Time to positivity of neonatal blood cultures: fast and furious?

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The aim of this study was to determine the time to positivity (TTP) of neonatal blood cultures, to investigate differences between early onset versus late-onset sepsis, and non-proven versus proven sepsis, and to examine differences in TTP by organism type using a retrospective observational study at the Neonatal Intensive Care Unit, Antwerp University Hospital, Belgium. The subjects were 1828 neonates with suspected sepsis who were treated with antimicrobials for at least 3 days. The TTP was recorded for all episodes of suspected sepsis in an approximately 6.5 year period. A total of 2916 blood cultures were collected, of which 437 (15 %) became positive. The overall TTP was 21.33 h (Q₁–Q₃ 13.17–32.46). The difference between the median TTP in early onset versus late-onset sepsis was 0.83 h (22.00 versus 21.17 h, $P=0.75$). The median TTP for Gram-negative organisms was 11.17 h (Q₁–Q₃ 8.84–15.67), whereas the median TTP for Gram-positive organisms was 23.59 h (Q₁–Q₃ 15.29–34.58, $P<0.001$). In Gram-positive isolates, the median TTP for coagulase-negative staphylococci (CNS) was 26.67 h (Q₁–Q₃ 19.00–38.17), whereas the median TTP for non-CNS was 12.83 h (Q₁–Q₃ 10.50–18.17, $P<0.001$). The median TTP in proven sepsis was 20.17 h (Q₁–Q₃ 13.00–30.37), whereas it was 29.67 h (Q₁–Q₃ 21.17–50.63, $P<0.001$) in non-proven sepsis. TTP of neonatal blood cultures was significantly shorter for Gram-negative organisms. We suggest shortening the total incubation time of neonatal blood cultures to a maximum of 3 days. However, blood cultures collected in infants <72 h of age might require a longer incubation time. According to our results, it may be safe to narrow the antimicrobial spectrum to solely target Gram-positive bacteria when the culture is still negative after 48 h, and to cease antimicrobial therapy when the culture is still negative after 72 h in clinically well infants.

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INTRODUCTION

Sepsis is a commonly encountered and potentially life-threatening problem in neonatal intensive care units (NICUs) (Gardner, 2009; Burke, 2009). During the past three decades, the incidence of early onset sepsis caused by group B streptococci decreased considerably (Van den Hoogen *et al.*, 2010). In contrast, the overall incidence of early onset sepsis has not decreased (Kristóf *et al.*, 2009). In addition, technological progress and sophisticated neonatal care have significantly increased the survival chances of premature and low-birth-weight newborns (Gibson, 2007). These critically ill infants are ever more exposed to invasive

medical devices, which are considered to be major portals of entry for (pathogenic) micro-organisms (Mullett *et al.*, 1998). Therefore, late-onset sepsis will continue to be a challenging complication in neonatal intensive care, affecting (co)morbidity, mortality, hospital stay and hospital costs (Kristóf *et al.*, 2009; Bizzarro *et al.*, 2005; Mahieu *et al.*, 2001; Leroyer *et al.*, 1997).

It is not easy to establish a definite diagnosis of sepsis in the neonate. Early clinical signs are mostly non-specific and inconclusive. However, outcome will indeed be jeopardized by delayed initiation of therapeutic action. Therefore, broad-spectrum antimicrobial therapy is started immediately if there is any suspicion of sepsis, while awaiting blood culture results. However, the majority of neonatal blood cultures will remain sterile, implying unnecessary

Abbreviations: CNS, coagulase-negative staphylococci; NICU, neonatal intensive care unit; TTP, time to positivity.

use of antibiotics and presumably triggering multidrug resistance.

