Does Monitoring Total and Free Polymyxin B1 Plasma Concentrations Predict Polymyxin B-Induced Nephrotoxicity? A Retrospective Study in Critically Ill Patients

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ABSTRACT

Introduction: The correlation between total and free polymyxin B (PMB including PMB1 and PMB2) exposure in vivo and acute kidney injury (AKI) remains obscure. This study explores the relationships between plasma exposure of PMB1 and PMB2 and nephrotoxicity, and investigates the risk factors for PMB-induced acute kidney injury (AKI) in critically ill patients.

Methods: Critically ill patients who used PMB and met the criteria were enrolled. The total plasma concentration and plasma binding of PMB1 and PMB2 were analysed by liquid chromatography–tandem mass spectrometry and equilibrium dialysis.

Results: A total of 89 patients were finally included, and AKI developed in 28.1% of them. The peak concentration of PMB1 (C_{max}(B1)) (adjusted odds ratio (AOR) = 1.68, 95% CI 1.08–2.62, \( p = 0.023 \)), baseline BUN level (AOR = 1.08, 95% CI 1.01–1.16, \( p = 0.039 \)) and hypertension (AOR = 3.73, 95% CI 1.21–11.54, \( p = 0.022 \)) were independent risk factors for PMB-induced AKI. The area under the ROC curve of the model was 0.799. When C_{max}(B1) was 5.23 \( \mu g/ml \) or more, the probability of AKI was higher than 50%. The ratio of PMB1/PMB2 decreased after PMB preparation entered into the body. The protein binding rate in critically ill patients indicated significant individual differences. Free C_{max}(B) and free C_{max}(B1) levels in the AKI group were significantly (\( p < 0.05 \)) higher than those in the non-AKI group. Total and free concentrations of PMB in patients showed a positive correlation.

Conclusions: Both the ROC curve and logistic regression model showed that C_{max}(B1) was a good predictor for the probability of PMB-induced AKI. Early therapeutic drug monitoring (TDM) of PMB should be considered in critically ill patients. Compared with C_{min}(B), C_{max}(B) and C_{max}(B1) may be helpful for the...
early prediction of PMB-induced AKI in critically ill patients.

**Keywords:** Drug exposure; Free concentration; Independent risk factors; Nephrotoxicity; Polymyxin B1; Polymyxin B

### Key Summary Points

- Data for the relationship between total and free PMB (PMB1 and PMB2) exposure in vivo and nephrotoxicity is still insufficient.
- Total and free concentrations of PMB, PMB1 or PMB2 may be used to predict PMB-induced nephrotoxicity.
- A predicted probability of developing AKI of 50% corresponds to a $C_{\text{max}}(B1)$ of 5.23 $\mu$g/ml.
- Early therapeutic drug monitoring of PMB should be considered in critically ill patients. Compared with $C_{\text{min}}(B)$, $C_{\text{max}}(B)$ and $C_{\text{max}}(B1)$ may be helpful for the early prediction of PMB-induced AKI in critically ill patients.

### INTRODUCTION

The incidence of multidrug-resistant (MDR) gram-negative bacterial (GNB) infections has increased dramatically over the last decade, and such infections have emerged as a major challenge in global public health. The mortality rate of MDR-GNB infections is 40% or higher [1–3]. Novel antibiotic agents for the treatment of MDR-GNB infections are limited. Owing to its potential activity against MDR-GNB infection, the “old” drug polymyxin has been repurposed and recommended as a last-resort therapy for MDR-GNB infections [4, 5].

Polymyxin B (PMB) and colistin, which have similar pharmacologically active moieties, are two different agents in the polymyxin class. PMB is a fermented mixture of more than 30 components from *Paenibacillus polymyxa* [6]. Nephrotoxicity is the major dose-limiting factor impacting the clinical use of PMB. Previous studies have indicated that the incidence of nephrotoxicity is 20–60% after intravenous administration of PMB [6–11]. Colistin is administered intravenously as a prodrug colistimethate sodium, affected by the ratio of prodrug lost to renal elimination prior to activation. Some studies showed the incidence of AKI of colistin is 30–76.1% [12–14]. Azad et al. found that PMB can noticeably accumulate in the renal cortex, especially in proximal tubular cells, and induce renal tubular epithelial cell apoptosis or necrosis [15], which indicates that the nephrotoxicity of PMB might be related to drug exposure in vivo. For critically ill patients, there are noticeable individual differences in the pharmacokinetics of polymyxin B [7, 8, 16], and the dose of PMB might not represent the actual amount of the drug to which the patient is exposed. Polymyxin B1 (PMB1) and polymyxin B2 (PMB2) are the two major components of polymyxin B (PMB), accounting for more than 60% of the total weight, and have been used to characterize the effects of PMB exposure in vivo for therapeutic drug monitoring (TDM) [16, 17].

Previous studies have reported some risk factors for PMB-induced nephrotoxicity, such as age, baseline SCr (serum creatinine) level, body mass index (BMI), concomitant use of vasoactive drugs and vancomycin, infection site, duration of therapy, and a daily dose of 200 mg or more [7, 8, 18–22]; however, PMB exposure in vivo was not assessed in these previous studies. According to the international consensus [23], the magnitude of polymyxin (i.e. PMB and colistin) exposure is the most important risk factor for polymyxin-associated acute kidney injury. Previously, for PMB, only Han et al. [22] and Wang et al. [24] found that the plasma trough concentration ($C_{\text{min}}$) of PMB and an AUC_{ss,24h} of at least 100 mg·h/L of PMB were independent risk factors for PMB-induced nephrotoxicity, respectively. Notably, the comorbidity variables included in their studies were limited. Evidence for the relationship between PMB exposure and nephrotoxicity is still insufficient with a limited sample size.
Furthermore, whether PMB1 and PMB2, the primary constituents of PMB, are associated with nephrotoxicity has not yet been reported. On the other hand, only the unbound fraction ($f_u$) of the drug is pharmacologically active, as the protein-bound drug cannot reach the site of infection. The reported protein binding rate of PMB ranges from 58% to 92.4% [25, 26]. The variation in protein binding can affect the volume of distribution and clearance of PMB and, thus, its efficacy and toxicity [27]. Given that pharmacological activity depends on the unbound protein concentration rather than the total plasma concentration, determination of the free protein concentration is essential. However, currently available reports on TDM of PMB did not directly assess the free drug concentration. The relationship between the free drug concentration and renal toxicity is still unknown.

