

Diagnosics of sepsis – molecular techniques



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Epidemiology of sepsis in children

- **Odetola FO et al. 2007**

2003- 21.448 hospitalizations for severe sepsis , age 0-19 years, „in hospital“ mortality 4,2%

- **Mangia KMF et al. 2011 PlosOne**

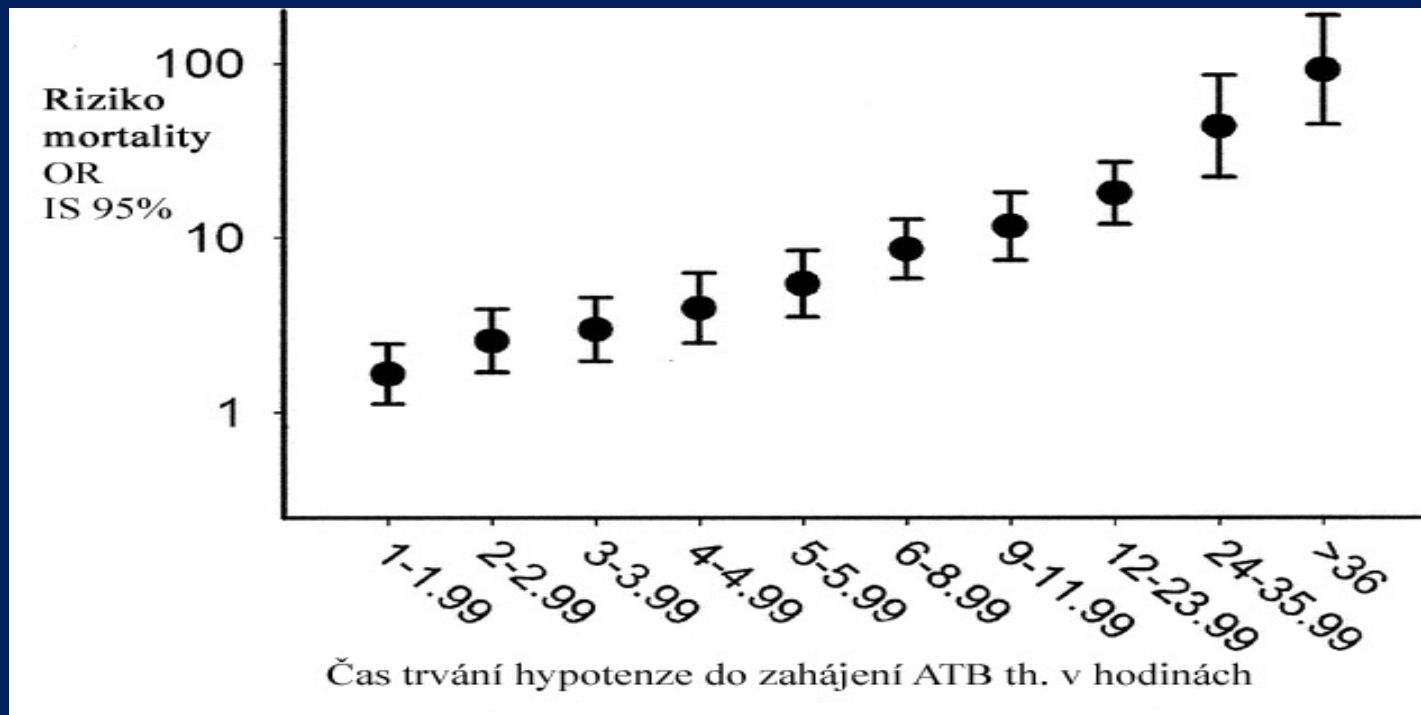
Trends 1992-2006 retrospectively, 556.093 cases of sepsis, mean mortality 19,9% ; a case reduction of 67% over 1992-2006 , the mortality rate remained unchanged (from 1992-1996, 20.5%; and from 2002-2006, 19.7%).

- **Inwald DP et al. 2009**, Arch Dis Child., Emergency management of children with severe sepsis in the United Kingdom: the results of the Paediatric Intensive Care Society sepsis audit.