Several studies on the time to positivity (TTP) of neonatal blood cultures have altered common practice and suggest discontinuing antibiotic therapy if blood cultures are still negative after 48–72 h in neonates without clinical or laboratory signs of infection (Rowley & Wald, 1986; Kurlat *et al.*, 1989; Hurst & Yoder, 1995). Other studies suggest a 24–36 h observation period to rule out sepsis in asymptomatic infants (Pauli *et al.*, 1999; Garcia-Prats *et al.*, 2000; Kumar *et al.*, 2001; Jardine *et al.*, 2006; Janjindamai & Phetpisal, 2006). Among these studies only two included a substantial number of positive blood cultures (Garcia-Prats *et al.*, 2000; Kumar *et al.*, 2001), and only two were published within the last 5 years (Jardine *et al.*, 2006; Janjindamai & Phetpisal, 2006). One recently published study recommends a 4 day observation period, based on the incubation times of 877 positive neonatal blood cultures, but did not consider organism type (Vinod Kumar & Neelagaud, 2005).

The objective of the present study was to determine the TTP of a large cohort of neonatal blood cultures collected in a tertiary care centre, and to investigate whether this differed by: (i) suspected early onset versus late-onset sepsis, (ii) Gram-positive versus Gram-negative organisms versus yeasts, and (iii) proven versus non-proven sepsis. The actual intention was to develop a practical strategy to diminish the incubation time of neonatal blood cultures in the microbiology laboratory and to guide antimicrobial therapy in the NICU in order to reduce antibiotic pressure.

METHODS

Patients and setting. This retrospective study was performed in a single 19 bed NICU, with a yearly admission rate of approximately 350 neonates, at Antwerp University Hospital, Belgium. All blood cultures obtained from NICU patients with suspected sepsis, and submitted to the hospital's microbiology laboratory between 1 January 2001 and 30 June 2007, were included.

Definitions. Suspected early onset sepsis was defined according to the three following criteria: (i) presence of clinical and/or laboratory signs of infection according to a nosocomial sepsis scoring system (Mahieu *et al.*, 2000), (ii) treatment with broad-spectrum antibiotics starting after blood culture collection, and (iii) onset of infection <72 h after birth (Haque, 2005). Suspected late-onset sepsis was defined according to the three following criteria: (i) presence of clinical and/or laboratory signs of infection according to a nosocomial sepsis scoring system (Mahieu *et al.*, 2000), (ii) treatment with broad-spectrum antibiotics starting after blood culture collection, and (iii) onset of infection ≥72 h after birth (Haque, 2005).

Definite pathogens, possible pathogens and contaminants were defined as described by Garcia-Prats *et al.* (2000). Briefly, definite pathogens are organisms known to cause disease in neonates, such as *Streptococcus agalactiae*, *Staphylococcus aureus* and Gram-negative organisms. Possible pathogens are organisms that can cause disease in neonates under special circumstances, such as coagulase-negative staphylococci (CNS), α -haemolytic streptococci and γ -haemolytic streptococci. Contaminants are organisms that rarely cause disease in

neonates, such as *Corynebacterium* species and *Propionibacterium* species.

A suspected sepsis episode was classified as proven when (i) a definite pathogen was isolated from a blood culture, or (ii) a possible pathogen was isolated in the presence of a central vascular catheter or from two blood samples taken from two different puncture sites (Guerti *et al.*, 2007). TTP was defined as the time between the start of culture incubation and the time the BacT/Alert system signalled the culture as being positive.

Data collection. In the microbiology laboratory, computerized data records were collected from the NICU patients who were screened for early onset or late-onset sepsis during the study period. These records contained for every sample: patient name, date and hour of sample collection, date and hour of sample loading, date and hour of sample detection and isolate(s) cultured. At the NICU, clinical, laboratory and demographic data were obtained using a computerized bedside data system (Clinicomp). All data were entered in the spreadsheet program Excel 2003 (Microsoft). For every episode the TTP was calculated as the difference between detection time and loading time.

Microbiological culture techniques. During the study period the non-quantitative BacT/Alert system (Organon Teknika) was used to automatically identify positive samples with a colorimetric, CO₂-reactive sensor. Throughout the study, two different types of culture bottles – the BacT/Alert PF bottle and the Pedi-BacT bottle – were in use. Detection times are the same for both bottle types (Krisher *et al.*, 2001). As described in the NICU protocol, culture bottles were inoculated with a minimum of 1 ml blood and incubated at 37 °C for a maximum of 6 days, with a maximum TTP of 144 h. All positive samples were examined by Gram stain, and cultured aerobically and anaerobically. Species identification was performed by conventional biochemical methods (Pattyn *et al.*, 1990; Ieven *et al.*, 1995). Blood cultures with recovery of multiple isolates were excluded from further analysis.

