

Late-onset lymphopenia during radiation is associated with an increased risk of tumor recurrence in newly-diagnosed pediatric medulloblastoma

Holly B. Lindsay¹, Michael Scheurer², Anthony K. Allam³, Susan McGovern⁴, Murali Chintagumpala², and Arnold Paulino⁴

¹University of Colorado Anschutz Medical Campus

²Texas Children's Cancer Center and Hematology Centers

³Baylor College of Medicine

⁴The University of Texas MD Anderson Cancer Center Division of Radiation Oncology

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Abstract

Background: Recent data found a correlation between lymphopenia occurring early during craniospinal irradiation (CSI) and risk of disease recurrence in newly-diagnosed childhood medulloblastoma. ¹ However, the population included patients that received myelosuppressive chemotherapy prior to or during RT. Here we investigate the effect of lymphopenia during RT in patients with newly-diagnosed pediatric medulloblastoma who did not receive myelosuppressive chemotherapy with RT. **Procedure:** We analyzed 54 patients with newly-diagnosed medulloblastoma (ages 2-21 years) treated between 1997-2013 with CSI. Log-rank tests were used to determine survival differences, and Cox proportional hazards regression was used to assess associations between patient characteristics and lymphopenia with disease recurrence risk. **Results:** 78% of patients (40/51) had grade [?]3 lymphopenia by RT week 3; 49% (23/47) improved to grade [?]2 lymphopenia by week 5. Similarly, the lowest median absolute lymphocyte count (ALC) occurred during RT week 3. Sixteen of 54 (30%) patients recurred an average of 30.2 months post-diagnosis. There was higher risk of disease recurrence in patients with grade [?]3 lymphopenia during weeks 4 (log-rank $p=0.015$; Cox $p=0.03$) and 5 (log-rank $p=0.0009$; Cox $p=0.004$) of RT. Recurrence-free survival was lower in patients with ALC. **Conclusions:** Lymphopenia during RT weeks 4 and 5 correlates with increased risk of tumor recurrence in pediatric patients with newly-diagnosed medulloblastoma. Future studies should correlate baseline numbers of tumor-infiltrating lymphocytes with risks of lymphopenia during RT and tumor recurrence.

INTRODUCTION

Lymphocytes are the most radiosensitive peripheral blood cells due to their inefficient DNA repair mechanisms. Lymphopenia secondary to radiation therapy (RT) is associated with poorer progression-free survival (PFS) and overall survival (OS) in patients with tumors of breast, brain, gastrointestinal tract, head and neck, lung, and pancreas.²⁻⁶ The poorer outcome in patients with RT-induced lymphopenia is hypothesized to be secondary to a decrease in tumor-infiltrating lymphocytes (TIL). It is known that medulloblastoma tumors have low numbers of TIL even prior to the initiation of RT.⁷⁻¹⁰ In patients with childhood medulloblastoma, a recent publication demonstrated a correlation between peripheral lymphopenia occurring during weeks 1 and 2 of craniospinal irradiation (CSI) and risk of disease recurrence.¹ However, many patients in this study received myelosuppressive chemotherapy prior to and/or concomitantly with RT; consequently, lymphopenia cannot be entirely attributed to RT alone.

The purpose of this study is to examine the effect of lymphopenia during RT in an independent popula-

tion of patients with newly-diagnosed pediatric medulloblastoma, none of whom received myelosuppressive chemotherapy concurrently with RT.

METHODS

This retrospective study was approved by our Institutional Review Board (IRB) prior to data abstraction or analysis. A waiver of informed consent was granted by the IRB as the study was entirely retrospective. The study population included patients 2 to 21 years old at tumor diagnosis treated for newly-diagnosed medulloblastoma at our tertiary children’s hospital between 1997-2013 with therapy that included CSI. Patients treated on clinical research studies SJMB96 (NCT00003211) or SJMB03 (NCT00085202)^{11,12}, and those treated for medulloblastoma outside of the context of a clinical trial were eligible for inclusion in our analyses. All patients treated prior to 2007 received photon beam RT while those treated in or after 2007 received proton beam RT.

Patients were classified as having high-risk disease, prior to the advent of molecular risk stratification, if they had metastatic disease (M stage) or post-operative residual disease $>1.5 \text{ cm}^2$; patients not meeting these criteria were classified as having clinically standard-risk disease.

The following data were abstracted from the medical record for each eligible patient: name, date of birth, gender, date of tumor diagnosis, age at diagnosis, tumor pathology, date and extent of surgical resection, M stage, start and end dates of RT, dose and modality (photon or proton beam) of RT, complete blood count (CBC) results at the start of/during/immediately after RT, post-RT chemotherapy regimen used, date of first recurrence (to allow calculation of recurrence-free survival (RFS), and survival status (alive or dead).

