RESEARCH ARTICLE

Serum IL-33 as a Diagnostic and Prognostic Marker in Non-small Cell Lung Cancer

Liang-An Hu*, Yu Fu, Dan-Ni Zhang, Jie Zhang

Abstract

Background: Interleukin-33 (IL-33) has recently been implicated in tumor immunity. The aim of this study was to explore the clinical role of serum IL-33 in patients with non-small-cell lung cancer (NSCLC). Methods: Sera collected from 250 healthy volunteers (HV), 256 patients with benign lung diseases (BLD) and 262 NSCLC cases were subjected to IL-33 ELISA and relationships between serum IL-33 and clinical characteristics were evaluated. Results: Circulating IL-33 levels were higher in the NSCLC group in comparison with the HV and BLD groups ($p<0.001$). Using a cut-off level 68 pg/ml (95% specificity in the HV group), IL-33 showed a good diagnostic performance for NSCLC. Multivariate survival analysis indicated that serum IL-33 was an independent prognostic factor in the entire NSCLC group [hazards ratio (HR) = 0.64 for low versus high IL-33 levels, 95% confidence interval (CI) 0.50–0.82; $p<0.001$] and in 165 selected patients with locally advanced or metastatic disease receiving chemoradiotherapy or chemotherapy (HR 0.70, 95% CI 0.52–0.94; $p=0.013$). Conclusions: IL-33 is a promising potential diagnostic and prognostic marker in NSCLC, independent of the therapeutic intervention.

Keywords: IL-33 - non-small-cell lung cancer - prognosis - differential diagnosis - biomarker

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Introduction

Lung cancer remains among the most prevalent and lethal cancers worldwide, accounting for more than a sixth of cancer deaths (Hoffman et al., 2000). Only 15 % of all patients with lung cancer are diagnosed at an early stage, and surgery is the treatment of choice for them (López-González et al., 2012). Non-small cell lung cancer (NSCLC) is the main type of lung cancer. NSCLC is often diagnosed at an advanced stage (Oguz,et al., 2013) so that patients have little prospect of effective and curative treatment, with 5-year survival rates of < 15% (Jemal et al., 2010).

Screening for early NSCLC predictive biomarkers is a key tool to establish the early diagnosis, which holds promise to further improve the outcome (Filipits et al., 2011). However, the existing biomarkers and predictors for NSCLC are not satisfying due to lack of adequate sensitivity and specificity (Indovina et al., 2011). The carcinoembryonic antigen (CEA) is one of the most widely-studied tumor markers in NSCLC, with an overall sensitivity of only approximately 40% (Tufman et al., 2010). Still, the use of CEA as a prognostic and predictive marker in patients with lung cancer is widely debated (Grunnet et al., 2012). Some new biomarkers are emerging and may be potentially important, but few have been established to be independent prognostic indicators (Bharti et al., 2007). Therefore, more studies are required to discover novel biomarkers in order to diagnose or screen early NSCLC.

Serum tumor markers are non-invasive tools for identifying malignant tumors, and are commonly used as a prognostic factor and an indicator of therapeutic efficacy in clinical practice. IL-33 is a novel member of the IL-1 family of cytokines, which includes IL-1α, IL-1β and IL-18 (Schmitz et al., 2005). Elevated serum IL-18 is involved in a wide variety of tumors (Srivastava et al., 2010). IL-33 was significantly increased in the lung tissues of K-ras TG mic related to cancer development (Lee et al., 2009). However, it is uncertain to date whether serum IL-33 has clinical significance in NSCLC. Therefore, the present study investigated the baseline serum levels of IL-33 in patients with NSCLC to determine its potential diagnostic and prognostic roles.

Materials and Methods

Patients

This study was approved by the Ethics Committee of First Affiliated Hospital of Chongqing Medical University, China and written informed consent was obtained from all patients and healthy controls.

