Cerebrospinal fluid neurofilament light chain as a potential prognostic biomarker for leptomeningeal metastasis

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Abstract. The present study aimed to evaluate whether the cerebrospinal fluid (CSF) neurofilament light chain (NfL) is a potential prognostic marker for patients with leptomeningeal metastasis (LM). NfL levels were measured in CSF using a single-molecule array assay. A total of 42 patients with LM who were treated with ventriculo-lumbar perfusion (VLP) chemotherapy and had available stored CSF samples from the lumbar subarachnoid space before VLP chemotherapy were included in the present study, in order to investigate the prognostic value of CSF NfL. The median CSF NfL level in patients with LM was 8.15 ng/ml; 30% of patients who had died at the time of analysis had CSF NfL levels higher than the calculated overall prognostic cut-off value (11 ng/ml). The median overall survival after initiation of VLP chemotherapy was significantly longer in patients with LM and low CSF NfL levels compared with in patients with LM and high CSF NfL levels (P<0.001). The statistical significance remained after adjusting for other known prognostic factors and in a subgroup analysis according to age. In conclusion, CSF NfL could be considered a putative prognostic marker in patients with LM treated with VLP chemotherapy.

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Introduction

Leptomeningeal metastasis (LM), defined as the invasion of metastatic tumour cells into the leptomeninges and cerebrospinal fluid (CSF) of the subarachnoid space, is a clinically devastating condition that occurs in the terminal stage of cancer (1). Approximately 10% of patients with metastatic cancer are diagnosed with LM during the course of disease (2). The longer survival achieved with more effective systemic cancer treatment and the improvement in neuroimaging technologies has led to an increase in the incidence of LM (3,4). Furthermore, several therapeutic advances, such as small molecular weight targeted therapies and immunotherapies, have led to prolonged survival of patients with LM (4-6). Predicting the therapeutic efficacy of active treatment in patients with LM has become increasingly necessary.

Ventriculo-lumbar perfusion (VLP) chemotherapy is a recently introduced treatment approach that alleviates CSF flow disturbances and can aid in prolonging the survival of patients with LM (7-10). Robust prognostic predictors for this population are needed to design personalised therapeutic strategies, and previous studies have reported several potential CSF biomarkers (11,12). However, CSF prognostic biomarkers are yet to become standardised in patients with LM, particularly those treated with VLP chemotherapy.

Neurofilament light chain (NfL) is a cytoskeletal protein that preserves the stability of neurons; NfL levels in the CSF reflect neuroaxonal damage from numerous neurological disorders, which results in neurological disability (13). A reliable quantification of the extent of baseline neuroaxonal damage is reported to be helpful for determining the prognosis in a wide spectrum of neurological disorders (13). However, the prognostic value of CSF NfL levels in LM has yet to be elucidated. This study aimed to evaluate the prognostic performance of CSF NfL in predicting survival in patients with LM treated with VLP chemotherapy.

Patients and methods

Patients. From 2014 to 2021, patients with LM who i) were included in a clinical trial for investigating the optimal perfusion rate of VLP chemotherapy at the National Cancer Center

in Korea (10,14), and ii) had available stored CSF samples obtained from the lumbar subarachnoid space immediately before initiation of VLP chemotherapy were enrolled. Patients with known central nervous system (CNS) disorders, including neurodegenerative and neurovascular diseases, which could result in elevated CSF NfL levels due to neuro-axonal damage, were excluded (13). The diagnosis of LM was made via either the cytological confirmation of malignant cells in the CSF or the presence of magnetic resonance imaging (MRI) features compatible with LM (1). In 42 participants with LM, VLP chemotherapy was administered with a perfusion rate of 15 ml/h, and a daily dose of methotrexate 24 mg (14). All participants completed the induction of VLP chemotherapy as previously described (14).

