Lymphocyte damage in four differently treated prostate radiotherapy cohort

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Abstract

Background: As the assessment of lymphocyte damage caused by radiotherapy gains importance as immunotherapies are used more frequently. Furthermore, there is scarce data to compare the biological dose of radiotherapy techniques in the same patient group.

Methods: Applying chromosome aberration technique, a five-year long (at 3, 6, 9, 12, 24, 36, 48, 60 months) prospective comparison of the biological impact of four different types of treatments for low- and intermediate-risk prostate cancer was performed (195 patients): conventional LINAC (linear accelerator) (70-78 Gy) and Cyberknife (40-37.5 Gy) teletherapy, LDR (low dose rate brachytherapy, 145 Gy) and HDR (high dose rate brachytherapy 19/21 Gy). Multivariate regression analyses were performed to analyze the predictive potential of the chromosome aberrations on side effects. The median follow-up was 48-60 months.

Results: We have found that teletherapy techniques (conv. LINAC and Cyberknife therapy) caused 1.7–3.2-fold more chromosomal aberrations than brachytherapies. At three months, 4.6%–12.7% of the lymphocytes were damaged. Five years after treatment, the total aberration values of conventional LINAC and LDR brachytherapy patients were still significantly higher than before the therapy (p=0.035 for LINAC and p=0.003 for LDR BT). We found significant regression models

suggesting chromosome aberration might predict side effects beside radiation dose and irradiated volumes ($V_{100\%}$): Late genitourinary (GU) side effects were dependent of chromosome fragments directly after conv. LINAC therapy (p=0.005).

Conclusions: We demonstrated long-term lymphocyte damage dependent on the type of radiotherapy. We also found less biological dose and side effects in brachytherapy patients.

Keywords: lymphocytes, radiotherapy, biological dose, dose of immune cells, chromosome aberrations

Highlights

- Fewer chromosomal aberrations arose after brachytherapies than after teletherapies
- 12% of the lymphocytes were damaged three months after conventional LINAC therapy
- Total aberrations were still higher at five years after LDR BT than at baseline

List of abbreviations

- BT - brachytherapy
- CTV - clinical target volume
- EORTC-RTOG - European Organization for Research and Treatment of Cancer-Radiation Therapy Oncology Group
- FBS - fetal bovine serum
- GI - gastrointestinal
- GU - genitourinary
- GS – gleason score
- HDR - high dose rate
- HT- hormone therapy
- IPSS - International prostate symptom score
- LDR - low dose rate
- LINAC - linear accelerator
- PROM – patient-reported outcome measure
- PTV - planning target volume
- SIB - simultaneous integrated boost
- QoL - quality of life
- $V_{100\%}$ - volume, which received the 100% of the prescribed dose

Introduction

Approximately half of tumor patients are treated using different radiotherapy techniques. However, the biological doses of the current radiotherapeutic techniques are not compared thoroughly (1). The biological dose describes the reaction of cells to the absorbed radiation dose. As the biological dose is mostly quantified by the damage of lymphocytes (micronucleus assay, Comet-assay, chromosomal aberration assay), it also describes the condition of the immune cells. As immunotherapy is administered to more and more patients, the damage to the targeted lymphocytes can be an important factor in the success rate even beside lymphocyte count. However, there is scarce information about how long it takes for the damaged cells to get eliminated (2).

Prostate cancer is the third most common cancer worldwide and radiotherapy is one of the leading treatment options for non-metastatic tumors. Therefore, we have selected low- and intermediate-risk prostate cancer patients (N=195), who can receive radiotherapy through four different techniques at our Institute. Teletherapy can be delivered by conventional linear accelerators (LINAC) or by hypofractionated Cyberknife LINAC with the precision of the robotic arm of the radiotherapy machine. The higher conformality allows higher doses per fraction and shorter overall treatment time. On the other hand, brachytherapy employs radioactive isotopes implanted into the tumor. The movement of the tumor causes less difficulties in this case and smaller safety margins around the clinical target volume (used for counteracting movement) can be applied. The high dose gradient also helps to prevent normal tissue toxicities. We compared the gold standard values of biological dosimetry – chromosomal aberration frequencies – after these four radiotherapy modalities in a five-year follow-up.

The chromosomal aberrations (CA) are also suggested to predict radiotherapeutic side effects (3-5), for which there is no method in clinical practice. We tested this hypothesis by comparing chromosomal aberrations and graded urogenital and gastrointestinal radiation toxicities.

Patients and Methods

Patients

Between April 2015 and June 2021, low and intermediate-risk prostate cancer patients (6) (N=195) were enrolled in our study. Patients with previous tumors were excluded. Blood samples were taken before the therapy and on the follow-up visits, when PSA measurement was also performed. The physicians evaluated patients' side effects according to the RTOG-EORTC grading system while the International Prostate Syndrome Score (IPSS) and the Quality of Life (QoL) questionnaires were completed by the patients themselves. Blood samples were taken once from healthy men (N=22) whose age matched that of the patient group. We performed the study according to the Helsinki guidelines with the approval of the Ethics Committee (document number 16738-2/2015/EKU on 24.03.2015, https://ett.okfo.gov.hu/tukeb/eng2015/). Both patients and volunteers signed informed consent forms.

