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Influence of Overall Treatment Time and Radiobiological Parameters on Biologically Effective Doses in Cervical Cancer Patients Treated with Radiation Therapy Alone

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The aim of the study was to examine the influence of overall treatment time (OTT) on the value of calculated biological effective doses (BEDs) for different biological variables. These variables were: tumour proliferation rate, different cell radiosensitivity ($\alpha = 0.2$, 0.3, and 0.4 / Gy), and different start time for repopulation (Tk = 21, 28, and 35 days). Also the influence of age ($\leq$ 50 years $>$), Hb level ($\leq$ 116 g/l $>$), tumor proliferation rate (bromodeoxyuridine labelling index; BrdUrdLI), and DNA ploidy on survival after shorter ($\leq$ 60 days) or longer ($>$60 days) OTT was investigated. The study included 229 patients with cervix carcinoma treated entirely by standard radiotherapy (RT) (external beam RT plus low-medium dose-rate (LDR/MDR) brachytherapy (BT) at the Center of Oncology in Krakow. The linear quadratic equation was used to calculate BED, which is proportional to log cell kill. BEDs 10 (for tumours) were calculated with consideration of OTT for each patient and tumour proliferation rate (standardized potential doubling time; standardized Tpot) based on BrdUrdLI assessed on biopsy material before RT. Median OTT was 90 days (range 30–210). The mean calculated total BED for point A for tumour and 'early reactions' was equal to 103.0 Gy10. The longest median survival time—52 months—was seen for patients treated with OTT $\leq$ 60 days. If OTT exceeded 90 days to more than 120 days, loss in BED10 for relatively radiosensitive tumours ($\alpha = 0.3$–0.4/Gy and Tk = 28 days) was equal to 0.37–0.26 Gy/day. However, for radioresistant tumours ($\alpha = 0.2$/Gy) it was 0.6 Gy/day. For fast proliferating tumours (BrdUrdLI $>$8.8%) BED loss was 1.4 Gy/day and for slowly proliferating tumours (BrdUrdLI $\leq$ 8.8%) it was 0.2 Gy/day. Assuming shorter (21 days) or longer (35 days) periods for Tk and relatively radiosensitive tumours similar BED loss of 0.38 Gy/day was observed. Kaplan–Meier analysis revealed that OTT $\leq$ 60 days was a significant prognostic factor for overall survival (OS) ($p = 0.019$), disease-free survival (DFS) ($p = 0.0173$), and local control (LC) ($p = 0.011$). BED10 had significant influence on survival ($p = 0.047$). Cox multivariate analysis revealed that for OTT shorter than 60 days the only favourable significant parameters were: age $>$50 years ($p = 0.003$) and high Hb level ($>$116 g/l) ($p = 0.041$). For longer treatments (OTT $>$60 days) the unfavourable parameters were: age $\leq$ 50 years ($p = 0.037$), BrdUrdLI $\leq$ 8.8% ($p = 0.003$), tumour aneuploidy ($p = 0.043$), and BED10 $\leq$ 103 Gy ($p = 0.017$). The examined tumour biological parameters should be taken into account for RT and provide a basis for adjuvant RT.

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Radiation oncologists have suspected for many years that prolongation of treatment time has an adverse effect on radiation therapy (RT) outcome. However, it was not until Denekamp (1) demonstrated compensatory proliferation in the epithelium of the skin of mice—which accelerates starting at 2 or 3 weeks after initiation of RT—that intensive studies of this problem began. Maciejewski et al. (2) and Withers et al. (3) described clinical observations documenting acceleration of repopulation of tumour cells during fractionated RT. Fowler (4) also emphasized rapid proliferation of malignant cells as an important factor influencing tumour control probability. Recently decreasing control of irradiated uterine cervix tumours with prolongation of treatment time has been reported (5–9). Some authors showed the influence of the overall time factor in 4- to 5-week treatments (6), while other authors could not demonstrate it for 7-week overall treatment time (OTT) (10).

There are at least four 'time factors' influencing the response of normal tissues and tumours to fractionated irradiation (11). Repair and redistribution across the cell cycle take place over relatively short time intervals, and are probably largely completed by the end of the approximately daily fractionation intervals used in most standard RT

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schedules. These time factors have the greatest significance for accelerated treatments in which fractionation intervals are shortened in order to deliver two or more doses per day. In contrast, reoxygenation and proliferation take place over more protracted times, and have the potential to lead to the largest differences in response after schedules of varying durations. For this reason, the phrase 'time factor' is currently most often used to refer to the effects of repopulation.

Before the 1980s, this 'time factor' in RT—repopulation—was thought to have little clinical relevance for the tumour response. This idea was based on the observation that human tumours have long pretreatment volume doubling times, with a median value of about 2 months (12). If this time factor continued during treatment, it would be offset by a single dose fraction of approximately 2 Gy, and therefore would have little detectable influence on tumour control. It was found from tumour cell kinetic studies, however, that proliferation after the start of treatment could be significantly greater than suggested and a large number of clinical studies have reported a significant overall time effect, amounting to as much loss of dose as about 1 Gy/day or 10% of local control (LC) per week in some series (3, 13, 14).

The optimal OTT for cervical cancer patients has not been established. Neither has the influence of patient age and tumour biological parameters (DNA ploidy, proliferation rate, or hypoxia) on overall survival (OS) of cervical cancer (SCC) patients after RT given in shorter or longer OTT been established.

The aim of the present study was to examine the influence of some biological variables—such as proliferation rate, different cell radiosensitivity ($\alpha = 0.2$, 0.3, and 0.4 /Gy)—and OTT, on the value of calculated total biological effective dose (BED 10). Also the influence of tumour proliferation rate, DNA ploidy, and haemoglobin level on OTT has been examined.

