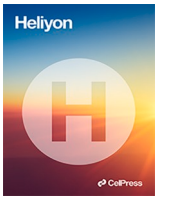
Heliyon 9 (2023) e18551

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Development and validation of a nomogram for predicting gram-negative bacterial infections in patients with peritoneal dialysis-associated peritonitis

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ARTICLE INFO

Keywords:
Peritoneal dialysis
Peritonitis
Gram-negative bacterial
Infection
Nomogram

ABSTRACT

Background: This study aimed to develop a nomogram for predicting gram-negative bacterial (GNB) infections in patients with peritoneal dialysis-associated peritonitis (PDAP) to identify patients at high risk for GNB infections.

Methods: In this investigation, hospitalization information was gathered retrospectively for patients with PDAP from January 2016 to December 2021. The concatenation of potential biomarkers obtained by univariate logistic regression, LASSO analysis, and RF algorithms into multivariate logistic regression was used to identify confounding factors related to GNB infections, which were then integrated into the nomogram. The concordance index (C-Index) was utilized to assess the precision of the model's predictions. The area under the curve (AUC) and decision curve analysis (DCA) was used to assess the predictive performance and clinical utility of the nomogram.

Results: The final study population included 217 patients with PDAP, and 37 (17.1%) patients had gram-negative bacteria due to dialysate effluent culture. After multivariate logistic regression, age, procalcitonin, and hemoglobin were predictive factors of GNB infections. The C-index and bootstrap-corrected index of the nomogram for estimating GNB infections in patients were 0.821 and 0.814, respectively. The calibration plots showed good agreement between the predictions of the nomogram and the actual observation of GNB infections. The AUC of the receiver operating characteristic curve was 0.821, 95% CI: 0.747–0.896, which indicates that the model has good predictive accuracy. In addition, the DCA curve showed that the nomogram had a high clinical value in the range of 1%–94%, which further demonstrated that the nomogram could accurately predict GNB infection in patients with PDAP.

Conclusions: We have created a new nomogram for predicting GNB infections in patients with PDAP. The nomogram model may improve the identification of GNB infections in patients with PDAP and contribute to timely intervention to improve patient prognosis.

Abbreviations: GNB, gram-negative bacterial; CHD, coronary heart disease; CRP, c-reactive protein; PCT, procalcitonin; WBC, white blood cell; BUN, blood urea nitrogen; iPTH, intact parathyroid hormone; PD, peritoneal dialysis.

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https://doi.org/10.1016/j.heliyon.2023.e18551
Received 5 March 2023; Received in revised form 14 July 2023; Accepted 20 July 2023
Available online 23 July 2023

1. Introduction

Peritoneal dialysis (PD) is one of the most significant alternative treatments for individuals with end-stage renal disease [1]. Peritoneal dialysis-associated peritonitis (PDAP) is a significant complication of PD that can lead to PD failure in approximately 20% of cases and death in 10% of patients [2–5]. Recently, peritonitis has dropped dramatically due to the advancement of dialysis equipment, enhanced disinfection procedures, and increasing knowledge of aseptic practices [2–5]. However, the increased use of antibiotics is altering the spectrum of pathogenicity associated with PDAP [6]. Several studies indicate that gram-positive bacterial peritonitis has declined dramatically over the past decade [7]. In contrast, the incidence and proportion of gram-negative bacterial (GNB) peritonitis have gradually grown [8]. In addition, the recurrence and relapse rates of GNB peritonitis are greater than those of other pathogens, accompanied by severe clinical symptoms and a bad prognosis [8]. Consequently, early identification of individuals with PDAP at high risk for GNB infections is crucial. It may help to guide the correct administration of antibiotics and improve these patients' prognosis.

Nomograms are a straightforward visual statistical tool that applies regression techniques to estimate the probability of a particular outcome. Nomograms are currently utilized extensively for diagnosing diseases and estimating mortality [9–12]. So far, however, no studies have utilized nomograms to predict GNB infections in PDAP patients. Therefore, the purpose of this study was to investigate risk factors for GNB infections in patients with PDAP, develop a nomogram for predicting the risk of GNB infections in these patients, and provide relevant clinical recommendations for the identification and treatment in patients at high risk of GNB infections.

