Proton therapy offers a reduction in dose to normal tissues compared to conventional photon therapy. Since the majority of pediatric cancer patients are expected to become long-term survivors [1], children are often referred to proton therapy to minimize radiation damage. While the overall incidence of brainstem injury following cranial proton therapy is relatively low [2–4], it is a very serious side effect that can lead to symptoms such as ataxia, dysphagia, respiratory difficulty, and in worst case death [2].

Protons have a higher relative biological effectiveness (RBE) compared to photons. Clinically, the RBE is set to a constant value of 1.1, implying that protons are uniformly characterized as 10% more biologically effective than photons. The RBE of 1.1 (RBE 1.1) was determined as a conservative value mainly based on animal experiments conducted in the 1970s [5]. While a conservative RBE increases the probability of ensuring tumor control, an underestimation of the RBE may lead to overdosage of healthy tissue. It is also well known that the RBE is not constant but varies as a function of the linear energy transfer (LET). Considering that the LET increases rapidly at the distal dose fall-off of the proton beam, elevated RBE values are of particular concern for organs at risk located in vicinity of the fall-off. Moreover, the RBE has also been shown to increase for lower (α/β) ratios in the linear quadratic model as well as for lower dose levels [6]. While these effects have been quantified through both in vitro [5,7,8] and in vivo experiments [9,10], the clinical consequences are less clear. In recent years, several reports have emerged indicating a potential correlation between toxicity and increased RBE [11–19]. Nevertheless, the evidence for correlation is not decisive [20], in particular for symptomatic toxicity, emphasizing the need for further study.
For pediatric brain tumor patients, the RBE variability in proton therapy may be particularly worrying for three reasons: (i) the brainstem is associated with low $(\alpha/\beta)_b$ ratios [21,22], (ii) fraction sizes are typically $\leq 2 \text{ Gy(RBE)}$ [23], and (iii) the LET increases for smaller modulation widths of the spread-out Bragg peak [5] which is often the case when using smaller sized treatment fields commonly applied for children treated with proton therapy.

There is great emphasis on keeping brainstem doses below established constraints. Furthermore, to reduce RBE and range uncertainties associated with proton beams, a common approach is to minimize the number of treatment fields ranging out within the brainstem [2,4,6]. There are, however, still persistent concerns about brainstem toxicity following cranial proton therapy, and regional differences in radiosensitivity of this vital brain structure have been indicated which might influence the incidence of toxicity [21,24,25]. The purpose of this study was therefore to investigate if symptomatic brainstem toxicity in pediatric brain tumor patients treated with proton therapy can be associated with a varying LET and RBE, and whether this effect is specific to anatomic subsites within the brainstem.

Materials and methods

Patient material

An anonymized cohort selected from 954 pediatric patients with brain tumors treated with double scattering proton therapy at the University of Florida Health Proton Therapy Institute (UFHPTI) between 2006 and 2017 were used in this institutional review board-approved case-control study. Symptomatic brainstem toxicity was defined as new or progressive symptoms not attributable to tumor progression, and further characterized as grade 2+ response according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Overall, 16 cases of the 954 patients experienced symptomatic brainstem toxicity. Seven cases were excluded either due to the lack of appropriate controls in the high dose region or due to intrinsic compromise of brainstem integrity. Each of the nine resulting cases was closely matched to three separate controls based on age (±1.5 years), diagnosis, adjuvant therapy, and brainstem RBE 1.1 dose parameters $(D_{10\%} \pm 2 \text{ Gy(RBE)}, D_{0.1cc} \pm 2 \text{ Gy(RBE)})$. All patients had clinical target volumes (CTVs) defined in addition to planning target volumes (PTVs) (CTV plus a 3 mm isotropic margin). The brainstem, including the brainstem core (brainstem cropped by 3 mm) and brainstem surface (3 mm edge of the brainstem), were delineated for treatment planning. For the purpose of this study, T1/T2 weighted magnetic resonance imaging (MRI) scans fused with computed tomography (CT) scans were used to define the substructures of the brainstem which included the midbrain, pons, and medulla oblongata (Fig. 1).

The patients had been diagnosed with either craniopharyngioma or ependymoma. The standard prescription doses ranged between 54.0 and 59.4 Gy(RBE), delivered in fractions of 1.8 Gy (RBE). An example of a dose distribution is shown in Fig. 1. The planning objectives were based on UFHPTI clinical protocols, where the CTV should be encompassed by the 99% isodose line and the PTV should be encompassed by the 95% isodose line of the prescribed dose. Clinically approved dosimetric constraints to the brainstem and organs at risk [24] were used during treatment planning in the Eclipse (Varian Medical Systems, Palo Alto, CA) treatment planning system (TPS).