MATERIAL AND METHODS

Selection of patients

The patient population consisted of 229 patients with FIGO stage IB–IIIB carcinoma of the cervix, treated 1987–1999 with primary definitive RT at the Center of Oncology in Krakow, Poland. Patients with incomplete treatments were excluded from the analysis. There were 9 stage IB, 86 stage IIA, 86 stage IIB, 2 stage IIIA, and 46 stage IIIB patients. Ages were similar for each stage group and ranged from 27 to 80 years (median 52.5 years). The study was approved by the Ethical Committee of the Center of Oncology, and each patient had given written consent.

Treatment

Detailed treatment descriptions have been given earlier (15). Briefly, patients with stage IB–IIA SCC of the cervix had three intracavitary applications—each of 13.9 Gy to point A (^{137}Cs irradiation, Selectron LDR) separated by 4–5 day intervals. After a 2- to 3-week break, external beam RT was administered (40 or 50 Gy in 20 or 25 fractions for 4 to 5 weeks, fraction dose 2.0 Gy). For stages IIB–IIIB the reverse RT schedule was applied—external beam being applied prior to curie-therapy. After a 3-week break, curie-therapy was administered as described above. Median OTT was 90 days with a range from 30 to 210 days. Average OTT was 89 days and 77.4% of the patients had completed their RT within 90 days.

Predictive potential doubling time, bromodeoxyuridine (BrdUrd) labelling index and DNA ploidy

In vitro incorporation of BrdUrd in tumour samples from a biopsy was carried out according to the high-pressure oxygen method. The BrdUrd staining procedure and flow cytometry have been described in detail elsewhere (16). The stained preparations were analysed with a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems, Sunnyvale, CA, USA) and $10–20 \times 10^3$ events were collected in each histogram. The BrdUrdLabelling index (BrdUrdLI) was calculated as a percentage of BrdUrd-labelled cells in a sample, which incorporated BrdUrd during one hour of incubation at 37°C (with discrimination of diploid subpopulation in aneuploid tumours). The tumour ploidy was calculated from the DNA profile with ModFit software running on a Macintosh computer. The tumour ploidy was estimated by evaluating the DNA index, i.e. the ratio of the modal DNA fluorescence of abnormal to normal $G_{1/0}$ cells. Aneuploidy was assessed in cases in which the normal and neoplastic cell populations gave two separate peaks. Human lymphocytes were used for the reference peak.

Tpot was calculated on the basis of the following formula: $\text{Tpot} = \lambda\text{Ts}/\text{BrdUrdLI}$, where λ is a correction factor for nonlinear distribution of cells in the cycle (assumed 0.8), Ts is assumed to be an average time for the duration of DNA synthesis in the cervix (15.8 hours), taken from the literature (17), and the BrdUrdLI is the percentage of S-phase cells that incorporated BrdUrd. This parameter was calculated for each analysed patient, and based on biopsy material taken before RT. This version of Tpot was simply proportional to 1/LI, since Ts was always assumed to be constant. It should be called a 'nominal' or 'standardized' Tpot. We proposed this based on the results of linear regression performed between Tpot and 1/LI, which yields to the simple relation: $\text{Tpot} = 52.46/\text{LI}$. Mean error estimation was 1.3 days and correlation of determination was 0.977. Therefore, for example BrdUrdLI = 8.8%, which corresponds to Tpot = 5.9 days.

BED[1] calculations

Defining parameters chosen. The linear quadratic (LQ) formula was used to calculate the BED to tumour (Gy10) and rectum (Gy3) (18). The parameters used for calculations involving the tumour were $\alpha/\beta = 10$ Gy with $\alpha = 0.3 \pm 0.1$ Gy^{-1}. For the rectum an α/β ratio of 3 Gy was used. The Gy10 or Gy3 values of BED were calculated assuming the α/β ratios to be 10 Gy and 3 Gy, respectively. BED for the tumour (point A) was calculated according to the following formula:

$$\mathrm{BED} = \mathrm{nd}\,[1 + \mathrm{d}/(\alpha/\beta)] \; - \; (\mathrm{T_D} - \mathrm{T_k})\ln 2/(\alpha \mathrm{Tpot}),$$

where n is the number of fractions of size d, α and β are the parameters of the LQ model, Tk is the delay in proliferation in tumours ('kick-off time' assume 28 days, or 21 or 35 days), Tpot is the potential doubling time of the tumour clonogenic cells, assumed constant beyond the 'kick-off time' T_k, and T_D is the duration of treatment considering that the first fraction was given on day 0.

BED for late rectal external beam irradiation was calculated according to the following formula:

$$\mathrm{BED3} = \mathrm{nd}\,(1 + \mathrm{d}/\alpha/\beta)$$

BED for brachytherapy

$$\mathrm{BED} = \mathrm{Total\ Dose} \times \left(1 + \frac{2\mathrm{R}}{\mu(\alpha/\beta)}\left[1 - \frac{1}{\mu \mathrm{T}}(1 - \mathrm{e}^{-\mu \mathrm{T}})\right]\right) \quad (19)$$

where R is dose rate in Gy/h, T is duration of irradiation (in hrs), μ-rate constant for DNA repair 0.46/h for the both late effects and the tumour and early effects, where $\mu = (\ln 2)/\mathrm{T1/2}$, and $\mathrm{T1/2} = 1.5$ h (18, 20, 21).

Statistical analysis

All statistical analyses were performed by the program STATISTICA version 5 (1997). OS, disease-free survival (DFS), and pelvic LC were calculated according to the actuarial method of Kaplan–Meier (22). Survival was measured from the first day of treatment to death or last follow-up. Patients who died of intercurrent disease, or who were lost to follow-up, were censored. Patient age, Hb level, tumour stage, BrdUrdLI, DNA ploidy, and OTT and BED10 (for $\alpha = 0.3$/Gy, Tk = 28 days) were the variable factors analysed. Apart from tumour stage and DNA ploidy, in univariate analysis minimal (the lowest) p-value was used as cut-off point. Risk of death was determined by Cox multiple regression analysis (23) for the whole series of patients, and separately for those treated with OTT <60 days and OTT >60 days (minimal cut-off point). Comparison of categorical variables was performed using the χ^2 test. Statistical significance was considered with p-values of less than 0.05 or 95% of significance.

