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RESEARCH ARTICLE

Serum superoxide dismutase, a potential predictor for radiation pneumonitis following chemoradiotherapy in non-small cell lung cancer patients

Dongqing Wang[1], Jingyu Zhu[2], Mingping Sun[1], Baosheng Li[1], Ling Wei[3], Zhongtang Wang[1], and Hongfu Sun[1]

[1]Department of Radiation Oncology, Shandong Cancer Hospital, Shandong Academy of Medical Sciences, Jinan, P.R. China, [2]Jinan Central Hospital affiliated to Shandong University, Jinan, P.R. China, and [3]Basic Research Center, Shandong Cancer Hospital, Shandong Academy of Medical Sciences, Jinan, P.R. China

Abstract

Purpose: To explore serum superoxide dismutase (SOD) for predicting radiation pneumonitis (RP) in non-small cell lung cancer patients following chemoradiotherapy.

Methods: Serum levels for SOD were measured by enzyme-linked immunosorbent assays prior to radiation therapy (Pre-RT) and post 40 Gy/4 weeks during the treatment (Pos-RT).

Results: SOD concentrations after delivery of 40 Gy/4 weeks was associated with the development of RP. The best predictive ability of SOD was observed for a cut-off value of 56 unit/ml, with a sensitivity of 0.80 (95% CI 0.28–0.99), and a specificity of 0.67 (95% CI 0.43–0.65) ($p = 0.040$).

Conclusion: Serum SOD may be a potential predictor for RP, which need to be further verified.

Keywords: Radiation therapy, radiation pneumonitis, non-small cell lung cancer, superoxide dismutase

Introduction

Radiation pneumonitis (RP) is an important dose-limiting toxicity during thoracic radiotherapy. According to recent data (Barriger et al. 2010, Phernambucq et al. 2011, Werner et al. 2011), clinical symptom RP (Grade ≥ 2) has been reported to occur in 7.0–32.0%, severe RP (Grade ≥ 3) 2.6–18.0%, and the lethal RP (Grade 5) 0–2.0%, for locally advanced non-small cell lung cancer (NSCLC) patients receiving concurrent chemoradiotherapy. Current studies suggest that many factors, such as dosimetric factors (De Jaeger et al. 2004, Evans et al. 2006, Hartsell et al. 2007, Kocak et al. 2007, Mazeron et al. 2010, Barriger et al. 2011), biomarkers (Anscher et al. 1997, Vujaskovic & Groen 2000, Chen et al. 2001, Chen et al. 2002, Novakova et al. 2004, Arpin et al. 2005, Chen et al. 2005, Hart et al. 2005, Evans et al. 2006, Hartsell et al. 2007, Zhao et al. 2008, Kim et al. 2009, Yuan et al. 2009, Hildebrandt et al. 2010, Zhang et al. 2010, Mak et al. 2011, Yang et al. 2011, Yin et al. 2011), and clinical factors (Hartsell et al. 2007, Kocak et al. 2007, Mazeron et al. 2010), contribute to the risks of development of RP, nonetheless, the occurrence of pneumonitis remain unpredictable accurately. Therefore, more reliable predictors in identifying individuals at a high risk of developing RP are most desirable for early treatment modifications in order to avoid serious complications.

Although the pathogenesis of pneumonitis has not yet been fully understood, this complex inflammatory process involves an interplay of cellular interactions between lung parenchymal cells and circulating immune cells mediated through a variety of cytokines including pro-inflammatory cytokines, chemokines, adhesion molecules, and pro-fibrotic cytokines, etc (Tsoutsou & Koukourakis 2006, Kong et al. 2008). Animal studies (Rube et al. 2000, Rübe et al. 2004) have shown an

The first two authors contributed equally to this work.

