A Novel Prognostic Model to Predict Prognosis of Patients With Osteosarcoma Based on Clinical Characteristics and Blood Biomarkers

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Abstract

**Background:** To develop a novel prognostic model based on clinical characteristics and blood biomarkers to estimate overall survival (OS) and progression-free survival (PFS) in osteosarcoma (OSC) patients.

**Methods:** A total of 71 patients with OSC from Sun Yat-sen University Cancer Center were retrospectively included. The novel prognostic model for predicting OS and PFS was established by using Lasso regression analysis based on blood biomarkers. The predictive accuracy and discriminative ability of the novel prognostic model was compared with TNM staging and clinical treatment using concordance index (C-index), time-dependent ROC (tdROC) curve, decision curve analysis (DCA), net reclassification improvement index (NRI), and integrated discrimination improvement index (IDI).

**Results:** Based on the Lasso regression analysis, we identified a 5 prognostic factors module (RBC, Ca\(^{2+}\), CRE, PNI, and LSR) as a novel predictive model for the OSC patients. The C-index of the novel prognostic model for predicting OS and PFS were 0.782 (95% CI = 0.658 - 0.905) and 0.741 (95% CI = 0.632 - 0.851), respectively, which were higher than that of TNM staging and clinical treatment. The tdROC curve and DCA also showed the novel model had good predictive accuracy and discriminatory power than TNM staging and treatment both in predicting OS and PFS. Moreover, the novel prognostic model performed well in all time frames (1, 3 and 5 years) in terms of the IDI and NRI when compared with the TNM staging, and clinical treatment.

**Conclusions:** The novel prognostic model showed favorable performance than TNM staging and clinical treatment for predicting OS and PFS in OSC patients.

**Background**

Osteosarcoma (OSC) is the most common bone cancer and one of the most common primary malignancies among children and adults\([1, 2]\), with an incidence of was 2–3/million/year in the general population, at the same time, statistics showed that the incidence and mortality of OSC had been increasing at a rate of approximately 1.4% per year\([3]\). OSC usually occurred in the long bone near the epiphyseal growth plate of the extremities. The most common sites were the distal femur, proximal tibia, and proximal humerus, characterized by a high propensity to metastasis and local recurrence\([4]\). Men were 1.4 times more likely to be infected than women\([5]\).

Despite multidisciplinary therapies such as surgical excision, radiotherapy and chemotherapy\([6]\), many OSC patients still experienced tumor recurrence and metastasis, resulting in poor prognosis and little improvement in survival in these patients\([7, 8]\). The presence or absence of metastasis, local recurrence, chemotherapy regimen, chemotherapy response, patient characteristics, tumor staging, tumor characteristics, and neoadjuvant tumor cell destruction percentage had effects on the prognosis\([7, 9]\). Metastatic disease had the greatest impact on prognosis. The overall survival rate for patients with metastatic disease was about 20% - 30%, compared with 70% - 80% for non-metastatic patients\([10]\).

Clinical staging of OSC was commonly used for risk assessment\([11]\). However, clinical staging systems for OSC, such as American Joint Committee on Cancer (AJCC) staging and Enneking staging, can only provide a rough assessment of the clinical risk of OSC based on the pathological grade, tumor size, and metastasis, but survival differs among patients with the same stage of tumor\([12]\). These results indicated that the traditional staging system was not adequate for predicting the survival of cancer patients without considering other prognostic factors (such as clinical characteristics or blood biomarkers). Thus, it is necessary to explore more reliable prognostic indicators to remedy the shortcomings of staging system and refine the prediction of clinical outcomes for patients with OSC.

Blood biomarkers can be determined simply, quickly, and stably, with negligible interference to the patient. A variety of blood biomarkers had been studied in the diagnosis and follow-up of OSC progression and recurrence, such as alkaline phosphatase (ALP) and lactate dehydrogenase, among which ALP had the most diagnostic value for OSC and it has been
shown to be positively correlated with tumor volume, which has additional useful prognostic significance[13]. So far, however, few researchers used combination of clinical characteristics and blood biomarker to predict the prognosis of OSC.

Thus, the aim of the present retrospective study was to develop a novel prognostic model based on clinical characteristics and blood biomarkers to estimate overall survival (OS) and progression-free survival (PFS) in patients with OSC and to assess its incremental value in traditional staging systems and clinical treatment of individual OS and PFS.

