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Article

Serum pro-surfactant protein B is correlated with clinical properties in osteosarcoma patients

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Abstract

It is critical to find efficient non-invasive prognostic factor for osteosarcoma. In this study, we demonstrated that serum protein of pro-surfactant protein B (pro-SFTPB) may be a potential diagnostic indicator in osteosarcoma. We found that serum pro-SFTPB was highly expressed in osteosarcoma patients and presented good diagnostic value to discern osteosarcoma patients from non-osteosarcoma control subjects. Serum pro-SFTPB was also significantly correlated with advanced clinical stage, distant metastasis, and shorter overall survival. In addition, serum pro-SFTPB was demonstrated to be an independent prognostic factor for osteosarcoma. Overall, our study demonstrated that serum pro-SFTPB may be a useful diagnostic factor for osteosarcoma.

Key words: osteosarcoma, pro-SFTPB diagnosis, survival, prognosis

Introduction

Osteosarcoma is one type of bone cancers that mostly occur in young people, including children and adolescents (Jafari et al. 2020; Sadykova et al. 2020). Osteosarcoma has a high tendency of lung metastasis (Jeffree et al. 1975) and early diagnosis methodologies are mostly lacking that most new cases are discovered at late stages with distance metastasis and poor prognosis (Allison et al. 2012; Anderson 2016; Sadykova et al. 2020). In addition, post-operative recurrences are observed in more than 50% patients with osteosarcoma, further reducing cancer patients' survival rate (Lindsey et al. 2017; Gazouli et al. 2021). Thus, the discovery of novel and efficient non-invasive, blood-based biomarkers is critical for early diagnosis and clinical management of osteosarcoma.

Several circulating biomarkers have been previously identified in osteosarcoma, including messenger RNA (mRNA), microRNA (miR), circulating tumor DNA (ctDNA), proteomics, metabonomics, metabolomics, and intact circulating tumor cells (Raimondi et al. 2017; Tan et al. 2019). Yet, a standard non-invasive osteosarcoma diagnosis protocol has never been established. Surfactant protein B (SFTPB) is a pulmonary surfactant–associated protein that was originally found to be mainly expressed in alveolar Type II epithelial cells and nonciliated bronchiolar epithelial cells (Clark et al. 1995; Stahlman et al. 2000). The pre-cleavage form of SFTPB is a hydrophilic 42-kD protein called pro-SFTPB, which undergoes C-/N- terminal cleavages by cysteine proteases in the endoplastic reticulum to form SFTPB (Guttentag et al. 2003). Recently, studies had demonstrated that serum expression level of pro-SFTPB was elevated in patients with non-small cell lung cancer (NSCLC) and closely associated with poor prognosis and shorter survival among patients with NSCLC, thus indicating a diagnostic role of serum pro-SFTPB for detecting human cancers (Sin et al. 2013; Taguchi et al. 2013; He et al. 2017; Wang et al. 2019; Lu et al. 2021).

In this study, we investigated whether serum levels of pro-SFTPB were different between osteosarcoma patients and non-osteosarcoma control subjects, and assessed the association between serum pro-SFTPB expression level and osteosarcoma patients' clinicopathological features and survival. We thus reported that serum pro-SFTPB may act as a potential biomarker for detection of osteosarcoma.

Materials and method

Patients

Between June 2011 and December 2021, a total of 233 osteosarcoma patients admitted to the Department of Second Orthopedics at the Affiliated Central Hospital of Shenyang Medical College were enrolled in this study. Patients were excluded if they had any other comorbidities or major brain/bone surgeries within 6 months, or life expectancy shorter than 6 months. All diagnosis was confirmed by patients' primary-care physicians and independent pathological examinations. Patients' clinicopathological features were determined according to the American Joint Committee on Cancer staging manual (Edge and Compton 2010), and the ESMO–PaedCan–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up for osteosarcoma

(Casali et al. 2018). In addition, all enrolled patients received neoadjuvant chemotherapy and (or) surgical treatment according to the ESMO–PaedCan–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up for osteosarcoma (Casali et al. 2018). Moreover, a total of 178 age- and sex-matched non-osteosarcoma control patients were recruited in this study.

