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Incremental value of multiplex real-time PCR for the early diagnosis of sepsis in the emergency department

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Summary

BACKGROUND: Delayed recognition of sepsis and inappropriate initial antibiotic therapy are associated with increased mortality and morbidity. The early detection of the causative organism in sepsis is an unmet clinical need. A novel multiplex real-time polymerase chain reaction (MRT-PCR) (Septi*Fast*®) technique may provide the microbiological diagnosis within six hours.

METHODS: We assessed the diagnostic accuracy of blood cultures and MRT-PCR in a comparative diagnostic cohort study in 110 consecutive adult patients presenting to the emergency department (ED) with suspected sepsis.

RESULTS: We collected 205 corresponding PCR samples and blood culture (BC) pairs from the 110 patients. There was moderate to high concordance between PCR and BC with 181 (88%) matching and 24 (12%) mismatching samples. The diagnostic accuracy of MRT-PCR in detecting sepsis and its causative organism was comparable to that of BCs. The additional use of MRT-PCR significantly reduced the time to microbiological diagnosis as compared to the use of conventional microbiological methods alone (mean time gained 3.9 hours, range 0–66 hours, p <0.001).

CONCLUSION: Diagnostic accuracy of BCs and MRT-PCR in the early diagnosis of sepsis and its causative organism in the ED are comparable. However, MRT-PCR reduces the time to microbiological diagnosis. Whether a more rapid detection of the organism by MRT-PCR could improve the outcome of patients has to be assessed in large prospective randomised trials.

Key words: *multiplex real-time PCR; sepsis; emergency department*

Abbreviations

BC	blood culture
CFU	colony-forming units
CNS	coagulase-negative staphylococci
ED	emergency department
MRT-PCR	multiplex real-time polymerase chain-reaction
NPV	negative predictive value
PPV	positive predictive value
SIRS	systemic inflammatory response syndrome
TTP	time to positivity

Introduction

Sepsis is a life-threatening condition caused by the uncontrolled systemic inflammatory response to bacterial, viral or fungal infection [1]. The incidence of sepsis and the number of sepsis-related deaths are increasing, making sepsis the leading cause of death in critically ill patients in Europe and the United States [2–6]. Delayed recognition of sepsis and inappropriate initial antibiotic therapy are associated with increased mortality and morbidity [7–9]. Rapid and accurate identification of sepsis and its causative organisms are a prerequisite for successful therapy, particularly in the emergency department (ED) [10].

The current gold standard for diagnosis of sepsis is culture of blood and other body fluids or tissues. However, even in severe sepsis, blood cultures (BCs) yield the causative microorganism in only 20%–40% of patients [9, 11]. For patients who have taken antimicrobial therapy prior to initial BCs and for fastidious microorganisms, the sensitivity of BCs is even lower. Moreover, at least 24 hours are needed to obtain preliminary information about the potential organism. Therefore, initial antimicrobial therapy is usually started empirically and consists of broad-spectrum antibiotics. Early diagnosis of sepsis and its causative organism in the ED would allow more rapid de-escalation from broad-spectrum towards targeted antimicrobial therapy and could therefore prevent development of antimicrobial resistance and super-infections.

The real-time multiplex polymerase chain-reaction (MRT-PCR) test Septi*Fast*® (Roche Diagnostics, Basel, Switzerland) is designed to detect the 25 most important bacteria

and fungi causing bloodstream infections from a single whole blood sample within six hours [12]. We tested the diagnostic accuracy of MRT-PCR in comparison with the current gold standard of BCs in the early diagnosis of sepsis and its causative organism in patients presenting to the ED. We also compared the time until definite microbiological diagnosis with MRT-PCR and BCs.

Materials and methods

Study design and population

From June 2007 to January 2009 this prospective single-centre study included 110 consecutive adult patients presenting to the ED of the University Hospital of Basel (Switzerland) with suspected sepsis. A member of the research team was present at the ED from 08:00 to 18:00. As soon as patients with fever (≥38.3 °C) or hypothermia (<36.0 °C) were admitted, they were screened for other systemic inflammatory response syndrome (SIRS) criteria. Patients who had at least one additional clinical SIRS criterion (tachycardia [heart rate >90/min], tachypnoea [respiratory rate >20/min]) were asked to participate in the study. Patients with fever or hypothermia and no tachygastria or tachypnoea were also asked to participate, but were excluded from the study if they did not have leucocytosis (>12,000 cells/µl) or leucopenia (<4,000 cells/µl) in the blood sample obtained at presentation. After having obtained written informed consent, a whole blood sample was drawn for MRT-PCR together with the first BC set. The only exclusion criterion was age <18 years. Both patients with and without prior antimicrobial therapy were included.

