CLINICAL STUDY

Pre-treatment lymphopenia and indication of tumor-induced systemic immunosuppression in medulloblastoma

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Abstract

The presence of tumor-induced systemic immune suppression, including lymphopenia, has been recognized in adult patients with glioblastoma for several decades, and pre-treatment neutrophil-to-lymphocyte count ratio (NLCR) is associated with inferior clinical outcome in patients with glioblastoma. Whether tumor-induced systemic immune suppression is also present in children with malignant brain tumors is not known. We performed a retrospective analysis of pretreatment neutrophil and lymphocyte counts in pediatric patients with medulloblastoma (MB) compared to a control group of children with posterior fossa pilocytic astrocytoma (PA). Compared to the control group, we observed statistically significantly lower absolute lymphocyte counts (ALCs) and higher NLCRs in the medulloblastoma group. Our findings suggest the presence of tumor-induced systemic immune suppression in MB patients already present at the time of diagnosis, with potential implications for the development of immune therapies in this population.

Keywords Medulloblastoma · Lymphopenia · Neutrophil/lymphocyte count ratio · Tumor-induced systemic immunosuppression

Introduction

Brain tumors represent the most common solid tumor in children. Immunosuppression has been associated with cancer at the time of diagnosis, even prior to subsequent cytotoxic therapies. The presence of tumor-induced systemic immune suppression, including lymphopenia, anergy, diminished CD4 T-cell compartment and cytokine shift toward regulatory T cells (T_{reg}), has been recognized in adult patients with glioblastoma for several decades [1–3]. In these patients, an elevated neutrophil-to-lymphocyte count ratio (NLCR) has been associated with worse prognosis [4, 5]. In contrast to the adult literature, there is a lack of data on the prevalence of tumor-induced systemic immune suppression in pediatric patients with malignant brain tumors. It is currently not known if tumor-induced systemic immune suppression is present at the time of diagnosis in pediatric patients with malignant brain tumors. Therefore, we retrospectively analyzed absolute lymphocyte counts (ALC) and absolute neutrophil counts (ANC) from pediatric patients with malignant brain tumors at the time of diagnosis, prior to treatment interventions, and compared to a control cohort.

We chose patients with medulloblastoma (MB), which represents the most common malignant brain tumor in children, for the study group. For the control group, we selected patients with posterior fossa pilocytic astrocytoma (PA). Posterior fossa PA is the most common low-grade pediatric brain tumor, with similar age, tumor location and size at presentation compared to MB.

The study protocol was approved by the NYU Langone Medical Center Institutional Review Board and was performed in accordance with institutional policies.

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Methods

Case selection and clinical review

From the electronic medical record, we retrospectively identified pediatric patients diagnosed with MB at ≤ 18 years of age and treated at NYU Langone Medical Center between 2000 and 2016. Patients were included in the study group if results of a complete blood count (CBC) including differential were available at the time of admission, and the CBC was collected prior to treatment intervention, surgery, and/or radiotherapy. Patients who received corticosteroids prior to the initial CBC were excluded, since corticosteroids are known to alter neutrophil and lymphocyte counts rapidly after administration [6]. For the study group, charts from 72 patients were reviewed, 16 of which met the inclusion criteria for further analysis.

For a control group, patients with a diagnosis of posterior fossa PA were identified, with otherwise identical inclusion criteria as in the study group. A total of 73 charts were reviewed, 20 of which fit the inclusion criteria.

Demographic, clinical and laboratory information, including CBC at first presentation prior to treatment intervention, were abstracted from the medical records from eligible patients in the study group and control group. NLCR was calculated as neutrophil count divided by lymphocyte count.

Genome-wide methylation profiling

Molecular subtype data was available for 6 of the 16 described MB cases. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue, and genome-wide methylation profiling was performed at NYU Department of Molecular Pathology using the Illumina Infinium Human Methylation 450 Bead-Chip (450 K array) according to the manufacturer's instruction (Illumina). MB subtype status was established and annotated as SHH, WNT, Group 3 or Group 4, as previously described [7].

Statistical analysis

To assess for age differences between sample populations, a Shapiro–Wilk's test was performed to determine data normality, followed by a Student's t test. The Mann–Whitney U test was performed for differences in CBC parameters, including NLCR, ANC and ALC. A Fisher Exact Test was used to assess differences in the proportion of lymphopenia among sample populations. Lymphopenia was established if ALC fell below normal age-adjusted ranges of lymphocyte count. Statistical significance was determined based on a p value of less than 0.05.

