RESEARCH ARTICLE

Thyroid Transcription Factor-1 Expression in Advanced Non-Small Cell Lung Cancer: Impact on Survival Outcome

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Abstract

Background: The prognostic role of thyroid transcription factor-1 (TTF-1) expression in lung cancer has been assessed but with inconsistent results. The present study aimed to evaluate the prognostic value of TTF1 expression in advanced non-squamous non-small cell lung cancer (NSCLC). Materials and Methods: In this retrospective study, patients with stage IIIB-IV non-squamous NSCLC were enrolled. Progression free survival (PFS) and overall survival (OS) were assessed according to TTF1 expression status, age categories (≤60 *vs* >60 years), gender, performance status (PS) (0-2 *vs* 3-4), type of 1st line chemotherapy (pemetrexed containing *vs* others) and EGFR status. Results: A total of 120 patients were included. In univariate analysis, PFS was improved in patients with PS 0-2 (7.0 *vs* 2.0 months, p=0.002) and those who received pemetrexed-containing chemotherapy (9.2 *vs* 5.8 months, p=0.004). OS was improved in female patients (23.0 *vs* 8.7 months, p<0.0001), PS 0-2 (14.4 *vs* 2.0 months, p<0.0001), those with pemetrexed-containing chemotherapy (17.0 *vs* 11.0 months, p=0.019), TTF1-positive (12.8 *vs* 5.8 months, p=0.011) and EGFR- mutant patients (23.0 *vs* 11.7 months, p=0.006). In multivariate analysis, male gender (HR=2.34, p=0.025) and non-pemetrexed containing therapy (HR=2.24, p=0.022) were independent predictors of worse PFS. Wild EGFR status (HR=2.49, p=0.015) and male gender (HR=2.78, p=0.008) were predictors of worse OS. Conclusions: Pemetrexed-containing therapy significantly improved PFS while OS was improved in EGFR mutant patients. Female patients had better PFS and OS. TTF1 expression was not a prognostic marker in advanced non-squamous NSCLC.

Keywords: TTF1 - prognosis - advanced - lung cancer

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Introduction

Lung cancer is the most common cause of cancer-related mortality worldwide and non-small cell Lung cancer (NSCLC) accounts for more than 85% of primary lung cancers (Li et al., 2012). However, the majority of patients present in advanced stage at time of diagnosis (Liu et al., 2013). Some clinical and disease-related factors have already been correlated with survival including performance status (PS), stage, age, gender and weight loss; however, for most of these factors, their discriminant values are insufficient to guide individual decision-making (Berghmans et al., 2006). Thyroid transcription factor-1 (TTF1) is a nuclear protein expressed in the thyroid and lung and plays a physiologic role in their development and morphogenesis during embryogenesis. In lung cancer, TTF1 is more frequently expressed in adenocarcinoma and small-cell lung cancer (Berghmans et al., 2006). Emerging evidence suggests that a subset of lung adenocarcinoma cell lines is dependent on persistent expression of TTF1 and its inhibition significantly induced growth inhibition and apoptosis (Tanaka et al., 2007).

The prognostic value of TTF1 expression in lung cancer patients has been evaluated in several studies, incorporating mainly local or loco-regional stages; however, results were conflicting and no consensus has been reached (Zhan et al., 2013). Data from these studies was limited by small sample size, patient heterogeneity and different immune-histochemical techniques used to detect TTF1 (Saad et al., 2004; Berghmans et al., 2006). For example, in stage I NSCLC patients, no survival difference was associated with TTF1 expression in one study while longer median overall survival (OS) was demonstrated in another one (Pelosi et al., 2001; Anagnostou et al., 2009). In a meta-analysis including mostly patients with early stage disease, TTF1 expression was associated with favourable prognosis (Zhan et al., 2013).

However, the prognostic value of TTF1 expression

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in advanced NSCLC in the recent era of modern chemotherapeutic and target therapies was not properly assessed. The present study aims to evaluate the value of TTF1 expression as a prognostic factor in advanced NSCLC patients and whether its potential prognostic value will be retained after adjusting for other prognostic variables.

Materials and Methods

Study population

Patients with histologically confirmed non-squamous NSCLC with stage IIIB-IV disease at the time of diagnosis were enrolled. The study group consisted of patients from three centres in Saudi Arabia who were diagnosed from May 2011 to December 2012. Enrolled patients must have available result of TTF1 expression.

