Procalcitonin-guided decision making for duration of antibiotic therapy in neonates with suspected early-onset sepsis: a multicentre, randomised controlled trial (NeoPIns)

Martin Stocker, Wendy van Herk*, Salhab el Helou, Sourabh Dutta, Matteo S Fontana, Frank A B A Schuerman, Rita K van den Tooren-de Groot, Jantien W Wieringa, Jan Janota, Laura H van der Meer-Kappelle, Rob Moonen, Sintha D Sie, Esther de Vries, Albertine E Donker, Urs Zimmerman, Luregn J Schlapbach, Amerik C de Mol, Angelique Hoffman-Haringsma, Madan Roy, Maren Tomaske, René F Kornelisse, Juliette van Gijsel, Eline G Visser, Sten P Willemsen, Annemarie M C van Rossum, and the NeoPInS Study Group†*

Summary

Background Up to 7% of term and late-preterm neonates in high-income countries receive antibiotics during the first 3 days of life because of suspected early-onset sepsis. The prevalence of culture-proven early-onset sepsis is 0·1% or less in high-income countries, suggesting substantial overtreatment. We assess whether procalcitonin-guided decision making for suspected early-onset sepsis can safely reduce the duration of antibiotic treatment.

Methods We did this randomised controlled intervention trial in Dutch (n=11), Swiss (n=4), Canadian (n=2), and Czech (n=1) hospitals. Neonates of gestational age 34 weeks or older, with suspected early-onset sepsis requiring antibiotic treatment were stratified into four risk categories by their treating physicians and randomly assigned [1:1] using a computer-generated list stratified per centre to procalcitonin-guided decision making or standard care-based antibiotic treatment. Neonates who underwent surgery within the first week of life or had major congenital malformations that would have required hospital admission were excluded. Only principal investigators were masked for group assignment. Co-primary outcomes were non-inferiority for re-infection or death in the first month of life (margin 2·0%) and superiority for duration of antibiotic therapy. Intention-to-treat and per-protocol analyses were done. This trial was registered with ClinicalTrials.gov, number NCT00854932.

Findings Between May 21, 2009, and Feb 14, 2015, we screened 2440 neonates with suspected early-onset sepsis. 622 infants were excluded due to lack of parental consent, 93 were ineligible for reasons unknown (68), congenital malformation (22), or surgery in the first week of life (3). 14 neonates were excluded as 100% data monitoring or retrieval was not feasible, and one neonate was excluded because their procalcitonin measurements could not be taken. 1710 neonates were enrolled and randomly assigned to either procalcitonin-guided therapy (n=866) or standard therapy (n=844). 1408 neonates underwent per-protocol analysis (745 in the procalcitonin group and 663 standard group). For the procalcitonin group, the duration of antibiotic therapy was reduced (intention to treat: 55·1 *vs* 65·0 h, p<0·0001; per protocol: 51·8 *vs* 64·0 h; p<0·0001). No sepsis-related deaths occurred, and 9 (<1%) of 1710 neonates had possible re-infection. The risk difference for non-inferiority was 0·1% (95% CI −4·6 to 4·8) in the intention-to-treat analysis (5 [0·6%] of 866 neonates in the procalcitonin group *vs* 4 [0·5%] of 844 neonates in the standard group) and 0·1% (−5·2 to 5·3) in the per-protocol analysis (5 [0·7%] of 745 neonates in the procalcitonin group *vs* 4 [0·6%] of 663 neonates in the standard group).

Interpretation Procalcitonin-guided decision making was superior to standard care in reducing antibiotic therapy in neonates with suspected early-onset sepsis. Non-inferiority for re-infection or death could not be shown due to the low occurrence of re-infections and absence of study-related death.

Funding The Thrasher Foundation, the NutsOhra Foundation, the Sophia Foundation for Scientific research.

Published **Online** July 12, 2017 http://dx.doi.org/10.1016/S0140-6736(17)31444-7

See **Online/Comment** http://dx.doi.org/10.1016/S0140-6736(17)31628-8

*Joint first authors

†A complete list of the members of the NeoPInS (Neonatal Procalcitonin Intervention Study) Study Group is provided in the appendix (p 3)

Department of Paediatrics, Neonatal and Paediatric Intensive Care Unit, Children's Hospital Lucerne, Lucerne, Switzerland (M Stocker MD, M S Fontana MD); **Department of Paediatrics, Division of Paediatric Infectious Diseases & Immunology, Erasmus MC University Medical Centre-Sophia Children's Hospital, Rotterdam, Netherlands** (W van Herk MD, E G Visser MA, A M C van Rossum PhD); **Division of Neonatology, McMaster University Children's Hospital, Hamilton Health Sciences, Hamilton, ON, Canada** (S el Helou MD, S Dutta PhD); **Department of Paediatrics, Flevo Hospital, Almere, Netherlands** (F A B A Schuerman MD); **Department of Paediatrics, Bronovo Hospital, 's Gravenhage, Netherlands** (R K van den Tooren-de Groot MD); **Department of Paediatrics, MC Haaglanden, 's Gravenhage, Netherlands** (J W Wieringa MD); **Department of Neonatology, Thomayer Hospital, Prague, Czech Republic** (Jan Janota PhD); **Institute of Pathological Physiology, First Medical Faculty, Charles University in Prague, Czech Republic** (Jan Janota PhD); **Department of Neonatology, Reinier de Graaf Gasthuis, Delft, Netherlands** (L H van der Meer-Kappelle MD);

Introduction

Neonatal sepsis is a leading cause of global mortality in children younger than 5 years.[1] Proven early-onset sepsis has mortality rates as high as 30% in high-income countries and up to 60% in low-income countries.[2,3] Prompt diagnosis and treatment of neonatal early-onset sepsis are crucial to prevent severe morbidity and mortality.[4] However, the initial, clinical presentation is often subtle and nonspecific, and commonly used biomarkers have low predictive values for early sepsis, which presents a daily challenge to clinicians involved in neonatal care.[5]

In high-income countries, between 4·0–7·4% of term and late-preterm neonates are given intravenous antibiotics within the first 3 days of life if they are suspected to have early-onset sepsis. However, the prevalence of culture-proven early-onset sepsis is less than 0·1%, which suggests that antibiotic treatment—with its ensuing hospital admission, neonatal and parental discomfort, medical costs, and use of resources—is unnecessary in many neonates.[6–9] Additionally, evidence is accumulating that antibiotic treatment in early life disturbs the microbial flora that

Department of Neonatology, Atrium Medical Centre, Heerlen, Netherlands (R Moonen MD); **Department of Neonatology, VU University Medical Centre, Amsterdam, Netherlands** (S D Sie MD); **Department of Paediatrics, Jeroen Bosch Hospital, 's-Hertogenbosch, Netherlands** (Prof E de Vries PhD); **Department of Paediatrics, Maxima Medical Centre, Veldhoven, Netherlands** (A E Donker MD); **Department of Paediatrics, Kantonsspital Winterthur, Winterthur, Switzerland** (U Zimmermann MD); **Department of Paediatrics, Bern University Hospital, Inselspital, University of Bern, Bern, Switzerland** (L J Schlapbach MD); **Paediatric Critical Care Research Group, Mater Research Institute, University of Queensland, Brisbane, QLD, Australia** (L J Schlapbach MD); **Paediatric Intensive Care Unit, Lady Cilento Children`s Hospital, Brisbane, QLD, Australia** (L J Schlapbach MD); **Department of Neonatology, Albert Schweitzer Hospital, Dordrecht, Netherlands** (A C de Mol PhD); **Department of Neonatology, Sint Franciscus Gasthuis, Rotterdam, Netherlands** (A Hofman-Haringsma MD); **Department of Neonatology, St. Josephs Healthcare, Hamilton Health Sciences, Hamilton, ON, Canada** (M Roy MD); **Department of Paediatrics, Stadtspital Triemli, Zürich, Switzerland** (M Tomaske MD); **Division of Neonatology, Erasmus MC University Medical Centre-Sophia Children's Hospital, Rotterdam, Netherlands** (R F Kornelisse PhD); **Julius Training General Practitioner, University Medical Centre Utrecht, Netherlands** (J van Gijsel MD); and **Department of Biostatistics, Erasmus MC University Medical Centre, Rotterdam, Netherlands** (S P Willemsen MSc)

