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Biology Contributions

Gamma-H2AX Foci Decay Ratio as a Stronger Predictive Factor of Late Radiation Toxicity Than Dose-Volume Parameters in a Prospective Cohort of Prostate Cancer Patients

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Abstract

Purpose: Late radiation toxicity is a major dose-limiting factor in curative cancer radiation therapy. Previous studies identified several risk factors for late radiation toxicity, including both dose-volume factors and genetic predisposition. Herein, we investigated the contribution of genetic predisposition, particularly compared with dose-volume factors, to the risk of late radiation toxicity in patients treated with highly conformal radiation therapy.

Methods and Materials: We included 179 patients with prostate cancer who underwent treatment with curative external beam radiation therapy between 2009 and 2013. Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 4.0. Transcriptional responsiveness of homologous recombination repair genes and γ-H2AX foci decay ratios (FDRs) were determined in ex vivo irradiated lymphocytes in a previous analysis. Dose-volume parameters were retrieved by delineating the organs at risk (OARs) on CT planning images. Associations between risk factors and grade $\geq$2 urinary and bowel late radiation toxicities were assessed using univariable and multivariable logistic regression analyses. The analyses were performed using the highest toxicity grade recorded during the follow-up per patient.

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Results: The median follow-up period was 31 months. One hundred and one patients (56%) developed grade ≥2 late radiation toxicity. Cumulative rates for urinary and bowel grade ≥2 late toxicities were 46% and 17%, respectively. In the multivariable analysis, factors significantly associated with grade ≥2 late toxicity were transurethral resection of the prostate (P = .013), γ-H2AX FDR <3.41 (P = .008), and rectum V70 >11.52% (P = .017).

Conclusions: Our results suggest that impaired DNA double-strand break repair in lymphocytes, as quantified by γ-H2AX FDR, is the most critical determining factor of late radiation toxicity. The limited influence of dose-volume parameters could be due to the use of increasingly conformal techniques, leading to improved dose-volume parameters of the organs at risk.

Introduction

After conventional external beam radiation therapy (EBRT) for prostate cancer, approximately 30% of patients develop moderate or severe late radiation toxicity.[1-5] Symptoms are often chronic and may significantly affect quality of life.[6,7] Common toxicities include urinary urgency and frequency, hematuria, urinary retention, and rectal bleeding.

Many risk factors for late radiation toxicity have been described. However, after decades of research, a reliable method to predict individual risk for radiation toxicity is still lacking. Accurate prediction would allow for personalized treatment and, consequently, might prevent the development of severe late side effects. With the help of personalized radiation therapy, patients at high risk for late radiation toxicity may receive lower doses of radiation or alternatively could be treated by surgery or brachytherapy, leading to reduced morbidity rates. By contrast, in patients expected to experience no or very limited toxicity, dose escalation should be considered to increase tumor control.

Both radiation dose and irradiated volume are important risk factors for the development of late radiation toxicity.[1,2,8] Suggested patient-related risk factors include higher age, prior transurethral resection of the prostate (TURP), previous abdominal surgery, and comorbidities such as diabetes mellitus and cardiovascular disease.[8-10] Of interest, clinicians have observed considerable variability in normal tissue toxicity between clinically comparable patients receiving the same regimen. In line with these observations, it is widely recognized that genetic factors influence radiation sensitivity. In fact, there is increasing evidence demonstrating that genetic predisposition is an important risk factor.[11-15]

DNA double-strand breaks (DSBs) are the most harmful lesions caused by ionizing radiation. The kinetics of DSBs can be monitored over time by the immunofluorescent detection of phosphorylated histone H2AX (γ-H2AX) because the phosphorylation of this histone is one of the earliest markers of DNA DSBs. The detection of γ-H2AX is a commonly used assay for determining DNA damage repair proficiency in experimental radiobiology research. However, in translational research, it has predominantly been applied in small studies wherein patients without symptoms were compared with patients with severe toxicity.[14,16-21]

In the prospective study of van Oorschot et al, radiation toxicity after EBRT for prostate cancer was related to a less efficient DNA DSB repair in ex vivo irradiated lymphocytes.[11] The efficiency of DSB repair was measured by the ratio of γ-H2AX foci found at 30 minutes and the number of γ-H2AX foci at 24 hours after irradiation. As a result, less efficient repair was reflected by a lower γ-H2AX foci decay ratio (FDR). Based on an FDR threshold of 3.41, as determined in a previous retrospective study, patients with severe radiation toxicity could be discriminated from patients without toxicity fairly accurately.[11,14] In line with this result, a less efficient DNA damage response was also reflected by a reduced transcriptional responsiveness to radiation of genes of the homologous recombination (HR) pathway.[11]

