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Monitoring of Patients Supported by Extracorporeal Membrane Oxygenation for Systemic Infections by Broad-Range rRNA Gene PCR Amplification and Sequence Analysis

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The rRNA gene PCR and sequencing test, SepsiTest, was compared with blood culture (BC) regarding the diagnosis of pathogens in 160 blood samples drawn from 28 patients during extracorporeal membrane oxygenation. With 45% of positive samples, SepsiTest was 13 to 75 h faster than BC. SepsiTest indicated bacteremias in 25% of patients who were BC negative.

Patients supported by extracorporeal membrane oxygenation (ECMO) are at high risk for microbial systemic infections (1, 2) and therefore are continuously monitored by clinical and inflammatory markers. Microbiological results are used to tailor antibiotic treatment to the most effective regimen. Unfortunately, etiologies are typically identified within 1 to 2 days, and up to 31% of cultures remain negative because of limited blood volume analyzed, growth failure of fastidious organisms, inhibition of growth of pathogens by antibiotics, and other unknown reasons (3, 4). Molecular methods are discussed as tools for improvement of the diagnosis of septicemia (5, 6). Among them, panbacterial and panfungal rRNA gene PCR followed by sequencing identifies the broadest range of pathogens (7–9). Here, we evaluated the usefulness of a broad-range PCR test, SepsiTest (6, 10–14), for the monitoring of patients for DNA of pathogens in the blood during ECMO.

The study was approved by the Ethics Committee of Hannover Medical High School. SepsiTest results were not considered for the administration of antibiotics. ECMO criteria included cardiac or respiratory failure with beginning or preexisting organ failure.

Single pairs of aerobic/anaerobic Bactec Plus bottles (BD, Heidelberg, Germany) and Bactec Mycosis-IC/F bottles were incubated each with 10 ml of blood collected from a venipuncture. Incubation was for 7 (bacteria) or 14 (fungi) days in case of negative results. Species were identified using Vitek 2 (bioMérieux, Marcy l'Etoile, France). Other samples were analyzed according to standard microbiological operations of the laboratory.

Duplicate samples (1 ml) of EDTA blood (from the same venipuncture as blood culture [BC]) were analyzed using SepsiTest (Molzym, Bremen, Germany) (6, 10–14), which supplies protocols and reagents for DNA extraction, 16S and 18S rRNA gene PCR, and negative, positive, and internal PCR controls. Amplicon sequencing was done by an overnight service. BLAST results (www.sepsitest-blast.net) were classified at the species (≥99% sequence identity) and genus (≥97%) levels (12). Corynebacteria, viridans streptococci, coagulase-negative staphylococci (CNS), and *Klebsiella oxytoca*/*Enterobacter cloacae* were not resolvable at the species level. Mixed sequences were resolved by Ripseq analysis (Isentio, Paradis, Norway).

Defined criteria were applied to interpret BC-negative, SepsiTest-positive results. "Probable" systemic infection was supposed if (i) results were supported by cultured material (12) before, during, or after ECMO and/or (ii) organisms were found in repeated blood draws within 48 h that were regarded clinically significant on the grounds of the chart reviews of the patients, including disease, inflammatory, and biochemical markers, leukocyte counts, and clinical course (15). "Possible" systemic infection was considered if a pathogen typical for ECMO patients was identified (2) but not cultured. Results were judged "indeterminate" if an organism was a rare pathogen or nonpathogenic species without correlation with culture.

From January 2010 to July 2011, 160 samples from 28 patients (18 males, 10 females) were collected at various times of ECMO. The median age was 47.7 years (range, 18.6 to 74.6), 6 blood samples were drawn per patient (range, 1 to 11), and ECMO lasted for 8 days (range, 1 to 18). Sixteen patients (57%) died during or within 2 months after ECMO.

