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Tumor metabolism and neurocognition in CNS lymphoma

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Abstract

Background. The mechanistic basis for neurocognitive deficits in central nervous system (CNS) lymphoma and other brain tumors is incompletely understood. We tested the hypothesis that tumor metabolism impairs neurotransmitter pathways and neurocognitive function.

Methods. We performed serial cerebrospinal fluid (CSF) metabolomic analyses using liquid chromatography-electrospray tandem mass spectrometry to evaluate changes in the tumor microenvironment in 14 patients with recurrent CNS lymphoma, focusing on 18 metabolites involved in neurotransmission and bioenergetics. These were paired with serial mini-mental state examination (MMSE) and MRI studies for tumor volumetric analyses. Patients were analyzed in the setting of the phase I trial of lenalidomide/rituximab. Associations were assessed by Pearson and Spearman correlation coefficient. Generalized estimating equation (GEE) models were also established, adjusting for within-subject repeated measures.

Results. Of 18 metabolites, elevated CSF lactate correlated most strongly with lower MMSE score ($P < 8E\text{-}8$, $\rho = -0.67$). High lactate was associated with lower gamma-aminobutyric acid (GABA), higher glutamate/GABA ratio, and dopamine. Conversely, high succinate correlated with higher MMSE scores. Serial analysis demonstrated a reproducible, time-dependent, reciprocal correlation between changes in lactate and GABA concentrations. While high lactate and low GABA correlated with tumor contrast-enhancing volume, they correlated more significantly with lower MMSE scores than tumor volumes.

Conclusions. We provide evidence that lactate production and Warburg metabolism may impact neurotransmitter dysregulation and neurocognition in CNS lymphomas. We identify novel metabolomic biomarkers that may be applied in future studies of neurocognition in CNS lymphomas. Elucidation of mechanistic interactions between lymphoma metabolism, neurotransmitter imbalance, and neurocognition may promote interventions that preserve cognitive function.

Key Points

1. Lymphoma Warburg metabolism impacts neurotransmitter balance in CSF and potentially impacts neurocognition.
2. Lesional size of lymphoma on MRI correlates with CSF concentrations of lactate, dopamine, and GABA.

Importance of the Study

The etiology of neurocognitive and neuropsychological deficits in cancer patients and cancer survivors is poorly understood. To date, there have been few studies of potential molecular factors elaborated by brain tumors that induce neurotransmitter imbalances linked to cognitive impairment. Neurocognitive function in central nervous system lymphoma is closely linked to quality of life outcomes. Insights into the pathogenesis of cognitive decline in this setting are needed to promote strategies to reverse brain injury. This is particularly important given that a significant fraction of patients currently experience long-term survival after completion of therapy. Our results suggest for the first time that tumor Warburg metabolism modulates selective neurochemical pathways that impact neurocognitive function. We anticipate that future application of biomarkers discovered in this analysis will help to dissect the relationship between tumor bioenergetics and neurotransmitter metabolism and that this may ultimately promote strategies to preserve cognitive function.

Because of ongoing improvements in therapy and advances in survival, the problem of cancer-associated cognitive dysfunction is highly significant. The etiologic basis for many of the neurocognitive and neuropsychological deficits in cancer patients and cancer survivors is poorly understood. While a major research focus during the past decade has been the characterization of the detrimental impact of treatment-related factors such as brain irradiation on neurocognitive function in brain tumor patients, an equally significant problem is the elucidation of intrinsic biological mechanisms of injury to the central nervous system (CNS), mediated directly by neoplasms as well as by the tumor-associated inflammatory response.

Primary CNS lymphoma (PCNSL) often presents with a devastating spectrum of neurologic symptoms including focal deficits related to tumor anatomical location as well as generalized neuropsychiatric symptoms. The basis for neurocognitive dysfunction has been thus far only partially explained. The propensity for growth in periventricular regions and dissemination along axonal projections including the corpus callosum are probable contributing factors. Tumor-associated T2 abnormalities on MRI, indicative of white matter disease, are strongly associated with neurocognitive outcomes.[1] Age and the impact of therapies such as brain radiotherapy and high-dose methotrexate have been implicated. While recent studies have documented improvement in selective domains of neurocognitive function that are associated with successful anti-lymphoma therapy, particularly strategies that avoid standard doses of whole-brain irradiation, a subset of neurologic deficits evident at presentation in PCNSL may improve only very slowly or remain fixed, despite apparent complete remission.[2] Given that neurocognitive function in PCNSL is closely linked to quality of life outcomes, insights into the pathogenesis of cognitive decline in this setting are needed, as are the development of strategies to reverse brain injury, particularly given that a significant fraction of patients currently experience long-term survival after completion of therapy.[1,3]

The neurological consequences of secondary CNS lymphomas (SCNSL) are heterogenous, with symptoms dictated by the presence of parenchymal vs leptomeningeal dissemination and/or cranial nerve involvement. To date, however, there have been few studies of molecular factors elaborated by lymphoma that impair synaptic function or that induce neurotransmitter imbalances linked to cognitive impairment.

The cerebrospinal fluid (CSF) reflects the composition of the extracellular fluid of the central nervous system. Therefore, the analysis of the CSF is a valuable approach to assess the neurochemical properties of the brain under normal and pathophysiological conditions. A variety of experimental data has demonstrated significant correlations between neurotransmitter concentrations in CSF and neuropsychiatric and neurodegenerative pathology.[4,5] This approach is particularly relevant for CNS lymphomas given their proclivity to grow in periventricular and leptomeningeal compartments.