Address for Correspondence: Baosheng Li, Shandong Cancer Hospital, 440 Jiyan Road, Jinan 250117, China. E-mail: baoshli@yahoo.com

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early overproduction of both pro-inflammatory and pro-fibrogenic cytokines during thoracic irradiation and have suggested an important role of the sustained production of serial cytokines in the development of pulmonary toxicity. Some clinical reports (Anscher et al. 1997, Vujaskovic & Groen 2000, Chen et al. 2001, Chen et al. 2002, Novakova et al. 2004, Arpin et al. 2005, Chen et al. 2005, Hart et al. 2005, Evans et al. 2006, Hartsell et al. 2007, Zhao et al. 2008, Kim et al. 2009) for lung cancer patients have shown changes in the plasma/serum concentrations of interleukin-6 (IL-6), and transforming growth factor-beta1 (TGF-β1) during radiation therapy (RT) and have suggested that these variations could identify patients at risk of RP. However, cytokines are also produced in tumors, this tumor-derived cytokines production may influence the circulating serum levels in cancer patients and may therefore confuse the results when investigating cytokines for predicting RP (Rübe et al. 2008).

At the molecular level, the initial process may involve direct action of reactive oxygen species (ROS) and ROS-driven oxidative stress, which causes DNA breaks and rapidly triggers the production of cytokines, biologic molecules, and more ROS, leading to the formation of oxidized substances, ultimately lung toxicity. To circumvent the damages caused by the ROS and ROS-driven oxidative stress, superoxide dismutase (SOD), glutathione peroxidase and catalase, together with glutathione, form the first-line of defense systems, which are present in human serum (Tamai et al. 2011) as well as erythrocytes (Park et al. 2007). Therefore, it is to be expected that serum antioxidant would act as early surrogate markers for lung damage. Previous study (Park et al. 2007) found the antioxidant activities in erythrocytes were respect to the development of pneumonitis. For patients developed pneumonitis showed higher SOD activities at baseline and the week 2, 4 and 6 during the treatment compared to those who did not suffer this complication. Recent report (Mak et al. 2011) have demonstrated that single nucleotide polymorphism (SNPs) in the antioxidant genes associate with RP for locally advanced lung cancer patients treated with thoracic RT. However, to our knowledge, there is no study focusing on the serum SOD for RP prediction following the chemoradiotherapy.

Therefore, the aim of present study was to identify the predictive factor of serum SOD for (also including serum TGF-β1 and IL-6, etc.) predicting radiation pneumonitis in NSCLC patients following chemoradiotherapy.

Methods and materials

Eligibility and patient population

Twenty-six patients with stage IIIA-IIIB NSCLC enrolled in a prospective study from March 2006 to April 2010 were analyzed. Eligibility criteria included biopsy-proven NSCLC, no prior chemotherapy or radiotherapy, no concurrent malignancy and no past history of lung cancer. The protocol was approved by our institutional review board, and written informed consent was obtained from all patients. To minimize potential confounding factors, only those patients receiving definitive radiotherapy with three dimensional radiotherapy technique and concurrent chemotherapy were included. Patients were excluded if: (i) Karnofsky Performance Status scale < 80; (ii) inductive radiotherapy and chemotherapy; (iii) patients with severe complications, such as chronic obstructive pulmonary disease.

Treatment description

Patients underwent dedicated ^{18}F-fluorodeoxyglucose position emission tomography and computed tomography scanning for cancer staging and treatment planning (^{18}F-FDG PET/CT, 4 slice Discovery LS, GE). Target volumes were defined according to the report of International Commission on Radiological Units. The gross tumor volume (GTV), including the primary disease plus any involved regional lymph node(s). The planning target volume (PTV) was considered to include the GTV plus a 10- to 15-mm margin. 95% isodose line encompassed the PTV. All plans adopted late-course accelerated hyperfractionated radiotherapy: the first phase was implemented with the conventional fractionated irradiation. This PTV was defined as receiving 40 Gy in total, 2 Gy per fraction, five fractions a week. In the second phase, accelerated hyperfractionated radiation was employed. The dose was delivered at 1.4 Gy per fraction, twice daily with a minimum interval of 6 h, 10 fractions a week to 19.6–28 Gy in 14–20 fractions. The total dose delivered of the two-phase irradiation would be 59.6–68 Gy/34–40 fractions in 5.4–6.0 weeks. Planning objective for total lung receiving > 20 Gy (V_{20}) limited to ≤ 35%. Treatment planning was optimized using Philips Pinnacle^3 planning system (Philips Radiation Oncology Systems, Milpitas, CA). The treatment plans were reviewed by peers and delivered using 6 MV beams on linear accelerators (21EX, 23EX or Trilogy; Varian Inc., CA). All patients were treated with concurrent definitive radiotherapy and chemotherapy with a cisplatin-based regimen. The chemotherapy regimen used in this study was known to have similar toxicity and effectiveness for treatment of NSCLC.