Study design and procedures

In this retrospective study, advanced non-squamous NSCLC patients were identified through review of medical records. Different patients and treatment data was collected including age at diagnosis, gender, ECOG PS, stage, TTF1 expression status, EGFR status, site of metastasis, type of 1st line therapy, number of cycles, response to 1st line therapy, receipt of maintenance therapy. In addition, dates of disease progression and death were also recorded. Approval from relevant institutional review board/ethical committee was obtained in each of the contributing centres.

Statistical analysis

The data was analysed using SPSS version 17 (SPSS Inc., Chicago, IL, USA) and has been subjected to descriptive analysis. We examined the distribution of patients and treatment characteristics according to TTF1 expression status to describe the study population and to identify possible associations with TTF1 expression. These categorical variables were compared using Chi-square test or fisher exact test as appropriate.

Progression free survival (PFS) and OS were assessed using survival analyses (Kaplan-Meier curves) and the differences in survival distributions according to TTF1 expression status, age categories (≤60 *vs* >60 years), gender, PS (0-2 *vs* 3-4), site of metastasis (single *vs* multiple and bone-only *vs* others), type of 1st line therapy, receipt of maintenance therapy and EGFR status were evaluated via Log Rank (Mantel-Cox) test. PFS was defined as the time from starting treatment to the first documented tumour progression or death from any cause. OS was defined as the time from date of diagnosis of NSCLC to the date of death due to any cause. Multivariate analyses by Cox proportional hazards models have been used to check for independent prognostic factors associated with PFS and OS. An alpha level of <0.05 has been considered significant for each analysis.

Results

Patient characteristics

One hundred and twenty patients diagnosed with advanced non-squamous NSCLC were identified (100 stage IV, 20 stage IIIB, 100 TTF1-positive, 20 TTF1-negative). 76.7% of the included patients were males and 51.7% were >60 years at diagnosis. 21.7% had poor PS (3-4) at the time of diagnosis and twenty patients (16.7%) did not receive any anticancer therapy due to poor PS. Eighty patients had available results of EGFR testing and 26.2% of them were mutant. Thirty three patients (27.5%) received maintenance therapy. At the time of analysis, 10% of patients did not develop disease progression and 32.5% were still alive. The median follow up was 22 months (range 15-31 months). Baseline patients and treatment characteristics were balanced between TTF1-positive and negative patients (Table 1).

Survival

In univariate analysis, PFS was significantly improved in patients with PS 0-2 (7.0 months, 95%CI=4.92-9.08 *vs* 2.0 months, 95%CI=0.50-4.99, p=0.002) and those who

Table 1. Distribution of Different Parameters in TTF1-Positive Compared to TTF1-Negative Patients

Parameters	TTF1-positive	TTF1-negative	p
Age			
≤60	50 (50%)	8 (40%)	0.47
>60	50 (50%)	12 (60%)	
Total	100	20	
Gender			
Male	76 (76%)	16 (80%)	0.47
Female	24 (24%)	4	(20%)
Total	100	20	
PS*[1]			
0-2	81 (81%)	13 (65%)	0.14
3-4	19 (19%)	7	(35%)
Total	100	20	
Bone-only metastasis	14 (16.9%)	1 (5.9%)	
Others	69 (83.1%)	16 (94.1%)	0.46
Total	83	17	
Site of metastasis			
Single	42 (50.6%)	11 (64.7%)	0.42
Multiple	41 (49.4%)	6 (35.3%)	
Total	83	17	
Type of 1st line therapy			
Pemetrexed-containing	35 (39.8%)	8 (44.4%)	
Non-pemetrexed	42(47.7%)	10 (55.6%)	0.79
Erlotinib	11(12.5%)	0 (0%)	
Total	88	18	
No of cycles of 1st line therapy			
≤4			
>4	52 (59.1%)	12 (66.7%)	0.83
Total	36 (40.9%)	6 (33.3%)	
88	18		
EGFR status			
Wild	50 (71.4%)	9 (90%)	0.28
Mutant	20 (28.6%)	1 (10%)	
Total	70	10	
Progression after 1st line			
Yes	90 (90%)	18(90%)	
No	10 (10%)	2 (10%)	0.68
Total	100	20	
Dead	65 (65%)	16 (80%)	
Alive	35 (35%)	4 (20%)	0.29
Total	100	20	

*[1]PS, ECOG performance status

received pemetrexed-containing 1st line chemotherapy (9.2 months, 95%CI=7.90-10.49 *vs* 5.8 months, 95%CI=4.49-7.10, p=0.004), (Figure 1, Table 2). Meanwhile, PFS was improved in patients with TTF1-positive tumours; however, it did not reach statistical significance (6.8 months, 95%CI=5.28-8.32 *vs* 2.3 months, 95%CI=0.40-4.20, p=0.17), (Figure 2, Table 2).