Weekly lymphocyte counts were analyzed both as a continuous variable and graded per the National Cancer Institute’s Common Terminology Criteria for Adverse Events (CTCAE) version 5.0: grade 1—absolute lymphocyte count (ALC) $<$ lower limit of normal - $0.8 \times 10^9/\text{L}$, grade 2— $\text{ALC} < 0.8 - 0.5 \times 10^9/\text{L}$, grade 3— $\text{ALC} < 0.5 - 0.2 \times 10^9/\text{L}$, and grade 4— $\text{ALC} < 0.2 \times 10^9/\text{L}$.

Descriptive statistics were used to characterize the study population. Kaplan-Meier survival curves were constructed, and the log-rank test was used to determine differences in survival curves by key clinical variables. Cox proportional hazards regression was used to assess the associations (hazards ratio and 95% confidence intervals) between patient characteristics and lymphopenia during RT with risk of disease recurrence, unadjusted and adjusted for risk group. A p-value of 0.05 was used as the cut-off for statistical significance. All analyses were conducted in Stata (SE version 17; Stata Corp, College Station, TX).

RESULTS

Clinical Characteristics of Cohort

Fifty-four patients diagnosed between 1997 and 2013 met our inclusion criteria; the clinical characteristics of the cohort are listed in **Table 1**. Seventy-four percent of patients in our cohort (40/54) had M0 disease. Two of the 54 patients (4%) received chemotherapy prior to RT: 1 of these patients was 2 years old at diagnosis and received chemotherapy treatment according to the Children’s Oncology Group ACNS0334 for patients with high risk infant medulloblastoma until he reached the age of 3 years at which time CSI was initiated, and the 2nd patient received 2 cycles of topotecan prior to starting RT.

All patients initiated RT within 35 days of tumor resection. Patients with standard-risk medulloblastoma received 23.4 Gray (Gy) CSI, while those with high-risk disease received 36-39.6 Gy CSI; both populations then received a boost to the tumor bed to a total dose of 54-55.8 Gy. Eighty percent of patients (43/54) received proton beam RT, while the remaining 11 patients received photon beam RT. For patients that were treated with proton therapy, the entire vertebral body was covered with the CSI prescription dose for skeletally immature patients, while for those who had achieved skeletal maturity, only the posterior portion of the vertebral body was treated during CSI.

One patient (2%) received concurrent chemotherapy during RT; however, the chemotherapy was single agent vincristine, which is not myelosuppressive. Following completion of RT, 82% of patients (44/54) received

chemotherapy either enrolled on or treated according to clinical trials SJMB96 or SJMB03 with vincristine, cisplatin, and cyclophosphamide.

Lymphopenia During RT

The number of patients with grade 3 or 4 lymphopenia during RT as well as the median ALC of all patients during RT is shown in **Figs. 1** and **2** and **Supplemental Table 1**. Seventy-eight percent of patients (40/51) had at least grade 3 lymphopenia by week 3 of RT, but 51% (24/47) had improved to grade 2 or lower lymphopenia by week 5. Similarly, the lowest median ALC occurred during week 3 of RT. Of note, not all 54 patients in our cohort had lymphocyte counts checked each week (see **Supplemental Table 1**). The degree of lymphopenia was greater in those patients who received a higher craniospinal dose (**Figs. 1** and **2**).

Impact of Clinical Factors and RT-induced Lymphopenia on Risk of Disease Recurrence

Sixteen of 54 (30%) patients developed a recurrence at an average of 30.2 months after diagnosis. As expected, patients with M+ disease had a significantly increased risk of disease recurrence (log-rank $p=0.0004$; Cox $p=0.001$). **Table 2** demonstrates the impact of clinical factors and RT-induced lymphopenia on the risk of disease recurrence. There was a higher risk of disease recurrence (lower RFS) in patients with grade 3 or lymphopenia during weeks 4 (log-rank $p=0.015$; Cox $p=0.03$) and 5 (log-rank $p=0.0009$; Cox $p=0.004$) of RT (**Fig. 3**). When considering the RFS of patients with ALC above or below the median during weeks 4 and 5 of RT, there was a significant difference during week 5 (log-rank $p=0.0026$; Cox $p=0.002$) (**Fig. 4B**) but not during week 4 (log-rank $p=0.123$; Cox $p=0.22$) (**Fig. 4A**). These results all held true when adjusted for risk group (**Table 2**). No correlation was found between risk of tumor recurrence and early lymphopenia (RT weeks 0-3) either when measured as CTCAE grade or as median ALC. Additionally, there was not a statistically significant difference between RFS in patients treated with proton compared to photon RT (Cox $p=0.09$) (**Supplemental Fig. 1**) or based on sex (Cox $p=0.810$).