Biologic markers measurement

In each patient, serial serum samples for IL-6, TGF-β1, intercellular adhesion molecule-1 (ICAM-1), macrophage inflammatory protein-1alpha (MIP-1α), cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), and SOD analysis were obtained prior to radiation therapy (Pre-RT) and post delivering 40 Gy/4 weeks during the treatment (Pos-RT). Circulating blood samples were collected in tubes, immediately chilled on ice, and then centrifuged at 3000*g* within 15 min for collection. All these biomarkers concentrations were measured by specific enzyme-linked immunoassay (ELISA) kits (Quantikine™, R&D Systems, Minneapolis, MN) according to manufacturer's protocol. All measurements were done in duplicate and mean values were used in further analysis.

Tumor response assessment

Some studies (Kong et al. 2008, Rübe et al. 2008) suggest that the tumor is the major source of circulating cytokines in NSCLC patients receiving radiation therapy. The changes of serum biomarkers may be correlated with the tumor responses following the treatment (Vujaskovic & Groen 2000, Rübe et al. 2008, Gupta et al. 2010). In order to elimination this confounding effect by the tumor responses, we examined the relationship between biologic markers and tumor responses following the treatment. Tumor response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (Therasse et al. 2006). The response criteria for the target lesions are complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) (Erasmus et al. 2003). Patients with an outcome of a CR or PR were subsequently classified as responder, whereas, those who had an outcome of SD or PD were defined as nonresponder.

Follow-up and RP evaluation

The clinical evaluation of patients was performed weekly during the course of RT. Follow-up examinations were performed at 1, 3, 6, and 9 months after completion of RT. Pre-RT assessments of lung function included symptom assessment, pulmonary function tests, single-photon emission computed tomography lung perfusion and PET/CT scan. Spiral CT-scans of the chest were performed at the end of treatment, and at every follow-up examination to monitor morphological changes in lung structure with respect to radiation-induced lung injury. In our analysis, the RP grade was defined according to the National Cancer Institute Common Toxicity Criteria (NCI CTC), version 3.0 (Trotti et al. 2003). The development of RP was considered as a binary variable: no-RP (Grade ≤ 1) and RP (Grade ≥ 2).

Statistical analysis

The nonparametric test of Wilcoxon procedure was used to compare serum levels of biomarkers between Pre-RT and Pos-RT. The ratios (defined as the serum levels at the time point of 40 Gy/4 weeks during the treatment divided by the pretreatment concentration, marked as "Pos/Pre-RT") for biologic markers between RP and no-RP groups were compared by Mann–Whitney *U* test. The clinical characteristics of patients, as well as the disease and treatment-related factors were related to the RP using Chi-square. The correlations of biologic markers with the development of RP and with the tumor responses were analyzed by univariate (Chi-square) and multivariate (backward stepwise logistic regression) analysis. Receiver operator characteristic (ROC) curves analysis was used to identify the optimal threshold of predictors and to assess the predictability of the predictors. A higher area under the ROC curve (AUC) indicates a more powerful predictor. Optimal threshold for predictor was defined as the point yielding the minimal value for $(1 - \text{senitivity})^2 + (1 - \text{sensitivity})^2$, which was the point on the ROC curve closest to the upper left-hand corner. All statistical tests were two-tailed and were performed using SPSS V.16.0 statistical software. The criterion for statistical significance was $p < 0.05$.