Methods. NfL levels were analyzed in the reserved CSF samples stored at -80˚C. CSF NfL levels were measured using a single molecular array assay (Simoa). CSF NfL concentrations were measured in duplicate by an independent investigator who was blinded to the clinical information. The mean inter-assay and intra-assay coefficients of variation were 7.4 and 5.3%, respectively.

The primary outcome was overall survival, which was defined as the time from the initiation of VLP chemotherapy to death. The previously known prognostic factors for LM, including Karnofsky performance status (KPS), CSF protein levels, presence of increased intracranial pressure (ICP), prior or concurrent radiotherapy, and prior systemic chemotherapy involving more than three different regimens, were evaluated at the beginning of VLP chemotherapy (1). NfL levels are influenced by neurodegenerative neuro-axonal injury and CSF turn-over reduction due to the aging process; therefore, we performed a sub-analysis with the patients classified according to age group (15).

The Institutional Review Board Committee at the National Cancer Center approved the current study (approval no. NCC2021-0162), and written informed consent was obtained from all participants.

Statistical analyses. The prognostic cut-off level was calculated using the method proposed by Contal and O'Quigley. After the CSF NfL was sorted, it was dichotomized at each threshold and the Q statistic was calculated based on the log-rank test statistics. The final cut-off was defined as the value that maximizes the Q statistics, meaning the maximal difference between the two groups (16). Kaplan-Meier survival curves of patients divided into groups according to CSF NfL cut-off levels are presented and were assessed using the log rank test. The Cox proportional hazards model was used to evaluate the prognostic performance of variables associated with survival, such as CSF NfL, protein levels, KPS, ICP, presence of prior or concurrent radiotherapy, and prior systemic chemotherapy over three different regimens, which were previously described prognostic factors for LM (1). The variables with P<0.2 in univariable analysis were pre-selected, and the final multivariable model was determined using backward selection method with an elimination criterion of P>0.05. The reported P-values are two-sided and statistical significance was defined at P<0.05. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.) and R project software (version 4.1.1).

Table I. Patient demographics (n=42).

Demographic information	Value
Median age at sampling, years (IQR)	55 (46-62)
Female/male (%)	27 (64.3)/15 (35.7)
Median overall survival, days (95% CI)	174 (104-237)
Median survival after initiation of VLP, days (95% CI)	151 (84-201)
Median KPS at the initiation of VLP (IQR)	70 (60-70)
Median ICP at the initiation of VLP (IQR)	200 (150-250)
Median CSF protein levels at the initiation of VLP, mg/dl (IQR)	40 (25-66)
Median CSF NfL levels at the initiation of VLP, ng/ml (IQR)	8.15 (2.11-14.30)
Primary cancer	
Lung (NSCLC + SCLC)	29 (28+1)
Breast	7
Ovarian	4
Melanoma	1
Unknown	1

IQR, interquatile range; CI, confidence interval; VLP, ventriculo-lumbar perfusion; KPS, Karnofsky performance score; ICP, intracranial pressure; CSF, cerebrospinal fluid; NfL, neurofilament light chain; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

Results

Demographics. The characteristics of the 42 patients with LM are summarised in Table I. There were 27 women and 15 men, and the median age at CSF sampling was 55 years. The median KPS at the initiation of VLP chemotherapy was 70. The most commonly observed primary tumour type was lung cancer (n=29, 69%), including 1 small cell lung cancer, followed by breast cancer (n=7, 17%) and ovarian cancer (n=4, 10%).

Prognostic performance of CSF NfL levels in patients with LM. At the time of the analysis, 40 (95%) of the 42 patients with LM treated with VLP chemotherapy died; 12 patients died from CNS-related causes (17), 10 died from systemic as well as CNS cancer progression, and the remaining 18 died from undetermined causes. The median overall survival after diagnosis was 174 days [95% confidence interval (CI): 104-237 days], and the median overall survival after initiation of VLP chemotherapy was 151 days (95% CI: 84-201 days). The median CSF NfL level was 8.15 ng/ml (interquartile range, 2.11-14.30). The estimated overall prognostic cut-off value was 11 ng/ml, and 12 (30%) of 40 patients had CSF NfL levels higher than this cut-off value. The median overall survival after initiation of VLP chemotherapy was longer in LM patients with low CSF NfL levels at the start of VLP chemotherapy than in those with

Table II. Multivariable analysis of the entire cohort and the different age groups [<60 years (n=24), ≥60 years (n=18)].

