Posttreatment plasma transforming growth factor beta 1 (TGF-beta1) level predicts for late morbidity in patients with advanced head and neck cancer

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Between 2001 and 2002, 29 patients with advanced inoperable squamous head and neck cancer treated with radiotherapy with or without simultaneous chemotherapy were evaluated for their plasma TGF-β1 levels prior to the treatment, in the middle of the radiotherapy course and at the end of the treatment. Patients were assessed for treatment response and late morbidity. Predictive value of TGF-β1 level on either of the assessed parameters was tested.

From 29 eligible patients (pts), 18 achieved complete response, 8 partial response and three pts progressed primarily. After a median follow-up of 16 months we recorded 16 cases of grade >1 late morbidity. We found that posttreatment elevated plasma TGF-β1 level predicts late morbidity grade >1 ($p=0.05$) rather than pretreatment level ($p=0.062$). Neither pretreatment nor posttreatment plasma TGF-β1 level has a predictive value to the treatment response (CR vs. no CR, $p=0.125$ and 0.252, respectively).

The posttreatment plasma TGF-beta 1 level can predict late morbidity grade >1 in advanced head and neck cancer treated with radio(chemo)therapy. This could make a basis for dose escalation in selected patients.

Key words: head and neck cancer, late morbidity, radiotherapy, TGF-beta1

Head and neck cancer makes about 5 per cent of all newly diagnosed invasive malignancies in Europe [7]. Curative treatment approaches include surgery or radiotherapy in early stages and radiotherapy in advanced stages. As these tumors are mostly squamous cancers with locoregional growth and late distant spreading, local control is of major concern. Consequently, very high doses of radiotherapy must be applied to achieve cure. Addition of chemotherapy is advocated by some authors to increase the cytotoxic effect of radiotherapy [1, 10, 12, 19] while accelerated radiotherapy schedules are preferred by others [2, 6, 13].

In high-dose radiotherapy, late radiation toxicity is the major factor limiting the therapeutic dose and thus the local control rate. Empirically we know that even the same doses, the same energies, the same target volumes and the same techniques produce very different degree of late morbidity in different patients. Therefore, there certainly is an intrinsic predisposition to late radiation damage of healthy tissues. Apart of very rare hereditary syndromes such as Ataxia - teleangiectasia or Fanconi's anemia, the intrinsic predictors of radiosensitivity are unknown.

Transforming growth factor beta 1 (TGF-β1) is a cytokine strongly involved in late radiation morbidity. It plays a crucial role in production of fibroid tissue via stimulation of extracellular matrix turnover and deposition as a reaction to trauma and inflammation of any origin, including damage with ionizing radiation [8, 22]. Because of this property, the endogenous TGF-β1 production might be one of the intrinsic factors responsible for higher grade of late morbidity in some patients.

In our study, we evaluated the plasma TGF-β1 level in squamous head and neck cancer patients and its variation during the radiotherapy course. We tested whether the level of this cytokine at various stages of anticancer treatment can predict treatment response or late morbidity and could be eventually used as factor allowing dose escalation without excessive late toxicity in selected subgroup of patients.

Patients and methods

Patients. Between 2001 and 2002, 29 patients with locally advanced nonmetastatic squamous head and neck cancer were treated with external beam radiotherapy (RT) with curative intent with or without simultaneous chemotherapy (CT). There were 24 men and 5 women, median age 55 years (range 42–72). The vast majority of patients suffered from laryngeal or oropharyngeal cancer. The patients and their basic characteristics are listed in Table 1. Staging was performed according to UICC, Fifth Edition, 1997.

There were 12 patients with very advanced disease (T4 and/or N3), these patients were considered as having high tumor burden, the rest (n=17) having low tumor burden.

Radiotherapy was carried out on a linear accelerator 5 MeV using two opposed lateral fields and a third posterior field with central spinal cord block to the dose of 50 Gy, then a boost was administered to macroscopic tumor and enlarged lymph nodes to the therapeutic dose of 70 Gy, 2 Gy daily dose. If the patient was in a good condition, performance status 0 or 1 (WHO) with serum kreatinine <100 mmol/l, neutrophil count >2.500/μl and platelet count >100.000/μl, cisplatin was added once weekly in a dose of 40 milligrams per meter squared body surface area.

