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Pilot study of DNA methylation-derived neutrophil-to-lymphocyte ratio and survival in pediatric medulloblastoma

Vidal M. Arroyo^a, Philip J. Lupo^{a,b}, Michael E. Scheurer^{a,b}, Surya P. Rednam^{a,b}, Jeffrey Murray^c, M. Fatih Okcu^{a,b}, Murali M. Chintagumpala^{a,b}, and Austin L. Brown^{a,b,*}

^aBaylor College of Medicine, One Baylor Plaza, Houston, TX, USA

^bTexas Children's Cancer and Hematology Centers, Houston, TX, USA

^cCook Children's Medical Center, Ft. Worth, TX, USA

Abstract

Introduction: Methylation-derived neutrophil-to-lymphocyte ratio (mdNLR) has been identified as a potential prognostic biomarker of outcomes in various cancers. We evaluated the prognostic value of blood-derived mdNLR within a retrospective cohort of pediatric medulloblastoma patients.

Materials and Methods: DNA methylation was measured in archival peripheral blood samples collected on 56 pediatric medulloblastoma patients. Hazard ratios (HR) and 95% confidence intervals (CI) for the association between mdNLR and survival were evaluated using Cox proportional hazard models.

Results: Compared to patients who were alive at last follow-up (n=43), the mean mdNLR value was slightly higher in deceased patients (n=13) (12.3 vs. 5.2, $P = 0.163$). Elevated log-transformed mdNLR was suggestively associated with an increased likelihood of death in unadjusted models (HR=1.43, 95%CI: 0.92–2.22) and significantly associated with mortality in adjusted models (HR=1.61, 95%CI: 1.01–2.58).

Discussion: Future work is warranted to investigate the relationship between mdNLR outcomes in specific pediatric medulloblastoma molecular subgroups.

* **Corresponding Author:** Austin L. Brown, PhD, MPH, Assistant Professor, Department of Pediatrics, Hematology-Oncology Section Baylor College of Medicine, One Baylor Plaza, MS: BCM305, Houston, TX 77030 Austin.Brown@bcm.edu, Phone: (713)798-6634, Fax: (713)798-8711.

Authorship Contribution

VMA, PJJ, and ALB conceived this study. ALB, SPR, VMA and MFO performed data collection and quality control. VMA and ALB produced all tables and figures. VMA, PJJ, and ALB analyzed data. All authors interpreted findings and identified discussion points. VMA and ALB drafted the manuscript, which was reviewed and modified with input from all authors over various versions. All authors saw and approved the final manuscript.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

Introduction

Improvements in the treatment of pediatric medulloblastoma, the most common malignant brain tumor diagnosed in children, have resulted in overall five-year survival rates exceeding 70% (1). Current risk stratification methods which account for tumor heterogeneity have identified molecular subgroups with differences in clinical outcomes (2); however, additional host factors likely contribute to interpatient variability in survival and outcomes. Therefore, the identification of prognostic biomarkers may inform clinical decision making in pediatric medulloblastoma. Numerous studies have demonstrated a link between inflammation and cancer progression and outcomes (3). Specifically, circulating neutrophil-lymphocyte ratio (NLR), a marker of systemic inflammation, has been associated with poor prognosis in multiple cancers, including adult brain tumors and pediatric solid tumors (4, 5). More recent studies have successfully applied cellular deconvolution methods to peripheral blood epigenome-wide DNA methylation array data to estimate methylation-derived NLR (mdNLR) (6). Elevated mdNLR values have been associated with an increased risk of death in adult solid tumor and brain tumor patients (7), but have not been evaluated among pediatric medulloblastoma patients. Therefore, the goal of this study was to explore the potential prognostic significance of peripheral blood mdNLR in pediatric medulloblastoma.

Materials and Methods

We retrospectively identified pediatric patients (<18 years of age at diagnosis) diagnosed and treated for medulloblastoma at Texas Children's Cancer Center between 1995 and 2015. Analyses were restricted to patients with available archival peripheral blood samples collected during routine clinical blood draws within five years of diagnosis. Clinical and demographic information for each patient was abstracted from medical records. This study was approved by the Institutional Review Board at Baylor College of Medicine.

