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Utility of plasma tumour necrosis factor-α and transforming growth factor-β1 as predictors of survival and treatment outcome in advanced non-small cell lung carcinoma

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Abstract

We hypothesized that plasma level of tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 may be a potential tool for diagnosis, prognosis and prediction of treatment outcome in non-small cell lung carcinoma (NSCLC). Plasma levels of TNF-α and TGF-β1 were quantified in 100 NSCLC patients and 100 controls. Association of TNF-α and TGF-β1 with response to therapy and survival was determined in 42 patients. An increased presence of TNF-α and TGF-β1 was observed in NSCLC compared with controls. TNF-α and TGF-β1 levels did not correlate with survival and response to chemotherapy. TNF-α and TGF-β1 do not appear to be reliable markers for predicting survival and response to therapy in advanced NSCLC.

Keywords: *Chemotherapy; diagnosis; prognosis; response*

Introduction

Lung cancer is the leading cause of cancer-related death in India and across the globe (Parkin et al. 2005). The low survival rates can be attributable to the fact that almost two-thirds of patients are diagnosed when locoregional and/or metastatic extension has already occurred. However, if diagnosed early, curative treatment may provide longer survival. Another major hurdle in the attempts to improve the survival of these patients has been the lack of a simple and effective test for early prediction of therapeutic efficacy. Much research is currently ongoing in the quest for such a reliable and minimally invasive tool; however, these have produced very little success. Thus, there is an urgent need for identifying biomarkers which can help in diagnosing and prognosticating the disease and predicting the therapeutic efficacy.

Cytokines are secreted or membrane-bound proteins that regulate the growth, differentiation and activation of immune cells. The cellular and molecular changes that initiate tumour formation also change the local cytokine expression (Dranoff 2004). Tumour necrosis factor (TNF)-α is a cytokine whose role in carcinogenesis has been explored (van Horssen et al. 2006). Transforming growth factor (TGF)-β1 is also believed to play a role in tumour transformation, progression and regression (Iyer et al. 2005). Despite the well-documented roles of TNF-α and TGF-β1 in carcinogenesis, their association with survival and response to therapy in non-small cell lung carcinoma (NSCLC) is not yet clearly defined. Another important gap in the literature is that none of the previous studies have reported on the association between levels of these cytokines and survival among Indian lung cancer patients, even though the lung cancer incidence

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and mortality rates in Indian population are very high (Parkin et al. 2005).

The present study was designed with an aim of analysing the efficacy of plasma TNF-α and TGF-β1 levels in discriminating lung cancer patients from controls (patients with benign pulmonary diseases). Additionally, the utility of plasma TNF-α and TGF-β1 as a prognostic marker of survival and in predicting response to therapy and disease progression was also assessed.

Materials and methods

Subjects

We evaluated 100 newly diagnosed and untreated patients with advanced-stage NSCLC and 100 age-matched controls (patients with benign pulmonary diseases, including 32 with chronic obstructive pulmonary disease, 26 pulmonary tuberculosis, 33 sarcoidosis, four bronchiectasis and five interstitial lung disease). All subjects were enrolled from the outpatient department of the Department of Medicine, All India Institute of Medical Sciences, New Delhi, India between the years 2006 and 2009. For all patients, a diagnosis of lung cancer was confirmed by the histological examinations of biopsy and/or cytology specimens obtained during fibreoptic bronchoscopy or with a computed tomography (CT)-guided procedure. Pretreatment assessment included evaluation of Eastern Cooperative Oncology Group performance status (ECOG), chest X-ray and CT scan of the chest and upper abdomen. If necessary, a CT or magnetic resonance imaging (MRI) scan of the brain and a radionuclide bone scan was performed. All the patients were staged according to the American Thoracic Society TNM classification (Mountain 1997). Epidemiological data including demographics, smoking status, stage, tumour size and histopathological data were also recorded. Forty-two patients received platinum-based chemotherapy for a minimum of three cycles. In these patients, response to therapy was classified according to the World Health Organization (WHO) guidelines defining 'complete remission' (CR) as compete disappearance of all tumour lesions, 'partial remission' (R) as tumour reduction ≥50%, 'progression' (P) as tumour increase ≥25% or appearance of new tumour manifestations and 'stable disease' (SD) as tumour reduction <50% or increase <25% (Miller et al. 1981). In this study, responders were defined as patients with complete remission (CR) or partial remission (PR), while non-responders were defined as patients with stable disease (SD) or progressive disease (PD). The radiology reviews performed for response measurements were independent and blinded. The study was approved by the Institute's Ethics Committee and informed written consent was obtained from all the patients.