The study was carried out in accordance with the principles of the Declaration of Helsinki and approved by the local ethics committee (Ethikkommission beider Basel, EK: 324/06). Written informed consent was obtained from all patients.

Routine clinical assessment

All patients underwent a clinical assessment that included history, physical examination, routine blood tests and – if needed – imaging studies. One pair of BCs was obtained at presentation (by venipuncture) and after 0.5–2 hours (again by venipuncture), and transported to the microbiology laboratory immediately. Simultaneously, a whole blood sample was drawn for MRT-PCR at both time points. Evaluation and treatment of patients were at the discretion of the attending physician and were performed according to standard practice of the hospital.

Primary endpoints

Reference standard

For each patient, two independent infectious disease specialists blinded to the results of the MRT-PCR analysis received all available records/results. Sepsis and its severity were defined according to the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference [13]. Patients were only considered to have sepsis if they had both SIRS and infection. Infection was defined as the injury caused by the invasion of normally sterile tissue or fluid or body cavity by pathogenic microorganisms. Typical clinical signs that were interpreted as an infection for the gold standard diagnosis were, for example: pulmonary infiltrate on chest X-ray, cholecystitis on ultrasound, significant leucocyturia in urinary analysis, visible skin rash, clinical signs of tonsillitis or otitis, etc. Accordingly, sepsis might have been only strongly suspected without being microbiologically confirmed. Therefore, we used an expanded reference standard (a combination of clinical and microbiological data) to compare BC and MRT-PCR. Patients were adjudicated as having bacterial or fungal sepsis, viral SIRS, or non-infectious SIRS. Cultures with low-virulence organisms that are part of the normal skin flora, such as coagulase-negative staphylococci (CNS), *Clostridium* spp. and *Bacillus* spp., were interpreted as contamination if only one bottle out of two was positive and the time to positivity (TTP) was >24 hours [14].

Diagnostic accuracy of MRT-PCR and blood cultures

Patients were categorised as "true positive" or "true negative" for bacterial sepsis on the basis of the expanded reference standard. This allowed for the calculation of sensitivities, specificities, negative predictive values (NPV) and positive predictive values (PPV) of MRT-PCR and BCs. Calculations were made both for the diagnosis of bacterial sepsis (n = 79) and for the diagnosis of the causative organism of sepsis (n = 36, patients who were adjudicated as having bacterial sepsis and were positive with any of the conventional microbiological methods (e.g. culture of blood/urine/sputum, rapid antigen testing in throat swabs [streptococcal rapid antigen test] or urine [*Streptococcus pneumoniae*, *Legionella pneumophila*]).

Time to diagnosis

The time to definite microbiological diagnosis was calculated on the basis of all patient cases (n = 110). If the microbiological method was negative or positive only after 72 hours, we used a cut-off of 72 hours to calculate the time to definite diagnosis. We chose a cut-off of 72 hours in order to reflect clinical practice that after a certain time period therapeutic and management decisions must be made even in the absence of a clear microbiological diagnosis. When MRT-PCR identified the causative organism earlier than the conventional microbiological method, we calculated the time (hours) gained by the use of MRT-PCR compared with the conventional microbiological methods.

Sampling and processing of blood cultures

Rapid processing of the blood cultures was ensured 24/7.

For each BC sample an average of 25 ml blood was inoculated into an aerobic and an anaerobic bottle and analysed in the BacT/Alert 3D (BioMérieux, Marcy l'Etoile, France) detection system. This system monitors carbon dioxide production within each bottle every ten minutes and records the time elapsed from the placement of each blood culture bottle in the system to the detection of microbial growth (e.g. Gram-negative rods, Gram-positive cocci, etc.). This time was considered to be the time to positivity (TTP). Bottles signalled as positive were removed from the system from 8:00 to 17:00 and an aliquot was taken for subculture on solid media.

Sampling and processing of MRT-PCR

Blood samples for MRT-PCR (LightCycler® SeptiFast® Test M^{GRADE}, Roche Diagnostics) analysis were collected into 2.7 ml tubes containing K-EDTA. After centrifugation, samples were frozen at –80°C and analysed according to the availability of the technician. The mechanical lysis of the specimens was performed using the Septi*Fast*® Lys Kit M^{GRADE}. Real-time PCR was performed in the LightCycler® 2.0 instrument (Roche Diagnostics GmbH, Mannheim, Germany). Three PCR amplification runs were performed simultaneously (for Gram-positive bacteria, Gram-negative bacteria, and fungi [yeasts and moulds]). The amplicon was then detected with fluorescence, which was measured with the LightCycler® 2.0 instrument. After completion of amplification, a melting curve analysis was performed. The melting temperature was automatically analysed by identification software.