RESULTS

Within the 10-year follow-up 89 (39.4%) out of 229 patients were still alive and 114 (49.8%) had died from LC, or metastasis. Ten patients (4.4%) had died from intercurrent disease and 26 (11.5%) were lost to follow-up. The median follow-up time was 30 months for all the analysable patients and 70 months for the surviving patients. One hundred and seventy-three patients had no metastases and median survival time 36 months, while those with metastasis (56) lived a median time of 18 months. One hundred and seventy-eight patients (78.8%) were locally controlled with a median time of 48 (range 4–132) months, and 51 (22.6%) patients were without LC (median survival time 9 months) (15).

Treatment factors for the whole series have been published before (15). We now studied the influence of different biological variables (see Material and Methods). The mean BED10 for external beam RT (exBED) and assumed medium tumour radiosensitivity ($\alpha = 0.3$/Gy) was 27.0 Gy, and for the late effects (exBED3) it was equal to 80.4 Gy (15). However, 24 (10.5%) out of 229 analysed patients apparently had no benefit from external beam RT, because their biological effective doses calculated for the tumours (exBED10) were negative (mean value $= -3.9$ Gy) due to their long OTT. Actually in these cases the OTT was very long (from 80 to 180 days) and these 24 patients had very fast proliferating tumours (mean standardized Tpot = 2.8 days). The mean value of standardized Tpot for the whole analysed group (229 patients) was 6.8 days and ranged from 1.4 to 75 days (SD = 7.4 days).

The mean BED10 for brachytherapy (BT) alone was 77.0 Gy, and for late effects (BED3) it was 150.0 Gy. Therefore, mean BEDs for total treatment with the two modalities were 103.0 Gy, and 229.0 Gy, for totBED10, and totBED3, respectively. The number for BED10 was obtained when we assumed Tk = 28 days, $\alpha = 0.3$/Gy, and considered the estimated standardized Tpot based on direct measurements of LI in each patient. This BED10 value was used for the further analysis. We also calculated BED for different α values (0.2 and 0.4/Gy) and different Tk (21 and 35 days). The mean values for different BEDs are given in Table 1.

The mean OTT was 89 days (median 90 days and range 30 to 210 days). One hundred and seventy-five (76.4%) out of 229 patients were treated with an OTT of 90 days. The most relevant reasons for extending the treatment time were: equipment failure, holidays, scheduling variations in treatment style, poor patient compliance, treatment complications, tumour complications, delayed intracavitary or over 2 intracavitaries, and parametrial boosts. The mean prolongation in treatment time was 40 days (range 0–131).

1 tot BED = total BED Ex BED = external BED BT BED = brachytherapy BED

Table 1

Biologically effective doses calculated for different cell radiosensitivity and different starting times assumed for tumour cell repopulation (Tk)

Variable	Overall survival	Biologically effective doses (Gy)		
	n	Mean	Median	Inter-quartile Range
TotBED10 ($\alpha = 0.3$ /Gy, Tk = 28 days)	229	103.0	105.2	35.7
TotBED10 ($\alpha = 0.2$ /Gy, Tk = 28 dys)	229	87.6	92.9	44.7
TotBED10 ($\alpha = 0.4$ /Gy, Tk = 28 days)	229	110.7	112.2	33.0
TotBED10 ($\alpha = 0.3$ /Gy, Tk = 21 days)	229	99.4	102.3	37.1
TotBED10 ($\alpha = 0.3$ /Gy, Tk = 35 days)	229	106.7	109.8	34.2
TotBED3	229	229.0	235.2	121.7

The longest median survival time—52 months—was observed for patients treated with short OTT ($\leq$ 60 days). In patients treated with OTT >60 days $\leq$ 90 days, >90 days $\leq$ 120 days, and >120 days, the survival figures were 27, 32, and 32 months, respectively, and were all shorter. All patients receiving RT with OTT >60 days survived approximately equally badly.

In the linear quadratic model we assumed for radiosensitive tumours $\alpha = 0.4$/Gy, for relatively radiosensitive ones $\alpha = 0.3$/Gy, and for radioresistant ones $\alpha = 0.2$/Gy. We used Tk of 21, 28, or 35 days for the start of repopulation. At OTT >90 days to >120 days, loss in early/tumour biological dose (BED10) was calculated to be from 107.2 to 77.9 Gy (29.3 Gy10 difference, Table 2). Almost the same magnitude of loss in BED was calculated assuming a Tk of 21, 28 or 35 days (Table 2). However, a higher loss in BED10 (39.2 Gy) was calculated for more radioresistant tumours ($\alpha = 0.2$/Gy) than for those with $\alpha = 0.3$/Gy, for the same OTT interval. For more radiosensitive tumours ($\alpha = 0.4$/Gy) a lower loss in BED10 than for the more radioresistant tumours, with an increase of OTT beyond 120 days, was calculated: 24.2 Gy10. For fast proliferating tumours (BrdUrdLI >8.8%) BED10 loss was equal to 1.4 Gy/day and for slowly proliferating tumours (BrdUrdLI $\leq$ 8.8%) it was only 0.2 Gy/day. Stage III tumour patients received no higher biological doses than stage I–II ones. This was nearly true for each OTT compartment. For OTT >120 days the calculated BED loss for stage III tumours was the highest (25.6 Gy) in comparison with I–II tumours (76.1 Gy).

Univariate analysis. A significant influence of tumour size on DFS was shown in our series of patients. Stage I–II patients had significantly ($p = 0.004$) better DFS than those with more advanced tumours (stage III). However, tumour stage had no significant influence on overall survival ($p = 0.287$) or tumour local control ($p = 0.398$).