Analysis of plasma TNF-α and TGF-β1 levels

Venous blood was collected in sterile EDTA-coated vials from all subjects. Within 1 h, samples were centrifuged (2500 *g*, 10 min), and the plasma was removed, aliquoted and stored at -80°C until further analysis. Plasma levels of TNF-α were quantified using the Human TNF-α ELISA Kit (Diaclone, Canton, MA, USA), according to the manufacturer's instructions. The limit of sensitivity of the TNF-α assay was <8 pg ml^{-1}. The coefficient of variation was less than 5.0%. TGF-β1 levels were quantified using the Human TGF-β1 ELISA Kit (RayBiotech Inc., Norcross, GA, USA) as per the manufacturer's instruction. The limit of sensitivity of the TGF-β1 assay was <80 pg ml^{-1}. The coefficient of variation was less than 7.0%.

Statistical analysis

Demographic information between NSCLC patients and controls was compared using the independent sample *t*-test for continuous variables and the χ^2 test for categorical variables. To evaluate the diagnostic performance of plasma TNF-α and TGF-β1 levels, the area under the receiver operating characteristic curve (AUC-ROC) was used to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), with 95% confidence interval (CI) at defined cut-offs (one at best sensitivity/best specificity and another one at 95% specificity). To assess the effect of plasma TNF-α and TGF-β1 as a risk factor for NSCLC, odds ratios (OR) and corresponding 95% CIs were calculated using multiple logistic regression analysis (adjusted for age, sex and smoking status). The correlation of plasma TNF-α and TGF-β1 levels with various clinicopathological factors in NSCLC was studied using an independent sample *t*-test. Survival analysis in 42 NSCLC patients was performed according to tertile stratification of plasma TNF-α and TGF-β1 distribution using the Kaplan–Meier method and Cox regression analysis (*p*-value not adjusted because of small sample size and as none of the variables correlated with survival (data not shown)).

The significance of plasma TNF-α and TGF-β1 in predicting response to therapy was analysed using the Wilcoxon rank sum test. Patients with stable disease are those where tumour reduction is not sufficient enough to be classified as 'remission' or an increase in tumour growth is not sufficient enough to be considered as 'progression'. Thus, it would be useful to find patients who are either (1) going to respond to the treatment, or (2) going to progress to a more advanced stage of disease. Therefore, we conducted two different evaluations of plasma TNF-α and TGF-β1 to address both clinical questions. In the first evaluation, pretreatment plasma TNF-α

and TGF-β1 levels in patients with complete remission and partial remission (CR+PR) were compared with those having stable and progressive disease (SD+PD) in an effort to predict the response to therapy. In the second evaluation, we compared pretreatment plasma TNF-α and TGF-β1 levels in patients with progressive disease with those having complete remission, partial remission and stable disease (CR+PR+SD) in order to predict disease progression. A value $p < 0.05$ was considered statistically significant. All statistical analyses were performed with the SPSS software program for Windows (SPSS 17.0; STATA Corp., TX, USA).

Results

Subject characteristics

The baseline characteristics of the patients and controls are given in Table 1. The median age of 100 patients with NSCLC was 56.0 years (range 33–80) and 56.5 years (range 35–86) in controls. Males comprised 92% and 78% of the patients and controls, respectively. Seventeen (17.0%) patients and 33 (33.0%) controls were never-smokers. The demographic characteristics of NSCLC patients and controls were significantly different for sex, smoking status and pack years, but not for age. The mean (± SD) TNF-α levels were 18.7 (± 7.3) pg ml^{-1} in patients with NSCLC and 11.0 (± 2.6) pg ml^{-1} in controls, the difference being highly significant (p <0.001; Table 1). The mean (± SD) TGF-β1 levels were 14.0 (± 5.4) ng ml^{-1} in patients with NSCLC and 8.6 (± 2.8) ng ml^{-1} in controls. This difference in TGF-β1 levels in case versus controls was highly significant (p <0.001; Table 1).