An accumulation of data supports the generalized hypothesis that aberrant mitochondrial bioenergetics in neurons and glia contribute to cognitive decline in a variety of neurodegenerative diseases including Parkinsonism, amyotrophic lateral sclerosis, and Alzheimer's disease. Elevated CSF lactate, indicative of mitochondrial dysfunction in neurons and glia, is linked to the pathogenesis of Alzheimer's dementia and to mini-mental state examination (MMSE) scores in this disease.[6,7] Similarly, elevated concentrations of extracellular lactate have been correlated with adverse outcomes in traumatic brain injury.[8,9]

Since the 1920s, it has been established that malignant transformation is associated with an acquired dependence on nonoxidative glycolysis for energy generation with increased lactate production as a consequence, the Warburg effect.[10,11] Elevated serum lactate dehydrogenase (LDH) is an important indicator of cancer aggressiveness in large B-cell lymphomas and is one of the core prognostic parameters of the scoring system used for PCNSL by the International Extranodal Lymphoma Study Group.[12]

We recently used metabolomic profiling based on differential gas chromatography/mass spectrometry of CSF to identify distinct metabolic constituents of the tumor microenvironment in patients with CNS lymphoma compared to patients with nonmalignant conditions. The most significantly upregulated metabolites associated with pathogenesis of CNS lymphoma were involved in bioenergetic pathways, including CSF lactate. We determined that elevated CSF lactate (>upper limit of normal, 2.8 mmol/L) correlated with shorter survival in three independent therapeutic trials evaluating CNS lymphoma patients with relapsed disease. In addition, we determined that lactate

concentration in CSF was 1.9-fold higher (median) than lactate concentration in plasma, supporting lactate generation from within the brain tumor microenvironment.[13] A weakness of the profiling platform used in this prior study was that as with the majority of global metabolomic profiling efforts of CSF,[14–16] it did not provide absolute quantification of metabolites, thus limiting inter-study comparisons.

Given these observations, the goals of this study are to: (1) quantitatively define for the first time selected components of the CSF metabolomic landscape of CNS lymphomas, with a focus on neurotransmitters and bioenergetics; (2) identify factors associated with impaired MMSE score and potentially with impaired neurocognition in CNS lymphomas. We hypothesize that lymphoma metabolism directly interferes with global neurocognitive function both by disrupting physiologic energy metabolism in neurons and glia and via the induction of imbalances in neurotransmitter pathways.

Materials and Methods

Patients

We performed this analysis within the context of the phase I trial of lenalidomide plus rituximab, in which a planned correlative study was to identify novel prognostic metabolomic biomarkers from within the tumor microenvironment and to evaluate their relationship to neurocognitive function. We performed MMSE at on-study consent, pre-treatment baseline, and at monthly time points simultaneously with collections of ventricular CSF for protocol-based analytical metabolomic studies. In addition, matched neuroimaging volumetric studies using MRI were performed for the majority of these analytical time points. Eligibility required age >18 years, recurrent/refractory lymphoma involving brain, CSF/meninges, and/or intraocular compartments, HIV negativity, survival >1 month, stable or no glucocorticoids. No patient received immunochemotherapy or investigational agents within 30 days of treatment. An Ommaya reservoir facilitated CSF collections in each patient in whom normal CSF flow was documented at baseline nuclear medicine studies. All patients were English-speaking and had at least high school level of education. All patients signed informed consent indicating awareness of potential risks and benefits of the study in accordance with national regulatory and review boards, the UCSF Committee on Human Research and the Declaration of Helsinki.

CSF Metabolomic Analysis

To identify candidate metabolomic biomarkers of neurocognition in CNS lymphoma, ventricular CSF samples from 14 patients were obtained at baseline pre-treatment and at monthly restaging time points, pre-lenalidomide, during the first 3 months of the study. In addition, for 10 patients, an additional baseline CSF specimen was obtained, after consent, during screening within 21 days of protocol-based therapy. CSF (atraumatic) was immediately processed by centrifugation and supernatants stored at –80°C for metabolomic analysis. Samples were assayed using an adaptation of Gertsman et al.[17]

Samples were analyzed by liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS) (Sciex API 4000) using both positive and negative ionization mode, using specific parent to daughter transitions, individually tailored by infusing the authentic standards and monitored by scheduling them at corresponding retention times.

Metabolite concentrations were calculated using the authentic standard in 6-8 non-zero levels calibration curves within 85%-115% back-calculated accuracies from nominal spanning physiological range concentrations, with 1/x of concentration weight, to compensate for different variance at low concentration, and coefficients of correlation, 0.99, or higher (Supplementary Table 1, Supplementary Figure 1). Quantification was conducted using MultiQuant 2.1 software.

MRI Acquisition and Analysis

Imaging studies were acquired on clinical MR scanners at either 1.5 or 3.0 Tesla field strength. MRI protocols varied between patients and time points, but studies included for image analysis had at a minimum diffusion-weighted imaging (DWI), T1-weighted pre- and post-gadolinium contrast, as well as T2-weighted fluid-attenuated inversion recovery (FLAIR) sequences.

All images were aligned to the T1-weighted post-contrast image. Contrast-enhancing lesion volumes were measured on the T1-weighted post-contrast images. T2 lesion volumes were measured on the T2 FLAIR images. The lesion volumes were defined using semi-automated software (3D Slicer 4; http://www.slicer.org). For DWI data, apparent diffusion coefficient (ADC) maps were generated, and the median voxel values within the T2 lesion on ADC maps were calculated and normalized to the mode of intensities in normal-appearing brain tissue (across the entire cerebrum, excluding the T2 lesion region of interest [ROI]).

Statistics

Clinical and demographic characteristics were summarized using descriptive statistics and Wilcoxon tests were used for comparisons on demographic and cognitive test scores. The associations between neurocognitive outcomes, metabolomic and neuroimaging outcomes were assessed by using the Pearson correlation coefficient (r) as well as Spearman correlation coefficient (ρ). Since each patient has repeated measurement on metabolomic and neuroimaging outcomes, to test for correlation in each biomarker over time with respect to MMSE score, generalized estimating equation (GEE) models[18] were established with biomarker as an independent variable and MMSE score as a dependent variable, adjusting for within-subject repeated measures using an independent correlation structure. To test for differences between the two groups (MMSE score $\geq$27 or $\leq$23) for each biomarker over time, GEE models[18] were established with group as an independent

variable and biomarker as a dependent variable, adjusting for within-subject longitudinal measures using an independent correlation structure. R Package geepack was used in R version 4.0.2.[19]

Results

Clinical characteristics of the 14 subjects in this study are summarized in Supplementary Table 2. Median age was 66 years (range, 47-79), including 5 males and 9 females. Median Eastern Cooperative Oncology Group score was 2 (range, 1-4). The mean baseline MMSE score on study was 25 and over the course of the first 3 months, the scores ranged from 0 to 30. Thirteen patients had large B-cell lymphoma and one had marginal zone lymphoma involving brain parenchyma. Subjects experienced disease progression after a median of 3 treatment regimens (range, 2-7). Twelve had received high-dose methotrexate, five had progressed after non-myeloablative therapy with etoposide/ara-C, and two progressed after autologous stem-cell transplant. Two had received irradiation to the eyes, four had received focal irradiation to brain lesions, and one had received whole-brain irradiation.

