Hypovascular Cellular Tumor in Primary Central Nervous System Lymphoma is Associated with Treatment Resistance: Tumor Habitat Analysis Using Physiologic MRI

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ABSTRACT

BACKGROUND AND PURPOSE: The microenvironment of lymphomas is known to be highly variable and closely associated with treatment resistance and survival. We tried to develop a physiologic MR imaging–based spatial habitat analysis to identify regions associated with treatment resistance to facilitate the prediction of tumor response after initial chemotherapy in patients with primary central nervous system lymphoma.

MATERIALS AND METHODS: Eighty-one patients with pathologically confirmed primary central nervous system lymphoma were enrolled. Pretreatment physiologic MR imaging was performed, and K-means clustering was used to separate voxels into 3 spatial habitats according to ADC and CBV values. Associations of spatial habitats and clinical and conventional imaging predictors with time to progression were analyzed using Cox proportional hazards modeling. The performance of statistically significant predictors for time to progression was assessed using the concordance probability index.

RESULTS: The 3 spatial habitats of hypervascular cellular tumor, hypovascular cellular tumor, and hypovascular hypocellular tumor were identified. A large hypovascular cellular habitat was most significantly associated with short time to progression (hazard ratio, 2.83; $P=0.017$). The presence of an atypical finding (hazard ratio, 4.41; $P=0.016$), high performance score (hazard ratio, 5.82; $P=0.04$), and high serum lactate dehydrogenase level (hazard ratio, 1.01; $P=0.013$) was significantly associated with time to progression. A predictive model constructed using the habitat score and other imaging parameters showed a concordance probability index for prediction of time to progression of 0.70 (95% CI, 0.54–0.87).

CONCLUSIONS: A hypovascular cellular tumor habitat is associated with treatment resistance in primary central nervous system lymphoma, and its assessment may refine prechemotherapy imaging–based response prediction for patients with primary central nervous system lymphoma.

ABBREVIATIONS: CE = contrast-enhanced; C-index = concordance probability index; ECOG = Eastern Cooperative Oncology Group; LDH = lactate dehydrogenase; MTX = methotrexate; nCBV = normalized cerebral blood volume; PCNSL = primary central nervous system lymphoma; TTP = time to progression

Primary central nervous system lymphoma (PCNSL) represents a histologically and immunohistochemically homogeneous type of lymphoma. However, the microenvironment of B-cell lymphomas was recently shown to be highly variable with regard to both the spatial arrangement and composition of cells, including immune and inflammatory cells, blood and lymphatic vascular networks, and the extracellular matrix. The tumor microenvironment is closely associated with treatment resistance and survival and partly reflects the tumor cell content and genetic aberrations. The mainstay of treatment for PCNSL is high-dose methotrexate (MTX) in combination with other chemotherapeutic agents. Because drug delivery, distribution, and interaction with the tumor are determined by the tumor microenvironment, depiction of the spatial arrangement of tumor cellularity and vascularity may provide valuable information for predicting treatment response. The tumor microenvironment can be depicted using imaging parameters derived from physiologic MR imaging such as ADC mapping, CBV mapping, and permeability parameters. In PCNSL, high cellularity with a low ADC is associated with poor
enable more precise prediction of treatment response, especially and vascularity-reflecting physiologic MR imaging parameters will hypovascularity. The combined spatial information from cellularity-regions of relative hyper- or hypocellularity and relative hyper- or hypervascularity. The combined spatial information from cellularity-and vascularity-reflecting physiologic MR imaging will enable more precise prediction of treatment response, especially identification of any tumor fraction with treatment resistance, which may exhibit high cellularity and low vascularity. Clustering methods can be applied to measurements of multiple parameters to separate different tumor habitats through parcellation. We hypothesized that physiologic MR imaging–based tumor parcellation could potentially provide spatial information on pretreatment PCNSL and identify regions of treatment resistance in patients with PCNSL. The purpose of this study was to develop spatial habitat analysis based on multiparametric physiologic MR imaging and identify regions associated with treatment resistance to facilitate the prediction of tumor response after initial chemotherapy in patients with PCNSL.

