Potential clinical utility of polymerase chain reaction in microbiological testing for sepsis

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Objectives: **To evaluate the potential improvement of antimicrobial treatment by utilizing a new multiplex polymerase chain reaction (PCR) assay that identifies sepsis-relevant microorganisms in blood.**

Design: **Prospective, observational international multicentered trial.**

Setting: **University hospitals in Germany (n = 2), Spain (n = 1), and the United States (n = 1), and one Italian tertiary general hospital.**

Patients: **436 sepsis patients with 467 episodes of antimicrobial treatment.**

Methods: **Whole blood for PCR and blood culture (BC) analysis was sampled independently for each episode. The potential impact of reporting microorganisms by PCR on adequacy and timeliness of antimicrobial therapy was analyzed. The number of gainable days on early adequate antimicrobial treatment attributable to PCR findings was assessed.**

Measurements and Main Results: **Sepsis criteria, days on antimicrobial therapy, antimicrobial substances administered, and microorganisms identified by PCR and BC susceptibility tests.**

Results: **BC diagnosed 117 clinically relevant microorganisms; PCR identified 154. Ninety-nine episodes were BC positive (BC+); 131 episodes were PCR positive (PCR+). Overall, 127.8 days of clinically inadequate empirical antibiotic treatment in the 99 BC+ episodes were observed. Utilization of PCR-aided diagnostics calculates to a potential reduction of 106.5 clinically inadequate treatment days. The ratio of gainable early adequate treatment days to number of PCR tests done is 22.8 days/100 tests overall (confidence interval 15–31) and 36.4 days/100 tests in the intensive care and surgical ward populations (confidence interval 22–51).**

Conclusions: **Rapid PCR identification of microorganisms may contribute to a reduction of early inadequate antibiotic treatment in sepsis. (Crit Care Med 2009; 37:3085–3090)**

Key Words: inadequate antimicrobials; rapid pathogen detection; adequate treatment; gainable days

Sepsis is the second leading cause of death in the noncoronary intensive care unit (ICU) (1). Early diagnosis, followed by prompt implementation of an appropriate treatment (2), improves the prognosis of septic patients (3–5). After early initiation of antimicrobial therapy, timely reassessment is important, because inappropriate antibiotic therapy negatively influences (6, 7) while adequate antibiotic therapy positively influences the outcome of patients with bacterial bloodstream infections (BSIs) and sepsis (8, 9).

Supplementing blood cultures (BC) for BSI testing by additional polymerase chain reaction (PCR)-based testing has been proposed (10). However, data for the clinical utility of PCR in microbiological testing for sepsis are still lacking.

Here, in a model analysis we retrospectively evaluate the potential clinical utility of supplemental PCR testing of sepsis patients to determine the impact in terms of gainable days on early adequate antimicrobial treatment. We used covariate analysis to identify independent factors.

METHODS

Study Design and Patients. The retrospective observational cohort study was conducted in four university hospitals and one tertiary general hospital. Inclusion criteria were ≥18 yrs of age, suspected sepsis (11), a blood culture drawn, and subsequent antibiotic treatment initiation or change. The ethics committees of the participating institutions approved the study, and written consent was obtained from each patient or relatives. Patients who met the inclusion criteria with suspected sepsis and blood culture drawn were serially enrolled as they presented in the unit or hospital. Data collected included indication for admission, recent medical history, vital signs and functions, antibiotics given at study inclusion, and laboratory test results. Study definitions are summarized in Table 1. The study was not designed for method comparison, therefore multiple BC tests per episode were allowed. Two study sites (Davis and Bergamo) included patients from the following services (ICU, emergency room, medical and surgical wards). Three sites included ICU patients only (Bonn, Frankfurt/M, Santiago de Compostela). Part of the data (Davis) have been analyzed for detection enhancement of bacteremia and fungemia (12).

