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Clinical Correlations Between Late Normal Tissue Endpoints After Radiotherapy: Implications for Predictive Assays of Radiosensitivity

Søren M. Bentzen, Marie Overgaard and Jens Overgaard

The discovery that certain genetic syndromes are associated with a high cellular radiosensitivity has stirred interest in the concept that radiosensitivity of persons in general should have a genetic component. This has motivated research into assays for prediction of cellular normal tissue radiosensitivity. If such an intrinsic factor were a major factor in the development of late normal tissue injury, this should be detectable as a correlation between the probability of developing injury in different tissues. This hypothesis is tested in a series of 229 patients treated with postmastectomy radiotherapy and evaluated with respect to a number of late endpoints 16 to 71 months after the end of treatment. In each patient, the presence of marked subcutaneous fibrosis and telangiectasia were evaluated in two different treatment areas: in a photon field underneath a 5-mm wax bolus and in an abutted electron field used for treating the chest wall. The use of two different doses per fraction and the fact that a single anterior photon field was used with the dose prescribed at the level of the mid-axilla, led to a substantial variation in total dose and dose per fraction in these patients. A non-tissue-specific patient-to-patient difference in radiosensitivity would cause higher than expected reactions in one treatment area to be correlated with higher than expected reactions in the other area. For each of the two endpoints, telangiectasia or subcutaneous fibrosis, patients experiencing stronger than expected reactions in one treatment area tended to do so in the other area as well. Thus, a strong host factor appears to exist for a specific endpoint. It is an open question whether this is explained by individual variability in intrinsic radiosensitivity, progression rate of injury or other. Contrary to this, no significant correlation was seen when pairing the two late end-points, fibrosis and telangiectasia. Thus, patients showing stronger than expected fibrosis developed on average marked telangiectasia with a probability well predicted from their total dose and dose per fraction. These findings suggest that an assay for clinical expression of late injury would have to be specific for that type of injury.
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INTRODUCTION

IN 1975, TAYLOR and colleagues [1] demonstrated that the *in vitro* radiosensitivity of skin fibroblasts from patients with ataxia telangiectasia (AT) was significantly increased compared to that of normal human fibroblasts. This observation, together with clinical case studies of unusual radiosensitivity of both normal tissues and tumours in patients with AT [2, 3], has led to the suggestion that the large patient-to-patient variability in response to radiotherapy might be due to individual differences in intrinsic radiosensitivity. Support for this idea is found in reports on *in vitro* radiosensitivity of fibroblasts from patients with an abnormally strong reaction to radiotherapy [4, 5]. However, the design of these studies in which patients were selected for radiosensitivity testing on the basis of atypically strong early radiation reactions, does not allow an evaluation of the clinical usefulness of fibroblast radiosensitivity as a predictor for normal tissue radiosensitivity in unselected patients.

If such an intrinsic factor was not tissue specific and if it indeed were a major determinant for the occurrence of normal tissue injury after radiotherapy, this should show up as a correlation between the probability of developing normal tissue injury in different tissues in individual patients. In other words, some patients would clinically appear to be unusually sensitive/resistant to normal tissue reactions in general. Relatively few clinical studies have analysed this problem and it is not straightforward to do so. The difficulties are illustrated in a study of normal tissue reactions in patients receiving intracavitary and/or external beam radiotherapy for cancer of the uterine cervix [6]. The authors showed that patients with severe early reactions after radiotherapy had a statistically significantly increased risk of developing late bowel complications. Yet, as this was a retrospective study, it was not possible to stratify according to the dose received or other treatment characteristics. Thus, it is possible that high risk of early and late sequelae were simply found in patients who had the highest total dose or dose rate. This is a general difficulty: in a population of patients treated with a variety of radiotherapy schedules, patients with a severe grade of one late endpoint are more likely also to present with another endpoint, simply because the incidence and severity of both endpoints increase with increasing dose/dose per fraction. When comparing two late endpoints there is yet another confounding factor: patients with a long follow-up are more likely to express any two late endpoints than patients with a short follow-up. All this means that both treatment intensity and follow-up time must be allowed for before looking for a possible association between two endpoints in individual patients.