Serum pro-SFTPB examination

The method of detecting serum pro-SFTPB expression levels among osteosarcoma patients was adapted from previous studies with slight modifications (Sin et al. 2013; Wang et al. 2019; Lu et al. 2021). Briefly, 4 mL peripheral blood was drawn from each patient, centrifuged at 3000 rpm at 4 °C for 30 min. The supernatant plasma was collected, either frozen at −80 °C until further analysis, or directly processed using an enzyme-linked immunosorbent assay (ELISA) kit for pro-SFTPB (Shanghai Enzyme-linked Biotechnology Co., Ltd, Shanghai, China). Quantification of serum pro-SFTPB expression levels was conducted using a Varioskan Lux multimode microplate reader (Thermo Fisher Scientific, Shanghai, China). The optical density of absorbance was measured at a wavelength at 455 nm and the exact serum pro-SFTPB expression levels were deduced from spectrophotometrically populated standard curves.

Statistical analysis

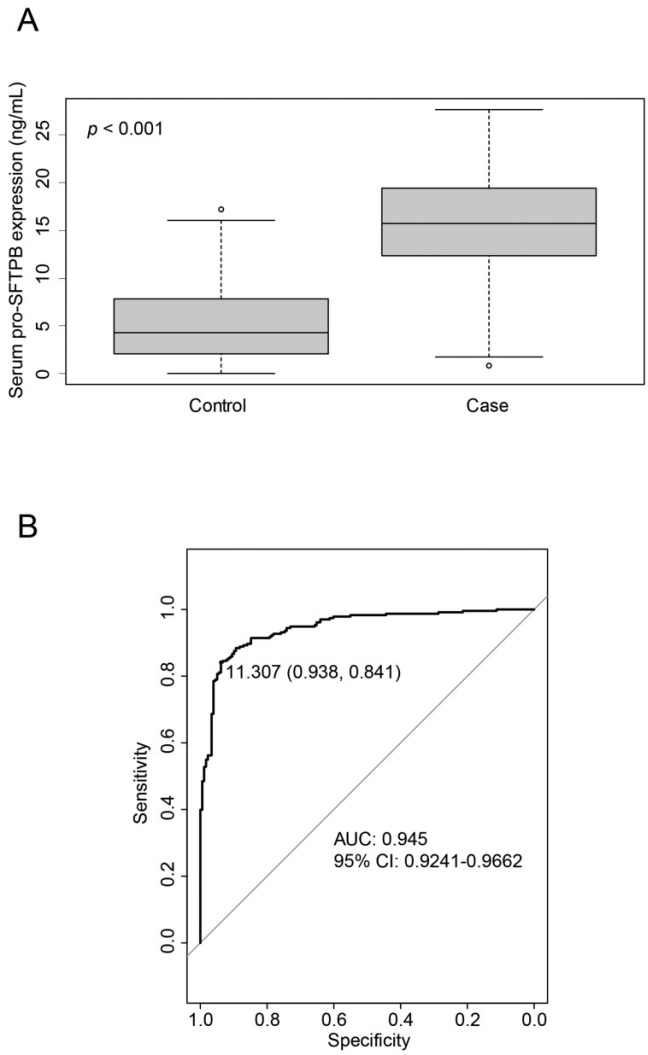
All statistical analyses were carried out using the RStudio Desktop IDE software (version 2022.02.1+461) (https://www.rstudio.com/). For direct comparisons of serum pro-SFTPB expressions between different subtypes, a Mann–Whitney test was used for comparisons between two groups, whereas a Kruskal–Wallis test was used for comparisons between three or more groups. Correlation between serum pro-SFTPB expression level and osteosarcoma patients' clinicopathological features was analyzed using a χ^2 test. Receiver operating characteristic (ROC) analysis was conducted and area under the curve (AUC) was calculated to estimate the clinical prediction value of serum pro-SFTPB. Overall survival (OS) was surveyed using the Kaplan–Meier curve with log-rank test. Predictability of OS was estimated using the Cox univariate/multivariate proportional hazard regression model. Significant difference was declared if $p < 0.05$.

Results

Serum pro-SFTPB expression level was highly expressed in osteosarcoma patients

Serum pro-SFTPB expression levels were compared between osteosarcoma patients (case) and non-osteosarcoma controls (control). The result demonstrated that serum pro-SFTPB was significantly upregulated in case group than in control group (Fig. 1A; $p < 0.001$). In addition, ROC analysis targeting serum pro-SFTPB expression indicated an AUC of 0.945 (95% confidence interval (CI): 0.9241–0.9662) and an optimal cut-off point with specificity of 0.938 and sensitivity of 0.841 (Fig. 1B; $p < 0.05$), suggesting that serum pro-SFTPB may be a potential biomarker to discern osteosarcoma patients from non-osteosarcoma subjects.