Radiotherapy

At our Institute, four types of radiotherapy are available for non-metastatic prostate cancer patients.

Brachytherapy techniques are suggested for patients whose prostate measured less than 60 cm^3, had more than 5 mm rectum–prostate distance, and no pubic arch interference. Patients with an initial IPSS score higher than 15 were advised against brachytherapy. Otherwise, it was considered whether patients preferred teletherapy or brachytherapy. Brachytherapy patients (LDR BT and one fraction HDR monotherapy) were randomized within a clinical trial (NCT02258087). The dose-volume constraints are in Supplementary Table 1. The Cyberknife machine was installed at our Institute in 2018. Before that, all teletherapy patients received LINAC therapy, after that, patients could opt for Cyberknife therapy as well.

LINAC therapy: For intermediate-risk patients, the prostate CTV was expanded with a 0.5 cm margin (except in posterior direction) and a 1.0 cm margin was added in cranial direction for seminal vesicle irradiation. Low-risk patients received 70-78 Gy on the prostate, and intermediate-risk

patients received 57.4-60 Gy on seminal vesicles as well. Simultaneous integrated boost (SIB: 2.5 Gy/fraction on the prostate, 2.05 Gy/fraction on seminal vesicle) and classical (2 Gy/fraction) fractionation were both accepted. The accelerator energy was 6, 10, or 18 MV (7). The treatment planning was performed by Eclipse v13.7 (Varian, Palo Alto, USA) or Pinnacle v9.8 (Philips, Eindhoven, The Netherlands) system. Dose volume constraints are shown in Supplementary Table 1.

Cyberknife therapy: For a precise setup, four gold markers were inserted into the prostate two weeks prior to the planning CT. The prescribed dose was 37.5-40 Gy to the prostate, and for intermediate-risk patients 30-32.5 Gy was given to the vesicle seminals CTV as well. Radiotherapy was delivered by Cyberknife machine (Accuray, Medison) in 5 fractions, administered on every second workday (8).

LDR BT (seed BT): Low dose rate brachytherapy was performed with stranded seeds (^{125}I isotope by Bebig). After anesthesia, catheters were inserted into the prostate with rectal ultrasound guidance in lithotomy position and the seeds were implanted (SPOT PRO 3.1 and Oncentra Prostate 3.2.2). 145 Gy was delivered on the surface of the prostate. The patients were discharged from hospital the next day. Four weeks after the seed implantation, a CT scan was performed and the final plan was adjusted to the final seed positions. (9, 10)

HDR BT: After Foley-catheter insertion, transrectal ultrasound-guided needle insertion was performed and an intraoperative plan was made (Oncentra Prostate 3.2.2). The HIPO algorithm (hybrid inverse planning and optimization) inverse optimization was applied. The dose delivery was executed with an afterloading machine (Elekta Brachytherapy, Veenendaal, The Netherlands), and the prescribed dose was 19 or 21 Gy (^{192}Ir) (11).

Supplementary Table 1. Dose volume constraints

conv. LINAC (2 Gy /frac)		**conv. LINAC (SIB)**		**Cyberknife**		**HDR**		**LDR**	
				Target volume		**Target volume**		**Target volume**	
				V_{100}	≥ 95%	V_{100}	≥ 95%	V_{100}	≥ 95%
						D_{90}	≥ 110%	D_{90}	≥ 110%
						V_{150}	≤ 50%	V_{150}	≤ 60%
Rectum		**Rectum**		**Rectum**		**Rectum**		**Rectum**	
V_{50Gy}	≤ 50%	$V_{45\,Gy}$	≤ 50%	V_{26Gy}	≤ 50%	D_{2cm3}	≤ 50%	D_{2cm3}	≤ 100%
V_{70Gy}	≤ 20%	$V_{63\,Gy}$	≤ 20%	V_{34Gy}	≤ 20%	$D_{0.1cm3}$	≤ 80%	$D_{0.1cm3}$	≤ 138%

					D_{20cm3}	≤	25 Gy				
					D_{max}	≤	38 Gy				
Bladder			**Bladder**		**Bladder**			**Urethra**		**Urethra**	
V_{50Gy}	≤	60%	V_{45}Gy	≤ 60%	V_{26Gy}	≤	65%	D_{10}	≤ 120%	D_{10}	≤ 150%
					D_{max}	≤	44 Gy	D_{30}	≤ 110%	D_{30}	≤ 130%
Hip bones			**Hip bones**		**Hip bones**						
V_{50Gy}	≤	10%	$V_{45\,Gy}$	≤ 10%	V_{26Gy}	≤	10%				
					D_{10cm3}	≤	30 Gy				

Dose volume constraints of the different radiotherapy methods applied to treat low and intermediate-risk prostate cancer patients.

Baseline characteristics

The distribution of Gleason scores and risk varied significantly between the groups, but there were no disparities observed in the T status. The patients in the HDR arm had significantly lower age and iPSA levels and risk compared to those in the conv. LINAC arm (Table 1).