To date, the relevance of genetic predisposition as a risk factor for late radiation toxicity has remained unclear. This could be because the contribution of genetic predisposition to late radiation toxicity compared with dose-volume parameters has not often been investigated. Specifically, the contribution of genetics to the development of radiation toxicity may be biased by unfavorable dose-volume parameters. In the past, dose-volume parameters were the dose-limiting determinants of radiation therapy; with increasingly conformal radiation therapy techniques, it is assumed that the role of dose-volume parameters will steadily diminish. In the present study, we aimed to unravel the importance of genetic predisposition to developing late radiation toxicity compared with other factors, particularly dose-volume parameters, in prostate cancer patients.

Methods and Materials

Patients

The present study was performed using patient data from a previous prospective study by van Oorschot et al.[11] This previous study was conducted to investigate correlations between clinical and biological parameters and toxicity after radiation therapy for prostate cancer. This study was approved by the local medical ethics committee. Between 2009 and 2013, 200 patients were recruited at the Academic Medical Center, and data from 198 patients were analyzed. Eligible patients had histologically confirmed prostate cancer and underwent EBRT with curative intent. Clinical

parameters recorded included baseline prostate-specific antigen (PSA), age, body mass index (BMI), Karnofsky Performance Status, Gleason score, T-stage, prostate volume, baseline medication, several comorbidities, hormonal treatment, presence of pretreatment symptoms, and smoking behavior. After written informed consent was obtained, blood was drawn from all patients before initiating treatment. Lymphocytes were isolated using Ficoll gradient separation and stored in liquid nitrogen.

The present analysis was limited to 179 patients. Excluded patients either underwent EBRT combined with brachytherapy or were lost to follow-up within the first 3 months after the end of treatment, and therefore were not available for late toxicity evaluations. All patients were treated with intensity modulated radiation therapy (IMRT), and the most frequently prescribed dose was 77 Gy in 35 fractions. To achieve consistent filling of the bladder and rectum during simulation and daily treatment, patients were instructed to empty their rectum and urinate 1 hour before radiation therapy followed by intake of 500 mL of water.

γ-H2AX foci assay

Lymphocytes were thawed and irradiated with 1 Gy γ-rays from a ^{137}Cs source. Induction and decay of radiation-induced γ-H2AX foci were assessed in unstimulated G(0) cells. The FDR was determined by dividing the number of γ-H2AX foci 30 minutes after irradiation by the number of γ-H2AX foci 24 hours after irradiation. Immunohistochemistry, scoring of foci, and FDR threshold determination were performed using previously published methods.[11,22]

Microarray analysis

Lymphocytes were cultured and stimulated with phytohemagglutinin. After 2 weeks, half of the cells were irradiated with 2 Gy γ-rays from a ^{137}Cs source, and the other half was left untreated. RNA isolation was performed 24 hours after irradiation. Biotin-labeled cRNA probes were generated, and RNA was hybridized to the HT HG-U133+ PM GeneChip arrays. Scanning of the array was conducted by the MicroArray Department of the University of Amsterdam, and images were processed to obtain an intensity value for each oligonucleotide probe. An HR gene set was used to determine differences in radiation response (ie, fold induction) between patients from 4 toxicity groups; specifically, patients with grade 0, 1, 2, or $\geq$3 adverse events. This HR gene set consisted of 9 genes: ATRX, LIG1, RAD51, RAD52, XRCC2, XRCC3, BRCA1, BRCA2, and MDC1. Microarray and gene set enrichment analyses were performed using previously published methods.[11] The microarray data have been deposited in the NCBI Gene Expression Omnibus in a MIAME compliant format and are accessible under GEO series accession number GSE85570.

Delineations and dose-volume histogram calculations

Delineation of the organs at risk (OARs) was performed by a trained physician to maintain consistency in volume definition. Delineation was performed on CT planning images using the RayStation (v8.99) software. The OARs included the anal canal, rectum, bowel bag, bladder, and penile bulb. The anal canal was defined from the anal verge to the level of the levator muscles. There the rectum started, and it ended superiorly before losing its round shape in the axial plane and connecting with the sigmoid. The rectum and bladder were both contoured as solid organs. The bowel bag was defined from the level of the most inferior bowel loop, or just above the rectum, whichever was the most inferior. The rectum was excluded as part of the bag when both a bowel loop and rectum were present in that slice. Anteriorly, contouring stopped at a level that was no longer exposed to any dose according to the plan evaluation. The penile bulb was defined according to the Radiation Therapy Oncology Group consensus definition, as the round-shaped portion of the bulbous spongiosum posterior to the urethra.[23,24] Dose-volume histograms (DVHs) of all OARs were calculated for the clinically approved dose distribution that was used for treatment. The bowel bag variables were expressed as absolute volumes; for instance, the amount of milliliter receiving 30 Gy or more (bowel bag V30). Other variables were expressed as relative volumes; for example, the percentage of the rectum receiving 60 Gy or more (rectum V60). For the analyses throughout the article we used physical dose.

