10
6	Polytrauma with sepsis	*Pseudomonas* sp.	+	>10
7	CVA, IHD, post-CPR, and sepsis	*Escherichia coli*	+	>10
8	SLE, seizures, and sepsis	*Escherichia coli*	+	>10
9	CVA, CRF, and sepsis	*Pseudomonas aeruginosa*	+	>10
10	ARDS and MODS	*Pseudomonas aeruginosa*	+	>10
11	Urosepsis	*S. epidermidis*	+	>10
12	CVA and sepsis	*Acinetobacter baumannii*	+	10
13	Urosepsis	*E. coli* and *Streptococcus mutans*	+	>10
14	ILD, bleeding duodenal ulcer, and sepsis	*Pseudomonas aeruginosa*	+	>10
15	CVA and sepsis	*Enterobacter cloacae*	+	>10
16	Renal failure and pneumonia	*E. coli*	+	>10
17	Cirrhosis, hepatorenal syndrome, and sepsis	*E. coli*	+	10
18	Colon cancer, myasthenia gravis, and sepsis	*Pseudomonas aeruginosa*	+	>10
19	Meningitis	*Klebsiella ozaenae*	+	10
20	CVA, COPD, and sepsis	*Klebsiella pneumoniae*	+	>10
21	CVA, pneumonia, and sepsis	*Klebsiella pneumoniae*	+	>10
22	CRF and sepsis	*Corynebacterium* sp.	+	10
23	Meningitis and sepsis	*Acinetobacter calcoaceticus*	+	10
24	Parkinson's disease and aspiration pneumonia	*Pseudomonas aeruginosa*	+	>2
25	IHD and urosepsis	*S. epidermidis*	+	>2
26	Lower limb cellulitis and ARDS	*Acinetobacter baumannii*	+	>10
27	CVA and sepsis	*Enterobacter cloacae*	+	>10
28	Lung abscess	*Enterobacter agglomerans*	+	>2
29	Fulminant hepatic failure and MODS	*E. coli*	+	10
30	Malaria and sepsis	*Klebsiella pneumoniae*	+	10

[a] Abbreviations: MODS, multiple organ dysfunction syndrome; GI, gastrointestinal; CVA, cerebrovascular accident; IHD, ischemic heart disease; CPR, cardiopulmonary resuscitation; SLE, systemic lupus erythematosus; CRF, chronic renal failure; ARDS, acute respiratory distress sydrome; ILD, interstitial lung diseases; COPD, chronic obstructive pulmonary disease.

our patients and the fact that only one culture was drawn could have resulted in a falsely low recovery rate of pathogens. Also, fastidious organism supplement (BD Bactec FOS; Becton Dickinson and Co., Sparks, MD) was not added to blood culture bottles; hence, we may have missed the occasional fastidious organism. In summary, in this study, if more blood cultures had been positive due to added volume and number of cultures and if these results matched the PCR and procalcitonin results, the specificities of these assays may have been improved.

In the current study, blood culture, PCR, and procalcitonin were all negative for eight patients who fulfilled the criteria for sepsis (group II). The negative PCR results in these patients could be due to low levels of detectable organisms, i.e., below 50 organisms per ml, the sensitivity threshold of our PCR assay.

Because of the potential limitation of the blood culture as a gold standard for detecting septicemia in our study, we performed second analyses for PCR and procalcitonin for resolution of some discordant results. Among the discordant false-positive PCR results, 22 of 34 were reclassified as true positive since these patients fulfilled the criteria for sepsis. The second analysis, using this reclassification, resulted in improved specificity and PPV. Of the remaining 12 discordant PCR results, 2 were from group III nonseptic ICU medical controls and 10 were from group IV nonseptic ICU surgical controls. The cause of the positive PCR result for two patients in group III could be the presence of vascular catheters and other indwelling devices such as nasogastric tubes or urinary catheters, which may cause transient bloodstream seeding with bacteria or bacterial products. Of the 10 PCR-positive, blood culture-negative patients in group IV (Table 4) 6 had elevated procalcitonin levels (in 4, >10 ng/ml, and in 2, >2 ng/ml) suggesting significant infection. Thus, these could be interpreted as truly positive results. Of the remaining four PCR-positive and procalcitonin-negative patients, two patients had undergone oropharyngeal and esophageal surgery and one had undergone coronary artery bypass grafting (CABG) on pump, procedures which are known to be associated with transient bacteremia (2, 17, 25). All 10 PCR-positive, blood culture-negative patients in group IV were on antibiotics, and the culture negativity in this group could be attributed to low and suppressed levels of bacteremia. In these patients PCR can play important role in detecting bacterial infections rapidly and at an early stage, thereby controlling the progression of sepsis by a correct antibiotic regimen.