Table 1: Baseline characteristics of the patients in our study

	conventional LINAC	**Cyberknife**	**LDT BT**	**HDR BT**	**p value**
Patients	25	51	67	52	
Age (Range)	68.5 (53-79)	67.4 (54-78)	66.9 (50-79)	65.1 (53-75)	LINAC-Cyber: 0.463; LINAC-LDR BT: 0.292; LINAC-HDR BT: **0.016**
iPSA (range)	11.2 (3.0-20.0)	9.6 (2.8-18.2)	9.6(4.4-20.0)	8.9 (4.9-18.5)	LINAC-Cyber: 0.095; LINAC-LDR BT: 0.068; LINAC-HDR BT: **0.028**
T status					
T1	7 (28.0%)	7 (13.7%)	16 (23.9%)	14 (26.9%)	0.310
T2	17 (68.0%)	43 (84.3%)	51 (76.1%)	38 (73.1%)	
n.a.	1 (4%)	1 (2.0%)	0	0	
Gleason score					
4-5	3 (12.0%)	1 (2.0%)	12 (17.9%)	2 (3.8%)	**0.003**
6	9 (36.0%)	20 (39.2%)	35 (52.2%)	34 (65.4%)	

7	13 (52.0%)	30 (58.8%)	17 (25.3%)	16 (30.8%)	
n.a.	0	0	3 (4.5%)	0	
Risk					
low/inter.	4/21	9/42	18/49	22/30	**0.019**
HT0/HT1	2/23	37/17	23/44	25/27	**<0.0001**
SIB/conv.	17/8	51/0	-	-	
19/21Gy	-	-	-	19/33	

Patients characteristics are shown according to the different treatment groups. (iPSA =initial PSA, LDR BT=low dose rate brachytherapy, HDR BT= high dose rate brachytherapy, Cyber.= Cyberknife therapy, Conv.=conventional LINAC based teletherapy, HT1= ADT receiving, SIB= Simultaneous integrated boost

L*ymphocyte sample preparation and chromosome analysis*

Blood sample collection was performed by venipuncture into Li-heparinized tubes. Lymphocytes were induced to proliferate with phytohemagglutinin (2% v/v, Gibco, cat: 10576015) in supplemented (15% FBS, 100 U/mL penicillin, 100 mg/mL streptomycin) RPMI media. After 48 hours of culture, colcemid (0.1 ug/mL, Gibco, cat: 15212012) was added to stop division (for additional 2 hours). The cells were treated with hypotonic KCl solution (0.1 M) for swelling, and chromosomes were fixed with methanol: acetic acid (3:1) for multiple times. The glass smears on glass slides were stained with Giemsa. A hundred metaphases on four coded slides were analyzed on 1000X magnification.

Chromosome aberrations were analyzed in metaphases with 44–47 chromosomes, according to international standards (12). Chromatid breaks and chromosome fragments, exchanges, translocations, rings and dicentrics (dicentrics+rings) were evaluated. Their summarized value is the total aberration value. Based on decades of practice, 5 aberrations /100 cells are considered as the normal range of total aberrations in our laboratory (13). The aberrant cell number is the number of cells with any aberration /100 cells, which means the frequency of the damaged cells.

Chromosomal aberrations were measured before radiotherapy, immediately after it, and 3, 6, 9, 12, 24, 36, 48, and 60 months after radiotherapy.

Statistical analysis

Chi-squared test was performed to compare the baseline characteristics of patients in different treatment arms. We applied the Mann–Whitney two-tailed U-test to compare the values of chromosomal aberrations of the treatment groups and to find differences between follow-up points and baseline values. One sample Wilcoxon test (two-tailed) was used to test if there is a difference between total aberration values and the normal range of our laboratory. We used multivariate regression analysis to investigate the effects of baseline characteristics on chromosome aberrations. Multivariate regression analysis was applied to investigate whether different chromosome aberrations are independent predictors of different side effects beside $V_{100\%}$ (the volume irradiated with 100% of the prescribed dose) and prescribed dose of the prostate. All tests and models were considered significant in the case of $p<0.05$. For data representation and analysis, StatSoft STATISTICA 12, GraphPad Prism (San Diego, CA, USA) and Origin Pro 8.5 were used.

Results

We followed 25 patients in the conventional LINAC treatment arm (LINAC) for 60 months (median), 51 patients in the Cyberknife group (48 months median follow-up, as it was installed later at our Centre), 67 patients in the low dose rate (seed) brachytherapy group (60 months median follow-up), and 52 patients in the high dose rate brachytherapy treatment group (HDR) (60 months median follow-up).

Change in PSA values after radiotherapy

In each treatment group, the average PSA concentration in patients' blood falls below the upper value of the reference range of our Institute (3 ng/mL). However, for HDR-treated patients, the

average was significantly higher than values after conv. LINAC therapy at all time points for four years (Figure 1).

