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Original Article

Establishment and validation of a nomogram model for preoperative prediction of the risk of cholangiocarcinoma with perineural invasion

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Abstract: Objective: To establish and validate a nomogram model for predicting the risk of cholangiocarcinoma with perineural invasion. Methods: We retrospectively collected the clinical data of 356 patients with surgically confirmed cholangiocarcinoma, including 98 cases of extrahepatic cholangiocarcinoma (eCCA), 197 cases of intrahepatic cholangiocarcinoma (iCCA), and 61 cases of perihilar cholangiocarcinoma (pCCA). Results: Based on these data, we determined the influencing factors of preoperative perineural invasion risk in patients with cholangiocarcinoma by forward multivariate regression analysis. Based on these variables, we established two nomogram models. The model variables for predicting perineural invasion of eCCA included prothrombin time, high-density lipoprotein and tumor size (all $P<0.05$). The consistency index (C-index) of internal and external validation was 0.845 and 0.806, respectively. In addition, the model variables for predicting perineural invasion of iCCA included carcinoembryonic antigen, carbohydrate antigen 19-9 and tumor size (all $P<0.05$). The internal and external validation of the C-index was 0.735 and 0.886, respectively. Both models have considerable results in terms of calibration accuracy and clinical decision-making. Kaplan-Meier survival analysis showed that the survival time of patients with perineural invasion was significantly reduced ($P=0.033$). Conclusions: We established a predictive model for preoperative perineural invasion in patients with iCCA and eCCA, and this model can provide good predictive value for clinicians. However, we have not obtained relevant predictive variables for predicting perineural invasion of pCCA, and the number of modeling cases was relatively small, so this study needs to be further explored.

Keywords: Cholangiocarcinoma, perineural invasion, preoperative prediction, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma

Introduction

Cholangiocarcinoma (CCA) is a malignant tumor originating from the epithelial cells of the bile duct [1] and is the second most common hepatobiliary and pancreatic tumor, accounting for 3% of all gastrointestinal malignancies [2, 3].

It is characterized by high malignancy and poor prognosis. The 5-year survival rate is usually less than 5%, and even after radical surgical resection, the 5-year survival rate is less than 20% [4, 5]. Based on their anatomical origin, they can be classified as intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA) or extrahepatic cholangiocarcinoma (eCCA) [6, 7]. The etiology of iCCA is not yet clear, but it may be closely related to hepatic schistosomiasis infection, intrahepatic bile duct stones, hepatitis virus infection, biliary malformation, non-biliary cirrhosis, and other factors [8]. Inducing factors of iCCA include chronic cholangitis, bile duct stones, and bile duct parasites. On the other hand, pCCA often occurs in patients with hepatitis B virus infection and cirrhosis [9]. Perineural invasion (PNI), an important feature of CCA, with high incidence and complex mechanism, is defined as the presence of tumor cells in any one of the epineurium, perineurium, and endoneurium or the tumor is close to the nerve or involves at least 1/3 circle of nerve circumference [10-12].

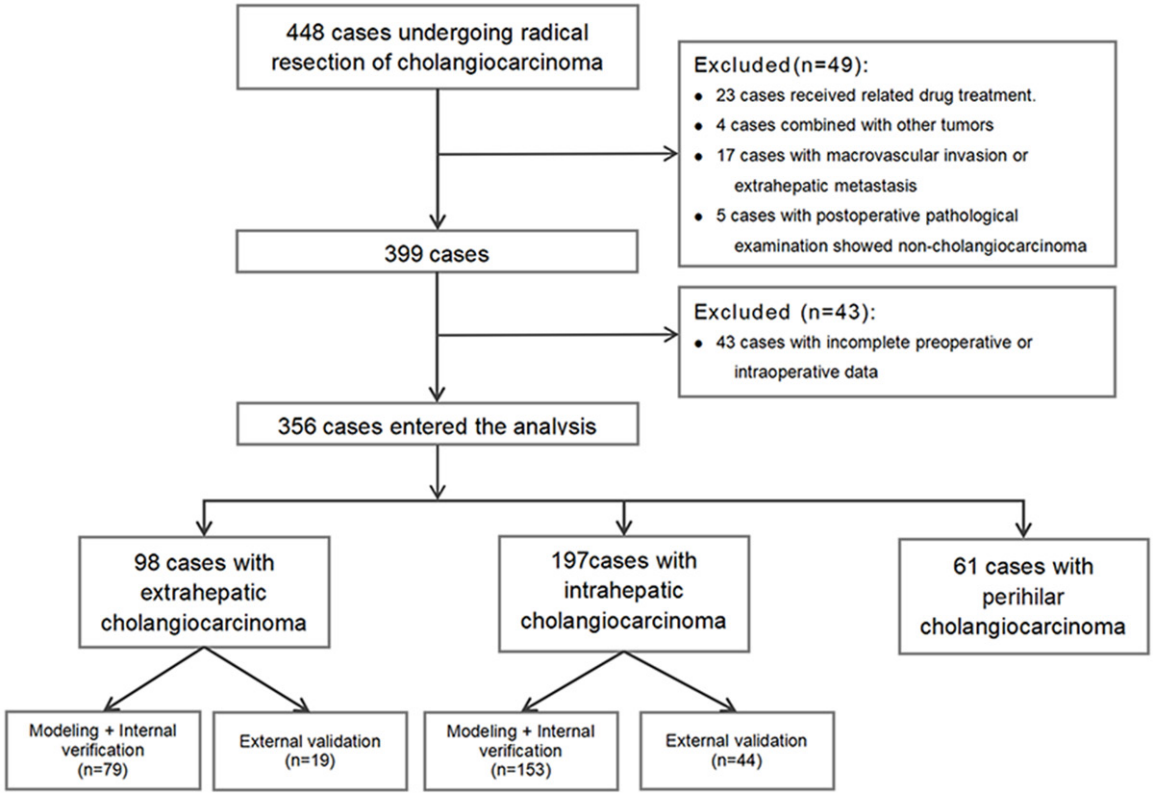

Figure 1. Process diagram details the selection of the included patients.