Kaplan–Meier analysis examining the correlation between OTT and patients' survival revealed that patients with

Table 2

Relationship between OTT and BED10, calculated for different biological parameters

	Overall treatment time (OTT)			
BEDtotal	$\leq$60 days	>60 $\leq$90 days	>90 $\leq$120 days	>120 days
$\alpha = 0.2$/Gy Tk = 28 days BED10	(41)[1] 96.1	(134)[1] 92.1	(43)[1] 74.5	(11)[1] 52.9
$\alpha = 0.3$/Gy Tk = 28 days BED10	103.7	107.2	96.0	77.9
$\alpha = 0.4$/Gy Tk = 28 days BED10	107.6	114.7	106.7	90.4
$\alpha = 0.3$/Gy Tk = 21 days BED10	100.1	103.9	92.5	75.2
$\alpha = 0.3$/Gy Tk = 35 days BED10	107.41	110.91	99.47	80.63

[1]Number of patients analysed.

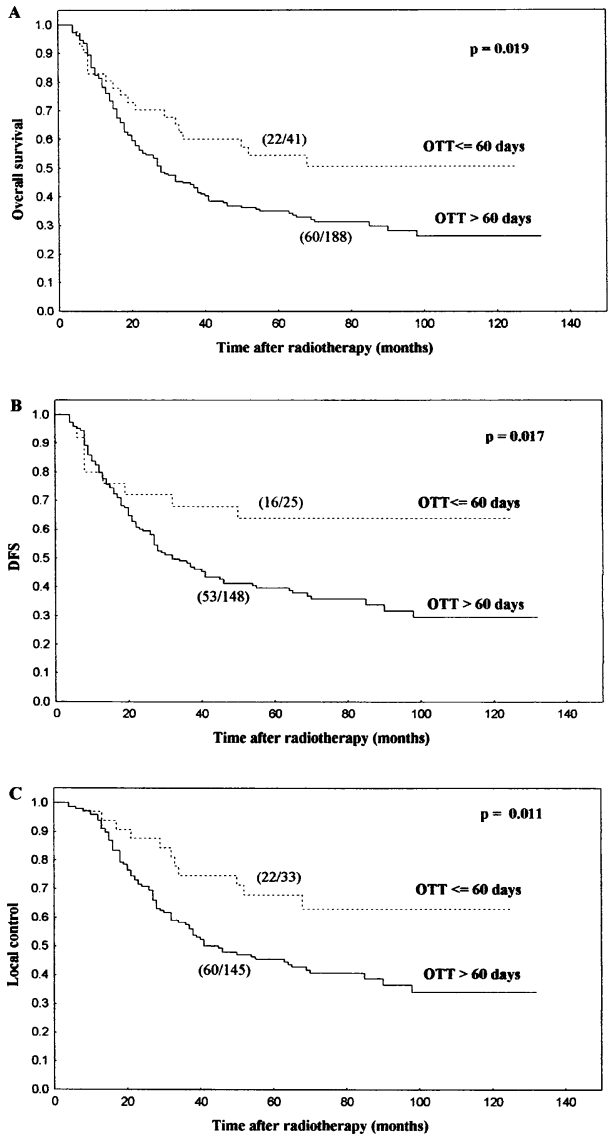

Fig. 1. The influence of OTT ($\leq$ 60 days $>$) on OS (A), DFS (B), and LC (C) for the whole series of 229 cervical SCC patients treated with RT alone. The relative number of patients surviving in each subgroup is given in parentheses.

a short treatment time $\leq$ 60 days achieved better OS ($p = 0.019$), DFS ($p = 0.017$), and LC ($p = 0.011$) (Fig. 1a, b, c, respectively). Patients treated with longer OTT did worse and approximately equally so at all the OTTs $>$60 days. The data reveal that only shorter OTTs, $\leq$ 60 days, were beneficial for survival (Fig. 1a, $p = 0.019$). This was also true for DFS (Fig. 1b, $p = 0.017$) and pelvic LC (Fig. 1c, $p = 0.011$). When we considered the influence of age and Hb level on OTT, we could see that younger patients ($\leq$ 50 years) survived equally badly after shorter and longer OTTs ($p = 0.655$). Also for patients with Hb $\leq$ 116 g/l OTT was not important and patients showed equally poor survival when treated with shorter or longer OTTs ($p = 0.742$). However, older patients ($>$50 years) did better after

Table 3

Prognostic value of clinical and biological parameters in cervical carcinoma in a univariate analysis

Parameter	p-value for 60 days $\leq$ OTT $>$60 days		
	Overall survival	DFS	Local control
Patient's age			
$\leq$50 years	0.655	0.876	0.622
$>$50 years	0.002[1]	0.009[1]	0.007[1]
Hb level			
$\leq$116 g/l	0.742	0.809	0.622
$>$116 g/l	0.005[1]	0.003[1]	0.007[1]
Tumours			
Diploid	0.449	0.144	0.816
Aneuploid	0.014[1]	0.036[1]	0.000[1]
BrdUrdLI			
$\leq$8.8%	0.020[1]	0.016[1]	0.129
$>$8.8%	0.261	0.215	0.026[1]

[1]Survival significantly worse for patients treated with OTT $>$60 days.

OTT $\leq$ 60 days ($p = 0.002$), (Table 3, Fig. 2a). Also patients having Hb $>$116 g/l survived significantly better after shorter treatments (OTT $\leq$ 60 days) than those after longer treatments (Fig. 2b). OTT $>$60 days was significantly worse also for patients with slowly proliferating (BrdUrdLI $\leq$ 8.8%; $p = 0.013$) (Fig. 2c) and aneuploid tumours ($p = 0.014$), (Fig. 2d, Table 3). The negative influence of OTT $>$60 days was observed not only for OS but also for DFS and LC (Table 3). Higher BED10 applied was beneficial for survival ($p = 0.047$) (15). Patients receiving higher BED10 than the mean—103 Gy—had higher OS.