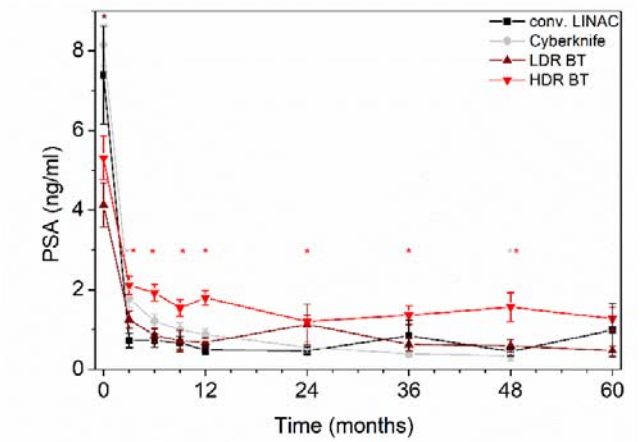

Figure 1: PSA decrease as a result of conventional LINAC (black) and Cyberknife (grey) therapy, LDR BT (dark red) and HDR BT (red). The significant differences compared to values after conv. LINAC therapy are indicated with asterisks. Error bars represent SEM.

Time-dependent chromosome aberrations

An increase in chromosome aberrations due to irradiation was observed in all groups, but we found 1.7–3.2 times more total aberrations in the groups undergoing teletherapy (17.2 ± 2.4 /100 cells LINAC, 11.5 ± 1.2 /100 cells Cyberknife three months after therapy) than in the brachytherapy groups (6.7 ± 0.5 /100 cells LDR, 5.4 ± 0.7 /100 cells HDR BT) (Figure 2a). HDR therapy caused the least aberrations. After an initial spike, aberrations started to be eliminated, but 60 months later, total aberrations still differed significantly from the baseline (p=0.003 for LDR BT; p=0.035 for LINAC). In our laboratory, 5 total aberrations /100 cells are the upper limit for a negative chromosomal aberration test when screening staff at risk of radiation exposure (13). The mean total chromosomal aberration of the groups reached this limit 24 months after Cyberknife therapy. Only 36 and 60 months after LINAC therapy, the total aberrations were below this limit (one sample test). Immediately after LDR BT, and 3, 6 and 12 months after LDR BT, total aberrations were higher than 5 aberrations /100 cells. Total aberrations after HDR were never higher than this threshold, even immediately after implantation. However, these values were significantly increased compared to the average total aberration values of the HDR patients before therapy. We also indicated the average

total aberrations of our twenty-two age-matched control subjects on the plots, (measured once, but plotted at every time-point, discussed later). The total aberration value of all radiotherapy modalities differed significantly from the value of our controls, even at baseline.

Aberrant cell frequencies showed similar time dependence as total aberration values (Figure 2b). Three months after LINAC and Cyberknife therapy, approximately 12.7 ± 1.6% and 8.7 ± 0.8% of the lymphocyte cells suffered chromosome damage. Brachytherapy techniques affected fewer lymphocytes (5.3 ± 0.4 % for LDR and 4.6 ± 0.6 % for HDR therapy, at 3 months).

Dicentric and ring aberrations (dicentrics+rings) are radiation-specific chromosomal aberrations which do not arise due to environmental conditions. Their values differed significantly from the baseline values at every time point for five years after LDR BT and conventional LINAC, for four years after Cyberknife, and for twelve months after HDR BT. The dicentric+ring value was 2.5–6.5-fold higher three months after teletherapy (7.8 ± 1.1 for conventional LINAC, 5.2 ± 0.6 for Cyberknife) than in the brachytherapy arms (2.1 ± 0.2 for LDR BT, 1.1 ± 0.2 for HDR BT). There was no difference between the baseline value of the patients (in any of the radiotherapies) and the dicentric+ring value of the controls (Figure 2c).

The chromosome break values show a similar trajectory to the values of total aberrations. These aberration values are lower after therapy, but persist for years (Figure 2d).