Results

Age range was 1–18 years in both the study group and the control group. The mean age at diagnosis was 7.75 ± 5.43 years in the study group, and 7.15 ± 3.86 years in the control group. There was no statistically significant difference in age between groups. The study group and control group included 11 males (68.8%) and 5 females (31.2%), and 11 males (55.0%) and 9 females (45.0%), respectively (Tables 1, 2, 3).

The median NLCR for the MB population was 2.50; the mean ANC and ALC were 4.84 ± 1.97 and 2.42 ± 1.72 K/µL, respectively. In the control group, the median NLCR was 1.45, with a mean ANC 4.80 ± 2.35 K/µL and mean ALC 3.19 ± 1.34 K/µL. In the MB group, NLCR was higher and ALC was lower compared to the PA group, reaching statistical significance (p < 0.05) (Table 3). We compared ALCs to published age-adjusted normal lymphocyte count ranges [8] to determine the gross prevalence of lymphopenia in each population. The proportion of lymphopenic patients in the MB population (43.75%) was statistically significantly higher (p < 0.05) compared to the PA group (5.0%). There was one patient with elevated ANC in the MB group, as well as the PA group; all other patients had ANC values within normal range for age (Tables 1, 2).

The four main subgroups of medulloblastoma (WNT, SHH, Group 3 and Group 4) express demographic, transcriptional, genetic and clinical differences. The WNT (Wingless)

Table 1 Study group (MB) patient demographics and CBC data

Age [years]	Sex	Molecular subtype	ANC [K/µL]	ALC [K/µL]	NLCR
1	F	n/a	2.1	6.8	0.31
1	M	SHH	3.4	3.7*	0.92
2	F	n/a	4.0	1.6*	2.5
3	M	n/a	3.4	1.4*	2.4
4	M	Group 3	7.6	4.4	1.7
4	M	Group 3	5.2	1.6*	3.3
5	M	n/a	5.9	1.3*	4.5
5	M	n/a	3.9	5.2	0.75
8	F	n/a	6.2	2.8	2.2
8	M	Group 4	2.4	0.9*	2.7
11	M	n/a	6.3	1.5	4.2
12	F	n/a	5.1	1.6	3.2
13	M	SHH	3.7	0.9*	4.1
14	M	Group 4	4.8	1.9	2.5
15	M	n/a	3.7	1.9	1.9
18	F	n/a	9.7**	1.2	8.1

NLCR neutrophil-to-lymphocyte count ratio, *ANC* absolute neutrophil count, *ALC* absolute lymphocyte count (*below or **above age-adjusted range of normal [8])

Table 2 Control group (PA) patient demographics and CBC data

Age [years]	Sex	ANC [K/µL]	ALC [K/µL]	NLCR
1	F	7.6	7.6	1.0
3	M	5.0	2.6	1.9
3	M	2.8	3.4	0.82
4	F	5.9	4.7	1.3
4	M	3.5	3.8	0.92
4	M	5.0	1.8*	2.8
6	M	2.3	2.6	0.88
6	M	7.9	2.8	2.8
6	F	1.9	2.8	0.68
6	F	4.6	1.5	3.1
7	F	4.3	3.1	1.4
7	M	6.2	3.7	1.7
8	F	5.0	1.5	3.3
8	F	1.9	3.3	0.58
10	F	4.8	3.1	1.5
10	M	11.7**	4.1	2.9
10	M	4.5	2.7	1.7
10	M	4.8	3.9	1.2
12	F	4.2	2.7	1.6
18	M	2.1	2.0	1.1

NLCR neutrophil-to-lymphocyte count ratio, *ANC* absolute neutrophil count, *ALC* absolute lymphocyte count (*below or **above age-adjusted range of normal [8])

subgroup offers favorable and long-term prognosis, while Group 3 patients often have a very poor prognosis. SHH medulloblastoma, named after the Sonic Hedgehog signaling pathway, and Group 4 medulloblastoma have a similar prognosis that is intermediate to WNT and Group 3 medulloblastoma [9]. We collected molecular subgroup data on the MB population when available to assess whether specific molecular subtypes correspond with observed changes in ALC. Six samples had available whole-genome methylation profiling data, of which two clustered as SHH, two as Group 3, and two as Group 4. Within these subsets, two SHH, one

Group 3, and one Group 4 MB patients had reduced lymphocyte counts (Table 1).

