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Published in:
Radiotherapy and Oncology

DOI:
10.1016/j.radonc.2004.01.019

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2004

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Novakova-Jiresova, A., van Gameren, MM., Coppes, RP., Kampinga, HH., & Groen, HJM. (2004). Transforming growth factor-beta plasma dynamics and post-irradiation lung injury in lung cancer patients. *Radiotherapy and Oncology*, *71*(2), 183-189. https://doi.org/10.1016/j.radonc.2004.01.019

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Radiotherapy and Oncology 71 (2004) 183–189

RADIOTHERAPY & ONCOLOGY
JOURNAL OF THE EUROPEAN SOCIETY FOR THERAPEUTIC RADIOLOGY AND ONCOLOGY

www.elsevier.com/locate/radonline

Transforming growth factor-β plasma dynamics and post-irradiation lung injury in lung cancer patients

Alena Novakova-Jiresova[a], Mieke M. van Gameren[b], Rob P. Coppes[a,b], Harm H. Kampinga[a], Harry J.M. Groen[c,*]

[a]*Department of Radiation and Stress Cell Biology, Faculty of Medical Sciences, University of Groningen, Ant. Deusinglaan 1, bldg 3215, 9713 AV Groningen, The Netherlands*

[b]*Department of Radiotherapy, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands*

[c]*Department of Pulmonary Diseases, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands*

Received 12 June 2003; received in revised form 26 November 2003; accepted 27 January 2004

Abstract

Purpose: To investigate the relevance of transforming growth factor-β (TGF-β) dynamics in plasma for identification of patients at low risk for developing pneumonitis as a complication of thoracic radiotherapy (RT).

Patients and methods: Non-small cell lung cancer patients undergoing conventional RT were included in the prospective study. Concentrations of TGF-β were measured in the patients' plasma prior to and weekly during 6 weeks of RT. The incidence of symptoms of early post-irradiation lung injury, i.e. symptomatic radiation pneumonitis, was correlated with TGF-β parameters.

Results: Forty-six patients were included in the study. Eleven patients (24%) developed symptomatic radiation pneumonitis. Absolute TGF-β plasma levels did not differ between the groups of patients without or with pneumonitis. However, patients who developed pneumonitis tended to show increases in TGF-β levels in the middle of the RT course relative to their pre-treatment levels while TGF-β plasma levels of patients who did not develop pneumonitis tended to decrease over the RT treatment. The difference in the relative TGF-β dynamics between the groups reached marginal significance in the third week of the treatment ($P = 0.055$) but weakened towards the end of the RT course. The utility of TGF-β testing was evaluated at each RT week based on the test's ability to yield more accurate estimate of complication probability in an individual patient compared to empirically expected probability in similar group of patients. The ratio of TGF-β level at week 3/week 0 being < 1 showed an ability to improve the prediction of freedom from pneumonitis, yet with a large degree of uncertainty (wide confidence intervals). The accuracy of prediction deteriorated at later time points (weeks 4, 5 and 6) rendering the end-RT ratios without predictive power.

Conclusions: We observed a trend of plasma TGF-β concentration to decrease below the pre-treatment value during the RT treatment in patients who did not develop pulmonary complications after the RT treatment. However, this trend was not consistent enough to warrant safe decision-making in clinical setting.

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Keywords: Lung cancer; Endocrine effects of TGF-β; Post-irradiation lung injury; Radiation pneumonitis

1. Introduction

A failure to maintain local control after the curative RT for non-small cell lung cancer (NSCLC) remains to be an important cause of poor survival in inoperable lung cancer patients [4]. It has been long accepted that the prescribed radiation doses in the range of 60–70 Gy delivered in the conventional RT are insufficient for eradicating the tumour [18]. The more recent estimates placed the dose required for achieving a significant probability of durable tumour control in the vicinity of 84 Gy [32]. Yet, already the conventional doses cause respiratory complications in as many as 20% of the treated patients [17,21,37]. A sub-acute, inflammatory phase of lung tissue injury, radiation pneumonitis, followed by chronic progressive fibrosis poses a great threat to the patient and limits the dose that can be safely delivered to the target volume. The possible approaches of how to obviate this obstacle include improvements of the physical parameters of the dose-delivery (IMRT, image guided radiotherapy) as well as biological optimisation of the treatment planning. An intensive search for biological variables that would identify patients suitable for dose-escalation at the

* Corresponding author.