Assessment of bowel and urinary toxicity

Toxicity was graded at the end of treatment and every 6 months thereafter by the attending physician according to the Common Terminology Criteria for Adverse Events version 4.0.[25] Toxicity grades were rated relative to baseline symptoms (ie, only new events were reported). One senior staff radiation oncologist reviewed all reported toxicities to adjust for possible interpretation differences among physicians. Erectile dysfunction was disregarded as possible radiation toxicity because most patients also received androgen deprivation therapy. Late side effects were defined as those appearing more than 3 months after the completion of radiation therapy.

Statistical analysis

The analyses were performed using the highest toxicity grade recorded during the follow-up per patient. Numeric values were analyzed using the independent samples t test or one-way analysis of variance, depending on the number of groups. Categorical data were assessed using the χ^2 test. Univariable logistic regression was performed to identify

factors associated with grade ≥2 toxicity. Patients were grouped according to the occurrence (or absence) of moderate to severe toxicity. Bowel and urinary toxicity was aggregated to provide sufficient power to allow a multivariable logistic regression analysis. Factors were selected based on a literature search and prior biological findings for this cohort. Continuous factors, except for BMI, were dichotomized as less than or equal to versus more than the mean value of the particular factor for the total cohort, as this would ensure comparable group sizes. As for BMI, patients with normal weight or underweight (BMI <25) were compared with those who were overweight or obese (BMI ≥25). Covariates that were potentially associated with grade ≥2 toxicity in univariable analyses (ie, $P \leq .10$) were evaluated in multivariable logistic regression analysis using the backward conditional method. The odds ratio (OR) was used to express the strength of the association between each factor and late toxicity. A 2-sided P value of ≤ .05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp. Released 2019. Armonk, NY).

Results

Patients and treatment

The median follow-up time was 31 months (interquartile range [IQR] 22-40). The median follow-up for the group without (n = 78) and with grade ≥2 toxicity (n = 101) was 29.5 months (IQR 19-38.25) and 33 months (IQR 26.5-43.5), respectively. Androgen deprivation therapy was administered to 86% (n = 154) of the patients. The most frequently (n = 170) prescribed radiation therapy dose was 77 Gy in 35 fractions (EQD2 80 Gy); 6 patients received a dose of 70 Gy in 35 fractions (EQD2 70 Gy), 2 patients received a dose of 64.6 Gy in 19 fractions (EQD2 83 Gy), and 1 patient received a dose of 46 Gy in 23 fractions on the pelvic area, followed by a prostate boost of 24 Gy in 12 fractions (EQD2 70 Gy). The correlation between patient characteristics and the incidence and severity of toxicity showed that prior TURP was not equally distributed among the groups of patients (P = .032; Table 1).

Late radiation toxicity

During follow-up, grade 2 and grade ≥2 late radiation toxicities were recorded in 91 (51%) and 101 patients (56%), respectively. Of the 101 patients with grade ≥2 late toxicity, 11% had both urinary and bowel grade ≥2 symptoms. The cumulative late urinary grade ≥2 toxicity was 46%, and the cumulative late bowel grade ≥2 toxicity was 17%. Among the 101 patients with grade ≥2 late toxicity, 17 patients developed late toxicity grade 3 and 1 of these 17 patients also developed late toxicity grade 4. Grade 2 and grade ≥3 toxicity events are shown in Table 2.

γ-H2AX foci decay ratios

The kinetics of radiation-induced DNA DSBs were determined in ex vivo irradiated lymphocytes by detecting γ-H2AX foci 30 minutes and 24 hours after irradiation, as illustrated in Fig. 1. At least 100 cells per patient per condition were counted. With the FDR threshold of 3.41 a Mantel-Haenszel linear-by-linear association χ^2 test revealed that the proportion of patients with a low FDR increases with severity of late toxicity (P = .001). The sensitivity and specificity of the FDR threshold of 3.41 were 79% and 42%, respectively. The odds of developing grade ≥2 toxicity were 2.79 times greater for patients with an FDR <3.41 versus patients with an FDR ≥3.41 (P = .002; Table 3).