200 children accepted for PICU admission with a discharge diagnosis of sepsis or suspected sepsis, 34/200 (17%) children died

overall fluid and inotrope management suggested by the 2002 ACCM-PALS guideline was not followed in 62% of shocked children

Sepsis – critical factor in diagnostics is time



Kumar A et al 2006

Each hour of delay in antimicrobial administration over the ensuing 6 hrs was associated with an average decrease in survival of 7.6%. Median time to effective antimicrobial therapy was 6 hrs (25-75th percentile, 2.0-15.0 hrs). Only 50% of septic shock patients received effective antimicrobial therapy within 6 hrs of documented hypotension.

Molecular methods in the diagnosis of neonatal sepsis

Hybridization methods

- FISH
- PNA FISH
- Probe hybridization
- Microarrays

Amplification methods

- PCR
- Broad-range PCR
- Pathogen-specific PCR
- Multiplex PCR

Post-amplification detection strategies

- PCR + sequencing
- PCR + pyrosequencing
- PCR + hybridization
- PCR + MALDI-TOF-MS

Non-nucleic acid methods

- Proteomics
- Spectroscopy
- Phage assays

Molecular assays in neonatal sepsis

- **Venkantesh M et al: Molecular microbiological methods in the diagnosis of neonatal sepsis, 2010**
- cohort of 6093 extremely low birthweight infants (ELBW; birthweight ≤ 1000 g)
- the sensitivity of molecular methods used to diagnose sepsis ranged from 41.1 to 100%, and specificity from 77.2 to 100%.
- The most widely studied method was broad-range PCR targeting sequences within the 16S rRNA gene
- The sensitivity of PCR improved with preamplification culture of samples for 5 h
- sensitivity was low (50%) in the only study that evaluated multiplex PCR targeting eight pathogens

Molecular techniques

Hybridization

- **FISH** (Fluorescence In Situ Hybridization) techniques – genus a species Gescher et al. 2008 , FISH probes were genus specific and species specific for G+ cocci; specificity 99%, sensitivity 98,7% (from BC specimens)
Kudo M et al: 2009: 60 patients with suspected sepsis (BC)
FISH – positive 41,7%, BC 11,7%, in patients treated ATB – FISH 61,9%, BC 11,7%
- **PNA –FISH technics** (peptide nucleic acid) with synthetic oligomers Forrest GN, Roghmann MC, Toombs LS, et al. Peptide nucleic acid fluorescent *in situ hybridization* for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy. Antimicrob Agents Chemother 2008;52(10):3558–3563. **Decreased 30-day mortality!!!!**