Fig. 1. Serum pro-SFTPB expression in osteosarcoma patients and its diagnosis potential. (A) Median serum pro-SFTPB expression levels were compared between non-osteosarcoma populations (Control) and osteosarcoma patients (Case). Each plot was composed with median (center line), upper and lower quartiles (box), 95% confidence interval (vertical lines), and outliers (points). (B) Receiver operating characteristic (ROC) analysis was conducted using serum pro-SFTPB as predicting factor. The area under the curve (AUC) was 0.945 with a 95% confidence interval between 0.9241 and 0.9662. At the optimal cut-off point, the specificity was 0.938 and sensitivity was 0.841 ($p > 0.05$).



Serum pro-SFTPB expression level was highly correlated with clinicopathological features of osteosarcoma patients

In this study, a total of 233 osteosarcoma patients were divided into a low serum pro-SFTPB expression group ($n = 116$) and a high serum pro-SFTPB expression group ($n = 117$), based on the median value of serum pro-SFTPB expression level. We

Table 1. The correlation between serum pro-SFTPB expression and osteosarcoma patients' clinicopathological features (*$p < 0.05$).

Clinicopathological feature	Low pro-SFTPB Total patients $N = 116$ N (%)	High pro-SFTPB Total Patients $N = 117$ N (%)	p value
Sex			
Male	63 (54.3%)	69 (59.0%)	0.5578
Female	53 (45.7%)	48 (4.0%)	
Age (years)			
<20	97 (83.6%)	90 (76.9%)	0.2629
>=20	19 (16.4%)	27 (23.1%)	
Tumor site			
Tibia/femur	69 (59.5%)	70 (59.8%)	0.972
Other	47 (40.5%)	47 (40.2%)	
Tumor size			
⇐8 cm	75 (64.7%)	83 (70.9%)	0.3754
>8 cm	41 (35.3%)	34 (29.1%)	
Histology			
Osteoblastic	55 (47.4%)	66 (56.4%)	
Chondroblastic	49 (42.2%)	41 (35.0%)	0.3889
Other	12 (10.4%)	10 (8.6%)	
Clinical stage			
IIA	93 (80.2%)	13 (11.1%)	<0.001*
IIB/III	23 (19.8%)	104 (88.9%)	
Distant metastasis			
Positive	84 (72.4%)	34 (29.1%)	<0.001*
Negative	32 (27.6%)	83 (70.9%)	
Response to chemotherapy			
Good	45 (38.8%)	50 (42.7%)	0.632
Poor	71 (61.2%)	67 (57.3%)	
Surgery			
Limb sparing	63 (54.3%)	83 (71.0%)	0.119
Amputation	9 (7.8%)	15 (12.8%)	

then investigated the statistical correlation between serum pro-SFTPB expression level and cancer patients' clinicopathological features (Table 1; *$p < 0.05$). As indicated, high serum pro-SFTPB expression level was highly correlated with clinical stage of IIB/III and distance metastasis (Table 1; $p < 0.001$). On the other hand, serum pro-SFTPB expression levels were not correlated with cancer patients' sex, age, tumor site, tumor size, histology, response to chemotherapy, or surgery treatments (Table 1; $p > 0.05$).

Serum pro-SFTPB expression level was higher in osteosarcoma patients of advanced clinical stages and with distance metastasis

Serum pro-SFTPB expression levels were then compared among different subtypes of osteosarcoma patients. The result showed that serum pro-SFTPB was not differentially expressed between male and female cancer patients (Fig. 2A; $p > 0.05$), between patients younger than 20-year and those not (Fig. 2B; $p > 0.05$), between patients with tumor sites at tibia/femur and those with other tumor sites (Fig. 2C; $p > 0.05$), among patients with osteoblastic osteosarcoma, chondroblastic osteosarcoma or other histological subtypes (Fig. 2D; $p > 0.05$), between patients with tumor sizes smaller than 8 cm and those with bigger (Fig. 2E; $p > 0.05$), or between patients with good or poor chemotherapy responses (Fig. 2H; $p > 0.05$).