Quantitative CSF metabolomic profiling for 18 metabolites was performed using 51 CSF specimens collected at planned clinic visits during which MMSE were also performed. These 51 combined MMSE/CSF collections were matched with baseline and restaging brain MRI studies that were assessable for brain volumetric studies in greater than 80% of the CSF/MMSE time points. Twenty specimens were obtained pre-protocol-based therapy and 31 were obtained after initiation of lenalidomide on protocol. The median interval between brain MRI studies and combined CSF/MMSE time points was 6 days. These data were collected at pre-treatment screening (10 patients), at study baseline (all patients), and at monthly follow-up examinations for up to the first 3 months of study. Overall, a median of four CSF specimens for metabolomic analyses with matched MMSE studies were available per patient.

Using CSF isolated from brain ventricles, we determined the concentration of selected metabolites that are likely integral to the bioenergetics of lymphoma and to normal brain, including lactate, and the TCA (tricarboxylic acid) cycle intermediates succinate, malate, fumarate, citrate, important in mitochondrial bioenergetics,[20] as well as neurotransmitters and metabolites that are engaged in synaptic transmission and neurocognitive function, including gamma-aminobutyric acid (GABA), glutamate, dopamine, L-Dopa, NAAG (N-acetyl-aspartyl-glutamate), NAA (N-acetyl-aspartate), choline, acetylcholine, epinephrine, serotonin, 5-HIAA (5-hydroxyindoleacetic acid), tryptophan, and caffeine (Table 1, Supplementary Table 3).

Lactate, choline, and NAA metabolites commonly detected in the clinical application of proton magnetic resonance spectroscopy used in the diagnostic and prognostic evaluation of malignant brain tumors,[21] were among the most concentrated metabolites detected (Figure 1). The mean CSF concentration of lactate was within normal limits, 2.24 mM, however, concentrations as high as 6.14 mM were detected. To confirm the quantitative accuracy of the LC-ESI-MS/MS approach used in this study, we compared the baseline CSF concentration of lactate in ventricular CSF in this analysis with lactate measurements from the same specimens, using a Beckman Coulter Unicell Dxc 800 Clinical Chemistry Analyzer, routinely applied in hospital-based, clinical decision-making. The correlation between the two methods for lactate determination was highly significant ($P = 2.1E-8$, $r = 0.97$, Pearson correlation) (Supplementary Figure 2).

Table 1 Concentration (μM) of Metabolites and Neurotransmitters in Ventricular CSF in Patients With Relapsed CNS Lymphoma

	Mean	SEM
Lactate	2241	167
GABA	0.20	0.01
Dopamine	0.07	0.01
Succinate	18.15	1.66
Glutamate	1.51	0.10
NAAG	3.09	0.41
ACh	0.13	0.00
L-Dopa	0.22	0.05
Malate	36.75	20.10
NAA	13.19	0.93
Fumarate	3.75	1.60
Citrate	122.6	6.2
5-HIAA	5.53	0.56
Choline	94.65	3.19
Epinephrine	0.03	0.00
Serotonin	0.04	0.00
Tryptophan	0.99	0.20
Caffeine	1.42	0.33

Abbreviations: ACh, acetylcholine; CNS, central nervous system; CSF, central nervous system; GABA, gamma-aminobutyric acid; 5-HIAA, 5-hydroxyindoleacetic acid; NAA, N-acetyl-aspartate; NAAG, N-acetyl-aspartyl-glutamate.

The mean overall CSF concentration of choline in the CNS lymphomas was 94.6 μM (range 41-143 μM), markedly elevated compared to CSF choline measurements obtained from control subjects, patients with movement disorders, medulloblastoma and glioma,[22,23] and thus likely a manifestation of rapid lymphoma growth. The mean CSF concentration of GABA in ventricular CSF from CNS lymphoma patients was slightly higher than concentrations reported in ventricular CSF in patients with intractable depression but lower than concentrations associated with head injury.[24] The mean CSF glutamate concentration in ventricular CSF in CNS lymphoma was markedly reduced compared to the highest glutamate concentrations reported for patients with traumatic brain injury or subarachnoid hemorrhage, in which concentrations may exceed 50 μM, but was similar overall to that reported for control subjects (1.2 ± 0.2 μM). However, for 10 patients,

