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
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.

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
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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 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 (Srividatha 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.

References