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Table 1. Study definitions

Sepsis	Sepsis was defined according to the SCCM/ACCP consensus conference guidelines of 1992 (11).
Episode of antimicrobial treatment	An episode started when an antimicrobial treatment was begun, extended, or changed. All antimicrobial treatments had to be kept for at least 24 hrs. Inclusion of multiple episodes per patient was possible.
Clinically inadequate antimicrobial treatment	Treatment episode under which an infection did not improve clinically, while a subsequent modification in antimicrobial treatment (based on culture and susceptibility finding) led to a clinical improvement.
Gainable days on early adequate treatment	Days by which a clinically adequate antimicrobial treatment could be given earlier when relying on a previously obtained PCR+ result. The days are counted from the time point of PCR+ result report to the time point when the adequate drug was actually given.
BC+ episode	Episodes in which a microorganism was identified by blood culture (BC+ episodes) or by PCR (PCR+ episodes). Episodes were followed daily until the end of the respective episode, which was defined by discontinuation of antimicrobials for more than 24 hrs, by hospital discharge, by death, or by start of a new episode.
PCR+ episode	
Positivity of BC	Identification of a classic BSI pathogen in a blood culture specimen, or the identification of a facultative pathogenic skin microorganism in at least two separate blood culture specimens from the same patient drawn from different sites.
Contamination of BC	Identification of a facultative pathogenic organism in only one out of two separate BC specimens from the same patient drawn from different sites.
Contamination of PCR	False positive PCR results due to either workflow or sample contaminations were reviewed and judged for the individual test result and episode by a panel of treating physicians and microbiologists based upon clinical information on the patient and all available supplementary laboratory data of the corresponding septic episode.

SCCM, Society of Critical Care Medicine; ACCP, American College of Chest Physicians; PCR, polymerase chain reaction; BC, blood culture; BSI, blood stream infection.

Table 2. Underlying diagnoses and identification of pathogens in 467 episodes

Underlying Diagnoses[a]	Totals	Polymerase Chain Reaction Positive	Blood Culture Positive
Intra-abdominal sepsis	136	51	39
Nosocomial pneumonia	112	46	38
Community-acquired pneumonia	19	4	4
Multiorgan dysfunction syndrome	13	10	9
Catheter-related sepsis	61	29	21
Neutropenic fever	47	17	9
Pyelonephritis	24	12	10
Genitourinary infection	13	3	3
Wound infection	10	0	0
Bone/joint infection	14	4	0
Other	102	17	14

[a]Underlying diagnoses do not add up to 467 due to dual conditions.

Multiplex PCR Method. The LightCycler Septi*Fast* PCR Test (Roche Diagnostics, Mannheim, Germany) was performed using ethylenediaminetetraacetic acid whole blood according to the manufacturer's recommendations. The design of the method and its analytical performance have been described previously (13). PCR workflow contamination was assessed by full process controls. Additional analysis for *Aspergillus fumigatus* workflow contamination was implemented. *Aspergillus fumigatus* workflow contamination was assumed if the patient had no clinical signs of pneumonia, negative chest radiogram, and negative galactomannan evaluation as assessed by three clinicians.

Blood Culture Methods. Samples for BC analysis were drawn from a fresh venipuncture site. Blood samples were inoculated into both aerobic and anaerobic bottles in parallel and analyzed subsequently using semiautomated blood systems, BACTEC (Becton Dickinson, Franklin Lakes, NJ) or BacT/ALERT (bioMerieux, Marcy l'Etoile, France). Rapid report (via phone or fax) of Gram-negative stain upon availability was standard of care at all participating study sites. Antimicrobial susceptibility tests were performed by broth microdilution and/or by agar diffusion and/or Etest (AB-biotest, Solna, Sweden) using Clinical and Laboratory Standards Institute breakpoints.

Analysis of Potential Effects of PCR Positive (PCR+) Findings. For data analysis we hypothesized that all microorganisms as recovered by PCR shall immediately be treated with an antimicrobial, or combination of two antimicrobials, that would give >90% coverage for any strains as documented locally in hospital antimicrobial resistance statistics. The adequacy of antimicrobial treatment was judged on the basis of all available clinical, pharmacological, and microbiological evidence (6). Gainable days on early adequate treatment were added up for the entire trial and for subgroups; normalization to 100 tests done was calculated.