Among the 14 BC-positive samples (8.8%; 7 patients), 12 (7.5%; 6 patients) were positive with bacteria and 2 with *Candida* spp. (1.3%; 2 patients). SepsiTest identified bacteria in 28 samples (17.5%; 13 patients) and *Candida glabrata* in 1 sample (0.6%). Mixed infections were observed by BC in 1 sample (0.6%) and by SepsiTest in 7 samples (4.4%; 5 patients). Compared with BC, the sensitivity, specificity, positive and negative predictive values, and concordance of positive and negative results of SepsiTest were 78.6, 88.4, 39.3, 97.7, and 87.5%, respectively.

Identical species were found in 9 samples (5 patients) (Table 1). Among the discordant results, SepsiTest identified *Staphylococcus aureus* (BC, CNS; patient 4, day 1), which corresponded to pre-ECMO and tracheal swab results and thus was probably true. Detection of both CNS and *Facklamia languida* lacked microbiological support (BC, *C. glabrata*; patient 5, pre-ECMO) and therefore probably reflects contaminations.

Received 11 September 2013 **Returned for modification** 16 September 2013
Accepted 17 October 2013
Published ahead of print 23 October 2013
Editor: A. B. Onderdonk
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doi:10.1128/JCM.02493-13

TABLE 1 Positive results of the diagnosis of blood from ECMO patients

Test result and patient no. (diagnosis)	Parameter	Result by sampling time[a]										
		Pre-ECMO	8 h	1 day	3 days	5 days	7 days	9 days	11 days	13 days	15 days	17 days
BC positive, SepsiTest positive												
1 (partial respiratory insufficiency, arterial/pulmonal hypertony)	BC	–	–	–	–	–	–	–	*E. faecium*	*E. faecium*	CNS	CNS
	SepsiTest	–	–	–	–	–	–	–	*E. faecium* (99.6)	*E. faecium* (99.8)	–	*E. faecium* (99.8), CNS (100)
	Other[b]											
	AB treatment	Cef [until 8 h]	Mox [until 11 days]			Una [until 11 days]		Flu [until 11 days]	Van [until 11 days], Cas, Mer, Dap, Tob [until 17 days]			
2 (ARDS,[c] lung emboly, infarctious pneumonia, pulmonary hypertonia, colitis ulcerosa)	BC	–	–	–	–	*Serratia marcescens*	*S. marcescens*	–	–	–	–	
	SepsiTest	–	–	–	–	*S. marcescens* (100)	*S. marcescens* (99.6)	–	–	–	–	
	Other	*S. marcescens*				*S. marcescens*, *E. cloacae*						
	AB treatment					Mer, Van [until 15 days]						
3 (ARDS, hypercapnia, respiratory acidosis, arterial acidosis)	BC	–	–	–	–	–	*S. aureus*	*S. aureus*, CNS				
	SepsiTest	–	–	–	–	–	*S. aureus* (99.5)	*S. aureus* (100)				
	Other	*S. aureus*, *Stenotrophomonas maltophilia*				*S. aureus*, *S. maltophilia*						
	AB treatment	Mer, Dif [until 7 days]				Mox [until 7 days]						
4 (ARDS, sepsis, pneumonia, cystic fibrosis, pancreas insufficiency)	BC	MRSA	ND	CNS	–	–	–					
	SepsiTest	*S. aureus* (99.3)	ND	*S. aureus* (99.2)	–	–	CNS (100)					
	Other	MRSA		MRSA		MRSA						
	AB treatment	Lin [until 7 days], Mer [until 5 days]	Tob [until 5 days], Ani [until 3 days]		Dif, Gen [until 7 days]							
5 (acute myocardial infarct, myocardial hypotrophy, renal failure, lymphoma, pneumonia)	BC	*C. glabrata*	–	*E. faecium*	–	–						
	SepsiTest	*F. languida* (99.3), CNS (99.8)	–	*E. faecium* (99.6), *C. glabrata* (100)	–	–						
	Other	*E. faecium*				*C. glabrata*, *C. albicans*						
	AB treatment	Ert, Cot [until 5 days]	Mox [until 5 days]	Van, Mer, Flu [until 5 days]								
BC positive, SepsiTest negative												
6 (ARDS, pneumothorax, open ankle fracture, renal failure)	BC	–	–	–	–	*C. albicans*						
	SepsiTest	–	–	–	–	–						
	Other	*C. albicans*										
	AB treatment	Van [until 5 days]	Ery [until 3 days]		Cef [until 5 days]							
7 (ARDS, multiorgan failure)	BC	–	ND	–	*E. faecium*	–						
	SepsiTest	–	ND	–	–	–						
	Other											
	AB treatment	Taz, Cas [until 5 days], Mox [until 8 h]				Lin						