2. Methods

2.1. Study population

This study includes adult patients diagnosed with PDAP for the first time at the Second Hospital of Anhui Medical University between January 2016 and December 2021. All PDAP patients met the 2016 ISPD diagnostic criteria [13]. All PDAP patients were administered 2 L of lactated peritoneal dialysis solution containing 1.5% and 2.5% glucose. Patients without dialysate effluent cultures documentation was eliminated. All participants submitted written expressions of consent. The research was conducted per the Declaration of Helsinki [14].

2.2. Ethical approval

This study complied with the Declaration of Helsinki and approved by the Ethics Committee of The Second Hospital of Anhui Medical University. The ethics number is YX2022-014.

2.3. Data collection

Based on standardized forms, we applied electronic medical records to collect patient data. Demographic factors include age, sex, height, and weight. Comorbidities include hypertension, diabetes, and coronary heart disease. Laboratory measurement results include c-reactive protein (CRP), procalcitonin (PCT), white blood cell (WBC), hemoglobin, platelet, potassium, calcium, magnesium, phosphorus, serum uric acid, creatinine, blood urea nitrogen (BUN), albumin, cholesterol, triglycerides, intact parathyroid hormone (iPTH) and the results of dialysate effluent cultures. The data of laboratory measurement results were obtained at the first onset of peritonitis. PD information includes the months after initiating PD. The International Classification of Diseases, Tenth Revision (ICD-10) criteria defined the comorbidities.

2.4. Bacterial culture

The first bag of PD effluent before the patient was admitted to the hospital for anti-infective treatment was used for bacterial culture. 5–10 ml of PD effluent was inoculated with blood medium using a sterile syringe and incubated at 35 °C. GNB were identified using the VITEK 2 Compact automated bacterial analysis system from bioMérieux, France.

2.5. Screening for potential biomarkers

We used univariate logistic analysis, LASSO analysis, and random forest (RF) algorithms to screen for potential biomarkers. The univariate logistic analysis used variables with $P < 0.05$ as potential biomarkers. For the LASSO analysis, we utilized the complete dataset for model development and employed cross-validation to choose the best tuning parameter (lambda). Specifically, we used 10-fold cross-validation, where the dataset was further divided into 10 subsets. The LASSO analysis was then applied iteratively on each fold, with the lambda parameter selected based on the minimum cross-validated error. Regarding the RF algorithms, we also conducted 10-fold cross-validation and selected the best hyperparameter (ntree, mtry, and nodesize), where the five variables with the highest importance were used as potential biomarkers.

2.6. Statistical analysis

The missing proportion of variables studied in this research was <5%. We employ multiple interpolation methods to fill in the missing data using the 'mice' package in R software.

In this research, continuous variables were expressed as the mean and standard deviation (SD), and the T-test or Mann-Whitney test was used to compare groups. The chi-square or Fisher's exact test was used to compare groups based on categorical variables expressed as numbers and percentages. Multivariate analysis included the concatenation of potential biomarkers obtained by univariate logistic analysis, LASSO analysis, and random forest algorithms. Backward stepwise regression was used to select the final logistic regression model. The collinearity between the final model variables was evaluated using the variance inflation factor (VIF). VIF $\leq$5 signified the absence of collinearity between the final model variables. Using the 'rms' package in R software, nomogram were generated based on the results of multivariate logistic regression on the dependent variable. In addition, we used the bootstrapping method with 1000 resamples to conduct internal validation. We used the concordance index (C-Index) to assess the model's discriminatory power and lessen its propensity for overfitting. To compare the predictive accuracy between our model and the two biomarkers (CRP and PCT), we assessed and compared their discrimination using the area under curve (AUC). We also used decision curve analysis (DCA) to assess the clinical utility of the model. Statistical analyses were performed using R software (version 4.2.1). *P*-values <0.05 was considered to be statistically significant.

3. Results

3.1. Characteristics of study participants

Between January 2016 and December 2021, a total of 220 patients were diagnosed with PDAP. Three patients were excluded due to a lack of results in dialysate effluent culture. In the end, 217 patients were included in the trial, and 37 (17.1%) individuals had GNB in their dialysate effluent culture (Fig. 1).