Table 1. Subject characteristics.

Characteristics	NSCLC (n=100)	Controls (n=100)	p-Value
Age (years), median (range)	56.0 (33–80)	56.5 (35–86)	0.812
Sex, *n* (%)			
Male	92 (92.0)	78 (78.0)	0.006
Female	8 (8.0)	22 (22.0)	
Smoking status, *n* (%)			
Never smokers	17 (17.0)	33 (33.0)	0.009
Smokers	83 (83.0)	67 (67.0)	
Pack years, median (range)	22.5 (3–125)	17.5 (5–70)	0.012
Plasma TNF-α (pg ml^{-1}), mean ± SD	18.7 ± 7.3	11.0 ± 2.6	<0.001
Plasma TGF-β1 (ng ml^{-1}), mean ± SD	14.0 ± 5.4	8.6 ± 2.8	<0.001

NSCLC, non-small cell lung cancer; TNF, tumour necrosis factor; TGF, transforming growth factor.

Sensitivity and specificity for plasma TNF-α levels as a diagnostic marker

To investigate the diagnostic potential of TNF-α level as a tumour marker, a threshold was chosen to define the specificity at 95%, which was determined by the distribution in control subjects. A ROC curve was generated for calculating the sensitivity, PPV, NPV and TNF-α cut-off value at 95% specificity. This resulted in a threshold for the TNF-α level at 15.3 pg ml^{-1} (area under the ROC curve 0.855; standard error 0.027; 95% CI 0.803–0.907; Figure 1). The sensitivity and specificity using this threshold were 67.0% (95% CI 56.8–75.9) and 95.0% (95% CI 88.2–98.1). Further, the predictive value of positive results and negative results were 93.1% (95% CI 83.9–97.4) and 74.2% (95% CI 65.6–81.4), respectively. When the cut-off was adjusted to 12.9 pg ml^{-1}, the sensitivity, specificity, PPV and NPV were 77.0% (95% CI 67.3–84.6), 76.0% (95% CI 66.2–83.7), 76.2% (95% CI 66.5–83.9) and 76.8% (95% CI 67.0–84.4), respectively. Further, an elevated plasma level of TNF-α (cut-off 12.9 pg ml^{-1}) was associated with a higher risk of NSCLC with an OR (adjusted for age, sex and smoking status) of 12.02 (95% CI 5.98–24.16; p <0.001).

Sensitivity and specificity for plasma TGF-β1 levels as a diagnostic marker

At 95% specificity (95% CI 88.2–98.1), plasma TGF–β1 reached a sensitivity of 39.0% (95% CI 29.5–49.3) for the detection of NSCLC (cut-off 13.6 ng ml^{-1}; area under the ROC curve 0.824; standard error 0.029; 95% CI 0.767–0.880; Figure 1). The predictive value of positive results

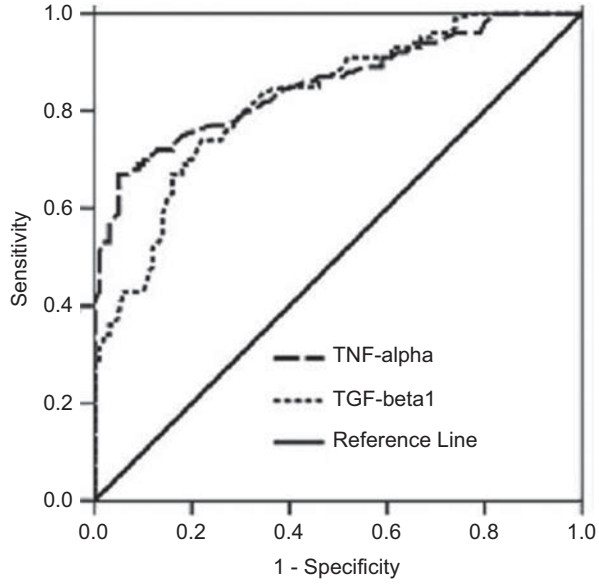

Figure 1. Receiver-operating characteristics (ROC) curve to calculate sensitivity and specificity of plasma tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 level as a tumour marker in non-small cell lung carcinoma (NSCLC).

and negative results were 88.6% (95% CI 74.6–95.7) and 60.9% (95% CI 52.7–68.5), respectively. When the cut-off was adjusted to 10.45 ng ml^{-1}, the sensitivity, specificity, PPV and NPV were 74.0% (95% CI 64.1–82.0), 78.0% (95% CI 68.4–85.4), 77.1% (95% CI 67.2–84.8) and 75.0% (95% CI 65.4–82.7), respectively. An elevated plasma level of TGF-β1 (cut-off 10.45 ng ml^{-1}) was associated with a higher risk of NSCLC with an OR (adjusted for age, sex and smoking status) of 10.13 (95% CI 5.17–19.83; p <0.001).

