

IMPACT OF RADIOTHERAPY PARAMETERS ON THE RISK OF LYMPHOPENIA IN UROLOGICAL TUMORS: A SYSTEMATIC REVIEW OF THE LITERATURE

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ABSTRACT

BACKGROUND

We investigated how radiotherapy (RT) parameters may contribute to the risk of lymphopenia in urological tumors and we discussed how this may impact clinical outcomes.

MATERIAL & METHODS

A systematic review was performed according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines. The PubMed, Embase and ISI Web Of Knowledge databases were searched. Study quality was assessed according to the Newcastle-Ottawa Scale.

RESULTS

Overall, 8 articles reporting on a total of 549 urological cancer patients met the inclusion criteria. The pooled mean incidence of acute severe lymphopenia (absolute lymphocyte count < 500 cells/ μ L) was 17.1%. Extended radiation volumes may lead to an increased risk of developing lymphopenia. Medium-high doses (\geq 40 Gy) to the whole pelvic (odds ratio (OR) = 1.01; 95% confidence interval (CI) 1.00-1.01; p-value = 0.025) and iliac (OR = 1.04; 95% CI 1.01-1.08; p-value = 0.009) bone marrow (BM) were associated with acute grade 3 and late grade 2 lymphopenia, respectively.

CONCLUSION

Multiple studies reported high and severe incidences of lymphopenia. Minimizing radiation volumes and unintentional irradiation of pelvic BM may reduce the incidence of lymphopenia, potentially improving clinical outcomes. More research is needed to further elucidate these findings and effectively implement recently developed new risk assessment tools.

KEYWORDS

Radiotherapy; Dosimetry; Lymphopenia; Lymphocyte count; Urologic neoplasms

HIGHLIGHTS

- RT may unintentionally cause lymphopenia.
- The mean pooled incidence of ASL (n = 474) was 17.1%.
- The extent of the target volume and dose to the pelvic BM should be limited.
- Tools to assess and limit the risk of lymphopenia are of particular interest.

INTRODUCTION

Improvements in radiotherapy (RT) planning and delivery have allowed for higher conformity of the dose distribution to the planning target volume (PTV) and a better sparing of the surrounding organs at risk (OAR). However, an important concern with the use of modulated RT is the increased spread out of non-target dose to normal tissue outside the PTV. This unintended dose may raise concern as it offers no therapeutic benefit and could cause adverse effects that negatively affect treatment outcomes and quality of life. The personalized dose distribution as calculated by the treatment planning system (TPS) is highly dependent on the preferred treatment approach in clinical practice, which in turn may be associated with different RT parameters [1].

Among lymphocytes, the CD3+ complex is involved in activating both CD8+ cytotoxic and CD4+ helper T-cells. CD4+ cells primarily mediate anti-tumor immunity by assisting CD8+ cells, promoting antibody responses and stimulating cytokine secretion. Lymphocytes are recognized as one of the most radiosensitive cells, with even doses as low as 150 cGy and 275 cGy for lymphocytes residing in lymph nodes *in vivo* and *in vitro* reported to be fatal for 50% of the lymphocyte cells ($D_{50\%}$) [2]. A more recent *in vitro* study showed a $D_{50\%}$ of approximately 2 Gy for isolated CD8+ lymphocytes [3]. Not only circulating lymphocytes are highly radiosensitive, the bone marrow (BM) is also prone to acute and chronic radiation toxicity [4]. The pelvic region represents 55.6% of the proliferating BM, of which 16.6% is distributed at the lumbar spine, 9.2% at the sacrum, 25.3% at the pelvis and 4.5% at the proximal femurs [5]. The level of RT-induced BM suppression is both time and dose dependent, with evidence of partial to complete recovery after one year [4, 6]. Together with lymphocytes residing in other critical organs, the pelvic BM – being the primary site of hematopoiesis – may be acknowledged as an important OAR for developing lymphopenia or other hematologic adverse events

The impact of radiation on the immune system has become a topic of particular interest in the search of new predictive and prognostic biomarkers. In this regard, lymphocytes are an appealing candidate immune-based biomarker of response to RT due to their accessibility, affordability and prognostic significance [7, 8]. With RT playing a prominent role in the management of urological cancers, a comprehensive search of the literature to identify relevant parameters that correlate with the risk and incidence of lymphopenia in any way seems justified.

MATERIAL AND METHODS

This systematic review was performed according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines [9]. The PubMed (National Institutes of Health), ISI Web of Knowledge (Clarivate Analytics) and Embase (Elsevier) databases were searched for relevant articles from inception until March 2021. The study protocol was registered on PROSPERO (National Institute for Health Research, University of York) [CRD42020159436]. Following in- and exclusion criteria were defined:

INCLUSION CRITERIA (1) Any clinical study of urological malignancies in humans; (2) RT was an integral part of the patient treatment – primary, salvage or (neo)adjuvant; (3) Documented RT-associated lymphocyte outcomes.