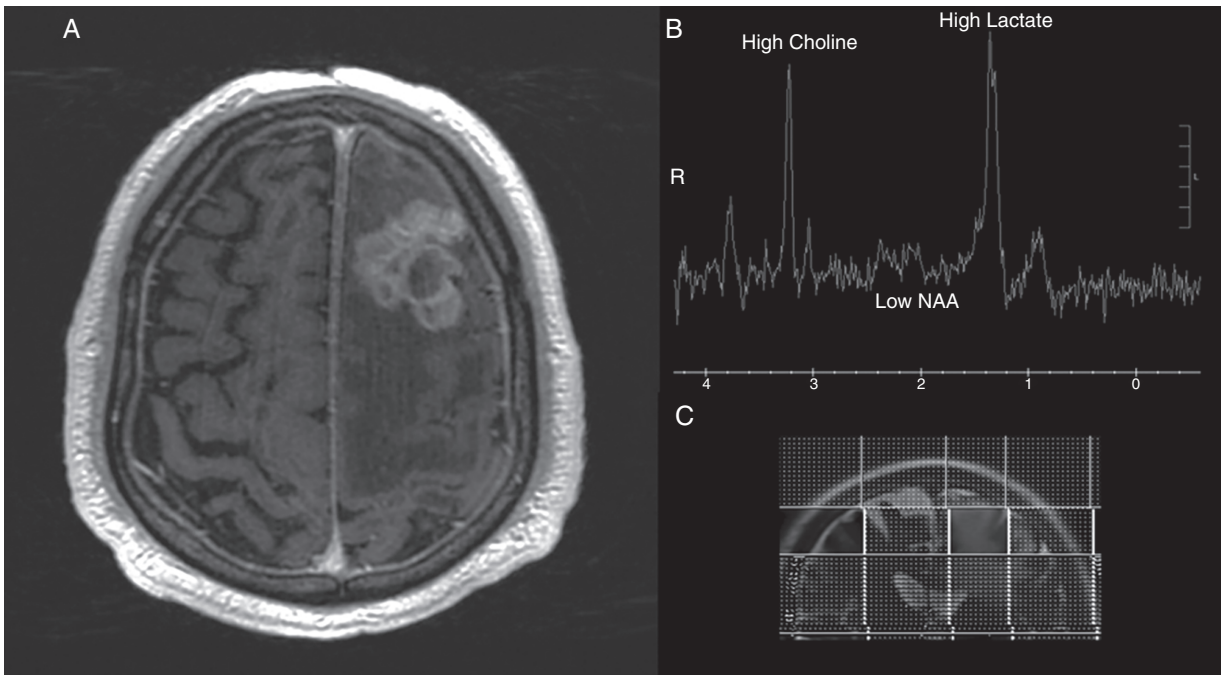

Fig. 1 Example of the application of proton magnetic resonance spectroscopy in the diagnostic evaluation of primary CNS lymphoma at presentation. MRI (T1 axial post-gadolinium) demonstrates a lobular, diffusely enhancing, cortical, and subcortical mass in the left superior frontal gyrus in an 81-year-old patient. Spectroscopy of the tumor demonstrates significantly elevated lactate and choline with suppression of NAA metabolites, a characteristic pattern of metabolites in primary and metastatic brain cancers. Abbreviations: CNS, central nervous system; MRI, magnetic resonance imaging; NAA, N-acetyl aspartate.

the maximum ventricular CSF glutamate concentration measured was greater than the concentration reported for controls and in four CNS lymphoma patients, CSF glutamate concentrations were within the range reported for subjects at between 1 and 10 h after severe head injury[24–26] Dopamine concentrations in CSF in CNS lymphoma were similar to concentrations reported in lumbar CSF previously.[27]

NAA concentrations in CSF in CNS lymphoma were markedly elevated compared to controls and to patients with multiple sclerosis.[28] While NAA is involved in energy metabolism in the brain, it also has roles in the production and maintenance of the myelin sheath, processes that are typically disrupted during the pathogenesis of PCNSL in which lymphomas typically invade along white matter tracts.

We proceeded to analyze potential relationships between relevant clinical parameters and the CSF metabolites in this study. First, we compared the pre-treatment baseline concentrations of the 18 CSF metabolites (N = 20 time points) with the concentrations from post-treatment time points (N = 31) and determined that overall, treatment with lenalidomide had no significant impact on any of the metabolite concentrations. Given that age has been linked both to prognosis as well as to cognitive decline in PCNSL,[12] we evaluated the relationship between CSF concentrations of energy metabolites and neurotransmitters with age as a continuous variable for the 14 subjects. Remarkably, the only metabolite whose concentration correlated with age in CNS lymphoma patients was citrate, an abundant TCA cycle intermediate ($P < .0053$, $\rho = 0.70$, Spearman correlation) (Figure 2).

Next, we determined the correlation between concentrations of the 18 metabolites with cognitive assessments, as determined by MMSE scores measured at baseline evaluations and over the first 3 months of the phase I trial of lenalidomide/rituximab (Figure 3A). We identified a striking correlation between high CSF lactate concentrations and lower MMSE score ($P < 8E\text{-}8$, $\rho = -0.67$, Spearman correlation; $P < .0071$ in GEE model, adjusting within-subject repeated measurement). Conversely, higher CSF concentration of succinate, another TCA cycle intermediate, was associated with higher MMSE score ($P < .0096$, $\rho = 0.36$). In addition, concentrations of dopamine ($P < .0004$, $\rho = -0.48$) and GABA ($P < .007$, $\rho = 0.37$) also were associated with MMSE score. Elevated ratio of glutamate to GABA, a metric associated with multiple psychosocial traits including autism, schizophrenia, alcohol withdrawal,[29–31] also correlated with lower MMSE scores ($P < .016$, $r = -0.34$; $P < .0022$ in GEE model, adjusting within-subject repeated measurement) (Figure 3B). Notably, in considering only the baseline, pre-treatment time point for each patient, the correlations of MMSE score with lactate and ratio of glutamate/GABA were still significant (Figure 3C). CSF lactate, GABA, and succinate concentrations were strongly associated with the extremes of cognitive dysfunction in this

A

Correlation with Age	Spearman p-value	Pearson p-value	Spearman correlation	Pearson correlation
Lactate	0.3701	0.5352	0.260	0.181
GABA	0.997	0.9171	0.001	–0.031
Dopamine	0.6754	0.9532	–0.123	0.017
Succinate	0.6914	0.4494	–0.117	–0.220
Glutamate	0.2328	0.7859	–0.341	–0.080
NAAG	0.1369	0.1681	0.418	0.390
ACh	0.7499	0.4142	–0.094	–0.237
L-Dopa	0.0977	0.8455	–0.460	0.057
Malate	0.9167	0.8504	0.031	0.056
NAA	0.6257	0.6967	–0.143	–0.114
Fumarate	0.8636	0.907	–0.051	–0.034
Citrate	0.0052	0.0148	0.701	0.634
5-HIAA	0.725	0.7118	0.103	0.109
Choline	0.2686	0.1467	0.318	0.409
Epinephrine	0.9936	0.59	–0.002	–0.158
Serotonin	0.2069	0.3912	–0.359	–0.249
Tryptophan	0.8108	0.8502	–0.070	–0.056
Caffeine	0.532	0.0651	–0.183	–0.506