Patients were evaluated for their plasma TGF-β1 levels before treatment, after three weeks of radiotherapy and at the end of the treatment.

Treatment response was evaluated one month after completion of the treatment. We used otorhinolaryngologic examination with indirect laryngoscopy, CAT scan of head and neck and chest X-ray. The response was scored according to RECIST criteria [24].

Late radiation morbidity was scored after a minimum follow up period of one year. RTOG scoring system was used for skin, mucous membranes, subcutaneous tissue, salivary glands and larynx.

TGF-β1 assay. For each sample, 5 ml of venous blood was collected into polypropylene tube and stored in –20 °C. Plasma TGF-β1 level was determined by capture ELISA according instruction of R&D Systems busing Monoclonal Anti-human TGF-β1 Antibody Catalog Number: MAB240, R&D Systems, Inc (USA). In brief, 100 μl of the capture antibody was transferred to an ELISA plate and incubated overnight at room temperature. Each well was then washed three

Table 1. The patients

Patient Nr.	Gender	Age	Tumor locality	Stage [TNM]	Treatment	Response	Late morbidity grade	Involved organs
1	M	52	oral cavity	T2N2bM0	RT	CR	3	subcutaneous tissues
2	F	46	larynx	T2N1M0	RT + CT	CR	2	subcutaneous tissues
3	F	72	nasopharynx	T3N2cM0	RT	CR	2	subcutaneous tissues
4	M	54	larynx	T3N0M0	RT	CR	2	subcutaneous tissues
5	M	71	larynx	T2N3M0	RT	CR	2	salivary glands, skin
6	M	55	oral cavity	T3N0M0	RT	CR	3	subcutaneous tissues
7	F	56	oropharynx	T3N1M0	RT + CT	CR	2	salivary glands
8	M	52	larynx	T3N0M0	RT	CR	2	subcutaneous tissues
9	M	51	larynx	T3N2cM0	RT	PR	1	
10	M	57	larynx	T2N2cM0	RT	PR	1	
11	M	54	nasopharynx	T4N1M0	RT + CT	PR	1	
12	M	65	oropharynx	T1N2cM0	RT	CR	1	
13	F	47	oropharynx	T4N2aM0	RT + CT	PR	3	larynx
14	M	67	oropharynx	T2N0M0	RT	CR	1	
15	M	68	larynx	T3N2cM0	RT	CR	1	
16	M	50	oropharynx	T4N1M0	RT + CT	CR	1	
17	M	52	oropharynx	T4N3M0	RT + CT	PD	2	subcutaneous tissues, skin, mucous membranes
18	M	42	hypopharynx	T4N0M0	RT + CT	CR	2	subcutaneous tissues, mucous membranes, larynx
19	M	54	hypopharynx	T4N1M0	RT + CT	PD	1	
20	M	59	larynx	T3N0M0	RT + CT	CR	1	
21	M	55	nasopharynx	T2N2cM0	RT + CT	CR	1	
22	M	57	oropharynx	T4N2aM0	RT + CT	PD	2	salivary glands, mucous membranes
23	M	50	maxillar sinus	T2N3M0	RT + CT	PR	2	salivary glands, subcutaneous tissues, larynx
24	M	51	oral cavity	T1N3M0	RT + CT	PR	1	
25	M	58	oropharynx	T2N2M0	RT + CT	CR	2	subcutaneous tissues, skin, mucous membranes
26	M	47	oropharynx	T2N2bM0	RT	CR	1	
27	M	58	oropharynx	T4N2cM0	RT + CT	PR	2	larynx, skin
28	M	64	larynx	T2N0M0	RT	CR	1	
29	F	61	oropharynx	T4N2cM0	RT + CT	PR	2	mucous membranes, larynx

times with wash buffer. After removal of the buffer, the plates were blocked by adding 300 µl of PBS containing 5% Tween 20, 5% sucrose and 0.05% NaN_3 to each well and incubated at room temperature for a minimum of 1 hour. 100 µl of blood plasma sample per well was added, the ELISA plate was covered with an adhesive strip and incubated 2 hours at room temperature. 100 µl of streptavidin HRP (R&D Systems, Catalog # DY998, 1/200 in appropriate diluent) was added to each well; the plate was covered and incubated for 20 minutes at room temperature. After subsequent addition of Substrate Solution and Stop Solution (both R&D Systems, Inc.), the optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm.