Recently, we have shown that patients experiencing early reactions, erythema or moist desquamation, in general, did not have a higher incidence of telangiectasia and subcutaneous

The authors are at the Danish Cancer Society, Department of Experimental Clinical Oncology and Department of Oncology, Aarhus University Hospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark.
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fibrosis than expected from their treatment characteristics. The exception was the association between moist desquamation and subsequent telangiectasia, which is probably explained as a consequential late reaction caused by the break-down of the epidermal protection of the endothelial cells [7]. These findings have later been supported by Tucker *et al.* [8] who in a reanalysis of the Gothenburg data found no significant association between the development of telangiectasia and previous erythema in individual patients. The question addressed in the current study is whether the expression of two late endpoints would show a within-patient correlation.

PATIENTS AND METHODS

A series of 229 patients treated with postmastectomy radiotherapy have been evaluated with respect to subcutaneous fibrosis and telangiectasia 16 to 71 months after the end of treatment. Evaluations were performed by the same person using an arbitrary four-point scale which has been described previously [9]. This report is concerned with marked subcutaneous fibrosis (grade ≥ 2) and severe telangiectasia (grade 3). Details of the radiotherapy technique and the dosimetry have been given previously [9, 10]. All patients were treated by an anterior 8 MV photon field covering the axillary and the infra- and supraclavicular areas. For the irradiation of the chest wall, an abutted electron field was used in order to reduce the dose to the underlying lung tissue. The area underneath the wax bolus (referred to as the axillary field) and the electron field (chest wall) provided two independent scores of early and late radiation reactions. Data from the axillary and the chest-wall fields were analysed jointly by including the relative biological effect of megavoltage electrons relative to high energy photons as a parameter in the fitting model [7], the value of which was estimated from the data in the direct analysis.

Beginning in 1981 the fractionation schedule was changed from two to five fractions per week. In the two fractions per week group, 88 patients received a maximum absorbed dose of 51.4 Gy and 75 patients received a minimum target dose, specified at the level of the mid-axilla, of 36.6 Gy. In both cases the dose was delivered in 12 fractions over a period of 37 to 46 days. In the five fractions per week group, 66 patients received a minimum target dose of 40.9 Gy in 22 fractions in an overall time of 29 to 35 days. Patient-to-patient variations in the absorbed dose to the skin and subcutis resulted from the two different dose prescriptions in the 12-fraction group, and from the interpatient variation in the depth of the mid-axilla.

Principles of the data analysis

As discussed above, a trivial association may be expected between two late endpoints because they both follow a positive dose–response relationship and because they both increase in severity over time. If groups of patients were treated with a standard dose fractionation schedule, this problem could be overcome by looking at the reaction in an individual patient compared with the average reaction in the group [8]. However, in the present series, where each patient had an individual dose, dose per fraction and follow-up period, another approach had to be taken. Here a dose fractionation model with allowance for latency has been fitted to all individual observations for each of the two endpoints, telangiectasia and subcutaneous fibrosis. The model obtained this way may be regarded as a description of the average relationship between occurrence of a specific endpoint and a specific set of treatment and follow-up characteristics. The idea is to look at the residuals (see [7, 11] for examples of this method), that is the difference between the observed and expected response for a given endpoint, scored in a specific treatment field. The expected response is estimated from the fitted model by inserting the individual values for total dose, dose per fraction and follow-up time. Residuals are positive for patients doing worse than predicted by the model and negative for patients doing better than expected. Graphically, positive and negative residuals correspond to (individual) observations lying above or below the fitted curve, respectively. The numerical value of the residual measures the vertical distance between the observation and the fitted curve. As an example, a patient with an estimated 10% probability of developing telangiectasia, who actually reaches this late endpoint, will have a residual of 0.9. This large value of the residual would indicate that the patient had a stronger than expected response to the given radiotherapy. By looking at the residuals it is possible to investigate, with allowance for dose fractionation details, the correlations between atypically good or bad responses for two endpoints or for the same endpoint in two treated areas. Note that the residual is zero when averaged over all patients. This is the result of the maximum likelihood fitting of the model to the data.