Methods And Materials

Patient selection and data collection

Patients hospitalized and treated in the Sun Yat-sen University Cancer Center (SYSUCC) between January 2010 and December 2017 consecutively enrolled into the present retrospective study. This study was approved by the Clinical Research Ethics Committee of the Sun Yat-sen University Cancer Center, and all patients provided written informed consent at the first visit to our center. The inclusion criteria for this study were as follows: (1) histologically confirmed OSC; (2) patients did not suffer from any cancer disease before OSC diagnosis; (3) patients did not receive any anti-cancer treatment; (4) complete clinical information, laboratory data, and follow-up data

Baseline clinical characteristics were collected from medical records including age, gender, smoking status, family history, tumor site, tumor size, tumor border, clinical treatment, and the eighth edition of the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging[14]. The pretreatment blood-routine biomarkers (within one week before anti-cancer treatment) including white blood cell (WBC), neutrophils (N), lymphocyte (L), monocyte (M), platelet (PLT), neutrophil / lymphocyte ratio (NLR), lymphocyte / monocyte ratio (LMR), platelet/lymphocyte ratio (PLR), derived neutrophil-to-lymphocyte ratio (dNLR), systemic immune-inflammation index (SII)[15], prognostic nutritional index (PNI)[16], red blood cell (RBC), hemoglobin (HGB), serum phosphorus (IP³⁺), serum calcium(Ca²⁺), serum magnesium (Mg²⁺), anion gap (Gap), alanine aminotransferase (ALT), aspartate aminotransferase (AST), ALT / AST ratio (LSR), alkaline phosphatase (ALP), lactic dehydrogenase (LDH), glutamyl transpeptidase (GGT), total protein (TP), albumin (ALB), globulin (GLOB), C-reactive protein (CRP), ALB / GLOB ratio (AGR), ALB / CRP ratio (ACR), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), total bile acid (TBA), urea, creatinine (CRE), cystatin C (Cys-C), uric acid (UA), total cholesterol (CHO), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), LDL-C / HDL-C ratio (LHR), apolipoprotein AI (APOA), apolipoprotein B (APOB), APOA / APOB ratio (ABR), the atherogenic index (AI)[17], glucose (GLU).

Patients Follow Up

The patients’ survival data follow-up was obtained by means of retrieving medical records, email, and direct telecommunication, all patients were followed up until death or July 2020, if still alive. Overall survival (OS) was defined as the time interval from diagnosis to the date of the patient's death or censored at the date of last follow-up. Progression-free survival (PFS) was calculated from the date of diagnosis to the date of the objective disease progression or death or the date of the last follow-up visit.

Statistical analysis

Statistical analysis was performed using R software (version 3.6.1, https://www.r-project.org). Continuous variables were presented as mean ± SD and tested by t-test or Wilcoxon test. Firstly, we utilized the LASSO regression algorithm (λ were determined by 10-fold cross validation with the error of the minimum criteria) to select the most useful prognostic factors out of all the OSC-associated blood biomarkers, and then established a novel predictive model. Subsequently, the predictive accuracy and discriminative ability of the novel prognostic model was compared with TNM staging and clinical treatment
using Harrell concordance index (C-index), time-dependent ROC (tdROC) curves[18], decision curve analysis (DCA)[19], net reclassification improvement index (NRI), and integrated discrimination improvement index (IDI)[20]. The larger C-index and area under the curve (AUC) of tdROC curves, the better the model is for the risk prediction. DCA was used to evaluate the clinical usefulness and net benefit of the predictive model[21]. The NRI assessed the ability of a new model to re-classify subjects compared to an old model into binary event or no-event categories. The IDI index quantified the improvement in average sensitivity without reducing the average specificity of a new model compared with an older model[22]. The correlation between the novel prognostic model, TNM staging and clinical treatment was evaluated by Pearson's correlation coefficient. Besides, a nomogram integrating the prognostic model risk score, TNM staging, and clinical treatment was developed that may assist in individual survival prediction of OSC patients. Internal validation and calibration of the nomogram were conducted by 1000-resample bootstrapping. Finally, we illustrated the discrimination by dividing the patients into low-risk groups and high-risk groups according to the novel predictive model scores. The Kaplan-Meier method was used to perform OS and PFS analysis, and the log-rank test was used to compare significance of the differences of survival distribution between groups. Generally, a P value of $\leq 0.05$ was considered as statistically significant for all analyses.

