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Population Pharmacokinetics and Dosing Optimization of Azlocillin in Neonates with Early Onset Sepsis: A Real-World Study

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ABSTRACT

Objectives. Nowadays, real-world data can be used to improve currently available dosing guidelines and to support regulatory approval of drugs for use in neonates by overcoming practical and ethical hurdles. The proof-of-concept study aimed to assess the population pharmacokinetics of azlocillin in neonates using real-world data, to make subsequent dose recommendations and to test these in neonates with early onset sepsis (EOS).

Methods. This prospective, open-label, investigator-initiated study of azlocillin in neonates with EOS was conducted using an adaptive two-step design. First, a maturational pharmacokinetic-pharmacodynamic model of azlocillin was developed, using an empirical dosing regimen combined with opportunistic blood sampling. Second, a phase II clinical trial (ClinicalTrials.gov-NCT03932123) of this newly developed model-based dosing regimen of azlocillin was conducted to assure optimised target attainment (70%fT>MIC, free drug concentration above MIC during 70% of the dosing interval), and to investigate the tolerance and safety in neonates.

Results. A one-compartment model with first order elimination, using 167 azlocillin concentrations from 95 neonates (31.7-41.6 weeks postmenstrual age), incorporating current weight and renal maturation, fitted the data best. For the second step, 45 neonates (30.3-41.3 weeks postmenstrual age) were subsequently included to investigate target attainment, tolerance and safety of the pharmacokinetic-

pharmacodynamic model-based dose regimen (100 mg/kg q8h). Forty-three (95.6%) neonates reached their pharmacokinetic target and only two neonates experienced adverse events (feeding intolerance, abnormal liver function), possibly related to azlocillin.

Conclusions. Target attainment, tolerance and safety of azlocillin was shown in neonates with EOS using a pharmacokinetic-pharmacodynamic model developed with real-world data.

1 Introduction

Off-label use of drugs in neonates is more the rule than exception and can therefore not be ignored.[1] The United States and China have issued guidelines for using real-world data to support drug development in 2019 and 2020, respectively,[2, 3] which is a great opportunity for neonatal drug development. Innovative methodologies could support the design and conduct of clinical studies with off-label drugs to provide evidence-based data for rational use as well as regulatory approval for use of these drugs in neonates.

Globally, early-onset sepsis (EOS), generally defined by onset of symptoms consistent with sepsis <72 h of life, is one of the most important causes of morbidity and mortality in neonates.[4-7] The incidence of EOS is reported to be 11.7 cases per 1000 admissions, accompanied by a case fatality rate as high as 19%.[7] Prompt diagnosis and treatment of neonatal EOS with antibacterial agents are crucial to reduce mortality and morbidity despite the challenge of confirming the diagnosis due to non-specific clinical symptoms and absence of specific biomarkers.[8, 9] So empiric antibiotic treatment is commonly used. Up to 7% of live-born term and late-preterm neonates and more than 70% of hospitalized neonates receive antibiotics on or before day 3 of postnatal life.[5, 6] Up to now, the combination of benzylpenicillin/ampicillin and aminoglycosides such as gentamicin is standard of care by international guidelines and based on many studies,[4, 10, 11] taking into consideration the main pathogens of EOS, *Group B*

Streptococcus and *Escherichia coli*.[12] However, the use of aminoglycosides is legally forbidden to be used in neonates in China because of a high risk of ototoxicity. Furthermore, the main pathogens of EOS in China are *Escherichia coli* and *Coagulase-negative Staphylococcus*, not *Group B Streptococcus* so that the existence of regional differences in the main pathogens causing EOS should be taken into account.[13] Currently, many broad-spectrum antibiotics such as cefotaxime, piperacillin-tazobactam, and amoxicillin have played an increasingly important role in the treatment of EOS.[14-18] In China, the three most frequently prescribed antibiotics in neonates are third-generation cephalosporins, penicillins plus beta-lactamase inhibitor, and carbapenems, a practice that is different from several other regions of the world.[19, 20] For many antibiotics, the pragmatic approach using available pharmacokinetic (PK) data of commonly used systemic antibiotics, has resulted in dosing regimens in neonates, but this approach does not yet cover drugs commonly used in China.[21] The use of real-world data from a given antibiotic like azlocillin is a feasible approach to improve quality of dosing guidelines and generate data to acquire regulatory approval for use in neonates.

Azlocillin, increasingly prescribed for EOS in China, is a semisynthetic penicillin with broad-spectrum antimicrobial activity against common pathogens associated with EOS.[22] Azlocillin is excreted principally via glomerular filtration and a small amount through the bile.[23] Approximately 60-75% of the dose is unchanged in the urine,

increasing with higher concentrations.[24] The CL value obtained from 12 premature and 13 term infants was 0.06-0.23 L/h/kg.[25] Currently, azlocillin is prescribed using a wide range of doses (100 mg/kg/dose, q12/8/6h). However, the dosing guidelines are not supported yet by maturational pharmacokinetics and pharmacodynamics (PK-PD) due to the limited well-designed PK studies in neonates. Efficacy and safety data are also very limited in neonates treated with azlocillin for EOS.

As a time-dependent antibiotic, the fT>MIC is an important pharmacodynamic parameter for efficacy. For adults, at least 40% to 50% fT>MIC of dosing interval is accepted.[26] However, neonates are considered immunocompromised[27, 28] and EOS is a serious disease with high mortality.[7] In order to avoid treatment failure and emergence of drug resistant bacteria, a higher fT>MIC target for effectiveness assessment is needed.[29, 30] In addition, a previous PK-PD study on beta-lactam antibiotics showed an bacteriostatic effect for 30%–40% fT>MIC *in vivo*, whereas maximum killing was reached from 60%–70% fT>MIC onwards.[31] Thus, 70% fT>MIC was selected as the PK target in this study.

To sum up, this proof-of-concept study using real-world data aimed to assess the population pharmacokinetics (PopPK) of azlocillin in neonates, and to make dose recommendations based on maturational PK-PD. The target attainment, tolerance and safety of this new model-based dosage regimen of azlocillin were subsequently evaluated in neonates with EOS.

2 Methods

2.1 Study design

This prospective, open-label, investigator-initiated study was conducted using an adaptive two-step design. First a developmental PK-PD study was performed using modeling and simulation techniques to provide dosing optimization of azlocillin in neonates with EOS. In a second step, a phase II trial was then designed and conducted to assess the target attainment, tolerance and safety of this new model-based dosage regimen (Figure 1).[32]

2.2 Ethics

The study was approved by Medical Ethics Committee of Tianjin Central Hospital of Gynecology Obstetrics (approval Number: 2019KY002) and was conducted according to the ethical principles of the Declaration of Helsinki. Parental written consent was obtained.

2.3 PK study and dose optimization of azlocillin

Design

The first part of this clinical investigation was a prospective, monocenter, open label PopPK study of azlocillin, conducted in Tianjin Central Hospital of Gynecology

Obstetrics, Tianjin, China. Preterm and term infants in the neonatal intensive care unit (NICU) who were treated with azlocillin monotherapy for suspected or proven EOS were eligible for enrollment in this study. Patients with the following characteristics were excluded: expected survival time less than the treatment cycle, major congenital malformations or certain organ dysfunctions, surgery within the first week of life, having received other systemic trial drug therapy, and poor compliance that the researcher considers unsuitable for inclusion.