Figure 1 illustrates the use of residuals. In the top panel, a dose–response relationship correcting for total dose and observation time has been fitted to the subcutaneous fibrosis data of all patients. But, for the sake of illustration, dose per fraction was omitted from the model. This is equivalent to fitting the linear quadratic model with just a linear term (with coefficient equal to the effective α). Therefore, when the deviations between observed and predicted response are plotted as a function of dose per fraction, it is evident that patients receiving doses-per-fraction less than about 3 Gy have less fibrosis than expected from the model, whereas patients receiving more than 3 Gy per fraction develop subcutaneous fibrosis with a higher probability than expected from the model. For example, with 2 Gy per fraction the chance of developing subcutaneous fibrosis is some 50–60% lower than expected from the model.

In the bottom panel of Fig. 1, dose per fraction is allowed for by including the quadratic term of the LQ model. There is no systematic deviation between the observed and predicted responses, indicating that in this example the LQ expression corrects for the effect of dose per fraction appropriately.

Analysis of residuals was used to investigate the correlation between two endpoints in the same patient. The basic model for the analysis of dose–response data with allowance for observation time was a single follow-up mixture model [7, 12] using the logistic formulation of the multifraction linear quadratic model. This model was fitted to the data for subcutaneous fibrosis and telangiectasia, separately. The non-parametric Spearmans rank correlation was used for judging the association between the residuals for two endpoints. For the graphical presentation, a two-dimensional scattergram with the relevant residuals was used. To this end, the residuals were binned on the x-axis in intervals of 0.1 units, and the averages of the y-values were plotted with an indication of $\pm$ 1 standard error of the mean. However, the Spearman rank correlation was calculated on the basis of all the individual pairs of residuals.

RESULTS

Correlations were estimated between residuals for fibrosis and telangiectasia scored in the chest wall or axillary fields. Figure 2 (top) shows the residuals for telangiectasia in the chest wall field vs. the axillary field. A highly statistically significant correlation

