# Pharmacokinetics and dosing of antibiotics in neonates

*Thesis Joyce Pullen* *26-4-2007*

BIOMEDICAL CHROMATOGRAPHY
*Biomed. Chromatogr.* **21**: 1259–1265 (2007)
Published online 29 June 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/bmc.881

ORIGINAL RESEARCH

# Microanalysis of amoxicillin, flucloxacillin, and rifampicin in neonatal plasma

**J. Pullen,[1]* L. M. L. Stolk,[1] C. Neef[1] and L. J. I. Zimmermann[2]**

[1]Department of Clinical Pharmacy and Toxicology of the University Hospital of Maastricht, Maastricht, The Netherlands
[2]Department of Paediatrics, Division of Neonatology of the University Hospital of Maastricht, PO Box 5800, NL-6202 AZ Maastricht, The Netherlands

*Received 19 March 2007; revised 7 May 2007; accepted 8 May 2007*

ABSTRACT: Simple and rapid reversed-phase high-performance liquid chromatographic assays with ultraviolet detection have been developed and validated for the determination of amoxicillin, flucloxacillin and rifampicin in neonatal plasma. Plasma samples were either precipitated with perchloric acid (amoxicillin) or methanol (rifampicin) or extracted with methylene chloride (flucloxacillin). Precision coefficients of variation and inaccuracy were less than 15% for all three assays. Only small sample volumes (20–40 μL) were required, making the assays suitable for therapeutic drug monitoring and pharmacokinetic studies in preterm and term neonates. The assays have successfully been applied to analysis of amoxicillin, flucloxacillin and rifampicin in previously published pharmacokinetic studies in neonates. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: amoxicillin; flucloxacillin; rifampicin; antibiotics; HPLC assay; liquid chromatography; neonatal plasma

## INTRODUCTION

Routine therapeutic drug monitoring (TDM) of antibiotics like gentamicin and vancomycin in preterm and term neonates is widely accepted. Other antibiotics like amoxicillin, flucloxacillin and rifampicin are also widely used in neonates. TDM of these antibiotics is not routinely performed, but could be useful in certain circumstances (Pullen *et al*., 2006a–c). In the literature, information about analytical assays of these antibiotics in neonates is either very briefly described in pharmacokinetic papers or absent (Pullen *et al*., 2006a–c; Huisman-de Boer *et al*., 1995; Tan *et al*., 1993). Abundant information is available about these assays for adults. However, TDM in neonates is complicated by the very small sample volume available. Phlebotomy has been reported as cause of anemia and transfusion in neonates (Lin *et al*., 2000). In this paper, we describe simple and rapid microanalytical assays for the determination of amoxicillin, flucloxacillin and rifampicin in neonatal plasma, based on reversed-phase high-performance liquid chromatography (RP-HPLC).

Various HPLC assays with UV detection have been developed to determine amoxicillin in adult human plasma (Bloemhof *et al*., 1988; Hoizey *et al*., 2002; Lindegårdh *et al*., 2005). However, these assays are not suitable for determination of amoxicillin in neonatal plasma, because they require a relatively large plasma volume. Therefore, we developed an RP-HPLC assay with UV detection for the determination of amoxicillin in small volume neonatal plasma samples, based on an RP-HPLC-UV method for the analysis of amoxicillin in adult plasma samples described earlier in literature (Bloemhof *et al*., 1988). The assay was validated for linearity, precision, accuracy and stability. Lindegårdh *et al*. (2005) evaluated long-term stability of amoxicillin in plasma at 8, –17 and –80°C over one month. Amoxicillin was found unstable in plasma stored at both 8 and –17°C. Amoxicillin was stable in plasma stored at –80°C for at least one month. Mascher and Kikuta (1998) also found that amoxicillin in human plasma was stable over a time period of at least 50 days at approximately –70°C, but not at –20°C.

Microbiological methods (Jalling *et al*., 1972; Sutherland *et al*., 1970) were used for the analysis of flucloxacillin in adult human plasma before more specific HPLC assays (Charles *et al*., 1994; Thijssen, 1980; Hung *et al*., 1988) became available. Herngren *et al*. (1987) used a microbiological method, based on agar diffusion and bacterial growth, to determine flucloxacillin concentrations in neonatal plasma in a study of the pharmacokinetics of flucloxacillin in newborn infants. An important reason for them to use a microbiological method instead of a more specific HPLC assay was the small plasma volume available for analysis from newborn infants (Herngren, 1989). For this microbiological assay, a plasma volume of 10–20 μL was sufficient. We

*Correspondence to: J. Pullen, Department of Clinical Pharmacy and Toxicology, University Hospital of Maastricht, PO Box 5800, NL-6202 AZ Maastricht, The Netherlands.
E-mail: joycepullen@hotmail.com

**Abbreviations used:** LLOQ, lower limit of quantification; TDM, therapeutic drug monitoring.

developed an RP-HPLC assay for the determination of flucloxacillin using only small plasma volumes (20 μL). This assay was based on a RP-HPLC method developed by Thijssen (1980) for flucloxacillin analysis in adults. The assay was validated for linearity, precision, accuracy, stability and recovery.

Many HPLC assays have been developed for the simultaneous determination of rifampicin and its main metabolite(s) in human plasma (Chandi *et al*., 1998; Ishii and Ogata, 1988; Oldfield *et al*., 1986; Ratti *et al*., 1981). However, none of these assays was specifically developed for neonates. We developed an RP-HPLC assay suitable for the simultaneous determination of rifampicin and 25-*O*-desacetylrifampicin in small volumes (40 μL) of neonatal plasma. The assay was based on a method for adult patients described by Chandi *et al*. (1998). The assay was validated for linearity, precision, accuracy and stability. Rifampicin was found to be stable in human plasma samples frozen at ≤–70°C, but unstable at room temperature and –20°C (Le Guellec *et al*., 1997; Peloquin, 1998).

## EXPERIMENTAL

### Determination of amoxicillin in neonatal plasma

**Chemicals.** The following chemicals were used: amoxicillin ($C_{16}H_{19}N_3O_5S$; Fluka, Buchs, Switzerland); sotalol hydrochloride ($C_{12}H_{20}N_2O_3S$ HCl; Bristol-Myers Squibb, New York, USA); and potassium dihydrogen phosphate ($KH_2PO_4$), dipotassium hydrogen phosphate ($K_2HPO_4$), perchloric acid ($HClO_4$) and methanol ($CH_4O$; Merck, Darmstadt, Germany).

**Chromatography.** The RP-HPLC system consisted of an automatic sample injector (model 717, Waters, USA), a pump (model 1050, Hewlett Packard, USA), a column, combined with a guard column, and a UV spectrophotometer detector (model 486, Waters, USA). The same chromatographic equipment was used for the determination of flucloxacillin and rifampicin in neonatal plasma. The mobile phase, consisting of 0.067 mol/L $KH_2PO_4$ (adjusted to pH 3.5 with phosphoric acid 25%), methanol and water (450:50:100), was pumped through a $C_8$ column (250 × 4.6 mm, 5 μm; Beckman, USA) after being degassed with helium. With a flow rate of 2.0 mL/min, amoxicillin and sotalol hydrochloride were detected at a wavelength of 225 nm.

**Preparation of calibration standards and quality controls.** Two independent stock solutions of amoxicillin (1000 mg/L) were prepared on the day of analysis by dissolving 23.1 mg amoxicillin in 20 mL water (factor of weight 1.155 for $H_2O$ bound to amoxicillin). One was used for the preparation of the calibration standards and the other was used for the preparation of the quality controls (50 mg/L). The working solution of amoxicillin (200 mg/L) was prepared by adding 600 μL stock solution to 2400 μL blank human plasma. Calibration standards were prepared by adding the required amount of working solution to blank human plasma to obtain concentrations of 10, 50, 100, 150 and 200 mg/L of amoxicillin. The stock solution of the internal standard sotalol hydrochloride (1000 mg/L) was prepared by dissolving 22.7 mg sotalol hydrochloride (HCl) in 20 mL ethanol (factor of weight 1.134 for HCl). The internal standard working solution (100 mg/L) was prepared by diluting the stock solution 10 times with water. The stock and working solutions of sotalol hydrochloride were stored at 4°C. The quality controls were stored in separate containers at –70°C until analysis.

**Sample preparation.** Aliquots (40 μL) of patient plasma, calibrations standards and quality controls were transferred into Eppendorf tubes and mixed with the internal standard working solution (40 μL) and perchloric acid 3% (80 μL) for protein precipitation. Following centrifugation at 10,500***g*** for 5 min, the clear supernatant (100 μL) was transferred into an Eppendorf tube, mixed with 1.2 mol/L $K_2HPO_4$ (40 μL), and centrifuged again. The clear supernatant (10 μL) was injected.

**Validation.** The RP-HPLC assay samples at six concentration levels of amoxicillin (8, 20, 40, 60, 80 and 160 mg/L) were prepared and analyzed on six separate days to determine the precision and accuracy of the RP-HPLC assay. The precision coefficient of variation (CV) determined at each concentration level should not exceed 15% except for the lower limit of quantification (LLOQ), where it should not exceed 20% (Shah *et al*., 1992). Accuracy should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20% (Shah *et al*., 1992). Long-term stability of amoxicillin in neonatal plasma during storage at –70°C was determined by analyzing three plasma controls of amoxicillin (25, 100 and 200 mg/L) stored at –70°C in routine assay runs for a period of one month. Desktop stability of amoxicillin in neonatal plasma at room temperature (light/dark) was evaluated by analyzing the three controls maintained at room temperature (light/dark) for 3 and 6 h. Post-preparative stability of amoxicillin in processed neonatal plasma samples during the run was checked by analyzing the three controls at the beginning of the run and at the end of the run after approximately 3 h.

### Determination of flucloxacillin in neonatal plasma

**Chemicals.** The following chemicals were used: flucloxacillin ($C_{19}H_{16}ClFN_3NaO_5S{\cdot}H_2O$; Synopharm, Barsbüttel, Germany), cloxacillin ($C_{19}H_{18}ClN_3O_5S$; SmithKline Beecham Pharmaceutical, Middlesex, UK), acetonitrile ($CH_3CN$), citric acid monohydrate ($C_6H_8O_7{\cdot}H_2O$), methylene chloride ($CH_2Cl_2$), hydrochloric acid (HCl) and sodium perchlorate ($NaClO_4{\cdot}H_2O$; Merck, Darmstadt, Germany).

**Chromatography.** Flucloxacillin and the internal standard cloxacillin were separated in a $C_8$ column (250 × 4.6 mm, 5 μm; Beckman, USA) at a flow rate of 2.0 mL/min. A mixture of 0.02 mol/L sodium perchlorate and acetonitrile (750:250) was used as mobile phase. Flucloxacillin and cloxacillin were detected at a wavelength of 220 nm.

**Preparation of calibration standards and quality controls.** For flucloxacillin, a stock solution (1000 mg/L) was freshly prepared by dissolving 11 mg sodium flucloxacillin in 10 mL



DOI: 10.1002/bmc

water (factor of weight 1.1 for sodium) and a working solution (100 mg/L) was freshly prepared by adding 200 µL stock solution to 1800 µL blank human plasma. The working solution was used to prepare the calibration standards by adding the required amount of working solution to blank human plasma to obtain concentrations of 10, 25, 50, 75 and 100 mg/L of flucloxacillin. An independent stock solution was prepared and used for the preparation of the quality controls (25 mg/L), which were stored in separate containers at –70°C until analysis. The stock solution of the internal standard cloxacillin (1000 mg/L) was prepared by dissolving 10 mg cloxacillin in 10 mL water. This stock solution was diluted 25 times with water to obtain the internal standard working solution (40 mg/L). Both cloxacillin stock and working solutions were stored at 4°C.

**Sample preparation.** In glass tubes, 20 µL sample volume was mixed with the internal standard working solution (20 µL), 0.5 mol/L citric acid monohydrate (40 µL) and 0.5 mol/L HCL (10 µL), extracted with methylene chloride (1 mL) for 2 min, and centrifuged at 1780***g*** for 5 min. After removal of the top water layer with vacuum, the organic phase was transferred into a glass tube and centrifuged again. After centrifugation, the organic phase was transferred into a glass tube and evaporated to dryness under a flow of nitrogen. The residue was dissolved in the mobile phase (100 µL) and injected (25 µL).

**Validation.** The RP-HPLC assay was validated for linearity, precision, accuracy, stability and recovery. To evaluate precision and accuracy, plasma samples at six concentration levels of flucloxacillin (5, 10, 20, 40, 60 and 100 mg/L) were prepared and analyzed on six separate days. Long-term stability of flucloxacillin in neonatal plasma during storage at room temperature (light/dark), 4, –20 and –70°C was determined by analyzing the quality controls over a period of 30 days at days 3, 7 and 30 ($n$ = 2). The recovery of flucloxacillin was determined by comparing the analytical results for extracted samples at two flucloxacillin concentrations (50 and 100 mg/L) with unextracted standards that represent 100% recovery.

## Simultaneous determination of rifampicin and 25-*O*-desacetylrifampicin in neonatal plasma

**Chemicals.** The following chemicals were used: rifampicin ($C_{43}H_{58}N_4O_{12}$; Yamanouchi Pharma, Leiderdorp, The Netherlands), 25-*O*-desacetylrifampicin ($C_{41}H_{56}N_4O_{11}$; Novartis Pharma, Arnhem, The Netherlands), ammonium sulfate [$(NH_4)_2SO_4$], methanol ($CH_4O$) and Titriplex® III ($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$; Merck, Darmstadt, Germany).

**Chromatography.** The mobile phase consisted of 0.005 mol/L disodium EDTA (0.93 g Titriplex® III in 500 mL water) and methanol (200:300). This mobile phase was pumped with a flow rate of 1.0 mL/min through a $C_{18}$ symmetry column (150 × 4.6 mm, 5 µm; Waters, USA). The detection was performed at a wavelength of 340 nm.

**Preparation of calibration standards and quality controls.** Stock solutions of rifampicin and 25-*O*-desacetylrifampicin (1000 mg/L) were prepared on the day of analysis by dissolving 20 mg rifampicin in 15 mL methanol and 5 mL water and by dissolving 20 mg 25-*O*-desacetylrifampicin in 6 mL methanol and 14 mL water. A solution containing both rifampicin and 25-*O*-desacetylrifampicin (100 mg/L) was prepared by adding 100 µL stock solution of rifampicin and 100 µL stock solution of 25-*O*-desacetylrifampicin to 800 µL blank human plasma. This solution was diluted 10 times with blank human plasma to obtain the working solution (10 mg/L). Calibration standards were prepared by adding the required amount of working solution to blank human plasma to obtain concentrations of 2, 4, 6, 8 and 10 mg/L of rifampicin and 25-*O*-desacetylrifampicin. Independent stock solutions of rifampicin and 25-*O*-desacetylrifampicin were made and used for the preparation of the quality controls, containing both rifampicin and 25-*O*-desacetylrifampicin (5 mg/L), which were stored in separate containers at –70°C until analysis.

**Sample preparation.** Small plasma volumes (40 µL) of patient samples, calibration standards and quality controls were transferred into Eppendorf tubes and mixed with ammonium sulfate 5% (10 µL) and methanol (80 µL) for protein precipitation. The mixture was centrifuged at 10,500***g*** for 5 min and the clear supernatant (30 µL) was injected.

**Validation.** The RP-HPLC assay was validated for linearity, precision, accuracy and stability. For rifampicin and 25-*O*-desacetylrifampicin, precision and accuracy were evaluated by measuring plasma samples at five concentration levels (2, 4, 6, 8 and 10 mg/L) on six separate days. Plasma samples at 12 concentrations levels of rifampicin and 25-*O*-desacetylrifampicin (0.1, 0.2, 0.3, 0.4, 1, 2, 4, 6, 8, 10, 25 and 50 mg/L) were measured on six separate days to determine the assay error patterns of rifampicin and 25-*O*-desacetylrifampicin. Rifampicin stability in neonatal plasma at room temperature (dark) was evaluated by analyzing plasma samples, containing 6 mg/L of rifampicin, stored at room temperature (dark) for 1, 2, 3 and 4 h. Post-preparative stability of rifampicin in processed neonatal plasma during the run was checked by analyzing plasma samples (6 mg/L) 1, 1.5 and 2 h after sample preparation.

# RESULTS

## Assay of amoxicillin in neonatal plasma

The calibration curves were linear. The correlation coefficients of the calibration curves were all higher than 0.99 ($r$ = 0.9985, $n$ = 6). The precision CV and accuracy determined at each concentration level of amoxicillin were less than 5%. The precision and accuracy of the amoxicillin RP-HPLC assay are presented in Table 1. The result of the quality control samples (50 mg/L), included in 48 routine assay runs over 3 months, was 53.7 ± 2.0 mg/L (precision CV 3.7%).

To determine the error pattern of the RP-HPLC assay, the relationship between the plasma concentration and the standard deviation (SD) with which it was determined was fitted with a polynomial of second order (Fig. 1; Jelliffe, 1994). The error pattern of the assay was: $SD = 0.1647 + 0.0009C + 0.0002C^2$ ($r^2$ = 0.992).

**Table 1. Precision and accuracy ($n$ = 6) of the amoxicillin RP-HPLC assay**

| True concentration (mg/L) | Measured concentration (mg/L) | SD (mg/L) | Precision CV (%) | Accuracy (%) |
|---|---|---|---|---|
| 8 | 8.13 | 0.24 | 3.0 | +1.6 |
| 20 | 20.66 | 0.32 | 1.5 | +3.3 |
| 40 | 38.25 | 0.44 | 1.2 | –4.4 |
| 60 | 61.08 | 0.74 | 1.2 | +1.8 |
| 80 | 79.04 | 1.89 | 2.4 | –1.2 |
| 160 | 158.50 | 5.71 | 3.6 | –0.9 |

CV, coefficient of variation; SD, standard deviation.

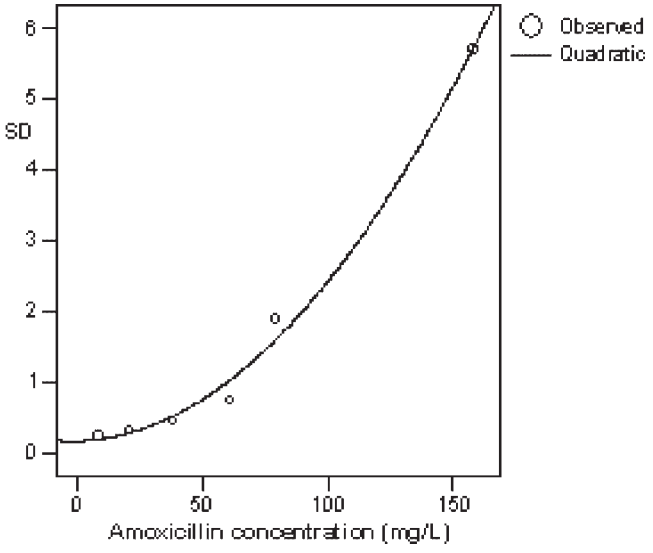


**Figure 1.** Error pattern of the amoxicillin RP-HPLC assay ($n$ = 6).

This polynomial equation can be used to calculate the SD corresponding with each plasma concentration. The LLOQ of the assay, the concentration that could just be determined with a precision of 20%, was 0.8 mg/L.

Amoxicillin was stable in neonatal plasma during storage at –70°C. Mean (±SD) amoxicillin plasma concentrations of the three controls (25, 100 and 200 mg/L), included in 28 routine assay runs during one month were 24.2 (±0.9), 97.7 (±4.8) and 195.9 (±8.5) mg/L, respectively. The desktop and post-preparative stability results are summarized in Table 2.

## Assay of flucloxacillin in neonatal plasma

All calibration curves were linear ($r$ = 0.9964, $n$ = 6). The precision CV and accuracy at all six concentration levels of flucloxacillin were <10 and <15%, respectively. The precision and accuracy of the flucloxacillin RP-HPLC assay are shown in Table 3. The results of the quality control samples (25 mg/L), included in 30 routine assay runs during 2 months, were 24.6 ± 1.2 mg/L (precision CV 4.7%).

**Table 2. Desktop and post-preparative stability of amoxicillin in neonatal plasma**

| Quality control (mg/L) | Desktop stability (light) | | Desktop stability (dark) | | Post-preparative stability |
|---|---|---|---|---|---|
| | 3 h | 6 h | 3 h | 6 h | 3 h |
| 25 | 95.8% | 85.0% | 105.8% | 95.0% | 98.1% |
| 100 | 97.7% | 97.5% | 98.7% | 97.5% | 107.8% |
| 200 | 101.2% | 98.9% | 106.1% | 97.1% | 99.8% |

**Table 3. Precision and accuracy ($n$ = 6) of the flucloxacillin RP-HPLC assay**

| True concentration (mg/L) | Measured concentration (mg/L) | SD (mg/L) | Precision CV (%) | Accuracy (%) |
|---|---|---|---|---|
| 5 | 5.61 | 0.38 | 6.8 | +12.2 |
| 10 | 10.70 | 0.77 | 7.2 | +7.0 |
| 20 | 20.37 | 0.76 | 3.7 | +1.9 |
| 40 | 39.82 | 1.87 | 4.7 | –0.5 |
| 60 | 58.00 | 1.29 | 2.2 | –3.3 |
| 100 | 101.08 | 3.82 | 3.8 | +1.1 |

CV, coefficient of variation; SD, standard deviation.



DOI: 10.1002/bmc

**Table 4. Long-term stability of flucloxacillin in neonatal plasma (25 mg/L; $n = 2$)**

| | 3 Days | 7 Days | 30 Days |
|---|---|---|---|
| Room temperature (light) | 90.8% | 80.8% | 15.5% |
| Room temperature (dark) | 92.3% | 95.4% | 2.7% |
| 4°C | 100.4% | 104.0% | 82.6% |
| –20°C | 101.3% | 122.3% | 100.0% |
| –70°C | 104.0% | 101.2% | 105.0% |

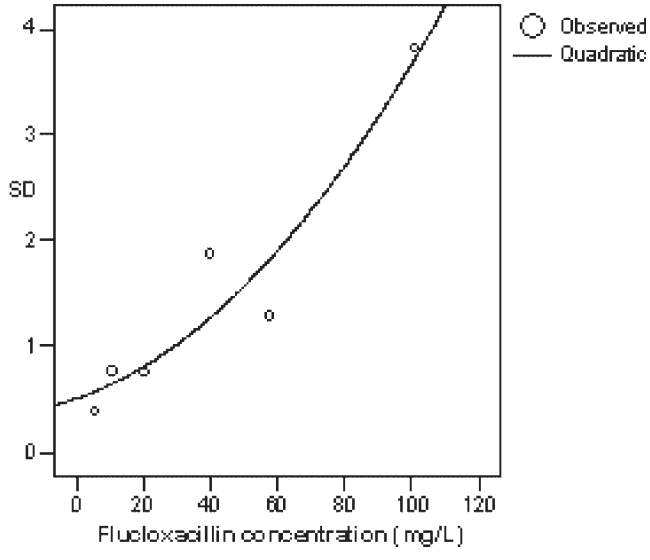


**Figure 2.** Error pattern of the flucloxacillin RP-HPLC assay ($n = 6$).

The quadratic relationship between the measured plasma concentration and the SD is presented in Fig. 2. The error pattern of the assay can be described by the following polynomial equation: $SD = 0.5117 + 0.0108C + 0.0002C^2$ ($r^2 = 0.907$). The LLOQ of the assay, as calculated from this polynomial equation, was 2.7 mg/L.

The long-term stability of flucloxacillin in neonatal plasma after storage at room temperature (light/dark) and 4, –20 and –70°C for 3, 7 and 30 days is presented in Table 4. Flucloxacillin appeared to be stable in neonatal plasma at –20 and –70°C for at least one month.

Mean recoveries of flucloxacillin at the two concentrations (50 and 100 mg/L) were 101.4 and 100.6% ($n = 2$), respectively.

## Simultaneous determination of rifampicin and 25-*O*-desacetylrifampicin in neonatal plasma

The calibration curves of rifampicin and 25-*O*-desacetylrifampicin were considered linear ($r = 0.995$, $n = 6$ and $r = 0.994$, $n = 6$, respectively). Precision CV and accuracy, measured at the five concentration levels, were <10 and <5%, respectively, for both rifampicin and 25-*O*-desacetylrifampicin (Table 5). The result of the quality control samples (5 mg/L), included in 30 routine assay runs over 2 months, was 4.7 ± 0.3 mg/L (precision CV 6.6%).

The plasma concentrations of rifampicin and 25-*O*-desacetylrifampicin and the corresponding standard deviations, used for the determination of the assay error patterns, are presented in Table 6.

In Fig. 3, the standard deviations are plotted against the measured plasma concentrations of rifampicin and 25-*O*-desacetylrifampicin and fitted with a polynomial of second order. The corresponding error patterns were: $SD = 0.0173 + 0.0455C + 0.0002C^2$ ($r^2 = 0.993$) for rifampicin and $SD = 0.0561 + 0.0323C + 0.0005C^2$ ($r^2 = 0.996$) for 25-*O*-desacetylrifampicin. The LLOQ was determined by analyzing plasma samples at low concentration levels (0.1, 0.2, 0.3 and 0.4 mg/L). The response at the LLOQ was at least five times the response compared with the blank response and was identifiable, discrete and reproducible with a precision of <20% and accuracy of 80–120%. The LLOQ was 0.1 mg/L for

**Table 5. Precision and accuracy ($n = 6$) of the RP-HPLC assays of rifampicin and 25-*O*-desacetylrifampicin**

| True concentration (mg/L) | Measured concentration (SD) (mg/L) | | Precision CV (%) | | Accuracy (%) | |
|---|---|---|---|---|---|---|
| | RIF | DES | RIF | DES | RIF | DES |
| 2 | 2.05 (0.17) | 2.04 (0.20) | 8.3 | 9.8 | +2.5 | +2.0 |
| 4 | 3.95 (0.26) | 3.98 (0.24) | 6.6 | 6.0 | –1.3 | –0.5 |
| 6 | 6.10 (0.21) | 6.09 (0.25) | 3.4 | 4.1 | +1.7 | +1.5 |
| 8 | 7.97 (0.14) | 7.99 (0.16) | 1.8 | 2.0 | –0.4 | –0.1 |
| 10 | 9.98 (0.26) | 9.96 (0.31) | 2.6 | 3.1 | –0.2 | –0.4 |

CV, coefficient of variation; DES, 25-*O*-desacetylrifampicin; RIF, rifampicin; SD, standard deviation.



