ORIGINAL RESEARCH

# Radiation-Associated Lymphopenia and Outcomes of Patients with Unresectable Hepatocellular Carcinoma Treated with Radiotherapy

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**Background:** The immune system plays a crucial role in cancer surveillance. Previous studies have shown that lymphopenia associated with radiotherapy (RT) portends a poor prognosis. We sought to differentiate the effects of proton and photon RT on changes in absolute lymphocyte count (ALC) for patients with hepatocellular carcinoma (HCC).

**Patients and Methods:** Patients with HCC treated with definitive RT from 2006 to 2016 were studied. Serial ALCs were graded according to CTCAE v4.0. Overall survival (OS), disease-free survival, and distant metastasis-free survival were analyzed using the Kaplan–Meier method. Univariable and multivariable Cox-proportional hazards analyses were used to identify predictors of OS. A cohort analysis matched for treatment volume was performed to investigate differences in ALC dynamics between photon and proton therapy.

**Results:** Of 143 patients identified, the median age was 66 (range, 19–90) years. The treatment modality was photon in 103 (72%) and proton in 40 (28%). Median follow-up was 17 months (95% confidence interval, 13–25 months). The median time to ALC nadir after initiation of RT was 17 days with a median relative decrease of 67%. Those who received proton RT had a higher median ALC nadir (0.41 vs 0.32 k/μL, p=0.002) and longer median OS (33 vs 13 months, p=0.002) than those who received photon RT. Matched cohort analyses revealed a larger low-dose liver volume in the photon group, which correlated with lower ALC. On multivariable Cox analysis, Grade 3 or higher lymphopenia prior to or after RT, portal venous tumor thrombus, larger planning target volumes, Child-Pugh (CP) Class B, and increased CP score after RT were associated with a higher risk of death, whereas the use of proton therapy was associated with lower risk.

**Conclusion:** Grade 3 or higher lymphopenia may be associated with poorer outcomes in patients receiving RT for HCC. Protons may mitigate lymphopenia compared with photons, potentially due to reduced dose exposure of sites of lymphopoiesis.

**Keywords:** lymphocyte count, circulating lymphocytes, splenic dose, liver dose, overall survival

## Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and one of the leading causes of cancer death globally.[1] The incidence of HCC is rapidly increasing and is disproportionately high in low- and middle-income countries and among groups of lower socioeconomic status in the United States.[2] Despite recent advances in treatment, the 5-year overall survival for liver cancers remains around 20%.[3] Advances in conformal photon and particle radiotherapy (RT) have bolstered the role of RT in the management of this disease.[4] Conformal photon-based

techniques such as intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT) have allowed clinicians to better optimize dose homogeneity at the expense of a larger volume of surrounding tissue receiving low doses of RT, termed the "low dose bath," which is hypothesized to increase the risk of toxicities or secondary malignancies.[5,6] Through the use of proton RT, however, clinicians are better able to escalate doses to malignant tissues while sparing surrounding parenchyma thereby limiting the risk for worsening of liver function.[4] Another potential benefit of proton RT is the reduced risk for high-grade lymphopenia.[7–9] Previous studies have shown that high-grade lymphopenia portends poorer outcomes in patients with pancreatic, cervical, esophageal, head and neck, lung, and central nervous system malignancies.[10–17] However, the effects of lymphopenia on the clinical course of patients treated with RT for unresectable HCC are not well characterized. Lower RT doses surrounding targets for the treatment of HCC may not directly injure organs at risk but may significantly deplete highly radiosensitive lymphocytes.[18] This study aims to characterize the impact of treatment-related lymphopenia on outcomes in patients with HCC and elucidate the impact of proton and photon RT on lymphocyte counts and clinical outcomes.

# Methods

## Patient Selection

The Institutional Review Board Committee at MD Anderson Cancer Center approved our request to review the medical records of these patients. The need for informed patient consent was waived, as this was a retrospective review and no identifiable patient information is included in this report. This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and its later amendments. We identified all patients aged ≥18 years with unresectable HCC treated with definitive RT between 2006 and 2016 at MD Anderson Cancer Center. Patients who received doses <30 Gy, did not complete RT, did not have lymphocyte count data available at least prior to and during RT, and those with the distant disease at the time of treatment were excluded. Patient characteristics, tumor features, treatment parameters, and clinical outcomes were recorded. Child-Pugh scores (CPS) prior to RT and at peak within 9 months of RT completion were calculated to assess for liver decompensation, defined as an increase by ≥2 points. Serial absolute lymphocyte counts (ALC) prior to,

during, and after RT were collected and graded according to Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Post-RT ALC data were recorded for patients with labs collected within 100 days of completing RT to capture patients returning for follow-up at approximately 3 months.

## Matched Cohort Analysis

To investigate differences in ALC between treatment modalities, a cohort analysis of 15 proton and 15 photon cases matched to treatment volume was conducted. For each patient, body dose-volume indices $V_x$ (the volume receiving a dose of X or higher, eg, $V_{1Gy}$, $V_{5Gy}$, $V_{10Gy}$) were extracted from treatment planning software. Associations of various body low dose $V_x$ with treatment modality (protons vs photons) and ALC nadir, defined as the lowest lymphocyte count recorded during treatment, were investigated. Similarly, spleen volumes and mean doses were obtained from treatment plans and compared for the matched cohort analysis.