The Gentra Puregene Blood Kit (Qiagen, Valencia, California, USA) was used to extract DNA from available peripheral blood samples. DNA methylation was evaluated using the Illumina Infinium Human Methylation450 Beadchip array (Illumina Inc, San Diego, CA). Preprocessing and quality control of methylation files were conducted to remove probes with a detection p-value >0.01 in >1% of samples, a bead count <3 in >5% of samples, cross-reactive probes or probes associated with single nucleotide polymorphisms. Quality-controlled methylation data were beta-mixture quantile normalized and batch-corrected. The estimated proportion of CD4+ T Cells, CD8+ T Cells, B cells, natural killer (NK) cells, monocytes, and granulocytes was used to compute mdNLR, as previously described (7).

Descriptive statistics of demographic and clinical characteristics were compared between patients who were deceased and alive at last follow-up. Kaplan-Meier survival curves and accompanying log-rank p-value were calculated to compare overall survival between patients above and below predetermined mdNLR cutoffs previously reported in the cancer literature: 2.5, 3.0, and 4.0. On the basis of log-rank tests, an mdNLR cutoff of 3.0 was selected as the optimal cutoff for the current study. Because NLR has been linearly associated with outcomes in previous studies (8), we also evaluated mdNLR as a continuous variable, log-transformed to improve normality. Cox proportional hazard regression models

were estimated to evaluate the association between mdNLR and overall survival in unadjusted models and models accounting for age at diagnosis, sex, race/ethnicity, and risk group. All statistical analyses were conducted in Stata Version 14 software (StataCorp LP, College Station, TX) at a p-value=0.05 significance level.

Results

We identified 56 eligible medulloblastoma patients with peripheral blood samples available (Table 1), of which 77% (n=43) were alive at last contact (median duration of follow-up: 6.0 years, range: 1.1 – 15.4 years). The study population was predominately male (64.3%), non-Hispanic white (64.3%), and treated on or according to St. Jude's Medulloblastoma (SJMB) 96/03 or Children's Cancer Group (CCG) 9961 protocols (80.4%). Demographic and clinical characteristics were similar between patients who were alive at last contact and those who died during follow-up (p >0.05).

We observed a slightly higher mdNLR among patients who did not survive (12.3) compared to those who were alive at the last follow-up (5.2), but the crude difference did not reach statistical significance (p=0.163). Of the 20 patients with a mdNLR ≥ 3 , 30.0% (n=6) died during study follow-up, compared to 19.4% (n=7) of the 36 patients with a mdNLR <3 (Figure 1, log-rank p-value=0.17). Similarly, in the unadjusted Cox proportional hazard regression model (Table 2), we found that a higher log-transformed mdNLR was suggestive of an association with poorer prognosis (HR=1.43, 95%CI: 0.92–2.22). The observed association between log-transformed mdNLR and overall survival was statistically significant in separate regression models accounting for age at diagnosis, sex, and race/ethnicity (HR=1.61, 95%CI: 1.01–2.58) as well as clinical risk group (HR=1.75, 95%CI: 1.01–3.04).

Discussion

This study is among the first to leverage epigenetic profiles from archival blood samples (median time from diagnosis to sample collection: 9.0 months; range: 0.0 – 58.6 months) to derive NLR in pediatric populations and evaluate the prognostic potential of mdNLR. Independent of clinical and demographic factors, we observed an increased risk of death with increases in log-transformed mdNLR. These findings are largely consistent with the adult literature, which has demonstrated a correlation between mdNLR and NLR and adverse outcomes in multiple cancers, including malignant brain tumors (7). Considered collectively, this evidence supports epigenetically-defined NLR as a potentially promising prognostic biomarker of inferior outcomes in pediatric medulloblastoma.

The biological basis underlying the association between high NLR and poor cancer survival have not been definitively elucidated. Higher levels of mdNLR may serve as a marker of host immune profiling in cancer, signaling a state of systemic inflammation and immune suppression (9). Elevated peripheral blood NLR is associated with greater neutrophil and lower T-cell tumor infiltration in adult glioblastoma. Neutrophil infiltration is thought to contribute to tumor progression and angiogenesis (10). In pediatric brain tumor patients, pre-operative blood neutrophil counts are inversely associated with histological grade (11),

suggesting more aggressive pediatric intracranial malignancies may be more likely to trigger a systemic immune response. Moreover, infiltrating neutrophils also appear to inhibit CD8+ T-cell activity (12). T-lymphocytes are a central component of the host defense against cancer and suppress proliferation of malignant cells through cytokine signaling and cytolytic activity (13). Compared to controls, pediatric medulloblastoma patients have depressed lymphocyte counts and elevated NLR at diagnosis (14). Additional work is warranted to investigate the link between systemic and tumor-localized inflammation and their impact on clinical outcomes.