Gene expression of HR DNA repair genes

The transcriptional responsiveness of the previously established HR gene set was examined in all patients. A (negative) correlation between mean fold induction and severity of toxicity was not found; the mean fold induction levels of the HR set as a single value per grade increase of toxicity were 0.032, −0.004, 0.030, and −0.038, respectively.

Dose-volume parameters

The average rectal V50 to V75 values were not significantly different between the group of patients without and those with grade ≥2 bowel toxicity (Table 4). When the patients were divided based on their rectal V70, either less than or equal to or more than the mean V70 of the total cohort (11.52%), the proportion of patients with grade ≥2 bowel toxicity was significantly higher in the group with V70 >mean (P = .020; Fig. 2). For the other parameters (ie, rectal V50, V55, V60, V65, and V75), this was not the case.

Analyses of the distribution of dose-volume parameters did not show an overrepresentation of patients with favorable dose-volume parameters in the protective γ-H2AX group (ie, FDR ≥3.41). For example, the mean rectum V70 was 10.7% among patients with FDR ≥3.41 versus 11.9% among patients with FDR <3.41 (P = .274). Furthermore, out of 54 patients with a favorable FDR, 20 patients had an unfavorable V70 (ie, >11.52%), and 34 patients had a favorable V70 (P = .081).

For the bladder, the average values of V50, V55, V60, V65, V70, and V75 were not significantly different between patients without and those with grade ≥2 urinary toxicity. Moreover, when groups were formed based on having a V value less than or equal to or more than the corresponding mean V value, the proportion of patients with grade ≥2 toxicity was not significantly different between the groups. Similarly, we did not find a correlation between late toxicity and dose-volume parameters for the other OARs: anal canal dose (V5, V10, V25, V30, V40, V65, mean dose), and grade of bowel toxicity; penile bulb dose (D70, D90, mean dose), and grade of urinary toxicity; and bowel bag dose

Table 1 Clinical characteristics of 179 patients with prostate cancer, displayed per maximum grade of toxicity as determined according to the CTCAE version 4.0

Variable		Grade 0 (n = 47)		Grade 1 (n = 31)		Grade 2 (n = 84)		Grade ≥ 3 (n = 17)		*P* value
		n (%)	Mean (range)	n (%)	Mean (range)	n (%)	Mean (range)	n (%)	Mean (range)	
Age (y)			67.7 (54-83)		69.8 (54-86)		70.0 (45-83)		68.6 (54-77)	.323
BMI (kg/m^2)			28.0 (21.3-38.3)		26.5 (19.4-31.5)		26.6 (17.4-38.0)		27.2 (21.3-42.9)	.247
KPS			98.0 (80-100)		97.9 (90-100)		96.8 (80-100)		97.1 (80-100)	.569
Gleason score			7.5 (6-9)		7.2 (6-9)		7.3 (6-10)		7.4 (6-9)	.643
PSA* (ng/mL)			34.8 (2.9-309.0)		33.4 (3.9-457.3)		27.3 (1.6-250.0)		38.5 (1.4-349.1)	.831
Prostate size (cc)			57 (26-108)		58 (22-154)		63 (20-141)		67 (28-195)	.478
EQD2† (Gy)	70	1 (2)		2 (7)		3 (4)		1 (6)		.789
	80	46 (98)		28 (90)		80 (95)		16 (94)		
	83	0 (0)		1 (3)		1 (1)		0 (0)		
Hormone therapy	Yes	42 (89)		27 (87)		70 (83)		15 (88)		.791
	No	5 (11)		4 (13)		14 (17)		2 (12)		
T-stage	T1	11 (24)		6 (19)		8 (10)		4 (24)		.542
	T2	16 (34)		11 (35)		40 (47)		7 (41)		
	T3	17 (36)		12 (39)		33 (39)		6 (35)		
	T4	3 (6)		2 (7)		3 (4)		0 (0)		
Abdominal surgery	Yes	15 (32)		6 (19)		17 (20)		7 (41)		.165
	No	32 (68)		25 (81)		67 (80)		10 (59)		
TURP	Yes	5 (11)		1 (3)		16 (19)		5 (29)		.048
	No	42 (89)		30 (97)		68 (81)		12 (71)		
Use of urinary medication*,§	Yes	8 (17)		5 (16)		25 (30)		3 (18)		.238
	No	39 (83)		26 (84)		59 (70)		14 (82)		
Diabetes mellitus	Yes	11 (23)		3 (10)		16 (19)		2 (12)		.406
	No	36 (77)		28 (90)		68 (81)		15 (88)		
Intestinal disease	Yes	1 (2)		0 (0)		5 (6)		1 (6)		.429
	No	46 (98)		31 (100)		79 (94)		16 (94)		
Cardiovascular disease	Yes	32 (68)		19 (61)		54 (64)		13 (76)		.721
	No	15 (32)		12 (39)		30 (36)		4 (24)		
Use of anticoagulants/ antiaggregants*	Yes	17 (36)		7 (23)		27 (32)		6 (35)		.631
	No	30 (64)		24 (77)		57 (68)		11 (65)		
Current smoking	Yes	9 (19)		3 (10)		16 (19)		2 (12)		.596
	No	38 (81)		28 (90)		68 (81)		15 (88)		