## Statistical Analysis

Patient baseline characteristics were compared with $t$-tests for continuous variables and χ2 tests for categorical variables. ALC counts were used to determine the change from baseline and compared using the Wilcoxon signed-rank test. Overall survival (OS), disease-free survival (DFS), and distant metastasis-free survival (DMFS) were measured from the start of RT, analyzed using the Kaplan–Meier method, and compared using the log-rank test. Correlation analysis with Bonferroni correction was performed using the Pearson method for continuous explanatory variables and the Spearman method for ordinal explanatory variables. Univariable and multivariable analyses were conducted using Cox proportional hazards. The proportional hazards assumptions were evaluated using a $\chi^2$ test of Schoenfeld residuals. To account for multicollinearity in the multivariable analysis, variable selection utilized stepwise elimination of the variable with the highest variance inflation factor (VIF) until the mean VIF of remaining variables was ≤2; these variables comprised the final model. The multivariable analysis utilized time-varying covariates for post-treatment parameters. Continuous variables, including age, total RT dose, dose per fraction, time to ALC nadir, percent change from baseline in ALC at nadir, and change in CPS were included directly in univariable and multivariable models. Statistical analysis was performed with Stata Version 13.0

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(StataCorp, College Station, TX, USA) and JMP Version 14.0 (SAS Institute, Cary, NC, USA).

# Results

## Patient Characteristics

A total of 143 patients were included in this study. Patient, treatment, and tumor characteristics are summarized in Table 1 and also stratified by treatment technique for comparison. The median age at treatment was 66 (range, 19–90) years. The majority of the cohort was male (78%) and of favorable performance status (90% Eastern Cooperative Oncology Group [ECOG] 0–1). There were 103 (72%) in the photon cohort and 40 (28%) in the proton cohort. Among all patients, 40% had American Joint Committee on Cancer (AJCC) 8th Edition Stage I or II disease and 60% had Stage III or IV disease. This differed between treatment groups as a greater portion of proton-treated patients had Stage I or II disease (65% vs 30%; p<0.001). The Barcelona Clinic Liver Cancer stages were early stage (A) in 10 (7%) patients, intermediate stage (B) in 2 (1%), and advanced stage (C) in 131 (92%).[19] Portal venous tumor thrombosis was present in 79 (55%) patients; a greater proportion of photon-treated patients had portal venous thrombus present at the start of RT (62% vs 38%; p=0.008). Child-Pugh A liver disease was noted in 114 (80%) patients, including 80 (56%) with A5 and 34 (24%) with A6 disease. The mean pre-treatment Child-Pugh score was 5.8 (range, 5–9) for patients receiving photon RT and 5.8 (range, 5–8) for those receiving proton RT. The median prescribed dose was 60 Gy (range, 30–100 Gy, interquartile range [IQR], 50.4–67.5 Gy) with a median of 15 (range, 3–34, IQR, 15–25) fractions. The median prescribed dose per fraction was 3.9 Gy (range, 1.8–20 Gy). The median biologically equivalent dose (BED) was 84 Gy (range, 44–180 Gy) and 97 GyE (range, 39–140 GyE) for patients treated with photons and protons, respectively. Prior to RT a total of 64 (45%) patients underwent one or more liver-directed local therapies: 56 (39%) with transarterial chemoembolization (TACE), 9 (6%) with radiofrequency ablation (RFA), 8 (6%) with surgical resection, 5 (3%) with portal vein embolization (PVE), and 4 (3%) with Yttrium-90 (Y-90) radioembolization. Following RT, a total of 16 (11%) patients underwent one or more liver-directed local therapies: 12 (8%) with TACE, 5 (3%) with a repeat course of RT, 3 (2%) with ethanol ablation, and 1 (1%) each with surgical resection, RFA, and Y-90 radioembolization.

## Changes in Absolute Lymphocyte Count During Radiotherapy

The median pre-RT ALC was 1.10 k/μL (range, 0.11–3.79 k/μL) and 10 patients (7%) had pre-RT ALC ≤ 0.5. Post-RT ALC data within 100 days of completing RT are available for 131 patients (92%). At a median of 42 days (range, 1–95 days, IQR, 24–60 days), the median post-RT ALC was 0.59 k/μL (range, 0.03–2.12 k/μL) and 59 patients (45%) had post-RT ALC ≤ 0.5. There was a significant decrease in ALC between pre-treatment and post-treatment periods (Figure 1A), with a median nadir reduction of 67% (IQR, 52–80%). During treatment, 45% of patients developed Grade 3 (G3) lymphopenia and 22% developed Grade 4 (G4) lymphopenia. Stratifying by technique, G3 or higher lymphopenia developed in 72% of patients receiving photons and 55% receiving protons (p=0.04) whereas G4 lymphopenia developed in 23% receiving photons and 8% receiving protons (p=0.007). The development of G3 or higher lymphopenia stratified by treatment technique is presented in Figure 1B. Univariable correlation analysis revealed an association between the percentage of ALC decrease during treatment and treatment modality (proton vs photon, p=0.04), pre-treatment ALC (p=0.02), dose per fraction (p=0.004), number of treatment fractions (p=0.0002), and use of IMRT/VMAT vs 3D conformal RT (p=0.03). There was no correlation between the percentage of ALC decrease during treatment with age, sex, pre- or post-treatment Child-Pugh score, change in Child-Pugh score, prior use of TACE, presence of portal vein tumor thrombus, total prescribed radiation dose, biologically effective dose (BED), volume of radiation target, or splenic volume.

As there was a significant correlation between pre-treatment ALC and the percentage of ALC decrease during RT, we sought to identify factors that may influence pre-treatment ALC. On univariable analysis, there was no correlation between pre-treatment ALC with age, presence of portal vein tumor thrombosis, or pre-treatment Child-Pugh score, but there was a significant inverse association with increasing splenic volume (r=−0.45, p<0.0001).