DOI: 10.1002/bmc

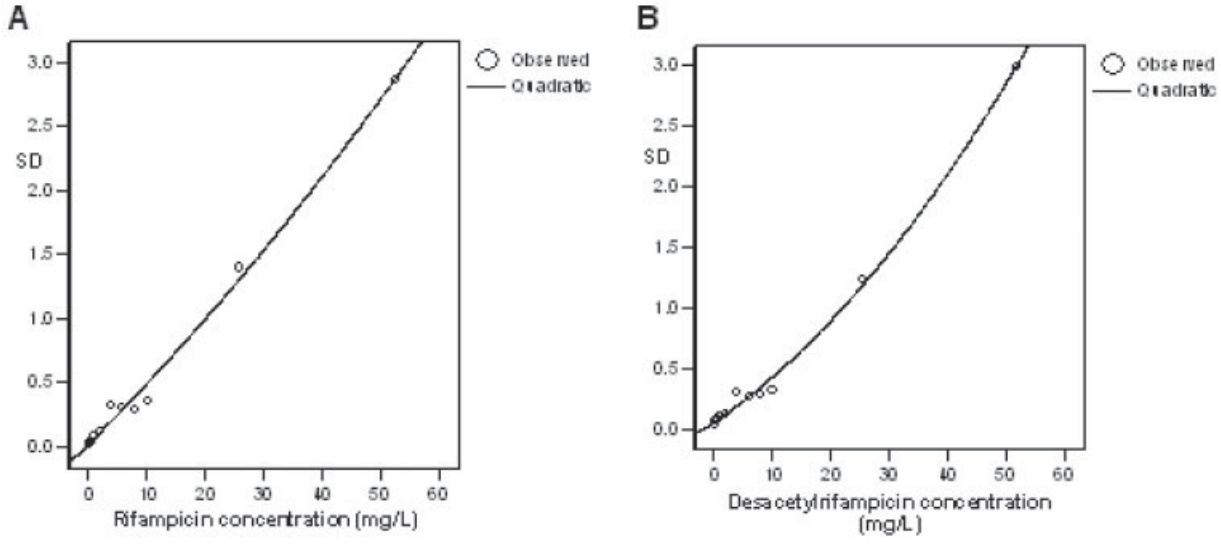


**Figure 3.** Assay error patterns of rifampicin (A) and 25-*O*-desacetylrifampicin (B) ($n = 6$).

**Table 6. Assay error patterns of rifampicin and 25-*O*-desacetylrifampicin ($n = 6$)**

| True concentration (mg/L) | Measured concentration (mg/L) | | SD (mg/L) | | Precision CV (%) | | Accuracy (%) | |
|---|---|---|---|---|---|---|---|---|
| | RIF | DES | RIF | DES | RIF | DES | RIF | DES |
| 0.1 | 0.10 | 0.12 | 0.01 | 0.02 | 10.8 | 20.8 | +3.7 | +15.0 |
| 0.2 | 0.18 | 0.25 | 0.03 | 0.07 | 17.6 | 27.4 | –8.6 | +23.5 |
| 0.3 | 0.29 | 0.36 | 0.04 | 0.08 | 12.6 | 22.1 | –4.3 | +20.7 |
| 0.4 | 0.39 | 0.45 | 0.03 | 0.07 | 7.5 | 15.0 | –2.2 | +11.9 |
| 1 | 0.99 | 1.05 | 0.08 | 0.10 | 8.4 | 10.0 | –0.6 | +5.0 |
| 2 | 1.99 | 2.07 | 0.11 | 0.12 | 5.6 | 5.9 | –0.6 | +3.6 |
| 4 | 3.83 | 3.93 | 0.32 | 0.31 | 8.2 | 7.8 | –4.2 | –1.8 |
| 6 | 5.85 | 5.95 | 0.31 | 0.27 | 5.2 | 4.6 | –2.4 | –0.8 |
| 8 | 8.00 | 8.02 | 0.29 | 0.29 | 3.7 | 3.6 | –0.0 | +0.3 |
| 10 | 10.2 | 10.0 | 0.36 | 0.32 | 3.5 | 3.2 | +1.6 | +0.3 |
| 25 | 25.8 | 25.4 | 1.40 | 1.24 | 5.4 | 4.9 | +3.3 | +1.4 |
| 50 | 52.8 | 51.9 | 2.87 | 2.99 | 5.4 | 5.8 | +5.7 | +3.8 |

DES, 25-*O*-desacetylrifampicin; RIF, rifampicin; SD, standard deviation.

rifampicin and 0.4 mg/L for 25-*O*-desacetylrifampicin (Table 6).

No decrease of rifampicin concentration in neonatal plasma stored at –70°C was observed during the entire period of two months. Rifampicin was stable in neonatal plasma at room temperature (dark) for 1 (103.8%), 2 (104.5%), 3 (105.5%) and 4 h (102.4%). After sample preparation, rifampicin was stable for 1 (96.7%), 1.5 (97.4%), and 2 h (96.1%).

## DISCUSSION AND CONCLUSIONS

Simple and rapid RP-HPLC assays with UV detection for the determination of amoxicillin, flucloxacillin and rifampicin in small volume neonatal plasma samples with satisfactory precision, accuracy and sensitivity have been developed. Considering the high precision and accuracy, the plasma volume required for amoxicillin and rifampicin analysis could be reduced from 40 to 20 µL or even 10 µL. It has been well documented that *in vitro* β-lactam antibiotics and aminoglycosides interact with each other resulting in the formation of two biological inactive compounds (Riff and Thomason, 1982; Tindula *et al*., 1983). Daly *et al*. (1997) studied the effect of varying concentrations of ampicillin on gentamicin concentration *in vitro* in pooled plasma obtained from umbilical cord blood, representing a medium analogous to neonatal plasma. Although inactivation of gentamicin by ampicillin did occur in this study, the degree of inactivation was small, especially at therapeutic concentrations of ampicillin. *In vivo* inactivation seems to occur



DOI: 10.1002/bmc

only when high nontherapeutic concentrations of the β-lactam antibiotic are present in plasma for prolonged periods. Nevertheless, we recommend plasma samples to be stored at −70°C as soon as possible after collection until analysis to avoid possible *in vitro* inactivation of amoxicillin and flucloxacillin, since the interaction between β-lactam antibiotics and aminoglycosides is dependent on temperature (Riff and Thomason, 1982; Tindula *et al*., 1983; Daly *et al*., 1997). Storage at −70°C is also recommended to avoid degradation of the antibiotics. The assays described are suitable for TDM and pharmacokinetic studies in neonates.

## REFERENCES


Bloemhof H, IJmker J and Uges DRA. De bepaling van enige xenobiotica in lichaamsvloeistoffen (The determination of some xenobiotics in body fluids). *Ziekenhuisfarmacie* 1988; **4**: 137.

Chandi LS, Sijs van der IH and Guchelaar HJ. Bepaling van rifampicine en desacetylrifampicine (Determination of rifampicin and desacetylrifampicin). *Ziekenhuisfarmacie* 1998; **14**: 71–72.

Charles BG, Foo CC and Gath J. Rapid column liquid chromatographic analysis of flucloxacillin in plasma on a microparticulate pre-column. *Journal of Chromatography B, Biomedical Applications* 1994; **660**: 186–190.

Daly JS, Dodge RA, Glew RH, Keroack MA, Bednarek FJ and Whalen M. Effect of time and temperature on inactivation of aminoglycosides by ampicillin at neonatal dosages. *Journal of Perinatology* 1997; **17**: 42–45.

Herngren L. Flucloxacillin kinetics in newborn infants. *European Journal of Clinical Pharmacology* 1989; **36**: 325.

Herngren L, Ehrnebo M and Broberger U. Pharmacokinetics of free and total flucloxacillin in newborn infants. *European Journal of Clinical Pharmacology* 1987; **32**: 403–409.

Hoizey G, Lamiable D, Frances C, Trenque T, Kaltenbach M, Denis J and Millart H. Simultaneous determination of amoxicillin and clavulanic acid in human plasma by HPLC with UV detection. *Journal of Pharmaceutical and Biomedical Analysis* 2002; **30**: 661–666.

Huisman-de Boer JJ, Anker van den JN, Vogel M, Goessens WH, Schoemaker RC and Groot de R. Amoxicillin pharmacokinetics in preterm infants with gestational ages of less than 32 weeks. *Antimicrobial Agents and Chemotherapy* 1995; **39**: 431–434.

Hung CT, Lim JK, Zoest AR and Lam FC. Optimization of high-performance liquid chromatographic analysis for isoxazolyl penicillins using factorial design. *Journal of Chromatography* 1988; **425**: 331–341.

Ishii M and Ogata H. Determination of rifampicin and its main metabolites in human plasma by high-performance liquid chromatography. *Journal of Chromatography* 1988; **426**: 412–416.

Jalling B, Malmborg AS, Lindman A and Boreus LO. Evaluation of a micromethod for determination of antibiotic concentrations in plasma. *European Journal of Clinical Pharmacology* 1972; **4**: 150–157.

Jelliffe RW. Effect of assumptions concerning assay error patterns upon pharmacokinetic parameter values and model behavior. *Journal of Veterinary Pharmacology and Therapeutics* 1994; **17**: 85–119.

Le Guellec C, Gaudet ML, Lamanetre S and Breteau M. Stability of rifampin in plasma: consequences for therapeutic monitoring and pharmacokinetic studies. *Therapeutic Drug Monitoring* 1997; **19**: 669–674.

Lin JC, Strauss RG, Kulhavy JC, Johnson KJ, Zimmerman MB, Cress GA, Connolly NW and Widness JA. Phlebotomy overdraw in the neonatal intensive care nursery. *Pediatrics* 2000; **106**: E19.

Lindegårdh N, Singtoroj T, Annerberg A, White NJ and Day NP. Development and validation of a solid-phase extraction-liquid chromatographic method for determination of amoxicillin in plasma. *Therapeutic Drug Monitoring* 2005; **27**: 503–508.

Mascher HJ and Kikuta C. Determination of amoxicillin in human serum and plasma by high-performance liquid chromatography and on-line postcolumn derivatisation. *Journal of Chromatography A* 1998; **812**: 221–226.

Oldfield S, Berg JD, Stiles HJ and Buckley BM. Measurement of rifampicin and 25-desacetylrifampicin in biological fluids using high-performance liquid chromatography with direct sample injection. *Journal of Chromatography* 1986; **377**: 423–429.

Peloquin CA. Rifampin stability. *Therapeutic Drug Monitoring* 1998; **20**: 450–451.

Pullen J, Rozario de L, Stolk LML, Degraeuwe PLJ, Tiel van FH and Zimmermann LJI. Population pharmacokinetics and dosing of flucloxacillin in preterm and term neonates. *Therapeutic Drug Monitoring* 2006a; **28**: 351–358.

Pullen J, Stolk LML, Degraeuwe PLJ, Tiel van FH, Neef C and Zimmermann LJI. Pharmacokinetics of intravenous rifampicin (rifampin) in neonates. *Therapeutic Drug Monitoring* 2006b; **28**: 654–661.

Pullen J, Stolk LML, Nieman FHM, Degraeuwe PLJ, Tiel van FH and Zimmermann LJI. Population pharmacokinetics and dosing of amoxicillin in (pre)term neonates. *Therapeutic Drug Monitoring* 2006c; **28**: 226–231.

Ratti B, Parenti RR, Toselli A and Zerilli LF. Quantitative assay of rifampicin and its main metabolite 25-desacetylrifampicin in human plasma by reversed-phase high-performance liquid chromatography. *Journal of Chromatography* 1981; **225**: 526–531.

Riff LJ and Thomason JL. Comparative aminoglycoside inactivation by beta-lactam antibiotics. Effects of a cephalosporin and six penicillins on five aminoglycosides. *The Journal of Antibiotics (Tokyo)* 1982; **35**: 850–857.

Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, Layloff T, Viswanathan CT, Cook CE, McDowall RD, Pittman KA and Spector S. Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies. *Journal of Pharmaceutical Sciences* 1992; **81**: 309–312.

Sutherland R, Croydon EAP and Rolinson GN. Flucloxacillin, a new isoxazolyl penicillin, compared with oxacillin, cloxacillin, and dicloxacillin. *British Medical Journal* 1970; **4**: 455–460.

Tan TQ, Mason EO, Ou CN and Kaplan SL. Use of intravenous rifampin in neonates with persistent staphylococcal bacteremia. *Antimicrobial Agents and Chemotherapy* 1993; **37**: 2401–2406.

Thijssen HHW. Analysis of isoxazolyl penicillins and their metabolites in body fluids by high-performance liquid chromatography. *Journal of Chromatography. B, Biomedical Applications* 1980; 183: 339–345.

Tindula RJ, Ambrose PJ and Harralson AF. Aminoglycoside inactivation by penicillins and cephalosporins and its impact on drug-level monitoring. *Drug Intelligence and Clinical Pharmacy* 1983; **17**: 906–908.

# Population Pharmacokinetics and Dosing of Amoxicillin in (Pre)term Neonates

*Joyce Pullen, MSc,* Leo M. L. Stolk, PhD,* Fred H. M. Nieman, PhD,† Pieter L. J. Degraeuwe, PhD,‡ Frank H. van Tiel, PhD,§ and Luc J. I. Zimmermann, PhD‡*

**Abstract:** Amoxicillin plasma concentrations, pharmacokinetic parameters, and the influence of demographic, anthropometric, and clinical covariates were investigated in 150 neonates. Gestational age (GA) ranged from 25 to 42 weeks and mean postnatal age (PNA) was 0.8 days. Amoxicillin concentrations were measured with reversed-phase HPLC in surplus plasma from routine assays of coadministered gentamicin. Mean total body clearance corrected for body weight (CL/W) was $0.096 \pm 0.036\,L\,kg^{-1}h^{-1}$, mean elimination half-life ($t^1/_2$) was $5.2 \pm 1.9$ hours, and mean volume of distribution corrected for body weight (V/W) was $0.65 \pm 0.13$ L/kg. Multiple regression equations were calculated for the prediction of CL/W amoxicillin. CL/W gentamicin, V/W gentamicin, and GA were significant predictors of CL/W amoxicillin. Amoxicillin peak and trough concentrations after the second dose and the time the concentration exceeds the minimum inhibitory concentration (T > MIC), reached with the current dosage regimen, were evaluated. Toxic plasma concentrations were reached in several patients. Therefore, the authors have proposed a lower dosage regimen, based on GA, population pharmacokinetic parameters, bacterial susceptibility (T > MIC), and possible toxicity: 15 mg/kg per 8 hours and 20 mg/kg per 8 hours for neonates with GA $\leq$ 34 and GA > 34 weeks, respectively. Simulation with this new dosage regimen indicated that satisfactory plasma concentrations were reached in all 150 neonates. Therefore, use of therapeutic drug monitoring and pharmacokinetic calculations for dosage adjustment is generally not necessary.

**Key Words:** population pharmacokinetics, amoxicillin, neonates, dosage regimen

(*Ther Drug Monit* 2006;28:226–231)

Received for publication July 4, 2005; accepted November 10, 2005.
From the *Department of Clinical Pharmacy and Toxicology, University Hospital of Maastricht, The Netherlands; †Department of Clinical Epidemiology and Medical Technology Assessment, University Hospital of Maastricht, The Netherlands; ‡Department of Paediatrics, Division of Neonatology, University Hospital of Maastricht, The Netherlands; and §Department of Medical Microbiology, University Hospital of Maastricht, The Netherlands.
Reprints: J. Pullen, Department of Clinical Pharmacy and Toxicology, University Hospital of Maastricht, PO Box 5800, NL-6202 AZ Maastricht, The Netherlands (e-mail: jpul@kfls.azm.nl).


Amoxicillin, an aminopenicillin, in combination with gentamicin, an aminoglycoside, is frequently used for the treatment of early-onset neonatal sepsis.[1] In contrast to gentamicin, therapeutic drug monitoring (TDM) of amoxicillin is not performed on a routine basis, because amoxicillin is considered to have a wide therapeutic window.

Despite the widespread use of amoxicillin in neonates, data on population pharmacokinetics in the literature are limited. The 4 studies available describe only a relatively small number of patients.[2–5] Charles et al[2] and Huisman-de Boer et al[3] limited their study population to neonates with gestational age less than 32 weeks. In the study by Autret et al[4] only term neonates were included.

The administration of excessive doses of amoxicillin is associated with a variety of adverse effects, such as rashes, urticaria, diarrhea, eosinophilia, elevated liver and muscle enzymes, seizures, and renal dysfunction.[6–9] Renal impairment and alterations in the blood-brain barrier are the 2 major risk factors for amoxicillin-induced neurotoxicity in preterm neonates.[6] The efficacy of penicillins, including amoxicillin, depends on the time the concentration exceeds the minimum inhibitory concentration (T > MIC).[10] A dosage regimen is suitable if it results in effective, nontoxic plasma concentrations.

The objectives of our study were to investigate whether the current dosage regimen of amoxicillin leads to satisfactory plasma concentrations and to devise a better dosage regimen if necessary. To accomplish these goals, our co-objectives were the establishment of the population pharmacokinetic parameters of amoxicillin and the identification of demographic, anthropometric, and clinical covariates that may significantly influence the individual pharmacokinetic parameters.

## MATERIALS AND METHODS

### Patients

The local ethical board approved the study. The population consisted of 150 (pre)term neonates at risk for or with (suspected) early-onset sepsis. The neonates were admitted to the neonatal ward at the University Hospital of Maastricht from July 2002 through February 2005. Inclusion criteria were: coadministration of amoxicillin and gentamicin, postnatal age (PNA) $\leq$9 days, availability of at least 1 plasma sample taken just before the administration of amoxicillin and gentamicin (trough

concentrations) and 1 plasma sample taken 1 hour after the administration (peak concentrations), and availability of reliable patient data with respect to gestational age (GA), PNA, dosing, and sampling times.

For each patient, the following demographic, anthropometric, and clinical characteristics were recorded: GA, PNA, body weight (W), gender, multiple pregnancy (P), length, head circumference (HC), arterial cord pH (ApH), 5-minute Apgar score (AP5), divided into 2 groups ( $< 7$ and $\geq 7$),[11] 1/plasma creatinine (1/Cr), plasma urea concentration (Ur), blood culture result (BC), C-reactive protein (CRP), total body clearance of gentamicin (CL gentamicin), total body clearance of gentamicin corrected for body weight (CL/W gentamicin), elimination half-life of gentamicin ($t^1/_2$ gentamicin), and volume of distribution of gentamicin corrected for body weight (V/W gentamicin).

## Gentamicin and Amoxicillin Administration and Plasma Sampling

The gentamicin dosage regimen was derived from results of our earlier study[12]: 5 mg/kg per 48 hours, 4.5 mg/kg per 36 hours, and 4 mg/kg per 24 hours for neonates with GA < 30, $30 \leq GA \leq 34$, and GA > 34 weeks, respectively. Gentamicin was administered intravenously by syringe pump during 30 minutes. Amoxicillin was administered by intravenous slow push according to dosing guidelines in literature[13]: 100 mg/kg per dose for (suspected) meningitis and 50 mg/kg per dose in other situations. Dosing interval was 12 hours in the first week of life, 8 hours in neonates 1 to 3 weeks old, and 6 hours in neonates $\geq 4$ weeks old.[13] Eleven neonates were treated with co-amoxiclav (Augmentin®), 133 neonates with amoxicillin, and 6 neonates with amoxicillin and co-amoxiclav. Amoxicillin and gentamicin were administered shortly after each other. Unless the mother was treated with indomethacin as a tocolytic, all neonates with GA < 32 weeks or birth weight < 1250 g were treated prophylactically with indomethacin during the first 3 days of life.[14]

Plasma samples were taken just before and 1 hour after the administration of amoxicillin and gentamicin. Surplus plasma samples from routine gentamicin assays were stored at $-70^{\circ}C$. Dosing and sampling times were recorded by nursing staff.

## Chemicals

Potassium dihydrogen phosphate ($KH_2PO_4$, CAS 7778-77-0), dipotassium hydrogen phosphate ($K_2HPO_4$, CAS 7758-11-4), perchloric acid ($HClO_4$, CAS 7601-90-3), and methanol ($CH_4O$, CAS 67-56-1) were obtained from Merck (Darmstadt, Germany). Amoxicillin ($C_{16}H_{19}N_3O_5S$, CAS 26787-78-0) was provided by Fluka (Buchs, Switzerland), and the internal standard sotalol hydrochloride ($C_{12}H_{20}N_2O_3S$ HCl, CAS 959-24-0) was obtained from Bristol-Myers Squibb (New York, NY). Ultrapure water was used in all preparations. All chemicals were of analytical grade. Human EDTA plasma for standard and control samples was provided by the Red Cross Bloodbank (Maastricht, The Netherlands).

## Amoxicillin HPLC Assay

Gentamicin plasma concentrations were measured with the COBAS INTEGRA 700 analyzer (Roche, Mannheim, Germany), using the principle of fluorescence polarization. The error pattern of the gentamicin assay was: $SD = 0.0129 + 0.0029\ C + 0.0034\ C^2$. The assay error pattern was determined according to a method described in literature.[15] The lower limit of determination was 0.1 mg/L. Amoxicillin plasma concentrations were determined with reversed-phase HPLC. An automatic sample injector (model 717, Waters, USA), a pump (model 1050, Hewlett Packard, USA), a C8 column, $250 \times 4.6$ mm, 5 μm (Beckman, USA), combined with a C8 guard column, and an UV spectrophotometer detector (model 486, Waters) were used. The eluent was composed of 0.067 mol/L potassium dihydrogen phosphate (pH 3.5), methanol, and distilled water (450:50:100). The separations were performed at room temperature and at a flow rate of 2 mL/minute. The detection occurred at a wavelength of 225 nm. The error pattern of the assay was: $SD = 0.1647 + 0.0009\ C + 0.0002\ C^2$. The lower limit of determination was 1 mg/L.

To 40 μL surplus plasma, 40 μL of 100 mg/L sotalol hydrochloride (internal standard) was added. While mixing the solution with the vortex, 80 μL of perchloric acid 3% was added. The solution was centrifuged at $10{,}500 \times g$ and 100 μL of the clear top layer was mixed with 40 μL of 1.2 mol/L dipotassium hydrogen phosphate. The solution was centrifuged again at $10{,}500 \times g$ and 10 μL of the clear top layer was injected. The standard samples, containing 10, 50, 100, 150, and 200 mg/L amoxicillin in plasma, were processed in the same way as the surplus plasma samples. A calibration curve was constructed and amoxicillin concentrations in surplus plasma samples were calculated. Two control samples, each containing 50 mg/L of amoxicillin in plasma, were measured in each run. Measurements were performed in duplicate, if sufficient surplus plasma was available.

## Data Analysis

Population pharmacokinetic parameters of amoxicillin and gentamicin were calculated with an iterative 2-stage Bayesian fitting procedure (IT2B) (MW\PHARM3.50, Mediware, The Netherlands). The Bayesian fitting procedure uses measured drug concentrations, population-based pharmacokinetic parameters, and the expected variability associated with each measurement to determine individualized pharmacokinetic parameters of a patient.[16] We used a 1-compartment open model. The initial pharmacokinetic parameters of amoxicillin were derived from other studies: $K_{el}$ $0.1 \pm 0.03$ hours$^{-1}$ and V/W $0.67 \pm 0.12$ L/kg for neonates with GA < 32 weeks[3] and $K_{el}$ $0.16 \pm 0.09$ hours$^{-1}$ and V/W $0.62 \pm 0.23$ L/kg for neonates with GA $\geq 32$ weeks.[4] The initial pharmacokinetic parameters of gentamicin were derived from the study of Stolk et al.[12]

Statistical analysis was started by checking CL/W and V/W of amoxicillin for normal distribution with the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) was used to test whether the factor variables (gender, P, AP5, and BC) had a significant effect on CL/W and V/W of amoxicillin. Pearson product-moment correlation coefficients ($r$) between pharmacokinetic parameters of amoxicillin and covariates were calculated. Multiple regression analysis with forward selection and backward elimination techniques was performed, using CL/W and V/W of amoxicillin as dependent variables. The significant (factor) variables, detected with one-way ANOVA and correlation analysis, were used as possible predictors. The significant predictors of the dependent variable were used to establish a direct-effects model. First-order interaction effects between the significant predictors were tested for significance by forced entering within a hierarchically constructed regression model. Residual analysis was performed to check the studentized residuals of the dependent variable for normality and outliers. Discrete factors were entered as dummy variables. Listwise deletion of missing cases was used during the entire regression analysis procedure. Statistical analysis was performed by using SPSS® 12.0 (SPSS Inc., Chicago, IL). $P < 0.05$ was considered to be statistically significant.

## Evaluation of Current and Proposed Dosage Regimen

The efficacy of penicillins, including amoxicillin, depends on the time the concentration exceeds the minimum inhibitory concentration (T > MIC).[10] In neonates, T > MIC should at least be 40% to 50%.[10] For the different species, identified as causative agents of bacteremia, the breakpoint MIC value of an antibiotic defines the highest MIC value still to be interpreted as indicating susceptibility to that antibiotic. This breakpoint MIC value of amoxicillin is 0.25 mg/L for *Streptococcus agalactiae* and 8 mg/L for Enterococcus species and species belonging to the enterobacteriaceae.[17] Therefore, amoxicillin plasma concentrations will have to exceed 8 mg/L during 40% to 50% of the time. To evaluate the efficacy of the current dosage regimen, we calculated T > MIC for each patient, using their individual pharmacokinetic parameters. Shaffer et al[6] reported that ampicillin concentrations higher than 140 mg/L may cause seizures in adults. To evaluate possible toxicity of the current dosage regimen, we calculated amoxicillin peak concentrations after the second dose of each patient, using their individual pharmacokinetic parameters.

Amoxicillin plasma concentrations, reached with the proposed dosage regimen, were simulated for all neonates in MW\PHARM 3.50 (Mediware, QJ;The Netherlands), using the individual pharmacokinetic parameters of amoxicillin.

# RESULTS

## Demographic, Anthropometric, and Clinical Characteristics

Demographic, anthropometric, and clinical characteristics of the study patients are presented in Table 1. The population consisted of 78 (52%) girls and 72 (48%) boys; 123 (82%) neonates were products of singleton pregnancies and 27 (18%) neonates of twin pregnancies. AP5 was $\geq 7$ in 122 of 148 (82.4%) neonates (2 missing data). Seven of 113 (6.2%) blood cultures (37 missing) were positive. There were 674 amoxicillin and gentamicin plasma concentrations with a mean of 4.5 ± 1.3.

## Population Pharmacokinetic Parameters

Mean total body clearance of amoxicillin corrected for body weight (CL/W) was 0.096 ± 0.036 $Lkg^{-1}h^{-1}$, mean amoxicillin elimination half-life ($t^1/_2$) was 5.2 ± 1.9 hours, and mean volume of distribution of amoxicillin corrected for body weight (V/W) was 0.65 ± 0.13 L/kg. Population pharmacokinetic parameters of gentamicin were: CL/W 0.043 ± 0.012 $Lkg^{-1}h^{-1}$, $t^1/_2$ 10 ± 3.2 hours, and V/W 0.59 ± 0.13 L/kg.

## Statistical Analysis: Prediction of CL/W Amoxicillin

The factor variables (gender, P, AP5, and BC) had no significant effect on CL/W amoxicillin. Significant Pearson correlations between the elimination parameters of amoxicillin (CL/W, CL, and $t^1/_2$) and the demographic, anthropometric, and clinical variables are presented in Table 2. Correlation between CL/W amoxicillin and GA is shown in Figure 1. Multiple regression analysis was performed by using CL/W amoxicillin (normally distributed) as dependent variable and GA, CL/W gentamicin, V/W gentamicin, and PNA as possible predictors. The variables 1/Cr and Ur were not used as possible

**TABLE 1.** Demographic, Anthropometric, and Clinical Characteristics of the Study Patients

| | Unit | No. of Patients | Mean (SD) | Range |
|---|---|---|---|---|
| GA | weeks | 150 | 34.6 (4.9) | 24.9–42.4 |
| PNA | days | 150 | 0.76 (1.57) | 0–9 |
| W | kg | 150 | 2.29 (1.08) | 0.57–4.7 |
| Length | cm | 104 | 43.7 (6.2) | 30–56 |
| HC | cm | 108 | 30.8 (4.7) | 21–55 |
| ApH | pH | 93 | 7.19 (0.14) | 6.7–7.48 |
| AP5 | points | 148 | 9 (median) | 1–10 |
| 1/Cr | L/μmol | 101 | 0.012 (0.004) | 0.006–0.036 |
| Ur | mmol/L | 100 | 5.05 (2.62) | 0.8–13.5 |
| CRP | mg/L | 141 | 14.7 (23.2) | 1–117 |
| CL gentamicin | L/h | 150 | 0.11 (0.065) | 0.02–0.28 |
| CL/W gentamicin | $Lkg^{-1}h^{-1}$ | 150 | 0.043 (0.012) | 0.023–0.082 |
| $t^1/_2$ gentamicin | h | 150 | 10 (3.2) | 5–19.7 |
| V/W gentamicin | L/kg | 150 | 0.59 (0.13) | 0.37–1.09 |

AP5, 5-minute Apgar score; ApH, arterial cord pH; CL, total body clearance; Cr, plasma creatinine level; CRP, C-reactive protein; GA, gestational age; HC, head circumference; PNA, postnatal age; $t^1/_2$, elimination half-life; Ur, plasma urea concentration; V, volume of distribution; W, body weight.