[a] Pre-ECMO, approximately 30 min before start of ECMO support; –, negative result; CNS, coagulase-negative staphylococci; MRSA, methicillin-resistant *Staphylococcus aureus*. Numbers in parentheses are percent sequence identities to the reference (unidirectional sequencing) of 16S (450-bp) and 18S (320-bp) rDNA amplicons. Duration of treatment is given in square brackets. The coverage of all sequences analyzed is 100%; read length is 380 to 400 bp (16S) and 240 to 260 bp (18S). Amp, ampicillin; Ani, anidulafungin; Cas, caspofungin; Cef, cefuroxime; Cot, cotrimoxazole; Dap, daptomycin; Dif, diflucan; Ert, ertapenem; Ery, erythromycin; Flu, flucloxacillin; Gen, gentamicin; Lin, linezolid; Mer, meropenem; Met, metronidazole; Mox, moxifloxacin; Taz, tazobactam; Tob, tobramycin; Una, sultamicillin (Unacid); Van, vancomycin.

[b] Other, swabs from tracheal exudates, perfusates, wounds, pharynx, catheter, urine, or bronchoalveolar lavage or cultures of tissue biopsies.

[c] ARDS, acute respiratory distress syndrome.

TABLE 2 BC-negative, SepsiTest-positive results

Patient no. (diagnosis)	Parameter	Result by sampling time[a]								Interpretation		
		Pre-ECMO	8 h	1 day	3 days	5 days	7 days	9 days	11 days	Probable	Possible	Indeterminate
8 (ARDS[c], pneumonia, pneumothorax)	SepsiTest	*Corynebacterium* spp. (99.7)	−	−	−	CNS (100)	CNS (99.7), *K. oxytoca/E. cloacae* (100)	CNS (99.6), *M. osloensis* (100)	CNS (100)	CNS	*K. oxytoca/E. cloacae*	*Corynebacterium* spp., *M. osloensis*
	Other[b]					*Enterococcus* spp.						
	AB treatment	Dif, Mox, Flu [until 11 days]				Mer, Van [until 11 days]	Met [until 11 days]					
9 (broncho-adeno carcinoma, pneumonia, sepsis/septic shock, pulmonal edema)	SepsiTest	−	−	−	−	−	CNS (99.7)	CNS (99.2)		CNS		
	Other											
	AB treatment	Mer, Van, Flu [until 9 days]										
10 (chronical heart insufficiency (endocarditis), colitis ulcerosa)	SepsiTest	*Corynebacterium* spp. (99.2)	−	−	*Corynebacterium* spp. (99.7)	−	ND	−				*Corynebacterium* spp.
	Other				*E. cloacae*		*S. maltophilia*					
	AB treatment				Dap [until 9 days]	Tob [until 7 days]						
11 (bronchiolitis obliterans, ARDS, pneumonia, pneumothorax)	SepsiTest	CNS (99.7)	−	CNS (99.7)	*Streptococcus* spp. (100)	−	−			CNS	*Streptococcus* spp.	
	Other				CNS		CNS, *Haemophilus parainfluenzae*					
	AB treatment	Cas, Mer, Mox [until 7 days]	Van, Met [until 7 days]				Tob					
12 (heart insufficiency, pulmonary hypertony)	SepsiTest	−	−	−	−	CNS (99.7), *Corynebacterium* spp. (100)	−			CNS		*Corynebacterium* spp.
	Other				CNS							
	AB treatment					Flu [until 7 days]	Van					
13 (heart and respiratory insufficiency, idiopathic pulmoarterial hypertony, renal failure)	SepsiTest	−	−	−	−	*S. aureus* (100)				*S. aureus*		
	Other					*S. aureus*						
	AB treatment					Mer, Tob, Flu						
14 (myocardial infarct, cardial shock, cardiovascular disease, renal failure)	SepsiTest	−	−	−	*Enterococcus cecorum* (99.4), *A. baumannii* (100)						*E. cecorum*, *A. baumannii*	
	Other	*Enterococcus* spp., *C. albicans*										
	AB treatment	Amp [until 8 h]	Mer [until 3 days]	Van [until 3 days]								
15 (prosthetic mitral valve endocarditis, pneumonia, renal failure)	SepsiTest	*E. faecalis* (99.6)								*E. faecalis*		
	Other[d]											
	AB treatment	Gen, Amp										