Therefore, the present study explores the relationship between exposure to total and free PMB (including PMB1 and PMB2) in the plasma and nephrotoxicity, and investigates the risk factors for PMB-induced nephrotoxicity in critically ill patients, providing possible reference data for clinical TDM.

METHODS

Patients and Data

A retrospective, observational cohort study was conducted at three general tertiary hospitals in China. As the central laboratory of the rational use of anti-infection agent’s technology demonstration base, the TDM laboratory of the Third Hospital of Changsha also undertook the TDM of polymyxin B for the other two hospitals. The Third Hospital of Changsha was the lead institution. The study was approved by the Ethics Research Committee of the Third Hospital of Changsha (No. CS3-KY-2021EC-008), and the requirement for written informed consent was waived. Ethics approval and the informed consent waiver were also accepted by the other two hospitals.

Critically ill patients (aged 18 years or more) with suspected or confirmed MDR-GNB infections who received intravenous injection of PMB sulfate (SPH No. 1, Biochemical and Pharmaceutical Co., Ltd. (Shanghai, China); PMB1/PMB2 ratio of approximately 5.18 ± 0.13) from May 1, 2019 to December 31, 2020 were enrolled. All patients received TDM of PMB. Patients were excluded if (a) they were undergoing haemodialysis and haemodiafiltration or extracorporeal membrane oxygenation; (b) they received PMB for a period of 48 h or less; or (c) the necessary medical data for the patient were lacking. The following medical information was collected: demographics, dosage regimen of PMB (including daily dosage, total dosage and duration days), comorbidities, type of infection, isolated microorganisms, concomitant drugs, duration of hospitalization, and laboratory test results. Creatinine clearance (CrCL) was calculated using the Cockcroft–Gault equation [28] and the CrCL before PMB treatment was used as an observed variable for PMB-induced AKI. The age-adjusted Charlson comorbidity index was used to evaluate the prognostic value of comorbidities [29, 30]. APACHE II scores were calculated on the basis of acute physiology measurements, age, and chronic health evaluation [31]. The definition of severe pneumonia was according to the 2019 ATS/IDSA Guidelines for the Diagnosis and Treatment of Adults with Community-Acquired Pneumonia [32].

PMB Administration and Sample Collection

Patient blood samples were collected at the sixth or later injection of PMB sulfate. The time for $C_{\text{max}}$ ($T_{\text{max}}$) of PMB1 and PMB2 was achieved immediately after the end of PMB infusion [17]. Considering the practical operability and the half-life of PMB, blood samples (2–3 ml) were collected 10 min after completion of PMB infusion to determine the $C_{\text{max}}$. In addition, the time of blood sample collection of trough PMB plasma concentration ($C_{\text{min}}$) was collected immediately before PMB injection [22, 33].

Blood samples were centrifuged immediately at 3500g and 2–8 °C for 10 min to obtain the plasma, and the plasma was collected in two
tubes (one for determination of total PMB1 and PMB2 levels and the other for determination of the plasma protein binding rates of PMB1 and PMB2) and then stored at –80°C until analysis.

Quantification of Total and Unbound PMB1 and PMB2 Concentrations

A validated liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method was used to quantify PMB1 and PMB2 levels at the TDM laboratory of the Third Hospital of Changsha. Reference standards of PMB1 sulfate (purity 94.078%) and PMB2 sulfate (purity 99.358%) used for the preparation of calibration standards and quality control samples were obtained from TOKU-E (Bellingham, WA, USA). A Shim-pack GIST C18 column (2.1 × 100 mm, 3 μm, Shimadzu, Japan) was used for liquid chromatographic separation. The mobile phase included solvent A (0.1% formic acid in water) and solvent B (acetonitrile). The following gradient elution was performed at a total flow rate of 0.4 ml/min for analyte elution: 5% B for 0.5 min, 5–50% B for 3.0 min, 50% to 60% for 2.0 min and 5% B for 4.5 min. The temperature of the autosampler was maintained at 6°C. The HPLC system was combined with a Shimadzu LC MS-8050 mass spectrometer and performed with multiple reaction monitoring (MRM) in positive ionization mode with an m/z of 402.1 → 101.15 (PMB1) and 397.4 → 101.15 (PMB2). The PMB concentration was calculated using the following equation:

\[ C(B) = \frac{C(B_1)/M(B_1) + C(B_2)/M(B_2)}{M(B)} \]  

where \( C \) represents the concentration and \( M \) is the molar mass.

For total PMB1 and PMB2 concentration analysis, 20 μl polymyxin E solution (2.66 μg/ml) was added to 100 μl of human plasma sample and vortexed for 30 s. Human plasma was extracted with 20 μl formic acid solution and 280 μl acetonitrile. After the extract was mixed for 2 min and centrifuged at 15,000 rpm at 4°C for 10 min, 150 μl supernatant was collected carefully and resuspended in 150 μl purified water. A 2-μl aliquot of this solution was injected into the LC-MS/MS system for analysis. The free PMB1 and PMB2 concentrations were analysed by equilibrium dialysis. The 48-well plate equilibrium dialysis plate was assembled with a semipermeable membrane (Thermo Fisher Scientific, Waltham, MA, USA) separating two reservoirs of the cell unit. The semipermeable membrane is selective for high molecular polymers, i.e., only small molecular substances can pass through the membrane while proteins and cells are excluded. Considering the adequacy of dialysis and stability of PMB in whole blood for 6 h at 37°C determined in our previous study [34], the plasma samples were dialyzed against isotonic phosphate buffer (pH 7.4) at 37°C for 5 h. For dialysis samples, 50 μl of blank plasma or phosphate buffer was added to the corresponding sample and processed as described above. The fraction of the plasma \( f_u \) value was calculated using the following equation:

\[ f_u = \frac{C_d}{C_p} \times 100 \]  

where \( C_d \) represents the concentration in the dialysate after completion of dialysis and \( C_p \) is the corresponding concentration in the plasma (μg/ml).

