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Does Transforming Growth Factor- β 1 Predict for Radiation-Induced Pneumonitis in Patients Treated for Lung Cancer?

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Abstract

The purpose of the study was to reassess the utility of transforming growth factor-beta-1 (TGF- β 1) together with dosimetric and tumor parameters as a predictor for radiation pneumonitis (RP). Of the 121 patients studied, 32 (26.4%) developed grade ≥ 1 RP, and 27 (22.3%) developed grade ≥ 2 RP. For the endpoint of grade ≥ 1 RP, those with V30 $> 30\%$ and an end-RT/baseline TGF- β 1 ratio ≥ 1 had a significantly higher incidence of RP than did those with V30 $> 30\%$ and an end-RT/baseline TGF- β 1 ratio < 1 . For most other patient groups, there were no clear associations between TGF- β 1 values and rates of RP. These findings suggest that TGF- β 1 is generally not predictive for RP except for the group of patients with a high V30.

Keywords

TGF- β 1; radiation pneumonitis

1. INTRODUCTION

Pulmonary injury following thoracic irradiation (RT) is fairly common. For patients treated for lung cancer, approximately 5% to 20% develop symptomatic lung injury, 50% to 100% develop radiologic evidence of regional injury, and 50% to 90% experience declines in pulmonary function [1–6]. Identification of the dosimetric and biologic determinants of RT-induced lung injury would be useful in developing risk profiles for individual patients; thereby tailoring treatment to maximize efficacy and minimize toxicity [1].

Several studies have successfully correlated the risk of developing RT-induced lung injury with 3D dose parameters such as mean lung dose (MLD) and the percentage of lung volume receiving ≥ 20 Gy (V20) [4,7–11]. These findings are encouraging, in that they demonstrate the ability of 3D tools to predict normal tissue risks [9].

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However, dosimetric parameters alone are not ideal predictors for lung injury [4,8–11]. Several recent studies have investigated transforming growth factor-beta-1 (TGF- β 1) as a predictor for RT-induced lung injury [1,3,7,12–15]. TGF- β 1 is a cytokine that has been suggested to play an important role in both tumor progression and normal tissue damage by suppressing anti-tumor immune responses, enhancing extracellular matrix production, and augmenting angiogenesis [16–18].

Anscher et al. [12–14] suggested that changes in plasma TGF- β 1 levels during RT might be useful in determining which patients were at risk for developing RP. Fu et al. [3] showed that plasma TGF- β 1 levels at the end of RT relative to pre-RT TGF- β 1 levels seemed to be an independent risk factor for developing symptomatic RT-induced lung injury, and that combining plasma TGF- β 1 levels with V30 appeared to facilitate stratification of patients into low-, intermediate-, and high-risk groups.

Complicating this line of investigation, De Jaeger et al. [7] observed that the TGF- β 1 value at the end of RT was related to the MLD. In addition, we and others have noted that TGF- β 1 can be produced by the tumor itself [17,19–23]. Thus, since tumor size might dictate RT field size (and hence MLD), and since MLD may have an impact on TGF- β 1, such analyses that consider each of these dosimetric, biologic, and tumor parameters are inherently complex.

Since our last publication [3], we have enrolled additional patients onto our studies. The goal of the present analysis is to reassess the utility of TGF- β 1 together with dosimetric and tumor parameters as a predictor for RP, in the context of a prospective clinical trial.

2. MATERIALS AND METHODS

2.1 Patient eligibility and characteristics

The records of 251 patients treated for lung cancer with thoracic RT with curative intent from 1991 to 2003 at Duke University Medical Center, and who were entered onto a series of prospective normal tissue injury studies, were reviewed. Of these, 121 patients meeting the following criteria were identified: 1) available TGF- β 1 data, 2) ≥ 6 months follow-up, 3) no surgery post-RT, 4) no intrathoracic disease progression < 6 mos post RT, and 5) no intraluminal brachytherapy. Patients who died within 6 months of RT were excluded. Causes of death were mostly due to complications from metastatic disease, as well as tumor progression, cardiovascular disease, infection, and leukemia. In 3 patients, the cause of death may have been, in part, related to RT-induced pulmonary complications. All studies were approved by the Duke University Medical Center Institutional Review Board. Patients gave written informed consent before enrollment. The patient, tumor, and treatment characteristics are shown in Table 1.