Studies have shown that PNI, as a special way of metastasis and invasion of CCA, is closely related to the poor prognosis and pain of CCA [13-15]. PNI of CCA is an important factor in predicting the prognosis and survival of CCA patients [16]. Whether iCCA, pCCA or eCCA combined with PNI is an important factor leading to poor prognosis of patients. However, no studies have been reported on preoperative prediction of the risk of CCA with PNI. The purpose of this study was to investigate the characteristics of of patients with CCA including iCCA, pCCA and eCCA combined with PNI, and to establish and verify a nomogram model for preoperative risk prediction of three types of CCA with PNI.

Data and methods

Ethics statement

This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University, and the report of this predictive study followed the TRIPOD statement [17] (Table S1).

Research object

Clinical data of 448 patients with CCA who underwent radical operation for CCA in the Department of Hepatobiliary and Pancreatic Surgery of the Affiliated Hospital of Qingdao University from June 2017 to September 2022 were retrospectively collected. Inclusion criteria: (1) Patients undergoing radical CCA surgery; (2) Patients with complete clinical data; (3) Patients with cholangiocarcinoma confirmed by postoperative pathology. Exclusion criteria: (1) Patients who had recovered from previous anti-tumor therapy; (2) Patients with other types of tumors; (3) Patients with macrovascular invasion or extrahepatic metastasis.

According to inclusion and exclusion criteria, 356 patients were included in the study, including 98 cases eCCA (79 cases were used for modeling and 19 cases for verification), 197 cases iCCA (153 cases were used for modeling and 44 cases for verification), and 61 cases pCCA. The enrollment flowchart of patients is shown in **Figure 1**.

Data collection

The patient's clinical information was obtained from the hospital's electronic case system, including (1) General information of patients: gender, age, etc.; (2) Preoperative imaging indicators: maximum tumor diameter, etc.; (3) The blood indexes of 1 day before operation: thrombin time (TT), prothrombin time (PT), microvascular infiltration (MVI), lymph node metastasis (N), the ratio of alanine aminotransferase to aspartate aminotransferase (ALT/AST), glutamyl transpeptidase (GGT), direct bilirubin (DBil), activated partial thromboplastin time (APTT), total bilirubin (TBil), total cholesterol (TC), carcinoembryonic antigen (CEA), carbohydrate antigen ca19.9 (CA19.9), D dimer (D-d), high-density lipoprotein (HDL-C), and alkaline phosphatase (ALP). Whether the patient had perineural invasion was confirmed by surgery.

Statistical methods

SPSS 24 software was used for statistical analysis. The count data were expressed as the number of cases with a percentage and analyzed using the Chi-square test. The measurement data of the study expressed as mean (SD) and analyzed using independent sample t-test. The independent risk factors of cholangiocarcinoma (including iCCA, eCCA and pCCA) complicated with PNI were screened by univariate and forward LR multivariate Logistic regression analysis method. P<0.05 was considered with statistical significance.

Among them, in the model predicting eCCA with PNI, data from 79 patients were utilized to develop the nomogram model. Internal validation of the model was performed using a non-parametric bootstrap resampling procedure with 1000 simulations. Additionally, external validation of the model was conducted using data from 19 patients. In the nomogram model predicting iCCA with PNI, data from 153 patients were used for modeling, and internal validation was completed using a bootstrap resampling procedure with 1000 simulations. External validation of the model was carried out using data from 44 patients. The C-index was employed to evaluate the internal and external validation results. The model's differentiation ability was assessed by calculating the area under the curve (AUC) using the receiver operating characteristic curve (ROC). The calibration of the model was evaluated using a calibration curve, and the clinical utility of the model was assessed through decision curve analysis (DCA). The nomogram model was established and verified using R-4.1.2 software. Statistical significance was defined as $P<0.05$.

Results

Factors associated with extrahepatic cholangiocarcinoma complicated with perineural invasion

Following surgical examination, it was determined that 58 out of 79 patients with eCCA had PNI, resulting in an incidence rate of 73.4% (58/79). We conducted a comparison of clinical data between the 58 patients with PNI and the remaining 21 patients without PNI.

Univariate logistic regression analysis indicated that thrombin time, prothrombin time, high-density lipoprotein and tumor size were the relevant factors for eCCA complicated with PNI ($P<0.05$). Based on this result, multivariate logistic regression analysis indicated that prothrombin time [odds ratio ((OR)] =9.526, 95% CI: 2.012-45.1), high-density lipoprotein (OR= 6.941, 95% CI: 1.187-40.599) and tumor size (OR=2.566, 95% CI: 1.116-5.902) were independent risk factors for eCCA complicated with PNI (all $P<0.05$) (**Table 1**).

Establishment and verification of the nomogram model of extrahepatic cholangiocarcinoma with perineural invasion

The nomogram model for PNI prediction in eCCA was established using R-4.1.2 software, incorporating independent factors associated with PNI (**Figure 2**). Each risk factor was assigned with a corresponding score, and the total score was then converted into the probability of eCCA with PNI using the nomogram. Internal validation of the model, using 1000 simulations, demonstrated a C-index of 0.845, indicating good agreement between the predicted and actual results. The model's discrimination ability was assessed through ROC curve analysis, yielding an AUC of 0.845 (95% CI: 0.732-0.958) (**Figure 3A**), indicating good discrimination. The calibration curve demonstrated the model's accuracy (**Figure 4A**). The reliability of the model was evaluated using