EXCLUSION CRITERIA (1) Studies published only as abstracts and reports from meetings, review articles, modeling studies and editorials; (2) (Pre)clinical *in vitro* and *in vivo* studies; (3) Studies reporting only on pre-treatment lymphocyte outcomes or associations with systemic therapy or surgery alone; (4) Radioactive isotope treatment only; (5) Studies reporting outcomes in HIV-positive or immunodeficient patients.

Article screening was done independently by two reviewers (WD and MS) using the Rayyan web application [10]. Any disagreement was resolved by mutual consensus. A total of 3168 articles were identified using the database search. Relevant articles were withheld based on title and/or abstract and further assessed for eligibility based on full text by two independent reviewers (WD and MS). Articles including patients treated with RT for urological tumors were selected manually during the screening process. Forward and backward snowballing of candidate articles was performed to identify additional relevant articles. A summary of the search process is shown in Figure S1.

All p-values < 0.05 were considered significant. Study quality was assessed by using the Newcastle-Ottawa Scale and applying the scoring algorithm as described by McPheeters et al. [11, 12] (Supplementary Table S1).

RESULTS

Overall, 8 articles met the inclusion criteria [13-20]. Table 1 summarizes all relevant details of these studies. In total, 549 patients were treated with RT for either prostate (n = 508) or bladder (n = 41) cancer. Four studies (n = 474) explicitly reported about the incidences of acute severe lymphopenia (ASL) (defined as absolute lymphocyte count (ALC) < 500 cells/ μ L). The pooled mean incidence of ASL was 17.1% [13, 16-18] (Figure 1). The incidence of late grade 2 (15%) and 3 (1%) lymphopenia persisting up to one year after the start of RT was only mentioned in one study [13]. The study of Miszczyk et al. – which was the only study to include both prostate (n = 74) and bladder (n = 41) cancer patients – could not demonstrate a significant difference in ALC decrease between either pathologies [16]. Of note, a summary of reported and back-calculated incidences of lymphopenia and other hematologic adverse events is provided in Table S2 [13, 16-18].

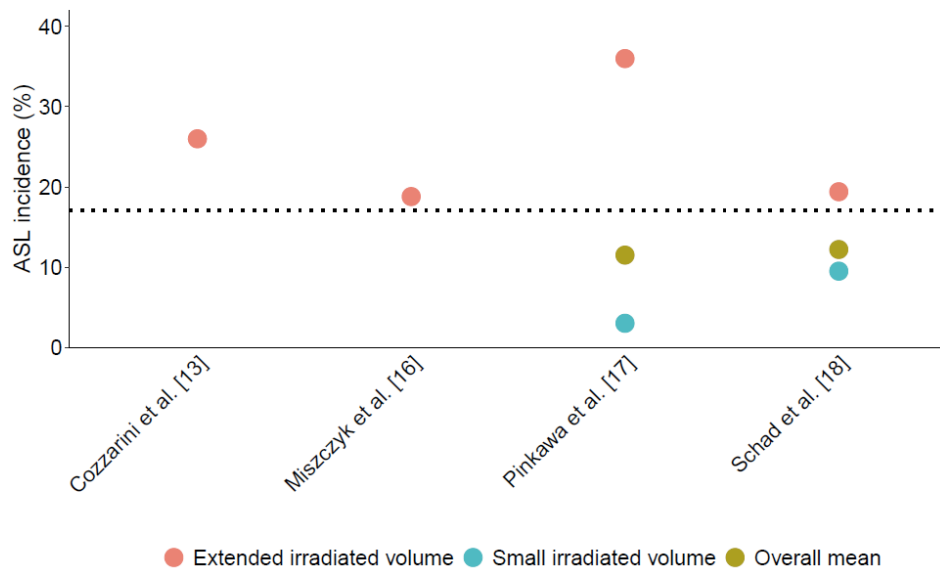


Figure 1 – Graphical summary of ASL incidence. To illustrate the impact of the target size, incidences per irradiated volume are displayed separately and as overall mean (if applicable). The study of Cozzarini et al. [13] and Miszczyk et al. [16] included only patients treated with extended radiation volumes (i.e. including the pelvic lymph nodes). The dotted line represents the pooled mean incidence (17.1%). ASL = acute severe lymphopenia.