The calibration curves showed acceptable linearity over 0.033–18.816 μg/ml for PMB1 and 0.034–19.872 μg/ml for PMB2. The interday and intraday precision were less than 12% and less than 9%, respectively. The accuracy was 99.8–110.4%.

Nephrotoxicity Definition

Acute kidney injury (AKI) was studied to assess PMB-induced nephrotoxicity. RIFLE criteria [35] were used to define AKI in this study according to SCr. Increased SCr × 1.5 was defined as risk stage of renal function; increased SCr × 2 was defined as injury; increased SCr × 3 was defined as failure. Persistent acute renal failure or loss of function for more than 4 weeks was defined as loss. Patients who met this criterion were defined as the AKI group. Assessment of SCr levels was performed at baseline, during PMB treatment, and at the end of PMB treatment.
Data Analysis

Statistical analysis was performed using R version 4.1.1 (R Foundation for Statistical Computing; Vienna, Austria; ISBN 3-000051-07-0, http://www.R-project.org) and SPSS for Windows (version 22.0). Continuous variables are presented as the mean ± standard deviation (SD) if the data were normally distributed and were compared using Student’s t tests. The median and interquartile range (IQR) are presented for non-normally distributed data, and the Mann–Whitney U test was used. Categorical variables are expressed as counts and percentages, and the chi-square test or Fisher’s exact test was used. Spearman’s rank correlation coefficient (r) was used to analyse the correlation between the total concentrations and free concentrations of PMB, PMB1 and PMB2. Univariate analysis was performed for all variables to identify possible risk factors for AKI. Variables found to be statistically significant (p < 0.05) were entered into the multivariate logistic regression models. A forward stepwise (likelihood ratio) method was used to determine the risk factors. Adjusted odds ratios (AOR), corresponding two-sided 95% confidence intervals (CIs) and p values are presented for the final logistic model. The Hosmer–Lemeshow test was used to determine the fitness of the model. The variance inflation factor (VIF) was used to test for multicollinearity among the risk factors in the final logistic models to ensure the independence of each variable. VIF values greater than 4.0 were considered to indicate an interaction among predictors [36]. A receiver operating characteristic (ROC) curve was drawn to evaluate the discriminatory power of the factors in the final multivariate logistic regression model. Discriminatory power was used as a measure of the model’s ability to distinguish between patients with AKI and without AKI. A p value less than 0.05 was considered statistically significant.

RESULTS

Characteristics

A total of 117 patients were included in the study. Twenty-eight patients were excluded; 11 patients had a PMB therapy duration less than 48 h, and necessary medical data was missing for 17 patients. Thus, 89 patients met the inclusion criteria (Fig. 1). The demographic characteristics of all patients are summarized in Table 1. The average APACHE II score of patients was 19.1 ± 7.4. The most common pathogenic bacteria were Acinetobacter baumannii (N = 59; 66.3%), followed by Klebsiella pneumoniae (N = 31; 34.8%) and Pseudomonas aeruginosa (N = 22; 24.7%). Sepsis (N = 26; 29.2%) and lung infection (N = 54; 60.7%) were the main types of infection. Seventeen patients had chronic renal dysfunction.

Total and Free Concentrations

The total and free concentrations of PMB1, PMB2 and PMB are shown in Table 3. The median protein binding rates of PMB, PMB1 and PMB2 in patients were 91.8% (range 65.7–96.0%), 95.1% (range 61.7–98.2%) and 82.8% (range 59.2–95.5%), respectively. Spearman’s rank correlation analysis showed that the total concentrations of PMB, PMB1 and PMB2 were positively correlated with their free concentrations (Fig. 2).

The ratio of the Cmin of PMB1 (Cmin (B1)) to the Cmin of PMB2 (Cmin (B2)) in patients ranged from 1.53 to 6.76, and the ratio of the Cmax of PMB1 (Cmax (B1)) to the Cmax of PMB2 (Cmax (B2)) ranged from 1.48 to 4.45. Only two patients had a PMB1/PMB2 ratio within the range of 5.18 ± 20% (the ratio of PMB1/PMB2 in the preparation was ± 20%). The concentrations of PMB1 and PMB2 in patient plasma are shown in Fig. 3.
AKI Analysis

According to the RIFLE criteria, 25 (28.1%) patients experienced AKI. The characteristics of the AKI group (N = 25) and the non-AKI group (N = 64) were analysed (Table 2). Patients in the AKI group were more likely to suffer from hypertension (p = 0.020), type 2 diabetes (p = 0.025) and chronic renal dysfunction (p = 0.011) group and had higher baseline BUN levels (p = 0.009) than patients in the non-AKI. The plasma concentrations (C\text{min} and C\text{max}) of PMB, PMB1 and PMB2 (p < 0.05) and the free C\text{max} (B) and free C\text{max} (B1) were significantly higher (p < 0.05) in the AKI group than in the non-AKI group (Table 3). On the basis of these results, a total of 12 possible risk factors were identified by the univariate analysis (Tables 2 and 3).