Correspondence to:
Dr Wendy van Herk, Department of Paediatrics, Division of Infectious Diseases and Immunology, Erasmus MC University Medical Centre-Sophia Children's Hospital, Rotterdam, 3015 CN, Netherlands
w.vanherk@erasmusmc.nl

Research in context

Evidence before this study

The National Institute for Health and Care Excellence (NICE) guideline CG149 Neonatal infection (early-onset): antibiotics for prevention and treatment, published in August, 2012, states that evidence to guide the decision to stop antibiotic treatment in neonates receiving antibiotics for suspected early-onset neonatal infection is scant. The guideline recommends initiating studies answering the question: What is the clinical effectiveness of laboratory investigations used individually or in combination to exclude early-onset neonatal infection in neonates receiving antibiotics for suspected infection? The ideal study design was described as a randomised controlled trial comparing clinical outcomes associated with particular investigation and treatment termination strategies. No literature search was done.

Added value of this study

To our knowledge, the Neonatal Procalcitonin Intervention Study (NeoPInS) is the first neonatal intervention study on suspected early-onset sepsis aiming to show superiority (duration of antibiotic treatment) and non-inferiority (re-infection or death in the first month of life) of biomarker-guided antibiotic therapy improving antimicrobial stewardship. NeoPInS is well aligned with the ideal study design as recommended by NICE clinical guideline 149 and with the need for pragmatic studies. In a group of 1710 neonates from high-income countries with a low prevalence of proven early-onset sepsis, we found that standardised risk assessment for suspected early-onset sepsis with procalcitonin-guided decision making reduces the duration of antibiotic therapy and hospital stay, with a low rate of re-infections and without study-related mortality.

Implications of all the available evidence

The results of NeoPInS can help to improve patient care and clinical practice in newborn babies' first days of life. Reducing unnecessary antibiotic treatment is important, as we now have accumulating evidence of the adverse effects of antibiotic resistance and the consequences of disturbing the microbiome in early life, such as eczema, allergies, inflammatory bowel diseases, and increased weight gain. Additionally, the reduction of hospital admission will improve families' experiences of this special period of life and might reduce the burden for health-care facilities and services.

colonises the neonate and might be associated with health problems such as eczema, allergies, inflammatory bowel diseases, and increased weight gain.[10]

Procalcitonin has the highest negative predictive value (87–100%) of all established biomarkers for severe, invasive bacterial infections in neonates.[11,12] The interpretation of procalcitonin values in neonates is complicated by a physiological increase up to 48 h post partum, and other perinatal factors—such as chorioamnionitis, hypoxaemia, perinatal asphyxia, and maternal pre-eclampsia—can also cause it to increase.[13] Reference values of procalcitonin in neonates with and without early-onset sepsis have been established.[14,15]

Procalcitonin-guided decision making has been used to safely reduce antibiotic treatment in critically ill adults and children with suspected or proven invasive bacterial infections.[16,17] To our knowledge, no neonatal intervention studies to reduce antibiotic treatment including a safety endpoint have been done. In a single-centre pilot intervention study[18] in term and near-term neonates with suspected early-onset sepsis, we previously showed that procalcitonin-guided decision making can reduce the duration of antibiotic treatment in this population. However, we could not assess safety due to the limited sample size, so we initiated a multicentre, multinational intervention study in both academic and non-academic hospitals to assess whether procalcitonin-guided decision making for neonates with suspected early-onset sepsis could safely reduce the duration of antibiotic treatment (superiority aspect) without increasing re-infection or death in the first month of life (non-inferiority aspect).

Methods

Study design

The Neonatal PCT Intervention Study (NeoPInS) is an investigator-initiated, superiority and non-inferiority, multicentre, randomised controlled intervention study, done by the Neonatal Sepsis Trial Network. Patients were enrolled in 18 hospitals in the Netherlands (n=11), Switzerland (n=4), Canada (n=2), and the Czech Republic (n=1). No financial incentive was provided to investigators and participants. The local institutional review board and national ethical committee of each site approved the protocol.

Participants

Neonates born after 34 weeks of gestational age who had suspected early-onset sepsis in the first 72 h of life and who required antibiotic therapy were eligible for inclusion. Suspected early-onset sepsis was based on risk factors, and/or clinical symptoms, and/or laboratory results (figure 1). Babies who underwent surgery within the first week of life and neonates with major congenital malformations that would have required hospital admission for the malformation alone were excluded. Written informed consent from the parents or guardians was obtained for all participants.

Randomisation and masking

Neonates were randomly assigned in a 1:1 ratio to receive procalcitonin-guided treatment or standard care by the treating physician (junior doctor or paediatrician on call). Parents, nursing staff, physicians, and local investigators were aware of group assignment. Principal

For more on the **Neonatal Sepsis Trial Network** see www.neonatalnet.org

A

Assessment of risk classification (on t=1–12 hours)		
Risk factors	Check box if positive	
	1 Mother Group B streptococcus positive ☐	
	2 Maternal chorioamnionitis (fever >38·5, fetal tachycardia) ☐	
	3 Premature rupture membranes >18 hours ☐	
	4 Gestational age <37 weeks ☐	**A=**
	0 boxes checked? Score 0 **≥1 box checked? Score 1→**	**0/1**
Clinical symptoms	Check box if positive	
	1 Respiratory distress or apnoea ☐	
	2 Tachycardia or bradycardia ☐	
	3 Arterial hypotension and/or poor perfusion ☐	
	4 Hypothermia or hyperthermia ☐	
	5 Seizure, floppy infant, irritability, or lethargy ☐	**B=**
	6 Vomiting or feeding intolerance or ileus ☐	
	0 boxes checked? Score 0 **≥1 box checked? Score 1→**	**0/1**
Laboratory findings	Check box if abnormal	
	1 White blood cells <5x10 E9 cells per L ☐	
	2 C-reactive protein >10 mg/L ☐	**C=**
	0 boxes checked? Score 0 **≥1 box checked? Score 1→**	**0/1**
	Total score A+B+C=	

	Culture	Total score	Category	Duration of antibiotic therapy	
				PCT group	Standard group
☐	Neg	0/1	Category 4 infection unlikely (low risk)	At least 24 h, stop after 2 consecutive procalcitonin values within range	36–72 h
☐	Neg	2	Category 3 infection possible (medium risk)		5–7 days
☐	Neg	3	Category 2 infection probable (high risk)	7–21 days	
☐	Pos	≥1	Category 1 infection proven		

