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ORIGINAL ARTICLE

Current management of late onset neonatal bacterial sepsis in five European countries

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Abstract Late onset neonatal sepsis (LOS) has a high mortality and the optimal management is poorly defined. We aimed to evaluate new expert panel-derived criteria to define LOS and characterize the current management and antibiotic susceptibility of LOS-causing organisms in Europe. A prospective observational study enrolled infants aged 4 to 90 days in five European countries. Clinical and laboratory findings as well as empiric treatment were recorded and patients were followed until the end of antibiotic therapy. Failure was defined as a change of primary antibiotic, no resolution of clinical signs, appearance of new signs/pathogens or death. Antibiotic therapy was considered appropriate if the organism was susceptible to at least one empiric antibiotic. 113 infants (median age 14 days, 62 % ≤1500 g) were recruited; 61 % were culture proven cases (28 CoNS, 24 Enterobacteriaceae, 11 other Gram-positives and 6 Gram-negative non-fermentative organisms). The predictive value of the expert-panel criteria to identify patients with a culture proven LOS was 61 % (95 % CI 52 % to 70 %). Around one third of Enterobacteriaceae were resistant to ampicillin + or

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cefotaxime + gentamicin but only 10 % to meropenem. Empiric treatment contained a total of 43 different antibiotic regimens. All-cause mortality was 8 % with an additional 45 % classified as failure of empiric therapy, mainly due to change of primary antibiotics (42/60). *Conclusions*: The expert panel—derived diagnostic criteria performed well identifying a high rate of culture proven sepsis. Current management of LOS in Europe is extremely variable suggesting an urgent need of evidence-based guidelines.

Keywords Meropenem · Antibiotic resistance · Neonatal meningitis · Observational study

Introduction

Late onset sepsis (LOS) is still one of the most common problems in neonatal intensive care units (NICU) with significant morbidity and mortality [9, 10]. Despite this, the efficacy and safety of antibiotics used in the treatment of LOS remains poorly studied. Almost all randomized controlled trials (RCTs) are more than 20 years old, usually with small sample size, include mixed population of early and late onset sepsis, and/or have evaluated antibiotics not in current use. Furthermore the methodology in most of these studies is below current standards of RCTs [8].

One of the major hurdles to be faced when planning studies in LOS is the lack of validated diagnostic and outcome criteria. In 2005, an International Pediatric Sepsis Consensus Conference established diagnostic criteria for term neonates [7]. A modification of these criteria was proposed by Wynn and Wong [23] for preterm neonates, but to the best of our knowledge, it has not been validated. To define LOS previous studies have used combinations of clinical and laboratory parameters together with positive cultures of sterile sites [4, 15, 17] or the presence of two clinical and laboratory criteria for clinical sepsis [16].

In 2010, at an expert meeting organized by the European Medicines Agency (EMA) the diagnostic criteria of LOS for neonates with postmenstrual age (PMA) of ≤44 weeks were agreed, but they have not yet been tested in clinical settings (www.ema.europa.eu/docs/en_GB/document_library/Report/2010/12/WC500100199.pdf).

Europe-wide data on the antimicrobial resistance (AMR) of LOS-causing organisms are almost entirely missing. Data from the Health Protection Agency's national bacteremia database have demonstrated that the great majority of organisms in England and Wales were susceptible to the UK currently recommended regimen of flucloxacillin + gentamicin or amoxicillin + cefotaxime; coagulase negative staphylococci (CoNS) were excluded [18]. However, resistance to, or MIC values of, individual antibiotics were not reported. In addition, the UK data are unlikely to be representative of all of Europe; Southern European countries are known to have greater resistance rates than Northern Europe (http://www.ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2011.pdf).

In preparation for a European multicenter RCT on the efficacy and safety of meropenem compared with the standard of care (gentamicin + cefotaxime or ampicillin) [13] we aimed to evaluate the appropriateness of the EMEA expert panel agreed diagnostic criteria of LOS, to estimate the proportion of patients with a favorable outcome at test of cure (TOC) visit, to characterize the AMR of infecting organisms and to describe the empiric antibiotic therapy of LOS in participating centers.

Methods

Study design

A prospective observational study was conducted between July 2010 and September 2011 in 18 NICUs in Estonia and Lithuania (Northern Europe) and Greece, Italy and Spain (Southern Europe).