Table 1. Univariate and multivariate analyses of extrahepatic cholangiocarcinoma

Characteristics	Univariate analysis		Multivariate analysis	
	OR	*P*-Value	OR (95% CI)	*P*-Value
Sex (male vs female)	1.433	0.514		
Age (>65 vs ≤65)	2.302	0.111		
TT (>19 or <12 vs ≥12 and ≤19)	0.185	0.017	0.373 (0.046-21.806)	0.356
PT (>13 or <11 vs ≥11 and ≤13)	12.273	<0.001	9.526 (2.012-45.1)	0.004
MVI (yes vs no)	0.885	0.853		
N (0 vs 1)	1.565	0.524		
ALT/AST (>2.5 or <1.5 vs ≥1.5 and ≤2.5)	0.757	0.594		
GGT (>54 vs ≥0 and ≤54)	0.917	0.941		
DBil (>6.8 vs ≥0 and ≤6.8)	0	0.999		
APTT (>37 or <23 vs ≥23 and ≤37)	1.481	0.732		
TBil (>17.1 vs ≥0 and ≤17.1)	0	0.999		
TC (>5.69 or <2.85 vs ≥2.85 and ≤5.69)	0.792	0.647		
CEA (>5 vs ≥0 and ≤5)	2.223	0.327		
CA19-9 (>37 vs ≥0 and ≤37)	0.767	0.754		
D-D (>200 vs ≥0 and ≤200)	1.005	0.993		
Tumor size*	1.989	0.036	2.566 (1.116-5.902)	0.027
HDL-C*	10.385	0.002	6.941 (1.187-40.599)	0.032
ALP*	0.999	0.528		
CEA*	1.001	0.974		
CA19-9*	1	0.521		

Abbreviations: TT, Thrombin time; PT, prothrombin time; MVI, microvascular infiltration; N, lymph node metastasis; ALT/AST, the ratio of alanine aminotransferase to Aspartate aminotransferase; GGT, glutamyl transpeptidase; DBil, direct bilirubin; APTT, activated partial thromboplastin time; TBil, total bilirubin; TC, total cholesterol; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen ca19-9; D-d, D dimer; HDL-C, high-density lipoprotein; ALP, alkaline phosphatase; OR, odds ratio; CI, confidence interval. Note: *Quantitative variables, the rest are categorical variables.

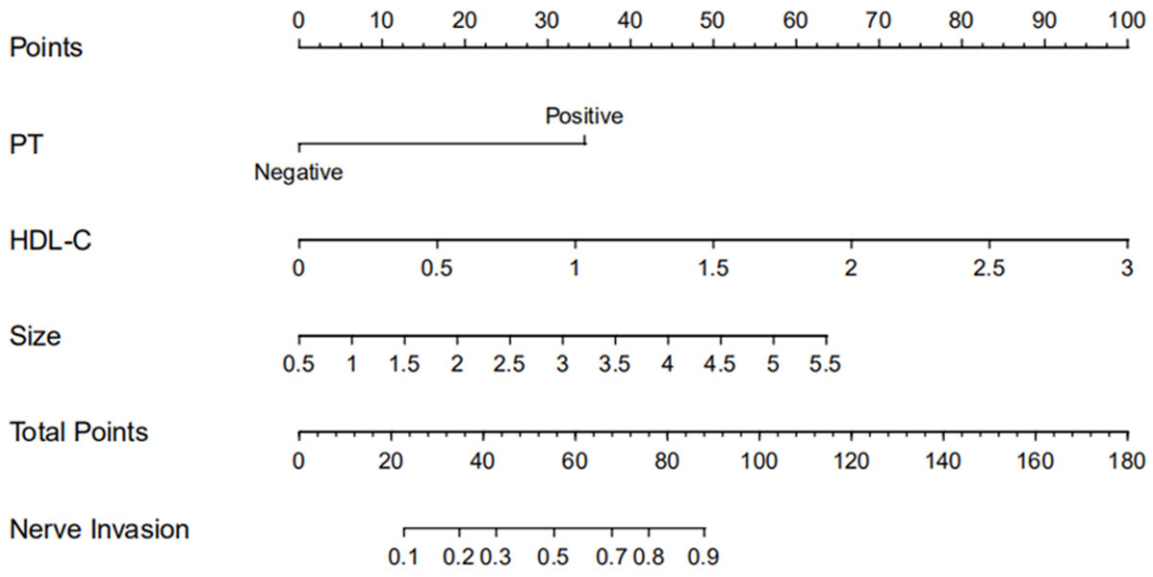

Figure 2. A nomogram model for predicting extrahepatic cholangiocarcinoma with perineural invasion. Abbreviations: PT, prothrombin time; HDL-C, high-density lipoprotein.

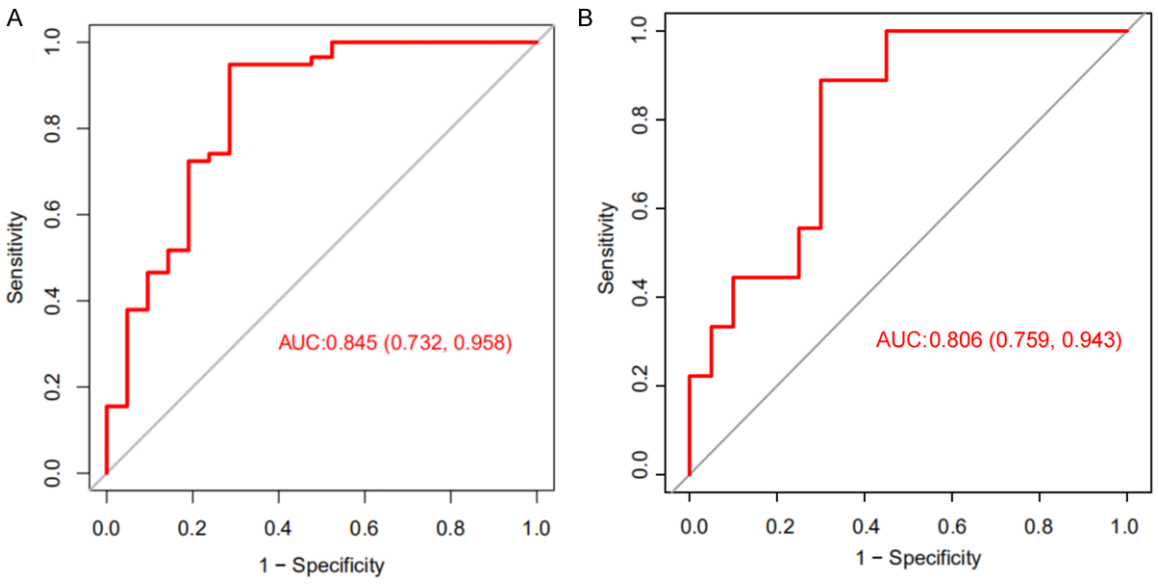

Figure 3. Internally and externally validated ROC curves of the risk prediction model for extrahepatic cholangiocarcinoma with perineural invasion. Note: A. Internal verification; B. External validation.