Statistical analysis. Non-parametric continuous data are presented as medians and interquartile ranges (Q₁–Q₃). The Mann–Whitney U test (Kruskal–Wallis test) was performed to analyse differences in non-parametric data between (more than) two distinct groups. The χ^2 test was used to analyse differences between proportions. All statistical analyses were performed with the statistical package SPSS 17.0 (SPSS).

Ethical approval. Ethical approval was obtained from the Institutional Review Board of Antwerp University Hospital. Because the study was retrospective and observational, not requiring extra blood sampling in the study population, the Institutional Review Board waived the need for parental informed consent.

RESULTS

Study population

A total of 2916 blood cultures were collected from 1828 neonates. Of these 2813 samples (96.5 %) were cultured in BacT/Alert PF bottles and 103 samples (3.5 %) were cultured in Pedi-BacT bottles. A total of 482 neonates had more than one blood culture taken, 1783 (61.1 %) blood cultures were obtained from 1685 infants <72 h after birth, and 1133 (38.9 %) blood cultures were obtained from 201 infants ≥72 h after birth (Table 1).

From the 2916 blood cultures collected throughout the entire study period, 437 (15.0 %) were positive from 249 neonates. In episodes of suspected early onset sepsis, 58 out

Table 1. Study population characteristics

Characteristic	All	Suspected EOS	Suspected LOS
Gender: no. of males (%)	1667 (57.2)	1016 (57.0)	651 (57.5)
Postnatal age (days), median [Q ₁ –Q ₃]	1 [1–11]	1 [0–1]	15 [8–32]*
No. of blood cultures	2916	1783	1133
No. of patients	1828	1685	201
No. of positive blood cultures (%)	437 (15.0)	58 (3.3)	379 (33.5)*
Median TTP [Q ₁ –Q ₃]	21.33 [13.17–32.46]	22.00 [11.00–35.67]	21.17 [13.42–31.59]
Multiple isolates (%)	25 (5.7)	3 (5.2)	22 (5.8)
Gram-positive isolates (%)	334 (76.4)	45 (77.6)	289 (76.3)
Gram-negative isolates (%)	57 (13.0)	10 (17.2)	47 (12.4)
Yeasts (%)	21 (4.8)	0 (0)	21 (5.5)

EOS, early onset sepsis; LOS, late-onset sepsis.

* $P<0.001$.

of 1783 (3.3 %) blood cultures were positive. In episodes of suspected late-onset sepsis 379 out of 1133 (33.5 %) blood cultures were positive.

Microbiology

During the study period, 437 samples were further processed for species identification and antibiotic susceptibility testing. This yielded 391 (89.5 %) bacterial isolates and 21 (4.8 %) yeasts. From 25 (5.7 %) blood cultures, multiple isolates were recovered. These samples were excluded from further analysis. Table 2 illustrates that most of the isolates were Gram-positive skin organisms. CNS accounted for 58.4 % of all isolates. Out of the 391 bacterial isolates, 334 (85.4 %) were Gram-positive isolates, and 57 (14.6 %) were Gram-negative isolates. A total of 358 positive blood cultures originated from infants with proven sepsis.

TTP

The median TTP of all positive blood cultures from which a single isolate was recovered ($n=412$), was 21.33 h (Q₁–Q₃ 13.17–32.46). A total of 90 (21.8 %) blood cultures became positive within the first 12 h after sample incubation, 231 (56.1 %) became positive within 24 h and 365 (88.6 %) within 48 h. At 72 h of sample incubation, 398 (96.6 %) blood cultures were positive (Table 3).

The median TTP in episodes of suspected early onset sepsis was 22.00 h (Q₁–Q₃ 11.00–35.67), as compared to 21.17 h (Q₁–Q₃ 13.42–31.59) in episodes of suspected late-onset sepsis (Mann–Whitney $U=9557.0$, $P=0.75$). In suspected early onset sepsis, 5 out of 55 blood cultures (9.1 %) became positive after 72 h of sample incubation as compared to 9 out of 357 (2.5 %) in suspected late-onset sepsis ($\chi^2=6.3$, $P=0.012$) (Fig. 1a).