Study subjects and treatment

Patients aged between 4 and 90 days with suspected LOS were screened for the presence of the expert panel-derived criteria of LOS presented in Table 1. Subjects with a PMA of >44 weeks were eligible if they met sepsis criteria as described by Goldstein et al. [7]. Those with PMA of ≤44 weeks were

Table 1 Clinical and laboratory parameters defining LOS in patients with PMA ≤44 weeks

Clinical parameters

1. Hyper- or hypothermia or temperature instability
2. Reduced urinary output or hypotension or mottled skin or impaired peripheral perfusion
3. Apnea or increased oxygen requirement or need for ventilatory support
4. Bradycardia spells or tachycardia or rhythm instability
5. Feeding intolerance or abdominal distension
6. Lethargy or hypotonia or irritability
7. Skin and subcutaneous lesions (such as petechial rash or sclerema)

Laboratory parameters

1. White blood cell count <4 or >20×10^9 cells/l
2. Immature to total neutrophil ratio >0.2
3. Platelet count <100×10^9/l
4. C-reactive protein >15 mg/l or procalcitonin ≥2 ng/ml
5. Glucose intolerance when receiving normal glucose amounts (8–15 g/kg/day) as expressed by blood glucose values >180 mg/dl or hypoglycemia (<40 mg/dl) confirmed on at least two occasions
6. Acidosis with base excess (BE) <−10 mmol/l or lactate above 2 mmol/l

required to have at least two clinical plus two laboratory parameters. Antibiotics and treatment duration were based on local practices.

Study visits, clinical and laboratory assessments

All patients were evaluated at screening and at TOC visit, ideally performed at 7±2 days after the end of antibiotic treatment. Microbiological samples were collected at baseline (ideally prior to initiation of antibiotics) and analyzed at local laboratories according to their guidelines. In post-hoc analysis available MIC values were re-evaluated centrally based on the EUCAST guidelines [12]. Otherwise, local criteria were used to define resistance.

Definitions

An LOS case with at least one positive culture from a normally sterile site prior to antibiotic therapy was considered culture-proven; all remaining cases were categorized as clinical sepsis. Antibiotic therapy was considered appropriate if the organism was susceptible or known to have class susceptibility to at least one antibiotic in the empiric regimen. The presence of all of the following was defined as a favorable outcome: the patient is alive and all abnormalities that defined LOS at baseline are resolved, and there is no new clinical or laboratory parameters that require antibiotic therapy, and baseline pathogen is eradicated (either confirmed or presumed), and assigned antibiotics have not been modified due to lack of efficacy.

Statistical considerations

The study primarily aimed to estimate the proportion of patients with culture proven LOS according to the new expert panel-derived criteria. Expecting a proportion of culture proven sepsis between 50 % and 70 %, a sample size of at least 100 evaluable patients was required to provide a standard deviation not greater than 5 %. This sample size would also provide similar precision for the estimate of the proportion of patients with a favorable outcome at TOC visit.

Statistical analyses were performed with SAS version 9.1.3. Survival was censored at the TOC visit. The probability of surviving at day 11 was derived using the Kaplan–Meier method. The predictive factors of a favorable outcome at TOC visit were first evaluated by logistic regression in a univariate model. All variables with $p<0.2$ were included in a multivariate model adjusting on age at onset.

Ethics

The study was approved by the local ethics committees. All parents/carers were informed of this study. Signed consent was obtained only if required by national regulations.

Results

Patients

Altogether, 166 patients with suspected LOS were screened in 15 NICUs; 113 met the inclusion criteria. The median number of patients per site was 7 (interquartile range [IQR] 2–12). Southern Europe contributed 80 and Northern Europe 33 patients. The reasons for the exclusion of 53 patients are presented in Fig. 1. The demographic data of patients are shown in Table 2. There were 109 patients with bacterial LOS with a median age at onset of 14 days (IQR 8–26 days). Of all patients, 108/113 (96 %) were at PMA ≤44 weeks and were included in the further analysis if not stated otherwise.