The turnaround time of MRT-PCR results was calculated on the assumption that the MRT-PCR could be performed 24/7 and using the reported turnaround time of the assay of approximately six hours. The limit of detection is 30 colony-forming units (CFU) per ml for most of the organisms detected by the Septi*Fast*®. Exceptions are CNS, *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Candida glabrata*, for which the limit of detection is 100 CFU/ml [15].

Statistical analysis

Continuous variables are presented as means ± standard deviations or medians (with interquartile range), and categorical variables as numbers and percentages. Continuous variables were compared with the Man-Whitney U-test, and categorical variables with the chi-square test. Patients were categorised as “true positive” or “true negative” for bacterial sepsis on the basis the compound reference standard. This allowed for the calculation of sensitivities, specificities, NPV and PPV based on 2 × 2 tables. Accuracy is defined as the proportion of true results (both true positives and true negatives) in the population. Parameters of diagnostic accuracy were compared with the chi-square test. All hypothesis testing was two-tailed and a p-value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS/PC Version 15.0 (SPSS Inc., Chicago, IL).

Results

Patient characteristics

Baseline characteristics of the 110 patients who were enrolled with suspected sepsis are shown in table 1. The adjudicated diagnosis was bacterial sepsis in 79 (72%), SIRS of suspected viral origin in 12 (11%) and non-infectious SIRS in 19 (17%) patients. The severity of sepsis was graded as sepsis without organ dysfunction in 61 patients (77%), severe sepsis in 13 (17%) and septic shock in 5 (6%). The two most common foci in patients with sepsis were the lungs (n = 33, 42%) and the urinary tract (n = 19, 24%). Sixteen (15%) patients were already on antibiotic therapy when admitted to the ED. There was no difference in the severity of sepsis in patients with or without prior antibiotic treatment. Of the 16 patients who were on prior antibiotic therapy, 11 were adjudicated has having sepsis and one patient as having severe sepsis. Eleven of 12 (92%) sepsis patients who were on prior antibiotic therapy had negative BCs, and all of these patients also had negative MRT-PCR. A microbiological diagnosis by any conventional microbiological method (culture of blood/urine/sputum/skin, rapid antigen testing in throat swabs and urine) was made in 36 (46%) of patients with bacterial sepsis (table 2). The or-

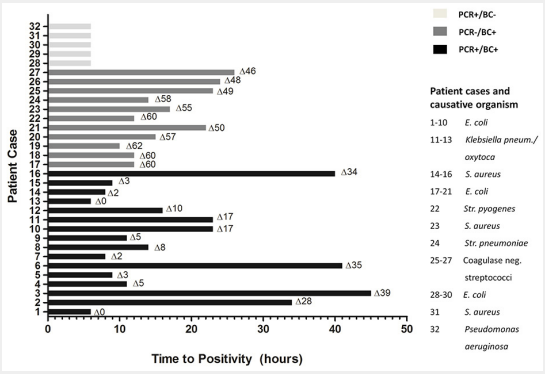

Figure 1

Time to positivity of the MRT-PCR and blood cultures.

Time to positivity (TTP) of blood cultures (BC) in the 32 patients who were either positive with BC alone (PCR–/BC+) or MRT-PCR alone (PCR+/BC–) or positive with both BC and MRT-PCR (PCR+/BC+). The 72 patients which were negative with both (PCR–/BC–) are not displayed.

TTP of MRT-PCR is 6 hours by definition. The difference (Δ hours) between TTP of BC and MRT-PCR and the causative organism are displayed in each patient. For example, if the BC was positive after 24 hours and the MRT-PCR was positive (thus after 6 hours), the ΔTTP was 18 hours. If the microbiological method was negative or positive only after 72 hours, we used a cut-off of 72 hours to calculate the TTP.

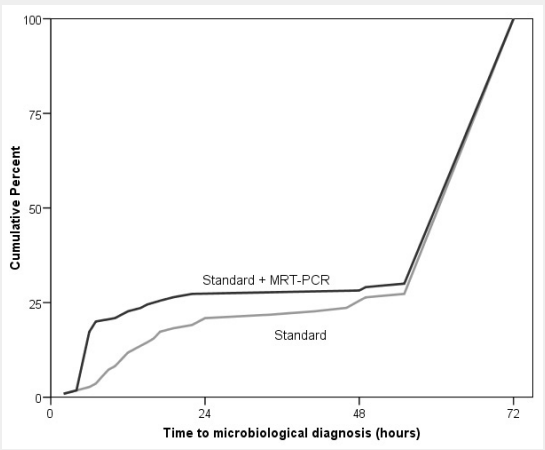

Figure 2

Time to microbiological diagnosis.