Results

Patient, tumor, and treatment characteristics

The characteristics of the patients are summarized in Table 1. For this limited patient population, there was no significant difference in the distribution of clinical parameters (gender, age, Karnofsky Performance Status, smoking history) between the two groups (no-RP vs. RP). Moreover, no differences were found between RP and no-RP groups on tumor-related (tumor location, clinical stage) and treatment-related factors (chemotherapy regimens) (all value of $p > 0.05$). The mean lung dose in no-RP group was 3.23–24.60 Gy, with a median of 15.64 Gy versus 18.35–20.13 Gy, with a median of 19.19 Gy in RP group ($p = 0.123$); The total lung V_{20} in no-RP group was 4.18–35.81%, with a median of 28.60% versus 25.58–34.00%, with a median of 32.58% in RP group ($p = 0.155$).

Treatment toxicity and tumor response

Of the 26 patients analyzed, five patients (19.2%) developed symptomatic RP according to the NCI CTC 3.0 scale with an median follow-up of 12 months. Twelve patients (46.1%) were asymptomatic but presenting with focal or minimal fibrosis on chest CT images. No patients suffered severe lung toxicity. In patients with symptomatic RP, RP was accompanied by worsening of respiratory symptoms and radiological changes in chest CT-scans.

After treatment, 17 patients (4 patients attained CR and 13 PR) were assessable for response and the overall response rate was 65.4%. Seven patients achieved SD and only two patients attained PD.

Serum levels for biomarkers before and during RT

Figure 1 displays the box-and-whisker diagram for serum levels of IL-6, TGF-β1, ICAM-1, MIP-1α, CYFRA21-1, and SOD in whole group of patients ($n = 26$), patients with

Table 1. Patient characteristic.

Characteristic	No. of patients (%)
Gender	
Male: female	19 (73.1%): 7 (26.9%)
Age (y)	
≥ 60: < 60	18 (69.2%): 8 (30.8%)
Histopathology	
Squamous cell carcinoma	12 (46.2%)
Adenocarcinoma	12 (46.2%)
Large cell carcinoma	2 (7.6%)
Clinical stage	
IIIA: IIIB	7 (26.9%): 19 (73.1%)
Smoking history	
No: Yes	8 (30.8%): 18 (69.2%)