Circulating plasma TNF-α and TGF-β1 levels in correlation with clinicopathological factors in NSCLC

The characteristics of patients with NSCLC, such as performance status, histology, stage, tumour size, nodal status, presence of metastasis, smoking and tobacco habits and their correlation with plasma TNF-α and TGF-β1 levels are shown in Table 2. Plasma TNF-α levels were significantly higher in patients with tumour size >3 cm (mean ± SD, 20.1 ± 7.4 pg ml^{-1}) compared with patients with tumour size <3 cm (15.1 ± 5.9 pg ml^{-1}; p = 0.002). There was no association between plasma TNF-α and TGF-β1 levels and various other clinicopathological factors.

Plasma TNF-α and TGF-β1 levels as a prognostic marker in NSCLC

The association of pretreatment plasma TNF-α and TGF-β1 levels and survival duration in 42 NSCLC patients was studied using three-tertile stratification. As is evident from Tables 3 and 4, plasma TNF-α and TGF-β1 levels did not correlate with survival.

Plasma TNF-α and TGF-β1 levels in correlation with response to therapy in NSCLC

The median follow-up time was 332.5 days ranging from 173 to 864 days. The median survival time was 367 days (95% CI 310.0–424.0). Staging investigations after the third cycle of chemotherapy showed that four (9.5%) of the 42 NSCLC patients achieved complete remission (CR), 12 (28.6%) had partial remission (PR), 14 (33.3%) showed progression (PD) and 12 (28.6%) had stable disease (SD). Alternatively, there were 16 responders (CR+PR) and 26 non-responders (SD+PD) to chemotherapy. Kaplan–Meier curves for overall survival showed highly significant differences in patients with remission (R), and stable disease (SD) or progression (P) (p <0.001) with median survival times of 528 (95% CI 430.7–625.3),

Table 2. Correlation between plasma tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 levels and clinicopathological factors in non-small cell lung carcinoma (n = 100).

Characteristics	n	TNF-α (pg ml^{-1}) Mean ± SD (range)	p-Value	TGF-β1 (ng ml^{-1}) Mean ± SD (range)	p-Value
Age (years)					
<60	62	18.4 ± 7.1 (8.5– 41.2)	0.521	13.8 ± 4.7 (6.4– 31.0)	0.616
≥60	38	19.3 ± 7.6 (8.6– 46.5)		14.3 ± 6.4 (6.3– 32.6)	
Sex					
Male	92	18.5 ± 7.2 (8.5– 46.5)	0.217	13.9 ± 5.2 (6.3– 31.0)	0.409
Female	8	21.8 ± 8.5 (9.5– 35.2)		15.5 ± 7.8 (9.5– 32.6)	
Smoking status					
Smokers	83	18.9 ± 7.4 (8.5– 46.5)	0.669	13.8 ± 5.1 (6.3– 31.0)	0.554
Non-smokers	17	18.0 ± 6.7 (8.6– 35.2)		14.7 ± 6.9 (8.3– 32.6)	
ECOG					
1	30	17.7 ± 7.1 (8.5– 33.2)	0.340	13.7 ± 4.6 (7.6– 27.6)	0.745
2–3	70	19.2 ± 7.4 (8.6– 46.5)		14.1 ± 5.7 (6.3– 32.6)	
Histology					
SCC	77	18.7 ± 7.7 (8.5– 46.5)	0.970	13.9 ± 5.6 (6.3– 32.6)	0.914
ADC	23	18.8 ± 5.7 (8.6– 33.2)		14.1 ± 4.9 (7.3– 27.6)	
Stage					
III	61	18.3 ± 7.2 (8.5– 46.5)	0.506	13.8 ± 4.9 (6.3– 32.6)	0.753
IV	39	19.4 ± 7.5 (8.8– 41.2)		14.2 ± 6.1 (6.4– 31.0)	
Tumour size					
<3 cm	28	15.1 ± 5.9 (8.6– 29.1)	0.002	14.2 ± 5.4 (8.3– 32.6)	0.794
>3 cm	72	20.1 ± 7.4 (8.5– 46.5)		13.9 ± 5.4 (6.3– 31.0)	
Lymph node					
N0–N1	44	19.5 ± 8.0 (8.5– 46.5)	0.368	13.9 ± 5.6 (6.3– 32.6)	0.884
N2–N3	56	18.2 ± 6.7 (9.3– 41.2)		14.1 ± 5.3 (6.4– 28.3)	