However, serum pro-SFTPB expression levels were significantly higher in cancer patients of IIB/III clinical stages than in those of IIA clinical stages (Fig. 2F; $p < 0.001$), and significantly higher in cancer patients with distance metastasis than in those without (Fig. 2G; $p < 0.001$).

Serum pro-SFTPB expression level had good diagnostic value

Further ROC analysis was conducted for osteosarcoma patients of different clinical stages, according to their serum pro-SFTPB expression levels. The result demonstrated, for stage IIA osteosarcoma patients, the AUC was 0.905 (95% CI: 0.8687–0.942) and the optimal cut-off point had specificity of 0.848 and sensitivity of 0.849 (Fig. 3A; $p < 0.05$). In addition, the result for stage IIB/III osteosarcoma patients was even better. The AUC was 0.978 (95% CI: 0.9619–0.9948) and the opti-

Fig. 2. Serum pro-SFTPB expressions among osteosarcoma subtype patients. Median serum pro-SFTPB expression levels were compared between different subtypes of osteosarcoma patients, based on their (A) sex, (B) age, (C) tumor site, (D) histology, (E) tumor size, (F) clinical stage, (G) distance metastasis, and (H) response to chemotherapy. Each plot was composed with median (center line), upper and lower quartiles (box), 95% confidence intervals (vertical lines), and outliers (points).

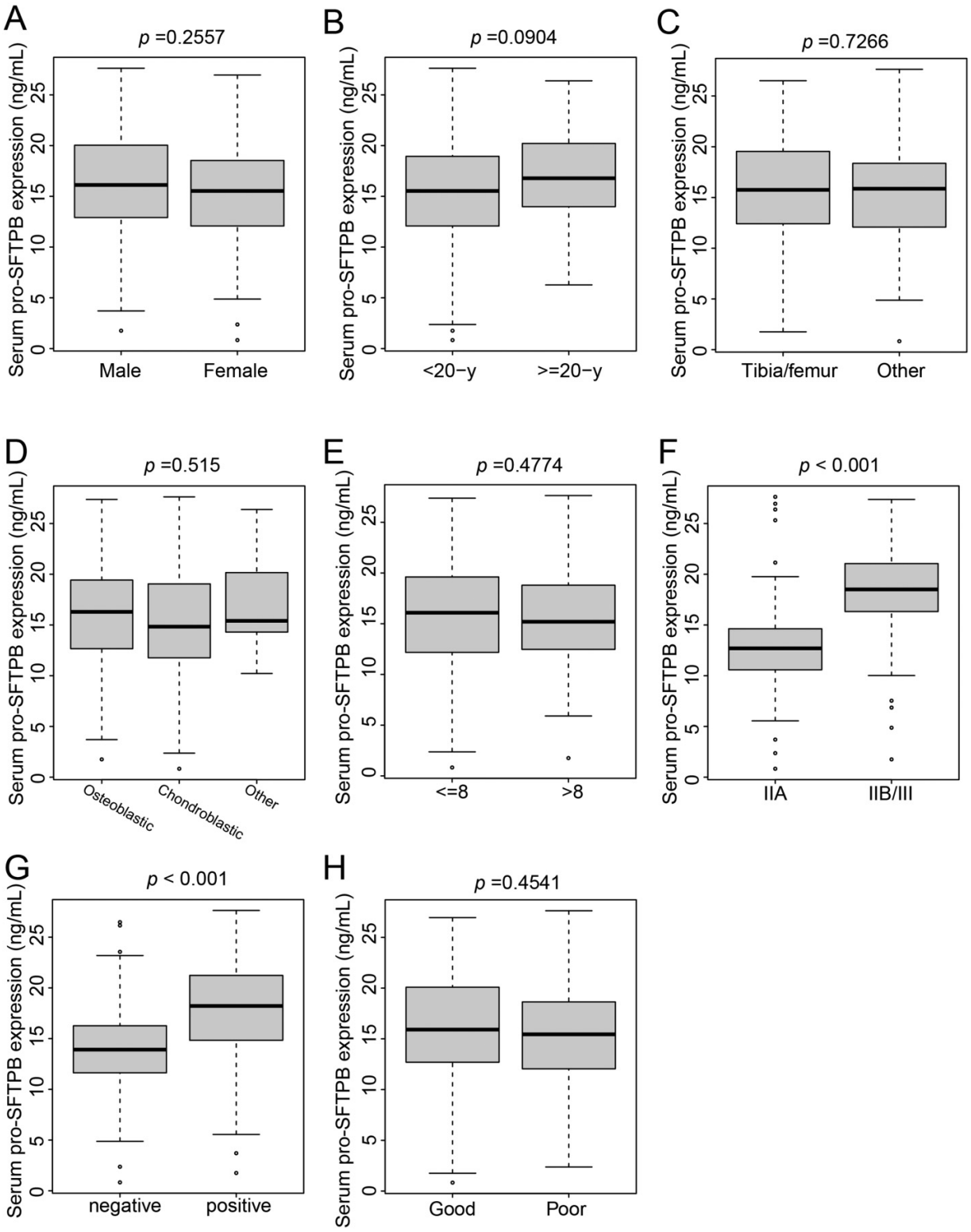
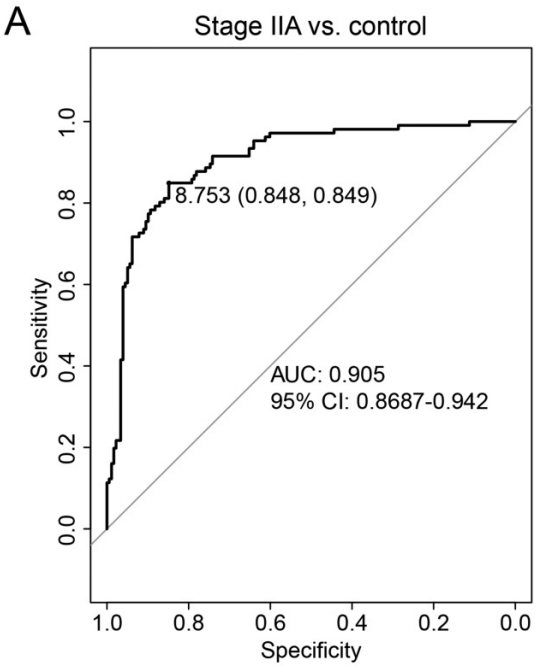