Results

Baseline clinical and characteristics

A total of 71 patients with osteosarcoma (OSC) were included for retrospective study. 39 (54.9%) of these patients were male, and 32 (45.1%) were female; the median age was 25 (range 3–83) years. According to 8th edition of the AJCC TNM staging criteria, the number of stages I&II and III&IV was 51 (71.8%) and 20 (28.2%), respectively. The median follow-up for OS and PFS were 34 months and 32 months respectively. The 1-, 3-, and 5-year OS rates were 70.4%, 38.0%, and 14.1%; the 1-, 3-, and 5-year PFS rates were 69.0%, 31.0%, and 11.3%. Baseline characteristics of the total OSC patients were shown in Table 1.
| Variable                | No. (%) or Mean ± sd |
|-------------------------|----------------------|
| **Characteristics**     |                      |
| Age (years)             | 28.0 ± 18.4          |
| **Gender**              |                      |
| Male                    | 39 (54.9%)           |
| Female                  | 32 (45.1%)           |
| **Smoke**               |                      |
| Yes                     | 4 (5.6%)             |
| No                      | 67 (94.4%)           |
| **Family history of cancer** |                |
| Yes                     | 2 (2.8%)             |
| No                      | 69 (97.2%)           |
| **Tumor site**          |                      |
| Extremities             | 40 (56.3%)           |
| Pelvis/Spine            | 4 (5.6%)             |
| Skull                   | 19 (26.8%)           |
| Other                   | 8 (11.3)             |
| **Tumor size (cm)**     | 6.6 ± 3.3            |
| **Tumor border**        |                      |
| Well-defined            | 21 (29.6%)           |
| Ill-defined             | 34 (47.9%)           |
| Unrecorded              | 16 (22.5%)           |
| **Treatment**           |                      |
| None                    | 2 (2.8%)             |
| Sur                     | 27 (38.0%)           |

a: The tumor maximum diameter;
b: TNM stage was classified according to the AJCC 8th TNM staging system;

Abbreviations: TNM: Tumor Node Metastasis stage; Sur: surgery; Rad: radiotherapy; Che: chemotherapy; PLT: platelet; NLR: neutrophil / lymphocyte ratio; LMR: lymphocyte / monocyte ratio; PLR: platelet / lymphocyte ratio; dNLR: derived neutrophil-to-lymphocyte ratio; SII: systemic immune-inflammation index; PNI: prognostic nutritional index; RBC: red blood cell; HGB: hemoglobin; IP\(^3+\): serum phosphorus; Ca\(^{2+}\): serum calcium; Mg\(^{2+}\): serum magnesium; Gap: anion gap; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LSR: ALT / AST ratio; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; GGT: glutamyl transpeptidase; TP: total protein; ALB: albumin; GLOB: globulin; CRP: C-reactive protein; AGR: ALB / GLOB ratio; ACR: ALB / CRP ratio; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; TBA: total bile acid; CRE: creatinine; Cys-C: cystatin C; UA: uric acid; CHO: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; LHR: LDL-C / HDL-C ratio; APOA: apolipoprotein A1; APOB: apolipoprotein B; ABR: APOA / APOB ratio; AI: The atherogenic index; GLU: glucose.
| Variable         | No. (%) or Mean ± sd |
|------------------|----------------------|
| Rad/Che          | 8 (11.3%)            |
| Sur and Rad/Che  | 32 (45.1%)           |
| Other            | 2 (2.8%)             |
| TNM stage<sup>b</sup> |                    |
| I&II             | 51 (71.8%)           |
| III&IV           | 20 (28.2%)           |
| Laboratory data  |                     |
| WBC (10<sup>9</sup>/L) | 7.6 ± 2.6          |
| Neutrophil (10<sup>9</sup>/L) | 4.7 ± 2.4          |
| Lymphocyte (10<sup>9</sup>/L) | 2.1 ± 0.6          |
| Monocyte (10<sup>9</sup>/L) | 0.5 ± 0.2          |
| PLT (10<sup>9</sup>/L) | 278.1 ± 86.4        |
| NLR              | 2.4 ± 1.5            |
| LMR              | 4.7 ± 3.3            |
| PLR              | 141.5 ± 59.1         |
| dNLR             | 1.7 ± 0.9            |
| SII              | 681.8 ± 453.9        |
| PNI              | 54.0 ± 7.6           |
| RBC (10<sup>12</sup>/L) | 4.8 ± 0.6           |
| HGB (g/L)        | 130.8 ± 20.9         |
| IP<sup>3+</sup> (mmol/L) | 1.49 ± 1.4        |
| Ca<sup>2+</sup> (mmol/L) | 2.5 ± 1.0          |
| Mg<sup>2+</sup> (mmol/L) | 0.9 ± 0.1          |
| Gap              | 13.7 ± 4.9           |

a: The tumor maximum diameter;

b: TNM stage was classified according to the AJCC 8th TNM staging system;