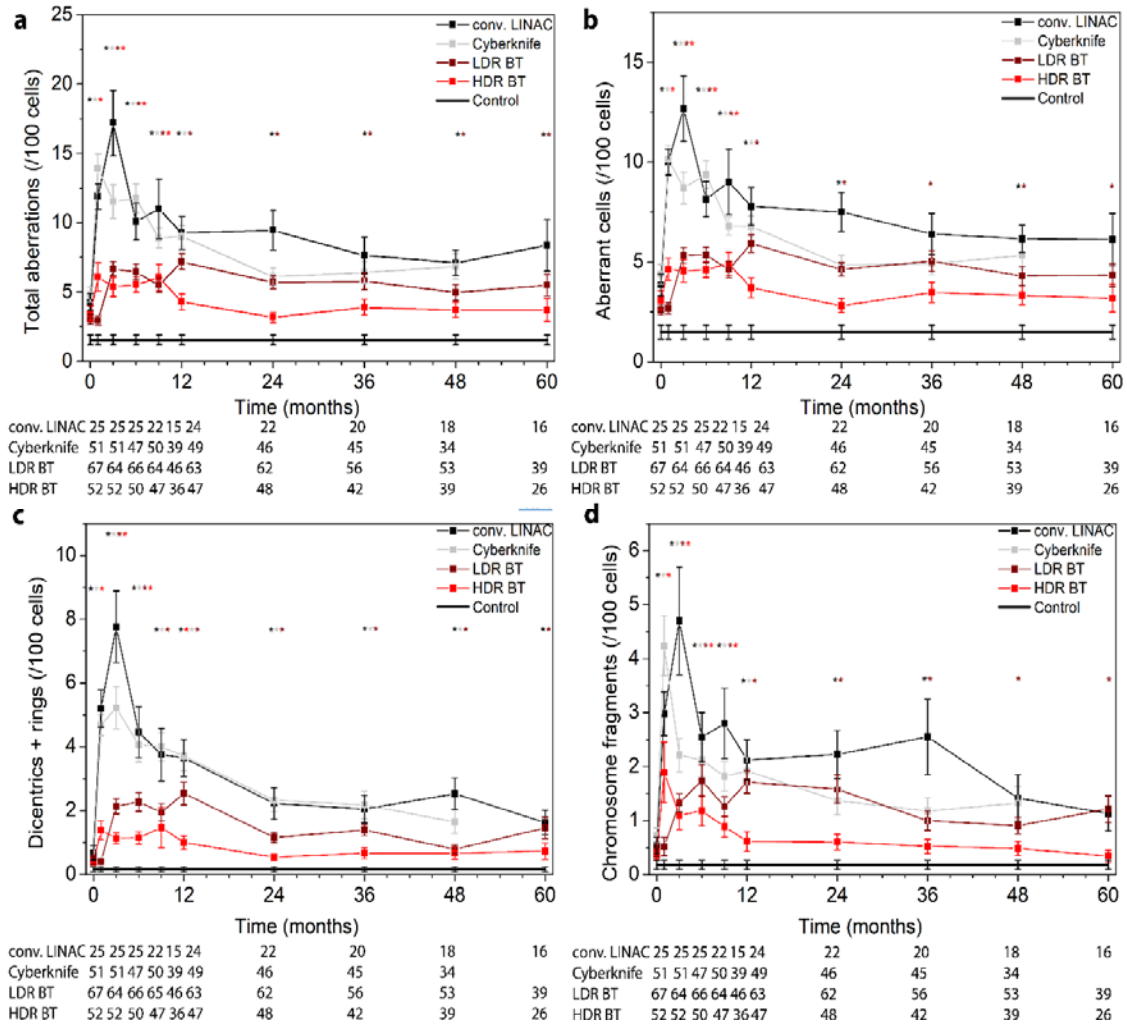

Figure 2: Chromosomal aberrations after conventional LINAC (black) and Cyberknife (grey) therapy, LDR BT (dark red) and HDR BT (red). Time dependence of a) total aberration b) aberrant cell c) dicentric+ring d) chromosome fragment frequencies are shown. The significant differences compared to the baseline value of each modality are indicated with asterisks. Values of age matched controls (one blood draw) are plotted at every time point and joined for comparison. Error bars represent SEM.

The role of baseline and treatment characteristics

We performed multivariate regression analysis to test the effect of baseline and treatment characteristics on chromosome aberrations. We decided to include all the characteristics, but omit risk as it is collinear with T status, iPSA and Gleason score, by definition. There wasn't any significant model in the analysis of chromosome aberrations at 3 months (Supplementary Table 2).

Side effects after different types of prostate cancer radiotherapy

EORTC-RTOG Grade 2 or more severe early urogenital side-effects occurred in 57.9, 64.9, 68.9, and 12.2% four years after LINAC, Cyberknife, LDR and HDR therapy, respectively. Of these, only 5.3%, 2.7% and 3.3% were Grade 3 toxicities (LINAC, Cyberknife, and LDR groups, respectively) (Figure 3a). There were no Grade 3 acute gastrointestinal side effects. Furthermore, the frequency of Grade 2 early gastrointestinal effects was 15.7% after LINAC, and 2.7% after Cyberknife therapy, and there were no Grade 2 side-effects after HDR and LDR BT (Figure 3b).

Regarding late urogenital side effects, there were 31.6% Grade ≥2 after LINAC, 35.1% after Cyberknife, 42.6% after LDR and 19.5% after HDR radiotherapy. The frequency of Grade 3 toxicities was 2.7%, 3.3% and 2.4% after Cyberknife, LDR and HDR irradiation, respectively (Figure 3c). There were only one (1.6%) grade 3 late gastrointestinal side effect among the LDR-BT patients, and Grade 2 late GI were 2.7% after Cyberknife therapy (Figure 3d).

We applied patient-reported questionnaires (PROMs) to further assess side effects. The International Prostate Syndrome Score questionnaire assessing urinary toxicities (score range: 0–35, where a higher score indicates worse side effects) was filled out by patients at each follow-up visit (Figure 3e). However, it was not expedient to administer the questionnaires directly after brachytherapy and Cyberknife treatments, because the questions ask about the symptoms of the past month. The scores increased immediately after conv. LINAC therapy (9.1 ± 1.5 to 14.5 ± 2.0). We measured an increase 3 months after LDR therapy (6.4 ± 0.6 to 14.8 ± 0.8) but no significant difference was found after HDR therapy compared to baseline (6.8 ± 0.6 to 7.1 ± 0.7) and Cyberknife (9.1 ± 0.8 to 8.0 ± 0.6). The IPSS scores decreased to values measured before therapy in the first year, except for conventional LINAC radiotherapy.