Cumulative frequency distribution curve for the time to microbiological diagnosis during the first 72 hours with conventional microbiological methods (“standard”) compared to the multiplex real-time polymerase chain reaction (MRT-PCR).

The microbiological diagnosis of the causative organism was achieved significant earlier with the combination of MRT-PCR and conventional microbiological methods compared to the conventional microbiological methods alone (Wilcoxon p ≤0.001). The median time gained until microbiological diagnosis was 3.9 hours (range 0–66).

ganisms and their corresponding detection methods are displayed in an appendix.

Concordance of MRT-PCR and BC samples

From the 110 patients we collected 205 corresponding MRT-PCR samples and BC pairs. Fifteen pairs of BC samples did not have a corresponding MRT-PCR sample owing to failure to draw blood (n = 12) or to technical failure of the MRT-PCR (internal control not detected, n = 3). Overall, there were 21 positive corresponding samples and 160 negative corresponding samples. The remaining 24 samples were either positive only by MRT-PCR or only by BC.

In total, 78 (71%) patients had negative MRT-PCR and negative BC (PCR–/BC–). Both positive MRT-PCR and BC (PCR+/BC+) were seen in 16 patients (15%) patients, 11 patients (10%) had negative MRT-PCR and positive BC (PCR–/BC+), and 5 patients (5%) had positive MRT-PCR and negative BC (PCR+/BC–). Among the 11 cases with PCR–/BC+, all pathogens should have been detected in the MRT-PCR panel and none of the patients had been on antibiotic treatment at admission: five cases had sepsis from a urogenital focus with detection of *E. coli* in BCs and/or urinary cultures, one case was pneumonia with positive BCs for *Str. pneumoniae* as well as positive urinary antigen testing for *Str. pneumoniae*, one case was cellulitis of the leg with positive BC for *Str. pyogenes* and one case was a prosthetic joint/bone infection with positive BC for *S. aureus* and identification of *S. aureus* in the bone biopsy. Among the 11 PCR–/BC+ cases there were three cases with identification of CNS in BCs. All three cases were adjudicated as contamination and BCs were accordingly judged as false-negative for the detection of the causative organism. Among the five PCR+/BC– cases all pathogens were compatible with the clinical condition but only one could be confirmed by other culture methods (*S. aureus* by BC drawn 5 days after presentation): *E. coli* in three patients with cholangitis, urinary tract infection and pneumonia, respectively, *S. aureus* in a patient with a possible syringe abscess and *P. aeruginosa* in a patient with a gastrointestinal bleeding. None of the PCR+/BC– cases was already on antibiotic treatment at admission.

Overall, the organisms of the 22 MRT–/PCR+ patient cases could be affirmed in 10 cases (45%) by other culture methods. The organisms of the 26 BC+ patient cases could be affirmed by other culture methods in 15 (58%) cases. There was no case in which discordant pathogens were found in MRT-PCR and BC.

Time to definite microbiological diagnosis

Median time to positivity (TTP) of BC was 16 hours (range 6–44 hours). If the three contaminations with CNS were excluded, median TTP was 15.5 hours (range 6–34 hours). The turnaround time for the result of the MRT-PCR was six hours. The difference (Δ hours) between TTP of BC and MRT-PCR is shown in figure 1. The time to definite microbiological diagnosis during the first 72 hours after presentation was significantly faster with the combined use of MRT-PCR and conventional microbiological methods as compared with conventional microbiological methods alone (Wilcoxon p ≤0.001; fig. 2). The overall mean time gained until definite microbiological diagnosis was 3.9 hours (range 0–66). MRT-PCR was faster than BC in 18 patients. Among these patients, the causative organism was *E. coli* in 12, *Klebsiella pneumoniae/oxytoca* in 3 and *S. aureus* in 3.

Diagnostic accuracy of MRT-PCR and BCs

Among the 79 patients with bacterial sepsis, the causative pathogen could be identified in 36 (46%) patients. The pathogen was detected by MRT-PCR in 20 (25%), by BC in 27 (34%), and by other microbiological methods in 27 (34%) patients.

Table 3 displays sensitivities, specificities, NPVs, PPVs and accuracies of BC and MRT-PCR in all patients adjudicated as having sepsis (n = 79), as well as in the patients adjudicated as having sepsis in whom the causative organism could be identified (by any conventional microbiological method) (n = 36). The sensitivity of both BCs and MRT-PCR was generally low, whereas the specificity was excellent. Accuracy of BCs and MRT-PCR was low for the diagnosis of patients with sepsis in general but good for the patients with sepsis in whom a causative organism was identified. None of the diagnostic parameters showed any significant differences between BCs and MRT-PCR when compared with chi-square analysis.