**Materials and Methods**

**Study Population**
This retrospective study was reviewed and approved by the institutional review board of Asan Medical Center (institutional review board No. 2021–0265), and the requirement for informed consent was waived. A review of the database of Asan Medical Center identified 103 patients who were diagnosed with PCNSL between July 2011 and September 2018. The inclusion criteria were as follows: 1) pretreatment acquisition of 3T MR imaging with a brain tumor protocol including anatomic MR imaging and physiologic MR imaging (DWI and DSC perfusion imaging); 2) histopathologic confirmation of PCNSL by stereotactic biopsy; and 3) adequate follow-up examinations (no longer than 2 weeks after completion of all planned therapy) to determine the treatment response according to clinicoradiologic consensus after MTX-based chemotherapy. Patients were excluded if they were lost to follow-up visits (n = 6), the tumor was surgically resected (n = 15), or any imaging was unreadable (because of artifacts, n = 1). Therefore, 81 patients were included in this study (age range, 29–80 years; mean age, 61.6 [SD, 11.8] years; 43 women). A flow chart of patient selection is shown in Fig 1.

**Clinical Variables and Treatment**
Clinical and pathologic data were retrieved from the medical records. Baseline characteristics including age, sex, Eastern Cooperative Oncology Group (ECOG) performance status at diagnosis, initial tumor volume, mean serum lactate dehydrogenase (LDH) level, mean CSF total protein, pretreatment imaging characteristics (location and presence of atypical findings), and initial posttreatment response based on a clinicoradiologic examination were collected.

All included patients had a pathologic diagnosis of diffuse large B-cell PCNSL and received MTX-based induction chemotherapy following 1 of 3 protocols: 1) MTX only (3–8 g/m²/day; n = 54); 2) a combination therapy with MTX and procarbazine, lomustine (CCNU), and vincristine regimen (PCV); procarbazine (0.1–0.2 g/m²/day)/vincristine (1.4 mg/m²/week; n = 15); or 3) a combination therapy with MTX, PCV, and rituximab (375 mg/m²/week; n = 12). After the patient was diagnosed with diffuse large B-cell PCNSL, dexamethasone was used in all patients for supportive care to minimize symptoms due to vasogenic edema or tumor mass effect.

**Response Assessment and Outcome Definition**
The posttreatment tumor response was assessed according to the International Primary CNS Lymphoma Collaborative Group criteria based on MR imaging, corticosteroid use, CSF cytology, and slit lamp examination. A complete response was defined as resolution of contrast-enhancing lesions on follow-up MR imaging and, if indicated, by CSF cytologic analysis (performed if CSF cytology was positive for malignant cells at the time of diagnostic staging). A partial response was defined as an interval decrease in contrast-enhancing lesion volume, and progressive disease was defined as an interval increase in contrast-enhancing lesion volume or development of new enhancing lesions on follow-up contrast-enhanced MR imaging or involvement of the eye or CSF. All follow-up imaging to assess the overall treatment response was performed within 2 weeks after completion of all planned chemotherapy of the first or second cycle. Clinicoradiologic diagnoses of posttreatment responses were made by consensus between a neurooncologist (Shin-kyo Yoon, with 10 years of experience in neuro-oncology practice) and a neuroradiologist (Ho Sung Kim, AJNR Am J Neuroradiol 43:40–47 Jan 2022 www.ajnr.org 41

**Fig 1.** Flow chart of patient recruitment.
with 18 years of experience in neuro-oncologic imaging) after complete imaging and medical chart review. Both complete response and partial response were used as a composite end point to define "treatment response," and both stable disease and progressive disease were used as a composite end point to define "treatment resistance." Time to progression (TTP) was defined as the interval between the initiation of induction therapy until documentation of resistance to induction therapy (MR imaging after 6 cycles or stable disease after at least 2 cycles and progression or death from any cause), relapse, or death from any cause after having achieved a partial or complete response.

**MR Imaging Acquisitions**

The brain tumor imaging protocol was acquired on a 3T scanner (Ingenia 3.0 CX; Philips Healthcare) and included conventional and advanced sequences, including T2WI, T2-weighted FLAIR, T1WI, DWI, DSC perfusion imaging, and contrast-enhanced (CE)-T1WI. The detailed imaging protocols are provided in the Online Supplemental Data, and the preprocessing procedures for the DWI and DSC imaging are summarized in the Online Supplemental Data.

**Conventional Image Analysis**

All MR images were read by an experienced neuroradiologist (S.Y. Jeong with 6 years of experience) who was blinded to the clinical outcomes. Conventional MR imaging parameters included deep or superficial location and the presence of atypical features of necrosis or a hemorrhagic component. A deep location meant involvement of the cerebral hemisphere with the exception of deep brain structures. The Online Supplemental Data show cases demonstrating atypical findings of PCNSL on conventional imaging.