We tested patients' subjective quality of life (QoL) (score range: 0–6, where a higher score indicates worse side effects) and the scores revealed an increase immediately after the last fraction of LINAC teletherapy (2.1 ± 0.4 to 2.7 ± 0.4) and 3 months later in case of seed therapy (1.3 ± 0.1 to 2.2 ± 0.1) similar to the IPSS results (Figure 3f). There was no increase for HDR or Cyberknife. The decrease occurred later for LDR and conventional LINAC therapy, but it happened in the first year.

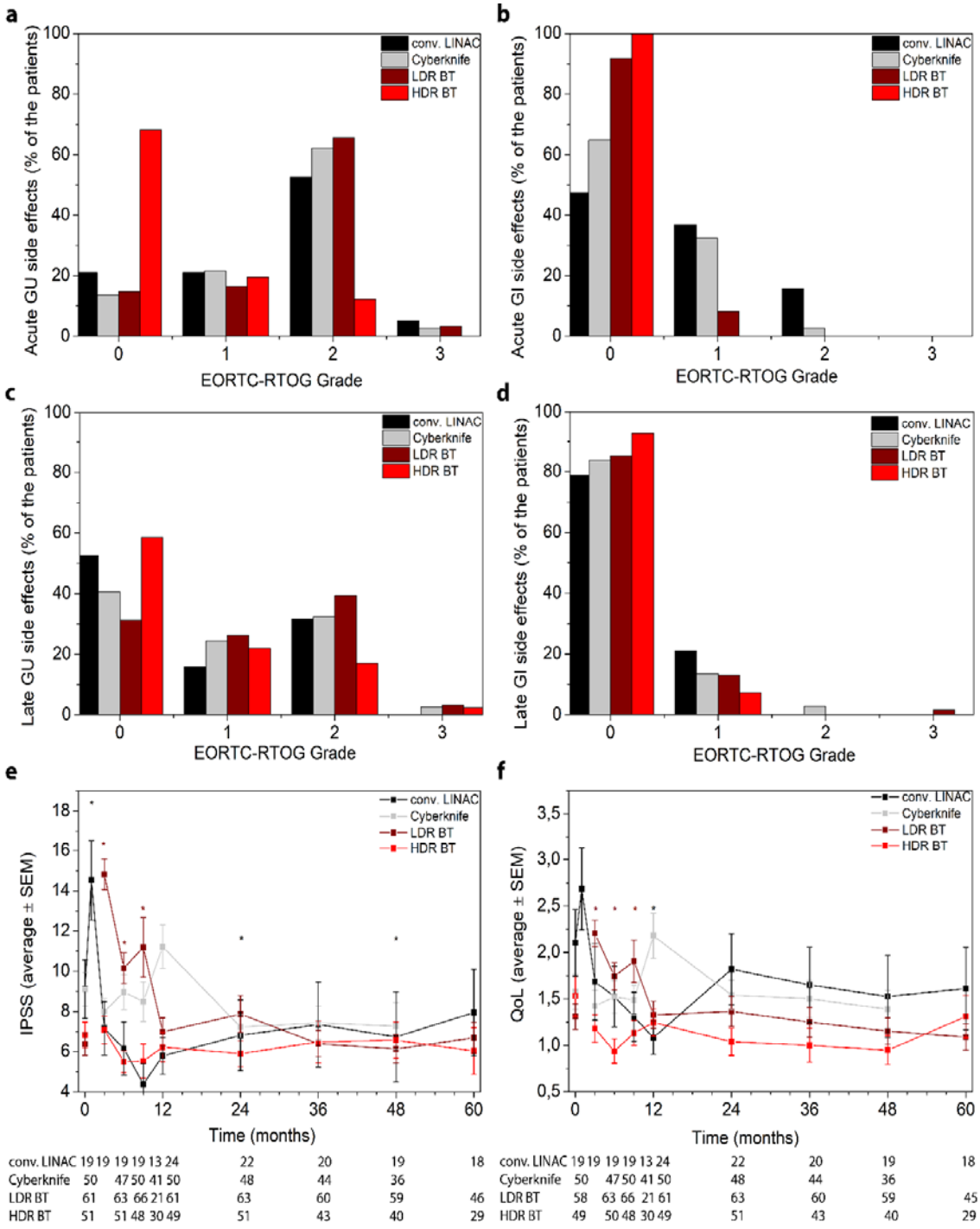

Figure 3: Side effects of conventional LINAC (black) and Cyberknife (grey) therapy, LDR BT (dark red) and HDR BT (red). a) Acute GU; b) acute GI; c) late GU; d) late GI graded side effects; e) International Prostate Syndrome Score; f) Quality of Life questionnaire scores are shown. The significant differences compared to the baseline value of each modality are indicated by asterisks. Error bars represent SEM.