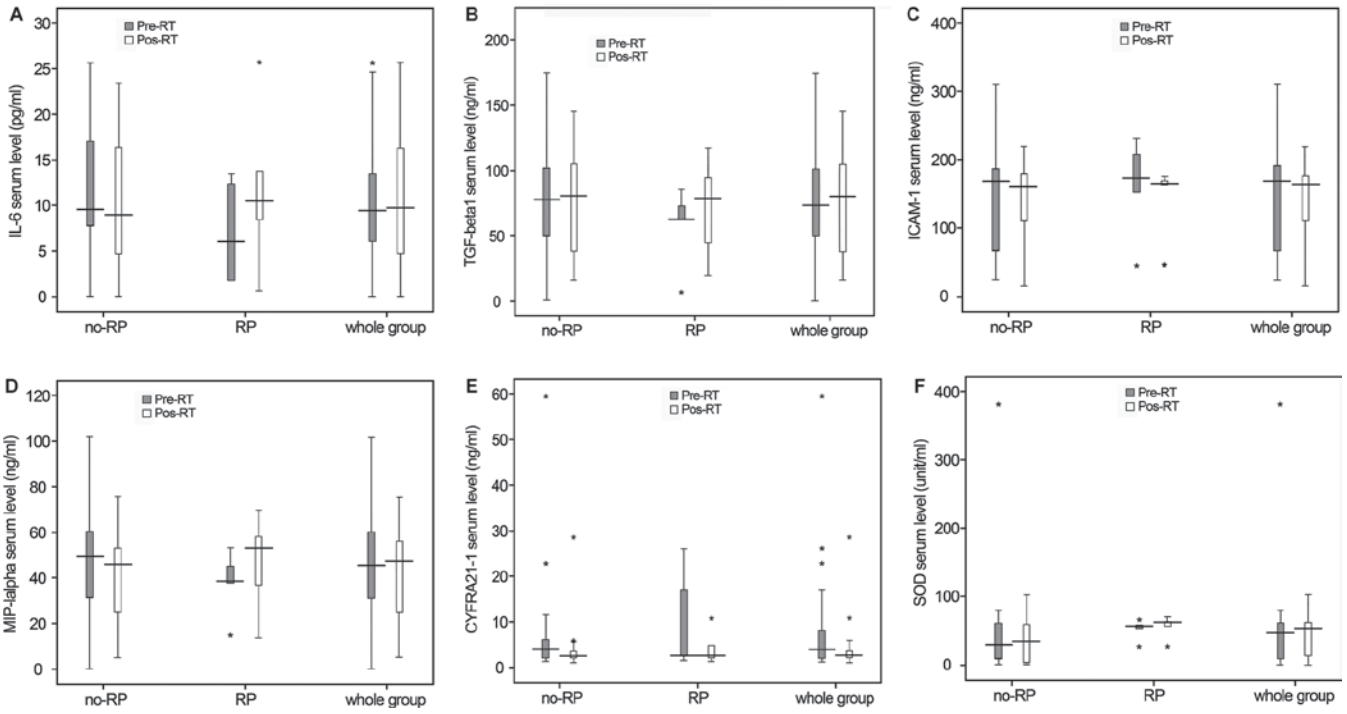

Figure 1. Serum levels of serial biomarkers (A) interleukin-6 [IL-6], (B) transforming growth factor-beta1 [TGF-β1], (C) intercellular adhesion molecule-1 [ICAM-1], (D) macrophage inflammatory protein-1alpha [MIP-1α], (E) cytokeratin 19 fragment antigen 21-1 [CYFRA21-1], (F) superoxide dismutase [SOD]) in whole group of patients ($n = 26$), patients with (RP, $n = 5$) and without radiation pneumonitis (no-RP, $n = 21$) before radiation therapy (Pre-RT) and post delivering 40 Gy/4 weeks during the treatment (Pos-RT).

(n = 5) and without RP (n = 21). The figure allows visualization of the expression level changes in serum for multi-biologic markers during the treatment. By statistical analysis, there was no significant difference of serum levels between "Pre-RT" and "Pos-RT" for multi-biologic markers in whole group of patients, except for CYFRA21-1. The median serum level of CYFRA21-1 concentrations after delivery of 40 Gy/4 weeks was lower than the onset of treatment (2.66 ng/ml vs. 3.92 ng/ml, p = 0.025). IL-6, TGF-β1, and MIP-1α serum levels measured after delivery of 40 Gy/4 weeks increased more strikingly for patients who experienced RP than those without RP. Also, SOD concentrations in serum after delivery of 40 Gy/4 weeks were higher in the RP group than in the no-RP group, but no significant difference was observed ($p > 0.05$). Table 2 reports the results for ratios of biomarkers between two group of patients with and without pneumonitis, which demonstrates no significant difference (all value of $p > 0.05$).

Table 2. Comparing results for the ratio of biomarkers between patients with and without radiation pneumonitis (RP). Data was presented with Median (Range).

Marker	no-RP (n = 21)	RP (n = 5)
IL-6 ratio	0.87 (0.22–3.02)	1.74 (0.31–4.84)
TGF-β1 ratio	0.99 (0.36–2.02)	0.77 (0.16–1.37)
ICAM-1 ratio	0.95 (0.30–2.53)	0.97 (0.76–1.06)
MIP-1α ratio	0.93 (0.32–3.35)	1.29 (0.91–1.38)
CYFRA21-1 ratio	0.83 (0.05–2.91)	0.63 (0.19–1.34)
SOD ratio	0.96 (0.14–2.19)	0.99 (0.94–1.32)

Note: ratio was defined as the serum levels at the time point of 40Gy/4weeks during the treatment divided by the pretreatment concentration.

Table 3. Results of biomarkers as predictors for radiation pneumonitis by univariate analysis.