Statistics. For statistical analyses, we used Prism 4 (GraphPad Software Inc.). The relationships between variables were obtained using Spearman's nonparametric test. Differences between variables were determined using the Mann-Whitney U-test. Statistical comparison of intra- and inter-individual variation in TGF-β1 levels was carried out using one-way analysis of variance (ANOVA).

Results

From the total of 29 eligible patients, sixteen were treated with combination of radiotherapy and chemotherapy while thirteen received radiotherapy only. All patients completed the full course of the treatment as prescribed and there were only minor delays never exceeding one week. In the first follow-up examination, we recorded 18 complete remissions, eight partial remissions and three primary progressions. Acute toxicity was acceptable with no grade 4 toxicity and no treatment-related death.

Initial tumor burden did not correlate with plasma TGF-β1 level. In fact, there was even a trend for higher plasma levels in low tumor burden patients, although this has not reached statistical significance (high versus low, median 6.1 and 15.3 ng/ml, respectively, p=0.07). The patients intended for radiotherapy only had significantly higher initial TGF-β1 level than those treated with simultaneous radiochemotherapy (median plasma level 15.8 and 6.1 ng/ml, respectively, p=0.044).

The treatment influenced very strongly the plasma TGF-β1 level. There was a significant decrease at the end of the treatment course when compared with the initial level (median 8.4 and 5.4 ng/ml, respectively, p=0.02) and with the middle-of-the-course level (median 9.8 and 5.4 ng/ml, respectively, p=0.018). Detailed analysis revealed, that this decrease was limited to patients treated with radiochemotherapy while in patients treated with radiotherapy only no such decrease was observed, see Figure 1. Treatment response showed no influence on TGF-β1 level; the posttreatment plasma levels were almost equal in patients achieving complete response and in those with partial response or progressive disease (median 5.8 and 4.6 ng/ml, respectively, p=0.25). Also, pretreatment plasma TGF-β1 level did not predict the degree of response (CR versus PR and PD, 9.1 and 6.0 ng/ml, respectively, p=0.12).

Late radiation morbidity grade 2 or 3 was observed in 16 patients after a median follow up of 18 months (range 12–33), mainly regarding salivary glands and subcutaneous tissues. (Tab. 1). Six out of the sixteen patients had radiotherapy only; the remaining ten underwent concurrent radiochemotherapy. We found that posttreatment high plasma TGF-β1 level predicts late morbidity grade 1 with borderline significance (morbidity yes vs. no, median level 16.4 and 6.9 ng/ml, respectively, p=0.05, see Fig. 2) rather than pretreatment level (median level 6.3 and 4.2 ng/ml, respectively, p=0.06).

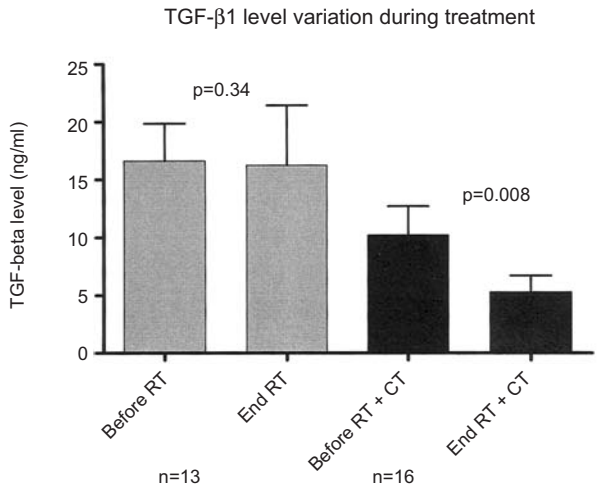

Figure 1. TGF-beta1 level variations during the treatment course.