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doi:10.1016/j.radonc.2004.01.019

end of the conventional treatment is ongoing. With regard to this, pre-RT pulmonary function tests [21,30,31], SPECT-based treatment planning [31] as well as cytokine effects [3–6,9,13,15,19,31,39,40] have been amply discus sed in the literature.

The cytokine TGF-β has been implicated in the development and perpetuation of the post-irradiation injury in various tissues, including the lung [33,36]. TGF-β is known to be upregulated in normal tissues damaged by radiation [2,34,36] as well as to be overproduced by tumours [14,23]. Although the basis for its autocrine and paracrine mode of action is well established, the endocrine effects have been debated. TGF-β is secreted as an inactive, latent complex with non-covalently bound latency associated peptide. This bond must be cleaved by proteolytic enzymes or presence of free radicals to enable signalling through the TGF-β membrane receptors on target cells [10,29]. Physiologically, TGF-β circulates in blood in its latent form or complexed with plasma proteins, the likely source being thrombocytes, white blood cells [20] or bone matrix [41]. Out of the three known isoforms, TGF-β1 is the predominant isoform in human plasma, TGF-β2 and β3 accounting for less than 5% of its total plasma concentration in healthy subjects [41]. Plasma TGF-β1 is also the most extensively studied isoform in cancer patients although, occasionally, elevations in the two other isoforms have also been detected [26,27,41]. Regarding the activity, increases in both, latent and active TGF-β1, have been reported in plasma of cancer patients [11,22–25,35]. While the 25 kD active TGF-β1 molecule has a plasma half-life of only 2–3 min and is rapidly sequestrated by and degraded in the liver [42], the evidence for ability of the large latent complex to cross the vascular endothelium in any direction is lacking [20]. However, a hypothesis has been put forward that damaged vasculature at the site of post-irradiation tissue injury or in a tumour stroma could allow leakage of the latent TGF-β1 between plasma and tissues providing a basis for its endocrine action [4].

In clinical setting, about half of the patients with NSCLC presents with elevated plasma TGF-β1 levels before treatment, most likely due to the TGF-β1 over-production by their tumours [3,24,25]. A normalisation of plasma TGF-β1 level by the end of RT has been linked to a lower risk of pulmonary complications after the treatment in several clinical trials [3,5,9,19,39]. Dynamics of TGF-β1 in plasma were suggested as a marker of RT-induced normal tissue injury as well as tumour response. It was proposed that this marker might be utilised in clinic for selecting patients at low risk of complications suitable for RT dose-escalation [3,7,8].

In this study, we assessed the value of TGF-β measurements in predicting freedom from symptomatic radiation pneumonitis in a group of NSCLC patients treated with conventional thoracic RT. A preliminary analysis of the subset of our data has been already published [39].

2. Patients and methods

2.1. Patient eligibility

Lung cancer patients from an outpatient clinic of the Department of Pulmonary Diseases at the University Hospital of Groningen, the Netherlands, were included in the prospective study. They had to fulfil the following criteria: age ≤ 76 years, locally unresectable stage IIIA or IIIB NSCLC proven either by mediastionoscopy, explorative thoracotomy, or clinically by involvement of the phrenic or recurrent nerve, Eastern Cooperative Oncology Group (ECOG) performance score ≤ 2, weight loss $< 10\%$, serum creatinine ≤ 120 μmol/l or creatinine clearence ≥ 60 ml/min, serum bilirubin ≤ 2.0 mg/dl, leukocytes $\geq 3.0 \times 10^9\,l^{-1}$ and thrombocytes $\geq 100 \times 10^9\,l^{-1}$. Ineligible were patients with prior chemo- or radiotherapy. Pulmonary function tests (PFT) including total lung capacity (TLC), vital capacity (VC), forced expiratory volume in 1 s (FEV_1), CO diffusing capacity corrected for alveolar volume (K_{CO}), pulmonary capillary blood volume (V_{cap}) and membrane diffusing factor (D_M) were obtained before RT. The measured values were expressed as percentage of the predicted value. The written informed consent was obtained from all patients. The study was approved by the Medical Ethical Committee of the University Hospital of Groningen.