Fig. 3. ROC curves of using serum pro-SFTPB expression levels to discern osteosarcoma patients of different clinical stages from controls. (A) Receiver operating characteristic (ROC) analysis was conducted using serum pro-SFTPB to discern stage IIA osteosarcoma patients from non-osteosarcoma controls. The area under the curve (AUC) was 0.905 with a 95% confidence interval between 0.8687 and 0.942. At the optimal cut-off point, the specificity was 0.848 and sensitivity was 0.849 ($p > 0.05$). (B) ROC analysis was conducted using serum pro-SFTPB to discern stage IIB/III osteosarcoma patients from non-osteosarcoma controls. The area under the curve (AUC) was 0.978 with a 95% confidence interval between 0.9619 and 0.9948. At the optimal cut-off point, the specificity was 0.961 and sensitivity was 0.945 ($p > 0.05$).



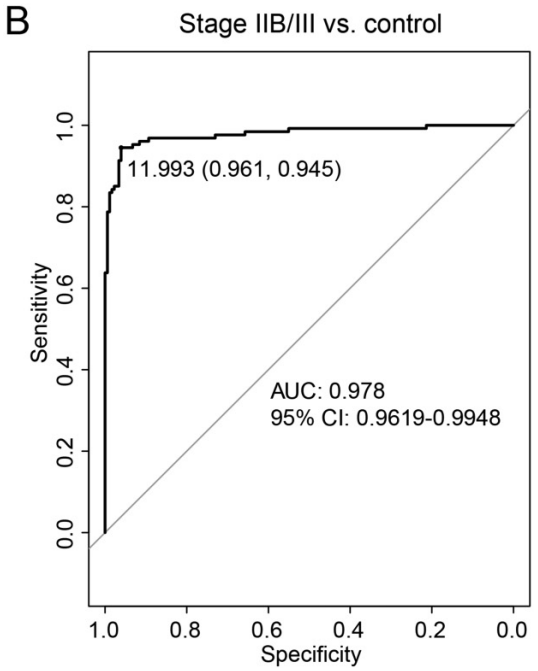
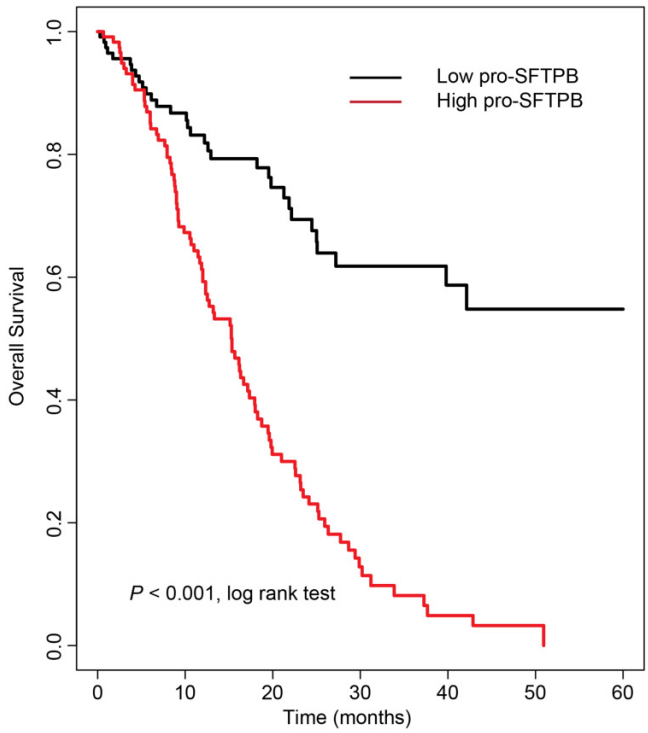


Fig. 4. Overall survival analysis of osteosarcoma patients by using serum pro-SFTPB expression levels. Kaplan–Meier analysis was conducted to estimate the predicting capability of serum pro-SFTPB for osteosarcoma patients' overall survival (log-rank test, $p = 0.008$).



mal cut-off point had specificity of 0.961 and sensitivity of 0.945 (Fig. 3B; $p < 0.05$).

Prognosis of serum pro-SFTPB expression level in osteosarcoma patients

We then investigated the prognostic implication of serum pro-SFTPB among osteosarcoma patients. Based on the results of Kaplan–Meier method and log-rank test, osteosarcoma patients with high serum pro-SFTPB expression levels had a significantly shorter OS than those with low serum pro-SFTPB expression levels (Fig. 4; $p = 0.008$, log rank test). In addition, using cox-regression analyses, the results of univariate estimation showed the clinical stage (HR = 2.033, 95% CI (1.381_2.994), $p = 0.003$) and serum pro-SFTPB expression level (HR = 3.952, 95% CI (2.618_5.968), $p < 0.001$) were significantly correlated with osteosarcoma patients' OS. Furthermore, the results of multivariate estimation suggested that serum pro-SFTPB expression level might act as an independent risk factor for survival outcome of osteosarcoma patients (HR = 4.6618, 95% CI (2.7551_7.888), $p < 0.001$) (Table 2).

Discussion

In this study, we presented novel data showing that serum pro-SFTPB was highly expressed in osteosarcoma patients than in non-osteosarcoma control subjects. Also,

Table 2. The prognostic analysis of osteosarcoma patients' clinicopathological features.

Clinicopathological feature	Comparison	Univariate estimation		Multivariate estimation	
		p value	HR (95% CI)	*p* value	HR (95% CI)
Sex	Male versus female	0.817	1.043 (0.7311–1.487)		
Age (years)	<20 versus ≥20	0.602	1.116 (0.7395–1.683)		
Tumor site	Tibia/femur versus other	0.323	0.8339 (0.5815–1.196)		
Tumor size	⇐8 cm versus >8 cm subtypes	0.287	0.8074 (0.5446–1.197)		
Histology	Osteoblastic versus other	0.441	0.8702 (0.611–1.24)		
Clinical stage	IIA versus IIB/III	<0.001*	2.033 (1.381–2.994)	0.312	0.7705 (0.4647–1.278)
Distant metastasis	Positive versus negative	0.005*	1.667 (1.165–2.386)	0.901	0.7753 (0.6912–1.521)
Response to chemotherapy	Good versus poor	0.598	0.9096 (0.6393–1.294)		
Serum pro-SFTPB expression level	Low and high	<0.001*	3.952 (2.618–5.968)	<0.001*	4.6312 (2.7081–7.92)