Dosage regimen, sampling and determination of azlocillin

Azlocillin (Zhejiang Jinhua Kangenbei Biological Pharmaceutical Co. Ltd, Zhejiang, China) was administered intravenously within 30 minutes using an empirical dose of 100 mg/kg twice daily. An opportunistic sampling design was selected to collect blood samples (see Supplementary Methods).[33] The concentrations of azlocillin were determined using high-performance liquid chromatography connected to an ultraviolet detector (HPLC-UV) (see Supplementary Methods).

Population pharmacokinetic modeling and validation

PK analysis was carried out using the nonlinear mixed effects modelling program NONMEM v7.4 (Icon Development Solutions, San Antonio, TX, USA). First order conditional estimation method with interaction was used to estimate PK parameters and inter-individual variability and residual variability. Covariate analysis followed a forward and backward selection process. Model validation was based on graphical

and statistical criteria, including goodness-of-fit plots, bootstrap and normalized prediction distribution errors (NPDE). The evaluation the predictive performance of the model to reproduce the observed data was assessed using by prediction-corrected visual predictive checks.[34] (see Supplementary Methods)

Simulation and dosage regimen optimization

As a time-dependent β-lactam antibiotic, the PK-PD index of azlocillin is the time period in which the free drug concentration is above MIC (fT>MIC).[35] The protein binding rate of azlocillin was reported to be about 35% for concentrations in the therapeutic range.[24] The MIC of 8 mg/L was selected as the PK-PD breakpoint which covered common pathogens for EOS (*Escherichia coli* and *Coagulase-negative Staphylococcus*).[36, 37] In addition, other different values of MIC (MIC=2/4/16/32 mg/L) were also used for Monte Carlo simulation to make it more useful for the cases where MIC in the actual culture can be established. Basing on a balance between maximal efficacy and minimal toxicity, as well as reduction of resistance, the desired target was that the free drug concentration of more than 70% of patients was above the MIC during 70% of the dosage interval.[17, 38, 39] The neonatal dosage simulation of azlocillin was on a mg/kg basis. Monte Carlo simulations were performed using parameter estimates obtained from the final model. One thousand simulations were performed using original dataset and the time above the MIC was calculated for each simulated patient. When the current dosage regimen resulted in underdosing, the virtual patient

was administered the optimised dosage regimen with increased frequency and/or dose. Therefore, different dosage regimens (100 mg/kg/dose, q24/12/8/6h) were simulated. For each dosage regimen, the probability of target attainment was calculated to optimize antimicrobial therapy.

2.4 Target attainment, tolerance and safety evaluation of azlocillin for EOS treatment using the new model-based optimised dosing regimen.

Design

A prospective, phase II trial was carried out in the same hospital center, which aimed at evaluating target attainment, tolerance and safety of this new model-based dosage regimen of azlocillin in neonates with EOS. Preterm and term infants ≤72 hours of life (PNA ⩽3 days) who met the standards for the use of antibiotics in the NICE guideline receiving intravenous azlocillin for treatment were eligible to be enrolled in the study. The standards for commencing antibiotics treatment in the NICE guideline specify that patients were qualified for antibiotic treatment, based on either one 'high risk factor' or more than one 'low risk factor' maternal factors or clinical indicators presented in Figure 2.[4] Exclusion criteria referred to PopPK study. The primary endpoint of the phase II trial was PK target attainment, and the secondary endpoint was the incidence of adverse events.

Dosage regimen and procedures

During the study, azlocillin was administered intravenously for EOS treatment using the model-based optimised dosage regimen (100 mg/kg, q8h) obtained from the PK-PD study. Discontinuation of azlocillin after 36h was decided by the pediatrician based on the following discontinuation criteria: negative blood cultures, C-reactive protein (CRP) ＜10 mg/liter, and absence of clinical signs of sepsis (Figure 3).[4] During treatment, tolerance and safety profile were well documented. The study process is presented in Figure 3.

Target attainment, tolerance and safety assessments

External model evaluation and PK target attainment analysis was performed using the PopPK model by NONMEM software. Tolerance and adverse events (AEs) including the adverse drug reactions mentioned in the drug labels and the general laboratory test outliers were recorded and assessed. (see Supplementary Methods)

3 Results

3.1 PK study and dose optimization of azlocillin

Study population

A total of ninety-five neonates were included for model building after the exclusion of four with incomplete dosing information from June to September 2018. The characteristics of included patients are summarized in Table 1.

Model building

For PK modeling, 167 azlocillin concentrations obtained from 95 neonates (range: 1-2 concentrations per patient) were available with the values ranging from less than lower limit of quantification (LLOQ) to 397.2 µg/mL. Six concentrations were lower than LLOQ and 1/2 LLOQ values were used in PK modelling. The concentration versus time profile is shown in Figure 4. Samples were collected over the full dosing interval. A one-compartment model with first-order elimination best fitted the data. The model was parameterized in terms of volume of distribution (V) and CL of azlocillin. A proportional model was best fitted for residual variability, while inter-individual variability was best described by an exponential model and then was used to estimating V and CL. The allometric size approach was used by incorporating a *priori* the CW into the basic model (allometric coefficients of 0.75 for CL, 1 for V), which caused a significant drop in the OFV of 30.3 points. PMA was identified as the most important covariate on CL, associated with a drop in the OFV of 31.4 units, while the ΔOFV values of PNA and GA were 18.8 and 29.9 units, respectively. However, BW and PNA together proved to be superior (ΔOFV 43.1 units) to PMA alone. The estimated PK parameters of the final model for neonates are presented in Table 2. The median (range) of estimated CL and volume distribution were 0.43 (0.13-0.81) L/h and 0.43 (0.13 - 0.75) L/kg, respectively. Azlocillin CL increased (allometric) with current weight in neonates.

Model evaluation

Model diagnostics showed acceptable goodness-of-fit for the final model of azlocillin. As shown in Figure 5(a)(b), predictions were unbiased. In the diagnostic plots of CWRES versus time and PRED, no trends were observed (Figure 5(c)(d)). In addition, the median parameter estimates resulting from the bootstrap procedure closely agreed with the respective values from the final population model, indicating that the final model was stable and could re-determine the estimates of PopPK parameters (Table 2). The NPDEs are presented in Figure 5(e)(f). NPDE distribution and histogram met well the theoretical N (0, 1) distribution and density, indicating a good fit of the model to the individual data. The mean and variance of NPDE were -0.031 (Wilcoxon signed rank test p=0.77) and 0.975 (Fisher variance test 0.846), respectively. The prediction-corrected visual predictive check confirmed that the model had good simulation properties (Figure 6).