These 12 variables (p < 0.05) were used to develop a multivariate logistic regression model. Finally, C\text{max} (B1) (AOR = 1.68, 95% CI 1.08–2.62, p = 0.023), the baseline BUN level (AOR = 1.08, 95% CI 1.01–1.16, p = 0.039) and hypertension (AOR = 3.73, 95% CI 1.21–11.54, p = 0.022) were included in the final model (Table 4). The predicted probability of AKI was calculated using the logistic function probability = 1 / (1 + e^{-\beta}), where \beta represents the summation of the model constant and the covariates. Therefore, the equation of the final obtained prediction model was

\[
\text{Probability (AKI)} = \frac{1}{1 + \exp(4.514 - 0.519 \times C\text{max}(B1) - 0.075 \times \text{baseline BUN} - 1.318 \times \text{hypertension})}
\]

The Hosmer–Lemeshow test showed that the model had favourable fitness (p = 0.60). The VIF values of the predictor variables in this model were all less than 1.2, indicating no multicollinearity. A ROC curve was used to calculate the discriminatory power of the model (Fig. 4). Figure 5 depicts that the model shows better discriminatory power for PMB-induced AKI than other single factors. The AUCs were as follows: model (0.799) > baseline BUN level (0.683) > C\text{max} (B1) (0.680) > C\text{max} (B) (0.674) > C\text{min} (B) (0.666).

In addition, a univariate logistic regression was developed to determine the correlation between the C\text{max} (B1) and the probability of developing AKI (Fig. 6). The results showed that

\[\]]
Table 1 Characteristics of patients

| Characteristics                              | Value               |
|----------------------------------------------|---------------------|
| **Demographics**                             |                     |
| Age (years) (mean ± SD)                       | 60.7 ± 15.1         |
| Gender (male) (n (%))                         | 63 (70.8%)          |
| Weight (kg)                                  | 55.0 (50.0–61.0)    |
| APACHE II scores                             | 19.1 ± 7.4          |
| Hospitalization days                         | 27 (21–35)          |
| **Comorbidities**                             |                     |
| ACCI (median (IQR))                           | 3 (1–4)             |
| Sepsis (n (%))                                | 26 (29.2%)          |
| Severe pneumonia (n (%))                      | 26 (29.2%)          |
| Sepsis + severe pneumonia (n (%))             | 12 (13.5%)          |
| Lung infection (n (%))                        | 54 (60.7%)          |
| Respiratory failure (n (%))                   | 18 (20.2%)          |
| Anaemia (n (%))                               | 12 (13.5%)          |
| Coronary heart disease (n (%))                | 22 (24.7%)          |
| Hypertension (n (%))                          | 43 (48.3%)          |
| Type 2 diabetes (n (%))                       | 13 (14.6%)          |
| Chronic liver disease (n (%))                 | 15 (16.9%)          |
| Chronic renal dysfunction (n (%))             | 17 (19.1%)          |
| Cerebral infarction (n (%))                   | 20 (22.5%)          |
| **Pathogenic bacteria**                       |                     |
| *Acinetobacter baumannii* (n (%))            | 59 (66.3%)          |
| *Klebsiella pneumoniae* (n (%))               | 31 (34.8%)          |
| *Pseudomonas aeruginosa* (n (%))              | 22 (24.7%)          |
| *Escherichia coli* (n (%))                    | 5 (5.6%)            |
| *Stenotrophomonas maltophilia* (n (%))        | 7 (7.9%)            |
| *Candida* (n (%))                             | 23 (25.8%)          |
| **PMB treatment**                             |                     |
| PMB daily dosage (mg) (median (IQR))          | 100 (100–100)       |

Table 1 continued

| Characteristics                              | Value               |
|----------------------------------------------|---------------------|
| PMB total dosage (mg) (median (IQR))         | 1000 (700–1300)     |
| PMB duration (days) (median (IQR))           | 10 (7–12)           |
| PMB monotherapy (n (%))                      | 14 (15.7%)          |
| Concomitant with nephrotoxic drugs (n (%))   | 35 (39.3%)          |
| Concomitant with β-lactam (n (%))            | 45 (50.6%)          |
| Carbapenem (n (%))                           | 62 (69.7%)          |
| Tigecycline (n (%))                          | 23 (25.8%)          |
| **Laboratory examination (before PMB treatment)** |               |
| WBC (× 10^9/L) (median (IQR))               | 10.9 (6.8–14.0)     |
| Percentage of neutrophils (%)                | 80.4 (71.2–86.1)    |
| Albumin (g/L) (median (IQR))                 | 32.4 (29.6–35.6)    |
| Serum creatinine (μmol/L) (median (IQR))     | 82.6 (57.7–124.0)   |
| BUN (mmol/L) (median (IQR))                  | 11.5 (7.3–18.4)     |
| Procalcitonin (ng/ml) (median (IQR))         | 1.2 (0.2–4.4)       |
| HCRP (mg/L) (median (IQR))                   | 96.0 (54.0–123.0)   |
| CrCL (ml/min)                                | 58.1 (36.4–86.0)    |

*APACHE* Acute Physiology and Chronic Health Evaluation, *ACCI* age-adjusted Charlson comorbidity index, *PMB* polymyxin B, *WBC* white blood cell count, *BUN* blood urea nitrogen, *HCRP* high-sensitivity C-reactive protein, *CrCL* creatinine clearance, *IQR* interquartile range

aFrom the clinic diagnosis of electronic medical records

bIncludes vancomycin, sulfamethoxazole, gentamicin, amikacin, ciprofloxacin and levofloxacin in this study
The \( C_{\text{max}} (B1) \) (OR = 1.68, 95% CI 1.15–2.47, \( p = 0.008 \)) was a significant predictor of AKI and that the predicted probability of AKI was higher than 50% when the \( C_{\text{max}} (B1) \) was 5.23 \( \mu g/ml \) or higher according to Eq. (4).

\[
\text{Probability (AKI)} = \frac{1}{1 + \exp(2.723 - 0.521 \times C_{\text{max}}(B1))}
\]