There were 69 (61 %; 95 % confidence interval [CI] 52–70 %) microbiologically proven LOS cases. CoNS (*Staphylococcus epidermidis* [*n*=18], *S. capitis* [*n*=4], *S. haemolyticus* [*n*=3], *S. warnerii* [*n*=2] and *S. hominis* [*n*=1]) and Enterobacteriaceae (*Klebsiella pneumoniae* [*n*=7], *Escherichia coli* [*n*=6], *Enterobacter cloacae* [*n*=5] and other [*n*=6]) were the most common bacteria isolated (25 % and 21 %, respectively). Other Gram-positive microorganisms (*S.aureus*, streptococci and enterococci) were seen in 10 % and non-fermentative Gram negative organisms (*Pseudomonas* spp. and *Acinetobacter* spp.) in 5 % of cases. In 39 % of LOS cases, the causative organism was not identified.

Criteria defining sepsis in patients with PMA ≤44 weeks

At baseline, a total of 21 clinical findings were recorded but none of them occurred in more than 40 % of patients. The most frequently observed clinical criteria were impaired peripheral perfusion, increased oxygen requirement and mottled skin, each seen in about 40 % (Fig. 2). There were 69 % of patients with three or more, 35 % with four or more and 19 % with five or more clinical criteria. Increased C-reactive protein (CRP; ≥15 mg/l) and reduced platelet count ($<100\times10^9$ cells/l) were the most common laboratory parameters, seen in 85 % and 42 % of patients, respectively (Fig. 3). Altogether, 44 % of patients had three or more, 19 % four or more and only 3 % five of the laboratory criteria. No differences in the presence of any of the clinical or laboratory criteria between those with culture proven or clinical sepsis were seen (data not shown). The predictive value of the expert-panel-derived criteria to identify patients with a culture proven LOS was 61 % (95 % CI 52–70 %).

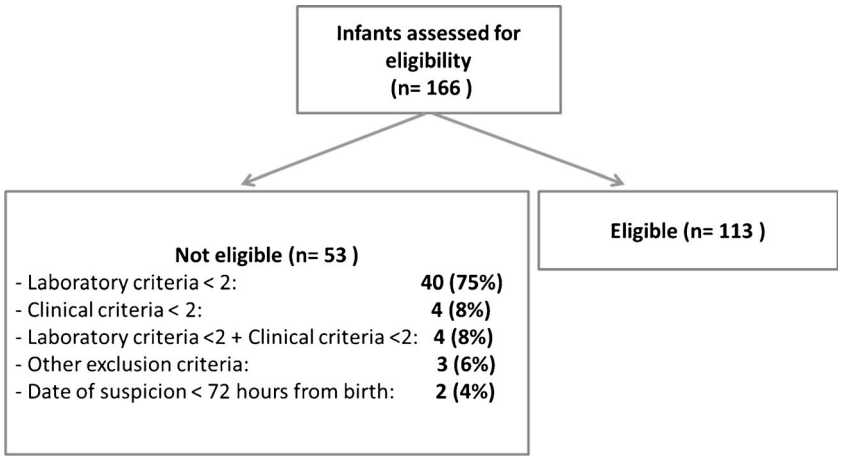

Fig. 1 Study flowchart and reasons for ineligibility in 53 of 166 screened patients

Antimicrobial susceptibility of infecting microorganisms

As presented in Table 3, 90 % of Enterobacteriaceae were susceptible to meropenem and at least two thirds to amikacin, cefotaxime, ciprofloxacin and the combination of cefotaxime and gentamicin. However, the median MIC values for cefotaxime and gentamicin were relatively low — 1 and 2 μg/ml, respectively. Almost all (95 %) Enterobacteriaceae were resistant to ampicillin with a median MIC value of 32 μg/ml. The number of non-fermentative Gram-negative organisms was low and their susceptibility to relevant antibiotics, including proposed NeoMero1 study regimens, did not exceed 50 %. The CoNS were predominantly susceptible to glycopeptides but resistant to all other tested antibiotics (Table 3).

Antibiotic treatment

A total of 43 different empiric antibiotic regimens were used in the 113 patients. Meropenem monotherapy or the combinations of cefotaxime plus gentamicin or ampicillin plus gentamicin were used infrequently, only in ten (9 %), one (1 %) and seven (6 %) patients, respectively (Table 4). However, 50 % of patients received meropenem or a third-generation cephalosporin or ampicillin, either alone, or in combination with aminoglycosides and/or glycopeptides. Use of empiric glycopeptides (mostly vancomycin in 51/58) and antifungals (mostly liposomal amphotericin in 26/46) was common and involved 51 % and 41 % of patients, respectively.