Note: HR, hazard ratio; CI, confidence interval. *, $p < 0.05$.

serum pro-SFTPB expression level may act as a valuable diagnostic indicator to discern osteosarcoma patients from non-osteosarcoma control subjects. In addition, we demonstrated that serum pro-SFTPB expression level was significantly associated with clinicopathological features of osteosarcoma patients, and highly expressed in osteosarcoma subsets of stage IIB/III patients (as compared to stage IIA patients) and patients with distance metastasis (as compared to patients without distant metastasis). Moreover, we showed that high serum pro-SFTPB expression level was significantly correlated with short OS among osteosarcoma patients. Therefore, we concluded that serum pro-SFTPB may act as a potential prognostic biomarker for osteosarcoma.

The prognostic values of pro-SFTPB in human diseases have been previously reported. In 2013, a Pan-Canadian Early Detection of Lung Cancer Study measured serum pro-SFTPB levels in 2485 individuals and it showed serum pro-SFTPB was an independent biomarker for lung cancer (Sin et al. 2013). In 2015, it was further confirmed that elevated serum pro-SFTPB expression level was significantly associated with declined lung functions among smokers (Leung et al. 2015). In addition, two independent studies, one in 2018 and one in 2021, confirmed that serum pro-SFTPB, along with other three serum cancer protein biomarkers, cancer antigen 125, carcinoembryonic antigen, and cytokeratin-19 fragment (CYFRA 21–1), may serve as an efficient circulating biomarker panel to predict lung cancer (Integrative Analysis of Lung Cancer et al. 2018; Lu et al. 2021). Interestingly, a 2018 study also demonstrated that serum pro-SFTPB expression levels were much elevated among patients with human immunodeficiency virus and were associated with immunosuppression and uncontrolled viremia (Shiels et al. 2018), suggesting that aberrant serum pro-SFTPB expression level may be associated with other human pathological conditions. In this study, we demonstrated that serum pro-SFTPB expression levels were significantly higher in osteosarcoma patients and markedly correlated with osteosarcoma patients' poor prognosis and shorter survival.

The limitation of this study is that aberrant serum pro-SFTPB expression level had been linked to human diseases (mentioned above) other than osteosarcoma that its prognostic effect may not be specifically associated with one single human disease. However, this pan-cancer or pan-human disease notion shall not stop us or other researchers to continuously explore the biomarker-potential of pro-SFTPB in auxiliary diagnosis of osteosarcoma. For example, if a patient has symptoms indicative of osteosarcoma, non-invasive blood test of serum pro-SFTPB expression level may assist clinicians to decide whether bone biopsy is warranted for a complete diagnosis. In addition, our data (Fig. 2G; Table 1) indicated that serum pro-SFTPB expression level was highly correlated with osteosarcoma patients with metastasis. This specific feature may potentially help to identify cancer patients with metastatic spread earlier than other currently available diagnostic methods. During the meantime, one shall not take the notion of serum pro-SFTPB being a metastatic indicator to underestimate its overall oncogenic implication in osteosarcoma, as our data demonstrated that, as compared to healthy control, serum pro-SFTPB expression levels were elevated in both metastatic and non-metastatic cancer patients (Figs. 1A and 2G). Moreover, recent advance in single-cell RNA sequencing revealed that distinct molecular identities were associated with treatment-native osteoblastic osteosarcoma cells (Liu et al. 2021). It would be very interesting to explore the origin of serum pro-SFTPB by investigating which osteoblastic subtype cells may be responsible for initiating the production or release of pro-SFTPB in the circulating systems among osteosarcoma patients.

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All data are available upon reasonable request.

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Project administration: LA
Resources: LA
Software: DF, YJ
Supervision: LA
Validation: DF, YZ, YJ, LA
Visualization: LZ, YJ
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Writing – review & editing: LA

Competing interests
The authors declare no conflict of interest.

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Ethics statement
The approval to conduct this study was granted by the Clinical Research and Ethics Committees at the Affiliated Central Hospital of Shenyang Medical College in Shenyang, Liaoning Province, China. Informal consent forms were signed by all participating patients. In addition, the guideline of the Declaration of Helsinki (2013) was followed throughout the study (General Assembly of the World Medical 2014). All authors consent to publication.

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