Fluorescence In Situ Hybridization

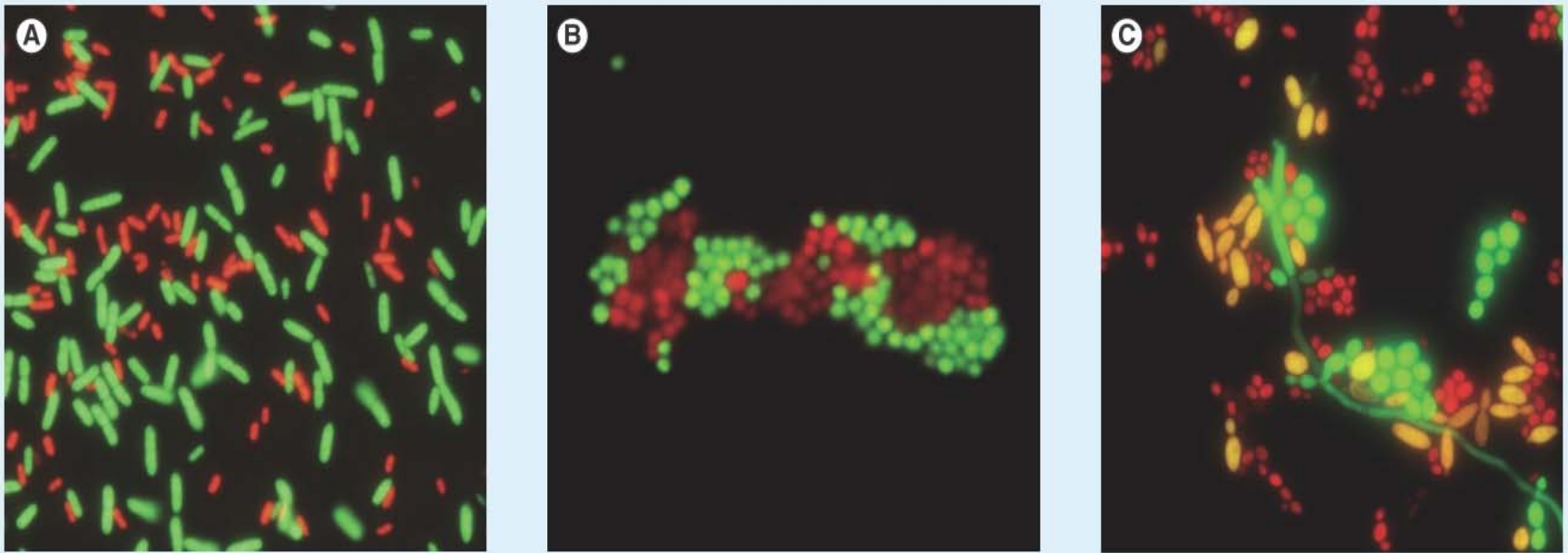


Figure 2. Peptide nucleic acid FISH. (A) A mixed culture of *Escherichia coli* and *Pseudomonas aeruginosa* was fixed to the slide and hybridized with the *E. coli*/*P. aeruginosa* probes. *E. coli* appears green and *P. aeruginosa* red. **(B)** A mixed culture of *Staphylococcus aureus* and *Staphylococcus epidermidis* was fixed to the slide and hybridized with specific PNA FISH probes. *S. aureus* appears green and *S. epidermidis* red. **(C)** A mixed culture of *Candida albicans*, *Candida tropicalis* and *Candida glabrata* was fixed to the slide and Yeast Traffic Light probe was applied. *C. albicans* appears green, *C. tropicalis* yellow and *C. glabrata* red. PNA FISH images were supplied by AdvanDx, MA, USA.

Table 1. Neonatal studies using molecular methods for the diagnosis of sepsis.

Study (year)	Population	Samples	Type of molecular assay	SEN (%)	SPEC (%)	PPV (%)	NPV (%)	Detection time (h)	Limitations	Ref.
Laforgia <i>et al.</i> (1997)	Neonates	Blood (n = 33)	Broad-range conventional PCR	100	93.1	66.6	100	Rapid detection	Small sample size	[35]
Jordan and Durso (2000)	Neonates	Blood (n = 548)	Broad-range conventional PCR and DNA dot blot analysis after 5 h preamplification culture	96	99.4	88.9	99.8	Rapid detection		[33]
Shang <i>et al.</i> (2001)	Neonates	Blood (22) and CSF (4) 30 healthy children were controls	Broad-range PCR followed by reverse hybridization with Gram-specific probes					≤6	Test indices not reported	[59]
Villaneuva-Uy <i>et al.</i> (2003)	Neonates with LOS	Blood (n = 61)	Broad-range 16S rRNA conventional PCR	78	100	100	83	9	Only abstract	[40]
Tong <i>et al.</i> (2004)	Neonates	Blood (n = 285)	16S rRNA-based PCR followed by hybridization to chips with 18 probes	100	96.8	47.1	100			[60]
Jordan and Durso (2005)	Neonates	Blood (n = 86)	Real-time 16S rRNA PCR	96	100	100	94.2	~4	Did not detect <i>Haemophilus influenzae</i> or enterococci	[80]
Yadav <i>et al.</i> (2005)	Neonates	Blood (n = 100)	Broad-range 16S rRNA PCR	100	95	69	100	Rapid detection		[36]
Makhoul <i>et al.</i> (2005)	Neonates with LOS	Blood (n = 215)	Staphylococcal 16S rRNA PCR (both <i>Staphylococcus aureus</i> and CONS)	69.2	100	100	98	<4	Low sensitivity	[48]
Makhoul <i>et al.</i> (2006)	Neonates with LOS	Blood (n = 148)	Staphylococcal 16S rRNA PCR (both <i>S. aureus</i> and CONS)	57.1	94.7	53.3	95.4	<4	Low sensitivity	[50]
Jordan <i>et al.</i> (2006)	Near term infants (>34 weeks)	Blood (n = 1233)	Conventional PCR based on 16S rRNA assay followed by pyrosequencing	41.1	97.5	18.9	99.2	Rapid detection	Low sensitivity	[34]
Wu <i>et al.</i> (2008)	Neonates	Blood (n = 600)	Real-time PCR with Gram-specific probes followed by sequencing	100	97.1	68	100	~3		[81]
Enomoto <i>et al.</i> (2009)	Newborn (23–41 weeks GA)	Blood, CSF, urine, BAL, skin, ascites, pharyngeal mucus (n = 130)	Multiplex PCR targeting eight pathogens	50	93	38	96	3.5–4.5	Low sensitivity	[51]
Reier-Nielsen <i>et al.</i> (2009)	Newborn (Bwt >1000 g and ≤7 days)	Blood (n = 48)	Broad-range 16S rRNA PCR followed by sequencing of PCR products	66.6	85.7	40	94.7	Rapid detection	Low sensitivity and PPV	[30]