Abbreviations: TNM: Tumor Node Metastasis stage; Sur: surgery; Rad: radiotherapy; Che: chemotherapy; PLT: platelet; NLR: neutrophil / lymphocyte ratio; LMR: lymphocyte / monocyte ratio; PLR: platelet / lymphocyte ratio; dNLR: derived neutrophil-to-lymphocyte ratio; SII: systemic immune-inflammation index; PNI: prognostic nutritional index; RBC: red blood cell; HGB: hemoglobin; IP<sup>3+</sup>: serum phosphorus; Ca<sup>2+</sup>: serum calcium; Mg<sup>2+</sup>: serum magnesium; Gap: anion gap; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LSR: ALT / AST ratio; ALP: alkaline phosphatase; LDH: lactic dehydrogenase; GGT:glutamyl transpeptidase; TP: total protein; ALB: albumin; GLOB: globulin; CRP: C-reactive protein; AGR: ALB / GLOB ratio; ACR: ALB / CRP ratio; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; TBA: total bile acid; CRE: creatinine; Cys-C: cystatin C; UA: uric acid; CHO: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; LHR: LDL-C / HDL-C ratio; APOA: apolipoprotein A1; APOB: apolipoprotein B; ABR: APOA / APOB ratio; AI: The atherogenic index; GLU: glucose.
| Variable   | No. (%) or Mean ± sd |
|------------|----------------------|
| ALT (U/L)  | 19.6 ± 14.8          |
| AST (U/L)  | 21.9 ± 11.1          |
| LSR        | 0.9 ± 0.5            |
| ALP (U/L)  | 460.5 ± 1187.6       |
| LDH (U/L)  | 259.6 ± 260.9        |
| GGT (U/L)  | 22.3 ± 11.3          |
| TP (g/L)   | 72.7 ± 6.4           |
| ALB (g/L)  | 43.4 ± 7.0           |
| GLOB (g/L) | 29.8 ± 4.4           |
| CRP (mg/L) | 7.7 ± 14.0           |
| AGR        | 1.48 ± 0.3           |
| ACR        | 45.2 ± 59.2          |
| TBIL (umol/L) | 10.3 ± 5.2         |
| DBIL (umol/L) | 3.4 ± 1.6              |
| IBIL (umol/L) | 6.8 ± 3.9             |
| TBA (umol/L) | 4.8 ± 6.3               |
| Urea (mmol/L) | 4.6 ± 1.7              |
| CRE (umol/L) | 57.1 ± 19.0           |
| Cys-C (mg/L) | 0.8 ± 0.2              |
| UA (umol/L)  | 352.3 ± 95.9         |
| CHO (mmol/L) | 4.4 ± 1.1              |
| TG (mmol/L)  | 1.1 ± 0.6             |
| HDL-C (mmol/L) | 1.2 ± 0.4              |
| LDL-C (mmol/L) | 3.4 ± 6.3              |
| LHR        | 2.8 ± 4.3            |

a: The tumor maximum diameter;
b: TNM stage was classified according to the AJCC 8th TNM staging system;

Abbreviations: TNM: Tumor Node Metastasis stage; Sur: surgery; Rad: radiotherapy; Che: chemotherapy; PLT: platelet; NLR: neutrophil / lymphocyte ratio; LMR: lymphocyte / monocyte ratio; PLR: platelet / lymphocyte ratio; dNLR: derived neutrophil-to-lymphocyte ratio; SII: systemic immune-inflammation index; PNI: prognostic nutritional index; RBC: red blood cell; HGB: hemoglobin; IP3+: serum phosphorus; Ca2+: serum calcium; Mg2+: serum magnesium; Gap: anion gap; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LSR: ALT / AST ratio; ALP: alkaline phosphatase; LDH: lactic dehydrogenase; GGT:glutamyl transpeptidase; TP: total protein; ALB: albumin; GLOB: globulin; CRP: C-reactive protein; AGR: ALB / GLOB ratio; ACR: ALB / CRP ratio; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; TBA: total bile acid; CRE: creatinine; Cys-C: cystatin C; UA: uric acid; CHOL: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; LHR: LDL-C / HDL-C ratio; APOA: apolipoprotein A1; APOB: apolipoprotein B; ABR: APOA / APOB ratio; AI: The atherogenic index; GLU: glucose.
Variable | No. (%) or Mean ± sd
--- | ---
APOA (g/L) | 1.2 ± 0.3
APOB (g/L) | 0.8 ± 0.3
ABR | 1.6 ± 0.6
AI | 2.7 ± 1.1
GLU (mmol/L) | 4.8 ± 0.8

a: The tumor maximum diameter;
b: TNM stage was classified according to the AJCC 8th TNM staging system;