OS was significantly improved in TTF1-positive (12.8 months, 95%CI=9.74-15.86 *vs* 5.8 months, 95%CI=2.13-9.47, p=0.011), (Figure 2, Table 3), those with pemetrexed-containing chemotherapy (17.0 months, 95%CI=12.87-21.13 *vs* 11.0 months, 95%CI=5.90-16.10,p=0.019), (Figure 1, Table 3), female patients (23.0 months, 95%CI=17.03-28.97 *vs* 8.7 months, 95%CI=5.16-12.24, p<0.0001), (Figure 3, Table 3), patients with PS 0-2 (14.4 months, 95%CI=10.66-18.14 *vs* 2.0 months, 95%CI=0.50-4.99, p<0.0001) and EGFR- mutant patients (23.0 months, 95%CI=16.41-27.39 *vs* 11.7 months, 95%CI=8.24-15.16, p=0.006) (Table 3).

In multivariate analysis, male gender (HR=2.34, p=0.025) and non-pemetrexed containing therapy (HR=2.24, p=0.022) were independent predictors of worse PFS. Wild EGFR status (HR=2.49, p=0.015) and male gender (HR=2.78, p=0.008) were independent predictors of worse OS. Furthermore, patients who received non-pemetrexed containing 1st line therapy had worse OS that approached statistical significance (HR=1.84, p=0.07). TTF1 expression status was not an independent prognostic factor for PFS (HR=1.54, p=0.41) or OS (HR=2.32, p=0.10).

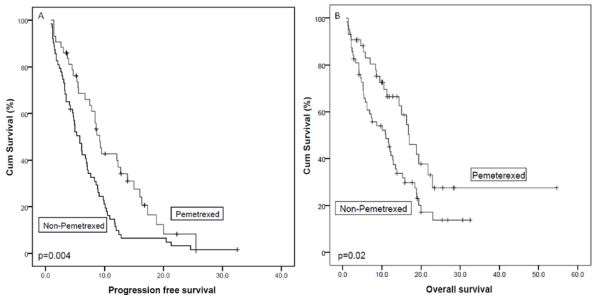

Figure 1. A) Progression free Survival (PFS) in Patients who Received Pemetrexed-containing 1st Line Chemotherapy *vs* Others B) Overall Survival (OS) in Patients who Received Pemetrexed- containing 1st Line Chemotherapy *vs* Others

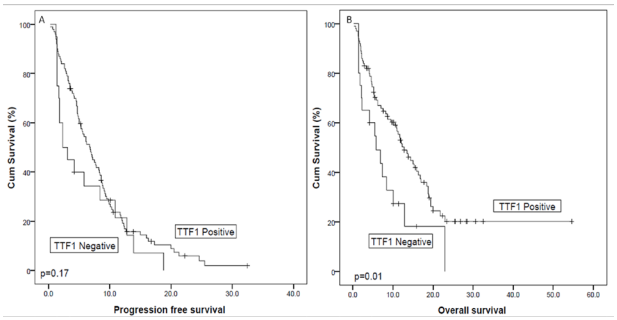

Figure 2. A) Progression Free Survival (PFS) in TTF1-Positive Compared to TTF1-Negative Patients B) Overall Survival (OS) in TTF1-Positive Compared to TTF1-Negative Patients