ECOG, Eastern Cooperative Oncology Group; SCC, squamous cell carcinoma; ADC, adenocarcinoma.

327 (95% CI 320.2–333.8) and 322 (95% CI 298.3–345.6) days, respectively.

There was no significant difference in pretreatment plasma TNF-α and TGF-β1 levels between responders and non-responders to chemotherapy (Table 5). Further, the plasma levels of TNF-α and TGF-β1 could not differentiate progressors from non-progressors of cancer when evaluated after the completion of three cycles of chemotherapy (Table 5).

Discussion

In the present study we evaluated the levels of TNF-α and TGF-β1 in the plasma of patients with advanced-stage NSCLC. The results show that both these biomarkers are elevated in the plasma of NSCLC compared with controls. However, we could not find any utility of these biomarkers for predicting survival or therapeutic efficacy.

TNF-α is a cytokine known to induce apoptosis through the activation of caspase in a wide variety of cells (Sidoti-de Fraisse et al. 1998). An antiproliferative effect of TNF-α has been demonstrated in various malignancies, such as colon (Schiller & Bittner 1990) and renal carcinoma (Skillings et al. 1992), as well as malignant melanoma (Lienard et al. 1992). *In vitro* studies have shown an antiproliferative effect of TNF-α against various NSCLC cell lines (Hong et al. 1987, Yang et al. 1989). Based on these data, it was speculated that TNF-α may be a predictor of response to chemotherapy and prognostic factor on survival in NSCLC. However, this hypothesis was not substantiated in the present study as plasma TNF-α proved to be a poor indicator of response to therapy. Similar results have also been reported by other studies as well on advanced-stage NSCLC patients receiving platinum-based combination chemotherapy (Derin et al. 2008, Tas et al. 2005). In contrast, significantly higher pretreatment serum TNF-α levels have been observed

Table 3. Survival analysis in non-small cell lung carcinoma according to three tertiles of plasma tumour necrosis factor (TNF)-α level.

Tertile (no.)	Plasma TNF-α range (pg ml^{-1})	Median survival time (days ± SE) (95% CI)	*p*-Value[a]	HR (95% CI)
Overall (42)	13.0– 46.5	367.0 ± 29.1 (310.0–424.0)	0.970[b]	
1st (14)	13.0– 20.5	348.0 ± 32.9 (283.5–412.5)		
2nd (14)	21.0– 25.1	367.0 ± 37.5 (293.5–440.5)	0.826[c]	1.1 (0.47–2.56)
3rd (14)	25.2– 46.5	333.0 ± 57.8 (219.6–446.4)	0.997[c]	1.0 (0.42–2.35)

CI, confidence interval; HR, hazard ratio.
[a]Unadjusted *p*-value by Cox regression analysis; [b]overall difference between three groups using log rank test for Kaplan–Meier analysis; [c]versus 1st tertile.

Table 4. Survival analysis in non-small cell lung carcinoma according to three tertiles of plasma transforming growth factor (TGF)-β1 level.

Tertile (no.)	Plasma TGF-β1 range (ng ml^{-1})	Median survival time (days ± SE) (95% CI)	*p*-Value[a]	HR (95% CI)
Overall (42)	6.3–31.0	367.0 ± 29.1 (310.0–424.0)	0.611[b]	
1st (15)	6.3–12.0	333.0 ± 12.9 (307.7–358.2)		
2nd (13)	12.6–16.1	394.0 ± 25.3 (344.4–443.6)	0.776[c]	1.1 (0.46–2.81)
3rd (14)	18.2–31.0	324.0 ± 18.3 (288.1–359.9)	0.332[c]	1.5 (0.66–3.45)

CI, confidence interval; HR, hazard ratio.
[a]Unadjusted *p*-value by Cox regression analysis; [b]overall difference between three groups using log rank test for Kaplan–Meier analysis; [c]versus 1st tertile.