In addition, the relationship between the \( C_{\text{max}} (B1) \) and the severity of AKI was assessed. Patients with AKI were stratified according to the RIFLE criteria (Table 5). Only one patient was classified as “failure”. To compare the difference of \( C_{\text{max}} (B1) \) among groups of “risk” and “injury”, a Student’s \( t \) test was performed. The result showed there was no significant difference between the “risk” group and the “injury” group (\( p = 0.52 \)), indicating that the \( C_{\text{max}} (B1) \) was not associated with the severity of AKI in this study.
| Characteristics                          | Non-AKI group (N = 64) | AKI group (N = 25) | p value |
|----------------------------------------|------------------------|-------------------|---------|
| Demographics                           |                        |                   |         |
| Age (years) (mean ± SD)                | 60.6 ± 14.8            | 61 ± 16.2         | 0.91    |
| Gender (male) (n (%))                  | 46 (71.9%)             | 17 (68.0%)        | 0.53    |
| Weight (kg) (median (IQR))             | 55.0 (49.5–60.3)       | 60.0 (52.0–61.0)  | 0.40    |
| APACHE II scores                       | 18.7 ± 7.1             | 19.9 ± 8.1        | 0.54    |
| Hospitalization days (median (IQR))    | 28 (19–36)             | 27 (24–33)        | 0.71    |
| Comorbidities*                         |                        |                   |         |
| ACCI (median (IQR))                    | 3 (1–4)                | 3 (2–5)           | 0.32    |
| Sepsis (n (%))                         | 16 (25.0%)             | 10 (40.0%)        | 0.16    |
| Severe pneumonia (n (%))               | 19 (29.7%)             | 7 (28.0%)         | 0.88    |
| Sepsis + severe pneumonia (n (%))      | 8 (12.5%)              | 4 (16.0%)         | 0.93    |
| Lung infection (n (%))                 | 37 (57.8%)             | 17 (68.0%)        | 0.43    |
| Respiratory failure (n (%))            | 15 (23.4%)             | 3 (12.0%)         | 0.36    |
| Anaemia (n (%))                        | 7 (10.9%)              | 5 (20.0%)         | 0.44    |
| Coronary heart disease (n (%))         | 15 (23.4%)             | 7 (28.0%)         | 0.65    |
| Hypertension (n (%))                   | 26 (40.6%)             | 17 (68.0%)        | 0.02*   |
| Type 2 diabetes (n (%))                | 6 (9.4%)               | 7 (28.0%)         | 0.03*   |
| Chronic liver disease (n (%))          | 9 (14.1%)              | 6 (24.0%)         | 0.26    |
| Chronic renal dysfunction (n (%))      | 8 (12.5%)              | 9 (36.0%)         | 0.01*   |
| Cerebral infarction (n (%))            | 14 (21.9%)             | 6 (24.0%)         | 0.83    |
| Pathogenic bacteria                    |                        |                   |         |
| *Acinetobacter baumannii (n (%))       | 41 (64.1%)             | 18 (72.0%)        | 0.48    |
| *Klebsiella pneumoniae (n (%))         | 23 (35.9%)             | 8 (32.0%)         | 0.73    |
| *Pseudomonas aeruginosa (n (%))        | 17 (26.6%)             | 5 (20.0%)         | 0.52    |
| *Escherichia coli (n (%))              | 4 (4.7%)               | 1 (4.0%)          | 1.00    |
| *Stenotrophomonas maltophilia (n (%))  | 6 (9.4%)               | 1 (4.0%)          | 0.68    |
| *Candida (n (%))                       | 20 (31.3%)             | 3 (12.0%)         | 0.11    |
| PMB treatment                          |                        |                   |         |
| PMB daily dosage (mg) (median (IQR))   | 100 (100–100)          | 100 (100–100)     | 0.90    |
| PMB total dosage (mg) (median (IQR))   | 1050 (780–1400)        | 900 (650–1100)    | 0.23    |
| PMB duration (days) (median (IQR))     | 11 (7–13)              | 9 (8–11)          | 0.32    |
| PMB monotherapy (n (%))                | 9 (14.1%)              | 5 (20.0%)         | 0.71    |

*p < 0.05"
The present study demonstrated that the $C_{\text{max}}(B1)$, hypertension and baseline BUN levels were independent risk factors for AKI development during PMB treatment in critically ill patients. To our knowledge, this is the first report showing that $C_{\text{max}}(B1)$ is related to PMB-induced nephrotoxicity. Furthermore, we were the first to explore the correlation between the free concentration of PMB ($\text{PMB1, PMB2}$) and PMB-induced nephrotoxicity in critically ill patients, and we found that the free $C_{\text{max}}(B)$ and free $C_{\text{max}}(B1)$ in the AKI group were significantly higher than those in the non-AKI group.

The ultrafiltration tube method and equilibrium dialysis are the two commonly used methods for determining free concentrations. The results of the ultrafiltration tube method are affected by multiple factors, such as temperature, centrifugal force, pH and the ultrafiltration membrane [37, 38]. Equilibrium dialysis is based on drug diffusion across a semipermeable membrane that separates the sample to be investigated from a buffer solution and is still considered the gold standard for monitoring free drug concentrations [39]. Equilibrium dialysis was adopted in our study, and the results showed that the median protein binding rates of PMB, PMB1 and PMB2 were 91.7% (range 65.7–96.0%), 95.2% (range 61.7–98.2%) and 82.7% (range 59.2–95.5%), respectively. It was reported that the plasma protein binding rate of PMB in eight critically ill patients (range 78.5–92.4%) was higher than that in healthy