B

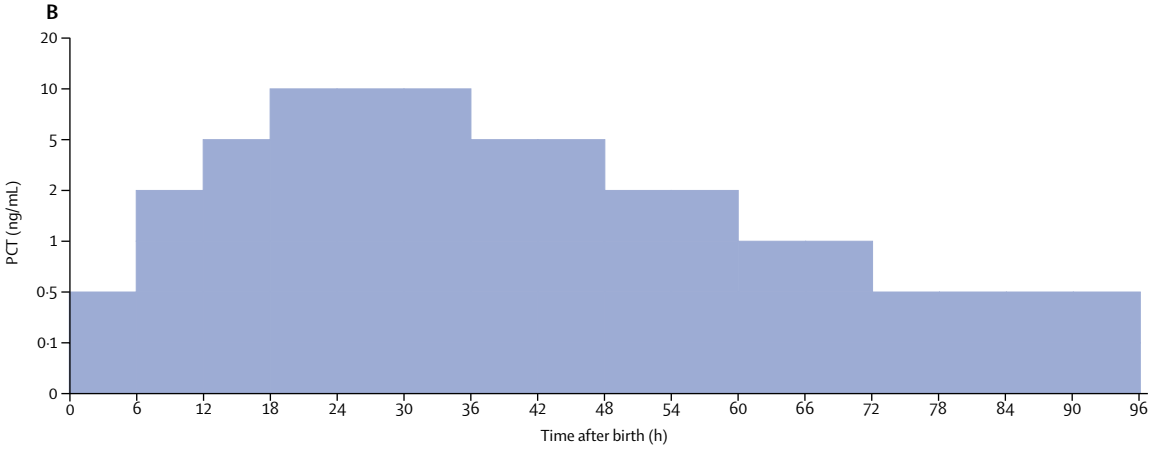

***Figure 1*: Risk classification and duration of antibiotic therapy using normal values of post-birth procalcitonin**
(A) Assessment of risk classification and duration of antibiotic therapy. (B) Normal hourly values of post-birth procalcitonin. PCT=procalcitonin. Neg=negative. Pos=positive.

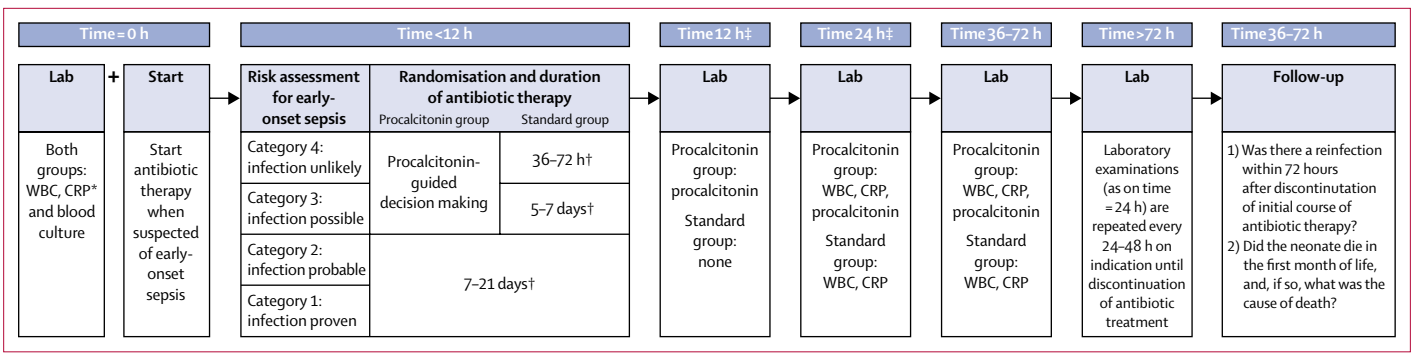

***Figure 2*: Study timeline**
Lab=laboratory measurements. WBC=white blood cell count. CRP=C-reactive protein. *If randomisation was known, procalcitonin was also measured in the procalcitonin group. †Antibiotic therapy was given according to local policy. ‡(6–18 h).

investigators were masked to group assignment. In Switzerland and the Czech Republic, randomisation was achieved by drawing group assignment cards at random. In the Netherlands and Canada randomisation was done by computer-based block randomisation with stratification for centre using blocks of four, six, and eight patients produced by the Department of Biostatistics, Erasmus MC University Medical Centre, Rotterdam, Netherlands. Allocation was concealed in all centres using sequentially numbered sealed opaque envelopes.

Procedures

The probability of infection was assessed using an easy-to-use scoring system based on risk factors, clinical symptoms, and conventional laboratory tests, independent of procalcitonin values (figure 1A).[18] The minimum score was 0, and the maximum score was 3 points. One point was given if one or more of the risk factors (maternal group B streptococci carriage, clinical signs of chorioamnionitis, premature rupture of membranes longer than 18 h, and gestational age less than 37 weeks) were positive. One point was given if one or more of the clinical symptoms (respiratory signs, heart rate abnormalities, perfusion problems, temperature deviations, neurological signs, or abdominal signs) were positive. One point was given for abnormal routine laboratory tests (leukocytopenia or C-reactive protein >10 mg/L).

The neonates were stratified into four risk categories within 12 h after initiation of antibiotic therapy: infection proven (category 1: neonates with positive blood culture, and total score ≥1); infection probable (category 2: neonates with risk factors, clinical signs, and abnormal routine laboratory values, total score 3); infection possible (category 3: neonates with abnormal findings in two of the three risk factors, clinical signs, and routine laboratory tests, total score 2); and infection unlikely (category 4: neonates with one or no abnormal finding of the three areas of risk factors, clinical signs, and routine laboratory tests, total score 0 or 1).

Neonates in categories 1 and 2 were given antibiotics and standard care for at least 7 days according to the local policy in each participating centre. For these babies, duration of antibiotic therapy was not guided by procalcitonin. We included these neonates in this study as part of our pragmatic approach to reflect daily clinical practice, where in patients except those in category 1 there is often a high level of uncertainty about whether or not they have an infection. In the standard treatment group, neonates in category 3 were treated for 5–7 days and neonates in category 4 were treated for 36–72 h. In the procalcitonin group, duration of therapy for patients in categories 3 and 4 was based on procalcitonin-guided decision making, for a minimum of 24 h, stop after 2 consecutive procalcitonin values within range, with a maximum treatment duration equal to the maximum treatment duration in the standard group (figure 1).

In the standard group, as in usual practice, the decision to discontinue antibiotic therapy was made by the treating physician on the basis of blood culture results, clinical signs, and routine laboratory test results. In the intervention group, the treating physician was advised to discontinue antibiotic therapy in neonates categorised as group 3 or 4 (infection possible or infection unlikely, corresponding to a score of 0, 1, or 2) after two consecutive procalcitonin measurements had been found within the normal range (figure 1). Physicians were always allowed to overrule the recommendation and continue antibiotic therapy if they felt that was appropriate (eg, on the basis of clinical symptoms or other laboratory investigations). The baby was discharged from hospital on the basis of the treating physicians' assessment. Reasons for prolonging hospital stay after discontinuation of antibiotic therapy were recorded as serious adverse events.

Laboratory examinations were done according to figure 2. Blood sampling was limited to normal frequencies already used in standard neonatal care, and one additional sample was obtained in the procalcitonin group 12 h (6–18 h) after inclusion. If possible, blood samples were obtained from existing intravenous lines to

minimise the burden on the child. Blood cultures were drawn before starting antibiotic therapy, and other cultures (eg, CSF culture) or other additional diagnostic tests (eg, radiography) were performed on indication.

Procalcitonin measurement was done on site at the central laboratory of each participating centre using the Roche Elecsys BRAHMS procalcitonin assay, with a functional sensitivity of 0·06 µg/L using Time Resolved Amplified Cryptate Emission (TRACE). Depending on the participating centre, a Kryptor machine (Kryptor PCT; Brahms, Hennigsdorf, Germany, with a minimum sample volume of 50 µL) or a suitable Roche Diagnostics Immunoanalyser (with a minimum sample volume of 30 µL) was used for the measurements. The measuring range of the assay was 0·02–100 µg/L with an automated dilution extending the upper range to 1000 µg/L.