2.2. Treatment

Curative conventional thoracic RT was delivered using a linear accelerator (6 MV photons) in 2 Gy daily fractions, 5 fractions a week, over a total treatment time of 6 weeks. The initial planning target volume (PTV1) encompassed all visible local and regional disease with a 2 cm margin based on the thoracic CT scan prior to the start of the treatment. Further, PTV1 included the mediastinum from 2 cm above the suprasternal notch to 5 cm below the carina, extending 2 cm across the midline. The reduced planning target volume (PTV2) contained only the local and regional disease with a 1 cm margin. The PTV1 and PTV2 received total doses of 40 and 60 Gy, respectively. The area of the initial anterior–posterior irradiation portal (till 40 Gy) minus blocks in cm^2 and the PTV2 volume in cm^3 were calculated for each patient.

2.3. Endpoint

The clinical evaluation of patients was performed weekly during the course of RT, 6 weeks after completion of RT, then every 3 months during the first year, every 6 months during the second and third year and once a year thereafter. The endpoint of the study was the development of symptomatic radiation pneumonitis. The scoring system of the National Cancer Institute Common Toxicity Criteria

(CTC) version 2 was used. Scoring was performed without a knowledge of the TGF-β levels in plasma.

2.4. Patient characteristics

Forty-six subsequent patients were included. All were tobacco smokers. In 30 patients, carboplatinum (Carboplatin, Bristol Meyers Squibb) was administered concurrently with radiotherapy as a radiosensitizer in a total dose of 860 mg/m^2 in a continuous infusion over 6 weeks. Eleven patients developed radiation pneumonitis and 35 showed no symptoms. The patient and treatment related characteristics of the two patient groups are presented in Table 1. None of the featured variables differed significantly between the groups.

2.5. Plasma TGF-β quantification

Blood samples were collected from patients prior to and then weekly during the 6 weeks of RT. Blood was drawn without placing a tourniquet on a patient's arm to prevent thrombocyte degranulation. Blood was collected in tubes containing 7.5% K_3 EDTA and immediately placed on ice. The samples were centrifuged at 4 °C for 30 min at 1000*g* within 1 h upon collection. The plasma for TGF-β determination was withdrawn from the middle of the plasma column avoiding the platelet interface. It was stored at −80 °C until analysis. TGF-β concentration in plasma was measured in a bioassay with mink lung epithelial cells (MLEC) permanently transfected with a reporter gene construct, plasminogen activator inhibitor-1 (PAI-1) promoter fused to the firefly luciferase gene [1,38]. This bioassay is based on the ability of TGF-β to specifically induce PAI-1 expression. The binding of TGF-β to the MLEC cells results in a dose-dependent increase in luciferase activity in the cell lysate. The assay cannot distinguish between the three TGF-β isoforms [1]. Therefore, we use the term TGF-β without the numeric index in our study although TGF-β1 is expected to account for the vast majority of the measured TGF-β concentration [38]. Since an acid-activation step was performed at the start of the assay, only total TGF-β (active + latent form) was detected. The detection limit was 0.1 ng/ml. All sequential samples from one particular patient were analysed simultaneously in one multiwell plate to ensure identical conditions of the assay procedure. The control group used for the determination of the physiologic plasma TGF-β value consisted of healthy volunteers described earlier [38]. The mean control plasma TGF-β value in our study was 7.2 ± SD 2.8 ng/ml. The mean control value +2 SD (= 12.8 ng/ml) was regarded as a cut-off between normal and pathologically elevated TGF-β levels.

The TGF-β levels in plasma of each individual patient were expressed both as absolute concentration (ng/ml) and as relative parameters, i.e. ratios of a value from a particular week of RT divided by the pre-RT value (w1/0, w2/0, etc.). The ratios were available only in 33 patients as 13 patients lacked week 0 value due to logistic reasons. The number of patients evaluable at each particular time-point are given in Table 2.