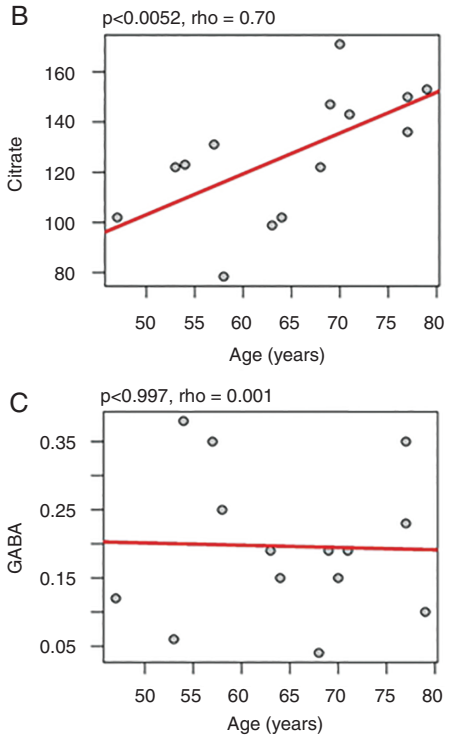

Fig. 2 Correlation of CSF citrate concentrations with increasing age in relapsed CNS lymphoma patients. (A) Of 18 metabolites, measured at the baseline, pre-treatment time point, only CSF citrate correlated with increasing age. (B) Citrate concentrations increased with patient age (range, 47-79 years). Spearman correlation *P* value <.0053, ρ = 0.70, and Pearson correlation *P* value <.015, *r* = 0.63. (C) There was no correlation between CSF, GABA, or other metabolites with age. Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid.

study (MMSE score ≤23) compared to higher scores of ≥27 (Figure 3D).

Next, we assessed clinical features that might be associated with impaired neurocognition in patients on this study. Given the established deleterious impact of brain irradiation on neurocognition in PCNSL,[2,32–34] we evaluated a potential impact of irradiation and compared CSF metabolite concentrations from the five patients that had been previously treated with any type of brain irradiation (four treated with focal brain irradiation plus one patient treated with whole-brain irradiation) vs the nine patients not treated with brain irradiation. Receipt of brain irradiation was associated with lower MMSE score ($P < .02$) and with elevated lactate ($P < .002$) and elevated dopamine ($P < .003$). As expected, patients with active leptomeningeal lymphoma had higher CSF concentrations of lactate ($P < 2E-6$) and lower MMSE scores ($P < 6E-5$) compared to patients without leptomeningeal disease. Nevertheless, the correlation between high CSF lactate and lower MMSE was significant in the subgroup of patients without leptomeningeal lymphoma, supporting an impact of lactate within the brain parenchyma. High lactate was also associated with lower MMSE in both PCNSL ($P < .005$, $\rho = –0.54$) and SCNSL ($P < .008$, $\rho = –0.52$) subgroups.

Given the correlation between CSF lactate and MMSE score (Figure 3), we tested the hypothesis that lactate may impact neurotransmitter metabolism. We determined that high CSF lactate correlates with lower CSF GABA concentration ($P < .0008$, $\rho = –0.46$, Spearman correlation; $P < .026$ in GEE model adjusting within-subject repeated measurement), with higher dopamine ($P < 8.4E-6$, $\rho = 0.58$; $P < .0039$ in GEE model), as well with higher glutamate/GABA ratios ($P < .022$, $\rho = 0.32$; $P < .013$ in GEE model) in CNS lymphoma (Figure 4A). This observation suggests that elevated CSF lactate may impact the enzymatic conversion of the excitotoxic neurotransmitter glutamate to GABA, via glutamic acid decarboxylase (GAD). Interestingly, for 10 patients on study, we observed a time-dependent, reciprocal correlation between CSF concentrations of lactate and GABA that reflected response to therapy (Figure 4B). These results support the hypothesis that perturbations of brain and tumor energy metabolism may reversibly modulate selective neurochemical pathways that impact neurocognitive function.

To control for the possibility that therapy with lenalidomide or lenalidomide/rituximab may impact the relationship between CSF lactate and neurotransmitter metabolism, we performed correlation analyses for the baseline, pre-treatment set of CSF samples, and MMSE scores for day 1 of each of the 14 patients. As above, not only were intrinsic relationships confirmed between high lactate and lower baseline MMSE score ($P < .02$, $r = –0.61$) as well as