At the time the neonate was discharged from hospital, their parents were informed about signs of a recurrent infection (either by a study-specific patient contact card or hospital-specific discharge procedures) and instructed to contact the hospital if their baby's condition deteriorated. For all patients, follow-up information for the first month of life regarding recurrence of infection, readmission to hospital, additional courses of antibiotics, and death was obtained by interviewing the parents during their follow-up visits, or by telephone interview at least 1 month after discharge.

Outcomes

The primary outcomes were duration of antibiotic treatment (superiority aspect) and re-infection or death in the first month of life (non-inferiority aspect). The secondary outcome was duration of hospital stay. Recurrence of infection was defined as a recurrence that required an additional course of antibiotic therapy within 72 h after completion of the initial course of antibiotic treatment.

During the trial, data were collected from individual patient records by the local investigators. A monitoring team did 100% source data verification through onsite visits to ensure data quality and completeness. Database access was restricted to the data management team until the end of the trial. An independent data and safety monitoring board reviewed masked data on patient safety. Serious adverse events were reported to the principal investigators within 48 h (except deaths, which were reported immediately) and subsequently reported to the data and safety monitoring board and the designated ethical committee in the Netherlands. All serious adverse events were verified by the monitoring team and followed up until they had abated or the patient's clinical condition had stabilised. To ensure quality, layout and randomisation improvements were made to the risk assessment during the trial (appendix pp 4–5). The trial was done in accordance with the International Conference on Harmonisation for Good Clinical Practice (ICH-GCP), the Declaration of Helsinki, and all applicable local regulations.

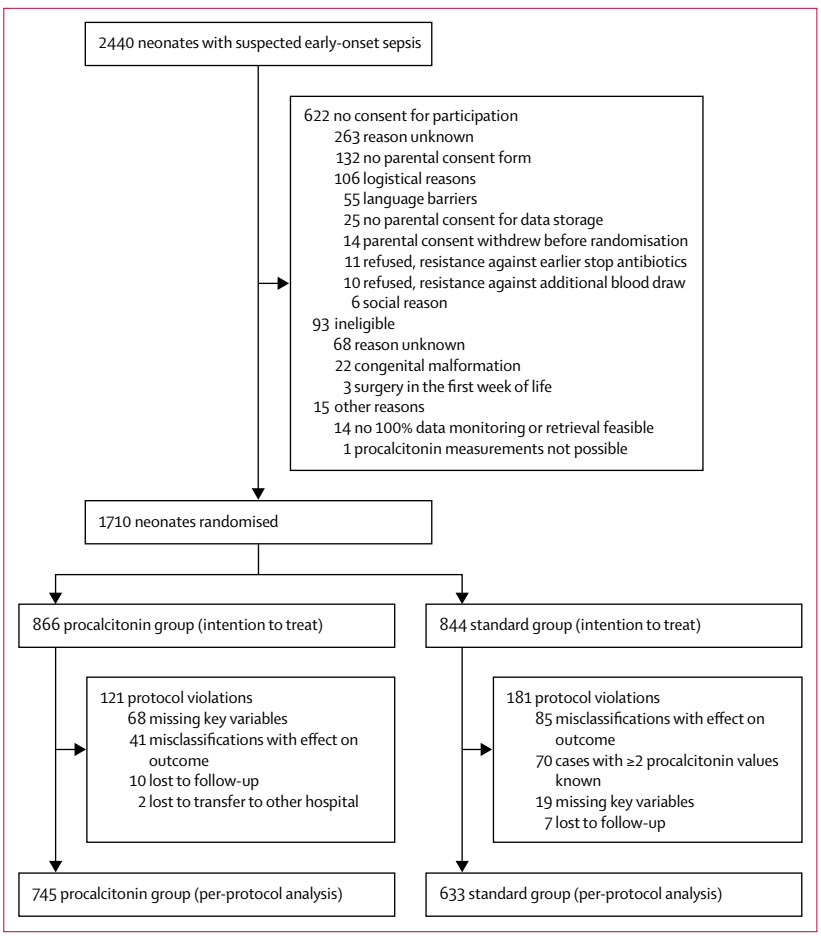

Figure 3: **Trial profile**

Statistical analysis

Primary analyses were done both according to the intention-to-treat principle and the per-protocol principle (figure 3). With the intention-to-treat analysis for superiority, we aimed to mimic daily practice, and with the per-protocol analysis for non-inferiority, we aimed to show the real potency of the protocol. The population for the per-protocol analysis was defined by excluding protocol violations (misclassification, missing key variable, lost to follow-up, lost to transfer, and cases in the standard group where 2 or more procalcitonin measurements were known). Any adjustments to the statistical methods compared with the published protocol are described in the statistical analysis plan (appendix pp 36–37). All secondary analyses were informal.

For the superiority outcome of efficacy, we compared the duration of antibiotic therapy (stratified by centre) and hospital stay with Wilcoxon-Mann-Whitney test. The death of any neonate was considered as the worst

See Online for appendix

outcome, and the duration of antibiotic treatment was set at the highest duration found. An exploratory multivariate analysis of the effect of various predictors (study group, participating centre, risk category, birthweight, and gestational age) on the durations of antibiotics and hospital stay was done by estimating a linear regression model. The response was log transformed. The variables study group and risk categories were expected to have an influence on the duration of antibiotic therapy and hospital stay. The participating centre gave information on the influence of local characteristics, because antibiotic use can depend on a unit's culture more than on clinical evidence.[19] Birthweight and gestational age are basic variables that characterised the analysed population with potential effects on antibiotic use.

For the non-inferiority outcome, we calculated 95% CI for the difference between the procalcitonin group and the standard group in probability of re-infection within less than 72 h after completion of the initial course of antibiotic treatment or death within the first month of life. When the upper bound of this 95% CI was less than 2%, we deemed the procalcitonin treatment non-inferior to the standard treatment. The margin of 2% was based on clinical and statistical reasoning. The probability of re-infection in less than 72 h after completion of the initial course of antibiotic treatment or death within the first month of life was 1·7% in our pilot study. On the basis of this non-inferiority margin, a total of 770 patients per group was required (with power 80% and significance 0·05, 2-sided). To allow for some unassessable cases, we included 800 patients per group, which was sufficient to detect a difference in the duration of antibiotic treatment of 10 h with 95% power. Stratification by centre was not possible for the analysis of the non-inferiority outcome.

We used SPSS version 21 (IBM, Armonk NY, USA) for the descriptive baseline statistics and basic variable transformations, SAS 9.3 (SAS Institute, Cary NC) for calculating the risks of re-infections or death and the exact CIs of the risk differences between the groups, and R 3.2 (R Foundation, Vienna, Austria) for all other statistics. Two-tailed p values of less than 0·05 were considered to be statistically significant. The trial was registered at ClinicalTrials.gov, number NCT00854932.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 21, 2009, and Feb 14, 2015, 2440 neonates with suspected early-onset sepsis were screened. Consent for participation was not obtained for 622 neonates, 93 were ineligible for reasons unknown (68), congenital malformation (22), or surgery in the first week of life (three). 14 neonates were excluded as 100% data monitoring or retrieval was not feasible, and one neonate was excluded as procalcitonin measurements were not possible. 1710 neonates receiving antibiotic therapy for suspected early-onset sepsis were enrolled and randomly assigned to receive either procalcitonin-guided decision making or standard care (figure 3). In the intention-to-treat population, 866 patients were included in the procalcitonin group and 844 patients in the standard group. In the per-protocol population, 745 patients were included in the procalcitonin group and 663 patients were included in the standard group. Baseline characteristics were similar in both study groups for both analyses (table 1). In our population, 710 (42%) of 1710 neonates had a low risk (infection unlikely) and 788 (46%) of 1710 neonates had a moderate risk (infection possible) of early-onset sepsis at the initial assessment. Different antibiotics were started at the moment of suspected early-onset sepsis, according to local policy in each participating centre. A β-lactam antibiotic (penicillin or cephalosporin) combined with an aminoglycoside was started in 1223 (73%) of 1674 neonates in the intention-to-treat population and 990 (71%) of 1400 neonates in the per-protocol population. In other cases, a combination of β-lactam antibiotics (penicillin and cephalosporin or penicillin and carbapenem) was started (451 [27%] of 1674 neonates in the intention-to-treat population, 410 [29%] of 1400 neonates in the per-protocol population; table 1).