Four studies investigated the impact of the target size, which could be considered as a surrogate for the irradiated volume. In the study of Schad et al., the incidence of lymphopenia (median follow-up total lymphocyte count (TLC) < 1000 cells/ μ L) was significantly increased in prostate cancer patients treated with pelvic lymph node (PLN) irradiation (61.1%) as compared to patients without PLN irradiation (26.3%) ($p < 0.001$). The incidence of severe lymphopenia (median follow-up TLC < 500 cells/ μ L) was also increased in the PLN-cohort, albeit non-significantly (19.4% versus 9.5%; p -value =0.12). Overall, an extended radiation volume was independently associated with an almost 3.5-fold risk of developing lymphopenia (hazard ratio (HR) = 3.42; 95% confidence interval (CI) 1.22-9.61; p -value =0.019) [18]. Indeed, treatment with prostate (bed) only RT (PBRT) resulted in higher lymphocyte counts ($p < 0.05$ for all time points after baseline) as well as a lower incidence of grade 2+ ($p < 0.01$) and grade 3 ($p < 0.01$) lymphopenia as compared to whole pelvis RT (WPRT). Of note, the incidence of grade 2 leukopenia (15% versus 2%; p -value =0.02) and grade 2 anemia (8% versus 0%; p -value =0.03) was also significantly higher in patients treated with WPRT [17]. Another study found that the ratio of CD8+/T_{reg} lymphocytes was significantly lower after RT including the PLN both at the end of RT (p -value =0.03) and until at least 3 months thereafter (p -value =0.03), indicating a stronger immunosuppressive effect [14]. An analysis of 125 prostate cancer patients treated with post-prostatectomy WPRT revealed that the PTV of the PLN – which included the obturator, hypogastric, internal and external iliac as well as presacral lymph-nodes anterior to the first sacral segment – failed to reach significance as a predictor for acute grade 2 (p -value =0.98), acute grade 3 (p -value =0.66) and late grade 2+ (p -value =0.94) lymphopenia on univariate analysis (UVA) [13].

Five studies investigated the association between lymphopenia and the applied radiation technique, but none of these studies could demonstrate a significant difference [13, 15, 16, 18, 19]. Elsworth et al. could not detect a significant difference in ALC decrease between patients treated with either three-dimensional conformal RT (3D-CRT) or tomotherapy. However, they did only one evaluation, half way through the trajectory after delivery of 34–38 Gy [15]. Similarly, the incidence of grade 3+ lymphopenia between patients treated with 3D-CRT (22.1%) or intensity modulated RT (IMRT) (17.1%) in the study of Miszczyk et al. was very comparable ($p > 0.05$) [16]. Another study confirmed that there was no apparent influence of different IMRT techniques (static versus rotational) for acute grade 2 (p -value =0.80), acute grade 3 (p -value =0.47) and late grade 2+ (p -value =0.81) lymphopenia [13, 19]. The study of Schad et al. included patients treated with brachytherapy, external beam RT (EBRT) or a combination of both. Although these techniques are associated with different treatment volumes, they did not impact the risk of lymphopenia (HR = 0.39; 95% CI 0.072-2.13; p -value =0.278 for high-dose rate brachytherapy versus HR = 0.50; 95% CI 0.12-2.07; p -value =0.338 for EBRT only) [18].

Cozzarini et al. found that the equivalent dose in fractions of 2 Gy (EQD2) ($\alpha/\beta = 3$ Gy) to the prostatic bed (median = 70.4 Gy) and PLN (median = 50.2 Gy) did not predict acute grade 2 (p -value =0.86 and p -value =0.63, respectively), acute grade 3 (p -value =0.88 and p -value =0.40, respectively) or late grade 2+ (p -value =0.29 and p -value =0.76, respectively) lymphopenia on UVA. In contrast, the EQD2 to the PLN was an independent predictor of acute thrombocytopenia (odds ratio (OR) = 0.84; 95% CI 0.73-0.97; p -value =0.0006). The number of RT fractions had no significant impact on the risk of acute grade 2 (p -value =0.17), acute grade 3 (p -value =0.18) and late grade 2+ (p -value =0.43) lymphopenia, but was significantly associated with acute neutropenia (OR = 3.40; 95% CI 1.09-10.66; p -value =0.03) [13].