The median TTP in episodes of suspected early and late-onset sepsis yielding a single Gram-negative isolate was 11.17 h (Q₁–Q₃ 8.84–15.67), as compared to 23.59 h

(Q₁–Q₃ 15.29–34.58) in episodes yielding a single Gram-positive isolate, and 26.17 h (Q₁–Q₃ 13.84–28.84) in episodes yielding a single yeast (Kruskal–Wallis $\chi^2=50.8$, $P<0.001$). The median TTP was significantly shorter in suspected sepsis episodes yielding a single Gram-negative isolate as compared to episodes yielding a single Gram-positive isolate (Mann–Whitney $U=3880.5$, $P<0.001$) or a single yeast (Mann–Whitney $U=300.0$, $P<0.001$), while there was no significant difference between the median TTP in suspected sepsis episodes yielding a single Gram-positive isolate and episodes yielding a single yeast (Mann–Whitney $U=3314.5$, $P=0.67$) (Fig. 1b). All 57 Gram-negative isolates and all 21 yeasts were recovered within the first 72 h of sample incubation. A total of 54 (94.7 %) Gram-negative isolates and 20 (95.2 %) yeasts were recovered within 48 h. A total of 14 out of 334 (4.2 %) Gram-positive isolates were recovered after 72 h of sample incubation (Table 3).

Within the group of Gram-positive isolates, the median TTP was 26.67 h (Q₁–Q₃ 19.00–38.17) for CNS, as compared to 12.83 h (Q₁–Q₃ 10.50–18.17) for non-CNS (Mann–Whitney $U=4711.0$, $P<0.001$) (Fig. 1c). Only 16 out of 255 (6.3 %) CNS were recovered within the first 12 h of sample incubation, as compared to 36 out of 79 (45.6 %) non-CNS ($\chi^2=70.6$, $P<0.001$) (Table 3).

The median TTP in episodes of proven sepsis was 20.17 h (Q₁–Q₃ 13.00–30.37), as compared to 29.67 h (Q₁–Q₃ 21.17–50.63) in episodes of non-proven sepsis (Mann–Whitney $U=6448.5$, $P<0.001$) (Fig. 1d). In episodes of proven sepsis, 9 out of 358 (2.5 %) blood cultures became positive after 72 h of sample incubation, as compared to 5 out of 54 (9.3 %) in episodes of non-proven sepsis ($\chi^2=6.5$, $P=0.011$) (Table 3).

DISCUSSION

In the present study we determined the TTP of a large cohort of neonatal blood cultures, and investigated

Table 2. TTP of 437 positive neonatal blood cultures

Isolate	No. of suspected EOS	No. of suspected LOS	No. of EOS and LOS	TTP (h)						
				<12	12–24	24–48	48–72	72–96	96–120	>120
Gram-positive bacteria										
<i>Staphylococcus aureus</i>	2	27	29	11	16	1	0	1	0	0
Non- <i>Staphylococcus aureus</i> species	21	234	255	16	89	116	28	5	1	0
<i>Streptococcus agalactiae</i>	9	1	10	8	2	0	0	0	0	0
<i>Streptococcus pyogenes</i>	0	1	1	1	0	0	0	0	0	0
<i>Streptococcus pneumoniae</i>	2	0	2	1	0	0	0	1	0	0
Other <i>Streptococcus</i> species	4	6	10	1	4	1	0	1	0	3
<i>Enterococcus</i> species	0	10	10	5	5	0	0	0	0	0
<i>Micrococcus</i> species	2	0	2	0	0	1	0	1	0	0
<i>Bacillus</i> species	1	10	11	7	3	0	0	1	0	0
<i>Corynebacterium</i> species	4	0	4	0	0	3	1	0	0	0
Gram-negative bacteria										
<i>Escherichia coli</i>	3	16	19	14	5	0	0	0	0	0
<i>Proteus</i> species	1	5	6	4	2	0	0	0	0	0
<i>Klebsiella</i> species	3	7	10	9	0	1	0	0	0	0
<i>Serratia</i> species	0	5	5	1	3	0	1	0	0	0
<i>Morganella</i> species	0	1	1	0	1	0	0	0	0	0
<i>Enterobacter</i> species	1	2	3	2	1	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	0	6	6	2	4	0	0	0	0	0
Other <i>Pseudomonas</i> species	1	2	3	0	1	1	1	0	0	0
<i>Haemophilus influenzae</i>	1	0	1	0	0	0	1	0	0	0
<i>Acinetobacter</i> species	0	2	2	0	2	0	0	0	0	0
<i>Sphingomonas</i> species	0	1	1	0	1	0	0	0	0	0
Yeasts										
<i>Candida albicans</i>	0	5	5	0	1	4	0	0	0	0
<i>Candida glabrata</i>	0	13	13	5	2	6	0	0	0	0
<i>Candida parapsilosis</i>	0	3	3	0	1	1	1	0	0	0
Multiple isolates	3	22	25	7	12	6	0	0	0	0
Total (%)	58	379	437	94 (22)	155 (35)	141 (32)	33 (8)	10 (2)	1 (0.2)	3 (0.7)