<u>Connections between chromosomal aberrations and side effects</u>

We performed a multivariate regression analysis to study the connection between chromosomal aberrations and side effects in order to investigate the predictive power of chromosomal aberrations. We analyzed the correlation between the aberrations in the time points of the first year and the cumulative side effects (graded GU and GI) because, in a former publication discussed below, we found connections between in vitro (irradiation of the patients' blood before therapy) and in vivo aberrations (blood of irradiated patients) in the first year (14). We tested if chromosome aberrations are independent predictors of side effects beside the radiation dose and $V_{100\%}$ (volume which received the 100% of the prescribed dose) in order to exclude the effect of the variabilities in radiotherapy. Significant models were found: Chromosome fragments directly after conv. LINAC therapy could predict late genitourinary (GU) side effects (p=0.005). Total aberrations directly after HDR BT predicted acute cumulative GU side effects (p=0.004). Three months after Cyberknife therapy both chromosome fragments, total aberrations and aberrant cell frequency were in relation with acute GU toxicities (p=0.044; p=0.028; p=0.044 respectively). On the other hand, dicentrics + rings six months after Cyberknife was an independent predictor of late GU beside $V_{100\%}$, and prescribed dose (p=0.007). Dicentrics+rings one year after LDR BT was in connection with late gastrointestinal (GI) side effects (p=0.008).

Supplementary Table 2: Effect of baseline characteristics on chromosome aberrations

	conv. LINAC		**Cyberknife**		**LDR BT**		**HDR BT**	
	total ab.	**dic+ring**	**total ab.**	**dic+ring**	**total ab.**	**dic+ring**	**total ab.**	**dic+ring**
Model	**0.375**	**0.363**	**0.654**	**0.765**	**0.221**	**0.684**	**0.364**	**0.364**
Age	0.029	0.099	0.521	0.957	0.978	0.754	0.900	0.460
iPSA	0.044	0.036	0.666	0.257	0.101	0.348	0.645	0.124
GS	0.968	0.695	0.355	0.250	0.842	0.945	0.288	0.345
T	0.743	0.614	0.933	0.320	0.561	0.980	0.170	0.198
HT	0.500	0.599	0.471	0.879	0.085	0.246	0.145	0.510
Dose pros.	0.391	0.510	0.748	0.987			0.569	0.324
Dose vesic.	0.705	0.500	0.211	0.987				
MV	0.929	0.609						
SIB	0.427	0.614						

Multivariate models of baseline and treatment characteristics and chromosome aberrations. P values are indicated.

Discussion

Prostate cancer has the third highest morbidity among cancers, and because it does not originate from a single, preventable cause like HPV-associated cancers, its incidence will not decrease in the near future. Our working group was the first to compare four types of radiotherapy modalities in prostate treatment regarding lymphocyte damage. We analyzed nearly 195,000 cells one by one under the microscope (195 patients, 10 samples each, at least 100 cells/sample). There are only a few chromosomal aberration studies (with a long follow-up period) in the literature because the method is highly labor-intensive. We described the effect of Cyberknife therapy as well, which has emerged as a new therapeutic modality and there is a need to find its niche in prostate therapy (15).

Lymphocytes were used as surrogate normal tissue markers and suggested to be used as predictive radiosensitivity markers (3-5, 16). However, the response to radiotherapy is also dependent on the prescribed dose. We found that HDR therapy, which caused the fewest chromosomal aberrations, also showed fewer side effects, but it caused slower PSA decrease as well (although our sample size and study set-up is not suitable to draw conclusions about survival). In this study, 19 or 21 Gy was given in one fraction as monotherapy, but since the start of our study 2 x 13.5 Gy was found to be superior and to provide excellent tumor control (17). This clearly a limitation of our study. However, randomized studies were not performed comparing 21 Gy HDR brachytherapy with other fractionation schedules or LDR brachytherapy. LDR brachytherapy also caused fewer chromosomal aberrations than teletherapies, but known to cause similar biochemical relapse free survival.

Lymphopenia is caused by bone-marrow damage in case of pelvic irradiations. Bone marrow cannot receive high dose irradiation in case of brachytherapies because of the high dose gradients. In case of teletherapies, the dose-volume constraints (Supplementary Table 1) define the hip bone dose. As there is no expanded pelvic node target volume (pelvic PTV) in case of low/intermediate risk patients, the bone marrow does not receive a high dose. The assessment of lymphocytopenia was not a goal of our study, but due to increasing interest of it in the literature, we analyzed the values

available. We found approximately 3.2% lymphocytopenia (defined <1000/ul in our Centre) in the blood tests any time after radiotherapy and approximately 1.0% Grade 1 white blood count toxicity (<4000 WBC/ul). We would like to add, that this is an elderly population with a mean age of 66.8 years. Roach et al. also found that without pelvic lymph node target volume there is much less risk of hematological toxicities (18). We suggest lymphopenia or bone-marrow irradiation may not influenced our chromosome aberration results.

The difference between baseline chromosome aberrations of prostate cancer patients can be explained by the diagnostic dose they received before their radiotherapy. Most of these patients had at least a diagnostic CT and teletherapy patients also undergo planning CT before therapy. There are also results suggesting that chromosome aberrations are elevated in the blood of cancer patients (19, 20).