Sini et al. evaluated the absolute average dose-volume histograms (DVHs) and identified dose constraints that were associated with acute grade 3 or late grade 2 lymphopenia. Contours of the pelvic bones – used as a surrogate for pelvic BM – were delineated on the planning computed tomography scan, as previously described by Mell et al. [21]. Medium-high doses (≥ 40 Gy) to the whole pelvic (OR = 1.01; 95% CI 1.00-1.01; p -value =0.025) and iliac (OR = 1.04; 95% CI 1.01-1.08; p -value =0.009) BM were associated with acute grade 3 and late grade 2 lymphopenia, respectively. Other important factors included baseline ALC and smoking [19]. In the study of Yang et al., correlations between carbon ion RT (CIRT) and variations in lymphocyte subsets at different time points were investigated. Rectum V_{20} correlated with CD8+ cells at post-radiotherapy (p -value =0.0463), rectum V_{47} correlated with CD4+ cells during radiotherapy (p -value =0.0421) and at follow-up (p -value =0.0445). Rectum V_{50} correlated with CD4+ cells during radiotherapy (p -value =0.0325) and at follow-up (p -value =0.0362), and with the CD4/CD8 ratio at follow-up (p -value =0.0459) [20].

Blood transfusions, excess infections or unexplained treatment breaks did not occur in the study of Pinkawa et al. [17]. Schad et al. reported no significant associations between lymphopenia and any treatment outcomes [18]. However, in the study of Yang et al. the CD4/CD8 ratio was significantly increased during ($p < 0.05$) and one-month after ($p < 0.05$) CIRT in patients with a complete or partial response as compared to those with stable disease, while the CD3+ and CD8+ cell count was significantly decreased ($p < 0.05$ and $p < 0.01$, respectively). The CD8+ cell count during CIRT was identified as an independent prognostic indicator of the short-term local

efficacy of CIRT (p-value =0.015), which was evaluated up to 3 months by prostate specific antigen (PSA) levels, and magnetic resonance and positron emission tomography imaging [20].

Aside from RT, systemic therapies may also be a part of the multimodal treatment of urological tumors. Hence, the risk of a possible confounding effect was more closely examined. Importantly, none of the included patients received chemotherapy. Androgen deprivation therapy (ADT) was no significant predictor of lymphopenia in both the study of Cozzarini et al. [13] (p-value =0.35 for acute grade 2, p-value =0.63 for acute grade 3 and p-value =0.43 for late grade 2+ lymphopenia) and Schad et al. (HR = 0.98; 95% CI 0.36-2.70; p-value =0.967) [18]. In the study of Pinkawa et al. a significant impact of neoadjuvant hormonal therapy on hemoglobin levels ($p < 0.01$) was reported, but not on leukocyte counts [17]. The high incidence of anemia (Table S2) might be partly attributed to the prescription of ADT ($n = 387$), which has been previously identified as an important risk factor [22]. It may be assumed that the effects of ADT on blood cells are unlikely of major clinical significance, although there is still room for further investigation. Of note, neither tumor stage nor Gleason score correlated with the risk of acute grade 2, acute grade 3 or late grade 2+ lymphopenia [13, 18]. The initial PSA value was a significant predictor of ASL (HR = 1.05; 95% CI 1.00-1.11; p-value =0.048) in one study, but this can be countered by the fact that administration of WPRT was only indicated in high-risk and unfavorable intermediate-risk prostate cancer [18].

DISCUSSION

The reviewed study cohort only included chemo-naïve urological cancer patients, thus representing an unique study population to scrutinize the interactions of RT with the immune system. Multiple studies reported high and severe incidences of lymphopenia. Although lymphopenia might be considered a secondary problem, a critical understanding of its etiology may be an important step towards improving clinical outcomes.

Although increasing evidence identifies lymphopenia as a significant predictor of poor outcomes across multiple tumor types [23, 24], no such associations were reported here [18]. This was probably due to a too low number of observations [18]. In contrast, the CD8+ cell count was associated with less effective responses after CIRT, suggesting that this parameter is a possible biomarker candidate for assessing prostate cancer progress and prognosis [20]. Indeed, previous research has also implicated peripheral CD8+ cells as an independent negative prognostic factor for bladder cancer recurrence [25]. Another important finding is the long lasting effect (up to several years) RT may exert on lymphocyte counts [18, 19]. O'Toole et al. demonstrated in a small cohort ($n = 34$) that lymphocyte counts returned to baseline levels within 3 years after RT in bladder cancer patients who were disease-free after a 5-year follow-up period. In contrast, this was not the case for patients with recurrent or residual disease [26]. In addition, with both encouraging as well as negative results of combined immune checkpoint inhibitors (ICIs) and RT strategies in genitourinary malignancies, peripheral blood biomarkers are of particular interest to predict durable disease control [7, 27]. Indeed, lymphocyte counts have recently been identified as a prognostic biomarker in metastatic urothelial cancer patients treated with ICIs [28]. RT-induced lymphopenia may also increase the platelet-to-lymphocyte and neutrophil-to-lymphocyte ratio, which have already been shown to negatively impact survival [29, 30]. Moreover, patients who are treated with extended

radiation volumes are highly susceptible to experiencing ASL, which might be of particular interest since data on the advantage of prophylactic PLN irradiation in high-risk prostate cancer patients are still mixed [31].