Statistical Analysis. Descriptive statistics were used on underlying diagnosis, pathogen identification (Tables 2 and 3), changes of antimicrobial treatment (Fig. 1), and potential impact on days of inadequate antimicrobial treatment (Fig. 2). Data are expressed as mean. PCR+ and BC+ rates are based on episodes. Subgroups of special interest are compared with the two-sample Student's *t* test (Table 4). To find independent covariates for gainable days on early adequate treatment, logistic regressions of PCR+ and BC+ episodes were performed with stepwise selection. Covariates included age, sex, department, kind of infection, and antimicrobial treatment. All *p*-values are two-tailed; $p < 0.05$ was set as the limit for the acceptance or removal of covariates and their interactions. Results are reported as adjusted odds ratios (AORs) with 95% confidence intervals (CIs).

RESULTS

Patients Included in the Study. Overall, 436 sepsis patients (168 female, 268 male), mean age 54.8 (range 18–92) with 467 episodes of antimicrobial treatment were included in the study in total. Of the patients, 92 were from a tertiary hospital and 344 were from university hospitals. Included patient episodes are characterized in Table 2.

Microorganisms Reported by BC and PCR. BC identified 117 clinically relevant

Table 3. Clinically significant microorganisms (n = 201) identified by polymerase chain reaction (PCR) (n = 154) and/or blood culture (BC) (n = 117) in 467 episodes, given in absolute numbers and as fraction of all episodes

	PCR Positive Only (%)	PCR and BC Positive (%)	BC Positive Only (%)[e]
Gram (+)	38 (8.2)	38 (8.2)	25 (5.4)
- *Staphylococcus aureus*	16	13	1
- Coagulase-negative staphylococci[a]	8	12	10
- *Enterococcus faecium* or *faecalis*	7	7	8
- *Streptococcus pneumoniae* or species	7	6	6
Gram (-)	35 (7.3)	26 (5.6)	14 (3.0)
- *Escherichia coli*	6	5	2
- *Proteus mirabilis*	1	2	1
- *Klebsiella* species	12	7	2
- *Enterobacter*	10	3	2
- *Serratia marcescens*	4	1	1
- *Stenotrophomonas maltophilia*	0	2	4
- *Pseudomonas aeruginosa*	2	6	2
Fungi	11 (2.4)	6 (1.3)	3 (0.6)
Candida[b]	4	6	3
Aspergillus fumigatus[c]	7	0	0
Not detectable by PCR assay[d]	—	—	5 (1.1)

[a]*Staphylococcus epidermidis* or other coagulase-negative species; [b]*Candida* were differentiated to following subspecies: *albicans, tropicalis, parapsilosis, krusei,* and *glabrata;* [c]seven additional PCR findings were judged as evident contamination with *Aspergillus fumigatus* (Table 1); [d]five findings (*Corynebacterium, Cryptococcus, Gemella, Morganella, Stomatococcus*); [e]twenty-eight positive BC results were judged as contaminations clinically (Table 1) and were excluded from the analysis: coagulase-negative *Staphylococcus* (n = 21), *Corynebacterium* species (n = 2), *Bacillus* species (n = 2), *Bacteroides* species (n = 1), *Pantoea agglomerans* (n = 1), and *Propionibacterium* (n = 1).

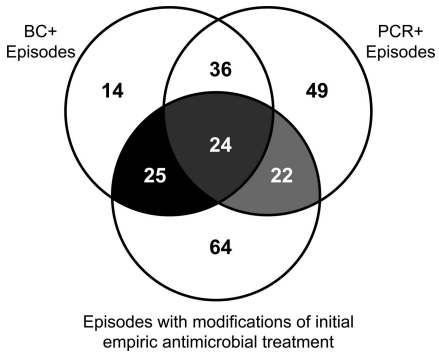

Figure 1. Overall, 135 antimicrobial modifications were observed in total (*lower circle*); 49 observed modifications (25 + 24) were blood culture (*BC+*) driven (*intersection with upper left circle*). Additionally, 86 (64 + 22) observed modifications were driven by findings of other microbiological specimen or clinical evidence. Intersections with *upper right circle* (131 polymerase chain reaction [*PCR+*] episodes) indicate how PCR+ utilization could accelerate 46 (24 + 22) of the observed antimicrobial changes.

microorganisms, obtained from 99 episodes. PCR identified 154 microorganisms in 131 episodes. Details are given in Table 3. Polymicrobial infections were detected by PCR in 21 episodes involving two pathogens. A single episode involved three pathogens. Table 3 summarizes all episodes depicting all microorganisms identified by method of detection.