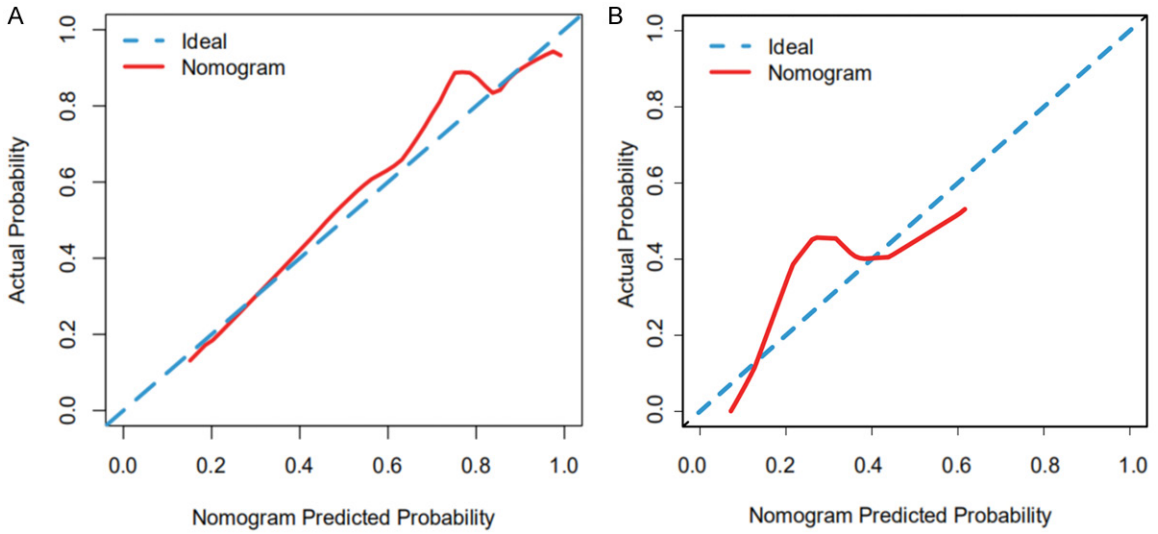

Figure 4. Internal and external validation calibration curves of the risk prediction model for extrahepatic cholangiocarcinoma with perineural invasion. Note: A. Internal verification; B. External validation.

decision curve analysis, which showed that the model provided clinical benefits within a specific threshold range (**Figure 5A**).

In addition, the data of 19 patients with eCCA further completed the external model verification of the model for predicting eCCA with PNI. Of the 19 patients, 11 had PNI, and the remaining 8 had no PNI. The external verification results of the model show that the C-index of the nomogram model is 0.806, indicating that the prediction results of the model are in good agreement with the actual results. Through ROC analysis, the AUC of the model was calculated to be 0.806 (95% CI: 0.759-0.943), as shown in **Figure 3B**, indicating that the model has good recognition ability. The calibration curve shows that the established model has good accuracy (**Figure 4B**). DCA results showed that the established model achieved good clini-

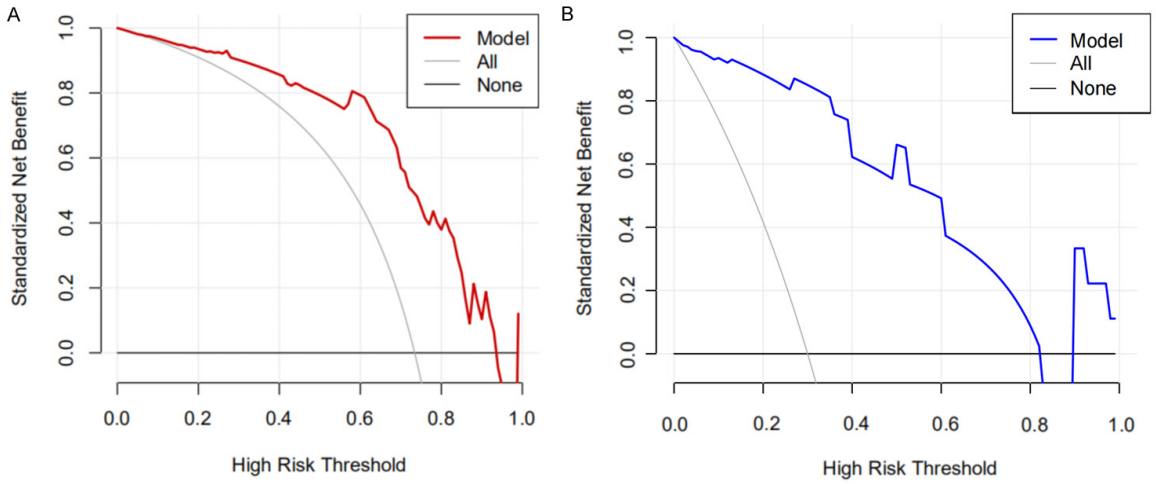

Figure 5. Preoperative prediction of extrahepatic cholangiocarcinoma with perineural invasion risk prediction model of internal and external validation of the decision curve. Note: A. Internal verification; B. External validation.

cal benefits within a certain threshold range (**Figure 5B**).

Factors associated with intrahepatic cholangiocarcinoma complicated with perineural invasion

After surgical examination, it was found that 85 out of 153 patients with iCCA had PNI, resulting in an incidence rate of 55.6% (85/153). We conducted a comparison of the clinical data between the 85 patients with PNI of the extrahepatic bile duct and the remaining patients without PNI.

In iCCA, univariate logistic regression analysis showed that lymph node metastasis, GGT, CEA, CA19.9 and tumor size were correlated with PNI (P<0.05). Multivariate logistic regression analysis showed CEA (OR=2.948, 95% CI: 1.213-7.165), CA19.9 (OR=2.318, 95% CI: 1.036-5.187) and tumor size (OR=1.287, 95% CI: 1.059-1.563) was an independent risk factor for the PNI of iCCA (P<0.05), as shown in **Table 2**.