**TABLE 2.** Significant Correlations of Demographic, Anthropometric, and Clinical Variables With CL/W, CL, and $t^1/_2$ of Amoxicillin

| | CL/W Amoxicillin | CL Amoxicillin | $t^1/_2$ Amoxicillin |
|---|---|---|---|
| GA | 0.656† | 0.857† | −0.655† |
| CL/W, CL, and $t^1/_2$ of gentamicin | 0.651† | 0.906† | 0.777† |
| W | 0.611† | 0.889† | −0.599† |
| 1/Cr | 0.521† | 0.352† | −0.394† |
| Ur | −0.42† | −0.277† | 0.446† |
| V/W gentamicin | −0.356† | −0.503† | 0.51† |
| PNA | 0.314† | 0.208* | −0.19* |

CL, total body clearance; Cr, plasma creatinine level; GA, gestational age; PNA, postnatal age; $t^1/_2$, elimination half-life; Ur, plasma urea concentration; V, volume of distribution; W, body weight.

*Correlation is significant at the 0.05 level (2-tailed).

†Correlation is significant at the 0.01 level (2-tailed).

predictors, because they had many missing data (49 and 50, respectively). The regression equation to predict CL/W amoxicillin was: CL/W amoxicillin ($Lkg^{-1}h^{-1}$) = 0.014 + 1.825 CL/W gentamicin ($Lkg^{-1}h^{-1}$) − 0.083 V/W gentamicin (L/kg) + 0.001 GA (weeks) ($r^2$ = 0.615, n = 150). The standardized beta coefficients, which represent the relative contribution of CL/W gentamicin, V/W gentamicin, and GA in the prediction of CL/W amoxicillin, were 0.580, −0.297, and 0.201, respectively. Because data on pharmacokinetics of gentamicin are usually not available at the start of the therapy, regression analysis also was performed without the pharmacokinetic parameters of gentamicin. The regression equation was: CL/W amoxicillin ($Lkg^{-1}h^{-1}$) = −0.067 + 0.005 GA (weeks) + 0.005 PNA (days) ($r^2$ = 0.479, n = 150). The standardized beta coefficients of GA and PNA were 0.623 and 0.223, respectively. The standardized studentized residuals of all models seemed to be normally distributed.

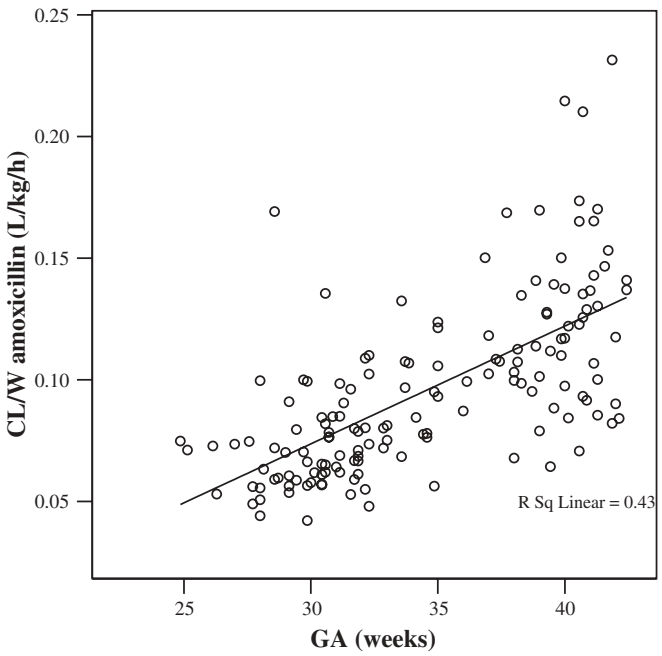


**FIGURE 1.** Correlation between total body clearance of amoxicillin corrected for body weight (CL/W amoxicillin) and gestational age (GA).

## Statistical Analysis: Prediction of V/W Amoxicillin

V/W amoxicillin differed significantly between singleton and twin pregnancies, $F_{(1,148)}$ 7.14 ($P$ = 0.008) and between neonates with AP5 < 7 and AP5 ≥ 7, $F_{(1,146)}$ 6.41 ($P$ = 0.012). Therefore, the factor variables P and AP5 were admitted as possible predictors of V/W amoxicillin to subsequent multiple regression analysis. Significant Pearson correlations were found between V/W amoxicillin and the variables CL/W gentamicin ($r$ = 0.216; $P$ = 0.008) and V/W gentamicin ($r$ = 0.183; $P$ = 0.025). However, these correlations are very low and, therefore, not relevant for use in clinical practice. The correlation between V amoxicillin and W was very strong (0.92), leading to the assumption that V is mainly predicted by W.

## Evaluation of Current and Proposed Dosage Regimen

Amoxicillin peak and trough concentrations after the second dose and T > MIC of each patient are presented in Table 3. With the current dosage regimen, T > MIC was much greater than 50% in all neonates. Peak concentrations were greater than 140 mg/L in 28 neonates. These results indicate that the current dosage regimen may result in toxic plasma concentrations. We recommend a reduction of the dose and dosing interval. Because of the high correlation between CL/W amoxicillin and GA and for practical reasons, neonates will be subdivided into the same 3 dosage GA subgroups as already used for gentamicin dosing. We recommend 15 mg/kg per 8 hours for neonates with GA < 30 and 30 ≤ GA ≤ 34 weeks and 20 mg/kg per 8 hours for neonates with GA > 34 weeks. With this new dosage regimen, simulated peak concentrations were < 140 mg/L in all neonates: 41.9 ± 8.4 mg/L (mean ± SD) with a range of 23 to 72.4 mg/L. Simulated T > MIC was greater than 50% in all neonates: 96.5 ± 8.7% (mean ± SD) with a range of 51.5% to 100%.

# DISCUSSION

## Population Pharmacokinetic Parameters

CL/W amoxicillin was approximately × 2 higher than CL/W gentamicin. This suggests the presence of other elimination pathways in addition to glomerular filtration, such as tubular secretion and/or metabolism, because gentamicin is completely cleared by glomerular filtration.[18] However, literature indicates that tubular secretion is very inefficient[19] and glomerular filtration is the major pathway for amoxicillin elimination[3] in (pre)term neonates.

The population pharmacokinetic parameters of amoxicillin found in the present study are similar to the

**TABLE 3.** Amoxicillin Peak and Trough Concentrations After the Second Dose and T>MIC

| Dose (mg/kg) | Interval (hr) | No. of Patients* | Trough Concentration (mg/L) (SD) | Peak Concentration (mg/L) (SD) | No. peak > 140 mg/L | T > MIC (%) (SD) |
|---|---|---|---|---|---|---|
| 25 | 6 | 1 | 15.9 | 53 | 0 | 100 |
| 50 | 8 | 2 | 15.3 (1.5) | 92.3 (12.6) | 0 | 100 (0) |
| 50 | 12 | 112 | 21.4 (13.1) | 101 (19.3) | 3 | 97.9 (6.8) |
| 75 | 12 | 6 | 24.2 (25.4) | 147.7 (28.2) | 5 | 95 (12.2) |
| 100 | 6 | 1 | 85.1 | 216.4 | 1 | 100 |
| 100 | 8 | 1 | 115.8 | 323.8 | 1 | 100 |
| 100 | 12 | 18 | 42.6 (24.3) | 212.2 (42.1) | 18 | 99.4 (2.4) |

T > MIC, the time the concentration exceeds the minimum inhibitory concentration; SD, standard deviation.
*Nine of 150 patients did not receive a dose of 25, 50, 75, or 100 mg/kg.

results of Autret et al,[4] CL/W 0.10 $Lkg^{-1}h^{-1}$ and V/W 0.62 L/kg. Huisman-de Boer et al[3] found a comparable V/W (0.67 L/kg), but a lower CL/W (0.07 $Lkg^{-1}h^{-1}$), which may be partially explained by the lower GA of the neonates. Peskine et al[5] found a much higher CL/W amoxicillin (0.21 $Lkg^{-1}h^{-1}$) and V/W amoxicillin (1.38 L/kg). These differences may be attributable to the relatively small study population and the use of the method of disk diffusion in nutrient agar instead of a more specific HPLC method.

## Prediction of CL/W Amoxicillin

According to literature, plasma creatinine in the first week of life does not reflect glomerular filtration rate (GFR) of the neonate.[20] Immediately after birth, plasma creatinine levels of the neonate represent maternal levels. Shortly thereafter, the tubular reabsorption of creatinine seems to be responsible for continuous high plasma creatinine levels in the neonate.[20] Therefore, we also included data of concomitant gentamicin monitoring for our prediction model. Gentamicin clearance as a predictor of GFR has been suggested earlier.[18] However, CL/W gentamicin as well as 1/Cr correlated significantly with CL/W amoxicillin. Our finding that CL/W gentamicin is a good predictor of CL/W amoxicillin might be useful in clinical practice as an alternative for 1/Cr. The study by Huisman-de Boer et al[3] indicated that inulin clearance, another marker of GFR, was highly correlated with amoxicillin clearance. Another important predictor of CL/W amoxicillin was GA. Amoxicillin clearance increases with gestational age, which probably reflects the development of renal function (both filtration and secretion). Other investigators found a positive correlation of CL amoxicillin with GA and/or W.[2,3,5]

## Dosage Regimen

Simulation with the proposed dosage regimen indicated that satisfactory plasma concentrations were reached for all 150 neonates. Therefore, we think therapeutic drug monitoring is generally not necessary. We recommend a reduction of the amoxicillin dose and dosing interval. Reduction of the amoxicillin dose in neonates has been recommended previously by other investigators. Charles et al[2] recommended 20 mg/kg per 12 hours or 50 mg/kg per day instead of 50 mg/kg per 12 hours for neonates with GA $\leq$ 32 weeks in the first week of life. Huisman-de Boer et al[3] recommended 25 mg/kg per 12 hours for neonates with GA < 32 weeks in the first week of life. Dose reduction reduces the risk of amoxicillin toxicity, but also lowers the costs and prevents unnecessary use of the antibiotic, which promotes bacterial overgrowth and resistance.

The proposed dosage regimen will result in amoxicillin plasma concentrations sufficiently high for the treatment of infections that are easily reached by antibiotics, such as bacteremia, urinary tract infection, and intravascular catheters-related infection. However, in patients who are suspected of infections that are not easily reached by antibiotics, such as meningitis, we recommend doses higher than our proposed dosage regimen. Some experts recommend 100 mg/kg per dose for treating meningitis.[21] However, our results indicate that a dose of 100 mg/kg will probably result in plasma concentrations > 140 mg/L, which may cause seizures. Moreover, literature indicates that inflammation of the meninges results in increased cerebrospinal fluid amoxicillin concentrations caused by the increased permeability of the blood-brain barrier and a decrease in drug clearance, jeopardizing the infant to amoxicillin neurotoxicity.[6] We suggest a maximum amoxicillin dose of 40 mg/kg per 12 hours for neonates with GA < 30 weeks and 45 mg/kg per 12 hours for neonates with 30 $\leq$ GA $\leq$ 34 and GA > 34 weeks, still resulting in plasma concentrations < 140 mg/L in all 150 neonates. However, the exact dosage regimen for treating meningitis still needs to be investigated.

## CONCLUSIONS

We recommend 15 mg/kg per 8 hours for neonates with GA $\leq$ 34 weeks and 20 mg/kg per 8 hours for neonates with GA > 34 weeks. Simulation with this dosage regimen indicated that satisfactory plasma concentrations were reached for all 150 neonates. Therefore, use of therapeutic drug monitoring of amoxicillin and pharmacokinetic calculations for dosage adjustment is generally not necessary.

## REFERENCES

1. Fanos V, Dall'Agnola A. Antibiotics in neonatal infections: a review. *Drugs*. 1999;58:405–427.

2. Charles BG, Preechagoon Y, Lee TC, et al. Population pharmacokinetics of intravenous amoxicillin in very low birth weight infants. *J Pharm Sci*. 1997;86:1288–1292.
3. Huisman-de Boer JJ, Anker van den JN, Vogel M, et al. Amoxicillin pharmacokinetics in preterm infants with gestational ages of less than 32 weeks. *Antimicrob Agents Chemother*. 1995;39:431–434.
4. Autret E, Laugier J, Marimbu J, et al. Comparaison des concentrations plasmatiques d'amoxicilline par voie orale et intraveineuse dans les colonisations bactériennes néonatales. *Arch Fr Pediatr*. 1988;45:679–682.
5. Peskine F, Despaux E, Rodiere M, et al. Étude pharmacocinétique et clinique de l'amoxicilline intra-veineuse chez le nouveau-né. *Pathol Biol (Paris)*. 1982;30:476–480.
6. Shaffer CL, Davey AM, Ransom JL, et al. Ampicillin-induced neurotoxicity in very-low-birth-weight neonates. *Ann Pharmacother*. 1998;32:482–484.
7. Saez-Llorens X, McCracken GH. Clinical pharmacology of antibacterial agents. In: Remington JS, Klein JO, eds. *Infectious Disease of the Fetus, Newborn and Infants*. Philadelphia: Saunders; 1995:1287–1336.
8. Baltimore RS. Neonatal sepsis: epidemiology and management. *Paediatr Drugs*. 2003;5:723–740.
9. Jones DP, Gaber L, Nilsson GR, et al. Acute renal failure following amoxicillin overdose. *Clin Pediatr (Phila)*. 1993;32:735–739.
10. Hoog de M, Mouton JW, Anker van den JN. New dosing strategies for antibacterial agents in the neonate. *Semin Fetal Neonatal Med*. 2005;10:185–194.
11. Wertz AW, Heeren MM. Delivery room resuscitation. In: Pomerance JJ, Richardson CJ, eds. *Neonatology for the Clinician*. Norwalk, CT: Appleton & Lange; 1993:93–121.
12. Stolk LML, Degraeuwe PLJ, Nieman FHM, et al. Population pharmacokinetics and relationship between demographic and clinical variables and pharmacokinetics of gentamicin in neonates. *Ther Drug Monit*. 2002;24:527–531.
13. Hey E, Hall C. *Neonatal Formulary*. 4th ed. London: Blackwell BMJ Books; 2003.
14. Fowlie PW, Davis PG. Prophylactic indomethacin for preterm infants: a systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal Ed*. 2003;88:F464–F466.
15. Dodge WF, Jelliffe RW, Zwischenberger JB, et al. Population pharmacokinetic models: effect of explicit versus assumed constant serum concentration assay error patterns upon parameter values of gentamicin in infants on and off extracorporeal membrane oxygenation. *Ther Drug Monit*. 1994;16:552–559.
16. Proost JH, Meijer DKF. MW\PHARM, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. *Comput Biol Med*. 1992;22:155–163.
17. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Standard M100-S12*. Wayne, Pa: NCCLS; 2002.
18. Koren G, James A, Perlman M. A simple method for the estimation of glomerular filtration rate by gentamicin pharmacokinetics during routine drug monitoring in the newborn. *Clin Pharmacol Ther*. 1985;38:680–685.
19. Morselli PL. Clinical pharmacology of the perinatal period and early infancy. *Clin Pharmacokinet*. 1989;17(Suppl 1):13–28.
20. Guignard JP, Drukker A. Why do newborn infants have a high plasma creatinine? *Pediatrics*. 1999;103:e49.
21. Kaplan JM, McCracken GH, Horton LJ, et al. Pharmacologic studies in neonates given large dosages of ampicillin. *J Pediatr*. 1974;84:571–577.

 

# Amoxicillin Pharmacokinetics in (Preterm) Infants Aged 10 to 52 Days: Effect of Postnatal Age

*J. Pullen, MSc,* M. Driessen, BSc,* L. M. L. Stolk, PhD,* P. L. J. Degraeuwe, MD, PhD,† F. H. van Tiel, PhD,‡ C. Neef, PhD,* and L. J. I. Zimmermann, MD, PhD†*

**Abstract:** The pharmacokinetic parameters of amoxicillin were determined in 32 newborn infants aged 10 to 52 days (mean postnatal age, 24.7 ± 12.4 days) to improve amoxicillin dosing in this age group. Amoxicillin plasma concentrations were determined using reversed-phase high-performance liquid chromatography in surplus plasma samples from routine gentamicin assays. Amoxicillin pharmacokinetic parameters (mean ± SD) were as follows: first-order elimination constant ($K_{el}$) = 0.27 ± 0.10 $h^{-1}$, volume of distribution corrected for body weight (V/W) = 0.66 ± 0.27 L/kg, total body clearance corrected for body weight (CL/W) = 0.18 ± 0.10 $Lkg^{-1}h^{-1}$, and elimination half-life ($t_{½}$) = 3.0 ± 1.3 hours. Amoxicillin body clearance was approximately twofold greater in our patients compared with published values in younger neonates (mean postnatal age, 0.76 ± 1.57 days). Simulation studies using the observed amoxicillin pharmacokinetic data suggest an amoxicillin dose of 40 mg/kg administered every 8 hours in infants older than 9 days postnatal age, independent of gestational age and postconceptional age, to achieve satisfactory target plasma amoxicillin concentrations less than 140 mg/L and time above minimum inhibitory concentration of at least 40%. Prospective evaluation of this suggested new dosage regimen is necessary before implementation in the care of ill neonates.

**Key Words:** pharmacokinetics, amoxicillin, therapeutic drug monitoring, infants, dosage regimen, postnatal age

(*Ther Drug Monit* 2007;29:376–380)

## INTRODUCTION

Amoxicillin is an aminopenicillin frequently used for the treatment of early-onset neonatal sepsis.[1] In an earlier study, we investigated amoxicillin pharmacokinetics, amoxicillin dosing, and the influence of covariates on the individual pharmacokinetic parameters in 150 (pre)term neonates aged 9 days or less (mean postnatal age [PNA], 0.76 ± 1.57 days).[2] Amoxicillin pharmacokinetics in neonates have also been investigated in four smaller studies.[3–6] In these studies, the pharmacokinetic parameters were established in the first week of neonatal life, underscoring the need for such data in older infants.

Postnatal development of renal function in (pre)term infants has been well documented.[7–11] The progressive postnatal increase in glomerular filtration rate (GFR) results mainly from an increase in glomerular perfusion pressure.[12] Because of this postnatal increase in GFR, it is expected that PNA will exert a major effect on the pharmacokinetics of drugs that are mainly eliminated by glomerular filtration. In our previous study, we found a high correlation between amoxicillin clearance and plasma creatinine and gentamicin body clearance, demonstrating the important role of glomerular filtration in the clearance of amoxicillin in neonates.[2] In previous studies, the effect of PNA on the pharmacokinetics of gentamicin,[13] ceftazidime,[14] and piperacillin[15] in neonates has been investigated. However, we were unable to identify any published data describing the pharmacokinetics of amoxicillin in neonates aged more than 9 days and the effect of PNA on amoxicillin pharmacokinetic characteristics [MEDLINE search terms: amoxicillin, amoxycillin, pharmacokinetic(s), pharmacology, postnatal (age), infant(s), newborn(s), neonate(s), and neonatal]. Thus, the main objective of the present study was to define an amoxicillin dosage regimen appropriate for (pre)term infants aged more than 9 days.

## MATERIALS AND METHODS

### Patients, Drug Administration, and Plasma Sampling

The present study was approved by the local Medical Ethical Committee. Thirty-two newborn infants aged more than 9 days, admitted to the neonatal intensive care unit at the University Hospital of Maastricht from June 2002 to March 2006, were identified for inclusion in the study. All infants were treated with amoxicillin in combination with gentamicin for (suspected) infection. Amoxicillin was administered by intravenous slow push according to published dosing guidelines: 100 mg/kg per dose for (suspected) meningitis and 50 mg/kg per dose in other situations.[16] Dosing interval was 12 hours in the first week of life, 8 hours in neonates 1 to 3 weeks old, and 6 hours in neonates 4 weeks or older.[16] Gentamicin (4 mg/kg) was administered intravenously by syringe pump during 30 minutes every 24 hours. Amoxicillin and gentamicin were administered shortly after each other. Most infants (53%) were treated with a combination of amoxicillin, gentamicin, and metronidazol for (suspected) necrotizing enterocolitis. Other indications for amoxicillin use were Gram-positive cocci

Received for publication January 23, 2007; accepted February 9, 2007.
From the *Departments of Clinical Pharmacy and Toxicology; †Paediatrics, Division of Neonatology; and ‡Medical Microbiology, University Hospital of Maastricht, Maastricht, The Netherlands.
Reprints: J. Pullen, MSc, Department of Clinical Pharmacy and Toxicology, University Hospital of Maastricht, PO Box 5800, NL-6202 AZ Maastricht, The Netherlands (e-mail: jpul@kfls.azm.nl).

in liquor (group B *Streptococcus* meningitis), increased C-reactive protein (CRP), and (suspected) urinary tract infection.

Therapeutic drug monitoring of gentamicin was performed on a routine basis. Plasma samples were obtained just before and 1 hour after the start of the infusion of gentamicin. Surplus plasma samples from routine gentamicin assays were collected and stored at −70°C until amoxicillin analysis. We collected 5.2 ± 2.1 plasma samples per infant during 3.5 ± 1.4 days (mean ± SD).

## Amoxicillin Assay

The determination of amoxicillin in plasma was performed using reversed-phase high-performance liquid chromatography with ultraviolet detection, as described previously.[2] With this reversed-phase high-performance liquid chromatography assay, total (free + protein-bound) plasma concentrations of amoxicillin are determined. In five infants, aged 10 to 73 days, we determined the extent of amoxicillin binding to plasma proteins.[17] To determine the free amoxicillin concentration in these five infants, we collected and combined several plasma samples of each infant to obtain a sufficiently large sample for analysis (150–1000 μL). Free amoxicillin was separated from protein-bound amoxicillin using ultrafiltration by a Centrifree micropartition device (Millipore Corporation, Bedford, MA). Fibrin was removed by centrifuging the sample at 4000 rpm for 25 minutes at room temperature before loading into the reservoir. For optimum solvent flow, a 33° fixed-angle centrifuge rotor was used.

## Data Analysis

Amoxicillin pharmacokinetic parameters were estimated from the analysis of the measured amoxicillin plasma concentrations using a one-compartment open model with iterative two-stage Bayesian fitting (MW\PHARM 3.60, Mediware, Groningen, The Netherlands), incorporating previously determined pharmacokinetic parameters[2] as initial parameters. In our previous study, we found a high correlation between amoxicillin clearance and gentamicin clearance. Because plasma creatinine may reflect GFR poorly in the first week(s) of life,[18] gentamicin clearance was also included as a measure of GFR.[19] Therefore, gentamicin pharmacokinetic parameters were also calculated using parameters published by Stolk et al.[20] The influence of covariates on the individual pharmacokinetic parameters was studied by calculating the Pearson correlation coefficients (r) with SPSS 12.0 (Chicago, IL). The covariates were gestational age (GA), PNA, postconceptional age (PCA), body weight (W), sex, multiple pregnancy, 1-minute Apgar score, 5-minute Apgar score, 1/plasma creatinine (1/Cr), plasma urea concentration, CRP, blood culture result, and the pharmacokinetic parameters of gentamicin. One-way analysis of variance was used to test whether the factor variables (sex, pregnancy, and blood culture result) had a significant effect on the pharmacokinetic parameters of amoxicillin.

## Dosage Regimen

The projected bacteriologic efficacy and safety of the amoxicillin dosage regimen used in our 32 infants was assessed. Projected bacteriologic efficacy was determined using standard antimicrobial pharmacokinetic-pharmacodynamic modeling,[21] and safety was projected by calculating the amoxicillin peak plasma concentrations after the second dose, which should not exceed 140 mg/L.[22,23] For bacteriologic efficacy, the amount of time (T) the free amoxicillin plasma concentration must remain above the minimum inhibitory concentration (T > MIC) of important neonatal pathogens (eg, target MIC = 0.25 mg/L for *Streptococcus agalactiae* and 8 mg/L for *Enterococcus* species and species belonging to *Enterobacteriaceae*)[24] should be at least 40% to 50% of the dosing interval for neonates.[25] In considering that amoxicillin plasma protein binding in adults is rather low (<20%)[26] and ampicillin protein binding in neonates averages 9.9% ± 1.5% (range, 8.3–11.5%, n = 4),[27] and our own data (see Results) suggest 11.7%, we elected to assume amoxicillin protein binding for our simulation studies to be 10%. Our target, optimal dosage regimen would result in free amoxicillin plasma concentrations that remain above the MIC for a minimum of at least 40% of the time, and total plasma concentrations had to be below 140 mg/L.

# RESULTS

The clinical characteristics of the infants included in the present study (8 girls and 24 boys; 7 infants were twins) and, for comparative purposes, the data obtained in neonates aged 9 days or less from our previous study[2] are presented in Table 1. The characteristics of both populations were very much alike, except for CRP, and the expected differences in PNA, PCA, and 1/Cr. The lower mean plasma creatinine concentration (45.0 vs. 90.7 μmol/L) probably reflects a higher GFR, and the higher mean CRP (128.0 vs. 14.7 mg/L) may indicate a higher incidence of bacterial infection (Table 1). Seven of 24 (29%) blood cultures were positive: coagulase negative staphylococci, n = 4, *Escherichia coli,* n = 2, and *Streptococcus mitis,* n = 1.

Amoxicillin pharmacokinetic parameters (mean ± SD) were first-order elimination constant ($K_{el}$) = 0.27 ± 0.10 $h^{-1}$, volume of distribution corrected for body weight (V/W) = 0.66 ± 0.27 L/kg, total body clearance corrected for body weight (CL/W) = 0.18 ± 0.10 $Lkg^{-1}h^{-1}$, and elimination half-life ($t_{½}$) = 3.0 ± 1.3 hours. Gentamicin pharmacokinetic parameters (mean ± SD) were $K_{el}$ = 0.11 ± 0.02 $h^{-1}$, V/W = 0.59 ± 0.13 L/kg, CL/W = 0.06 ± 0.02 $Lkg^{-1}h^{-1}$, and $t_{½}$ = 6.8 ± 1.9 hours. The mean (±SD) amoxicillin protein binding in the 5 infants aged 10 to 73 was 11.7% ± 2.7% (range, 8.0–14.1%).