## Overview of Clinical Outcomes

At a median follow-up of 16.7 months (range, 1–117 months), 29% of all patients were alive at the last follow-up. Recurrences were local in 17% at median 8.5 months (95% CI, 3.8–11.9 months), out-of-field liver only in 39% at median 6.3 months (95% CI 5.1–8.2), and distant metastasis in 31% at median 6.1 months (95% CI, 3.9–7.4 months). After RT, the

**Table 1** Baseline Patient, Tumor and Treatment Characteristics

| Parameter | All Patients (n=143) | Photon-Treated Patients (n=103) | Proton-Treated Patients (n=40) | p-value |
|---|---|---|---|---|
| **Median age (range)** | **66 (19–90)** | **63 (19–90)** | **73 (51–89)** | **<0.001** |
| Sex | | | | 0.360 |
|   Male | 111 (78%) | 82 (80%) | 29 (73%) | |
|   Female | 32 (22%) | 21 (20%) | 11 (28%) | |
| ECOG | | | | 0.210 |
|   0 | 27 (19%) | 21 (20%) | 6 (15%) | |
|   1 | 101 (71%) | 85 (72%) | 27 (68%) | |
|   2 | 15 (10%) | 8 (8%) | 7 (18%) | |
| **AJCC 8th Edition Stage** | | | | **<0.001** |
|   **I–II** | **57 (40%)** | **31 (30%)** | **26 (65%)** | |
|   **III–IV** | **86 (60%)** | **72 (70%)** | **14 (35%)** | |
| Median baseline ALC in k/µL (range) | 1.10 (0.11–3.79) | 1.09 (0.37–3.79) | 1.23 (0.11–2.83) | 0.925 |
| Pre-treatment Child-Pugh score | | | | 0.745 |
|   A5 | 80 (56%) | 55 (54%) | 24 (60%) | |
|   A6 | 34 (24%) | 26 (25%) | 8 (20%) | |
|   B7 | 18 (13%) | 14 (14%) | 4 (10%) | |
|   B8 | 10 (7%) | 6 (6%) | 4 (10%) | |
|   B9 | 1 (1%) | 1 (1%) | 0 (0%) | |
| **Portal venous tumor thrombosis** | | | | **0.008** |
|   **Yes** | **79 (55%)** | **64 (62%)** | **15 (38%)** | |
|   **No** | **64 (45%)** | **39 (38%)** | **25 (63%)\*** | |
| Median splenic volume (IQR)** | 322 cm³ (197–551 cm³) | 333 cm³ (219–551cm³) | 248 cm³ (187–537 cm³) | 0.328 |
| Median dose (range) | 60 Gy (30–100 Gy) | 60 Gy (35–100 Gy) | 68 GyE (30–100 GyE) | 0.050 |
| Median # of fractions (range) | 15 (3–34) | 15 (3–34) | 15 (10–30) | 0.343 |
| Median planning target volume (IQR) | 297 cm³ (143–541 cm³) | 286 cm³ (129–529 cm³) | 339 cm³ (177–573 cm³) | 0.491 |

**Notes:** Attributes for photon-treated and proton-treated patients are compared with *t*-test for continuous variables and χ2 test for categorical variables. Significant differences between patients treated with photons and protons are highlighted in bold. *Percentages do not sum to 100% due to rounding. **Spleen volumes were available for 108 patients (76%), including 73 (71%) treated with photons and 35 (88%) treated with protons.
**Abbreviations:** IQR, interquartile range; AJCC, American Joint Commission on Cancer; ECOG, Eastern Cooperative Oncology Group; ALC, absolute lymphocyte count.

mean change in Child-Pugh score was +1.5 (95% CI, 1.1–1.8). A worsening of Child-Pugh score by ≥ 2 developed in 42 patients (29%). Among all patients, the median OS was 13.8 months (95% CI, 11.1–16.7 months), the median DFS was 7.4 months (95% CI, 5.4–8.8 months), and the median DMFS was 30.0 months (95% CI, 20.6–45.7).

Factors on univariable analysis significantly associated with worse overall survival (Table 2) included the presence of portal venous tumor thrombus, increasing pre-RT Child-Pugh score, pre-RT ALC ≤ 0.5, post-RT ALC ≤ 0.5, G3 or higher lymphopenia during treatment, use of IMRT/VMAT, increasing volume of planning target volume (PTV), and increase in Child-Pugh score after RT. Factors significantly

associated with improved OS included increasing total RT dose and the use of proton therapy.