Abbreviations: TNM: Tumor Node Metastasis stage; Sur: surgery; Rad: radiotherapy; Che: chemotherapy; PLT: platelet; NLR: neutrophil / lymphocyte ratio; LMR: lymphocyte / monocyte ratio; PLR: platelet / lymphocyte ratio; dNLR: derived neutrophil-to-lymphocyte ratio; SII: systemic immune-inflammation index; PNI: prognostic nutritional index; RBC: red blood cell; HGB: hemoglobin; IP3+: serum phosphorus; Ca2+: serum calcium; Mg2+: serum magnesium; Gap: anion gap; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LSR: ALT / AST ratio; ALP: alkaline phosphatase; LDH: lactic dehydrogenase; GGT: glutamyl transpeptidase; TP: total protein; ALB: albumin; GLOB: globulin; CRP: C-reactive protein, AGR: ALB / GLOB ratio; ACR: ALB / CRP ratio; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirekt bilirubin; TBA: total bile acid; CRE: creatinine; Cys-C: cystatin C; UA: uric acid; CHO: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; LHR: LDL-C / HDL-C ratio; APOA: apolipoprotein A1; APOB: apolipoprotein B; ABR: APOA / APOB ratio; AI: The atherogenic index; GLU: glucose.

**Construction Of Prognostic Model For Os And Pfs**

Firstly, the LASSO regression analysis was performed to extract significant predictors associated with overall survival (OS). Figure 1A showed the change in trajectory of each predictor was analyzed. Afterwards, the optimal value for $\lambda$ was determined using 10-fold cross-validation with the minimum criteria (Fig. 1B). According to the criteria, the optimal value of the $\lambda$ was 0.076 in this study, and its corresponding predictors were considered to be the significant prognostic factors for OS, which included RBC, Ca2+, CRE, PNI, and LSR. Finally, a prognostic model was constructed for predicting OS and PFS based on the coefficients of significant predictors derived from the LASSO regression, with a risk score was calculated by using the following formula: The prognostic model risk score = 0.191 - (0.077 × RBC) + (0.048 × Ca$^{2+}$ - (0.007 × CRE) - (0.006 × PNI) - (0.726 × LSR). In this formula, each variable value represents their respective serum levels.

**Assessing the performance between the novel prognostic model, TNM staging, and clinical treatment**

In order to determine the incremental predictive value of the novel prognostic model to traditional TNM staging and clinical treatment for OS and PFS. Hence, we introduced C-index, tdROC curve, DCA, NRI, and IDI to evaluate the predictive accuracy of the novel prognostic model, TNM staging, and clinical treatment.

Firstly, we calculated the C-index in the three predictive signatures, as shown in Table 2. For OS, the C-index of the novel prognostic model was 0.782 (95% CI = 0.658–0.905), which was higher than that of the TNM staging [0.593 (95% CI = 0.458–0.728), P = 0.088] and clinical treatment [0.521 (95% CI = 0.400–0.643), P < 0.001]. For PFS, the C-index of the novel prognostic model was 0.741 (95% CI = 0.632–0.851), which was significantly higher than that of the TNM staging [0.544 (95% CI = 0.433–0.656), P = 0.013] and clinical treatment [0.505 (95% CI = 0.392–0.617), P < 0.001]. Secondly, we plotted tdROC curves and calculated the corresponding AUCs in the three predictive signatures. Results showed the dynamic AUC levels of the novel prognostic model estimated exceeding 0.75 both in OS and PFS, which were higher than TNM staging and clinical treatment (Fig. 2). Thirdly, the DCA showed the novel prognostic model had a higher overall net benefit than TNM staging and clinical treatment across the majority of the range of reasonable threshold probabilities both in OS and PFS (Fig. 3). Finally, both the NRI and IDI calculations were obtained at 1, 3 and 5 years and used to compare the
alternative prognostic indices of our model with the TNM staging and clinical treatment. The results were presented in Table 3. For OS, NRI analysis revealed that the accuracy of the novel prognostic model was higher than that of the TNM staging [for 1-year survival (0.538, P = 0.030), 3-year survival (0.324, P = 0.289), and 5-year survival (0.211, P = 0.706)] and clinical treatment [for 1-year survival (0.385, P = 0.070), 3-year survival (0.367, P = 0.149), and 5-year survival (0.216, P = 0.478)]. IDI analysis showed that the discrimination ability the novel prognostic model was also higher than that of the TNM staging [for 1-year survival (0.193, P = 0.050), 3-year survival (0.165, P = 0.139), and 5-year survival (0.153, P = 0.468)] and clinical treatment [for 1-year survival (0.212, P = 0.010), 3-year survival (0.202, P = 0.070), and 5-year survival (0.207, P = 0.189)]. In addition, the similar results also showed that the novel prognostic model had a good performance in predicting the PFS for OSC patients than others.