The mean age of the GNB infections group (61.57 $\pm$ 9.39 years) was 9.42 years higher than that of the non-GNB infections group (52.15 $\pm$ 12.23 years). In terms of laboratory tests, there were significant differences in PCT, hemoglobin, calcium, and albumin between the two groups ($p < 0.05$). Table 1 provides a summary of the basic characteristics of the data set.

3.2. Screening for potential biomarkers

We used univariate logistic analysis, LASSO analysis, and RF algorithms to screen potential biomarkers. Univariate logistic analysis screened five potential biomarkers (age, PCT, hemoglobin, calcium, and albumin) (Table 2). For the LASSO analysis, Fig. 2A shows a regression coefficient plot for the model. Each curve represents one variable. At each of the different inputs, the factors with nonzero coefficients and the corresponding nonzero coefficients constitute a LASSO model. The LASSO feature selection process is shown in Fig. 2B. We chose 10-fold cross-validation to further determine the optimal model. When $\lambda = 0.0791$, the model showed the lowest cross-validation error. The final LASSO regression model includes three potential biomarkers (age, PCT, and hemoglobin). For the RF algorithms, Fig. 2C shows the ranking of the importance of the variables in the RF model, and RF algorithms screened five potential biomarkers (age, PCT, hemoglobin, CRP, and serum uric acid).

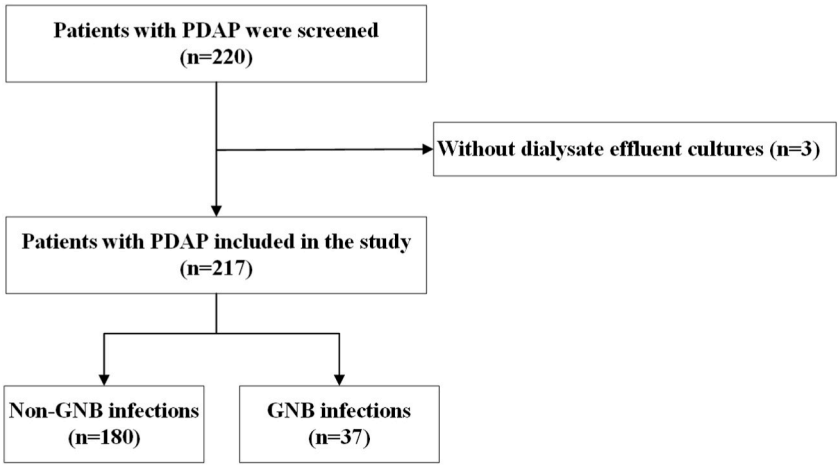

Fig. 1. Flow chart. Abbreviations: PDAP: peritoneal dialysis-associated peritonitis, GNB: gram-negative bacterial.

Table 1
Baseline characteristics.

Variables	Total (n = 217)	Non-GNB infections group (n = 180)	GNB group (n = 37)	*P* value
Age (years)	53.76 ± 12.30	52.15 ± 12.23	61.57 ± 9.39	<0.001
Sex, male	120 (55.3)	100 (55.6)	20 (54.1)	1.000
Height (cm)	161.09 ± 8.00	161.19 ± 8.16	160.59 ± 7.24	0.679
Weight (kg)	60.93 ± 11.53	61.05 ± 11.94	60.32 ± 9.40	0.729
Hypertension, n (%)	186 (85.7)	157 (87.2)	29 (78.4)	0.253
Diabetes, n (%)	44 (20.3)	37 (20.6)	7 (18.9)	0.999
Coronary heart disease, n (%)	70 (32.3)	57 (31.7)	13 (35.1)	0.827
CRP (mg/L)	94.29 ± 85.29	90.08 ± 81.41	114.80 ± 100.84	0.109
PCT (ng/ml)	8.55 ± 18.89	5.71 ± 14.04	22.38 ± 30.40	<0.001
WBC (× 10^9/L)	8.38 ± 4.02	8.32 ± 3.94	8.69 ± 4.44	0.604
Hemoglobin (g/L)	92.95 ± 17.61	90.99 ± 16.91	102.46 ± 18.10	<0.001
Platelets (× 10^9/L)	205.65 ± 85.33	210.08 ± 87.68	184.08 ± 69.84	0.091
Potassium (mmol/L)	3.67 ± 0.69	3.68 ± 0.69	3.61 ± 0.67	0.583
Calcium (mmol/L)	2.09 ± 0.22	2.07 ± 0.22	2.16 ± 0.20	0.019
Magnesium (mmol/L)	0.70 ± 0.14	0.71 ± 0.14	0.69 ± 0.12	0.528
Phosphorus (mmol/L)	1.35 ± 0.45	1.37 ± 0.46	1.25 ± 0.42	0.144
Serum uric acid (μmol/L)	344.98 ± 89.00	345.84 ± 89.00	340.81 ± 90.12	0.755
Creatinine (μmol/L)	828.73 ± 277.90	828.52 ± 283.23	829.73 ± 253.97	0.981
BUN (mmol/L)	16.40 ± 5.70	16.34 ± 5.89	16.71 ± 4.70	0.714
Albumin (g/L)	27.03 ± 5.87	26.66 ± 5.96	28.86 ± 5.13	0.038
Cholesterol (mmol/L)	4.13 ± 1.00	4.14 ± 1.01	4.09 ± 0.95	0.770
Triglycerides (mmol/L)	1.42 ± 0.87	1.45 ± 0.87	1.26 ± 0.87	0.240
iPTH (pg/ml)	258.68 ± 271.17	251.22 ± 269.72	295.01 ± 279.00	0.372
Months after initiating PD (month)	38.29 ± 32.48	37.56 ± 32.29	41.81 ± 33.61	0.470