It is hypothesized that RT causes lymphopenia by impairment of local BM regeneration and unintentionally depleting the reservoir of lymphocytes [32, 33]. Since the magnitude of BM damage depends on both radiation dose and volume [4], it is worth mentioning that the literature reporting on dose constraints for lymphopenia or other hematologic adverse events in urological patients is very sparse. To our knowledge, only one article defined BM dose constraints, though they may still require external validation [19]. In addition, lymphocyte-dense structures have also been identified as important modulators [33-36]. Yang et al. demonstrated that sparing the lymphoid reflux around the rectum may be advantageous [20]. Although it is highly unlikely that the spleen will receive such high doses when treating the pelvic region, large pelvic vessels may expose a substantial blood volume to irradiation. Discovery of RT parameters that may influence the spatiotemporal blood dose distribution is a critical step in moving towards “immunity-sparing RT”, which is hypothesized to improve clinical outcomes [37]. Recently, mathematical frameworks have been developed to assess the impact of RT on the immune system, but future research is required to assess the clinical utility and implementation of novel therapeutic strategies in this regard [7, 35, 36].

Despite resulting in highly different spatial dose distributions, none of the included studies could demonstrate a significant impact of the RT technique on the immune system. One possible explanation is the similar size of the PTVs between the investigated groups [1]. Dose fractionation may also play an important role, as hypofractionated regimens have been associated with a decreased incidence of lymphopenia, probably due to a lower cumulative exposure of the blood volume with fewer fractions [38, 39]. However, the currently reviewed data could not confirm this, possibly due to only one article investigating this parameter and the relatively wide range in the total number of fractions given (median = 28, range 28 to 43) [13]. Although lack of volumetric data and primary field borders in the included studies limit a firm conclusion, an association between the radiation volume and incidence of ASL may be assumed. Of note, Cozzarini et al. used an α/β of 3 Gy for calculating the EQD2, which is not typically used for describing acute toxicities [13]. In this context, it would be more appropriate to use an α/β of 10 Gy, which is commonly used to specify the high radiosensitivity of BM [39, 40].

More prospective data is required in order to thoroughly assess the clinical importance of lymphopenia in urological cancers. Concluding remarks about the predictive power of DVH-parameters remain ambiguous and need further validation. Clinical trials investigating how dedicated strategies – such as BM-sparing RT – may reduce lymphopenia are currently lacking. Publishing details on volumetric data and primary field borders is encouraged, especially in those studies investigating the effect of PLN irradiation. In addition, nearly all studies need longer follow-up periods, and more elaborate reporting of survival and response rates.

There were some limitations to this analysis. The majority of the included articles were retrospective studies or post hoc analyses, potentially introducing selection and reporting bias of patients with a (predefined) number of available counts. Most of the reported results were based on single-institutional small study cohorts. Although the scope of this systematic review was confined to a specific anatomical region, the analyzed study population

was heterogeneous in terms of disease stage and RT setting. Most importantly, there was high inter-study heterogeneity with regards to measurement time points and outcome definitions.

CONCLUSION

Despite some heterogeneity across the included studies, high incidences and severe grades of lymphopenia were reported. Minimizing radiation volumes and unintentional irradiation of pelvic BM may reduce the incidence of lymphopenia, potentially improving clinical outcomes. Although the identified risk factors seem rational, as of yet it is unlikely that more inferences can be made, while drawing attention to the issue seems warranted. More research is needed to further elucidate these findings and effectively implement recently developed new risk assessment tools.

CONFLICT OF INTEREST

The authors do not report any conflict of interest.

APPENDIX

Supplementary Material.