BAL: Bronchoalveolar lavage; Bwt: Birthweight; CONS: Coagulase-negative staphylococci; CSF: Cerebrospinal fluid; FQ: Fluorescence quantitative; GA: Gestational age; ITS: Internal transcribed spacer regions; LOS: Late-onset sepsis; NICU: Neonatal intensive-care unit; NPV: Negative predictive value; PICU: Pediatric intensive care unit; PPV: Positive predictive value; SEN: Sensitivity; SPEC: Specificity.

PCR and invasive candidiasis

- **Avni T 2011: PCR Diagnosis of Invasive Candidiasis: Systematic Review and Meta-Analysis**
- Were included **54 studies** with 4,694 patients, 963 of whom had proven/probable or possible IC.
- Perfect (100%) sensitivity and specificity for PCR in whole-blood samples was observed when patients with cases had candidemia and controls were healthy people
- When PCR was performed to evaluate patients with suspected invasive candidiasis, the pooled sensitivity for the diagnosis of candidemia was 0.95 (confidence interval, 0.88 to 0.98) and the pooled specificity was 0.92
- **PCR positivity rates among patients with proven or probable IC were 85% (78 to 91%), while blood cultures were positive for 38% (29 to 46%)**

PCR and viral infections

Van de Pol AC 2006: Diagnostic value of real-time polymerase chain reaction to detect viruses in young children admitted to the paediatric intensive care unit with lower respiratory tract infection

Table 1

Demographic and clinical characteristics of children with lower respiratory tract infection on admission to the PICU

Characteristic	Value
Demographics	
Age, median months (range)	2.6 (0.5–26.5)
Male	10 (43%)
Admissions from outside hospital	20 (87%)
Underlying conditions	
Preterm birth (<37 weeks)	11 (48%)
Underlying disease	11 (48%)
Pulmonary	3
Cardiac	4
Other	5
Severity	
ICU stay (days; median [range])	10 (2–33)
Mechanically ventilated at PICU	20 (87%)
Deaths due to LRTI	1

A total of 23 patients were included in the study. LRTI, lower respiratory tract infection; PICU, paediatric intensive care unit.

Viruses Identified by conventional methods and real-time PCR

Pathogen	Viral culture (n = 21)	Immunofluorescence (n = 22)	Real-time PCR (n = 23)
RSV A/B	4	7	16 (9)
Influenzavirus A/B	0	2	3 (1)
Rhinoviruses	0		6 (2)
Adenoviruses	2	2	3 (1)
Coronavirus OC43, 229E, NL63			3
hMPV			1 (1)
PIV 1/3	0	0	1
PIV 2/4	0	0	0
<i>Chlamydia pneumoniae</i>			0
<i>Mycoplasma pneumoniae</i>			0
Indeterminate		4	
Total positive	6*	11*	33

Numbers in parentheses indicate single infections. *Single infections. hMPV, human metapneumovirus; PCR, polymerase chain reaction; PIV, parainfluenzavirus; RSV, respiratory syncytial virus.