2.2 Clinical evaluation, treatment planning, and follow-up

Pretreatment evaluation included a history and physical examination, automated blood count, kidney, and liver function tests. Chest X-ray and computed tomography (CT) scans of the chest and upper abdomen were routinely performed for staging purposes. Patients with locally advanced disease also had bone scans and a brain CT. Patients were staged according to the American Joint Committee on Cancer 1997 staging criteria [3].

One hundred one patients had a CT scan in the treatment position for 3D treatment planning. 3D dose distributions were calculated using tissue inhomogeneity corrections [24–27]. Lung contours were defined using an automatic threshold method and edited as appropriate to exclude the gross tumor and the bronchus [3]. Dosimetric data (MLD, V20, V30) was available for 98 patients and unavailable for 3 patients due to technical difficulties.

Gross tumor volume (GTV) data was available for 70 patients. The GTV was identified as the radiographically abnormal area, based on a review of the planning CT as well as the diagnostic imaging, which typically included a CT and/or PET. For uniformity, the GTV was reviewed and approved by several investigators.

Following RT, patients had follow-up 6 weeks post RT, and generally every 3 months thereafter as clinically indicated. At follow-up, a history, physical examination, chest X-ray, and often a CT were obtained. Other tests, such as bone scans and brain CT scans, were done if required by protocol or by the clinical situation [3].

2.3 Plasma TGF- β 1 analysis

The methods used for analysis of plasma TGF- β 1 samples prior to 1998 have been previously described [3]. Elevated levels were defined as > 7.5 ng/ml, which is 2 standard deviations above the mean for normal controls [3]. After 1998, plasma TGF- β 1 samples were generally run using a modified ELISA method previously described in rat models [28]. According to the new ELISA method, elevated levels were redefined as > 5 ng/ml.

TGF- β 1 levels were generally to be drawn pre-RT and at regular intervals during RT. As patients were enrolled on a variety of different studies, the exact timing of the TGF- β 1 varied. Furthermore, due to logistical issues, many TGF- β 1 specimens were not analyzable, either because blood samples were not drawn, or because samples were lost or ruined. Therefore, we do not have complete TGF- β 1 on all patients.

For the purpose of this study, three different TGF- β 1 levels were considered: baseline, mid-RT, and end-RT. The baseline TGF- β 1 level was generally drawn on the first day of treatment; however, in 50% of patients, this level was actually drawn prior to the commencement of treatment (1–38 days pre-RT). These values were considered an acceptable representation of the baseline level, provided that no anti-cancer therapy had been administered at that time. In 12 patients (10%), the baseline level was drawn within the first 7 days of treatment. Mid-RT values represented those TGF- β 1 levels obtained at ≈ 35 Gy (range 25–45 Gy). In the event where TGF- β 1 samples had not been drawn at ≈ 35 Gy, but had been drawn within the above range at approximately equal intervals above and below 35 Gy (i.e., at 30 Gy and 40 Gy), the values of these samples were averaged in order to obtain a TGF- β 1 level at ≈ 35 Gy. End-RT values represented those TGF- β 1 levels drawn at ≈ 70 Gy (range 45–80 Gy), during the later course of treatment, and the same rules for averaging applied when necessary. For the TGF- β 1 parameter, both absolute values and ratios (mid-RT/baseline, end-RT/mid-RT, end-RT/baseline) were considered. Baseline, Mid-RT, and End-RT TGF- β 1 values were available for 121, 114, and 104 patients, respectively.

2.4 Endpoints and statistical analysis

The endpoint of this study was the development of RP at any time before 6 months post-RT. The presence or absence of RP was assessed by both the treating physician, as well as by a panel of investigators. An event in the current analysis was defined as RP based on clinical symptoms. Using this modification of the NCI common toxicity criteria, patients with clinical symptoms were required to demonstrate a worsening of ≥ 1 grade from baseline on the NCI scale to make a positive diagnosis of RP [3]. The rates of RP in patient subgroups, defined based on dosimetric (MLD, V20, V30), tumor (GTV), and TGF- β 1 defined parameters, were compared using the two-tailed Fisher's exact test.