REFERENCES

- [1] Kry SF, Bednarz B, Howell RM, Dauer L, Followill D, Klein E, et al. AAPM TG 158: Measurement and calculation of doses outside the treated volume from external-beam radiation therapy. *Med Phys*. 2017;44:e391-e429.
- [2] Trowell OA. The sensitivity of lymphocytes to ionising radiation. *J Pathol Bacteriol*. 1952;64:687-704.
- [3] Nakamura N, Kusunoki Y, Akiyama M. Radiosensitivity of CD4 or CD8 positive human T-lymphocytes by an in vitro colony formation assay. *Radiat Res*. 1990;123:224-227.
- [4] Mauch P, Constine L, Greenberger J, Knosp W, Sullivan J, Liesveld JL, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. *Int J Radiat Oncol Biol Phys*. 1995;31:1319-1339.
- [5] Hayman JA, Callahan JW, Herschtal A, Everitt S, Binns DS, Hicks RJ, et al. Distribution of proliferating bone marrow in adult cancer patients determined using FLT-PET imaging. *Int J Radiat Oncol Biol Phys*. 2011;79:847-852.
- [6] Rubin P, Landman S, Mayer E, Keller B, Ciccio S. Bone marrow regeneration and extension after extended field irradiation in Hodgkin's disease. *Cancer*. 1973;32:699-711.
- [7] Grassberger C, Ellsworth SG, Wilks MQ, Keane FK, Loeffler JS. Assessing the interactions between radiotherapy and antitumour immunity. *Nat Rev Clin Oncol*. 2019;16:729-745.
- [8] Cho Y, Park S, Byun HK, Lee CG, Cho J, Hong MH, et al. Impact of Treatment-Related Lymphopenia on Immunotherapy for Advanced Non-Small Cell Lung Cancer. *Int J Radiat Oncol Biol Phys*. 2019;105:1065-1073.
- [9] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.

- [10] Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev.* 2016;5:210.
- [11] (2011). The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
- [12] McPheeters ML, Kripalani S, Peterson NB, Idowu RT, Jerome RN, Potter SA, et al. Quality Improvement Interventions To Address Health Disparities. Evidence Report/ technology Assessment. Rockville (MD): Agency for Healthcare Research and Quality (US). <http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0049222/pdf/TOC.pdf>. 2012.
- [13] Cozzarini C, Noris Chiorda B, Sini C, Fiorino C, Briganti A, Montorsi F, et al. Hematologic Toxicity in Patients Treated With Postprostatectomy Whole- Pelvis Irradiation With Different Intensity Modulated Radiation Therapy Techniques Is Not Negligible and Is Prolonged: Preliminary Results of a Longitudinal, Observational Study. *Int J Radiat Oncol Biol Phys.* 2016;95:690-695.
- [14] Eckert F, Schaedle P, Zips D, Schmid-Horch B, Rammensee HG, Gani C, et al. Impact of curative radiotherapy on the immune status of patients with localized prostate cancer. *Oncoimmunology.* 2018;7:e1496881.
- [15] Elsworthy M, Plowman PN. Peripheral blood lymphocyte counts during standard conformal radiotherapy and TomoTherapy/IMRT for prostate cancer. *JRP.* 2008;7:223-227.
- [16] Miszczyk M, Majewski W. Hematologic Toxicity of Conformal Radiotherapy and Intensity Modulated Radiotherapy in Prostate and Bladder Cancer Patients. *Asian Pac J Cancer Prev.* 2018;19:2803-2806.
- [17] Pinkawa M, Djukic V, Klotz J, Petz D, Piroth MD, Holy R, et al. Hematologic changes during prostate cancer radiation therapy are dependent on the treatment volume. *Future Oncol.* 2014;10:835-843.
- [18] Schad MD, Dutta SW, Muller DM, Wijesooriya K, Showalter TN. Radiation-related Lymphopenia after Pelvic Nodal Irradiation for Prostate Cancer. *Adv Radiat Oncol.* 2019;4:323-330.
- [19] Sini C, Fiorino C, Perna L, Noris Chiorda B, Deantoni CL, Bianchi M, et al. Dose-volume effects for pelvic bone marrow in predicting hematological toxicity in prostate cancer radiotherapy with pelvic node irradiation. *Radiother Oncol.* 2016;118:79-84.
- [20] Yang ZR, Zhao N, Meng J, Shi ZL, Li BX, Wu XW, et al. Peripheral lymphocyte subset variation predicts prostate cancer carbon ion radiotherapy outcomes. *Oncotarget.* 2016;7:26422-26435.
- [21] Mell LK, Kochanski JD, Roeske JC, Haslam JJ, Mehta N, Yamada SD, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients treated with concurrent cisplatin and intensity-modulated pelvic radiotherapy. *Int J Radiat Oncol Biol Phys.* 2006;66:1356-1365.
- [22] Hicks BM, Klil-Drori AJ, Yin H, Campeau L, Azoulay L. Androgen Deprivation Therapy and the Risk of Anemia in Men with Prostate Cancer. *Epidemiology.* 2017;28:712-718.
- [23] Venkatesulu BP, Mallick S, Lin SH, Krishnan S. A systematic review of the influence of radiation-induced lymphopenia on survival outcomes in solid tumors. *Crit Rev Oncol Hematol.* 2018;123:42-51.
- [24] Ray-Coquard I, Cropet C, Van Glabbeke M, Sebban C, Le Cesne A, Judson I, et al. Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. *Cancer Res.* 2009;69:5383-91.
- [25] Lin CT, Tung CL, Tsai YS, Shen CH, Jou YC, Yu MT, et al. Prognostic relevance of preoperative circulating CD8-positive lymphocytes in the urinary bladder recurrence of urothelial carcinoma. *Urol Oncol.* 2012;30:680-687.