Abbreviations: GNB: gram-negative bacterial, CRP: c-reactive protein, PCT: procalcitonin, WBC: white blood cell, BUN: blood urea nitrogen, iPTH, intact parathyroid hormone, PD: peritoneal dialysis.

Table 2
Logistic regression analysis of predictors for gram-negative bacterial infections of patients with PDAP.

Variables	Univariate			Multivariate		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Age (years)	1.081	1.041–1.122	<0.001	1.084	1.039–1.130	<0.001
Sex, male	0.941	0.463–1.915	0.867			
Height (cm)	0.991	0.947–1.036	0.677			
Weight (kg)	0.994	0.964–1.026	0.727			
Hypertension, n (%)	0.531	0.217–1.302	0.167			
Diabetes, n (%)	0.902	0.367–2.215	0.822			
Coronary heart disease, n (%)	1.169	0.555–2.461	0.681			
CRP (mg/L)	1.003	0.999–1.007	0.111			
PCT (ng/ml)	1.034	1.017–1.052	<0.001	1.036	1.017–1.055	<0.001
WBC (× 10^9/L)	1.023	0.939–1.115	0.602			
Hemoglobin (g/L)	1.040	1.017–1.063	0.001	1.038	1.013–1.064	0.003
Platelets (× 10^9/L)	0.996	0.991–1.001	0.094			
Potassium (mmol/L)	0.862	0.510–1.459	0.581			
Calcium (mmol/L)	2.727	1.337–3.841	0.021			
Magnesium (mmol/L)	0.428	0.031–5.874	0.526			
Phosphorus (mmol/L)	0.536	0.232–1.239	0.145			
Serum uric acid (μmol/L)	0.999	0.995–1.003	0.754			
Creatinine (μmol/L)	1.000	0.999–1.001	0.981			
BUN (mmol/L)	1.012	0.951–1.076	0.713			
Albumin (g/L)	1.067	1.003–1.135	0.039			
Cholesterol (mmol/L)	0.948	0.662–1.357	0.769			
Triglycerides (mmol/L)	0.747	0.458–1.217	0.241			
iPTH (pg/ml)	1.001	0.999–1.002	0.372			
Months after initiating PD (month)	1.004	0.993–1.014	0.469			

Abbreviations: PDAP: peritoneal dialysis-associated peritonitis, CRP: C-reactive protein, PCT: procalcitonin, WBC: white blood cell, BUN: blood urea nitrogen, iPTH, intact parathyroid hormone, PD: peritoneal dialysis.