Table 5. Pretreatment plasma tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 levels in relation to response to therapy.

Response to chemotherapy (*n* = 42)	TNF-α (pg ml^{-1}) Median (range)	*p*-Value	TGF-β1 (ng ml^{-1}) Median (range)	*p*-Value
Responders (*n* = 16)	22.4 (15.9–35.0)		14.4 (9.5–22.5)	
CR (*n* = 4)	24.5 (22.3–26.5)		14.6 (9.5–19.6)	
PR (*n* = 12)	20.2 (15.9–35.0)		14.5 (10.2–22.5)	
Non-responders (*n* = 26)	23.1 (13.0–46.5)	0.577[a]	13.2 (6.3–31.0)	0.632[a]
SD (*n* = 12)	23.2 (15.8–46.5)		11.7 (6.3–27.6)	
PD (*n* = 14)	22.8 (13.0–41.2)		14.4 (10.5–31.0)	
Disease progression (*n* = 14)	22.8 (13.0–41.2)	0.915	14.4 (10.5–31.0)	0.157
Non-progression[b] (*n* = 28)	22.8 (15.8–46.5)		13.2 (6.3–27.6)	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. [a]*p*-Value for the difference in the median plasma TNF-α and TGF-β1 levels in responders and non-responders to chemotherapy; [b]group constitutes patients with CR+PR+SD.

in non-responders compared with those who responded to chemotherapy (O'Brien et al. 2005). We also could not find any association between pretreatment plasma TNF-α levels and survival. Existing data regarding the prognostic relevance of pretreatment TNF-α level in lung cancer are scant and conflicting. Based on previous studies, it was believed that serum levels of this cytokine were not a reliable indicator of survival in patients with NSCLC (Derin et al. 2008, Tas et al. 2005, Martin et al. 1999, Kaminska et al. 2006, Kayacan et al. 2006). However, in a recent study on Caucasians with NSCLC, high serum TNF-α was associated with worse survival after adjusting for stage (Enewold et al. 2009). Possible explanations for the discrepancy in survival data may be the variation in patient selection combining NSCLC and SCLC together (Martin et al. 1999), and/or different methods of TNF-α quantification. Because NSCLC and SCLC differ in biology, treatment and prognosis, we included only NSCLC patients.

To evaluate the utility of TNF-α as a tumour marker, we compared plasma TNF-α levels in 100 NSCLC patients and 100 controls. We observed significantly higher TNF-α levels in patients with NSCLC compared with the controls (patients with benign pulmonary diseases). Most of the published literature has also demonstrated elevated serum TNF-α levels in patients with NSCLC compared with controls (Derin et al. 2008, Tas et al. 2005, Martin et al. 1999, Kaminska et al. 2006, Kayacan et al. 2006, Dalaveris et al. 2009, Guadagni et al. 2004). We also tried to evaluate the utility of plasma TNF-α levels as a diagnostic marker and calculated sensitivity at 95% specificity using a ROC curve. At 95% specificity, plasma TNF-α could detect NSCLC with a sensitivity of 67% at a cut-off of 15.3 pg ml^{-1}. When the cut-off was adjusted to 12.9 pg ml^{-1}, the sensitivity and specificity were 77.0% and 76.0%, respectively. Further, an elevated plasma level of TNF-α was associated with a higher risk of NSCLC (OR 12.02; 95% CI 5.98–24.16; p <0.001). As none of the previous studies have established a cut-off level for TNF-α, further studies are needed to define the exact potential of TNF-α as a diagnostic marker.