30-Day Mortality Rate According to BC and PCR Testing. The 30-day mortality associated with all BC+ episodes was 30.3% compared with 26.7% for all PCR+ episodes. 30-day mortality of BC+ *and* PCR+ episodes was 33.8%. In episodes with negative BC *and* PCR findings the 30-day mortality was 14.1%.

Episodes and Change of Antimicrobial Treatment. In 135 of 467 episodes empirical antimicrobial treatment was changed, providing coverage of different species or resistances. In 49 of these episodes, change was driven by BC+ results (Fig. 1). The sum of days on inadequate antimicrobial treatment from these 49 BC+ episodes was 127.8 days in total, or 2.6 days for the average BC+ episode, respectively. In another 86 (64 + 22) episodes adjustments were made using positive findings of other microbiological specimen, such as bronchoalveolar lavage fluids, swabs, urine, or clinical evidence (Fig. 1).

Potential Impact of Utilizing PCR Results on Inadequate Antimicrobial Treatment Days. In 46 (24 + 22) episodes (Fig. 1) the PCR results suggested antimicrobial adjustments equivalent to those actually made. The associated total potential reduction of inadequate treatment was 106.5 days. In 24 (dark gray area, Fig. 1) of these episodes, PCR results preceded results from concurrent blood cultures by a total of 49.5 days (Fig. 2*A*). In 22 episodes (light gray area, Fig. 1) PCR results showed increased sensitivity over concurrent blood cultures, with a potential to reduce inadequate treatment by a total of 57.0 days (Fig. 2*B*).

Of 131 PCR+ episodes, 85 (36 + 49) were at time of PCR+ reporting adequately covered by the empirically selected antimicrobials (Fig. 1). No correction of antimicrobial therapy was made. Of note, nine of these 85 episodes had a change of antimicrobial treatment executed based on Gram-negative stain results before the time of PCR reporting, rendering the PCR result of no additional use. Finally, utilizing PCR information could trigger potential overtreatments in 18 of these episodes.

Gainable Days on Early Adequate Treatment per 100 PCR Tests. On average, in the five participating centers, 22.8 days on early adequate treatment could be gained per 100 tests done. This effect was significantly higher in episodes of surgical and ICU patients, than in episodes managed in emergency departments or in other wards (Table 4). Furthermore, the potential benefit from PCR utilization was significantly increased in episodes in which initial empirical antimicrobial therapy included at least one of the following agents: carbapenem, vancomycin, oxazolidinone, piperacillin/tazobactam, or an antifungal (Table 4).

In covariate analysis, the strongest independent factor for gainable days on early adequate treatment per 100 tests was the underlying diagnosis. Episodes with intra-abdominal sepsis, nosocomial pneumonia, catheter-related sepsis, multiorgan dysfunction, pyelonephritis, or neutropenic fever showed significantly elevated impact regarding gainable days on early adequate treatment (AOR 3.9; CI 1.4–11.3). Finally, potential benefit was significantly higher in patients ≥56 yrs old than younger ones (AOR 2.8; CI 1.4–5.9).