PCR and viral infections

- **A total of 23 patients were included, of whom 11 (48%) were positive for a respiratory virus by conventional methods.** Real-time PCR confirmed all of these positive results. In addition, real-time PCR identified 22 more viruses in 11 patients, yielding a total of 22 (96%) patients with a positive sample. More than one virus was detected in eight (35%) children.
- Real-time PCR for respiratory viruses was found to be a sensitive and reliable method in PICU patients with lower respiratory tract infection, **increasing the diagnostic yield twofold compared to conventional methods.**

Table 4. Associations between immunosuppressive agents and specific infections

Agent	Mechanism of Action	Infection	Comments
Corticosteroids	Multiple: on neutrophils and macrophages (inhibiting phagocytosis), T cells and antigen presenting cells	Bacterial infection most common; herpes virus, fungal infections (<i>Candida</i> , <i>Aspergillus</i> , <i>Cryptococcus Pneumocystis</i>), and <i>Strongyloides</i> superinfection well documented	No increased risk with <10 mg of prednisone equivalent per day or <700 mg cumulative; Aspergillosis risk after allogeneic BMT increases with ≥ 1 mg/kg prednisone equivalent for ≥ 1 wk
Calcineurin inhibitors (Cyclosporine A, Tacrolimus)	Cyclosporine binds to cyclophilin; tacrolimus binds to FKBP12; for both, this results in inhibition of calcineurin and inhibition of interleukin 2 gene transcription	No specific association with severe infection	Role of cyclosporine and tacrolimus alone is difficult to quantitate, but they seem to be associated with the smallest risk of infection from all these agents
Sirolimus (rapamycin)	Inhibits lymphocyte proliferation	Possible decrease in CNV compared with cyclosporine	One study reported increased risk of invasive aspergillosis
Cyclophosphamide	As immunomodulator, inhibits lymphocyte proliferation	Bacterial complications of neutropenia, herpes zoster	
Azathioprine	As immunomodulator, inhibits lymphocyte proliferation	Bacterial complications of neutropenia	No difference in infection rate between methotrexate with azathioprine in rheumatoid arthritis [Boerbooms, 1995 #1240]
MMF	Antilymphoproliferative agent (purine synthesis inhibition)	Increased incidence of CMV disease, VZV	No increase in bacterial or fungal infections; less frequency of <i>Pneumocystis</i> [Husain, 2002 #1234]
Methotrexate	As immunomodulator, inhibits lymphocyte proliferation	Histoplasmosis, <i>Listeria</i> , <i>Pneumocystis</i>	<i>Pneumocystis</i> may be the most common opportunistic infection associated with low-dose methotrexate
Anti-TNF agents (etanercept, infliximab)	Infliximab is a chimeric antibody against human TNF- α , and it can fix complement and lyse target cells; etanercept is a modified soluble TNF- α receptor	Bacteremia, <i>Listeria</i> , Tuberculosis, cryptococcosis, aspergillosis, CMV	Most cases of severe infection reported with infliximab; besides the risk of infections, the lack of signs and symptoms of infections until they are very advanced has been a consistent feature of the descriptions
Anti-CD25 antibodies (daclizumab, basiliximab)	Blocks the high-affinity interleukin-2 receptor	No increase in bacterial, fungal or viral infections.	Delayed wound healing described by manufacturer
Purine analogues (2-chlorodeoxyadenosine, fludarabine)	Inhibition of DNA synthesis	<i>Cryptococcus</i> , <i>Listeria</i> , Herpesvirus	Used in hematologic malignancies that carry their own immunosuppression
Alemtuzumab (campath)	anti-CD52, targets T and B lymphocytes, monocytes	Respiratory virus, adenovirus, CMV	Reactivation of CMV is common; CMV disease is rare except in the setting of allogeneic HSCT

BMT, allogeneic bone marrow transplantation; CNV, choroidal neovascularization; CMV, cytomegalovirus; VZV, varicellazoster virus; TNF, tumor necrosis factor; HSCT, hemopoietic stem cell transplantation (see Refs. 1-30).

The late phase of sepsis – different diagnostics and different therapy

- **Otto GP et al: 2011:** The late phase of sepsis is characterized by an increased microbiological burden and death rate
- In a retrospective trial, 16,041 patient charts from a university intensive care unit were screened, and 999 patients with severe sepsis or septic shock were identified.
- Three phases were established: phase I (days 1 to 5), phase II (days 6 to 15) and phase III (days 16 to 150).
- Out of 999 enrolled patients, 308 died during the course of sepsis presenting a characteristic mortality rate (30.8%) with three distinct mortality peaks (at days 2, 7 and 17).
- Overall 36.7% of all deaths occurred in the early phase (phase I) and 63.3% during the later phases (phase II + III).