Establishment and verification of the nomogram model of intrahepatic cholangiocarcinoma with perineural invasion

In the case of iCCA, we also established a nomogram model consisting of independent factors related to PNI by using R-4.1.2 (**Figure 6**), corresponding the values of each risk factor to the corresponding score, and converted the total score into the probability of iCCA combined with PNI by nomogram. The results of the internal verification of the model using the auxiliary program of 1000 simulations are shown: The C-index of the model is 0.735, indicating that the predicted results of the model are in good agreement with the actual results. Through ROC curve analysis, the AUC of the model was calculated to be 0.735 (95% CI: 0.6577-0.813), as shown in **Figure 7A**, indicating that the model also had good discriminant ability. The calibration curve shows that the established model has good accuracy (**Figure 8A**). The reliability of the model was evaluated by DCA. The results showed that the established model achieved good clinical benefits within a certain threshold range (**Figure 9A**).

Furthermore, external validation of the model for predicting iCCA with PNI was conducted using data from 44 patients, of which 17 had PNI and 27 did not. The external validation results indicated that the C-index of the nomogram model was 0.886, demonstrating its ability to distinguish between iCCA with and without PNI. The ROC curve analysis yielded an AUC of 0.886 (95% CI: 0.759-0.943) (**Figure 7B**), indicating excellent recognition ability. The calibration curve demonstrated that the established model had high accuracy (**Figure 8B**). The DCA results showed that the model provided good clinical benefits within a certain threshold range (**Figure 9B**).

Factors associated with perihilar cholangiocarcinoma complicated with perineural invasion

After surgical examination, it was determined that 47 out of 61 patients with pCCA had PNI,

Table 2. Univariate and multivariate analyses of intrahepatic cholangiocarcinoma

Characteristics	Univariate analysis		Multivariate analysis	
	OR	*P*-Value	OR (95% CI)	*P*-Value
Sex (male vs female)	0.877	0.703		
Age (>65 vs ≤65)	1.147	0.683		
TT (>19 or <12 vs ≥12 and ≤19)	1.115	0.827		
PT (>13 or <11 vs ≥11 and ≤13)	1.147	0.682		
MVI (yes vs no)	1.940	0.054		
N (0 vs 1)	2.7	0.016	1.810 (0.734-4.462)	0.197
ALT/AST (>2.5 or <1.5 vs ≥1.5 and ≤2.5)	1.069	0.881		
GGT (>54 vs ≥0 and ≤54)	2.148	0.023	1.352 (0.584-3.310)	0.482
DBil (>6.8 vs ≥0 and ≤6.8)	1.449	0.286		
APTT (>37 or <23 vs ≥23 and ≤37)	1.074	0.9		
TBil (>17.1 vs ≥0 and ≤17.1)	1.436	0.274		
TC (>5.69 or <2.85 vs ≥2.85 and ≤5.69)	1.922	0.111		
CEA (>5 vs ≥0 and ≤5)	4.370	<0.001	2.948 (1.213-7.165)	0.017
CA19-9 (>37 vs ≥0 and ≤37)	3.474	<0.001	2.318 (1.036-5.187)	0.041
D-D (>200 vs ≥0 and ≤200)	1.321	0.495		
Tumor size*	1.312	0.003	1.287 (1.059-1.563)	0.011
HDL-C*	0.958	0.533		
ALP*	1.003	0.028	1.002 (0.998-1.005)	0.302
CEA*	0.999	0.557		
CA19-9*	1	0.837		

Abbreviations: TT, Thrombin time; PT, prothrombin time; MVI, microvascular infiltration; N, lymph node metastasis; ALT/AST, the ratio of alanine aminotransferase to Aspartate aminotransferase; GGT, glutamyl transpeptidase; DBil, direct bilirubin; APTT, activated partial thromboplastin time; TBil, total bilirubin; TC, total cholesterol; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen ca19-9; D-d, D dimer; HDL-C, high-density lipoprotein; ALP, alkaline phosphatase; OR, odds ratio; CI, confidence interval. Note: *Quantitative variables, the rest are categorical variables.

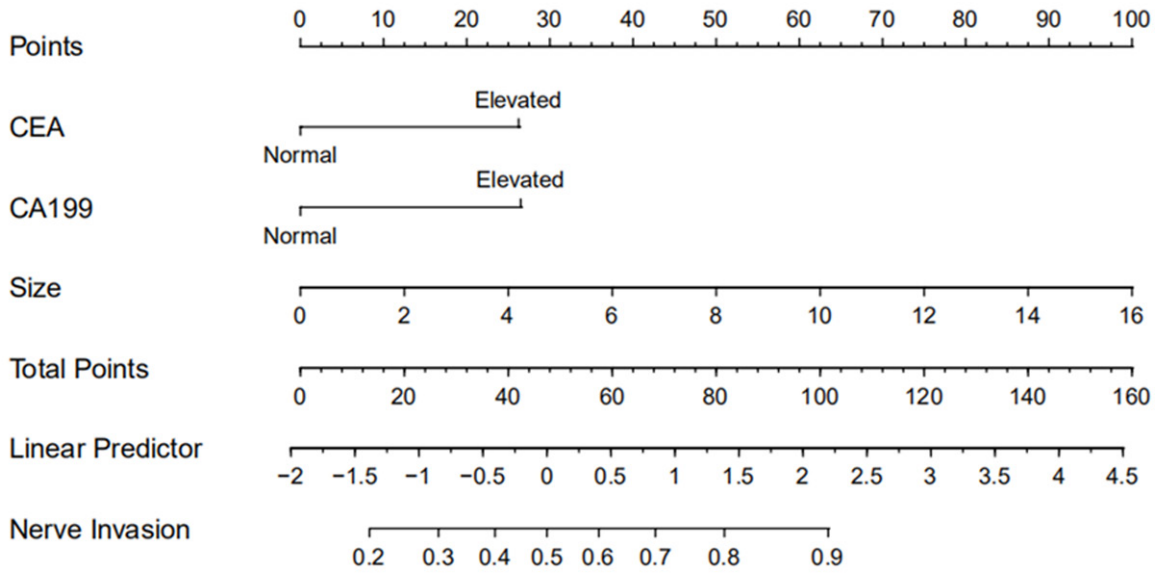

Figure 6. A nomogram model for predicting intrahepatic cholangiocarcinoma with perineural invasion. Abbreviations: CEA, carcinoembryonic antigen; CA199, carbohydrate antigen ca199.

resulting in an incidence rate of 77.0% (47/61). We conducted a comparison of the clinical data between the 47 patients with PNI of the pCCA and the remaining patients without PNI.