2.6. Statistical analysis

Univariate and multivariate methods were applied to study the relation between the pneumonitis incidence (as binary variable) and various potentially predictive variables. We used the non-parametric Mann–Whitney *U*-test for the univariate evaluation of continuous predictive variables (age, pre-RT PFT, initial radiation field area, PTV2, absolute TGF-β levels and TGF-β ratios at each individual time point) and the Pearson chi-squared test for the univariate evaluation of categorical predictive variables (PS, stage, carboplatinum administration). The combined effects of the predictive variables were evaluated in

Table 1
Patient characteristics

Variable	Without pneumonitis $n = 35$	With pneumonitis $n = 11$
Median age (years)	62 (range 45–76)	65 (range 44–76)
Sex (male/female)	34/1	10/1
PS (= 0/1/2)	14/18/3	4/7/0
Stage (IIIA/IIIB)	15/20	7/4
Histology SSC/AC/LCC	16/8/11	7/3/1
Mean TLC (± SD)	88.7 (± 15.4)	84.9 (± 10.9)
Mean VC (± SD)	89.7 (± 20.5)	81.6 (± 14.8)
Mean FEV_1 (± SD)	69.7 (± 17.5)	61.8 (± 17.2)
Mean K_{CO} (± SD)	112.1 (± 18.5)	107.8 (± 37.1)
Mean V_{cap} (± SD)	74.1 (± 19.7)	64.8 (± 13.8)
Mean D_M (± SD)	87.2 (± 20.4)	72.6 (± 17.2)
Carboplatinum	22	8
Mean field area (cm^2)	236 (range 104–352)	227 (range 176–293)
Mean PTV2 (cm^3)	776 (range 198–4445)	570 (range 161–1028)

PS, performance score; SCC, sqaumous cell carcinoma; AC, adenocarcinoma; LCC, large cell carcinoma; Mean TLC, VC, FEV_1, K_{CO}, V_{cap}, D_M, mean percentage predicted values of PFT prior to RT; Mean field area, the area of the initial anterior–posterior irradiation portal (till 40 Gy) minus blocks.

Table 2
TGF-β variables—comparison between patients developing or not developing pneumonitis

Variable	Without pneumonitis	With pneumonitis	*P* value
Median absolute TGF-β concentration (with range) from *n* evaluable patients			
w0	39.9 (7.1–85.7) $n = 24$	45.8 (10.6–106.4) $n = 9$	0.65
w3	40.9 (2.2–118.5) $n = 30$	36.1 (13.1–147.0) $n = 9$	0.88
w6	36.6 (10.4–115.1) $n = 15$	38.2 (7.3–74.7) $n = 6$	0.97
Median TGF-β ratio (with range) from *n* evaluable patients			
w1/0	0.98 (0.38–3.27) $n = 21$	1.12 (0.16–2.91) $n = 8$	0.76
w2/0	1.01 (0.18–4.03) $n = 20$	1.38 (0.25–1.86) $n = 7$	0.31
w3/0	1.02 (0.09–1.65) $n = 20$	1.41 (0.32–3.45) $n = 7$	0.055
w4/0	0.91 (0.19–1.76) $n = 20$	0.95 (0.24–4.17) $n = 7$	0.73
w5/0	0.88 (0.07–1.42) $n = 18$	0.98 (0.13–6.49) $n = 7$	0.66
w6/0	0.73 (0.32–1.71) $n = 8$	0.61 (0.18–3.29) $n = 5$	0.72

w0, w3, w6, median absolute pre-, mid- and end-RT concentrations of TGF-β in plasma (ng/ml); w1/0–w6/0, median ratios of the TGF-β plasma concentration at a particular week relative to the pre-RT concentration; *P*-value (exact) obtained from a comparison by the Mann–Whitney *U*-test.

multivariate analysis (multiple logistic regression). The number of cases included in the regression varied from analysis to analysis depending on the missing values of TGF-β parameters and thus its results are to be viewed as explorative. We used 5% as the nominal level of statistical significance. Exact *P*-values were calculated wherever its appropriate. Calculations were performed in the SPSS software package version 10.0.

Further, to describe the predictive properties of the plasma TGF-β dynamics (i.e. the ratios being ≥ 1 or < 1 and presence or absence of pneumonitis), we calculated sensitivity, specificity, positive and negative predictive values and the corresponding 95% confidence intervals separately for each week of the RT treatment. The 95% confidence intervals (95% CI) were calculated by the exact method.