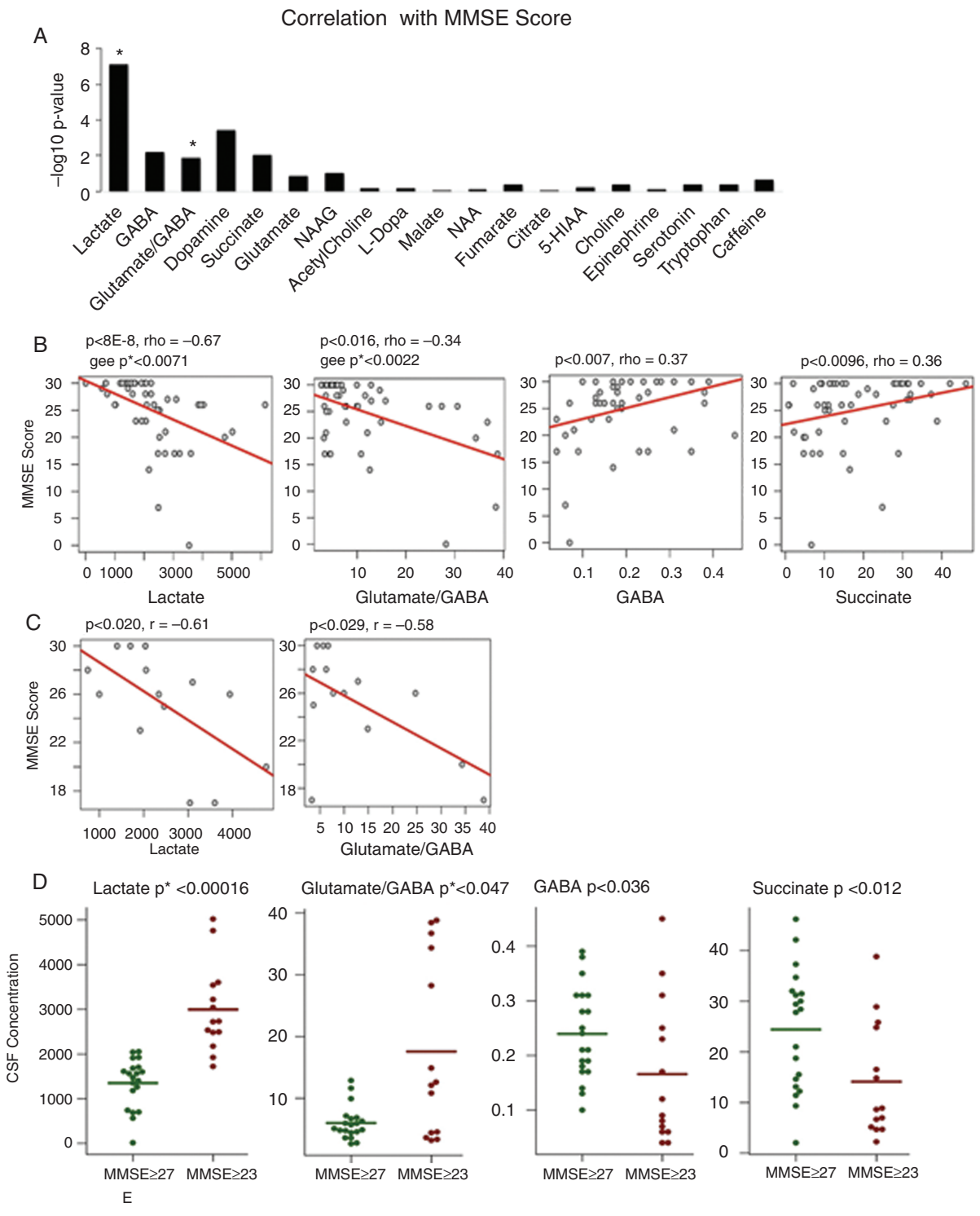

Fig. 3 Correlation of metabolites with mini-mental state examination (MMSE) scores. Mean (±SEM) concentration of metabolites and neurotransmitters in ventricular CSF in patients with relapsed CNS lymphoma. (A) The concentrations of 18 CSF metabolites from 51 collections of ventricular CSF were correlated with paired MMSE scores determined at pre-treatment visits and up to the first 3 months of study. *P* values from Spearman correlation. (B) CSF lactate concentrations strongly correlated with lower MMSE score ($P < 8E\text{-}8$, $\rho = -0.67$). GABA concentrations in CSF positively correlated with MMSE score ($P < .007$, $\rho = 0.37$). Generalized estimating equation (GEE) models were established with biomarker as an independent variable and MMSE score as a dependent variable, adjusting for within-subject repeated measures. Significant GEE *P* values were indicated by *p** for lactate and ratio of glutamate/GABA, while GEE *P* values were not significant for GABA and succinate. (C) CSF lactate

high glutamate/GABA ratio and lower baseline MMSE score ($P < .029$, $r = –0.58$) (Figure 3C) using only the 14 pre-treatment samples, the relationships between high lactate and higher glutamate/GABA ratio ($P < .0033$, $r = 0.73$) and high lactate and dopamine ($P < .045$, $\rho = 0.55$) were significant as well, prior to study therapy (Supplementary Figure 3).

White matter disease, as assessed by quantitative T2 signal measurements on MRI, is reproducibly associated with impaired neurocognitive function in PCNSL. We therefore tested the hypothesis that the CSF concentrations of metabolites associated with MMSE scores might correlate with tumor and brain volumetric parameters of CNS lymphoma that were determined by MRI in serial imaging studies obtained at baseline and over the course of the study (Table 1). We assessed the following imaging parameters: tumor contrast-enhancing volume, T2 volume, T2 mean ADC, and T2 mean FLAIR intensity (Table 2). As expected, of these parameters, T2 signal was associated with neurocognitive impairment by MMSE score ($P < .0036$, $\rho = –0.46$) (Supplementary Figure 4). Lesional contrast-enhancing volume of CNS lymphoma also correlated with MMSE score ($P < .0013$, $\rho = –0.5$) (Supplementary Figure 5). Interestingly, tumor enhancing volume strongly correlated with reduced CSF GABA concentrations ($P < 7.1E\text{-}5$, $\rho = –0.59$), as well as with elevated glutamate/GABA ratio ($P < .0005$, $\rho = 0.53$) and with higher CSF lactate ($P < .0063$, $\rho = 0.43$) (Supplementary Figure 5). T2 volume also correlated with lower GABA concentration ($P < .016$, $\rho = –0.39$), higher glutamate/GABA ($P < .0059$, $\rho = 0.43$) but only trended with CSF lactate ($P < .073$, $\rho = 0.29$) (Supplementary Figure 4). Notably, distinct from CSF lactate, the correlations between baseline neuroimaging parameters with baseline MMSE score, glutamate/GABA ratio, and dopamine were not statistically significant (Supplementary Figures 4 and 5). Neither lesional contrast-enhancing volume nor T2 volume correlated significantly with CSF succinate concentration, supporting a non-tumor origin.

Discussion

To our knowledge, we provide the first quantitative assessment of the landscape of CSF metabolites involving brain bioenergetics as well as neurotransmission in patients with PCNSL and SCNSL. This is also likely the first data evaluating cancer metabolism, neurotransmitter dysregulation, neurocognitive deficits, as assessed by MMSE score, and tumor volumetrics assessed by MRI in a brain tumor patient population. We demonstrate that the correlation between elevated CSF lactate concentration and impaired MMSE test scores is more significant than size of the brain tumor, as quantified by volumetric analysis of tumor T2 signal and lesional contrast-enhancing volume. We provide evidence for significant relationships between lesional size of lymphoma and the CSF concentrations of classical neurotransmitters such as GABA and dopamine, as well as CSF lactate.

Given the relationship between aberrant lactate metabolism and neurocognitive deficits in Alzheimer's dementia and other neurodegenerative conditions, our results suggest overlapping mechanisms of cognitive decline in CNS lymphoma. Of note, isolated "cerebral" lactic acidosis, due to genetic defects in pyruvate metabolism, and without systemic acidosis, has previously been correlated with profound cognitive decline in neonates and young infants.[35]

Lower concentrations of GABA in brain and CSF are associated with cognitive impairment in Alzheimer's disease and with a variety of neuropsychiatric conditions including psychosis, depression, and mania.[36,37] Given the spectrum of neurocognitive deficits in PCNSL, it is logical to speculate that lower GABA concentrations in CSF may contribute to deleterious neurocognitive plus neuropsychiatric complications associated with the pathogenesis of this disease. Lactate-associated imbalances in the glutamate/GABA ratio may also contribute to the induction and spread of seizures and seizure-induced secondary neural injury.