Antibiotic therapy prior to presentation at ED had been started in 14 (18%) patients with bacterial sepsis. Although the number of patients was too small to reach statistical significance, there seemed to be no difference in the diagnostic accuracy of MRT-PCR and BC when comparing patients with or without prior antimicrobial therapy. Most notably, none of the 5 PCR+/BC– patients had received prior antimicrobial therapy.

Discussion

This prospective study examined the clinical utility of MRT-PCR in the early identification of sepsis and its causative organism in 110 unselected patients presenting to the ED with suspected sepsis. First, there was moderate to high concordance between MRT-PCR and BC with 84% matching results. Second, the diagnostic accuracy of MRT-PCR was comparable to that of BC. Third, the additional use of MRT-PCR significantly reduced the time to microbiological diagnosis as compared with the use of conventional microbiological methods alone (mean time gained 3.9 hours, range 0–66 hours, p <0.001). Overall, our findings may have important clinical implications, as early diagnosis is essential to improve outcome in patients with suspected sepsis.

Clinical symptoms of sepsis are non-specific and also seen in non-infectious conditions. Definite aetiological diagnosis of sepsis requires isolation of the microorganism from blood or a local site of infection. However, BCs have several limitations, including low sensitivity, especially for slow-growing and fastidious organisms, contamination and long TTP. Because of the high morbidity and mortality associated with sepsis, it is essential rapidly to diagnose sepsis and identify its causative organism to ensure appropriate antibiotic therapy during the first hours [7-9]. Therefore, more sensitive and specific diagnostic techniques with

a shorter turnaround time are needed. Direct molecular detection of organisms in the blood with PCR is a promising method. The utility of broad-range (directed against eubacterial 16S or 23S rDNA/RNA or panfungal 8S or

Table 1: Baseline characteristics.

	All patients	Bacterial sepsis	Viral and non-infectious SIRS	p-value
	n = 110	n = 79	n = 31	
Age – median years	64	65	61	0.20
Male gender – no. (%)	56 (60)	38 (48)	18 (58)	0.35
Inclusion criteria – no. (%):				
Fever (temperature ≥38.3 °C)	107 (96)	78 (99)	29 (94)	0.14
Hypothermia (temperature <36.0 °C)	3 (3)	1 (1)	2 (7)	0.14
Tachycardia (heart rate >90/min.)	84 (76)	59 (75)	25 (81)	0.65
Tachypnoea (respiratory rate >20/min)	28 (26)	24 (30)	4 (13)	0.40
Leucocytosis (>12 x109/l)	58 (53)	47 (60)	11 (36)	0.02
Leucopenia (<4 x109/l)	8 (7)	6 (6)	2 (9)	0.55
History – no. (%):				
Diabetes mellitus (Type 1 or Type 2)	26 (24)	17 (22)	9 (29)	0.42
Chronic renal impairment	22 (20)	16 (20)	6 (20)	0.84
Immunosuppression (drug-induced or HIV)	15 (14)	14 (18)	1 (3)	0.05
Antibiotic therapy at admission – no. (%)	16 (15)	14 (18)	2 (7)	0.15
Vital status at presentation (median):				
Temperature – °C	38.9	38.9	39.1	0.45
Systolic blood pressure – mm Hg	136	136	138	0.26
Diastolic blood pressure – mm Hg	78	77	79	0.19
Heart rate – beats per minute	103	103	104	0.74
Oxygen saturation – %	97	97	96	0.83
Laboratory assessment at presentation (median):				
Leucocytes – x109/l	12.4	13.2	10.5	0.02
Neutrophils – x109/l	10.8	11.4	8.0	0.02
CRP – mg/l	75	91	42	0.14
Procalcitonin – ng/ml	0.344	0.565	0.122	0.01
Infectious focus – no. (%):				
Pulmonary	38 (35)	33 (42)	5 (16)	
Urogenital	19 (17)	19 (24)	0 (0)	
Abdominal	8 (7)	6 (8)	2 (7)	
Musculoskeletal	3 (3)	3 (4)	0 (0)	
Skin	7 (6)	7 (9)	0 (0)	
Ear-nose-throat	5 (5)	5 (6)	0 (0)	
Other	3 (3)	3 (4)	0 (0)	
Systemic	5 (5)	0 (0)	5 (16)	
No focus found	3 (3)	3 (4)	0 (0)	
Length of hospital stay (median days)	11	12	9	0.27

CRP = C reactive protein; HIV = human immunodeficiency virus; SIRS = systemic inflammatory respone syndrome

Table 2: Overview of organisms detected with multiplex real-time polymerase chain reaction (MRT-PCR), blood cultures (BC) and other microbiological methods and time to positivity (TTP) of BC in patients with bacterial sepsis (n = 79).