Dosage regimen evaluation and optimization

The target attainment rates as functions of simulated dose for standard MIC susceptibility breakpoint of 8 mg/L is shown in Figure 7. With the empirical dosage regimen prescribed in the study (100 mg/kg q12h), only 63.1% of neonates achieved target (70% fT>MIC) at steady state, reflecting underdosing in a relevant portion of cases. By Monte Carlo simulation, the dosing regimen (100 mg/kg q8h) could achieve target in 91.2% of neonates and was recommended. The target attainment rates for other different values of MIC (MIC=2/4/16/32 mg/L) are also shown in Figure 7 for the

cases where MIC in the actual culture can be established. No safety differences between these dosage regimens are shown in the drug labels.

3.2 Target attainment, tolerance and safety evaluation of azlocillin for EOS treatment using the model-based optimised dosing regimen.

Study population

From May to October, 2019, another 50 neonates were screened. Of these, 49 neonates fulfilled the inclusion and exclusion criteria and were enrolled. Four neonates had major protocol violations and 45 neonates completed an azlocillin treatment course and were included in tolerance and safety analyses (Figure 8). Baseline characteristics of the 45 neonates are shown in Table 3. A dosage regimen of 100 mg/kg q8h was administered in all patients.

Target attainment assessments

For target attainment evaluation, model validation was conduct using the second dataset of these 45 patients. The MPE% and MAPE% median values were -15.1% (range: -85.6% - 40.0%) and 18.7% (range: 0.3%-85.6%), respectively, which showed that the model fitted the second dataset. The proportion of patients with MPE% and MAPE% within ±20% and ±30% were 56.2% and 82.2%, respectively. The visual predictive check is presented in Figure 6. The PK target calculated by the PK model was reached in 43 (95.6%) neonates.

Tolerance and safety assessments

All patients tolerated the intravenous administration of azlocillin without any discomfort or any sign of local irritation. For safety evaluation, no patient discontinued the treatment of azlocillin or had dosage regimen adjusted due to AEs. The adverse reactions data are shown in Table 4. AEs possibly related to azlocillin included mild feeding intolerance (n=1) and moderate abnormal liver function (n=1). The AST and ALT values of the latter were 347 U/L and 556 U/L on day 2 of azlocillin treatment, respectively. Without interruption of treatment, AST and ALT values reduced to 193 U/L and 498 U/L next day, respectively. Eventually, liver function of the neonate returned to normal according to the test on Day 9. AEs not related to azlocillin included mild feeding intolerance (n=1) and moderate cerebral ultrasound abnormalities (n=4). No infection-related death occurred in the first month of life.

4 Discussion

To the best of our knowledge, the work reported in this paper is the first PopPK, tolerance and safety study of azlocillin performed in neonates with suspected or documented EOS. The results showed that a one-compartment model incorporating size and renal maturation with first-order elimination best fitted the PK data of azlocillin. In the subsequent validation study, azlocillin was safe for EOS treatment with high

target attainment and good tolerance, using a maturational PK-PD model-based dosage regimen (100 mg/kg q8h).

In our PK analysis, the estimated median CL and weight-normalized CL value of azlocillin were 0.43 (range 0.13-0.81) L/h and 0.13 (range 0.07-0.20) L/h/kg, respectively. This was in agreement with previously published data: 0.10 (0.06-0.23) L/h/kg.[25] Azlocillin is excreted principally via glomerular filtration.[23] Thus, renal anatomical and functional maturation is considered of important influence on azlocillin clearance and dosing in neonates. Based on our results, the combination of BW and PNA had the greatest effect on azlocillin clearance compared with PMA alone, which had the similar results for aminoglycosides in neonates,[40] and showed that antenatal and postnatal renal maturation together had a crucial impact on azlocillin clearance, which was consistent with the pattern on renally cleared antibiotics previously reported.[41]

According to existing studies, the protein binding rate of azlocillin is more closely related to the drug concentrations, which is different from the drugs with high protein binding rate of about 95%.[24, 42] Thus, the total concentrations were mearsured and converted to unbound concentrations using the protein binding rate of 35% at therapeutic concentrations for dose optimization. The dosage regimen initially prescribed in the study (100 mg/kg, q12h) only resulted in target attainment in 63.1% of the included neonates using the MIC value of 8 mg/L. Insufficient empirical

antimicrobial therapy can easily lead to treatment failure and drug resistance. The optimized dosing regimen (100 mg/kg q8h) could achieve target in 91.2% of infants. Therefore, the model-based dose regimen (100 mg/kg q8h) is more applicable to treat EOS. For the cases where MIC in the actual culture can be established, the target attainment rates for other values of MIC (MIC=2/4/16/32 mg/L) (Figure 7) could be useful for dose selection.

In the phase II trial, considering the characteristics of EOS including low positive rate of blood cultures, unclear clinical symptoms and the need for early diagnosis and treatment, we conducted a trial designed in accordance with the NICE guidelines which included maternal factors or clinical indicators for treatment, to evaluate the tolerance and safety of azlocillin used to treat EOS with a model-based dosage regimen (100 mg/kg q8h). In our study, there was no neonate with a culture-proven infection. However, this is rather common if we perform this type of studies as the majority of blood culture remains negative, especially in EOS setting. In the study of Blackburn *et al*, the positive rate of blood culture on the day of birth is 0.8%,[43] reflecting the fact that the positive rate of blood culture in EOS patients is quite low. In our study, the mean time interval between birth and start of azlocillin in 45 neonates was less than 3 hours, which confirms the role of maternal factors for the occurrence of EOS. Not coincidentally, the top reason to start antibacterial treatment was maternal factor 'suspected or confirmed rupture of membranes for more than 18 hours in a preterm

birth' which occurred in 60% of neonates. In general, maternal factors that led to the initiation of antibiotics in neonates were more common than clinical indicators. In our study, duration of antibiotic administration (98 hours) was shorter than the findings of Fjalstad's study in which the median duration of antibiotic therapy in term neonates was 6 days for culture-negative EOS.[44] This could be partly explained by the fact that early discontinuation evaluation reduced the use of antibiotics. Stocker *et al*. reported a shorter antibiotic treatment duration (65h) in standard antibiotic treatment group, and that may be explained by the fact that there were more than 40% neonates with a low risk of EOS.[6]

For target attainment, tolerance and safety evaluation of azlocillin for EOS treatment using the model-based optimised dosing regimen, azlocillin showed a high target attainment, excellent tolerance and very few safety issues. The PK target was reached in 95.6% of neonates. AEs determined to be possibly related to azlocillin therapy occurred in only 4.4% neonates. Azlocillin treatment was not discontinued nor adjusted due to the documented AEs in this study.

There were several limitations in our study. Our data cannot simply be extrapolated to late-onset sepsis (LOS) or EOS in very low birth weight ($<$ 1500 g) or preterm neonates < 32 weeks. Further studies are needed with azlocillin therapy for neonatal populations with other gestational and/or postnatal ages. In addition, our study evaluated the safety of azlocillin in the treatment of EOS and azlocillin performed well.