DISCUSSION

Modern molecular diagnostic approaches in patients with clinical sepsis include broad-range PCR (14, 15), targeted PCR (16, 178), fluorescence *in situ* hybridization (18), and microarray technology (19). Such methods are also reviewed elsewhere (10, 20–22). In this retrospective analysis we demonstrate that only 21.2% of episodes are characterized

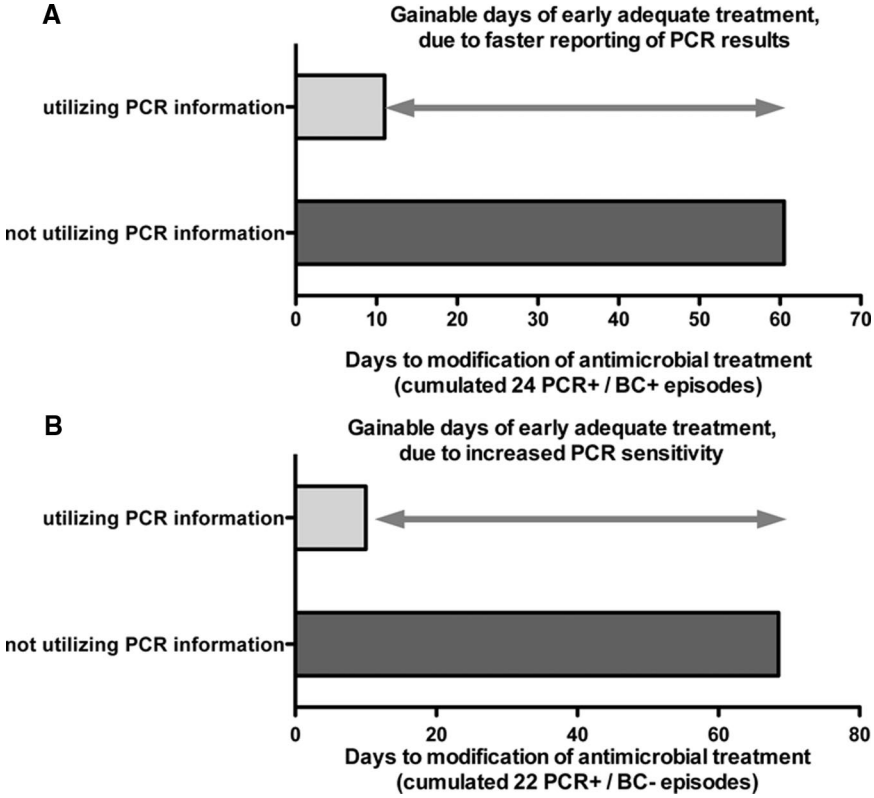

Figure 2. Potential impact of polymerase chain reaction (*PCR*) utilization on days of inadequate antimicrobial treatment. *A,* Episodes in which concurrently drawn PCR and blood culture (*BC*) pointed to an inadequately covered microorganism. *B,* Episodes in which PCR pointed to an inadequately covered microorganism, and another culture result and/or actual clinical course supported the potential clinical significance of the PCR result.

Table 4. Subgroup analysis of gainable early adequate treatment days (gainable days are normalized to 100 polymerase chain reaction tests done)

	Subgroup (Episodes)	Gainable Days	*p*
All episodes	— (467)	22.8	—
Site of service attended	Intensive care unit or surgical ward (221)	36.4	.002
	Emergency room, other (246)	10.6	
Underlying diagnosis	Invasive group[a] (327)	30.7	<.001
	All other (140)	4.3	
Patients' age	Age ≥56 (231)	37.0	<.001
	Age ≤55 (236)	8.9	
Initial antimicrobial treatment	Extended antibiotic coverage[b] (251)	35.1	<.001
	All other (216)	8.6	

[a]Intra-abdominal sepsis, nosocomial pneumonia, catheter-related sepsis, multiorgan dysfunction, pyelonephritis, and neutropenic fever; [b]involving at least one of vancomycin, oxazolidinone, piperacillin/tazobactam, a carbapenem, or an antifungal agent.

by positive blood culture findings alone, which is in close agreement to findings of other multicentered studies (23). When complementing blood cultures with one PCR test per episode, the number of clinically relevant positive microbiological findings increased by 72% (from 117 to 201, calculated from Table 3).