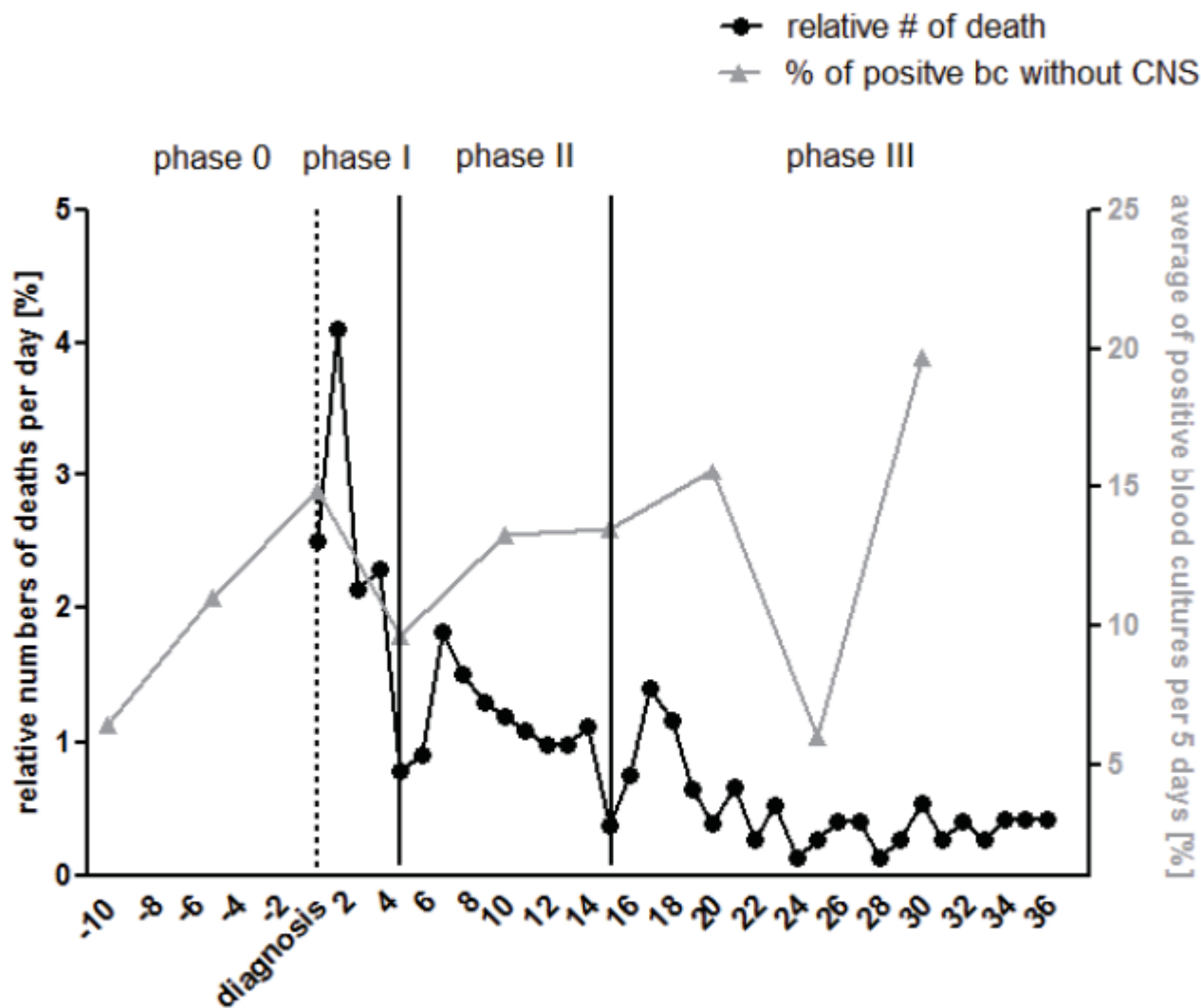


Figure 1 Distribution of non survivors and positive blood cultures during sepsis. Relative numbers of deaths per day from 999 patients with severe sepsis or septic shock according to ACCP/SCCM criteria are shown from the day of onset/diagnosis until observation Day 36. Three phases were defined, characterized by the nadir at Day 5 and Day 15. Also, the average rates of positive blood cultures without CNS in a five-day period with respect to sampling times are shown. bc, blood cultures; CNS, Coagulase negative staphylococci; #, numbers; %, relative number.