However, the long-term safety of azlocillin in larger samples needs further study. While target attainment has been confirmed, the necessary next step will be a prospective assessment of safety in larger groups. At last, our study used MIC data reported in published articles and CLSI dataset, because culture specific MIC data were not available in the local hospital. Taking these limitations into account, we are convinced that the current azlocillin study may serve as a template on how high-quality dosing guideline and model-based drug evaluation can be developed and validated in neonates and young infants using real-word data.

5 Conclusions

The off-label and poorly validated use of antibiotic treatment for EOS in the vulnerable neonatal population is a worrisome problem that cannot be ignored. We conducted a proof-of-concept study to provide evidence-based real-world data for EOS treatment in neonates. With an adaptive two-step design, the results indicated that an adapted dosing regimen for azlocillin was well tolerated and safe for EOS treatment. Innovative methodologies should be promoted to evaluate off-label drugs in neonates to support rational use and regulatory approval.

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Transparency declarations

None to declare.

Author contributions

Y-E. W. and T. W. contributed equally to the interpretation of the data for the work, conception and design of study, drafting of the initial manuscript, and revising of the manuscript. T. W., H-L. Y., L. K., X. L. and Q. G. acquired and checked data: laboratory or clinical. Y-E. W., B-H. T., X. L., B-F. Y., H-Y. S., X. H. and W-Q. W. checked and analyzed data. E. J-A., K. A. and J. A. interpreted of data for the work, provided advice,

410 critically reviewed and revised the manuscript. X-Y. T. and W. Z. contributed equally

411 to the conception and design of the work, supervising the data, critically reviewing and

412 revising the manuscript.

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Table Legends

Table 1. Baseline characteristics in 95 neonates in PopPK analysis.

Table 2. Population pharmacokinetic parameters of azlocillin and bootstrap results.

Table 3. Baseline characteristics in 45 neonates in the phase II trial.

Table 4. Target attainment, tolerance and safety outcome measures.

Figure Legends

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Figure 7. Results of the simulations for dosing regimens (100 mg/kg/dose, q24/12/8/6h).

Figure 8. Trial profile.

552 **Table 1**. Baseline characteristics in 95 neonates in PopPK analysis.

	Number	Median (Range)
Patients	95	
Sex (male/female)	49 M / 46 F	
Preterm / full-term	16 / 79	
Race	95 Chinese	
GA (weeks)		39.6 (31.6–41.4)
PMA (weeks)		39.6 (31.7–41.6)
PNA (days)		1.0 (1.0–3.0)
BW (g)		3390 (1800–4850)
CW (g)		3390 (1820–4810)
Azlocillin treatment		
Dose (mg/dose)		340.0 (180.0–485.0)
Dose (mg/kg/dose)		99.0 (95.7–127.4)
Azlocillin sampling and determination		
TAD (h)		4.6 (1.2–22.4)
Concentrations (μg/mL)		73.5 (LLOQ–397.2)

553 **PopPK:** population pharmacokinetics; **GA:** gestational age at birth; **PMA:**
554 postmenstrual age at enrollment; **PNA:** postnatal age at enrollment; **BW:** birth weight;
555 **CW:** current weight at enrollment; **TAD:** time after dose; **LLOQ:** lower limit of
556 quantification.

557 **Table 2**. Population pharmacokinetic parameters of azlocillin and bootstrap results.

Parameters	Allometic scaling base model estimate	RSE(%)	Full dataset		Bootstrap	
			Final estimate	RSE(%)	Median	5th – 95th
V (L)						
V=θ1× (CW/3335)						
θ1	1.32	7.20	1.38	6.70	1.39	1.23 – 1.53
CL(L/h)						
$CL=\theta2\times(CW/3335)^{0.75}\times F_{age}$						
θ2	0.418	5.70	0.440	4.60	0.439	0.406 – 0.476
$F_{age}=(BW/3390)^{\theta3}\times(PNA/3)^{\theta4}$						
θ3	-	-	0.907	17.0	0.901	0.635 – 1.15
θ4	-	-	0.367	26.0	0.367	0.0988 – 0.520
Inter-individual variability (%)						
$\theta_i= \theta_{mean}*e^{\eta i}$						
V	47.3	28.4	42.3	12.5	41.8	27.8 – 50.2
CL	27.9	19.1	22.6	27.6	22.4	4.80 – 31.1
Residual variability (%)	37.4	10.9	32.2	9.70	32.0	26.3 – 37.1

558 **V**: volume of distribution; **CL**: clearance; **CW**: current weight in gram; **BW**: birth weight in gram; **PNA**: postnatal age in days. In our

559 population, 3335 gram and 3 days were the median values of current weight and postnatal age at the time of first sampling, respectively.

560 **Table 3.** Baseline characteristics in 45 neonates in the phase II trial.

	Median (Range)	Number
Patients		45
Male/female		26/19
GA (weeks)	36.6 (30.1-41.1)	
PNA (days)	1.0 (1.0-2.0)	
PMA (weeks)	36.7 (30.3-41.3)	
BW (g)	2680.0 (1770.0-4070.0)	
CW (g)	2680.0 (1770.0-4070.0)	
CRP (mg/L)	1.8 (0-24.4)	
WBC (x 10^9/L)	20.64 (10.3-43.8)	
Factors contributing to the initiation of antibiotic therapy		
Maternal factors		
• Maternal group B streptococcal colonisation, bacteriuria or infection in the current pregnancy		1
• Prelabour rupture of membranes		17
• Preterm birth following spontaneous labour (before 37 weeks' gestation)		17
• Suspected or confirmed rupture of membranes for more than 18 hours in a preterm birth		27
• Intrapartum fever higher than 38°C, or confirmed or suspected chorioamnionitis		12
• Parenteral antibiotic treatment given to the woman for confirmed or suspected invasive bacterial infection (such as septicaemia) at any time		4

during labour, or in the 24-hour periods before and after the birth [This does not refer to intrapartum antibiotic prophylaxis]*

Clinical indicators

- Altered behaviour or responsiveness 1
- Abnormal heart rate (bradycardia or tachycardia) 1
- Signs of respiratory distress 4
- Respiratory distress starting more than 4 hours after birth* 2
- Hypoxia (for example, central cyanosis or reduced oxygen saturation level) 2
- Jaundice within 24 hours of birth 1
- Apnoea 1
- Altered glucose homeostasis (hypoglycaemia or hyperglycaemia) 2
- Metabolic acidosis (base deficit of 10 mmol/litre or greater) 3

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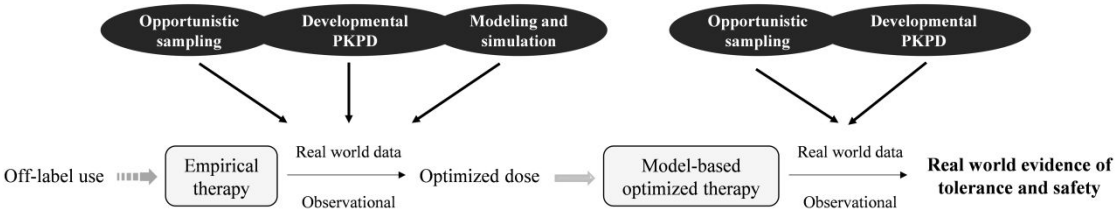
561 **GA:** gestational age at birth; **PMA:** postmenstrual age at enrollment; **PNA:** postnatal
562 age at enrollment; **BW:** birth weight; **CW:** current weight at PK analysis; **CRP:** C-
563 reactive protein; **WBC:** white blood cell count.