**Deep Learning Segmentation**

The overall process of the deep learning segmentation and tumor habitat analysis is shown in Fig 2. The 3D CE-T1WI of each patient was registered and resampled into $\frac{1}{16}$ mm isometric voxel sizes. T2WI, ADC, and normalized CBV (nCBV) maps were then coregistered and resampled to the isovoxel 3D enhanced T1WI using rigid transformations with 6 df in the SPM package (Version 12; http://www.fil.ion.ucl.ac.uk/spm/).

Deep learning segmentation was performed using an implementation of a 3D UNet-based method (https://github.com/MIC-DKFZ/nnUNet) applied to the FLAIR and 3D CE-T1WI using the PyTorch package, Version 1.1 in Python 3.7 (http://www.python.org). This deep learning segmentation package is optimized for processing heterogeneous MR imaging results with various pathologic characteristics. The segmentation process included identification of contrast-enhancing lesions, necrosis, and peritumoral edema, with the contrast-enhancing lesions being chosen for subsequent investigation.

**Tumor Habitat Analysis**

The final voxel classifications based on ADC and nCBV values were implemented using a K-means clustering module in the scikit-learn Python package (https://scikit-learn.org/stable/index.html). The individual voxels in each cluster were grouped according to their similarities and differences using a K-means clustering algorithm with squared Euclidean distances between voxel
intensities as the similarity metric. The cluster number was set to 3 to depict 3 different habitats according to the combinations of ADC and nCBV parameters, with the intention of avoiding over-parameterized models and producing a model that was easily explainable. For example, low or high ADC and CBV values reflected “hyper-/hypovascularity” and “cellularity/hypocellularity,” respectively, and 3 clusters was the lowest number to demonstrate the biologic differences.

Population-Level Clustering. Three clusters were set using ADC and nCBV values: Cluster 1 represented hypervascular cellular tumor with high CBV values and low ADC values; cluster 2 represented hypovascular cellular tumor with low CBV values and low ADC values; and cluster 3 represented hypovascular hypocellular tumor with low CBV values and high ADC values. All voxels were allocated to 1 of the 3 clusters and were displayed as spatial habitats in the original image space. The parameter value ranges for the 3 clusters was the lowest number to demonstrate the biologic differences.

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The optimal tumor habitat cutoff for stratifying patients according to treatment response and treatment resistance was >9000 voxels in the hypovascular cellular habitat. This cutoff value stratified patients according to treatment response and treatment resistance with a significant difference in the log-rank test \((P = .001)\). Figure 4 shows the Kaplan-Meier survival curve and the risk table generated on the basis of the hypovascular cellular habitat. A habitat risk score based on discrete increments in the hypovascular cellular habitat was assigned for the patients.

### Performance of the Prediction Model for TTP

The results of the prediction of TTP in PCNSL using tumor habitat and single physiologic MR imaging parameters are summarized in Table 4. The C-index for prediction of TTP using tumor habitats was 0.65 (95% CI, 0.52–0.78), which was significantly higher than that of the single physiologic MR imaging parameters.

A predictive model was constructed using the habitat score, the presence of an atypical finding, the ECOG score, and the serum LDH level. The C-index of this model combining selected clinical predictors, conventional imaging predictors, and tumor habitat for predicting TTP was 0.73 (95% CI, 0.67–0.80), which was higher than that of models based on clinical predictors only (C-index, 0.68; 95% CI, 0.54–0.82) or conventional imaging predictors only (C-index, 0.63; 95% CI, 0.50–0.76). The Hosmer-Lemeshow test indicated that the model had good calibration \((P > .5)\). The C-index of the combined model of clinical predictors and conventional imaging predictors for predicting TTP was 0.71 (95% CI, 0.54–0.78), which was slightly lower than the combined habitat score model, but it did not reach statistical significance \((P = .10)\).

### Discussion

In this study, we demonstrated that in PCNSL, the TTP after initial chemotherapy could be predicted by physiologic MR imaging–based tumor habitat analysis. A hypovascular cellular habitat with both low CBV and ADC values was significantly associated with treatment resistance, with a large extent of hypovascular cellular habitat being associated with a short TTP. Furthermore, a model combining selected clinical parameters, conventional imaging predictors, and tumor habitat analysis had great predictive power for identifying treatment resistance to initial chemotherapy.