Table 2
The C-index of OS and PFS for our model, TNM stage, and treatment.

| Models for survival prediction | C-index  | 95 CI %  | P      |
|-------------------------------|----------|----------|--------|
| For OS                        |          |          |        |
| Our model                     | 0.782    | 0.658–0.905 | 0.088  |
| TNM stage                     | 0.593    | 0.458–0.728 | < 0.001|
| Treatment                     | 0.521    | 0.400–0.643 |        |
| Our model vs TNM stage        |          |          |        |
| Our model A vs Treatment      |          |          |        |
| For PFS                       |          |          |        |
| Our model                     | 0.741    | 0.632–0.851 | 0.013  |
| TNM stage                     | 0.544    | 0.433–0.656 | < 0.001|
| Treatment                     | 0.505    | 0.392–0.617 |        |
| Our model vs TNM stage        |          |          |        |
| Our model A vs Treatment      |          |          |        |

C-index = concordance index; P values are calculated based on normal approximation using function rcorrp.cens in Hmisc package.
Table 3
A comparison of discriminatory ability of Our model with TNM stage and Treatment using NRI and IDI for OS and PFS.

|            | 1-Year | 3-Year | 5-Year |
|------------|--------|--------|--------|
|            |  NRI   |  P     |  IDI   |  P     |  NRI   |  P     |  IDI   |  P     |  NRI   |  P     |  IDI   |  P     |
| For OS     |        |        |        |        |        |        |        |        |
| Our model vs TNM stage | 0.538  | 0.030  | 0.193  | 0.050  | 0.324  | 0.289  | 0.165  | 0.139  | 0.211  | 0.706  | 0.153  | 0.468  |
| Our model vs Treatment  | 0.385  | 0.070  | 0.212  | 0.010  | 0.367  | 0.149  | 0.202  | 0.070  | 0.216  | 0.478  | 0.207  | 0.189  |
| For PFS    |        |        |        |        |        |        |        |        |
| Our model vs TNM stage | 0.528  | 0.040  | 0.127  | 0.050  | 0.287  | 0.259  | 0.136  | 0.129  | 0.401  | 0.239  | 0.139  | 0.169  |
| Our model vs Treatment  | 0.374  | 0.050  | 0.135  | 0.010  | 0.333  | 0.129  | 0.154  | 0.050  | 0.276  | 0.219  | 0.143  | 0.139  |

Construction of a predictive nomogram based on prognostic model risk score, TNM staging, and clinical treatment

The nomogram incorporating the prognostic model risk score, TNM staging, and clinical treatment to predict the probability of 1-, 3-, and 5-year OS (Fig. 4A) and PFS (Fig. 4B) in OSC patients. Each patient would assign one point for each prognostic variable, the estimated probability of 1-, 3- and 5- year OS and PFS was determined by summing all of the point, and the higher number of total points indicated a worse outcome for the patient. In addition, the calibration curve showed good agreement between prediction and observation in 1-, 3-, and 5-year OS (Fig. 4C, 4E, 4H) and PFS (Fig. 4D, 4F, 4I). The C-index of the nomogram for OS and PFS was 0.784 and 0.737, respectively.

The correlation between the prognostic model, TNM staging and clinical treatment

Currently, AJCC TNM staging system remains the most valuable tool to predict prognosis for OSC. Next, we assessed the correlation between the prognostic model, TNM staging and clinical treatment (Fig. 5). In the plot, the blue represented positive correlation, and the red represented negative correlation. The color intensity and the size of the circle were proportional to the correlation coefficients. Significant linear dependence between variables was identified using Pearson's correlation coefficient (PCC). The results showed that the prognostic model was positive correlation with TNM staging (PCC = 0.17, P = 0.150) and clinical treatment (PCC = 0.10, P = 0.423).