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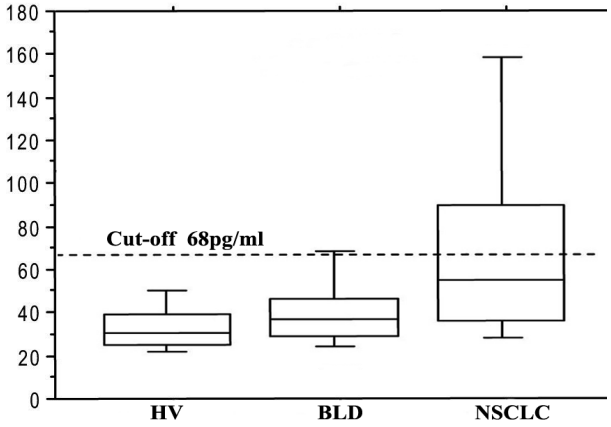

Figure 1. Box Plots of IL-33(pg/ml) and Displays 10%, 25%, Median, 75%, 90%. Healthy volunteers (HV), benign lung diseases (BLD) and non-small-cell lung cancer (NSCLC) patients

Three separate groups were included. The first group included 262 NSCLC patients (stages I-IV). We selected cases from our hospital based on availability of serum sample and adequate follow-up for survival analyses between 2006 and 2011. Median age was 67 years (range 41-94 years). On data analysis, patients were classified as subgroup 1 (aged >70 years, 105 cases) or subgroup 2 (<70 years, 157 cases). The demographic and clinicopathologic features of patients from this group are shown in Table 1. Survival was calculated from the date of sample collection until death from any cause (events) or the last follow-up (censors). Median follow-up of 96 censor cases (36.6%) was 34 months (range 18-52 months). In 166 subjects with locally advanced or advanced NSCLC, the median follow-up of 20 censor cases (12%) was 38 months (range 28-49 months).

The second group enrolled 256 sex- and age-matched consecutive cases with benign lung diseases (BLD). Diagnoses were pulmonary or pleural infections (146 cases) and benign lung infiltrates or lung nodules (110 cases). In this group the median age was 66 years (range 16-87).

The third patient group included 250 healthy volunteers (HV). Serum samples from this subject group were offered from Chongqing Blood Bank.

Sample collection and ELISA

Sera were collected from 250 healthy volunteers (HV), 256 patients with benign lung diseases (BLD) and 262 NSCLC patients. Serum levels of IL-33 were determined using a Quantikine ELISA according to the manufacturer's instructions (R&D Systems).

Statistical analysis

Levels of IL-33 are expressed as median and interquartile range (IQR). Due to non-normal distribution of these parameters in all groups, the non-parametrics Kruskal-Wallis test was used to analyze the correlation between the serum IL-33 levels with clinicopathologic characteristics. Spearman correlation analysis was used to examine the relationship between continuous variables. To determine the diagnostic accuracy of IL-33, receiver operating characteristic (ROC) curves were retrieved from logistic regression analysis and the area under the curve (AUC) was calculated. Univariate survival analysis was performed using the Kaplan-Meier method and the log-rank test. Multivariate analysis was conducted to determine an independent impact on survival using the Cox proportional hazard method. $P<0.05$ was considered statistically significant. Statistical analyses were conducted using the SPSS 16.0.

Table 1. L-33 Levels in the Three Groups and Clinical Features in the NSCLC Group

Group	IL-33 level			
	N (%)	Median	IQR	*P*
HV	250	23	10–39	<0.001
BLD	256	36	21–59	
NSCLC	262	54	34–103	
Clinical featuresin NSCLC Group				
Gender				0.08
Male	118 (45)	63	38–96	
Female	144 (55)	45	29–80	
Age				0.7
≤70	157 (61)	50	32–90	
>70	105 (39)	56	35–94	
Stage				0.005
I-IIIA	97 (37)	43	32–69	
IIIAbulky-IIIB	81 (31)	62	33–87	
IV	84 (32)	74	47–139	
Smoking history				0.9
Current	102 (39)	48	33–99	
Former	79 (30)	60	31–94	
Never	81 (31)	75	35–136	
Histology				0.2
Non-squamous	181 (69)	49	32–96	
Squamous	81 (31)	64	34–121	