The highest Pearson correlation coefficient was found between amoxicillin clearance and PCA (r = 0.718, $P < 0.001$), which showed a logarithmic rise with PCA (r = 0.717). The correlation between amoxicillin clearance and PCA, on linear and semilogarithmic scales, is shown in Figure 1. Amoxicillin clearance was also significantly related to GA (r = 0.655, $P < 0.001$), 1/Cr (r = 0.552, $P = 0.002$), and gentamicin clearance (r = 0.490, $P = 0.004$). However, no significant relationship was observed between amoxicillin clearance and PNA (r = 0.318, $P = 0.076$). V/W amoxicillin was significantly correlated with GA (r = 0.455, $P = 0.009$), PCA (r = 0.463, $P = 0.008$), and CL/W gentamicin (r = 0.444, $P = 0.011$).

**TABLE 1.** Characteristics of the Infants in the Present Study and our Previous Study[2]

| | | Present Study | | | Previous Study | | |
|---|---|---|---|---|---|---|---|
| | Unit | n | Mean (SD) | Range | n | Mean (SD) | Range |
| GA | weeks | 32 | 33.7 (4.6) | 26.3–41.4 | 150 | 34.6 (4.9) | 24.9–42.4 |
| PNA | days | 32 | 24.7 (12.4) | 10–52 | 150 | 0.76 (1.57) | 0–9 |
| PCA | weeks | 32 | 37.2 (4.9) | 28–46.1 | 150 | 34.7 (4.9) | 24.9–42.6 |
| W | kg | 32 | 2.27 (1.02) | 0.78–4.25 | 150 | 2.29 (1.08) | 0.57–4.7 |
| AP5 | points | 30 | 8 (median) | 4–10 | 148 | 9 (median) | 1–10 |
| 1/Cr | L/μmol | 30 | 0.025 (0.009) | 0.011–0.050 | 101 | 0.012 (0.004) | 0.006–0.036 |
| Ur | mmol/L | 30 | 3.70 (2.06) | 0.6–11.5 | 100 | 5.05 (2.62) | 0.8–13.5 |
| CRP | mg/L | 31 | 128.0 (100.2) | 3.6–343.0 | 141 | 14.7 (23.2) | 1–117 |

AP5, 5-minute Apgar score; Cr, plasma creatinine level; CRP, C-reactive protein; GA, gestational age; PCA, postconceptional age; PNA, postnatal age; Ur, plasma urea concentration; W, body weight.

We could not find a relationship between infection and amoxicillin pharmacokinetics. Furthermore, no relationship or differences were observed between amoxicillin pharmacokinetics and infants suffering from an infectious disease (defined by a positive blood culture) and infants having negative blood cultures. Moreover, no relationship between CRP and amoxicillin pharmacokinetic parameters was observed.

The amoxicillin T > MIC (8 mg/L) was greater than 40% in all 32 infants: T > MIC = 85.4% ± 18.3% (mean ± SD), range 41% to 100%. The average (±SD) peak amoxicillin plasma concentration was 80.1 (±37.9) mg/L with a range of 20.1 to 167.8 mg/L, and the average (±SD) trough amoxicillin plasma concentration was 10.2 (±8.4) mg/L, with a range of 0.6 to 34.8 mg/L. In 3 of the 32 infants, amoxicillin peak plasma concentrations were higher than 140 mg/L (140.6, 158.3, and 167.8 mg/L). Simulation studies using the determined amoxicillin pharmacokinetic parameters to determine the dose to achieve a target T > MIC of at least 40% of the dosing interval and peak amoxicillin concentrations less than 140 mg/L suggest a new dosage regimen of 40 mg amoxicillin per kilogram every 8 hours, independent of GA, PNA, and PCA, for infants aged more than 9 days. On the basis of these simulations, this dosage regimen yielded satisfactory amoxicillin plasma concentrations in all infants with the T > MIC greater than 40% in all 32 infants (mean T > MIC = 87.3% ± 19.1%; range, 43–100%) and greater than 50% in 31 of the 32 infants. Furthermore, the peak amoxicillin plasma concentrations were below 140 mg/L in all 32 infants: 70.0 ± 27.4 (range, 29.7–134.5) mg/L; average trough plasma concentration was 10.8 (±7.6) mg/L, with a range of 1.0 to 32.6 mg/L.

## DISCUSSION

The amoxicillin body clearance in infants aged more than 9 days (mean PNA, 24.7 ± 12.4 days) determined in the present study (0.18 $Lkg^{-1}h^{-1}$) is approximately twofold greater than the amoxicillin body clearance observed in younger neonates (0.10 $Lkg^{-1}h^{-1}$) aged 9 days or less (mean PNA, 0.76 ± 1.57 days). In addition, the amoxicillin $t_{½}$ was much shorter (3.0 hr vs. 5.2 hr), whereas the V/W was approximately the same (0.66 L/kg vs. 0.65 L/kg) in the older and younger infants, respectively.[2] These results confirm our expectations about the effect of PNA on amoxicillin pharmacokinetics. Glomerular filtration and tubular secretion are less mature during the first week of life.[28] Therefore, amoxicillin clearance is considerably reduced in the newborn infant compared with that in older infants.

Amoxicillin clearance showed a logarithmic rise with PCA (Fig. 1). For infants aged more than 9 days, amoxicillin clearance hardly appears to increase until the infant reaches a PCA of 34 weeks. After that, there is a rapid linear increase in amoxicillin clearance with PCA (r = 0.863). This finding is consistent with results in the literature.[11,29] Coulthard[11] reported a logarithmic rise of absolute GFR with PCA, which was independent of PNA, on the basis of his own study in 39 infants (GA, 27–40 wk; PNA, 2–63 days) and 14 other studies.[11] Arant[10] reported a slight increase in GFR before the time an infant reaches a PCA of 34 weeks and a more rapid increase thereafter. A PCA of 34 weeks appears to be the point in renal development from which the absolute GFR increases gradually to mature values.[29] So, GFR and thus amoxicillin clearance are dependent upon the infant's PCA, which differs between our first study (34.7 wk) and the present study (37.2 wk). This may help to explain the difference in amoxicillin clearance observed between the two studies.

Amoxicillin clearance was also highly related to GA (r = 0.655). In our previous study, we found the same relationship between amoxicillin clearance and GA (r = 0.656), which probably reflects the development of renal function.[2] In the present study, too, we found a relationship between amoxicillin clearance and the predictors of GFR, 1/Cr (r = 0.552 versus r = 0.521), and gentamicin clearance (r = 0.490 versus r = 0.651), indicating the important role of glomerular filtration in the clearance of amoxicillin in (pre)term infants.[2]

The amoxicillin body clearance observed in our 32 infants was, on average, three times higher than the observed gentamicin clearance. This observation indicates the additive role of tubular secretion in amoxicillin clearance because gentamicin is completely cleared by glomerular filtration.[19] In younger infants, amoxicillin body clearance exceeded the gentamicin clearance by approximately twofold[2] reflecting the dependence upon age for the maturation of the proximal renal tubular weak acid transport system.

Dose simulation assessments incorporating the amoxicillin pharmacokinetic data determined in the present study to

 

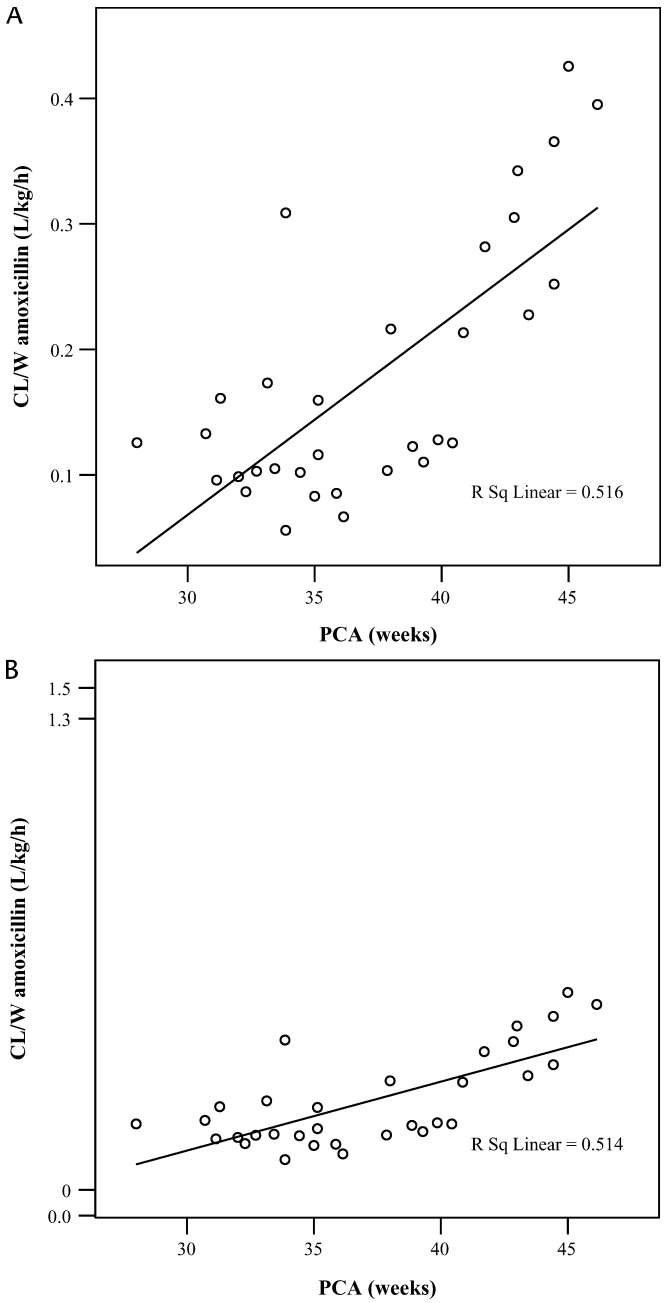


**FIGURE 1.** Correlation between total body clearance of amoxicillin corrected for body weight (CL/W amoxicillin) and postconceptional age (PCA) on linear scale (A) and semilogarithmic scale (B).

achieve specific targets, that is, T > MIC of at least 40% (MIC = 8 mg/L) and peak amoxicillin concentrations less than 140 mg/L, suggest an amoxicillin dose of 40 mg/kg administered every 8 hours in neonates more than 9 days of age, independent of GA and PCA. It should be noted that this dosage regimen will result in plasma concentrations sufficiently high only for the treatment of infections that are easily reached by beta-lactam antibiotics, such as bacteriemia, urinary tract infection, and intravascular catheter-related infection, but not for meningitis. Last, when performing simulation assessments with expected free amoxicillin concentrations, as we did in this study, it might be possible that higher T > MIC values (eg, T > MIC 60%) are necessary for optimal treatment of neonates who suffer from age-dependent deficiencies in immunologic functions. However, prospective validation of this new dosage regimen is necessary.

## CONCLUSIONS

Postnatal alterations in amoxicillin pharmacokinetics, particularly an increase in amoxicillin body clearance, require adjustment of the dosage regimen after the first week of life. We suggest an amoxicillin dose of 40 mg/kg every 8 hours for infants aged more than 9 days. The validity of this projected dose regimen must be evaluated before routine implementation in the care of older neonates.

## REFERENCES

1. Fanos V, Dall'Agnola A. Antibiotics in neonatal infections: a review. *Drugs*. 1999;58:405–427.
2. Pullen J, Stolk LML, Nieman FHM, et al. Population pharmacokinetics and dosing of amoxicillin in (pre)term neonates. *Ther Drug Monit*. 2006; 28:226–231.
3. Huisman-de Boer JJ, Anker van den JN, Vogel M, et al. Amoxicillin pharmacokinetics in preterm infants with gestational ages of less than 32 weeks. *Antimicrob Agents Chemother*. 1995;39:431–434.
4. Peskine F, Despaux E, Rodiere M, et al. Étude pharmacocinétique et clinique de l'amoxicilline intra-veineuse chez le nouveau-né. *Pathol Biol (Paris)*. 1982;30:476–480.
5. Charles BG, Preechagoon Y, Lee TC, et al. Population pharmacokinetics of intravenous amoxicillin in very low birth weight infants. *J Pharm Sci*. 1997;86:1288–1292.
6. Autret E, Laugier J, Marimbu J, et al. Comparaison des concentrations plasmatiques d'amoxicilline par voie orale et intraveineuse dans les colonisations bactériennes néonatales. *Arch Fr Pediatr*. 1988;45:679–682.
7. Aperia A, Broberger O, Elinder G, et al. Postnatal development of renal function in pre-term and full-term infants. *Acta Paediatr Scand*. 1981;70: 183–187.
8. Heijden van der AJ, Grose WF, Ambagtsheer JJ, et al. Glomerular filtration rate in the preterm infant: the relation to gestational and postnatal age. *Eur J Pediatr*. 1988;148:24–28.
9. Fawer CL, Torrado A, Guignard JP. Maturation of renal function in full-term and premature neonates. *Helv Paediatr Acta*. 1979;34:11–21.
10. Arant BS. Developmental patterns of renal functional maturation compared in the human neonate. *J Pediatr*. 1978;92:705–712.
11. Coulthard MG. Maturation of glomerular filtration in preterm and mature babies. *Early Hum Dev*. 1985;11:281–292.
12. Jose PA, Fildes RD, Gomez RA, et al. Neonatal renal function and physiology. *Curr Opin Pediatr*. 1994;6:172–177.
13. Knight JA, Davis EM, Manouilov K, et al. The effect of postnatal age on gentamicin pharmacokinetics in neonates. *Pharmacotherapy*. 2003;23: 992–996.
14. Anker van den JN, Hop WC, Schoemaker RC, et al. Ceftazidime pharmacokinetics in preterm infants: effect of postnatal age and postnatal exposure to indomethacin. *Br J Clin Pharmacol*. 1995;40:439–443.
15. Kacet N, Roussel-Delvallez M, Gremillet C, et al. Pharmacokinetic study of piperacillin in newborns relating to gestational an postnatal age. *Pediatr Infect Dis J*. 1992;11:365–369.
16. Hey E, Hall C. *Neonatal Formulary*, ed 4. London: Blackwell BMJ Books, 2003.
17. Notarianni LJ. Plasma protein binding of drugs in pregnancy and in neonates. *Clin Pharmacokinet*. 1990;18:20–36.
18. Guignard JP, Drukker A. Why do newborn infants have a high plasma creatinine? *Pediatrics*. 1999;103:e49.

19. Koren G, James A, Perlman M. A simple method for the estimation of glomerular filtration rate by gentamicin pharmacokinetics during routine drug monitoring in the newborn. *Clin Pharmacol Ther*. 1985;38: 680–685.
20. Stolk LM, Degraeuwe PL, Nieman FH, et al. Population pharmacokinetics and relationship between demographic and clinical variables and pharmacokinetics of gentamicin in neonates. *Ther Drug Monit*. 2002; 24:527–531.
21. Andes D, Anon J, Jacobs MR, et al. Application of pharmacokinetics and pharmacodynamics to antimicrobial therapy of respiratory tract infections. *Clin Lab Med*. 2004;24:477–502.
22. Shaffer CL, Davey AM, Ransom JL, et al. Ampicillin-induced neurotoxicity in very-low-birth-weight neonates. *Ann Pharmacother*. 1998;32: 482–484.
23. Hodgman T, Dasta JF, Armstrong DK, et al. Ampicillin-associated seizures. *South Med J*. 1984;77:1323–1325.
24. National Committee for Clinical Laboratory Standards. *Performance Standards forAntimicrobial Susceptibility Testing; Twelfth Informational Standard M100-S12*. Wayne, PA: NCCLS, 2002.
25. Hoog de M, Mouton JW, Anker van den JN. New dosing strategies for antibacterial agents in the neonate. *Semin Fetal Neonatal Med*. 2005;10: 185–194.
26. Working Party of the British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother*. 1991;27(Suppl D): 1–50.
27. Ehrnebo M, Agurell S, Jalling B, et al. Age differences in drug binding by plasma proteins: studies on human foetuses, neonates and adults. *Eur J Clin Pharmacol*. 1971;3:189–193.
28. Heimann G. Drug disposition during the perinatal period. *Int J Biol Res Pregnancy*. 1981;2:1–14.
29. Arant BS. Postnatal development of renal function during the first year of life. *Pediatr Nephrol*. 1987;1:308–313.

 

# Population Pharmacokinetics and Dosing of Flucloxacillin in Preterm and Term Neonates

*Joyce Pullen, MSc,* Lindsay de Rozario, MSc,* Leo M. L. Stolk, PhD,**
*Pieter L. J. Degraeuwe, PhD,† Frank H. van Tiel, PhD,‡ and Luc J. I. Zimmermann, PhD†*

**Abstract:** In total 235 flucloxacillin total (free + protein bound) plasma concentrations were determined in 55 neonates (gestational age 26 to 42 weeks, postnatal age 0 to 44 days) with reversed-phase HPLC in surplus plasma samples from routine gentamicin assays. Population pharmacokinetic parameters were calculated according to an one compartment open model with iterative two-stage Bayesian fitting (MW\PHARM 3.50, Mediware, The Netherlands). Mean clearance corrected for weight was $0.18 \pm 0.10\,L\,kg^{-1}h^{-1}$ and volume of distribution corrected for weight was $0.54 \pm 0.17\,L/kg$. Pearson correlations between the individual pharmacokinetic parameters and covariates, like gestational age, plasma creatinine, and gentamicin clearance, were low and therefore not relevant for use in clinical practice. Total plasma concentrations above 200 mg/L were considered toxic and $T >$ MIC (time above minimum inhibitory free plasma concentration) of more than 40% was considered effective. Protein binding was assumed to be 86.3% in all neonates, based on literature. The current dosage regimen, 25 or 50 mg/kg every 8 or 12 hours, did not result in effective plasma concentrations for the treatment of *Staphylococcus aureus* in 17 (31%) of the 55 neonates. Therefore, the authors suggest an initial dose of 25 mg/kg/4 h for all neonates, irrespective of their age, based on the breakpoint MIC value of flucloxacillin for *Staphylococcus aureus* (2.0 mg/L). After isolation of the causative agent of infection, flucloxacillin administration ought to be reconsidered based on the expected susceptibility pattern of the isolate. When oxacillin sensitive coagulase negative staphylococci are isolated, the initial dose should be reduced to 10 mg/kg/6 h, based on the breakpoint MIC value of 0.25 mg/L. Simulation with these new dosage regimens indicated that satisfactory plasma concentrations were reached in 52 of the 55 neonates. However, the regimens need prospective verification. Moreover, the exact role of neonatal protein binding needs to be further investigated.

**Key Words:** population pharmacokinetics, flucloxacillin, dosage regimen, neonates

(*Ther Drug Monit* 2006;28:351–358)

Received for publication November 15, 2005; accepted March 28, 2006.
From the Departments of *Clinical Pharmacy and Toxicology, †Paediatrics, Division of Neonatology, and ‡Medical Microbiology, University Hospital of Maastricht, The Netherlands.
Reprints: J. Pullen, MSc, Department of Clinical Pharmacy and Toxicology, University Hospital of Maastricht, PO Box 5800, NL-6202 AZ Maastricht, The Netherlands (e-mail: jpul@kfls.azm.nl).


Late-onset neonatal sepsis is relatively common in hospitalized neonates, especially in those who are extremely premature. Preterm infants frequently need invasive interventions, such as prolonged ventilation and vascular access, which may increase the risk of infection.[1] Most late-onset infections are caused by Gram positive organisms. Coagulase negative staphylococci (CoNS) account for half of all late-onset infections.[1] It is not clear which antibiotic regimen is most suitable for initial treatment of suspected late-onset sepsis.[2] Flucloxacillin, a narrow spectrum penicillin, in combination with gentamicin, an aminoglycoside, is the drug of choice in the vast majority in Dutch hospitals.[3,4] In contrast to gentamicin, therapeutic drug monitoring (TDM) of flucloxacillin is not performed on a routine basis.

Data concerning population pharmacokinetics of flucloxacillin in adults are limited to a few studies.[5–8] Considering the large differences in body composition and metabolic functions between neonates and adults, pharmacokinetic data from studies in adults cannot be applied to newborn infants. In a small study flucloxacillin pharmacokinetics in nine newborn infants of various gestational (mean 36.6 weeks) and chronological (mean 7.2 days) ages were investigated.[9] Flucloxacillin pharmacokinetics were also investigated in another study with eleven neonates aged between 3 and 60 days undergoing cardiopulmonary bypass surgery.[10] The elimination half-life was considerably longer, the total body clearance was much lower, and the apparent volumes of distribution in the central and peripheral compartments were much larger than in healthy adults.

The present study was designed to investigate the efficacy of the current dosage regimen of flucloxacillin and to develop a better dosage regimen if necessary. Therefore, flucloxacillin plasma concentrations of a large population of 55 neonates were determined with a specific HPLC method, the population pharmacokinetic parameters of flucloxacillin were calculated, and demographic, anthropometric, and clinical covariates, that may influence the individual pharmacokinetic parameters of flucloxacillin, were investigated for significance.

## MATERIALS AND METHODS

### Patients

The study was approved by the local Ethical Board. Fifty-five (pre)term neonates, admitted to the neonatal

ward at the University Hospital of Maastricht from May 2002 through March 2005, participated in the study. Inclusion criteria were: coadministration of flucloxacillin and gentamicin, postnatal age (PNA) $\leq 2$ months, availability of reliable patient data with respect to gestational age (GA), PNA, dosing and sampling times, and availability of at least one plasma sample taken shortly before the administration of gentamicin and flucloxacillin (trough concentration) and one plasma sample taken 1 hour after the administration (peak concentration).

For each patient, the following demographic, anthropometric, and clinical characteristics were recorded: GA, PNA, postconceptional age (PCA), birth weight (BW), body weight (W), gender, multiple pregnancy (P), arterial cord pH (ApH), 1-min Apgar score (AP1), 5-min Apgar score (AP5), divided into two groups ($< 7$ and $\geq 7$), 1/plasma creatinine (1/Cr), plasma urea concentration (Ur), blood culture result (BC), and C-reactive protein (CRP). Because literature[11] indicates that plasma creatinine in the first week of life does not reflect glomerular filtration rate (GFR) of the neonate, data on gentamicin clearance were also collected. Gentamicin clearance as an estimate of GFR in neonates has been suggested previously.[12] The following pharmacokinetic parameters of gentamicin were calculated: total body clearance corrected for body weight (CL/W gentamicin), elimination half-life ($t1/2$ gentamicin), and volume of distribution corrected for body weight (V/W gentamicin).

## Drug Administration and Plasma Sampling

Gentamicin dosage regimen was not 5.0, 4.5, and 4.0 mg/kg every 24 h, but gentamicin dosage regimen was 5.0 mg/kg/48 h, 4.5 mg/kg/36 h, and 4.0 mg/kg/24 h for neonates in the first week of life with GA $< 30$, $30 \leq$ GA $\leq 34$, and GA $> 34$ weeks, respectively.[13] Neonates older than one week received 4 mg/kg/24 h.[14] Gentamicin was administered intravenously by syringe pump over 30 min. The flucloxacillin dosage regimen, 25–50 mg/kg every 8 or 12 hours, depending on GA and PNA, was based on dosing guidelines in literature.[4,15] Flucloxacillin was administered by intravenous slow push (0.1 hours). Usually, flucloxacillin and gentamicin were administered shortly after each other.

Plasma samples were taken just before and 1 hour after the infusion of gentamicin. Dosing and sampling times were recorded by nursing staff. Surplus plasma samples from routine gentamicin assays were stored at $-70^{\circ}$C.

## Chemicals

Acetonitrile ($CH_3CN$, CAS 75-05-8), citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$, CAS 5949-29-1), methylene chloride ($CH_2Cl_2$, CAS 75-09-2), hydrochloric acid (HCl, CAS 7647-01-0), and sodium perchlorate ($NaClO_4 \cdot H_2O$, CAS 7601-89-0) were obtained from Merck (Darmstadt, Germany). Flucloxacillin ($C_{19}H_{16}ClFN_3NaO_5S \cdot H_2O$, CAS 1847-24-1) was provided by Synopharm (Barsbüttel, Germany) and the internal standard cloxacillin ($C_{19}H_{18}ClN_3O_5S$, CAS 61-72-3) was obtained from SmithKline Beecham Pharmaceutical (Middlesex, United Kingdom). Ultrapure water was used in all preparations. All chemicals were of analytical grade. Human EDTA plasma for standard and control samples was provided by the Red Cross Bloodbank (Maastricht, The Netherlands).

## Analytical Techniques

Gentamicin plasma concentrations were measured with the COBAS INTEGRA 700 analyzer (Roche, Mannheim, Germany), using the principle of fluorescence polarization. The error pattern of the gentamicin assay was: $SD = 0.0129 + 0.0029\ C + 0.0034\ C^2$. The assay error pattern was determined according to a method described in literature.[16] The lower limit of determination was 0.1 mg/L. Total (free + protein bound) flucloxacillin concentrations were determined with reversed-phase HPLC in surplus plasma from routine gentamicin assays. The HPLC assay method was based on a method described by Thijssen.[17] An automatic sample injector (model 717, Waters, USA), a pump (model 1050, Hewlett Packard, USA), a C8 column, $250 \times 4.6$ mm, 5 µm (Beckman, USA), combined with a C8 guard column, and an UV spectrophotometer detector (model 486, Waters, USA) were used. The eluent was composed of 0.02 mol/L sodium perchlorate in water and acetonitrile (750:250). The separations were carried out at room temperature and at a flow rate of 2.0 mL/min. The detection occurred at a wavelength of 220 nm. The error pattern of the assay was: $SD = 0.5117 + 0.0108\ C + 0.0002\ C^2$. The lower limit of determination was 3 mg/L.

To 20 µL surplus plasma, consecutively 20 µL 40 mg/L cloxacillin internal standard solution, 40 µL 0.5 mol/L citric acid monohydrate, 10 µL 0.5 mol/L HCl, and 1 mL methylene chloride were added. The solution was extracted for 2 minutes and centrifuged at 1780*g* for 5 minutes. The top water layer was removed with vacuum and the organic layer containing flucloxacillin was centrifuged at 1780*g* for 5 minutes. The organic layer was evaporated with nitrogen at room temperature. After evaporation, 100 µL eluent was added and the solution was mixed for 60 s. This solution (25 µL) was injected into the HPLC. Standard solutions, containing 10, 25, 50, 75, and 100 mg/L flucloxacillin, were freshly prepared on the day of analysis. The standard plasma samples were processed in the same way as the surplus plasma samples. A calibration curve was constructed and total flucloxacillin concentrations in surplus plasma samples were calculated. Plasma samples containing more than 100 mg/L flucloxacillin were diluted two times with blank plasma. Control plasma samples, containing 25 mg/L flucloxacillin, were prepared and stored in separate containers at $-70^{\circ}$C until analysis. Two control plasma samples were measured in each run.

## Validation

The HPLC assay method was validated on linearity, precision, accuracy, and extraction recovery.

The correlation coefficient of the calibration curves (n = 6) was > 0.99. Interday precision and accuracy, measured at 10, 25, 50, 75, and 100 mg/L (n = 6), were < 10% and < 5%, respectively. Extraction recovery, measured at 50 and 100 mg/L, was 100%.