Variable selection for the multivariable model began with all variables shown in Table 2. Stepwise selection yielded a mean VIF of 1.74; variables in the final multivariable model included are listed in Table 3. The final model included 110 patients to account for patients with one or more missing values. A test of the proportional hazards assumption yielded $\chi^2(19, n=110)=16.62$, p=0.616 and thus the null hypothesis that the hazards are proportional cannot be rejected. The presence of portal venous tumor thrombus, Child-Pugh Class B, pre-RT ALC ≤ 0.5, post-RT ALC ≤ 0.5, increasing volume of PTV, and increased Child-Pugh score after RT were independently
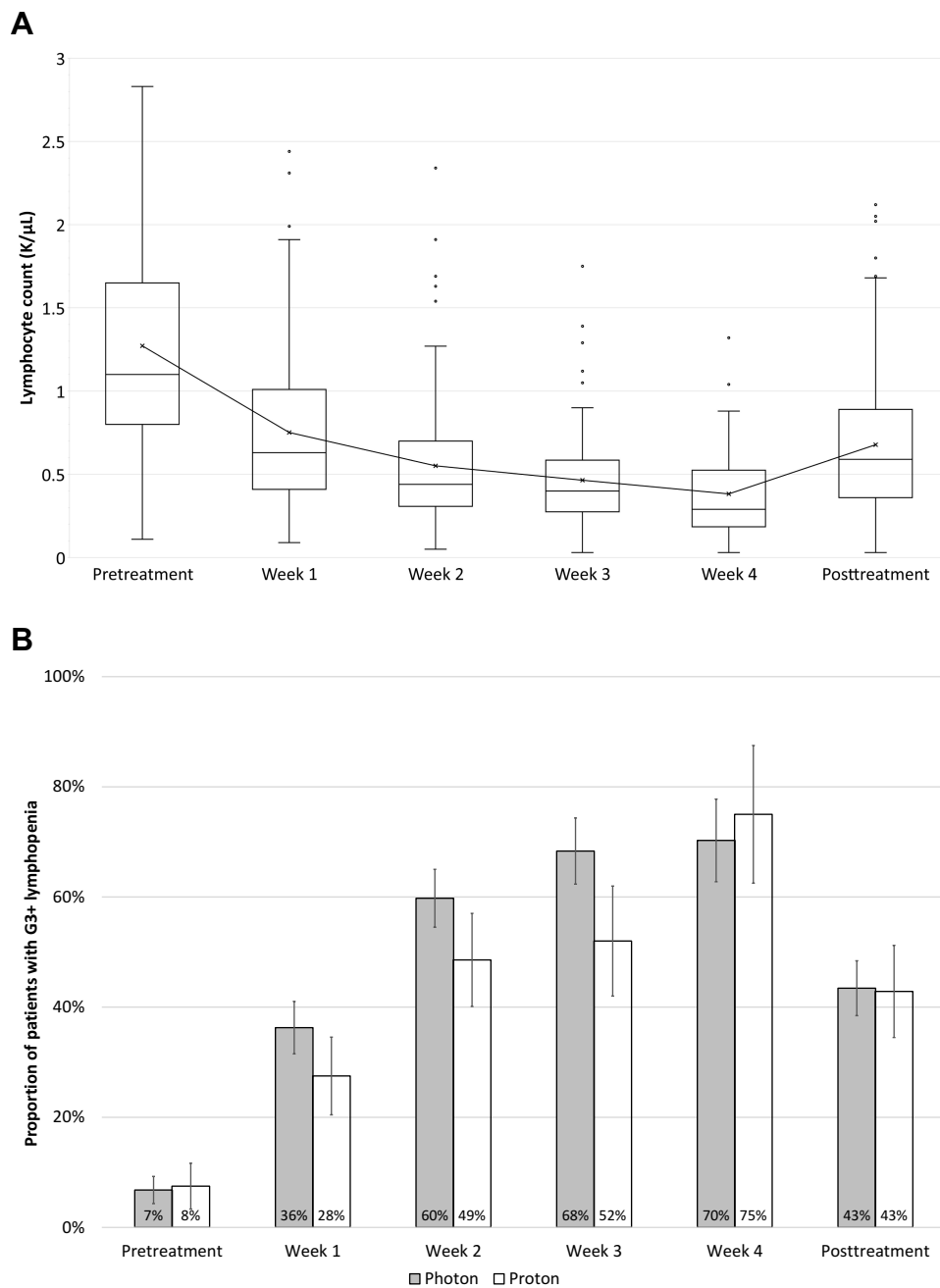
**A**



**B**



**Figure 1** (**A**) The kinetics of lymphocyte counts during radiotherapy and (**B**) the proportion of patients developing G3 or higher lymphopenia stratified by treatment technique.

associated with a significant increased hazard of death, whereas the use of proton RT was independently associated with a reduced risk of death.

## Relationship Between Grade 3 or Higher Lymphopenia and Clinical Outcomes

Patients with pre-RT ALC $\leq 0.5$ had significantly shorter OS (median 7 vs 20 months, p=0.03) and DFS (median 7 vs 28 months) but not DMFS (11 vs 30 months, p=0.71). Patients who had G3 or higher lymphopenia during RT had inferior OS (median 13 vs 31 months, p<0.001) and DMFS (median 16 vs 51 months, p=0.003) but not DFS (median 25 vs 37 months, p=0.0502) compared with their counterparts (Figure 2). Splenic volume did not correlate with OS or DMFS. G3 or higher lymphopenia during treatment was significantly associated with the inferior OS on univariate analysis. On multivariable analysis, ALC $\leq 0.5$