When comparing the use of empiric antibiotics between Northern and Southern Europe, the data indicate that cefotaxime, piperacillin/tazobactam and gentamicin were more often used in the Northern and teicoplanin exclusively in the Southern European countries (data not shown). The median duration of empiric and overall antibacterial therapy were 15 days (IQR 10–24) and 16 days (IQR 10–27), respectively. Of 69 patients with culture proven LOS, appropriate empiric antibiotics were given to 42 (61 %) patients, inappropriate to 16 (23 %) patients and no assessment was available in the 11 (16 %) remaining cases.

Outcome

A favorable outcome at TOC was seen in only 53 of 113 patients (47 %; 95 % CI 38–56 %). No differences in

Table 2 Demographic parameters of 113 patients included in the study

	Clinical sepsis (*N*=40)	Culture proven sepsis (*N*=69)	All included patients[a] (*N*=113)
Age at onset (days)	14.9 (7.3–30.2)	14.0 (8.1–25.0)	14.0 (8.0–16.0)
GA (w)	28.7 (26.8–33.6)	29.1 (26.7–33.6)	29 (26.9–33.6)
>37 weeks, *n* (%)	6 (15)	13 (19)	19 (17)
PMA (w)	32.2 (29.6–37.8)	32.1 (29.7–36.3)	32.4 (29.7–36.6)
>44 weeks, *n* (%)	1 (3)	4 (6)	5 (4)
Male gender, *n* (%)	26 (65)	43 (62)	70 (62)
BW (g)	988 (780–2,162)	1,230 (815–1,850)	1,190 (0.815–1,850)
≤1,000 g, *n* (%)	21 (53 %)	29 (42 %)	50 (44 %)
≤1,500 g, *n* (%)	26 (65 %)	44 (64 %)	70 (62 %)

Data are presented as median with IQR in parenthesis

[a] Four patients met the inclusion criteria but had viral or fungal (non-bacterial) LOS

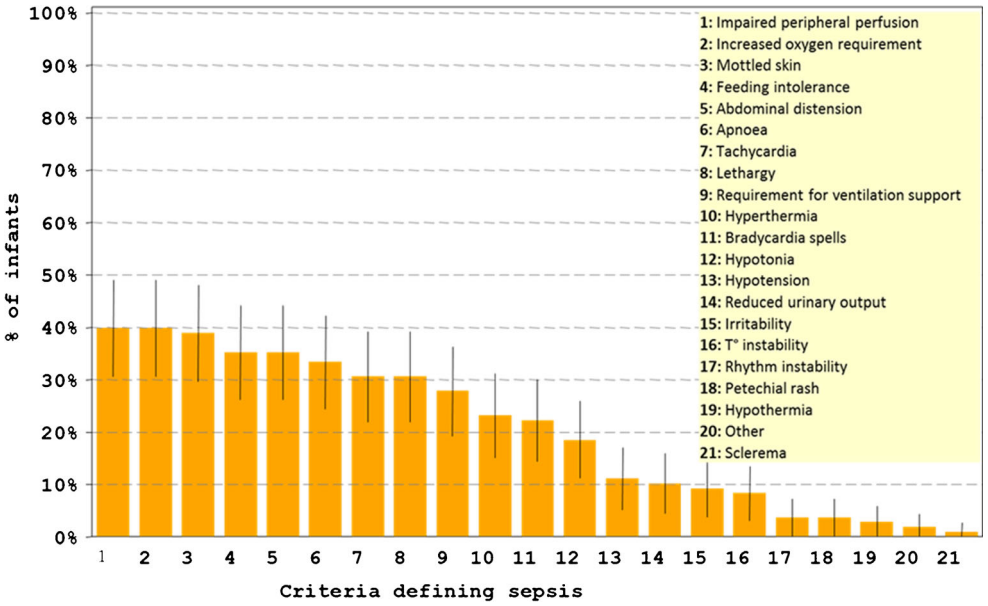

Fig. 2 Proportion of patients with PMA ≤44 weeks who had the expert panel defined clinical criteria of LOS (n=108). The error bars indicate 95 % confidence intervals

favorable outcome between culture proven (36/69 [52 %; 95 % CI 40–64 %]) and clinical sepsis (15/40 [38 %; 95 % CI 23–53 %]) were observed. The most frequently documented reason for failure was modification of antibiotics for lack of efficacy (42/60; 70 %) followed by no resolution of clinical features or of laboratory parameters (30/60; 50 %).