EOS, Early onset sepsis; LOS, late-onset sepsis.

Table 3. Numbers (and percentages) of blood cultures that became positive within different incubation periods compared by sepsis type and organism type

	TTP (h)				
	<12	<24	<48	<72	>72
Suspected early onset sepsis	17 (30.9)	33 (60.0)	45 (81.8)	50 (90.9)*	5 (9.1)*
Suspected late-onset sepsis	73 (20.4)	198 (55.5)	320 (89.6)	348 (97.5)*	9 (2.5)*
Gram-positive isolates	52 (15.6)†	170 (50.9)†	291 (87.1)	320 (95.8)	14 (4.2)
Gram-negative isolates	33 (57.9)†	52 (91.2)†	54 (94.7)	57 (100)	0 (0)
Yeasts	5 (23.8)†	9 (42.9)†	20 (95.2)	21 (100)	0 (0)
Proven sepsis	83 (23.2)	213 (59.5)†	326 (91.1)†	349 (97.5)*	9 (2.5)*
Non-proven sepsis	7 (13.0)	18 (33.3)†	39 (72.2)†	49 (90.7)*	5 (9.3)*
Total	90 (21.8)	231 (56.1)	365 (88.6)	398 (96.6)	14 (3.4)

* $P<0.05$.† $P<0.001$.

whether this differed by: (i) suspected early onset versus late-onset sepsis, (ii) Gram-positive versus Gram-negative organisms versus yeasts, and (ii) proven versus non-proven sepsis. In contrast to previous studies, we have relied upon objective and robust definitions to bound our groups, and not on the experience and expertise of a limited number of specialized clinicians.

The positivity rate of neonatal blood cultures in our study was 15 %, which compares well with the positivity rate found in most other recent studies (Pauli *et al.*, 1999; Kumar *et al.*, 2001; Janjindamai & Phetpaisal, 2006). We noticed a significant difference between the positivity rate of blood cultures collected in infants <72 h of age (3 %) and infants ≥ 72 h of age (33 %). This might reflect a less

Fig. 1. Proportion of positive neonatal blood cultures according to the TTP (%) in: (a) suspected early onset sepsis (black bars) and suspected late-onset sepsis (hatched bars); (b) Gram-positive isolates (black bars), Gram-negative isolates (hatched bars) and yeasts (spotted bars); (c) CNS (black bars) and non-CNS (hatched bars) within Gram-positive isolates; and (d) proven sepsis (black bars) and non-proven sepsis (hatched bars). *, $P<0.05$; †, $P<0.001$.

restrictive policy as to the collection of blood cultures in younger infants. Jardine and colleagues reported a total positivity rate of only 6% (Jardine *et al.*, 2006), whereas Vinod Kumar and Neelagaud reported a positivity rate of more than 50% (Vinod Kumar & Neelagaud, 2005). The lower positivity rate in the former study might be the result of a higher amount of suspected early onset sepsis in this study (75 versus 61% in our study). The latter study did not report any details on the (pre-)analytical aspects of the blood culture processing procedure that could explain this extremely high positivity rate.