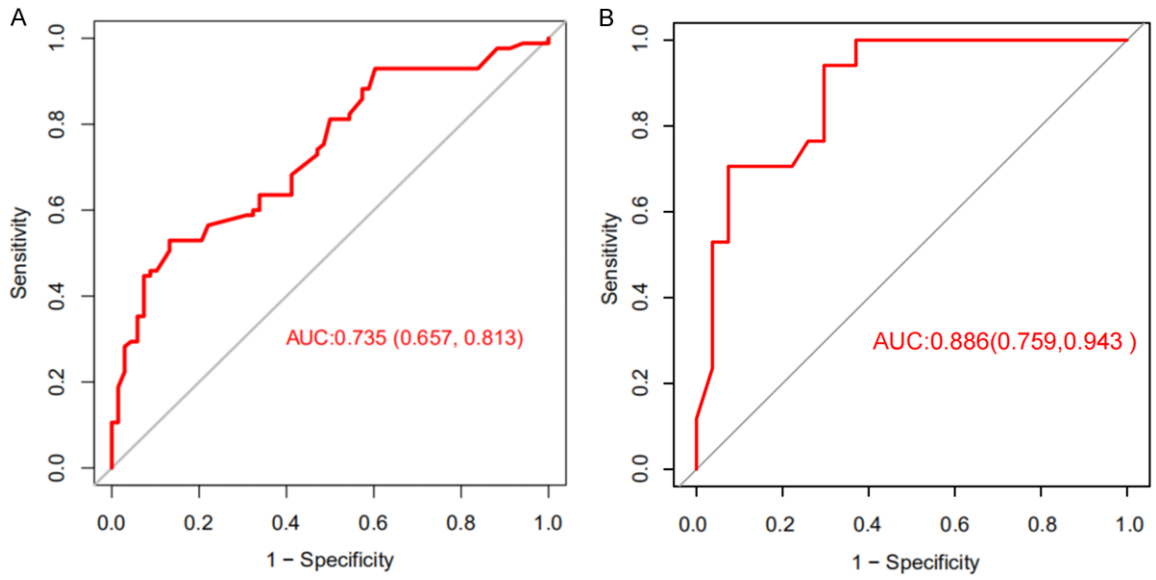

Figure 7. Internally and externally validated Roc curves of the risk prediction model for intrahepatic cholangiocarcinoma with perineural invasion. Note: A. Internal verification; B. External validation.

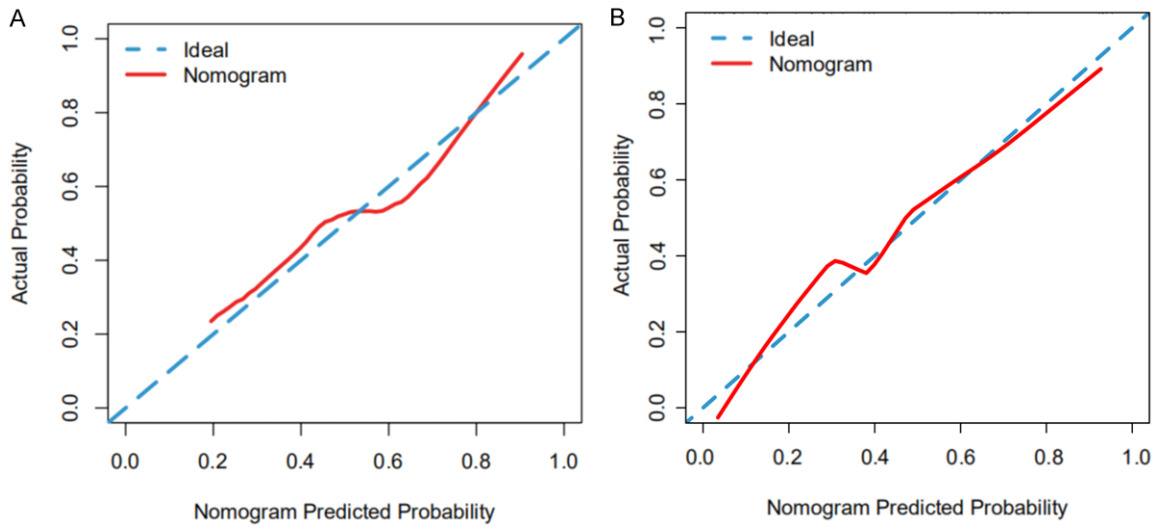

Figure 8. Internal and external validation calibration curves of the risk prediction model for intrahepatic cholangiocarcinoma with perineural invasion. Note: A. Internal verification; B. External validation.

In the univariate logistics analysis of pCCA, only tumor size is a relevant factor ($P<0.05$), and the other factors are meaningless, so it is impossible to analyze independent risk factors of pCCA and establish a model (**Table 3**).

Survival of cholangiocarcinoma with perineural invasion

Postoperative follow-up was conducted on a cohort of 56 patients diagnosed with CCA. Among these patients, 25 were found to have PNI, with a median survival time of 515 days. Conversely, the remaining 30 patients without PNI had a median survival time of 655.50 days. Kaplan-Meier survival analysis revealed a significant reduction in survival time for patients with PNI ($P=0.033$, **Figure 10**).