Procalcitonin-guided decision making resulted in a significant reduction in the duration of antibiotic therapy. In the intention-to-treat analysis, the effect size was a median difference of –9·9 h between the procalcitonin group and the standard group (55·1 h, 95% CI 50·5–60·0 in the procalcitonin group *vs* 65·0 h, 63·0–69·0 in the standard group; p<0·0001), and in the per-protocol analysis the median difference between groups was –12·2 h (51·8 h, 48·2–56·0 in the procalcitonin group *vs* 64·0 h, 61·0–68·1 in the standard group; p<0·0001). Length of hospital stay was significantly shorter in the procalcitonin group. In the intention-to-treat analysis, the effect size was a median difference of 3·5 h between the procalcitonin group and the standard group (123·0 h, 95% CI 113·0–134·5 in the procalcitonin group *vs* 126·5 h, 117·5–144·3 in the standard group; p=0·0019), and in the per-protocol analysis it was –5·2 h (115·8 h, 107·5–126·0 in the procalcitonin group *vs* 121·0 h, 111·2–138·0 in the standard group; p=0·0039). Multivariate regression analysis showed that the duration of antibiotic therapy and hospital stay depended on study group, risk category, gestational age (term or late preterm), and participating centre. Duration of hospital stay also depended on birthweight (appendix p 39).

In the procalcitonin group, five (0·7%) of 745 neonates in the per-protocol analysis (95% CI 0·2–1·6) and five (0·6%) of 866 neonates in the intention-to-treat analysis (0·2–1·3) had a suspected re-infection. In the standard group, one

	Intention-to-treat population		Per-protocol population	
	Procalcitonin group (n=866)	Standard group (n=844)	Procalcitonin group (n=745)	Standard group (n=663)
Sex				
Male	508/866 (59%)	492/844 (58%)	433/745 (58%)	378/663 (57%)
Female	358/866 (41%)	352/844 (42%)	312/745 (42%)	285/663 (43%)
Gestational age, weeks	39 (36–40)	39 (37–40)	39 (37–40)	39 (38–40)
Birthweight, kg	3·4 (2·8–3·7)	3·4 (2·9–3·7)	3·4 (2·8–3·7)	3·4 (3·0–3·7)
Method of delivery*				
Spontaneous vaginal	425/862 (49%)	387/836 (46%)	363/743 (49%)	300/662 (45%)
Vacuum or forceps	125/862 (15%)	139/836 (17%)	118/743 (16%)	114/662 (17%)
Primary caesarean section	62/862 (7%)	80/836 (10%)	49/743 (6%)	63/662 (10%)
Secondary caesarean section	250/862 (29%)	230/836 (27%)	213/743 (29%)	185/662 (28%)
Arterial cord pH	7·22 (7·15–7·29)	7·22 (7·15–7·29)	7·22 (7·15–7·29)	7·22 (7·15–7·29)
Apgar score				
1 min post partum	7 (5–9)	7 (5–9)	8 (5–9)	7 (5–9)
5 min post partum	8 (7–9)	8 (7–9)	9 (7–9)	8 (7–9)
10 min post partum	9 (8–10)	9 (8–10)	9 (8–10)	9 (8–10)
Risk factors				
Group B streptococcus-positive mother	119/863 (14%)	127/836 (15%)	97/745 (13%)	94/663 (14%)
Chorioamnionitis	165/863 (19%)	163/836 (20%)	155/745 (21%)	140/663 (21%)
Premature rupture of membranes 18 h or longer before birth	207/863 (24%)	188/836 (23%)	184/745 (25%)	148/663 (22%)
Gestational age less than 37 weeks	188/863 (22%)	163/836 (20%)	158/745 (21%)	115/663 (17%)
Clinical symptoms				
Respiratory distress or apnoea	514/863(60%)	508/836 (61%)	422/745 (57%)	373/663 (56%)
Tachycardia or bradycardia	95/863 (11%)	83/836 (10%)	77/745 (10%)	62/663 (9%)
Arterial hypotension or poor perfusion	79/863 (9%)	77/836 (9%)	55/745 (7%)	51/663 (8%)
Hypothermia or hyperthermia	154/863(18%)	127/836 (15%)	139/745 (19%)	107/663 (16%)
Seizure and/or floppy infants and/or irritability and/or lethargy	74/863 (9%)	91/836 (11%)	66/745 (9%)	70/663 (11%)
Vomiting and/or feeding intolerance and/or ileus	59/863 (7%)	56/836 (7%)	46/745 (6%)	46/663 (7%)
Laboratory findings <12 hours				
White blood cell count <5 x 10 E9 cells per L	21/858 (2%)	14/834 (2%)	14/740 (2%)	12/661 (2%)
C-reactive protein >10 mg/L	232/863(27%)	207/836 (25%)	173/745 (23%)	141/663 (21%)
Infection likelihood				
Infection proven	12/862 (1%)	15/837 (2%)	8/745 (1%)	13/663 (2%)
Infection probable	85/862 (10%)	76/837 (9%)	59/745 (8%)	44/663 (7%)
Infection possible	405/862 (47%)	383/837 (46%)	352/745 (47%)	293/663 (44%)
Infection unlikely	350/862 (41%)	360/837 (43%)	326/745 (44%)	313/663 (47%)
Unknown†	10/862(1%)	3/837 (0%)	..	..
Time between birth and start of antibiotic therapy, h	2·0 (1·0–12·0)	2·0 (1·0–11·0)	2·0 (1·0–10·0)	2·0 (1·0–10·0)
Combination of antibiotics given at the moment of suspected early-onset sepsis				
β-lactam antibiotic (penicillin or cephalosporin) combined with an aminoglycoside	633/857 (74%)	590/817 (72%)	534/742 (72%)	456/658 (69%)
β-lactam antibiotics (penicillin + cephalosporin or penicillin + carbapenem)	224/857 (26%)	227/817 (28%)	208/742 (28%)	202/658 (31%)

Data are median (IQR) or n/total (%). *There were no significant differences (p<0·05) between groups. †In these cases, the risk classification was a missing key variable. Numbers are less than n in each group where data were not available.