The spectrum of isolates in our study clearly illustrates the changing microbiology of neonatal blood stream infections (Zaidi *et al.*, 2009). About 60% of positive blood cultures yielded CNS, which is comparable with most other recent studies (Pauli *et al.*, 1999; Garcia-Prats *et al.*, 2000; Kumar *et al.*, 2001; Jardine *et al.*, 2006). In the 1989 study of Kurlat and colleagues only 28% of blood culture isolates were CNS (Kurlat *et al.*, 1989). The increasing proportion of CNS isolated from neonatal blood cultures most probably reflects an inevitable side-effect of today's more advanced (and invasive) neonatal care. Surprisingly, in their 2005 study, Vinod Kumar and Neelagaud isolated only 13% CNS from 877 positive blood cultures (Vinod Kumar & Neelagaud, 2005). They also reported a 54% share of Gram-negative isolates, which is much higher than the 10–20% reported in other studies (Pauli *et al.*, 1999; Garcia-Prats *et al.*, 2000; Kumar *et al.*, 2001; Jardine *et al.*, 2006). The authors did not however provide any details on their study population, making it impossible to reveal the true cause of this aberrant bacteriological profile.

The median TTP in our study was similar to the findings of previous recent studies that used automated blood culture systems (Pauli *et al.*, 1999; Garcia-Prats *et al.*, 2000; Kumar *et al.*, 2001; Jardine *et al.*, 2006). The sensitivities for bacteraemia after an incubation time of 24, 48 and 72 h were 56, 89 and 97%, respectively. These results are similar to the results of Kumar *et al.* (2001) (46, 93 and 97%) and the results of Garcia-Prats *et al.* (2000) (77, 93 and 96%). In 1989, Kurlat and colleagues could only detect 88% of positive blood cultures within 72 h. However, they did not use a fully automated blood culture system and, therefore, could not continuously detect positive samples (Kurlat *et al.*, 1989). Out of the 14 isolates recovered after 72 h of sample incubation, 4 were classified as definite pathogens. These included one *Staphylococcus aureus* and one *Streptococcus pneumoniae*, both recovered at day 4. Two group C streptococci were not recovered until day 6. The other 10 isolates that were recovered after 72 h of sample incubation were classified as possible pathogens ($n=8$) or contaminants ($n=2$).

We did not find a statistically significant difference in median TTP between the early and late-onset groups. This result is in accordance with the findings of Jardine *et al.* (2006). Despite the nearly identical median detection times in both groups, we did observe a statistically significant

difference between the percentages of blood cultures that became positive after 72 h of incubation (Table 3). We found a higher proportion of blood cultures with a prolonged TTP in episodes of suspected early onset sepsis. A possible explanation could be interference by antimicrobials that were administered prior to blood culture collection or prenatal antimicrobial prophylaxis. This hypothesis is, however, contradicted by the study of Garcia-Prats *et al.* (2000). Out of the five isolates recovered after 72 h of sample incubation in early onset sepsis, three were classified as definite pathogens (one *Streptococcus pneumoniae*, two group C streptococci), one as a possible pathogen (*Staphylococcus epidermidis*) and one as a contaminant (*Micrococcus* species). All three definite pathogens yielded a significant increase in C-reactive protein on the day of blood culture collection. This finding might justify a longer incubation time for blood cultures collected in infants <72 h of age with laboratory signs of infection.