DISCUSSION

Almost all patients in our study were treated uniformly, with only 2/54 (4%) receiving chemotherapy prior to RT and 1/54 (2%) receiving non-myelosuppressive chemotherapy (vincristine) during RT. The majority of the patients (80%, 43/54) also received proton beam RT. Typically, for patients receiving 23.4 Gy CSI, the CSI portion of the RT course is completed by the end of week 3, with CSI extended through week 4 for those receiving [?] ≥ 36 Gy; by week 5, CSI is normally complete, and the RT field shrinks to cover only the tumor bed. Consequently, late lymphopenia during weeks 4 and 5 may have more clinical significance than lymphopenia that develops earlier in the RT course, as the exposure to CSI is completed at the end of week 3 or later.

As previously mentioned, RT-induced lymphopenia is associated with worse PFS and OS in numerous adult tumors, a phenomenon presumed secondary to RT causing a decrease in TIL.^{2-6,13-15} Among CNS tumors, higher numbers of both CD3+ T cells generally and specifically CD8+ cytotoxic TIL at the time of tumor diagnosis are associated in the majority of studies with improved prognosis in adult high grade glioma and atypical meningioma.^{13,14,16-19}

Unfortunately, medulloblastoma tumor samples characteristically have low numbers of TIL even prior to the start of RT.^{7-10,20,21} These findings suggest that medulloblastoma has an immunosuppressive tumor microenvironment. Overall, CD8+ T cells are the largest component of the TIL in medulloblastoma; additionally, there are differences in numbers of CD8+ T cells and TIL in general among medulloblastoma molecular groups, subgroups, and even down to the subtype level.^{8,9,20,22-24} The few published investigations assessing for a correlation between survival and TIL/CD8+ T cell numbers in pediatric medulloblastoma have had inconsistent results, demonstrating that larger studies are clearly needed.^{23,24}

Our data showed no statistically significant difference in RFS between patients treated with protons vs. photons and in fact showed a trend toward lower RFS in patients that received proton RT. However, there is increasing awareness that proton beam RT may induce less lymphopenia than photons.²⁵⁻²⁷ When proton

vertebral body sparing techniques are utilized for CSI, which in our cohort occurred for skeletally mature patients, data shows that lymphopenia is lessened^{28,29}, as the vertebral bodies contain >25% of the total bone marrow.³⁰ The majority of our patients had their entire vertebral body covered by the CSI dose due to receiving photon beam RT or being skeletally immature during proton beam RT.

To our knowledge, there are no published data regarding the association of molecular group with lymphopenia during RT. It is of value to control for molecular group when assessing for an association between risk of recurrence and lymphopenia over the course of RT which unfortunately we were unable to do in our study as the vast majority were diagnosed prior to the routine use of molecular grouping as standard diagnostic practice.

While our study shows the influence of lymphopenia during week 4 and 5 of RT on risk of medulloblastoma, other groups have shown that early lymphopenia (weeks 1 and 2 of RT) is what impacts the risk of recurrence in medulloblastoma¹ or that there is no effect at all of lymphopenia on RFS in childhood medulloblastoma³¹. More data are clearly needed from additional patients with newly-diagnosed childhood medulloblastoma and ideally should be collected in a prospective fashion. Future studies should consider analyzing lymphocyte subsets throughout the RT course to characterize changes in specific lymphocyte populations over time. It would also be worthwhile to correlate the baseline number of TIL from the initial tumor resection with the risk both of lymphopenia throughout the RT course and tumor recurrence.

In summary, lymphopenia during weeks 4 and 5 of RT measured as CTCAE grade 3-4 and during week 5 of RT measured as below median ALC correlates with an increased risk of tumor recurrence in patients with newly-diagnosed childhood medulloblastoma.

CONFLICT OF INTEREST STATEMENT: SLG serves on the advisory board for Chimerix, which has no relationship to this manuscript. HBL, MES, AKA, MC, and ACP have no conflicts of interest to disclose.