***Table 1*: Baseline characteristics**

neonate died because of the consequences of severe perinatal asphyxia, three (0·6%) of 663 neonates in the per-protocol analysis (95% CI 0·2–1·5) and three (0·5%) of 844 neonates in the intention-to-treat analysis (0·1–1·2) had suspected re-infection. The risk difference for re-infection within 72 h after completion of the initial course of antibiotic treatment or death within the first month of life between the two groups was 0·1% (95% CI –5·2 to 5·3)

	Procalcitonin group (n=745)	Standard group (n=663)
Total number of patients with prolonged antibiotic therapy	180 (24·2%)	73 (11·2%)
Physicians' decision based on:	89 (11·9%)	45 (6·9%)
Clinical presentation	39 (5·2%)	22 (3·3%)
Laboratory test results: C-reactive protein value	29 (3·9%)	14 (2·1%)
Laboratory test results: other laboratory test results	4 (0·5%)	2 (0·3%)
Culture results	12 (1·6%)	2 (0·3%)
Combination of clinical presentation and C-reactive protein value	5 (0·7%)	3 (0·5%)
Combination of clinical presentation and other laboratory test results	0 (0%)	1 (0·2%)
Combination of clinical presentation and culture results	0 (0%)	1 (0·2%)
Combination of culture results and C-reactive protein value	0 (0%)	0 (0%)
Combination of culture results and other laboratory test results	0 (0%)	0 (0%)
Other reasons:		
(Misinterpretation of) procalcitonin nomogram	35 (4·7%)	..
Practical issues (eg, change of staff shifts)	22 (3·0%)	3 (0·5%)
Reason unknown	34 (4·6%)	25 (3·8%)
Total	91 (12·3%)	28 (4·3%)
Total number of patients with shorter antibiotic therapy	156 (20·9%)	198 (29·9%)
Physicians' decision based on:	102 (13·7%)	117 (17·6%)
Clinical presentation	14 (1·9%)	8 (1·2%)
Laboratory test results: C-reactive protein value	4 (0·5%)	0 (0%)
Laboratory test results: other laboratory test results	1 (0·1%)	0 (0%)
Culture results	3 (0·4%)	3 (0·5%)
Combination of clinical presentation and C-reactive protein value	5 (0·7%)	4 (0·6%)
Combination of clinical presentation and other laboratory test results	4 (0·5%)	1 (0·2%)
Combination of clinical presentation and culture results	6 (0·8%)	34 (5·1%)
Combination of culture results and C-reactive protein value	25 (3·4%)	43 (6·5%)
Combination of culture results and other laboratory test results	4 (0·5%)	1 (0·2%)
Combination of clinical presentation, culture results, and C-reactive protein	36 (4·8%)	23 (3·5%)
Other reasons		
(Misinterpretation of) procalcitonin nomogram	10 (1·3%)	..
Failed intravenous-access	6 (0·8%)	3 (0·5%)
Reason unknown	38 (5·1%)	78 (11·8%)
Total	54 (7·2%)	81 (12·3%)

Data are n (%).

***Table 2*: Reasons for changes in recommended antibiotic therapy in the per-protocol population**

in the per-protocol analysis and 0·1% in the intention-to-treat analysis (–4·6 to 4·8). None of the neonates suspected of re-infection in the standard group or the procalcitonin group had a culture-proven bacterial infection. A detailed description of all nine cases with a re-infection within 72 h after completion of the initial course of antibiotic treatment or deceased within the first month of life and an overview of all serious adverse events reported during the study can be seen in the appendix (pp 40–41).

According to the protocol, physicians were allowed to overrule the algorithm if they felt that was appropriate. We named these overruled cases non-adherent and compared them in an informal analysis with all cases treated strictly according to the algorithm. The recommendations on the duration of antibiotic treatment were overruled by the treating physician in 191 (25·6%) of 745 neonates in the procalcitonin group and in 162 (24·4%) of 663 neonates in the standard group (table 2). Physicians decided to continue antibiotic treatment when antibiotic therapy could have been stopped according to the protocol significantly more often in the procalcitonin group (89 [11·9%] of 745 neonates) than in the standard group (45 [6·9%] of 663 neonates, p<0·0010). Physicians decided to stop antibiotic treatment earlier than recommended in 102 (13·7%) of 745 neonates in the procalcitonin group and in 117 (17·6%) of 663 neonates in the standard group (p=0·0409). An informal analysis showed that the duration of antibiotic therapy was significantly reduced when comparing adherent with non-adherent cases: mean duration of antibiotic therapy of adherent cases was 40·0 h (95% CI 36·0–46·5) in the procalcitonin group and 61·5 h (59·0–64·5) in the standard group. Similarly, length of hospital stay was significantly reduced when comparing adherent cases with non-adherent cases (appendix p 45). Five non-adherent subjects had suspected re-infection: four in the procalcitonin group and one in the standard group. Of the four non-adherent cases in the procalcitonin group, the antibiotic therapy was stopped earlier than recommended in three cases and prolonged in one case. For the non-adherent case in the standard group, the antibiotic therapy was stopped earlier than recommended (appendix p 45).

Discussion

We report that procalcitonin-guided decision making led to a significant reduction in duration of empirical antibiotic therapy and hospital stay in term and near-term neonates with suspected early-onset sepsis, with a low rate of re-infections and with no study-related mortality. Combining serial procalcitonin measurements with initial assessment based on perinatal risk factors, the neonate's clinical signs and symptoms, and conventional laboratory variables support antimicrobial stewardship and help physicians to decide to discontinue antibiotic treatment sooner in neonates classified as having low or moderate risk of infection.

In this era of globally increasing antibiotic resistance rates, WHO have highlighted the urgent need for enhanced antimicrobial stewardship to address this issue.[20,21] Compliance with antimicrobial stewardship is difficult to obtain and rarely reported in neonatology.[22–24] Increasing evidence suggests, however, that every dose of antimicrobial therapy counts in the emergence of antimicrobial resistance and in changing the human microbiome, and other evidence suggests that changes in the microbiome in early life are particularly important in shaping the individual's immune system and future health.[19,25–27] A cornerstone of antimicrobial stewardship,

therefore, is to lower the emergence of antimicrobial resistance by reducing use of antimicrobials.[28–30] In our cohort, with a short overall duration of antibiotic therapy in the control group compared with the literature, we were able to show a statistically significant reduction in duration of antibiotic treatment (55 h *vs* 65 h) between the intervention and the standard group.[9] Although this duration of antibiotic treatment has been calculated at group level, the difference might be much larger at the individual level.

Procalcitonin seems to be the best intervention to reduce duration of antibiotic treatment in neonates suspected of early-onset sepsis, because procalcitonin has the highest negative predictive value of all established biomarkers for infection.[11,12] One could argue that proven neonatal infection is so uncommon that any intervention that enables clinicians to reduce the duration of antibiotic treatment will have a similar effect. This is true, but to our knowledge no other intervention studied in neonates suspected of early-onset sepsis reduces the duration of antibiotic treatment while showing low morbidity and mortality. The only other studies on biomarkers that reported an attempted reduction of the duration of antibiotic treatment in neonates were done more than a decade ago by Philip and coworkers[31] and Ehl and colleagues[32] using C-reactive protein (CRP)-guided decision making. Neither study was powered to prove the safety of this approach. To our knowledge, all other published studies assessing biomarkers for neonatal infection are observational studies, most including the biomarkers: immature to total neutrophil counts, CRP, or interleukin-6.[33] Observational studies for neonatal infections are difficult to interpret due to the absence of a gold standard for proven infection.

The start and duration of antibiotic treatment is often more dependent on the physician's beliefs or the unit's culture than on objective variables.[34] A comparison of 127 neonatal intensive care units in California, USA, showed a 40-times variation in patient-days of antibiotic use with similar rates of proven infections.[35] Our multivariate regression analysis shows that the duration of antibiotic therapy depends on the participating centre, which is in line with the findings in California. A risk stratification scheme that aimed to reduce the number of neonates started on antibiotic treatment was developed using retrospective data from a population of more than 600 000 term and late preterm neonates born in 12 hospitals in the USA,[8] which recommended a number needed to treat (NNT) of 118 for one culture-proven infection as a reasonable cutoff to start antibiotic treatment.[8] A population-based study in Norway showed that 91 of 3964 term neonates given antibiotics had a proven bacterial infection (NNT=44).[9] In our international study population we found 27 proven bacterial infections of 1710 neonates given antibiotics (NNT=63), which is within the reported range from the USA and Norway.