A, Entire cohort (n= 42)

	Univariable analysis		Multivariable analysis	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
KPS				
≥70	1		1	
<70	2.60 (1.32-5.13)	0.006	2.22 (1.09-4.50)	0.028
ICP				
<200	1			
≥200	1.07 (0.56-2.03)	0.837		
CSF protein, mg/dl				
≤50	1			
>50	1.06 (0.55-2.03)	0.865		
Prior/concurrent RTx				
No	1			
Yes	0.75 (0.39-1.45)	0.391		
NSCLC				
No	1			
Yes	0.48 (0.24-0.93)	0.031		
Number of chemotherapy regimen				
≤3	1			
>3	1.11 (0.58-2.14)	0.750		
NfL, ng/ml				
≤11	1		1	
>11	7.26 (3.10-17.02)	<0.001	6.63 (2.76-15.94)	<0.001

B, <60 years (n=24)

	Univariable analysis		Multivariable analysis	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
KPS				
≥70	1		1	
<70	2.54 (1.04-6.20)	0.041	2.97 (1.14-7.78)	0.027
ICP				
<200	1			
≥200	1.48 (0.64-3.40)	0.360		
CSF protein, mg/dl				
≤50	1			
>50	0.98 (0.38-2.52)	0.962		
Prior/concurrent RTx				
No	1		1	
Yes	0.57 (0.25-1.33)	0.195	0.31 (0.12-0.80)	0.015
NSCLC				
No	1			
Yes	0.51 (0.21-1.23)	0.133		
Number of chemotherapy regimen				
≤3	1			
>3	1.28 (0.55-2.97)	0.570		

Table II. Continued.

B, <60 years (n=24)

	Univariable analysis		Multivariable analysis	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
NfL, ng/ml				
≤7.85	1		1	
>7.85	2.68 (1.02-7.01)	0.045	3.73 (1.32-10.57)	0.013

C, ≥60 years (n=18)

	Univariable analysis		Multivariable analysis	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
KPS				
≥70	1			
<70	3.28 (1.06-10.18)	0.039		
ICP				
<200	1			
≥200	0.68 (0.24-1.96)	0.473		
CSF protein, mg/dl				
≤50	1			
>50	0.90 (0.33-2.50)	0.841		
Prior/concurrent RTx				
No	1			
Yes	0.87 (0.27-2.78)	0.820		
NSCLC				
No	1			
Yes	0.33 (0.11-1.03)	0.055		
Number of chemotherapy regimen				
≤3	1			
>3	1.53 (0.40-5.83)	0.532		
NfL, ng/ml				
≤11	1		1	
>11	24.44 (2.80-213.72)	0.004	24.44 (2.80-213.72)	0.004

HR, hazard ratio; CI, confidence interval; KPS, Karnofsky performance score; ICP, intracranial pressure; CSF, cerebrospinal fluid; RTx, radiotherapy; NSCLC, non-small cell lung cancer; NfL, neurofilament light chain.

high CSF NfL levels [201 (95% CI: 143-334) vs. 56 (95% CI: 29-84) days, P<0.001; Fig. 1A].

In the univariable analysis, CSF NfL >11 ng/ml, KPS <70, and a primary cancer type other than non-small cell lung cancer showed a significant association with unfavourable survival after initiation of VLP chemotherapy (Table IIA). The ICP at the initiation of VLP chemotherapy, CSF protein level >50 mg/dl, presence of prior or concurrent radiation therapy, and systemic chemotherapy involving over three different regimens did not show significant associations. In the multivariable analysis, CSF NfL >11 ng/ml and KPS <70 continued to be significant variables.