Flucloxacillin stability in plasma samples during storage at −70°C was determined by analyzing the control plasma samples stored at −70°C over a period of 30 days at day 3, 7, and 30. We did not observe decrease of flucloxacillin concentration. In each run, six standard plasma samples, two control plasma samples, and six surplus plasma samples, if possible in duplicate, were included. The duration of each run was about three hours. Flucloxacillin is reported to be stable at 4°C and at room temperature for up to 24 hours.[18]

## Data Analysis

Population pharmacokinetic parameters of flucloxacillin and gentamicin were calculated according to an one compartment open model with an iterative two-stage Bayesian fitting procedure (IT2B) (MW\PHARM 3.50, Mediware, The Netherlands). Individual pharmacokinetic parameters were calculated by maximum a posteriori Bayesian fitting (MW\PHARM 3.50). The Bayesian fitting procedure uses measured drug concentrations, population-based pharmacokinetic parameters, and the expected variability associated with each measurement to determine individualized pharmacokinetic parameters of a patient.[19] The initial pharmacokinetic parameters of flucloxacillin were derived from Herngren et al[9]: $K_{el}$ $0.15 \pm 0.05\,h^{-1}$ and V/W $0.28 \pm 0.05$ L/kg. The initial pharmacokinetic parameters of gentamicin were derived from the study of Stolk et al[13] The population pharmacokinetic model was validated with Monte Carlo analysis.[20,21] With the MW\PHARM program, 100 sets of 55 simulated patients were generated, based on our own original 55 patients. The individual and population pharmacokinetic parameters of these 5500 simulated patients were calculated with iterative two-stage Bayesian fitting. The calculated individual pharmacokinetic parameters were compared with the "real" (generated) individual pharmacokinetic parameters and the bias (mean error) and the root mean square error (RMSE) were determined. The smaller the bias and the RMSE, the better the performance of the model. Also, the calculated population pharmacokinetic parameters of the 5500 simulated patients were compared with the original population pharmacokinetic parameters.

Statistical analysis was started by checking CL/W and V/W of flucloxacillin for normal distribution with the Kolmogorov-Smirnov test. One-Way Analysis of Variance was used to test if the factor variables (gender, P, AP5, and BC) had a significant effect on CL/W and V/W of flucloxacillin. Pearson product-moment correlation coefficients ($r$) between pharmacokinetic parameters of flucloxacillin and covariates were calculated. Multiple regression analysis with forward selection and backward elimination techniques was done with CL/W and V/W of flucloxacillin as dependent variables. The significant variables, detected with correlation analysis, were used as possible predictors. The significant predictors of the dependent variable were used to establish a direct-effects model. First-order interaction effects between the significant predictors were tested for significance by forced entering within a hierarchically constructed regression model. Residual analysis was performed to check the studentized residuals of the dependent variable for normality and outliers. Discrete factors were entered as dummy variables. Listwise deletion of missing cases was used during the entire regression analysis procedure. Statistical analysis was performed using SPSS 12.0 (Chicago, IL). A $P$ value of $< 0.05$ was considered to be statistically significant.

## Evaluation of Current and Proposed Dosage Regimen

The efficacy of penicillins, including flucloxacillin, depends on the time the free (not protein bound) plasma concentration exceeds the minimum inhibitory concentration ($T > MIC$).[22] In neonates, $T > MIC$ should be at least 40%.[22] The breakpoint MIC value of an antibiotic defines the highest MIC value still to be interpreted as indicating susceptibility to that antibiotic. In neonates, flucloxacillin is frequently used for the treatment of infections caused by CoNS, including *Staphylococcus epidermidis*, and less frequently by *Staphylococcus aureus*. For these two bacterial species, the breakpoint MIC values of flucloxacillin are 0.25 and 2.0 mg/L, respectively.[23] Therefore, when treating staphylococcal infections, plasma concentrations of free flucloxacillin should exceed 0.25 or 2.0 mg/L, depending on the species determination of the blood isolate, during at least 40% of the time. To evaluate the efficacy of the current dosage regimen, we calculated $T > MIC_{2.0}$ and $T > MIC_{0.25}$ for all patients, using their individual pharmacokinetic parameters. The expected level of free flucloxacillin activity was calculated, assuming that our neonates had levels of protein binding of 86.3%, according to Herngren et al.[9]

High intravenous doses of isoxazolyl penicillins have been considered to increase the risk of kernicterus in neonates due to displacement of bilirubin at plasma levels above 200 mg/L.[9,24] According to Rolinson et al,[25] saturation of albumin with penicillins in adults occurs at plasma concentrations of approximately 200 mg/L, when a substantial proportion of available sites on the albumin molecules become occupied by antibiotic molecules. This situation may result in displacement of bilirubin. Therefore, total flucloxacillin plasma concentrations should not exceed the possibly toxic limit of 200 mg/L. To evaluate possible toxicity of the current dosage regimen, we calculated flucloxacillin peak concentrations after the second dose of all patients, using their individual pharmacokinetic parameters.

Flucloxacillin plasma concentrations, reached with the proposed dosage regimen, were simulated in MW\PHARM 3.50 for all neonates, using their individual pharmacokinetic parameters.

**TABLE 1.** Demographic, Anthropometric, and Clinical Characteristics of the Study Patients

| | Unit | N | Mean (SD) | Range |
|---|---|---|---|---|
| GA | wk | 55 | 31.2 (4.1) | 26.0 to 42.0 |
| PNA | d | 55 | 11.1 (9.3) | 0 to 44 |
| PCA | wk | 55 | 32.7 (4.2) | 27.1 to 42.9 |
| BW | kg | 55 | 1.39 (8.60) | 0.37 to 4.06 |
| W | kg | 55 | 1.49 (0.82) | 0.43 to 4.11 |
| ApH | | 36 | 7.19 (0.15) | 6.74 to 7.46 |
| AP1 | Points | 55 | 7 (median) | 0 to 10 |
| AP5 | Points | 55 | 9 (median) | 0 to 10 |
| Cr | μmol/L | 43 | 64.5 (23.9) | 21 to 115 |
| Ur | mmol/L | 40 | 4.01 (2.45) | 0.10 to 10.00 |
| CRP | mg/L | 55 | 40.0 (49.0) | 1.0 to 234.0 |
| CL/W gentamicin | L/kg/h | 55 | 0.057 (0.016) | 0.029 to 0.096 |
| $t_{1/2}$ gentamicin | h | 55 | 9.1 (3.5) | 5.0 to 19.8 |
| V/W gentamicin | L/kg | 55 | 0.69 (0.16) | 0.43 to 1.18 |

AP1 indicates 1-min Apgar score; AP5, 5-min Apgar score; ApH, arterial cord pH; BW, birth weight; CL, total body clearance; Cr, plasma creatinine level; CRP, C-reactive protein; GA, gestational age; PCA, postconceptional age; PNA, postnatal age; $t_{1/2}$, elimination half-life; Ur, plasma urea concentration; V, volume of distribution; W, body weight.

## RESULTS

### Patients

Demographic, anthropometric, and clinical characteristics of the population are presented in Table 1. The population consisted of 21 (38.2%) girls and 34 (61.8%) boys. Forty-four (80%) neonates were products of singleton pregnancies and 11 (20%) neonates of twin pregnancies. Eighteen (33.3%) out of 54 blood cultures (1 missing) were positive. Only staphylococcus species were isolated: CoNS (88.9%), including *S. epidermidis* (25%), and *S. aureus* (11.1%).

### Population Pharmacokinetic Parameters

In total 235 flucloxacillin plasma concentrations (mean 4.3 ± 1.4 per patient) and 267 gentamicin plasma concentrations (4.9 ± 1.4 per patient) were measured. An example of a modelled concentration-time profile of a patient with iterative two-stage Bayesian fitting is shown in Figure 1. A plot of the actual measured concentrations versus the estimated concentrations with iterative two-stage Bayesian fitting is presented in Figure 2.

Mean total body clearance of flucloxacillin corrected for body weight (CL/W) was $0.18 \pm 0.10\,\mathrm{L\,kg^{-1}h^{-1}}$, mean flucloxacillin elimination half-life ($t_{1/2}$) was 2.6 ± 1.6 hours, and mean volume of distribution of flucloxacillin corrected for body weight (V/W) was 0.54 ± 0.17 L/kg. Mean population pharmacokinetic parameters of gentamicin were: CL/W $0.057 \pm 0.016\,\mathrm{L\,kg^{-1}h^{-1}}$, $t_{1/2}$ 9.1 ± 3.5 h, and V/W 0.69 ± 0.16 L/kg.

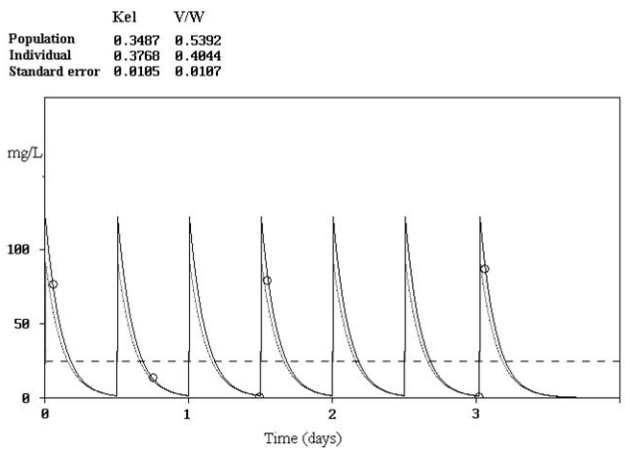


**FIGURE 1.** Example of a modelled concentration-time profile of a patient with iterative two-stage Bayesian fitting (MW\PHARM 3.50, Mediware, The Netherlands).

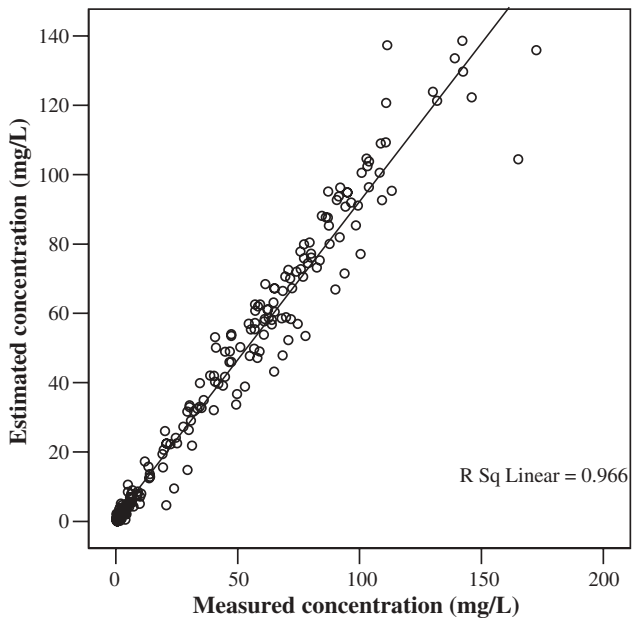


**FIGURE 2.** Correlation between actual measured concentration (mg/L) and estimated concentration (mg/L) with iterative two-stage Bayesian fitting (MW\PHARM 3.50, Mediware, The Netherlands).

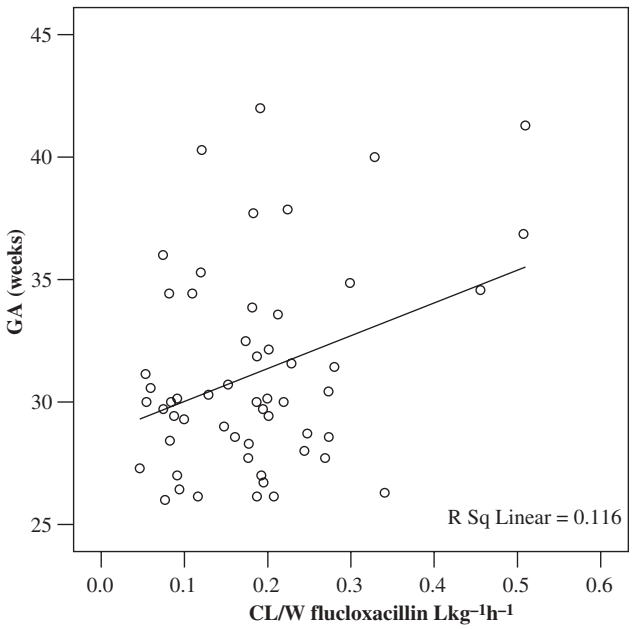


**FIGURE 3.** Correlation between total body clearance of flucloxacillin corrected for body weight (CL/W) ($L\,kg^{-1}\,h^{-1}$) and gestational age (GA) (weeks).

The population pharmacokinetic parameters of flucloxacillin estimated with Monte Carlo analysis ($K_{el}$ $0.3417 \pm 0.1559\,h^{-1}$ and V/W $0.5486 \pm 0.1735$ L/kg) were almost identical to the population pharmacokinetic parameters of our original population ($K_{el}$ $0.3486 \pm 0.1662\,h^{-1}$ and V/W $0.5392 \pm 0.1698$ L/kg). The bias and the RMSE of the Monte Carlo analysis were low, respectively, $-2.2\%$ and 3.1% for $K_{el}$ flucloxacillin and 0.9% and 1.5% for V/W flucloxacillin.

## Statistical Analysis

Few statistically significant Pearson correlations were found between covariates and pharmacokinetic parameters. CL/W flucloxacillin correlated significantly with the covariates GA ($r = 0.340$, $P = 0.011$) and PCA ($r = 0.319$, $P = 0.018$). The correlation between CL/W flucloxacillin and GA is shown in Figure 3. V/W flucloxacillin correlated significantly with the covariates V/W gentamicin ($r = 0.397$, $P = 0.003$) and AP1 ($r = -0.266$, $P = 0.049$). Multiple regression analysis was done with CL/W flucloxacillin (normally distributed) as dependent variable and GA and PCA as possible predictors and with V/W flucloxacillin as dependent variable and V/W gentamicin and AP1 as possible predictors. GA was a significant predictor of CL/W flucloxacillin ($r^2 = 0.116$, n = 55), and V/W gentamicin was a significant predictor of V/W flucloxacillin ($r^2 = 0.158$, n = 55). The standardized studentized residuals of all models seemed to be normally distributed.

## Evaluation of Current and Proposed Dosage Regimen

Flucloxacillin peak and trough concentrations after the second dose and $T > MIC_{0.25}$ and $T > MIC_{2.0}$ of each patient are shown in Table 2. With the current dosage regimen, $T > MIC_{0.25}$ was much higher than 40% in all neonates, except one (36%). $T > MIC_{2.0}$ was lower than 40% in 17 (31%) of the 55 neonates. None of the neonates had peak concentrations above 200 mg/L. Thus, the current dosage regimen resulted in ineffective plasma concentrations in several patients, especially for the treatment of *S. aureus*. *S. aureus* is a less common, but virulent pathogen isolated in late-onset infections. Therefore, the initial flucloxacillin dose should result in free plasma concentrations exceeding 2.0 mg/L during at least 40% of the time. We suggest an initial dose of 25 mg/kg/4 h. After isolation of the causative agent of infection, the initial dose should be either reduced to 10 mg/kg/6 h, when CoNS are isolated, or maintained, when *S. aureus* is isolated.

With the suggested initial dose of 25 mg/kg/4 h, simulated peak concentrations did not exceed 200 mg/L: $76.4 \pm 31.1$ mg/L (mean ± SD) with a range of 31.8 to 161.2 mg/L (Fig. 4). Simulated $T > MIC_{2.0}$ was at least 40% in 52 neonates with a mean of $86.3 \pm 19.5\%$ (mean ± SD) with a range of 29% to 100% (Fig. 4). With the dose of 10 mg/kg/6 h, simulated peak concentrations were all < 200 mg/L with a mean of $25.2 \pm 8.8$ mg/L (range 11.8 to 48.8 mg/L). Simulated $T > MIC_{0.25}$ was satisfactory ( > 40%) in all neonates with a mean of $94.1 \pm 12.7\%$ (range 52% to 100%).

**TABLE 2.** Flucloxacillin Peak and Trough Concentrations After the Second Dose and the Time the Free Concentration Exceeds the Minimum Inhibitory Concentration of 0.25 ($T>MIC_{0.25}$) and 2.0 mg/L ($T>MIC_{2.0}$)

| Dose (mg/kg) | Interval (h) | No. Patients | Trough (mg/L) (SD) | Peak (mg/L) (SD) | $T > MIC_{0.25}$(%) (SD) | $T > MIC_{2.0}$(%) (SD) |
|---|---|---|---|---|---|---|
| 25 | 12 | 4 | 1.4 (2.2)<br>0.4 (0.1/3.6)* | 38.4 (5.5) | 66.0 (26.7) | 24.5 (16.2) |
| 35 | 8 | 1 | 0.3 | 82.0 | 70 | 33 |
| 35 | 12 | 2 | 0.8 (0.1) | 70.9 (19.2) | 76.5 (2.1) | 34.0 (5.7) |
| 50 | 8 | 9 | 10.8 (16.9)<br>3.8 (1.5/14.6)* | 114.4 (38.8) | 94.9 (12.6) | 64.1 (28.6) |
| 50 | 12 | 39 | 8.0 (11.2)<br>2.2 (0.9/13.3)* | 106.3 (31.0) | 91.4 (12.8) | 59.3 (26.0) |

*Median (percentile 25/75).

## DISCUSSION

### Population Pharmacokinetic Parameters

We found a high interindividual variability in the pharmacokinetic parameters. CL/W flucloxacillin was about three times higher than CL/W gentamicin in our neonates. This suggests the presence of other elimination pathways in addition to glomerular filtration, such as tubular secretion and nonrenal clearance (metabolism), because gentamicin is completely cleared by glomerular filtration.[12] However, tubular secretion is believed to be very inefficient in neonates.[26] For isoxazolyl penicillins, including flucloxacillin, about 30% to 40% of their total plasma clearance in healthy adults is by a nonrenal route.[27,28] The formation of inactive penicilloic acids seems to be the main route by which these penicillins are metabolized.[29] In addition, as the result of other metabolic pathways, active metabolites are formed.[30] Because the formation of active metabolites is small (about 10% in adults) and their antibacterial activities are of the same order of magnitude as the parent compounds, it is not to be expected that their formation will have any influence on the outcome of therapy.[30]

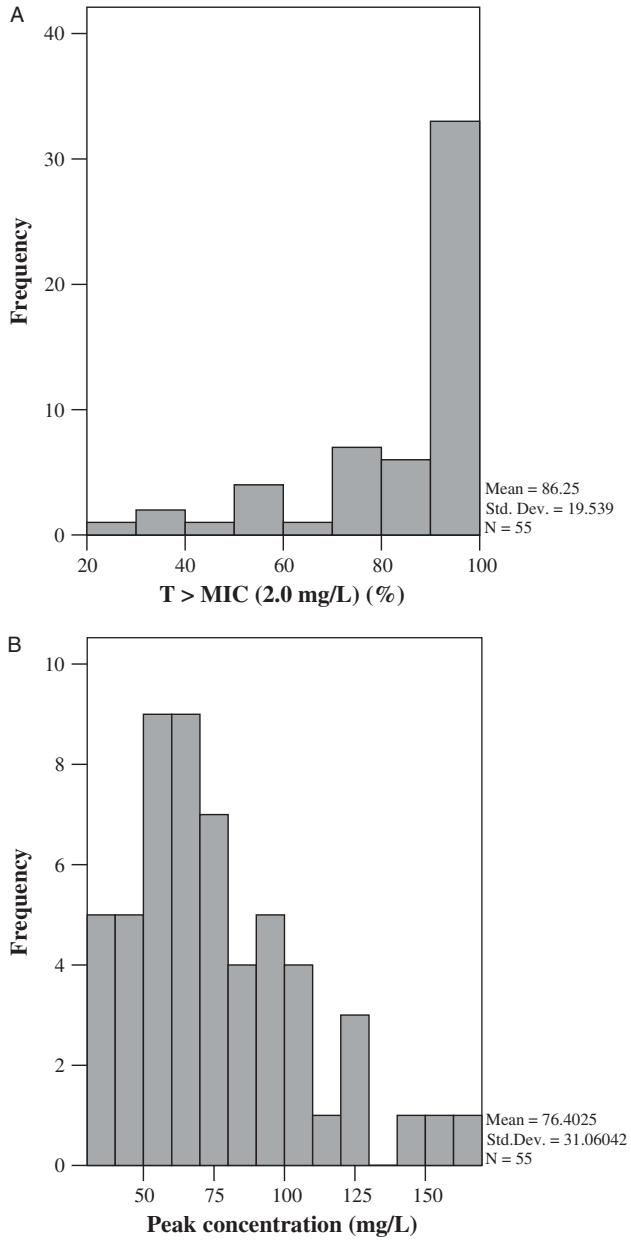


**FIGURE 4.** Histogram at the time above the minimum inhibitory concentration (*T*>MIC) of 2.0 mg/L (%) (A) and histogram at the peak concentration (mg/L) (B) with the proposed dosage regimen (25 mg/kg/4 h).

The population pharmacokinetic parameters of flucloxacillin in the present study differ from the population pharmacokinetic parameters found by Herngren et al.[9] CL/W (0.04 L $kg^{-1}h^{-1}$) and V/W (0.28 L/kg) were low and $t_{1/2}$ (4.6 h) was high compared with our results. These differences may be attributable to their relatively small study population (n = 9) and the use of the method of disk diffusion in nutrient agar instead of the more specific HPLC method. The population pharmacokinetic parameters found by Adrianzén Vargas et al[10] were: CL/W 0.12 L $kg^{-1}h^{-1}$, $t_{1/2}$ 2.6 hours, and V/W 0.45 L/kg. They studied older neonates (n = 11), as a result of which we would expect a higher CL/W, a shorter $t_{1/2}$, and a lower V/W. However, cardiopulmonary bypass has an effect on drug pharmacokinetics and leads to a decreased CL/W, a prolonged $t_{1/2}$, and an increased V/W.[10]

### Predictors of Individual Pharmacokinetic Parameters

The investigated covariates had no or very low predictive power for the pharmacokinetic parameters of flucloxacillin. The age parameters GA and PNA had low predictability for CL/W flucloxacillin in contrast with our earlier study of amoxicillin.[31] Herngren et al[9] found a significant negative correlation between elimination half-life of flucloxacillin and gestational age in 9 neonates ($r = -0.661$, $P < 0.05$). The correlation between elimination half-life of flucloxacillin and gestational age in our population was not statistically significant ($r = -0.257$, $P = 0.058$). This difference may be associated with the size of the study population, 9 versus 55 neonates, and with the range of gestational age of the population, 33 to 41 versus 26 to 42 weeks.

In our previous study we found that CL/W gentamicin and 1/Cr were very good predictors of CL/W amoxicillin in (pre)term neonates.[31] In the present study we did not find a significant correlation between CL/W flucloxacillin and the covariates CL/W gentamicin and 1/Cr. Therefore, we think that the relative contribution of glomerular filtration to the elimination of flucloxacillin is small compared with that of gentamicin and amoxicillin. Glomerular filtration of flucloxacillin may be lowered by its high protein binding.[32]

## Dosage Regimen

The efficacy of the current and proposed dosage regimen was investigated by calculating the time the free plasma concentration of flucloxacillin exceeds the MIC. The free plasma concentration was assumed as constant in all neonates. However, there are a lot of factors, such as circulating substances (albumin, bilirubin, free fatty acids), disease, albumin structure, plasma volume, and GA, that may influence protein binding and cause interpatient variability in protein binding.[33] Herngren et al[9] found a significant positive correlation between protein binding (mean 86.3%) and GA (mean 36.6 weeks, range 33 to 41 weeks). Therefore, we expect part of our neonates (mean GA 31.2 weeks, range 26 to 42 weeks) to have protein binding below 86.3% and a favorably higher $T > \text{MIC}$. Knowledge of the free fraction would be beneficial in estimating the required dose. However, there are ethical and practical problems in obtaining a sufficiently large sample for analysis from neonates and the assay is time-consuming and complicated.

We suggest one dose for all neonates, irrespective of their age, because of the low correlation between CL/W flucloxacillin and the covariates GA and PNA. This is in contrast with the existing dosing schemes. For initial therapy, we suggest a higher dose and a more frequent administration than is customary now to reach sufficiently high plasma concentrations. Continuous infusion could be considered as an alternative for frequent intermittent administration, because flucloxacillin is reported to be stable at room temperature for 24 hours.[18] We suggest an initial dose of 25 mg/kg/4 h. This initial dose should be reduced to 10 mg/kg/6 h when CoNS are isolated. Dose reduction lowers the costs and prevents unnecessary use of the antibiotic, which promotes bacterial overgrowth and resistance. These new dosage regimens need prospective verification, because they have been tested only by the original data from which the regimens derived.

Simulation with the new dosage regimens indicated that satisfactory plasma concentrations were reached in 52 of the 55 neonates. A large interindividual variability of $T > \text{MIC}$ and peak concentrations was observed with these dosage regimens. With the suggested initial dose of 25 mg/kg/4 h, simulated $T > \text{MIC}_{2.0}$ did not exceed 40% in three neonates (29%, 35%, and 35%). With a higher initial dose, eg 30 mg/kg/4 h, simulated $T > \text{MIC}_{2.0}$ did exceed 40% in all 55 neonates, but simulated peak concentrations came very close to the toxic limit of 200 mg/L in several patients. We think it is safer to have $T > \text{MIC}_{2.0}$ somewhat below the desired 40% in a few patients instead of risking toxic levels in several patients. Flucloxacillin is always administered in combination with gentamicin for the initial treatment of suspected late-onset sepsis. Moreover, the toxic limit of 200 mg/L found in adults might even be lower in neonates, because of the lower binding capacity of albumin in neonates.[34]

The suggested dosage regimens will result in plasma concentrations sufficiently high for the treatment of infections which are easily reached by antibiotics, such as bacteraemia, urinary tract infection, and intravascular catheters-related infection. However, in patients who are suspected of infections which are not easily reached by antibiotics, such as meningitis, higher doses should be necessary. In these patients, flucloxacillin administration ought to be reconsidered, because doses higher than our proposed dosage regimens may result in plasma concentrations exceeding 200 mg/L.

## CONCLUSIONS

We suggest an initial flucloxacillin dose of 25 mg/kg/4 h for all neonates, irrespective of their GA and PNA. This dose is higher than the customary dose. After isolation of the causative agent of infection, the initial dose should be either reduced to 10 mg/kg/6 h, when oxacillin sensitive CoNS are isolated, or maintained, when *S. aureus* is isolated. Simulation with these new dosage regimens showed that satisfactory plasma concentrations were reached in 52 of the 55 neonates. However, the regimens need prospective verification. Moreover, the exact role of neonatal protein binding needs to be further investigated.

## REFERENCES

1. McGuire W, Clerihew L, Fowlie PW. Infection in the preterm infant. *BMJ*. 2004;329:1277–1280.
2. Gordon A, Jeffery HE. Antibiotic regimens for suspected late onset sepsis in newborn infants. *The Cochrane Database of Systematic Reviews* 2005.
3. Janknegt R. The treatment of staphylococcal infections with special reference to pharmacokinetic, pharmacodynamic and pharmacoeconomic considerations. *Pharm World Sci*. 1997;19:133–141.
4. Hey E, Hall C. *Neonatal Formulary*. 4th ed. London: Blackwell BMJ Books; 2003.
5. Adam D, Koeppe P, Heilmann HD. Pharmacokinetics of amoxicillin and flucloxacillin following the simultaneous intravenous administration of 4 g and 1 g, respectively. *Infection*. 1983;11:150–154.
6. Thijssen HHW, Wolters J. The metabolic disposition of flucloxacillin in patients with impaired kidney function. *Eur J Clin Pharmacol*. 1982;22:429–434.
7. Røder BL, Frimodt-Møller N, Espersen F, et al. Dicloxacillin and flucloxacillin: pharmacokinetics, protein binding and serum bactericidal titers in healthy subjects after oral administration. *Infection*. 1995;23:107–112.
8. Gath J, Charles B, Sampson J, et al. Pharmacokinetics and bioavailability of flucloxacillin in elderly hospitalized patients. *J Clin Pharmacol*. 1995;35:31–36.
9. Herngren L, Ehrnebo M, Broberger U. Pharmacokinetics of free and total flucloxacillin in newborn infants. *Eur J Clin Pharmacol*. 1987;32:403–409.
10. Adrianzén Vargas MR, Danton MH, Javaid SM, et al. Pharmacokinetics of intravenous flucloxacillin and amoxicillin in neonatal and infant cardiopulmonary bypass surgery. *Eur J Cardiothorac Surg*. 2004;25:256–260.
11. Guignard J, Drukker A. Why do newborn infants have a high plasma creatinine? *Pediatrics*. 1999;103:e49.
12. Koren G, James A, Perlman M. A simple method for the estimation of glomerular filtration rate by gentamicin pharmacokinetics during routine drug monitoring in the newborn. *Clin Pharmacol Ther*. 1985;38:680–685.
13. Stolk LML, Degraeuwe PLJ, Nieman FHM, et al. Population pharmacokinetics and relationship between demographic and clinical variables and pharmacokinetics of gentamicin in neonates. *Ther Drug Monit*. 2002;24:527–531.