Table 2. Progression Free Survival (PFS) According to Different Parameters

Parameters	Univariate analysis		Multivariate analysis	
	Median PFS in months (95% CI[1])	p value	HR[2] (95% CI)	p value
Age				
≤60	6.8 (4.18-9.42)	0.67	-	-
>60	5.3 (3.02-7.57)			
Gender				
Male	5.5 (4.22-6.78)	0.12	2.34 (1.11-4.94)	0.025
Female	7.5 (5.57-9.43)			
PS[3]				
0-2	7.00 (4.92-9.08)	0.002	-	-
3-4	2.00 (0.00-4.99)			
Site of metastasis				
Bone-only	5.30 (1.01-9.59)	0.59	-	-
Others	5.80 (4.47-7.13)			
Site of metastasis				
Single	6.10 (3.73-8.47)	0.18	-	-
Multiple	5.00 (3.73-6.27)			
Type of 1st line therapy				
Pemetrexed-containing	9.20 (7.90-10.49)	0.004	2.24 (1.13-4.45)	0.022
Non-pemetrexed	5.80 (4.49-7.10)			
No of cycles of 1st line therapy				
≤4	6.90 (5.15-8.65)	0.77	-	-
>4	7.20 (5.23-8.57)			
Maintenance therapy				
Yes	5.8 (4.37-7.23)	0.52	-	-
No	4.7 (0.99-8.41)			
TTF1-positive	6.80 (5.28-8.32)	0.17	-	-
TTF1-negative	2.30 (0.40-4.20)			
EGFR status				
Wild	6.80 (4.60-8.99)	0.35	-	-
Mutant	6.90 (2.41-11.39)			

*[1]CI: confidence interval, [2]HR: hazard ratio, [3]PS: ECOG performance status

Table 3. Overall Survival (OS) According to Different Parameters

Parameters	Univariate analysis		Multivariate analysis	
	Median OS in months (95% CI[1])	P-value	HR[2] (95%CI)	P-value
Age				
≤60	14.4 (11.05-17.75)	0.09	-	-
>60	8.2 (3.29-13.11)			
Gender				
Male	8.7 (5.16-12.24)	<0.0001	2.78 (1.30-5.95)	0.008
Female	23.0 (17.03-28.97)			
PS[3]				
0-2	14.40 (10.66-18.14)	<0.0001	-	-
3-4	2.00 (0.00-4.99)			
Site of metastasis				
Bone-only	5.30 (0.00-12.87)	0.66	-	-
Others	10.50 (7.17-13.83)			
Site of metastasis				
Single	10.90 (5.15-16.65)	0.33	-	-
Multiple	8.70 (3.58-13.82)			
Type of 1st line				
Pemetrexed-containing	17.00 (12.87-21.13)	0.02	-	-
Non-pemetrexed	11.00 (5.90-16.10)			
No of cycles of 1st line therapy				
≤ 4	6.90 (5.15-8.65)	0.77	-	-
>4	7.20 (5.23-8.57)			
Maintenance therapy				
Yes	8.4 (5.49- 11.31)	0.28	-	-
No	8.2 (3.03- 13.37)			
TTF1-positive	12.80 (9.74-15.86)	0.01	-	-
TTF1-negative	5.80 (2.13-9.47)			
EGFR status				
Wild	11.70 (8.24-15.16)	0.006	2.49 (1.19 -5.19)	0.015
Mutant	23.00 (16.41-27.39)			

*[1]CI: confidence interval, [2]HR: hazard ratio, [3]PS: ECOG performance status

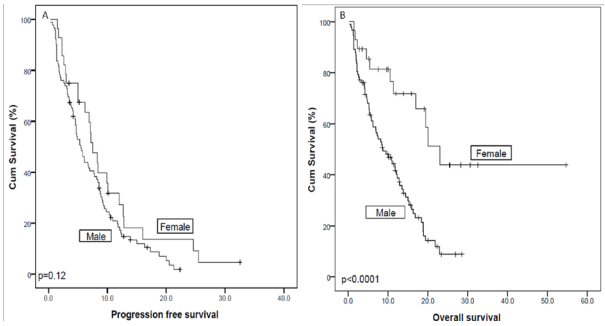

Figure 3. A) Progression free Survival (PFS) in Male Compared to Female Patients B) Overall Survival (OS) in Male Compared to Female Patients

Discussion

Thyroid transcription factor-1 (TTF1) is critical for the regulation of expression of multiple genes involved in lung development (Anagnostou et al., 2009). In addition, TTF1 is preferentially expressed in adenocarcinoma of the lung and it is most commonly used to distinguish primary lung adenocarcinoma from tumours that have metastasized to the lung (Stenhouse et al., 2004). Although the role of TTF1 in differential diagnosis of primary lung adenocarcinoma has been well documented, its prognostic value for patients with lung cancer has been less rigorously studied (Berghmans et al., 2006).