3. Results

Of the 46 included patients, 11 (24%) developed radiation pneumonitis within 6 months after RT. The severity of pneumonitis was of grade 1 in 5 patients, grade 2 in 3 patients and grade 3 in 3 patients. The median follow-up was 51 weeks (range 11–230 + weeks). The pre-RT TGF-β level in plasma was elevated above the cut-off in 30 (91%) out of 33 measured patients.

The TGF-β variables compared between the groups of patients not developing or developing pneumonitis are summarised in Table 2. Regarding the absolute TGF-β plasma levels from week 0 to 6 (only w0, w3 and w6 values shown in the table), there were no significant differences between the groups. Also, most of the relative TGF-β parameters did not differ with an exception of week 3/week 0 ratio (w3/0) that was borderline significantly higher in the pneumonitis group ($P = 0.055$, Mann–Whitney *U*-test).

The dynamics in relative TGF-β changes are visualised in Fig. 1. A striking observation was the large variability of the ratio values in the pneumonitis group. Between weeks 1 and 5, the 75th and the 97.5th percentiles of the pneumonitis group encompassed higher ratio values than the same percentiles in the group without symptoms. This suggested that the plasma TGF-β concentrations in patients subsequently developing pneumonitis tended to rise above the pre-RT value during the first 5 weeks of the treatment, while patients not developing pneumonitis had much narrower spread of the ratio values fluctuating around or just below 1. The difference disappeared at 6 weeks. Nevertheless, due to the large variability, the difference between the groups reached only borderline significance and that only at week 3 (Table 2).

Therefore, we tested the clinical utility of the relative TGF-β parameters for detection of patients at low risk of radiation pneumonitis in our set of patients. Ratios from w3/0 to w6/0 were assessed separately (Table 3). Patients were considered a true positive if their ratio was ≥ 1 and they developed pneumonitis. They were considered a true negative if their ratio was < 1 and they did not develop pneumonitis. It is apparent from Table 3 that the best prediction of freedom from pneumonitis was achieved when

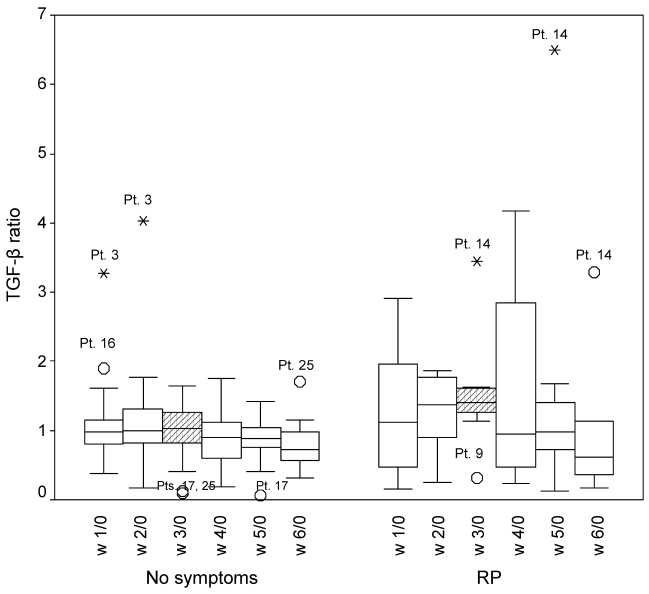

Fig. 1. Plasma TGF-β levels during 6 weeks of radiotherapy relative to the pretreatment TGF-β level expressed as ratios of the value at a particular week over the pre-RT value (i.e. w1/0–w6/0). The ratios were plotted separately for the group without pneumonitis ('No symptoms') and with pneumonitis ('RP'). The boxes indicate the 25th and the 75th percentile (the lower and upper edge, respectively), the central line represents the median. Median numeric values and number of patients evaluable at each time point can be found in Table 2. The points at the ends of the whiskers are the 2.5th and the 97.5th percentiles. Outliers (circles) and extreme values (stars) are plotted individually with a patient serial number. The difference between the groups was borderline significant ($P = 0.055$, Man–Whitney *U*-test) only for the ratio w3/0 (dashed boxes).