We subsequently calculated specific cut-off values according to patient age. In the <60- and ≥60-year subgroups, the prognostic cut-off values were estimated as 7.85 and 11 ng/ml, respectively. Using these cut-off values, the median overall survival after initiation of VLP chemotherapy was longer in LM patients with low CSF NfL levels than in those with high CSF NfL levels in both age groups [213 (95% CI: 60-483) vs. 145 (95% CI: 77-187) days, P=0.037 in the <60 group; 293 (95% CI: 76-361) vs. 54.5 (95% CI: 18-71) days, P<0.001 in the ≥60 group; Fig. 1B and C]. In the multivariable analysis, CSF NfL levels continued to show a significant association with survival in both age groups (Table IIB and C).

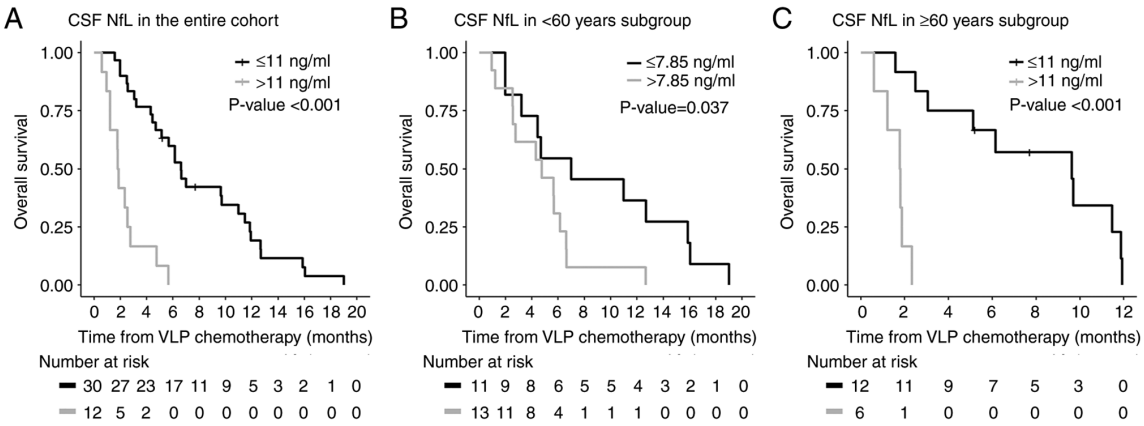

Figure 1. Kaplan-Meier curves for the (A) entire cohort, and (B) <60 and (C) ≥60 years age subgroups. CSF, cerebrospinal fluid; NfL, neurofilament light chain; VLP, ventriculo-lumbar perfusion.

Discussion

The median overall survival after commencement of VLP chemotherapy in LM patients with low CSF NfL levels was longer than that in those with high CSF NfL levels. In the multivariable analysis with diverse potential prognostic factors and sub-analysis of different age groups, statistical significance was maintained. CSF NfL levels at the initiation of VLP chemotherapy may be a potential prognostic factor for predicting overall survival in patients with LM, and it would be helpful to design individualized therapeutic strategies.

In previous studies, the most consistent prognostic factor in LM has been performance status at diagnosis (1). In line with the previous results, clinical performance status represented by the KPS score was a significant prognostic factor in patients with LM from the multivariable analysis of the current study. Prior and/or concurrent radiotherapy was a significant prognostic factor in an age group under 60 years but not significant for those over 60 years, and there was a discrepancy in whether radiotherapy was associated with overall survival in previous studies (1). To the best of our knowledge, this study is the first to demonstrate the prognostic significance of CSF NfL as a quantitative and objective marker in LM.