3.3. Multivariate logistic regression analysis

We included the concatenation of potential biomarkers obtained by univariate logistic analysis, LASSO analysis, and RF algorithm into multivariate analysis. To determine the most accurate predictors of GNB infections in individuals with PDAP, we employed backward stepwise regression in a multivariate logistic regression. The multivariate logistic model's mean VIF of 1.02 indicates the

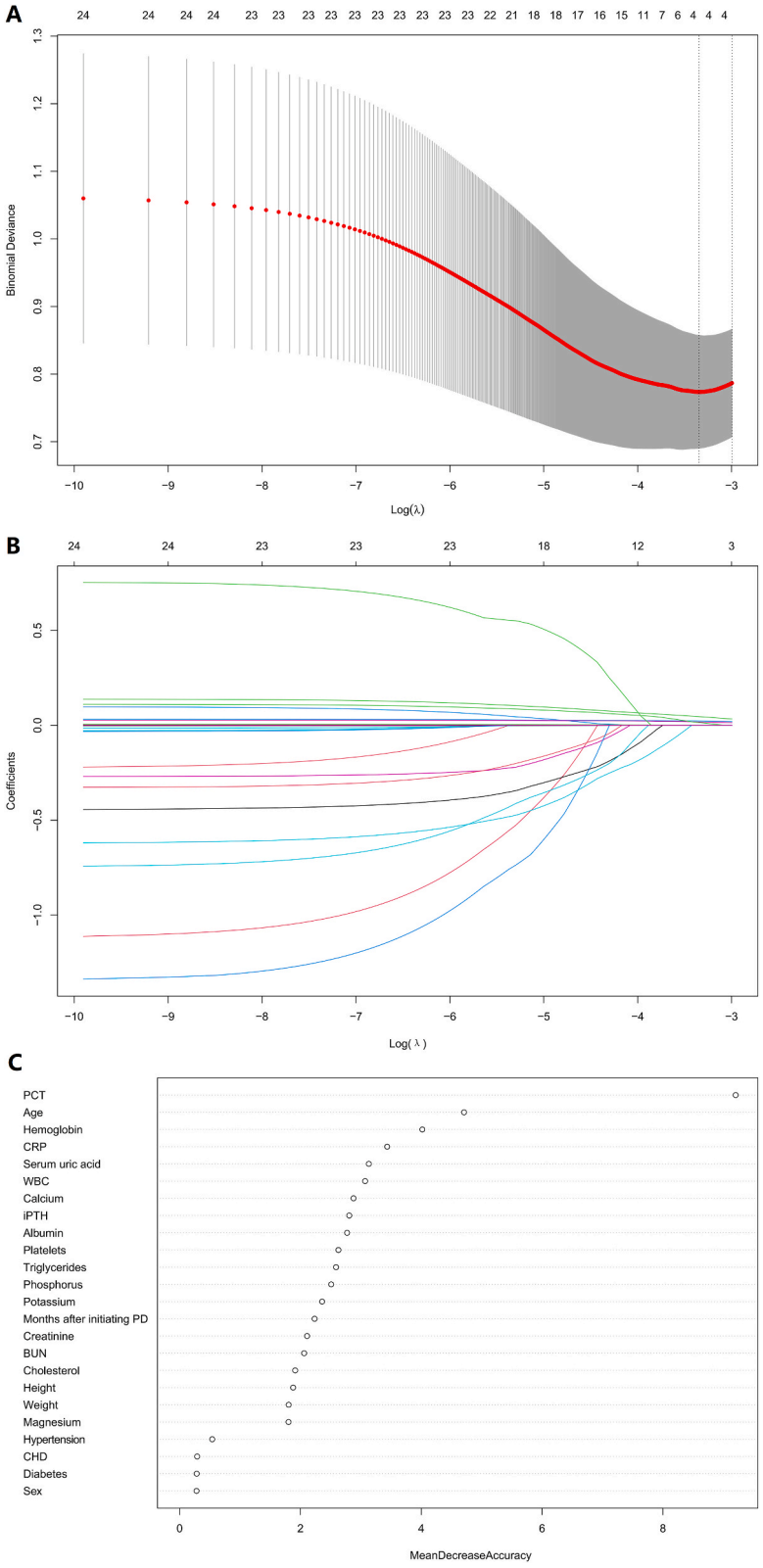

Fig. 2. Lasso regression and random forest algorithm to identify potential biomarkers.

direct absence of multicollinearity among variables. Multivariate logistic regression revealed that age (OR 1.084, CI 1.039–1.130; $p < 0.05$), PCT (OR 1.036, CI 1.017–1.055; $p < 0.05$), and hemoglobin (OR 1.038, CI 1.013–1.064; $p < 0.05$) were significant risk variables for GNB infections (Table 2).