The median duration of antibiotic therapy of 55·1 h for the procalcitonin group in the intention-to-treat-analysis, including neonates of all infection risk categories, is low compared with that suggested by previous literature. In a population-based study in Norway,[9] the median duration of antibiotic therapy in term neonates was reported to be 8 days for culture-positive early-onset sepsis, 6 days for culture-negative early-onset sepsis, and 4 days for so-called ruled-out sepsis situations. Whereas guidelines regarding duration of antibiotic therapy for low-risk situations or ruled-out sepsis situations uniformly recommend re-assessing the need for antibiotic therapy after 48 h, observational studies indicate that prolonged antibiotic therapy for low-risk situations is common. An intervention study showed antibiotic use could be reduced safely in the neonatal intensive care unit in ruled-out sepsis situations and culture-negative sepsis therapy.[36]

The superiority effect size was dependent on protocol adherence. The reduction of duration of antibiotic therapy was 9·9 h in the intention-to-treat analysis and 12·2 h in the per-protocol analysis. The recommendations on antibiotic treatment duration were overruled by the treating physician in around 25% of the neonates in the per-protocol population. When comparing adherent versus non-adherent cases in the procalcitonin group of the per-protocol population in an informal analysis, a further reduction of duration of antibiotic therapy was shown, with an increased superiority effect size of 21·5 h. Non-inferiority results were also compromised by non-adherence: five of eight possible re-infections were observed in the non-adherent group; three of four in the procalcitonin group with shortened antibiotic therapy.

The effect size of the reduction of duration of hospital stay (median duration of 123 h in the intervention group versus 126 h in the standard group) was smaller than the reduction of antibiotic therapy. Feeding difficulties, hyperbilirubinaemia, and apnoea or bradycardia were frequently reported in the late-preterm infants of our cohort; these are known to increase the duration of hospital stay independently of treatment for possible sepsis. Indeed, multivariate analysis showed that preterm birth and lower birthweight significantly increased the duration of hospital stay. This is in accordance with a study[37] using the USA national database, which showed that more than 50% of late-preterm infants needed prolonged hospital admission, mainly because of feeding difficulties. Not surprisingly, the studied intervention has only a limited benefit for the duration of hospital stay in neonates that also require hospital admission for reasons other than suspicion of infection. It would be interesting to analyse duration of hospital stay separately for neonates with or without other reasons for hospital admission, but this was not possible in our study design. We do not know, therefore, the effect of the study intervention on duration of hospital stay for neonates with a low or moderate risk of infection without other reasons for hospital admission.

Non-inferiority for re-infection or death could not be shown due to the low occurrence of re-infections and the absence of study-related deaths. With a possible re-infection rate of less than 1%, no culture-proven bacterial re-infection in both groups, and no study-related mortality, we believe that the procalcitonin-guided approach used in our study can be introduced safely in countries with a similar population. Prolongation of hospital stay due to feeding difficulties, hyperbilirubinaemia, and apnoea or bradycardia were the most frequently reported adverse events. Beside the eight patients with possible re-infection, none of the other reported adverse events were deemed by the data and safety monitoring board and the ethical committee to be possibly study related.

An important limitation of our study is that the results cannot be extrapolated to pre-term neonates, populations with larger proportions of neonates with a high risk of early-onset sepsis, countries with a higher incidence of proven early-onset sepsis, or countries with a different safety-netting system. The neonates in the participating countries all had easy and low-threshold access to health care. We have not investigated the extent of the role played by the safety-netting systems in the participating countries. Other limitations of our study include the use of a non-validated assessment tool for the risk of early-onset sepsis and the inevitable absence of non-masked procalcitonin values in the procalcitonin group, which could have influenced clinicians' decisions to prolong antibiotic therapy due to high procalcitonin values—possibly resulting in less reduction of antibiotic therapy for the entire intervention group.

A strength of our study is its pragmatic approach, as is promoted for paediatric trials.[38] Although the trial did not include an intervention for the neonates in the high-risk categories (those newborn babies with proven and probable early-onset sepsis), they were included in the study because the definition of sepsis in neonates is difficult, and whether or not the neonate is truly infected is rarely known at the moment antibiotic treatment is started.[39,40] We aimed to include our target population as closely as possible by using broad inclusion criteria, emphasising a pragmatic approach. It is important to underline that this study was not designed to find out whether or not to start antibiotic therapy, but aimed to reduce the duration of antibiotic treatment. Our population consisted of high proportions of neonates with a low risk (infection unlikely) and a moderate risk (infection possible) of early-onset sepsis at the initial assessment. The infection risk distribution of the studied population highly influenced the non-inferiority aspect of the study.

In conclusion, standardised risk assessment for suspected early-onset sepsis and procalcitonin-guided decision making reduced the duration of antibiotic therapy and hospital stay, with a low rate of re-infection and without study-related mortality, in a large cohort of neonates from high-income countries with a low incidence of proven early-onset sepsis.

Contributors

AMCvR and MS were the principal investigators. AMCvR, MS, WvH, EGV, and JvG did the study concept and design, which was approved by all authors. SeH, SD, MSF, FABAS, RvdT-dG, JWW, JJ, LHvdM-K, RM, SDS, EdV, AED, UZ, LJS, ACdM, AH-H, MR, MT, and RFK enrolled patients and did data collection. WvH, MS, and AMCvR were responsible for study supervision. WvH was responsible for supervision and monitoring of data entry and checking database for accuracy. WvH and SPW did the statistical analysis. WvH, AMCvR, MS, and SPW analysed and interpreted the data. AMCvR, MS, and WvH obtained funding. All authors read, critically revised, and approved the manuscript; approved the final version; and agree to be accountable for all aspects of the work.

Declaration of interests

We declare no competing interests.

Acknowledgments

The trial was financially supported by the Thrasher Foundation, the NutsOhra Foundation (1101-059) and the Sophia Foundation for Scientific Research (681). Thermofisher provided procalcitonin kits and provided an unrestricted grant for the organisation of four investigator meetings (2008, 2009, 2013, and 2015). All sponsors had no involvement in other aspects of the trial. We thank the patients and their families for participating in this trial. We thank our Data and Safety Monitoring Board for their expertise and supervising this trial: R Oostenbrink (Erasmus MC University Medical Centre—Sophia Children's Hospital, Rotterdam, Netherlands); Prof Emmanuel Lesaffre (Leuven Biostatistics and Statistical Bioinformatics Centre, Leuven, Belgium); Prof G Schubiger (Children's Hospital Lucerne, Lucerne, Switzerland); and C Hagmann (University Hospital Zurich). We thank Dr Wim Hop for his contribution to the design of the study. We thank Mirjam van Weissenbruch for facilitating the study in Amsterdam. We thank Jurgen Reimer, all physicians, physician assistants, and nursing staff for their commitment to the trial. None of those listed received any financial incentives for their contributions. We thank Badies Manai, Sonja Hoekstein, Satu Siiskonen, David Pogorzelski, Ahmed Bakry, and Nadia Lanz for doing the monitoring. Further thanks go to Mijke Hofhuis, Lindsay de Ligt, Ivar Gondrie, Justine Asch van Wijk, Jose van der Velden, and Saron Donker, who worked on the trial as medical students.

References

1 Collaborators GCM. Global, regional, national, and selected subnational levels of stillbirths, neonatal, infant, and under-5 mortality, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388:** 1725–74.

2 Stoll BJ, Hansen NI, Sanchez PJ, et al. Early onset neonatal sepsis: the burden of group B Streprococcal and E. Coli disease continues. *Pediatrics* 2011; **127:** 817–26.