The all-cause mortality rate was 8 % (9/113; 95 % CI 3–13 %), with no significant difference between those with culture proven (7/69; 10 % [95 % CI 4–20 %]) or clinical sepsis (2/40; 5 % [95 % CI 1–17 %]). The median time to death from screening was 7 days (range 1–42 days). At day 11 (recommended treatment duration in NeoMero1), the probability of survival was 94 % (95 % CI 87–97 %). Among the seven cases of culture proven sepsis who died, three had *K. pneumoniae* isolated. *E. cloacae*, *S. haemolyticus*, *Acinetobacter* spp. and *Streptococcus pyogenes* were identified in the remaining four cases. The mortality rate in patients with Gram-negative sepsis was 17 % (95 % CI 6–35 %) as compared to 5 % in Gram-positive sepsis (95 % CI 1–17 %). Two microorganisms, *S. haemolyticus* and *K. pneumoniae*, were resistant to empiric antibiotic regimens of gentamicin and ampicillin plus gentamicin, respectively.

In multivariate analysis none of the factors included in the model (birth weight ≤1,000 g, age at sepsis onset, gestational age, number of clinical or laboratory criteria, gender, type and adequacy of empiric antibiotic regimen) were predictive of a favorable outcome.

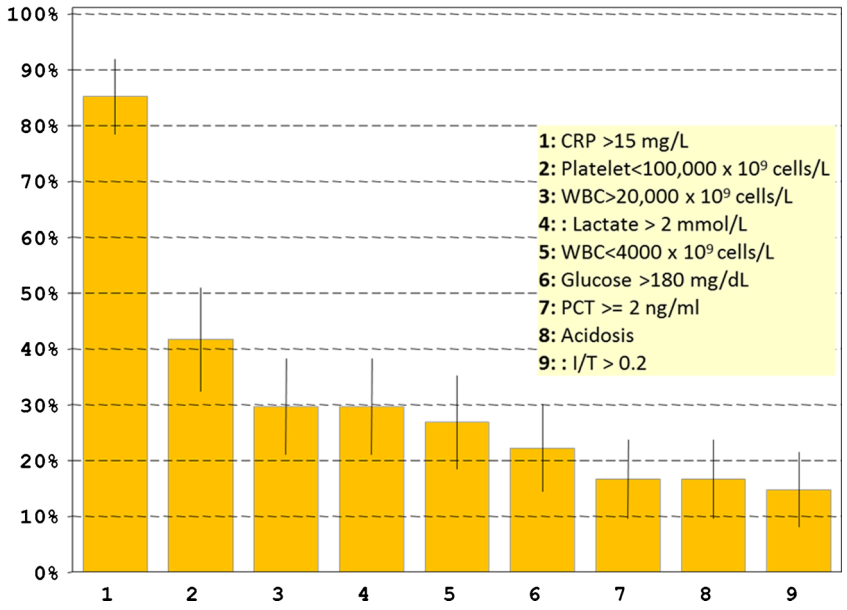

Fig. 3 Proportion of patients with PMA ≤44 weeks who had the expert panel-defined laboratory criteria of LOS (n=108). The error bars indicate 95 % confidence intervals

Table 3 Antimicrobial resistance (*R*) based on EUCAST criteria and relevant MIC values of Enterobacteriaceae, non-fermentative Gram-negative microorganisms and CoNS