Table 2 continued

| Characteristics | Non-AKI group ($N = 64$) | AKI group ($N = 25$) | $p$ value |
|-----------------|--------------------------|---------------------|-----------|
| Concomitant with nephrotoxic drug$^b$ ($n$ (%)) | 24 (37.5%) | 11 (44.0%) | 0.57 |
| Concomitant with $\beta$-lactam ($n$ (%)) | 33 (51.6%) | 12 (48.0%) | 0.76 |
| Carbapenem ($n$ (%)) | 47 (73.4%) | 15 (60.0%) | 0.22 |
| Tigecycline ($n$ (%)) | 16 (25.0%) | 7 (28.0%) | 0.77 |
| Laboratory examination (before PMB treatment) | | | |
| WBC ($\times 10^9$/L) (median (IQR)) | 10.2 (6.7–13.9) | 12.8 (7.6–14.2) | 0.29 |
| Percentage of neutrophils (%) | 80.0 (70.8–87.1) | 81.0 (75.4–85.0) | 0.06 |
| Albumin (g/L) (median (IQR)) | 32.7 (30.2–36.3) | 32.1 (28.5–35.0) | 0.32 |
| Serum creatinine (\text{\mu}mol/L) (median (IQR)) | 92.9 (60.9–127.1) | 73.9 (51.9–102.5) | 0.13 |
| BUN (mmol/L) (median (IQR)) | 10.8 (7.3–14.5) | 18.7 (12.6–23.8) | 0.01* |
| Procalcitonin (ng/ml) (median (IQR)) | 1.3 (0.2–5.2) | 1.3 (0.7–3.7) | 0.89 |
| HCRP (mg/L) (median (IQR)) | 92.0 (42.5–119.0) | 101 (81.7–133) | 0.25 |
| CrCL (ml/min) | 53.6 (36.7–81.7) | 77.4 (34.1–104.3) | 0.15 |

$APACHE$ Acute Physiology and Chronic Health Evaluation, $ACCI$ age-adjusted Charlson comorbidity index, $PMB$ polymyxin B, $WBC$ white blood cell count, $BUN$ blood urea nitrogen, $HCRP$ high-sensitivity C-reactive protein, $CrCL$ creatinine clearance, $IQR$ interquartile range

$^a$From the clinic diagnosis of electronic medical records

$^b$Includes vancomycin, sulfamethoxazole, gentamicin, amikacin, ciprofloxacin and levofloxacin in this study

*Denotes $p < 0.05$, differences between the AKI group and the non-AKI group were tested for statistical significance

DISCUSSION

The present study demonstrated that the $C_{\text{max}}(B1)$, hypertension and baseline BUN levels were independent risk factors for AKI development during PMB treatment in critically ill patients. To our knowledge, this is the first report showing that $C_{\text{max}}(B1)$ is related to PMB-induced nephrotoxicity. Furthermore, we were the first to explore the correlation between the free concentration of PMB (PMB1, PMB2) and PMB-induced nephrotoxicity in critically ill patients, and we found that the free $C_{\text{max}}(B)$ and free $C_{\text{max}}(B1)$ in the AKI group were significantly higher than those in the non-AKI group.

The ultrafiltration tube method and equilibrium dialysis are the two commonly used
Table 3  Total concentration and free concentration of PMB in patients’ plasma

|                  | All \((N = 89)\) | Non-AKI group \((N = 64)\) | AKI group \((N = 25)\) | \(p\) value |
|------------------|-----------------|--------------------------|---------------------|-------------|
| **Total concentration (median (IQR))** |                 |                          |                     |             |
| \(C_{\text{min}}\) (B) \((\mu g/ml)\) | 1.39 (0.97–1.91) | 1.25 (0.92–1.87)          | 1.70 (1.39–1.94)    | 0.02*       |
| \(C_{\text{max}}\) (B) \((\mu g/ml)\) | 3.99 (3.01–5.18) | 3.82 (2.92–4.84)          | 4.55 (3.73–5.96)    | 0.01*       |
| \(C_{\text{max}}\) (B1) \((\mu g/ml)\) | 3.07 (2.30–3.82) | 2.89 (2.22–3.68)          | 3.45 (3.00–4.50)    | 0.01*       |
| \(C_{\text{max}}\) (B2) \((\mu g/ml)\) | 0.91 (0.75–1.24) | 0.89 (0.69–1.17)          | 0.99 (0.85–1.44)    | 0.04*       |
| \(C_{\text{min}}\) (B)/\(C_{\text{min}}\) (B2) | 3.28 (2.90–3.66) | 3.27 (2.91–3.65)          | 3.37 (2.85–3.68)    | 0.98        |
| \(C_{\text{max}}\) (B1)/\(C_{\text{max}}\) (B2) | 3.27 (3.02–3.70) | 3.23 (2.97–3.69)          | 3.46 (3.13–3.78)    | 0.08        |
| **Protein binding rate (median (IQR))** |                 |                          |                     |             |
| \(C_{\text{min}}\) (B) (%) | 89.7 (83.4–92.7) | 88.6 (83.4–92.1)          | 90.1 (84.5–93.2)    | 0.21        |
| \(C_{\text{min}}\) (B1) (%) | 94.5 (88.9–96.1) | 94.3 (88.7–96.1)          | 94.7 (89.6–96.1)    | 0.58        |
| \(C_{\text{min}}\) (B2) (%) | 78.5 (73.3–83.0) | 77.9 (71.6–81.9)          | 80.6 (76.1–85.1)    | 0.10        |
| \(C_{\text{max}}\) (B) (%) | 92.7 (91.1–93.7) | 92.4 (91.0–93.7)          | 92.9 (91.1–93.8)    | 0.43        |
| \(C_{\text{max}}\) (B1) (%) | 95.6 (94.3–96.5) | 95.8 (94.3–96.5)          | 95.5 (94.3–96.3)    | 0.80        |
| \(C_{\text{max}}\) (B2) (%) | 84.4 (82.2–86.4) | 83.9 (82.1–86.0)          | 85.9 (83.2–87.1)    | 0.10        |
| **Free concentration (median (IQR))** |                 |                          |                     |             |
| Free \(C_{\text{min}}\) (B) \((\mu g/ml)\) | 0.16 (0.12–0.23) | 0.16 (0.12–0.21)          | 0.14 (0.11–0.35)    | 0.54        |
| Free \(C_{\text{min}}\) (B1) \((\mu g/ml)\) | 0.07 (0.04–0.11) | 0.07 (0.04–0.10)          | 0.05 (0.04–0.19)    | 0.51        |
| Free \(C_{\text{min}}\) (B2) \((\mu g/ml)\) | 0.07 (0.05–0.10) | 0.07 (0.05–0.10)          | 0.07 (0.06–0.09)    | 0.69        |
| Free \(C_{\text{max}}\) (B) \((\mu g/ml)\) | 0.31 (0.23–0.38) | 0.29 (0.22–0.37)          | 0.33 (0.29–0.47)    | 0.02*       |
| Free \(C_{\text{max}}\) (B1) \((\mu g/ml)\) | 0.14 (0.10–0.20) | 0.14 (0.11–0.18)          | 0.19 (0.12–0.26)    | 0.02*       |
| Free \(C_{\text{max}}\) (B2) \((\mu g/ml)\) | 0.15 (0.12–0.19) | 0.14 (0.11–0.18)          | 0.16 (0.14–0.19)    | 0.13        |