	Number of isolates positive with				
	BC only	MRT-PCR only	MRT-PCR and BC	Other microbiological detection method only	Median TTP of BCs (hours, range)
True positive causative organisms					
Escherichia coli	5	3	10	2	14.5 (6–41)
Klebsiella pneumoniae/oxytoca	0	0	3	0	22 (16–24)
Enterococcus spp.	0	0	0	2	–
Campylobacter jejuni	0	0	0	1	–
Legionella pneumophila	0	0	0	2	–
Streptococcus pneumoniae	1	0	0	4	14
Streptococcus pyogenes	1	0	0	3	12
Staphylococcus aureus	1	1	3	0	9 (8–17)
Oral flora	0	0	0	2	–
False positive organisms (contaminations)					
Coagulase negative staphylococci	3	0	0	0	26 (23–44)
Pseudomonas aeruginosa	0	1	0	0	–
Total	11 (14%)	5 (6%)	16 (20%)	16 (20%)	16 (6-44)

18S rDNA/RNA) and multiplex (addressing a set of different pathogens typical for a certain infection type) PCR has been investigated in various settings. Most studies were performed in distinct patient populations such as neonates, intravenous drug users, neutropenic and critically ill patients and showed wide ranges in sensitivity (54%–100%) and specificity (58%–99%) [16–23]. Our data extend and corroborate two recent studies in patients with suspected sepsis presenting to the ED [24, 25]. The diagnostic accuracy of MRT-PCR in our cohort were comparable to previous studies. The low sensitivity of both BC and MRT-PCR in our cohort may be due to the definition of our compound reference standard, which differs from many of the studies cited above. In the small subgroup of patients who were already on antibiotic treatment at admission to the ED, MRT-PCR did not offer any diagnostic benefit in comparison with BCs. To our knowledge, our study is the first study to describe the actual gain in time by using MRT-PCR. We showed that the additional use of MRT-PCR significantly reduced the time to microbiological diagnosis as compared with the use of conventional microbiological methods alone. This gain in time is based on the assumption that the MRT-PCR is available 24/7 and the median time gained of 3.9 hours (range 0–66 hours) is relatively low. Empirical antibiotic therapy has to be started during the first hours after presentation, and earlier detection of the organism would allow more rapid alteration from broad-spectrum to targeted therapy. Since most empirical antibiotic treatments are administered 4, 6 or 8/hourly, the time gain of 3.9 hours would in theory only have an influence on a single dose of antibiotics. Larger prospective randomised studies are needed to address the question as to whether a more rapid detection of the organism with MRT-PCR could improve the outcome of patients, and reduce costs and drug resistance.

Several disadvantages of MRT-PCR have to be taken into account. The amplification-based assays may potentially lead to detection of transient bacteraemia and fungaemia due to translocation from naturally colonised surfaces and even non-replicating bacteria. Such results are medically irrelevant but may be misleading and difficult to judge. In our cohort, we judged one MRT-PCR+ result (*Pseudomonas aeruginosa*) as contamination as a result of possible translocation from the intestine during gastrointestinal bleeding. The reported detection limit of MRT-PCR is 30–100 CFU/ml (detection limit of blood cultures: 1 CFU/ml), which is above the usual bacterial burden in sepsis (<10 CFU/ml). MRT-PCR may be not sensitive enough to detect adult bacteraemia, but may be particularly useful in paediatric patients where higher bacterial loads are found (around 100 CFU/ml). In addition, it is important to note that MRT-PCR does not offer broad susceptibility testing. SeptiFast® for example, can only detect mecA DNA of methicillin resistant *S. aureus* strains.

Potential limitations of the current study merit consideration. The patient cohort is relatively small, and of the 110 patients only 79 were adjudicated as having sepsis and only 36 had a positive microbiological diagnosis. The small number of patients may explain why we did not detect any statistically significant findings for the diagnostic accuracy. Our analysis was based on the reported turnaround time of six hours for the MRT-PCR and on the assumption that MRT-PCR is integrated as a routine test in clinical practice of a hospital available 24/7. Therefore, our calculations should apply to most large hospitals, but not necessarily to smaller and medium sized hospitals because the case load might be too small to warrant processing of MRT-PCR 24/7. In those institutions, the time gain will be smaller.

Conclusion

In conclusion, diagnostic accuracy of BCs and MRT-PCR in the early diagnosis of sepsis and its causative organism in the ED are comparable. However, MRT-PCR reduces the time to microbiological diagnosis. Whether a more rapid detection of the organism by MRT-PCR could improve the outcome of patients has to be assessed in large prospective randomised trials. Until now MRT-PCR does not offer broad susceptibility testing and therefore can currently be used only as an adjunct method to BCs.