3. RESULTS

3.1 Patient subgroups

Patients were segregated by TGF- β 1 level (baseline, mid-RT, end-RT) and ratio (mid-RT/baseline, end-RT/mid-RT, end-RT/baseline) according to whether their levels were $>$ or $\leq$ normal (7.5 ng/ml and 5 ng/ml for samples collected pre-1998 and post-1998, respectively), and whether their TGF- β 1 ratios were $\geq$ or $<$ 1. Patients were further divided according to MLD, V20, V30, and GTV. The median values for MLD, V20, and V30 were 18.5 Gy, 31.5%, and 26%, respectively. Thus, 20 Gy, 30%, and 30% were used as cut-points for these three metrics, respectively, to define the patient subgroups in this study. These cut-points are consistent with prior analyses [3,4,8,10]. The cut-point used for GTV was 100 cm³, which corresponded with the median value. Additional analyses were performed, omitting the 12 patients whose baseline TGF- β 1 values were obtained between day 1 and day 7 of RT, as well as using the actual median values for MLD, V20, and V30 as cut-points (data not shown). The results were largely similar.

3.2 Incidence of RP based on patient subgroups

3.2.1 Incidence of grade $\geq$ 1 RP—Thirty-two patients (26.4%) developed grade 1 or higher RP. Patients with V30 $>$ 30% and an end-RT/baseline TGF- β 1 ratio $\geq$ 1 had a significantly higher incidence of RP (6/13; 46.2%) than did those with V30 $>$ 30% and an end-RT/baseline TGF- β 1 ratio $<$ 1 (2/17; 11.8%; $p=0.049$). For the patients with V30 $<$ 30%, the incidence of RP was similar between patients with normal versus elevated TGF- β 1 levels or TGF- β 1 ratios $>$ or $<$ 1 ($p=0.37$ –1). Besides V30, no other dosimetric parameters significantly predicted for RP. GTV was not a predictive factor for RP when considered with TGF- β 1 levels or ratios. The rates of grade $\geq$ 1 RP in patients based on TGF- β 1, MLD, V20, V30, and GTV are listed in Table 2a.

3.2.2 Incidence of grade $\geq$ 2 RP—Twenty-seven patients (22.3%) developed grade 2 or higher RP. Patients with mid-RT TGF- β 1 levels $\leq$ normal had a significantly higher incidence of RP (20/71; 28.2%) than did patients with mid-RT TGF- β 1 levels $>$ normal (4/43; 8.3%; $p=0.018$). In contrast to the results for the endpoint of grade $\geq$ 1 RP, no dosimetric parameter significantly predicted for $\geq$ grade 2 RP when segregating patients by TGF- β 1 level or ratio. Again, GTV was not a predictive factor for RP when considered with TGF- β 1 levels or ratios. The rates of grade $\geq$ 2 RP in patients based on TGF- β 1, MLD, V20, V30, and GTV are listed in Table 2b.

4. DISCUSSION

Since 1991, we have been prospectively evaluating the utility of TGF- β 1 as a predictor of radiation-induced lung injury [3]. The mechanisms through which TGF- β 1 performs are complex, and involve both the inhibition of epithelial cell proliferation and the development of tissue fibrosis in response to irradiation [3,15,29–31]. The role of TGF- β 1 in the progression of human disease and in tissue response to therapy has already been described in great detail [3,7,12–18,20,22,32–34].