**Table 2** Univariable Analysis for Overall Survival

| Parameters | Hazard Ratio | 95% Confidence Interval | p-value |
|---|---|---|---|
| Age | 0.998 | 1.019–1.086 | 0.002 |
| Sex | | | |
|    Female | (Reference group) | | |
|    Male | 1.017 | 0.643–1.610 | 0.942 |
| AJCC Stage | | | |
|    I | (Reference group) | | |
|    II | 1.055 | 0.534–2.085 | 0.878 |
|    IIIA | 1.803 | 0.741–4.386 | 0.194 |
|    IIIB | 1.448 | 0.884–2.372 | 0.141 |
|    IVA | 1.272 | 0.629–2.573 | 0.504 |
|    IVB | 1.660 | 0.720–3.824 | 0.234 |
| ECOG Performance Status | | | |
|    0 | (Reference group) | | |
|    1 | 1.460 | 0.825–2.584 | 0.194 |
|    2 | 1.532 | 0.706–3.321 | 0.280 |
| **Portal venous tumor thrombus present** | **1.551** | **1.043–2.305** | **0.030** |
| **Pre-RT Child-Pugh Class** | | | |
|    **A** | **(Reference group)** | | |
|    **B** | **1.846** | **1.164–2.929** | **0.009** |
| Viral hepatitis etiology | 0.837 | 0.563–1.244 | 0.378 |
| Prior transarterial chemoembolization | 0.742 | 0.493–1.115 | 0.151 |
| **Pre-RT Child-Pugh score > 5** | **1.691** | **1.138–2.513** | **0.009** |
| **Pre-RT ALC $\leq$ 0.5** | **2.096** | **1.052–4.177** | **0.035** |
| **Post-RT ALC $\leq$ 0.5** | **1.681** | **1.119–2.525** | **0.012** |
| Time to ALC nadir | 1.020 | 0.999–1.041 | 0.059 |
| % change from baseline in ALC at nadir | 1.634 | 0.599–4.460 | 0.338 |
| **Grade $\geq$ 3 lymphopenia during RT** | **2.201** | **1.380–3.511** | **0.001** |
| Use of breath hold | 1.135 | 0.763–1.688 | 0.531 |
| **Use of IMRT/VMAT** | **1.680** | **1.110–2.542** | **0.014** |
| **Use of proton therapy** | **0.498** | **0.315–0.786** | **0.003** |
| **Total RT dose (Gy)** | **0.978** | **0.961–0.995** | **0.012** |
| Dose per fraction (Gy) | 0.947 | 0.861–1.041 | 0.258 |
| **Volume of PTV (in cc)** | **1.0001** | **1.0000–1.0001** | **0.001** |
| **Change in Child-Pugh score after RT** | **1.765** | **1.541–2.022** | **<0.001** |

**Note:** Significant associations are highlighted in bold.
**Abbreviations:** AJCC, American Joint Commission on Cancer; ECOG, Eastern Cooperative Oncology Group; ALC, absolute lympho-cyte count; IMRT, intensity-modulated radiation therapy; VMAT, volumetric modulated arc therapy; PTV, planning target volume.

prior to or after RT – but not during – was found to be significantly associated with inferior OS. Overall, those who received proton RT had a higher median ALC nadir (0.41 vs 0.32 k/μL, p=0.002) than those who had photon RT.

## Relationship Between Splenic Volume and Lymphopenia

The development of lymphopenia is often multifactorial, and in this population of HCC patients treated with radiotherapy,

one potential confounder may be splenomegaly secondary to portal hypertension. To further explore this, we measured the splenic volume on planning CT and found that splenic volume correlated with the development of G3 or higher lymphopenia (p<0.001) during RT (Figure 3A). To investi-gate whether there were treatment-related effects on lympho-cytes and patient outcomes, we compared the splenic volumes of those treated with photons to those who had proton therapy. As shown in Figure 3B, there was no

**Table 3** Multivariable Analysis for Overall Survival Using Selected Variables After Adjustment for Multicollinearity

| Parameters | Hazard Ratio | 95% Confidence Interval | p-value |
|---|---|---|---|
| **Age** | **1.043** | **1.013–1.074** | **0.004** |
| Sex | | | |
|   Female | (Reference group) | | |
|   Male | 0.756 | 0.399–1.431 | 0.391 |
| AJCC Stage | | | |
|   I | (Reference group) | | |
|   II | 1.234 | 0.468–3.256 | 0.671 |
|   IIIA | 0.882 | 0.241–3.220 | 0.849 |
|   IIIB | 0.447 | 0.153–1.306 | 1.141 |
|   IVA | 0.542 | 0.174–1.679 | 0.288 |
|   IVB | 2.171 | 0.642–7.344 | 0.213 |
| ECOG Performance Status | | | |
|   0 | (Reference group) | | |
|   1 | 0.963 | 0.462–2.007 | 0.920 |
|   2 | 0.831 | 0.282–2.454 | 0.739 |
| **Portal venous thrombus present** | **3.576** | **1.472–8.683** | **0.005** |
| Pre-RT Child-Pugh Class | | | |
|   A | (Reference group) | | |
|   **B** | **2.330** | **1.041–5.210** | **0.039** |
| Pre-RT Child-Pugh score >5 | 0.889 | 0.442–1.785 | 0.740 |
| **Pre-RT ALC ≤ 0.5** | **2.677** | **1.057–6.779** | **0.039** |
| ***Post-RT ALC ≤ 0.5** | **1.031** | **1.001–1.062** | **0.043** |
| Total RT dose (Gy) | 0.9999 | 0.9996–1.0001 | 0.471 |
| **Volume of PTV (cc)** | **1.001** | **1.000–1.001** | **<0.001** |
| ***Change in Child-Pugh score after RT** | **1.037** | **1.021–1.053** | **<0.001** |
| Time to ALC nadir (days) | 1.010 | 0.979–1.042 | 0.526 |
| **Use of proton RT** | **0.456** | **0.236–0.881** | **0.019** |

**Notes:** Variables specified as time-varying are indicated by *. Significant associations are highlighted in bold.
**Abbreviations:** AJCC, American Joint Commission on Cancer; ECOG, Eastern Cooperative Oncology Group; ALC, absolute lymphocyte count; PTV, planning target volume.

significant difference in the splenic volume between the two groups.