NSCLC, non-small-cell lung cancer; HV, healthy volunteers; BLD, benign lung diseases; IQR, interquartile range

Results

Serum levels of IL-33 were elevated in the NSCLC group

As demonstrated in Figure 1, the concentration of IL-33 showed non-normal distribution in all the three groups. Median levels of circulating IL-33 were significantly higher in the NSCLC group compared with the BLD and the HV groups ($p<0.001$) and correlated with tumor stage (Table 1). No correlation was observed between serum IL-33 levels and patient age, gender and smoking history or tumor histology (Table 1).

Diagnostic performance of IL-33 in NSCLC

We further explored the potential diagnostic value of IL-33 in NSCLC. To determine the diagnostic potential of the ELISA assays, we first calculated which concentration corresponded to 95% specificity in the HV group. IL-33 cut-off level was found to be 68 pg/ml and used to estimate the sensitivity, specificity, negative and positive predictive values of IL-33 in the NSCLC and BLD groups.

The performance of all serum samples was summarized with an ROC curve. The predictive performance of IL-33 level was determined by plotting sensitivity (true positive) against 1-specificity (false positive) values. For each possible cut-point, the resulting sensitivity and specificity

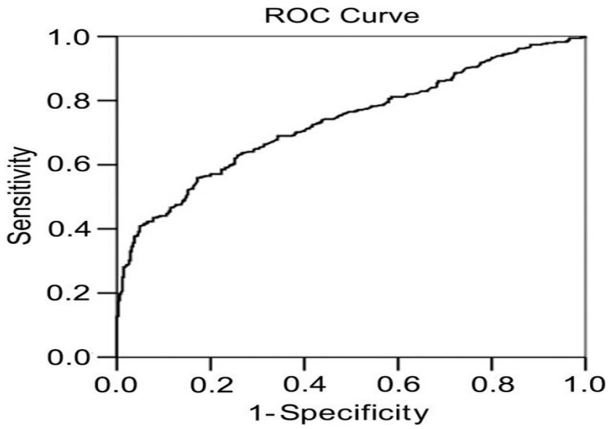

Figure 2. Receiver Operating Characteristic Curves for IL-33 Level to Discriminate Between NSCLC or BLD. Serum concentrations of IL-33 among 256 patients with benign lung diseases (BLD) and 262 non-small-cell lung cancer (NSCLC) patients. were determined by ELISA. The diagnostic potential of IL-33 was assessed by ROC curves. The area underneath the curve (AUC) was 0.736

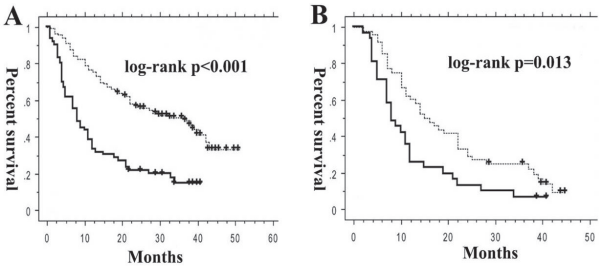

Figure 3. Kaplan–Meier Curves for IL-33 in All NSCLC Cases (A) and in the Subgroup of Patients with Stages III and IV NSCLC Receiving Either Combination Chemoradiotherapy or First-line Palliative Chemotherapy (B). (High IL-33 levels straight lines; low levels dotted lines)

were indicated as a point on the graph. The area underneath the curve (AUC) was 0.736 (Figure 2), suggesting that the IL-33 is a potential diagnostic and differentially diagnostic marker for NSCLC.