3.4. Nomogram construction and validation

The results of multivariate logistic regression were applied to construct a nomogram of GNB infections in patients with PDAP, and scores were assigned to the screened variables according to their regression coefficients (Fig. 3). According to the nomogram, patients with older age, high PCT, and hemoglobin were more likely to be infected with GNB.

Internal validation was conducted to evaluate the model's discrimination and reduce overfitting bias. The results demonstrated a C-Index of 0.821 for the primary cohort and 0.814 for the internal validation cohort. The calibration plots showed good agreement between the predictions of the nomogram and the actual observation of GNB infections (Fig. 4). The AUC of the receiver operating characteristic curve for the nomogram was 0.821, 95% CI: 0.747–0.896, which indicates that the model has good predictive accuracy (Fig. 5). Moreover, Fig. 5 shows that the nomogram performed better than the two biomarkers (CRP and PCT). In addition, the DCA curve showed that the nomogram model had a high clinical value in the range of 1%–94%, which further demonstrated that the nomogram model could accurately predict GNB infection in patients with PDAP (Fig. 6).

4. Discussion

Predictive models, such as nomograms, can be helpful to physicians through tailored assessment and communication. Thus, the nomogram, widely used in clinical practice, is the most user-friendly tool now available in clinical medicine, allowing doctors to assess patients at high risk of developing poor outcomes by providing a total score [15,16]. In addition, it is simple to comprehend for patients, allowing them to have a deeper understanding of their illnesses. Although numerous nomograms have been developed to estimate the prevalence or risk of various diseases, nomograms have been employed infrequently to predict GNB infections in patients with PDAP.

Logistic regression analysis identified age, PCT, and hemoglobin as risk factors for GNB infections in PDAP patients. For the GNB infections of these patients, a nomogram containing these predictors demonstrated excellent discrimination and calibration for predictive purposes. To our knowledge, this is the first study to establish a nomogram to predict the risk of GNB infections in patients with PDAP, and it may provide clinical guidance for the early identification and empirical use of antibiotics in patients with PDAP who are at high risk for GNB infections.

Numerous research is currently investigating the risk factors for GNB infections in PDAP patients. However, few studies predict GNB infections in patients with PDAP. According to the nomogram, age, PCT, and hemoglobin were the three predictors of GNB infections in PDAP patients. There are two plausible explanations for the older age of GNB peritonitis patients. First, it has been demonstrated that GNB peritonitis is related to immunocompromised states [17,18]. They are particularly prone to GNB infections due to their often compromised immune systems. Second, GNB peritonitis is a typical intestinal infection. The higher occurrence of gastrointestinal diseases, such as constipation, mesenteric ischemia, diverticulosis, and malignancy, among older patients on peritoneal dialysis may further raise the risk of infection with GNB [19]. PCT is a relatively specific biomarker for severe bacterial infections and sepsis. It is a product of bacterial endotoxins and inflammatory cytokines [20]. GNB and gram-positive bacterial (GPB) trigger separate Toll-like receptor signaling pathways, leading to the generation of proinflammatory cytokines that induce PCT release [21]. Thus, infections caused by various bacteria can result in varying PCT production amounts. GNB can create endotoxins that can be released during cell death, resulting in persistently elevated PCT concentrations [22,23]. Recent studies have also established the