## Outcomes Stratified by Treatment Technique

After RT, the mean change in Child-Pugh score was +1.8 (95% confidence interval [CI], 1.3–2.2) for photon patients vs +0.8 (95% CI, 0.3–1.2) for proton patients (p=0.006). A worsening of Child-Pugh score by ≥ 2 developed in 34% of photon patients and 18% of proton patients (p=0.03). The median OS with photon RT was 13.2 months (95% CI, 9.6–16.7 months) and with proton RT was 33.2 months (95% CI, 19.8–50.7 months). The median DFS with photon RT was 19.6 months (95% CI, 12.7–28.0 months) and with proton

RT was 36.2 months (95% CI, 29.2–56.0 months). Median DMFS with photon RT was 45.7 months (95% CI, 11.5 months – upper bound undefined) vs 30 months for proton RT (95% CI, 21.7–42.5 months). Kaplan-Meier survival curves for OS and DFS stratified by treatment technique are displayed in Figure 2.

## Matched Cohort Dosimetric Analyses of Proton versus Photon Cases

Matched cohort dosimetric analyses revealed that there were significantly higher body volumes of low-dose bath in the photon group ($V_{1Gy}$ and $V_{10Gy}$, p<0.0001 for both; $V_{5Gy}$, p=0.002) compared to the proton group (Figure 4). Higher body volume of low-dose bath correlated with lower ALC (r= −0.34, p=0.06 for $V_{1Gy}$; r =
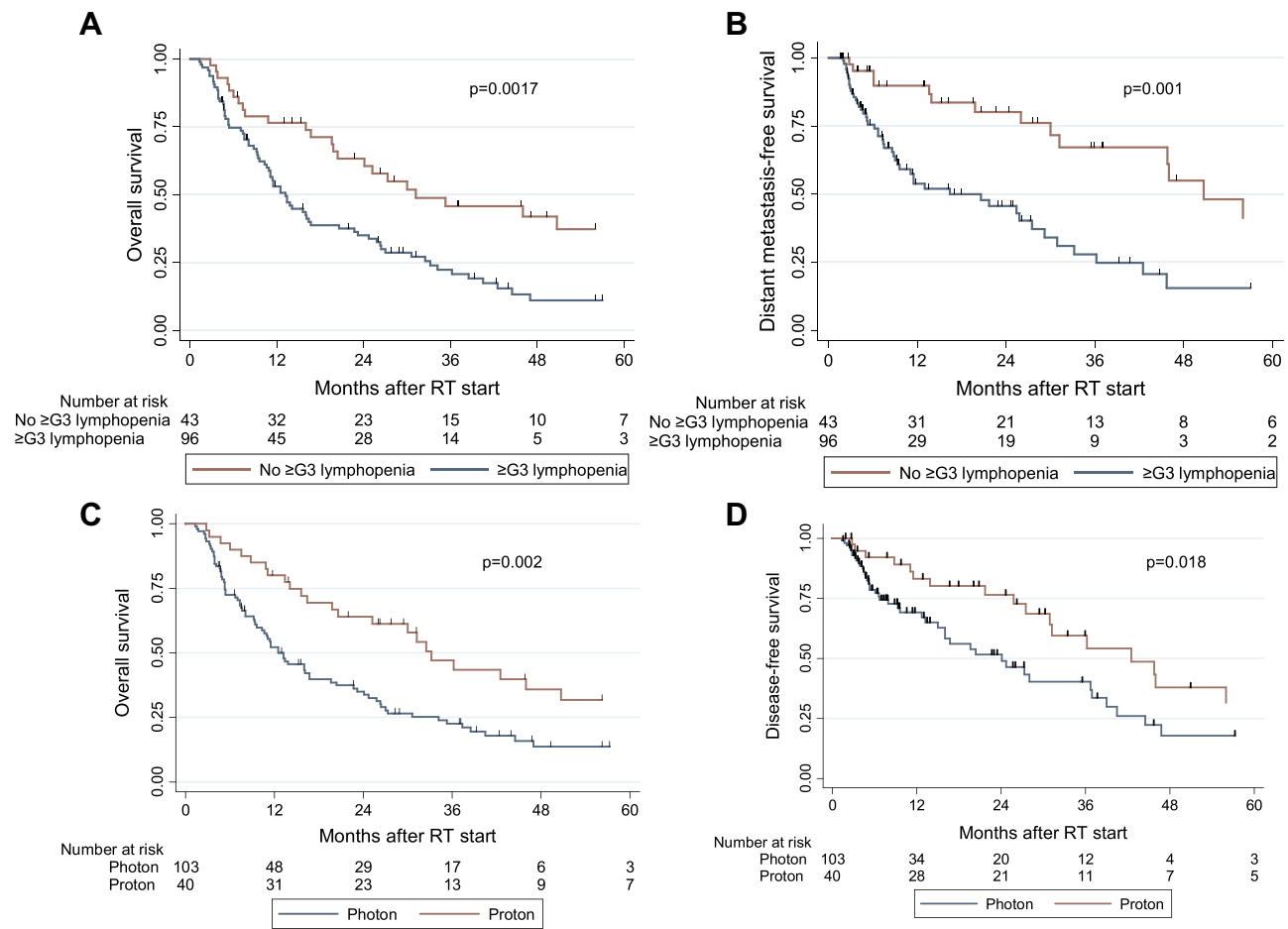
**Figure 2** Survival outcomes stratified by development of grade 3 or higher lymphopenia after RT start (**A** and **B**) and treatment technique (**C** and **D**).
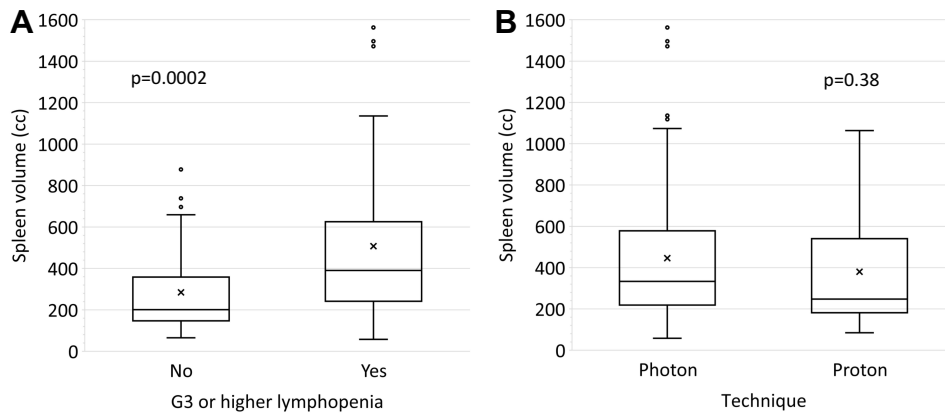