Abbreviations: BMI = body mass index; CTCAE = Common Terminology Criteria for Adverse Events; EQD2 = equivalent dose in 2 Gy fractions; KPS = Karnofsky Performance Status; PSA = prostate-specific antigen; TURP = transurethral resection of the prostate.

* Before radiation therapy.

† We used an alpha/beta ratio of 3 Gy.

§ Selective alpha-1 blockers, 5-alpha-reductase inhibitors, urinary antispasmodics, and combination drugs.Patients are grouped based on their highest toxicity grade during follow-up. Erectile dysfunction was disregarded as possible radiation toxicity because most patients also received hormonal therapy.

Table 2 Type of grade 2 and grade ≥3 adverse events as determined according to the CTCAEv4, in 91 and 17 patients, respectively

Toxicity	Grade 2 (n = 91)	Grade ≥3 (n = 17)
Bowel		
Rectal hemorrhage	5	7
Diarrhea	2	1
Proctitis	15	
Bladder		
Urinary tract obstruction	3	
Hematuria	2	3
Frequency	12	
Retention	33	6
Dysuria	20	
Urinary incontinence	6	1
Cystitis	4	1
Bladder spasms	1	

Abbreviation: CTCAEv4 = Common Terminology Criteria for Adverse Events version 4.0.

One hundred and three grade 2 events were recorded in 91 patients and 19 grade ≥3 events were recorded in 17 patients. A repeated symptom was counted as a single event. Of the 17 patients with grade ≥3 toxicity, 7 also had one or more grade 2 toxicity adverse events.

(V5, V10, V15, V20, V25, V30, V35, V40, V45), and grade of toxicity (data not shown).

Risk factors according to univariable and multivariable analysis

In the univariable logistic regression analysis, risk factors for the development of grade ≥2 toxicity were T2-stage (P = .044), TURP (P = .019), γ-H2AX FDR <3.41 (P = .002), and rectum V70 >11.52% (P = .010; Table 3). Independent risk factors for the development of grade ≥2 toxicity identified in the multivariable analyses were TURP (P = .013), γ-H2AX FDR <3.41 (P = .008), and rectum V70 >11.52% (P = .017; Table 3).

Discussion

Late radiation toxicity is a major dose-limiting factor in curative cancer radiation therapy. In this prospective study of patients irradiated for prostate cancer, we found that impaired DNA DSB repair, corresponding to a lower γ-H2AX FDR in ex vivo irradiated lymphocytes, was the most significant risk factor for late bowel and urinary toxicity grade ≥2, more so than dosimetric risk factors or prior TURP.

In a previous prospective study of patients irradiated for prostate cancer, late radiation toxicity was found to be associated with impaired DNA DSB repair.[11] A limitation of this prior study was that the contribution of radiation dose and volume to the OARs may have been underestimated, particularly for the bowel and bladder, and therefore the role of genetic predisposition could have been overestimated. Therefore, in this study, we carefully redelineated the OARs on the original simulation CT scans and superimposed the delivered radiation plans to combine detailed dosimetric factors and clinical factors into the genetic analysis.

In accordance with the previous study, the multivariable analysis performed herein confirmed that the ability of lymphocytes to repair radiation-induced DNA DSBs correlates with the presence of toxicity. Specifically, an FDR below the threshold of 3.41 was correlated with the presence of grade ≥2 bowel and urinary toxicity. Originally, the FDR threshold was determined in a retrospective patient cohort. This retrospective study included patients with either severe late radiation toxicity (grade ≥3) or no radiation toxicity (grade 0).[14] In a subsequent prospective cohort, 82% of grade ≥3 and 64% of grade 0 patients could be correctly classified based on this threshold.[11] However, to be of use in the clinic, the threshold should discriminate between subgroups of the entire population. For this reason, we decided to transform toxicity into a binary outcome with patients having either grade ≥2 or grade <2 toxicity. Despite the different application of the previously established γ-H2AX FDR threshold, we found that an FDR below the threshold was associated with a higher incidence of grade ≥2 toxicity.