Discussion

CCA is a highly malignant tumor that can originate in any part of the bile duct [18], and in recent years, the incidence of CCA has been on

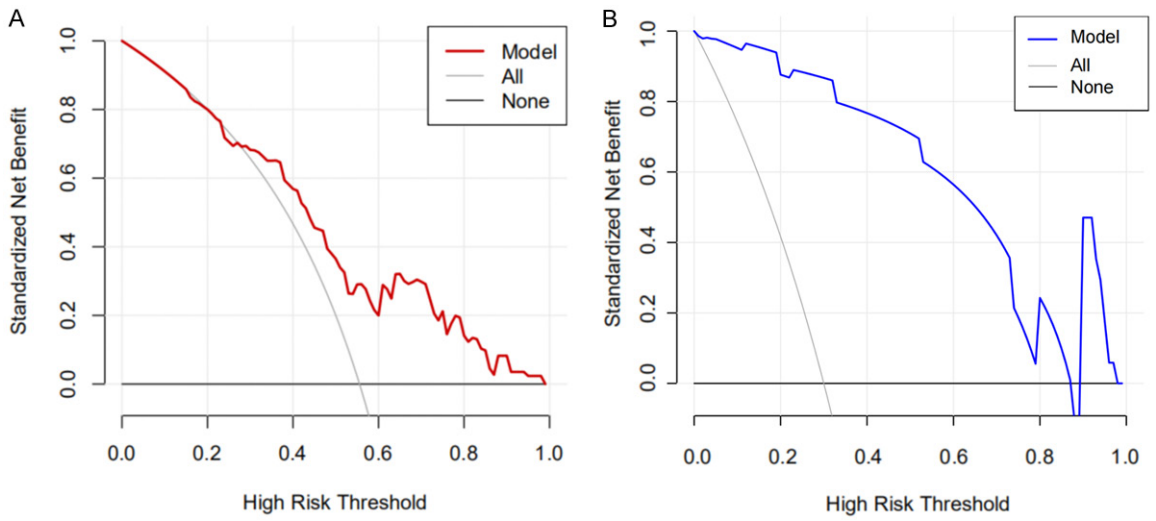

Figure 9. Preoperative prediction of intrahepatic cholangiocarcinoma with perineural invasion risk prediction model of internal and external validation of the decision curve. Note: A. Internal verification; B. External validation.

Table 3. Univariate and multivariate analyses of perihilar cholangiocarcinoma

Characteristics	Univariate analysis		Multivariate analysis	
	OR	*P*-Value	OR	*P*-Value
Sex (male vs female)	0.643	0.542		
Age (>65 vs ≤65)	1.173	0.795		
TT (>19 or <12 vs ≥12 and ≤19)	551625556.1	0.999		
PT (>13 or <11 vs ≥11 and ≤13)	1.324	0.654		
MVI (yes vs no)	1.892	0.376		
N (0 vs 1)	1.719	0.454		
ALT/AST (>2.5 or <1.5 vs ≥1.5 and ≤2.5)	1.768	0.364		
GGT (>54 vs ≥0 and ≤54)	5840567208	1		
DBil (>6.8 vs ≥0 and ≤6.8)	1.792	0.529		
APTT (>37 or <23 vs ≥23 and ≤37)	0.714	0.708		
TBil (>17.1 vs ≥0 and ≤17.1)	1.792	0.529		
TC (>5.69 or <2.85 vs ≥2.85 and ≤5.69)	0.489	0.256		
CEA (>5 vs ≥0 and ≤5)	1.290	0.702		
CA19-9 (>37 vs ≥0 and ≤37)	1.4	0.708		
D-D (>200 vs ≥0 and ≤200)	4.3	0.065		
Tumor size*	0.662	0.042		
HDL-C*	2.846	0.052		
ALP*	0.999	0.539		
CEA*	1.051	0.415		
CA19-9*	1	0.42		

Abbreviations: TT, Thrombin time; PT, prothrombin time; MVI, microvascular infiltration; N, lymph node metastasis; ALT/AST, the ratio of alanine aminotransferase to Aspartate aminotransferase; GGT, glutamyl transpeptidase; DBil, direct bilirubin; APTT, activated partial thromboplastin time; TBil, total bilirubin; TC, total cholesterol; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen ca19-9; D-d, D dimer; HDL-C, high-density lipoprotein; ALP, alkaline phosphatase; OR, odds ratio; CI, confidence interval. Note: *Quantitative variables, the rest are categorical variables.

the rise [8]. Only 1/3 of patients can remove the tumor through surgery, while most of the remaining patients have only non-surgical options such as chemotherapy and targeted

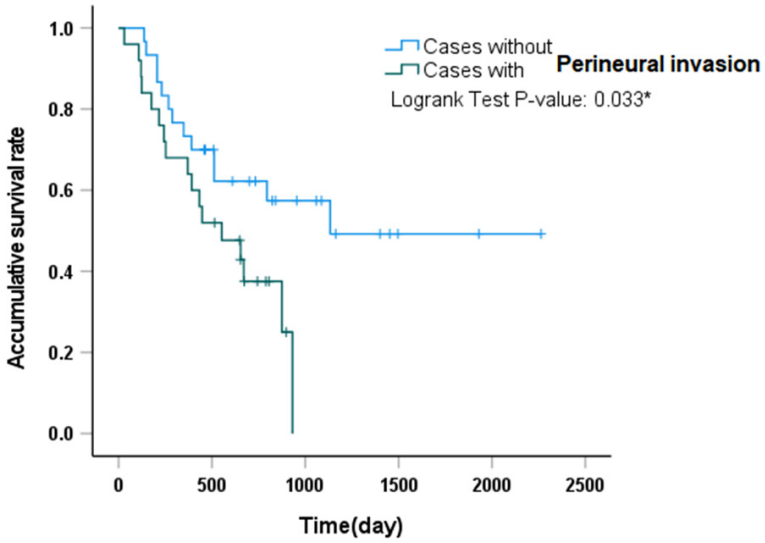

Figure 10. Survival of cholangiocarcinoma with perineural invasion.

drugs [19]. Therefore, CCA is a tumor with a very poor prognosis and a very low five-year survival rate [20]. PNI is the process of tumor invasion of peripheral nerves, which is considered the fifth form of cancer metastasis different from direct invasion, hematologic metastasis and lymphatic metastasis. In the pathological results of postoperative CCA, PNI has become a routine detection index, and it has become an important indicator of prognosis [21]. PNI increases the risk of tumor recurrence and distant metastasis [22]. Therefore, preoperative prediction of PNI is particularly important to judge the prognosis of patients.