Discussion

In contrast to adult patients with glioblastoma, there is a paucity of data in regards to tumor-induced systemic immune suppression in children with malignant brain tumors. Comparing pediatric MB patients to a control group of posterior fossa PA patients, we identified statistically significantly reduced ALCs and elevated NLCRs in the MB patients. Furthermore, the incidence of lymphopenia at initial presentation in the MB population was statistically significantly higher in the MB population. These findings suggest the presence of tumor-induced systemic immune suppression and represent a potential novel finding that has not been previously reported in pediatric brain tumors and should be verified in prospective studies.

While the NLCR has been recognized as a prognostic biomarker in adult glioblastoma [4, 5], no such data exist in malignant pediatric brain tumors. In a recent retrospective series including pediatric patients less than 3 years of age, Tumturk et al. found an association between the presence of a central nervous system (CNS) tumor and set of hematological parameters including pre-operative white blood cell count (WBC), mean platelet volume (MPV) and NLCR [10]. In the study, WBC, MPV and NLCR were higher in the study group, and regression analyses showed a positive association between these parameters and the presence of a CNS tumor. The study group was limited to patients less than 3 years of age and included a wide range of malignant and benign tumor types. No relationship between MPV, WBC, NLCR, and histological subgroups was observed.

To our knowledge, this study is the first to investigate the prevalence of pre-treatment lymphopenia in pediatric brain tumors, suggesting the presence of tumor-induced systemic immunosuppression in medulloblastoma. A major strength of our study is that we excluded patients who prior to their

Table 3 Study group (MB) and control group (PA) comparison

	Study group (n = 16)		Control group (n = 20)		
Sex	11 male, 5 female		11 male, 9 female		
Mean age ± SD (range)	7.75 ± 5.43 years (1–18)		7.15 ± 3.86 years (1–18)		p = 0.70
	Mean ± SD	*Median*	*Mean ± SD*	*Median*	
NLCR	2.83 ± 1.86	2.50	1.66 ± 0.86	1.45	p < 0.05 (U = 92.5)*
ANC [K/µL]	4.84 ± 1.97	4.40	4.80 ± 2.35	4.70	(U = 153)*
ALC [K/µL]	2.42 ± 1.72	1.60	3.19 ± 1.34	2.95	p < 0.05 (U = 92.5)*
Lymphopenia (%)	n = 7 (43.75)		n = 1 (5.0)		p = 0.012

SD standard deviation, *NLCR* neutrophil-to-lymphocyte count ratio, *ANC* absolute neutrophil count, *ALC* absolute lymphocyte count

*Critical U at p < 0.05 = 98

initial CBC had received corticosteroids, which are known to rapidly alter peripheral leukocyte counts [6].

There are notable limitations to our study. First, this was a single-site retrospective cohort study, and findings should be confirmed in other cohorts. Second, our analysis limited the prevalence of lymphopenia to MB, the most common malignant pediatric brain tumor. In addition, sample size was limited primarily due to incidences of missing data and/or administration of dexamethasone prior to CBC collection. Therefore, prospective studies should interrogate the presence of tumor-induced systemic immunosuppression in MB and other pediatric brain tumors and on a larger cohort.

Available molecular information on the MB samples was reported to determine associations between molecular subtype and ALC. We observed reduced lymphocyte counts in all SHH patients, and in half of the Group 3 and Group 4 patients. Due to the small sample size of each subpopulation, comprehensive analysis was limited. Future studies should assess the molecular characterization of the reduced lymphocyte population and whether systemic immunosuppression varies by medulloblastoma subtype.

Our findings have implications for future research, including the development of immune therapies for children with MB, which generally have an unfavorable local immunologic environment, with a paucity of infiltrating myeloid and lymphoid cells [11], and lack of programmed death ligand 1 (PD-L1) expression [12]. The apparent combination of local and systemic immune suppression present in MB represents a challenge for the development of immune therapies, and the underlying mechanisms should be further investigated in future studies.

Compliance with ethical standards

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