Svensson et al found a clear association between the development of late radiation toxicity and the gene expression responses of ex vivo irradiated lymphocytes.[13] A prediction model based on affected gene sets correctly classified 55% of the patients with high certainty. This retrospective study was restricted to the extreme responders (ie, prostate cancer patients with either severe late radiation toxicity [n = 21] or no toxicity at all [n = 17]). Inspired by this study, we have retrospectively and prospectively compared transcriptional responsiveness between patients without (grade 0) and patients with severe late radiation toxicity (grade ≥3), in both single genes and gene sets of the HR and nonhomologous end joining repair pathways.[11,14] In both studies, a significantly stronger induction of the HR repair gene set was observed in patients without toxicity compared with those with severe late radiation toxicity.[11,14] In the present analysis, we unexpectedly did not find a correlation between toxicity grade and the induction level of the HR repair gene set in ex vivo irradiated lymphocytes. This may be explained by inclusion of patients with all toxicity grades. Because there was no trend in mean fold induction per grade increase in toxicity, we could not identify fold induction as a risk factor.

Opposed to messenger RNA, proteins represent the functional effectors of radiation induced damage. The advantage of a functional assay, such as the γ-H2AX-assay, is that it reflects the net effect of all genetic and other cell-intrinsic

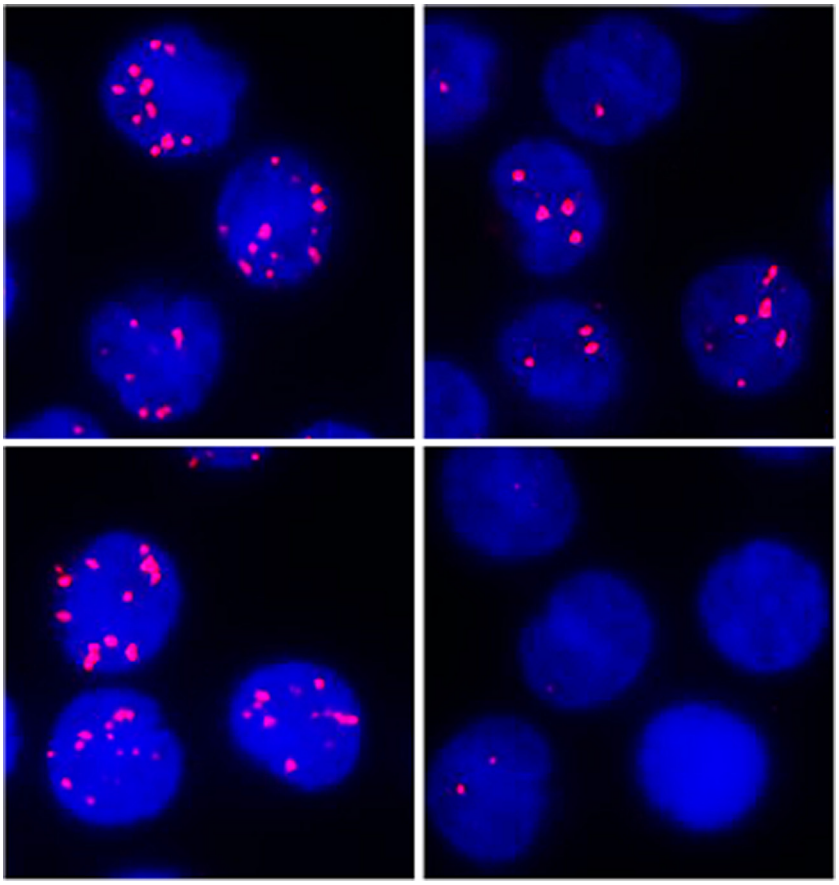

Fig. 1. Visualization of γ-H2AX foci (red) in lymphocytes at 30 minutes (left panel) and 24 hours (right panel) after 1 Gy of ex vivo irradiation. Each focus represents a single DNA DSB. At 24 hours after ionizing radiation, more γ-H2AX foci were observed in patients with severe late toxicity (upper row) compared with patients without late toxicity (lower row).

factors, including gene expression differences. This may explain why the γ-H2AX FDR is a more discriminating factor for late radiation toxicity than the expression of the HR gene set.