Multivariate analysis. Multivariate regression analysis performed on the whole series of patients showed that the following variables were significant for the risk of death: age $>$50 years ($p = 0.002$), Hb level $>$116 g/l ($p = 0.041$), fast proliferation of tumour cells ($p = 0.004$), DNA ploidy ($p = 0.040$), and BED10 $>$103 Gy ($p = 0.013$) (Table 4). Tumour stage was not a statistically significant variable in this series ($p = 0.550$). In the Cox analysis for DFS important parameters were: age ($p = 0.010$), tumour proliferation ($p = 0.006$), and BED10 ($p = 0.013$). The following parameters were significant for LC: tumour proliferation ($p = 0.033$), OTT ($p = 0.020$), and DNA ploidy ($p = 0.042$) (Table 4). As in the univariate analysis OTT was a significant prognostic factor, and therefore we performed Cox analysis separately for shorter and longer OTT. For shorter treatments the favourable significant parameters were age $>$50 years ($p = 0.003$) and higher Hb level ($p = 0.041$; Table 4). However, for longer treatments the unfavourable parameters were: age $<$50 years ($p = 0.037$), BrdUrdLI $\leq$ 8.8% ($p = 0.003$), tumour aneuploidy ($p = 0.043$) and BED10 $\leq$ 103 Gy ($p = 0.017$; Table 4).

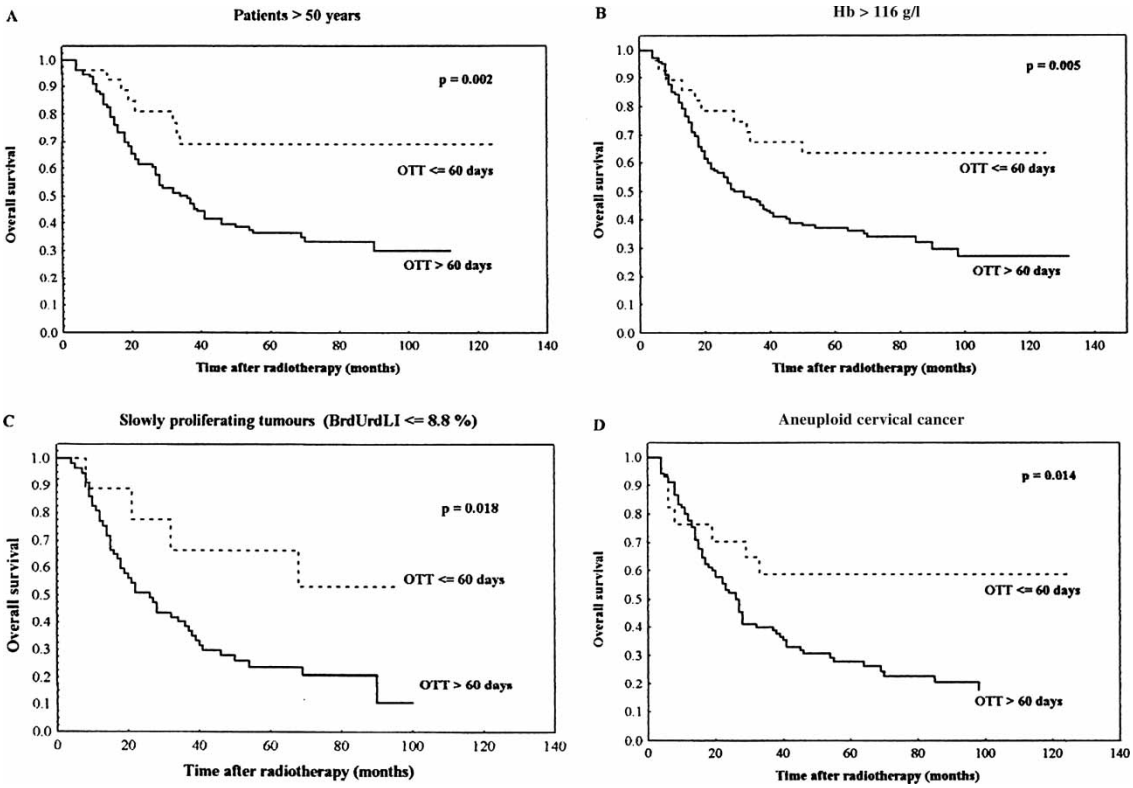

Fig. 2. Negative biological factors for the survival of cervical SCC patients treated with long OTT (>60 days): age (>50 years; A), Hb level (>116 g/l; B), slow tumour proliferation rate (BrdUrdLI $\leq$ 8.8%; C), and tumour aneuploidy (D).

DISCUSSION

For the present series of patients it was shown previously that ExBED10 alone had less influence on survival than the BT BED10 or tot BED10 (15). Patients who were treated with Ex BED10 >27.0 Gy10 did not exhibit better 10-year survival ($p = 0.712$). However, patients treated with BT at BED10 >77.0 Gy10 ($p = 0.008$) had a higher incidence of long-term survival in comparison with patients treated with lower doses (15). The size of total BED for ExRT plus BTRT influenced the results of treatment. Patients treated with tot BED10 >103.0 Gy10 had a higher survival rate than those treated with lower doses ($p = 0.045$) (15).

We analysed the influence of OTT on BED. Patients treated with OTT $\leq$ 60 days had better OS, DFS, and LC. In our earlier study young age ($\leq$ 50 years), slow proliferation of tumour cells (16) and low Hb level (24) were negative factors for survival. In that study OTT was not considered. In the present study we were able to show by Cox analysis that only young age ($\leq$ 50 years) and Hb >116 g/l were significant factors for patients receiving shorter treatments (OTT $\leq$ 60 days). Surprisingly, tumour proliferation had no impact for short treatments. The analysis showed the following unfavourable parameters for longer treatments: age ($\leq$ 50 years), slow tumour proliferation (BrdUrdLI $\leq$ 8.8%), tumour aneuploidy, and BED10 <103 Gy.