NfL is a biomarker of acute and chronic neuro-axonal damage, and baseline CSF NfL levels are associated with neurological deterioration and/or survival in various neurological diseases including dementia, amyotrophic lateral sclerosis, and multiple sclerosis (13). Similar to the context of previous reports, in this study, we found that baseline CSF NfL levels are associated with overall survival after treatment in LM. A caveat is that NfL levels are influenced by the aging process; therefore, we needed the cut-off value estimated per individual age groups (15). Given the small sample size in the present study, we could only divide patients into two different age groups; the sub-analysis showed that CSF NfL levels were significant prognostic factors in both age groups. Further large studies including sufficient number of patients from all age groups are required to establish universal standardized age-dependent prognostic cut-off values.

VLP is a recently introduced development in the treatment of LM that can help overcome disruption of CSF flow, found in up to 50% of patients (8-10,18). In order to increase drug delivery but reduce drug toxicity, the VLP method was used, and several previous clinical trials showed the efficacy of VLP chemotherapy, using a small dosage of chemo-agent and slow perfusion rate to improve survival of LM patients (10,14). However, no robust data on the prognostic factors in patients with LM treated with VLP chemotherapy are available. The prognostic value of CSF CYFRA 21-1 has been reported in previous studies (11,12), and herein we observed the prognostic value of CSF NfL in LM. Combining these biomarkers would be helpful in building an integrated prognostic system that aids in making personalised therapeutic decisions. Unfortunately, we could not simultaneously estimate the levels of CSF CYFRA 21-1 because of the shortage of CSF samples in the current study. Additional studies are required, including simultaneous measurement of such biomarkers and their combination with clinical prognostic factors, to establish a more reliable system for predicting the prognosis of LM.

One limitation of this study is that the retrospective design and single referral centre setting may carry the risk of unintentional selection bias. Additionally, we could not analyse the patients with CNS-related causes of death separately because of the small sample size (17). Finally, to demonstrate the universal usefulness of CSF NfL as a prognostic biomarker, external validation in larger LM patient cohorts with individual specific primary tumour types and other therapeutic strategies beyond VLP chemotherapy, including conventional intrathecal chemotherapy or targeted/immunotherapies, is needed.

In conclusion, CSF NfL could be a putative prognostic biomarker in patients with LM undergoing VLP chemotherapy. On the basis of current results, we could move one step forward to predict the therapeutic outcome of LM, which would facilitate the selection of candidates who might benefit most from active treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JWH conceived the study. JWH and EYP confirm the authenticity of all the raw data. JWH, YK, KHK, SHK, EYP, JHY, HY, HSG and HJK interpreted and/or analysed the data. JWH, EYP and HJK wrote the manuscript. JWH, YK, KHK, SHK, EYP, JHY, HY, HSG and HJK revised the manuscript. JWH, HSG and HJK supervised the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Institutional Review Board Committee at the National Cancer Center approved the current study (approval no. NCC2021-0162), and written informed consent was obtained from all participants.

Patient consent for publication

Consent for publication was covered by the written informed consent.

Competing interests

Kim Y, Kim KH, Park EY, Youn JH, Yoo H, Gwak HS report no conflict of interest. Hyun JW has received a grant from the National Cancer Center and the National Research Foundation of Korea. Kim SH has lectured, consulted, and received honoraria from Bayer Schering Pharma, Biogen, Genzyme, Merck Serono, and UCB and received a grant from the National Research Foundation of Korea. Kim HJ received a grant from the National Research Foundation of Korea and research support from Aprilbio and Eisai; received consultancy/speaker fees from Alexion, Aprilbio, Biogen, Celltrion, Daewoong, Eisai, GC Pharma, HanAll BioPharma, MDimune, Merck Serono, Novartis, Roche, Sanofi Genzyme, Teva-Handok, UCB, and Viela Bio; and is a co-editor for the Multiple Sclerosis Journal and an associated editor for the Journal of Clinical Neurology.

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