3 Thaver D, Zaidi AK. Burden of neonatal infections in developing countries: a review of evidence from community-based studies. *Pediatr Infect Dis J* 2009; **28**(1 suppl)**:** S3–9

4 Weiss SL, Fitzgerald JC, Balamuth F, et al. Delayed antimicrobial therapy increases mortality and organ dysfunction duration in pediatric sepsis. *Crit Care Med* 2014; **42:** 2409–17.

5 National Institute for Health and Clinical Excellence (NICE). Antibiotics for early-onset neonatal infection: antibiotics for the prevention and treatment of early-onset neonatal infection (Clinical Guideline CG149). August 2012. URL: https://www.nice.org.uk/guidance/cg149 (accessed Jan 23, 2017).

6 Vergnano S, Menson E, Kennea N, et al. Neonatal infections in England: the NeonIN surveillance network. *Arch Dis Child Fetal Neonatal Ed* 2011; **96:** F9–14.

7 Cohen-Wolkowiez M, Moran C, Benjamin DK, et al. Early and late onset sepsis in late preterm infants. *Pediatr Infect Dis J* 2009; **28:** 1052–56.

8 Escobar GJ, Puopolo KM, Wi S, et al. Stratification of risk of early-onset sepsis in newborns >/= 34 weeks' gestation. *Pediatrics 2014*; **133:** 30–36.

9 Fjalstad JW, Stensvold HJ, Bergseng H, et al. Early-onset Sepsis and Antibiotic Exposure in Term Infants: A Nationwide Population-based Study in Norway. *Pediatr Infect Dis J* 2016; **35:** 1–6.

10 Schulfer A, Blaser MJ. Risks of antibiotic exposures early in life on the developing microbiome. *PLoS Pathog 2015*; **11:** e1004903.

11 van Rossum AM, Wulkan RW, Oudesluys-Murphy AM. Procalcitonin as an early marker of infection in neonates and children. *Lancet Infect Dis* 2004; **4:** 620–30.

12 Vouloumanou EK, Plessa E, Karageorgopoulos DE, Mantadakis E, Falagas ME. Serum procalcitonin as a diagnostic marker for neonatal sepsis: a systematic review and meta-analysis. *Intensive Care Med* 2011; **37:** 747–62.

13 Chiesa C, Pellegrini G, Panero A, et al. C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. *Clin Chem* 2003; **49:** 60–68.

14 Assumma M, Signore F, Pacifico L, Rossi N, Osborn JF, Chiesa C. Serum procalcitonin concentrations in term delivering mothers and their healthy offspring: a longitudinal study. *Clin Chem* 2000; **46:** 1583–87.

15 Chiesa C, Panero A, Rossi N, et al. Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. *Clin Infect Dis* 1998; **26:** 664–72.

16 de Jong E, van Oers JA, Beishuizen A, et al. Efficacy and safety of procalcitonin guidance in reducing the duration of antibiotic treatment in critically ill patients: a randomised, controlled, open-label trial. *Lancet Infect Dis* 2016; **16:** 819–27.

17 Baer G, Baumann P, Buettcher M, et al. Procalcitonin guidance to reduce antibiotic treatment of lower respiratory tract infection in children and adolescents (ProPAED): a randomized controlled trial. *PLoS One* 2013; **8:** e68419.

18 Stocker M, Fontana M, El Helou S, Wegscheider K, Berger TM. Use of procalcitonin-guided decision-making to shorten antibiotic therapy in suspected neonatal early-onset sepsis: prospective randomized intervention trial. *Neonatology* 2010; **97:** 165–74.

19 Ruppe E, Andremont A. Causes, consequences, and perspectives in the variations of intestinal density of colonization of multidrug-resistant enterobacteria. *Front Microbiol* 2013; **4:** 129.

20 Centres for Disease Control and Prevention (CDC). CDC 12-step program to prevent antimicrobial resistance in health care settings. April 19, 2002. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5115a5.htm (accessed Jan 23, 2017).

21 World Health Organization (WHO). World Health Organization Global Strategy for Containment of Antimicrobial Resistance. 2001. http://www.who.int/drugresistance/WHO_Global_Strategy.htm/en/ (accessed Jan 23, 2017).

22 Cantey JB, Patel SJ. Antimicrobial stewardship in the NICU. *Infect Dis Clin North Am* 2014; **28:** 247–61.

23 Patel SJ, Rosen E, Zaoutis T, Prasad P, Saiman L. Neonatologists' perceptions of antimicrobial resistance and stewardship in neonatal intensive care units. *Infect Control Hosp Epidemiol* 2010; **31:** 1298–300.

24 Hersh AL, Beekmann SE, Polgreen PM, Zaoutis TE, Newland JG. Antimicrobial stewardship programs in pediatrics. *Infect Control Hosp Epidemiol* 2009; **30:** 1211–17.

25 Armand-Lefevre L, Angebault C, Barbier F, et al. Emergence of imipenem-resistant gram-negative bacilli in intestinal flora of intensive care patients. *Antimicrob Agents Chemother* 2013; **57:** 1488–95

26 Poignant S et al. Risk factors and outcomes for intestinal carriage of AmpC-hyperproducing Enterobacteriadeae in intensive care unit patients. *Antimicrob Agents Chemother* 2016; **60:** 1883–87;

27 Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science* 2016; **352:** 539–44.

28 Holmes AH. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* 2016; **387:** 176–87

29 De Santis V, Gresiou M, Corona A, Wilson AP, Singer M. Bacteraemia incidence, causative organisms and resistance patterns, antibiotic strategies and outcomes in a single university hospital ICU: continuing improvement between 2000 and 2013. *J Antimicrob Chemother* 2015; **70:** 273–78.

30 Ruppe E, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Ann Intensive Care* 2015; **5:** 61.

31 Philip AG, Mills PC. Use of C-reactive protein in minimizing antibiotic exposure: experience with infants initially admitted to a well-baby nursery. *Pediatrics* 2000; **106:** E4.

32 Ehl S, Gering B, Bartmann P, Hogel J, Pohlandt F. C-reactive protein is a useful marker for guiding duration of antibiotic therapy in suspected neonatal bacterial infection. *Pediatrics* 1997; **99:** 216–21.

33 Bhandari V. Effective biomarkers for diagnosis of neonatal sepsis. *J Pediatr Infect Dis Soc* 2014; **3:** 234–45.

34 Soll RF, Edwards WH. Antibiotic use in neonatal intensive care. *Pediatrics* 2015; **135:** 928–29.

35 Schulman J, Dimand RJ, Lee HC, Duenas GV, Bennett MV, Gould JB. Neonatal intensive care unit antibiotic use. *Pediatrics* 2015; **135:** 826–33.

36 Cantey JB, Wozniak PS, Pruszynski JE, Sanchez PJ. Reducing unnecessary antibiotic use in the neonatal intensive care unit (SCOUT): a prospective interrupted time-series study. *Lancet Infect Dis* 2016; **16:** 1178–84.

37 Aly H, Hoffman H, El-Dib M, Said L, Mohamed M. Factor affecting length of stay in late preterm infants: an US national database study. *J Matern Fetal Neonatal Med* 2015; **28:** 598–604.

38 Randolph AG. Pragmatic trials in critically ill children are CATCHing on. *Lancet* 2016; **387:** 1697–98.

39 Chiesa C, Pacifico L, Osborn JF, Bonci E, Hofer N, Resch B. Early-onset neonatal sepsis: still room for improvement in procalcitonin diagnostic accuracy studies. *Medicine* 2015; **94:** e1230.

40 Escobar GJ. What have we learned from observational studies on neonatal sepsis? *Pediatr Crit Care Med* 2005; **6:** S138–45.