The first finding in this study is that *lower* mid-RT TGF- β 1 levels correspond to a *higher* rate of grade $\geq$ 2 RP. This finding is similar to those of De Jaeger et al. [7]. Anscher et al. [12] reported slightly lower baseline TGF- β 1 levels in patients with RP versus those without RP (18.7 ng/ml vs. 26.0 ng/ml, respectively), but the difference was not statistically significant. In subsequent studies, Anscher et al. [13,14] found that changes in TGF- β 1 levels over the course of therapy appeared to be useful in identifying patients at risk for developing RP. For example, Anscher et al. [12–14], Vujaskovic et al. [34], and Fu et al. [3] have reported that

patients with a TGF- β 1 ratio (end-RT/baseline TGF- β 1) ≥ 1 are more likely to develop RP than are patients with a ratio < 1 . Our current report, largely an update of the prior work, is consistent with this, but only for the endpoint of grade 1 RP in one patient subset. In most of the patient subsets, the TGF- β 1 level was not predictive for RP. We do find, however, that when patients with poor pre-RT pulmonary function test parameters (FEV1 $< 40\%$ of predicted value) are excluded, that dosimetric parameters are predictive for RP [26]. The observation that a low baseline TGF- β 1 level is associated with increased rate of RP may have a simple explanation. Elevated post-RT levels result in a ratio > 1 for patients with low baseline levels, but may be associated with a ratio $< \text{or} > 1$ for patients with an elevated baseline level. In other words, a low baseline level has mostly upside potential, while a high baseline value has both upside and downside potential.

Stromal tumor cells may produce TGF- β , perhaps accounting for elevated pre-treatment levels [35]. We hypothesize that tumors not associated with elevations in plasma TGF- β may retain some responsiveness to the growth-inhibitory effects of TGF- β . As a result, the normal growth-inhibitory feedback controls may not be altered sufficiently to result in an overproduction of TGF- β to the degree necessary to produce increased plasma levels. Alternatively, some patients may increase the degradation of TGF- β such that plasma levels are not elevated, even when production of TGF- β is increased and released into circulation [35]. Thus, the TGF- β levels before, during, and after RT likely reflect complex tumor- and treatment-related factors. In our analysis, the GTV alone was never a significant predictor for the TGF- β 1 levels, nor RP.

The identification of biological markers predictive for RT-induced lung injury remains challenging. While there is conflicting *clinical* data regarding the predictive ability of TGF- β 1, there is additional *pre-clinical* data to suggest that TGF- β 1 plays a role in the evolution of RT-induced normal tissue injury. Animal studies by Anscher et al. have shown that the administration of anti-TGF- β antibodies can decrease the inflammatory response and reduce TGF β activation several weeks after thoracic irradiation, further suggesting that targeting the TGF- β pathway may be a useful strategy to prevent RT-induced lung injury [36].

In addition to TGF- β 1, the roles of other cytokines in RT-induced lung injury are being investigated (Table 3). In studies from the University of Rochester, Chen et al. [33] suggested that interleukin 1 α (IL-1 α) and interleukin 6 (IL-6) might also be predictive for RP. The production of cytokines (TGF- β 1, among others) that occurs in the tumor may be affected by treatment. Therefore, plasma cytokine levels may not accurately reflect what is happening locally in the tissues, and what is seen is a balance between tumor production and that which is produced in response to irradiation. Another factor that can affect the bioavailability of TGF- β is the M6P/IGF2 receptor, a tumor suppressor gene that is important in both the activation and degradation of TGF- β . Kong et al. have shown that the loss of the M6P/IGF2 gene in patients with carcinoma of the lung is highly correlated with the development of RP after thoracic RT, and that a loss of heterozygosity are more likely to have increased plasma TGF β , suggesting an inability to normally process this cytokine [37]. Recent analysis from our group suggests that screening pretreatment plasma samples for the presence of a panel of inflammatory and immunomodulating cytokines may permit the construction of a cytokine profile for risk assessment of lung injury [38].

In conclusion, we have found that TGF- β 1 is generally not predictive for RP except for the small group of patients with V30 $> 30\%$. Due to the relatively low incidence of RP ($< 30\%$) in our present analysis), large numbers of patients might be necessary in order to better study this issue. Further experimental and clinical investigation on the role of TGF- β 1 in RT-induced lung injury is warranted.