The PCR method used in this study was an open system; however, negative controls with each run were negative, indicating no amplicon contamination. In the absence of a closed-system PCR assay (e.g., real-time PCR) or DNA sequencing of

TABLE 3. Results of blood culture, eubacterial PCR, and procalcitonin for group II sepsis patients with negative blood cultures

Patient no.	Clinical diagnosis[a]	Result for:		
		Blood culture	Eubacterial PCR	Procalcitonin (ng/ml)
1	Convulsions and urosepsis	−	+	2
2	Asthma with chest infection	−	−	<0.5
3	Postoperative abdominal and hepatobiliary sepsis	−	−	<0.5
4	Postcraniotomy meningitis	−	+	>10
5	CVA with secondary sepsis	−	+	<0.5
6	IHD and chest infection	−	+	>10
7	CVA, CCF, acute renal failure, and sepsis	−	−	<0.5
8	CVA, diabetic foot, cellulites, and sepsis	−	−	<0.5
9	Dengue and ARDS	−	+	>2
10	Pulmonary TB and pneumonia	−	+	>2
11	Bilateral bronchopneumonia	−	+	>10
12	CVA with sepsis	−	+	<0.5
13	CCF with sepsis	−	+	10
14	Lymphoma and perianal abscess	−	+	10
15	Moyamoya disease with sepsis	−	−	<0.5
16	Pulmonary embolism and sepsis	−	+	<0.5
17	SLE and pneumonia	−	+	>10
18	TB meningitis and sepsis	−	+	<0.5
19	Splenic abscess	−	+	>2
20	Pneumonia and MODS	−	+	2
21	Diabetes, renal failure, and sepsis	−	+	2
22	Head injury with sepsis	−	−	<0.5
23	Pyogenic meningitis	−	+	>10
24	Pneumonia and MODS	−	−	<0.5
25	Post-CPR and sepsis	−	+	2
26	Pneumonia and MODS	−	+	<0.5
27	Wegener granulomatosis with mucormycosis	−	−	<0.5
28	Viral encephalitis and MODS	−	+	>10
29	COPD and pneumonia	−	+	>2
30	Postoperative LVF and sepsis	−	+	10

[a] Abbreviations: CCF, carotid-cavernous fistulas; TB, tuberculosis; LVF, left ventricular failure. Other abbreviations are as defined for Table 2.

TABLE 4. Results of eubacterial PCR and procalcitonin in nonseptic patients from groups III and IV

Group[a] and patient no.	Diagnosis	Result for:	
		Eubacterial PCR	Procalcitonin (ng/ml)
III			
1	Chronic liver disease with epistaxis	−	<0.5
2	Myocardial infarction	−	<0.5
3	Ischemic heart disease with hypertension	−	<0.5
4	Myasthenia gravis	−	<0.5
5	Cerebrovascular accident (infarction)	−	<0.5
6	Bronchitis and left ventricular failure	−	<0.5
7	Coronary artery disease	−	<0.5
8	Postictal loss of consciousness	−	<0.5
9	Acute interstitial pneumonia	+	<0.5
10	Ischemic heart disease with left ventricular failure	−	<0.5
11	Ischemic heart disease with hypertension	−	<0.5
12	Myocardial infarction	+	<0.5
13	Coronary artery disease	−	<0.5
14	Coronary artery disease	−	<0.5
15	Multivalvular heart disease	−	<0.5
IV			
1	Mitral and aortic valve replacement	+	10
2	Repeat of mitral valve replacement	−	<0.5
3	Craniotomy: aneurysm clipping	+	10
4	CABG (off pump)	−	<0.5
5	CABG (off pump)	−	<0.5
6	Tibia interlock	+	>2
7	CABG (on pump)	+	10
8	Hemiglossectomy	+	<0.5
9	Total esophagotomy	+	<0.5
10	CABG (off pump)	−	<0.5
11	Craniotomy; cerebellar hemorrhage decompression	+	10
12	Craniotomy; posterior fossa abscess evacuation	−	<0.5
13	CABG (on pump)	+	>2
14	CABG (on pump)	+	<0.5
15	Total hip replacement	+	<0.5

[a] Groups are defined in Materials and Methods.

amplification products, it is difficult to speculate about or preclude the positive PCR results for nonseptic patients in groups III and IV. Additionally PCR was also positive for samples growing *Candida* spp. in blood culture (group I). The positive PCR result could be due to dual infection with a bacterium since, in the prestudy validation of the PCR technique, the eubacterial primer did not show cross-reactivity with fungi, including *Candida* spp.

The procalcitonin assay demonstrated excellent sensitivity (100%) compared with blood culture. Two studies have reported that procalcitonin has low diagnostic performance in differentiating between sepsis and SIRS (24, 26). In contrast to this, a few other studies have reported that procalcitonin was a better marker to estimate the severity, prognosis, or further course of the sepsis (6, 22, 23). There are currently no data comparing PCR and procalcitonin in sepsis cases.

In our study concordance between PCR and procalcitonin was 88.88%. All PCR-negative patients were procalcitonin negative. Though in this study procalcitonin was observed to be useful for rapid diagnosis of sepsis, it could not differentiate between bacterial and fungal sepsis. We currently use a combination of blood cultures and procalcitonin, along with clinical judgment, in the diagnosis of early sepsis in our practice. Moreover the average time taken for eubacterial PCR was 10 h, that for blood culture was 33 h (final identification), and that for procalcitonin estimation was 45 min in the present study. The availability of real-time PCR techniques has direct clinical application for rapid, early, and accurate diagnosis of sepsis even before culture results are available (28), whereas procalcitonin

determination serves as a point-of-care test to diagnose sepsis, specially in crucial settings like the ICU.

To summarize, both PCR and procalcitonin determination may be useful as rapid tests for detecting septicemia but compared with blood cultures lacked specificity.

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