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Table 3: Diagnostic accuracy of blood cultures and MRT-PCR*.

		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
Bacterial sepsis (n = 79)	**Blood cultures**	0.30 (0.20–0.41)	1.00 (0.86–1.0)	1.00 (0.83–1.0)	0.35 (0.26–0.46)	0.49 (0.40–0.58)
	MRT-PCR	0.25 (0.16–0.36)	0.97 (0.81–1.0)	0.95 (0.74–1.0)	0.33 (0.24–0.44)	0.45 (0.36–0.54)
Bacterial sepsis with positive microbiology (n = 36)**	**Blood cultures**	0.59 (0.42–0.73)	1.00 (0.93–1.0)	1.00 (0.83–1.0)	0.81 (0.71–0.88)	0.85 (0.77–0.90)
	MRT-PCR	0.44 (0.29–0.60)	0.99 (0.91–1.0)	0.95 (0.72–1.0)	0.75 (0.65–0.83)	0.79 (0.70–0.85)

CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value
* p-values for all comparisons of BC and MRT-PCR with chi-square analysis were not significant
** any conventional microbiology method (blood cultures, urinary culture, antigen testing in sputum etc.)

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Appendix

Causative organisms and corresponding microbiological detection methods.

Causative microorganisms	**Microbiological detection methods**							
	Blood culture	Urine culture	Urine serology	Sputum/BAL culture	Stool culture	Skin swab culture	Throat swab culture	Other material
Escherichia coli	14	10	0	0	0	0	0	0
Klebsiella pneumoniae / oxytoca	3	3	0	0	0	0	0	0
Enterococcus spp.	0	2	0	0	0	0	0	0
Campylobacter jejuni	0	0	0	0	1	0	0	0
Legionella pneumophila	0	0	2	1	0	0	0	0
Streptococcus pneumoniae	1	0	3	1	0	0	0	0
Streptococcus pyogenes	1	0	0	0	0	1	2	0
Staphylococcus aureus	3	0	0	0	0	0	0	0
Coagulase negative staphylococci	3	0	0	0	0	0	0	0
Other bacteria	0	0	0	1[1]	0	0	0	0
Varicella zoster virus	0	0	0	0	0	0	0	1[2]
Dengue virus	0	0	0	0	0	0	0	1[3]

[1] oral flora, [2] polymerase chain-reaction (PCR) from broncho-alveolar lavage (BAL), [3] PCR from blood

Figures (large format)