In the present study, including advanced non-squamous NSCLC, TTF1- positive patients had better OS in univariate analysis which was lost in multivariate analysis involving different prognostic factors. Our results refer to the importance of the type of 1st line systemic treatment as a prognostic factor of PFS while it approaches statistical significance for OS. In a Chinese study, pemetrexed-containing 1st line chemotherapy significantly improved PFS as well as OS in univariate and multivariate analyses (Wang and Cai, 2013). Noteworthy, in another study, the response and progression free survival offered by first-line chemotherapy affect the response, PFS and OS with second-line chemotherapy (Cao et al., 2014). Furthermore, in our study, female patients had better PFS and OS compared to males. This favourable outcome may be explained by lower smoking habit in Saudi females with different disease biology that may confer better response to therapy and improved survival.

Several studies have assessed the prognostic value of TTF1 in NSCLC, however, most of them included early stage patients. Only one study has demonstrated a negative impact of TTF-1 expression on survival (Tan et al., 2003) while four studies showed no impact (Haque et al., 2002 ; Hoffman et al., 2000; Hotta et al., 2004; Saad et al., 2004). Meanwhile, positive impact of TTF-1 expression on survival was demonstrated in several studies (Zhan et al., 2013).

In a meta-analysis including 10 studies, high TTF-1 protein expression was associated with better survival in NSCLC, mainly in early-stage NSCLC (Berghmans et al., 2006). Favourable survival outcome of TTF1 expression in early-stage NSCLC may be explained by

the lack of adjuvant therapy in most of these patients, and subsequently survival was less likely to be affected by treatments other than surgical resection (Berghmans et al., 2006). In a more recent meta-analysis including 17 studies with 2,235 patients, TTF1 overexpression had a favourable impact on survival of patients with NSCLC. However, several important prognostic parameters were not taken in consideration in that meta-analysis such as types of therapy, PS and EGFR status (Zhan et al., 2013).

Furthermore, four studies (Barlesi et al., 2005; Martins et al., 2009; Sun et al., 2011; Chung et al., 2012) have evaluated the impact of TTF1 expression in Stage IIIB-IV NSCLC that revealed positive impact on survival. In these studies, several relevant parameters were not included in multivariate analyses such as site, number of metastases, type of therapy, EGFR mutation status. In a Korean study involving patients treated with pemetrexed-containing therapy, TTF1-positive patients had better survival outcome than negative patients (Sun et al., 2011). However, OS of both cohorts of patients (25.4 versus 14.2 months, respectively) are nearly double that found in our study. The poor OS in our study may contribute to the lack of survival difference between TTF1-positive and negative patients in multivariate analysis.

The percentage of positive TTF1 expression reported in our study (83.3%) is similar to that of asian patients. In a Chinese trial, 89.3% of advanced patients were TTF1-positive (Chung et al., 2012) which is superior to the prevalence of TTF1 expression in western population (66% in French patients) (Barlési et al., 2005). This may point to the possibility of biologic similarity of NSCLC in Saudi Arabia with that of Asian patients. Noteworthy, TTF1 positivity was found to correlate with higher prevalence of epidermal growth factor receptor (EGFR) mutation in lung adenocarcinoma (Chung et al., 2012). In our study, 40% of TTF1- positive patients were EGFR-mutant.

Several factors can explain the lack of survival improvement with TTF1 expression in our study. Considerable percentage of patients did not receive any systemic therapy due to poor PS at the time of presentation to our institute. This is may be related to late diagnosis and delayed referral due to health system logistics. In addition, adjusting for multiple prognostic parameters in multivariate analysis is another factor.

Our study has some limitations. Weight loss and smoking history were not included in the analysis as they were not adequately reported in medical records for most of the patients. Treatment was heterogeneous among the study group (40.5% received pemetrexed-containing therapy, 17 % received erlotinib, other types of chemotherapy in the others). Only half of EGFR-mutant patients received 1st line erlotinib due to delayed EGFR testing results. This may account for lack of PFS benefit in EGFR-mutant patients.

In conclusion, female patients and those who received pemetrexed-containing 1st line chemotherapy had significantly improved PFS while OS was improved in females and EGFR-mutant patients. Survival outcome was improved in TTF1-positive compared to negative patients in univariate but not in multivariate analysis. The potential prognostic value of TTF1 expression was lost after adjusting with several clinical and therapeutic parameters; some of them were not considered in previous studies.

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