Table 3
Test-performance characteristics of the TGF-β ratios in relation to radiation pneumonitis

Ratio *n* evaluable patients	w3/0 $n = 27$	w4/0 $n = 27$	w5/0 $n = 25$	w6/0 $n = 13$
Sensitivity	86%	43%	43%	40%
Specificity	50%	55%	72%	75%
Positive predictive value (with 95% CI)	38% (15–65%)	25% (5–57%)	38% (9–76%)	50% (7–93%)
Negative predictive value (with 95% CI)	91% (59–100%)	73% (45–92%)	77% (50–93%)	67% (30–93%)

Numbers were rounded up.

using the mid-RT parameter (the ratio of w3/0) as documented by the negative predictive value 91%. However, a large uncertainty was associated with this value (95% CI ranging from 59 to 100%). Thus, if the overall pneumonitis probability in this set of patients was 24%, the negative test outcome at the third week of the RT treatment could revise the probability to 9% (i.e. 100 minus 91), or perhaps to 41 or 0% (i.e. 100 minus 59 or 100). The accuracy of the prediction diminished further at the later time points (weeks 4, 5 and 6). This confirmed the observation made already in Table 2 and Fig. 1: that only the w3/0 TGF-β ratio was able to make some distinction between patients developing or not developing pneumonitis.

Finally, the relationship between pneumonitis incidence and variables presented in Tables 1 and 2 (age, PS, stage, pre-RT PFT, initial radiation field area till 40 Gy, PTV2, absolute plasma concentrations of TGF-β and TGF-β ratios from all weeks) was evaluated in multivariate analysis. None of the results reached statistical significance.

In conclusion, the transience and weakness of the observed link between plasma TGF-β and pulmonary symptoms in this set of patients would imply that the power of TGF-β dynamics to predict radiation pneumonitis (or freedom from it) is rather low. Moreover, closer examination of the only time point that demonstrated some predictive power revealed more reasons for caution. Three patients with the highest w3/0 ratios (>1.5) in the pneumonitis group tended to have a low pre-treatment TGF-β values (w0 10.6, 16.9 and 11.7 ng/ml) below or close to the cut-off of 12.8 ng/ml. In contrast, the pre-treatment values were generally elevated in remaining patients from both groups (only 2 more patients had initial TGF-β <20 ng/ml). Thus, the three highest ratios in the pneumonitis group at week 3 were, in fact, the result of only minor absolute increases in plasma TGF-β concentration in two out of three cases. This questions the mechanism behind the putative association between the pneumonitis risk and relative increases in TGF-β plasma levels.

4. Discussion

Many efforts in radiotherapy focus on the determination of clinically useful indicators that would discriminate between patients with high or low risk of pulmonary complications after RT treatment of thoracic tumours. This would allow selection of patients in whom the radiation dose to the tumour could be safely escalated to ensure a cure.

TGF-β1 is a ubiquitous immunomodulatory and profibrotic cytokine that plays a major role in tissue responses to irradiation. A link between rising plasma TGF-β1 level during the thoracic RT treatment and the subsequent development of radiation pneumonitis has been presented in the literature [4,40]. Two possible sources of rising plasma TGF-β1 have been suggested by these authors. First, the tumour stroma could be a source. When a tumour would not respond to therapy, its stroma would keep on producing increasing amounts of the cytokine. Second, the gradually accumulating normal tissue damage within the irradiation field would be expected to launch TGF-β1 production in the injured site. Under physiological circumstances, the locally produced TGF-β1 would not be likely to transit into the circulation [20]. However, the vasculature is often defective in both the tumours and normal tissue damaged by radiation. Therefore, it is believed to be possible for the excessive TGF-β1 to leak into the circulation and to be detected in plasma as a possible marker of tumour response and normal tissue injury [4,40]. At the same time, the TGF-β1 is believed to leak back into the site of the injury, become activated and augment local processes leading later to the manifestation of radiation pneumonitis and fibrosis. This hypothesis is based on the clinically observed association between rising plasma TGF-β1 levels towards the end of thoracic RT and increased incidence of pulmonary complications in several studies involving mostly lung cancer patients [3,5,9,19,39]. Also for radiation-induced fibrosis of the breast, an association with high TGF-β1 levels in plasma (pre-treatment) was reported [28].