**Figure 3** Distribution of splenic volumes stratified by (**A**) the development of G3 or higher lymphopenia during treatment and (**B**) photon vs proton therapy. Spleen volumes were available for 108 patients (76%), including 73 (71%) treated with photons and 35 (88%) treated with protons.

−0.44, p=0.01 for $V_{5Gy}$; r = −0.51, p=0.004 for $V_{10Gy}$). In addition, splenic irradiation dose for the paired cohort was correlated with ALC nadir, and proton cases had significantly less splenic radiation dose (p<0.0001).

## Discussion

In the current study, we demonstrate that patients with unresectable HCC who developed G3 or higher radiation-related lymphopenia had significantly poorer OS and DMFS than those who did not. The presence of G3
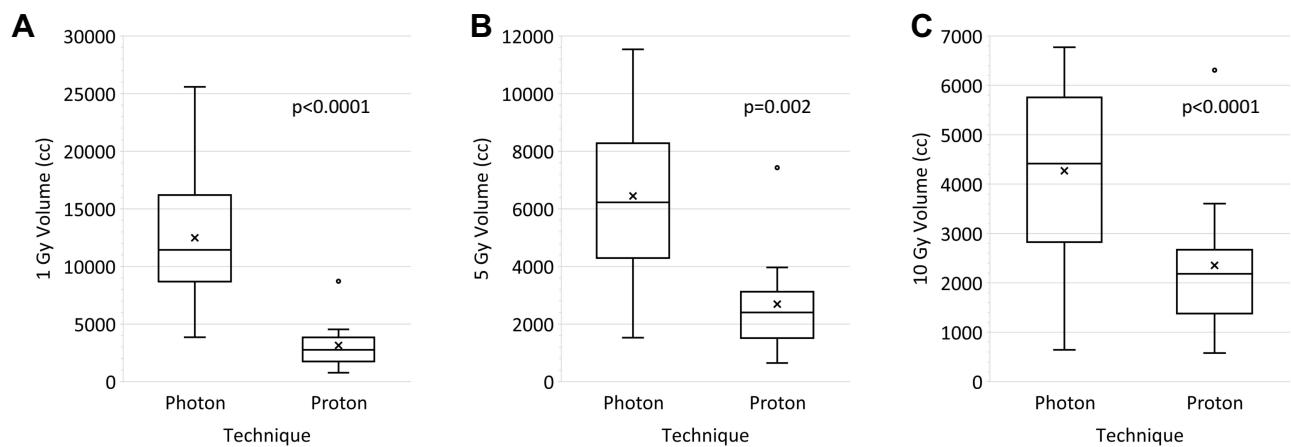
**Figure 4** Matched cohort dosimetric analysis comparing volumes between photon versus proton groups receiving (**A**) 1 Gy, (**B**) 5 Gy, and (**C**) 10 Gy. A representative comparison of proton (top panel) and photon (bottom panel) treatment plans is shown.

or higher lymphopenia before the start of RT and after RT completion were found to be independent predictors of poorer survival. While G3 or higher lymphopenia can develop during both photon- and proton-based RT, proton therapy was associated with significantly lower rates of high-grade lymphopenia. In addition, on matched cohort analysis, our analysis demonstrated that proton-treated patients had significantly less low-dose radiation volume and splenic irradiation, which may help to explain the lower rates of high-grade lymphopenia in this cohort.

Radiation-associated lymphopenia has been associated with poorer patient outcomes in a variety of cancer types. A combined cohort of various newly diagnosed solid tumors demonstrated that high-grade lymphopenia was associated with an increased risk of death in all pathology types studied (hazard ratio [HR] 2.1, 95% CI, 1.54–2.78; $p<0.0001$).[20] A study at the MD Anderson Cancer Center demonstrated the adverse effects of high-grade lymphopenia on outcomes in patients with esophageal cancer.[21] In another study of patients treated for non-small cell lung cancer, lower lymphocyte counts during RT correlated

with larger gross tumor volumes and significantly reduced OS (HR 0.51 per $10^3$ lymphocytes/μL; p=0.01).[14] In a study of limited-stage small cell lung cancer, median OS was found to be 19 months in the lymphopenia group and 132 months in the group that did not develop high-grade lymphopenia.[15] Among patients treated with RT for locally advanced rectal cancer, those with higher ALC nadir had improved response rates (89.7% vs 67.6%, p=0.006) and OS (HR 0.24, CI, 0.08–0.69, p=0.004).[22] In a cohort of patients with HCC treated with RT, patients with high-grade lymphopenia had decreased OS as compared to those without lymphopenia (13.6 vs 46.7 months, p<0.001).[23] Further studies of the effects of radiation therapy in HCC have characterized the effects of RT fractionation on lymphocyte depletion and the relevance of specific T-cell populations in antitumor immunity.[18,24] Overall, these previous studies are consistent with our current findings that G3 or higher lymphopenia was associated with poorer OS (13 vs 31 months).