Antibiotic	*Enterobacteriaceae* (*N*=24)		Non-fermentative Gram-negatives[a] (*N*=6)		CoNS (*N*=28)	
	Resistant *n*/*N* (%)	Median MIC (IQR) (μg/ml)	Resistant *n*/*N* (%)	Median MIC (IQR) (μg/ml)	Resistant *n*/*N* (%)	Median MIC (IQR) (μg/ml)
Ampicillin	21/22 (95)	32 (32; 32)	NA	NA	28/28	8 (8; 8)
Oxacillin	NA	NA	NA	NA	27/28 (96)	4 (2; 4)
Cefotaxime/ceftazidime	7/21 (33)	1 (1; 36)	3/5 (60)	16 (16; 16)[b]	27/28 (96)	ND
Meropenem	2/20 (10)	0.25 (0.25; 2)	3/6 (50)	12 (4; 16)	27/28	ND
Amox/clavulanic acid	13/21 (62)	16 (4; 32)[c]	NA	NA	ND	ND
Piperacillin/tazo-bactam	8/20 (40)	4 (4; 16)[c]	4/6 (67)	128 (128; 128)[c]	ND	ND
Gentamicin	8/21 (38)	2 (1; 16)	4/6 (67)	16 (16; 16)	22/27 (81)	8 (8; 8)
Amikacin	5/21 (24)	4 (2; 16)	3/5 (60)	64 (48; 64)	6/6 (100)	32 (16; 32)
Ciprofloxacin	6/18 (33)	0.25 (0.25; 1)	3/5 (60)	4 (4; 4)	4/5 (80)	ND
Vancomycin	NA	NA	NA	NA	2/26 (8)	2 (0.9; 2)
Teicoplanin	NA	NA	NA	NA	5/19 (26)	4 (1; 8)
Ampicillin + gentamicin	8/21 (38)	NA	4/6 (67)	NA	22/27 (81)	NA
Cefotaxime + gentamicin	7/22 (32)	NA	4/6 (67)	NA	22/27 (81)	NA

n number of susceptible isolates, *N* total number of isolates with available MIC values, *NA* not applicable, *ND* not done

[a] Includes *Pseudomonas* spp. and *Acinetobacter* spp.

[b] MIC values are presented for ceftazidime

[c] MIC values are presented for amoxillin or piperacillin, respectively

Discussion

To the best of our knowledge, this is one of the very few studies to prospectively assess the current management of LOS in a multinational setting. In a study predominantly enrolling premature neonates we observed first, that the EMA expert-panel-derived criteria had a predictive value of 61 % to identify patients with culture-proven LOS; second, that the currently employed antibiotic regimens used in participating EU countries are extremely variable and third, that the resistance rates of Enterobacteriaceae to ampicillin and of CoNS to oxacillin and gentamicin, commonly recommended for the empiric treatment of LOS by experts [1, 3], are high.

The conduct of RCTs in LOS is complicated by the large variety of clinical and laboratory findings that can be observed and reflected in the definition of LOS by the expert panel. The fact that none of the clinical findings was seen in more than 40 % of subjects confirms the non-specificity of these criteria as observed in previous publications [21]. A smaller selection of clinical symptoms (n=12) has been proposed by Modi et al. [17] for monitoring bloodstream infections. However, these criteria only apply for culture proven and not for clinical (i.e., culture negative) infections. Mahieu et al. [14] have developed the NOSEP bedside scoring system composed of five parameters (CRP, neutrophil fraction, low platelet count, fever and parenteral nutrition) for early identification of neonatal sepsis. With the exception of CRP other components of this scoring system were infrequent in our study and seen in less than 30 % of patients. In a more recent study increased respiratory support, capillary refill, pallor/grey skin were identified as most predictive signs of clinical and culture proven LOS in preterm neonates, but in this study Gram-negative organisms only accounted for 6 % of blood culture proven sepsis cases [2]. One of the secondary aims of the NeoMero1 study is to develop and validate these criteria to facilitate further clinical studies in LOS.

The significant variability in the choice of empiric antibiotic treatment for LOS despite only a few antibiotics being recommended by experts, was surprising [19, 22]. We did not aim to evaluate reasons for the lack of compliance of practicing neonatologists with "expert" recommendations. However, one could speculate that the lack of RCTs in this area, regional customs including different views on acceptable risks and the specifics of local antibiotic resistance patterns, could be just a few of them. Similarly, high variability in local neonatal guidelines was reported for the UK/Ireland [6].