\(C_{\text{min}}\) trough concentration, \(C_{\text{max}}\) peak concentration, B polymyxin B, B1 polymyxin B1, B2 polymyxin B2

*Denotes \(p < 0.05\), differences between the AKI group and the non-AKI group were tested for statistical significance
humans (55.9% ± 4.7%) [26], which is consistent with our study. However, Sandri and colleagues found that the median protein binding rate in 23 critically ill patients was 58% (36–74%) [25], which is quite different from that observed in our study. Special pathophysiological and iatrogenic factors in critically ill patients can affect protein concentrations by altering synthesis and catabolism or promoting protein movement from the plasma to extravascular sites [40]. Alterations in protein levels vary significantly among individuals and might result in variability in the free concentration of PMB in critically ill patients. Total concentrations and published protein binding values are usually used to predict the unbound drug concentration in clinical practice in general. However, the measured total concentration is not an adequate surrogate for the free concentration in some antibiotics studies [41, 42]. In the present study, a positive correlation between the total and free concentrations was found (Fig. 2). We attempted to use the clinical data of patients to develop a multivariate linear regression equation (stepwise regression) to predict the free concentration. However, these variables were not included in the final equation. Furthermore, although the free $C_{\text{max}}$ (B1) was not independently associated with AKI in this study, the significant difference between the two groups in the univariate analysis (Table 3) suggests that the free $C_{\text{max}}$ (B) and $C_{\text{max}}$ (B1) may also be associated with AKI. Our results indicated that monitoring of free drug concentrations should be considered in the management of critically ill patients administered PMB.

In the present study, we preliminarily analysed the in vivo PMB1/PMB2 ratio. The PMB1/PMB2 $C_{\text{min}}$ ratio and PMB1/PMB2 $C_{\text{max}}$ ratio in patients ranged from 1.53 to 6.76 and from 1.48 to 4.44, respectively. The ratio of PMB1/PMB2 was less than 80% of that in the preparation in most patients and exhibited noticeable individual differences (Fig. 3). The PMB1/PMB2 ratio in the plasma was related not only to the ratio in the preparation but also to PK parameters such as volume of distribution ($V_d$) and $t_{1/2}$. Reports on the PK parameters of PMB1 and PMB2 in patients are limited. Wang et al. reported the PK parameters of PMB1 and PMB2 in 15 patients, and a significant interindividual difference in $V_d$ was observed [16]. $V_d$ is influenced by plasma protein binding [43]. For PMB, there were significant individual differences in the plasma protein binding rate among patients [25, 26]. We speculated that interindividual differences in the PK parameters of PMB1 and PMB2 in different populations could be a reasonable explanation for the discrepancy. Considering that $C_{\text{max}}$ (B1) was independently related to AKI, TDM of PMB1 and PMB2 should be warranted.

The clinical risk factors for PMB-induced nephrotoxicity reported in previous studies include age, baseline SCr levels, body mass index (BMI), concomitant use of medications such as vasoactive drugs and vancomycin, and the infection site [18–22]. Notably, the identification of risk factors had yielded mixed results from different studies. For example, Mendes et al. [19] and Han et al. [22] found that the baseline SCr level is a risk factor for PMB-induced nephrotoxicity. However, three other reports [21, 44, 45] involving critically ill patients indicated that the baseline SCr level was not associated with PMB-induced nephrotoxicity. This difference might be related to differences in the severity of illness in the patients included in the different studies. For instance, the in-hospital mortality rate (61.4%) of patients in the study by Mendes et al. [19] was higher than that (23–42%) of patients in the other three studies [21, 44, 45] that reported

| Risk factors   | Adjusted OR | 95% CI       | p value |
|----------------|-------------|--------------|---------|
| $C_{\text{max}}$ (B1) | 1.68        | 1.08–2.62    | 0.023*  |
| Baseline BUN   | 1.08        | 1.01–1.16    | 0.039*  |
| Hypertension   | 3.73        | 1.21–11.54   | 0.022*  |

$BUN$ blood Urea nitrogen, $C_{\text{max}}$ (B1) peak concentration of polymyxin B

*Denotes $p < 0.05$, differences between the AKI group and the non-AKI group were tested for statistical significance.
that the SCr level is not associated with PMB-induced nephrotoxicity. CrCL is calculated according to age, weight and sex and may be a more accurate index for evaluating renal function than the SCr level. However, it was not identified as a risk factor in our research or two other studies [20, 43]. The BUN level is another indicator of renal function and was found to be associated with kidney injury induced by other antibiotics, such as vancomycin-related nephrotoxicity [46]. It should be noted that BUN may be too nonspecific for kidney injury [47], and elevated BUN may be caused by non-renal factors such as protein intake, catabolic state, upper gastrointestinal bleeding, volume status and therapy with high-dose steroids [48–51]. In the present study, although BUN eventually entered a multivariate logistic regression model, the ROC curves (Fig. 5) showed the predictive power of BUN is lower than that of the multivariate logistic regression model, which indicated that the predictive power of BUN may be limited when it was used

Table 5 Relationship between patients and severity of AKI

| Category criteria | Number of patients (%) | $C_{\text{max}}(B_1)$ (mean ± SD) |
|-------------------|------------------------|----------------------------------|
| Risk (R)          | 16 (18.0%)             | 3.65 ± 1.26                     |
| Injury (I)        | 8 (9.0%)               | 4.04 ± 1.59                     |
| Failure (F)       | 1 (1.1%)               | 6.17                             |
| Loss (L)          | 0                      | NA                               |

NA not available
as a single indicator for predicting drug-related AKI.