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Table 1
Patient, Tumor, and Treatment Characteristics (N=121)

Age (y)	
Median (Range)	65 (33–88)
Gender (% Male/Female)	55/45
History of Tobacco Use (%)	97
Stage (%)	
I	14
II	17
III	64
IV	0.8
Histologic Type (%)	
Adenocarcinoma	24
Adenosquamous cell	2
Bronchioalveolar	1
Large cell	4
NSCLC NOS, mixed	28
Small cell	6
Squamous cell	35
Gross Tumor Volume (cm ³)	
Mean (Range)	103 (7–527)
Prescribed Tumor Dose (Gy)	
Mean (Range)	66 (36–86.4)
Mean Lung Dose (Gy)	
Mean (Range)	18.5 (5.5–36.3)
V20 (%)	
Mean (Range)	31.5 (8.2–62.9)
V30 (%)	
Mean (Range)	26 (4.9–48.5)
Chemotherapy (%)	60
Pre-RT surgery (%)	31

Abbreviations: NSCLS=non-small-cell lung carcinoma; NOS=not otherwise specified.

1 patient with both adenocarcinoma and squamous cell carcinoma

Table 2a

Incidence of grade ≥ 1 RP in patient subgroups based on TGF- $\beta 1$, dosimetric parameters, and gross tumor volume

TGF- $\beta 1$	All Patients	MLD <20 Gy	MLD >20 Gy	V20 $<30\%$	V20 $>30\%$	V30 $<30\%$	V30 $>30\%$	GTV $<100\text{cm}^3$	GTV $>100\text{cm}^3$
Baseline Level									
$\leq$ normal	31% (67*)	39% (28)	35% (26)	32% (22)	41% (32)	39% (31)	35% (23)	38% (21)	27% (22)
$>$ normal	20% (54)	21% (28)	31% (16)	24% (21)	26% (23)	28% (32)	17% (12)	25% (16)	27% (11)
p-value	0.22	0.24	1	0.74	0.39	0.43	0.43	0.49	1
Mid-RT Level									
$\leq$ normal	30% (71)	34% (35)	33% (24)	30% (27)	38% (32)	34% (38)	33% (21)		
$>$ normal	16% (43)	22% (18)	20% (15)	27% (15)	17% (18)	21% (19)	21% (14)		
p-value	0.12	0.52	0.48	1	0.20	0.37	0.70		
End-RT Level									
$\leq$ normal	27% (70)	34% (32)	28% (25)	31% (26)	32% (31)	34% (38)	26% (19)		
$>$ normal	21% (34)	19% (16)	33% (12)	10% (10)	33% (18)	24% (17)	27% (11)		
p-value	0.63	0.33	1	0.39	1	0.54	1		
Mid-RT/Baseline									
Ratio									
<1	30% (61)	26% (31)	25% (20)	23% (26)	28% (25)	30% (33)	17% (18)	17% (18)	29% (14)
≥ 1	26% (53)	36% (22)	32% (19)	38% (16)	32% (25)	29% (24)	41% (17)	40% (15)	22% (18)
p-value	0.83	0.55	0.73	0.48	1	1	0.15	0.24	0.70
End-RT/Mid-RT									
Ratio									
<1	20% (54)	29% (21)	23% (22)	29% (17)	23% (26)	23% (26)	29% (17)		
≥ 1	27% (44)	29% (24)	31% (13)	22% (18)	37% (19)	33% (24)	23% (13)		
p-value	0.48	1	0.70	0.71	0.34	0.53	1		
End-RT/Baseline									
Ratio									
<1	21% (63)	27% (30)	21% (24)	24% (25)	24% (29)	30% (37)	12% (17)	30% (20)	21% (19)
≥ 1	32% (41)	33% (18)	46% (13)	27% (11)	45% (20)	33% (18)	46% (13)	31% (13)	33% (12)
p-value	0.25	0.75	0.14	1	0.21	1	0.049	1	0.68

* Numbers in parentheses are sample sizes

Table 2b

Incidence of grade ≥ 2 RP in patient subgroups based on TGF- $\beta 1$, dosimetric parameters, and gross tumor volume