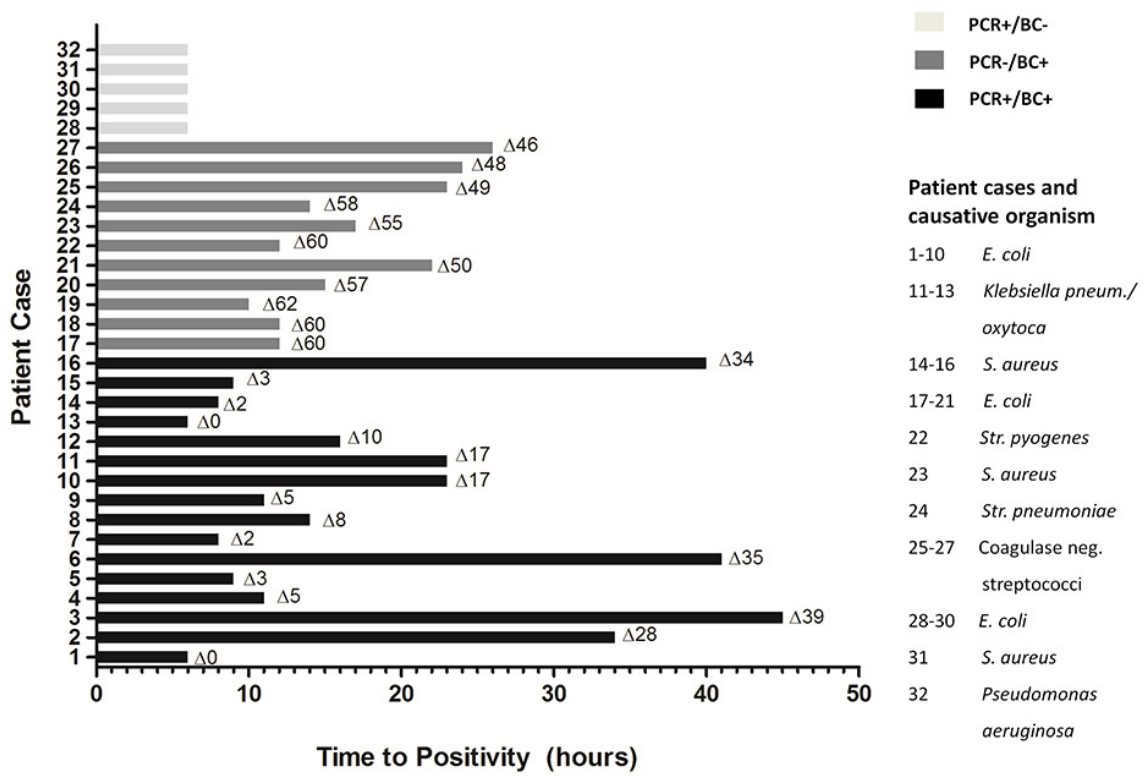

Figure 1

Time to positivity of the MRT-PCR and blood cultures.

Time to positivity (TTP) of blood cultures (BC) in the 32 patients who were either positive with BC alone (PCR–/BC+) or MRT-PCR alone (PCR+/BC–) or positive with both BC and MRT-PCR (PCR+/BC+). The 72 patients which were negative with both (PCR–/BC–) are not displayed. TTP of MRT-PCR is 6 hours by definition. The difference (Δ hours) between TTP of BC and MRT-PCR and the causative organism are displayed in each patient. For example, if the BC was positive after 24 hours and the MRT-PCR was positive (thus after 6 hours), the ΔTTP was 18 hours. If the microbiological method was negative or positive only after 72 hours, we used a cut-off of 72 hours to calculate the TTP.

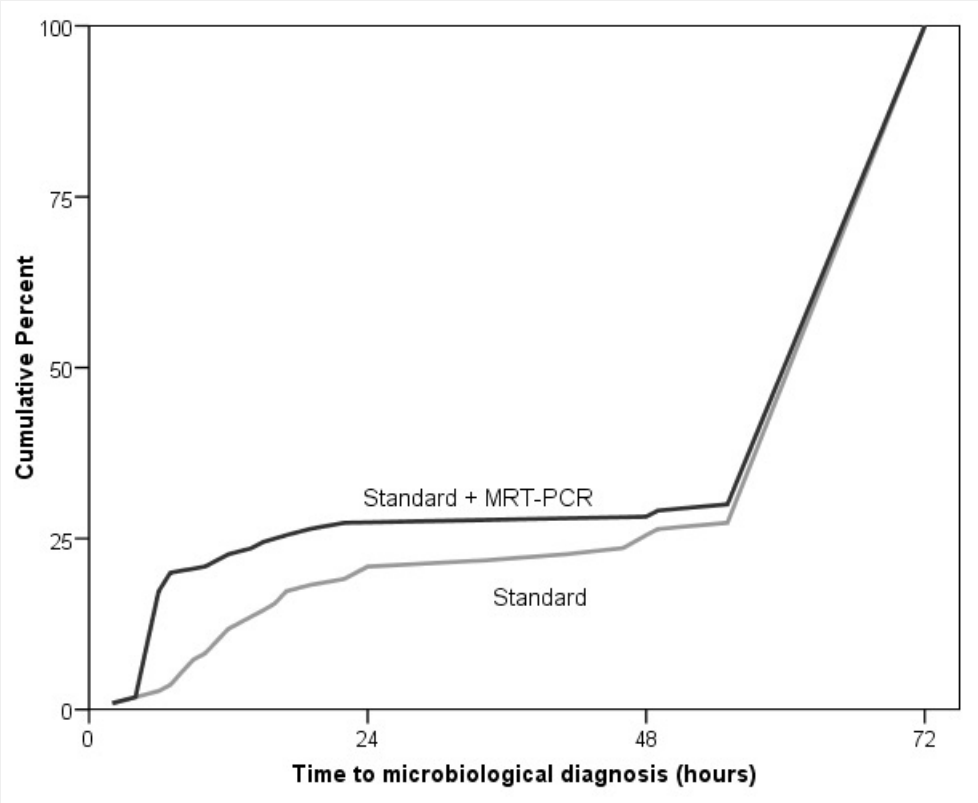

Figure 2

Time to microbiological diagnosis.

Cumulative frequency distribution curve for the time to microbiological diagnosis during the first 72 hours with conventional microbiological methods ("standard") compared to the multiplex real-time polymerase chain reaction (MRT-PCR).

The microbiological diagnosis of the causative organism was achieved significant earlier with the combination of MRT-PCR and conventional microbiological methods compared to the conventional microbiological methods alone (Wilcoxon p ≤0.001). The median time gained until microbiological diagnosis was 3.9 hours (range 0–66).