14. Stolk LML, Degraeuwe PLJ. Gentamicin pharmacokinetics in preterm infants of less than 30 weeks gestational age at birth: effect of postnatal age [abstract]. *Br J Clin Pharmacol*. 1999;48:767P.
15. Young TE, Mangum B. *Neofax®: A Manual of Drugs Used in Neonatal Care*. 17th ed. Raleigh, North Carolina: Acorn Publishing; 2004.
16. Dodge WF, Jelliffe RW, Zwischenberger JB, et al. Population pharmacokinetic models: effect of explicit versus assumed constant serum concentration assay error patterns upon parameter values of gentamicin in infants on and off extracorporeal membrane oxygenation. *Ther Drug Monit*. 1994;16:552–559.
17. Thijssen HHW. Analysis of isoxazolyl penicillins and their metabolites in body fluids by high-performance liquid chromatography. *J Chromatogr*. 1980;183:339–345.
18. Thalhammer F, Maier-Salamon A, Jäger W. Prüfung von stabilität und kompatibilität von flucloxacillin (Floxapen®) und ceftazidim (Fortum®) in zwei infusionslösungen: relevanz für die klinische praxis. *Wien Med Wochenschr*. 2005;155:337–343.
19. Proost JH, Meijer DKF. MW\PHARM, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. *Comput Biol Med*. 1992;22:155–163.
20. Bonate PL. A brief introduction to Monte Carlo simulation. *Clin Pharmacokinet*. 2001;40:15–22.
21. Mediware: software for healthcare. Available at: http://www.mediware.nl.
22. Hoog de M, Mouton JW, Anker van den JN. New dosing strategies for antibacterial agents in the neonate. *Semin Fetal Neonatal Med*. 2005;10:185–194.
23. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Standard M100-S12*. Wayne, Pa: NCCLS; 2002.
24. Bratlid D. Pharmacologic aspects of neonatal hyperbilirubinemia. *Birth Defects*. 1976;12:184–191.
25. Rolinson GN, Sutherland R. The binding of antibiotics to serum proteins. *Br J Pharmacol*. 1965;25:638–650.
26. Morselli PL. Clinical pharmacology of the perinatal period and early infancy. *Clin Pharmacokinet*. 1989;17:13–28.
27. Dittert LW, Griffen WO, LaPiana JC, et al. Pharmacokinetic interpretation of penicillin levels in serum and urine after intravenous administration. *Antimicrob Agents Chemother*. 1969;9:42–48.
28. Nauta EH, Mattie H. Pharmacokinetics of flucloxacillin and cloxacillin in healthy subjects and patients on chronic intermittent haemodialysis. *Br J Clin Pharmacol*. 1975;2:111–121.
29. Cole M, Kenig MD, Hewitt VA. Metabolism of penicillins to penicilloic acid and 6-aminopenicillanic acid in man and its significance in assessing penicillin absorption. *Antimicrob Agents Chemother*. 1973;3:463–468.
30. Thijssen HHW, Mattie H. Active metabolites of isoxazolyl penicillins in humans. *Antimicrob Agents Chemother*. 1976;10:441–446.
31. Pullen J, Stolk LML, Nieman FHM, et al. Population pharmacokinetics and dosing of amoxicillin in (pre)term neonates. *Ther Drug Monit*. 2006;28:226–231.
32. Bowman RH. Renal secretion of $^{35}$S-furosemide and its depression by albumin binding. *Am J Physiol*. 1975;229:93–98.
33. Notarianni LJ. Plasma protein binding of drugs in pregnancy and in neonates. *Clin Pharmacokinet*. 1990;18:20–36.
34. Ehrnebo M, Agurell S, Jalling B, et al. Age differences in drug binding by plasma proteins: studies on human foetuses, neonates and adults. *Europ J Clin Pharmacol*. 1971;3:189–193.

 

# Protein Binding of Flucloxacillin in Neonates

*Joyce Pullen, MSc,* Leo M. L. Stolk, PhD,* Pieter L. J. Degraeuwe, MD, PhD,† Frank H. van Tiel, PhD,‡ Cees Neef, PhD,* and Luc J. I. Zimmermann, MD, PhD†*

**Abstract:** The isoxazolyl penicillins, including flucloxacillin, have the highest levels of plasma protein binding among the semisynthetic penicillins. Because only the free fraction of the penicillin is pharmacologically active, it would be useful to measure both protein-bound and free flucloxacillin to determine its protein binding. Until now, flucloxacillin protein binding in newborn infants has been investigated in only two studies with relatively small populations. In the present study, flucloxacillin protein binding was investigated in 56 (preterm) infants aged 3 to 87 days (gestational age, 25–41 weeks). Surplus plasma samples from routine gentamicin assays of each infant were collected and combined to obtain a sufficiently large sample for analysis. Free flucloxacillin was separated from protein-bound flucloxacillin using ultrafiltration. Reversed-phase high-performance liquid chromatography with ultraviolet detection was used to measure free flucloxacillin concentrations in ultrafiltrate and total flucloxacillin concentrations in pooled plasma. Flucloxacillin protein binding was 74.5% ± 13.1% (mean ± standard deviation) with a high variability among the infants (34.3% to 89.7%). High Pearson correlations were found between protein binding and the covariates–plasma albumin concentration ($r = 0.804$, $P < 0.001$, $n = 18$) and plasma creatinine concentration ($r = -0.601$, $P < 0.001$, $n = 45$). Statistically significant but less striking correlations were found between protein binding and gestational age, postconceptional age, body weight, and triglyceride concentration. Because of the high variability of protein binding among infants, it is difficult to devise a flucloxacillin dosage regimen effective for all infants. Individualized dosing, based on free flucloxacillin concentrations, might help to optimize treatment of late-onset neonatal sepsis, but practical obstacles will probably prevent analysis of free flucloxacillin concentrations in newborn infants on a routine basis.

**Key Words:** protein binding, flucloxacillin, therapeutic drug monitoring, infants, ultrafiltration, albumin

(*Ther Drug Monit* 2007;29:279–283)

## INTRODUCTION

Flucloxacillin is used frequently for the initial treatment of late-onset neonatal sepsis in Dutch hospitals despite limited information about protein binding.[1] In an earlier study, we investigated flucloxacillin population pharmacokinetics, flucloxacillin dosing, and the influence of covariates on the individual pharmacokinetic parameters in 55 (pre)term neonates.[2] To evaluate the efficacy of the dosage regimen, the time the free (nonprotein-bound) plasma concentration exceeded the minimum inhibitory concentration ($T > MIC$) was calculated.[2] A dosage regimen, based on a $T > MIC$ of at least 40%, as advised in the literature,[3] was devised. Based on previous studies, it was assumed that all neonates had a protein binding level of 86.3%.[4] However, there are many covariates that affect protein binding, including circulating substances (albumin, bilirubin, free fatty acids), disease, presence of other drugs, plasma volume, and gestational age (GA).[5]

The isoxazolyl penicillins, including flucloxacillin, have the highest levels of plasma protein binding among the semisynthetic penicillins.[6] Plasma protein binding is the major factor determining extravascular penetration of antimicrobial agents into tissues.[7] Zeitlinger et al[8] showed a significant decrease in bacterial killing by penicillins after the addition of albumin, suggesting that only the free fraction of the penicillin is available for bacterial killing. Therefore, knowledge of the plasma protein binding of flucloxacillin and the covariates affecting its protein binding is essential for estimating the flucloxacillin dose required for effective treatment of late-onset neonatal sepsis.

Plasma protein binding of flucloxacillin has already been studied in healthy adults,[9–11] patients with pacemakers,[12] postpartum mothers,[13] and patients with impaired kidney function.[10] Protein binding values ranged from 91% in pacemaker patients[12] to 95.7% in healthy adults.[9] In neonates, plasma protein binding of drugs is generally lower than in adults.[5] In two studies, flucloxacillin protein binding was investigated in relatively small populations of newborn infants. Herngren et al[4] found a mean protein binding value of 86.3% (range, 82.7–89.7%) in nine newborn infants aged 7.2 days (mean GA, 36.6 weeks). In another study, plasma protein binding of flucloxacillin was determined in vitro by equilibrium dialysis in newborn infants at delivery and during their first postnatal week.[13] Median protein binding values of 93.4% (range, 90.5–95.8%) and 88.6% (range, 86.5–90.6%) were reported in 22 infants immediately after delivery (mean GA, 40.1 weeks) and in two infants during the first week (fifth to seventh day) after delivery (mean GA, 40.7 weeks), respectively.[13] Free flucloxacillin was separated from protein-bound flucloxacillin using an ultrafiltration method[9,11] or equilibrium dialysis.[4,10,12,13] Protein binding values of flucloxacillin, as determined by equilibrium dialysis and ultrafiltration, are comparable.[14] Flucloxacillin concentrations were measured with a microbiologic assay[4,9,11–13] or a more specific high-performance liquid chromatography (HPLC) method.[10]

Received for publication December 11, 2006; accepted February 9, 2007.
From the Departments of *Clinical Pharmacy and Toxicology; †Paediatrics, Division of Neonatology; and ‡Medical Microbiology of the University Hospital of Maastricht, Maastricht, The Netherlands.
Correspondence: Joyce Pullen, MSc, Department of Clinical Pharmacy and Toxicology, University Hospital of Maastricht, PO Box 5800, NL-6202 AZ Maastricht, The Netherlands (e-mail: jpul@kfls.azm.nl).

In this study, plasma protein binding of flucloxacillin was investigated in a large population of (preterm) infants who were treated with flucloxacillin for (suspected) late-onset sepsis. Several covariates possibly affecting flucloxacillin protein binding were tested for significance and the implications for flucloxacillin dosing of variation in free fraction were explored.

## MATERIALS AND METHODS

### Patients

The study was approved by the local medical ethics committee. Plasma of 56 (pre)term infants was investigated. All infants had been admitted to the neonatal ward at the University Hospital of Maastricht and received flucloxacillin in combination with gentamicin for (suspected) late-onset sepsis.

### Sample Preparation of Nonprotein-Bound Flucloxacillin

Free flucloxacillin was separated from protein-bound flucloxacillin in samples from 56 infants (Table 1) by ultrafiltration, using a Centrifree micropartition device (Millipore Corp., Bedford, MA).[15] This system required a minimum plasma volume of 150 μL for analysis.[15] Unused portions of plasma samples from routine gentamicin assays were collected and combined for each infant to obtain a sufficiently large sample. A total of 5.5 ± 2.1 plasma samples were collected per infant that had been obtained over a period of 3.4 ± 1.3 days (mean ± standard deviation [SD]). For more efficient flow rates, fibrin was first removed by centrifugation at 1780 × *g* for 5 minutes at room temperature. Micropartition devices were then centrifuged at 4000 rpm for 25 minutes at room temperature using a 33° fixed-angle centrifuge rotor for optimum solvent flow.

### Assay of Free Flucloxacillin

Flucloxacillin was provided by Synopharm (Barsbüttel, Germany). A stock solution of flucloxacillin (1000 mg/L) was freshly prepared on the day of analysis by dissolving 11 mg sodium flucloxacillin in 10 mL water. This stock solution was diluted 10-fold with 0.9% saline solution to obtain the working solution (100 mg/L). Calibration standards were prepared by adding the required amount of working solution to 0.9% saline solution to obtain concentrations of 10, 25, 50, 75, and 100 mg/L of flucloxacillin. A quality control sample, containing 100 mg/L of flucloxacillin in plasma, was prepared and ultrafiltrated. The calibration standards, the ultrafiltrate of the quality control, and the ultrafiltrates of the infants were directly injected (25 μL) into a RP-HPLC with ultraviolet detection.[2] Mean concentration of free flucloxacillin in the ultrafiltrates of the quality controls was 8.1 ± 0.6 mg/L (n = 11) (mean ± SD). Thus, mean protein binding of flucloxacillin in quality control adult plasma was 91.9%. Coefficient of variation was 7.8%. The RP-HPLC assay was validated for linearity, precision, and accuracy. The correlation coefficients of the calibration curves (n = 6) were greater than 0.99. To determine interday precision and accuracy, three concentrations of flucloxacillin (0.5, 1, and 25 mg/L) were prepared in blank neonatal ultrafiltrate and each of them was analyzed six times. Coefficients of variation were 14.0%, 9.3%, and 3.3%, respectively. Inaccuracy, measured at the three concentrations, was less than 15% (+13.0%, −1.5%, and +3.2%, respectively). The lowest free flucloxacillin concentration measured in the ultrafiltrates from infants was 0.6 mg/L.

**TABLE 1.** Demographic, Anthropometric, and Clinical Characteristics of 56 Infants

| | Unit | No. | Mean (SD) | Range |
|---|---|---|---|---|
| GA | Weeks | 55 | 31.2 (4.7) | 25.0–41.4 |
| PNA | Days | 56 | 20.4 (18.7) | 3–87 |
| PCA | Weeks | 55 | 34.1 (4.6) | 27.7–42.7 |
| Weight | kg | 56 | 1.63 (0.83) | 0.67–3.90 |
| Creatinine | μmol/L | 45 | 57.3 (26.8) | 20–139 |
| Total bilirubin | μmol/L | 33 | 95.7 (50.7) | 3–193 |
| Unconjugated bilirubin | μmol/L | 13 | 68.1 (51.7) | 7–163.6 |
| Albumin | g/L | 18 | 17.5 (5.1) | 10.1–27.8 |

SD, standard deviation; GA, gestational age; PCA, postconceptional age; PNA, postnatal age.

### Total Flucloxacillin Concentrations

Analysis of total flucloxacillin concentrations in pooled plasma was performed as described previously.[2] RP-HPLC with ultraviolet detection was used. The mobile phase consisted of perchlorate buffer and acetonitrile. Cloxacillin was used as internal standard and methylene chloride was used for extraction.

### Protein Binding

The binding of flucloxacillin to plasma proteins was determined using the following equation: $(C_p - C_u)/C_p \cdot 100\%$, where $C_p$ and $C_u$ are the concentrations in plasma (total) and ultrafiltrate (free), respectively.

### Covariates Affecting Protein Binding

Pearson correlation coefficients (r) between flucloxacillin protein binding and covariates possibly affecting this protein binding were calculated with SPSS 12.0 (Chicago, IL) considering a $P$ value of $<0.05$ as being statistically significant. Covariates possibly affecting flucloxacillin protein binding were: plasma albumin concentration, unconjugated plasma bilirubin concentration, plasma concentration of free fatty acids, and plasma creatinine concentration.[5,16] Unfortunately, the plasma concentration of free fatty acids was measured in only one infant. However, plasma triglyceride concentrations were measured in 38 infants. Pearson correlation coefficients were also calculated between protein binding and GA, postnatal age (PNA), postconceptional age (PCA), and body weight. Coadministered drugs with a high protein binding might also affect flucloxacillin protein binding. Therefore, complete concomitant medication of each infant was registered. One-way analysis of variance was used to test if the coadministered drugs with high protein binding had a significant influence on flucloxacillin protein binding. Because of the large number of missing values, mainly of plasma albumin concentration, regression analysis was not performed.

## RESULTS

### Patients

The main characteristics of the infants are presented in Table 1. The population consisted of 30 boys (54%) and 26 girls (46%). In 53 infants (three missing), blood cultures were taken. Twenty-six (49%) of the 53 blood cultures were positive, most frequently with coagulase-negative staphylococci (CoNS) (77%).

### Protein Binding

Free and total flucloxacillin plasma concentrations of all infants are illustrated in Figure 1. Plasma protein binding of flucloxacillin was 74.5% ± 13.1% (mean ± SD) (median 79.4%) with a range of 34.3% to 89.7%. The distribution of the free fraction of flucloxacillin among the infants is illustrated in Figure 2.

### Covariates Affecting Protein Binding

Statistically significant Pearson correlation coefficients were found between flucloxacillin protein binding and plasma albumin concentration ($r = 0.804$, $P < 0.001$, $n = 18$), plasma creatinine concentration ($r = -0.601$, $P < 0.001$, $n = 45$), body weight ($r = 0.475$, $P < 0.001$, $n = 56$), PCA ($r = 0.450$, $P = 0.001$, $n = 55$), GA ($r = 0.374$, $P = 0.005$, $n = 55$), and plasma triglyceride concentration ($r = -0.326$, $P = 0.046$, $n = 38$). The relationship between flucloxacillin protein binding and plasma albumin concentration is shown in Figure 3. The regression equation to predict flucloxacillin protein binding from plasma albumin concentration was: flucloxacillin protein binding (%) = 23.05 + 2.58·plasma albumin concentration (g/L) ($r^2 = 0.647$, $n = 18$). The standard error of the estimate (SEE), which is a measure of the accuracy of the predictions made with the regression line, was 9.92. The correlation between flucloxacillin protein binding and plasma creatinine concentration is presented in Figure 4. Fifteen infants received coadministered drugs with a protein binding greater than 90% (eg, furosemide, hydrochlorothiazide/triamterene, and indomethacin). We did not observe a significant difference in protein binding between these 15 infants and the other 41 infants.

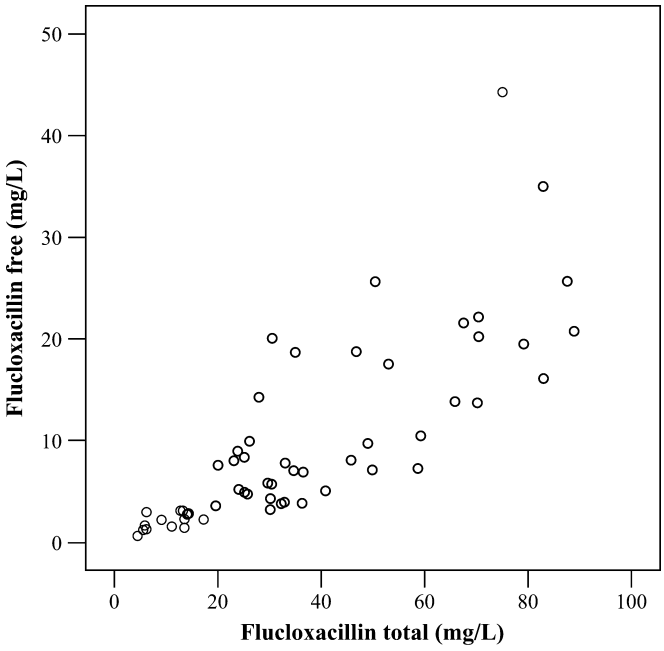


**FIGURE 1.** Free versus total flucloxacillin plasma concentrations of 56 infants.

## DISCUSSION

In the present study, flucloxacillin has been shown to be highly bound to plasma proteins in neonates (median, 79.4%). However, even higher protein binding values were found by Herngren et al.[4,13] The high interinfant variability in protein binding found in the present study (34.3–89.7%) and the differences between our results and the results reported by Herngren et al[4,13] might be explained by difference in covariates affecting protein binding.

A highly significant relationship was found between the plasma protein binding of flucloxacillin and plasma albumin concentration ($r = 0.804$, $P < 0.001$, $n = 18$). Herngren et al[13] found a correlation coefficient of 0.612 ($P < 0.01$) between the molar concentration of plasma albumin and plasma binding ratio (bound flucloxacillin/unbound flucloxacillin) in 22 newborn infants. Mean plasma albumin concentration (17.5 g/L) measured in 18 of our 56 infants (mean GA,

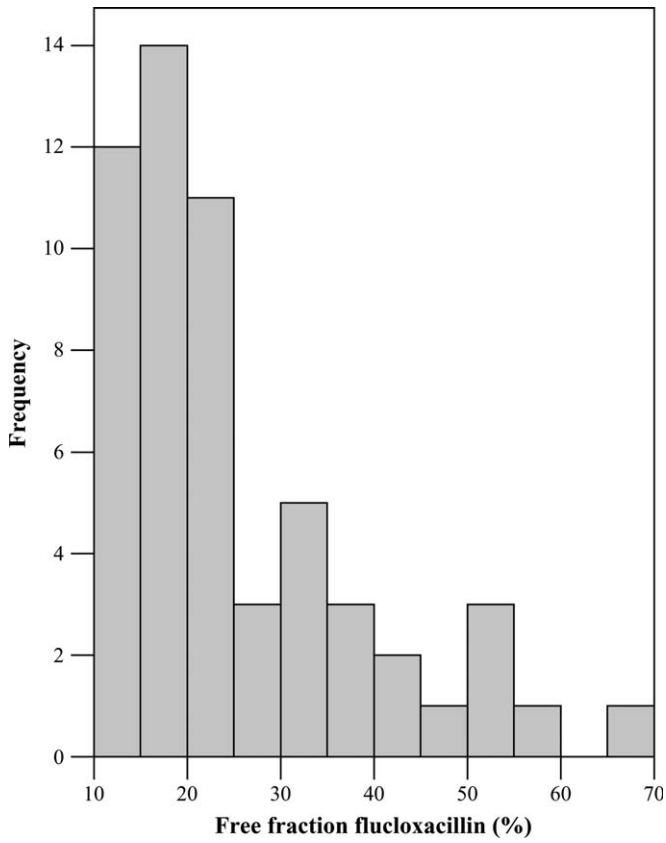


**FIGURE 2.** Distribution of free flucloxacillin among 56 infants.

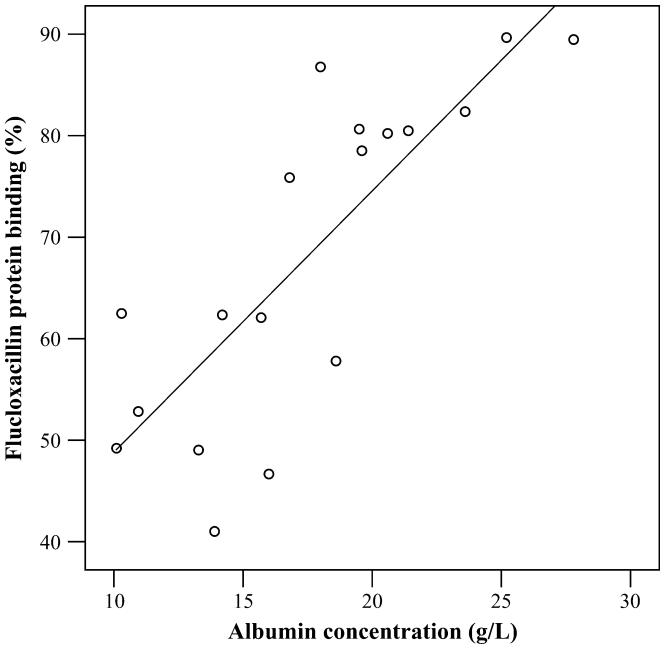


**FIGURE 3.** Correlation between flucloxacillin protein binding and plasma albumin concentration (n = 18). The equation of the regression line was y = 23.05 + 2.58x and the coefficient of correlation was r = 0.804 ($P < 0.001$).

30.2 weeks; mean PNA, 27.1 days) was somewhat lower than that generally seen in healthy infants of the same age.[17] It should be noted that in our neonatal unit, plasma albumin concentrations are only measured in sick infants usually experiencing edema or liver dysfunction. Mean protein

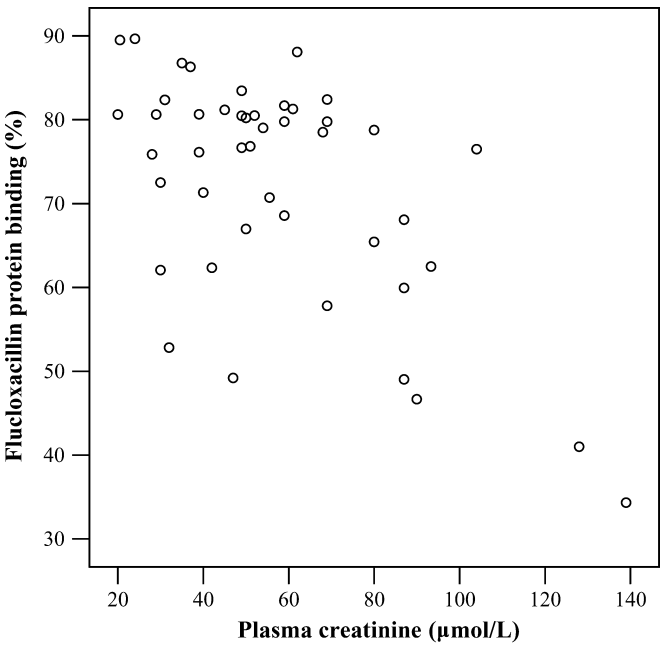


**FIGURE 4.** Correlation between flucloxacillin protein binding and plasma creatinine concentration (n = 45).

binding of these 18 infants was 68.2% and was considerably lower than the protein binding found by Herngren et al,[4,13] which may partly be explained by the difference in plasma albumin concentration, 17.5 g/L versus 33 g/L[4] and 39.0 g/L.[13]

We also noticed a significant relationship between flucloxacillin protein binding and plasma creatinine concentration ($r = -0.601$, $P < 0.001$, $n = 45$) as noted for phenytoin, theophylline, methotrexate, and diazepam ($r = -0.87$, $-0.80$, $-0.79$, and $-0.67$, respectively, $P < 0.001$) in chronic renal failure.[16] This relationship supports earlier findings that endogenous acidic ligands, which accumulate in renal failure, act as binding inhibitors. These ligands are responsible for decreased plasma protein binding in renal failure of many acidic drugs, including the isoxazolyl penicillins.[10,18–21] In addition, decreased drug binding in renal failure may be at least partly the result of altered albumin composition.[22] Moreover, the relationship between flucloxacillin protein binding and plasma creatinine concentration may partly be explained by the relationship between protein binding and GA, PCA, and body weight, because plasma creatinine concentration is inversely related to these parameters.