Based on a retrospective analysis of the five-year data of CCA patients from the Affiliated Hospital of Qingdao University from 2017 to 2022, and the data were segmented according to the tumor site, we established and validated the preoperative prediction model of PNI for intrahepatic and eCCA, providing a method for clinicians to judge the PNI and prognosis of CCA.

In our study of eCCA data, we found that prothrombin time (PT), high-density lipoprotein (HDL-C), and tumor size were strongly correlated with eCCA perineuronal invasion. A positive prothrombin time, large tumor size, and high HDL were associated with a higher risk of PNI of Ecca [23]. We think that PNI is a manifestation of CCA progression, and CCA progression can lead to liver dysfunction that affects prothrombin time. There is some evidence that higher levels of HDL-C are associated with an increased risk of non-endometrium-like endometrial cancer, but the relationship between HDL-C and cancer risk is still controversial [24]. Moreover, the tumor size of CCA has also become an important indicator of prognosis [25]. In our study, we established and validated a nomogram model of eCCA perineuronal infiltration, and the factors involved were found to be relevant in other similar analyses.

In addition, in the relevant data of iCCA, we verified that CEA, CA19-9 and tumor size were significantly correlated with PNI, when CEA and CA19-9 were positive and the tumor size was large, patients with eCCA were more likely to develop PNI. CEA and CA19-9 were the most commonly used tumor markers in the diagnosis of CCA patients during the clinical time [26]. In some studies, tumor size has been shown to correlate with aggressiveness. For example, the formation of portal vein cancer thrombus in liver cancer is closely related to tumor size [27]. Based on the above three factors, we established a nomogram model of the PNI of iCCA, which can be a reliable basis for our results.

However, in the data analysis of pCCA, we have not found meaningful results, only that tumor size may be related to the PNI. pCCA originates in the hilar bile duct and has a five-year survival rate of less than 40% [28]. Surgery for pCCA is difficult and risky, and only half of patients are suitable for surgery [29]. This is also the reason for the small amount of data on patients undergoing surgery for pCCA.

In recent years, the prevalence of CCA has gradually increased, but at present, there are very few studies on CCA and even fewer studies on the PNI of CCA. At present, there are no relevant studies on the treatment of PNI of CCA as the main target, which may become the focus of our future research. However, the adverse effects of PNI on the prognosis of CCA have been confirmed. Our follow-up results also con-

firmed the correlation between PNI and poor prognosis of patients. At the same time, our data are all based on the results of routine blood tests and imaging examinations. These data are convenient to obtain and reliable, which also provides convenience for clinicians to use this model.

However, looking back at our research, we believe that there are also some shortcomings. There may be some bias in our data. For example, some indicators, such as HDL, may be associated with other medical conditions in patients, which can also bias the data. In addition, the retrospective study itself has its limitations, and the small number of patients in the study will affect the results, so it is very necessary for us to conduct a multi-center prospective study in the future. Also, there are some limitations in our study, which can only guide the prognosis of CCA but cannot guide the treatment, which also reminds us of the focus of our future work.

In summary, we established and verified the preoperative nomogram model of PNI in patients with iCCA and eCCA and predicted the postoperative prognosis of patients with iCCA through PNI. This study deepens the previous understanding of CCA and opens up this unknown area of PNI, which will benefit clinicians.

Acknowledgements

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Disclosure of conflict of interest

None.

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Table S1. PRISMA 2020 checklist

Section and Topic	Item #	Development or validation?	Checklist item	Page
Title or abstract				
Title	1	D; V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1-2
Abstract	2	D; V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	28-52
Introduction				
Background and objectives	3a	D; V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	56-79
	3b	D; V	Specify the objectives, including whether the study describes the development or validation of the model, or both.	79-82
Methods				
Source of data	4a	D; V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	98-102
	4b	D; V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	89-987
Participants	5a	D; V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	None
	5b	D; V	Describe eligibility criteria for participants.	92-97
	5c	D; V	Give details of treatments received, if relevant.	None
Outcome	6a	D; V	Clearly define the outcome that is predicted by the prediction Clearly define the outcome that is predicted by the prediction.	104-114
	6b	D; V	Report any actions to blind assessment of the outcome to be predicted.	None
Predictors	7a	D; V	Clearly define all predictors used in developing the multivariable prediction model, including how and when they were measured.	116-137
	7b	D; V	Report any actions to blind assessment of predictors for the outcome and other predictors.	None
Sample size	8	D; V	Explain how the study size was arrived at.	None
Missing data	9	D; V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	None
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	116-137
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	116-137
	10c	V	For validation, describe how the predictions were calculated.	116-137
	10d	D; V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	116-137
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	None
Risk groups	11	D; V	Provide details on how risk groups were created, if done.	None

Development vs validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	None
Results				
Participants	13a	D; V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	98-102
	13b	D; V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	None
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	None
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	141-144, 179-182, 215-218
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	145-151, 183-188, 219-221
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	None
	15b	D	Explain how to use the prediction model.	None
Model performance	16	D; V	Report performance measures (with CIs) for the prediction model.	154-176, 191-212
Model updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	None
Discussion				
Limitations	18	D; V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	290-298
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	None
	19b	D; V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	290-298
Implications	20	D; V	Discuss the potential clinical use of the model and implications for future research.	299-303
Other information				
Supplementary information	21	D; V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	None
Funding	22	D; V	Give the source of funding and the role of the funders for the present study.	11-13

Note: Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D; V. We recommend using the TRIPOD Checklist in conjunction with the TRIPOD explanation and elaboration document. *From: Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. BMJ. 2015 Jan 7;350:g7594. doi: 10.1136/bmj.g7594. PMID: 25569120.*