[a] Pre-ECMO, approximately 30 min before start of ECMO support; −, negative result; CNS, coagulase-negative staphylococci; MRSA, methicillin-resistant *Staphylococcus aureus*. Numbers in parentheses are percent sequence identities to the reference (unidirectional sequencing) of 16S (450-bp) and 18S (320-bp) rDNA amplicons. Duration of treatment is given in square brackets. The coverage of all sequences analyzed is 100%; read length is 380 to 400 bp (16S) and 240 to 260 bp (18S). Amp, ampicillin; Ani, anidulafungin; Cas, caspofungin; Cef, cefuroxime; Cot, cotrimoxazole; Dap, daptomycin; Dif, diflucan; Ert, ertapenem; Ery, erythromycin; Flu, flucloxacillin; Gen, gentamicin; Lin, linezolid; Mer, meropenem; Met, metronidazole; Mox, moxifloxacin; Taz, tazobactam; Tob, tobramycin; Una, sultamicillin (Unacid); Van, vancomycin.

[b] Other, swabs from tracheal exudates, perfusates, wounds, pharynx, catheter, urine, or bronchoalveolar lavage or cultures of tissue biopsies.

[c] ARDS, acute respiratory distress syndrome.

[d] *E. faecalis* in BC 3 weeks before ECMO support.

TABLE 3 Comparison of BC and SepsiTest time to results

Patient no.	Sample time[a]	Organism		BC TTP (h)[b]	Minimum time gain by SepsiTest (h)[c]
		BC	SepsiTest		
1	11 days	*E. faecium*	*E. faecium*	11.1/10.9	−
	13 days	*E. faecium*	*E. faecium*	6.0/6.7	−
	17 days	CNS	CNS, *E. faecium*	51.6/96.6	27
2	5 days	*S. marcescens*	*S. marcescens*	6.4/6.4	−
	7 days	*S. marcescens*	*S. marcescens*	Neg/37.3	13
3	7 days	*S. aureus*	*S. aureus*	12.1/13.4	−
	9 days	*S. aureus*, CNS	*S. aureus*	11.0/15.1	−
4	Pre-ECMO	*S. aureus*	*S. aureus*	Neg/99.8	75
	1 day	CNS	*S. aureus*	Neg/50.1	26
5	Pre-ECMO	*C. glabrata*	*F. languida*, CNS	121	NA
	1 day	*E. faecium*	*E. faecium*, *C. glabrata*	Neg/13.5	−/73[d]

[a] Refer to Table 1.
[b] Results for aerobic/anaerobic culture; Neg, negative culture; TTP, time to positive.
[c] Values are the differences between the shortest time to positivity of pairs of aerobic and anaerobic cultures and the SepsiTest turnaround time (24 h); −, SepsiTest was slower; NA, not applicable (contamination).
[d] − refers to *E. faecium*; value, considering the TTP of *C. glabrata* at pre-ECMO and SepsiTest analysis at day 1.