TGF- $\beta 1$	All Patients	MLD<20Gy	MLD>20Gy	V20<30%	V20>30%	V30<30%	V30>30%	GTV<100cm ³	GTV>100cm ³
Baseline Level									
$\leq$ normal	28% (67*)	39% (28)	27% (26)	32% (22)	34% (32)	36% (31)	30% (23)	38% (21)	18% (22)
> normal	15% (54)	14% (28)	25% (16)	14% (21)	22% (23)	22% (32)	8.3% (12)	13% (16)	27% (11)
p-value	0.084	0.068	1	0.28	0.38	0.27	0.22	0.14	0.66
Mid-RT Level									
$\leq$ normal	28% (71)	34% (35)	29% (24)	30% (27)	34% (32)	34% (38)	29% (21)		
> normal	8.3% (43)	11% (18)	13% (15)	13% (15)	11% (18)	11% (19)	14% (14)		
p-value	0.018	0.10	0.44	0.29	0.098	0.065	0.43		
End-RT Level									
$\leq$ normal	27% (70)	34% (32)	28% (25)	31% (26)	32% (31)	34% (38)	26% (19)		
> normal	12% (34)	13% (16)	17% (12)	0% (10)	22% (18)	18% (17)	9.1% (11)		
p-value	0.085	0.17	0.69	0.076	0.53	0.34	0.37		
Mid-RT/Baseline									
Ratio									
<1	23% (61)	26% (31)	25% (20)	23% (26)	28% (25)	30% (33)	17% (18)	17% (18)	29% (14)
≥ 1	19% (53)	27% (22)	21% (19)	25% (16)	24% (25)	21% (24)	29% (17)	37% (15)	17% (18)
p-value	0.65	1	1	1	1	0.55	0.44	0.67	0.67
End-RT/Mid-RT									
Ratio									
<1	19% (54)	29% (21)	18% (22)	29% (17)	19% (26)	23% (26)	24% (17)		
≥ 1	23% (44)	25% (24)	23% (13)	17% (18)	32% (19)	29% (24)	15% (13)		
p-value	0.62	1	1	0.44	0.49	0.75	0.67		
End-RT/Baseline									
Ratio									
<1	21% (63)	27% (30)	21% (24)	24% (25)	24% (29)	30% (37)	12% (17)	30% (20)	21% (19)
≥ 1	24% (41)	28% (18)	31% (13)	18% (11)	35% (20)	28% (18)	31% (13)	23% (13)	25% (12)
p-value	0.81	1	0.69	1	0.52	1	0.36	1	1

* Numbers in parentheses are sample sizes

Table 3

Studies investigating the association between TGF- β 1 and other cytokines and the development of radiation pneumonitis

Author Institution	Number of Patients	Cytokine	Cytokine time-point(s) of Interest	Elevated Level Associated with Radiation Pneumonitis
Chen [33] University of Rochester	24	IL-1 alpha	before, during, after RT	Yes
		IL-6	before, during, after RT	Yes
		TGF- β 1		No
		b FGF		No
		MCP-1		No
		E/L selectin		No
DeJaeger [7] Netherlands Cancer Institute	68	TGF- β 1	before, during, after RT	No
Novakova-Jiresova [15] University Hospital Groningen Fu, Anscher, current study [3,14] Duke University Hart [38] Duke University	46	TGF- β 1	mid-RT (relative to pre-RT)	Weakly
	103, 73, 121	TGF- β 1	end-RT (relative to pre-RT)	Yes
	55	Panel of 17 cytokines	before RT	No*
Gridley [39] Loma Linda University	12	TGF- β 1	end-RT (relative to pre-RT)	No
		b FGF	before RT	No
		TNF-alpha	before RT	No
		IL-1 beta	before RT	No
		IL-6	before RT	No
		IL-10		No
Barthelemy-Brichant [40] Sart-Tilman University Hospital (Belgium)	11	P III P	before RT	No
		TGF- β 1	after RT	Yes
		IL-6	after RT	Yes

Abbreviations: IL=interleukin; TGF=transforming growth factor; b FGF=basic fibroblast growth factor; MCP=monocyte chemoattractant protein; TNF=tumor necrosis factor; P III P=procollagen III peptide

* Lower levels of plasma IL-8 before RT were significantly associated with radiation-induced lung injury.