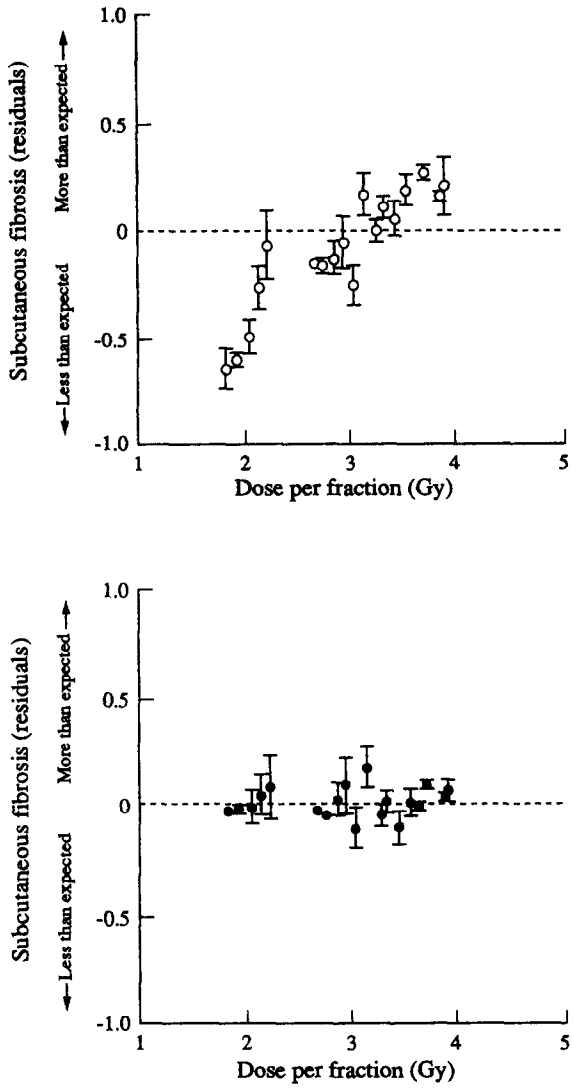

Fig. 1. Average residuals for marked subcutaneous fibrosis as a function of dose per fraction. In the top panel dose–response data were fitted to a model correcting for total dose but without a dose-per-fraction term. It is evident that patients treated with low doses per fraction do considerably better (i.e. they have less fibrosis) than expected from the model and that patients treated with high dose per fraction do worse than expected from the model. After inclusion of a 'dose × dose-per-fraction' term in the model there is no systematic deviation between the observed and predicted incidence of subcutaneous fibrosis.

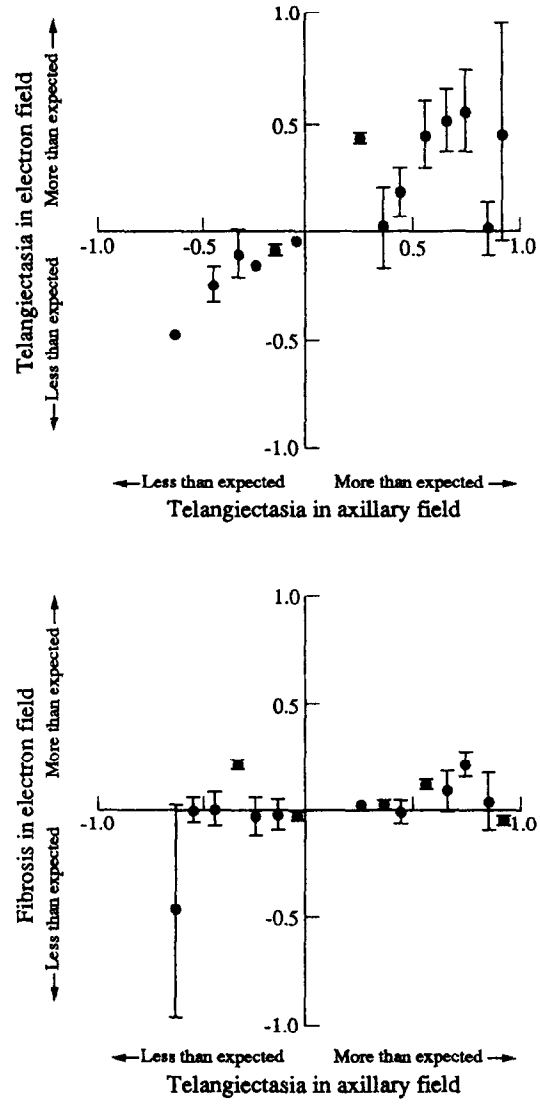

Fig. 2. Average residuals for severe telangiectasia in the chest wall field (top) and marked subcutaneous fibrosis in the chest wall field (bottom) vs. the residual for severe telangiectasia in the axillary field. Negative or positive values in this plot correspond to groups of patients that appear to be more resistant or sensitive, respectively. Thus, in the top panel, there is a within-patient correlation between the deviations between the observed and expected incidence of telangiectasia in the chest-wall field and in the axillary field. In the bottom panel, the average residuals for subcutaneous fibrosis in the chest wall field are close to zero independently of the residuals for telangiectasia in the axillary field.