The tumor microenvironment is composed of both cellular and molecular components that continuously interact. The tumor microenvironments in lymphomas are highly variable with regard to both the spatial arrangement and composition of cells. The tumor microenvironment plays critical roles in the regulation of tumor cell survival, proliferation, and immune escape, which leads to treatment resistance.\(^{2,4,8}\) Imaging techniques such as texture analysis, fractal analysis, and habitat analysis can quantify these spatial heterogeneities of microenvironments within tumors,\(^{12}\) and in this study, we used tumor habitat analysis to identify tumor subregions and then used them to predict treatment resistance. Tumor habitat analysis, which parcellates similar voxels using a clustering method, can reflect the heterogeneity of tumor cellularity and vascularity and identify subregions that can be used to predict the response to chemotherapy in PCNSL.

A significant inverse correlation exists between tumor cellularity and ADC values,\(^{23}\) suggesting that ADC values can be used as a surrogate marker of tumor proliferation.\(^{24–26}\) ADC values have shown potential as a prognostic imaging biomarker for the response to treatment of non-Hodgkin lymphoma.\(^{27}\) In our study,
low ADC values in PCNSL with a cellular habitat indicating a high tumor burden correlated with adverse clinical outcomes.

CBV measurements obtained using DSC MR imaging are one of the indirect modalities used to evaluate the functional vascularity of the tumor and its oxygenation status, which are closely related to the response to chemotherapy or radiation therapy. The hypovascular habitat identified in our study may reflect a hypoxic microenvironment that is resistant to treatment, with changes in gene and molecular expression and evolution toward increased malignancy and an increasingly aggressive phenotype. Furthermore, low CBV values may represent a decrease in the patent vessels that deliver the chemotherapeutic agent to the tumor bed, which, therefore, induces resistance to treatment. This is supported in a previous study that found that high CBV values were associated with a good prognosis with longer overall survival and progression-free survival in PCNSL. The hypovascular cellular habitat showed the strongest correlation with overall tumor burden, and it may indicate the region of tumor core to be treated rather than the region responding well to therapy.

Five clinical parameters are well-known to correlate with a worse prognosis in PCNSL: elevated LDH, age older than 60 years, poor performance status, elevated CSF protein, and location of tumor within the deep regions of the brain such as periventricular regions, basal ganglia, brainstem, and/or cerebellum. The presence of atypical imaging findings that reflect necrosis or a hemorrhagic component is associated with short TTP. These findings also indicate poor tumor perfusion and decreased perfusion-related drug delivery because of necrosis and hemorrhage within the tumor. This study has several limitations. The first is the small sample size resulting from the rarity of the disease and the lack of external validation. Second, the chemotherapy regimens

FIG 3. A, Demonstration of the 3 spatial habitats defined by clustering of voxels using normalized ADC and nCBV maps in a 53-year-old male patient. The hypervascular cellular habitat (red) shows high nCBV and low ADC, the hypovascular cellular habitat (green) shows low nCBV and low ADC, and the hypovascular hypocellular habitat (blue) shows low nCBV and high ADC. The tumor exhibits a large hypovascular cellular habitat (green), and a persistent enhancing mass was associated with a short TTP after initial chemotherapy. B, Demonstration of the 3 spatial habitats defined by clustering of voxels using normalized ADC and nCBV maps in a 57-year-old male patient. The hypervascular cellular habitat (red) shows high nCBV and low ADC, the hypovascular cellular habitat (green) shows low nCBV and low ADC, and the hypovascular hypocellular habitat (blue) shows low nCBV and high ADC. The tumor has a small hypovascular cellular habitat (green) and showed a complete response at 53 days after initial chemotherapy.
used in this study were not unified. Because there is no consensus regarding the other agents to be used in combination with MTX, several combinations of MTX-based chemotherapy were tried. We did not perform subgroup analysis per regimen because there were only a small number of patients in each combination regimen. Also, although high-dose radiation therapy plus consolidation chemotherapy or radiation therapy or both are used, there is no consensus on their combination with MTX.34 Our results should be confirmed by other studies with strict control of the chemotherapy regimen. Finally, the prediction performance was not high, with a C-index of only 0.70. Nonetheless, our study may be meaningful because it demonstrates the heterogeneity of PCNSL using physiologic MR imaging and the possibility of a future imaging biomarker–directed therapeutic plan. A well-designed prospective study is warranted.

**CONCLUSIONS**

The analysis of spatial habitats derived from multiparametric physiologic MR imaging may provide added value for predicting treatment response after initial chemotherapy. A hypovascular cellular tumor habitat was associated with treatment resistance in PCNSL and may be useful for refining imaging-based prediction for patients with PCNSL before they undergo chemotherapy.

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Disclosure forms provided by the authors are available with the full text and PDF of this article at www.ajnr.org.
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