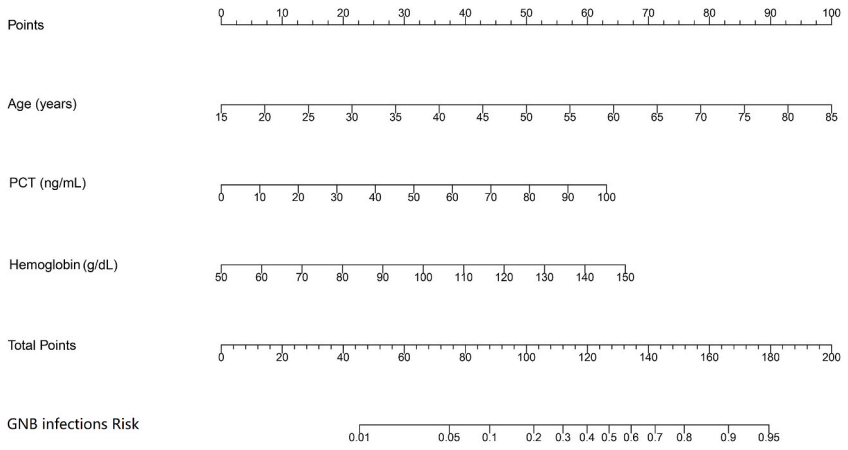

Fig. 3. Nomogram for predicting GNB infections of patients with PDAP. Abbreviations: PDAP: peritoneal dialysis-associated peritonitis, GNB: gram-negative bacterial.

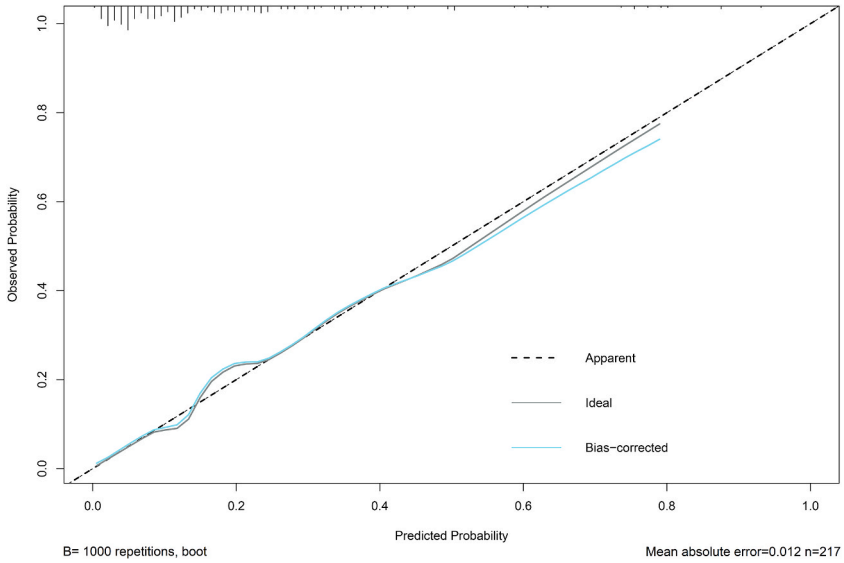

Fig. 4. Calibration plots of internal validation.