Rectum V70 was identified as a significant dosimetric risk factor. Remarkably, no significant associations between other dose-volume parameters and late toxicity were observed. The volume of the rectum receiving high doses (≥60 Gy) has consistently been associated with a risk of grade ≥2 rectal toxicity.[26,27] Dose-volume tolerance data for other OARs are scarce; however, urinary toxicities also seem to occur mainly in high-dose areas.[28-30] The lack of significant dose-volume parameters other than rectum V70 may be partly explained by the relatively low rectum and bladder volumes receiving high doses. The use of increasingly conformal treatment techniques enables irradiation of the prostate at a higher dose, and the doses to the OARs remain minimal. For example, only 4 patients in our study had a "high-risk rectal DVH," as defined by Valdagni et al as V70 >25% and V50 >60%.[31] For the bladder, the number of patients with V75 >25%, V70 >35%, or V65 >50% was 0, 2, and 1, respectively (solid bladder constraints from the conventional fractionation arm of the Radiation Therapy Oncology Group 0415 study).[30,32]

In the present study, we identified prior TURP as a significant clinical risk factor for late radiation toxicity. In our previous study, this factor was not statistically significant.[11] One explanation could be that, in the present study, toxicity was transformed into the outcome of either the presence or absence of grade ≥2 toxicity. Prior TURP has been described to be associated with late (urinary) toxicity in several studies[8,33,34]; therefore, in most centers, EBRT is not planned directly after TURP. For other factors, there is a lack of consistent evidence in independent studies.[8,9,35] This may be partly due to the large variety of methods used to evaluate toxicity. Other explanations for the conflicting results are the retrospective nature of many studies, small sample sizes, and a short follow-up period.

With a median follow-up of 31 months (IQR 22-40), a relatively short follow-up period was also a limitation of our study. Toxicities can take up many years to develop; therefore, the rates may have been underestimated.[36,37] Nevertheless, the median follow-up for the separate groups did not differ significantly; therefore, a higher incidence of toxicities cannot be attributed to follow-up duration. A second limitation is that DVHs were obtained from a single CT scan. Due to inter- and intrafractional variations in rectal and bladder filling as well as setup variability, one

Table 3 Univariable and multivariable analysis of (potential) risk factors for moderate to severe late bowel and urinary toxicity after EBRT for prostate cancer

	Univariable			Multivariable		
Factor	OR	95% CI	*P*	OR	95% CI	*P* value
Age, y: >69 (vs age ≤69)	1.62	0.89-2.94	.112			
Smoking: current smokers (vs never smoked or quitted)	1.17	0.53-2.61	.699			
BMI: <25 (vs BMI ≥25)	1.61	0.87-2.97	.129			
Prostate size, cc: >61 vs ≤61)	1.39	0.75-2.59	.298			
T-stage (vs stage 1)						
T2	2.47	1.03-5.93	.044			
T3-T4	1.75	0.74-4.16	.205			
TURP: yes (vs no)	3.15	1.20-8.24	.019	3.60	1.31-9.89	.013
Urinary medication: yes (vs no)	1.92	0.92-4.01	.084			
Abdominal surgery: yes (vs no)	0.85	0.43-1.67	.629			
Diabetes mellitus: yes (vs no)	0.99	0.46-2.14	.982			
Cardiovascular disease: yes (vs no)	1.04	0.56-1.95	.894			
Anticoagulants/antiaggregants: yes (vs no)	1.09	0.58-2.06	.786			
γ-H2AX FDR: <3.41 (vs ≥3.41)	2.79	1.45-5.39	.002	2.53	1.27-5.04	.008
Log_2 fold change HR set: <0.018* (vs ≥0.018)	0.92	0.51-1.67	.788			
Bladder V65: >17.20%* (vs ≤17.20%)	1.48	0.80-2.74	.209			
Bladder V70: >11.42%* (vs ≤11.42%)	1.45	0.78-2.69	.241			
Rectum V60: >34.64%* (vs ≤34.64%)	1.51	0.83-2.75	.180			
Rectum V65: >25.56%* (vs ≤25.56%)	1.56	0.86-2.86	.142			
Rectum V70: >11.52%* (vs ≤11.52%)	2.22	1.21-4.08	.010	2.18	1.15-4.14	.017
Rectum V75: >0.61%* (vs ≤0.61%)	1.58	0.81-3.05	.178			
Anal canal V25: >40.20%* (vs ≤40.20%)	1.36	0.75-2.47	.305			
Penile bulb D90: >13.22 Gy* (vs ≤13.22 Gy)	1.26	0.66-2.42	.489			
Bowel bag V10: >70.70 mL* (vs ≤70.70 mL)	1.19	0.64-2.20	.584			