Marker	Cut-off	Chi-Square	P value	Odds Ratio	95% CI for Odds Ratio Lower	Upper
IL-6 ratio	1.0	3.287	0.070	7.429	0.690	79.957
TGF-β1 ratio	0.8	2.252	0.133	0.222	0.028	1.736
SOD (unit/ml)	56.2	5.634	0.018	12.800	1.149	142.577

Note: ratio was defined as the serum levels at the time point of 40Gy/4weeks during the treatment divided by the pretreatment concentration.

Correlation between biomarkers and the incidence of RP

As demonstrated in Table 3, the SOD concentrations in serum after delivery of 40 Gy/4 weeks seemed to be association with the risks of developing RP by univariate analysis (OR = 12.800, 95.0% confidence interval [CI] 1.149–142.577, p = 0.018). Based on this small cohort of patients, IL-6 ratio was observed marginally significant to the development of pneumonitis (OR = 7.429, 95.0% CI 0.690–79.957, p = 0.070), however, TGF-β1 ratio was not significantly association with this complication (OR = 0.222, 95.0% CI 0.028–1.736, p = 0.133). The multivariate analysis confirmed that serum SOD was highly correlated with the risks of developing pneumonitis (p = 0.036, Table 4). In order to further examine the

predictability for RP using these biologic markers, ROC curve analysis was adopted. By ROC analysis, serum SOD was confirmed a potential predictor for RP. The best predictive efficacy of serum SOD concentrations after delivery of 40 Gy/4 weeks was observed for a cut-off value of 56 unit/ml, with a sensitivity of 0.80 (95% CI 0.28–0.99), and a specificity of 0.67 (95% CI 0.43–0.65) (AUC = 0.733, 95% CI 0.525–0.886, p = 0.040), whereas, SOD concentrations before treatment was not a predictor of RP (AUC = 0.638, 95% CI 0.428–0.816, $p = 0.193$) (Figure 2). The pro-fibrotic cytokines TGF-β1 was not a good predictor for RP. The AUC was 0.620 (95% CI 0.406–0.805, $p = 0.513$) for TGF-β1 ratio with a best cut-off value 0.8 as a predictor, the predictive sensitivity and specificity was 60.0% and 75.0%, respectively. Furthermore, the pro-inflammatory cytokine IL-6 ratio had a moderate predictive power with predictive sensitivity and specificity of 80.0% and 70.0%, respectively, the best cut-off value was defined as 1.0, however, it failed to identify patients who were at risk of developing RP accurately (AUC = 0.720, 95% CI 0.506–0.879, $p = 0.166$) (Figure 3).

Table 4. Results of biomarkers as predictors for radiation pneumonitis by multivariate analysis.

Marker	B	S.E.	Wald	*p* value	Exp (B)	95% CI for EXP (B) Lower	Upper
IL-6 ratio	2.720	1.506	3.259	0.071	15.176	0.792	290.724
SOD	3.118	1.489	4.385	0.036	22.601	1.221	418.435

Note: ratio was defined as the serum levels at the time point of 40 Gy/4 weeks during the treatment divided by the pretreatment concentration.

Correlation between biomarkers and tumor response

Serum SOD concentrations after delivery of 40 Gy/4 weeks was not correlated to the tumor responses. On the contrary, the decline of CYFRA21-1 concentration during the treatment was a reliable surrogate marker to tumor responses (AUC = 0.752, 95% CI 0.544–0.899, $p = 0.012$).

Discussion

Although complicated predictive models were employed, Kocak failed to accurately segregate patients into high and low risk group of developing RP prospectively (Kocak et al. 2007). This consequences might be influenced by serial biologic markers, which determine the intrinsic susceptibility to toxicity of each individual and add to the complexity of RP prediction. Recent insights into the pathogenesis of radiation-induced lung injury revealed a number of biologic markers, including cytokines, protein molecules, and SNPs were significantly correlated with the incidence of RP.