Monte Carlo simulations

To obtain LET and variable RBE-weighted doses, the CT images as well as treatment plan information for the 36 patients were imported into the FLUKA Monte Carlo (MC) code [26–28] version 2011.2x. We have previously developed a framework that allows for recalculation of proton plans in FLUKA. This framework includes translation of treatment plan information and semi-automated setup of the recalculation system, as well as methods to obtain LET [29] and variable RBE-weighted doses from multiple RBE models [30]. To allow for an accurate recalculation of the proton therapy plans, a detailed model of the double scattering treatment nozzle at the UFHPTI was implemented and commissioned in an earlier publication [31].

The number of treatment fields for each proton plan ranged between two and five. Each field was simulated separately with 600 million primary protons, and scoring files were combined during post-processing. We scored the physical dose, dose-averaged LET (LET$_a$), as well as the LET spectra on a voxel-by-voxel basis using the same scoring grid specifications as in the clinical treatment plans. Using proton stopping power ratios, the dose and LET were converted to dose-to-water and LET-to-water,
respectively (details found in [31]). The dose was calculated taking all particles into consideration, while the LET calculations were based on primary and secondary protons only. To maintain consistency with the clinically calculated TPS dose, and not focus on differences in dose calculation algorithms, the MC recalculated physical dose distributions were in post-processing normalized to the median CTV dose that was obtained during the initial treatment planning, with normalization factors ranging between 3.2% and 2.4%. The normalized dose distributions were used in the analysis of both RBE$_{1.1}$ and variable RBE-weighted doses, while the reported LET values were unaffected by the normalization.

### Results

In areas receiving doses of 54 Gy(RBE) or higher, the median LET$_{10}$, L$_{10}$, and L$_{0.1cc}$ showed trends towards higher average values for the symptomatic brainstem necrosis cases compared to the controls in the brainstem (Fig. 2), with cases having an average median LET$_{10}$ of 2.7 keV/µm (95% CI: 2.5–2.9 keV/µm) compared to controls with an average value of 2.4 keV/µm (95% CI: 2.2–2.6 keV/µm) (P = .08). The trends became more obvious when smaller volumes were considered with differences in case-control means for L$_{1cc}$ at 3.1 keV/µm (95% CI: 2.8–3.5 keV/µm) vs. 2.8 keV/µm (95% CI: 2.7–2.9) (P = .05) and L$_{0.1cc}$ at 3.4 keV/µm (95% CI: 2.9–3.8 keV/µm) vs. 3.0 keV/µm (95% CI: 2.9–3.2 keV/µm) (P = .06). The trend towards higher metrics for cases compared to controls was less evident when applying a dose cutoff of only 1 Gy(RBE), where in the case of the median LET$_{10}$ in the brainstem an average of 3.3 keV/µm (95% CI: 2.8–3.8 keV/µm) was found for cases and 3.1 keV/µm (95% CI: 2.8–3.3 keV/µm) for controls (P = .3).

### Discussion

In this case-control study we investigated the impact of variable RBE-weighted doses and LET$_{10}$ on brainstem toxicity for 36 pediatric patients treated with proton therapy. The case-control differences were generally small for both RBE-weighted dose and LET$_{10}$, with high heterogeneity, wide confidence intervals and insignificant P values. Nevertheless, the average case typically trended towards higher LET$_{10}$ to the brainstem for similar doses, as well as for most brainstem substructures. There was also a minor trend between cases and controls also giving an indication of the statistical significance of the results.
towards increased RBE-weighted dose differences between cases and controls when comparing variable RBE models to RBE\textsubscript{1.1} doses.

Multiple published studies have found a correlation between image changes, i.e., CTCAE grade 1 toxicity and LET/RBE [11,14,16–19], while others have been unable to identify a significant correlation [36–38]. While the degree to which image changes clinically impact patients is unclear [39], a potential advantage of including patients with asymptomatic toxicity is that such patients

Fig. 2. Median LET\textsubscript{d} (a), LET\textsubscript{d} at 10\% volume (\textit{L\textsubscript{10\%}}) (b), and LET\textsubscript{d} at 0.1 cc volume (\textit{L\textsubscript{0.1cc}}) (c) with a 54 Gy(RBE) dose cutoff for cases (red circles) and controls (green squares) in the brainstem and brainstem substructures. Horizontal lines show average values for cases (red solid lines) and controls (green dashed lines), while vertical error bars depict 95\% confidence intervals. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).
are more abundant compared to individuals diagnosed with symptomatic toxicity. For example, the incidence of symptomatic brainstem toxicity for pediatric brain tumor patients following proton have been reported to be approximately 2% [4]. While the low incidence is fortunate, the serious nature of these side effects calls for investigation. Nevertheless, clinically applied efforts to reduce LET in vital organs [2,4,6] coupled with the low incidence of symptomatic brainstem necrosis as well as the difficulty of distinguishing between symptomatic toxicity and disease progression [22], complicates the task of acquiring a sufficient amount of patients to draw definitive conclusions regarding the clinical effects of the RBE variability [40], particularly for this clinical endpoint. For instance, in a recently published study, a power analysis was conducted for head and neck cancer patients treated with intensity-modulated proton therapy. The authors estimated that a data set consisting of over 15,000 patients would be required to determine a definitive correlation between a variable RBE and toxicity for this patient group [41]. Nonetheless, the trends observed in this study coupled with previous evidence should warrant further investigation and clinical precautions with regard to LET.