564 **Table 4.** Target attainment, tolerance and safety outcome measures.

	Patients （n=45）
Azlocillin treatment	
Time to begin azlocillin therapy after birth (h)	2.7 (4.6, 0.4-28.8)
Duration of azlocillin treatment (h)	98.4 (31.6, 46.8-166.5)
Duration of azlocillin treatment (days)	4.1 (1.3, 2.0-6.9)
Length of hospitalization (days)	7 (4, 2-25)
PK target attainment	95.6%
Mortality (%)	
1-month mortality#	0 (0%)
Adverse events	
Definitely related	0 (0%)
Probably related	0 (0%)
Possibly related	2 (4.4%)
Not related	5 (11.1%)
Unable to determine	0 (0%)

565 Data are mean (SD, range), n (%). # Infection-related death in the first month of life.



566

567 **Figure 1**. Diagram depicting the process to generate real-world evidence. PK-PD:

568 pharmacokinetics - pharmacodynamics.

High risk factor

Maternal factors

- Parenteral antibiotic treatment given to the woman for confirmed or suspected invasive bacterial infection (such as septicaemia) at any time during labour, or in the 24-hour periods before and after the birth [This does not refer to intrapartum antibiotic prophylaxis]
- Suspected or confirmed infection in another baby in the case of a multiple pregnancy

Clinical indicators

- Respiratory distress starting more than 4 hours after birth
- Seizures
- Need for mechanical ventilation in a term baby
- Signs of shock

Low risk factor

Maternal factors

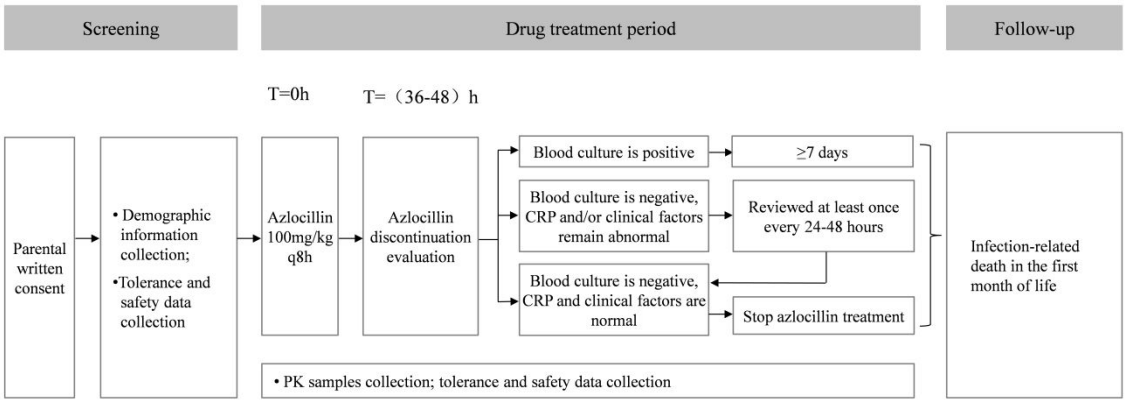
- Invasive group B streptococcal infection in a previous baby
- Maternal group B streptococcal colonisation, bacteriuria or infection in the current pregnancy
- Prelabour rupture of membranes
- Preterm birth following spontaneous labour (before 37 weeks' gestation)
- Suspected or confirmed rupture of membranes for more than 18 hours in a preterm birth
- Intrapartum fever higher than 38°C, or confirmed or suspected chorioamnionitis

Clinical indicators

- Altered behaviour or responsiveness
- Altered muscle tone (for example, floppiness)
- Feeding difficulties (for example, feed refusal)
- Feed intolerance, including vomiting, excessive gastric aspirates and abdominal distension
- Abnormal heart rate (bradycardia or tachycardia)
- Signs of respiratory distress
- Hypoxia (for example, central cyanosis or reduced oxygen saturation level)
- Jaundice within 24 hours of birth
- Apnoea
- Signs of neonatal encephalopathy
- Need for cardio–pulmonary resuscitation
- Need for mechanical ventilation in a preterm baby
- Persistent fetal circulation (persistent pulmonary hypertension)
- Temperature abnormality (lower than 36°C or higher than 38°C) unexplained by environmental factors
- Unexplained excessive bleeding, thrombocytopenia, or abnormal coagulation (International Normalised Ratio greater than 2.0)
- Oliguria persisting beyond 24 hours after birth
- Altered glucose homeostasis (hypoglycaemia or hyperglycaemia)
- Metabolic acidosis (base deficit of 10 mmol/litre or greater)
- Local signs of infection (for example, affecting the skin or eye)

569

570 **Figure 2.** The standards for initiation of antibiotics treatment.