We also looked for a correlation between plasma TNF-α levels with various clinicopathological factors in NSCLC. We observed a significant association between plasma TNF-α levels and tumour size. However, no such correlation has been observed in previous studies (Kaminska et al. 2006, Guadagni et al. 2004). Further, plasma TNF-α levels did not correlate with age, gender, stage, histology, performance status, lymph node involvement and smoking habits. Previous studies have also failed to correlate TNF-α levels with stage (Derin et al. 2008, Tas et al. 2005, Martin et al. 1999, Kaminska et al. 2006, Dalaveris et al. 2009), histology (Derin et al. 2008, Tas et al. 2005, Martin et al. 1999, Kaminska et al. 2006, Dalaveris et al. 2009, Guadagni et al. 2004), age (Derin et al. 2008, Tas et al. 2005, Dalaveris et al. 2009) and gender (Derin et al. 2008, Tas et al. 2005, Dalaveris et al. 2009).

A considerable number of reports have documented the tumour-promoting role of TGF-β through its effects on tumour cell invasion and alterations in the tumour microenvironment. TGF-β1 inhibits the growth of normal epithelial cells, whereas the neoplastic cells are resistant to its inhibitory effect and rather proliferate in response to TGF-β1 (Hsu et al. 1994, Massague 1990). The TGF-β gene, mRNA and protein have been detected in lung cancer cell lines (Söderdahl et al. 1988, Jakowlew et al. 1995, Nørgaard et al. 1996). Previous studies have observed a strong correlation between increased TGF-β1 levels and disease status in lung cancer patients after radiotherapy (Kong et al. 1996, 1999). In lung cancer, plasma TGF-β1 levels at follow-up after radiotherapy have also been used to monitor the course of the disease (Kong et al. 1996, 1999). Further, an elevated plasma TGF-β1 level in breast cancer patients after surgical removal of the primary tumour has been found to be associated with a high risk of having residual disease (Kong et al. 1995). All these studies suggest that quantifying TGF-β1 levels may help in prognosticating the disease status and in predicting therapy outcome. However, we could not find any significant difference in the plasma TGF-β1 levels between responders and non-responders to chemotherapy. Similarly, plasma TGF-β1 levels could not distinguish among patients with cancer progression from those without progression. Further, pretreatment plasma TGF-β1 levels were not associated with survival in NSCLC. In the literature, studies measuring the effect of TGF-β1 levels on overall survival in NSCLC patients are lacking. Thus, more studies will be needed on larger sample size to know the exact potential of plasma TGF-β1 levels as a marker to predict survival and therapy outcome in lung cancer.

The plasma levels of TGF-β1 were compared between 100 NSCLC patients and 100 controls to evaluate its diagnostic potential. We observed significantly higher plasma TGF-β1 levels in patients with NSCLC compared with controls. Most of the other published data have also demonstrated elevated serum TGF-β1 levels in patients with NSCLC compared with controls (Kong et al. 1996, 1999, Zhao et al. 2008). In contrast, no difference in plasma TGF-β1 levels was observed when levels were compared between lung cancer patients and patients with non-malignant pulmonary diseases (Barthelemy-Brichant et al. 2002). At 95% specificity, plasma TGF-β1 could detect NSCLC with a sensitivity of 39% at a cut-off of 13.6 ng ml^{-1}. When the cut-off was adjusted to 10.45 ng ml^{-1}, the sensitivity and specificity were 74.0% and 78.0%, respectively. Further, an elevated plasma level of TGF-β1 was found to be associated with a higher risk of NSCLC (OR 10.13; 95% CI 5.17–19.83; p <0.001). As none of the previous studies have established a cut-off level

for TGF-β1, more studies are needed to know the exact potential of TGF-β1 levels as a diagnostic marker.

We also looked at correlation between plasma TGF-β1 levels with various clinicopathological factors. Plasma TGF-β1 levels did not correlate with age, gender, stage, tumour size, histology, performance status, lymph node involvement or smoking habits. Previous studies have also failed to demonstrate any correlation of TGF-β1 levels with stage (Kong et al. 1996, Barthelemy-Brichant et al. 2002), histology (Kong et al. 1999), age (Kong et al. 1996, Barthelemy-Brichant et al. 2002) and gender (Kong et al. 1996).

In conclusion, the present study showed that plasma levels of TNF-α and TGF-β1 were elevated in NSCLC. However, pretreatment plasma TNF-α and TGF-β1 levels cannot predict therapy outcome in advanced NSCLC. Further, survival was not influenced by plasma TNF-α and TGF-β1 levels. Although small sample size was a limitation, we feel that identification of other, more specific and more sensitive serum- or plasma-based biomarkers will be required for the better management of lung cancer patients.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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