Two other endogenous compounds that may affect plasma protein binding of flucloxacillin in neonates are free fatty acids and unconjugated bilirubin. Unfortunately, the plasma concentration of free fatty acids was measured in only one infant. However, we found a significant relationship between flucloxacillin protein binding and plasma triglyceride concentration. Flucloxacillin protein binding decreased when the plasma concentration of triglyceride, which is split into glycerol and free fatty acids in the intestine, increased. Most neonates have high levels of unconjugated bilirubin in plasma, because bilirubin production is increased and bilirubin removal is decreased in the first weeks of life.[5] Unconjugated bilirubin is capable of binding to plasma albumin. Isoxazolyl penicillins may displace bilirubin from albumin binding sites.[4,23] Bilirubin itself may also displace drugs from their binding to albumin and thereby increase the free fraction of the drug.[24] However, we did not find any relationship between flucloxacillin protein binding and unconjugated plasma bilirubin concentration. In contrast, a significant correlation between total (conjugated + unconjugated) bilirubin/albumin molar concentration ratio and flucloxacillin binding ratio (bound/unbound flucloxacillin) was reported by Herngren et al.[4] We did not observe a significant correlation between total bilirubin/albumin ratio, which could be calculated for only 12 of our 56 infants, and flucloxacillin binding ratio. It should be noted that total bilirubin concentration varies considerably in the first few days/weeks of life.[5] In most newborn infants, total plasma bilirubin concentration increases during the first week after birth and then decreases because metabolic processes become more efficient in its removal.[5] Mean total bilirubin concentration in our infants (mean PNA, 16.5 days) was low compared with the concentration in the much younger infants (mean PNA, 7.2 days) investigated by Herngren et al[4] (95.7 versus 131 μmol/L).

The plasma protein binding of flucloxacillin was also significantly correlated with GA, PCA, and body weight. Herngren et al[4] also showed a significant correlation between flucloxacillin protein binding and GA. This may partly be

 

explained by the quantitative and qualitative changes in plasma proteins during foetal and postnatal growth.[25] Plasma albumin concentration and binding affinity increases with gestational and postnatal age.[5] Moreover, plasma volume and, therefore, the absolute number of drug-binding sites increase during gestational and postnatal growth.[5] GA, PCA, and body weight of our population are lower than those of the populations investigated by Herngren et al,[4,13] which may partly explain the lower protein binding found in the present study.

In an earlier study, we investigated flucloxacillin population pharmacokinetics, flucloxacillin dosing, and the influence of covariates on the individual pharmacokinetic parameters in 55 (pre)term neonates.[2] An initial flucloxacillin dose of 25 mg/kg every 4 hours was suggested for all neonates. This initial dose should be either reduced to 10 mg/kg every 6 hours, when oxacillin sensitive CoNS are isolated, or maintained, when *Staphylococcus aureus* is isolated.[2] The free flucloxacillin fraction was assumed to be 13.7% in all neonates based on the literature.[4] Patient characteristics were comparable to the characteristics in this study, except for PNA (11.1 days versus 20.4 days). In the present study, the free flucloxacillin fraction was higher than 13.7% in most infants, but in nine infants, it was lower than 13.7%. To reach effective plasma concentrations in all infants, including those having a low free flucloxacillin fraction, the earlier suggested dosage regimens should probably be increased. However, higher doses may result in plasma concentrations exceeding the possibly toxic limit of 200 mg/L.[2] Thus, because of the high variability of protein binding among infants, caused by many covariates, it is difficult to devise a flucloxacillin dosage regimen effective for all infants. Individual measurement of the free flucloxacillin fraction would be beneficial in estimating the required dose, but methods for separating free and protein-bound drug are labor-intensive and there are ethical and practical problems in obtaining a sufficiently large sample for analysis from newborn infants.[5] Moreover, the literature[5] indicates that newborns display a continuingly changing plasma composition, which might affect protein binding. Subsequently, frequent dosage adaptation is required in newborn infants. Perhaps, plasma albumin measurement could be helpful in predicting protein binding or use of alternative antibiotics could be considered.

## CONCLUSIONS

Predicting flucloxacillin protein binding in newborn infants, aimed at optimal dosing of this drug, is very difficult, because many covariates affect protein binding. A high variability of flucloxacillin protein binding was demonstrated among 56 infants with (suspected) late-onset sepsis. Effective treatment with flucloxacillin should take this variability into account. Individualized dosing, based on free flucloxacillin concentrations, might help to optimize treatment of late-onset neonatal sepsis, but practical obstacles will probably prevent their analysis in newborn infants on a routine basis. We suggest further studies or efforts to develop an inexpensive and efficient test to measure free flucloxacillin concentrations in neonates.

## REFERENCES


1. Janknegt R. The treatment of staphylococcal infections with special reference to pharmacokinetic, pharmacodynamic and pharmacoeconomic considerations. *Pharm World Sci*. 1997;19:133–141.
2. Pullen J, Rozario de L, Stolk LML, et al. Population pharmacokinetics and dosing of flucloxacillin in preterm and term neonates. *Ther Drug Monit*. 2006;28:351–358.
3. Hoog de M, Mouton JW, Anker van den JN. New dosing strategies for antibacterial agents in the neonate. *Semin Fetal Neonatal Med*. 2005;10: 185–194.
4. Herngren L, Ehrnebo M, Broberger U. Pharmacokinetics of free and total flucloxacillin in newborn infants. *Eur J Clin Pharmacol*. 1987;32:403–409.
5. Notarianni LJ. Plasma protein binding of drugs in pregnancy and in neonates. *Clin Pharmacokinet*. 1990;18:20–36.
6. Ullmann U. The binding of isoxazolyl penicillins to human serum proteins. *Arzneimittelforschung*. 1977;27:2136–2138.
7. Bergan T, Engeset A, Olszewski W. Does serum protein binding inhibit tissue penetration of antibiotics? *Rev Infect Dis*. 1987;9:713–718.
8. Zeitlinger MA, Sauermann R, Traunmüller F, et al. Impact of plasma protein binding on antimicrobial activity using time-killing curves. *J Antimicrob Chemother*. 2004;54:876–880.
9. Røder BL, Frimodt-Møller N, Espersen F, et al. Dicloxacillin and flucloxacillin: pharmacokinetics, protein binding and serum bactericidal titers in healthy subjects after oral administration. *Infection*. 1995;23: 107–112.
10. Thijssen HHW, Wolters J. The metabolic disposition of flucloxacillin in patients with impaired kidney function. *Eur J Clin Pharmacol*. 1982;22: 429–434.
11. Sutherland R, Croydon EAP, Rolinson GN. Flucloxacillin, a new isoxazolyl penicillin, compared with oxacillin, cloxacillin, and dicloxacillin. *BMJ*. 1970;4:455–460.
12. Anderson P, Bluhm G, Ehrnebo M, et al. Pharmacokinetics and distribution of flucloxacillin in pacemaker patients. *Eur J Clin Pharmacol*. 1985;27:713–719.
13. Herngren L, Ehrnebo M, Boréus LO. Drug distribution in whole blood of mothers and their newborn infants. *Eur J Clin Pharmacol*. 1982;22: 351–358.
14. Craig WA, Suh B. Protein binding and the antimicrobial effects: methods for the determination of protein binding. In: Lorian V, ed. *Antibiotics in Laboratory Medicine,* 2nd ed. Baltimore: Williams & Wilkins; 1986: 500–504.
15. Millipore Corporation. Centrifree Micropartition Devices for in Vitro Diagnostic Use and MPS Micropartition Devices Not for Use in Diagnostic Procedures. Available at: www.millipore.com/userguides.nsf/docs/pr02272. Accessed April 12, 2006.
16. Vanholder R, Landschoot van N, de Smet R, et al. Drug protein binding in chronic renal failure: evaluation of nine drugs. *Kidney Int*. 1988;33: 996–1004.
17. Cartlidge PHT, Rutter N. Serum albumin concentrations and oedema in the newborn. *Arch Dis Child*. 1986;61:657–660.
18. Depner TA, Gulyassy PF. Plasma protein binding in uremia: extraction and characterization of an inhibitor. *Kidney Int*. 1980;18:86–94.
19. Robertz GM, Dengler HJ. Endogenous ligand(s) decrease drug-protein binding in uremic sera: a fluorescence probe study. *Klin Wochenschr*. 1983;61:649–653.
20. McNamara PJ, Lalka D, Gibaldi M. Endogenous accumulation products and serum protein binding in uremia. *J Lab Clin Med*. 1981;98:730–740.
21. Gulyassy PF, Bottini AT, Stanfel LA, et al. Isolation and chemical identification of inhibitors of plasma ligand binding. *Kidney Int*. 1986;30: 391–398.
22. Boobis SW. Alteration of plasma albumin in relation to decreased drug binding in uremia. *Clin Pharmacol Ther*. 1977;22:147–153.
23. Friedman LA, Lewis PJ. The effect of semisynthetic penicillins on the binding of bilirubin by neonatal serum. *Br J Clin Pharmacol*. 1980;9:61–65.
24. Bratlid D. Pharmacologic aspects of neonatal hyperbilirubinemia. *Birth Defects*. 1976;12:184–191.
25. Ehrnebo M, Agurell S, Jalling B, et al. Age differences in drug binding by plasma proteins: studies on human foetuses, neonates and adults. *Eur J Clin Pharmacol*. 1971;3:189–193.

# Pharmacokinetics of Intravenous Rifampicin (Rifampin) in Neonates

*Joyce Pullen, MSc,* Leo M. L. Stolk, PhD,* Pieter L. J. Degraeuwe, PhD,† Frank H. van Tiel, PhD,‡ Cees Neef, PhD,* and Luc J. I. Zimmermann, PhD†*

**Abstract:** Few reports have addressed neonatal rifampicin plasma concentrations and data on neonatal rifampicin pharmacokinetics are completely lacking. Therefore, plasma concentrations of rifampicin and its main metabolite 25-*O*-desacetylrifampicin (DES) were measured in 123 surplus plasma samples from routine vancomycin monitoring in 21 neonates using reversed-phase HPLC. Rifampicin peak and trough plasma concentrations were 4.66 ± 1.47 mg/L and 0.21 ± 0.20 mg/L, respectively, after a dose of 8.5 ± 2.1 (mean ± SD) mg/kg per day. A significant linear relationship between rifampicin dose and peak plasma concentrations was found, but inter-patient variability was high. Pharmacokinetic parameters of rifampicin were calculated according to a one-compartment open model with iterative two-stage Bayesian fitting (MW\PHARM 3.60, Mediware, The Netherlands). First-order elimination constant, volume of distribution corrected for weight, total body clearance corrected for weight (CL/W), and elimination half-life were 0.16 ± 0.06 $h^{-1}$, 1.84 ± 0.59 L/kg, 0.28 ± 0.11 $Lkg^{-1}h^{-1}$, and 4.9 ± 1.7 h, respectively. A high Pearson correlation was found between CL/W rifampicin and the covariates plasma creatinine and CL/W gentamicin of a preceding gentamicin treatment course, $r = 0.728$ (n = 17) and $r = 0.837$ (n = 12), respectively. DES was detected in each plasma sample. Therefore, rifampicin seems to be eliminated by both renal and metabolic pathways in neonates. In 8 study patients, plasma concentrations of rifampicin and DES were measured again after two weeks of therapy. CL/W rifampicin was significantly higher (67 ± 50%). The authors suggest maintaining the current dose regimen of 10 mg/kg once a day. Because of the large inter-patient variability in rifampicin plasma concentrations and CL/W increase during therapy, the authors suggest monitoring rifampicin peak and trough plasma concentrations to avoid low plasma concentrations. More research is needed to determine well-founded dosing guidelines.

**Key Words:** pharmacokinetics, rifampicin, rifampin, neonates, plasma concentrations, therapeutic drug monitoring

(*Ther Drug Monit* 2006;28:654–661)

From the Departments of *Clinical Pharmacy and Toxicology; †Pediatrics, Division of Neonatology; and ‡Medical Microbiology of the University Hospital of Maastricht, The Netherlands.

Reprints: Joyce Pullen, MSc, Department of Clinical Pharmacy and Toxicology, University Hospital of Maastricht, PO Box 5800, NL-6202 AZ Maastricht, The Netherlands (e-mail: jpul@kfls.azm.nl).



The initial antibiotic choice in suspected late-onset neonatal infections depends on the local susceptibility patterns. In most Dutch hospitals, flucloxacillin, in combination with gentamicin, is used for this indication.[1] After isolation of the causative agent of infection, antibiotic choice ought to be reconsidered based on the expected or determined susceptibility pattern of the isolate. Flucloxacillin is used for the treatment of infections caused by methicillin-susceptible staphylococci, whereas vancomycin is the drug of choice for infections caused by methicillin-resistant coagulase-negative staphylococci (CoNS), mainly *Staphylococcus epidermidis*.[2] However, some neonates do not respond to treatment with vancomycin.[2] The addition of rifampicin is a safe and effective option in the treatment of these persistent staphylococcal infections in neonates.[2–5] However, rifampicin and vancomycin have been shown to be synergistic, indifferent, or antagonistic, depending on the rifampicin concentration. Rifampicin and vancomycin seem to be antagonistic when the concentrations of rifampicin are high.[6,7]

Rifampicin has a wide spectrum of antibacterial activity and is the most active anti-staphylococcal agent known.[4] Rifampicin is highly soluble in lipids and has the unique ability to penetrate phagocytic leukocytes and kill intracellular staphylococci.[8] Plasma protein binding of rifampicin in adults is about 80%.[9] Rifampicin cannot be used as monotherapy because resistance may develop during therapy.[10]

Several studies have investigated the pharmacokinetics of IV rifampicin in children[11,12] and adults.[13–15] In neonates, rifampicin pharmacokinetics have not been studied yet. Only one small study investigated peak and trough plasma concentrations after IV rifampicin administration in four neonates (gestational age 30 to 40 weeks, mean 35 weeks).[2] However, rifampicin pharmacokinetic parameters were not reported. Because very little is known about the pharmacokinetics of rifampicin in neonates, the current dosage regimen[5] is based on pharmacokinetic data from studies in children and adults. Knowledge about neonatal rifampicin pharmacokinetics is essential for optimizing rifampicin dosing in neonates. Therefore, we have determined plasma concentrations of rifampicin in surplus plasma samples from routine vancomycin monitoring in 21 neonates, who received cotreatment with IV rifampicin and vancomycin for persistent staphylococcal bacteremia, in order to determine the pharmacokinetic parameters of rifampicin. We also investigated the influence of several demographic, anthropometric, and clinical covariates on the individual pharmacokinetic parameters of rifampicin.

## MATERIALS AND METHODS

### Patients

The study was approved by the local Ethical Board. Twenty-one neonates, admitted to the neonatal ward at the University Hospital of Maastricht from July, 2002 through November, 2005, participated in the study. They were treated concurrently with IV rifampicin and vancomycin for persistent staphylococcal infections. To calculate the individual pharmacokinetic parameters of rifampicin, at least two surplus plasma samples from routine vancomycin assays of each neonate had to be available. Patient information concerning gestational age (GA), postnatal age (PNA), postconceptional age (PCA), birth weight (BW), body weight (W), gender, multiple pregnancy (P), 1-minute Apgar score (AP1), 5-minute Apgar score (AP5), plasma creatinine (Cr), plasma urea concentration (Ur), C-reactive protein (CRP), and blood culture result (BC) was obtained from patient records.

### Demographic, Anthropometric, and Clinical Characteristics

The demographic, anthropometric, and clinical characteristics of the neonates are described in Table 1. Of the eight patients who were treated with rifampicin for more than two weeks, GA and PNA were 28.4 ± 2.3 weeks and 24.8 ± 15.9 days (at the beginning of the rifampicin therapy), respectively. Seven neonates (33%) were immature (GA < 28 weeks) and 12 neonates (57%) were premature (28 ≤ GA < 37 weeks). These 19 preterm neonates had a low birth weight (<2500 g) and 18 of them had a very low birth weight (<1500 g). Seventeen neonates (81%) were male. Fourteen neonates (67%) were products of singleton pregnancies and 7 neonates (33%) were products of twin pregnancies. Seventeen out of the 19 blood cultures (89%) (2 missing) were positive. In all cases, CoNS, including *Staphylococcus epidermidis* (41%), were isolated.

### Drug Administration and Sample Collection

Rifampicin was dosed at 5 to 10 mg/kg intravenously on a once-a-day schedule: 5 mg/kg (n = 4), 7 mg/kg (n = 1), 9 mg/kg (n = 3), and 10 mg/kg (n = 12). One neonate received 5 mg/kg every 12 hours. Rifampicin was infused over 30 minutes using an electronic infusion pump.[5] Neonates with a PCA of <30, 30 to 33, 34 to 37, 38 to 44, and >44 weeks received 20, 20, 20, 15, and 10 mg/kg vancomycin with an interval of 24, 18, 12, 8, and 6 hours, respectively. Vancomycin was infused over 60 minutes using an electronic infusion pump.[5] Dosing times of vancomycin and rifampicin were different. Dosing times were recorded in the patient's medical record by nursing staff each time rifampicin and vancomycin were administered.

Twelve neonates were treated with a combination therapy of IV flucloxacillin and gentamicin for 4.5 ± 2.3 days (range 2 to 9 days) preceding rifampicin therapy. However, this combination therapy turned out to be unsuccessful in all 12 neonates and therapy with IV vancomycin was started. The other nine neonates were treated from the beginning with IV vancomycin. After 3.3 ± 2.6 days (range 0 to 8 days), IV rifampicin was added. The duration of the rifampicin course was 16.4 ± 7.1 days (4 to 29 days). After the addition of rifampicin, 14 out of the 17 positive blood cultures were sterile after 6.6 ± 5.0 days (1 to 16 days). The other three positive blood cultures also became sterile, but it was not known after how many days.

Therapeutic drug monitoring of vancomycin was performed on a routine basis. Plasma samples were drawn by heel prick just before and two hours after the end of the vancomycin infusion. Sampling times were recorded in the patient's medical record by nursing staff each time a blood sample was taken for vancomycin analysis. Surplus plasma samples from routine vancomycin assays were collected and frozen at –70°C until the rifampicin assay was performed. Heparin was used as anticoagulant. We collected 5.9 ± 3.0 plasma samples per neonate during 3.7 ± 1.8 days (mean ± SD). Fourteen of the 21 neonates were treated with rifampicin for more than two weeks. Surplus plasma samples after 2 weeks of treatment were available for only 8 of these 14 neonates. In this second study period, we collected 5.9 ± 2.6 plasma samples per neonate during 3.4 ± 1.6 days.

### Chemicals

Ammonium sulphate ($(NH_4)_2SO_4$, CAS 7783-20-2), methanol ($CH_4O$, CAS 67-56-1), and Titriplex® III

**TABLE 1.** Demographic, Anthropometric, and Clinical Characteristics of the Neonates

| | Unit | N | Mean (SD) | Median (percentile 25/75) | Range |
|---|---|---|---|---|---|
| GA | weeks | 21 | 29.9 (4.1) | 28.6 (26.9/31.5) | 26.0–41.3 |
| PNA | days | 21 | 24.8 (13.4) | 18.0 (15.0/34.5) | 11–55 |
| PCA | weeks | 21 | 33.5 (5.0) | 31.4 (30.4/36.2) | 28.3–48.0 |
| BW | kg | 21 | 1.24 (1.02) | 0.88 (0.68/1.30) | 0.37–4.30 |
| W | kg | 21 | 1.58 (1.14) | 1.25 (0.92/1.50) | 0.49–4.78 |
| AP1 | points | 21 | 6.6 (2.9) | 8 (5/9) | 0–9 |
| AP5 | points | 21 | 8.6 (1.8) | 9 (9/9) | 2–10 |
| Cr | μmol/L | 17 | 52.1 (13.4) | 52.0 (42.5/58.0) | 33.0–85.0 |
| Ur | mmol/L | 17 | 3.5 (2.6) | 2.7 (1.9/5.3) | 0.4–11.1 |
| CRP | mg/L | 20 | 74.7 (45.8) | 62.0 (38.3/116.8) | 2.9–158.0 |

AP1, 1-minute Apgar score; AP5, 5-minute Apgar score; BW, birth weight; Cr, plasma creatinine level; CRP, C-reactive protein; GA, gestational age; PCA, postconceptional age; PNA, postnatal age; Ur, plasma urea concentration; W, body weight.

($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$, CAS 6381-92-6) were obtained from Merck (Darmstadt, Germany). Rifampicin ($C_{43}H_{58}N_4O_{12}$, CAS 13292-46-1) was provided by Yamanouchi Pharma (Leiderdorp, The Netherlands) and its main metabolite, 25-*O*-desacetylrifampicin ($C_{41}H_{56}N_4O_{11}$), was a gift from Novartis Pharma (Arnhem, The Netherlands). Ultra-pure water was used in all preparations. All chemicals were of HPLC grade. Human EDTA plasma for standard and control samples was provided by the Red Cross Blood Bank (Maastricht, The Netherlands).

## High-performance Liquid Chromatography

Rifampicin and 25-*O*-desacetylrifampicin plasma concentrations were measured with reversed-phase high-performance liquid chromatography (RP-HPLC). The RP-HPLC assay method was based on a method described by Chandi et al.[16] The RP-HPLC system consisted of an automatic sample injector (model 717, Waters, USA), a pump (model 1050, Hewlett Packard, USA), a C18 symmetry column, 150 × 4.6 mm, 5 μm (Waters, USA) combined with a C18 guard column, and an UV spectrophotometric detector (model 486, Waters, USA). The effluent was composed of 0.005 mol/L disodium EDTA (0.93 g Titriplex® III in 500 mL ultra-pure water) and methanol (200:300). The separations were carried out at room temperature and at a flow rate of 1.0 mL/min. The detection was performed at a wavelength of 340 nm. The assay error pattern was determined according to a method described in the literature.[17] To determine the error pattern, we measured plasma samples at twelve concentration levels (0.1, 0.2, 0.3, 0.4, 1, 2, 4, 6, 8, 10, 25, and 50 mg/L) on six separate days. The relationship between the measured plasma concentration and SD was fitted with a second-order polynomial function. The error pattern of the rifampicin assay was: $SD = 0.0173 + 0.0455\ C + 0.0002\ C^2$. The error pattern of the 25-*O*-desacetylrifampicin assay was: $SD = 0.0561 + 0.0323\ C + 0.0005\ C^2$. The lower limit of quantification (LLOQ) of rifampicin was 0.1 mg/L and the LLOQ of 25-*O*-desacetylrifampicin was 0.4 mg/L. The rifampicin and 25-*O*-desacetylrifampicin response at the LLOQ was at least 5 times the response compared to blank response and was identifiable, discrete, and reproducible with a precision of <20% (11 and 15%, respectively) and accuracy of 80 to 120% (104 and 112%, respectively) according to guidelines in the literature.[18]

Ammonium sulphate (10 μL, 5%) and 80 μL methanol were added to 40 μL surplus plasma, and the solution was mixed for 1 minute. The solution was centrifuged at 10,500 × g for 5 minutes. Following centrifugation, chromatography of the supernatant (30 μL) was accomplished. Standard solutions, containing 2, 4, 6, 8, and 10 mg/L rifampicin and 25-*O*-desacetylrifampicin, were freshly prepared on the day of analysis. The standard plasma samples were processed in the same way as the surplus plasma samples. A calibration curve was constructed and concentrations of rifampicin and 25-*O*-desacetylrifampicin in surplus plasma samples were calculated. Control plasma samples, containing 5 mg/L rifampicin and 25-*O*-desacetylrifampicin, were prepared and stored in separate containers at –70°C until analysis.

## Validation

The RP-HPLC assay method was validated for linearity, precision, and accuracy. The correlation coefficient of the calibration curves was >0.99 (n = 6). Inter-day precision and inaccuracy, measured at 2, 4, 6, 8, and 10 mg/L (n = 6), were <10% and <5%, respectively, for both rifampicin and 25-*O*-desacetylrifampicin.

Rifampicin stability in plasma samples during storage at −70°C was determined by analyzing the control plasma samples stored at −70°C in each run during the entire measurement period of approximately 2 months. We did not observe any decrease of rifampicin concentration. This has been found earlier by Peloquin.[19] We also measured rifampicin plasma concentrations after 1, 2, 3, and 4 hours at room temperature (in the dark). We observed no loss under these conditions although Peloquin[19] found rifampicin plasma extracts began to decay after approximately 4 hours at room temperature (under fluorescent ceiling lights). In each run, six standard plasma samples, one control plasma sample, and five surplus plasma samples were included. The duration of each run was about two hours. We checked rifampicin stability during the run by analyzing one control plasma sample at the beginning of the run and one control plasma sample at the end of the run after approximately two hours. We did not see any difference in rifampicin concentration between the two control plasma samples.

## Data Analysis

The individual pharmacokinetic parameters of rifampicin and vancomycin were calculated by maximum a posteriori Bayesian fitting (MW\PHARM 3.60, Mediware, The Netherlands). The population pharmacokinetic parameters were calculated with an iterative two-stage Bayesian fitting procedure (IT2B) (MW\PHARM 3.60). The Bayesian method uses measured drug concentrations, population-based parameters, and the expected variability associated with each measurement to determine individualized pharmacokinetic parameters of a patient.[20] Analysis took place according to a one-compartment open model. The initial pharmacokinetic parameters of rifampicin, $K_{el}$ 0.10 ± 0.05 $h^{-1}$ and V/W 1.60 ± 0.80 L/kg, were extrapolated from rifampicin pharmacokinetic data in adults. The initial pharmacokinetic parameters of vancomycin were derived from the study by De Beer et al.[21] The population pharmacokinetic model was validated with Monte Carlo analysis.[22,23] With the MW\PHARM program, 100 sets of 21 simulated patients were generated, based on our own original 21 patients. The individual and population pharmacokinetic parameters of these 2,100 simulated patients were calculated with iterative two-stage Bayesian fitting. The calculated individual pharmacokinetic parameters were compared with the "real" (generated) individual pharmacokinetic parameters and the bias (mean error) and the root mean square error (RMSE) were determined. The smaller the bias and the RMSE, the better the performance of the model. Also, the calculated population pharmacokinetic parameters of the 2,100 simulated patients were compared with the original population pharmacokinetic parameters.

We calculated peak (1 h after the beginning of the rifampicin infusion) and trough (just before the infusion)

 

plasma concentrations of rifampicin after the second dose of each neonate in MW\PHARM 3.60, using the individual pharmacokinetic parameters.

Statistical analysis was performed using SPSS 12.0 (Chicago, IL). $P < 0.05$ was considered to be statistically significant. The paired-samples *t* test was used to investigate the differences between the individual pharmacokinetic parameters at the beginning of the rifampicin therapy and the individual pharmacokinetic parameters after two weeks of rifampicin therapy. While it is known that statistical evaluation of estimates such as pharmacokinetic parameter values is not nearly as accurate as that of direct observations, nevertheless the paired-samples *t* test was used. To investigate the influence of several demographic, anthropometric, and clinical covariates on the individual pharmacokinetic parameters of rifampicin, we calculated Pearson product-moment correlation coefficients. The investigated covariates were: GA, PNA, PCA, BW, W, gender, P, AP1, AP5, 1/Cr, Ur, CRP, BC, pharmacokinetic parameters of vancomycin, and pharmacokinetic parameters of gentamicin of a preceding gentamicin treatment course. Gentamicin clearance is an ideal exogenous marker for estimation of the glomerular filtration rate in neonates, since gentamicin is eliminated almost entirely by the kidney and gentamicin clearance can easily be calculated during routine therapeutic drug monitoring.[24,25] One-way ANOVA was used to test if the factor variables (gender, P, and BC) had a significant effect on the individual pharmacokinetic parameters of rifampicin.

## RESULTS

### Plasma Concentrations

We measured plasma concentrations of rifampicin and 25-*O*-desacetylrifampicin in 123 plasma samples. These plasma concentrations are plotted against the time after rifampicin administration in Figure 1. Calculated rifampicin peak and trough plasma concentrations after the second dose were 4.66 ± 1.47 mg/L (range 2.06 to 6.87 mg/L) and 0.21 ± 0.20 mg/L (range 0.01 to 0.75 mg/L), respectively, after a dose of 8.5 ± 2.1 mg/kg. A significant linear relationship between rifampicin dose and peak plasma concentrations was found ($r = 0.556$, $P = 0.009$), but inter-patient variability was high (Figure 2).