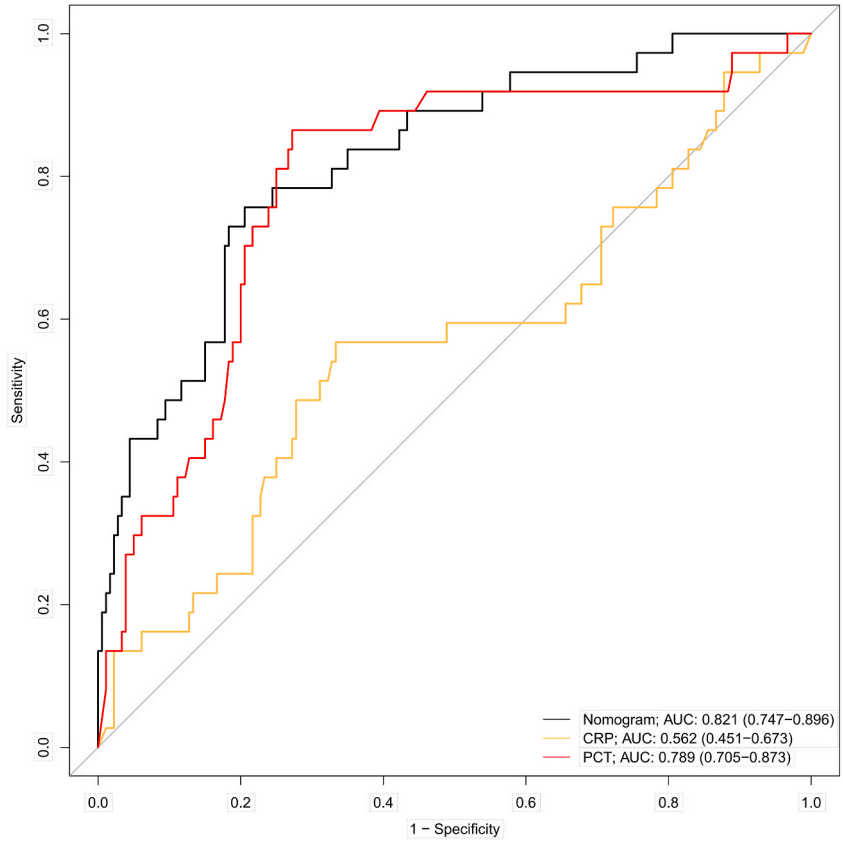

Fig. 5. Receiver operating characteristic curve of the nomogram, CRP, and PCT. Abbreviations: AUC: area under curve, CRP: c-reactive protein, PCT: procalcitonin.

usefulness of PCT levels in discriminating between GNB and GPB infections [24,25]. Similarly, our study identified PCT as a risk factor for Gram-negative infection in PDAP patients, indicating that plasma PCT levels may be a biomarker for Gram-negative infection in PDAP patients, allowing for the estimation of the microbial type and, consequently, the selection of the optimal antibiotic therapy.

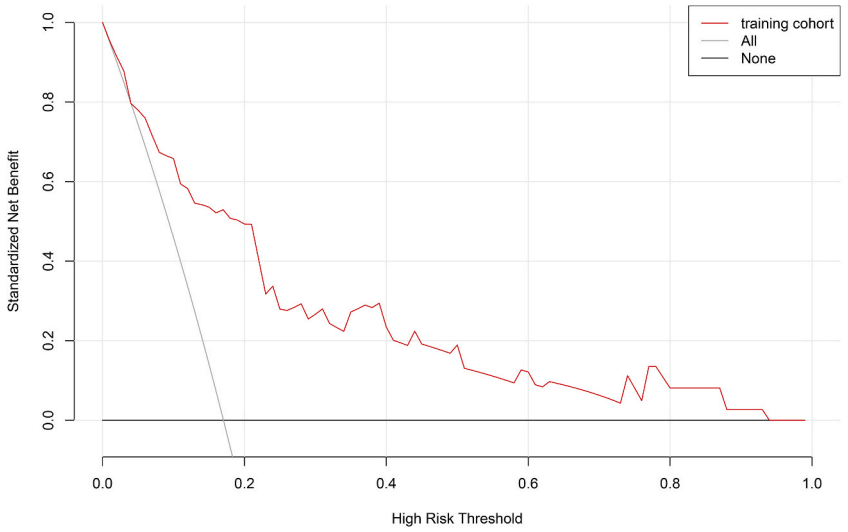

Fig. 6. Decision curve analysis curve of the nomogram.

Another element influencing GNB infections is hemoglobin. Previous research has demonstrated a correlation between greater hemoglobin and a higher prevalence of GNB infections [26].

This study has some significant drawbacks. First, because this was a retrospective modeling study conducted at a single location, we were unable to determine the causal association between features and outcomes. To verify the precision of our approaches, we require additional prospective randomized clinical trials. Second, our study's retrospective and observational approach may result in selection bias. Finally, although our work employed bootstrap approaches for internal validation, future research will require external validation.

5. Conclusions

In conclusion, age, PCT, and hemoglobin are predictors of GNB infection in PDAP patients. In addition, we developed a new nomogram model to predict GNB infection based on the above predictors. This model may serve as a promising predictive tool to improve the identification of GNB infection in patients with PDAP.

Ethical approval

This study complied with the Declaration of Helsinki and approved by the Ethics Committee of The Second Hospital of Anhui Medical University. The ethics number is YX2022-014.

Author contribution statement

Guiling Liu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Xunliang Li: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Wenman Zhao; Rui Shi; Yuyu Zhu; Zhijuan Wang: Analyzed and interpreted the data.

Haifeng Pan: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Deguang Wang: Conceived and designed the experiments.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e18551.

(A) LASSO coefficient profiles of the variables. Each line represented a variable, and the end of the parameter pointed to a vertical coordinate, which was the coefficient. (B) A tenfold cross-validation approach was used to select the tuning parameter (lambda) in the LASSO regressions. (C) Ranking the importance of variables in the random forest model. The larger the mean decrease in accuracy, the greater the importance of the variable.

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