The size of BED10 only had no influence on pelvic LC. If OTT lasted from >90 days to >120 days, a loss in biological dose (BED10) from 107.2 to 77.9 Gy (29.2 Gy10 difference) was observed. Nearly the same magnitude of loss in BEDs was calculated for Tk of 21, 28, and 35 days. Calculation of BED10 revealed that if OTT exceeded 90 days to 120 days, loss in biological dose for relatively radiosensitive tumours ($\alpha = 0.3$/Gy) was equal to 0.26–0.32 Gy/day. For radioresistant tumours ($\alpha = 0.2$/Gy), however, it was 0.6 Gy/day. The α values were taken from the literature. We think that pretreatment assessment of inherent radiosensitivity of tumour cells might be helpful in a clinical setting and would allow for more accurate BED calculations. The usefulness of radiosensitivity assessment has already been indicated by West et al. (25).

When we calculated BED loss for treatments longer than 120 days—separately for slowly (standardized Tpot >5.9 days or BrdUrdLI $\leq$ 8.8%) and fast proliferating (standardized Tpot $\leq$ 5.9 days or BrdUrdLI >8.8%) tumours—then the dose appeared to be significantly higher for fast proliferating (1.4 Gy/day) than for slowly proliferating (0.2 Gy/day) tumours, based on our model. This would imply that the faster tumours should be more resistant in general. However, in reality, the observed fast tumour response was more sensitive than predicted by the model.

Table 4

Overall survival by Cox multivariate analysis for cervical cancer patients treated with radiotherapy alone (final results). Model 1 for 229 patients treated within the whole range of OTT. Model 2 for patients treated with shorter (OTT ≤60 days) and longer treatments (OTT >60 days)

		All OTT survival		
	Variable	RR	95% CI	p-value
Model 1				
	Patient age			
	≤50 years	1.72	1.22–2.44	0.002
	>50 years	1.00	Reference	
	Hb			
	≤116 g/l	1.52	1.02–2.22	0.041
	>116 g/l	1.00	Reference	
	BrdUrdLI			
	≤8.8%	1.75	1.19–2.56	0.004
	>8.8%	1.00	Reference	
	Tumour			
	Diploid	1.00	Reference	
	Aneuploid	1.44	1.02–2.05	0.040
	BED10			
	≤103 Gy	1.53	1.10–2.27	0.013
	>103 Gy	1.00	Reference	
	OTT			
	≤60 days	1.00	Reference	
	>60 days	1.50	0.93–2.62	0.091
Model 2		OTT <60 days		
	Patient age			
	≤50 years	5.88	1.81–20.00	
	>50 years	1.00	Reference	0.003
	Hb			
	≤116 g/l	2.86	1.04–7.69	
	>116 g/l	1.00	Reference	0.041
	OTT >60 days			
	Patient age			
	≤50 years	1.49	1.02–2.17	0.037
	>50 years	1.00	Reference	
	BrdUrdLI			
	≤8.8%	1.89	1.25–2.86	0.003
	>8.8%	1.00	Reference	
	Tumour			
	Diploid	1.00	Reference	
	Aneuploid	1.47	1.01–2.14	0.043
	BED10			
	≤103 Gy	1.61	1.09–2.38	0.017
	>103 Gy	1.00	Reference	

It is generally assumed that the main reason for poor results of extended RT is the accelerated repopulation of clonogenic cells surviving irradiation. Cell loss is an important factor in tumour regression, and it is not clear how soon after starting treatment spontaneous cell loss falls to low values. According to Fowler and Lindstrom (26), it can occur either when three or four fractions of 2 Gy have been delivered or when the tumour has shrunk (1 or 2 weeks from start of RT for carcinomas). The study by Tan et al. (27) performed on cervical SCC demonstrates that the exponential tumour volume regression commences during the first week of teletherapy and that the tumour volume regression rate can range from 0.6% per day to 20.7% per day.

Evidence of 'accelerated repopulation' in clinical head and neck cancer data sets was first deduced by Maciejewski et al. (2) and later by Withers et al. (3). It was suggested by Withers et al. (3) that the phenomenon may commence approximately 4 weeks after conventionally fractionated RT starts and may proceed at a rate sufficient to offset 0.6 Gy

of the daily dose delivered. Much discussion has then been devoted to the questions of whether a decrease in tumour cell doubling time during RT occurs or whether changes in growth fraction and cell loss factor simulate an apparent acceleration in repopulation (28). If the repopulation rate in cervical cancer does increase at around 21–28 days after starting external beam RT, due to changes in cell loss factor or in growth factor, then, as suggested (19), BT should ideally be performed at around this time because cells would be more sensitive.

In general, more extensive tumours are given necessarily longer OTTs and they have a higher local failure rate. A greater total dose used (and in particular the large intracavitary dose) may overcome a greater proportion of accelerated clonogen proliferation. However, heterogeneity of repopulation in tumours may cause a disparity in the tumour response. If significant heterogeneity exists this will dilute the OT effect in large groups of patients (6). Indeed, in the cervix we observe a wider range of values for Tpot or BrdUrdLI than for other tumours (i.e. head and neck tumours).

Investigators interpret their data on OTT using various methods of linear regression analysis, specifying the results in terms of the percentage decrease in OS or LC per day. These models assume that each additional day, from the normal length of treatment onwards, has the same effect on outcome. However, none of the studies provides enough data to justify this linearity assumption (29). Keane et al. (30) were the first to present a report where the time factor in cervix SCC was shown (for stages I and II 0.7% per day and for stages III and IV $<1.2\%$ per day of decreased pelvic LC). Fyles et al. (6) demonstrated the adverse effect of increased duration on pelvic LC in 830 patients with SCC treated with RT alone. Loss of tumour control approximated 1% per day of treatment prolongation beyond 30 days. Lanciano et al. (31), in an analysis of 837 patients treated with doses ≥ 66.0 Gy, also reported a highly significant decrease in pelvic tumour LC and survival with prolongation of treatment times from 6 weeks or less to 10 weeks ($p = 0.000$). In the study of Petereit et al. (8) survival at 5 years decreased by 0.6% (confidence interval 0.17–0.98) and pelvic control by 0.7% (confidence interval 0.20–1.27), respectively, for each additional day of treatment beyond 55 days. Girinsky et al. (7) in a study of 386 stage IIB–III patients treated with RT (45 to 50 Gy whole pelvis and 10 Gy intracavitary curietherapy), also observed loss of LC and OS when the treatment time exceeded 52 days. In the Girinsky study (7), the 10-year local recurrence-free survival rate decreased rapidly when OTT was extended from 52 to >62 days. A 1.1% loss of pelvic LC per day was observed in their regression analysis, similarly to our results and those of Perez et al. (32). Recently, Ferrigno et al. (5) have shown that OTT with 45 Gy to the whole pelvis combined with 4 fractions of 6 Gy with HDR BT is an effective and safe fractionation schedule, if realized in 50 days.