In the present study, we investigated prospectively the changes of serum levels for serial cytokines, CYFRA21-1,

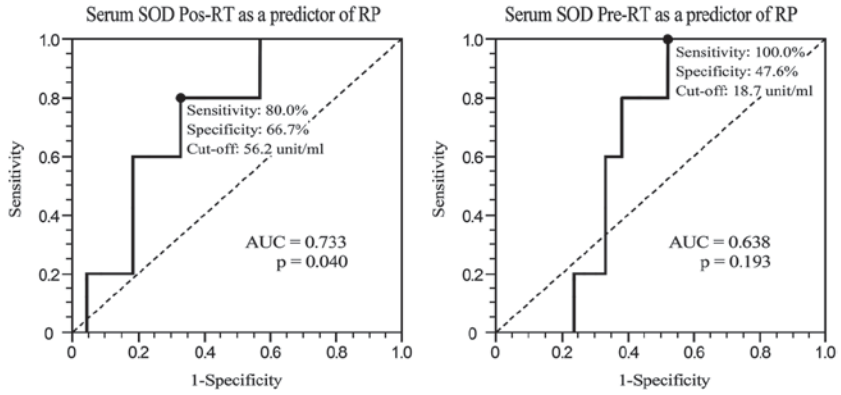

Figure 2. Serum levels of superoxide dismutase (SOD) before radiation therapy (Pre-RT) and post delivering 40 Gy/4 weeks during the treatment (Pos-RT) as predictors for radiation pneumonitis (RP).

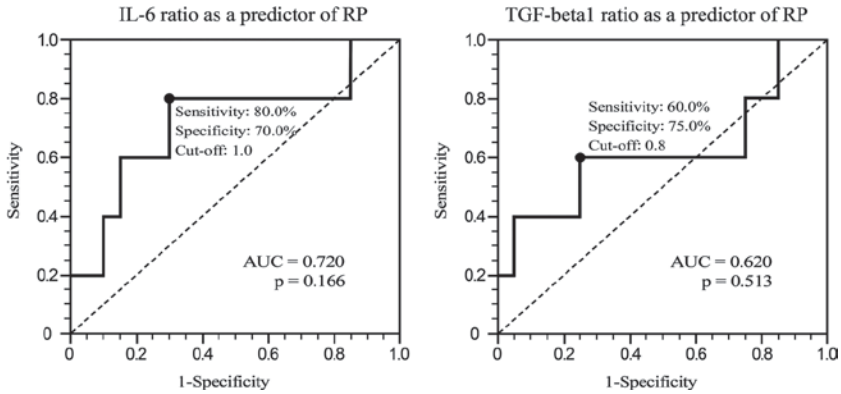

Figure 3. Ratios (serum levels at the time point of 40 Gy/4 weeks during the treatment divided by the pretreatment concentrations) for interleukin-6 (IL-6) and transforming growth factor-beta1 (TGF-β1) as predictors for radiation pneumonitis.

and SOD between the onset of radiation and after delivery of 40 Gy/4 weeks during the course of radiation in eligible NSCLC patients to ascertain their prognostic value to predict RP. Furthermore, an attempt has been made to evaluate the tumor responses related biomarkers which may impair the prospective identification of patients at risk for RP. Based on this small cohort of patients, our study indicated a significant association of elevated SOD serum levels after delivery of 40 Gy/4 weeks with the development of RP. Whereas, CYFRA21-1 was identified as a reliable surrogate marker to tumor responses for NSCLC patients treated with chemoradiotherapy. In addition, present data displayed that IL-6 ratio was observed marginally significant to the development of RP, which might indicate that the IL-6 is also probably associated with RP, if considering our small sample size. However, TGF-β1 ratio was not a reliable predictor for RP. Our results did not confirm that ICAM-1 or MIP-1α serum levels, neither their absolute nor their ratios, may identify patients at risk for RP.