- [26] O'Toole C, Unsgaard B. Clinical status and rate of recovery of blood lymphocyte levels after radiotherapy for bladder cancer. *Cancer Res.* 1979;39:840-843.
- [27] Solanki AA, Bossi A, Efstathiou JA, Lock D, Mondini M, Ramapriyan R, et al. Combining Immunotherapy with Radiotherapy for the Treatment of Genitourinary Malignancies. *Eur Urol Oncol.* 2019;2:79-87.
- [28] Elumalai T, Aversa C, Buijtenhuijs B, Conroy R, Croxford W, Das A, et al. Predicting survival in urothelial cancer patients after immunotherapy using real-world data. *Ann Oncol.* 2020;31:S590.
- [29] Li DY, Hao XY, Ma TM, Dai HX, Song YS. The Prognostic Value of Platelet-to-Lymphocyte Ratio in Urological Cancers: A Meta-Analysis. *Sci Rep.* 2017;7:15387.
- [30] Luo Y, She DL, Xiong H, Fu SJ, Yang L. Pretreatment Neutrophil to Lymphocyte Ratio as a Prognostic Predictor of Urologic Tumors: A Systematic Review and Meta-Analysis. *Medicine.* 2015;94:e1670.
- [31] Tharmalingam H, Choudhury A, Van Herk M, McWilliam A, Hoskin PJ. New approaches for effective and safe pelvic radiotherapy in high-risk prostate cancer. *Nat Rev Urol.* 2019;16:523-538.
- [32] Sacks EL, Goris ML, Glatstein E, Gilbert E, Kaplan HS. Bone marrow regeneration following large field radiation: influence of volume, age, dose, and time. *Cancer.* 1978;42:1057-65.
- [33] Yovino S, Kleinberg L, Grossman SA, Narayanan M, Ford E. The etiology of treatment-related lymphopenia in patients with malignant gliomas: modeling radiation dose to circulating lymphocytes explains clinical observations and suggests methods of modifying the impact of radiation on immune cells. *Cancer Invest.* 2013;31:140-144.
- [34] Chadha AS, Liu G, Chen HC, Das P, Minsky BD, Mahmood U, et al. Does Unintentional Splenic Radiation Predict Outcomes After Pancreatic Cancer Radiation Therapy? *Int J Radiat Oncol Biol Phys.* 2017;97:323-332.
- [35] Ladbury CJ, Rusthoven CG, Camidge DR, Kavanagh BD, Nath SK. Impact of Radiation Dose to the Host Immune System on Tumor Control and Survival for Stage III Non-Small Cell Lung Cancer Treated with Definitive Radiation Therapy. *Int J Radiat Oncol Biol Phys.* 2019;105:346-355.
- [36] Shin J, Xing S, McCullum L, Hammi A, Pursley J, Correa CA, et al. HEDOS-a computational tool to assess radiation dose to circulating blood cells during external beam radiotherapy based on whole-body blood flow simulations. *Phys Med Biol.* 2021;66.
- [37] Lambin P, Lieverse RIY, Eckert F, Marcus D, Oberije C, van der Wiel AMA, et al. Lymphocyte-Sparing Radiotherapy: The Rationale for Protecting Lymphocyte-rich Organs When Combining Radiotherapy With Immunotherapy. *Semin Radiat Oncol.* 2020;30:187-193.
- [38] Wild AT, Herman JM, Dholakia AS, Moini S, Lu Y, Rosati LM, et al. Lymphocyte-Sparing Effect of Stereotactic Body Radiation Therapy in Patients With Unresectable Pancreatic Cancer. *Int J Radiat Oncol Biol Phys.* 2016;94:571-579.
- [39] Terrones-Campos C, Ledergerber B, Vogelius IR, Specht L, Helleberg M, Lundgren J. Lymphocyte Count Kinetics, Factors Associated with the End-of-Radiation- Therapy Lymphocyte Count, and Risk of Infection in Patients with Solid Malignant Tumors Treated with Curative-Intent Radiation Therapy. *Int J Radiat Oncol Biol Phys.* 2019;105:812-823.
- [40] Dörr W. Pathogenesis of normal-tissue side-effects. *Basic Clinical Radiobiology.* Fourth ed: Taylor & Francis Ltd; 2009. p. 179-181.

Table 1 – Summary of included studies.