The mechanism by which lymphopenia develops during RT is hypothesized to be due to the effect of the integral radiation dose on the regional lymph nodes, neighboring organs, and circulating blood volume, suggesting a benefit for use of protons over photons. Indeed, we demonstrated a reduced low-dose bath with protons as compared to photons in a matched cohort analysis. The suggested lymphocyte-sparing capabilities of proton RT in the present study are in line with what previous studies have found in other malignancies. An esophageal cancer study found that 40.4% of patients treated with photons developed grade 4 lymphopenia compared to 17.6% in the proton group (p<0.0001).[9] Similarly, a propensity-matched cohort analysis conducted at the Mayo Clinic found that photon RT was associated with higher rates of G4 lymphopenia than proton RT in patients with esophageal cancer (56% vs 22%; p<0.01).[25] Additionally, a propensity-matched analysis of 144 patients treated with proton RT or photon RT for esophageal cancer demonstrated that photon RT resulted in higher rates of high-grade lymphopenia as compared to proton RT (56% vs 22%; p < 0.01).[25] Previous studies have explored other RT modalities, such as stereotactic body radiotherapy (SBRT) as a means of mitigating lymphopenia. SBRT has been associated with a lower risk for lymphopenia in treating pancreatic ductal adenocarcinoma and has been shown to have a normal liver-sparing effect for smaller HCC lesions.[26] Hypofractionated treatment in HCC has been shown to

lead to decreased lymphocyte depletion and faster recovery following treatment.[18]

Several studies have shown encouraging data for immunotherapy in the treatment of HCC, either as monotherapy or in combination with other therapies, such as targeted agents or RT.[27] However, the impact of lymphopenia on these novel agents and combinations remains a nascent area of investigation. A recent retrospective study of advanced non-small cell lung cancer demonstrated that peri-immunotherapy lymphopenia was associated with significantly poorer PFS and OS, and that multiple courses, multiple irradiated sites, and higher dose were correlated with RT-associated lymphopenia.[28] Similarly, an analysis of Phase I/II lung cancer trials showed that SBRT was associated with an attenuated decrease in ALC in patients receiving combined immunotherapy with RT.[29] The impact of lymphopenia is particularly important to consider when deciding upon RT target volume and technique; in addition to its direct association with poorer outcomes, treatment-related lymphopenia may potentially limit the efficacy of promising immunotherapy agents in HCC.[24] In this context, further investigations into optimal selection, sequencing, and timing of treatments, as well as therapeutic strategies to enable recovery of lymphocytes, are needed.[30]

Our data show that proton RT was significantly associated with improved OS and DFS. The OS benefit was also demonstrated on multivariable analysis, which showed that the use of proton therapy was independently associated with a decreased risk of death by more than half. Data from other institutions are concordant with our findings. The Massachusetts General Hospital reported that proton therapy was associated with a decrease in the development of the non-classic radiation-induced liver disease, as defined by an increase in baseline CP score by ≥ 2 points at 3 months posttreatment. In addition, they reported a median OS benefit in patients with HCC treated with proton RT (31 vs 14 months), findings which are similar to those of the current study (33 vs 13 months).[31,32] A prospective study of proton therapy for HCC and intrahepatic cholangiocarcinoma reported promising local control and OS rates as compared to historical data using photons, leading to a randomized study of protons versus photons for HCC (NRG GI003, NCT03186898).[32] A trial at the University of Tsukuba reported that proton RT was both safe and effective for the treatment of HCC and demonstrated a 5-year OS of 44.5%.[33]

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This study has some limitations. The small size of the proton cohort may limit robust statistical analysis to more completely characterize the impact of proton therapy on lymphocytes and survival outcomes. Additionally, as with any retrospective study, there may be an element of selection bias. In this study, venous tumor thrombosis also emerged as an independent prognostic factor. A greater proportion of patients treated with photons had portal venous tumor thrombosis and had Stage III or IV disease. Though these attributes were controlled for in our multivariable analysis, these differences may nonetheless confound interpretation of outcomes by technique. Patients who received proton RT in this group tended to have right-sided liver disease thereby resulting in reduced dose to the portal/splenic system. Lastly, splenic dose, which may affect the degree of lymphopenia, was not reported for the entire cohort as it was not routinely recorded prior to 2016. To mitigate this potential bias, a re-calculation of the splenic dose was performed in the matched cohort analysis; however, the sample size for this calculation was limited to 15 per cohort.

Given the hypothesis that RT-associated lymphopenia arises from the irradiation of circulating blood based on the results of this study, it will be important to examine the correlation between the time of delivery of RT and ALC. We hypothesize that reducing the time of exposure to RT and dose to the portal/splenic system may reduce the overall ALC decrease observed during radiation, and one strategy to achieve this may be the use of proton therapy given its dosimetric advantages over photon therapy.[34] Monitoring ALC during RT as a potential predictive biomarker of outcome may allow clinicians to modify their management in order to produce better outcomes. Overall, our study demonstrates that lymphopenia in the peritreatment period is associated with poorer outcomes for patients with unresectable HCC. We have also shown that the use of proton therapy may be associated with mitigation of lymphopenia, reduced frequency of liver decompensation as measured by the change in CPS, and increased OS. These results indicate that the development of strategies to mitigate lymphopenia is warranted.

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