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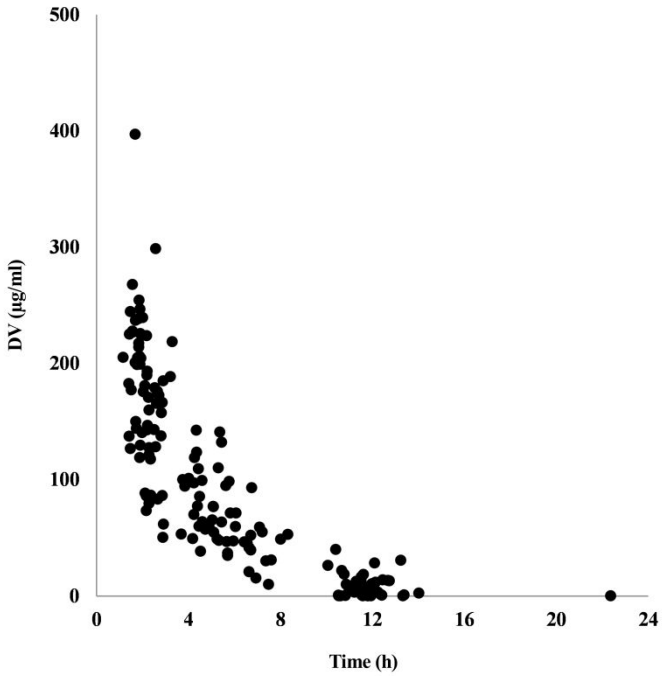
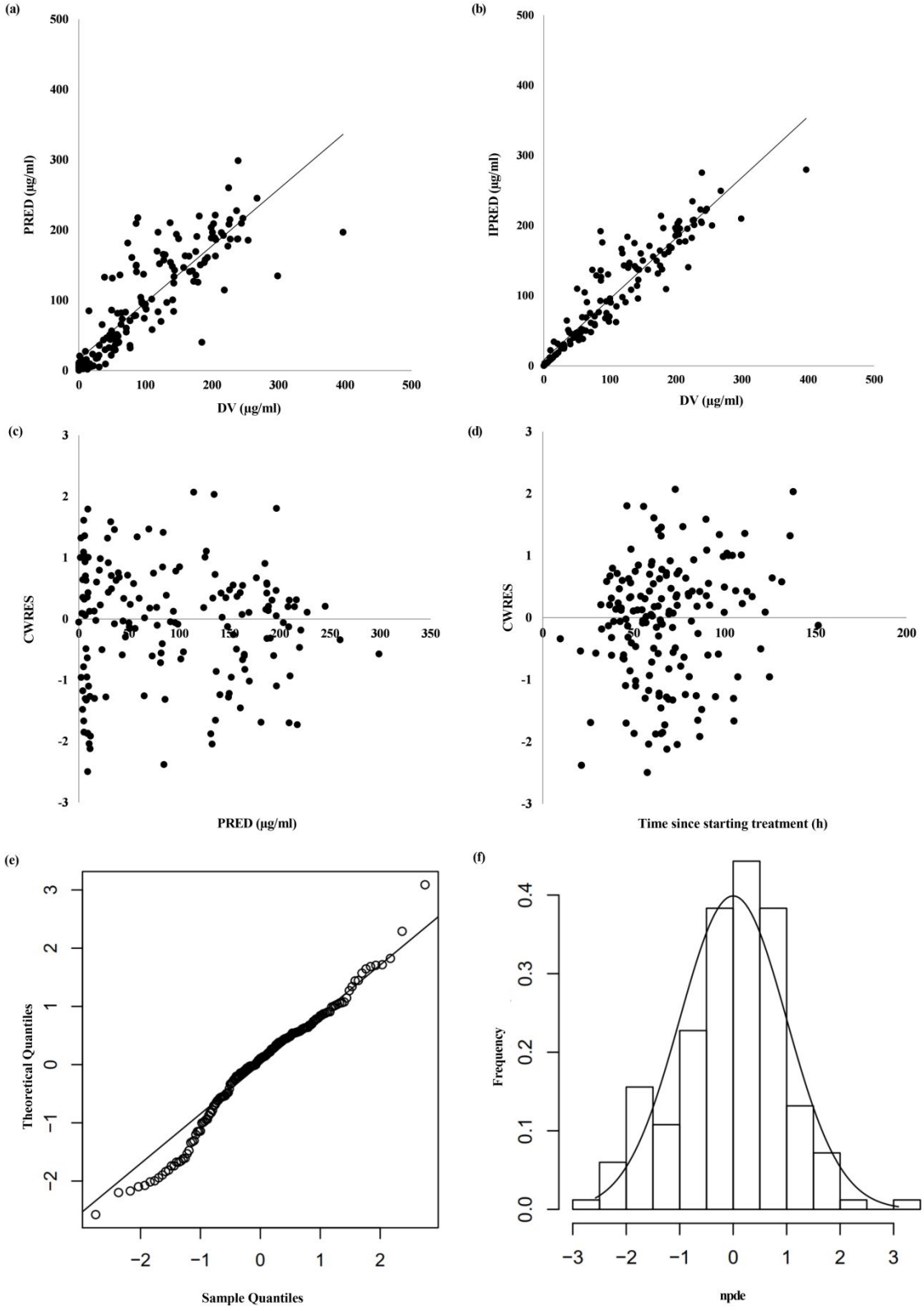
572 **Figure 3.** Flow chart describing the validation study.



573

574 **Figure 4.** Azlocillin concentrations versus time since last dose.



575

576 **Figure 5.** Model evaluation and simulation for azlocillin. (a) Population predicted

577 (PRED) versus observed concentrations (DV); (b) Individual predicted (IPRED) versus

578 DV; (c) Conditional weighted residuals (CWRES) versus time; (d): CWRES versus
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580 (NPDE) versus the theoretical N (0,1) distribution; (f) Histogram of the distribution of
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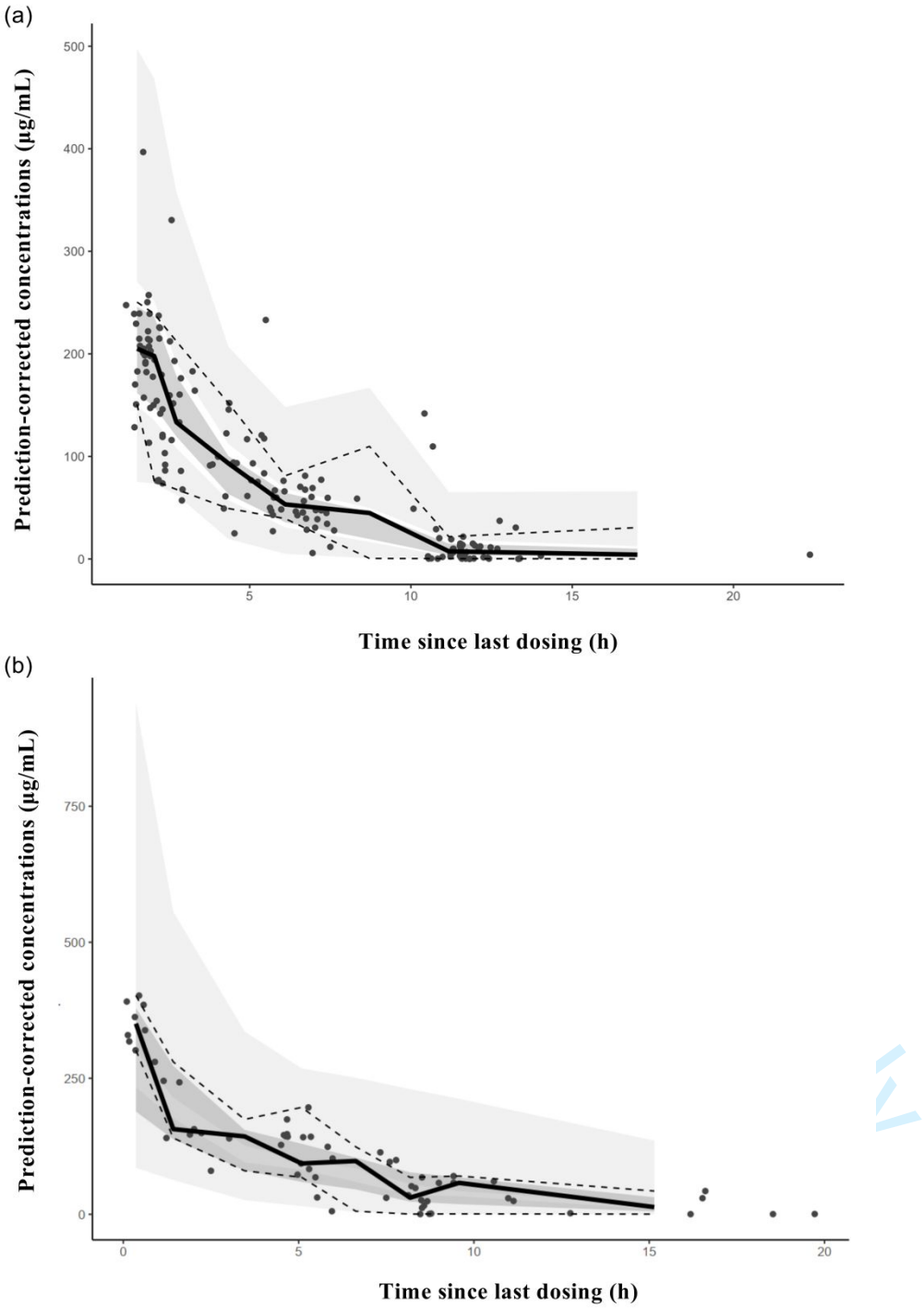