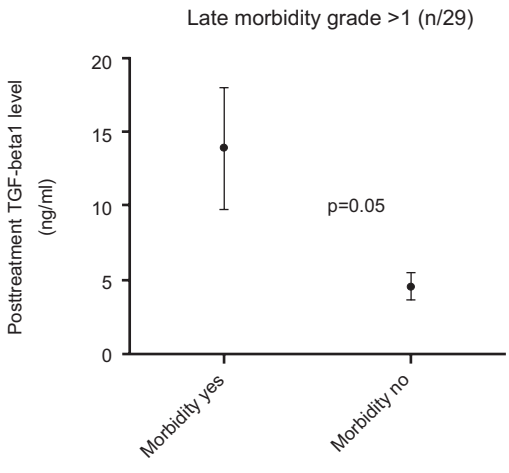

Figure 2. Predictive power of posttreatment TGF-beta1 level for late morbidity.

Discussion

Predictive power of TGF-β1 for either treatment response or posttreatment morbidity was tested in several small studies for different malignant tumors. Probably most advanced results have been published for non-small cell lung cancer, where posttreatment elevated plasma TGF-β1 level predicted for increased late morbidity, results very similar to our study [3]. This led the investigators to an approach called "TGF-β1-guided radiotherapy" [4], i.e. to dose escalation in patients with normal posttreatment TGF-β1 level. In these patients, no increase in late toxicity was reported. These impressive results, however, could not be confirmed by recent IVANOVIC study [14], where no relationship was found between plasma TGF-β1 level and late morbidity. Certainly, more data are needed to make definite conclusions.

In breast cancer, similar relationship was found between plasma TGF-β1 level and postradiotherapy chest wall fibrosis [16]. Although interesting, these data are, in our opinion, of limited importance for radiotherapy practice, since chest wall fibrosis is not the morbidity that matters most in breast cancer. Much more interesting would be the influence of TGF-β1 elevation on lung or heart toxicity, where it could have an immediate impact on decision making in this very common malignant tumor. Regarding treatment outcome, according to some preliminary data [14,20] elevated TGF-β1 might be an independent predictor of disease progression.

Conflicting results are reported in radiotherapy for cervical cancer. Here, some authors did not find any correlation between plasma TGF-β1 level and late morbidity, but there was rather negative predictive power of elevated TGF-β1 for treatment response and survival [11]. Again, the results could not be confirmed by another study [26], so we really don't know whether there's any potential for this cytokine to predict anything in cervical cancer.

To the best of our knowledge, our study is the first to show the predictive power of TGF-β1 for late morbidity in head and neck cancer. At the same time, we did not find relationship between plasma TGF-β1 level and tumor burden and neither to treatment response. Thus, it can be hypothesized that healthy tissues and immune system make the major part of TGF-β1 production and cancer cells have only minor contribution to the total plasma TGF-β1 level. Similar results were obtained for non-small cell lung cancer [5]. In contrary, gastric, colorectal, prostate, renal and liver cancers did show a positive relationship between tumor burden and TGF-β1 level [15, 17, 18, 21, 23, 25, 27]. If the missing correlation between plasma TGF-β1 level and disease extension is the property of squamous cell cancers remains to be answered.

Interestingly, patients treated with radiotherapy without chemotherapy, i.e. those with poor kidney functions, presented an initially elevated TGF-β1 levels. This is very likely to be contributed to preexistent nephropathy, which typically shows increase in plasma TGF-β1 level [28].

Although the kinetics of TGF-β1 in the blood differ between patients treated with radiochemotherapy and those treated with radiotherapy only, the predictive power of posttreatment TGF-β1 level for delayed toxicity is equal for both treatment modalities. This is a good result, because the praxis of adding chemotherapy simultaneously to radiotherapy differs among big centers, but prediction of late morbidity using TGF-β1 might be further independently investigated for both approaches. In our study, the predictive power of plasma TGF-β1 level has reached borderline significance, probably due to a limited number of patients. We believe that with an increasing number of patients the differences will be more pronounced and, if confirmed, can make a basis for "TGF-β1-guided high-dose radiotherapy" in advanced head and neck cancer.

In summary, posttreatment plasma TGF-β1 level can predict late morbidity grade 1 in advanced head and neck cancer treated with curative radio(chemo)therapy.

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