Survival analyses of OSC patients according to prognostic model risk score

Using the R package “survminer” and “survival”, we classified patients into low -risk patients and high-risk patients based on the prognostic model risk score, and make the Kaplan-Meier curve. The results showed that patients with higher risk scores (risk score > -0.94) had a significantly lower OS (Fig. 6A, P < 0.001) and PFS (Fig. 6B, P = 0.001) rate than their low-risk counter-parts (risk score ≤ -0.94). In order to test whether the prognostic model could remedy the current deficiencies of AJCC TNM stage. Patients were factitiously stratified into early stage (stage I/II) and late stage (stage III/IV). Kaplan-Meier curve showed that high-risk patients in the early stage had significantly shorter OS (Fig. 6C, P < 0.001) and PFS (Fig. 6D, P < 0.001) than low-risk patients, but in the late stage, the OS (Fig. 6E, P = 0.220) and PFS (Fig. 6F, P = 0.450) in low-risk patients and high-risk patients displayed no significant difference.
The serum levels for the 5 selected predictors in the low-risk and high-risk patients

Figure 7 showed the pretreatment serum values of RBC, Ca\(^{2+}\), CRE, PNI, and LSR in the low-risk and high-risk patients. The serum values of RBC (4.87 ± 0.53 \(10^{12}/\text{L}\)), CRE (60.75 ± 17.09 umol/L), PNI (54.66 ± 7.54), and LSR (0.99 ± 0.43) in the low-risk patients, were significantly higher than high-risk patients (RBC (4.07 ± 0.79 \(10^{12}/\text{L}\)), CRE (32.26 ± 9.44 umol/L), PNI (49.31 ± 6.11), and LSR (0.35 ± 0.17). But the low-risk patients had a lower Ca\(^{2+}\) levels (2.34 ± 0.10 mmol/L) compared with high-risk patients (3.23 ± 2.65 mmol/L).

Discussion

In the present study, we had analyzed individual clinical characteristics and blood biomarkers based on survival analysis. The Lasso regression algorithm was used to establish a novel prognostic model for predicting OS and PFS in OSC patients. Compared with traditional TNM staging and clinical treatment, our prognostic model had better predictive accuracy and discriminatory ability. The prognostic model successfully classified those patients into high-risk and low-risk subgroups that were significantly different in terms of OS and PFS.

According to the results of LASSO regression analysis, we identified a 5 prognostic factors module (RBC, Ca\(^{2+}\), CRE, PNI, and LSR) as a novel predictive model for the OSC patients. RBC count was one of the erythrocyte parameters, the reduced preoperative RBC count might reflect worse liver function, it was well known that liver function impacts patients’ survival\[23, 24]\). Lu et al. had reported that preoperative RBC counts lower than normal had worse OS rates than those without reduced preoperative RBC counts in primary liver cancer patients\[25]. Ca\(^{2+}\) played a pivotal role in cancer cells growth, migration, and death, serving as a principal signalling agent and the expression of Ca\(^{2+}\) channel transcripts had been highlighted as a potential biomarker in the growing number of cancers\[26, 27]\). CRE as a marker of kidney function, which had been investigated as a prognostic parameter in colorectal\[28]\), liposarcoma\[29]\), and prostate cancer\[30]\). PNI could reflect the immune and nutritional status of human body, previous studies had suggested the predictive and prognostic value of it in a variety of tumors\[31, 32]\). Huang et al. found that preoperative low PNI was significantly correlated with OSC tumor size, tumor staging, pathologic fractures, local recurrence, and metastasis, suggesting that PNI may be an important prognostic parameter in patients with OSC\[33]\). LSR was often used in the assessment of liver injury, the increased serum LSR in patients with gastric adenocarcinoma was associated with better prognosis\[34]\). Chen et al. found that alginate oligosaccharide treatment reduced the progression of OSC, and decreased levels of IL-1, IL-6, and the ratios of AST/ALT, which may be related to the improvement of antioxidant and anti-inflammatory capabilities in OSC patients\[35]\). All of the above research showed that the 5 prognostic factors were closely related to the occurrence or development of tumors. These suggested that our analysis results had credible prognostic value.

In order to determine whether our model could remedy the deficiencies of TNM staging in the prognostic assessment of OSC patients, we divided the patients into low-risk and high-risk subgroups based on the prognostic model risk scores. Kaplan-Meier survival curves showed that the high-risk groups were associated with shortened survival in OSC patients with stage I&II and stage III&IV. Thus, the results reminded us that even patients in the same stage, high-risk patients required more intensive treatment. Moreover, the results also suggested that our model could remedy the deficiencies of TNM staging and enhance the predictive power of TNM stage. Improved prediction of individual prognosis would help clinicians in counseling patients, selecting personalized treatment, and scheduling patient follow-up.