To summarize, we can state that the overall time factor in cervical SCC treatment is very important. We believe that it is essential to initiate RT as soon as possible and complete it as quickly as acute tolerance allows. For conventional RT with MDR BT an overall time of 50–60 days for OTT seems to be reasonable. In our instance, the response was better when RT started with BT ($p = 0.252$). This finding, however, may be connected to lower tumour stage rather than to sequence of the treatment. Continuous low dose rate BT has an advantage of tumour overkill occurring very close to the radiation sources, while more distant normal structures are preserved (33). Irradiation of normal tissues at lower dose rates than of the tumour allows additional radiobiological dose sparing. Short treatments of only a few days limit the repopulation of tumour clonogens during treatment, regardless of cell cycle check point blocks. Therefore, we probably have better results after BT than after external beam RT. However, in the cervix, OTT has a less pronounced effect than for the head and neck tumours (3, 34).

Cox analysis showed that tumour proliferation is not important for short OTT (< 60 days). This may perhaps in part explain why in many studies performed previously, with short OTT, the significance of tumour proliferation rate was not obtained. However, this result is in contradiction to the Tsang et al. (35) study, which indicated that the lower tumour proliferation rate in the cervix was a prognostic factor for patients treated with OTT ≤ 70 days (median 45 days). We obtained different results for longer treatments (OTT >60 days)—slow tumour proliferation was a negative prognostic factor. In our series of patients the only statistically important parameters for short OTT remains age >50 years and Hb level.

A better cell redistribution at OTT ≤ 60 days may cause a higher incidence of apoptosis after irradiation. Another explanation for better tumour response (see Dale and Jones, 19), could be that fast proliferating tumours shrink faster than slowly proliferating ones. More tumour cells will then be reached by higher doses from the implant. This effect would counteract the effect of repopulation. However, we also observed better survival for patients with slowly proliferating tumours treated with short OTT. It seems that the element of BT in treatment improves the RT results independently of tumour proliferation status, as we have observed a significant influence of BT dose and not external beam dose on survival (15).

Our data confirm the current concept of tumour response to RT (36), saying that slowly proliferating tumours might have a greater propensity to recruit cells (from G0 phase) into rapid cycle response to treatment. Rapidly proliferating cells, on the other hand, have little reserve capacity for further accelerating their cell cycle. Our study also confirms

the current opinion that the LI might be a better prognostic factor than Tpot (37). Begg, Haustermans et al. (37)—although they found that Tpot had no significant correlation to clinical outcome—demonstrated that LI was well correlated. Our data also support the Chappell and Fowler (38) suggestion on the analysis of Tpot value in clinical data sets. They have proposed using an inverse scale (1/Tpot) model for Tpot analysis. This formulation may be important, because LI is directly proportional to proliferation rate, while Tpot loses precision at short Tpots. Using this concept we have shown a correlation between 1/LI and standardized Tpot and we can show that 1/Tpot can give a good correlation with outcome, because LI with a 'constant Ts' is exactly proportional to the reciprocal of Tpot.

We indicate further improvements in RT results by assessment of such parameters as: age, Hb level, tumour proliferation, and DNA ploidy. We suggest these because, in our series, survival of younger patients was equally poor after shorter (≤ 60 days) or longer (>60 days) OTT. However, older patients (>50 years) survived significantly better after shorter OTTs than after longer treatments. A higher Hb level was also important for survival when OTT was ≤ 60 days. However, OTT longer than 60 days was detrimental for patients with aneuploid or slowly proliferating tumours (BrdUrdLI $\leq 8.8\%$), and for those treated with BED10 <103 Gy. Therefore, we can suggest that the above-mentioned biological parameters should be taken into account before RT and provide a basis for adjuvant treatments.