582

583 **Figure 6.** Prediction corrected visual predictive checks. (a) Internal validation; (b)
584 external evaluation. The circles represent the prediction-corrected observed
585 concentrations. The solid line represent the median prediction-corrected observed
586 concentrations and semitransparent gray field represents simulation-based 95%
587 confidence intervals for the median. The observed 5th and 95th percentiles are
588 indicated by dashed lines, and the 95% intervals for the model-predicted percentiles
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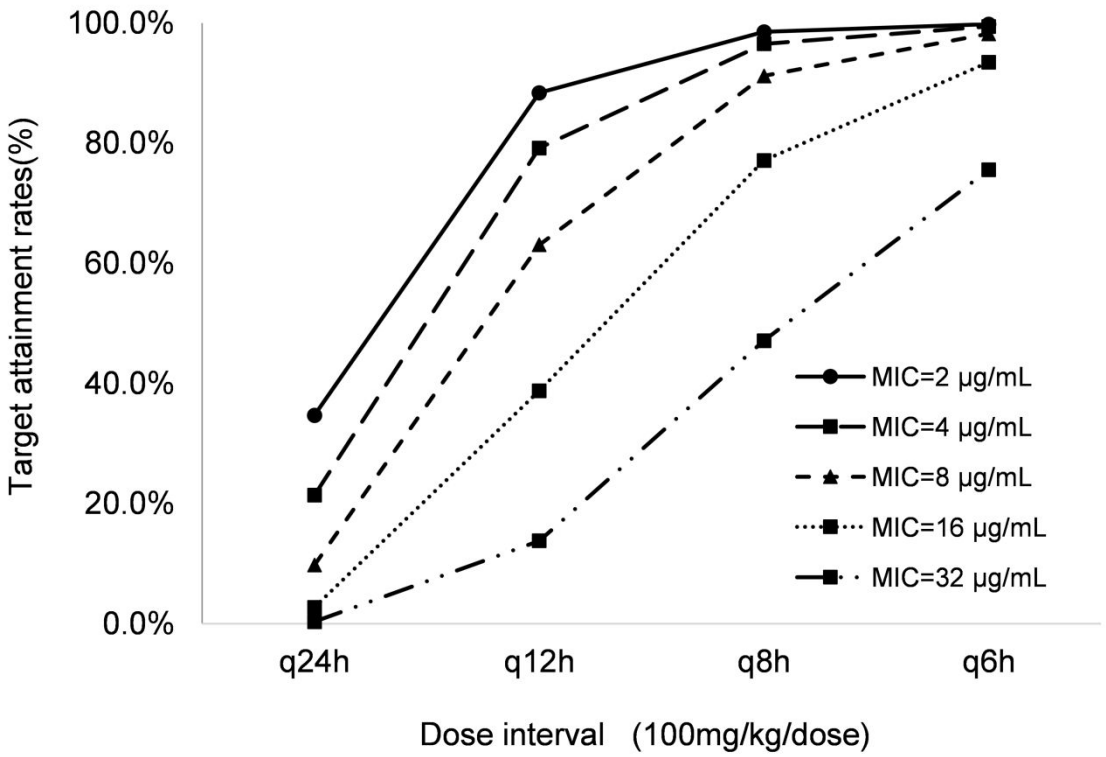

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591 **Figure 7.** Results of the simulations for dosing regimens (100 mg/kg/dose,
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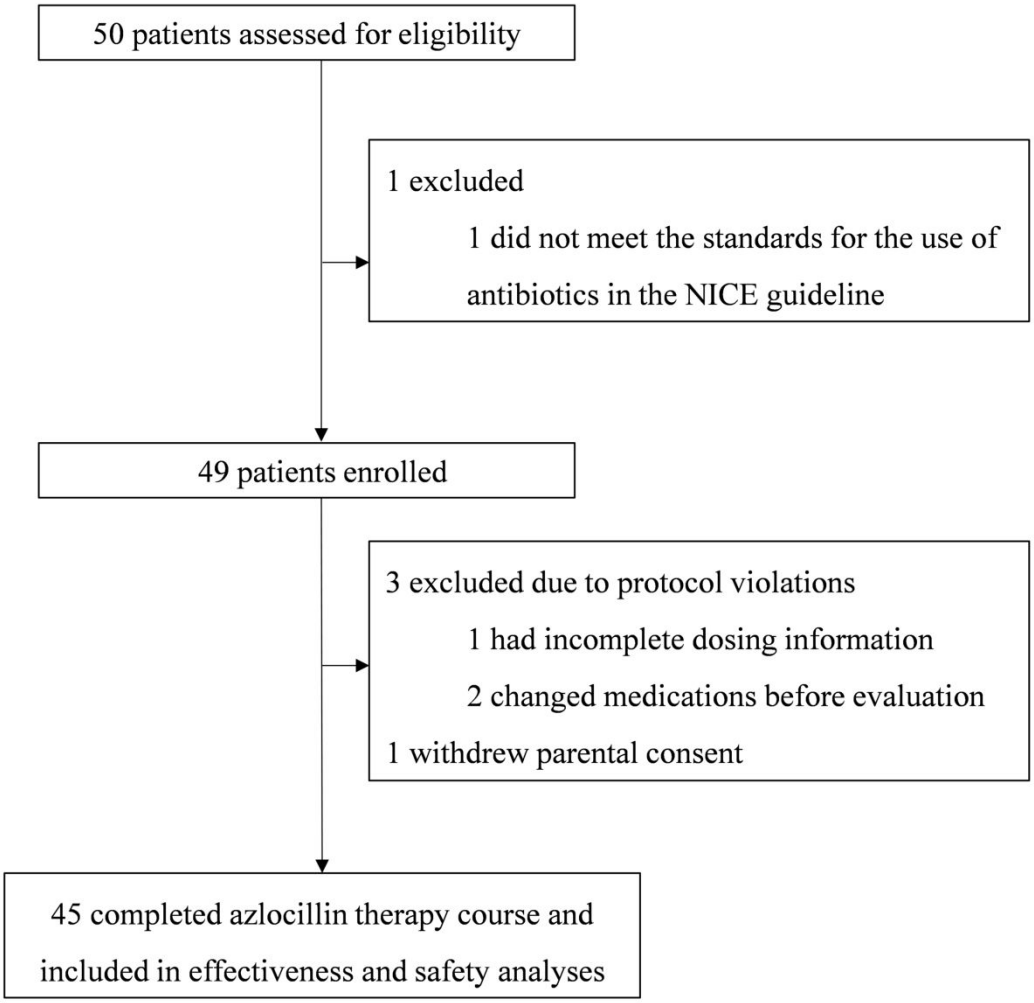

593

594 **Figure 8.** Trial profile.