We identified mdNLR as a significant prognostic marker in pediatric medulloblastoma. The use of peripheral blood samples is an appealing, minimally invasive biospecimen for biomarker discovery, while the use of DNA methylation array data to estimate NLR is an attractive avenue of research in settings where cytologic evaluation of NLR is not always possible. This pilot study is limited by the lack of systematic peripheral blood sample collection and information on medulloblastoma molecular subgroups. Because immunologic differences may exist between molecular subgroups (15), the association between NLR and outcomes should be reevaluated in prospective studies of medulloblastoma molecular subgroups with standardized sample collection. Nonetheless, mdNLR seems to hold promise as a prognostic biomarker for pediatric medulloblastoma that captures immune-specific information. If replicated in future prospective studies, mdNLR has the potential to guide the delivery of clinical interventions to patients who are most likely to benefit from adjuvant therapy.

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Highlights

- Neutrophil-to-lymphocyte ratio (NLR) can be estimated from DNA methylation data
- Elevated methylation-derived NLR linked to mortality in pediatric medulloblastoma
- This association was independent of demographic and clinical factors

Figure 1. Kaplan-Meier curve comparing overall survival for pediatric medulloblastoma patients with peripheral blood mdNLR ≥ 3 and mdNLR < 3

Table 1.

Demographic characteristics of study participants

	Alive (N=43)	Deceased (N=13)	P-val
Mean age at diagnosis, year(SD)	8.1 (4.6)	7.1 (5.4)	0.527
Median time to sample (Range), year	0.8 (0.0, 4.9)	0.4 (0.0, 4.1)	0.800
Median follow up time (Range), year	6.0 (1.1, 15.4)	2.6 (0.1, 11.3)	0.006
Sex			0.752
Male	27 (62.8)	9 (69.2)	
Female	16 (37.2)	4 (30.8)	
Race/ethnicity, N(%)			0.304
White	34 (79.1)	8 (61.5)	
Black	6 (13.9)	3 (23.1)	
Other	3 (7.0)	2 (15.4)	
Chemotherapy Regimen, N(%)			0.455
SJMB96/03orCCG-9961	30 (83.3)	7 (70.0)	
VCR, CDDP, CTX or CCNU +/- VP-16	5 (13.9)	2 (20.0)	
Other without these agents	1 (2.8)	1 (10.0)	
Treatment-associated risk, N(%)			0.660
Average/Standard	22 (57.9)	5 (50.0)	
High	10 (26.3)	3 (30.0)	
Age < 3 years	6 (15.8)	2 (20.0)	
Mean mdNLR (SD)	5.2 (11.7)	12.3 (25.4)	0.163
Mean estimated cell proportion (SD) *			
CD8+ T cells	0.069 (0.062)	0.066 (0.055)	0.862
CD4+ T cells	0.071 (0.052)	0.075 (0.068)	0.826
Natural Killer	0.065 (0.054)	0.036 (0.047)	0.092
B cells	0.072 (0.044)	0.065 (0.057)	0.681
Monocytes	0.142 (0.058)	0.137 (0.041)	0.782
Granulocytes	0.599 (0.118)	0.629 (0.157)	0.456

Abbreviations: Standard Deviation, SD; P-value from t-test or Fischer's exact test, P-val; Number, No.; Vincristine, VCR; Cisplatin, CDDP; Cyclophosphamide, CTX; Lomustine, CCNU; Etoposide, VP-16; methylation-derived Neutrophil-to-Lymphocyte Ratio, mdNLR

* Cell proportions estimated from cellular deconvolution methods using DNA methylation array data

Missing or incomplete information on risk (n=8) and chemotherapy regimen (n=10)

Table 2.

Cox proportional hazards models for association between log-transformed mdNLR and survival

	Number of Patients (Alive, Deceased)	HR (95% CI)	P-val
Unadjusted Model	69 (43, 13)	1.43 (0.92, 2.22)	0.110
Adjusted Model A *	69 (43, 13)	1.61 (1.01, 2.58)	0.049
Adjusted Model B **	49 (38, 11)	1.75 (1.01, 3.04)	0.048

* Adjusted for age at diagnosis, sex, and race/ethnicity

** Adjusted for age at diagnosis, sex, race/ethnicity, and treatment-associated risk