Previously, numerous studies emphasized the important role of TGF-β1 (Anscher et al. 1997, Vujaskovic & Groen 2000, Novakova-Jiresova et al. 2004, Evans et al. 2006, Zhao et al. 2008, Kim et al. 2009) and IL-6 (Chen et al. 2001, Chen et al. 2002, Arpin et al. 2005, Chen et al. 2005, Hartsell et al. 2007) in the prediction of RP. However, in our study TGF-β1 failed to accurately identify patients who are at risk of developing pneumonitis (AUC = 0.620, p = 0.513). Another pro-inflammatory cytokine, IL-6 was investigated borderline significant to the development of pneumonitis (Tables 3 and 4), pitifully, also failed to identify the risks of developing RP (AUC = 0.720, p = 0.166) with a best cut-off value of 1.0 defined by ROC curve. This result was possibly due to (i) there was a small cohort of patient analyzed, (ii) the predictive value of cytokines is confounded by the tumor effect, as cytokines are produced not only in normal lung tissue after irradiation, but are also over-expressed in tumor cells of NSCLC specimens (Rübe et al. 2008). Considering our small sample size and those positive data, we could not deny its potential predictive value categorically.

According to recent research, the cellular antioxidant response seems to play an important role in the development of lung toxicity (Tsoutsou & Koukourakis 2006). SOD gene therapy studies in animals have shown a protective effect following irradiation by decreasing expression of mRNA for irradiation-induced inflammatory cytokines. It is well known that three types of SOD exist: MnSOD (SOD2), Cu-ZnSOD (SOD1), and extracellular SOD (EC-SOD), which is also referred to as SOD3. All the three forms of SOD protect against radiation injury. The EC-SOD is highly expressed in the extracellular matrix of lung tissue and is believed to protect the lung from oxidative damage. Previous studies (Fattman et al. 2001, Bowler et al. 2002) confirmed that EC-SOD played an important role in attenuating bleomycin-induced lung injury. One research found the antioxidant activities in erythrocytes was related to the development of pneumonitis (Park et al. 2007). Recent study (Mak et al. 2011) has demonstrated that SNPs in the antioxidant genes associate with RP for locally advanced lung cancer patients treated with thoracic RT. However, little is known about the role of serum SOD levels for predicting RP following the chemoradiotherapy.

In the present study, the association between SOD serum levels and risk of RP was investigated. The SOD concentrations in serum after delivery of 40 Gy/4 weeks were relatively higher for patients who developed RP than those who did not develop this complication. A significant association between SOD serum levels at time point of 40 Gy/4 weeks during irradiation and the development of RP was observed, which was in accordance with the findings of Park's (Park et al. 2007). In Park's study, the mean values of SOD activities in red blood cells were clearly higher before, and through each week of treatment in the pneumonitis group. Moreover, pre-treatment SOD activities had increased significantly in the group with pneumonitis (p = 0.019). The week 2, 4 and 6 values of SOD activities were also borderline statistical significance (p = 0.054, 0.072, 0.052, respectively). By further analysis, our results indicate that using 56 unit/ml as a threshold, elevated serum level of SOD can predict RP with a sensitivity, specificity, and accuracy of 80.0%, 66.7%, and 73.3%, respectively (p = 0.040) in our cohort of patients. However, pretreatment SOD was not a predictor of RP (p = 0.193). Gupta et al. (2010) reported that SOD activity after chemotherapy in NSCLC patients was also association with treatment response. In order to elimination this confounding effect by the tumor response, we examined the relationship between SOD concentration and tumor response following the treatment. Our results confirm that SOD is not association with tumor response following chemoradiotherapy. Hopefully, serum SOD could act as a reliable predictor for RP, which is released into the blood circulation as a result of radiation-induced lung injury and it would not be superimposed by the overproduction of the tumor's response to the chemoradiotherapy.

In summary, SOD serum level after radiation of 40 Gy/4 weeks seems to be a potential predictor for RP. However, the conclusions should be moderated by several limitations in our study. First, the sample size is not large enough. Second, it must be noted that the threshold of predictors in the present study analyzed by ROC curve would be changed with different patient population recruited. Further studies involving larger numbers of patients are required to further assess the value of serum SOD for predicting RP.

Declaration of interest

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