is seen between telangiectasia in the two fields, indicating that patients who responded better or worse than expected tended to do so in both fields. However, plotting the residuals for marked subcutaneous fibrosis in the chest wall field vs. the residuals for telangiectasia in the axillary field revealed no statistically significant trend (Fig. 2, bottom). Other combinations were tried as well. Table 1 shows the value of Spearmans rank correlation coefficient applied to the set of residuals in individual patients for various combinations of endpoints/fields. For each of the two endpoints, telangiectasia or subcutaneous fibrosis, patients experiencing stronger than expected reactions in one treatment area tended to do so in other area as well. Thus, a strong host factor appears to exist for a specific endpoint. It is an open question whether this is explained by individual variability in intrinsic radiosensitivity, progression rate of injury, or other. Contrary to this, no significant correlation was seen when pairing the two late endpoints, fibrosis and telangiectasia. Thus, patients showing stronger than expected fibrosis, developed on average marked telangiectasia with a probability well predicted from their total dose and dose per fraction.

DISCUSSION

The lack of correlation between atypical responses to radiotherapy in two different tissues is in agreement with the findings for early and late endpoints reported by our group [7] and by Tucker *et al.* [8].

It is interesting to compare these results with the *in vitro* studies published so far. Two studies have investigated the possible correlation between the radiosensitivities of resting T-lymphocytes and fibroblasts from the same individuals [13, 14].

Table 1. Intrapatient correlations between late radiation reactions*

	Telangiectasia		Fibrosis	
	Axillary	Chest wall	Axillary	Chest wall
Telangiectasia				
Axillary	1	0.58†	0.03	−0.02†
		$P < 10^{-8}$	ns	ns
Chest wall		1	−0.06	−0.11
			ns	ns
Fibrosis				
Axillary			1	0.28
				$P = 2 \times 10^{-5}$
Chest wall				1

*Spearmans rank correlation and its associated statistical significance (ns: not significant at $P = 0.05$ level). The table shows whether an unexpectedly strong (or weak) expression of injury for a specific endpoint/treatment field is statistically significantly correlated with a strong (or weak) expression for another combination of endpoint/treatment field. †Data shown in Fig. 1.

Both studies showed no demonstrable correlation between the sensitivities of the two cell types. Similarly, in a small study on radiosensitivities of keratinocytes from 6 patients and fibroblasts from 4 of these [15], there appeared to be very low variability in the sensitivity of the keratinocytes compared to that of the fibroblasts, again suggesting the lack of any strong correlation between the two.

At the time of writing, the only study that seems to be at variance with this overall picture is that of Burnet and colleagues [16], who measured the *in vitro* radiosensitivity of skin fibroblast from 6 of the patients from Gothenburg. Apparently, several measures of radiosensitivity were estimated, but the authors chose the dose required to reduce survival to 1% (typically in the order of 6.5–8 Gy) for presentation in their paper. Although no formal statistical test was used, the authors suggested that there was a correlation of this quantity with both the grades of early erythema and telangiectasia at 10 years, although the correlation with grade of telangiectasia was weaker than for erythema. In view of the study by Tucker *et al.*[8] of all the patients from Gothenburg, it seems unlikely that a strong correlation exists between the early and late endpoint on one hand and a common measure of radiosensitivity on the other. However, a more critical analysis of the relationship between *in vitro* radiosensitivity and clinical response awaits the accumulation of data from more patients from this series.

CONCLUSION

The expression of a specific endpoint in two different treatment fields shows a highly significant intrapatient correlation after allowing for treatment characteristics and observation time. However, when telangiectasia and subcutaneous fibrosis are compared, either in the same field or in two different fields, there is no significant within-patient correlation between stronger than expected expression of one endpoint and of the other. Cellular radiosensitivity has a genetic component, which indeed dominates the overall response to radiotherapy in certain inheritable syndromes. But the lack of correlation between atypical responses to radiotherapy in two different tissues strongly suggests that this intrinsic radiosensitivity may not be a dominating feature in an unselected patient population. Therefore, this study suggests that a predictive assay for clinically expressed radiation injury should be aimed at a specific tissue or even a specific endpoint.

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