A key question for this study was whether the selected comparator regimens would ensure adequate coverage of LOS causing organisms in participating centers in the NeoMero1 study. In the absence of clinical data we had to rely on in vitro breakpoints. Except for non-fermentative microorganisms which rarely cause neonatal sepsis [10],

Table 4 Empiric antibiotic regimens used for patients included in the study

Antibiotic regimen	*N*=113 (%)
Ampicillin based regimens (*n*=11)	
Ampicillin/aminoglycoside	8 (7)
Ampicillin	1 (1)
Ampicillin sulbactam	1 (1)
Ampicillin sulbactam/netilmicin	1 (1)
Third-generation cephalosporin based regimens (*n*=20)	
Ceftazidime/glycopeptide	9 (8)
Cefotaxime	4 (4)
Cefotaxime/aminoglycoside	3 (2)
Cefotaxime/gentamicin/Penicillin G	2 (2)
Ceftazidime	1 (1)
Cefotaxime/vancomycin	1 (1)
Meropenem based regimens (*n*=30)	
Meropenem/glycopeptide	14 (12)
Meropenem	10 (9)
Meropenem/amikacin	2 (2)
Meropenem/glycopeptide/aminoglycoside	2 (2)
Meropenem/vancomycin/cefuroxime	1 (1)
Meropenem/piperacillintazobactam/gentamicin	1 (1)
Other regimens[a]	52 (46)
- Vancomycin or teicoplanin	10 (9)
- Aminoglycoside + glycopeptide	16 (14)
- Others[a]	26 (23)

[a] Includes cefepime alone or in combination with glycopeptides (*n*=7), aminoglycoside monotherapy (*n*=5), piperacillin/tazobactam in combination with aminoglycosides or glycopeptides (*n*=5)

about two thirds of Enterobacteriaceae were susceptible to ampicillin + gentamicin or cefotaxime + gentamicin. However, the median cefotaxime MIC value was much lower than that of ampicillin (1 μg/ml vs. 32 mcg/ml). The high resistance rate of CoNS to oxacillin and gentamicin should not be an issue as the empiric use of vancomycin is allowed in NeoMero1 [13].

The low rate of favorable outcome (47 %), mainly due to changes of antibiotic regimen, should also be noted. However, these data need to be treated with caution as in this observational study the antibiotic stopping or changing criteria were not pre-defined, neither were the reasons for treatment modifications recorded. One should also note that some of the empiric regimens may have been suboptimal in treating LOS (e.g., vancomycin monotherapy in patients with Gram-negative sepsis). Despite the low rates of favorable outcome, strict enrollment criteria and high antibiotic resistance rates, the all-cause mortality of 8 % in this study is comparable or even slightly lower than demonstrated in recently conducted surveys of culture-proven and clinical sepsis [10]. The relatively low all-cause mortality rate of LOS supports our choice of a composite endpoint for the NeoMero1 study. For similar reasons this composite endpoint was recently also suggested by Ambrose et al. [11] for antibiotic studies of drug resistant infections.

The main limitation of this study was its observational nature without proper monitoring and without unified criteria for antibiotic change and/or resistance testing. One should also note that the data on antibiotic therapy and AMR were collected from five European countries and thus are not necessarily representative for the entire Europe. Still, we believe that these limitations did not prevent us from achieving the main aim of the study which was to describe the current management of LOS in centers that participate in NeoMero1 study, and in drawing adequate conclusions.

Thus, we conclude that the EMA expert panel defined diagnostic criteria of LOS for subjects with PMA ≤44 weeks performed reasonably well in identifying a high rate of culture-proven sepsis and that the currently recommended regimens for NeoMero1 study are, in general, adequate. We highlight the large variability of empiric antibiotic regimens of LOS in these five European countries. A recently completed meropenem safety and effectiveness study [5], the ongoing studies NeoMero1 [13] and NeoMero2 and the ARPEC project (collecting Europe-wide data on AMR and antibiotic use in neonatal sepsis (www.arpecproject.eu), are just the first steps for improving the evidence base for the management of neonatal sepsis.

These data suggest an urgent need for improved evidence-based guidelines for the empiric therapy of LOS based on locally derived AMR data and RCTs. We believe that RCTs, however, should be complemented by neonatal antimicrobial PK/PD modeling [20], as the PK of antibiotics and clinical course of LOS in neonates is different to that seen in older children and adults.

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