The median TTP was significantly shorter in suspected sepsis episodes yielding a single Gram-negative isolate, as compared to episodes yielding a single Gram-positive isolate or a single yeast. The sensitivities for Gram-negative bacteraemia after an incubation time of 24, 48 and 72 h were 91, 95 and 100%, respectively, whereas in the study of Garcia-Prats and colleagues the sensitivities for Gram-negative bacteraemia were only 77, 79 and 85%, respectively (Garcia-Prats *et al.*, 2000). However, they used a different type of culture bottles (ESP 80A aerobic bottle), which might explain these lower sensitivities. According to a 1998 comparative study, the BacT/Alert PF paediatric FAN aerobic blood culture bottle yields enhanced detection rates of various Gram-negative bacteria as compared to the ESP 80A aerobic blood culture bottle (Doern *et al.*, 1998). The sensitivities for fungal bacteraemia were 43, 95 and 100%, respectively. These sensitivities are higher than the sensitivities found in earlier studies (Kurlat *et al.*, 1989; Garcia-Prats *et al.*, 2000; Kumar *et al.*, 2001). All Gram-negative and fungal isolates were detected within 72 h. This finding might justify narrowing the antimicrobial spectrum to solely target Gram-positive bacteria when the culture is still negative after 48 h. The sensitivities for Gram-positive bacteraemia after an incubation time of 24, 48 and 72 h were 51, 87 and 96%, respectively, whereas in the study of Garcia-Prats and colleagues the sensitivities for Gram-positive bacteraemia were 80, 93 and 98%, respectively (Garcia-Prats *et al.*, 2000). This finding is in accordance with the study of Doern *et al.* (1998) who described a faster recovery of streptococci and enterococci with the ESP 80A bottle. Indeed, in the study of Garcia-Prats *et al.* (2000) only 3 out of 70 (4%) streptococci/enterococci were recovered after 48 h, whereas in our study 5 out of 33 (15%) streptococci/enterococci were recovered after 48 h.

Within the group of Gram-positive isolates the median TTP was significantly longer for CNS, as compared to non-CNS. The sensitivities for bacteraemia due to CNS after an incubation time of 24, 48 and 72 h were 41, 87 and 98%,

respectively, whereas the sensitivities for non-CNS were 84, 91 and 93 %, respectively.

The median TTP was significantly shorter in episodes of proven sepsis, as compared to episodes of non-proven sepsis. As we did not consider outcome or mortality in our study, this could be an interesting focus for future studies. Is a shorter TTP a premonition of adverse or fatal outcome, and therefore a trigger for extended care?

A possible weakness of our study is its retrospective design, resulting in some uncontrolled factors, such as the volumes of blood inoculated in the culture bottles, the use of different culture media during the study period, and the possible administration of antibiotics prior to blood culture collection. We do not routinely keep records of the exact amounts of blood inoculated in culture bottles. However, as described in the NICU protocol, culture bottles should be inoculated with a minimum of 1 ml blood. Hence we have no reason to suspect any differences in blood volumes between our groups or during the course of the study. Throughout the study, two different types of culture media were in use. It has been described that the overall detection times are comparable for both media types (Krisher *et al.*, 2001). According to our NICU protocol, blood cultures should be collected prior to initiation of antimicrobial therapy. In routine daily practice it is inevitable that now and then this will happen the wrong way round. It is reassuring, however, that Garcia-Prats and colleagues did not find a significant difference in TTP between pre-therapy and post-therapy obtained positive blood cultures, and that prenatal administration of antimicrobials did not seem to affect the TTP of blood cultures collected on the first day of life (Garcia-Prats *et al.*, 2000). In spite of these concerns, the large number of neonatal blood cultures in our study (2916), the large number of positive blood cultures (437), the length of our study period (6.5 years), and the conscious use of objective and robust definitions, have convinced us of the significance and usefulness of our results.

Conclusions

This retrospective review demonstrates that the TTP of neonatal blood cultures differs significantly by organism type. As 97 % of positive blood cultures are detected within 72 h, we suggest shortening the total incubation time of neonatal blood cultures to a maximum of 3 days. However, blood cultures collected from infants <72 h of age with laboratory signs of infection might require a longer incubation time.

According to our results, it may be safe to narrow the antimicrobial spectrum to solely target Gram-positive bacteria when the culture is still negative after 48 h, and to cease antimicrobial therapy when the culture is still negative after 72 h in clinically well infants. However, our results do not support the recommendation of a 24–36 h observation period as proposed in earlier studies (Pauli

et al., 1999; Garcia-Prats *et al.*, 2000; Kumar *et al.*, 2001; Jardine *et al.*, 2006; Janjindamai & Phetpisal, 2006).

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