Compared to previous studies\[33, 36, 37]\), this study had the following advantages: 1. The prognostic factors of OSC in the past were mostly single indicators, but our study included more potential prognostic factors than previous studies. The simplicity, cheapness and availability of the five prognostic biomarkers fully reflect the advantages of their combined application. 2. We used the new algorithm LASSO regression analysis to develop a prognostic model as a statistical method for filtering variables to establish a prognostic model, which allows to adjust the overfitting of the model to avoid extreme predictions, so the prediction accuracy could be significantly improved, and this method has been applied in many
In this study, we used multiple methods to compare the predictive accuracy and discriminative ability of the novel prognostic model with TNM staging and clinical treatment. And these results all showed that our model outperformed than others. The endpoint of this study were OS and PFS, so this model could achieve better clinical application.

However, there still presented some limitations in this study. 1. This was a retrospective analysis, its selection bias may be unavoidable, so its calculated predictive value was for clinicians' reference only. 2. We only analyzed the data from a single cancer center, with a small sample size. Therefore, it is necessary to conduct multi-center and large-scale studies in the future to further verify the generalizability of the prognostic model established in this study. 3. This study had shown that RBC, Ca\(^{2+}\), CRE, PNI and LSR were related to the prognosis of OSC patients, but the molecular mechanisms behind the above-mentioned effect have not been clarified. 4. Although these five predictors were easy to obtain, it was undeniable that these predictors were all non-specific OSC predictors and may lack certain specificity. Some biomarkers may be incorporated into these prognostic models to improve specificity, such as immunohistochemical markers, radiomics, and recently newly applied non-coding RNAs. 5. We collected data only for the initial diagnosis and did not dynamically monitor the entire course of the patient, so we could not know the significance of biomarkers for the prognosis of the patient after each treatment. Despite the above shortcomings, the prognostic model was effective and may help predict the prognosis of OSC patients, providing clinicians with a more practical and convenient tool for individualized treatment decision making and survival assessment at the initial diagnosis.

**Conclusions**

In conclusion, we successfully established a novel prognostic model based on clinical characteristics and blood biomarkers, which showed outperform TNM staging and clinical treatment in predicting OS and PFS in OSC patients. The low cost, precise, secure stability and understandable prognostic model may act as a potential tool for clinicians in counselling, personalizing treatment, and scheduling patients' follow-ups for OSC patients. But the wide practical application of this model required more clinical data to verify the accuracies of our model for predicting prognosis of OSC patients.

**Abbreviations**

TNM: Tumor Node Metastasis stage; Sur: surgery; Rad: radiotherapy; Che: chemotherapy; PLT: platelet; NLR: neutrophil / lymphocyte ratio; LMR: lymphocyte / monocyte ratio; PLR: platelet / lymphocyte ratio; dNLR: derived neutrophil-to-lymphocyte ratio; SII: systemic immune-inflammation index; PNI: prognostic nutritional index; RBC: red blood cell; HGB: hemoglobin; IP\(^{3+}\): serum phosphorus; Ca\(^{2+}\): serum calcium; Mg\(^{2+}\): serum magnesium; Gap: anion gap; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LSR: ALT / AST ratio; ALP: alkaline phosphatase; LDH: lactic dehydrogenase; GGT:glutamyl transpeptidase; TP: total protei; ALB: albumin; GLOB: globulin; CRP: C-reactive protein, AGR: ALB / GLOB ratio; ACR: ALB / CRP ratio; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; TBA: total bile acid; CRE: creatinine; Cys-C: cystatin C; UA: uric acid; CHO: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; LHR: LDL-C / HDL-C ratio; APOA: apolipoprotein A1; APOB: apolipoprotein B; ABR: APOA / APOB ratio; AI: The atherogenic index; GLU: glucose.

**Declarations**

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**Authors’ contributions**
All authors contributed to this manuscript, including conception and design (PQS, CXX), acquisition of data (LRT, SLC), analysis and interpretation of data (DMZ, YYC, TFZ), writing (LRT, SLC, CL), material support (XMY, ZHL, WTT), study supervision (PQS, CXX), and all authors participated in review and revision of the manuscript.

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Availability of data and materials

The datasets analyzed during the current study are not publicly available due to patient privacy concerns, but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committee of the Sun Yat-sen University Cancer Center, and all patients provided written informed consent at the first visit to our center.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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