The kidney is an essential organ for blood pressure regulation and one of the main target organs damaged by hypertension [52]. A previous study has reported that hypertension may be a potential risk factor for colistin-induced nephrotoxicity [53]. In our study, hypertension was identified as another risk factor for PMB-induced nephrotoxicity. The mechanism of this damage may be related to oxidative stress and haemodynamics [54]. In addition, previous studies have found that kidney injury molecule 1 (KIM-1), a factor associated with diabetic nephropathy, may be associated with colistin-induced nephrotoxicity [55–57]. In our study, we found that the incidence rates of type 2 diabetes and chronic renal dysfunction were different between the two groups in the univariate analysis (Table 2). Although the difference became weak in the multivariate analysis, it did not increase the discriminative ability of the final logistic regression model. The meta-analysis [58] reported that underlying diabetes mellitus was a risk factor for polymyxin-induced nephrotoxicity. Although this meta-analysis focused on polymyxins, and polymyxin B and colistin were not analysed separately, the possible risk of AKI in patients with diabetes should be considered.

In our study, the onset of nephrotoxicity in patients ranged from day 3 to day 12. Four patients (16%) experienced nephrotoxicity on day 3. Considering that early PMB-induced nephrotoxicity on day 3 is a predictive factor for later nephrotoxicity [44], early monitoring of renal function during PMB treatment is necessary.

The area under the ROC curve (AUC) of the final multivariate logistic regression model reached 0.799, which is similar to the AUC (0.813) of Han et al.’s combined predictor ($C_{\text{min}}$ (B) and baseline SCr level) [22]. Sörli and colleagues also found that trough plasma level is an independent risk factor for colistin-induced AKI [33]. The optimal cut-off trough concentrations for predicting PMB-related and colistin-related nephrotoxicity in these two studies were 3.55 mg/L and 3.33 mg/L, respectively. However, $C_{\text{min}}$ (B) was not included in the final logistic model in our study. This may have been in part related to the relatively low $C_{\text{min}}$ (B) in patients included in our study. Considering that the $C_{\text{min}}$ (B) showed a significant difference in univariate analysis (Table 3), we attempted to develop a univariate logistic regression model to observe the correlation between $C_{\text{min}}$ (B) and the probability of AKI development. The results showed that the predicted risk of AKI reached 50% when the $C_{\text{min}}$ (B) was 3.63 mg/L or higher. However, when the $C_{\text{min}}$ (B) was used alone to distinguish patients with AKI, the area under the ROC curve was normal (0.666). The predictive value of the $C_{\text{min}}$ (B) for AKI should also be considered in future research. The multivariate model (Eq. 3) is convenient for calculating the predicted risk probability of AKI according to the $C_{\text{max}}$ (B1), presence of hypertension and baseline BUN levels. When data such as baseline BUN levels are missing, Eq. 4 can also provide a preliminary method for predicting AKI risk. Because samples of $C_{\text{max}}$ (B) were collected 10 min after completion of PMB infusion and $C_{\text{min}}$ (B) was obtained immediately before infusion in our study, we contend that $C_{\text{max}}$ (B) and $C_{\text{max}}$ (B1) may be helpful for the early prediction of PMB-induced AKI in critically ill patients. Overall, our model might provide a helpful method for early identification of patients with a high risk of AKI and formulate corresponding intervention strategies.

The study had several limitations. First, this was a retrospective study. We could not analyse other possible risk factors, such as more sensitive indicators reflecting early renal function injury. Second, we were unable to determine the PK parameters in patients to assess the relationship between the AUC and AKI risk of PMB, and the difference in PK parameters between PMB1 and PMB2 was not further assessed. Third, although the enrolled patients came from three medical centres, the sample size was relatively small, limiting the generalizability of our results. Fourth, although the TDM of PMB was recommended by the guideline, the quantification method of PMB1 has not yet been performed in numerous laboratories, which might limit the application of our findings in clinical routine practice.
CONCLUSIONS

The present work identified the $C_{\text{max}}$, baseline BUN levels, and hypertension as independent risk factors for PMB-induced AKI. The ratio of PMB1/PMB2 may decrease after PMB preparation entered into the body. Both the ROC curve and logistic regression model showed that $C_{\text{max}}$ (B1) was a good predictor for the probability of PMB-induced AKI. Early therapeutic drug monitoring of PMB should be considered in critically ill patients. Compared with $C_{\text{min}}$, $C_{\text{max}}$ and $C_{\text{max}}$ (B1) may be helpful for the early prediction of PMB-induced AKI in critically ill patients.

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Author Contributions. All authors contributed to the study conception and design. The manuscript was written by Yang Deng and Jun-Yuan Gu; the research was designed by Xin Li and Yang Deng; the research was performed by Yang Deng, You Li, Si-Wei Guo and Bing Xu; data analysis was performed by Yang Deng, Bi-Kui Zhang, Ying Li and Hai-Ying Huang; new reagents/analytical tools was contributed by Jun-Yuan Gu, Huan Tong and Gui-Ying Xiao. All authors approved the final manuscript.

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Compliance with Ethics Guidelines. As the central laboratory of the rational use of anti-infection agent’s technology demonstration base, the TDM laboratory of the Third Hospital of Changsha also undertook the TDM of polymyxin B for the other two medical institutions, and the Third Hospital of Changsha was the lead institution of this retrospective study. According to the “Deepening the Reform of the Review and Approval System and Encouraging the Innovation of Drugs and Medical Devices” issued (on October 8, 2017) by the State Council of China [59], this study was only approved by the Ethics Research Committee of the Third Hospital of Changsha (No. CS3-KY-2021EC-008), and the requirement for written informed consent was waived. Ethics approval and the informed consent waiver were also accepted by the other two hospitals.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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