Plasma concentrations of rifampicin and 25-*O*-desacetylrifampicin were also measured after 2 weeks of rifampicin therapy in 8 neonates (Figure 3). Both rifampicin and 25-*O*-desacetylrifampicin concentrations were lower after two weeks of rifampicin therapy.

### Population Pharmacokinetic Parameters

An example of a modelled concentration-time profile of a patient with iterative two-stage Bayesian fitting is shown in Figure 4. A plot of the actual measured concentrations versus the estimated concentrations with iterative two-stage Bayesian fitting is presented in Figure 5. Rifampicin first-order elimination constant ($K_{el}$), volume of distribution corrected for body weight (V/W), total body clearance corrected for body weight (CL/W), and elimination half-life (t½) were 0.16 ± 0.06 $h^{-1}$, 1.84 ± 0.59 L/kg, 0.28 ± 0.11 $Lkg^{-1}h^{-1}$, and 4.9 ± 1.7 h, respectively.

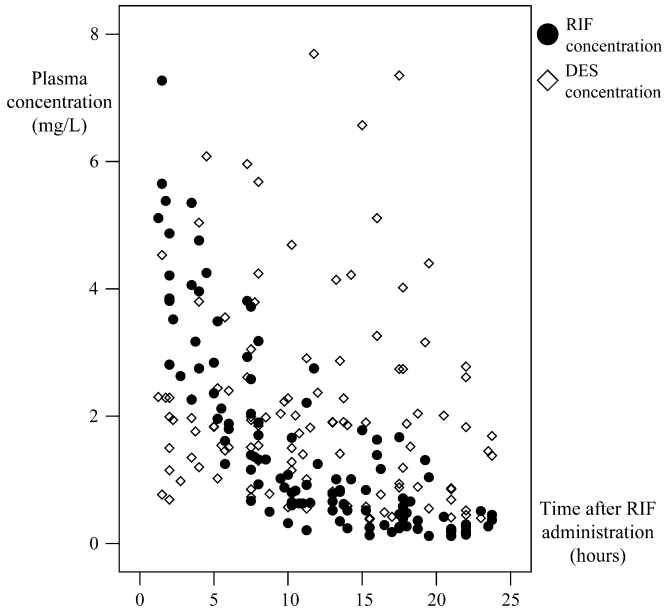


**FIGURE 1.** Plasma concentrations of rifampicin (RIF) and 25-*O*-desacetylrifampicin (DES) plotted against the time after rifampicin administration.

The population pharmacokinetic parameters of rifampicin, estimated with Monte Carlo analysis ($K_{el}$ 0.1600 ± 0.0562 $h^{-1}$ and V/W 1.7949 ± 0.5998 L/kg), were almost identical to the population pharmacokinetic parameters of our original population ($K_{el}$ 0.1592 ± 0.0570 $h^{-1}$ and V/W

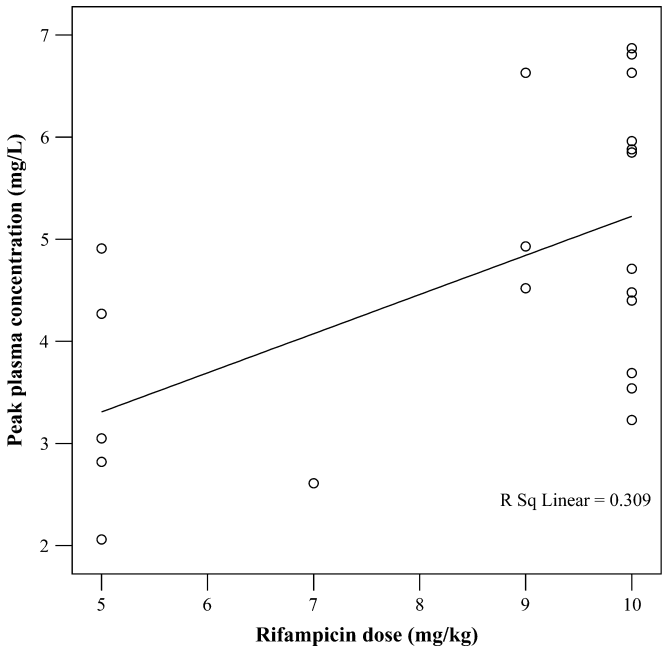


**FIGURE 2.** Rifampicin peak plasma concentrations after the second dose plotted against the rifampicin dose.

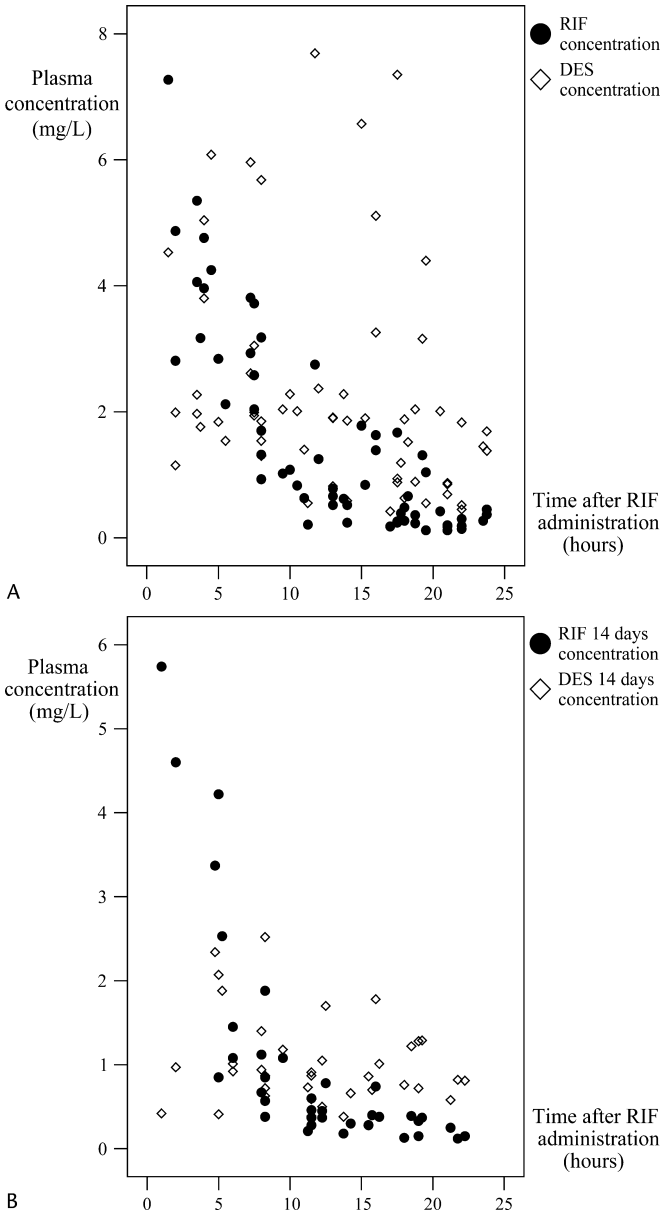


**FIGURE 3.** Plasma concentrations of rifampicin (RIF) and 25-*O*-desacetylrifampicin (DES) plotted against the time after rifampicin administration at the beginning of the rifampicin therapy (A) and after two weeks of therapy (B) in eight study patients.

1.8425 ± 0.5888 L/kg). The bias and the RMSE of the Monte Carlo analysis were low, 0.3 and 2.3%, respectively, for $K_{el}$ rifampicin and –1.6 and 4.3%, respectively, for V/W rifampicin.

We also calculated the individual pharmacokinetic parameters of rifampicin after 2 weeks of rifampicin therapy in 8 of the 21 neonates. Mean pharmacokinetic parameters at the beginning of the rifampicin therapy, mean pharmacokinetic parameters after two weeks of rifampicin therapy, and the results of the paired-samples *t* test of these eight neonates are presented in Table 2. The difference between CL/W rifampicin at the beginning and after 2 weeks of rifampicin therapy was

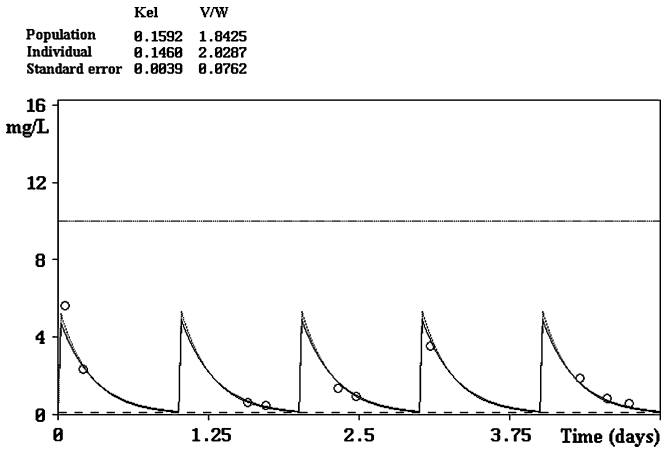


**FIGURE 4.** Example of a modelled concentration-time profile of a patient with iterative two-stage Bayesian fitting (MW\PHARM 3.60, Mediware, The Netherlands).

statistically significant ($P = 0.007$; n = 8). CL/W rifampicin increased significantly (67 ± 50%). These results suggest that CL/W rifampicin increases during the first two weeks of rifampicin therapy.

## Influence of Covariates on Individual Pharmacokinetic Parameters

We found statistically significant Pearson correlations between CL/W rifampicin and the covariates CL/W gentamicin, 1/Cr, GA, PCA, CL/W vancomycin, BW, and W (Table 3).

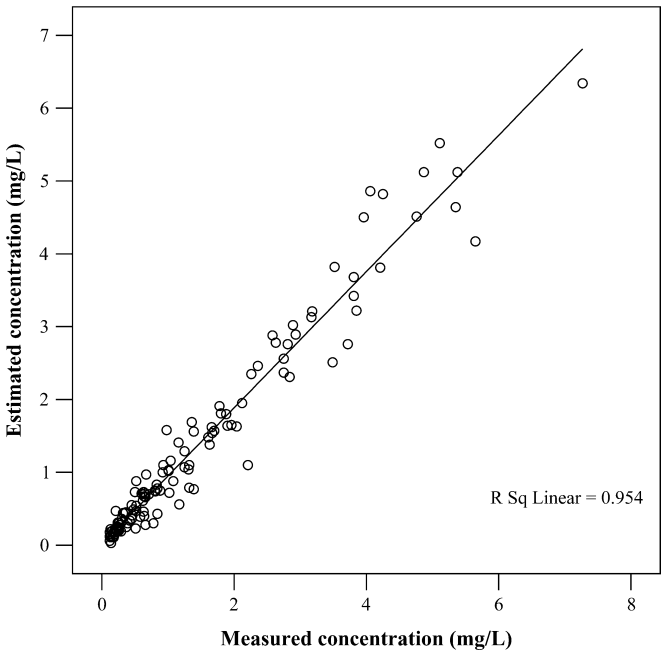


**FIGURE 5.** Correlation between rifampicin actual measured concentration and estimated concentration with iterative two-stage Bayesian fitting (MW\PHARM 3.60, Mediware, The Netherlands).

**TABLE 2.** Differences between Mean Pharmacokinetic Parameters at the Beginning and after Two Weeks of Rifampicin (RIF) Therapy (n = 8)

| Pharmacokinetic Parameter | Begin RIF Therapy | After Two Weeks RIF Therapy | Paired-samples *t* test |
|---|---|---|---|
| $K_{el}$ ($h^{-1}$) | 0.13 (±0.06) | 0.18 (±0.06) | −1.55 ($P = 0.165$) |
| V/W (L/kg) | 1.77 (±0.31) | 2.33 (±1.19) | −1.30 ($P = 0.236$) |
| CL/W ($Lkg^{-1}h^{-1}$) | 0.22 (±0.07) | 0.36 (±0.15) | −3.74 ($P = 0.007$) |
| t½ (h) | 6.1 (±1.9) | 4.4 (±1.7) | 1.91 ($P = 0.099$) |

CL, total body clearance; $K_{el}$, first-order elimination constant; t½, elimination half-life; V, volume of distribution; W, body weight.

The highest correlations were found between CL/W rifampicin and the covariates CL/W gentamicin and 1/Cr, $r = 0.837$ and $r = 0.728$, respectively (Figure 6).

## DISCUSSION

### Plasma Concentrations

We calculated rifampicin peak and trough plasma concentrations after the second dose of each neonate. Rifampicin peak and trough plasma concentrations after IV rifampicin administration have been investigated earlier in neonates. Tan et al[2] investigated peak and trough plasma concentrations in four neonates (GA 30 to 40 weeks, mean 35 weeks), who received 5 mg/kg rifampicin. Rifampicin peak concentration was 4.02 ± 1.22 mg/L (range 3.13 to 5.82 mg/L). Five of our 21 neonates also received a rifampicin dose of 5 mg/kg. Rifampicin peak concentration of these neonates was 3.42 ± 1.15 mg/L (range 2.06 to 4.91 mg/L). The plasma concentrations seem to be effective in the treatment of persistent staphylococcal bacteremia, since all blood cultures became sterile.

After two weeks of therapy rifampicin concentrations were lower than at the beginning of therapy. This might be explained by auto-induction of metabolism of the liver, which metabolizes rifampicin more rapidly to 25-*O*-desacetylrifampicin.[4] However, 25-*O*-desacetylrifampicin concentrations were also lower after two weeks of rifampicin therapy. Probably, the elimination of 25-*O*-desacetylrifampicin also increases during rifampicin therapy. Mouton et al[26] also found decreasing 25-*O*-desacetylrifampicin concentrations during therapy in adults. We did not observe any change in plasma creatinine after two weeks of rifampicin therapy. This suggests that the increase in the elimination of rifampicin and 25-*O*-desacetylrifampicin during rifampicin therapy is caused by an increase of non-renal clearance.

**TABLE 3.** Statistically Significant Pearson Correlations between the Individual Pharmacokinetic Parameters of Rifampicin (RIF) and the Demographic, Anthropometric, and Clinical Covariates

| | CL/W RIF | V/W RIF | $K_{el}$ RIF | t½ RIF |
|---|---|---|---|---|
| CL/W/V/W/$K_{el}$/t½ gentamicin (n = 12) | 0.837** | NS | NS | NS |
| 1/Cr (n = 17) | 0.728** | NS | 0.506* | −0.580* |
| GA (n = 21) | 0.600** | 0.468* | NS | NS |
| PCA (n = 21) | 0.557** | 0.477* | NS | NS |
| CL/W/V/W/$K_{el}$/t½ vancomycin (n = 21) | 0.552** | NS | NS | 0.508* |
| BW (n = 21) | 0.510* | 0.568** | NS | NS |
| W (n = 21) | 0.449* | 0.560** | NS | NS |

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).
BW, birth weight; CL, total body clearance; Cr, plasma creatinine level; GA, gestational age; $K_{el}$, first-order elimination constant; PCA, postconceptional age; t½, elimination half-life; V, volume of distribution; W, body weight; NS, not significant.

### Population Pharmacokinetic Parameters

Pharmacokinetic data of rifampicin are only available of older children and adults. Koup et al[12] and Nahata et al[11] both investigated the pharmacokinetics of IV rifampicin in older pediatric patients. Koup et al[12] studied 12 pediatric patients aged 3 months to 12.8 years (mean 4.6 years). $K_{el}$, V/W, CL/W, and t½ of rifampicin were 0.37 $h^{-1}$, 0.63 L/kg, 0.16 $Lkg^{-1}h^{-1}$, and 1.94 h, respectively. Nahata et al[11] investigated the pharmacokinetics of IV rifampicin in 9 pediatric patients aged 1 day to 18 years (mean 5.6 years), who received a single dose of rifampicin (20 mg/kg). $K_{el}$, V/W, CL/W, and t½ of rifampicin were 0.25 $h^{-1}$, 1.1 L/kg, 0.29 $Lkg^{-1}h^{-1}$, and 2.8 h, respectively. In the present study, V/W was higher. This difference is possibly due to a larger total body water compartment in neonates compared to infants and older children. In the present study, t½ was longer and $K_{el}$ lower. The elimination of rifampicin seems to be less efficient in neonates compared to infants and older children.

We compared the pharmacokinetic parameters of rifampicin at the beginning of the rifampicin therapy with the pharmacokinetic parameters after two weeks of therapy. Our results suggest that CL/W rifampicin increases during the first two weeks of rifampicin therapy. This has been found earlier by other investigators in children and adults.[9,12,14] Rifampicin induces its own metabolism (auto-induction) and, as a result, clearance increases markedly during the first two weeks of treatment.[4] In adults, equilibrium is reached after one to two weeks of treatment, after which no more changes occur.[27] Therefore, it seems prudent to consider adaptation of the rifampicin dose after two weeks of therapy in neonates. For future research, it would be useful to develop a one-compartment open model integrated with a correction for auto-induction in MW\PHARM.

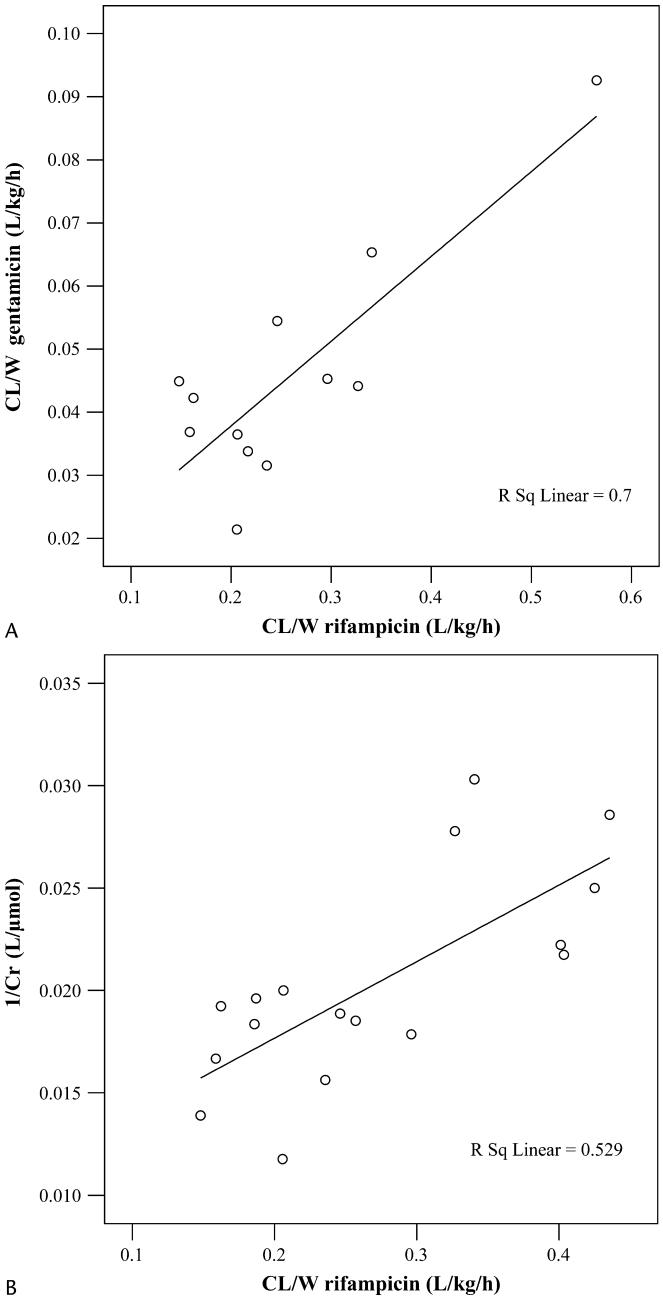


**FIGURE 6.** Correlation between total body clearance of rifampicin corrected for body weight (CL/W) and the covariates CL/W gentamicin (n = 12) (A) and 1/plasma creatinine (1/Cr) (n = 17) (B).

## Predictors of Individual Pharmacokinetic Parameters

We investigated the influence of several demographic, anthropometric, and clinical covariates on the individual pharmacokinetic parameters of rifampicin. The correlation between CL/W rifampicin and the covariates CL/W gentamicin and 1/Cr was high. About 70% of the inter-patient variance in CL/W rifampicin could be explained by CL/W gentamicin and more than 50% could be explained by 1/Cr. CL/W gentamicin and plasma creatinine are both predictors of the glomerular filtration rate in neonates.[24,25] Therefore, we conclude that glomerular filtration rate plays an important role in the elimination of rifampicin in neonates. Holdiness[28] reported that 37% of the rifampicin dose (10 mg/kg) was recovered in urine in the first 12 hours in neonates compared with 2.5% of the dose in older children. In each plasma sample, 25-*O*-desacetylrifampicin was found. Therefore, both non-renal clearance (metabolism) and renal clearance are involved in the elimination of rifampicin in neonates.

## Rifampicin Dosing

It is not possible to provide a definite dosing scheme for rifampicin in neonates at this stage. Information about effective pharmacodynamics of rifampicin, the role of protein binding, the free-fraction, and pharmacodynamic interaction with co-administered vancomycin or flucloxacillin is insufficient at this moment. For now, we suggest maintaining the current dose regimen of 10 mg/kg intravenously on a once-a-day schedule, until more information is available. The rifampicin plasma concentrations achieved with this dose seem to be effective in the treatment of persistent staphylococcal bacteremia, since all blood cultures became sterile. It may be appropriate to shorten the dosing interval from 24 to 12 h to avoid prolonged intervals of low plasma concentrations. Tan et al[2] and Shama et al[3] both found prompt clearance of persistent staphylococcal infections in neonates within only five days after the addition of rifampicin to vancomycin. In both studies, the neonates received rifampicin (5 mg/kg and 10 mg/kg, respectively) twice a day. More research is needed to determine well-founded dosing guidelines.

# CONCLUSIONS

We suggest maintaining the current rifampicin dose regimen of 10 mg/kg on a once-a-day schedule, until information about effective pharmacodynamics of rifampicin, the role of protein binding and the free-fraction, and pharmacodynamic interaction with co-administered vancomycin or flucloxacillin is available. Because of the large inter-patient variability in rifampicin plasma concentrations and in CL/W increase during rifampicin therapy, we suggest monitoring of peak and trough plasma concentrations to avoid low plasma concentrations. More pharmacokinetic work is required to give a solid base for neonatal rifampicin dose guidelines.

## REFERENCES

1. Janknegt R. The treatment of staphylococcal infections with special reference to pharmacokinetic, pharmacodynamic and pharmacoeconomic considerations. *Pharm World Sci*. 1997;19:133–141.
2. Tan TQ, Mason EO, Ou C-N, et al. Use of intravenous rifampin in neonates with persistent staphylococcal bacteremia. *Antimicrob Agents Chemother*. 1993;37:2401–2406.
3. Shama A, Patole SK, Whitehall JS. Intravenous rifampicin in neonates with persistent staphylococcal bacteraemia. *Acta Paediatr*. 2002;91:670–673.
4. Hey E, Hall C. *Neonatal Formulary*. 4 ed. London: Blackwell BMJ Books; 2003.
5. Young TE, Mangum B. *Neofax®: A Manual of Drugs Used in Neonatal Care*. 17 ed. Raleigh, North Carolina: Acorn Publishing; 2004.

6. Zinner SH, Lagast H, Klastersky J. Antistaphylococcal activity of rifampin with other antibiotics. *J Infect Dis*. 1981;144:365–371.
7. Acar JF, Goldstein FW, Duval J. Use of rifampin for the treatment of serious staphylococcal and gram-negative bacillary infections. *Rev Infect Dis*. 1983;5:502S–506S.
8. Mandell GL, Vest TK. Killing of intraleukocytic *Staphylococcus aureus* by rifampin: in-vitro and in-vivo studies. *J Infect Dis*. 1972;125:486–490.
9. Acocella G. Clinical Pharmacokinetics of Rifampicin. *Clin Pharmacokinet*. 1978;3:108–127.
10. Wehrli W. Rifampin: mechanisms of action and resistance. *Rev Infect Dis*. 1983;5:407S–411S.
11. Nahata MC, Fan-Havard P, Barson WJ. Pharmacokinetics, cerebrospinal fluid concentration, and safety of intravenous rifampin in pediatric patients undergoing shunt placements. *Eur J Clin Pharmacol*. 1990;38: 515–517.
12. Koup JR, Williams-Warren J, Weber A, et al. Pharmacokinetics of rifampin in children. I. Multiple dose intravenous infusion. *Ther Drug Monit*. 1986;8:11–16.
13. Nitti V, Virgilio R, Patricolo MR, et al. Pharmacokinetic study of intravenous rifampicin. *Chemotherapy.* 1977;23:1–6.
14. Loos U, Musch E, Jensen JC, et al. Pharmacokinetics of oral and intravenous rifampicin during chronic administration. *Klin Wochenschr*. 1985;63:1205–1211.
15. Peloquin CA, Jaresko GS, Yong C-L. Population pharmacokinetic modeling of isoniazid, rifampin, and pyrazinamide. *Antimicrob Agents Chemother*. 1997;41:2670–2679.
16. Chandi LS, Sijs van der IH, Guchelaar HJ. Bepaling van rifampicine en desacetylrifampicine [Determination of rifampicin and desacetylrifampicin]. *Ziekenhuisfarmacie*. 1998;14:71–72.
17. Dodge WF, Jelliffe RW, Zwischenberger JB, et al. Population pharmacokinetic models: effect of explicit versus assumed constant serum concentration assay error patterns upon parameter values of gentamicin in infants on and off extracorporeal membrane oxygenation. *Ther Drug Monit*. 1994;16:552–559.
18. US Food and Drug Administration. *Guidance for Industry: Bioanalytical Method Validation*. Rockville: Center for Drug Evaluation and Research (CDER); 2001.
19. Peloquin CA. Rifampin stability. *Ther Drug Monit*. 1998;20:450–451.
20. Proost JH, Meijer DKF. MW\PHARM, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. *Comput Biol Med*. 1992;22:155–163.
21. Beer de EFG, Stolk LML, Degraeuwe PLJ, et al. Pharmacokinetics of vancomycin in (pre)term neonates and influence of covariates. *Br J Clin Pharmacol*. 2002;54:555.
22. Mediware: Software for healthcare. Available at: http://www.mediware.nl.
23. Bonate PL. A brief introduction to Monte Carlo simulation. *Clin Pharmacokinet*. 2001;40:15–22.
24. Koren G, James A, Perlman M. A simple method for the estimation of glomerular filtration rate by gentamicin pharmacokinetics during routine drug monitoring in the newborn. *Clin Pharmacol Ther*. 1985; 38:680–685.
25. Sonntag J, Prankel B, Waltz S. Serum creatinine concentration, urinary creatinine excretion and creatinine clearance during the first 9 weeks in preterm infants with a birth weight below 1500 g. *Eur J Pediatr*. 1996;155: 815–819.
26. Mouton RP, Mattie H, Swart K, et al. Blood levels of rifampicin, desacetylrifampicin and isoniazid during combined therapy. *J Antimicrob Chemother.* 1979;5:447–454.
27. Acocella G. Pharmacokinetics and metabolism of rifampin in humans. *Rev Infect Dis*. 1983;5:428S–432S.
28. Holdiness MR. Clinical pharmacokinetics of the antituberculosis drugs. *Clin Pharmacokinet*. 1984;9:511–544.

 