Survival analysis

Finally, we determined whether the baseline serum concentration of IL-33 would be a prognostic marker in NSCLC. NSCLC patients were dichotomized using the same cut-off. Median survival time of patients with IL-33>68 pg/ml (n=109) was 7.6 months in comparison with 35 months in cases with IL-33 ≤68 pg/ml (n=153) (log-rank $p<0.001$) (Figure 3A). Multivariate Cox regression analysis was adjusted by gender, age, histology, stage (I-IIIA versus IIIAbulky-IIIB versus IV), history of smoking and whether patients received chemotherapy or not. As a result, IL-33 was confirmed as an independent prognostic factor, with hazard ratio (HR) 0.64 for low versus high IL-33 levels [95% confidence interval (CI) 0.50-0.82; $p<0.01$].

We further sought to explore whether the baseline serum concentration of IL-33 would effectively predict overall survival in the selected population of 165 patients with locally advanced or advanced NSCLC (stage IIIAbulky-IIIB or IV). Patients with IL-33 levels < 68 pg/ml had a median survival of 14.8 months, whereas patients with a concentration above the cut-off had a median survival time of 7.7 months. Multivariate analysis, adjusted by gender, age, smoking history, histology, stage (III versus IV) and treatment setting (curative chemoradiotherapy versus 1st-line palliative chemotherapy) indicated IL-33 was an independent prognostic factor (HR 0.70, 95% CI 0.52-0.94; log-rank $p=0.013$) in the advanced NSCLC patients (Figure 3B).

Discussion

In the present study, we determined the serum IL-33 levels in NSCLC patients with detailed information including clinical parameters and follow-up and evaluated the clinical role of IL-33. We show that circulating levels of IL-33 were elevated in patients with NSCLC when compared to patients with BLD and HV. At a cut-off value 68 pg/ml, serum IL-33 showed an excellent diagnostic performance. Interestingly, baseline serum IL-33 was an independent prognostic factor in NSCLC and in the subgroup of patients who received active treatment for locally-advanced or metastatic disease.

IL-33 is a new member of the IL-1 family and recent publications imply that vascular endothelial cells are the dominant IL-33-expressing cell population in vivo (Küchler et al., 2008). Recent data suggest that IL-33 promotes angiogenesis and endothelial permeability involved in tumorigenesis (Choi et al., 2009). Expression of IL-33 has been observed in various organs including stomach and lung, as well as in cells including pancreatic cancer cells and activated macrophages (Carriere et al., 2007). A recent study has shown IL-33 protein was elevated in gastric cancer cell lines and gastric carcinoma tissues in comparison with matched normal tissues (Sun et al., 2011). Serum IL-33 may be a useful indicator for prognosis of gastric cancer (Sun et al., 2011). In line with these findings, our data showed that serum IL-33 was a potential diagnostic and prognostic marker in non-small cell lung cancer, adding support to the role of IL-33 in clinical significance in tumor.

IL-33 signaling is mediated via its receptor ST2L. The role of IL-33/ST2 axis has recently been implicated in cancer, but with limited data. Deletion of ST2 signaling may enhance anti-tumor immune response in a mouse model of metastatic breast carcinoma (Jovanovic et al., 2011). In addition, IL-33 is most closely related to IL-18 of the IL-1 family (Dinarello et al., 1998). Both precursor forms of IL-18 and IL-33 are cleaved by caspase-1 to generate mature and biologically active cytokines (Dinarello et al., 1998). Serum IL-18 levels have been found to be markedly up-regulated in cancer patients (Srivastava et al., 2010), which indicate that there may be a close relation between serum IL-33 and tumor. The results of our study showed that serum levels of IL-33 in patients with NSCLC were significantly higher than that of healthy people and suggested that serum IL-33 was related to prognosis, distant metastasis and advanced stage.

In conclusion, our data suggest that serum IL-33 may be a useful diagnostic biomarker and shows a promising potential as prognostic marker in NSCLC patients,

independently of the therapeutic intervention. More large-scale prospective studies are warranted to confirm the findings.

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