AUTHOR	PATHOLOGY	NUMBER OF PATIENTS	STUDY DESIGN	RADIOTHERAPY DETAILS	SYSTEMIC THERAPY	LYMPHOCYTE PARAMETER	OUTCOME
Cozzarini et al. [13] 2016	Prostate	125	Observational	Post-prostatectomy WPRT SS-IMRT (15.2%), RA (48.0%) and TT (36.8%) Median EQD2 to prostate bed: 70.4 Gy Median EQD2 to PLN: 50.2 Gy	ADT (34%)	Lymphopenia as per CTCAE v4.03	Acute G1+ = 100% G2+ = 87% G3+ = 26% Late G2 = 15% G3 = 1%
Eckert et al. [14] 2018	Prostate	18	Prospective	PBRT/WPRT Prescribed dose for PBRT: 70–78 Gy in 35–39 fractions Prescribed dose for WPRT: 50.4 Gy in 28 fractions	ADT (83.3%)	Lymphocyte subset changes	PBRT versus WPRT <ul style="list-style-type: none"> • ↓ fraction of T lymphocytes and CD8+/Treg ratio • No statistically significant differences for B-, CD4+ and CD8+ cells
Elsworthy et al. [15] 2008	Prostate	28	Prospective	PBRT ± seminal vesicles 3D-CRT (46.4%) or TT (53.6%)	N/A	Changes in ALC	3D-CRT Baseline: 2020 ± 620 cells/μL Mid-point: 1170 ± 470 cells/μL TT Baseline: 1560 ± 590 cells/μL Mid-point: 1040 ± 290 cells/μL

Miszczyk et al. [16] 2018	Prostate Bladder	115	Retrospective	Primary RT + prophylactic PLN irradiation Bladder: 60-70 Gy Prostate: 76 Gy PLN: 44-45 Gy (+ additional boost in 2 cases)	ADT for prostate cancer patients No chemotherapy for bladder cancer patients	Lymphopenia as per CTCAE v4.0	G0 = 15.2% G1 = 19.6% G2 = 46.4% G3 = 18.8% G4 = 0.0%
Pinkawa et al. [17] 2014	Prostate	113	Prospective	PBRT or WPRT 5-field IMRT (primary or adjuvant/salvage) Prescribed dose for PBRT 66 or 76 Gy ± boost up to 80 Gy Prescribed dose for WPRT 45 Gy	ADT (23.9%)	Lymphopenia as per CTCAE v3.0	PBRT-cohort G2+ = 33% G3 = 3% WPRT-cohort G2+ = 91% G3 = 36%
Schad et al. [18] 2019	Prostate	131	Retrospective	PBRT or WPRT (EBRT ± brachytherapy)	ADT (52.8%)	Lymphopenia: median follow-up TLC < 1000 cells/μL Severe lymphopenia: median	Lymphopenia PBRT-cohort: 61.1% WPRT-cohort: 26.3% Severe lymphopenia PBRT-cohort: 19.4% WPRT-cohort: 9.5%

						follow-up TLC < 500 cells/ μ L	
Sini et al. [19] 2016	Prostate	121	Observational	Post-prostatectomy WPRT SF-IMRT (16%), RA (47%) and TT (37%) Prescription dose to PTV (range): 70–75.6 Gy; 65.5–71.4 Gy Prescription dose to pelvis (median, range): 51.8 Gy; 50.4–52.5 Gy	ADT (34%)	Lymphopenia as per CTCAE v4.03 Variation in percentage with respect to baseline	Acute G1 = 100% G2 = 61% G3 = 25% Late G2+ = 15.7%
Yang et al. [20] 2016	Prostate	19	Prospective	Carbon ion (^{13}C) RT Prescription dose to PTV: 63-66 GyE in fractions of 2.74 GyE	N/A	Lymphocyte subset changes	<ul style="list-style-type: none"> • Rectum V₂₀ correlated with CD8+ cells at post-radiotherapy • Rectum V₄₇ correlated with CD4+ cells during radiotherapy and at follow-up • Rectum V₅₀ correlated with CD4+ cells during radiotherapy and at follow-up, and with the CD4/CD8 ratio at follow-up

Abbreviations: RT = radiotherapy; EBRT = external beam RT; 3D-CRT = three-dimensional conformal RT; IMRT = intensity modulated RT; SS-IMRT = step-and-shoot IMRT; RA = RapidArc; TT = tomotherapy; PBRT = prostate bed (only) RT; WPRT = whole pelvis RT; PTV = planning target volume; PLN = pelvic lymph nodes; CTCAE = common terminology for adverse events; ADT = androgen deprivation therapy; EQD2 = equivalent dose in fractions of 2 Gy; ALC = absolute lymphocyte count; TLC = total lymphocyte count: Gx = grade x; Gy = gray; GyE = gray equivalent.