Abbreviations: Anal canal V25 = volume (%) of the anal canal receiving 25 Gy or more; bladder V65 = volume (%) of the bladder receiving 65 Gy or more; BMI = body mass index; bowel bag V10 = volume (mL) of the bowel bag receiving 10 Gy or more; CI = confidence interval; EBRT = external beam radiation therapy; FDR = foci decay ratio; HR = homologous recombination; OR = odds ratio; penile bulb D90 = the minimum dose received by 90% of the penile bulb volume; TURP = transurethral resection of the prostate; rectum V65 = volume (%) of the rectum receiving 65 Gy or more.

* Mean value for the total cohort.For the univariable analysis, only dose-volume parameters with *P* values < .3 are presented. When all parameters for a certain OAR had *P* values >.3, the parameter with the lowest *P* value was presented.For the multivariable analysis, only significant risk factors are presented.

(simulation) CT scan is unlikely to represent the true dose distributions to the OARs during the treatment course. Yet, a strength of this study is that all OARs were delineated by the same physician to avoid interobserver variability. A

Table 4 Comparison of rectal dose and grade of bowel toxicity as determined according to the CTCAEv4

Variable	Grade 0-1 patients (n = 149)	Grade ≥2 patients (n = 30)	*P* value
V50	51.00 ± 14.59	50.24 ± 16.67	.799
V55	42.83 ± 13.93	43.32 ± 15.57	.863
V60	34.43 ± 13.03	35.70 ± 12.72	.626
V65	25.37 ± 10.90	26.49 ± 8.40	.593
V70	11.39 ± 6.43	12.19 ± 5.86	.529
V75	0.60 ± 0.98	0.62 ± 0.83	.945

Abbreviations: CTCAEv4 = Common Terminology Criteria for Adverse Events version 4.0; V50 = volume of the rectum receiving 50 Gy or more; V55 = volume of the rectum receiving 55 Gy or more.

All data represent mean volumes shown as percentage with standard deviation.

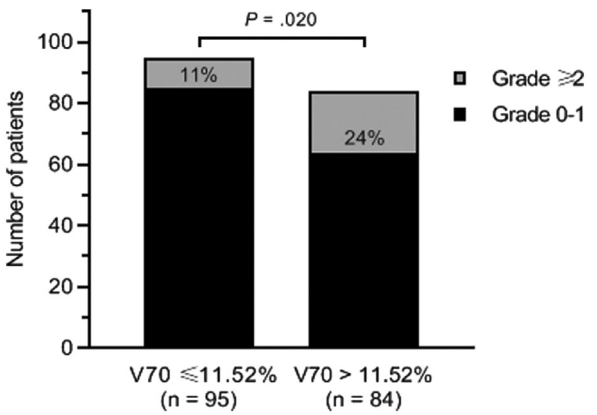

Fig. 2. Association between rectum V70 and bowel toxicity according to Common Terminology Criteria for Adverse Events version 4.0. When patients are divided into 2 groups, V70 less than or equal to or >11.52% (mean V70 of the total cohort), grade ≥2 bowel toxicity was significantly more frequent in the group with V70 >11.52%.

third limitation is that radiation toxicity as a primary outcome is somewhat difficult to determine in general because symptoms attributed to late radiation toxicity could originate from other genitourinary and gastrointestinal causes. Despite these limitations, we were able to identify several significant risk factors for moderate to severe late urinary or bowel toxicity in patients with prostate cancer treated with EBRT.

Conclusions

We showed that genetic predisposition independently affects the risk of late radiation toxicity. In particular, our results show that a less efficient repair of DNA DSBs, as quantified by the γ-H2AX FDR, is an independent risk factor for the development of late bowel and urinary toxicity. In this specific cohort of prostate cancer patients, γ-H2AX FDR was a stronger predictor of late radiation toxicity than the dose and volume parameters of the OARs; only rectum V70 was identified as a significant dosimetric risk factor.

To the best of our knowledge, our study is the first to show that γ-H2AX FDR measurement is a useful tool for predicting late toxicity risk in individuals when considering other important risk factors such as dose-volume parameters. The γ-H2AX assay is a robust, inexpensive, and reproducible technique. To further validate γ-H2AX FDR as a predictive marker, a new prospective study involving the analysis of risk factors is currently in progress. In addition to patients with prostate cancer, this study will also include patients with cervical cancer. We hope to validate our findings within this broader population of patients, with the final aim of developing a reliable predictive model to support decision making in radiation therapy practice.

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