We also confirmed markedly elevated concentrations of NAA in CSF of patients with CNS lymphomas. Notably, elevated NAA in CSF has recently been reported as a biomarker of traumatic brain injury, suggesting overlapping mechanistic features with this disease mechanism as well,[38] particularly with respect to diffuse axonal injury.

Our data suggest that tumor-associated aberrations in GABA metabolism in CNS lymphoma can be reversible, and that pharmacologic antagonism of Warburg metabolism-mediated lactate production, potentially via inhibition of LDH, may contribute to preservation and/or improvement of neurocognitive function. Interestingly, the observation that succinate is associated with favorable cognitive function in this population also supports a novel approach for intervention, as succinate has recently been shown to support oxidative metabolism in astrocytes and macrophage-derived succinate demonstrated to suppress chronic neuroinflammation.[39] Succinate administration has been proposed as a novel treatment for traumatic brain injury,[40,41] a strategy that may also be relevant to CNS lymphoma.

While not linked to neurocognitive impairment, our dataset indicates that citrate concentrations in the CSF correlate with increasing age in our study population. Of note, citrate accumulation has previously been linked to the selective decreased activity of aconitase, a citric acid enzyme that converts citrate to isocitrate, during aging in the housefly. It has been suggested that selected age-related decreases in aconitase activity contribute to diminished efficiency of mitochondrial bioenergetics in flies as well as in

concentrations and ratio of glutamate/GABA at baseline, pre-treatment time point, were strongly correlated with MMSE score. (D) CSF lactate and ratio of glutamate/GABA concentrations were strongly associated with the extremes of cognitive dysfunction in this study (MMSE score ≤23) compared to higher scores of ≥27. *P* values were calculated from 2-sided Wilcoxon test. *p** indicates significant *P* values from GEE models, with group as an independent variable and biomarker as a dependent variable, adjusting for within-subject repeated measures. Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid.