Table 3 Epidemiology of isolated microorganisms

microorganisms	phase 0	phase I	phase II	phase III
number of positive bc without CNS, #	26	143	56	90
typically opportunistic bacteria (TOB), % (#)	7.7 (2)	9.1 (13)	14.3 (8)	17.8 (16)*
<i>Candida spp.</i> overall, % (#)	7.7 (2)	12.6 (18)	35.7 (19)§	30 (27)§
pathogenic bacteria, % (#)	88.5 (23)	78.3 (112)	51.8 (29)	52.2 (47)
CNS <i>spp.</i> overall, #	28	52	53	78

Isolated microorganisms from blood cultures of patients with severe sepsis or septic shock are demonstrated. The absolute as well as relative numbers are given in dependency of defined phases. Others include all other isolated pathogen and presented separately in the online supplement (Additional file 1: Table S1). CNS are also presented but excluded from relative analyses. CNS, Coagulase negative staphylococci; bc, blood cultures; *, indicates statistically significant difference compared to phase I ($P \leq 0.05$; χ^2 test); §, indicates statistically significant difference compared to phase 0 and phase I ($P \leq 0.05$; χ^2 test).

Table 2 Characteristics of phase-dependent outcome and microbiological diagnosis

phases in relation to diagnosis	phase 0	phase I	phase II	phase III
days prior to diagnosis or during sepsis	Day -10 to Day -1	Day 1 to Day 5	Day 6 to Day 15	Day 16 to Day 150
# of patients alive at onset of phase, #	999	999	886	791
# of non survivors during phase, #		113	95	100
relative # of non survivors wrt all non survivors, # (%)		36.7 (113/308)	30.8 (95/308)	32.5 (100/308)
relative numbers of non survivors during phase, # (%)		11.3 (113/999)	10.7 (95/886)	12.6 (100/791)
# of drawn bc during phase, #	250	882	461	524
relative # of bc per patient alive, # (%)	25.0 (250/999)§	88.3 (882/999)§	52 (461/886)§	66.2 (524/791)§
# of all positive bc during phase, #	49	173	96	146
relative # of positive bc during phase, # (%)	19.6 (49/250)	19.6 (173/882)*	20.8 (96/461)	27.9 (146/524)*
# of positive bc without CNS during phase, #	24	131	32	80
relative # of positive bc without CNS during phase, # (%)	9.6 (24/250)	14.9 (131/882)*	11.3 (52/461)	15.3 (80/524)*

Outcome of patients enrolled ($n = 999$) with severe sepsis or septic shock is shown corresponding to the distinct phases as defined in Figure 1, starting with phase 0 including a period of 10 days prior to diagnosis of sepsis up to the end of phase III at Day 150. Also, the absolute numbers of drawn blood culture samples, the rate of blood culture samples per number of patients, the rate of all positive blood cultures (including CNS) and the rate of positive blood cultures without CNS are presented. CNS, Coagulase negative staphylococci; §, indicates statistically significant difference between all phases ($P \leq 0.05$; χ^2 test); *, indicates statistically significant difference compared to phase 0 and II ($P \leq 0.05$; χ^2 test); #, absolute numbers; %, relative number; bc, blood cultures; wrt, with respect to.

Summary

- Targets groups of patients
- The time of sepsis – early phase, late phase
- Analyte – blood, BAL, cerebrospinal fluid
tissue

Summary

- **Molecular methods may offer advantages over blood cultures in the diagnosis of sepsis.**
- **Molecular assays are rapid and require small sample volumes.**
- **Molecular assays may be automated, enabling high throughput, and reduce microbiological workload compared with blood cultures.**
- **Molecular methods may evaluate virulence and antibiotic resistance markers that may inform antibiotic therapy.**
- **Positive results of more sensitive molecular methods (detection of pathogen DNA) in the face of a negative blood culture (absence of viable organisms) need to be interpreted carefully in a clinical setting.**

Summary

- **False-negative results from molecular assays may be due to inefficient DNA extraction, presence of low levels of pathogen DNA or the presence of inhibitors.**
- **High negative predictive values of a diagnostic test may be clinically useful in ruling out sepsis and avoiding unnecessary antibiotics.**
- **Costs, availability of equipment and technical skills in the microbiological laboratory are important considerations.**
- **Cost–effectiveness of the newer molecular assays should be established before widespread acceptance in clinical practice.**