Early diagnosis followed by adequate antimicrobial therapy is key for a favorable outcome in patients with ICU-acquired bloodstream infection (8). For our PCR+ subpopulation, the results suggest that some 35.1% (46 PCR+ episodes/131 PCR+ episodes) of episodes were not adequately covered at the time of PCR reporting. Inadequate rates of over 30% have been reported for nosocomial infections before (24–26). Indeed, the inadequate initial antimicrobial therapy was changed in a total of 28.9% (n = 135) of 467 episodes. The potential information gain from PCR+ results for the clinician was observed in 46 episodes, either by faster reporting (n = 24) or by increased sensitivity (n = 22).

Nevertheless, the present study also revealed limitations of the PCR method as used and its potential clinical utility. In all, 64 episodes of evident infection, as detected by conventional culture methods followed by subsequent adjustment of initial empirical antimicrobial therapy, were not picked up by PCR (Fig. 1). Furthermore, 39 episodes of bloodstream infection were not picked up by PCR, 25 with subsequent adjustment of initial empirical antimicrobial therapy, and 14 without (Fig. 1). False-negative PCR findings may result from possible variability of the DNA target affecting the binding of primers or probes (12, 13). Furthermore, the sensitivity for coagulase-negative staphylococci and streptococci is artificially limited by introduction of a cross-contamination threshold by the assay manufacturer and by a reduced sensitivity for *Candida glabrata* (13). In this study, 10 coagulase-negative staphylococci clinically relevant findings by BC were unmatched by PCR. This clearly limits the diagnostic capabilities of the assay and potentially underestimates clinical utility. Omission of this threshold would certainly generate more, but potentially also false-positive, results. False-positive PCR results may also originate from amplification of free DNA released from nonviable or killed bacteria and fungi, thereby mimicking ongoing infection. Consequently, overtreatment triggered by utilization of false-positive PCR results may occur. At present, a further reason for possible overtreatment is the currently missing incorporation of susceptibility testing into the PCR method, potentially triggering unnecessary modifications of antimicrobial treatment. Indeed, analyzing the possible impact of PCR findings as obtained in our study suggested an unnecessary rule-in of extended antimicrobials in 18 of 467 episodes. Therefore, clinicians should not solely rely on PCR results to rule in or rule out true infection, but should always include additional corroborating clinical and laboratory evidence in their decision-making process. Furthermore, the test procedure is sophisticated and labor intensive, and rapid PCR-based diagnostics suffer from a lack of standardization (21) and a risk for workflow contamination (27, 28). Therefore, assay application

seems most appropriate in core diagnostic facilities such as university hospitals.

The benefit of molecular diagnostics is already accepted for specific diagnostic indications, such as the rapid diagnosis of fastidious or slow-growing organisms or pathogen detection during ongoing antimicrobial treatment (16, 17, 29). Similarly, PCR detection of microbial DNA from blood can be achieved in patients with sepsis within a few hours, and may become a helpful tool to bypass some of the disadvantages of conventional BC when used as an diagnostic adjunct (12, 13). Recent data from Garnacho-Montero et al (30) report an association of inadequate antimicrobial therapy with a significant hospitalization increment of 15 days in surviving patients. For the assay as used in this study, cost is estimated at €220 per PCR test (€170 for reagents and disposables plus €50 for labor). The mean cost to eliminate one day of early inadequate empirical antimicrobial treatment in our study calculates to €965 (467 episodes × €220 costs per test/106.5 inadequate treatment days saved).

In summary, our present model investigation is clearly limited and cannot provide a "proof of principle" when it comes to clinical utility of therapy adjustments guided by the timely report of PCR results. As outlined in our retrospective analysis, however, the supplementary inclusion of PCR-derived information on bloodstream pathogens in rapid clinical decision-making may pave the way to improving appropriate antimicrobial coverage in clinical sepsis. Consequently, the urgent need for conducting prospective interventional trials with valid clinical end points to establish a benefit of molecular diagnostics in patients with clinical sepsis is underlined.

CONCLUSIONS

As suggested by the present retrospective model analysis, PCR detection of microbes in septic patients' blood holds promise for improving antimicrobial treatment by reducing the number of days on inadequate antibiotic treatment. However, prospective interventional studies with valid clinical end points are urgently needed to definitely prove the benefit and cost-effectiveness of multiplex PCR-based diagnosis in the clinical setting.

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