Several dose cutoffs for the LET were applied in order to explore the isolated clinical effect of the LET, while maintaining the context of biological damage which requires a certain dose level. As a result, metrics based on relative volumes for the LET were only calculated for voxels with doses above the applied cutoff, and not for the full structure. It should therefore be kept in mind that the reported LET metrics at 50% (median) and 10% volumes are only considering the subvolume of voxels above the dose cutoff, hence leading to decreased absolute volumes. A consequence was therefore a higher \( L_{50\%} \) and/or \( L_{10\%} \) compared to \( L_{0.1cc} \) for certain high dose cutoffs (Fig. 2 and Supplementary Fig. S1), due to the relative volumes reaching below an absolute value of

Fig. 3. LET volume histograms for the brainstem for each matched group with cases (red solid lines) and controls (green dashed lines). No dose cutoff has been applied. The x-axes vary between different patient groups. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).
Fig. 4. LET₄ distributions for all patients in voxels with RBE₄ doses of 50 Gy(RBE) or above. Boxes in the bottom right corners list median LET₄ as well as median doses from RBE₄, ROR, and LWD in the brainstem. Midbrain, pons, and medulla oblongata (from top to bottom) are delineated in red. The sagittal plane is centred in the pons and cropped window sizes have been normalized for all patients.
Fig. 5. Median doses from RBE$_{1.1}$ (a), LWD (b) and ROR (c) for cases (red circles) and controls (green squares) in the brainstem and brainstem substructures. Horizontal lines show average values for cases (red solid lines) and controls (green dashed lines), while vertical error bars depict 95% confidence intervals. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).
0.1 cc. Nevertheless, the trend of higher average LETₐ metrics for cases vs. controls was generally consistent regardless of the applied dose cutoff, evaluated metric or structure. It is also important to note that the LETₐ was scored using only primary and secondary protons, in agreement with the majority of previously published papers on LETₐ in proton therapy [42]. Including heavier particles would increase the calculated LETₐ [43,44], in particular in the entrance region of the proton beam [45]. Nevertheless, until there is a consensus in the scientific community regarding which particles to include for LETₐ calculation [46], the most important measure is to precisely report the method of LET calculation [42].

The substructures were separately evaluated in order to identify any trends in LETₐ or RBE-weighted dose to specific sections of the brainstem. While there was a certain variance in both LETₐ and RBE-weighted dose to the substructures, no obvious trends were identified, with the uncertainty in the origin of the brainstem necrosis also contributing towards the inconclusiveness of the substructure analysis. As the necrosis for a case should hypothetically originate from a single substructure, regarding all patients with symptomatic brainstem toxicity as cases for all substructures could introduce ambiguity. This could have been resolved if the precise location of the origin of the necrosis were known with certainty.

In our study, suitable follow-up MRI images were not available, therefore only dosimetric trends related to symptomatic brainstem toxicity as an endpoint could have been discovered through this analysis. Identifying regional differences in radiosensitivity within the brainstem could have been merged with such follow-up MRI images and potential image changes related to toxicity could have been analyzed in relation to the specific substructures. It should, however, be emphasized that image changes are associated with significant uncertainties, especially regarding the origin of necrosis [19,38]. Hence, a study of grade 2+ brainstem necrosis focuses more on the general organ volume of the patient where a voxel-wise analysis of image changes (grade 1) might take away from this focus on symptomatic disease, which additionally is of increased clinical relevance due to their severity and potential lethality compared to the asymptomatic nature of image changes. Furthermore, all structures were evaluated based on the same (∝/□) value, of 2.1. If a significant regional difference in radiosensitivity within the brainstem exists, it would have to be reflected through different (∝/□) values for each substructure, which further would have affected the doses calculated by the phenomenological ROR model.

In conclusion, we identified very minor trends towards increased RBE-weighted dose to cases compared to controls. Case-control trends were more apparent when considering LETₐ as the average case received higher LETₐ than the average control for nearly all dose levels and brainstem substructures. There was, however, a substantial interpatient variability leading to wide confidence intervals and case-control differences that generally could not be considered statistically significant. Nevertheless, due to trends observed in this study we believe that individual assessment of LET in clinics should be explored further and successful application may provide safer delivery of proton therapy for patients at risk of brainstem toxicity.

Conflict of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2022.07.022.

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