Three BC-positive samples (CNS; patient 1, day 15; *Enterococcus faecium*, *Candida albicans*; Table 1) were SepsiTest negative. Correct results from internal control runs excluded PCR inhibition (not shown). Also, the organisms are detectable by SepsiTest. Perhaps microbial counts, which are reported to be low in the blood (1 to 30 CFU/ml) (16, 17), were beneath the detection limit of SepsiTest (10 to 20 CFU/ml, according to the manual). Analysis of blood volumes higher than used here may be an option of increasing the diagnostic sensitivity of SepsiTest (18).

Eight BC-negative patients (16 samples) were SepsiTest positive (Table 2). CNS were found in successive blood draws from patients 8 (days 5 to 11), 9 (days 7 and 9), and 11 (pre-ECMO and day 1). In patient 11, CNS was supported by cultures of a lung biopsy specimen (day 3), BAL fluid (day 7), and blood drawn 4 days after ECMO (not shown). CNS and *S. aureus* in patients 12 and 13 (both day 5) were in accordance with cultures of tracheal secretion (day 3) and lung perfusate (day 5), respectively. *Enterococcus faecalis* (patient 15) matched results of a BC 3 weeks before (not shown). All above-given results were considered probable bacteremias according to the definitions described above, including chart review (Table 2). Nonetheless, in particular in the case of CNS, further discrimination on the strain level by analysis of other genes more variable than the 16S rRNA gene would be necessary to exclude contamination.

In patient 14 (day 3), results from SepsiTest and a cultured operative wound swab at pre-ECMO corresponded on the *Enterococcus* species level, which, like *Streptococcus* spp. (patient 11, day 3), a *K. oxytoca* or *E. cloacae* strain (patient 8, day 7), and *Acinetobacter baumannii* (patient 14, day 3), represent pathogens typical for ECMO patients (2). These results were judged possible bacteremias (Table 2). *Corynebacterium* spp. (patients 8, 10, and 12) and *Moraxella osloensis* (patient 8) were considered indeterminate.

Considering available data, including a turnaround time of 24 h until sequence identification and the time to positivity of BC, SepsiTest results were obtained 13 to 75 h earlier than BC with 5 of the 11 positive samples (45%) (Table 3). Another benefit of SepsiTest was the detection of culture-negative infections, which applied to 7 of 28 patients (25%) (Table 2). The reason why BC were negative is unclear but may be explained by growth inhibition as a cause of antibiotic treatment during ECMO. Notably, SepsiTest indicates bacteremia rather than DNAemia, because floating DNA is removed during extraction (10, 11).

The usefulness of monitoring for systemic infections is of debate in view of prophylactic antibiotic treatment in the management of ECMO patients (19). Kühn et al. (20) discussed persistent septic complications as an indication for the exchange of the membrane oxygenator (MO). They found 45% of membranes colonized by bacteria, fungi, or multiple strains which were discussed as potential sources of bloodstream infections associated with an increased risk for clinical worsening. Interestingly, MOs of patients 1, 3, and 13 (Tables 1 and 2) were colonized by the same bacteria (not shown) found by SepsiTest in the blood. This finding supports the hypothesis of dispersal of microorganisms from MOs into the bloodstream.

Clearly, molecular tests add a further economic burden to the generally high costs of ECMO patient monitoring. However, considering the facts that in a considerable fraction of samples SepsiTest presented results up to days earlier than BC and detected clinically relevant bacteremias that were missed by BC, the additional costs can be put into perspective.

ACKNOWLEDGMENTS

This work was supported by the German Ministry of Economy (Bundeswirtschaftsministerium) under the program ZIM 2202601 AJ9.

We declare that we do not have conflicts of interest.

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