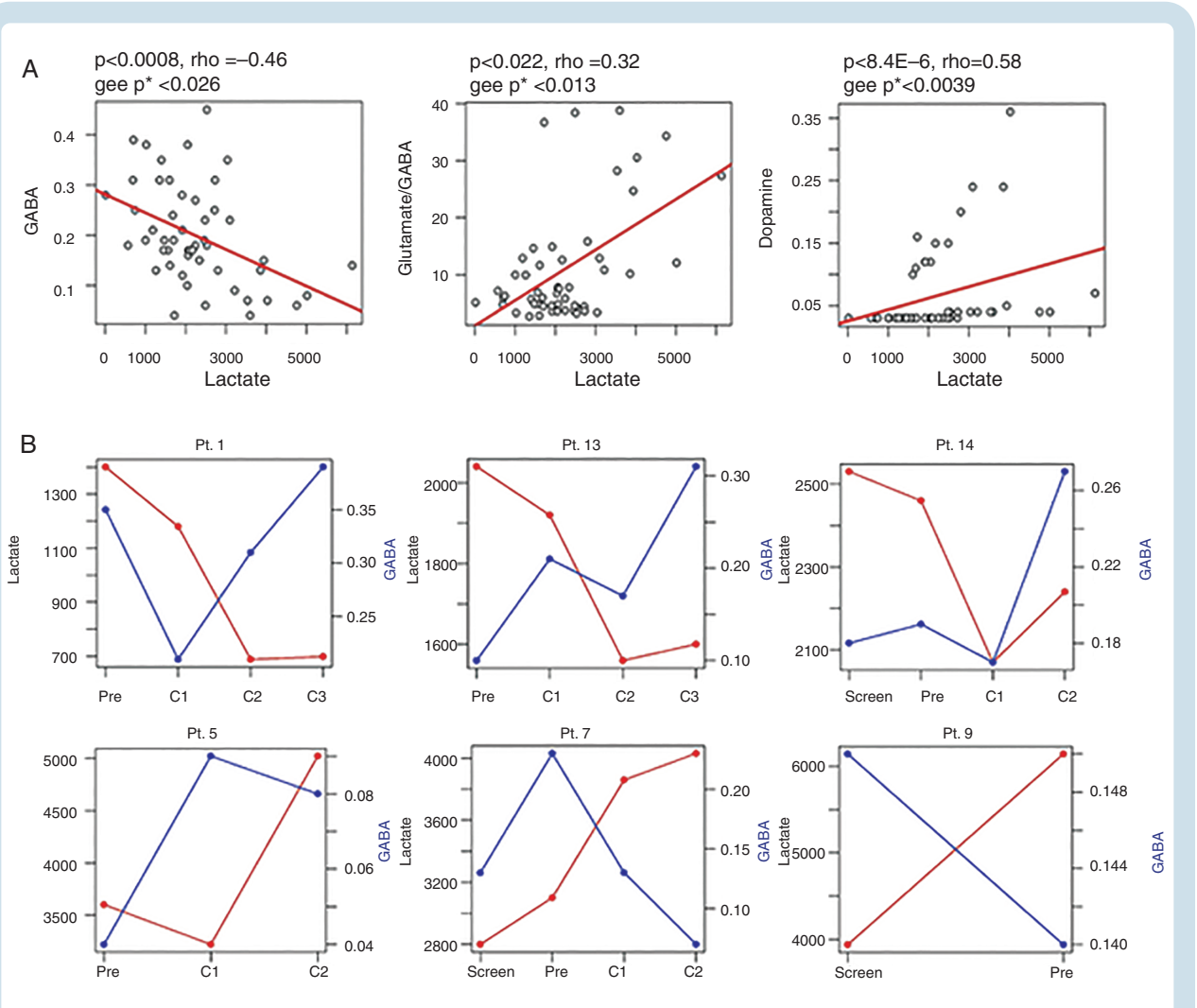

Fig. 4 (A) Correlation of CSF lactate with concentrations of neurotransmitters and succinate. High CSF lactate correlates with lower CSF GABA concentration ($P < .0008$, $\rho = -0.46$) and with higher glutamate/GABA ratios in CNS lymphoma ($P < .022$; $\rho = 0.32$). Lactate also positively correlated with increased dopamine concentration in CSF ($P < 8.4E\text{-}6$, $\rho = 0.58$). Significant P values from GEE model were indicated by p^*. (B) Time-dependent inverse correlations between CSF lactate and CSF GABA. Patient 1 demonstrated stable disease at restaging at Cycles 1 and 2 with lenalidomide. Decreases in lactate were associated with increases in GABA. Patients 13 and 14 responded to lenalidomide. Decreases in lactate were associated with increases in GABA. Patient 5 exhibited initial response to lenalidomide, with a decrease in lactate and increase in GABA at 1 month. After 2 months, lymphoma progression was associated with reciprocal increase in lactate and decrease in GABA. Patient 7 exhibited disease progression at month 2 of lenalidomide. Reciprocal increases in lactate and decreases in GABA were detected. Patient 9 exhibited early disease progression shortly after screening and was removed from the study. Reciprocal increase in lactate and decrease in GABA were detected. Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid.

murine kidney.[42,43] Citrate contributes to histone acetylation events via acetyl-CoA production and thus may promote an epigenetic pathway implicated in Alzheimer's disease.[44]

Notably, single nucleotide polymorphisms involving the dopamine regulation pathway involving catechol-*O*-methyltransferase (COMT) and DRD1, have been recently associated with cognitive impairment in patients with brain tumors, including patients with low- and high-grade gliomas as well as PCNSLs.[45,46]

This study has several limitations, including the small numbers of patients and the limited sensitivity and range of neurocognitive domains that are assessed by the MMSE compared to more detailed neurocognitive testing.[47] Nevertheless, the MMSE is a highly validated screening test for dementia and pre-dementia that is used in studies of brain tumor patients including PCNSL.[2,48–50] We identified a significant and novel correlation between CSF lactate and lower MMSE score as well as with neurotransmitter regulation, evident in both PCNSL as well as SCNSL. We confirmed the anticipated correlations between tumor size on T2 imaging as well as the effects of prior irradiation and lower cognitive scores. The strength of correlations between neuroimaging parameters and MMSE scores in our study are similar to those reported for other neurocognitive endpoints previously.[1] We provide evidence for a relationship between prior irradiation and tumor-associated lactate production.

Table 2 Correlation of MMSE, CSF Lactate, Succinate, GABA, Glutamate/GABA with Tumor Volumetric Analysis

Spearman Correlation *P* value	MMSE Score	Lactate	GABA	Glutamate/ GABA	Dopamine	Succinate	T2 Volume	Enhancing Volume	Mean ADC	Mean T2
MMSE score		**–7.9E-8**	**6.9E-3**	**–0.0147**	**–3.9E-4**	**9.5E-3**	**–3.5E-3**	**–1.2E-3**	0.458	–0.924
Lactate	**–7.9E-8**		**–7.8E-4**	**0.0211**	**8.3E-6**	**–0.0126**	0.072	**6.2E-3**	–0.387	–0.926
GABA	**6.9E-3**	**–7.8E-04**		**–2.9E-13**	**–0.0101**	–0.87	**–0.015**	**–7.0E-5**	0.922	0.356
Glutamate/GABA	**–0.0147**	**0.0211**	**–2.9E-13**		**3.2E-4**	0.59	**0.006**	**4.9E-4**	–0.689	0.667
Dopamine	**–3.9E-4**	**8.3E-6**	**–0.0101**	**3.2E-4**		0.33	**0.049**	**1.1E-3**	–0.245	–0.741
Succinate	**9.5E-3**	**–0.0126**	–0.87	0.59	0.33		0.605	0.580	0.522	–0.123
T2 volume	**–3.5E-3**	0.072	**–0.015**	**0.006**	**0.049**	–0.605		**5.4E-3**	–0.985	0.742
Enhancing volume	**–1.2E-3**	**6.2E-3**	**–7.0E-5**	**4.9E-4**	**1.1E-3**	–0.580	**5.4E-3**		0.555	–0.989
Mean ADC	0.458	–0.387	0.922	–0.689	–0.245	0.522	–0.985	0.555		**0.047**
Mean T2	–0.924	–0.926	0.356	0.667	–0.741	–0.123	0.742	–0.989	**0.047**	

Abbreviations: ADC, apparent diffusion coefficient; CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid; MMSE, mini-mental state examination.
Two-sided Spearman correlation *P* value was calculated for each pair of variables. Bold text indicates significant *P* values ($P < .05$); a positive number indicates a positive correlation and a negative number indicates a negative correlation.

The highly statistically significant overall correlation between lactate and MMSE, relevant to both PCNSL and SCNSL patients on study, remained statistically valid even in assessing solely the baseline pre-treatment cognitive scores in 14 patients. Larger studies are required to assess in greater detail the neurochemical pathways that are different in the CSF in PCNSL vs SCNSL as well as their relationship to more subtle cognitive domains that are not addressed by the MMSE.

These results identify CSF lactate, succinate, GABA, glutamate, dopamine, and others as candidate biomarkers to be implemented in future studies that investigate neurocognitive function in CNS lymphoma and potentially in other types of brain tumors. We anticipate that quantitative assessment of these biomarkers in larger studies that also apply anatomical metabolic neuroimaging plus detailed neuropsychiatric evaluations will provide opportunities to dissect further the mechanistic relationships between bioenergetic pathways, neurochemical imbalances, and neurocognitive dysfunction in PCNSL and SCNSL. Future studies may also evaluate CSF citrate as a potential biomarker associated with normal mitochondrial aging in the CNS. Further elucidation of molecular pathways involving lymphoma and brain metabolism, as well as neurotransmitter imbalance, in studies that apply more comprehensive neurocognitive testing, may facilitate important mechanistic insights. These studies may facilitate the development of interventions that preserve cognitive function in CNS lymphoma and potentially other types of brain tumors.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

Keywords

lymphoma metabolism | neurocognition | neurotransmitter pathways

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