Supplementary Methods

Sampling and determination of azlocillin

An opportunistic sampling design which refers to the use of samples collected from blood remaining after routine laboratory tests as part of clinical care was selected to collect blood samples.[1] Azlocillin was stable and had no problems throughout the opportunistic sampling process.[2] The predefined suggested pharmacokinetic sampling scheme was 5-10 minutes, 0.5-10 hours (PopPK study) or 0.5-6 hours (phase II study) after the end of infusion and 2 hours before the next dose. From each neonate 1-2 blood samples (no more than 0.3 mL per sample) of the above sampling scheme were taken, after any one dose. Clinicians could adjust the time of routine laboratory test according to the scheme. The administration and sampling information were accurately recorded according to standard operating procedure. Samples were centrifuged at 4,000 rpm at 4℃ for 10 minutes, and plasma samples were stored at -70℃ until the concentration of azlocillin was quantitated.

The concentrations of azlocillin were determined using high-performance liquid chromatography connected to an ultraviolet detector (HPLC-UV) with mezlocillin as internal standard. HPLC separation was achieved with a Insustain C18 column (250*4.6mm, 5μm, Shimadzu, Japan) at 210 nm wavelength, using methanol and 0.067 mol/L dipotassium hydrogen phosphate solution at a flow rate of 1.3 mL/min at 35℃. Acetonitrile protein precipitation and dichloromethane reverse extraction were

used to deal with plasma samples (100 µL). The calibration curve ranged from 0.2 to 300 µg/mL. The inter- and intra- day coefficients of variation were 1.7%-3.1% and 0.8%-4.5%, respectively, using quality controls at low (0.5 µg/mL), medium (40 µg/mL) and high (200 µg/mL) concentrations. The lower limit of quantification (LOQ) was 0.2 µg/mL.

Population pharmacokinetic modeling

Inter-individual variability of CL and V was estimated using an exponential model and was expressed as follows:

$\theta_i = \theta_{mean} * e^{\eta i}$ (1)

where θ_i represents the parameter value of the i^{th} subject, θ_{mean} the typical value of the parameter in the population and ηi the variability between subjects which is assumed to follow a normal distribution with a mean of zero and variance ω2.

In covariate analysis, due to the collinearity of the covariates, size correction was necessary and current weight (CW) was typically incorporated first into the basic model by the allometric size approach.[3] In addition, the effects of birth weight (BW), gestational age (GA), postnatal age (PNA) and postmenstrual age (PMA) were also investigated as potential variables affecting PK parameters. During the forward selection process, the likelihood ratio test was used to quantify the effect of each variable on model parameters. A covariate was included if a significant ($p<0.05$, χ^2

distribution with one degree of freedom) decrease (reduction>3.84) in the objective function value (OFV) from the basic model was obtained with reduction in the variability of the PK parameter. After each round, the most statistically significant covariate was added. Covariates were separately implemented in the model, using the following allometric equation. This process would be repeated until all the statistically significant covariates were added into a "full" model.

$$P_i = P_P * (Cov/Cov_{Median})^k$$

where P_i represents the individual parameter estimate of the ith subject, P_p represents the population parameter estimate, Cov is the covariate, and k is the exponent.

During the backward elimination process, each covariate was independently removed from the full model. If the increase in the OFV was higher than 6.635 ($p<0.01$, χ^2 distribution), the covariate was considered as significantly impacting with the PK parameter and was therefore retained in the final model. Like the forward selection, this process would be repeated until the removal of each covariate from the model resulted in a statistically significant detriment to the fit. A randomization test was also be performed to determine if covariates had both statistical and physiological significance.

Population pharmacokinetic model validation

Goodness-of-fit plots, including observed (DV) versus population prediction (PRED); DV versus individual prediction (IPRED); conditional weighted residuals (CWRES) versus time and CWRES versus PRED were initially used for diagnostic purposes. The stability and performance of the final model was also assessed by means of a nonparametric bootstrap with re-sampling and replacement. Re-sampling was repeated 1000 times and the values of estimated parameters from the bootstrap procedure were compared with those estimated from the original data set. The entire procedure was performed in an automated fashion, using PsN (v5.0.0).[4] One thousand datasets were simulated using the final population model parameters. Normalized prediction distribution errors (NPDE) results were summarized graphically by default as provided by the NPDE R package (v1.2):[5] (i) QQ-plot of the NPDE; (ii) histogram of the NPDE. The NPDE is expected to follow the N (0, 1) distribution. The prediction-corrected visual predictive checks were made using 1000 simulations by Pirana software (V2.9.9), observed and simulated dependent variables were normalized on the basis of typical population prediction of the median independent variable in the bin. For the median and the 5th and 95th percentiles of the prediction-corrected simulated concentrations, the 95% confidence intervals were calculated, plotted against the time, and compared to the prediction-corrected observed concentrations.

External model evaluation and PK target attainment evaluation

The independent dataset obtained from this prospective, phase II trial was used for external evaluation of the model and assessment of PK target attainment. Mean absolute prediction error percent (MAPE%) and mean prediction error percent (MPE%) were applied to calculate the bias and imprecision of the predictive performance.[6] (Eqs. 2 and 3). In addition, the number of patients with MPE% and MAPE% within ±20% and ±30% were calculated.[6, 7] PK target was defined as the azlocillin free concentration above MIC during 70% of the dosing interval (70% fT > MIC). An external prediction-corrected visual predictive checks were made using 1000 simulations for an additional check.

$$\mathrm{MAPE\%} = \frac{1}{N}\sum\left|\frac{\mathrm{PRED_i} - \mathrm{OBS_i}}{\mathrm{OBS_i}}\right| \times 100\% \quad (2)$$

$$\mathrm{MPE\%} = \frac{1}{N}\sum\frac{\mathrm{PRED_i} - \mathrm{OBS_i}}{\mathrm{OBS_i}} \times 100\% \quad (3)$$

Tolerance and safety assessments

Tolerance was primarily related to the acceptance of the intravenous administration of azlocillin and phlebitis assessed using the Phlebitis Scale of Infusion Therapy Standards of Practice.[8] The focus of adverse events (AEs) monitoring included the adverse drug reactions mentioned in the drug instructions and the general laboratory test outliers. The adverse drug reactions in the drug label included diarrhea, vomiting, fever, prolonged bleeding time, leukopenia, electrolyte disturbance (hypernatremia). Clinical signs and laboratory tests including liver function tests, renal function tests,

blood cell analysis, blood gas were monitored according to the study timelines (Figure 3) as routine part of standard clinical care. All AEs data were documented and the causal relationship between AEs and azlocillin was determined by the local pediatrician and clinical pharmacist at five levels: definitely related, probably related, possibly related, not related, or unable to determine. The infection-related death occurred in the first month of life was also documented.

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