Our current data, however, do not support the notion that TGF-β values in plasma could be used to identify patients at low risk for developing radiation pneumonitis. Although we found a mid-treatment rise in the relative plasma TGF-β values in patients who developed pneumonitis and not in those who did not develop symptoms of pulmonary injury, the predictive power of this observation was rather low. Further, no relation was detected between pneumonitis and absolute pre-treatment TGF-β values or other than mid-treatment relative changes. Especially the end/pre-RT TGF-β ratio was suggested as the most reliable identifier of patients at low risk of radiation-induced pulmonary symptoms in earlier studies [3,5,7–9,19]. Our finding that

we particularly lose significant relationship at these end/pre-RT ratios rises questions about the importance of the timing of TGF-β dynamics assessment that would ensure clinical reliability of the parameter.

Our study is in agreement with studies of Marks et al. [31] and Chen et al. [13] that also failed to find relationship between TGF-β parameters and radiation-induced pulmonary symptoms. Whereas the data by Chen et al. [13] may have been confounded by the use of heparin as an anticoagulant in blood collection tubes, with a higher risk of platelet degranulation [38,41], this cannot explain the negative results of Marks et al. [31] or ourselves. Lack of association between plasma TGF-β1 levels and late morbidity following radiotherapy for stage I-III cervical carcinoma [16] or abnormal wound healing in skin [12] further questions the power of this parameter to discriminate between patients at risk or not at risk of radiation-induced complications.

So, what could be the reason for the conflicting results with regards to the TGF-β plasma levels and radiation-induced complications? We can only speculate. First, for the time point of week 3 of the RT treatment where we found a weak association, the results were dominated by patients with low pre-treatment values. Here, even small changes in absolute TGF-β concentration could have spuriously large effects on ratio calculations. So far, the notion that a minimal rise in plasma TGF-β level predisposed some patients to pneumonitis while massively elevated but persistent levels had no consequences in other patients lacks a pathophysiological explanation. Secondly, in case of respiratory function, the clinical outcome may be influenced by treatment related dysfunction of other organs (e.g. the heart) on which TGB-β plasma levels might have less impact. In that aspect, selecting patients for dose-escalation based on one sole parameter like TGF-β dynamics might be dangerously simplistic approach. Finally, as mentioned above, changes in plasma levels of TGF-β can be caused by two, partly counteractive, mechanisms. On one hand, plasma levels may decrease due to the death of the TGF-β producing tumour stromal cells following radiation. On the other hand, radiation may increase the plasma TGF-β pool due to elevated production of the cytokine by cells in injured normal tissues from which leakage to the circulating system may occur depending on the level of damage to the endothelium. With respect to the latter, a recent dose escalation study for lung cancer by de Jaeger et al. [15] is worth mentioning. Like in the current study, no relation between changes in circulating TGF-β1 and the development of symptomatic radiation pneumonitis was found. However, a clear relation between mean lung dose and plasma TGF-β1 levels at the end of radiotherapy was detected. The increases in the cytokine concentration could be linked only to the radiation-induced normal tissue injury as patients with non-responding tumours ('progressive disease') were excluded from the study. Such a finding corroborates our conclusion that, although the extent of radiation-induced pulmonary injury may be reflected in rising plasma TGF-β levels, the strength of the association is too variable to serve as a reliable predictor of manifestation of symptoms.

We could not evaluate the influence of radiation dose because all patients were treated to a constant total dose of 60 Gy. However, the irradiated volume ranged considerably. Despite that, no relationship between irradiated volume and incidence of pneumonitis was detected in this study. This may be a consequence of unavailability of 3D dosimetry data.

In conclusion, we report a weak predictive association between mid-treatment plasma TGF-β dynamics and low risk of symptomatic radiation pneumonitis in the set of lung cancer patients treated by conventional RT. However, together with the existing conflicting data from the literature, we feel it is justified to conclude that the predictive power of the sole TGF-β parameter is too unstable to warrant a reliable use in clinical decision-making.

Acknowledgements

The authors thank Dr Vaclav Fidler for his indispensable advice regarding the statistical analysis of the data and Mrs M.A.W.H. van Waarde and Mrs A.J. van Assen for an excellent technical support.

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