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REFERENCES

1. Denekamp J. Changes in the rate of repopulation during multifraction irradiation of mouse skin. Br J Radiol 1973; 46: 381–7.
2. Maciejewski B, Preuss-Bayer G, Trott KR. The influence of the number of fractions and of overall treatment time on local control and late complication rate in squamous cell carcinoma of the larynx. Int J Radiat Oncol Biol Phys 1983; 9: 321–8.
3. Withers HR, Taylor JMG, Maciejewski B. The hazard of the accelerated tumor clonogen repopulation during radiotherapy. Acta Oncol 1988; 27: 131–46.
4. Fowler JF. Potential for increasing the differential response between tumors and normal tissues: can proliferation rate be used? Int J Radiat Oncol Biol Phys 1986; 12: 641–5.
5. Ferrigno R, Ribeiro dos Santos Novaes PE, Pellizzon ACA, et al. High-dose-rate brachytherapy in the treatment of uterine cervix cancer. Analysis of dose effectiveness and late complications. Int J Radiat Oncol Biol Phys 2001; 50: 1123–35.
6. Fyles A, Keane TJ, Barton M, Simm J. The effect of treatment duration in local control of cervix cancer. Radiother Oncol 1992; 25: 273–9.
7. Girinsky T, Rey A, Roche B, et al. Overall treatment time in advanced cervical carcinoma: a critical parameter in treatment outcome. Int J Radiat Oncol Biol Phys 1993; 27: 1051–6.
8. Petereit DG, Sarkaria JN, Chappell R, et al. The adverse effect of treatment prolongation in cervical carcinoma. Int J Radiat Oncol Biol Phys 1995; 32: 1301–7.
9. Roberts SA, Hendry JH. The delay before onset of accelerated tumour cell repopulation during radiotherapy: a direct maximum-likelihood analysis of a collection of worldwide tumour-control data. Radiat Oncol 1993; 29: 69–74.
10. Erridge SC, Kerr GR, Downing D, Duncan W, Price A. The effect of overall treatment time on the survival and toxicity of radical radiotherapy for cervical carcinoma. Radiother Oncol 2002; 63: 59–66.
11. Withers HR. The four R's of radiotherapy. In: Lett JT, Adler H, eds. Advances in radiation biology. 5th ed. New York: Academic Press, 1975. p. 241–7.
12. Steel GG. Growth kinetics of tumours. Oxford: Clarendon Press; 1977.
13. Fowler JF, Lindstrom MJ. Loss of local control with prolongation in radiotherapy. Int J Radiat Oncol Biol Phys 1992; 23: 457–67.
14. Parsons JT, Mendenhall WM, Stringer SP, Cassisi NJ, Millon RR. An analysis of factors influencing the outcome of postoperative irradiation for squamous cell carcinoma of the oral cavity. Int J Radiat Oncol Biol Phys 1997; 39: 137–48.
15. Urbanski K, Gasinska A, Pudelek J, Fowler JF, Lind B, Brahme A. Biologically effective doses in radiotherapy of cervical carcinoma. Neoplasma; 2004; 51: 228–38.
16. Gasinska A, Urbanski K, Jakubowicz K, Klimek M, Biesaga B, Wilson GD. Tumour cell kinetics as a prognostic factor in squamous cell carcinoma of the cervix treated with radiotherapy. Radiother Oncol 1999; 50: 77–84.
17. Wilson GD. Limitations of the bromodeoxyuridine technique for measurement of tumour proliferation. Medical Radiology. Current Topics in Clinical Radiobiology of Tumours. Berlin: Springer, 1993.
18. Fowler JF. The linear-quadratic formula and progress in fractionated radiotherapy. Br J Radiol 1989; 62: 679–94.
19. Dale RG, Jones B. The clinical radiobiology of brachytherapy. Brit J Radiol 1998; 71: 465–83.
20. Barendsen GW. Dose fractionation, dose rate and isoeffect relationships for normal tissue responses. Int J Radiat Oncol Biol Phys 1982; 8: 1981–97.
21. Fowler JF. Dose reduction factors when increasing dose rate in LDR or MDR brachytherapy of carcinoma of the cervix. Radiother Oncol 1997; 45: 49–54.
22. Kaplan EL, Meier P. Non-parametric estimation for incomplete observations. J Am Stat Assoc 1956; 53: 457–81.
23. Cox DR. Regression models and life tables. J R Stat Soc 1972; 34: 187–220.
24. Gasinska A, Urbanski K, Adamczyk A, Pudelek J, Lind BK, Brahme A. High intratumour microvessel density and high hemoglobin level are associated with high survival in carcinoma of the uterine cervix. Acta Oncol 2002; 41: 437–43.
25. West CML. Invited review: Intrinsic radiosensitivity as a predictor of patient response to radiotherapy. Br J Radiol 1995; 68: 827–37.
26. Fowler JF. Lindstrom MJ. Loss of local control with prolongation in radiotherapy. Int J Radiat Oncol Biol Phys 1992; 23: 457–67.
27. Tan LT, Jones B, Green JA, Kingston RE, Clark PI. Treatment of carcinomas of the uterine cervix which remain bulky after external beam radiotherapy: a pilot study using integrated

cytotoxic chemotherapy prior to brachytherapy. Br J Radiol 1996; 69: 165–71.
28. Fowler JF. Rapid repopulation in radiotherapy: a debate on mechanism: the phantom of tumour treatment—continually rapid proliferation unmasked. Radiother Oncol 1991; 22: 156–8.
29. Eifel PJ, Thames HD. Has the influence of treatment duration on local control of carcinoma of the cervix been defined? Int J Radiat Oncol Biol Phys 1995; 32: 1527–9.
30. Keane T, Fyles A, O'Sullivan B, Barton M, Maki E, Simm J. The effect of treatment duration on local control of squamous carcinoma of the tonsil and carcinoma of the cervix. Sem Radiat Oncol 1992; 2: 26–8.
31. Lanciano RM, Pajak TF, Martz K, Hanks GE. The influence of treatment time on outcome for squamous cell cancer of the uterine cervix treated with radiation: a patterns of care study. Int J Radiat Oncol Biol Phys 1993; 25: 391–7.
32. Perez CA, Grigsby PW, Castro-Vita H, Lockett MA. Carcinoma of the uterine cervix, I: Impact of prolongation of overall treatment time and timing of brachytherapy on outcome of radiation therapy. Int J Radiat Oncol Biol Phys 1995; 32: 1275–88.
33. Scalliet P, Gerbaulet A, Dubray R. HDR versus LDR gynecological brachytherapy revisited. Radiother Oncol 1993; 28: 118–26.
34. Skladowski K, Law MMG, Maciejewski B, Steel GG. Planned and unplanned gaps in radiotherapy: the importance of gap position and gap duration. Radiother Oncol 1994; 30: 109–20.
35. Tsang RW, Wong CS, Fyles AW, et al. M. Tumor proliferation and apoptosis in human uterine cervix carcinoma, II: Correlations with clinical outcome. Radiother Oncol 1999; 50: 93–101.
36. Wilson GD. A new look at proliferation. Acta Oncol 2001; 40: 989–94.
37. Begg AC, Haustermans K, Hart AAM, et al. The value of pretreatment cell kinetics parameters as predictors for radiotherapy outcome in head and neck cancer: a multicenter analysis. Radiother Oncol 1999; 50: 13–23.
38. Chappell R, Fowler JF. How to efficiently model Tpot and why we should care. Int J Radiat Oncol Biol Phys 2002; 54 (Suppl 2): 54.