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LEGENDS

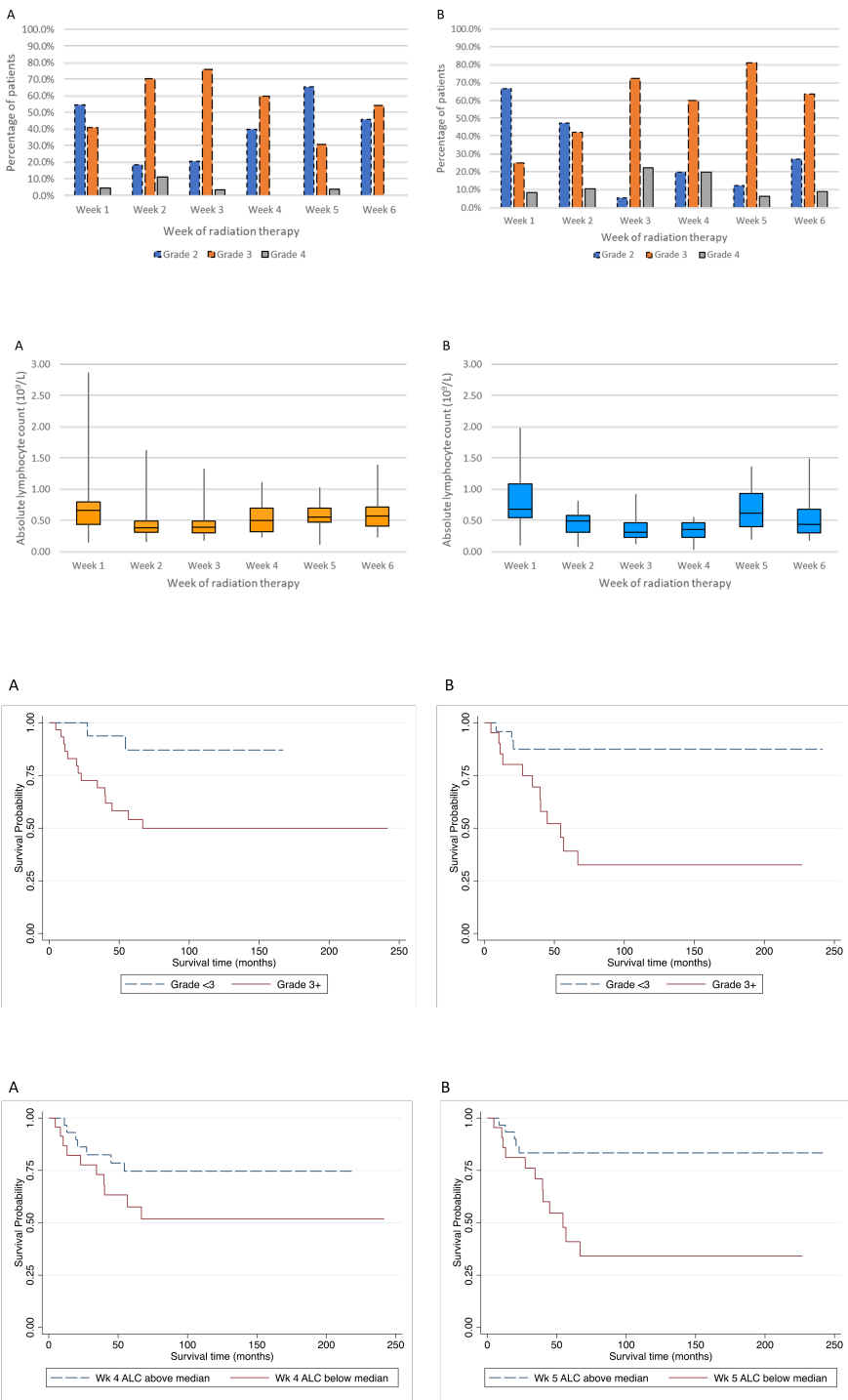
FIGURE 1 Percent of patients that received 23.4 Gray (**A**) or 36-39.6 Gray (**B**) craniospinal irradiation who developed grades 2-4 lymphopenia during radiation therapy

FIGURE 2 Absolute lymphocyte count during radiation therapy in patients that received 23.4 Gray (**A**) or 36-39.6 Gray craniospinal irradiation (**B**)

FIGURE 3 Higher risk of disease recurrence (lower recurrence-free survival) with grade 3 or 4 lymphopenia in weeks 4 (**A**) and 5 (**B**) radiation therapy (log-rank p=0.015 and Cox p=0.03 for week 4; log-rank p=0.0009 and Cox p=0.004 for week 5)

FIGURE 4 Low absolute lymphocyte count (ALC) during week 5 of radiation therapy is associated with lower recurrence-free survival (higher risk of disease recurrence) (**B**); this association was significant during week 4 of radiation (**A**) (log-rank p=0.123 and Cox p=0.22 for week 4; log-rank p=0.0026 and Cox p=0.002 for week 5) (*low/high split on median ALC value of 0.4368 for week 4 and 0.4905 for week 5)

SUPPLEMENTAL FIGURE 1 No difference between recurrence-free survival in patients treated with proton vs. photon radiation therapy (hazard ratio=0.40, p=0.09)



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