We investigated the connection between chromosomal aberrations and side effects to shed light on their predictive value. We corrected the analysis with prescribed dose and irradiated volume ($V_{100\%}$). The irradiated volume mirrors the size of prostate, but also the technique (because of the different safety margins). We analyzed the volumes irradiated with different doses in a previous publication and find, that the mean $V_{100\%}$ of LDR brachytherapy was 2.4 times less than conv. LINAC based therapy $V_{100\%}$-s, but $V_{10\%}$ (volume which received the 10% of the prescribed dose) of LDR was 9.3 times smaller than the value of LINAC based therapy (21). This suggests, that not the smaller prostate volumes of brachytherapy patients but the volume of healthy tissue, the different dose gradient, safety margins, etc. causes the less chromosome aberrations of brachytherapy techniques.

According to our previous publication, there is a direct correlation between in vivo aberrations measured after radiotherapy and the aberrations in the in vitro irradiated blood of these patients (taken before therapy). We found correlations between in vitro and in vivo irradiated chromosomal aberrations only in the first year. Therefore, we compared the aberrations with the cumulative side effects in the first year. We found two significant model of late genitourinary toxicities and one model of late gastrointestinal toxicities predicted by dicentrics+rings and chromosome fragments.

Furthermore, there were four model suggesting connection between acute side effects and chromosome aberrations. The results suggest that the chromosomal aberration assay may be suitable to select radiosensitive patients. On the other hand, the method is not a high-throughput technique, and other predictive tests may be more likely to be introduced into clinical use (22).

To our knowledge, there is no complex analysis of immune cell damage after different radiotherapy modalities. We found that the damage of the lymphocytes did not clear from the blood over a period from nine months up to five years (depending on the type of therapy). There have been few publications applying chromosomal aberration technique and even fewer applied long-term follow-up of radiotherapy patients because the method is highly labor-intensive. However, there are already published studies with similar conclusions, but mostly with shorter follow-up: Hille et al. found significantly more chromosomal aberrations one year after prostate LINAC radiotherapy compared to values before therapy (2). They also measured total aberration values after LINAC therapy comparable with our results. Hartel et al. found that one year after radiotherapy of prostate cancer not just translocations, which are considered long persisting aberrations, but non-transmissible aberrations detected by FISH technique (dicentrics and non-transmissible exchanges) were higher than at baseline (23). Formerly, it was found that in highly exposed plutonium workers complex chromosomal aberrations persisted for decades (24). Furthermore, non-cancer patients irradiated with old radiotherapy techniques and analyzed with a highly similar chromosomal aberrations method showed higher aberration values than the controls approximately 20–40 years after irradiation (25, 26). These studies included a higher number of patients (max. 143), but the persistence of chromosomal aberration could be interpreted as the effect of bone marrow dose.

It is to be considered that the chromosomal aberration value did not decrease in our study because the patients got older. However, according to our long-term cohort (2145 healthy people, tested with the chromosomal aberration method between 1989 and 2018) the increase is less than 0.1 total aberrations/year, which could not cause the persistence of aberrations in our follow-up

(27). Therefore, we plotted the chromosome aberration values of the healthy controls at every time point, however, measured only once.

As we mentioned above, the excess chromosomal aberration can persist in the blood for as long as 5 years. The frequency of damaged cells in the case of teletherapies is above 10% at 3 months, and around 6% after 3–5 years. On the other hand, the clinical significance of these findings needs further investigations. The chromosomal aberration method is performed on lymphocytes, which are induced to divide by phytohemagglutinin M (PHA). According to the literature, mostly T lymphocytes are sensitive to PHA (28). Furthermore, we have shown before that there is still a significant increase 3 years after LDR BT in NK cells and 2 years after seed implantation in lymphoid dendritic cells compared to the frequency before therapy (29). However, the most important implication is the impact of radiotherapy on the efficacy of immunotherapy. If immune cells targeted by immunotherapy are not present or are suppressed (in another way that can be rescued by the drug), therapeutic failure can occur. Further studies are needed to investigate which immune cell subpopulations are affected. Our work suggests that bracytherapies may hinder immunotherapy less than teletherapies. The time course of immune cell damage is also important; therefore, the schedule of complex therapies might be adjusted accordingly in the future.

Conclusion

We found that teletherapies increase the biological dose of prostate radiotherapy patients by 1.7–3.2 times compared to brachytherapies. The number of affected lymphocytes can be as high as 13% in the first 3 months. In our study, radiation-induced chromosomal aberrations in lymphocytes did not diminish even 5 years after therapy.

Declarations

Ethics approval and consent to participate

The ethical permit number is 16738-2/2015/EKU, approved by ETT TUKEB (Medical Research Council) on 03.24.2015. Both the patients and volunteers signed informed consents.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

There is no financial or non-financial competing interest.

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Authors' contributions

ZSK, PÁ, CsP and CsP conceptualized the study, GyF, GS and GYOS are participated in the investigation. KJ, LG, AH, GS and GF performed data curation. TM, ZT-N and ZJ acquired funding and supervised the study.

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